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Full Length Research Paper

Biological efficacy of the ecotoxically favourable insecticides and their mixture in the control of gypsy moth

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Forest certification is one of the ways of adapting forestry to modern ideas of "sustainable management", by meeting demands of Forest Stewardship Council (FSC) standard. By joining this process, Serbia obligated to follow FSC pesticides policy. Almost all insecticides registered in Serbia and applied for control of the most dangerous outbreaking defoliating species in forests, are on the list of prohibited ones. In certificated forest ecosystems in Serbia, microbiological insecticides (active ingredient: Spores and protein-crystals of the bacterium *Bacillus thuringiensis* ssp. *kurstaki*) are most widely used pesticides for the control of one of the most important economically harmful defoliators – gypsy moths, in progragation phase, when the number of pests is relatively small. When the number is greater, it is assumed that the so-called "soft" ecotoxicologically favourable preparations Avaunt®, Alverde®, Coragen®, registered for application in agriculture, but not in forestry can be used for inhibition of multiplication. Studies of biological efficacy of the aforementioned preparations and their mixtures with biological insecticide Foray® showed that they have preconditions for application in forest ecosystems. The high biologic efficacy, mechanism of action, resistance to water rinsing, high selectivity, and small quantities of application, anticipated a bright future for them. Since results of researches of biological efficacy of insecticides in laboratory and field conditions are statistically different, studies done in natural conditions should be favored.

Key words: Insecticides, gypsy moth larvae, efficacy, validity of different research methods.

INTRODUCTION

The sudden growth of the world population, accelerated industrialization and urbanization, impose the need of thinking about the forest resources and forest management in a completely new way. Forest certification is one of the most rapidly developed concepts which can be used for adapting forestry to modern ideas of "sustainable management", by meeting the demands of FSC Standards. In Serbian forestry, certification based on the principles of the sustainable forest management was adopted in 2006. During the preparation of it, the FSC Criteria (FSC-STD-01-001, 2004), relating to pesticide use (Criterion 6.6, 10.7, 10.8), proved to be particularly interesting. FSC has a list of chemicals that are prohibited. A company applying for certification would normally have to stop using these chemicals before it can receive an FSC certificate.

In Serbia, broad-leaf forests which are particularly endangered by the outbreaking insect species from the group of defoliators, cover 1988800 ha, or 88.3% of the area covered by forests (Bankovic et al., 2009) and almost all previously registered and applied chemical insecticides are on the list of prohibited ones. First, this paper was aimed at the study of the biological efficacy of the insecticides which are ecologically acceptable, and based on FSC's Criteria for Chemical Pesticides, permitted to be used in certificated forests. Microbiological insecticides, which are used for the control of the most economically harmful insect species in Serbian forests – gypsy moth, show the best efficacy on the younger larval

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Table 1. Doses of the applied preparations used in the biological efficacy tests.

Code	Name	Dose
A_1	distilled water	1000 L/ha
A_2	Foray® 48B	3000 ml/ha Foray + water up to 1000 L/ha
A_3	Wormox® SC	2000 ml/ha Wormox + water up to 1000 L/ha
A_4	Dimilin® SC-48	150 ml /ha Dimilin + 850 ml/ha white oil + water up to 1000 L/ha
A_5	Avaunt® 15 SC	250 ml /ha Avaunt + 750 ml/ha white oil + water up to 1000 L/ha
A_6	Coragen® 20 SC	200 ml /ha Coragen + 800 ml/ha white oil + water up to 1000 L/ha
A_7	Alverde® 240 SC	100 ml /ha Alverde + 900 ml/ha white oil + water up to 1000 L/ha
A_8	Foray® 48B + 10% Alverde® 240 SC	3000 ml /ha Foray + 10 ml/ha Alverde + water up to 1000 L/ha
A_9	Forey® 48B + 10% Coragen® 20 SC	3000 ml /ha Foray + 20 ml/ha Coragen + water up to 1000 L/ha
A_{10}	Forey® 48B + 10% Avaunt® 15 SC	3000 ml /ha Foray + 25 ml/ha Avaunt + water up to 1000 L/ha

A – insecticides.

instars (L_1 and L_2). The older instars require higher lethal doses, so very often the applied rates of the preparation cause sublethal effects (Tabakovic-Tosic, 2005). It is hypothesized that the earlier stated problem can be satisfactorily solved by using the synergetic effect of microbiological and chemical insecticides.

