Review Article

Scientific Approaches for Mass Production, Cold Storage and Release of *Trichogramma*: Their Role in Insect Pest Management

ABSTRACT

Having suitable storage methods for parasitoids is a valuable asset within biological control programs. The quality of *Trichogramma* is affected by cold storage with an acclimation period. Prepupae were subjected to storage for 50, 75, and 100 days at 5°C, preceded by acclimation periods of 10 or 20 days at 12°C. The research revealed the potential to halt the development of *Trichogramma*. Treatments with a 10-day acclimation period resulted in emergence values below 10%, rendering them unsuitable for establishing a cold storage protocol. In contrast, a 20-day acclimation period had a beneficial impact on cold storage tolerance for the 50- and 75-day storage periods. The storage of *Trichogramma,* using a 20-day acclimation period and up to 50 days under cold temperatures, did not adversely affect adult emergence, emergence time, sex ratio, parasitism, or progeny quality.

Key words: *Corcyra cephalonica,* Cold storage, *Trichogramma* and Trichocard.

INTRODUCTION:

The genus *Trichogramma* has a global distribution, being found in various terrestrial habitats. It comprises approximately 145 described species and is mainly known for primary egg parasitizing of Lepidoptera insects [1] but they also parasitize eggs from other insect orders, including Coleoptera, Diptera, Hemiptera, Hymenoptera, and Neuroptera. This unique behavior involves adult female *Trichogramma* wasps laying their eggs inside the eggs of other insects. The hatched *Trichogramma* larvae then consume the host egg from the inside. This natural occurrence of *Trichogramma* and its effectiveness in biological control, achieved through large-scale releases, make it a vital component of plant protection strategies [2]

About *Trichogramma*:

Since the late 1970s, *Trichogramma* wasps have been intentionally released to manage numerous economically damaging pests affecting agricultural and fruit crops [3]. The fragility of their body structures and the absence of well-preserved reference wasp specimens (type specimens stored in museums or university collections) have led to numerous misidentifications of species within the Trichogrammatidae family [4,5].

The development process in all *Trichogramma* spp. follows a remarkably similar pattern. Being egg parasites, the females create a hole through the chorion (eggshell) of the host's egg and place their own eggs within it. This action causes a small amount of yolk to be pushed out due to internal pressure. The females then consume this yolk, which enhances their lifespan especially in controlled laboratory conditions [6,7]. Female *Trichogramma* parasitize anywhere from one to ten eggs per day, totaling from ten to 190 eggs during their entire lifespan. Larger females tend to parasitize more eggs than smaller ones. However, in cases involving sugarcane, where the moth borer eggs are small, typically only 1 or 2 parasites develop per egg [4,8]. (19, 27)

Trichogramma fuentesi, one of the frequently encountered species within this genus, originates from the USA, Mexico, Peru, Barbados, Argentina, Venezuela, and Cuba [6,9]. In the United States, it has been documented in states such as Alabama, California, New Jersey, South Carolina, Florida, Texas, and Louisiana [10] *Trichogramma fuentesi* can be found in most annual crop and fruit tree habitats [9].

A female parasitoid possesses the ability to differentiate between already parasitized eggs, thereby preventing super parasitism or multiple parasitism in natural settings. The number of eggs a female can produce, ranging from 20 to 200, depends on factors such as species, host type, and adult longevity. Eggs in the early developmental stages are more conducive to parasite growth. Around the 3rd instar (3 to 4 days post-parasitization of the host egg), dark melanin granules accumulate on the inner surface of the egg chorion, causing the host egg to adopt a black appearance. This distinctive feature serves as a valuable diagnostic marker, allowing differentiation from unparasitized eggs.

The egg, larval, and pupal stages of *Trichogramma* at a temperature of 28 ± 2°C are completed in approximately 1 day, 3 to 4 days, and 4 to 5 days, respectively. Consequently, the entire life cycle takes 8 to 10 days to complete, but this duration can extend at lower temperatures or be impeded under very high temperatures. The adult wasps have a relatively short lifespan of 6 to 8 days. Mating and oviposition occur immediately after emergence, generally resulting in a 1:1 sex ratio.

