

ORIGINAL PAPERS

APPLICATION OF KAIROMONS IN INCREASING BIOLOGICAL INDICES OF THE ENTOMOPHAGUS *TRICHOGRAMMA EVANESCENS*

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Key words: protection, efficacy, pests, pheromone, monitoring, biological indices, prolificacy

INTRODUCTION

The newness of the topic and the argument of the research direction

Auxiliary insect entomophage (parasitoids and predators of phytophagous insects) evolved in a multitrophic context. They are part of the third trophic level, and their behavior and physiology are implicitly influenced by elements of the second trophic level (phytophagous insects) and the first trophic level (host plant). The complex interactions between phytophagous and the host plant, on the one hand, and between phytophagous and entomophagous, on the other hand, can only be understood within this triotrophic complex (Price et al., 1980; Dicke, et al., 1999).

A wide range of research has been carried out with the use of chemical mediators (special pheromones, kairomones, synomones and allomonies), as a way of use in monitoring, managing or combating many harmful insect species, in parallel with reducing to eliminating at all treatments with pesticides and their replacement with biopesticides (Bakthavatsalam, 2013; Bigler 2008).

The kairomonal effect of saturated hydrocarbons on the egg parasitoids, *Trichogramma brasiliensis* (Ashmead) and *Trichogramma exiguum*, (Hym., *Trichogrammatidae*) was described by Paul AV, 1980 and experiments were performed by the authors Padmavathi Ch., Paul AV (2009).

The role of kairomons in biological pest control in crops has been described by the authors Ramasamy Kanagaraj, Murali-Baskaran, Kailash Chander Sharma, (2017). The impact of plant phenols as semiachemics on the performance of *Trichogramma chilonis* Ishii, was described by the authors Pathipati Usha Rani, Pratyusha Sambangi, Kurra Sandhyarani, 2017.

The authors Fatouros Nina, Marcel Dicke, Roland Mumm, (2008), [15] conducted research using kairomons. To compensate for the limited ability to fly and to have access to the newly laid host eggs of egg parasitosis, several strategies have been developed such as the use of kairomons. The kairomonal effect of saturated hydrocarbons on egg parasitosis, *Trichogramma brasiliensis* (Ashmead) and *Trichogramma exiguum*, *T. pintoi*, Paul, A

Singh, S., Singh A.K., Pathipati, 2017, Padmavathi, 2017, Ramasamy, 2017, Stefano Colazza, 2010, *Trichogramma brasiliensis* and *T. exiguum* with linear chain hydrocarbons, showed that pentacosan and hexacosan recorded an index of activity of the large parasite and parasitism for both species of what indicates a high kairomonal activity and tetracosan, which can be grouped as favorable hydrocarbons, which show different levels of kairomonal activity for *T. brasiliensis*. Hexane extract and lepidopteran eggs, which increase the ability to search for insects, were used as a source of kairomon. (Stan Gh., 1993). A kairomon is a messaging substance for the transfer of information between different species (so-called allochemicals), which only benefits the recipient organism (Bakthavatsalam, N. P. L. Tandon P. L., Deepa, (2013).

Biological protection can be defined as the use of living organisms and the products of their biological activity in order to regulate pest populations in field crops. The efficiency of the application of entomophagous in the integrated protection of agricultural crops has been demonstrated by several scientists in different countries, Fatouros, 2008, Ksentini, 2011.

The extensive use of entomophagous *Trichogramma* spp. In plant protection is related to its quality in mass propagation and field application.

The aim of the research is: Estimation of the effect of biologically active substances with attractive properties on the ethological aspects of *Trichogramma* spp.

MATERIALS AND METHODS

The fundamental research was carried out in the Institute of Genetics, Physiology and Plant Protection, Phytopharmacy and Ecotoxicology laboratory, of Republic of Moldova.

The problem solved by the given process is the increase of the quality (biological indices) of *Trichogramma* spp. as a result of the influence of the biologically active substances with attractive properties of the cereal moth (*Sitotroga cerealella* Ol.) at the egg stage.

To determine the search capacity of the entomophage at one end of the tube was fitted a

buffer soaked in biologically active substances with attractive properties of moth eggs, aged 24-26 hours previously treated in a solution of 10, 20, 30% alcohol for 5, 10, 15 minutes each, at the other end of the tube, the females of *Trichogramma evanescens* W. were launched one by one, a total of 50 females in each variant. The first stage is the preparation of eggs of cereal moth (*Sitotroga cerealella* Ol.) aged 24-26 hours as a laboratory host, which are used for the treatment and mass growth of the entomophagous *Trichogramma*, to improve the production technology of *Trichogramma* spp., which allows the increase its quality and effectiveness in the field.

