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TRICHOGRAMMA ENTOMOPHAGE IN INTEGRATED PLANT PROTECTION AS MEANS TO REDUCE PESTS' POPULATION DENSITY ON ANNUAL CROPS

During the 2000 to 2017 years 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 76%. Pests attack on agricultural crops researched varied from 2% to 10%, after Trichogramma launching. In the untreated field, the same index varied from 16% to 90%.

***T. evanescens*, *T. pintoi*, *H. armigera*, *S. cerealella* Ol., experiments, biological efficacy.**

A very important role in integrated plant protection belongs to biological measure for plant protection. Conservation and activation of the natural mechanisms in harmful organisms density regulation must be based on profound knowledge of all the factors and biocenotic 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 biocenotic 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 of plant protection. Therefore it is necessary to create favorable conditions to entomophage organisms for their development.

Trichogramma species parasitize many species of pests and are used in biological plant protection. Considering [1]. data from year 2000, annually 45 million hectares are being treated with *Trichogramma* sp. in the world. Considering, Knutson, 2001 [2]. information, *Trichogramma* is the most used entomophage in the world, which was launched annually on approximate 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 vast gamma of cultures. *Helicoverpa 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. entomophage in combating (controlling) these pests helps reduce the number of pest's density from 60 to 80% 1995 [3].

Pest *Helicoverpa armigera* Hübner (*Lepidoptera: Noctuidae*) control with *Trichogramma evanescens* Westwood (*Hymenoptera: Trichogrammatidae*) in cotton cultures (*Malvaceae*) in Turkey. *Helicoverpa armigera* H. 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 [4].

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 [10]. 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 [11]. 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*, Fiodorencu et al., 2009 [9]. Use of the biological methods for agricultural cultures 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 [5].

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 Institute of Genetics, Physiology and Plant Protection of the Republic of Moldova, in 1976 was created the “*Trichogramma* laboratory”, under the initiative of the academician Popusoi Ion, directed by Dr. habilitat Grinberg Alexandru and succeeded by Dr. Lidia Gavrilita. The main goal of the laboratory is to research, analyze, elaborate data in rearing and application of the *Trichogramma* entomophage in pest's control.

In order to apply *Trichogramma* in plant protection, profound knowledge in Ecology, Biology of entomophage 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's control leads to variables continuous analysis and results driven focuses knowing the role and place of entomophage for regulating pest's density.

Materials and methods. Researches were done in field and laboratory conditions during various institutional and state projects with the Innovation and Technological Transfer Agency. Implementation took place in various farms in the Republic of Moldova and on the 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 according [6]. Rearing of the laboratory host — grain moth for *Trichogramma* production was done by Abaschin et al., 1979, [7] author's methods. Mathematical data elaboration was done according to Mencer & Zimerman, 1986 [8].

Steps to obtaining maternal cultures of entomophage: 1. For the reason that the *Trichogramma* entomophage is applied several times at different stages of pest's maturity for agricultural production protection, it is necessary to collect annually from the natural environment the entomophage for its geophone renewal, because from reared several generations in laboratory conditions, it is known that *Trichogramma* loses from 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 it in the field; 5. Biological indexes (quality) analysis before launching in field for protection of crops.

Steps and periods for entomophage field application: 1. To make accurate assessment of pest density in field, records necessary to determine the specific pest for each species and crop, which depend largely on their bioecological peculiarities, must be taken. Field pest monitoring with pheromone traps; 2. Deciding the rules and terms of release of *Trichogramma* entomophage, 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; 3. 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 entomophage in different cultures; 4. During the launch of the *Trichogramma* a strict record of the “control” (no launches) takes place which gives knowledge about the natural density of the entomophage, which helps in tracking and taking decisions for further

launches; 5. Entomophage 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, which covered only 30 to 663 thousands hectares.

From 1994 to 2002, in the Republic of Moldova, the biological agent *Trichogramma* has not been produced. Starting from 2002 the production process 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.

Trichogramma spp. entomophage 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 accumulate fatly because of its short development period of a generation. *Trichogramma* entomophage 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 augmenting its efficacy. One of the procedures with high success is rearing *Trichogramma* on preliminarily gamma rays irradiated eggs. The results are presented in the following Table 1.

In the Research Institute for of Genetics, Physiology and Plant Protection of Repub. Moldova, 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 brassicae* L. eggs treated with different factors, ranged from 33.1 to 60.4 eggs/female. In untreated host eggs the results were the following: 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 kuehniella*'s 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*'s 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

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

Hosts	<i>Mamestra brassicae</i> L.	<i>Ostrinia nubilalis</i> H.	<i>Ephestia kuhniella</i> Z.	<i>Sitotroga cerealella</i> Ol.
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 ₃ O 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

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 non treated eggs — 17.8. On *Ephestia kuhniella* Z. irradiated beforehand with gamma rays — 14.4, whereas non-treated eggs 11.4. On *Sitotroga cerealella* Ol. 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 ($T_r = 2.4 - 3.3 > T_{0.05} = 1.96$).