LIFE CYCLE

Trichogramma spp. experience a full metamorphic process, encompassing four distinct life stages: egg, larva, pupa, and adult. A period of dormancy can disrupt the life cycle of numerous insects, offering protection to a susceptible stage against unfavorable environmental circumstances. In the case of most parasitoids, this dormancy can be achieved through either quiescence or diapausee [11,12]. The duration from egg to adult spans seven to ten days, leading to typically more generations per year compared to their host species. *Trichogramma* wasps function as endoparasitoids, signifying that they finalize their development inside the body of their host. Once they complete the pupal phase within the host's egg, a newly matured adult *Trichogramma* wasp emerges from the emptied egg case of the host and takes flight to parasitize (deposit additional eggs) in the eggs of other hosts [4]. The family Trichogrammatidae, comprising egg parasitoids, has achieved global success in utilizing inundative biological control methods to effectively manage various Lepidoptera pests [13, 14].

EGGS:

Adult female *Trichogramma* wasps create a minute opening in a recently laid host egg, where they place one or more of their own eggs. They use their ovipositor to apply a chemical marker to eggs that have already been parasitized [15]. There is evidence to suggest that in certain Trichogramma species, females also inject venom into the host egg they parasitize. This venom initiates the predigestion of the egg's contents, making it easier for *Trichogramma* larvae to feed [16].

LARVAE:

Upon entering the host egg, *Trichogramma* eggs undergo hatching, transforming into larvae and progressing through three distinct larval instars while utilizing the embryo and yolk within the host's egg as their nutritional source. It typically takes 3-4 days after parasitism for a larva to reach the third and final instar, during which it deposits dark melanin granules on the inner surface of the host egg's lining [17, 9].

PUPAE:

After completing the third larval instar, *Trichogramma* larvae will have consumed the contents of the host egg and will pupate inside the host egg. Although this insect undergoes multiple generations per year, the pupae may spend cooler winter months within the host's egg, during which they slow their metabolic activity. Overwintering pupae can tolerate and survive subfreezing temperatures during the cold months of the year [18,19].

Adults leave the host eggs as they emerge from their pupae. Males emerging from a parasitized host egg will remain at the host egg, awaiting the emergence of nearby adult females to mate with.

ADULTS:

During May and June, the pupae that overwintered within parasitized host eggs typically emerge as adult *Trichogramma*, ready to target new host eggs. *Trichogramma* adults are extremely petite, measuring 1 mm or less in size, with elongated antennae and small hairs proportional to their body size [10]. Distinguishing between *Trichogramma* species is quite challenging due to their similar morphology. Their forewings exhibit distinctive sections and venation, while the hindwings have less prominent veining. Several species, such as *Trichogramma fuentesi*, feature a light yellow or yellow hue with tallow-brown to black markings as adults. In females, the ovipositor is slightly shorter than the antennae [6].

Mated females tend to stop parasitizing after five days as an adult [18]. Adult females of *Trichogramma fuentesi* show a preference for laying their eggs in host eggs that are in the early developmental stages, and their offspring usually exhibit a bias towards females in terms of sex ratio [20]. Research indicates that numerous parasitoid wasps, including *Trichogramma* spp., appear to adjust the sex of their offspring depending on the quality of the egg hosts [21]. For instance, they tend to place unfertilized eggs (which develop into males) in lower quality host eggs, reserving fertilized eggs (leading to females) for higher quality host eggs [22]. *Trichogramma* species will also consume the eggs of their host to obtain additional nutrients to fuel their egg production (4).