The study of the biological efficacy in forestry, by contrast to the same researches in agriculture, is faced up with many difficulties and problems, the biggest of which lies in the fact that, in the natural conditions it is only possible when the outbreak of the target species occurs on a huge area of land. Therefore, the last aim of these researches was to check whether there was statistically significant difference in the achieved biological efficacy, if it is studied in the laboratory or natural conditions, and whether these differences occurred; if the insecticide was applied on synthetic or natural food of the target insect species in the experiment.

MATERIALS AND METHODS

The main characteristics of the observed insecticides

During the period of 2009 to 2010, the biological efficacy of the two biological (Foray® 48 B, Wormox®) and four chemical insecticides (Dimilin® SC-48, Avaunt® 15 SC, Coragen® 20 SC, Alverde® 240 SC) and their mixtures, were tested in the laboratory and field conditions.

Based on the technical documentation, Wormox® and Foray® 48 B potencies are 16.000 and 10.600 IU/mg. They are highly-selective microbiological insecticides active by ingestion. Their pathogenicity is based on the character of *Bacillus thuringiensis* ssp. *kurstaki* to produce crystalline proteins with toxic effects (causing the host toxicosis and septicaemia). Dimilin® SC-48 (active ingredient – diflubenzuron), Avaunt® 15 SC (active ingredient – indoxacarb), Coragen® 20 SC (active ingredient - chlorantraniliprole) and Alverde® 240 SC (active ingredient - metaflumizone) are modern chemical non-systemic pesticides of the third generation, active by ingestion, less by contact.

Dimilin is a restricted use pesticide due to its toxicity to aquatic invertebrate animals. Treatment of susceptible larvae with this insecticide generally results in an inability to moult. The larvae are unable to escape from the exuviae and often lethally injure the weak new cuticle in the attempt. Even when moulting is successful, they usually die soon afterwards. Indoxacarb and metaflumizone inhibit the sodium ions entry into nerve cells, which paralyze the larvae and cause the cessation of feeding and insect death. This mode of action requires no metabolism for toxicity to target insects. Chlorantraniliprole is a diamide insecticide. The mode of action of chlorantraniliprole is the activation of insect ryanodine receptors. This activation stimulates the release of calcium from the internal stores of smooth and striated muscle which causes impaired muscle regulation, paralysis and insect death. Chemical insecticides Avaunt® 15 SC, Coragen® 20 SC and Alverde® 240 SC are highly potent and active at low rates on target species, such as Lepidoptera and some Coleoptera and Diptera too.

Studies of biological efficacy of insecticides on the gypsy moth

The laboratory experiment on biological efficacy of pesticides presented in Table 1 was established in the two-year-period (2009 to 2010), during the third larval instar of the gypsy moth. From the beginning of feeding till the end of the experiment, the caterpillars were fed with the natural (Pedunculate oak leaves) and synthetic food (Gypsy Moth Diet produced by MP Biomedicals, LLC – Aurora, Ohio, USA), but from the third instar, the food was shortly soaked in water solutions of the analyzed doses of the preparation. During the experiment, temperature and light conditions were constant (temperature 21°C, light regime 14/10 h day/night). The potency was controlled 72, 144 and 216 h after the establishment of the experiment.

