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APPLICATION OF BIOLOGICALLY ACTIVE SUBSTANCES TO ENHANCE THE VITALITY OF THE ENTOMOPHAGOUS *TRICHOGRAMMA DENDROLIMI* Mats. AND *TRICHOGRAMMA PINTOI* Voeg.

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KEYWORDS

T. dendrolim
T. pintoi
Percentage of parasitism
Sitotroga cerealella Ol.

ABSTRACT

The fecundity of *Trichogramma pintoi* (Voegele,1982) was 24.6 eggs/female, while that of *T. dendrolimi* (Matsumura, 1926) was 23.0 eggs/female. *T. pintoi* proved to be slightly more prolific than *T. dendrolimi*. The hatching rate for *T. pintoi* was 90.3%, and for *T. dendrolimi* 88.5%. Both species showed a high hatching rate; however, *T. pintoi* demonstrated a more favorable performance. The female emergence rate was 53.4% for *T. pintoi* and 52.3% for *T. dendrolimi*, indicating a slight superiority of *T. pintoi*, which is important for biological efficiency. The searching capacity in the mini-olfactometer for *T. pintoi* was 8.7%; *T. pintoi* exhibited better olfactory orientation ability, which is essential for host localization. The searching time in glass tubes (min) was 19.4 min for *T. pintoi* and 20.6 min for *T. dendrolimi*. These values are close; however, the shorter time recorded for *T. pintoi* may indicate a higher speed in host finding. The parasitism rate for *T. pintoi* was 78.7%, and for *T. dendrolimi* 78.2%. *T. pintoi* showed a significantly higher parasitism capacity, representing a key parameter for efficiency in biological control.

INTRODUCTION

The control of insect pests within modern plant protection concepts represents one of the important links in trophic chains and trophic networks, ensuring improved environmental quality and enabling the production of ecologically safe agri-food products. A wide range of studies has been conducted using chemical mediators (especially pheromones, kairomones, synomones, and allomones) as tools for monitoring, management, or control of numerous pest insect species, alongside the reduction and even elimination of pesticide treatments and their replacement with biopesticides (Bakthavatsalam et al., 2013; Paul et al., 2008). The kairomonal effect of certain saturated hydrocarbons on egg parasitoids *Trichogramma* brasiliensis (Ashmead, 1904), *Trichogramma exiguum* (Pinto & Platner, 1978), and *Trichogramma chilonis* (Ishii, 1941) (Hym., *Trichogramma*) has been described by several authors (Rani et al., 2017). A kairomone enhances the fitness of the receiver and, in this respect, differs from an allomone, which has the opposite effect, being beneficial to the producer and detrimental to the receiver. Initially, research focused mainly on the study of sex pheromones from a fundamental perspective, due to their role in shaping reproductive behavior; however, subsequent studies revealed other particularly interesting aspects. These substances, released by one sex (most often the female) in extremely small quantities, transmit highly important informational messages for survival and species perpetuation and can also be exploited for species control. The role of kairomones in the biological control of agricultural pests has been described by several authors (Murali-Baskaran et al., 2018). To compensate for the limited flight capacity of egg parasitoids and to enable access to freshly laid host eggs, multiple strategies have been developed, including the use of kairomones. The kairomonal effect of certain saturated hydrocarbons on egg parasitoids *Trichogramma brasiliensis*, *Trichogramma exiguum*, and *Trichogramma pintoi* has also been reported (Paul et al., 2002). The author's experiments (Vasileva, 2010) demonstrated that extracts obtained from the mandibular glands of certain Lepidoptera insects possess attractive properties influencing the population density of Lepidoptera pests in stored cereal products. These

