

## EVALUATING THE EFFICIENCY OF BIOLOGICALLY ACTIVE SUBSTANCES WITH KAIROMONAL PROPERTIES ON THE BEHAVIOR OF THE ENTOMOPHAGOUS *TRICHOGRAMMA EVANESCENS* W.

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### KEYWORDS

Cereal moth  
Percentage of parasitism  
*Sitotroga cerealella* Ol.  
*T. evanescens* Kairomone

### ABSTRACT

The research conducted during the years 2020-2023 has demonstrated that in the experimental variant (in the presence of biologically active substances), the prolificity of *Trichogramma evanescens* females increased by approximately 1.4-1.5 times compared to the prolificity of females in the control variant. As a result, there is a quantitative saving of *Sitotroga cerealella* eggs in the reproduction process by 20-25%, and of the elite entomophage *Trichogramma* spp. by about 15-20%. The searching capacity of the entomophage *Trichogramma evanescens*, as a result of the influence of biologically active substances with kairomonal properties, showed results approximately 1.6 times higher in the control, indicating that females in the control move more slowly than in the experimental variant. The obtained results demonstrate a significant activation of the entomophage females in the presence of biologically active substances with kairomonal properties. Due to the influence of kairomonal substances, the females of the entomophage *Trichogramma evanescens* are more active in searching for eggs for parasitization, leading to approximately a 10% increase in the rate of parasitized eggs compared to the control variant. In the case of experiments conducted under field conditions, the action of kairomonal substances was confirmed to significantly increase the rate of parasitized eggs compared to the control variant ( $T_d = 2.5-4.8 > 1.96 = T_{0.05}$ ). Biologically active substances play a crucial role in the biological protection of plants.

### INTRODUCTION

Kairomones play a crucial role in enhancing reproductive behavior, but recent studies have revealed additional intriguing aspects. These substances, typically emitted in minimal quantities by one sex, often the female, carry vital informational messages essential for the survival and perpetuation of the species. Furthermore, they can be employed for the control of other species. Kairomones function as messenger substances, facilitating information transfer between different species to benefit the receiving organism. Unlike alomones, which benefit the producer and harm the recipient, kairomones enhance the recipient's capabilities.

Initially, research primarily focused on the fundamental role of sexual pheromones, especially in shaping reproductive behavior. However, subsequent studies have unveiled diverse and fascinating aspects of these substances. Notably, their role extends beyond reproductive behavior to include crucial messages for survival and potential applications in the destruction of other species.

The involvement of kairomones in the biological control of agricultural pests has been documented by various authors such as Fatouros et al. (2008); Murali-Baskaran et al. (2017); Rani et al. (2017). Impact of Plant Phenolics as Semiochemicals on the Performance of *Trichogramma chiloni* have conducted studies utilizing kairomones. To overcome the limited flight capacity and gain access to freshly deposited host eggs of egg parasitoids, various strategies, including the use of kairomones, have been developed. Our research results significantly contribute to

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solving challenges in the application of the entomophagous *Trichogramma* spp. in combating economically important pest species in the agriculture of the Republic of Moldova.

The natural population of the entomophagous *Trichogramma* spp. plays a crucial role in reducing pest population densities. However, the population density of the entomophagous organism alone is insufficient for the protection of agricultural crops from pests. Therefore, supplementary releases are carried out in the agroecosystems of various crops. The biological agent *Trichogramma* spp. is applied at the egg stage of pests in orchards, vineyards, forests, technical crops, grasses, vegetables, and leguminous crops.

The scientifically well-founded methodological application has led to a reduction of up to 70-80% in pest egg populations and a significant increase in agricultural crop yields. The procedures and methods for the production and application of *Trichogramma* with enhanced qualities have been developed and implemented in the field, covering an area of approximately 3000 hectares. These procedures have been tested in various agricultural zones across the Republic of Moldova.

## MATERIALS AND METHODS

Field research was conducted on soybean crops at the Institute of Genetics, Physiology, and Plant Protection in Chisinau, Moldova, to assess the efficacy of *Trichogramma evanescens* Westw. in the presence of kairomones.

Alcohol extract and eggs of the Angoumois grain moth (*Sitotroga cerealella* Ol) were utilized as the source of kairomones (Biologically Active Substance). The impact of the fractions extracted from moth scales on the searching capacity of *T. evanescens* W, an active component enhancing the quality of the entomophage and reducing the density of harmful insects, was evaluated. In the 2020-2023 experiments, the kairomone with optimal properties obtained in 2020 using the "Optimclas" program and following Box 3 Plan was employed.

