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TRICHOGRAMMA ENTOMOPHAGE IN INTEGRATED PLANT PROTECTION AS MEANS TO REDUCE PESTS' POPULATION DENSITY CROPS IN REPUBLIC OF MOLDOVA

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Key words: *Trichogramma, pest control, parasites, plant protection, biological methods, entomophagous, phytophagous*

INTRODUCTION

A very important role in integrated plant protection refers to biological measures for plant protection. Conservation and activation of the natural mechanisms in harmful organism density regulation must be based on profound knowledge of all the factors and biocoenotic bounding. In general it is noticed a very notable trend in ambient protection in the activities that depend on environment.

The basic principle of the biological control is the biocoenotic balance through which the population of a species (prey, host) is conditioned by other species (predators, parasites and pathogens). But this balance is swinging, has dynamic character and can be disrupted by agro technical practices as well as plant protection. Therefore it is necessary to create favorable conditions to entomophagous organisms for their development.

Trichogramma species parasitize many species of pests and are used in biological plant protection. Considering (Lenteren, 2000) the data from 2000 year, annually, 45 million hectares are treated with *Trichogramma* sp. in the world. Considering (Knutson,.2001) this information, *Trichogramma* is the most used entomophage in the world, which was launched annually on approximately 32 million hectares on agricultural cultures and forests in 30 different countries.

A pest example against which *Trichogramma* is used is *Helicoverpa armigera Hb*, which brings harm to a wide range of cultures. *H.armigera Hb.* is a polyphagous species, attacking over 120 species of crops and wild, causing significant damage to nature. Annual harvest losses of vegetables, corn and other crops are 15-80%. Correct and appropriate time launching of *Trichogramma* spp. entomophages in combating (controlling) these pests helps us reduce the number of pest density from 60 to 80% (Mureşan and Mustea, 1995).

The pest *H. armigera* Hübner (*Lepidoptera*: *Noctuidae*) is controlled with *Trichogramma evanescens*Westwood *Hymenoptera*: *Trichogrammatidae*) in cotton cultures (*Malvaceae*) in Turkey. *H. armigera* Hb. has 5 generations per year. *Trichogramma* launching was done twice for each of the three generations of *H. armigera* in 2004 and 2005 years. For every release of *Trichogramma* in field, 120.000 parasitoids were used per hectare. Percentage of parasitized eggs was – 62.9% and 71.6%.The percentage of larvae of *H. armigera* was reduced to 76.8% and 80.6%, respectively (Oztemiz et al., 2009). The "Biotop" Company from Europe produces and sells the *Trichogramma* entomophage, which is used against Lepidoptera pests. *Trichogramma* is produced for farmers in biological protection against *Ostrinia nubilalis* (corn) and *Tuta absoluta* (tomatoes) and is used yearly on 100.000 hectares in France, Germany, Switzerland and Czech Republic, (Frandon, 2012)**.** About *Trichogramma brassicae's* use as parasite on pests eggs in Germany, which was reared in laboratory conditions and applied in field against *O. nubilalis* for corn and other cultures like (apple, plum, grapes and some crops in greenhouses) on a total surface of 11.000 hectares, several papers were written by the author, (Zimermmann, 2004). In Ukraine, in present time, biological methods are used on an overall area of about 1.2 million hectares, where 65 laboratories are functioning and where 35-40% inclusively of the reared material is *Trichogramma* (Fiodorenco et al., 2009). The use of the biological methods for agricultural culture protection, inclusively with *Trichogramma* in Latin America (Brazil, Colombia, Costa Rica, Cuba, Ecuador, Panama, Venezuela) emerged to a total area of about 9 million hectares in 2002 (Bueno, Lenteren, 2002).

In the Republic of Moldova, as well as in other countries, specialists' goal is to minimize yield losses caused by illnesses and pests without influencing negatively the fauna in biocoenosis and to obtain ecologically pure yield.

Within the Research Institute of Genetics and Physiology of Plant Protection of the Republic of Moldova, in 1976, it was created the "*Trichogramma* laboratory". The main goal of the laboratory is to research, analyses, elaborate data in rearing and application of the *Trichogramma* entomophage in pest control. Nowadays, researches are still been carried out with great success. In order to apply *Trichogramma* in plant protection, profound knowledge in Ecology, Biology of entomophagous and phytophagous organisms is required. There are several factors to be considered, which influence the efficacy in field. Obtaining satisfactory results in the *Trichogramma* application for pest control leads to the continuous analysis of variables and the driven results focuses knowing the role and place of entomophages for regulating pest density.

MATERIALS AND METHODS

The research studies were done in field and laboratory conditions during various institutional and state projects with Innovation and Technological Transfer Agency. Implementation took place in various farms in the Republic of Moldova and on Institute's agricultural territory along the 2006-2012 years. *Trichogramma* has been collected from annual cultures (cabbage, tomatoes, apple, plum, maize, peas, sugar beet and soybean) *Trichogramma evanescens* Westw. cu *Trichogramma embryophagum* Htg., was reared on different host eggs preliminarily irradiated and non-irradiated with gamma rays. Collecting, identification, storage and accumulation of *Trichogramma* species were done using (according to) (Diurici, 2008). Rearing of the aboratory host – grain moth (*Sitotroga cerealella* Ol.), for *Trichogramma* production was done by (Abaschin et al., 1979) authors' methods. Mathematical data elaboration was done according to Mencer, Zimerman (1986). Investigation objectives represented in Figure 1-22.

RESULTS AND DISCUSSION

Trichogramma spp. entomophagous is one of the most important biological agents in biological plant protection and in integrated plant protection, which rears easily in the laboratory conditions and accumulates fatly because of its short development period of a generation. *Trichogramma* entomophagous is used at its initial development stage (eggs).