The studies of the biological efficacy were conducted in May 2009 and 2010, on one site, in the nursery for the production of the forest seedling material, on ten-year-old Pedunculate oak seedlings where over the previous five-year-period no agricultural-technical measures, regarding the chemical control of the economically harmful insect species, had been applied. By the detailed survey of the site, the presence of gypsy moths was not reported, so the method of infestation of larvae (15 individuals) was applied on some branches, after the application of the preparations on the whole. Prevent migration, as well as the predator and parasitoid effect on their mortality, the input groups were protected with sacks made of dense tulle. Given the fact that the efficacy of the application of pesticides in natural conditions to a large extent depends on the meteorological conditions, the lowest, highest and mean daily temperatures and the relative air humidity for May 2009

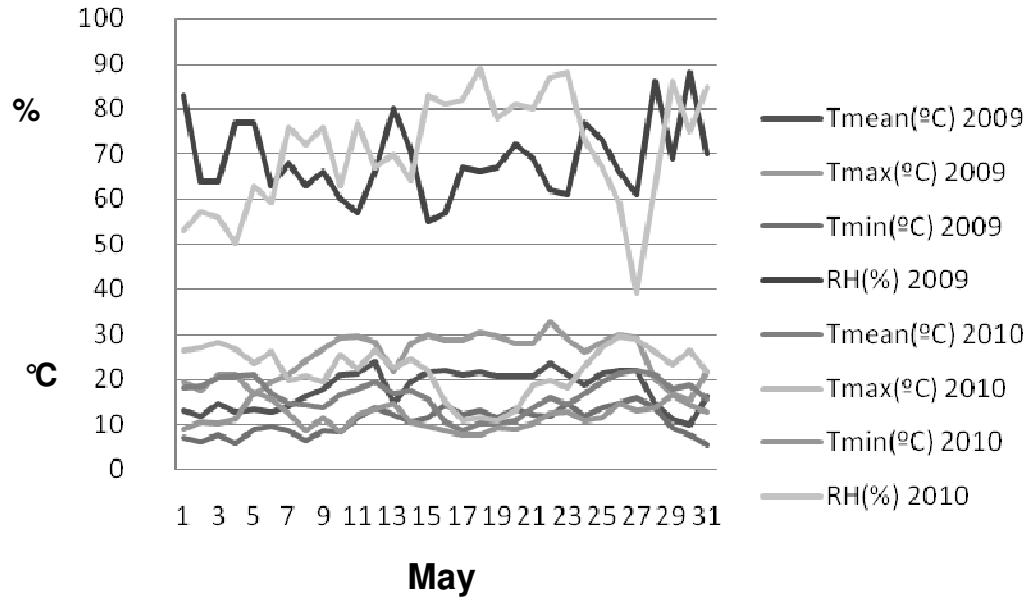

Figure 1. Meteorological data.

and 2010 (Figure 1) are presented. The data are obtained from the Republic Hydrometeorological Service of Serbia and refer to the weather station which is located close to the nursery (The City of Kragujevac). During the assessment of the biological efficacy, Abbott's formula (Abbott, 1925) was used since there was the constant number of the inviduals of the target insect in some elementary units and repetations.

Statistical analysis of the experiment

The factorial experiments (plans of the experiment in which two or more factors are introduced at the same time with all combinations, and each factor have two or more treatments, that is levels of observation) was used for this study. The factorial combinations were repeated in the blocks or Latin squares. They are frequently used in the biological researches, and particularly in agriculture and forestry (Jeffers, 1960; Hadzivukovic, 1977). The experiment was set up in order to study the biological efficacy of the selected insecticides and their mixtures which had three factors with the different number of treatments, repeated in four blocks (repetitions):

Factor A – preparation (A_1 to A_{10} from Table 1);
Factor B – type of food (B_1 – synthetic food in laboratory conditions, B_2 - Pedunculate oak leaves in laboratory conditions, B_3 - Pedunculate oak leaves in natural conditions);
Factor C – exposure of the larvae to the harmful effect of the preparations (C_1 - 72 h, C_2 - 144 h, C_3 – 216 h).

