ENZYME ACTIVITIES IN BROWN FOREST SOILS AFTER INTRODUCTION OF BACILLUS THURINGIENSIS-BASED BIOINSECTICIDES

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INTRODUCTION

Much attention in the complex of forest pest control methods nowadays is devoted to the application of biological preparations, especially to bacterial formulations produced on the base of Bacillus thuringiensis (BT) that in addition to their high biological effectiveness against injurious insects are safe for man, homoiotherms, beneficial insects and fish. The usage norm of modern bacterial preparations produced on the base of BT makes up to 0.5-5.0 kg ha^{-1} and 1g of preparation powder contains 45-100 milliards of viable spores. According to the research data, during the implementation of pest insects control only 20-40% of sprayed preparation influences directly on pests while its 60-80% by different ways (during spraying, precipitations and exfoliation) eventually penetrates into the soil. As follows from scientific research data, in studies in the field of plant protection the biological and economic efficacies of applying preparations are emphasized very often, whereas the influence of insecticides introduced into the soil as a result of spraying on factors defining soils fertility particularly on enzymatic activity is disregarded. The evaluation of aforesaid characteristics will enable the prevention of undesirable aftereffects of using preparations.

Taking into consideration the above-mentioned the goal was set to determine the impact of some separately applied domestic insecticides of BT species (BT кб-1, BT кб-2, BT(SAR)-49, BT(SAR)-54, BT(SAR)-86, BT subsp. thuringiensis), introduced into the brown forest soils after spraying, on soil enzymatic activity (invertase, urease) defining its fertility.

The lack of any negative influences of bacterial insecticides of BT species on brown forest soils' biological activity will create the opportunities for large-scale application of domestic preparations against injurious insects in woodlots of the Republic of Armenia.

MATERIALS AND METHODS

Studies were conducted in 2010 under laboratory conditions. Insecticide strains BT(SAR)-49, BT(SAR)-54, BT(SAR)-86, isolated by us from dead injurious insects (natural mortality), bacterial insecticide BT subsp. thuringiensis-202, serving as the basis for the production of commercial preparation BTB (Bitoxibacillin), museum bacterial insecticides BT кб-1, BT кб-2 of the Institute of Biotechnology of Republic of Armenia as well as brown forest soil type, by area making up to 79% of Armenia total forest soils, were the materials of our study. Titer of experimental liquid made up to 600 million spore ml^{-1}, consumption consisted 1litre per 10 m^2 of soil layer. Activities of brown forest soils' invertase and urease were determined according to workbook. Variants sprayed and non-sprayed (control) by bacterial insecticides had 5 repetitions. Results of study were subjected to mathematical analysis according to operating instructions.

RESULTS AND DISCUSSION

By our previous study results it has been proved, that during the pest control implementation by separate application of BT species insecticides (BT(SAR)-49, BT(SAR)-54, BT(SAR)-86, BT кб-1, BT кб-2) against leaf-eating insects in woodlots, bacterial stimulants introduced into forest biocenosis are being preserved in brown forest soils (with tendency of quantity decline) during 3-4 months.

In this connection the impact of insecticide crystal-forming spore bacteria introduced into the brown forest soils after spraying on soil enzymatic activity (invertase, urease), defining its fertility, was determined from May to August.

It is typical that invertase is the enzyme widely distributed in nature and occurring almost in all soil types. Hydrolyzing sucrose, raffinose, gentianose and stachyose, contained in soil, invertase forms monosaccharides easily assimilating by plants and soil bacteria. These sugars serve as energy source and initial substances for the biosynthesis of organic acids (aminoacids, fatty acids) for rootage and microflora.

Changes of invertase activity in sprayed by bacterial insecticides and non-sprayed (control) variants from May to August are presented in Fig. 1. Evidently invertase activity during vegetation is subjected to dynamic alterations. Thus the average amount of glucose formed per day in soil has increased in experimental variants from May (19.10 (BT кб-2) – 22.41 (BT кб-1)) mg $C_6H_{12}O_6$ g^{-1}, has reached the maximum in June (24.01 (BT(SAR)-49) – 27.07 (BT(SAR)-86)) mg $C_6H_{12}O_6$ g^{-1} and has gradually decreased in July (22.01 (BT(SAR)-54) – 24.68 (BT subsp. thuringiensis)) mg $C_6H_{12}O_6$ g^{-1} and in August (20.34 (BT(SAR)-86) – 23.34 (BT(SAR)-49)) mg $C_6H_{12}O_6$ g^{-1}. Similar pattern of glucose release was also registered in non-sprayed (control) variants, and the minimal quantity of released glucose in soil was recorded in May (20.86 mg $C_6H_{12}O_6$ g^{-1}), the maximal – in June (25.68 mg $C_6H_{12}O_6$ g^{-1}) at that.