***Trichogramma* use for annual and multi annual crops protection:**

1. Vegetables – cabbage, tomatoes, peppers, eggplant, etc. – Noctuidae complex: *Mamestra brasssicae* L., *Helicoverpa armigera Hb., Agrotis exclamationis* L, *Amathes-S-nigrum* L., *Agrotis Ypsilon* L, moth complex: cabbage moth (*Plutella maculipenis),* Pieridae complex: *Pieris brassicae, Pieris rapa*, etc.
2. Legumes - peas, soybeans, lentils, etc. – Noctuidae complex: *Mamestra brasssicae L., Helicoverpa armigera Hb., Agrotis exclamationis* L*., Amathes-S-nigrum L., Agrotis silon* L. and moth complex..
3. Technical - sugar beet, sunflower, etc., Noctuidae complex: *Mamestra brasssicae* L., *Helicoverpa armigera Hb., Agrotis exclamationis* L*., Amathes-S-nigrum* L., *Agrotis Ypsilon* L.), moth complex, etc.
4. Grasses - corn, wheat, barley, oats, etc. – Noctuidae complex: *Mamestra brasssicae L., Helicoverpa armigera* L*., Agrotis exclamationis* L*., Amathes-S-nigrum* L*., Agrotis Ypsilon* L.moth complex, and corn *Ostrinia nubilalis* L.
5. Floriculture – Noctuidae complex: *Helicoverpa armigera Hb., Agrotis exclamationisL.*, moth complex.

6. Floriculture – Noctuidae complex: *Helicoverpa armigera Hb., Agrotis exclamationisL.*, moth complex

Medicinal – Noctuidae complex: *Helicoverpa armigera Hb., Agrotis exclamationis L.*, moth complex.

7. Fruits – Noctuidae complex; *Cydia pomonella L., Grapholitha funebrana Tr. Grapholitha molesta* Br.

8.Vineyard – Noctuidae complex: *Lobesia botrna L.*

In the Republic of Moldova, it was organized and functioned until recently an integrated system for producing biological resources for biological plant protection. For some years now the system is stationary, currently working partly, only two biological laboratories (Cahul, Soroca) out of 14. *Trichogramma* spp. is one of the most important biological agents in plant protection. In the Republic of Moldova, the volume of utilization of *Trichogramma* in field constituted 80% to 85% of the agricultural territory (1984-1992 years). Nowadays, the volume of production decreased considerably, covering only 30 to 663 thousands hectares (Figure 23). From 1994 to 2002 in Rep. of Moldova the biological agent *Trichogramma* has not been produced. Starting with 2002 the production process has had been reinitiated..

Investigation objectives

Figure 1. *M. brassicae* parasitizing process (original)

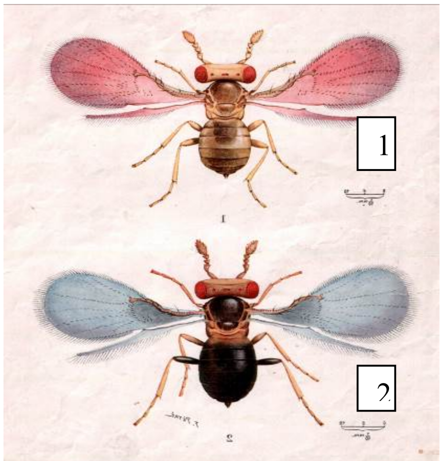

Figure 2. 1. *T. evanescens;*
2. *T. pintoi*

Figure 3. *Ostrinia nubilalis.* parasitizing process (original)

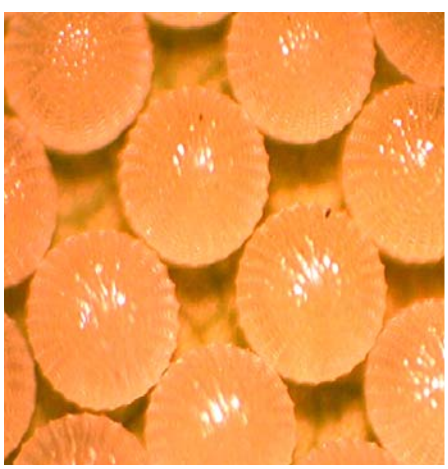

Figure 4. *M. brassicae* eggs (original)

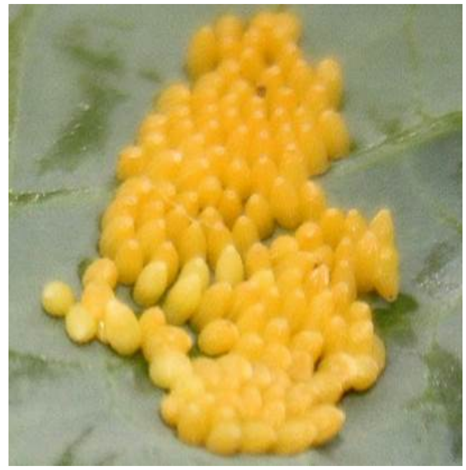

Figure 5. *Pieris brassicae* eggs at cabbadge (original)

Figure 6 **.** *Ostrinia nubilalis* eggs corn (original)

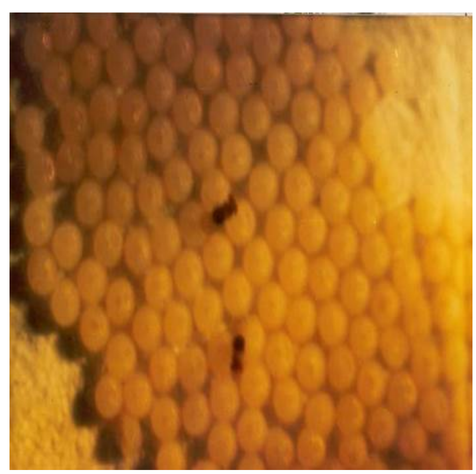

Figure 7. Parasitizing *M. brassicae* by *Trichogramma* (original)

Figure 8. *Pieris rappaie* egg at cabbadge. (original)

Figure 9. *Ostrinia nubilalis* eggs a parasitized by *Trichogramma.* (original)

Figure 10. *M. brassicae eggs parasitized by Trichogramma* (original)

Figure 11. *H. armigera parasitizing process* (original)

Figure 12. *Sitotroga cerealella* Ol. Parasitizing process (original)

Figure13. *M. brassicae* eggs parasitized by *Trichogramma* on corn (original)

Figure 14. *H. armigera* parasitized egg oat corn. (original)

Figure 15. *H. armigera* egg at cabbage (original)

