INFLUENCE OF INTRASPECIFIC AND INTERSPECIFIC COMPETITION ON FITNESS OF TWO *TRICHOGRAMMA* EGG PARASITOIDS

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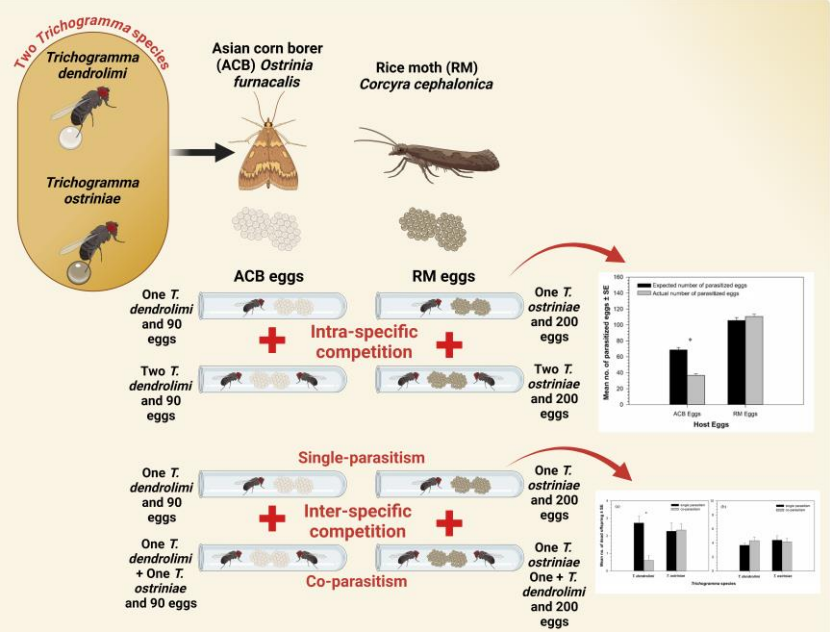
GRAPHICAL ABSTRACT



ABSTRACT

The Asian corn borer (ACB), *Ostrinia furnacalis* Guenee, is a destructive and economically important pest of corn in Asia and particularly in China. *Trichogramma* wasps are the most successfully employed biological control agents against lepidopterous pests. These parasitoids are effectively mass reared on the eggs of rice moth (RM), *Corcyra cephalonica* Stainton, and are frequently used to control

economically significant lepidopterous pests such as ACB. *T. dendrolimi* Matsumura and *T. ostriniae* Pang and Chen are regarded as the most promising biocontrol agents for the control of ACB. Multiparasitism is the most promising approach to enhancing *Trichogramma*'s efficiency as a biocontrol agent. However, multiparasitism can lead to intra- and interspecific competition, which influences parasitoid fitness. This study determined the intraspecific and interspecific competition among *T. dendrolimi* and *T. ostriniae* for parasitizing the eggs of ACB and RM by assessing the number of parasitized host eggs and the number of emerged and dead progeny. Results of intraspecific competition did not indicate any significant difference between the expected and actual numbers of parasitized eggs of both lepidopterous hosts by both *Trichogramma* species. While bioassays of interspecific competition showed ACB eggs as the most promising host than RM eggs. *T. ostriniae* exhibited higher parasitization, successful offspring emergence and survival than *T. dendrolimi* on both type of host eggs. Compared to the co-parasitism by *T. dendrolimi* and *T. ostriniae*, the single parasitism by *T. dendrolimi* on ACB eggs was more advantageous. This might be due to decreased offspring emergence of *T. dendrolimi* influenced by the interspecific competition among both *Trichogramma* parasitoids. Furthermore, the higher number of dead offsprings of *T. dendrolimi* was observed in single parasitism compared to co-parasitism. No influence of single and co-parasitism of *Trichogramma* species on RM eggs regarding parasitization, offspring emergence, and death was observed. Nevertheless, the assessment of competitive ability of *T. ostriniae* over *T. dendrolimi* under field conditions constitutes the future perspectives of this study.

