BIOLOGICAL PROTECTION OF STORED GRAIN PRODUCTS AGAINST THE MOTHS COMPLEX

GAVRILIŢA Lidia

Abstract. Before depositing in the warehouse, there were determined the biological indexes of *Trichogramma pintoi*. The determined indexes were: prolificacy of females of 31.9 eggs / female hatching of the individuals 91.6%, female share 60.4%, static criterion of the quality 17.4. In order to determine the number of *Sitotroga cerealella* eggs parasitized by *T. pintoi* in the grain warehouse from Chişinău, an artificial fond was created to have a more precise evidence, by fixing 120 cards on the walls of the warehouse, windows, equipments with an amount of 12,000 eggs of *Sitotroga cerealella* preliminarily irradiated with gamma rays. In this warehouse *T. pintoi* was launched in capsules, with a rate of 1:1 (host: parasite), meaning 12.000 moth eggs to 12.000 *Trichogramma* females. The cards were then gathered and analysed, in order to emphasize the share of parasitized and exposed eggs. The the percent of parasitized eggs of *T. pintoi* varied between 61.8 to 80.24%.

Keywords: biological indices, prolificacy, effectiveness, *Trichogramma*, biological efficacy.

Rezumat. Protecţia biologică a produselor cerealiere depozitate în combaterea complexului de molii. Înainte de lansare în depozit s-au determinat indicii biologici a speciei *Trichogramma pintoi*. Aceşti indici sunt: prolificitatea femelelor de 3,.9-ouă/femelă, ecloziunea indivizilor de 91,6%, cota femelelor 60,4%, criteriul static al calităţii 17,4. În scopul determinării numărului de ouă de *Sitotroga cerealella* parazitate de *T. pintoi* în depozitul de cereale din Chişinău, a fost creat un fond artificial, pentru a face o evidenţă mai exactă. Pe pereţi, ferestre, utilajul din depozit, s-au fixat 120 de cartonaşe cu 12.000 de ouă de *Sitotroga cerealella* iradiate cu raze gama şi încleiate pe aceste cartonaşe. În acest depozit s-a lansat *T. pintoi* în capsule, cu raportul de 1:1 (gazdă: parazit), adică la 12.000 de ouă de *S. cerealella* s-au lansat 12.000 de femele incluse în capsule. Cartonaşele au fost scoase (culese) şi efectuată evidenţa procentului de ouă parazitate şi expuse în depozit. Procentul de ouă parazitate de *T. pintoi* a variat între 61,8-80,24%.

Cuvinte cheie: indici biologici, prolificitate, eficacitate, *Trichogramma*, eficacitate biologică.

INTRODUCTION

The damage caused by the insects to the agricultural products has a great economic importance. There have been undertaken numerous and various investigations, related to this issue; therefore, a series of synthesis papers of general and systematic nature concerning this group of insects were realized. Studies on the biology and ecology of the insect pests of the stored cereal products have been published by EVANS, 1987.

A major concern in the protection of the stored agricultural products is the biological control of pests. Scientific papers have been elaborated by many authors on the use of the parasitic hymenoptera in the biological control of the insect pests of the stored agricultural products.

To control the complex of flour pests there were performed experiments with three species of *Trichogramma* - *T. deion, T. ostriniae, T. pretiosum* in the laboratory conditions for control of *Plodia interpunctella* (MATTHEEW, 2008). The researches on the application of the biological protection of stored pests control with the different species of entomophages - *Trichogramma pretiosum* and *Bracon hebedor* have been realized by (BROWE, 1988; BROWER & PRESS, 1990). The works of these authors demonstrated the real possibility of application of the entomophages *Trichogramma* and *Bracon*) in the control of the moth product pests as one of the main elements of the integrated protection of the stored food products. The approach to the integrated protection strategy of the stored food products is well reflected in the work of the authors (TODIRAŞ et al., 2009). There is a large complex of pests in the warehouses: *Calandra granaria* L., *Sitophilus granarius* L., *Acarus siro* L., *Sitotroga cerealella* Ol., *Tinea granella* L., *Plodia interpunctella* Hubn., *Ephestia elutella* Hubn., *Ephestia кuhniella* Zell., etc. Against these pests different control methods are used: biological, chemical controls, fumigation, the use of pheromones, acoustic methods, thermal methods, application of plant products, gamma radiation, animal pests, etc.