The formula for determining the amount of water required to dilute the alcohol to the necessary strength is as follows:  $X = P * (N/M - 1)$ . Here, X represents the amount of water needed to dilute ethyl alcohol to the required strength, P is the amount of ethyl alcohol for dilution in each variant, N is the initial strength of ethyl alcohol (96%), and M is the required strength of ethyl alcohol (30%).

The research was conducted in laboratory conditions (Phytopharmacy and Ecotoxicology) at the Institute of Genetics, Physiology, and Plant Protection during the years 2020-2023. The subject of the study was the entomophagous *Trichogramma evanescens* W. and the laboratory host *Sitotroga cerealella* Ol. The research plan for the years 2020-2023 is presented in Table 1.

**Table 1. The research plan for the period 2020-2023**

| Years | Prolificity,<br>eggs/female | Parasitized egg quantity, % |    |    |   |   |    | Searching capacity (mini olfactometer) |    |   |    |                                          |    |   |  |
|-------|-----------------------------|-----------------------------|----|----|---|---|----|----------------------------------------|----|---|----|------------------------------------------|----|---|--|
|       |                             |                             |    |    |   |   |    | Rectangular type<br>(parasitized eggs) |    |   |    | Tubular type<br>(distance traveled, min) |    |   |  |
|       | 24                          | 24                          | 48 | 72 | L | A | 24 | 48                                     | 72 | L | 24 | 48                                       | 72 | L |  |
| 2020  | x                           | x                           | x  | x  | x | x | x  | x                                      | x  | x | x  | x                                        | x  | x |  |
| 2021  | x                           | x                           | x  | x  | x | x | x  | x                                      | x  | x | x  | x                                        | x  | x |  |
| 2022  | x                           | x                           | x  | x  | x | x | x  | x                                      | x  | x | x  | x                                        | x  | x |  |
| 2023  | x                           | x                           | x  | x  | x | x | x  | x                                      | x  | x | x  | x                                        | x  | x |  |

24, 48, 72 hours – Age of eggs from deposition; L – Larvae; A – Adult

## RESULTS AND DISCUSSION

Variant I: Four plants in each corner of the plots were treated with a 30% concentration of *Sitotroga cerealella* Ol. kairomone extract for 5 min. The search capacity of *Trichogramma evanescens* was determined as a result of the influence of (Biologically Active Substances) with kairomonal properties of *Sitotroga cerealella* Ol. at the egg stage. Variant II: (Control) - the plants in the plots were not treated with kairomone, but *Trichogramma evanescens*.

### Determining the prolificity of entomophagous females of *Trichogramma evanescens* W. in the presence of biologically active substances with kairomonal properties

It was observed that in the presence of substances with kairomonal properties, prolificity varied from 28.5 to 32.6 eggs/female. In the Control variant, the prolificity of females ranged from 19.1 to 22.8 eggs/female. Thus, in the experimental variant, the prolificity of females increased by approximately 1.4-1.5 times compared to the prolificity of females in the Control variant. As a result, there is a quantitative saving of *Sitotroga cerealella* eggs in the reproduction process by 20-25% and of the elite entomophage *Trichogramma* spp. by about 15-20% (Table 2).

**Table 2. Prolificity of *Trichogramma evanescens* females determined in the presence of biologically active substances with kairomonal properties**

| Years               | Prolificity, parasitized eggs         |                            |
|---------------------|---------------------------------------|----------------------------|
|                     | The variant with kairomone (24 hours) | Control, without kairomone |
| 2020                | 31.8±1.0                              | 22.8±0.3                   |
| 2021                | 30.5±0.8                              | 20.4±0.4                   |
| 2022                | 32.6 ±2.3                             | 19.1±1.4                   |
| 2023                | 28.5±2.2                              | 22.8±2.1                   |
| media               | 30.8±0.9                              | 20.9±1.1                   |
| DEM <sub>0,05</sub> | Td=1.98-3.7>1.96=T <sub>0,05</sub>    |                            |

**Behavior of the entomophage *Trichogramma evanescens* W. in the presence of biologically active substances with kairomonal properties in a tubular-shaped mini-olfactometer**

During the years 2020-2023, the searching capacity of the entomophage *Trichogramma evanescens* was determined as a result of the influence of the biologically active substance with kairomonal properties in two types of olfactometers with a length of 1.5 m. At one end of the tubular-shaped mini-olfactometer, a pad soaked with biologically active substances with kairomonal properties obtained from eggs of the grain moth at the age of 24, 48, 72 h, and larvae was attached. At the other end of the mini-olfactometer, 160 females of *Trichogramma evanescens* W. were released. The time it took for the females to cover the distance of 1.5 m ranged from 29.8 to 36.2 min. Concurrently, in the control variant, this index ranged from 46.7 to 54.9 min, which is approximately 1.6 times higher than in the experimental variant. The obtained results demonstrate a significant activation of the entomophage females in the presence of biologically active substances with kairomonal properties.

