INFLUENCE OF STERILIZATION OF *Sitotroga cerealella* Ol. EGGS AND OF PASSAGES ON BIOLOGICAL INDICES AND EFFICACY OF *Trichogramma* spp.

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Abstract. According to available data, the variant where *Sitotroga cerealella* Ol. eggs were irradiated with gamma rays of 150 Gy dose and kept within one month, the state quality criterion was 2.6 higher, irradiated and stored in within two months – 1.8 times greater, held within three months - 1.5 times higher, held within four months - 1.5 times higher, held within five months 1.34 times higher, compared to the control sample. The higher the terms of storage, the lower the biological indices of *Trichogramma* spp. Mathematical processing, analysis of variance indicated that, according to the criterion – T., statistics are accurate at 95%. Static quality criterion *Trichogramma* spp. difference, multiplied by *Sitotroga cerealella* Ol. irradiated eggs kept for five months, for further propagation of *Trichogramma* compared to the control is essentially higher.

Keywords: biological indices, prolificacy, *Trichogramma* spp., *Sitotroga cerealalla*, biological efficacy.

Rezumat. Influenţa sterilizării ouălor de *Sitotroga cerealella* Ol. şi a pasajelor asupra indicilor biologici şi a eficacităţii *Trichogramma* spp. Conform datelor obţinute, în prima variantă, unde ouăle de *Sitotroga cerealella* Ol. au fost iradiate cu raze gama cu doza de 150 Gy (Grey) şi păstrate în decurs de o lună, criteriul static al calităţii a fost de 2,6 mai mare, iradiate şi păstrate în decurs de două luni – de 1,8 ori mai mare, păstrate în decurs de trei luni – de 1,5 ori mai mare, păstrate în decurs de patru luni – de 1,5 ori mai mare, păstrate în decurs de cinci luni – de 1,34 ori mai mare, decât în martor. Cu cât termenii de păstrare sunt mai mari, cu atât mai mici sunt indicii biologici a *Trichogramma* spp. Prelucrarea matematică, efectuarea analizei dispersionale indică faptul, că conform criteriului – T – datele statistice sunt veridice la nivelul de 95%. Deosebirea criteriului static al calităţii *Trichogramma spp*. înmulţită pe ouă *Sitotroga cerealella* Ol. iradiate, care s-au păstrat cinci luni, pentru înmulţirea ulterioară a *Trichogramma* în comparaţie cu martorul este esenţial mai mare.

Cuvinte cheie: indicii biologici, prolificitatea, *Trichogramma* spp*., Sitotroga cerealalla*, eficacitatea biologică.

INTRODUCTION

One of the procedures to increase *Trichogramma* vitality is obtaining biological material of sterile insect eggs. There are several factors of host eggs' sterilization that allow improving insect development oofage: using low temperatures, thermo procedure, ultraviolet irradiation (VOEGELE & DAUMAL, 1974), irradiation with gamma rays (GAVRILIŢA, 1993, 1995, 1996, 2002; GAVRILIŢA & GREENBERG, 1996; BUTNARU & GAVRILIŢA 2011; LYSIKOVA, 1985; FIRU et al., 2003), etc. At our discretion obtaining anytime host eggs as a result of their longer storage has favored settling the issue. Agnomens grain moth (*Sitotoga cerealella* Ol.) cannot be stored for a long time in a refrigerator. This problem is particularly acute in biological laboratories where it is necessary to prepare big quantities of host eggs parasitized by *Trichogramma* (BURZINSKI & KOT, 1963; MENCHER et al., 1980) and it has been shown that the irradiation of *Sitotoga cerealella* Ol. eggs has a positive effect on the reproductive indices of *Trichogramma* developing on them. Similar indices have been obtained when *Trichogramma* developed on cabbage moth eggs irradiated with X-rays (dose of 15 krad) (DEGTYAREV & YANISHEVSKAYA, 1985).

Special prolongation of shelf life for irradiated eggs for their parasitizing by *Trichogramma* (up to 12 days) as compared to 2-3 days of non-irradiated eggs allowed reducing the number of eggs required for parasitation with *Trichogramma* (GAVRILIŢA, 1995). There still lack data on the usage of fresh *Sitotoga cerealella* Ol. eggs, gamma irradiated, with the aim of their long – term storage and possibility of subsequent *Trichogramma* spp. development on them.