The objective of the studies: Evaluation of the application technology of *Trichogramma pintoi* V. and other elements of biological protection in the control of moths in stored products.

MATERIAL AND METHODS

The objectives of the research during 2009-2010 were the entomophage *Trichogramma pintoi* and the hosts *S. cerealella* Ol., *P. interpunctella* L. and *E. kuehniella Zell*. The researches were conducted in the laboratory conditions of the Institute of Genetics, Physiology and Plant Protection and in the grain warehouse from Chisinau. The biological indices of the entomophagous *Trichogramma pintoi* were determined with the goal of using the entomophagous insects against a complex of moths present in the warehouse.

The collecting, determination, maintaining and accumulation of the species of *Trichogramma* sp. were performed according to the author methods (DYURICH, 2008), which are to be recommended.

Rearing the laboratory host grain moth (*Sitotroga cerealella* Ol.), determining biological indices of *T. pintoi*, determination of the biological efficacy of *Trichogramma* were performed according to the traditional methods of the authors (ABASCHIN et al., 1979). Breeding of grain moth and *Trichogramma*, the evaluation of biological effectiveness of the entomophages *by pest and mathematical data processing were conducted according to the relevant procedures and guidelines for mass breeding and use of Trichogramma* (ABASCHIN et al., 1979*;* MENCER & ZIMERMAN, 1986; EVANS, 1987). The biological efficacy of *T. pintoi* after each launching was determined by taking into consideration the number of moth larvae from the samples of wheat flour. The biological efficacy of *T. pintoi* was determined by the formula: E = 100 – B/A x 100, where: E - biological efficacy expressed in % compared with the control; A – the average number of pest in control; B – the average number of pest in experiments („Îndrumări metodice pentru testarea produselor chimice si biologice, 2002).

RESULTS AND DISCUSSIONS

1. **Evaluation of the percentage of infestation and biological effectiveness of *T. pintoi* as a result of different tests conducted at different substrates of cereal products in the warehouse.**

Experiments were held in the thermostat at the temperature of 25±1ºC and relative humidity of the air of 80-85 %, with a repetitiveness of 5 times. Experiments were mounted in a glass desiccator with a cereal product substrate of a thickness of 5-6 cm, in an amount of 1 kg of each substrate of wheat, barley, maize, beforehand infested with grain moth (for each kg of cereal substrate a gram of moth eggs was used). The period of development of a grain moth generation lasted 32-34 days; then, the butterflies started flying and precisely 5 days after the interval when the butterflies laid their eggs) *T. pintoi* was launched with a report of parasite: host of 1:10 (100 females to 1,000 eggs) to control the grain moth. Five days after the launching of *Trichogramma*, the biological effectiveness was determined for *T. pintoi* (number of eggs parasitized) on each substrate of cereal product. In Control, the experiments were carried out without substrates (Table 1, Fig. 1).

Table 1. The biological efficacy of *T. pintoi* at different layers of cereal products in the warehouse.

No. of variants	Layer-types	% of infested grains	% of *S. cerealella* parasitized eggs on layers
1	barley	90.8±3.9	78.2±3.0
2	wheat	82.4±3.5	72.5±2.6
3	corn	78.4±3.0	70.0±2.0
5	Control (no layers)	0	86.7±3.7