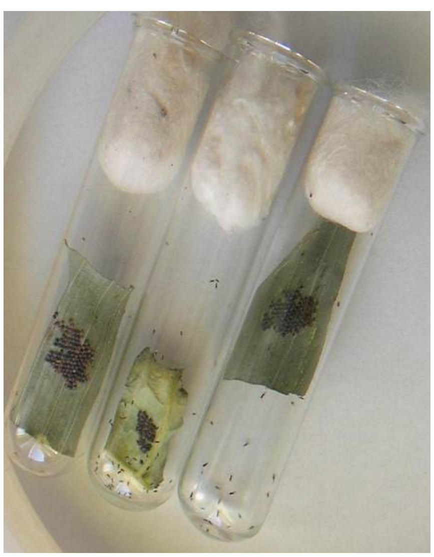

Figure 16. *M. brassicae* hatched eggs parasitized by Trichogramma (original)

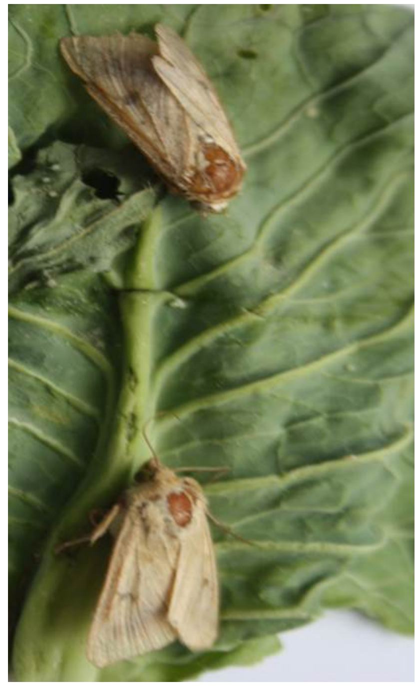

Figure 17. *H. armigera* eggs and adults at cabbage (original)

Figure 18. *H. armigera* parasitized eggs by Trichogramma on tomato (original)

Figure 19. *H. armigera* egg on plums (original)

Figure 21. *Grapholitha funebrana* parasitized egg at plum. (original)

Figure 20. *Laspeyresia pomonella* parasitized eggs apple culture (original)

Figure 22. *Trichogramma* development of one generation from host eggs parasitizing to larvae development stages, to hatching

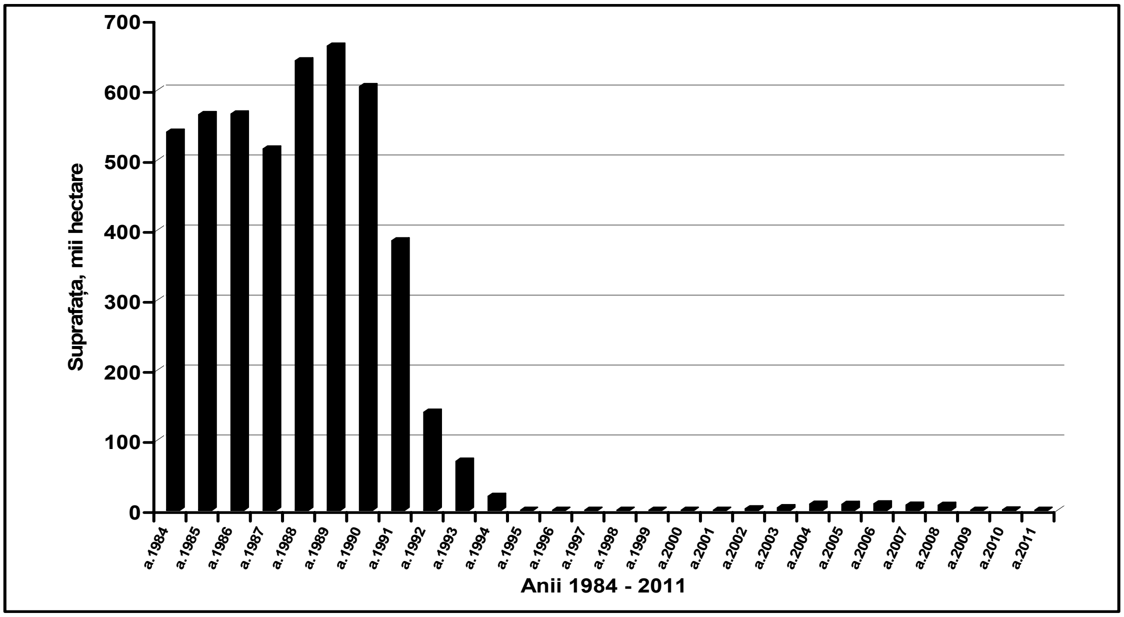

Figure 23. Agricultural filed area protected by *Trichogramma* in Republic of Moldova during the 1984 to 2011 years. (Information taken from the Inspectorate for general sanitary surveillance and seeds control. Plant Protection Division, Department of Monitoring

Table 1. Methods for increasing the quality of *Trichogramma evanescens* Westw. grown on different hosts eggs

Hosts	*Mamestra brassicae*	*Ostrinia nubilalis*	*Ephestia кuhniella*	*Sitotroga cerealella*
Procedures	Prolificacy, eggs/female			
1. Passages in natural hosts	40.4±1.8	23.0±1.0	30.8±1.3	30.0±2.0
2.Supplementary feeding with honey	34.5±1.8	26.6±1.5	32.3±1.7	32.5±1.5
3.Supplementary feeding with 20% sugar syrup	33.1±1.8	25.5±1.5	32.6±1.8	33.4±1.9
4. Gamma rays, grei	60.4±2.9	23.1±1.6	50.8±2.9	50.0±2.5
5. Ultraviolet, hours	46.0±2.8	21.3±1.5	29.3±1.8	27.6±1.3
6. Fe_3O_4 magnetic fluid	-	-	30.3±1.5	37.0±1.7
7. „In vitro" medium	-	-	24.8±1.6	25.0±1.6
8. Untreated hosts (control)	23.2±2.9	13.3±1.0	25.3±1.4	20.3±1.2

Protected crops areas with *Trichogramma* spp, in Moldova, during the 2002-2011 years constituted a total of 47.7 thousands hectares at different annual crops (cabbage, tomatoes, corn, peas, sugar beet and soybean), from 2002 to 2008 years, and ranged from 29. thousand hectares One of the most significant problems in mass rearing of *Trichogramma* is its quality, which decreases easily while rearing several generations consecutively on host laboratory eggs, respectively its field efficacy decreases. For this reason several researches were made for augmentation its efficacy. One of the procedures with high success is rearing *Trichogramma* on preliminarily gamma rays irradiated eggs. The results are presented in Table 1.