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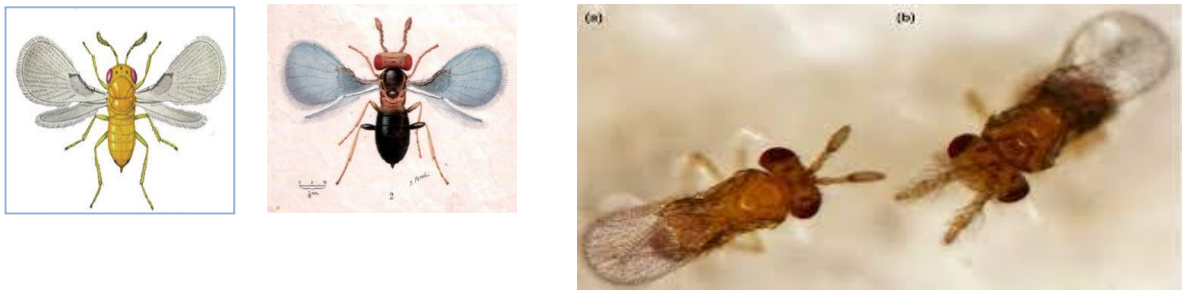
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insects negatively affect both larval development and the egg-laying process of adults, while simultaneously attracting parasitoids and predators due to their kairomonal activity. Annually, crop yield losses in agricultural production range between 15% and 80%. The widespread use of the entomophagous *Trichogramma* spp. in plant protection is closely related to its quality in mass rearing and field application. The objective of the research conducted in 2025 was to assess the efficiency of biologically active substances with kairomonal properties on the behavior of the entomophagous *Trichogramma dendrolimi* Mats. and *Trichogramma pintoi* V., with the aim of increasing their parasitism potential on pest eggs.

MATERIALS AND METHODS

Objects of the study

The objects of the research were *Trichogramma* species, with the laboratory host *Sitotroga cerealella* (Olivier, 1789). Fundamental research was conducted under laboratory and field conditions at the Institute of Genetics, Physiology, and Plant Protection, Chișinău. The collection of entomophagous insects was carried out in various annual crops—soybean, maize, tomato, sunflower—as well as in perennial crops—plum, apple, and pear.



a. T. *dendrolimi* Mats. b. T. *pintoi* Voeg.

Figure 1. Female *Trichogramma* (a), Male – *Trichogramma* (b)

Tabel 1. Experimental plan, 2025

Species	*T. pintoi*		*T. dendrolimi*	
Variants	I with SBA	II without SBA	I with SBA	II without SBA
Fecundity, eggs/female	X	X	X	X
Searching capacity, %	X	X	X	X
Percentage of parasitized eggs	X	X	X	X
Distance traveled by females, cm	X	X	X	X
Static quality criterion	X	X	X	X

To assess the behavior of the entomophagous *Trichogramma dendrolimi* and *Trichogramma pintoi* under the influence of biologically active substances with kairomonal properties, and to compensate for the limited flight capacity of *Trichogramma*, several quality-enhancing strategies were developed, including the use of kairomones. As a kairomone source (biologically active substance, SBA), alcoholic extracts and eggs of the grain moth *Sitotroga cerealella* were used. The activity of fractions extracted from moth scales on the searching capacity of *Trichogramma dendrolimi* and *Trichogramma pintoi* was evaluated. These fractions represent an active component for improving entomophagous quality and reducing pest insect density. In the experiments conducted in 2025, a kairomone with optimal properties was used, obtained in 2020 using the "Optimclas" software and according to the Box 3 experimental design. For the preparation of biologically active substances with attractive properties, a 30% alcohol solution was used, into which host eggs were introduced and left for an exposure period of 5 minutes [1]. The studies carried out in 2025 followed the experimental design presented in Table 1. Determination of the searching capacity of *Trichogramma dendrolimi* and *Trichogramma pintoi* under the influence of the kairomone from *Sitotroga cerealella* at the egg stage (mini-olfactometers). The mini-olfactometer is 1.5 m long; therefore, the experiments were conducted under laboratory conditions, with daily recording of temperature and comparison against a control.

Variant 1 (treatment)

The searching capacity of *Trichogramma dendrolimi* and *Trichogramma pintoi* as determined separately using a mini-olfactometer with a length of 1.5 m. To evaluate the interaction between biologically active substances with kairomonal properties from *Sitotroga cerealella* and the behavior of entomophagous species, bean plants grown under laboratory conditions were treated with the SBA extract (optimal variant) at a concentration of 30% and an

exposure time of 5 minutes. Egg cards containing eggs of the grain moth were fixed onto the treated plants. After the kairomone applied to the plants had dried, the entomophagous insects were released.

Variant 2 (control)

The plants were not treated with SBA, and *Trichogramma dendrolimi* and *Trichogramma pintoi* V. were released separately. In each variant, the entomophagous insects were introduced at the optimal parasitoid–host ratio of 1:30, meaning that one female corresponded to 30 grain moth eggs. After 4–5 days, the number of parasitized eggs was recorded, and the percentage of parasitism was determined. The experiments were conducted in three replicates. Determination of the searching capacity (time required) of *Trichogramma dendrolimi* and *Trichogramma pintoi* under the influence of BAS with kairomonal properties from *Sitotroga cerealella* (glass tubes, is 1.5 m long).