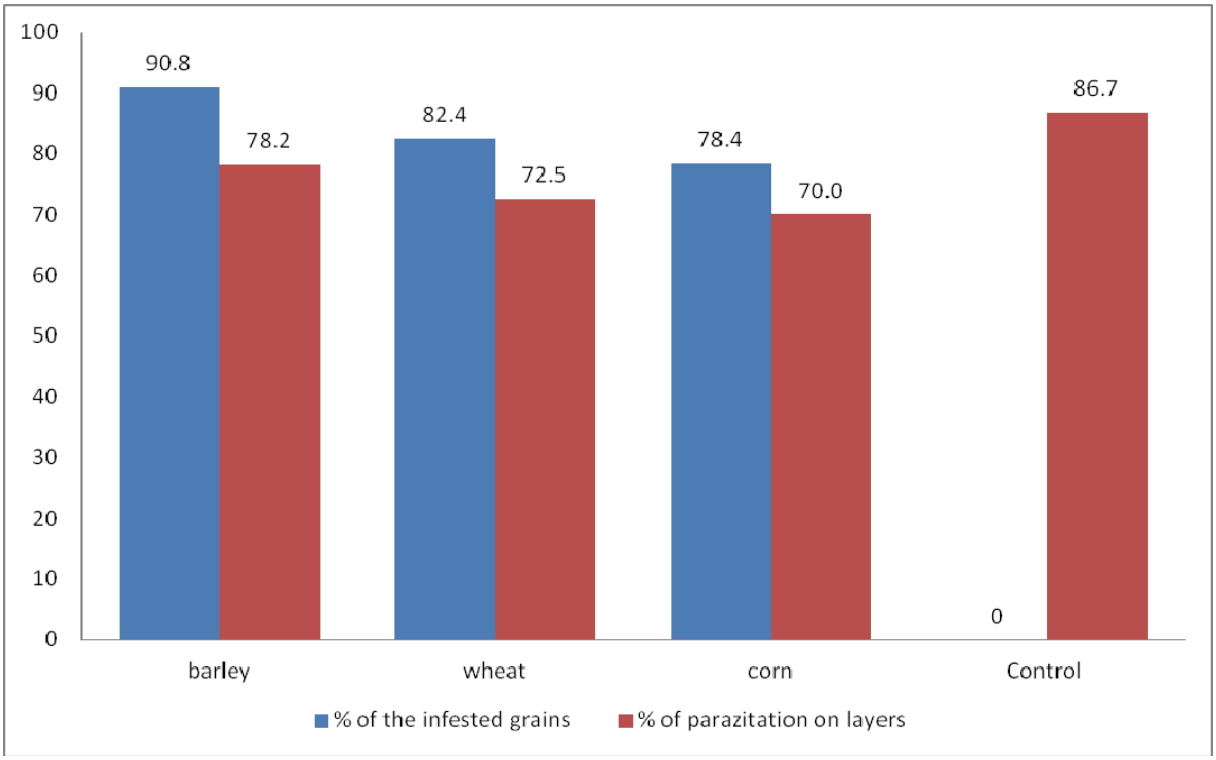


Figure 1. Percentage of the grains infested by the grain moth *S. cerealella* and the brown moth *T. pintoi.*

Analyzing the number of infested grains on variants, it was revealed that the substrate of barley grains was infested up to 90.8%. The number of eggs parasitized by the *T. pintoi* on the barley substrate was of 78.2%. The infestation on the wheat substrate reached 82.4% and on corn the infestation percentage was of 78.4%. Parasitizing

percentage on the wheat layer reached 72.5%, on corn though it was of 70%. In Control experiment, the researches were conducted without substrates and the number of parasitized eggs registered a percentage of 86.7% i

2a. **The estimation of biological effectiveness of *T. pinto*i with varying amounts of flour on the surface of the eggs of *S. cerealella* and *E. kuehniella*.**

The experiments were carried out in the thermostat at a temperature of 25 ± 1°C and relative humidity 80-85%, in 5 replicates. The amount of 2,000 eggs were placed in the Petri dish, in one single layer, then the eggs were covered with various amounts of flour on substrates (1, 2, 3, 4, 5, 6, 7, 8 grams), after which *T. pintoi* was launched with a norm of 40 females at 2.000 eggs of *S. cerealella* (ratio 1:50). The launch was carried out in semi capsules placed in the centre of the Petri dish. In the Control experiment, non-covered eggs were used (no flour). Five days after the launching, we recorded the number of parasitized eggs by *T. pintoi* in controlling *S. cerealella* and *E. kuehniella* on variants (Table 2; Figs. 2, 3).

Table 2. Evaluation of the percentage of parasitation of *T. pintoi* with varying amounts of flour on the surface of the grain moth eggs.

No. of variants	**The amount of flour on the surface of the moth eggs (g) and thickness of the flour layer (mm).**								**Control**
	1/0.5	2/1	3/1.5	4/2	5/2.5	6/3	7/3.5	8/4	0
Number of *S. cerealella* Ol. eggs parasitized with *T.pintoi*, %	88.3 ±3.5	80.9 ±3.1	72.5 ±2.8	59.6 ±2.4	40. ±2.1	20. ±1.9	9.0 ±1.0	2 ±0.3	92.5±3.9
Number of *E. kuehniella* Zell. eggs parasitized with *T. pintoi*, %	90.0 ±3.9	84.0 ±3.6	76.5 ±3.4	60.0 ±2.7	42.4 ±2.3	22. ±2.0	10.0 ±1.2	2.5 ±0.3	95.0±4.2

