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Control strategies against invasive pest Box Tree Moth - *Cydalima perspectalis* in Georgia

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Abstract – The Box Tree Moth (BTM) - *Cydalima perspectalis* (Walker, 1859) (Lepidoptera; Crambidae) was introduce 2012 in Georgia and in the next year it began to defoliate *Buxus* spp in large quantities. Today the situation is quite alarming in that BTM damage *Buxus colchica,* which is endemic species of Caucasian flora and threatened by habitat loss. The larvae feed on leaves and shoots, caused serious damages, defoliating box trees, causing economic, social and environment problems. The pest have 2 generations in Georgia.

2017-2018, WitaTrap® Funnel trap system, with pheromone CYDAWIT® were used for the monitoring and control of BTM and in total approximately 93000 (2017) and 74000 (2018) adult were captured.

Biological pesticides based on entomopathogenic bacteria, *Bacillus thuringiensis* var. kurstaki , sold as Lepidocid CK-M (Georgian Production) and DiPel® against L2-L5 instars larvae were used. Efficiencies achieve 60,6-88,6% respectively. More then 350 insect were dissected and studied. Only finding exemplars in August, 2017 were observed develop fungal symptoms on the body of adults insects in natural habitats of boxwood forest. To isolation pathogens on different artificial media (SDAY, PDA), of entomopathogenic fungus *Beauveria bassiana* were identified.

Keywords – Cydalima perspectalis, Buxus colchica, pheromone trap, Bacillus thuringiensis, Beauveria bassiana

I. Introduction

Alien invasive species have been described as an outstanding global problem. Numbers of species introductions to new habitats have been accelerated all over the world due to the increasing mobility of people and goods over the past decades. Numerous alien insect species, many of them introduced only in the last century have become successfully established in various ecosystems in Georgia.

The Box Tree Moth (BTM) - *Cydalima perspectalis* (Walker, 1859) (Lepidoptera; Crambidae) is an insect of Asian origin [1]-[3]) from the humid subtropical regions of East Asia, India [4], China [5], Japan [6], Korea [7] and Far East Russia [8]. Within a few years it has already invaded at least 16 European countries, in which it has become a serious pest of ornamental box trees (*Buxus* spp.) [9]-[10]-[11]-[12]-[13]-[14]-[15]-[16]-[17]-[18].

In Georgia BTM was introduce in 2012. During the preparation to the 2014 Winter Olympics was introduced from Italy to Sochi with the planting stock of *Buxus sempervirens*, then arrived in Georgia and in the next year it began to defoliate *Buxus colchic* in large quantities, as a species of Buxus native to Georgia (country), Azerbaijan [19]-[20]-[21], that recently invaded Georgia, Turkey and the Caucasus, causing serious damage to ornamental box (Buxus sp.) shrubs and trees [19]-[22]-[23]. *Buxus colchica* is endemic species of Caucasian flora and threatened by habitat loss. BTM larvae cause damage not only in native habitats of box trees, also in public and private gardens and parks [21].

Today the situation is quite alarming in Western Georgia, that BTM damage Buxus colchica, which is endemic species of Caucasian flora and threatened by habitat loss [24]. The larvae feed on leaves and shoots [25] caused serious damages, defoliating box trees, causing economic, social and environment problems [26].

The development of management strategies against C. perspectalis, are urgently needed to preserve the natural box tree forests in Georgia..

II. Materials and Method

Site of Investigation

The study of spreading of BTM - Cydalima perspectalis individuals was conducted in the summer of 2016, in nature distribution of Buxus colchica of different regions and locations in Georgia: Imereti, Racha, Tsageri, Samegrelo and Adjara [21].

2017-2018, Different instars of living larvae and moth of C. perspectalis were collected manually from boxwood forest of West Georgia

In every location are made visual assessments at least 50 individuals of box trees and hedges. Then, evaluated plants are distributedin 5 different levels of damage, basing on assess for the degree of defoliation of box trees by pest larvae, using its own 5 point scale. [27]

Pheromon trap

The determation of the population densities of the BTM 2017-2018, WitaTrap® Funnel trap system, with pheromone CYDAWIT® (Witasek, Pflanzenschutz, GmbH, Austria), were installed .