Urease, hydrolyzing urea, contained in soil, forms carbon dioxide and ammonia. The latter serves as nitrogen source for plants and bacteria during nutrition process. As follows from research data, soil is mainly enriched with urea by bacteria and by residues of dead plants. Urease activity rates of brown forest soils sprayed by insecticides of BT species and non-sprayed (control) from May to August are presented in Fig. 2. As follows from data the maximum quantity of released per day ammonia in discussed variants was recorded in June (11.32 (BT(SAR)-86) – 13.16 (BT subsp. thuringiensis)) mg NH_3 g^{-1} and minimal – in August (8.36 (BT(SAR)-49) – 10.20 (BT(SAR)-54)) mg NH_3 g^{-1}. The mentioned pattern was similar in sprayed by bacterial insecticides and non-sprayed variants.

In the process of decline of enzyme (invertase, urease) activities from June to August most probably toxins synthesized by soil bacteria and comparatively unfavorable hydrothermal conditions of soil have immediate concern.

The error of experiment for invertase and urease in sprayed by bacterial insecticides and non-sprayed variants was generally fluctuating in the limits 1.0-5.3% and this proves the validity of obtained data (Tables 1 and 2).

With the help of Student's t confidence coefficient (t_c) it has been established that there aren't any significant differences between indices of enzyme (invertase, urease) activities in separately sprayed by bacterial insecticides (BT кб-1, BT кб-2, BT(SAR)-49, BT(SAR)-54, BT(SAR)-86, BT subsp. thuringiensis) and non-sprayed (control) brown forest soils (in case of $P_{0.95}$ and n=5 the estimated indices of Student's t confidence coefficient (0.177-2.190) are less than its tabular index (2.571).

CONCLUSIONS

Study results have led us to the assumption that introduced into the soil after spraying domestic bacterial insecticides (BT кб-1, BT кб-2, BT(SAR)-49, BT(SAR)-54, BT(SAR)-86) as well as BT subsp. thuringiensis insecticide, serving as the basis for the production of commercial preparation BTB, don't influence adversely on enzyme activities of brown forest soils. These results will facilitate the application of aforesaid effective bacterial insecticides in the field of plant protection.

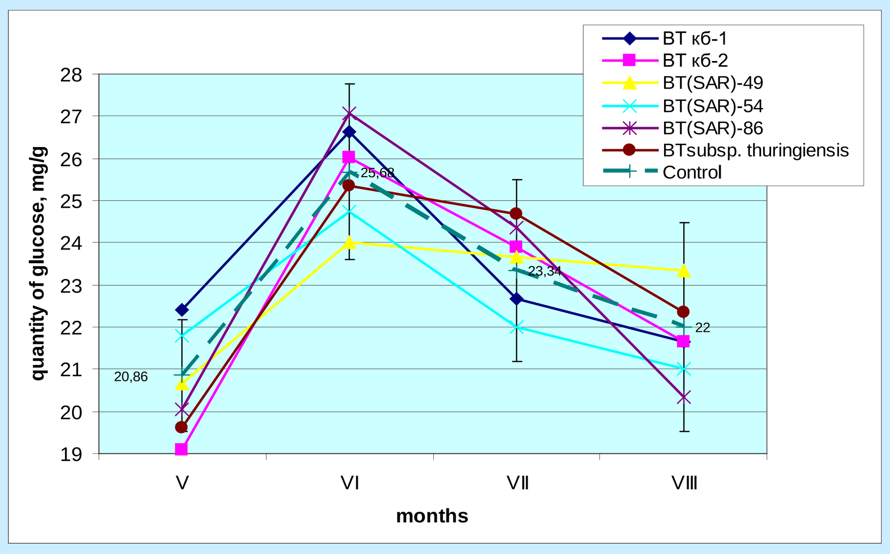

Fig.1. Invertase activity indices after introduction of BT species insecticides into brown forest soils

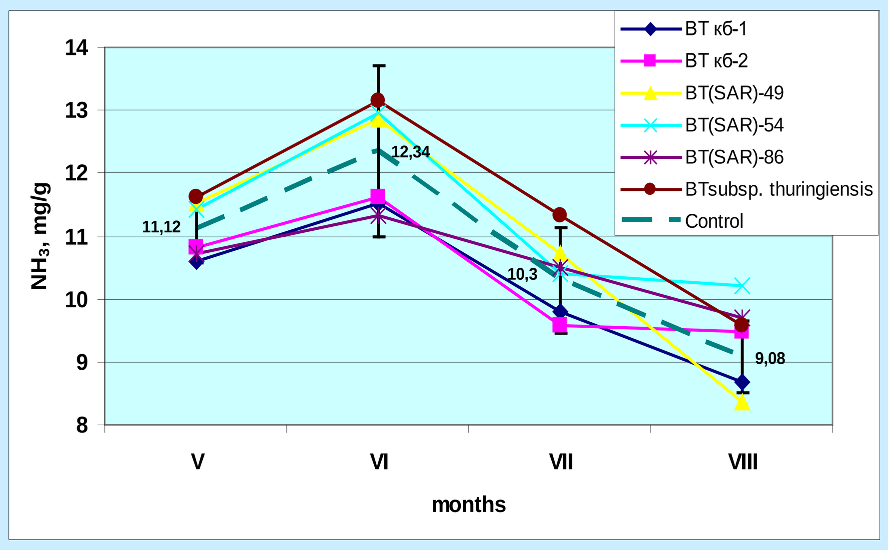

Fig.2. Urease activity indices after introduction of BT species insecticides into brown forest soils

