

Research Article

Enhancing the Effectiveness of *Trichogramma* Wasps through Innovative Formulations

Seyed Reza Fani^{1a*}, Hanif Amrulloh^{2b}, Golbanoo Azizpoor^{3c}, Hamed Kioumars^{3d}

¹ Plant Protection Department, Gilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran.

² Department of Islamic Primary Teacher Education, Universitas Ma'arif Lampung, Metro, Indonesia

³ Plant Protection Management, Agricultural Jihad Organization of Gilan Province, Rasht, Iran

⁴ Department of Animal Science Research, Gilan Agricultural and Natural Resources Research and Education Center, AREEO, Rasht, Iran

Email: ^{a)*}rezafani52@gmail.com; ^{b)}amrulloh.h@umala.ac.id; ^{c)}rezafani52@gmail.com;
^{d)}h_kioumars@yahoo.com

Submitted: 2025-03-05

Revised: 2025-03-20

Accepted: 2025-03-21

Abstract

The mass of Trichogramma wasp larvae, recognized as a vital agent for pest biocontrol in Iran, is produced primarily in the form of Trichocards. These bacteria are utilized for the biocontrol of key and significant pests, such as rice stem borers, corn stem borers, apple worms, and pomegranate fruit worms. However, the application of this formulation presents several drawbacks, including the difficulty of installing cards across fields and orchards, the destruction of larvae by predatory insects, and the sensitivity of larvae to dryness and high temperatures, which can impair the effectiveness of the method. Alternative formulations such as capsules, balls, and liquids have been introduced in various countries to address these application issues. These alternatives offer advantages such as increased durability of larvae under adverse conditions, increased lifespan and efficiency of wasps, reduced production costs, faster operational speed, and greater willingness among farmers and orchardists to adopt biological methods. Given that the method of supplying Trichogramma larvae has remained unchanged in its production stages over the past 30 years in Iran, a continuous dynamic growth trend in the application of Trichogramma wasps will undoubtedly require a reassessment of various supply stages and an improvement in the production process.

Keywords: Biological Control; Efficiency; Formulation; Trichogramma.

Copyright © 2025. The authors (CC BY-SA 4.0)

Introduction

Trichogramma species are considered among the most important natural enemies of pests and have the potential to reduce pest populations. They exhibit a wide variety of searching behaviors, host preferences, and responses to environmental conditions; therefore, their stability and durability for use in biological control programs differ. Failure to control agricultural pests through the parasitism of pest eggs by wasps may be due to the use of inappropriate *Trichogramma* strains [1]. It is essential to adopt a more comprehensive perspective on agricultural science across its various disciplines. Furthermore, there is a need to implement innovative methods and procedures, moving away from traditional approaches that have been employed for many years, to transform the current situation and achieve improved and more sustainable outcomes [2–5].

Numerous efforts have been made over the past decade to improve the efficacy of *Trichogramma* wasps; however, the outcomes appear to be insufficient at this time [6]. Various researchers have reported differences between strains of a species and emphasized the importance of selecting a specific strain. Other limitations include the need for vegetation cover and humidity in the orchard environment, difficulties in release operations, adherence to transport and release standards, and damage from predators such as ants and beetles from the *Coccinellidae* family [7]. There are serious problems and needs for the practical use of these egg parasitoids in different countries, which are very common and include low quality and high production costs, short shelf life, mixing of species or unwanted strains with the main species, the need for appropriate training and promotion, standardization of quality control methods for producing *Trichogramma*, insufficient studies evaluating the impact of using *Trichogramma*, distinguishing the role of these factors from other factors that reduce pest damage, a lack of suitable release equipment, and losses caused by predators [8], [9].

Materials and Methods

The *Trichogramma* wasp is released in the form of pupae, which are transferred and released inside the host egg. The parasitized pupae are distributed just before the emergence of adult wasps in the field. In some Latin American countries, wasps are also released after their emergence [10]. The parasitized host eggs can be mixed with a carrier material for distribution or glued onto cards or inside paper capsules that are later placed on or attached to the crop, or they can be placed inside small balls and scattered over the field or orchard [1].

