Trichogramma ENTOMOPHAGE IN INTEGRATED PLANT PROTECTION AS MEANS TO REDUCE PESTS' POPULATION DENSITY ON ANNUAL CROPS IN THE REPUBLIC OF MOLDOVA

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Abstract. During 2006-2010, average biological efficacy after 4 and respectively 6 treatments with *Trichogramma* 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%.

Keywords: *Trichogramma*, pest control, parasites, plant protection, biological methods, entomophagous, phytophagous.

Rezumat. Entomofagul *Trichogramma* în protecţia integrată a plantelor ca mijloc de reducere a densităţii populaţiilor dăunătorilor culturilor anuale în Republica Moldova. În perioada anilor 2006–2010 în culturile anuale cercetate (varză, porumb, tomate, mazăre, sfeclă de zahăr şi soia), după 4 - 6 lansări cu *Trichogramma* în câmp, eficacitatea biologică medie a entomofagului în prima variantă a fost cuprinsă între 74 şi 90,0%, iar în varianta a doua, între 60 şi 81%. Atacul dăunătorilor, în culturile agricole anuale cercetate în această perioadă, a variat de la 2 la 10% în variantele cu *Trichogramma.* În cultura Martor, procentele au variat între 16 şi 90%.

Cuvinte cheie: *Trichogramma*, reducerea dăunătorilor, parazitoizi, protecţia plantelor, metode biologice, entomofagi, fitofagi.

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 favourable 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* HÜBNER., which brings harm to a wide range of cultures. *Helicoverpa armigera* 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% (MUREŞEAN & MUSTEA, 1995).

The pest *Helicoverpa armigera* (Lepidoptera: Noctuidae) is controlled with *Trichogramma evanescens* WESTWOOD (Hymenoptera: Trichogrammatidae) in cotton cultures (Malvaceae) in Turkey. *Helicoverpa armigera* 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 *Ostrinia 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, (ZIMMERMANN, 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 for Plant Protection and Ecological Agriculture of the Republic of Moldova, in 1976, it was created the "*Trichogramma* laboratory", under the initiative of the academician Popusoi Ion, directed by Dr. habilitat Grinberg Alexandru and succeeded by Dr. Lidia Gavriliţa. The main goal of the laboratory is to research, analyse, elaborate data in rearing and application of the *Trichogramma* entomophage in pest control.

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.

MATERIAL 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 2006-2010 years. *Trichogramma* has been collected from annual cultures (cabbage, tomatoes, maize, peas, sugar beet and soybean). *Trichogramma evanescens* 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 laboratory host – grain moth, for *Trichogramma* production was done by (ABAŞCHIN *et al*., 1979) authors' methods. Mathematical data elaboration was done according to MENCER *&* ZIMERMAN (1986).

RESULTS AND DISCUSSIONS

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

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

Steps to obtaining maternal cultures of entomophages:

1. For the reason that the *Trichogramma* entomophage is applied several times at different stages of pest maturity for agricultural production protection, it is necessary to collect annually from natural environment the entomophage for its geophone renewal, because reared several generations in laboratory conditions it is known that *Trichogramma* loses certain of its qualities.
2. The annual natural collecting is done from the same cultures to which it is going to be applied afterwards.
3. It follows: identification, rearing and maintenance along the year of the species collected from nature.
4. Use of different procedural methods for quality increase during rearing process of the *Trichogramma* entomophage before launching in field.
5. Biological indexes (quality) analysis before launching in field for protection of crops.

Steps and periods for entomophages field application:

1. To make accurate assessment of pest density in the field, records necessary to determine the specific pest for each species and crop, which depend largely on their bioecological peculiarities must be taken.
2. Field pest monitoring with pheromone traps.
3. Deciding the rules and terms of release of *Trichogramma* entomophages, which have to be taken depending on the pest density at different developmental stages (egg, larvae, pupae, adults), also must take into account the damaging economic threshold for each pest and crop.
4. Determination of the biological efficacy in field, made after each launching of *Trichogramma*, based on the period of pest development (1-3 generations), to which 4-6 launches can be made with entomophages in different cultures.
5. During the launch of the *Trichogramma* a strict record of the "martor" (no launches) takes place which gives knowledge about the natural density of the entomophage, which helps in tracking and taking decisions for further launches.
6. Entomophages releases in our experiments were performed in capsules, to be protected by various predators.