Materials required for rearing of *Trichogramma* spp:

The list of items includes: Corcyra eggs, Nucleus culture of *Trichogramma*, Plastic bags, Elastic bands, Cutting tools, Adhesive, Bristled tool, Mesh strainer, *Trichogramma* release cards, Honey solution (50%), Stapler, Refrigeration unit, and Ultraviolet lamp/LED light

Hosts used for *Trichogramma* spp:

Trichogramma wasps exhibit a broad parasitic range, targeting various host species. Among the hosts that *Trichogramma* spp. parasitize are the tomato hornworm (*Manduca quinquemaculata* Haworth), tomato pinworm *(Keiferia lycopersicella* Walshingham), imported cabbageworm (*Pieris rapae* Linnaeus), diamondback moth (*Plutella xylostella* Linnaeus), Oriental fruit moth (*Grapholita molesta* Busck), codling moth (*Cydia pomonella* Linnaeus), and spongy moth (*Lymantria dispar* Linnaeus) [4].

ECONOMIC IMPORTANCE

Given their swift maturation and ability to parasitize a wide variety of hosts, *Trichogramma* wasps have been recognized as valuable candidates for commercial biological control programs aimed at mitigating economically significant insect pests in plants. Numerous *Trichogramma* species are cultivated by private enterprises and marketed for the purpose of bolstering biological control efforts [23].

Certain companies offer individual *Trichogramma* species for sale, either singly or in combination with several other species. The large-scale production of *Trichogramma* necessitates a supply of host eggs for the adult wasps to parasitize, often utilizing insects like the Angoumois grain moth (*Sitotroga cerealella* Olivier) and the Mediterranean flour moth (*Ephestia kuehniella* Zeller that are raised on wheat and other grain-bearing host plants [9]. Despite the widespread accessibility and ongoing efforts, there remains some debate regarding the efficacy of Trichogramma wasps as agents for biological pest control [24].

Cold storage of *Trichogramma:*

In the mass rearing of *Trichogramma* spp., two primary cold storage approaches have been employed: one involving prior diapause induction and the other without it [25,12]. In the large-scale cultivation of *Trichogramma* spp., there are two primary cold storage methods used one includes the induction of diapause beforehand [26], while the other does not involve diapause induction [27,1]. In parasitoids, acclimation has a positive impact on cold storage tolerance [28,29]. However, some cases with detrimental effects of acclimation have also been recorded [30]. Prolonged exposure to cold could have negative effects on both the host and the parasitoid, with lethal or nonlethal results [31]. Thus, it is expected that the fitness cost related with either survival or reproduction will be affected by the reduction of energy stores, particularly lipids, during cold exposure [32]. Immediately subsequent storage, later in development, or even in next generations, a decline of fitness-related features in surviving individuals could be seen [33]. Although there are lot of studies concerning storage of *Trichogrammatidae* species [34,35] it is important to focus on the amenability to cold store of certain particular species since not all of them are able to be cold stored [36].

Table 1: Concerning storage of *Trichogrammatidae* species.

Crop/insect	***Trichogramma* spp.**	**Recommendations**
Rice Leaf folder	*T. chilonis*	1 lakh adults /ha 20/30 DAT at 10 days interval
Tomato: *H. armigera*	*T. pretiosum*	1.5 lakh adults /ha at 10 days interval
Sugarcane: *Chilo spp.*	*T.chilonis*	0.5 lakh ad/ha
Rice Yellow stem bore	*T. japonicum*	1 lakh adults /ha 20/30 DAT at 10 days interval
Cotton bollworm	*Trichogrammatoidea bactrae*	1.0 lakh adults /ha at 10 days interva
Cabbage: Diamond back moth	*T. bactrae*	1.0 lakh ad/ha at 10daysinterval

Across the last few decades, there have been numerous instances of introducing *Trichogramma* wasps into the United States, with the aim of utilizing classical biological control against economic pests [23]. These introductions are listed below:

- ❖ *Trichogramma evanescens* Westwood was introduced from Europe to southern California and Missouri in 1968, specifically to manage imported cabbageworm and cabbage looper populations on cabbage crops [37].

