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QUANTITIES OF AMMONIFIERS IN BROWN FOREST SOILS AFTER INTRODUCTION OF CRYSTALLOPHORES

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By statistical analysis of study results it has been established that there are not any significant differences between indices of ammonifiers in brown forest soils sprayed and non-sprayed by tested biopesticides. The study indicates that BT-based insecticides, introduced into forest biocenosis, are preserved in brown forest soils during four (BT E-3, BT CM-25) or five (BT AP-8, BT AM-22) months, do not influence adversely on soil-inhabiting ammonifiers of mentioned soils and, therefore, can be widely used in plant protection.

Keywords: soil microorganisms, spraying, persistence of bioinsecticides, dynamics of entomopathogenes.

Introduction. The implementation of pest control measures, that are safe for environment and effective against injurious insects, is one of urgent problems in preservation and protection of forests [1].

As is generally known, pesticides in contrast to other organic toxic compounds that may enter the environment as waste products and through accidental release are used intentionally [2]. The huge amount of applied insecticides and fungicides (97–99%) and 60–95% of herbicides penetrate aimlessly into soil, water reservoirs and air even in case of satisfaction of all demands concerning the spraying operation [3]. As is reported in [4], during the implementation of pest insects control only 20–40% of sprayed insecticides influences directly on pests while its 60–80% by different ways (during spraying, precipitations and exfoliation) eventually passes into the soil.

Nowadays it is paid much attention to the complex of forest pest control methods, especially to the application of formulations, produced on the base of bacterial insecticides of *Bacillus thuringiensis* (BT) species. 1 *g* of such safe for man and environment preparation powder contains 45–100 milliards of viable spores [5].

As follows from many facts, biological efficacy of used preparations very often is emphasized in the area of plant protection research neglecting the detection of the influence of insecticides introducing into the soil as a result of spraying on

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main indices determining the fertility of soils. Via determination of these indices it will be possible to prevent unacceptable impact of applied preparations.

It is well known that plants during their nutrition process amongst numerous chemical combinations of nitrogen generally utilize its mineral compounds – mainly ammonia salts and nitrates. Ammonia salts are generated during ammonification process, which is the significant indicator of soil fertility [6–7].

In this connection, the study is aimed to reveal the residual quantities of BT-based bacterial stimulants, introduced into the soils as a result of spraying, in brown forest soils (the total area of this type of soils makes 75% of all forest soils in Armenia) as well as to determine the impact of bacterial insecticides on quantity of ammonifiers – index, determining the fertility of soils. By determination of these parameters it will be feasible to predict undesirable shifts in forest soils and to prevent these changes. In case of lack of any negative manifestations the application of bacterial insecticides of BT species will be facilitated in the Republic of Armenia.

Material and Methods. Studies were conducted in 2011 from May to October under laboratory conditions (Centre of Biotechnology and Deposit of Microorganisms) and in the woodlots of Ttoujour (Aragatsotn Marz).

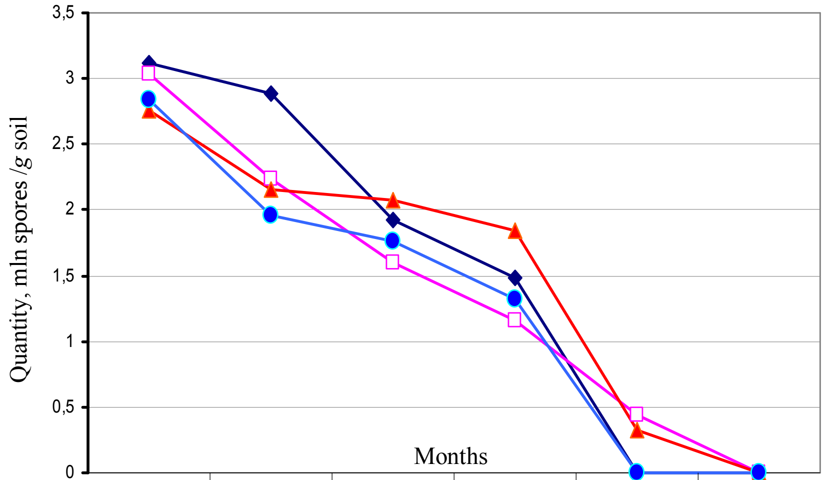
Brown forest soil type, soil-inhabiting ammonifiers and crystal-forming insecticides BT E-3, BT AP-8, BT AM-22, BT CM-25 isolated by us from caterpillars died by natural death were the materials of our study. Titer in culture liquid amounted to 300 mln spores/*ml*, consumption – to 5 *l* per 50 m^2 of soil layer, woodlot area for each variant was 50 m^2. Sprayings were performed by knapsack sprayer of Ozdesan mark. Microbiological studies were conducted by Petri-dish assay on meat infusion agar by dilution method [8].

For determination of bacterial insecticides' persistence their initial quantities introduced into the soil in May served as control and for ammonifiers – the soils non-sprayed by BT insecticides. The 0–10 *cm* soil layer was studied (humus content – 12%). Staining of vegetative cells, spores and proteic crystals (endotoxin) of insecticide bacteria was carried out according to recommendations [8].