The experiment which was set, is the combination of the qualitative (insecticides, type of food) and quantitative (exposures) treatments and can be abbreviated as 10 x 3 x 3 treatments. The research lasted for two years, and the number of survived gypsy moth larvae, that is of their combinations was registered upon the application of the aforementioned treatments. The statistical analysis of the factorial experiment was conducted in the laboratory of the Institute of Forestry in Belgrade. The program STATGRAPHICS, version 5.0, was used. By dividing the total sums of the squares of variance of the observed characteristic into the sums of the squares of the treatment variance, that is their combinations and the sums of the squares of the experiment error, the direct influence of the observed factors and treatments (A, B, C) on the survival of the larvae was determined, as well as the influence of their interaction of the first (AB, AC, BC) and second levels (ABC). F-test was used for the assessment of the statistical importance of these influences, t-test for the assessment of the statistical significance of the differences between the treatment mean rates, that is the least significant difference (LSD) and Duncan's multiple range test (Hadzivukovic, 1991).

RESULTS AND DISCUSSION

During the period of 2009 to 2010, the biological efficacy of the biological (Foray®, Wormox®) and modern (third generation) chemical insecticides (Dimilin®, Avaunt®, Alverde®, Coragen®) and their mixtures, was studied in the Institute of Forestry in Belgrade, in the aim of their registration for use in the certificated forests in Serbia. The study results are presented in Table 2. During the first assessment (C_1), the following total mean values of biological efficacy (from the highest to the lowest one) of some preparations were registered: Foray® - 62.08%; Foray® + 10% Alverde® - 60,39%; Wormox® - 56.77%; Forey® + 10% Coragen® - 54.88%; Forey® + 10% Avaunt® - 49.92%; Avaunt® - 43.54%; Coragen® - 37.36%; Dimilin® - 34.52%; and Alverde® - 34.49%. Individually, the best results (96.59%) were achieved by the biological insecticide Foray® during the phase in which the experiment was conducted under laboratory conditions when the preparation was applied on the natural food of the gypsy moth. The insecticide Alverde® had the lowest efficiency (7.61%) when the caterpillars fed on the treated synthetic food (Table 2).

With respect to factor B – type of food, the highest

Table 2. Biological efficacy of the tested insecticides and their mixtures in the control of the third larval instar of the gypsy moth (for the period 2009 to 2010).

Combination of treatment			Average number of alive larvae per repetition				X_{mean}	E (%)by Abbott
			I	II	III	IV		
		C_1	14.85	14.35	14.65	15.00	14.71	
	B_1	C_2	14.70	14.20	14.65	15.00	14.64	
		C_3	14.70	14.20	14.65	14.85	14.60	
		C_1	14.85	15.00	14.85	14.00	14.67	
A_1	B_2	C_2	14.85	15.00	14.85	14.00	14.67	
		C_3	14.85	15.00	14.85	14.00	14.67	
		C_1	14.30	14.50	14.70	14.15	14.41	
	B_3	C_2	14.15	13.65	14.70	13.65	14.04	
		C_3	14.15	13.65	14.70	13.65	14.04	
		C_1	5.70	4.65	4.15	4.65	4.79	67.44
	B_1	C_2	1.35	1.00	0.70	1.00	1.01	93.10
		C_3	0.35	0.15	0.15	0.15	0.20	98.63
		C_1	1.15	0.50	0.00	0.35	0.50	96.59
A_2	B_2	C_2	0.00	0.00	0.00	0.35	0.09	99.39
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	11.65	11.50	10.35	11.35	11.21	22.21
	B_3	C_2	6.35	4.30	5.50	7.00	5.79	58.76
		C_3	4.15	3.30	4.00	4.50	3.99	71.58
		C_1	12.00	11.70	10.70	9.70	11.02	25.08
	B_1	C_2	1.30	0.70	0.00	1.00	0.75	94.88
		C_3	0.70	0.70	0.00	0.70	0.52	96.44
		C_1	1.35	1.15	1.15	0.70	1.09	92.57
A_3	B_2	C_2	0.00	0.00	0.15	0.35	0.12	99.18
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	3.30	8.70	10.00	5.30	6.82	52.67
	B_3	C_2	1.30	5.70	8.70	4.30	5.00	64.39
		C_3	1.00	3.30	4.70	2.00	2.75	80.41
		C_1	10.30	13.35	13.65	12.50	12.45	15.36
	B_1	C_2	4.15	5.50	9.35	5.65	6.16	57.92
		C_3	0.00	1.00	0.65	0.85	0.62	95.75
		C_1	8.50	5.35	4.85	6.70	6.35	56.71
A_4	B_2	C_2	0.15	0.00	0.35	0.00	0.12	99.18
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	9.85	9.35	10.80	9.50	9.87	31.50
	B_3	C_2	6.35	4.35	4.85	6.70	5.56	60.40
		C_3	1.85	1.15	0.65	2.85	1.62	88.46
A_5	B_1	C_1	11.15	12.20	10.15	10.00	10.87	26.10