Results and Discussion

1. *Trichocard*

Pupae of different ages are glued onto cardboard, known as *Trichocard*, which can be installed on fruit tree branches or agricultural plants. The parasitoids gradually emerge over a period of 14 days and begin searching for eggs by walking or flying. The cards should be kept in a warm, humid place away from direct sunlight to complete the insect growth cycle (Figure 1 and figure 2). Depending on the ambient temperature, wasps appear within 2–5 days, with the optimal temperature being between 26–32 degrees Celsius; lower temperatures (up to 4.5 degrees Celsius) delay emergence. Wasps typically emerge in the morning. To increase efficiency before release, parasitized grain moth eggs are incubated in glass containers, paper bags, or other sealed containers. This can be accomplished by cutting the cards into squares and placing them inside a slit of a paper cup. The cup's opening is folded twice on both sides and sealed, when the adult *Trichogramma* begins to emerge and crowd, the cups can be used. They can be opened and placed on plants or have small holes made on each side to protect unhatched eggs from predators and moisture. A source of honey that is dry enough that wasps do not stick to it helps improve honey efficiency [11]. The simplest method is to use a sharp tool such as a scalpel to create a very thin layer of honey inside containers with wasps. Excessive amounts of this substance cause adult insects to stick to it. Another method involves placing honey-coated papers inside glass containers or paper cups. Adding honey to emerging wasps increases their ability to search for pest eggs, potentially increasing their lifespan up to four times and increasing the number of eggs they lay [12].

2. *Liquid formulation*

In this type of formulation, a mixture of water and a thickening agent (*polyacrylic*) surrounds the pupae. The presence of this thickening agent minimizes the movement of the pupae. This mixture can be applied to foliage at a specific dosage. The tools used for distributing the liquid must be gentle on the pupae. The simplest tools are liquid soap dispensers that can be attached to 2–5 liter bottles. The cost of liquid formulations is lower and offers advantages such as higher emergence rates and greater survival of *Trichogramma* in the environment [13]. Wasps

emerge within 2 to 5 days depending on the environmental temperature, with approximately 2000 eggs present per milliliter of this liquid formulation [14].



Figure 1. Rearing *Trichogramma* wasps: A step-by-step process: The process of rearing *Trichogramma* wasps for their production involves several distinct stages. First, the Angoumois grain moth (*Sitotroga cerealella*) is reared on barley to produce the host eggs necessary for rearing. Next, the eggs of the Angoumois grain moth are extracted, and *Trichogramma* wasps are reared on these eggs. The pupae of Angoumois grain moth parasitized by *Trichogramma* wasps are subsequently separated and prepared for release in agricultural fields or orchards.



Figure 2. Illustration of a *Trichogramma* adult wasp (left) and a Trichocard employed in a rice field (right).

3. Capsules

Using capsules is an easy and effective method for distributing *Trichogramma*. This type of formulation is particularly useful in areas with high rainfall or where parasitized eggs need

protection from predators. Each capsule contained at least 1000 parasitized moth eggs. If the wasps are stored at the temperature indicated on them, wasps will start emerging from the time they are packaged [15]. Cooler temperatures prolong emergence, whereas higher temperatures accelerate emergence. The package containing capsules may include small transparent bubbles containing parasitized eggs as indicators for wasp emergence; wasps inside these bubbles will appear approximately 24 hours before those inside capsules, indicating when capsules should be placed in orchards [9], [16].

4. Balls

These balls are easily transported and can be quickly scattered over fields or gardens. Additionally, this type of formulation protects *Trichogramma* from predation by animals such as birds and certain beetles (*Coccinellidae* family) as well as green lacewings (*Chrysoperla carnea*) [17]. These balls maintain their shape even after heavy rain or irrigation. Furthermore, the materials used in these packages prolong the hatching time and duration of *Trichogramma* activity, leading to better treatment outcomes. Each ball has a diameter of 2 centimeters and is suitable for aerial or tractor distribution because of its good flight characteristics [18].

