ORIGINAL PAPERS

INFLUENCE OF STERILIZATION OF *SITOTROGA CEREALELLA* Ol. EGGS ON BIOLOGICAL INDICES AND EFFICACY OF *TRICHOGRAMMA*

Lidia Gavrilita

Key words: *biological indices, prolificacy, pests, Trichogramma, Sitotroga cerealalla, entomophagous, biological efficacy*

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 (GAVRILITA, 1993, GAVRILITA L.,1995, GAVRILITA, 1996, GAVRILITA, Greenberg, 1996, GAVRILITA, 2002, Lysikova, 1985) 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.) can not 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, Rusnak, Taritsa, 1980) and it has been shown that irradiation of *Sitotoga cerealella* Ol. eggs has a positive effect on 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 has allowed reducing the number of eggs required for parasitation with *Trichogramma* (GAVRILITA, 1995). Still lacked are data on usage of fresh *Sitotoga cerealella* Ol. eggs gamma irradiated with the aim of their long – term storage and possibility of subsequent *Trichogramma* development on them.

Scientific research conducted at the Institute of Genetics, Physiology and Plant Protection has allowed us establishing that a technique for improving *Trichogramma* quality consists in rearing it on gamma irradiated eggs of *S. cerealella* (GAVRILITA, Greenberg, 1996). Taking into consideration research tasks the developed technique has demonstrated prospects of rearing the entomophagy on gamma radiated eggs of the *S. cerealella*. In this connection rearing the parasitoid on gamma radiated eggs of *S. cerealella* has improved biological indices of the entomophage by 1.5-2 times. Research results obtained by Lidia Gavrilita have shown 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 3oC up to 4-5 months for subsequent *Trichogramma* rearing. Such rearing of *Trichogramma* on gamma radiated eggs of *S. cerealella* has contributed to improvement of its biological indices by 1.5-2 times. The sex ratio has played an important role in regulating population density. Bibliography on the subject has shown that changing *Trichogramma* spp. sex ratio impacts a number factors such as temperature, humidity, a number of developing larvae per egg, host species and their age, term of storing *Trichogramma* in diapauses and many others (GAVRILITA, GREENBERG, 1996).

Bibliography on the subject shown, proves that biological indices of the parasitoid are directly proportional to the host species, its age and egg number (GAVRILITA, 1996). Each species has a high capacity for selecting the host to develop on them. Some differences have been 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. While conducting biological and ecological research and evaluating results traditional methods have been used as well as specific techniques designed for specific experience while collecting and identifying *Trichogramma* species and *Trichogramma* rearing, determining biological indices and biological efficacy of the entomophage.

MATERIALS AND METHODS

Determination of shelf life of irradiated *Sitotroga cerealella* Ol. eggs in plastic bags.

Mathematical data processing has been done using variance analysis method (Mencer, Zemshman, 1986). In experiments have been 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 3OC ± 1, biological indices have been determined for *T. evanescens* (prolificacy, hatching, females' rate) reared on these eggs. The best results with prolificacy of *Trichogamma* have been obtained applying the irradiation dose of 150 Gy to the eggs of the *S. cerealella*. After determining the optimal dose for irradiating eggs of the *Sitotroga cerealella* in subsequent experiments the dose of 150 Gy has been applied to eggs.

Collecting, identification, storage and accumulation of *Trichogramma* species were done according to Diurici G. (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

The table 1 and fig. 1 shows results of storing irradiated eggs of the Sitfor 157 days in plastic bags (5 x 5 cm) and check where eggs of the *Sitotroga cerealella* Ol. , that have been neither irradiated nor stored. After irradiating 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* have been determined.

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

The obtained results have shown that 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^{O}C. Embryo in the irradiated Angoumois grain moth has died and this has allowed parasitizing and rearing *Trichogramma* for a longer time and increasing its prolificacy**.** It has been found that female longevity increased by 2-3 times (Table 2) in the variant, where *Trichogramma* has been reared on irradiated eggs as compared to the check when *Trichogramma* has been reared without radiation.

When *Trichogramma* has been developed on irradiated *S. cerealella* eggs and stored in the refrigerator, its average longevity has equaled to 7 days, in the check – to 2.1 days, when stored for 82 days, respectively – to 4.8 days in the variant, in the check – to 2.2 days, when stored for 103 days – 2.2 days in the variant and 1.7 days, respectively. The Table 3 shows 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) has been reared on irradiated *S. cerealella* eggs, while subsequent six generations have been reared on non-irradiated eggs. When comparing F_0, F_1, F_2, F_3, F_4, with the check increase by 1.5-2 times has been found in the static criterion and quality, in general, while generations F_5, F_6 have demonstrated no essential differences. *Trichogramma* quality has 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*) eggs

Irradiated *Sitotroga cerealella* eggs have been stored for five months in small glasses with the volume of 50 ml. Every month irradiated and stored eggs have been 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 stored irradiated *S. cerealella* (variant) and on non-stored Angoumois grain moth eggs (check).

According to the obtained data, quality static criterion of eggs irradiated and stored for one month has been by 2.6 times higher and when storing for two months – by 1.8 times higher, for three months – by 1.5 times higher, for four months – by 1.5 times higher, for 5 months – by 1.34 times higher. The longer is the storage period the less is the biological index of *Trichogramma*. Mathematical processing and analysis of variance have shown that according to T – criterion data have been accurate at level of 95 %. The difference of quality static criterion of *Trichogramma* reared on irradiated *S. cerealella* eggs stored for five months as compared to the check has been essential and $T_{0,05}$=2,23<T_f=6.587-15.428. Irradiated *S. cerealella* eggs can be stored in glasses for five months with essential differences as compared to the check.

