

**IMPLEMENTATION OF SOME BIOPESTICIDES
IN INTEGRATED PEST MANAGEMENT (IPM)
OF LARCH APHID *ADELGES LARICIS* VALLOT
(*F. ADELGIDAE* – *HOMOPTERA*) AND THE HORSE
CHESTNUT LEAF MINER *CAMERARIA OHRIDELLA*
DESCH & DIMIC (*F. GRACILLARIIDAE* –
LEPIDOPTERA)**

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ABSTRACT - The larch wooly adelgid *Adelges laricis* is an important pest due to his attacks, especially against the nurseries and young trees and the horse chestnut leaf miner *Cameraria ohridella* which attacks the leaves of the *Aesculus hippocastanum* L., that produces trees defoliation, there growth slowing down and finally there drying. In the management of the larch wooly adelgid, in the lab tests, the microbial products Laser 240 SC and Milbeknock and the vegetal one NeemAzal T/S, had a high efficacy against the young larvae of *Adelges laricis* (98-100%) and more reduced against the older larvae (60-98%). The product Laser 240 SC, that acts more by ingestion as by contact, has lesser efficacies against older larvae. In the experiments on trees all bio pesticides had high efficacies. It is recommended the application of the treatments at the beginning of the infestation when the young larvae appear. In the management of the chestnut tree leaf miner

Cameraria ohridella the use of Laser 240 SC and NeemAzal T/S by spraying, the leaves attack was between 20-30%, comparatively with 80% for the untreated witness. Because they are 3-4 generations/year, the treatments must be fulfilled in the eggs laying periods and during the neonate larvae apparition, in order to obtain high efficacies. The treatments by painting with NeemAzal T/S reduce the mining larvae number and also the attack's degree on the leaves. After 35 days from the treatment its take place a rising of the attack due to the pressure of the next generation therefore another treatment is necessary. The treatment on the bark is recommended for the residential areas and parks because the spraying treatments are harmful for the mean's health.

Key words: Bio pesticides; Azadyrachtin; Spinosad; Milbecmectin; Larch wooly adelgid; Chestnut tree leaf mine.

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REZUMAT – Implementarea unor pesticide în managementul integrat (IPM) al păduchelui laricii (*Adelges laricis* Vallot (F. *Adelgidae* – *Homoptera*) și al moliei miniere a castanului (*Cameraria ohridella* Desch. & Dimic (F. *Gracillariidae* – *Lepidoptera*)). Păduchele laricii (*Adelges laricis*) este un important dăunător, prin atacurile produse, în special, în pepiniere și pe arborii tineri, iar molia minieră (*Cameraria ohridella*), care atacă frunzele castanului ornamental (*Aesculus hippocastanum* L., cauzează defolierea arborilor, încetinirea creșterilor și, în final, uscarea acestora. În controlul păduchelui laricii, în testarea de laborator, produsele microbiene Laser 240 SC, Milbeknock și vegetal NeemAzal T/S au avut o eficacitate ridicată asupra larvelor tinere de păduche (98-100%) și mai redusă asupra larvelor în vârstă (60-98%). Biopesticidul Laser 240 SC, care acționează mai mult prin ingestie decât prin contact, a avut eficacități mai reduse asupra larvelor în vârstă. În experimentările pe arbori, toate biopesticidele au avut eficacități ridicate. Se recomandă aplicarea tratamentelor la începutul infestării, la apariția larvelor tinere. În controlul moliei castanului (*Cameraria ohridella*), prin aplicarea stropirilor cu biopesticidele Laser 240 SC și NeemAZAL T/S, gradul de atac la frunze produs de minările omizilor a fost cuprins între 20 și 30%, comparativ cu varianta netratată, de 80%. Având în vedere numărul de 3-4 generații pe an, se impune aplicarea unor tratamente la depunerile masive de ouă și la apariția larvelor neonate, când pot acționa mai eficient. Aplicarea tratamentului prin pensulare cu NeemAzal T/S a redus numărul de larve care minează și gradul de atac pe frunze. După 35 de zile de la tratament a avut loc o creștere a gradului de atac, datorită presiunii generației următoare, ceea ce a necesitat reluarea tratamentului. Aplicarea tratamentului pe scoarță se recomandă la arborii ornamentalii din zonele rezidențiale, parcuri, unde, prin aplicarea

tratamentelor foliare, este afectată sănătatea oamenilor.