Scientific research conducted at the Institute of Genetics, Physiology and Plant Protection allowed us to establish that a technique for improving *Trichogramma* spp. quality and it consists in rearing it on gamma irradiated eggs of *S. cerealella* (GAVRILIŢA & GREENBERG, 1996). Taking into consideration research tasks, the developed technique demonstrated prospects of rearing the entomophage on gamma radiated eggs of the *S. cerealella*. In this connection, rearing the parasitoid on gamma irradiated eggs of *S. cerealella* improved biological indices of the entomophage 1.5-2 times. The obtained research results showed that gamma irradiation of *S. cerealella* eggs at the age of 24 hours allows increasing the term of host eggs storage at the temperature of 3^0C up to 4-5 months for subsequent *Trichogramma* rearing. Such rearing of *Trichogramma* on gamma radiated eggs of *S. cerealella* contributed to the improvement of its biological indices 1.5-2 times. Sex ratio played an important role in regulating population density. Bibliography on the showed that changing *Trichogramma* spp. sex ratio impacts a number of factors such as temperature, humidity, a number of developing larvae per egg, host species and their age, the term of storing *Trichogramma* in diapauses and many others (GAVRILIŢA & GREENBERG, 1996).

Bibliography on the subject proves that biological indices of the parasitoid are directly proportional to the host species, its age and egg number (GAVRILIŢA, 1996). Each species has a high capacity for selecting the host to develop on them. Some differences were revealed in one and the same *Trichogramma* species from different hosts. *Trichogramma* successfully develops with high biological indices when rearing is made on host eggs in early stages of embryonic development. Biological and ecological research has been conducted and the results are evaluated using traditional methods.

For specific experiences, the *Trichogramma* species were collected, multiplied and identified, then the biological indices and biological effectiveness of the entomophagus were determined.

MATERIALS AND METHODS

Determination of shelf life of irradiated *Sitotroga cerealella* Ol. eggs in plastic bags. Mathematical data processing was done using variance analysis method (MENCER & ZEMSHMAN, 1986). In the experiments, there were used fresh eggs of the Angoumois grain moth (*S. cerealella* Ol.) at the age of (24-28 hours), if possible, under mass rearing conditions, gamma irradiated at the dose of 150 Gy.

After a certain term of storing eggs of the *S. cerealella* in the refrigerator at the temperature of 3^0C ± 1, biological indices were determined for *T. evanescens* (prolificacy, hatching, females' rate) reared on these eggs. The best results with prolificacy of *Trichogamma* spp. were obtained when applying the irradiation dose of 150 Gy to the eggs of the *S. cerealella*. After determining the optimal dose for irradiating the eggs of *S. cerealella*, in subsequent experiments, the dose of 150 Gy was applied to eggs.

Collection, identification, storage and accumulation of *Trichogramma* species were done according to DIURICI (2008). Rearing of the laboratory host – grain moth (*Sitotroga cerealella* Ol.), for *Trichogramma* production was done by (ABASCHIN et al., 1997) authors' methods.

RESULTS AND DISCUSSIONS

In Table 1 and Fig. 1 shows the results of storing *Sitotroga cerealella* irradiated eggs for 157 days in plastic bags (5 x 5 cm) and the control, where the eggs of *S. cerealella* Ol., were neither irradiated nor stored.

After irradiating the eggs of the *S. cerealella* at the dose of 150 Gy and placing them into plastic bags to be stored for 32, 65, 102, 136, and 157 days, biological indices of *T. evanescens* were determined.

Prolificacy of *Trichogramma* spp. reared on *S. cerealella* eggs soon after irradiation constituted 43.0 eggs per female, in the – 24.7, prolificacy in the trial with irradiation was two times higher than in the. It eloquently explains that irradiated eggs of the Angoumois grain moth contributed to improving *Trichogramma* prolificacy. In 32 and 65 days of storing eggs of the *S. cerealella*, prolificacy of *Trichogramma* spp. remained at almost the same level as at the beginning (41.8; 38.5), though considerably higher than in the (21.7; 21.3). According to criterion T., static data were accurate to the level of 95 %. In 102 days, no essential differences were revealed between the irradiation variant and the. In 136-157 days, *Trichogramma* prolificacy in the variant with essential irradiation was much lower than in the and equaled respectively to 17.8 and 4.76 eggs per female, in the – 22.2 and 18.0 eggs per female.