In the Research Institute of Genetics and Physiology of Plant Protection to increase the quality and effectiveness of *Trichogramma*, different rearing methods were used on various hosts for plant protection. *T. evanescens* prolificacy reared on *M. brassicae L.* eggs treated with different factors, ranged from 33.1 to 60.4 eggs/female. In untreated host eggs the results were 23.2 eggs/female. Reared on *O. nubilalis*' eggs, the results ranged from 21.3 to 26.6 eggs/female whereas in untreated host eggs – 13.3 eggs/female. Reared on *E. kuhniella* eggs the results ranged from 24.8 to 50.8 eggs/female, whereas in untreated eggs the results were – 25.3 eggs/female. Reared on *S. cerealella* eggs the results ranged from 25.0 to 50.0 eggs/female whereas in untreated eggs – 20.3 eggs/female. Biological indexes: prolificacy, individual hatching, rate of females are higher in the variants where *Trichogramma* was reared on treated hosts eggs rather than untreated eggs having a 1.5 to 2.5 times higher results and effectiveness in field increased from 7% to 10%.

Static criteria of the quality (including: prolificacy, individual hatching, female rate) in the variant where *Trichogramma* was reared on *M. brassicae* L. constituted 23.4; on untreated eggs – 17.8. On *E. kuhniella* Z. irradiated beforehand with gamma rays – 14.4 whereas untreated eggs – 11.4. On *S. cerealella* Oliv. treated eggs – 13; untreated 8.7. On *O. nubilalis* Hb. treated eggs – 11; untreated – 6. Comparing the variants of the treated and untreated eggs under the T-Student criteria, statistical data are veridical at a 95% level (T_f = 2.4-3.3 > $T_{0,05}$ = 1.96).

Determination of biological indices of the *Trichogramma* spp.

During the 2006 to 2010 years, in laboratory conditions, after prolonged storage (diapause) – 6 months, *Trichogramma* was reared for 3-4 generations each year. Later on, the biological indices were determined for *Trichogramma* grown on grain moth irradiated beforehand with gamma rays (I Variant) and non-irradiated (II Variant) - prolificacy, number of female, individuals hatching at the temperature of 26 ± 1 °C and relative humidity of 80-85%. Results are presented in Table 2.

For *T. evanescens, T. pintoi* (tomato, corn), biological indicators in the first variant varied as it follows: female prolificacy – from 30.9 to 36.8 eggs/female, individuals hatching from 88.3% to 94%, female number – 56.7 to 60.0%, static criteria of quality – from 16.2 to 19.6. In the second variant these indices ranged as it follows: prolificacy of females – from 18.0 to 21.5 eggs/female, individuals hatching from – 79.6% to 86.0%, female number – 53.5 to 56.0%, static quality criteria – 8.4 to 9.4.

During the development period of 2-3 generations of the annual researched crops (cabbage, corn, tomatoes, peas, sugar beet and soybean), average biological efficacy of *T. evanescens* in pest control during the 2000-2014 years, the percentages varied from 74 to 90% in the first variant and from 60- to 81% in the second one (Table 3).

Pest density of the researched annual crops during the 2006-2014 years varied from 1 to 60 eggs/plant. Pest attack on agricultural cultures in 2000-2014 years varied from 2 to 30% in *Trichogramma* applied variant, whereas in control (untreated crops by *Trichogramma*) the same index varied from 16 to 90%. The percentage of different pest eggs parasitized by *Trichogramma* in nature on different cultures in the same period of time varied from 1 to 9% (at the end of vegetation period).

Table 2. Biological indexes of *Trichogramma spp*., applied on annual crops in 2006 - 2010

Years	*Trichogramma* species	Variants*	Biological indexes			
			Prolificay eggs/female, (P)	Individual hatching, % (α1)	Female rate, % (α2)	Static criteria of the quality, (γ*1*)
2006	*T. evanescens (tomatoes)*	I	36.8±0.7	94.0±1.3	56.7±2.7	19.6±0.7
		II	21.5±0.1	79.6±3.2	54.5±2.5	94±2.5
2007	*T. evanescens (corn)*	I	34.0±1.5	90.0±3.2	56.0±3.2	17,1±0,7
		II	20.0±1.3	80.0±1.6	53.0±2.1	8.4±0.5
2008	*T. evanescens (tomatoes)*	I	34.6±1.9	88.3±2.3	60.0±2.3	18.3±0.8
		II	18.0±1.6	85.0±2,5	55.0±2.5	8.4±0.1
2009	*T. pintoi (corn)*	I	30.9±2.5	88.6±3.2	59.4±3.2	16.2±0.3
		II	20.0±2.1	86.0±1.3	55.0±2.5	9.4±0.6
2010	*T. pintoi (corn)*	I	31.9±0.9	91.6±1.9	60.4±3.2	17.4±0.6
		II	21.0±0.8	84.0±1.8	56.0±2.5	9.8±0.7
	DEM					2.1-5.2

*I variant – reared on irradiated host eggs, II variant – reared on different non irradiated host eggs

Table 3. Biological efficacy of *Trichogramma evanescens* in pest control on annual crops for 2000 to 2014 years.

Farms	Culture	Pest name	Area, (hectares)	*Trichogramma* species	Variants*	Parasitized eggs, (%)
Gura Bâcului, Sărata Galbenă, Băcioi, Sîngera Chetrosu	Cabbage	*Mamestra brassicae L. Helicoverpa armigera* Hb.	36,5	*T. evanescens*	I II	74.0 - 90.0 60.0 - 81.0
Sărata Galbenă, Băcioi, Coşniţa, Mărăndeni, Bălţi, Căuşeni	Corn	*Helicoverpa armigera* Hb. *Ostrinia nubilalis* Hb.	1512	*T. evanescens*	I II	80.0 - 88.0 73.0 - 80.0
Sărata Galbenă, Chişinău, Gura Bâcului, Sângera	Tomatoes	*Helicoverpa armigera* Hb.	288	*T. evanescens*	I II	83.0 - 90.0 74.0 - 80.8
Sărata Galbenă, Chişinău	Peas	*Helicoverpa armigera* Hb.	210	*T. evanescens*	I II	80.0 - 86.0 75.0 - 80.0
Pohoarna, Mărăndeni	Sugar beet	*Helicoverpa armigera* Hb.	350	*T. evanescens*	I II	84.0 - 85.0 76.0 -77.0
Mărăndeni	Soya	*Helicoverpa armigera* Hb.	580	*T. evanescens*	I II	83.0 - 86.0 75.0 - 76.0
		Sum: 2976.5		(Td=1.0-1.90 <1.96=To.o5).		