Cuvinte cheie: biopesticide; azadirachtin; spinosad; milbecmectin; păduchele laricii; molia minieră a castanului.

INTRODUCTION

The decrease of the abusive use of chemical insecticides imposes the development of other aspects of the integrated pest management by using prevention measures and - as repressive methods - the use of biological methods. Here we can count on the “bio-rational” insecticides with a reduced harmfully impact against the non-targeted organisms and against the environment.

The Larch wooly adelgid *Adelges laricis* is an important pest due to his attacks, especially against the nurseries and young trees. In the Suceava County he produces strong attacks that affects the annual growths due to the premature loose of needles.

The horse chestnut leaf miner *Cameraria ohridella* which attacks the leaves of the *Aesculus hippocastanum* L., appeared for the first time in Europe in 1984, in Macedonia. It appears in the Banat area in 1996 and now it is spreaded in all Romania (Perju, Oltean, 2001; Perju *et al.*, 2003). Due to the presence of 3-4 generations and the lack of entomofagous insects, on one single foliole there can be identified 40-50 galleries, that produces trees defoliation, there growth slowing down and finally there drying. In the

IPM there are used synthetic pyrethroids, neonicotinoides, and a.o. The treatments applied in the parks are pollutants and affects the entomofagous fauna.

The paper presents some characteristics of the bio pesticides used in the experiments. Spinosad is a secondary metabolite produced 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 to its action mode, the low toxicity against useful insects, animals and environment. Spinosad links itself 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). Its 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 translocates, 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, 1997, 2000).

Azadirachtines – 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. Its efficacy was tested against more than 110 insects; for 75% of them the control was successful. It controls sucking insects and acarids due to its systemic effects. The azadirachtines degrade 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 in concentration of 0,5% has a good efficacy – 91.7-96.5%, after 7 days, in the control of aphides *Brevicoryne brassicae* (cabbage), *Macrosiphon euphorbiae* (potato), *Aulocorthum solani* (tomatoes) and the beetle *Leptinotarsa decemlineata* (potato) (Călin *et al.*, 2004). 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 azadirachtin has destroyed the eggs and larvae of miner moth

(*Leucoptera coffella*) from the coffee leaves (Venzon *et al.*, 2004).

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 the larch wooly adelgid and the horse chestnut leaf miner, namely: Laser 240 SC (spinosad), NeemAzal T-S (azadirachtin), Milbeknock (milbecmectin), in order to replace the chemical insecticides.

MATERIALS AND METHOD

The researches on the larch wooly adelgid were fulfilled in the forest range Marginea; on the leaves there were larvae of all ages and from there appeared parthenogenetic females with or without wings. On each needle there were 2-3

larvae, in one macroblast there were approximately 90 eggs and larvae. In the lab the tests were fulfilled in growth jars, each variant in three repetitions; the stems were sprayed with a small pump. Periodically the observations were fulfilled by analyzing 10 macroblasts with a stereomicroscope. The treatment was performed in 9.05.2007. The efficacy (E %) was calculated with the Schneider-Orelli formula, according to the mortality percentage. The treatments on the tree were performed with a Kioritz device that sprayed the solution until 10 m of height. For each variant the treatment were applied on three trees. Periodically, in order to observe the infestation and mortality, stems were harvested and studied with the stereomicroscope. The majority of the larvae were of first ages.