Table 1

Invertase activity indices after introduction of BT species insecticides into brown forest soils (2010 year)

Variants	Months	Average quantity of glucose formed in soil per day (mg/g)	Quadratic deviation	Coefficient of variation, %	Average error	Error of experiment, %	Estimated index of Student's t confidence coefficient (t_c)
1	2	3	4	5	6	7	8
BT кб-1	May	22.41	1.891	8.44	0.846	3.8	1.343
	June	26.62	1.321	4.96	0.591	2.2	0.762
	July	22.67	2.416	10.66	1.080	4.8	0.414
	August	21.66	1.221	5.64	0.546	2.5	0.245
BT кб-2	May	19.10	2.031	10,63	0.908	4.7	1.452
	June	26.01	0.871	3,36	0.389	1.5	0.292
	July	23.89	1.205	5.04	0.539	2.2	0.446
	August	21.66	1.060	4.89	0.474	2.2	0.251
BT(SAR)-49	May	20.67	1.133	5.48	0.507	2.4	0.218
	June	24.01	1.514	6.30	0.677	2.8	1.296
	July	23.67	2.591	10.95	1.159	4.9	0.196
	August	23.34	1.788	7.66	0.800	3.4	0.875
BT(SAR)-54	May	21.80	1.555	7.13	0.695	3.2	1.034
	June	24.75	1.257	5.08	0.562	2.3	0.764
	July	22.01	2.488	11.30	1.113	5.0	0.809
	August	21.00	1.293	6.16	0.578	2.7	1.000
BT(SAR)-86	May	20.06	0.698	3.48	0.312	1.5	1.069
	June	27.07	2.226	8.22	0.995	3.7	0.912
	July	24.35	2.233	9.17	0.999	4.1	0.771
	August	20.34	2.220	10.91	0.993	4.9	0.996
BT subsp. thuringiensis	May	19.60	1.221	6.23	0.546	2.8	1.399
	June	25.35	1.693	6.68	0.757	3.0	0.246
	July	24.68	1.791	7.26	0.801	3.2	0.957
	August	22.34	0.968	4.33	0.433	1.9	0.255
Non-sprayed (control)	May	20.86	1.323	6.34	0.592	2.8	
	June	25.68	2.084	8.11	0.932	3.6	
	July	23.34	2.150	9.21	0.961	4.1	
	August	22.00	2.488	11.31	1.112	5.0	

Table 2

Urease activity indices after introduction of BT species insecticides into brown forest soils (2010 year)

Variants	Months	Average quantity of NH_3 formed in soil per day (mg/g)	Quadratic deviation	Coefficient of variation, %	Average error	Error of experiment, %	Estimated index of Student's t confidence coefficient (t_c)
1	2	3	4	5	6	7	8
BT кб-1	May	10.61	0.321	3.02	0.143	1.3	1.213
	June	11.53	1.005	8.72	0.449	3.9	0.956
	July	9.79	0.627	6.40	0.280	2.9	0.972
	August	8.67	0.721	8.32	0.322	3.7	0.895
BT кб-2	May	10.81	0.630	5.83	0.282	2.6	0.738
	June	11.63	1.017	8.74	0.455	3.9	0.834
	July	9.59	0.561	5.85	0.251	2.6	1.404
	August	9.49	0.712	7.50	0.318	3.3	0.903
BT(SAR)-49	May	11.53	0.272	2.36	0.122	1.0	1.324
	June	12.85	1.439	11.20	0.643	5.0	0.514
	July	10.71	1.163	10.86	0.520	4.8	0.571
	August	8.36	0.981	11.73	0.439	5.2	1.273
BT(SAR)-54	May	11.42	0.392	3.43	0.175	1.5	0.882
	June	12.95	0.946	7.30	0.423	3.3	0.735
	July	10.40	0.749	7.20	0.335	3.2	0.177
	August	10.20	0.853	8.36	0.381	3.7	2.190
BT(SAR)-86	May	10.71	0.559	5.22	0.250	2.3	1.040
	June	11.32	0.903	7.98	0.404	3.6	1.247
	July	10.50	1.247	11.80	0.558	5.3	0.266
	August	9.69	1.117	11.53	0.499	5.1	0.975
BT subsp. thuringiensis	May	11.63	0.718	6.17	0.321	2.8	1.123
	June	13.16	0.847	6.44	0.379	2.9	1.021
	July	11.32	1.062	9.38	0.475	4.2	1.506
	August	9.59	0.723	7.54	0.323	3.4	1.274
Non-sprayed (control)	May	11.12	0.556	5.00	0.249	2.2	
	June	12.34	1.364	11.05	0.610	4.9	
	July	10.30	0.841	8.16	0.376	3.6	
	August	9.08	0.563	6.20	0.252	2.8	

Note: the tabular index of Student's t confidence coefficient (t_c) for Tables 1 and 2 makes 2.571 in case of $P_{0.95}$ and n=5.