A long term trapping was conducted at two location of boxwood forest in Tsageri - Ambrolauri (South slop of Grate Caucasian mountain range) in West Georgia. 450 pheromone trap were set up on the 150 hectare (ha) at least ten day before the pest is expected to emerge and at the proper height above the ground or in the plant canopy. Three trap per ha were placed, where prevailing winds was carry the pheromone into the forest area.

Bioassay of target pest

Two Bt products against and on entomopathogenic fungus *Beauveria bassiana* on the larva of BTM were tested. Both biological comertial pesticides based on the entomopathogenic bacteria *Bacillus thuringiensis* var. kurstaki: Lepidocid CK-M(Georgian Production) and DiPel® (Valent BioSciences Corporation) [28]. *Beauveria bassiana* - MB-103, were isolated from the infected adult of *C.perspectalis*, founded in nature on Boxwood forest in

The 2nd to 5th (L2-L5) instars of larvae target insect - *C.perspectalis* performed for the bioassay in laboratory. The design of experiment was arranged with three biopesticiedes treatments plus control treated with water.

Bush of the *Buxus* sp. was treated by *Btk* fresh culture suspension with 3 concentration: 0,1%, 0,5%, 1%, with 4 replication each treatment. 30 larvae of *C.perspectalis* were put on the each treated bush and placed in the cage and kept at room temperature ~23 ºC (day) /18 ~ ºC (night), with RH 57-85%, 14/10 light/dark regime [29]. Mortality of larvae was recorded on 3,5,7,9,12 days after treatment [30].

Plot for semi-field experiments were selecteed the boxwood bushes. We are choose middle size of bush, placed larvae of different stage (L2-L5), treated by biopesticides and banding them by cotton nets, that larvae can not speaded. As a control we are used water treatment [31].

Analyses of data

Mortality data were analyzed using SPSS program of variance (Mixed Design, 2 way ANOVA) and Turkey test, was used to compare means the average survival time (AST) during the assismentperiodwas analyzed with Kaplan-Meier survival test. Statistical analyses were performed using Statistic PSS 17 for Windows.

III. Results

Study to establish the degree of damages caused from C. *perspectalis* of *Buxus* spp. was conducted in locations with confirmed presence of this pest in 2016. The results of the research are presented in Table 1.

The results show that *C. perspectalis* is a serious pest on the box trees in the majority of studied localities. The percentages of defoliation ranged from 1 to 4. In general has been observed that the damage level of box trees varieted between weak to very strong (15% -100%).

Trapping

2017-2018, funnel trap system, with pheromone were installed for the monitoring and control of BTM in following regions and are. In Figure 1, is given area (in hectares) of distribution native population of *Buxsus* sp. different region of Georgia.

Table 1. *C. perspectalis* damage level in different locations of Georgia (2016)

#	Region /Area	Defoliation (%)	Damage level	Damage in %	Damage Significance
1.	Imereti	1-25	1	15,7-24.8	weak
2.	Racha	26-50	2	30,5-47,6	middle
3.	Tsageri	51-75	3	65,5-72,3	strong
4.	Samegrelo	51-75	3	52,5-69,5	strong
5.	Adjara	≤ 75	4	75-100	very strong

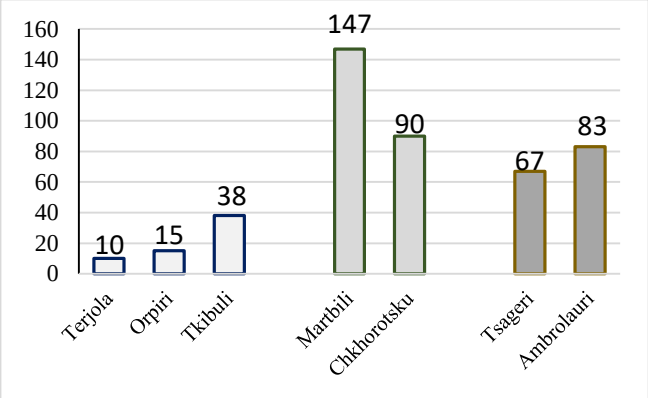

Fig.1. The area (in ha) distribution of *C.perspectalis* and hanging pheromone trap for monitoring and controlling in different region of Georgia, 2017-2018.