Determining efficacy of *Trichogramma* generations

The Table 5 and Fig. 2 gives graphical representation of biological indices of *T. evanescens* reared on irradiated and non – irradiated *Sitotroga cerealella* Ol. eggs. While comparing biological indices of *Trichogramma*, reared on *S. cerealella* eggs from the stock generation (F.) with 12 consecutive generations reared on irradiated and non-stored it has been 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. Comparison of variant generations F_1 – F_{12} with those in the check have shown considerable difference, where $T_{0,05}$ = 2.78; $T_{1\text{-}12}$ = 5.9-50.9 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	Storage time, days	Average prolificacy, eggs / female	Hatching per individual, %	Confidence interval	Stiudent criteris of assessment - 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 Check	48±2.36 0	7.0±0.8 2.1±0.1
150 Gy Check	82 ±3.32 0	4.8±0.6 2.2±0.2
150 Gy Check	103±3.36 0	2.1±0.1 1.7±0.3

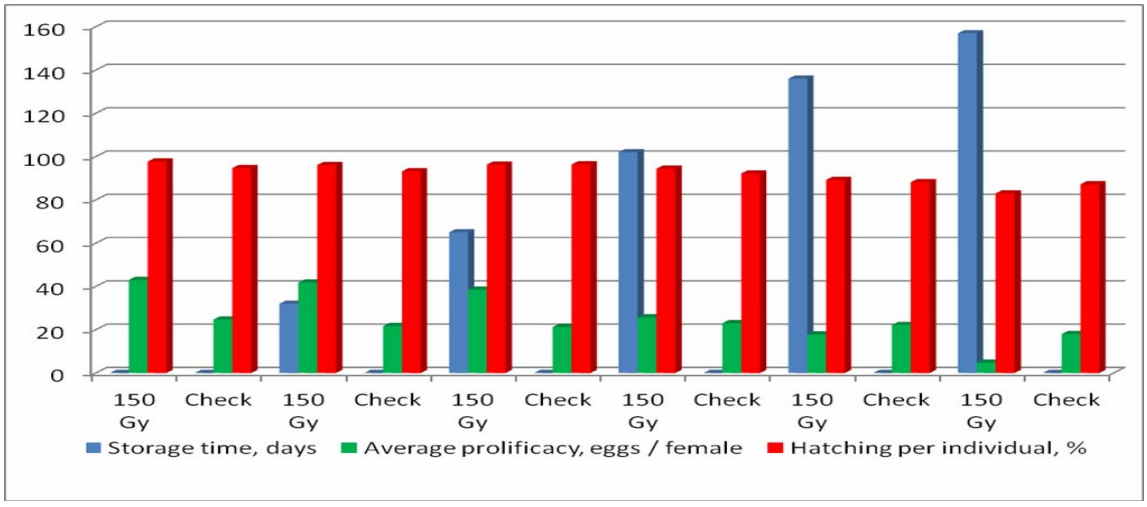


Fig. 1. Influence of gamma radiation of the *Sitotroga cerealalla* Ol. eggs on biological indices of *Trichogramma evanescens.*

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

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

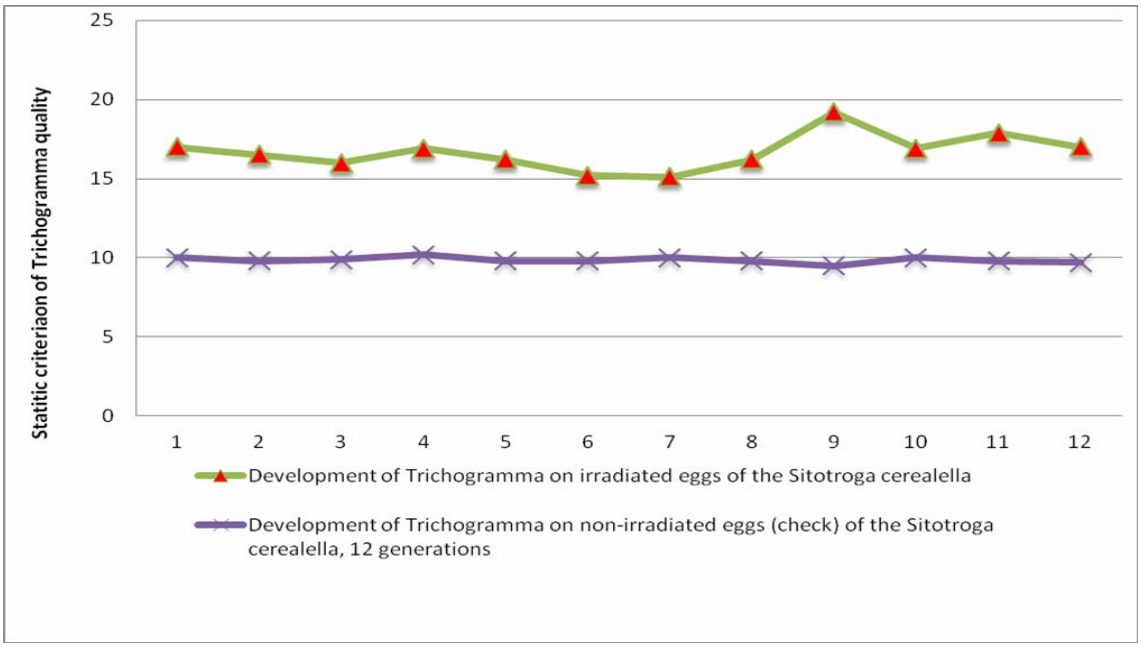


Fig. 2. Static criteria of *Trichogramma* quality (Y_1) by generations.

