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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

PARASITISATION POTENTIAL OF EGG AND LARVAL PARASITOIDS AGAINST LESSER WAX MOTH *Achroia grisella* F. (LEPIDOPTERA: PYRALIDAE) UNDER STORED CONDITION

Yumurta ve Larval Parazitoidlerinin Küçük Mum Güvesi *Achroia grisella* F. (Lepidoptera: Pyralidae) Karşısında Saklanmış Şartlarda Parazitlenme Potansiyeli

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ABSTRACT

The study conducted to evaluate the parasitisation potential of egg (*Trichogramma chilonis* Ishii) and larval parasitoids (*Bracon brevicornis* Wesmael and *Apanteles galleriae* Wilkinson) on the developmental stages of lesser wax moth, *Achroia grisella* F. The results indicated that a maximum parasitisation of 46.67% was noticed on egg masses released with seven pairs of T*. chilonis*, with an overall mean of 28.89 % and also the adult emergence was recorded about 59.49 %. Among the two larval parasitoids taken, the per cent parasitisation of lesser wax moth larvae by *A. galleriae* was maximum (38.68% overall mean) than by *B. brevicornis* (33.14%) with a mean adult emergence of 71.16% and 64.78% respectively.

Key words: Lesser wax moth, *Achroia grisella*, Bio-control agents, Parasitisation

ÖZ

Yumurta (*Trichogramma chilonis* Ishii) ve larva parazitoitlerinin (*Bracon brevicornis* Wesmael ve *Apanteles galleriae* Wilkinson) küçük mum güvesi *Achroia grisella* F'nin gelişim evreleri üzerindeki parazitlenme potansiyelini değerlendirmek için yürütülen bir çalışlma yapılmıştır. Sonuçlar maksimum parazitlenmenin %46,67 olduğunu göstermektedir. Yedi çift *T. chilonis* ile salınan yumurta kütlelerinde toplam ortalama %28.89 ile fark edilmiş ve ayrıca ergin çıkışı yaklaşık %59.49 olarak kaydedilmiştir. Alınan iki larva parazitoidi arasında, küçük mum güvesi larvalarının *A. galleriae* tarafından parazitlenme yüzdesi, *B. brevicornis*'e (%33.14) göre maksimum (toplam ortalama %38.68), sırasıyla ortalama %71.16 ve %64.78'lik bir ergin çıkış oranı belirlenmiştir.

Anahtar kelimeler: Küçük mum güvesi, *Achroia grisella*, Biyo-kontrol ajanları, Parazitlenme

GENİŞLETİLMİŞ ÖZET

Çalışmanın Amacı: Balmumu güvesi *Achroia grisella* Fabricius (Lepidoptera: Pyralidae), bal arısı kolonileri için ciddi tehditlerden biridir ve kolonilerin tamamen tahrip olmasına ve bal veriminin düşmesine neden olur. Daha küçük mum güvelerine karşı sentetik pestisitlerin kullanılması, balın kirlenmesine ve bal arısı kolonileri ve hedef olmayan organizmalar üzerinde zararlı etkilere neden olur. Bu sorunların üstesinden gelmek için, böcek zararlılarını kontrol etmek için biyolojik kontrol ajanları kullanılmaktadır. Bu amaçla, yumurta (*Trichogramma chilonis* Ishii) ve larva parazitoitlerinin (*Bracon brevicornis* Wesmael ve *Apanteles galleriae* Wilkinson) daha az çevre dostu ve uygun maliyetli mum güvesine karşı parazitleme potansiyeli üzerinde deneyler yapılmıştır.

Gereç ve yöntemler: Küçük balmumu güvesinin ve olgunlaşmamış aşamalarının toplu kültürü, böcek kafeslerinde bir yıllık bal toplanmış peteklerde sürdürüldü. *T. chilonis*'in parazitlenme potansiyeli, farklı güçlerde (bir çift ila yedi çift) yetişkin parazitoidlerin replikasyon başına 50 yumurtadan oluşan mum güvesi yumurta kütleleri üzerine salınmasıyla değerlendirildi. Larva parazitoitlerinin potansiyelinin değerlendirilmesi için "sandviç yöntemi" izlendi; bu yöntemde gebe dişi parazitoitler, besin kaynağı görevi gören bal ve suya (1:1 oranında) batırılmış bir pamuklu çubukla plastik kaplara aktarıldı.