Against *Cameraria ohridella*, the treatment by spraying were applied in 3.07.2007 with a portable pump when in the leaves there were mature larvae, pupae and the fly of the first generation adults was intense. The larvae mortality and the degree of attack on leaves were analyzed. In the year 2008, at the recommendation of the **Trifolio M** firm from Germany, the NeemAzal T/S bio pesticide were applied by painting on the tree's trunk; the doses were established according to the trunk's diameter. The treatment was applied during the maximal flight of the first generation (2.06.2008). The advantage of this method is that applied in parks or residential areas affects less the habitants which are allergic to insecticides.

RESULTS AND DISCUSSION

Adelges laricis. The fundatrix females hibernates at the basis of the spruce needles and in the spring they lay eggs; the larvae produces galls

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(1.5 - 3 cm) at the end of June – July they appears the winged females that colonizes the larch trees. The larvae from the parthenogenetic generations sucks the needles in the middle, bend them, and the needles become gray and wither. On the larch and also on the spruce the larvae produce the debilitation and withering of the

spruces and also the slowing down of the growths (*Fig. 1*). The insect attack combined with the one of fungus produces the needles fall (Skarmoutsos and Mullar, 2007) and are considered important pest in China (Sanyang, Shanchun, 1997) and in the UK where the larch tree can be destroyed (Strouts, 2003).



Attack produced by larvae on larch macro blasts



Galls produces by fundatrix females on spruce



Larva



Eggs laid by the aptere female

Figure 1 - Attack produced by *Adelges laricis* on the larch needles and galls on spruce (original)

In the lab, from the first day after the treatment we found a high mortality at all the used bio pesticides (*Table 1*). We must mention that Laser 240 SC and NeemAzal T/S acts better by ingestion than contact. In these conditions, high mortalities are

observed to young larvae that feed intensively than to older ones. Milbeknock acts well by contact too, also affecting the non-feeding stages. After five days from the treatment, in the growth jars, they appeared 3-5% winged adults.

Table 1 - Efficacy (E%) of some bio pesticides against the larch wooly adelgid *Adelges laricis*, 2007

Variant	Concentration %	1 day		3 days		5 days	
		Young larvae	Older larvae	Young larvae	Older larvae	Young larvae	Older larvae
Laser 240 SC	0.016	90	51	95	55	98	60
Laser 240 SC	0.033	92	50	95	60	100	65
NeemAzal T/S	0.41	97	70	100	95	100	95
NeemAzal T/S	0.83	97	90	98	100	100	93
Milbeknock	0.075	100	90	100	90	100	98
Untreated witness		0	0	0	0	0	0

All the products presented a high efficacy against young larch wooly adelgid larvae (98-100%), but more reduced against the older ones that stopped the feeding (60-98%); the lowest efficacy against mature larvae was observed at Laser 240 SC. Other references indicates an insignificantly activity of spinosad against sucking insects, excepting the trips (Bret, 1997; Thompson, 2000) but in our experiments we observed high

efficacies against young larvae. The NeemAzal T/S product presented high efficacies even at lower dose (0.41 %), thus it is more economical. In order to obtain a higher efficacy it is recommended to apply the treatments by lesser infestations.

Considering the foliar treatments of the trees we obtained high efficacies for all the tree products' facts that make them useful in the management of that pest (*Table 2*).

Table 2 - Efficacy of some bio pesticides against the larch wooly adelgid *Adelgis laricis*, (forest range Marginea, Suceava county)

Variant	Concentration %	E%, after number of days:	
		5 days (21.05.2007)	12 days (28.05.2007)
Laser 240 SC	0.033	100	100
Milbeknock	0.075	100	100
NeemAzal T/S	0.37	100	95
Untreated witness	-	-	-

Cameraria ohridella. In 2007 we applied the products by tree spraying; after 10 days the larvae mortality was 16% for Laser 240 SC and 25% for NeemAzal T/S. After 20 days the second product presented a higher mortality (*Table 3*). The

mortalities were small, even for the Neem product, because the older larvae are more resistant.