*I variant – reared on irradiated host eggs *Sitotroga cerealella* Ol., II variant – reared on different non irradiated host eggs.

Application *Trichogramma embryophagum* in the apple tree orchards

During the period 2002-2009, experiments were carried out in apple orchards with different densities of apple moth populations in the Institute of Protection of Plants and Ecological Agriculture of the ASM, and agricultural enterprises Mereni and Puhoy, applying *T. embryophagum* for the development of two full and part-time third-generations of apple moth pest. The launch scheme of *T. embryophagum* in the field in small bags is represented in Figure 24. The results of the efficacy of *T. embryophagum* against apple moth in the period from 2002 to 2009 are presented in Table 4,. Research was conducted in three variants: the 1st version of *T. embryophagum* was bred on grain moth eggs irradiated with gamma rays, in the 2nd variant *T. embryophagum* was bred on the non-irradiated grain moth eggs; the 3rd option - control - without *Trichogramma* releases, where it was the detection of entomophage presence in nature.

To monitor the pest the pheromone traps were set in the garden. Direct recording of density apple moth eggs and the percentage of infected *Trichogramma* was carried on there. The density of eggs according to generations and years ranged from 8.0 - 90.0 eggs per 100 fruits and leaves. The total area of the orchids where entomophage issues were conducted was 33.15 hectares. After six issues of *T. embryophagum* the biological efficacy ranged from 68.2 to 90% in the 1st variant and 64-84.7% in the 2nd version. Control check reflected that the number of pest-parasitized eggs ranged from 2.0 to 21.5% and fruit-damage ranged from 45.0-93.0%.

Therefore flooding issues of the entomophagous *T. embryophagum* to reduce the number of codling moth, the major pest of apple, can become one of the main methods of integrated garden protection, allowing the reduction of the number of insecticide treatments to a minimum.

The entomophagous *Trichogramma* is one of the most important biological agents which may by utilized against a complex of pests both in biological control and in the system of integrated plant protection. The utilization of the eggs of *Sitotroga cerealella* Oliv. irradiated preliminary by gamma rays essentially increases *Trichogramma* biological indices and the efficiency in the field. The biological efficacy of *Trichogramma* and the degree of damage in the first version (on irradiated eggs of grain moth) when compared with the second option (on the non-irradiated eggs) are significantly different.

The priorities of entomophagous in plant protection are: to reduce the financial expenses for protection, to save useful organisms in nature, to increase the biological effectiveness in the field, to increase the volume of agricultural production to provide food quality, to reduce the number of chemical treatments to a minimum of integrated system that prevents contamination of the environment, low cost, safe, good-quality, easy usage, ecological cleanliness.

The entomophagous *Trichogramma* spp. is one of the most important biological agents which may be used against a complex of pests both in biological control and in the Integrated Pests Management. The biological indicators of *T. evanescens* rearing on the eggs of different hosts irradiated with gamma rays increased by 2-2.5 times and its biological effectiveness in the field is more than 10-15%.

Table 4. Biological efficiency of *Trichogramma embryophagum* on the apple tree crop

Year, area	Variants	Biological effectiveness, %	Apple fruit damage,%
2002 Mereni	I II III	75.5±2.5 64.0±1.9 3.5 ±0.2	4.7±0.3 6.8±0.2 45.0±1.1
2003 Chisinău	I II III	90.0±2,8 84.7±2.5 4.0±0.3	11.0±1.6 16.0±1.80 81.7±2.9
2005 Chisinău	I II III	86.0±3.0 80.0±2.8 5.0±0.1	9.0±0.8 15.0±0.9 80.0±2.9
2006 Chisinău	I II III	80.0±3.0 73.3±2.1 5.0±2.0	11.2±0.8 18.7±1,0 70.0±2,1
2007 Chisinău	I II III	83.3±3.5 78.1±2.5 5.7±0.7	25.5±1,9 27.8±1.0 90.1±3.5
2007 Puhoi	I II III	83.3±2.7 78.1±2.1 2.0±0.1	3.5±0.3 4.4±0.4 34.0±1.2
2008 Chisinău	I III	68.2±2.0 8.8 ±1.0	42.3±1.7 92.5±3.6
2009 Chisinău	I III	78.5±2.8 21.5±0.9	38.0±1.5 93.0±3.6

Legend: Variant I - *T. embryophagum* bred on irradiated grain moth eggs (*Sitotroga cerealella* Oliv.) Variant II - *T.embryophagum* bred on non-irradiated grain moth eggs; Variant III - control, without the release of *Trichogramma.*

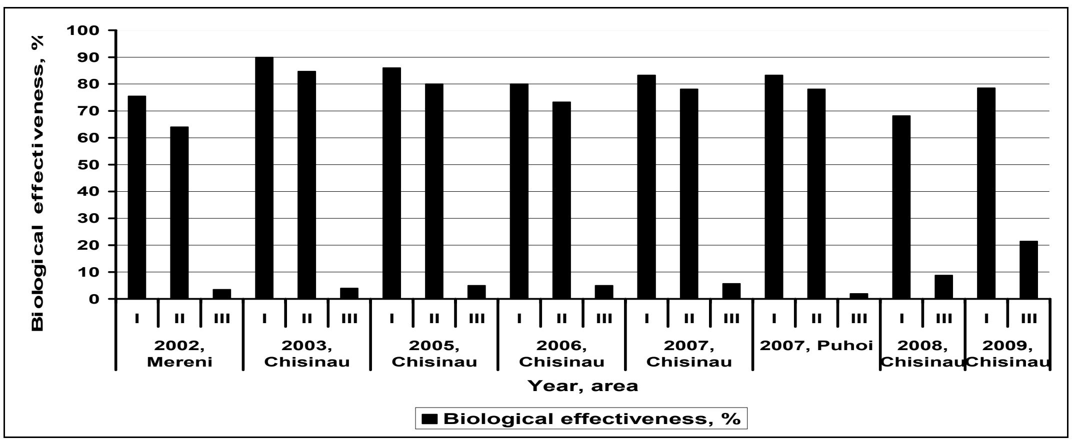

Figure 24. Biological effectiveness of *Trichogramma embryophagum* biological control over the *Laspeyresia pomonella* L. in orchards

Determination of species, biological indices and conservation of collected populations of *Trichogramma* for plum crop protection.