For the preparation of biologically active substances with attractive properties (caïromon): different concentrations of alcohol 10%, 20% and 30% were used, with which 1g, 2g, 3g of host eggs were treated (*Sitotroga cerealella* Ol.) at each concentration and treated (exposure) for 5, 10, 15 minutes each. At each factor level there are 9 variants x 3 levels in total 27 variants. The planning conditions and the results of the Box-3 experiments are presented in Table 2. In each variant, 50 females were checked, where the prolificacy and search capacity of a female were determined, according to the Box-3 experiments plan.

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Determination of the search capacity of *T. evanescens*, as a result of the influence of biologically active substances with attractive properties of *Sitotroga cerealella* Ol. at the egg stage was performed in laboratory conditions in different variants at a temperature of 24-26°C.

The experiments were performed according to the Box-3 plan, where the level of factors is presented: concentrations (X1) -10%, 20%, 30%; Egg weight, g, (X2) -1g, 2g, 3g; Exposure, (X3) - 5, 10, 15 minutes. The conditions for planning Box-3 experiments are presented in Table 1.

Table 1. Box-3 Experiment Planning Conditions, 2020

Nr.	Factor levels	Concentrations, % (X1)	The weight of the eggs, g, (X2)	Exposition, min. (X3)
1	-1	10	1	5
2	0	20	2	10
3	+1	30	3	15

Formula for determining the amount of water required to dilute ethyl alcohol to the required strength: $X = P * (N / M - 1)$

X- the amount of water required to dilute the ethyl alcohol to the required strength; P - 5 ml the amount of ethyl alcohol for dilution by variants; N- 96 degrees - the strength of the initial ethyl alcohol; M-10; 20; 30 degrees - the strength of ethyl alcohol required.

Table 2. The conditions for planning and conducting the Box-3, 2020 experiments

Nr.	Factors Level			Concentrations, %, (X1)	The weight of the eggs, g, (X2)	Exposition, min. (X3)
	X1	X2	X3			
1	-1	-1	-1	10	1	5
2	-1	-1	1	10	2	5
3	-1	-1	0	10	3	5
4	-1	1	-1	10	1	10
5	-1	1	1	10	2	10
6	-1	1	0	10	3	10
7	-1	0	-1	10	1	15
8	-1	0	1	10	2	15
9	-1	0	0	10	3	15
10	1	-1	-1	20	1	5
11	1	-1	1	20	2	5
12	1	-1	0	20	3	5
13	1	1	-1	20	1	10
14	1	1	1	20	2	10
15	1	1	0	20	3	10
16	1	0	-1	20	1	15
17	1	0	1	20	2	15
18	1	0	0	20	3	15
19	0	-1	-1	30	1	5
20	0	-1	1	30	2	5
21	0	-1	0	30	3	5
22	0	1	-1	30	1	10
23	0	1	1	30	2	10
24	0	1	0	30	3	10
25	0	0	-1	30	1	15
26	0	0	1	30	2	15
27	0	0	0	30	3	15

RESULTS AND DISCUSSIONS

In order to compensate for the limited ability to fly and to have access to the newly laid host eggs of parasites, several strategies have been developed such as the use of "kairomons". As a source of kairomon (SBA) the extract of alcohol and eggs of cereal moth (*Sitotroga cerealella* Ol.) will be used, where the action of the fractions extracted from scales from moths will be appreciated on the search capacity of *T. evanescens* W., which is an active component to reduce the density of harmful insects.

Table 3 presents the results of *T. evanescens* prolificacy (search capacity) of *T. evanescens*, in different combinations of conditions - Concentration, Exposure, Weight.

For the preparation of biologically active substances with attractive properties: alcohol of different concentrations 10%, 20% and 30% were used, with which 1g, 2g, 3g of host eggs (*Sitotroga cerealella* Ol.) were treated at each concentration and processed (exposure) for 5, 10, 15 minutes each.

The prolificacy of *T. evanescens*, at a concentration of 10%, and different combinations of factors, ranged from 9.2-11.4 eggs / female, the average -10.37 eggs / female for 24 hours.

The prolificacy of *T. evanescens*, 20% concentration, and various combinations of factors ranged from 8.6-11.20 eggs / female, the average - 9.9 eggs / female for 24 hours.

The prolificacy of *T. evanescens*, at a concentration of 30%, and different combinations of factors, ranged from 10.0-13.70 eggs / female, the average -11.77 eggs / female for 24 hours.

In the control, the prolificacy of *T. evanescens* without treatment varied from 6.6-8.20 eggs / female, the average -7.6 eggs / female for 24 hours.