As a result of the carried out experiments, it was noticed that the eggs of *S. cerealella* and *E. kuhniella* placed in the Petri dish and covered with flour up to a 0.5 mm (one gram of flour) were parasitized by *T. pintoi* up to 88.3% and 90% respectively; for a higher amount of flour (two grams) and the thickness of 1 mm, the indices are as it follows: 80.9% and 84% respectively, for 1.5 mm (three grams of flour) the results are: 72.54% and 76.5% respectively. The indices of parasitation for 2 mm layer of flour (4 grams) are 59.57% and 60%, for 2.5 mm layer of flour (5 grams) the percentage is 40.5% for *S. cerealella* and 42.4% for *E. kuhniella*, for the layer of 3.0 mm (six grams) of flour the indices are 22.2-20.5% and for a layer of 3.5 mm (seven gram) of flour: 9.0% and 10.0%, for the layer of 4 mm of flower (eight grams) the indices are 2.0% and 2.5% respectively.

In control, there were used uncovered eggs. The number of eggs parasitized by *T. pintoi* amounted 92.5% at *S. cerealella* and 95.0% at *E. kuhniella*. The entomophagous *Trichogramma* can penetrate through the layers of flour from 0.5 mm to 4 mm and parasitize the eggs of *S. cerealella* from 88.3% to 2.0%, *E. kuehniella* 90.0 to 2.5%.

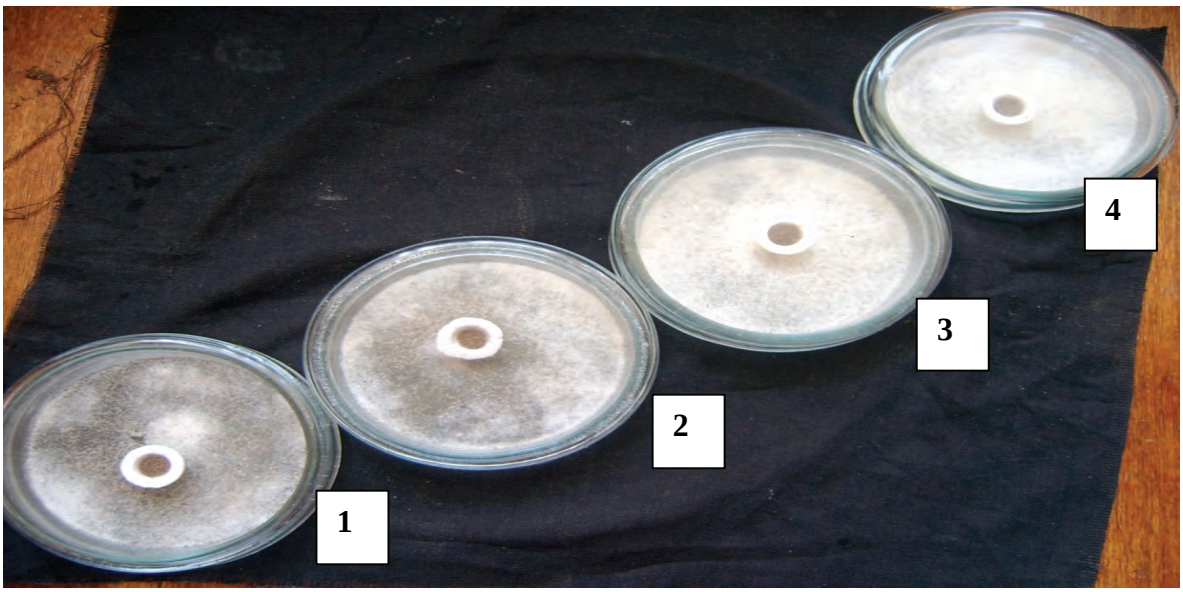
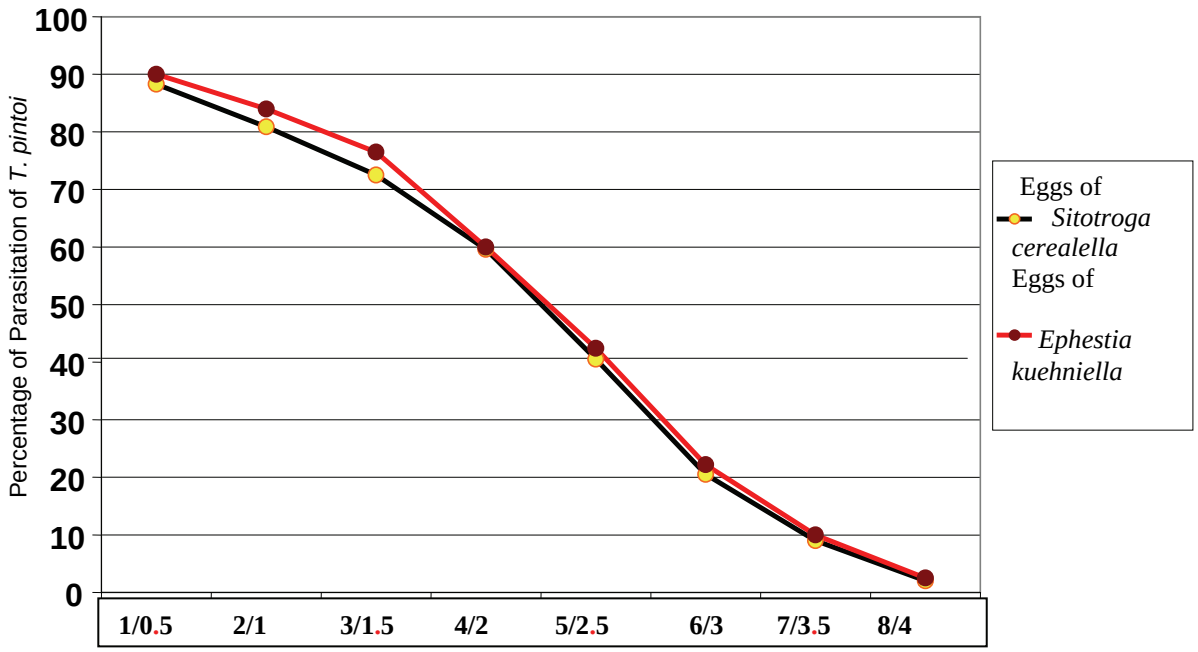