Developing *Trichogramma* stock culture for entomophage mass rearing.

Decrease in the size of females under long-term mass rearing of *Trichogramma* on eggs of the Angoumois grain moth has been proportional to the weight, prolificacy and other biological indices. Increasing the number of *Trichogramma* stock colonies by passaging through the cabbage moth (*Mamestra brassicae*) is very important for preserving heterogeneous genetical characteristics of the natural population. To effectuate the above-mentioned it is necessary to settle the following issues:

Developing *Trichogramma* stock colonies*;*
Defining the time for making passages;
Quantitative evaluation of passages efficacy;
Determining passages after effects

Developing *Trichogramma* original colonies has been effectuated through collecting eggs of different hosts parasitized under natural conditions and distributing eggs of the Angoumois grain eggs (laboratory host) placed onto cards in the field, identification of appurtenance of each eggs batch separate and in group on the base of dominating species to set up an original colony.

A starting point has been establishing the size (amount) of the original colony. With this reference it is necessary to take into consideration numerical efficacy i.e. the number of individuals that may have a real share when reproducing the colony. It has been established that the best result has been achieved when in the original colony after passage there is no less than 2000 females that oviposit in eggs of the Angoumois grain moth. This allows keeping the gene pool and evitating genetic elimination.

- To determine terms and number of passages under laboratory conditions experimens have been made with *T. evanescens* and *T. pintoi* on eggs of the cabbage moth according to the following scheme:

Variant 1 – making passage in autumn before diapause;
Variant 2 – making passage in spring after diapause;
Variant 3 – making one passage in autumn and one passage in spring (before and after diapause);
Variant 4 – making two passages in spring after diapause;
Variant 5 – without any passages (check)

Biological indices of the species have been determined under laboratory conditions according to the variants. When comparing variants it has been found that the most efficient is variant 4 with two passages in spring, static criterion of quality for *T. evanescens* has equalled to 27.50 (confidence interval – 18.92; 36.07). In the check biological indices have been lower and constituted 8.03 (confidence interval – 5.26; 10.78). Static criterion of quality for *T pintoi* has equalled to 26.00 (confidence interval – 17.00; 32.00). In the check static criterion of quality has been considerably lower and constituted 7.00 (confidence interval – 6.10; 9.60).

Trials have been continued with two spring passages on eggs of the cabbage moth (*Mamestra brassicae*) and with three passages on eggs of the Angoumois grain moth (*Sitotroga cerealella*). In check *Trichogramma* has been reared only on eggs of the Angoumois grain moth. Biological indices of *Trichogramma* have been determined before and after diapause and after passage.

Results of *T.evanescens* and *T. pintoi* analyses have been summarized in Table 7. Static criterion of quality for *T. evanescens* before diapause has equalled to 7.09, on eggs of the Angoumois grain moth after diapause – 9.5, while after diapause and after passage on eggs of the cabbage moth and after three generations on eggs of the Angoumois grain moth – 19.4. Female longevity has equalled respectively to 1.60, 2.37 and 4.11 days.

Static criterion of quality for *T. pintoi* before diapause has equalled to 19.0 on eggs of the Angoumois grain moth and after diapause decreased up to 6.66, while after making two passages on eggs of the cabbage moth and three passages on eggs of the Angoumois grain moth – 30.78. Females longevity equalled, respectively, to 2.76; 2.88 and 4.21 days.

In the check static criterion of quality for *T. pintoi* has equalled to 16.10 after five passages on eggs of the Angoumois grain moth and after diapause. Female longevity has constituted 2.14 days. For *T. evanescens* these indices have equalled respectively to 13.48 and 2.41 days. When comparing variants with passages on the cabbage moth eggs (experiment) and the Angoumois grain moth eggs (the check) has been observed considerable increase of prolificacy, static criterion of quality and female longevity after making two passages on eggs of *Mamestra brassicae* **(**Table 8).

Passage on cabbage moth eggs has positively affected all biological indices of *T. evanescens* and *T. pintoi*. Confidence interval for γ_1 has demonstrated impact of the passage and diapause on *Trichogramma* quality. Considerable difference has been revealed between variants. No essential peculiarities have been observed between female longevity of these species.

Field verification of *T. evanescens* and *T. pintoi*, Băcioi village, Ialoveni region, Moldova.

After determining biological indices under laboratory conditions field releases of *Trichogramma* have been made against the cabbage moth to determine entomophage biological efficacy (Table 9**.**). *T. pintoi* and *T. evanescens* with different passages have been tested in cabbage field against the second generation of the cabbage moth (*Mamestra brassicae*) în Băcioi village in year 1977 in the area of 2 hectares in five variants: variant 1 – spring passage before entering in diapauses when biological effectiveness after three releases of *T. pintoi and T. evanescens* has constituted, respectively, 62% and 587%; variant 2 – spring passage after diapause when biological effectiveness after three releases of *T. pintoi and T. evanescens* has constituted, respectively, 55.8% and 60.1%; in variant 3 – autumn and spring passages (before and after diapauses) when biological effectiveness of *T. evanescens* and *T. pintoi* has equalled to 47.0% and 63.3%; variant 4 – two spring passages after diapause when biological effectiveness of *T. evanescens* and *T. pintoi* has equalled, respectively, to 64.0% and 86.6%; variant 5 – releases without any passages when biological effectiveness of *T. evanescens* and *T. pintoi* has constituted 33.3and 35.6%. More effective have been results obtained in the variant where two spring passages have been effectuated. In the check where the entomophage has not been relied no parasitized eggs have been reported (Table 6). During the entomophage development and field tests with *Trichogramma* average temperature during 24 hours has varied from 17.2 to 24.1^{O}C and relative air humidity – from 56.7% to 62.9%. The pest egg density during this period has varied from 0.8 to 3.6 eggs/sq.m.