**Table 3. The effect of modifying the behavior of the entomophage *Trichogramma evanescens* in the presence of biologically active substances in a tubular mini-olfactometer**

| Years               | Experiment (different ages of moth eggs) |      |      |        | Control (different ages of moth eggs) |      |       |        |
|---------------------|------------------------------------------|------|------|--------|---------------------------------------|------|-------|--------|
|                     | 24                                       | 48   | 72   | larvae | 24                                    | 48   | 72    | larvae |
| 2020                | 21.9                                     | 25.0 | 27.7 | 18.1   | 41.8                                  | 42.0 | 43.4  | 38.2   |
| 2021                | 40.5                                     | 45.5 | -    | -      | 50.5                                  | 52.0 | -     | -      |
| 2022                | 24.4                                     | 30.7 | -    | -      | 38.4                                  | 44.5 | -     | -      |
| 2023                | 32.5                                     | 33.0 | 44.7 | 41.0   | 56.2                                  | 45.4 | 66.3  | 55.1   |
| media               | 29.8                                     | 33.5 | 36.2 | 29.5   | 46.7                                  | 45.9 | 54.89 | 46.7   |
| DEM <sub>0,05</sub> | Td=1.8-2.7>1.9=T <sub>0,05</sub>         |      |      |        |                                       |      |       |        |

**Behavior of the entomophage *Trichogramma evanescens* W. in the presence of biologically active substances with kairomonal properties in a rectangular-shaped mini-olfactometer**

Results obtained in the corresponding experiment also demonstrated a significant increase in the rate of parasitized eggs by the females of the entomophage *Trichogramma evanescens* under the influence of substances with kairomonal properties. Thus, the rate of parasitized eggs in the rectangular-shaped mini-olfactometer with obstacles varied from 69.6% to 89.9%. In the tested variant in the rectangular mini-olfactometer without obstacles, the rate of parasitized eggs ranged from 63.1% to 91.2%. Simultaneously, it was observed that in the control variant, the rate of parasitized eggs ranged from 64.1% to 74.3%, and correspondingly from 58.4% to 79.2%. Thus, in this case as well, it was demonstrated that due to the influence of kairomonal substances, the females of the entomophage *Trichogramma evanescens* are more active in searching for eggs for parasitization, leading to an increase of about 10% in the rate of parasitized eggs compared to the control variant (Table 4).

**Table 4. The effect of modifying the behavior of the entomophage *Trichogramma evanescens* in the presence of kairomonal substances in a rectangular-shaped mini-olfactometer**

| Years               | Experiment (different ages of moth eggs) |       |      |        | Control (different ages of moth eggs) |      |      |        |
|---------------------|------------------------------------------|-------|------|--------|---------------------------------------|------|------|--------|
|                     | 24                                       | 48    | 72   | larvae | 24                                    | 48   | 72   | larvae |
| 2020                | 88.5                                     | 81.5  | -    | -      | 71.4                                  | 69.9 | -    | -      |
| 2021                | 86.8                                     | 78.54 | -    | -      | 70.4                                  | 66.4 | -    | -      |
| 2022                | 89.7                                     | 88.4  | 80.0 | 70.2   | 78.0                                  | 70.0 | 70.0 | 67.9   |
| 2023                | 94.4                                     | 88.0  | 75.2 | 69.0   | 77.3                                  | 71.9 | 68.2 | 62.0   |
| media               | 89.9                                     | 84.1  | 77.6 | 69.6   | 74.3                                  | 69.5 | 69.1 | 64.9   |
| DEM <sub>0,05</sub> | Tf = 2.4-3.3 > T <sub>0,05</sub> = 1.96  |       |      |        |                                       |      |      |        |

It has been demonstrated that the searching capacity of eggs for parasitization by the females of the entomophage *Trichogramma evanescens* depends to a large extent on abiotic factors: temperature and relative humidity of the air, where the parasitization percentage is higher when the temperature is within the optimal values for the development of the parasitoid.