KEYWORDS

Asian corn borer, co-parasitism, egg parasitoids, rice moth, single parasitism,

INTRODUCTION

The Asian corn borer (ACB), *Ostrinia furnacalis* Guenee (Crambidae: Lepidoptera), is an important agricultural pest of corn in Asia (Zhang et al. 2013, Le et al. 2019, Iqbal et al. 2021). ACB causes an average of 6 – 9 million tons of corn yield loss annually in China (Wang et al. 2024). The ACB larvae feed on silks and tassels before boring into the stalk and tunneling and causing fallen ears or stalk lodging, leading to a 30% yield loss (Chen et al. 2015, Guo et al. 2022). The ineffectiveness of insecticides used to control ACB is attributed to the difficulty in scheduling their appropriate applications before the ACB larvae penetrate corn stalks (Chen et al. 2013). Additionally, extensive use of synthetic insecticides is characterized by a variety of issues, including the induction of hormesis in insects (Sial et al. 2018, Gowda et al. 2021, Guedes et al. 2022), the development of resistance (Pu & Chung

2024), the detrimental effects on non-target insects (Sánchez-Bayo 2021, Serrão et al. 2022) and human health (Ahmad et al. 2024).

The rice moth (RM) *Corcyra cephalonica* Stainton, is an economically important stored grain pest worldwide (Atwal & Dhaliwal 2015). RM larvae feed and infested the stored grains and their faeces, exuviae, and cadavers render the grains unfit for human consumption (Vincent et al. 2021). The global loss of stored grains owing to stored grain pest infestation is more than 10%, with China alone accounting for 30% (Zhang et al. 2021). Besides controlling RM with synthetic insecticides (Gowda et al. 2021, Nath et al. 2023), plant-based insecticides (Coelho et al. 2007, Attia et al. 2020), and pathogens (Kaur et al. 2014), Hong-xing et al. (2017) and Xue et al. (2023) advocated that natural enemies would be the promising, sustainable, and non-chemical management strategy for controlling agricultural pests including RM in China.

The *Trichogramma* wasps are among the most often used natural enemies in biological control programs worldwide (Romeis et al. 2005). It is the most extensive genus in the Trichogrammatidae family containing 230 described species worldwide (Jalali et al. 2016, Zang et al. 2021). *Trichogramma* parasitoids are effectively mass-reared on artificial host egg, rice moth (RM), *Corcyra cephalonica* Stainton (Wang et al. 2014), and are frequently used to control economically significant lepidopterous pests such as ACB (Zang et al. 2021). According to Wang et al. (2014), *Trichogramma* has been subjected to significant mass rearing on RM since 1970s, and has been dispersed on a large scale in China to control ACB. RM is a highly prospective factitious host for the propagation of *Trichogramma* parasitoids (Vincent et al. 2021, Wang et al. 2014, Chaudhuri & Senapati 2017). Nevertheless, short shelf life is the limiting factor of this RM host (Masry & El-Wakeil 2020).

Trichogramma dendrolimi Matsumura and *T. ostriniae* Pang and Chen are regarded as the most promising biocontrol agents for ACB (Zang et al. 2021, Wang et al. 2022). For enhancing the efficiency of *Trichogramma*, multiparasitism is the most promising approach (Zhang et al. 2021), which allows the oviposition of more than one parasitoid species in the same host individual (Resh & Carde 2009). Iqbal et al. (2021) recently reported the feasibility of culturing of *T. ostriniae* on eggs of *Antheraea pernyi* Guerin-Meneville through multiparasitism with *Trichogramma chilonis* Ishii. The findings demonstrated that *Trichogramma* parasitoids could effectively manage the ACB population that reproduced through multiparasitism on *A. pernyi* hosts. Additionally, the *T. ostriniae* parasitoids that were reared in the factitious host *A. pernyi* had large size, higher fecundity, lived for longer period, and parasitized more ACB eggs than the *T. ostriniae* that were raised on the RM. Surprisingly, multiparasitism is a corresponding interspecific competition that impacts

the parasitoid's fitness (Cusumano et al. 2016). Furthermore, the parasitoid progeny engages in interspecific competition for resources throughout their larval and adult life stages, which results in reduced survival and reproductive output (Cusumano et al. 2016). Besides, interspecific competition, the intraspecific competition also exists in parasitoids (Couchoux & van Nouhuys 2014). Self-superparasitism occurs when trichogrammatid females deposit more than one egg per host, thereby exposing their progeny to intraspecific competition for resources (DaSilva et al. 2016). Morris & Blackwood (2015) posited that intraspecific competition is more intense than interspecific competition because individuals of the same species possess comparable biological requirements. Furthermore, intraspecific competition substantially influences the biological traits of *Trichogramma atopovirilia* Oatman & Platner's offspring (DaSilva et al. 2016). Consequently, the survivorship of *T. atopovirilia* progeny that emerges from the super-parasitized host is lower than that of offspring from the singly parasitized host.