As a described in methods, 450 pheromone trap were installed on 150 ha. Attracted moth by the pheromone fall into capture container and cannot fly out (Fig.2).

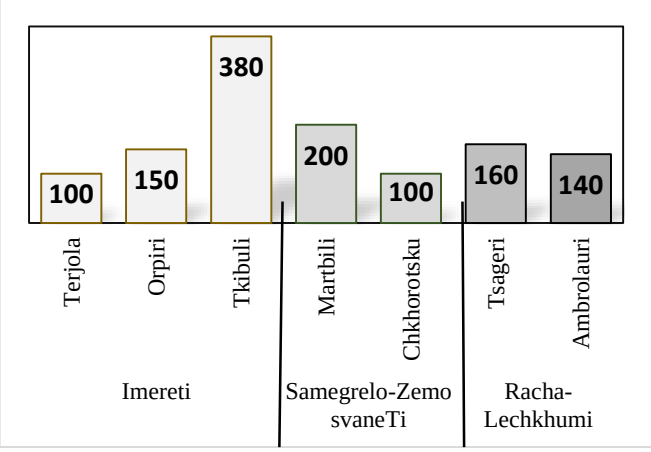

Fig.2 .Pheromone traps set up different region of Georgia for monitoring *C.perspectalis*

The pheromone traps were emptied and new dispensers were added two time at the during the flying period of C.perspectalis. The number of captured adults were variated from 11 to 176 moth from one trap. In total approximat. 93000 (2017) and 74000 (2018) of moth captured in Tsageri – Ambrolauri region (Fig.3, 4).

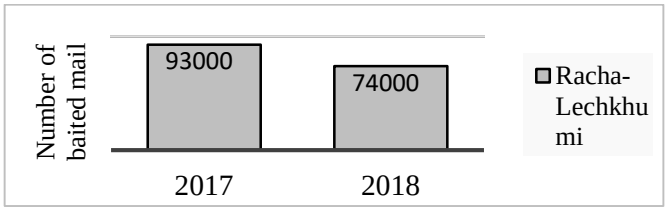

Fig. 3. Total number of *C.perspectalis captured* in Tsageri – Ambrolauri region 2017-2018

Fig.4. *C.perspectalis* captured in in pheromone trap

Fungal distribution and isolation

Adult of BTM, infected with entomopathogenic fungi were found in the nature, boxwood forest during field trip in the Tsageri District. The fungus was identified using microscopic preparations made directly from mycelia developing on the moth. After morphological analysis of monocultures, individual isolates of *Beauveria* sp. was identificated. Conidia dimensions were between (1.5) 2.0 – 3.0 (4.0) x (1.5) 2.0 – 2.5 (- 3.0) µm.

Bioassay

Results of the laboratory bioassay and field trial, bacterial biopesticides and fungus strain for the control of *C.perspectalis*, after 12 days are presented in figures 5 and 6.

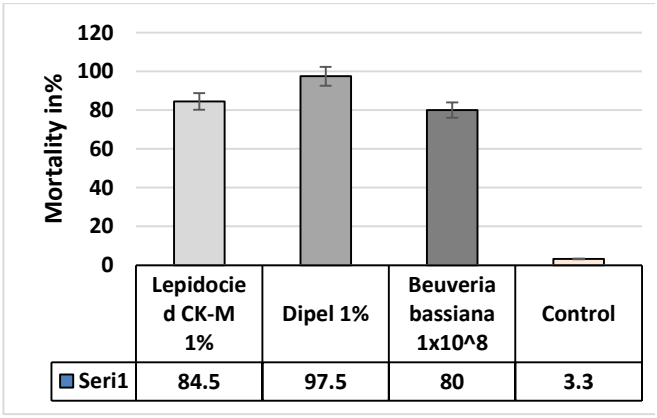

Fig. . Effectives of biooesticides agains *C.perspectalis* larvae in Laboratory after 12 day.