Variant 1 (kairomone)

The searching capacity of *Trichogramma dendrolimi* and *Trichogramma pintoi* was determined separately in glass tubes with a length of 1.5 m. The experiments were conducted under laboratory conditions. A cotton plug impregnated with kairomone was placed at one end of the tube, while *Trichogramma dendrolimi* and *Trichogramma pintoi* were released separately at the opposite end. The time required for females to cover the distance of 1.5 m was recorded.

Variant 2 (control)

The searching capacity was determined in glass tubes with a length of 1.5 m under laboratory conditions. A cotton plug soaked with distilled water was placed at one end of the tube, while *Trichogramma dendrolimi* and *Trichogramma pintoi* V. were released separately at the opposite end. The time required for females to cover the distance of 1.5 m was recorded.
All experiments were conducted in three replicates, and significant differences between variants were determined.

RESULTS AND DISCUSSION

The experiments were conducted at a temperature of 23–25°C, and humidity 70-75%

1. Determination of the searching capacity of *Trichogramma dendrolimi* and *Trichogramma pintoi* as a result of the influence of the kairomone of *Sitotroga cerealella* at the egg stage (mini-olfactometer).
2. Searching capacity of *T. dendrolimi* and *T. pintoi*, second generation after diapause, in the mini-olfactometer, 2025.

Table 2. Searching capacity of *T. dendrolimi*, second generation after diapause (mini-olfactometer)

***Trichogramma* species**	**Parasitized eggs in treatments, %**		**Parasitized eggs in treatments, %**	
	With SBA	**Control (without SBA)**	**With SBA**	**Control (without SBA)**
T. pintoi	2614	1511	87.13±2.8	50.3±2.8
T. dendrolimi	2489	1336	83.0±3.7	44.5±2.5
DEM (Td=2.0-3.0>1.96=To.o5)				

The searching capacity of *T. pintoi* in the kairomone treatment in a mini-olfactometer with obstacles: out of 3,000 cereal moth eggs offered for parasitism, 87.13% were parasitized. In the control (without kairomone) in a mini-olfactometer with obstacles, the parasitism rate from 3.000 eggs was 50.3%. The searching capacity of *T. dendrolimi*: 83.0% of the 3,000 moth eggs offered for parasitism were parasitized. In the control (without kairomone) in a mini-olfactometer with obstacles, the parasitism rate was 44.5% out of the 3,000 eggs offered. In the kairomone treatments, the parasitism rate was significantly higher compared to the control.
For *T. pintoi*: Time difference: 50.1 − 17.3 = 32.8 min. For *T. dendrolimi*: Time difference: 42.5 − 19.8 = 22.7 min.
2. Searching capacity of *T. pintoi* and *T. dendrolimi*, second generation after diapause (in glass tubes), average temperature −23 to −25 °C.
The analysis of the searching capacity of *Trichogramma* females (*T. pintoi and T. dendrolimi*) in glass tubes was conducted. The orientation and searching ability of females of the two *Trichogramma* species (*T. pintoi and T. dendrolimi*) were evaluated in the presence and absence of a bio-attractive substance (SBA).

Table 3. Searching capacity, second generation after diapause, in glass tubes No. 2, average temperature

Trichogramma species	Number of females in treatments		Time taken by females in treatments, minutes	
	With SBA	Control (without SBA)	With SBA	Control (without SBA)
T. pintoi	80	40	17.3±1.4	50.1±2.8
T. dendrolimi	80	40	19.8±1.7	42.5±2.5
DEM	(Td=1.8-2.9>1.96=To.o5)			

The number of females oriented toward the SBA source was 80, while in the control it was 40 for both species. The time required for females to reach the source was significantly shorter in the SBA treatment: for *T. pintoi*, 17.3 min with SBA compared to 50.1 min in the control without SBA; for *T. dendrolimi*, 19.8 min with SBA compared to 42.5 min in the control without SBA.

For *T. pintoi*: time difference: 50.1 − 17.3 = 32.8 minutes.

For *T. dendrolimi*: time difference: 42.5 − 19.8 = 22.7 minutes.