The obtained results showed that the eggs of the *S. cerealella* at the age of 24 hours, gamma irradiated to accumulate eggs, can be stored up to 102 days (3-4 months) in the refrigerator at the temperature of T=3^0C. The embryo in the irradiated Angoumois grain moth died and this allowed parasitizing and rearing of *Trichogramma spp.* for a longer time, thus increasing its prolificacy**.** It was found that female longevity increased 2-3 times (Table 2) in the variant, where *Trichogramma* was reared on irradiated eggs as compared to the when *Trichogramma* was reared without radiation.

When *Trichogramma* developed on irradiated *S. cerealella* eggs and stored in the refrigerator, its average longevity equaled to 7 days, in the – to 2.1 days, when stored for 82 days, respectively – to 4.8 days in the variant, in the – to 2.2 days, when stored for 103 days – 2.2 days in the variant and 1.7 days, respectively. Table 3 shows the biological indices of *Trichogramma* reared on irradiated Angoumois grain moth eggs stored for 2 months in plastic bags (5 x 10 cm). *Trichogramma* stock generation (F_0) was reared on irradiated *S. cerealella* eggs, while subsequent six generations were reared on non-irradiated eggs. When comparing F_0, F_1, F_2, F_3, F_4, with the, an increase by 1.5-2 times was found in the static criterion and quality, in general, while generations F_5, F_6 demonstrated no essential differences. *Trichogramma* quality increased in generations (F_1 to F_6) reared on irradiated eggs compared to F_0, that may be explained by physiological changes in *S. cerealella* eggs caused by gamma irradiation at the doze of 150 Gy.

Determining storage period of the Agnomens grain moth (*Sitotroga cerealella* Ol.) eggs.

Irradiated *Sitotroga cerealella* eggs were stored for five months in small glasses with the volume of 50 ml. Every month irradiated and stored eggs were exposed to parasitizing by *Trichogramma* followed by determining biological indices in order to reveal optimal terms for storage of irradiated eggs. Table 4 shows static criterion of *Trichogramma* quality reared on the stored irradiated *S. cerealella* (variant) and on non-stored Angoumois grain moth eggs.

According to the obtained data, quality static criterion of eggs irradiated and stored for one month was 2.6 times higher and when storing for two months –1.8 times higher, for three months –1.5 times higher, for four months - 1.5 times higher, for 5 months –1.34 times higher. The longer the storage period, the lower the biological index of *Trichogramma*. Mathematical processing and analysis of variance showed that, according to T – criterion, data were accurate at the level of 95 %. The difference of quality was essential and $T_{0.05}=2.23<T_f=6.587\text{-}15.428$. Irradiated *S. cerealella* eggs can be stored in glasses for five months with essential differences as compared to the.

Determining efficacy of *Trichogramma* generations

Table 5 and Fig. 2 give graphical representation of the biological indices of *T. evanescens* reared on irradiated and non – irradiated *Sitotroga cerealella* Ol. eggs. While comparing the biological indices of *Trichogramma*, reared on *S. cerealella* eggs from the stock generation (F.) with 12 consecutive generations reared on irradiated and non-stored

eggs, it was shown that they are two times higher in the variant with radiation than those in the check. Prolificacy has varied in 12 generations from 31.1 to 42.1 in the variant with radiation and from 19 to 22 in the check and the static criterion of quality in the variant with irradiation from 15.5 to 19.6 and from 8.7 to 9.8 in the check. The comparison of variant generations $F_1 - F_{12}$ with those in the check showed considerable difference, where $T_{0,05} = 2.78$; $T_{1-12} = 5.9\text{-}50.9$ at the level of 95% accuracy $F_{practical} = 58 > F_{theoretixal} = 7.7$.

Table 1. Influence of gamma radiation of the *Sitotroga cerealella* Ol. eggs on biological indices of *T. evanescens.*