Discussion

In Iran, *Trichogramma* is used exclusively through the preparation and application of trichocards. These cards are highly sensitive to adverse environmental conditions such as high temperatures and rainfall and can easily be destroyed by predators such as ants. Unfortunately, since production began using this type of formulation, few changes have been made to address its issues. Farmers face specific difficulties related to the transportation, storage, and installation of *Trichocards*, which negatively impacts the expected results, increases consumption per hectare, and ultimately challenges widespread adoption. Improving current Trichocard formulations and enhancing emerged insects with honey strips while studying and utilizing other forms used globally—such as liquids, capsules, and balls—could attract attention from technical research sectors in the country [19]. These favorable results could lead to mass production aimed at improving the efficacy of this natural enemy against pests such as apple worms, pomegranate fruit borers, and rice stem borers, addressing gaps between research and implementation in biocontrol strategies for various agricultural pests that require more effective technical interventions [20].

Future studies

The effectiveness of *Trichogramma* wasps as biological control agents has been widely studied, there remains a significant need for future research to increase their application in pest management strategies [21]. However, despite their potential, the commercial use of these wasps in agricultural settings, such as rice paddies, is still limited because several factors warrant further investigation. *Trichogramma* wasps can achieve high parasitism rates under laboratory conditions. This discrepancy highlights the challenges faced when transitioning from laboratory to field applications. Factors such as environmental conditions, the presence of competing species, and the physical characteristics of pest egg masses can influence the effectiveness of wasps [22]. To address these limitations, future studies should focus on several key areas, such as field trials across diverse conditions, optimization of release strategies, behavioral studies, and integration with other control methods. While *Trichogramma* wasps present a viable option for biological pest control, ongoing research is vital to overcome existing challenges and optimize their use in agriculture. By addressing the gaps in current knowledge through targeted studies on effectiveness, release strategies, behavior, integration with other methods, and ecological impacts, researchers can better harness the potential of these parasitoids for sustainable pest management [23].

Conclusion

The study findings indicate that the application of *Trichogramma* wasps through innovative formulations significantly enhances their effectiveness as natural biocontrol agents for agricultural pests. The approach fills the gap between research and practice in biocontrol interventions by addressing the need for more effective technical interventions. Therefore, applying *Trichogramma* wasps through such formulations is strongly recommended.

Acknowledgement

This research supported by Agricultural Research, Education and Extension Organization (AREEO), Iran which is the largest National Agricultural Research System (NARS) in the Middle East.