The presence of the biologically active substance (SBA) with kairomonal properties proved to be attractive, leading to a marked increase in female attack activity and a significant reduction in orientation time. *T. pintoi* showed better orientation efficiency compared to *T. dendrolimi* in both experimental treatments. The results highlight the effectiveness of the bio-attractive substance in stimulating the searching behavior of *Trichogramma pintoi* females, which demonstrated faster orientation than *T. dendrolimi*, making it a promising species for use in attractant-assisted biological control programs. *Trichogramma pintoi* is a species that, due to its biological characteristics, preferentially parasitizes eggs of moth complexes associated with annual crops, whereas *T. dendrolimi* preferentially parasitizes eggs of moth complexes found in perennial crops.

The experiments were conducted at a temperature of 26–30 °C

The following biological indices were determined: fecundity, percentage of adult emergence (%), percentage of female emergence (%), searching capacity of females in the mini-olfactometer (%), searching capacity of females in glass tubes (minutes), and percentage of moth egg parasitism (%). The results are presented in Table 4.,5.

Table 4. Biological indices of *T. pintoi*

Biological indices	Treatment (with SBA)	Control (C)
Fecundity, parasitized eggs	24.6±1.2	21.8±2.4
Adult emergence rate (%)	90.3±3.4	86.0±3.7
Female emergence rate (%)	53.4±3.1	50.8±2.8
Searching in mini-olfactometer (%)	88.7±3.8	73.0±2.9
Searching in glass tubes (min)	19.4±1.4	39.1±2.4
Parasitism rate (%)	88.7±3.6	73.0±3.4
DEM	(Td=1,94-4,9>1,96=To,o5)	

Table 5. Biological indices of *T. dendrolimi*

Biological indices	I.V. (with SBA)	Control (C)
Fecundity, parasitized eggs	23.0±1.4	21.4±2.1
Adult emergence rate (%)	88.5±3.8	80,0±3.9
Female emergence rate (%)	52.3±2.4	50,0±3.3
Searching in mini-olfactometer (%)	83.4±3.6	69.0±3.6
Searching in glass tubes (min)	20.6±1.6	41.8±2.5
Parasitism rate (%)	78.2±3.2	43.5±2.6
DEM	(Td=1.98-3,9>1,96=To,o5)	

Table 6. Comparison of biological indices between *T. pintoi* and *T. dendrolimi* in treatments with SBA

Biological indices	*T. pintoi*	*T. dendrolimi*
Fecundity, parasitized eggs	24.6±2.8	23.0±1.6
Adult emergence rate (%)	90.3±4.6	88.5±4.2
Female emergence rate (%)	53.4±2.6	52.3±2.4
Searching in mini-olfactometer (%)	88.7±4.1	83.4±4.6
Searching in glass tubes (min)	19.4±1.6	20.6±1.6
Parasitism rate (%)	88.7±3.8	78.2±3.4
DEM	(Td=1.8-8.9>1.96=To.o5)	

Comparative biological analysis of the results, Table 6

1. **Adult emergence rate:** *T. pintoi*: 90.3%; *T. dendrolimi*: 88.5%. Both have a high level, but *T. pintoi* performs slightly better.
2. **Female emergence rate:** *T. pintoi*: 53.4%; *T. dendrolimi*: 52.3%. Slight superiority of *T. pintoi*, important for biological efficiency.
3. **Searching capacity in mini-olfactometer:** *T. pintoi*: 88.7%. *T. pintoi* shows better olfactory orientation capacity, essential for host location.
4. **Searching capacity in glass tubes (min):** *T. pintoi*: 19.4 min; *T. dendrolimi*: 20.6 min. Values are close; the shorter time for *T. pintoi* may indicate faster host finding.
5. **Parasitism rate:** *T. pintoi*: 88.7%; *T. dendrolimi*: 78.2%. *T. pintoi* has significantly higher parasitism capacity, a key parameter for biological control efficiency.

CONCLUSIONS

Comparing the two datasets — for *T. pintoi* and *T. dendrolimi*, each with six biological indices: 1. Fecundity (parasitized eggs); 2. Adult emergence rate (%); 3. Female emergence rate (%); 4. Searching capacity (%) in mini-olfactometer; 5. Searching capacity (min) in glass tubes; 6. Parasitism rate (%) — in the treatments with SBA, it is observed that *T. pintoi* exhibits superior biological indices compared to *T. dendrolimi*.

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