The ability to search for *T. evanescens* was determined as a result of the influence of biologically active substances with attractive properties of *Sitotroga cerealella* Ol. at the egg stage.

In order to conduct research on the interaction of biologically active substances with attractive properties on the behavior of the entomophagous *Trichogramma evanescens* W., the laboratory host of the cereal moth (*Sitotroga cerealella* Ol. glass tubes with a length of 1.5 m, a diameter of 1.5 cm.

The time taken by the females of the distance of 1.5 m at the concentration of 10%, 20%, 30% at different levels of the factors the weight of the eggs (1, 2, 3g) and the exposure (5, 10, 15 min.) varied from 20.42-22.06-23.36 min, the age of the eggs from the cereal moth 24 hours.

The time taken by the females of the distance of 1.5 m in the control varied from 40.72-41.82-42.84 min., the age of the eggs 24 hours. The optimization area was: alcohol with a concentration of 30%, egg weight 1 gr, exposure 5 minutes. In all 27 variants, the time spent (search capacity) by the females was determined. of eggs is shown in Table 4.

To increase the quality (biological indices) of the entomophagous *Trichogramma* spp. Produced on laboratory host eggs cereal moth (*Sitotroga cerealella* Ol.) Is used to treat eggs with the optimal extract with alcohol concentration 30%, egg weight 1 gr, exposure 5 minutes old of 24-26 hours.

As a result, biological indices increase (female prolificacy 1.51-1.67 times, search capacity is 1.83-2.0 times) and saving eggs of cereal moth and entomophagous - *Trichogramma* spp.

The time traveled by females of the distance of 1.5 m in the control (without treatment) is essentially longer than in the treatment version, where the speed traveled by females is higher. The results obtained on the search capacity of *Trichogramma evanescens* with attractive properties of *Sitotroga cerealella*, 24-hour egg age and Bioclass Program, determined the optimization area, which is: host concentration 30%, egg weight 1 gr, exposure 5 minutes, where biological indices are maximum.

As the closest solution was used and demonstrated the advantages of pre-treatment of eggs with cereal moth (*Sitotroga cerealella* Ol.), Where the results are maximum. The significant increase of the biological indices of *Trichogramma* spp. Obtained according to the given procedure, gives the possibility to save *Trichogramma* spp. Of elite by 20-25%.

In Fig.1. the ability to search for *Trichogramma evanescens* with attractive properties of *Sitotroga cerealella* is presented, where the time spent by females in different combinations of factors is observed, which varies differently in the range of combinations of factors, where is presented the search capacity of *Trichogramma evanescens* with attractive properties of *Sitotroga cerealella*, egg age - 24 hours, where they are presented - A-Factors and results of experiments: different concentrations (10, 20, 30%), weight of eggs and larvae treated (1, 2, 3g), duration of treatment (5, 10, 15 min), where the results of the treatment and control variant (without treatment) are represented, which vary with different combinations of factors.

B- The graph of efficacy, which represents the values of time spent by females in the variant with treatments and control - without treatment. In the control, the time spent by the females is essentially longer than in the treatment variant, where the time traveled by the females is essentially shorter.

C- The Graph of Performance of the results, indicates the degree of performance, which varies from 0-0.2, which corresponds to the values - very well and the time of females traveled from 1-10 minutes;

The degree of performance - well, varies from 0.2-0.4, and the time traveled by females from 10-20 minutes; the sufficient degree of performance-0.4-0.6 and the time of females traveled was 20-30 minutes; the degree of poor performance-0.6-0.8, and the time traveled by females was 30-40 minutes;

Degree of performance -0.6-0.8, very bad- 0.6-0.8, and the time traveled by females greater than 40 minutes;

D – Optimization area – all factor combinations were studied, then the optimization area was determined,

which was determined and constitutes the concentration-30%, weight of treated eggs (1g), duration of treatment (5 min), where the time spent by females is minimal .

Table 3. Searching ability, Prolificity of *T. evanescens*, under different conditions

Date	Concentrations, %	Exposition, min.	The weight of the eggs, g	Average Prolificity, eggs/female
03.06.20	10	5	1	9.2±1.0
05.06.20	10	5	2	11.4±1.1
09.06.20	10	5	3	10.37±0.1
Average				10.20
12.06.20	20	10	1	11.1±1.2
15.06.20	20	10	2	8.60±1.3
17.06.20	20	10	3	11.20±1.3
Average				9.90
22.06.20	30	15	1	13.7±1.9
25.06.20	30	15	2	11.1±1.6
27.06.20	30	15	3	10.0±1.7
Average				11.77
DEM	(Td=1.8-2.0>1.96=To,05)			

Table 4. Assessment of the behavior of the entomophagous *Trichogramma evanescens* Westw to the action of biologically active substances with attractive properties of the laboratory host *Sitotroga cerealella* Ol. at the egg' stage (24 hours).