Concerning the efficacy of those products we can observe that after 20 days from the treatment, although the larvae mortality from the

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mesophyllum is not very high, the intensity of the leaf attack is very reduced, according to the mining area. The treatments applied during the eggs laying influenced the neonate larvae in the leaves mesophyllum, due to the semi-systemic character and the digestive action of the products so that, face to the untreated

witness which presented almost mature larvae (23.07.2007), the treated variants presented only young larvae with a slow development and a reduced number of galleries in the leaves. The attack's degree of the leaves were 20-30% at the treated variants, face to 80% for the untreated witness.

Table 3 - Efficacy of some bio pesticides applied onto the horse chestnut trees against the leaf miner *Cameraria orhridella*

Variant	Concentration	Mortality (%) after number of days		
		10	20	G.A.% on leaves
Laser 240 SC	0.033	16	35.1	30
NeemAzal T/S	0,5	25	70	20
Untreated witness	-	-	-	80

Those bio pesticides can be used in the management of the chestnut tree leaf miner in the urban areas without impacts against man and environment but they must be applied during the eggs lying period, especially by the neonate larvae hatching, before the leaves mining.

In 2008, NeemAzal T/S was applied by painting on the tree's trunk. Analyzing five leaves from each tree, after 8 and 18 days from the treatment date, we observed a reduced number of caterpillars by the treated trees, face to the untreated ones. For the first NeemAzal T/S concentration we found, in average, 3.4 caterpillars / leaf, for the second concentration 5.4 caterpillars / leaf and 24.7 caterpillars/leaf for the untreated trees. After 18 days there were 3.8, 11 and 45.8 caterpillars / leaf, respectively. This situation was also

reflected in the attack's degree that was between 8.6-11% for the treated variants and 35% for the untreated witness, after 32 days from the treatment (*Table 4*).

It can be estimated that the treatment applied by painting reduced the leaves infestation with caterpillars but, after 35 days, the attack's degree is rising due to the pressure of the second generation of butterflies. In order to protect the leaves system it is also necessary the application of the second treatment. The Chinese literature mentions that injecting imidaclopride in the trunk the effect remains for more years (Buszko, 2006). The fight against larvae was also fulfilled with chitin inhibitors (diflubenzurone, triflumurone) (Blümel and Hausdorf, 1997; Krehan, 1997).

Table 4 - The efficacy of NeemAzal T-S (azadirachtin 1%) in the management of the leaf miner *Cameraria ohridella*

Variant	Dose/ 10 cm diameter	Infestation with larvae/leaf after number of days and G.A.%				
		Before the treatment	8 days (10.06)	18 days (20.06.)	G.A.%, (20.06.)	GA%, (4.07.)
NeemAzal T/S	25 ml	0.45	3.4	3.8	2	8.6 ^{***}
NeemAzal T/S	40 ml	1.5	5.4	11	2.5	11 ^{***}
Untreated	-	3.2	24.7	45.8	17.5	35

Anova Test $P < 0,05\%$

At least in our country the recommendations in the chestnut tree leaf miner management refers only to conventional insecticides (pyrethroids and neonicotinoides), that presents well known side effects against the useful insects (Perju *et al.*, 2003).

Those researches for fighting against the chestnut tree leaf miner are attempts to use bio products with secondary metabolites from microbes and plants with a lesser side impact against the urban microclimate, the useful organisms and the man.

CONCLUSIONS

In the management of the larch wooly adelgid *Adelges laricis*, in the lab tests, the microbial products Laser 240 SC and Milbeknock and the vegetal one NeemAzal T/S, had a high efficacy against the young larvae of *Adelges laricis* (98-100%) and more reduced against the older larvae (60-98%). In the experiments on trees all bio pesticides had high efficacies.

In the management of the chestnut tree leaf miner *Cameraria ohridella* the use of Laser 240 SC and

NeemAzal T/S by spraying, the leaves attack was between 20-30%, comparatively with 80% for the untreated witness. The treatments by painting with NeemAzal T/S reduce the mining larvae number and also the attack's degree on the leaves. After 35 days from the treatment its take place a rising of the attack due to the pressure of the next generation therefore another treatment is necessary.

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