Field verification of *T. evanescens* and *T. pintoi*, Costesti village, Hincesti region, Moldova.

Testing of *T. evanescens* and *T. pintoi* with different passages has been effectuated in cabbage field against the second generation of the cabbage moth in Costeşti village, Hincesti region, Moldova in year 1978 in the area of 4 hectares. Three releases of *Trichogramma* have been made. After each record *Trichogramma* species have been 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 has been made on eggs of *M. brassicae* and subsequently released into the field, where biological efficacy after three releases of *T. evanescens* has varied from 77.9 to 90.7% and after three releases of *T. pintoi* – from 64.1% to 80.7% (Table 10).

In variant II *T. evanescens* and *T. pintoi* have been reared on eggs of *Sitotroga cerealella* after diapauses, and then three releases have been made into the field on late cabbage. Biological effectiveness after three releases of *T. evanescens* has varied from 62.4 to 82.7% and for *T. pintoi* – from 5.1% to 72.7%. Pest density records have revealed presence in the field of *Trichogramma* from nature; hence, the result has taken into account percentage of parasitizing by laboratory *T. evanescens* together with *Trichogramma* from nature. In the check density of parasitized eggs has varied from 3.2 to 6.0%.

During pest development and verification of *Trichogramma* efficacy in the field average temperature per 24 houses has varied from 19.8 to 20.2^{O}C and relative air humidity during this period has varied from 61.0 % to 62.0%. Density of pest eggs during this period has varied from 1.0 to 4.0 eggs/sq.m.

Table 6. Percentage of parasitizing *Mamestra brassicae* L. eggs by *T. pintoi and T. evanescens* in Băcioi village, Ialoveni

VARIANTS	*T. pintoi*						*T. evanescens*					
	Ist release 22.07		II-nd release 27.07		III-rd release 04.08		I-st release 22.07		II-nd release 27.07		III-rd release 04.08	
	% of parasityzing	Confidence interval	% of parasityzing	Confidence interval	% of parasityzing	Confidence interval	% of parasityzing	Confidence interval	% of parasityzing	Confidence interval	% of parasityzing	Confidence interval
I	2.7	1.9-3.6	3.8	2.8-4.9	62.0	54.4-63,4	10.7	8.7-14,8	37.7	34.2-41.2	58.7	48.7-60.7
II.	3.5	2.3-5,0	10.0	8.1-12.1	55.8	52.2-61.4	12.1	10.3-14.1	42.6	37.0-48.1	60.1	55.1-65.1
III.	5.7	4.3-7,2	11.7	9.6-14.0	47.0	41.9-52.1	15.0	12.4-6.4	61.4	56.5-66.3	63.3	56.7-68.9
IV	10.7	9.3-12.1	25.2	22.5-27.9	64.0	60.8-67.2	20.2	17.8-22.7	77.5	74.3-80,7	86.6	82.6-90.6
V	2.6	1.6 -3.8	2.3	1.3-3.6	33.3	29.7-36.9	8.7	6.5-11.2	20.8	15.7-25.9	35.6	30.4-40.8
Check	0		0		0		0		0		0	
T ^{O}C,	21.6		24.1		17.2		21.6		24.1		17.2	
W,%	61.3		56.7		62.9		61.3		56.7		62.9	
Density eggs / sq.m	0.8		3.4		2.2		3.6		2.2		1.2	

Note: T – average day temperature,^{O}C; W – humidity, %; D – density of pest eggs, eggs/sq/m$^{2;}$ Variant 1 – autumn passage before diapause; Variant 2 – spring passage after diapause; Variant 3 – autumn and spring passages (before and after diapauses); Variant 4 – yowl spring passages after diapauses: Variant 5 – release without any passages (check).

Table 7. Biological indices of *T. evanescens* and *T. pintoi* after passage on the natural host eggs of *Mamestra brassicae and Sitotroga cerealella*.

No.	Biological indices	*T. pintoi*			*T. evanescens*		
		Before diapaus	After diapause	Two passages on *Mamastra brassicae* and three passages on *Sitotroga cerealella* eggs	Before diapause	After diapause	Two passages on *Mamastra brassicae* and three passages on *Sitotroga cerealella* eggs
1.	Prolificacy, (P)	13.3	23.8	44.0	34.5	23.7	52.1
2.	Individual hatching, % (α_1)	86.3	83.5	78.0	90.0	46.0	92.5
3.	Female hatching, % (α_2)	61.8	48.5	56.8	61.1	61.5	63.8
4.	Number of unstraibed individuals, % (α_3)	98.8	100	100	94.1	98.6	100
5.	Static criteria of quality, (γ_1)	7.0	9.5	19.4	19.0	6.6	30.7
6.	Female longevity / days, (γ_2)	1.6	2.37	4.1	2.78	2.88	4.21
7.	Average error per sq.m. (S γ_1)	0.861	1.195	2.00	2.689	0.772	2.508
8.	95% Confidence interval, $\gamma_1 \pm 1{,}96$	(5.40;8,78)	(7.57;11.46)	(15.45; 23.33)	(13.73; 24,28)	(5.15; 8,17)	(25.86; 35.70)