Results. It has been found experimentally that the quantities of above-mentioned four subspecies of BT (BT E-3, BT AP-8, BT AM-22 and BT CM-25) introduced into the soil in May (2.76–3.12 mln spores/*g*, and used as control) during vegetation underwent changes in June, July and August with declining trend and constituted 1.96–2.88, 1.60–2.08, 1.16–1.84 mln spores/*g* respectively (Fig. 1).

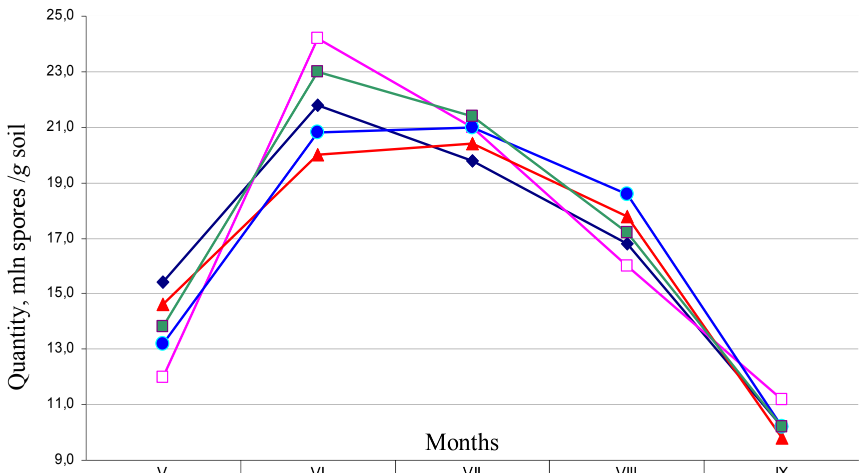
The decrease of quantities of insecticides BT E-3, BT AP-8, BT AM-22 and BT CM-25 in August comparing with control constituted respectively 52.6, 61.9, 33.3 and 53.5%. The insecticides BT E-3 and BT CM-25 in September as well as BT AP-8 and BT AM-22 in October were not preserved in soils.

By laboratory studies it was determined that above-mentioned strains during their stay within a period of 4–5 months in brown forest soils preserved the morphological characteristics of native (non-introduced into the soil) strain as well as its ability to synthesize spores and proteic crystals (endotoxin). With the help of Student's confidence coefficient (t_c) it has been established that from June to September the quantities of the mentioned bacterial insecticides, introduced into the soil, significantly differed (decreased) in comparison with initial quantity introduced into the soil in May, so long as in case of $P_{0.95}$ and n=5, the estimated summarized t_c indices from June to September (3.638–21.088) were greater than its tabular index (2.571).



	V	VI	VII	VIII	IX	X
BT E-3	3,12	2,88	1,92	1,48	0	0
BT AP-8	3,04	2,24	1,6	1,16	0,44	0
BT AM-22	2,76	2,16	2,08	1,84	0,32	0
BT CM-25	2,84	1,96	1,76	1,32	0	0

Fig. 1. Residual quantity of BT insecticides in brown forest soils after spraying by culture liquids.



	V	VI	VII	VIII	IX
BT E-3	15,4	21,8	19,8	16,8	10,2
BT AP-8	12,0	24,2	21,0	16,0	11,2
BT AM-22	14,6	20,0	20,4	17,8	9,8
BT CM-25	13,2	20,8	21,0	18,6	10,2
Control	13,8	23,0	21,4	17,2	10,2

Fig. 2. Quantity of ammonifiers in brown forest soils after introduction of BT insecticides.

The study results indicate (Fig. 2) that in separately sprayed by BT-based bioinsecticides and non-sprayed brown forest soils the quantities of ammonifiers undergo changes: they began increasing in May (12.0–15.4 mln spores/*g*, in whole), reach their maximum in June (20.0–24.2 mln spores/*g*) and gradually decrease from July (19.8–21.4 mln spores/*g*) to September (9.8–11.2 mln spores/*g*). In control (non-sprayed) variants the recorded indices in above-mentioned months constituted 13.8, 23.0, 21.4, 17.2 and 10.2 mln spores/*g* respectively. The error of experiment in test patterns was fluctuating in the limits 2.2–5.7% and this proves the reliability of obtained data.

By statistical analysis of study results it has been established that there are not any significant differences between quantities of ammonifiers in brown forest soils separately sprayed and non-sprayed (control) by tested biopesticides (from May to September, in case of $P_{0.95}$ and $n = 5$, the estimated t_c indices (0–2.238) were less than its tabular index (2.571)).

Conclusions. The study indicates that BT-based insecticides introduced into forest biocenosis after spraying are preserved in brown forest soils with tendency of quantity decline during four (BT E-3, BT CM-25) or five (BT AP-8, BT AM-22) months and do not influence adversely on soil-inhabiting ammonifiers defining the fertility of the mentioned soils. It will facilitate the application of the aforesaid effective bacterial insecticides in plant protection.

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