References

- [1] A. Ivezić dan B. Trudić, “Parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae), natural enemies of European corn borer *Ostrinia nubilalis* (Hübner, 1796) (Lepidoptera: Crambidae),” *J. Cent. Eur. Agric.*, vol. 22, no. 4, hlm. 787–797, 2021, doi: [10.5513/JCEA01/22.4.3247](https://doi.org/10.5513/JCEA01/22.4.3247).
- [2] S. Morrone, C. Dimauro, F. Gambella, dan M. G. Cappai, “Industry 4.0 and Precision Livestock Farming (PLF): An up to Date Overview across Animal Productions,” *Sensors*, vol. 22, no. 12, hlm. 4319, Jun 2022, doi: [10.3390/s22124319](https://doi.org/10.3390/s22124319).
- [3] S. M. Mahdavian, F. Askari, H. Kioumars, R. Naseri Harsini, H. Dehghanzadeh, dan B. Saboori, “Modeling the linkage between climate change, CH₄ emissions, and land use with Iran’s livestock production: A food security perspective,” *Nat. Resour. Forum*, hlm. 1477–8947.12532, Jun 2024, doi: [10.1111/1477-8947.12532](https://doi.org/10.1111/1477-8947.12532).
- [4] L. Kalavari, N. Nasiri, F. Ahmadian, dan H. Kioumars, “Enrichment of Doogh with Olive Leaf Extract and Investigation of Its Physicochemical, Microbial, and Sensory Properties during Storage at Room Temperature and Refrigerator,” *J. Multidiscip. Appl. Nat. Sci.*, vol. 3, no. 1, hlm. 34–42, Agu 2022, doi: [10.47352/jmans.2774-3047.143](https://doi.org/10.47352/jmans.2774-3047.143).
- [5] A. Khakpour, N. A. Shadmehri, H. Amrulloh, dan H. Kioumars, “Antibacterial Effect of *Juglans regia*, *Citrus sinensis*, *Vicia faba*, and *Urtica urens* Extracts under In vitro Conditions,” *Bioactivities*, vol. 1, no. 2, hlm. 74–80, Okt 2023, doi: [10.47352/bioactivities.2963-654x.195](https://doi.org/10.47352/bioactivities.2963-654x.195).
- [6] A. Al-Riyami dan I. C. W. Hardy, “Conspicuous by their absence: extremely rare field parasitism by *Trichogramma* wasps imported to control pest butterflies in a pomegranate agro-ecosystem,” *Biocontrol Sci. Technol.*, vol. 34, no. 1, hlm. 65–78, Jan 2024, doi: [10.1080/09583157.2023.2297163](https://doi.org/10.1080/09583157.2023.2297163).
- [7] M. G. Solomon *et al.*, “Biocontrol of Pests of Apples and Pears in Northern and Central Europe - 3. Predators,” *Biocontrol Sci. Technol.*, vol. 10, no. 2, hlm. 91–128, Apr 2000, doi: [10.1080/09583150029260](https://doi.org/10.1080/09583150029260).
- [8] Ch. Ulrichs dan I. Mewis, “Evaluation of the efficacy of *Trichogramma evanescens* Westwood (Hym., Trichogrammatidae) inundative releases for the control of *Maruca vitrata* F. (Lep., Pyralidae),” *J. Appl. Entomol.*, vol. 128, no. 6, hlm. 426–431, Jul 2004, doi: [10.1111/j.1439-0418.2004.00867.x](https://doi.org/10.1111/j.1439-0418.2004.00867.x).
- [9] L.-S. Zang, S. Wang, F. Zhang, dan N. Desneux, “Biological Control with *Trichogramma* in China: History, Present Status, and Perspectives,” *Annu. Rev. Entomol.*, vol. 66, no. 1, hlm. 463–484, Jan 2021, doi: [10.1146/annurev-ento-060120-091620](https://doi.org/10.1146/annurev-ento-060120-091620).
- [10] K. Kraaijeveld, “Penetrance of symbiont-mediated parthenogenesis is driven by reproductive rate in a parasitoid wasp (v0.1)”, doi: [10.7287/peerj.3505v0.1/reviews/1](https://doi.org/10.7287/peerj.3505v0.1/reviews/1).
- [11] H. Xu *et al.*, “Supplementary sugars enhance the production efficiency and parasitism performance of the egg parasitoid *Trichogramma dendrolimi* (Hymenoptera:

- Trichogrammatidae),” *J. Econ. Entomol.*, vol. 117, no. 5, hlm. 1729–1738, Okt 2024, doi: [10.1093/jee/toae168](https://doi.org/10.1093/jee/toae168).
- [12] G. M. Gurr dan H. I. Nicol, “Effect of food on longevity of adults of *Trichogramma carverae* Oatman and Pinto and *Trichogramma nr brassicae* Bezdenko (Hymenoptera: Trichogrammatidae),” *Aust. J. Entomol.*, vol. 39, no. 3, hlm. 185–187, Jul 2000, doi: [10.1046/j.1440-6055.2000.00159.x](https://doi.org/10.1046/j.1440-6055.2000.00159.x).
- [13] J. D. Cluever, C. W. Beiermann, N. C. Lawrence, dan J. D. Bradshaw, “Assessing the toxicity of selected pesticides to *Trichogramma ostrinae* (Hymenoptera: Trichogrammatidae) pupae as a first step in the development of a potential novel deployment programme,” *Biocontrol Sci. Technol.*, vol. 33, no. 11, hlm. 1065–1084, Nov 2023, doi: [10.1080/09583157.2023.2275116](https://doi.org/10.1080/09583157.2023.2275116).
- [14] R. M. Goulart, H. X. Volpe, A. M. Vacari, R. T. Thuler, dan S. A. De Bortoli, “Insecticide selectivity to two species of *Trichogramma* in three different hosts, as determined by IOBC/WPRS methodology,” *Pest Manag. Sci.*, vol. 68, no. 2, hlm. 240–244, Feb 2012, doi: [10.1002/ps.2251](https://doi.org/10.1002/ps.2251).
- [15] J. Pizzol, B. Pintureau, O. Khoualdia, dan N. Desneux, “Temperature-dependent differences in biological traits between two strains of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae),” *J. Pest Sci.*, vol. 83, no. 4, hlm. 447–452, Des 2010, doi: [10.1007/s10340-010-0327-0](https://doi.org/10.1007/s10340-010-0327-0).
- [16] M. Askari Seyahooei, A. Mohammadi-Rad, S. Hesami, dan A. Bagheri, “Temperature and Exposure Time in Cold Storage Reshape Parasitic Performance of *Habrobracon hebetor* (Hymenoptera: Braconidae),” *J. Econ. Entomol.*, vol. 111, no. 2, hlm. 564–569, Apr 2018, doi: [10.1093/jee/toy004](https://doi.org/10.1093/jee/toy004).
- [17] W. T. Oehmichen *et al.*, “Temperature based differences in biological parameters of some potential species/strains of *Trichogramma*,” *J. Biol. Control*, vol. 31, no. 2, hlm. 82–89, Nov 2017, doi: [10.18311/jbc/2017/16338](https://doi.org/10.18311/jbc/2017/16338).
- [18] D. M. Firake dan M. A. Khan, “Alternating Temperatures Affect the Performance of *Trichogramma* Species,” *J. Insect Sci.*, vol. 14, no. 41, hlm. 1–14, Mar 2014, doi: [10.1673/031.014.41](https://doi.org/10.1673/031.014.41).
- [19] S. Bhandari, K. R. Pandey, Y. R. Joshi, dan S. K. Lamichhane, “An overview of multifaceted role of *Trichoderma* spp. for sustainable agriculture,” *Arch. Agric. Environ. Sci.*, vol. 6, no. 1, hlm. 72–79, Mar 2021, doi: [10.26832/24566632.2021.0601010](https://doi.org/10.26832/24566632.2021.0601010).
- [20] J. Karimi *et al.*, “Analytical Approach to Opportunities and Obstacles of Iranian Biological Pest Control,” dalam *Biological Control of Insect and Mite Pests in Iran*, vol. 18, J. Karimi dan H. Madadi, Ed., dalam *Progress in Biological Control*, vol. 18. , Cham: Springer International Publishing, 2021, hlm. 601–621. doi: [10.1007/978-3-030-63990-7_17](https://doi.org/10.1007/978-3-030-63990-7_17).
- [21] R. Tang, D. Babendreier, F. Zhang, M. Kang, K. Song, dan M.-L. Hou, “Assessment of *Trichogramma japonicum* and *T. chilonis* as Potential Biological Control Agents of Yellow Stem Borer in Rice,” *Insects*, vol. 8, no. 1, hlm. 19, Feb 2017, doi: [10.3390/insects8010019](https://doi.org/10.3390/insects8010019).
- [22] J. Gardner, M. P. Hoffmann, S. A. Pitcher, dan J. K. Harper, “Integrating insecticides and *Trichogramma ostrinae* to control European corn borer in sweet corn: Economic analysis,” *Biol. Control*, vol. 56, no. 1, hlm. 9–16, Jan 2011, doi: [10.1016/j.biocontrol.2010.08.010](https://doi.org/10.1016/j.biocontrol.2010.08.010).
- [23] E. Vindas-Reyes, R. Chacón-Cerdas, dan W. Rivera-Méndez, “*Trichoderma* Production and Encapsulation Methods for Agricultural Applications,” *AgriEngineering*, vol. 6, no. 3, hlm. 2366–2384, Jul 2024, doi: [10.3390/agriengineering6030138](https://doi.org/10.3390/agriengineering6030138).