Ref. No.	Variants, gamma radiation, Grey	Storage time, days	Average prolificacy, eggs / female	Hatching per individual, %	Confidence interval	Student comparison criterion - T
1.	150 Gy	0	43.0±14.8	97.7±13.8	(36.38; 49.52)	$t_{f = 4.13} > t_{0.05 = 2.04}$
2.	Check	0	24.7±10.4	94.7±12.8	(19.37; 30.04)	
3.	150 Gy	32	41.8±12.6	96.1±10.8	(36.53; 47.2)	$t_{f = 6.40} > t_{0.05 = 2.04}$
4.	Check	0	21.7±3.4	93.2±9.8	(20.01; 23.38)	
5.	150 Gy	65	38.5±9.9	96.3±10.6	(33.84; 43.16)	$t_{f = 6.52} > t_{0.05 = 2.04}$
6.	Check	0	21.3±3.8	96.4±10.7	(19.44; 23.16)	
7.	150 Gy	102	25.7±5.6	94.4±9.8	(23.13; 28.27)	$t_{f = 1.3} > t_{0.05=2.02}$
8.	Check	0	23.0±4.5	92.2±9.3	(21.10; 24.80)	
9.	150 Gy	136	17.8±3.1	89.2±9.2	(16.56; 9.03)	$t_{f = 2.31} > t_{0.05=2.01}$
10.	Check	0	22.2±6.4	88.2±8.8	(19.47; 24.92)	
11.	150 Gy	157	4.7±1.36	83.0±7.2	(3.84; 4.96)	$t_{f = 2.89} > t_{0.05=2.01}$
12	Check	0	18.0±6.3	87.2±7.8	(15.45; 20.55)	

Table 2. Longevity of *Trichogramma* females reared on irradiated and stored eggs of the *Sitotroga cerealella* Ol.

Variant	Storage period	Female longevity (days)
150 Gy	48±2.36	7.0±0.8
Check	0	2.1±0.1
150 Gy	82 ±3.32	4.8±0.6
Check	0	2.2±0.2
150 Gy	103±3.36	2.1±0.1
Check	0	1.7±0.3

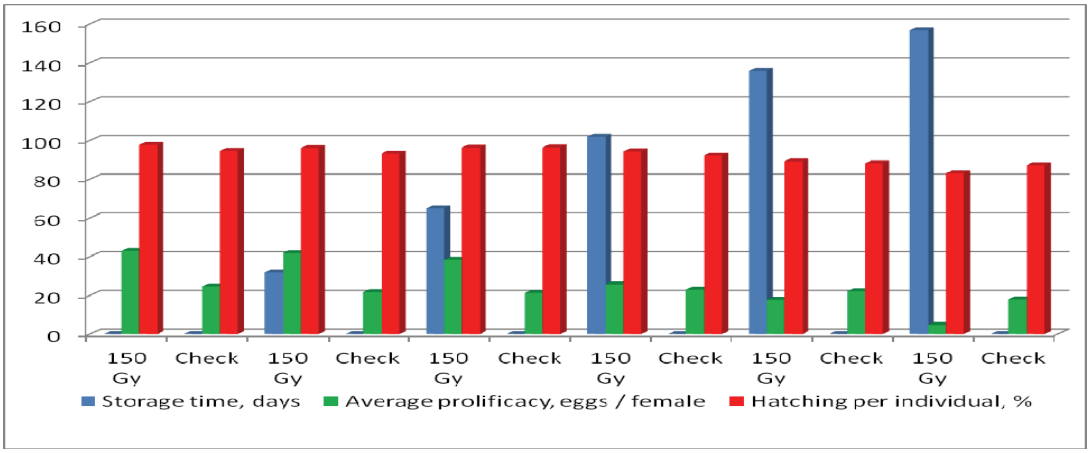

Figure 1. Influence of gamma radiation of the *Sitotroga cerealalla* Ol. eggs on biological indices of *Trichogramma evanescens.*

Table 3. Biological indices of *T. evanescens* reared on eggs of the *Sitotroga cerealella* Ol. stored for 2 months.

Biological indices	Value of biological indices by generations													
	F_0	M	F_1	M	F_2	M	F_3	M	F_4	M	F_5	M	F_6	M
Prolificacy, egg/female, (P)	30.0	22.0	34.2	22.1	35.8	22.1	28.6	20.0	32.4	20.0	25.7	20.0	18.7	18.0
Individual hatching, % (α_1)			85.0	85.5	87.6	85.0	88.1	85.5	84.6	80.0	81.3	80.0	80.6	81.0
Error	1.1	0.001	0.9	4.6	2.6	4.6	2.8	0.43	2.0	0.43	0.88	0.43	6.5	0.51
Females hatching, % (α_2)			53.0	53.0	53.4	52.9	52.5	51.0	53.6	53.7	54.0	53.7	54.0	52.0
Static criteria of quality (γ_1)			15.4	10.0	17.2	10.0	13.2	8.6	14.2	8.6	11.2	8.6	8.0	7.6
Error			0.68	0.001	0.19	0.002	0.9	0.17	0.2	0.17	0.41	0.1	0.17	0.2
Searching capacity, % (γ_2)			33.4	20.4	33.0	22.8	28.0	20.8	22.0	20.4	24.0	20.4	25.0	21.0
General criteria of quality, (*D*)			0.55	0.25	0.59	0.26	0.44	0.24	0.31	0.24	0.34	0.24	0.33	0.23
Error			0.006	0.001	0.01	0.03	0.2	0.005	0.1	0.005	0.005	0.005	0.01	0.003