During the year 2011-2012, under laboratory conditions after prolonged storage (diapauses) for 6 months, *Trichogramma* was multiplied for 3 generations and there were determined the biological indices of *T. embryophagum* bred on the grain moth eggs – prolificacy, the share of females, hatching individuals.

T.embryophagum biological indices (collected from plum) were: prolificacy – 25.0 eggs per female, individuals hatching – 87.0%, the share of females – 100%, static criterion of quality – 21.7.

For research conducting reason, entomophagous *Trichogramma* was collected from the nature. The reason for this was to have and work with species that dominate the plum crop and for gene pool population renewal, because the multiplication under laboratory conditions of many generations in a row (years) leads to the decrease of the entomophagous quality. In the plum orchard from "AGRO BRIO" Bacioi farm", where experiments were conducted, 12 records were performed. Entomophagous *Trichogramma* presence in nature was reported at a 6.0% quote. The most common species of *Trichogramma* collected from plum crop are: *T. embryophagum* – 50%, *T. dendrolimi* – 30%, and 20% of *T. evanescens*. From these three species, *T. embryophagum* predominates. These species were collected and were induced in diapauses for further research.

We determined the pest (*Grapholitha funebrana*) dynamics of eggs lay during the development period to optimize pest terms and rules of launches. In Bacioi plum orchard 12 records were carried out for the determination of egg density of *G. funebrana* on plum, before and after the release of *Trichogramma*. In the first pest generation the average density of plum moth eggs ranged from 1 to 8 eggs per 100 fruits, on variants. During the development of the second generation, pest average density of plum moth eggs ranged from 10 to 16 eggs per 100 fruits, on variants.

Determining the spatial distribution of the pest *G. funebrana* density in the field, to optimize terms and rules of entomophagous *T. embryophagum* launch in pest control during the development of the plum crop.

During the pest (*G. funebrana*) development period, worm eggs laying dynamics was determined to optimize the launch terms and conditions. Density determination of the pest eggs (*G. funebrana*), was done by the traditional method. In Bacioi farm plum orchard 6 launches and 12 records were performed to determine egg density (*G. funebrana*) in plum, before and after the launch with *Trichogramma*. In each variant, records of the number of pest eggs were taken, with 100 fruits per tree, where pheromone traps were installed. The first variant includes rows 1, 4, 7 recording a total of 27 trees and 2700 fruits. The second variant includes row number 12, for a total of 15 trees and 1500 fruits. Third variant (raw 18) comprises a total of 14 trees and 1400 fruits. Fourth variant (Witness -control) contains row 24 in total 4 trees recorded for 500 fruits. Records were conducted at 60 plum trees uniformly distributed in the field.

During the first generation of the pest, the average density of plum worm eggs ranged from 1 to 8 eggs per 100 fruits per variants. Pest eggs density was lower than the economic damage threshold (2-5 eggs/100 fruits, or 2-3% damage). During the development of the second generation of the pest, average density of plum worm eggs ranged from 1 up to 16 eggs per 100 fruits per variants (Figure 25). With the “Bio Class” program – multi-classification system criteria of information optimization regarding the initial density in the field of *G. funebrana* there were built digital maps of the spatial (Figure 30) distribution of eggs of *G. funebrana* during the development of two generations of this pest and where the uneven distribution was determine and outbreaks detected locations.

Determination of the release norms of entomophagous *T. embryophagum* in combating plums worm (*G. funebrana*) at plums.

For the determination of the optimal norms of the launch of the entomophagous *Trichogramma* against plum moth *G. funebrana* at plums, several variants were analyzed as it follows: 450 thousand individuals per hectare in the first variant, 350 thousand/ha in the second variant and 250 thousand individuals per hectare in the third variant. In the fourth variant (witness) there were no launches performed. The number of parasitized eggs was determined on variants and witness.

Determination of the parasitic capacity of the plum moth eggs (*G. funebrana*) by the entomophagous *T. embryophagum* at plum crops.

The launch of the entomophagous *T. embryophagum* was conducted after the accumulation, determination of the biological indices and monitoring with pheromone traps of the plum moth butterfly in the field. The launch scheme of *T. embryophagum* in the field in small bags is represented in Figure 26. In the plum orchard from Bacioi farm 6 launches were conducted for (*G. funebrana*) egg density determination. Evidences were performed before and after each the launch with *Trichogramma*. The density of the pest eggs was initially small and this is the reason why two launches were prophylactic.

In the first generation – eggs parasitized by *T. embryophagum* varied on variants from 16.6% to 25.0 %, after the first two launches. During the second generation 4 launches were conducted up to the date of August 2012 in dependence on the pest presence in the field. Later on the number of eggs parasitized by *T. embryophagum* was determined on variants and it oscillated between 16.6% and 75% (Table 5, Figure 26). During the two generation development of the pest *G. funebrana*, biological efficacy of *T. embryophagum* in the field varied from 22% to 75% in first variant and from 20% to 69.2% in the second variant. In the third variant efficacy in the field varied from 16.6% to 66.6%. In case of the control plot the parasitized eggs during the entire period varied from 3.8% to 6%.

During the two generation development period of the pest *G. funebrana* in the plum orchard the pest attack was determined as it follows: in the first generation on variants it varied from 1.8 to 2.2%, during the second generation on variants it varied from 1.3% up to 2.2%. In case of the control plot, the pest attack on fruits varied from 3.0% to 4.4%. On variants, fruit attack ranged from 0.6 to 2.0% in the first variant; in the second variant ranged from 0.9 to 2.3%, in the third attack it ranged from 1.0 to 2.5%. In control, fruit attack ranged from 1.8 to 4.4%. Figure 27 summarizes *G. funebrana*’s eggs on fruits parasitized by entomophagous *T. embryophagum* in the plum orchard. When comparing the results of the first with the third variant and third with the witness - the difference in biological effectiveness of the mean values is essential.