Table 8. Biological indices of *T. evanescens* and *T. pintoi* (check)

Biological indices	Symbols	*T. pintoi*			*T. evanescens*		
		Before diapauses	After diapause First generation	Five generations on *Sitotroga cerealella* (after diapause)	Before diapauses	After diapause first generation	Five generations on *Sitotroga cerealella* (after diapause
Prolificacy	P	13.30	23.81	38.5	34.57	23.78	39,75
Individual hatching, %	α_1	86.3	83.50	70	90.0	46.0	78,0
Female hatching, %	α_2	61.8	48.5	50	61.1	61.5	51,6
Number of unstraibed individuals, %	α_3	98.8	100	100	94.1	98.6	100
Static criterion of quality	γ_1	7.09	9.5	13.48	19.01	6.66	16,10
Female longevity / days	γ_2	1.60	2.37	2.41	2.78	2.88	2,14
Average error per sq.m	S γ_1	0.861	1.195	1.637	2.689	0.772	1,835
95% confidence interval	$\gamma_1 \pm 1.96$	(5.40; 8,78)	(7.35; 11.03)	(10.27; 16.69)	(13.74;24.2)	(5.15;8.17)	(12,50; 19,70)

Table 9. Percentage of *Mamestra brassicae* eggs parasitized by *Trichogramma* in the field of Costesti, village, Hancesti region, Moldova

Variant	Percentage of parasitized eggs of *Mamestra brassicae*					
	Trichogramma evanescens W.			*Trichogramma pintoi V.*		
	Firest release, 25.07	Second release, 02.08	Third release, 08.08	First release, 25.07	Second release, 02.08	Third release, 08.08
Varianta 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

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 on ovipositing of the pest and the process of parasitizing cabbage moth eggs in the field (Table 10). The average temperature and humidity during this period have varied, respectively, from 19.8 to 20.2^{O}C, and from 61.0 to 62.0%.

- **Quantitative evaluation of passages**

Many indices (survival – vitality, sex index, prolificacy) that currently characterize *Trichogramma* do not create any possibility to evaluate identically its quality. The situation is even worse as it does not take into account capacity of searching natural host eggs by *Trichogramma*.

With this reference the scientifically grounded criterion for assessing efficacy of *Trichogramma* passages on eggs of the main host shall be based on one integral index, i.e. the general criterion of quality that comprises all the above-mentioned characteristics. The general criterion of quality in the original culture of *T. evanescens* in experiments has equaled to 0.26-0.28, that according to the existing standard does not allow to assign to a non-standard biological material. Closed vessels, provided they contain big numbers of cabbage moth eggs, transition of *Trichogramma,* reared on the eggs of the Angoumois moth, onto eggs of the main host is satisfactory (the number of parasitized eggs in average constitutes from 71.2 to 92 %, while the number of ovipositing females equals to 90-100 %. When transferring *Trichogramma* from cabbage moth eggs on eggs of the Angoumois grain moth these indices equal, respectively, to 30 to 41,5 % and 23 to 33 % in the first generation (Table 11). This transition has been accompanied by "compressing population" and substantial impoverishment of the gene pool. Preservation of the heterogeneous gene pool of the population from nature is achieved at the expense of growth of the number of original colonies The procedure of making passages depends on the physiological state of insects, peculiarities of the metabolism in the organism, food assimilation, host eggs content quality and quantity. It is done for comparing biological indices of *Trichogramma* reared on eggs of the cabbage moth and the Angoumois grain moth. In the first case (when reared on eggs of the cabbage moth) integral indices – static criterion of quality and capacity of searching host eggs are by 2.2–2.3 times higher than when reared on eggs of the Angoumois grain moth. After making five passages on cabbage moth eggs general criterion of quality has varied from 0.74 to 0.90, that corresponds to the Ist class quality, while the exact rearing speed has equaled to 41.8; in the check, in the variant where *Trichogramma* has been reared on the eggs of the Angoumois grain moth these indices have varied from 0.28 to 0.32 and rearing speed has equaled to 17.0 that corresponds to non-standard *Trichogramma* (Table 12).

Data analysis has shown that for increasing *Trichogramma* vitality it is enough to make 1 - 2 passages on eggs of the cabbage moth (*Mamestra brassicae).*It has been mentioned that *Trichogramma* reared for many sequent generations on eggs of the Angoumois grain moth followed by passage on eggs of the cabage moth has increased its vitality. At subsequent rearing of *Trichogramma* on eggs of the laboratory host - the Angoumois grain moth during six generations, general criterion of the entomophage quality has equalled to 0.47-0.7 (1-2 passages on cabbage moth eggs) to 0.5-0.73 (4-6 passages on cabbage moth eggs). All trial variants have demonstrated increase of this index from the first to the third, the fourth at rearing on the Angoumois grain moth eggs with subsequent decrease after the Vth –VIth generation. These changes have been less obvious after 3-4 passages of *Trichogramma* on eggs of the main host. Improved quality of *Trichogramma* has been achieved at the expense of imcreasing capacity and efficacy of host eggs searching capacity and *Trichogamma* prolificacy. In this case the share that refers to the first index has equaled to 67%. When rearing *Trichogramma* on the Angoumois grain moth eggs, hatching usually lasts for 4 days, including 18.4 % – on the Ist day, 36.1 % on the II nd day, 30.6 % - on the III-rd day and 13.9 % – on th VI-th day, while after passages during the first 2 days release from 75 to 80 % of individuals.