**The rate of parasitized eggs by females of *Trichogramma evanescens* W. in the presence of biologically active substances with kairomonal properties under controlled laboratory conditions.**

Evaluation of the influence of kairomonal substances on the degree of parasitization of eggs by females of *Trichogramma evanescens* was analyzed under controlled laboratory conditions. Two variants were set up - experimental and control.

**Experimental variant:** On the inner surface of glass vessels (volume 2 liters), kairomonal substance (obtained similarly to previous experiments) was applied, and eggs of the grain moth were then attached. Females of the entomophage *Trichogramma evanescens* were released into the vessels with the optimal parasitoid: host ratio of 1:30, meaning 1 female per 30 eggs. The analysis performed after 5 days revealed that the rate of parasitized eggs ranged from 74.6% to 87.6% on average.

**Control variant:** On the inner surface of glass vessels (volume 2 liters), distilled water was applied, and eggs of *Sitotroga cerealella* (age 24 hours) were then attached. Females of the entomophage *Trichogramma evanescens* were released into the vessels with the optimal parasitoid: host ratio of 1:30, meaning 1 female per 30 eggs. The analysis performed after 5 days revealed that the rate of parasitized eggs ranged from 67.3% to 76.8% on average. Thus, in the case of the corresponding experiments, the action of kairomonal substances on increasing the rate of parasitized eggs compared to females in the control variant was confirmed by approximately 10%.

**Table 5. Evaluation of the influence of kairomonal substances on the degree of parasitization of eggs by females of *Trichogramma evanescens***

| Years               | Experiment (different ages of moth eggs) |      |      |        |       | Control (different ages of moth eggs) |      |      |        |       |
|---------------------|------------------------------------------|------|------|--------|-------|---------------------------------------|------|------|--------|-------|
|                     | 24                                       | 48   | 72   | larvae | imago | 24                                    | 48   | 72   | larvae | imago |
| 2020                | 84.8                                     | 80.0 | 77.2 | 70.0   |       | 76.4                                  | 70.0 | 65.3 | -      | -     |
| 2021                | 86.8                                     | 74.1 | -    | -      | 85.7  | 73.7                                  | 69.2 | -    | -      | 72.9  |
| 2022                | 89.1                                     | 85.6 | -    | -      | -     | 78.6                                  | 74.1 | -    | 70.9   | -     |
| 2023                | 89.7                                     | 78.0 | 74.9 | 73.5   | 79.2  | 78.5                                  | 71.8 | 69.3 | 68.2   | 70.4  |
| media               | 87.6                                     | 79.4 | 76.0 | 74.6   | 82.5  | 76.8                                  | 71.3 | 67.3 | 69.5   | 71.6  |
| DEM <sub>0,05</sub> | Tf = 2.4-3.3 > T <sub>0,05</sub> = 1.96  |      |      |        |       |                                       |      |      |        |       |

Thus, in the case of experiments conducted under field conditions, the action of kairomonal substances on a considerable increase in the rate of parasitized eggs compared to the control variant was confirmed ( $T_d = 2.5 - 4.8 > 1.96 = T_{0.05}$ ). Biologically active substances play a crucial role in the biological protection of plants.

## CONCLUSION

During the years 2020-2023, it was demonstrated that in the experimental variant (in the presence of biologically active substances), the prolificity of *Trichogramma evanescens* females increased by approximately 1.4-1.5 times compared to the prolificity of females in the control variant. As a result, there is a quantitative saving of *Sitotroga cerealella* eggs in the reproduction process by 20-25%, and of the elite entomophage *Trichogramma* spp. by about 15-20%. It was demonstrated that the searching capacity of the entomophage *Trichogramma evanescens*, as a result of the influence of biologically active substances with kairomonal properties during the years 2020-2023, the results obtained in the control are approximately 1.6 times higher, meaning that in the control, females move more slowly than in the experimental variant. The obtained results demonstrate a significant activation of the entomophage females in the presence of biologically active substances with kairomonal properties.

It was demonstrated that due to the influence of kairomonal substances, the females of the entomophage *Trichogramma evanescens* are more active in searching for eggs for parasitization, with an approximately 10% increase in the rate of parasitized eggs compared to the control variant.

In the case of experiments conducted under field conditions, the action of kairomonal substances was confirmed to significantly increase the rate of parasitized eggs compared to the control variant ( $T_d = 2.5 - 4.8 > 1.96 = T_{0.05}$ ). Biologically active substances play a crucial role in the biological protection of plants.

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