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 (Fig. 1).

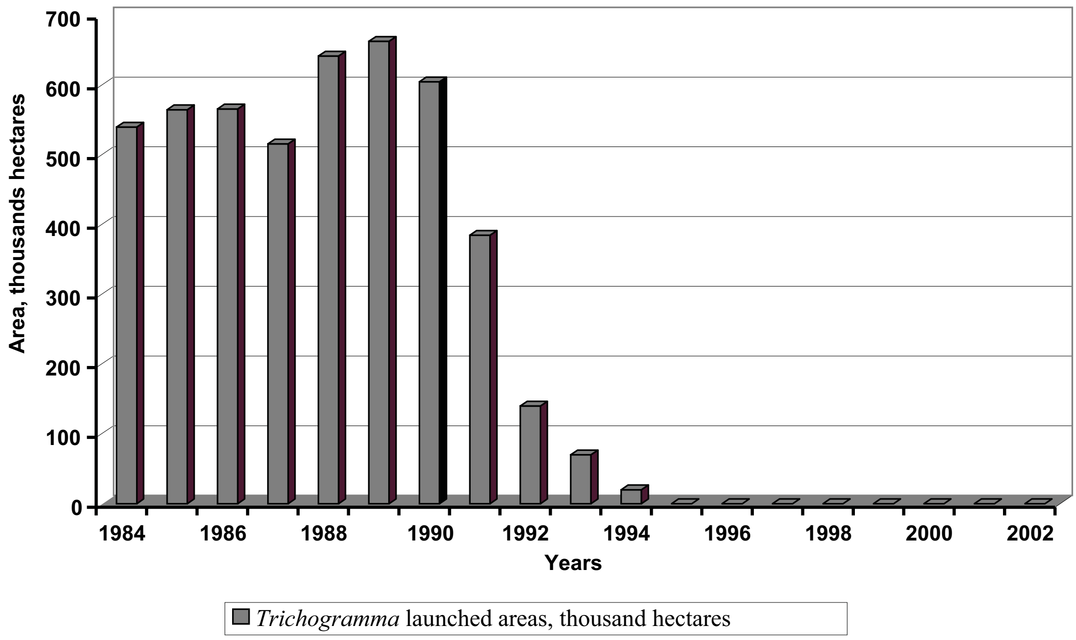

Figure 1. Areas of protected crops in the Republic of Moldova by *Trichogramma* spp. 1984-2002 (Information taken from the General Inspectorate for health surveillance and control. Plant Protection, Monitoring Department). / Figura 1. Suprafeţele culturilor agricole protejate cu *Trichogramma* spp. în Republica Moldova, 1984-2002 (Informaţii preluate de la Inspectoratul General pentru supravegherea sănătăţii şi controlul de protecţie a plantelor, Departamentul de monitorizare).

From 1994 to 2002 in Republic of Moldova the biological agent *Trichogramma* has not been produced. Starting with 2002 the production process has had been reinitiated. 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 2-9 thousand hectares (Fig. 2).