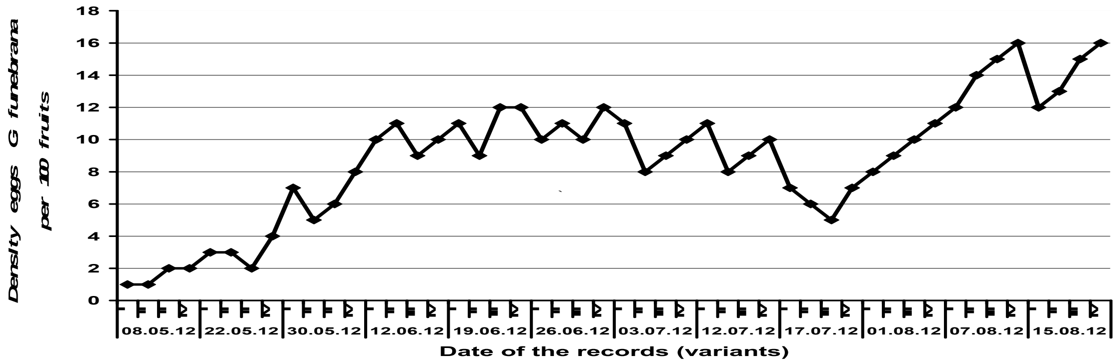

Figure 25. *G. funebrana* eggs density on plum crop, Bacioi, 2012

Table 5. Percentage of parasitisation of *G. funebrana* eggs by *T. embryophagum* as a result of the release of entomophagous on plum crop, Bacioi, 2012

Date of the record	Variant	Percentage of parasitized eggs, %	Date of the record	Variant	Percentage of parasitized eggs, %
08 May	I	0	03 July	I	42.8
	II	0		II	37.5
	II	0		III	30.5
	IV	0		IV	3.5
22 May	I	0	12 July	I	45.5
	II	0		II	37.5
	II	0		III	33.3
	IV	0		IV	3.8
30 May	I	22.4	17 July	I	57.1
	II	20.0		II	50.0
	III	16.6		III	40.0
	IV	0		IV	4.0
12 June	I	25.0	01 August	I	62.0
	II	22.2		II	55.5
	III	20.0		III	50.0
	IV	0		IV	4.5
19 June	I	30.0	07 August	I	66.6
	II	26.3		II	64.2
	III	22.7		III	60.0
	IV	2.4		IV	5.0
26 June	I	34.6	15 August	I	75.0
	II	28.0		II	69.2
	III	25.0		III	66.6
	IV	3.5		IV	6.0
Td=1.36-5.79 > tb o.o5=1.96					

I- variants were analyzed as follows: 450 thousand individuals per hectare in the first variant, II- 350 thousand/ha in the second variant and III-250 thousand individuals per hectare in the third variant, IV- Control

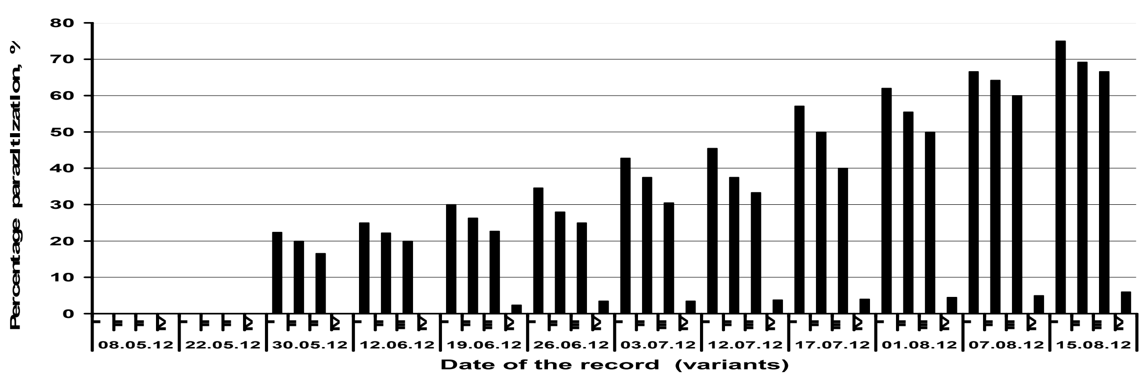

Figure 27. Percentage of parasitisation of *G. funebrana* eggs by *T. embryophagum* as a result of the release of the entomophagous on plum crop, Bacioi, 2012

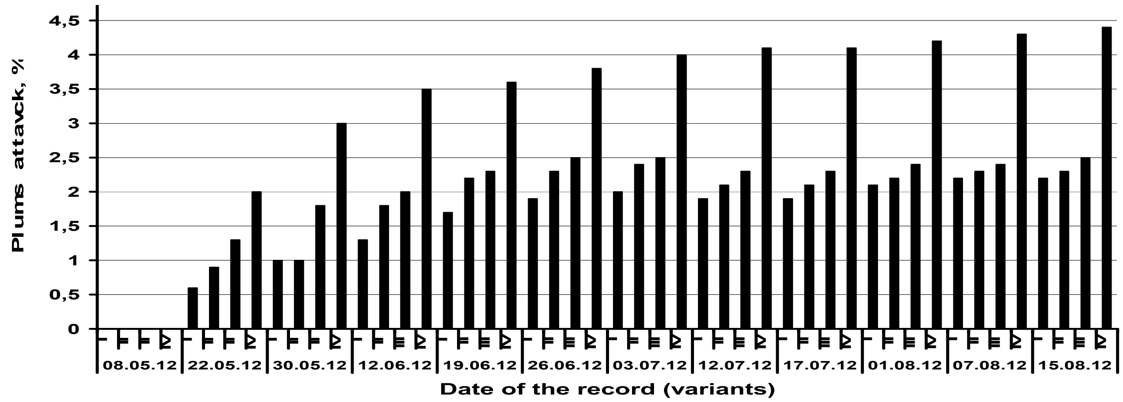

Figure 28. Plums attack by *G. funebrana*, in the plums tree orchard from Bacioi, 2012

Figure 29. *T. embryophagum* launch in Bacioi plum orchard (original)