Bulgular: Toplam ortalama %28.89 ve %59.49 parazitoid ergin çıkış ile yedi çift *T. chilonis* ile salınan yumurta kütlelerinde %46.67'lik maksimum parazitlenme fark edildi. Yüzde parazitlenme ve ergin çıkışının salınan parazitoitlerin sayısına karşılık geldiği belirlendi. Denenen iki larval parazitoit arasında, küçük mum güvesi larvalarının *A. galleriae* tarafından parazitlenme yüzdesi, sırasıyla %71.16 ve %64.78'lik ortalama ergin çıkışıyla *B. brevicornis*'e (%33.14) göre maksimum (toplam ortalama %38.68) olarak bulunmuştur.

Sonuç: Mevcut çalışmamız, biyolojik kontrol ajanlarının, depolanmış durumda *Achroia grisella* Fabricius'u kontrol etmek için en iyi alternatif olabileceğini, çünkü hedef olmayan organizmalara ve insanlara zararlı bir etki oluşturmadığını ortaya koymaktadır.

INTRODUCTION

According to Steffan-Dewenter *et al*. (2006), honey bees (*Apis* sp.) are dependable pollinators who pollinate nearly 70% of the world's important crops. They are also resourceful insects who produce honey, wax, resin, royal jelly, and other products. According to Raina (2006), honey bees are essential for ecosystem stability, poverty reduction, and food security. Honey bee pests *viz*., small hive beetle, *Aethina tumida* Murray (Coleoptera: Nitidulidae), large hive beetle, *Oplostomus haroldi* Witte (Coleoptera: Cetoniidae), the greater wax moth (GWM), *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), lesser wax moth (LWM), *Achroia grisella* Fabricius and an invasive mite, *Varroa destructor* Anderson and Trueman (Parasitiformes: Varroidae) have all been reported to cause significant economic losses in honey bee colonies (Shimanuki et al., 1980).

Wax moths are the potential threat to bee keeping due to its prolific development of larvae which devour wax, pollen and larvae of bees. Similar to the greater wax moth *G. mellonella* L. the coexistence and invasion by lesser wax moth, *Achroia grisella* F. is an additional concern to the beekeepers due to their extensive damage, resulting in heap of webs (*Galleriasis*) both under field and stored conditions. Both chemical and non-chemical management strategies applied to lessen the losses associated with wax moths' infestation are limited by various challenges due to the high degree of sensitive nature of honey bees and hive environment (Flint and Merkle, 1983 and Jafari *et al*., 2010).

An exploitation of synthetic pesticides against lesser wax moth results in the contamination of honey, and detrimental effects on honey bee colonies and non-target organisms. However, it's essential to note that pesticides are not the sole treatment option for managing the lesser wax moth. There are three primary alternative methods such as, Biological control: *Bacillus thuringiensis*, a bacterium with commercially available spore preparations can be applied using a hand sprayer through which bacteria effectively invade and eliminate all wax moth larvae. Physical control: Chilling or freezing can be employed by placing combs in a freezer or relying on winter frosts in cold regions. Chemical control: Fumes of sulfur and strong acetic acid can be placed above boxes containing drawn comb, which are then sealed. These fumes penetrate the combs and help control the wax moth population. Additionally,

chemical volatile oils and gaseous treatments like carbon dioxide, ozone, and nitrogen have been explored as treatment options (Ellis *et al*., 2013 and Ghimire and Phillips, 2010). Hence, in order to find a suitable alternative to contain lesser wax moth infestation under stored conditions, the parasitisation potential of egg parasitoid, *Trichogramma chilonis* Ishii and two larval parasitoids *Bracon brevicornis* Wesmael and *Apanteles galleriae* Wilkinson were evaluated.