Legend: F_0 – storage of eggs of the *S. cerealella* irradiated for 2 months; $F_1 - F_6$ – *Trichogramma* reared on non-irradiated *S. cerealella* eggs, six generations; M – check.

Table 4. Static criteria of quality of *Trichogramma evanescens* reared on irradiated eggs of *Sitotroga cerealella*.

Variant	**Storage term (months)**				
T. evanescens reared on irradiated eggs of *Sitotroga cerealella*	1	2	3	4	5
Static criteria of quality of *Trichogramma evanescens* reared on irradiated eggs	23.4	17.1	13.5	12.7	11.6
Error	1.74	0.94	0.29	0.25	0.20
Dispersion	9.60	2.63	0.26	0.19	0.12
Check (on non-irradiated eggs)	1	2	3	4	5
Static criteria of quality of *Trichogramma evanescens* reared on non-irradiated non-stored eggs	9.02±0.8	9.2±0.9	8.6±0.7	8.4±0.8	8.7±0.8
Error	0.005	0.10	0.45	0.025	0.10
Dispersion	0.0001	0.02	0.40	0.001	0.02

Table 5. Static criteria of *Trichogramma evanescens* quality.

Static criteria of quality	**Generations**											
	1	2	3	4	5	6	7	8	9	10	11	12
Development of *Trichogramma* on irradiated eggs of the *Sitotroga cerealella*	17.0 ±1.8	16.5 ±1.3	16.0 ±1.1	16.9 ±1.2	16.2 ±1.4	15.2 ±1.2	15.1 ±1.1	16.2 ±1.0	19.2 ±1.8	16.9 ±1.5	17.9 ±1.2	17.0 ±1.4
Development of *Trichogramma* on non-irradiated eggs (check) of the *Sitotroga cerealella*, 12 generations	10 ±1.4	9.8 ±1.0	9.9 ±1.1	10,2 ±.5	9.8 ±1.0	9.8 ±1.1	10 ±1.3	9.8 ±1.2	9.5 ±1.1	10 ±1.0	9.8 ±1.3	9.7 ±1.2

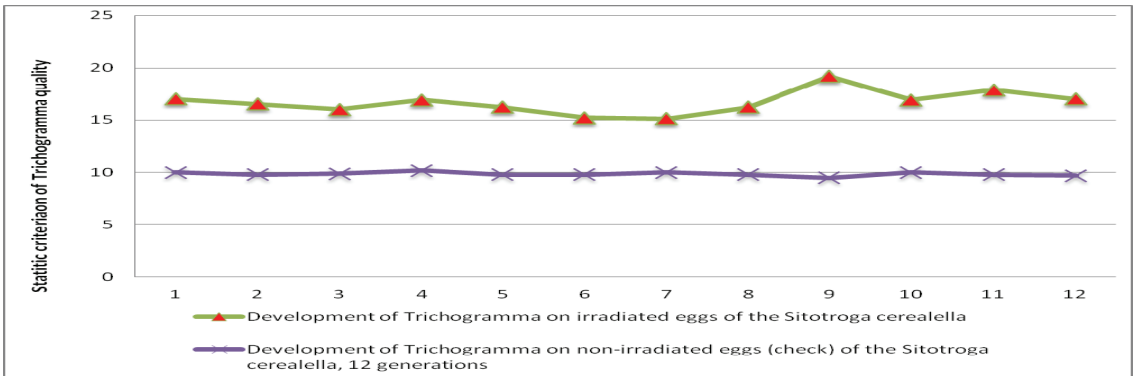

Figure 2. Static criteria of *Trichogramma* quality (Y_1) by generations.

Field verification of *T. evanescens* and *T. pintoi*, Costeşti village, Hinceşti region, Moldova.