Figure 2. Percentage of parasitation by *T. pintoi* of *S. cerealella* eggs covered with different quantities of flower: 1 gram; 2 grams; 3 grams; 4 grams.



Quantity of flour on the surface of *S. cerealella* eggs in (gr.) and *E. kuhniella* and the thickness of the flour layer (mm)

Figure 3. Percentage of parasitation *of S. cerealella* and *E. kuhniella* eggs covered by flour by *T. pintoi.*

2b. **Determination of the biological efficiency of *Trichogramma pintoi* in the ecological space room and within the warehouse.**

In order to elaborate the technology based on *T. pintoi*, for the integrated protection of the cereal products and control of the moth complex in the warehouses, artificial conditions were created, but similar to the ones in warehouses, where preliminary results were estimated for later *Trichogramma* control technology implementation and examination.

Laboratory experiments were carried out in the ecological room at the temperature of 25±1ºC and relative humidity of 80-85%, in 50 repetitions (50 cards). Thermostat volume is 2m x 2m x 2m = 8 m^3, where eggs of *S. cerealella* and *E. kuhniella* were placed on small sized cards. In the given space, *T. pintoi* was launched in 10 capsules with the launching norm of 1,000 females at 20,000 eggs of *S. cerealella and E. kuhniella* with the rate of parasite: host of 1:20. After 7 days, we conducted the tracking of the number of host eggs parasitized (blackened) on cards by the entomophagous *T. pintoi*, where the biological efficacy in controlling hosts by *T. pintoi* varied from 60.4 to 80%.

Under the same laboratory conditions of the ecological room, experiments with the same hosts were carried out with the optimal rate of 1:1 (parasite: host). The number of parasitized eggs of *S. cerealella* and *E. kuhniella* in the area of 8m^3 varied from 62.5 to 88.6%. The entomophages searched for the moth eggs in the given area and parasitized them. In a warehouse, which is much bigger, the eggs of the hosts can be searched and parasitized by *Trichogramma*, but with a smaller rate parasite: host.