Table 2. cont.

		C_2	6.50	5.50	7.00	5.65	6.16	57.92
		C_3	0.65	0.85	0.65	0.65	0.70	95.20
		C_1	4.85	5.35	4.65	6.00	5.21	64.48
	B_2	C_2	0.00	0.00	0.15	0.00	0.04	99.73
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	10.30	10.30	7.30	6.65	8.64	40.04
	B_3	C_2	4.85	7.00	3.85	2.85	4.64	66.95
		C_3	3.65	3.70	2.50	1.00	2.71	80.70
		C_1	13.65	12.50	13.15	13.35	13.16	10.54
	B_1	C_2	1.20	2.50	4.20	2.85	2.69	81.62
		C_3	0.00	0.65	0.50	0.00	0.29	98.01
		C_1	5.85	6.70	6.65	8.50	6.92	52.83
A_6	B_2	C_2	0.15	0.15	0.35	0.15	0.20	98.64
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	9.35	9.50	5.35	5.35	7.39	48.72
	B_3	C_2	0.15	0.35	0.00	0.00	0.12	99.14
		C_3	0.15	0.00	0.00	0.00	0.04	99.71
		C_1	12.50	14.85	13.50	13.50	13.59	7.61
	B_1	C_2	4.85	6.85	6.80	6.80	6.32	56.83
		C_3	0.70	0.50	0.35	0.30	0.46	96.85
		C_1	5.50	6.00	0.65	4.35	4.12	71.91
A_7	B_2	C_2	1.15	1.15	0.00	0.65	0.74	94.95
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	11.50	12.20	8.85	11.30	10.96	23.94
	B_3	C_2	6.50	6.50	5.70	3.20	5.47	61.04
		C_3	4.80	2.80	4.15	1.50	3.31	76.42
		C_1	5.65	5.80	5.85	5.65	5.74	60.98
	B_1	C_2	1.20	0.65	1.35	0.65	0.96	93.44
		C_3	0.00	0.15	0.50	0.15	0.20	98.63
		C_1	3.15	1.65	2.50	2.00	2.32	84.18
A_8	B_2	C_2	0.85	0.00	0.00	0.00	0.21	98.57
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	11.70	9.35	9.50	6.35	9.22	36.02
	B_3	C_2	8.50	5.70	4.30	3.30	5.45	61.18
		C_3	2.00	3.30	1.00	1.00	1.82	87.04
		C_1	5.15	6.35	6.35	6.35	6.05	58.87
	B_1	C_2	0.35	1.35	0.80	1.00	0.87	94.06
A_9		C_3	0.00	0.00	0.35	0.15	0.12	99.18
	B_2	C_1	3.35	4.20	2.70	3.50	3.44	76.55

Table 2. cont.