Testing *T. evanescens* and *T. pintoi* with different passages was effectuated in cabbage field against the second generation of the cabbage moth in Costesti village, Hincesti region, Moldova, in the year 1978, within an area of 4 hectares. Three releases of *Trichogramma* were made. After each record, *Trichogramma* species was identified and the share of eggs parasitized by *T. evanescens* and *T. pintoi* from nature and laboratory populations. In variant I, one passage of the entomophage was made on eggs of *M. brassicae* and subsequently released into the field, where biological efficacy after three releases of *T. evanescens* varied from 77.9 to 90.7% and after three releases of *T. pintoi* – from 64.1% to 80.7% (Table 6).

Table 6. Percentage of *Mamestra brrassicae* eggs parasitized by *Trichogramma* in the field of Costeşti village, Hincesti region, Moldova.

Variant	**Percentage of parasitized eggs of *Mamestra brrassicae***					
	Trichogramma evanescens Westw.			*Trichogramma pintoi* Vegele		
	First release, 25.07	Second release, 02.08	Third release, 08.08	First release, 25.07	Second release, 02.08	Third release, 08.08
Variant I Passage on eggs of *M. brrassicae*	77.9 ± 2.9	84.6 ± 3.0	90.7 ± 3.4	64.1 ± 2.0	70.4 ± 2.2	80.7 ±3.3
Variant II Passage on eggs of *Sitotroga cerealella*	62.4 ± 2,3	79.7 ± 2.9	82.7 ± 2.9	54.1 ± 1.6	66.0 ± 1.6	72.7 ± 2.5
Average day temperature (T, ^{O}C)	20.1	20.2	19.8	20.1	20.2	19.8
Average daily humidity (W, %)	61.0	61.2	62.0	61.0	61.2	62.0

In variant II, *T. evanescens* and *T. pintoi* were reared on eggs of *Sitotroga cerealella* after diapause, then three releases were made into the field on late cabbage. Biological effectiveness after three releases of *T. evanescens* varied from 62.4 to 82.7% and for *T. pintoi* – from 5.1% to – 72.7%. Pest density records revealed the presence of *Trichogramma* in the field from nature, hence, the result took into account the percentage of parasitizing by laboratory *T. evanescens* together with *Trichogramma* from nature. In the check, the density of parasitized eggs varied from 3.2 to 6.0%. During pest development and verification of *Trichogramma* efficacy in the field, the average temperature per 24 house varied from 19.8 to 20.2ºC and relative air humidity from 61.0 % to 62.0%. The density of pest eggs during this period varied from 1.0 to 4.0 eggs/sq.m

Observations have shown that *T. pintoi* in cabbage field is low active, does not aggregate and gradually disappears. The picture is different when rearing *T. evanescens* and *T. pintoi* on eggs of the Angoumius grain moth (*Sitotroga cerealella*) under laboratory conditions. At mass rearing of both entomophage species there has take place their mixture and subsequent substitution of *T. evanescens* by *T. pintoi*.

Average temperature and humidity during experiments have positively influenced ovipositing of the pest and the process of parasitizing cabbage moth eggs in the field (Table 6). The average temperature and humidity during this period have varied, from 19.8 to 20.2^{O}C, and from 61.0 to 62.0%.

CONCLUSIONS

1. According to available data, the get variant where *Sitotroga cerealella* Ol. eggs were irradiated with gamma rays of 150 Gy dose and kept within one month, the state quality criteria was 2.6 higher, irradiated and stored in within two months – 1.8 times higher, held within three months –1.5 times higher, held within four months –1.5 times higher, held within five months – 1.34 times higher, compared to the control sample. The higher the terms of storage, the smaller the biological indices of *Trichogramma* spp.

2. Mathematical processing, analysis of variance indicated that the criterion – T– statistics are accurate at 95%. Static quality criterion *Trichogramma* spp. difference, multiplied by *Sitotroga cerealella* Ol. eggs irradiated were kept five months, further propagation of *Trichogramma* compared to control is essentially higher.

3. In variant I, one passage of the entomophage was made on eggs of *M. brassicae* and subsequently released into the field, where biological efficacy after three releases of *T. evanescens* varied from 77.9 to 90.7% and after three releases of *T. pintoi* – from 64.1% to 80.7%. In variant II, *T. evanescens* and *T. pintoi* have been reared on eggs of *Sitotroga cerealella* after diapause, then three releases were made into the field on late cabbage. Biological effectiveness after three releases of *T. evanescens* varied from 62.4 to 82.7% and for *T. pintoi* – from 5.1% to – 72.7%.

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