3**. Determination of the percentage of parasitized eggs by *T. pintoi* in the grain warehouse from Chişinău.**

Before launching, in the warehouse *T. pintoi* biological indexes were determined. The determined indexes were: prolificacy of females of 31.9 eggs /female hatching of the individuals 91.6%, female share 60.4%, static criterion of the quality 17.4. In order to determine the number of eggs parasitized by *T. pintoi* in the grain warehouse from Chisinau, an artificial fond was created to have a more precise evidence, by fixing 120 cards on the walls of the warehouse, windows, equipments with an amount of 12,000 eggs of *Sitotroga cerealella* preliminarily irradiated with gamma rays. In this warehouse *T. pintoi* was launched in capsules, with a rate of 1:1 (host: parasite), meaning 12,000 moth eggs to 12,000 *Trichogramma* females. The cards were then gathered and analysed, in order to emphasize the share of parasitized and exposed eggs. The percent of parasitized eggs of *T. pintoi* varied between 61.8 to 80.24%. It can be affirmed that in the grain warehouses the entomophagous *Trichogramma* can be used as one of the main elements in integrated protection.

During the years 2009-2010 the experiments were carried out in the grain warehouse from Chisinau. The evidence of the pests in warehouse was carried out on the entire surface of the investigated territory by means of application of the main techniques for monitoring inspections: inspections, sampling, temperature monitoring, and usage of pheromone traps. In order to determine the presence and numerical density of various species of moths in the grain store, there were mounted pheromone traps for: *E. kuhniella* Zell., *P. interpunctella Hubn.* and *E. elutella Hubn.* The monitoring of the moths by pheromone traps (Fig. 4) during the storage of cereal products was carried out from the 18th of May till the 20th of May 2010. The analysis of the moths captured in traps revealed the presence of the following species in the warehouse: *E. kuhniella*, which varied between 8.0 and 80.0 individuals and *P. interpunctella* between 1.0 and 5.0 individuals in a pheromone trap at the Mill No. 1. At the mill No. 2, *E. kuhniella* between 7.0 and 86.0

individuals were caught in a trap; *P. interpunctella* varied between 1.2 and 3.0 individuals. In the elevator (grain store), 2 to 10 individuals of *E. κuhniella* were caught in a trap in average, while *P. interpunctella* varied between 5.0 and 79.0 individuals. In the flour storage, there were caught in a trap from 17.7 to 109.0 *E. κuhniella* individuals, but *P. interpunctella* varied between 3.0 and 7.0 individuals. In elevator No. 2 - wheat warehouse, which served as a control – 3 to 9 *E. κuhniella* individuals were caught in a trap from, *P. interpunctella* varied between 19.0 and 139.0 individuals. The difference of the average is essential. *E. elutella* was not captured in the pheromone traps. In the flour mills, it predominates *E. κuhniella*, but in the elevator – *P. interpunctella*. The average temperature from the 18th of May till the 20th of August 2010, varied from 18.5ºC to 28°C.

4. Determination of the numerical density of moths in cereal products warehouse.

To identify the strategies that would ensure the protection of the stored cereals stocks, the first requirement is the knowledge of the diversity of pests present in warehouses. To realize this program of moths control with *T. pintoi*, it was necessary to determine the species and numerical density of the moth complex from the grain warehouse from Chişinău.

To determine the density of the moth complex (number of larvae) within the grain warehouse and the wheat elevators, there were collected 9 samples from different places in each store for every record. The determination of the numerical density of moths in the grain mill was made according to (TODIRAŞ et al., 2009).