		C_2	0.00	1.00	0.30	0.65	0.49	96.66
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	10.35	11.50	7.30	11.65	10.20	29.21
	B_3	C_2	5.70	6.35	5.30	8.70	6.51	53.63
		C_3	3.30	4.15	4.50	1.00	3.24	76.92
		C_1	8.85	6.50	9.65	8.50	8.37	43.10
	B_1	C_2	2.15	2.00	1.20	2.30	1.91	86.95
		C_3	0.15	0.15	0.15	0.65	0.27	98.15
		C_1	5.85	6.65	7.00	7.50	6.75	53.99
A_{10}	B_2	C_2	1.85	2.65	1.35	1.65	1.87	87.25
		C_3	0.00	0.00	0.00	0.00	0.00	100
		C_1	3.30	8.70	10.00	5.30	6.82	52.67
	B_3	C_2	1.30	5.70	8.70	4.30	5.00	64.39
		C_3	1.00	1.00	3.30	2.00	1.82	87.04

B – Type of food (B1 – synthetic food in laboratory condition, B2 - Pedunculate oak leaves in laboratory conditions, B3 - Pedunculate oak leaves in natural conditions); C - exposure (C1 - 72 h, C2 - 144 h, C3 – 216 h).

rates, ranging from 52.83 (Coragen®) to 96.59% (Foray®), were reported in the phase of the experiment which was conducted in the laboratory and where the observed preparations were applied on the natural food of the gypsy moth. Significantly lower rates were reported during the study of biological efficacy in the natural conditions (ranging from 22.21% for Foray®, to 52.67 for Wormox® and the mixture Forey® + 10% Avaunt®). The lowest rates were reported when the preparations were applied on the synthetic food for the gypsy moth (ranging from 7.61% for Alverde®, to 60.98% for its mixture with the biological insecticide Foray®). In the following are ratios of the total mean values of biological efficacy of the insecticides and their mixtures to the type of food: 35.01% (B_1) : 72.20% (B_2) : 37.44% (B_3) (Table 2). When the larvae were grown in the semi-controlled laboratory conditions, and the preparations were applied on the natural food of the gypsy moth, 144 h exposure to the observed insecticides and their mixtures were sufficient for all of them without exception, to achieve absolute, maximum efficacy (100%).

After the second assessment, the experiment was continued in the laboratory, in the phase which refers to the groups of larvae fed on the treated synthetic food, as well as in the natural conditions of study. In the natural conditions, 216 h after the application of the insecticides, the biological efficacy in the control of gypsy moth larvae ranged from 71.58 (Foray®) to 99.71% (Coragen®), and in the laboratory conditions, in the narrow range from 95.20 (Avaunt®) to 99.18% (Forey® + 10% Coragen®). The results presented in the Table 2 show that all the selected insecticides can be used for the control of gypsy moth larvae in forest ecosystems, but attention should be paid to the fact that the application of Dimilin, is still prohibited in the certificated forests. As the remaining three chemical preparations (Avaunt®, Alverde®, Coragen®) achieved the maximum rates of efficacy and are not on the list of the prohibited ones, it is recommended that the possibility of their registration is for the control of some economically significant harmful species of the defoliating insects in the broadleaf forests. The biological efficacy of the studied mixture was the same (under laboratory conditions) or considerably higher (in natural conditions) compared to the cases when microbiological preparation are applied independently. Their mixture did not cause the inactivation of the spores and protein crystals of the bacterium *B. thuringiensis* ssp. *kurstaki*.