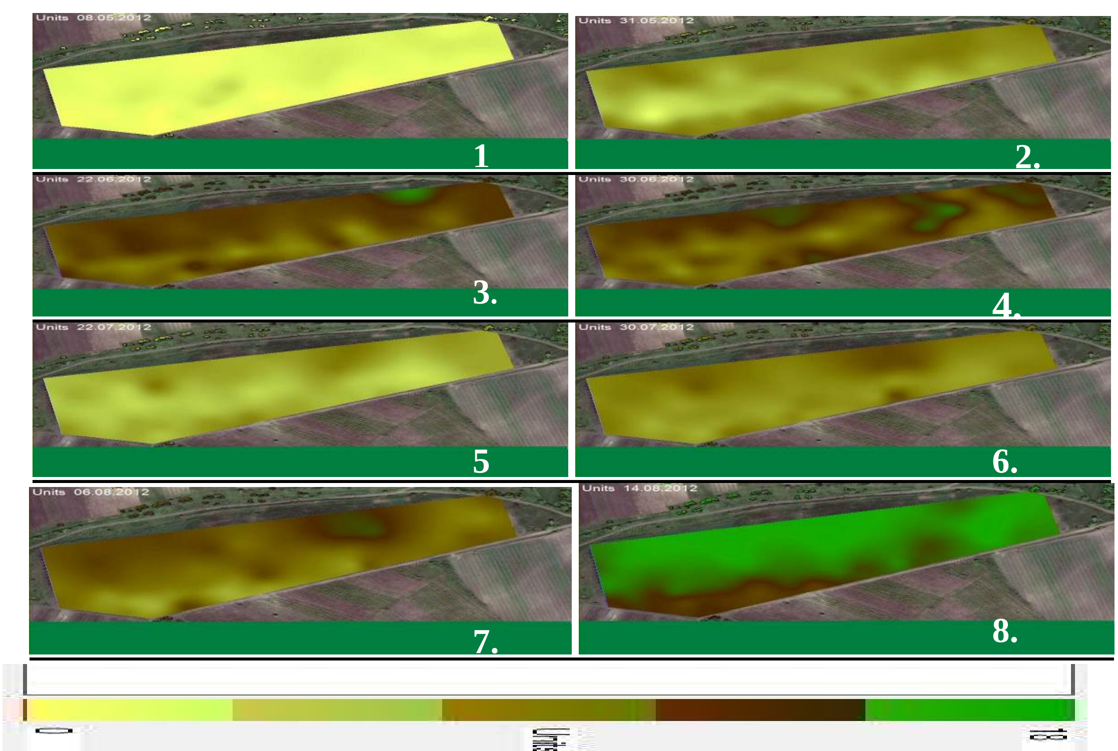

Figure 30. Digital maps (space representation) of *G. funebrana* eggs density on plum culture, Bacioi 2012. The darker the color the higher the density

CONCLUSIONS

During the 2006 to 2010 years, the average biological efficacy after 4 and respectively 6 treatments with *Trichogramma*, on annual corps such as cabbage, corn, tomatoes, peas, sugar beet and soybean, varied from 74% to 90% in the first variant, whereas in the second one, from 60- to 81%. Pests attack on the researched agricultural crops varied from 2% to 30% after *Trichogramma* launching. The same index varied from 16% to 90% in the untreated field.

During the development period of 2-3 pests' generations on annual crops (cabbage, tomatoes, corn, sugar beet, peas, soy, etc.), a very important role in reducing pest density is played by entomophages. For this reason the following steps have to be performed in crop protection.

Grain moth eggs *(Sitotroga cerealella* O.*)* irradiated with gamma radiation provides higher biological indicators of *Trichogramma* than its cultivation on non-irradiated eggs.

4.Consequential issues (5-6) – *T. embryophagum* in the garden provide a higher level of eggs parasitism thus significantly reducing the percentage of damaged fruit.

After six issues of *T. embryophagum* the biological efficacy ranged from 68.2 to 90% in the 1st variant and 64.0 - 84.7% in the 2nd variant. Control check reflected that the number of pest-parasitized eggs ranged from 2.0 to 21.5% and fruit-damage ranged from 45.0 to 93.0%.

As a result of the researches, it was achieved a complete data base regarding the laid eggs on the basis of which it was rendered the spatial distribution (digital maps were built in time) of the pest *Grapholitha funebrana* eggs density, for norms and terms optimization of entomophagous *T. embryophagum* launch in the field against pest density reduction at plum crop.

Digital maps were created (spatial distribution) of *G. funebrana* eggs density at plum crop.

Parasitizing capacity of plum moth eggs (*G. funebrana*) by *T. embryophagum* was determined by applying different norms of launch (450.000, 350.000, 250.000), where efficacy per variants varied from 66.6% to 75%.The immediate results of the research was the elaboration of methodological principles of application of entomophagous *T.embryophagum* for diminishing the pest *G. funebrana* density.

As final result, the application procedures for entomophagous *T. embryophagum* were implemented, as an important element in the integrated protection meant to reduce the pest population density of *G. funebrana* in the plum orchard from Bacioi farm on a surface of 10.8 ha in 2012.

The role of entomophages' application as element in integrated plant protection is the following: Economic effect: Cost reduction for plant protection by 3-4 times relative to chemical treatments.

Ecological effect: Preservation of useful organisms in nature, minimize the number of chemical treatments in integrated system, creating conditions to reduce environmental pollution and the production of organic farming.

Creating conditions to reduce environmental pollution, getting organic ecological and qualitative products.

ABSTRACT

During 2006-2014, average biological efficacy after 4 and respectively 4-6 launches with *Trichogramma evanescens Westw.* on annual crops (such as cabbage, corn, tomatoes, peas, sugar beet and soybean) varied in the first variant from 74% to 90.0%, whereas in the second variant varied from 60 to 81%. Pest attack on the researched agricultural crops varied from 2% to 10%, after *Trichogramma* launching. In the untreated field, the same index varied from 16% to 90%. During the period 2011-2012, research studies with *Trichogramma embryophagum* Htg. it was applied on plum cultures in AGRO BRIO farm from rep. Moldova on a surface of 10.8 hectares against the pest *Grapholitha funebrana* TR. In the plum orchard, there were performed 6 launches with *T. embryophagum.* During the development of two generations of the plum moth (*G. funebrana*), it was determined the dynamics of pest eggs laying and there were constructed digital maps of the spatial distribution of eggs of *G. funebrana* Tr. in the field, as well as the biological efficacy of *T. embryophagum*. which varied from 66.6% up to 75.0%. During the 2002 to 2009 years on apple culture the *T. embryophagum* average efficacy in field after 4-6 treatments varied in the first variant from 74 to 90%, in the second though efficacy varied from 60 to 81%.

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