Box-3 Experiment Planning Conditions, 2020 Age of <i>S. cerealella</i> eggs 24 hours								
Nr.	Level of factors			Concentrations %. (X1)	The weight of the eggs, g, (X2)	Exposition, min. (X3)	Time traveled by females, mins	Time traveled by females, min.
	X1	X2	X3	X1	X2	X3	X4	X5
1	-1	-1	-1	10	1	5	26.00±5.6	49.55±12.4
2	-1	0	-1	10	2	5	21.48±1.1	48.55±6.7
3	-1	1	-1	10	3	5	17.58±2.8	40.00±1.8
4	-1	-1	0	10	1	10	23.52±2.1	41.04±0.7
5	-1	0	0	10	2	10	17.96±2.4	40.00±1.8
6	-1	1	0	10	3	10	20.24±0.2	42.00±0.2
7	-1	-1	1	10	1	15	19.76±0.7	42.80±0.9
8	-1	0	1	10	2	15	17.10±3.3	40.00 ±1.82
9	-1	1	1	10	3	15	20.14±0.3	32.44±9.3
Average							20.42	41.82
10	0	-1	-1	20	1	5	25.80 ±2.1	38.36 ±2.3
11	0	0	-1	20	2	5	23.60±3.7	48.55±7.8
12	0	1	-1	20	3	5	17.64±2.1	38.36±2.3
13	0	-1	0	20	1	10	23.72±1.7	42.8±2.1
14	0	0	0	20	2	10	25.96±3.9	41.04±0.3
15	0	1	0	20	3	10	19.96±2.1	38.08±2.6
16	0	-1	1	20	1	15	22.20±0.1	38.08±2.1
17	0	0	1	20	2	15	20.68±2.6	42.84±2.1
18	0	1	1	20	3	15	19.20±2.8	38.44±2.2
Average							22.06	40.72
19	1	-1	-1	30	1	5	17.8±5.2	38.44±4.4
20	1	0	-1	30	2	5	21.26±2.1	40.04±2.8
21	1	1	-1	30	3	5	29.16±5.8	40.44±2.4
22	1	-1	0	30	1	10	18.36±5.0	38.44±4.4
23	1	0	0	30	2	10	22.14±0.8	30.00±12.8
24	1	1	0	30	3	10	24.04±0.7	41.04±1.8
25	1	-1	1	30	1	15	28.50±5.1	42.8±2.0
26	1	0	1	30	2	15	26.40±2.1	30.0±12.8
27	1	1	1	30	3	15	23.32±0.1	42.84±4.62
Average							23.36	38.22
(DEM)				(Td=2,8-3,7>1,96=To,05)				