In order to get a more accurate answer to the third question which is defined in this paper, the mean rates of survival of gypsy moth larvae in the experiment conducted over the period of 2009 to 2010, presented in Table 2, were subject to detailed statistical analysis. First, the variance analysis, in which the direct effect of the three aforestated factors (A,B,C) and the effect of the blocks (D) were studied, was conducted. F-test showed that the effect of the blocks was statistically random ($p > 0.72$), whereas the effect of all factors was statistically significant at the level $p < 0.01$. In further analyses, the blocks were regarded as the usual repetitions. Then, we carried out a variance analysis with the interaction of factors. The aim was to test the statistical significance of the interaction between the observed factors, that is to minimize the occurrence of the error in the experiment. The results of the variance analysis are shown in Table 3.

Table 3. Variance analysis of the direct and combined effects of factors on the survival of larvae.

Source of variance	Sum of square	Degree of freedom	Mean square	F- ratio	p - value
A - insecticide	3918.82	9	435.424	329.88	0.0000
B - food	768.58	2	384.29	291.14	0.0000
C – exposure	2297.45	2	1148.73	870.27	0.0000
AB	531.834	18	29.5463	22.38	0.0000
AC	439.698	18	24.4277	18.51	0.0000
BC	226.301	4	56.5753	42.86	0.0000
ABC	245.933	36	6.83147	5.18	0.0000
Error	356.389	270	1.31996		
Total	8785.00	359			

The result of F-test shows that the separate or combined influence of all factors on the number of survived larvae in the experiment is of high statistical significance. The variance of error is S^2_p = 1.31996, and its standard deviation or error of experiment is S_p = 1.14889. By including the interaction of factors in the analysis, the error of experiment was reduced by 49.63%. All combinations of insecticides with the type of food and duration of exposure of the larvae are statistically significant. The differences in the mean rates of the survived larvae for the factor – preparation are $LSD_{0.05}$ = 0.53314 and $LSD_{0.01}$ = 0.70249. Out of the 45 possible comparisons of the mean rates, in the first instance 32 or 71.1% are significant, and in the second instance 27 or 60%. Based on the differences of the mean rates at the level $p < 0.05$, the preparations were divided into six homogenous groups, and at the level $p < 0.01$ in five groups (Table 4). The differences of the mean rates of the survived larvae for the factor, the type of food and the mean values for the factor, exposures, are $LSD_{0.05}$ = 0.29066 and $LSD_{0.01}$ = 0.38261. The mean rates of survival for the factor, type of food are: B_1 = 5.33667, B_2 = 2.82083 and B_3 = 6.28333. By comparing the difference of mean rates and LSD, it was determined that the difference between all three types of food of the larvae was statistically significant at the level $p < 0.01$, and the highest in the comparison with the treatment B_2 – natural food in the laboratory. The mean rates for the factor of exposures of larvae are the following: C_1 = 8.25708, C_2 = 3.91625 and C_3 = 2.06750. By comparing the differences of the mean rates and LSD, it was determined that the difference between all three durations of exposures of the larvae was significant at the level $p < 0.01$. Based on the Duncan's test, at the level $p < 0.05$, preparations were divided into five homogenous groups, and at the level $p < 0.01$ in four groups (Table 4). Out of 45 possible comparisons of the mean rates, in the first instance 29 or 64.4%, and in the second one 26 or 57.8% were significant.

During this study, no significant difference in the determination of the statistical significance of the difference between the mean rates of the treatments based on the LSD and Duncan's test was reported. Other authors came to the same conclusion during the experimental studies of the different tests for the simultaneous comparison of several mean rates (Hadzivukovic et al., 1973). The statistical analyses of the results of the experiments was set up in order to study the biological efficacy of the selected biological and chemical insects on the gypsy moth larvae as the test insect, which undoubtedly showed that all the previously stated factors (type of insecticide, type of food and duration of exposure) to a greater or lesser extent affected the achieved rates (Table 3). In regards to the first factor – insecticide, the mechanism of action had a crucial influence on the efficacy of the mechanism of action, that is considerably longer time was needed to pass from the moment of their taking up of biological preparations in the organism of the target insect to their action to start, than in the case of the observed chemical insecticides. In addition, Foray and Wormox, the active ingredient of which is *B. thuringiensis* ssp. *kurstaki*, were most efficient on the younger larva instars (L_1 and L_2), whereas for the older ones (experiment performed on L_3) the higher lethal dose from the applied one or the longer exposure was needed, in contrast to the selected chemical insecticides. In this way, the influence of the third observed factor – duration of exposure of the larvae to the harmful effect of the preparation is also explained.