Determination of biological indexes of the *Trichogramma* spp.: During the 2006 to 2017 years, in laboratory conditions, after prolonged storage (diapause) — 6 months, *Trichogramma* was reared for 3—4 generations each year. Later on, the biological indexes 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 $26 \pm 1^\circ\text{C}$ 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 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 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 re-

2. Biological efficacy of *Trichogramma evanescens* in pests' control on annual crops for 2000 to 2017 years

Farms	Culture	Pests' name	Area, (hect-ares)	<i>Trichogramma</i> species	Variants	Parasitized eggs, (%)
Gura Bacului, Sărata Galbena, Bacioi, Sangera Chetrosu	Cabbage	<i>Mamestra brassicae</i> L. <i>Helicoverpa armigera</i> Hb.	55.5	<i>T. evanescens</i>	I II	74.0 — 90.0 60.0 — 81.0
Sarata Galbena, Bacioi, Coșnița, Marandeni, Balți, Caușeni	Corn	<i>Helicoverpa armigera</i> Hb. <i>Ostrinia nubilalis</i> Hb.	1615	<i>T. evanescens</i>	I II	80.0 — 88.0 73.0 — 80.0
Sarata Galbena, Chișinau, Gura Bacului, Sangera	Tomatoes	<i>Helicoverpa armigera</i> Hb.	290	<i>T. evanescens</i>	I II	83.0 — 90.0 74.0 — 80.8
Sarata Galbena, Chișinău	Peas	<i>Helicoverpa armigera</i> Hb.	210	<i>T. evanescens</i>	I II	80.0 — 86.0 75.0 — 80.0
Pohoarna, Marandeni	Sugar beet	<i>Helicoverpa armigera</i> Hb.	450	<i>T. evanescens</i>	I II	84.0 — 85.0 76.0 — 77.0
Marandeni Chișinau	Soya	<i>Helicoverpa armigera</i> Hb.	336	<i>T. evanescens</i>	I II	83.0 — 86.0 75.0 — 76.0 (Td=1.0—1.90 1.96=To.05)
Sum:			2956.5			

I variant — reared on irradiated host eggs.

II variant — reared on different non irradiated host eggs.

searched crops (cabbage, corn, tomatoes, peas, sugar beet and soybean), average biological efficacy of *T. evanescens* in pests control during the 2000—2017 years, the percentages varied from 74 to 90% in the first variant and from 60- to 76.0% in the second one (Table 2).

Pest density of the researched annual crops during the 2006—2017 years varied from 1 to 8 eggs/plant. Pests' attack on agricultural cultures in 2006—2017 years varied from 2 to 10% in *Trichogramma* applied variant, whereas in “control” (non treated crops by *Trichogramma*) the same index varied from 16 to 90%. The percentage of different pests' 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 an 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, minimizing 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 crops 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 agricultural crops researched 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 pest's generations on annual crops (cabbage, tomatoes, corn, sugar beet, peas, soy, etc.), a very important role in reducing pest's density belongs to entomophage. 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 butterflies;
- In the pest's 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 entomophage can be released — Bracon, Apanteles;
- Treatments with biological compositions 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, crop's 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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Гаврилита Л.Ф. Ентомофаг трихограма в інтегрованому захисті рослин як засіб зниження щільності популяції шкідників однорічних культур

Протягом 2000—2017 рр. у досліджуваних однорічних культурах (капуста, кукурудза, томати, горох, цукровий буряк і соя) після 4—6 польових випусків трихограми середня біологічна ефективність ентомофага в першому варіанті була в межах від 74 до 90%, а в другому варіанті — від 60 до 76%. Пошкодженість однорічних культур шкідниками протягом цього періоду варіювала від 2 до 10% у варіантах з *Trichogramma*. У контролі пошкодженість рослин становила 16—90%.

Гаврилита Л.Ф. Энтомофаг трихограмма в итегрированной защите растений как средство снижения плотности популяции вредителей однолетних культур

В течение 2000—2017 гг. в исследуемых однолетних культурах (капуста, кукуруза, томаты, горох, сахарная свекла и соя) после 4—6 полевых выпусков с трихограммой средняя биологическая эффективность энтомофага в первом варианте колебалась в пределах от 74 до 90%, а во втором варианте — от 60 до 76%. Поврежденность однолетних культур от вредителей, в течение этого периода, варьировала от 2 до 10% в вариантах с *Trichogramma*. В контроле поврежденность растений составляла 16—90%.