MATERIALS AND METHODS

Experimental location

An experiment was done at the Apiary of the Department of Entomology under Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Mass culturing of lesser wax moth

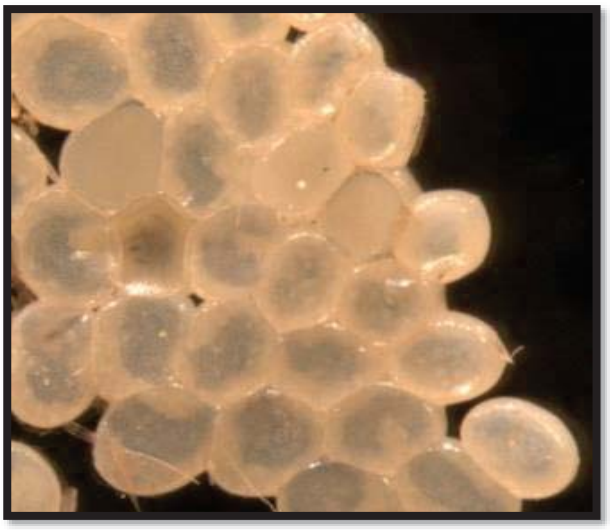
Lesser wax moths were mass cultured, together with their embryonic stages, on honey harvest combs from a year prior in insect cages (Fig. 2a), in a lab setting with a constant temperature of 27°C. *A. grisella* was mass cultured in plastic insect rearing boxes (Fig. 2b). The several instars of the larvae were distinguished based on their size and length. According to Paddock (1918), the diameter and length of the larvae were 1-30mm and 0.12-7.0mm, respectively. The larvae of the later instar (Fig. 1d) start to spin the cocoon (Fig. 1e), which they afterwards pupate (Fig. 1f) inside the cocoon. Development of the larvae takes 6-7 weeks. The plastic lid's edges were where the pupation was located. The last two instars were when growth and size increased most frequently. Throughout its development, the larva goes through seven moults (Ellis *et al*. 2013). Moths were moved into a separate plastic container for the purpose of mating after becoming adults (Fig. 1g&h). To offer a surface for egg laying in the mating cage, paper scraps were introduced. Clusters of eggs are laid (Fig. 1a&b). In order to determine the parasitisation capacity of parasitoids on the developmental phases of the lesser wax moth, the newly hatched larvae were cultured and allowed to grow continuously (Fig. 1c).

Experimental setup for Parasitisation potential assessment

The parasitisation potential of *T. chilonis* was assessed by releasing the adult parasitoids in different strengths (one pair to seven pairs) on the wax moth egg masses comprising of 50 eggs per replication and the observations on percent egg parasitized and mean adult parasitoids emerged from wax moth eggs were recorded.

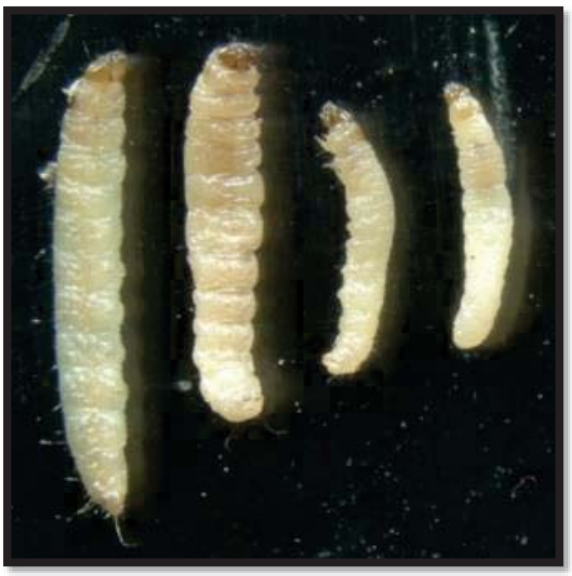
For assessing the potential of larval parasitoids “sandwich method” (Fig. 3) was followed, in which gravid females parasitoids were transferred to plastic containers with a cotton swab soaked in honey and water (at 1:1 ratio) which acted as a food source. The container was covered with khada cloth and secured with the rubber bands and the wax moth larvae were placed above the khada cloth and then covered with another layer of khada cloth. This setup was kept undisturbed and observations were taken on third, fifth and seventh day after release.

$$\% \text{ Parasitisation} = \frac{\text{Number of larvae parasitized}}{\text{Total number of larvae taken}} \times 100$$



1a. Egg mass

1b. Egg mass (Magnified)



1c. Larval instars

1d. Larva (Magnified)

1e. Pupal cocoon

1f. Pupa

1g. Adult moth

1h. Adult (Magnified)

Figure 1. Developmental stages of Lesser Wax Moth

2a. Wax moth culture under Rearing cage

2b. Lesser Wax Moth larvae feed on honey comb

Figure 2. Mass Culturing of Lesser Wax Moth

Figure 3. Sandwich method

Statistical Analysis

The experiments were set up using a Completely Randomised Block Design (CRBD) with three replications, and the means were sorted using Duncan's Multiple Range Test (DMRT) after the data were statistically analysed using Analysis of Variance (ANOVA) methods (Khan & Khanum, 1994). Furthermore, F test is used to compare the variance.