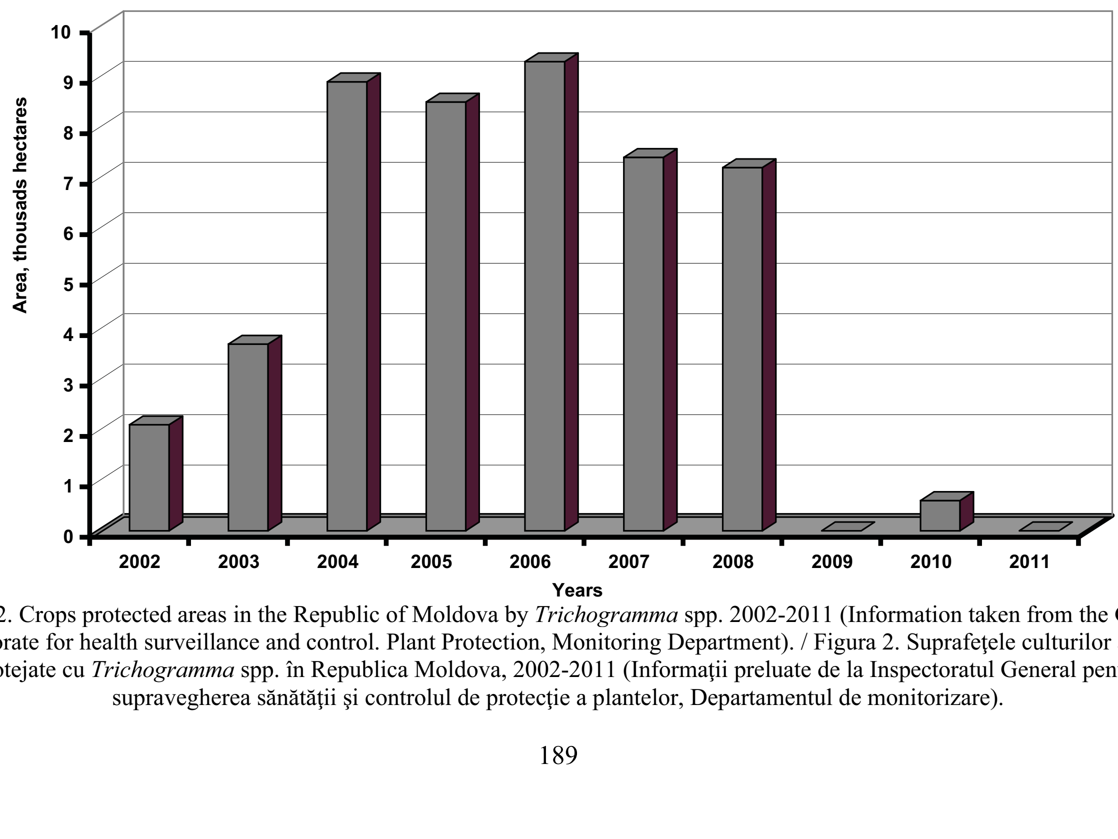

Figure 2. Crops protected areas in the Republic of Moldova by *Trichogramma* spp. 2002-2011 (Information taken from the General Inspectorate for health surveillance and control. Plant Protection, Monitoring Department). / Figura 2. Suprafeţele culturilor agricole protejate cu *Trichogramma* spp. în Republica Moldova, 2002-2011 (Informaţii preluate de la Inspectoratul General pentru supravegherea sănătăţii şi controlul de protecţie a plantelor, Departamentul de monitorizare).

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). 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.

Table 1. Methods for increasing the quality of *Trichogramma evanescens* W. grown on different hosts eggs.
Tabel 1. Procedee de sporire a calităţii la *Trichogramma evanescens* W. crescută pe ouă de diferite gazde.

Hosts	***Mamestra brasssicae* L.**	***Ostrinia nubilalis* H.**	***Ephestia кuhniella* Z.**	***Sitotroga cerealella* O.**
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 (martor)	23.2±2.9	13.3±1.0	25.3±1.4	20.3±1.2

In the Research Institute for Plant Protection and Ecological Agriculture to increase the quality and effectiveness of *Trichogramma*, different rearing methods were used on various hosts for plant protection. *T. evanescens* prolificacy reared on *Mamestra brasssicae* 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 *Ostrinia nubilalis*' eggs, the results ranged from 21.3 to 26.6 eggs/female whereas in untreated host eggs – 13.3 eggs/female. Reared on *Ephestia 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 *Sitotroga 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 *Mamestra brassicae* L. constituted 23.4; on untreated eggs – 17.8. On *Ephestia kuhniella* Z. irradiated beforehand with gamma rays – 14.4 whereas untreated eggs 11.4. On *Sitotroga cerealella* O. treated eggs – 13; untreated 8.7. On *Ostrinia nubilalis* H. 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).