Table 10. Biological indices of *T. evanescens* produced on eggs of the cabbage moth (*Mamestra brassicae*)

Number of passages on eggs of (*Mamestra brassicae*)	Biological indices			
	Parasitized eggs, %		Share of ovipositing females, %	
	Sitotroga cerealella	*Mamestra brassicae*	*Sitotroga cerealella*	*Mamestra brassicae*
I st generation	30.0±1.4	71.2±2.4	28.0±1,3	90.0±3.3
II-nd generation	31.2±1.5	90.0±2.6	30.0±1.5	100
III-rd generation	41.5±1.8	90.0±2.9	30.0±1,3	100
IV-th generation	37.0±1.5	92.0±2.8	33.0±1.6	100
V-th generation	32.4±1,3	91.8±2.7	23.0±1.2	100

Table 11. Biological indices of *T. evanescens* reared on eggs of the cabbage moth (*Mamestra brassicae)*and the Angoumois grain moth *(Sitotroga cerealella)*

Biological indices	*Mamestra brassicae L.*					*Sitotroga cerealella* Ol.				
	F1	F2	F3	F4	F5	F10	F11	F12	F13	F14
Hatching of individ, %,(α_1)	93.2± 2.5	95.0± 2.8	94.6± 2.1	100± 1.0	98.1± 1.9	82.4± 1.92, 2	81.6± 2.3	76.3± 2,6	80.7± 2.3	83.3± 2.2
Female hatching, %,(α_2)	68.9± 3.6	65.4± 3.4	67.1± 3.4	66.6± 3.7	61.0± 3.4	58.3± 3.4	62.0± 3.4	60.3± 3.2	63.6± 3,1	57.2± 3.1
Eggprolificacy/ female	35.5± 1,6	34.9± 2,0	35.1± 2,1	44.0± 3.3	54.0± 3.4	24.7± 2.2	24.8± 4,8	25.4± 3.7	27.7± 4,5	21.4± 3.1
Static criterion of quality (γ_1)	22.8± 1.0	21.6± 1.0	22.2± 1.1	29.3± 1.3	32.3± 1.4	11.6± 1.2	12.5± 1,8	11.7± 1,7	14.2± 1,5	10.2± 1.1
Searching capacity, % (γ_2)	40.3± 5.5	43.3± 5.4	49.4± 5.1	50.5± 3.3	52.0± 4.4	21.4± 2.4	21.9± 4.0	20.9± 3.4	20.4± 2.4	20.7± 3.0
General criterion of quality (*D*)	0.75± 0.1	0.74± 0,1	0.80± 0.1	0.86± 0,1	0.90± 0,1	0.28± 0,04	0.29± 0,04	0.27± 0.02	0.32± 0.04	0.25± 0.03

Impact of passages on *Trichogramma* quality

According to obtained data impact of passages has lasted for 6 – 7 reproductions when rearing *Trichogramma* on the Angoumois grain moth eggs. When making passages on eggs of the cabbage moth and further on rearing eggs of the Angoumois grain moth for 6-7 generations, biological material quality decreases to the 2nd class level that being released under optimal hydrothermal conditions provides biological efficacy within 60-80 %. Then after two passages on eggs of the natural host and subsequently 12 generations on the eggs of the Angoumois grain moth, general criterion of the parasitoid quality decreases till the VII ith generation from 0.51 to 0.65, that corresponds to class II of *Trichogramma* quality (Table 13, fig. 3.), while the speed of rearing varies from 16.8 to 27.9, subsequently *Trichogramma* class has corresponded to class III quality.

In the check after rearing of *Trichogramma* for 12 generations on eggs of the Angoumois grain moth, these indices have equalled 0.3 to 13 reproductions and –0.13 after 24 reproductions on the laboratory host (Table 14), however the speed of *Trichogramma* rearing equalled, respectively, to 18.0 and 16.0. It has been found that the above – mentioned happens when dealing with original colonies after passages on eggs of the cabbage moth with no less that 2.000 females that oviposit into eggs of the Angoumois grain moth. It allows determining the number of passages. The results of field evaluation of passages are given in Table 14 where it is seen that the number of eggs parasitized by *Trichogramma* after two passages on eggs of the cabbage moth and 6 – on the Angoumois grain moth have been two – three times bigger as compared to not long rearing on eggs of the Angoumois grain moth. Difference between the variants has been considerable. No essential changes have been observed after the 12th generation.