- *Trichogramma euproctidis* Girault was introduced from Russia to Georgia in 1975 with the primary goal of controlling cotton bollworm infestations on cotton plants [4].
- In 1993, *Trichogramma bactrae* Nagaraja was brought from Australia to California and Arizona, aimed at managing pink bollworm populations in cotton fields [4].
- In 1993 and 1996, *Trichogramma ostriniae* Pang and Chen were introduced from China to New York, specifically for the purpose of managing European corn borer populations in sweet corn crops [38].
- In addition to the *Trichogramma* species introduced historically, certain species are currently in use within biological control programs [39].
- *Trichogramma minutum*, originally found in North America, is employed for managing pests such as grape berry moth, cabbage looper, and codling moth across diverse settings, including ornamentals, orchards, grape production, and forests [17].
- *Trichogramma platneri* - native to North America used for control of codling moth and leafrollers in avocados, ornamentals, orchards, and grape production [40].
- *Trichogramma brassicae*, a species native to Europe, finds application in controlling lepidopteran vegetable pests, notably the European corn borer [41].
- *Trichogramma pretiosum* – native to North America for lepidopteran cotton and vegetable pests (e.g. bollworms and budworms) [42].
- *Trichogramma* wasps are generally susceptible to a wide range of insecticides (such as lambda-cyhalothrin, bifenthrin, and indoxacarb), in addition to certain fungicides and herbicides [39]. The impact of these pesticides on Trichogramma varies based on the specific life stage exposed to the pesticide and the application rate, potentially leading to reduced effectiveness when integrating Trichogramma into Integrated Pest Management (IPM) programs [39].

Preparation of Trichocards:

- The parasitisation of *Trichogramma* spp., in laboratory condition on one cc eggs of Corcyra cephalonica, which are uniformly spread and pasted on a card measuring 15 cm x 10 cm, is called as Tricho card. The card has 12 demarcations (stamps).
- Apply gum on the card and sprinkle the cleaned eggs uniformly in a single layer with the aid of a tea strainer.

- The excess eggs pasted are removed by gently passing a shoe brush over the card after sufficient air drying under fan.
- Treat the eggs under UV lamp for 30 minutes to kill the embryo (at present price of UV light in market is Rs. 1,121)
- Take polythene bag, insert UV treated “Trichocard” and nucleus card at the ratio of 6:1 (6 Corcyra egg cards: 1 Trichogramma nucleus card) and provide 50% honey with vitamin E in a soaked cotton swab.
- Remove the Tricho cards after 24 hours. On fourth day, the Corcyra eggs changes to black in colour and indicates the parasitization of eggs.
- Release the tricho cards in the fields when at least 5 % adult emergence (pharate stage) is observed. During each release trichocards should be cut into 12 or 16 bits and staple to the lower side of leaf of plant during morning or evening hours.
- At fourth or fifth day tricho cards can be stored in refrigerator/fridge at 10 degree centigrade up to 21 days. (if we will not store Trichocard in refrigerator then we should immediately release Trichocard in field.