RESULTS

The results showed that when compared to the experimented strength of egg parasitoid, a maximum parasitisation of 46.67% was noticed on egg masses released with seven pairs of *T. chilonis*, with an overall mean of 28.89 % and 59.49 % of parasitoid adult emergence. It was noticed that the per cent parasitisation and adult emergence were corresponding to the number of parasitoids released (Table 1). Among the two larval parasitoids tried, the per cent parasitisation of lesser wax moth larvae by *A. galleriae* was maximum (38.68% overall mean) than by *B. brevicornis* (33.14%) (Fig. 4) with a mean adult emergence of 71.16% and 64.78% respectively (Fig. 5). Among different larval instars, the late instars (IV-VII) were preferred for higher level of parasitisation. There was nil per cent parasitisation in control which is maintained without the release of parasitoids. The study inferred the possible use of egg and larval parasitoids for the successful management of lesser wax moth under the storage conditions.

4a. Pupae of *B. brevicornis* on host larva

4b. Parasitized wax moth larva with punctures

Figure 4. Parasitisation of Lesser Wax Moth larva by *Bracon brevicornis*

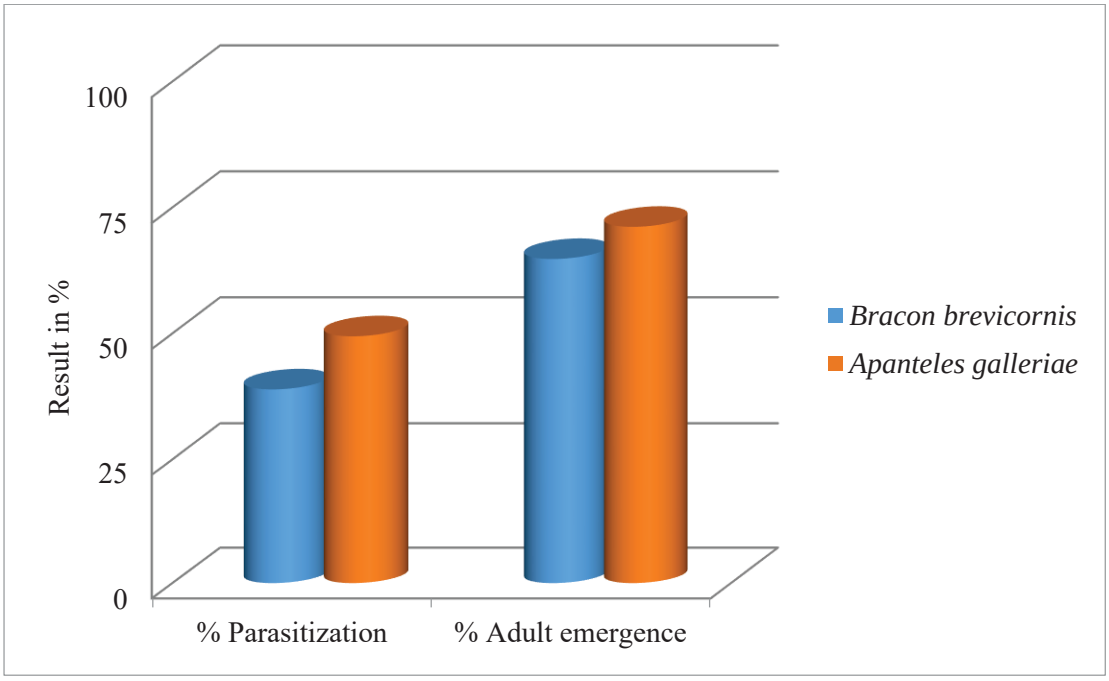


Figure 5. Evaluation of bio-control agents against lesser wax moth larvae

Table 1. Parasitisation potential of parasitoids on egg and larvae of wax moths *A. grisella*