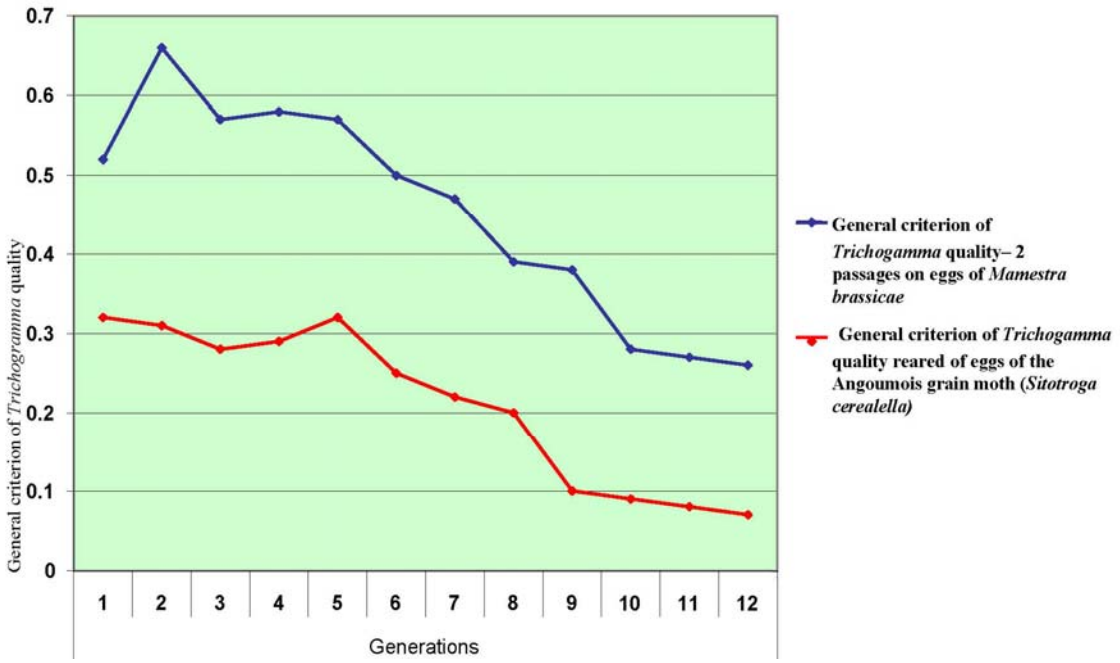
As far as commercial production of *Trichogramma* is exorcized on Angoumois grain moth eggs, passages are important for improving quality of the biological material. In the future we recommend rearing *Trichogramma* on eggs of the natural main host. Basis for the above said is development of technologies and equipment for rearing different moth and tortricid species on

artificial nutritive diets carried out in many national and foreign research institutions.

Scientific studies and analysis of biological loboratories' performance have resulted in obtaining data for justifying new procedures of mechanizing and improving the existing equipment for determining production optimal regimes, volumes and structure that may serve as the basis for setting up biological laboratories of the II-nd generation for *Trichogramma* rearing on more efficient equipment with less nomenclature and higher degree of procedures mechanization and automation.

Table 12 Biological indices of *T.evanescens* after passages on eggs of the cabbage moth (*Mamestra brassicae*)

Number of generations on eggs of (*Sitotroga cerealella* Ol.	Hatching of individuals, % (α_1)	Hatching of females, %, (α_2)	Egg prolificacy, P		Static criterion of quality (γ_1)	Searching capacity, % (γ_2)	General criterion of quality (*D*)	Confidence interval of static criterion of quality $\gamma_1 \pm$
			Angoumois grain moth	Cabbage moth				
1	90.6 ± 5.5	66.9 ± 11,5	21.1 ± 5	45.7 ± 6.4	12.80	32.2 ± 3,3	0.51	(10.543;16.051)
2	90.3 ± 5.2	56.4 ± 1,.0	25.1 ± 1	44.7 ± 5.4	12.70	33.6 ± 4.8	0.52	(9.743; 15.687)
3	88.6 ± 3.5	64.5 ± 9.1	36.9 ±10.8	48.1 ± 8.8	21.10	33.0 ± 4.2	0.65	(18.63; 23.560)
4	85.3 ± 0.2	56.6 ± 1.2	31.3 ± 5,2	49.6± 10.3	14.30	31.4 ± 2.6	0.54	(11.836; 6.763)
5	85.7 ± 0.6	53.0 ± 2.4	31.6 ± 5.5	39.5 ± 0.2	14.35	33.0 ± 4.2	0.56	(11.886;16.760)
6	84.6 ± 0.5	52.7 ± 2.7	29.5 ± 3.4	42.9 ± 3.6	13.15	32.2 ± 3,4	0.54	(11.060;15.934)
7	87.7 ± 2.6	54.4 ± 1.0	29.6 ± 3.5	42.3 ± 2.7	14.12	28.3 ± 0.1	0.48	(11.500;15.000)
8	84.6 ± 0.5	56.3 ± 0.9	26.4 ± 0.3	34.7 ± 4.6	12.60	28.5 ± 0.1	0.45	(10.205;14.995)
9	82.0 ± 3.1	51.2 ± 4.2	22.3 ± 3.8	32.6 ± 6.7	9.36	25.0 ± 3,8	0.38	(7.84; 10.88)
10	82.3 ± 2.8	52.2 ± 3.2	22.2 ± 3.9	30.8 ± 8.5	9.53	24.2 ± 4.6	0.37	(8.00; 11.200)
11	81.3 ± 3.8	50.8 ± 4.6	19.7 ± 6.4	31.5 ± 7.8	8.13	22.6 ± 6.2	0.30	(7.00; 9.200)
12	79.0 ± 6.1	50.6 ± 4.8	17.4 ± 8.7	30.4 ± 8.9	6.96	22.2 ± 6.6	0.26	(5.480; 8.420)



Fig, 3. General criterion of *Trichogramma* quality

Table 13. Biological indices of *T. evanescens* reared on eggs of the Angoumois grain moth (*Sitotroga cerealella* Ol.).