Table 2. Biological indexes of *Trichogramma* spp., applied on annual crops in 2006 – 2010.
Tabel 2. Indicii biologici la *Trichogramma* spp., aplicate la culturile anuale în 2006 – 2010.

Years	***Trichogramma species***	**Variants ***	**Biological Indexes**				**DEM**
			Prolificacy 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	3.0
		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	3.7
		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	5.2
		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	3.5
		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	2.1
		II	21.0±0.8	84.0±1.8	56.0±2.5	9.8±0.7	

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

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.

Table 3. Biological efficacy of *Trichogramma evanescens* in pest control on annual crops for 2000 to 2010 years.
Tabel 3. Eficacitatea biologică la *Trichogramma evanescens* în combaterea complexului de dăunători la culturile anuale în anii 2000-2010.

Farms	**Culture**	**Pest name**	**Area, (hectares)**	***Trichogramma* species**	**Variants***	**Parasitized eggs, (%)**
Gura Bâcului, Sărata Galbenă, Băcioi, Sîngera Chetrosu	Cabbage	*Mamestra brasssicae* *Helicoverpa armigera*	36,5	*T. evanescens*	I II	74.0 – 90.0 60.0 – 81.0
Sărata Galbenă, Băcioi, Coşniţa, Mărăndeni, Bălţi, Căuşeni	Corn	*H. armigera* *Ostrinia nubilalis*	1190	*T. evanescens*	I II	80.0 – 88.0 73.0 – 80.0
Sărata Galbenă, Chişinău, Gura Bâcului, Sîngera	Tomatoes	*H. armigera*	288	*T. evanescens*	I II	83.0 – 90.0 74.0 – 80.8
Sărata Galbenă, Chişinău	Peas	*H. armigera*	210	*T. evanescens*	I II	80.0 – 86.0 75.0 – 80.0
Pohoarna, Mărăndeni	Sugar beet	*H. armigera*	350	*T. evanescens*	I II	84.0 – 85.0 76.0 – 77.0
Mărăndeni	Soya	*H. armigera*	270 Sum: **2344.5**	*T. evanescens*	I II	83.0 – 86.0 75.0 – 76.0 **(Td=1.0-1.90 1.96=To.o5).**

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

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 2000-2010 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 2006-2010 years varied from 1 to 8 eggs/plant. Pest attack on agricultural cultures in 2006-2010 years varied from 2 to 10% in *Trichogramma* applied variant, whereas in "martor" (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).

The role of entomophages' application as element in integrated plant protection is the following:

1. **Economic effect:** Cost reduction for plant protection by 3-4 times relative to chemical treatments.
2. **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.

CONCLUSIONS

1. 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 10% after *Trichogramma* launching. The same index varied from 16% to 90% in the untreated field.

2. 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:

- In the adult stage, pheromone traps are mounted to monitor and capture in mass the butterflies;
- In the pests' egg stage, 5 to 6 *Trichogramma* releases must be applied, having the norm of 200,000 to 300,000 individuals per hectare;
- In the larval stage of pests, other entomophages can be released - Bracon, Apanteles;
- Treatments with biological composes in pest control can be applied;
- 1 – 2 treatments with fungicides to be carried in combating diseases;
- 1 – 2 treatments with selective insecticides to be carried in combating the complex of pests (if necessary);
- Mechanical and physical means, crops' rotation;
- Preservation of useful organisms in nature;
- Minimizing (or avoiding) the number of chemical treatments in integrated protection;
- Creating conditions to reduce environmental pollution;
- Getting organic ecological and qualitative products.

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