Representation of results in the Optimclass Program

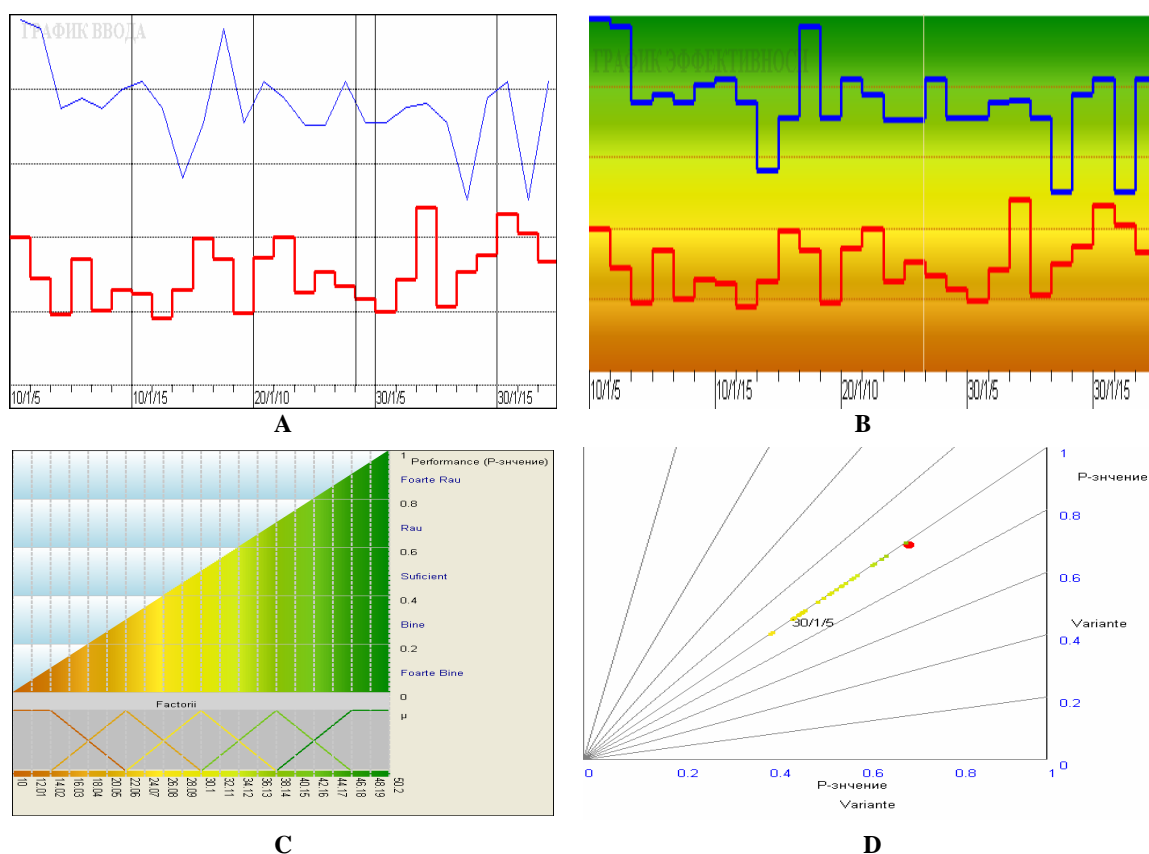


Fig.1 *Trichogramma evanescens* search capability with attractive properties of *Sitotroga cerealella*, egg age - 24 hours. A-Factors and results of experiments; B- Efficiency chart; C- Performance of results; D-Optimization area.

CONCLUSIONS

1. The prolificacy of *T. evanescens*, at different combinations of conditions at concentrations of 10% -30%, varied from 9.2-13.70 eggs / female, average - 11.55 eggs / female for 24 hours. In the control, the prolificacy of *T. evanescens*, without treatment varied from 6.6-8.20 eggs / female, the average -7.6 eggs / female for 24 hours.
2. The time taken by the females of the distance of 1.5m at the concentration of 10%, 20%, 30% at different levels of the factors the weight of the eggs (1, 2, 3g) and the exposure (5, 10, 15 min) varied from 20.42-22.06-23.36 min, the age of the eggs from the cereal moth of 24 hours, at the stage. The time traveled by females of the distance of 1.5m in the control varies from 40.72-41.82-42.84, the age of the eggs 24 hours, to the egg stage.
3. Based on the research results obtained in 27 variants of the *Trichogramma evanescens* Search Capacity with attractive properties of *Sitotroga cerealella*, the age of the eggs of the cereal moth (*S. cerealella* Ol.) Of 24 hours and the Optimclass program, the graphs were constructed.

4. Factors and results of experiments; Efficiency chart; Performance of results; allowed us to determine the area of optimization, at the age of eggs of cereal moth (*S. cerealella* Ol.) of 24 hours, concentration 30%, egg weight 1 gr, exposure 5 minutes.

ABSTRACT

Based on the results of research obtained in 27 variants of the *Trichogramma evanescens*' search capacity, with attractive properties of *Sitotroga cerealella* at the age of cereal moth eggs (*S. cerealella* Ol.) of 24 hours, were built the following graphs in Optimclass: Factors and Results of the experiments; Efficiency chart; Performance of results; The graphs allowed us to determine the area of optimization, at the age of eggs of cereal moth (*S. cerealella* Ol.) of 24 hours, concentration 30%, egg weight 1 gr, exposure 5 minutes. The prolificacy of *T. evanescens*, at different combinations of conditions at concentrations of 10%, 20%, 30%, varied from 9.2-13.70 eggs/female, average -11.55 eggs/female for 24 hours. In the control, the prolificacy of *T. evanescens*, without treatment

varied from 6.6-8.20 eggs/female, the average -7.6 eggs/female for 24 hours.

The time taken by the females to reach the distance of 1.5m at the concentration of 10%, 20%, 30% at different levels of the factors the weight of the eggs (1, 2, 3g) and the exposure (5, 10, 15 min), varied from 20.42- 22.06-23.36 min, the age of the eggs of the cereal moth being 24 hours. The time traveled by females of the distance of 1.5m in the control varies from 40.72-41.82-42.84, the age of the eggs 24 hours, to the egg stage.

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