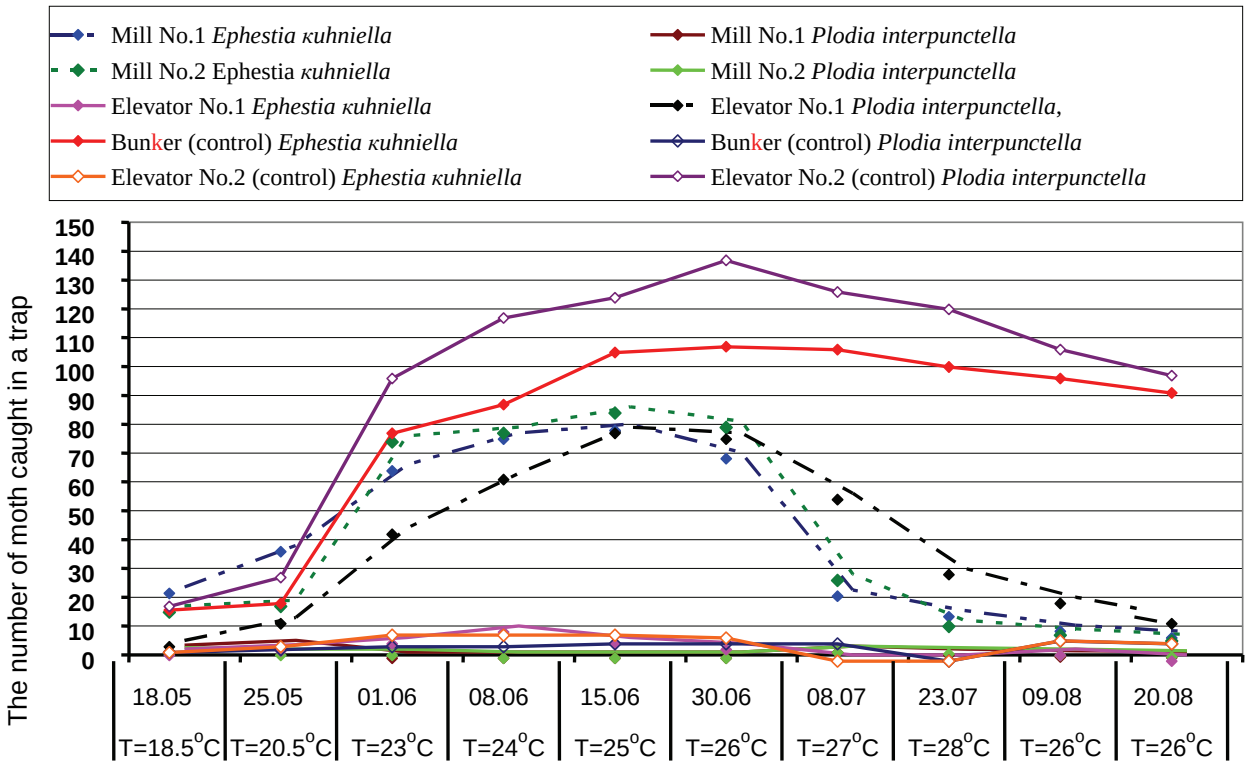


Figure 4. The dynamics of moths caught in the traps and the temperature.

After determining of the pest density it was determined the biological efficacy of *T. pintoi* in the control of the moths *E. κuhniella* and *P.interpunctella* in warehouses. The entomophagous *T. pintoi* was used as one of the important elements in the integrated protection of cereal products. The entomophagous *T. pintoi* was launched in capsules. The records of the bags of cereal products, walls, windows, equipment in the warehouse were realized, and then *T. pintoi* launched. During the experiments, *T. pintoi* was launched six times and there were realized seven records before and after the launching of the moths *Ephestia κuhniella* and *Plodia interpunctella*.

The biological efficacy after six launches of *T. pintoi* varied from 16.67% to 71.4% within the Mill No. 1. The biological efficacy of *T. pintoi* varied from 20.0% to 68.5% within the, Mill No. 2. Within the elevator No. 1 (grain warehouse), the biological efficacy of *T. pintoi* varied from 27.50% to 73.3%. In the control (flour mill and elevator No. 2), where the entomophagous insect has not been launched, the efficacy was not reported. The biological efficacy of *T. pintoi* increased after each start until the end of the experiments.

When comparing the efficacies of *T. pintoi* at the mills No. 1, No. 2, Elevator No. 1 and control (where the entomophagous insect was not launched), the difference of the average was significant: (T_d = 2.88-10.40)> ($T_{0.05}$ = 2.12) after sixth launches. Our results demonstrate that the release of *T. pintoi* into commodity storages can play an important role in the elimination of moth populations from warehouses, especially as part of an integrated control program.

CONCLUSIONS

As a result of the evaluation of the percentage of infestation of *Sitotroga cerealella* and *Ephestia kuhniella* Z, in laboratory conditions in different sublevels of barley, wheat, corn warehouses, the values of infestation varied from 70% to 90.8%.

As a result of estimating the biological efficacy of *T. pintoi* with different amounts of dust on the surface of *S. cerealella* and *E. κuhniella* eggs in the Petri dish and covered with flour, *T. pintoi* can parasitize the moth eggs under the layers the thickness of which varies from 0.5 mm (one gram) to 4 mm (eight grams), with 2.0% to 90.0% indices. In the control, number of eggs parasitized by *T. pintoi* was 92.5% in *S. cerealella* and 95.0% in *E. kuhniella.*

The density of larvae in the warehouse varied from 2 to 3. 4 individuals at mill No. 1 and mill Nr. 2. In the control (warehouse) the density of larvae varied from 3 to 7.5 individuals. In the elevator No. 1 (wheat storage), the larvae density varied from 2.7 individuals to 4.4 individuals. In the control at elevator No. 2 the number of larvae varied from 4 to 10.2 individuals. There is a very big difference compared to the control. The most encountered pests at the cereal warehouses from Chişinău were *E. κuhniella* and *P. interpunctella*; against these pests, *T. pintoi* has been launched six times.