Number of reoroductions on eggs of the *Sitotroga cerealella*	Hatching of individuaks, % (α_1)	Hatching of females, %, (α_2)	Eggs prolificacy, P		Static criterion of quality (γ_1)	Searching capacity, % (γ_2)	General criterion of quality (*D*)	Confidence interval of static criterion of quality $\gamma_1 \pm$
			Angoumois grain moth	Cabbage moth				
1	84.5 ± 3,2	54.5 ± 2,4	20.2 ± 0,6	38.8 ± 8.5	9.30	22.2 ± 2.0	0.30	(8.044; 10.550)
2	85.3 ± 3.7	53.3 ± 1.2	25.5 ± 1.0	31.1 ± 0.8	9.28	23.0 ± 3.0	0.31	(8.000; 10.540)
3	84.3 ± 3.0	53.0 ± 0.9	23.7 ± 4.2	37.8 ± 7.5	10.6	21.4 ± 1.4	0.31	(9.805; 11.395)
4	81.3 ± 0	53.0 ± 0.9	19.2 ± 0.3	35.4 ± 5.1	8.30	21.0 ± 1.0	0.28	(6.349; 10.250)
5	81.3± 0	51.6 ± 1.4	22.2 ± 2.7	34.5 ± 4.2	9.31	19.0 ± 1.0	0.30	(7.340; 10.500)
6	85.3 ± 4.0	50.4 ± 1.7	21.8 ± 2.3	31.5 ± 1.2	9.40	21.0 ± 1.0	0.31	(7.450; 10.600)
7	82.3 ± 1.0	52.4 ± 0.3	21.7 ± 2.2	26.3 ± 4.0	8.80	19.1 ± 0.9	0.25	(6.817; 10.783)
8	83.3 ± 2.0	53.2 ± 1.1	18.3 ± 1.5	24.4 ± 5.9	8.11	20.4 ± 0.4	0.22	(6.700; 9.800)
9	82.0 ± 3.1	51.6 ± 0.5	17.5 ± 2.0	25.9 ± 4.4	7.34	19.3 ± 0.7	0.22	(6.409; 8.843)
10	75.5 ± 5.8	52.0 ± 0.1	18.0 ± 1.5	29.4 ± 0.9	7.06	20.4 ± 0.4	0.21	(6.320; 8.700)
11	77.0 ± 4.3	51.1 ± 1.0	16.1 ± 3.4	30.4 ± 0.1	6.33	16.7 ± 3.3	0.15	(5.500; 7.300)
12	75.3 ± 6.0	50.4 ± 1.7	15.0 ± 4.5	18.0 ± 12.3	5.7	16.9 ± 3.1	0.13	(4.578; 6.822)

Table 14 Comparative assessment of passages impact on vital capacity of *Trichogramma evanescens* after one field release

Variants	Percentage of parasitized eggs	Essential difference of diets
T = 25 ^{O}C, W = 60 %		
2 passages of *Trichogramma* on eggs of the cabbage moth and 6 passages on eggs of the Angoumois grain moth	68.6 ± 2.19	$t_{d = 2.57 > 1.96 = t_{0.05}}$
18 generations of *Trichogramma* on eggs of the Angoumois grain moth	30.0 ± 2.17	
Check (without *Trichogramma* releases)	0	
T = 23^{O}C, W = 70 %		
2 passages of *Trichogramma* on eggs of the cabbage moth and 12 passages on eggs of the Angoumois grain moth	34.0 ± 2,22	$t_{d = 2.57 > t_{0.05}=1.96}$
24 reproductions of *Trichogramma* on eggs of the Angoumois grain moth	25.4 ± 2,08	
Check (without *Trichogramma* releases)	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, state quality criteria was 2.6 higher, irradiated and stored in within two months – 1.8 times greater, held within three months – by 1.5 times, held within four months – by 1.5 times, held within five months – 1.34 times higher, as the witness. The terms of storage are higher, the smaller the biological indices of *Trichogramma*.

2. Mathematical processing, analysis of variance indicated that the criterion – T– statistics are accurate at the 95%. Static quality criterion *Trichogramma* difference, multiplied by *Sitotroga cerealella* Ol. eggs. irradiated were kept five months, further propagation of *Trichogramma* compared to control is essential higher ($T_{0,05} = 2.23 < T_f = 6.58$ to 15.43).

3. In variant I one passage of the entomophage has been made on eggs of *M. brassicae* and subsequently released into the field, where biological efficacy after three releases of *T. evanescens* has 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 have been made into the field on late cabbage. Biological effectiveness after three releases of *T. evanescens* has varied from 62.4 to 82.7% and for *T. pintoi* – from 5.1% to 72.7%.

4. Then after two passages on eggs of the natural host and subsequently 12 generations on the eggs of the Angoumois grain moth (*Sitotroga cerealella* Ol.), general criterion of the parasitoid quality decreases till the VII ith generation from 0.51 to 0.65, that corresponds to class II of *Trichogramma* quality, while the speed of rearing varies from 16.8 to 27.9, subsequently *Trichogramma* class has corresponded to class III quality.

ABSTRACT

According to available data, the Get variant where au *Sitotroga cerealella* Ol. eggs were irradiated with gamma rays of 150 Gy dose and kept within one

month, state quality criteria was 2.6 higher, irradiated and stored in within two months – 1.8 times greater, held within three months - by 1.5 times, held within four months - by 1.5 times, held within five months 1.34 times higher, as the witness. The terms of storage are higher, the lower are the biological indices of *Trichogramma*. Mathematical processing, analysis of variance indicated that the criterion – T. statistics are accurate at the 95%. Static quality criterion *Trichogramma* difference, multiplied by *Sitotroga cerealella* Ol. eggs. irradiated were kept five months, further propagation of *Trichogramma* compared to the control is essential higher ($T_{0,05}$ = 2.23 <T_f = 6.58 - 15.43).

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AUTHOR'S ADDRESS

GAVRILITA LIDIA - Institute of Genetics, Physiology and Plant Protection of ASM, e-mail: lidia_gavrilita@yahoo.com