Egg parasitoid									
Treatment / larval instars	**Per cent Larval parasitisation (*A. grisella)****							**Mean (% parasitisation)**	**Mean adult emergence (%)**
	I	**II**	**II**	**IV**	**V**	**VI**	**VII**		
Larval parasitoids									
Bracon brevicornis	0.00 (4.06)	10.00 (18.43)^{ab}	30.00 (33.21)^{ab}	43.33 (41.17)^{b}	63.33 (52.73)^{ab}	46.73 (43.13)^{ab}	40.00 (39.23)^{ab}	33.14	64.78
Apanteles galleriae	0.00 (4.06)	16.67 (24.09)^{a}	40.00 (39.23)^{a}	60.00 (50.77)^{a}	70.00 (56.81)^{a}	63.33 (52.73)^{a}	46.67 (43.09)^{a}	38.68	71.16
mean	4.06	21.26	36.22	45.97	54.77	47.93	41.16	-	
SD	0.00	4.72	7.07	11.79	4.72	11.74	4.72		
Egg parasitoid									
	1 pair	**2 pairs**	**3 pairs**	**4 pairs**	**5 pairs**	**6 pairs**	**7 pairs**	**Mean parasitisation (%)**	**Mean adult emergence (%)**
T. chilonis	4.23 (8.48)^{c}	6.67 (10.71)^{c}	23.33 (28.56)^{b}	26.67 (30.79)^{b}	33.33 (34.98)^{b}	36.67 (43.02)^{ab}	46.67 (43.05)^{a}	28.89	59.49
F test for larval parasitoids Vs larval instars									
CD at 5%	**Treatment** (Larval parasitoids)							0.46	
	Larval instars							0.87	
	T * L							1.23	

Note: *Mean of five replications. Figures with brackets represent values that have undergone arc sine transformation; SD–Standard Deviation, CD–Critical Difference; those without the same alphabetical letters in the same column differ significantly from one another at $p < 0.05$. Here, alphabets used for DMRT test.

DISCUSSION

In general, reports on the application of bio-control agents against wax moths are sporadic and limited due to the fact that employing parasitoids in a live colony has least scope. However, attempts were made to evaluate the parasitisation efficiency of egg and larval parasitoids under storage conditions due to its practicality.

In addition, the present experiment exhibited a positive correlation between host size and per cent parasitisation by *B. brevicornis* which deposited supplementary eggs on superior hosts (later instar) than the smaller ones (early instar), which is in conformity with the findings of Taylor (1988). Presumably, the parasitoid adjusts their clutch size as per the nutritional value of the host insect, thereby avoiding larval competition among progeny (Taylor, 1988). The oviposition rate and percent emergence of *B. brevicornis* was observed to be maximum on 5th instar larvae of lesser wax moths (63.33%) and the successful parasitisation decreased drastically with host age after 5th instar. Accordingly, the parasitisation by *A. galleriae* was observed to be minimum on early stage larvae (1st-3rd instar) as the parasitoids require long time to complete their larval development (Acevedo-Gonzalez *et al*., 2019). The 5th larval instar of lesser wax moth was found to be most suitable for parasitisation due to its optimum size.

The parasitisation potential of *T. chilonis*, inferred that it could be a potential biological control agent against eggs of wax moths under storage conditions. The rate of parasitisation increased with respect to the enhanced number of pairs released on the target pest. Release of seven pairs of *T. chilonis* resulted in higher parasitisation of 46.67% on *A. grisella* eggs. The role of egg parasitoids for the pest management was well established and reported as a dependable and safer method (Hood *et al*., 2003).

The present study could be the best one among all other control measures when compared to the outcome of Kumar and Khan (2020) who stated that management of the wax moth in order to reduce losses in storage conditions, it is recommended that the combs should be treated with Neem oil (3%) or Sulphur fumigation. These control measure will lead to the detrimental effect on honey bee colonies in terms of inducing feeding deterrence.

Conclusion

Our present study revealed that biological control agents could be the best alternative to control *Achroia grisella* Fabricius when compared to chemical methods under stored condition as it does not pose any harmful effect to non target organisms and also human beings. Furthermore, *Trichogramma chilonis* (egg parasitoid) and *Apanteles galleriae* (larval parasitoid) which can be highly recommended as bio-control agents among other egg and larval parasitoids for the successful management of lesser wax moths under stored condition.

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Author contribution: P. Sabatina: Data collection, analysis and result interpretation; Dr. G. Umapathy: Study conception and manuscript correction; Dr. P.A. Saravanan: Study design and manuscript editing.

Conflict of interest: The authors have no conflict of interest to declare.

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Ethical issues: Not applicable

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