In regards to the second factor – type of food, that is nutrition by the natural or synthetic food, the maximum biological efficacy of all insecticides achieved in the semi-controlled laboratory or natural conditions when they were applied on Pedunculate oak leaves and when the gypsy moth larvae were grown in laboratory conditions can be explained by the tendency of larvae to prefer the natural food to the synthetic one, regardless of the fact whether it contains the feeding stimulators, as well as by the exclusion of the influences of the unfavorable conditions of the natural microclimate (Figure 1). The ranged from 20 to 30°C. Due to the cold and rainy weather immediately after the application of preparations,

Table 4. Homogenous groups of insecticides based on LSD and Duncan's tests.

Code*	Mean	Homogeneous group																			
		p < 0.05										p < 0.01									
		LSD Test					Duncan's test					LSD Test					Duncan's test				
A_8	2.88194	x					x					x					x				
A_2	3.06389	x	x				x	x				x	x				x				
A_3	3.12083	x	x	x			x	x				x	x				x				
A_6	3.42361		x	x			x	x				x	x				x				
A_9	3.43611		x	x			x	x				x	x				x				
A_{10}	3.64861			x				x					x	x			x	x			
A_5	4.33056				x				x					x	x			x	x		
A_4	4.75278				x	x			x	x					x				x		
A_7	4.98194					x				x					x				x		
A_1	14.4958						x				x					x					x

A – insecticides.

the larvae fed less, so the dose of the consumed insecticide is sublethal, that is the amount of introduced active ingredient is not sufficient to cause the lethal effect (Tabakovic-Tosic, 2008). The considerably lower rates of the biological efficacy obtained during the studies conducted in the natural conditions can be explained by the unfavorable meteorological conditions (precipitation, wind, low temperatures) during the period of the study of the biological efficacy – May 2009 and 2010 (Figure 1).

The considerably lower rate of efficacy achieved when the biological preparations Foray and Wormox were applied in natural conditions, and in comparison with the chemical ones, can be also explained by the destructive effect of sunlight on them. Salama et al. (1983) found that, one day of direct sunlight could inactivate over 90% of *B. thuringiensis ssp. kurstaki* spores on potted white spruce. The trees themselves in the dark can inactivate 78% of the spores in 14 days. Endotoxin activity also was reduced; however, it required about 8 times more light exposure (3.8 h) to obtain a 50% loss in insecticidal activity (Ignoffo, 1992). Wavelengths in the 300/380 nm range of the solar spectrum are largely responsible for loss of toxicity in purified *Btk* crystals. Sunlight radiation has been proved to cause tryptophan destruction in protein crystals (Pozsgay et al., 1987).

Conclusion

The results of the studies of the biological efficacy of the selected chemical insecticides of the third generation, which are not on the list of preparations that are prohibited in the certificated forests, have shown that they have all the necessary properties (high biological efficacy, mechanism of action, resistance to watering and a small amount of application) for use in forest ecosystems. Also, they are compatible with biological insecticides, the active ingredient of which is *B. thuringiensis* ssp. *kurstaki*, so they can be safely mixed in the aim of increasing the biological efficacy and safe application. In spite of the fact that the experiments is set in order to study the biological efficacy of insecticides, aimed at obtaining the permission for their use in the forest stands, can be conducted in the laboratory or field conditions, attention should be paid to the fact that the results are statistically different and the ones performed under natural conditions should be favored as, upon the registration, the preparations will be applied there.

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