In laboratory experiments two *Bt* commercial product and one of entomopathogenic fungus strain were used against larvae L2-L5 instars of BTM. The mortality in the control reached 3.3% and the treated groups the corrected mortality achieved 97,5% (DiPel®), 84,5% (Lepidocid CK-M) and 80% (local isolation *Beauveria bassiana* -MB-103). To conclude this laboratory test showed a high level of insecticidal activity of the commercial production and local fungus isolates on the BTM.

In the field experiments, suspension of Bt pesticiedes Lepidocid CK-M and DiPel® with 1%, and local isolation *Beauveria bassiana* -MB-103 $1x10^8$ concentration were spraying. The efficiency were calculated on 12 day after application and compared to the control, where mortality of larvae from Lepidocid CK-M - 60.6%, DiPel® -88.6%, *Beauveria bassiana*-MB-103 -60%, in control 17.3% were observed.

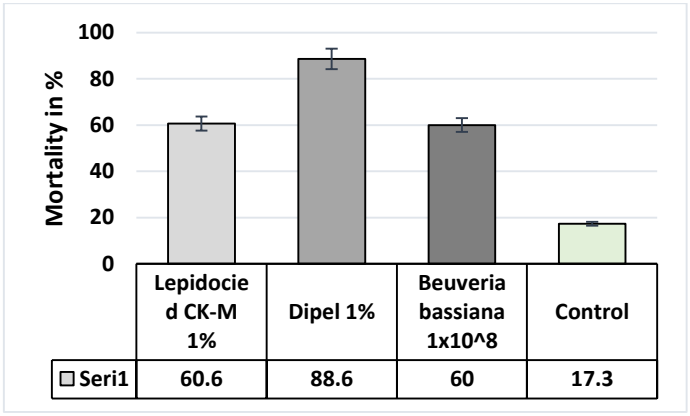

Fig. Effectives of biooesticides agains *C.perspectalis* larvae in Laboratory after 12 day.

IV. DISCUSSION

The moth trapping in the untreated control indicate that the pest population density was in numbers that cause serious damage (Fig.1). Monitoring with pheromone traps showed that for trap installation until to remove total number of captured males in 2017 was 93000 and 2018 – 74000 and during one years, number of captured mails reduce on 19000 adults which 20.5 %. The majority of males was caught during in the second decade of May (first generation) and in first decade of August (second generation).

Assessing the potential of biocontrol agents against BTM under the field conditions, Bt pesticiedes Lepidocid CK-M, DiPel® and local isolation *Beauveria bassiana* -MB-103 were statistically superior compared to the control. In the treatment with DiPel® real damage caused by BTM was estimated at 11,4%, with Lepidocid CK-M -39,4% and in case of *Beauveria bassiana* -MB-103 achieved -40%.

The continues use of broad spectrum insecticides in Georgia can lead to resistance, environmental contamination, health hazards and dangerous for human [32]. The results indicate that infestation can be significantly reduced through tree successive applications with *Bt* and *Beauveria bassiana*.

V. CONCLUSION

1.The box-tree moth has shown rapid active spread since 2012 when it first appeared in the West Georgia.

2. Pest defoliate endemic species of Caucasian flora *Buxus colchica* and other Buxus spp in large quantities.

3. The larvae feed on leaves and shoots, caused serious damages, defoliating box trees, causing economic, social and environment problems.

4. 2017-2018, WitaTrap® Funnel trap system, with pheromone CYDAWIT® were used for the monitoring and control of BTM and in total approximately 93000 (2017) and 74000 (2018) adult were captured.

5. Adult (Moth) of BTM, infected with entomopathogenic fungi were found in the nature, boxwood forest d in the Tsageri District. The fungus was identified and established that it was *Beauveria bassiana* (strain MB-103).

6. Biological pesticides based on entomopathogenic bacteria, *Bacillus thuringiensis* var. kurstaki Lepidocid CK-M, DiPel® and *Beauveria bassiana*-MB-103 against II-V caterpillars of BTM were used. Mortality in laboratory 84,5%, 97,5% and 80%, and in field 60.6%, 88.6%, 60%, respectively were observed.

7. The insecticidal activity of biopesticids based on *Bt*, particularly two different company Lepidocid CK-M, DiPel®, demonstrated in the control of boxwood forest defoliators.

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