The number of eggs of *S. cerealella* and *E. κuhniella* parasitized by *T. pintoi* in the area of 8 m^3 where the optimal ratio (P:H) is 1:1 ranged from 60.4 to 88.6%. The percentage of eggs parasitized by *T. pintoi* in the deposit was 61.8%-80.24%.

As a result of the launching the *T. pintoi* in the ecological room and storage spaces on cards, it was determined – the biological efficacy of *T. pintoi*. Analyzing range parasite: host relationships, it was found that the optimum ratio (P:H) is 1:1, where the maximum efficacy in controlling the moth complex in the ecological room is 88.66% and 80.24% in deposits.

In the control option from the bunker, which was held in elevator No. 1 (flour warehouse), No. 2 (wheat warehouse) and where the entomophagous insect was not launched, the efficacy has not been reported. The mean differences is critical, (DEM), (Td = 2.88 to 10.40> 2.12 = T 0.05), after the second – the sixth release.

The density of larvae in the storage ranged from 2.0 to 3.4 individuals, mill No. 1, No. 2 flour storage, the control – bunker (flour storage), the density of larvae ranged from 3 to 7.5 individuals. In case of elevator No. 1 (wheat storage), larval density ranged from 2.7 to 4.4 individuals, in the control - (Elevator No. 2 - wheat warehouse the density of larvae ranged from 4.0 to 10.2 individuals. This difference is essential compared to the control.

The results obtained in the 2009-2010 were used for the elaboration of the technology used for *T. pintoi*, for integrated protection of the cereal products used against the moth complex in warehouses.

REFERENCES

ABASHKIN A., VOROTYNTSEVA S., GREENBERG A. 1979 *Guide for mass decreases and the use of Trichogramma*. Methodological Guide. Moscow. Vasihnil. 133 pp. [In Russian].

BROWER, J. H. 1988. The natural occurrence of the egg parasite *Trichogramma*, on almond moth eggs in peanut storages. *Journal of the Georgia Entomological Society*. Georgia. **19**: 285-290.

BROWER J. H., & PRESS J. W. 1990. Interaction of *Bracon hebetor* (*Hymenoptera: Braconidae*) and *Trichogramma* pretiosum (*Hymenoptera: Trichogrammatidae*) in suppressing stored product moth populations in small in-shell peanut storages. *Journal of Economic Entomology*. **83**(3): 1096-1101.

DYURICH G. F. 2008. *Determination, collecting and maintaining live cultures of the genus Trichogramma* Westw. (*Hymenoptera, Trichogrammatidae*). Methodological Guide. CEP Chişinău-Prim. 36 pp. [In Russian].

EVANS D. E. 1987. The influence of rate of heating on the mortality of Rhyzopertha dominica (F.) (*Coleoptera: Bostrichidae*). *Journal of Stored Products Research*. **23**: 73-77.

MATTHEEW, J., M. GREISHOP, P. FLINN, J. NECHOLS, F. JAMES. 2008. *Effect of fine-grain habitat complexity on egg parasitism by tree species of Trichogramma Kansas State University*. Department of Entomology. 123 West Waters Hall. Manhattan. USA. 34-38.

MENCHER, E. & ZEMSHMAN A. 1986. Basics to planning experiences with elements of mathematical statistics in viticulture research. Chișinău. 20-27. [In Russian].

TODIRAȘ V., TRETIACOV TATIANA., BALAN IU. 2009. Integrated protection and quality assurance of stored food products, Chişinău: 10-16.

Methodical guidelines for chemical and biological plant protection products from pests, diseases and weeds in Moldova testing. 2002. State Center for Certification of Chemical and Biological Protection and plant growth stimulation, Chişinău, (F.E.P**.** "Central Printing"): 31-45.

Gavriliţa Lidia
Institute for Genetic, Physiology and Plant Protection, Academy of Science of Moldova
Chişinău, Str. Pădurii , No. 20, Republic of Moldova.
E-mail: lidia_gavrilita@yahoo.com

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