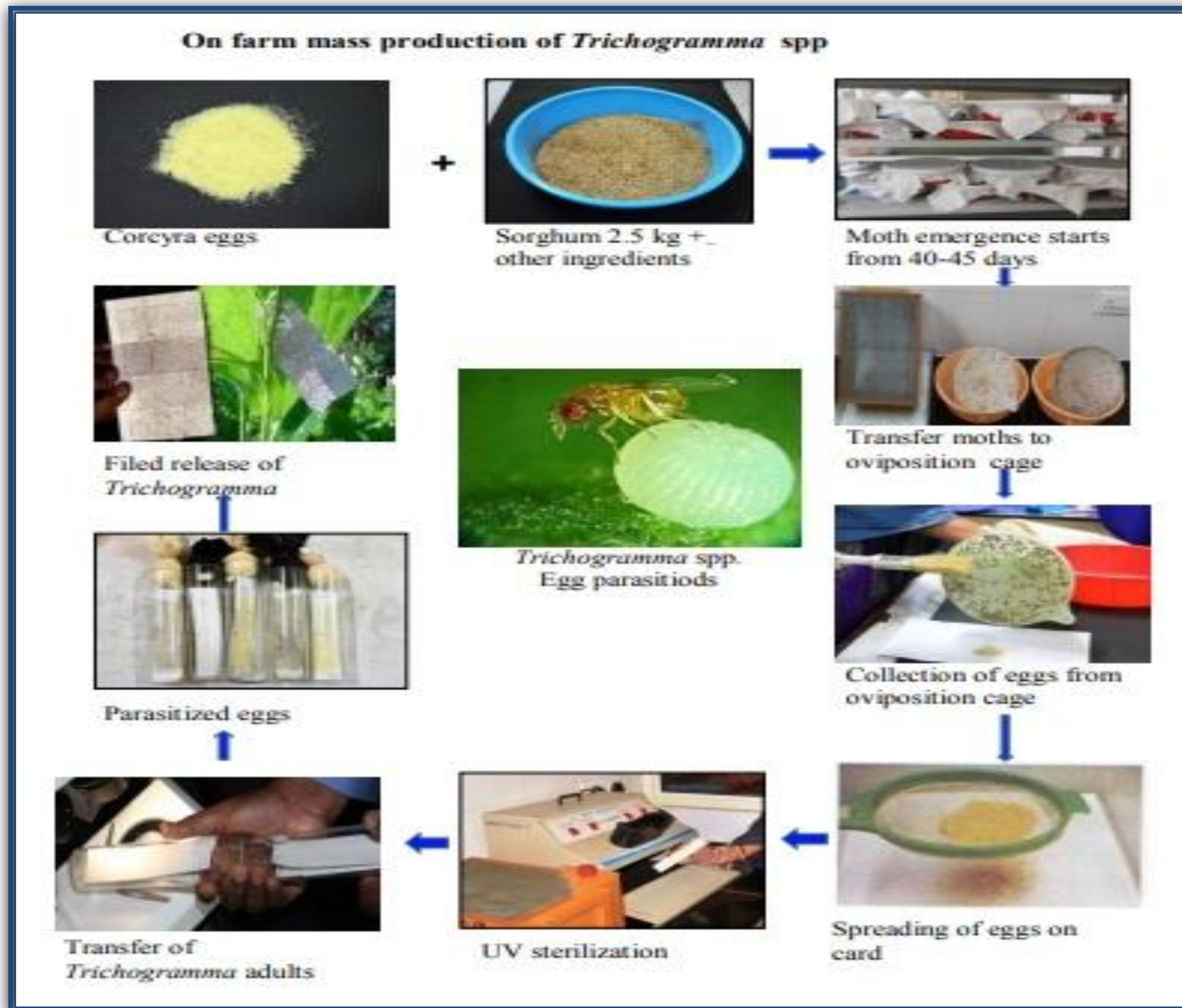

Fig 1: On farm mass production of *Trichogramma Spp.*

Conclusion

The final emphasis of this abstract lies in the strategic release of Trichogramma into pest-infested areas. Scientific approaches have been developed to determine optimal release rates, timings, and distribution patterns. Factors such as pest population dynamics, crop phenology, and environmental conditions are considered to maximize the impact of Trichogramma on target pest populations while minimizing non-target effects. In conclusion, scientific advancements in mass production, cold storage, and strategic release techniques have propelled Trichogramma-based insect pest management to the forefront of sustainable agricultural practices. The integration of these approaches into broader pest management strategies has the potential to significantly reduce reliance on chemical pesticides, mitigate environmental impact, and enhance overall crop yields. Continued research and refinement of these methodologies are vital to unlocking the full potential of Trichogramma in shaping the future of agriculture.

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