This laboratory study determined the intraspecific and interspecific competition between *T. dendrolimi* and *T. ostriniae* for the eggs of ACB and RM, with a particular emphasis on the competition between the two parasitic wasps and to find out how this intra or interspecific competition can influence the fitness of parasitoids. Future studies could focus on field-based experiments to assess intraspecific and interspecific competition under natural conditions and explore the genetic and environmental factors influencing parasitoid fitness. Long-term assessments could help understand the broader ecological impacts on parasitoid populations.

MATERIALS AND METHODS

PARASITOIDS

Trichogramma dendrolimi and *T. ostriniae* adults were collected from the parasitized ACB eggs found in maize fields in Changchun, Jilin Province, China. Electronic microscope micrographs were employed to identify both species from the male genital capsule (Pinto 1992) and the identification was subsequently confirmed through rDNA-ITS2 sequence analysis (the gene bank accession numbers for *T. dendrolimi* and *T. ostriniae* are FR750279 and HE648326, respectively) (Stouthamer et al. 1999). The voucher specimens were deposited at the Institute of Biological Control, Jilin Agricultural University (China). The initial parasitization capacity of these insects was maintained by rearing *Trichogramma* parasitoids on RM eggs in an incubator (MLR-351H; Sanyo Corporation, Osaka, Japan) under controlled conditions (70 ± 5% RH, 25 ± 2 °C, L14: D10). After five generations of

continuous rearing on RM eggs, *T. dendrolimi* and *T. ostriniae* colonies were maintained on their native host (ACB eggs) till F_1 generation.

HOSTS

ASIAN CORN BORER (ACB), *OSTRINIA FURNACALIS*

To obtain ACB host eggs for the experiment, rearing of the moths was carried out at the Biological Control Laboratory, College of Agriculture, Jilin Agricultural Science and Technology University, China. ACB larvae were kept under laboratory conditions whitin a climate chamber set at 25 ± 2 °C, 70 ± 5% RH and a photoperiod of L14:D10 h. The artificial diet for rearing ACB larvae was consisted of 300 g of wheat bran, 100 g of yeast, 8 g of methyl 4-hydroxybenzoate, 8 g of sorbic acid, 8 g of ascorbic acid, 50 µL of linoleic acid, 28 g of sucrose, 30 g of agar, and 1500 ml of water. The artificial diet was provided to the ACB larvae in plastic containers (23 × 23 × 23 cm). After pupation, the larvae were housed in a cage (35 × 35 × 35 cm; Bugdorm-I, Taiwan, China) under stable conditions (65 ± 5% RH, 25 ± 2 °C, with a photoperiod of L14: D10 h). After the moths emerged, 10% honey solution was put in the cage along with hanging strips of wax paper for oviposition. After being cut with a scissor, the egg masses were put in a climate room until they reached a specific age (4 h) needed for the experimentation.

RICE MOTH (RM) *CORCYRA CEPHALONICA*

To obtain RM host eggs for the experiment, a moth rearing was developed at the biological control laboratory, College of Agriculture, Jilin Agricultural Science and Technology University, 132101 Jilin, China. RM larvae were kept under laboratory conditions, using a climate chamber with 25 ± 1 °C, 70 ± 5% RH, and a photoperiod of L14:D10 h. The artificial diet for rearing RM larvae consisted of 500 g of maize flour, 142 g of wheat bran, 21 g of yeast, 49 g of sucrose, and 200 ml of distilled water (Pinto et al. 2019). The artificial diet was provided to the RM larvae in plastic containers (23 × 23 × 23 cm). After pupation, the larvae were housed in a cage (35 × 35 × 35 cm; Bugdorm-I, Taiwan, China) under stable conditions (65 ± 5% RH, 25 ± 2 °C, with a photoperiod of L14: D10 h). After emergence, the RM adults were collected and moved to mesh cages (35 × 22 × 44 cm; Bugdorm-I, Taiwan, China) for oviposition. The cages were put on an egg collecting tray. The oviposition was monitored regularly, and eggs on the cage walls were swept into the tray using a collecting brush. The obtained RM eggs were screened using a 0.5 mm screening

net to remove debris and scales, yielding clean eggs. These eggs were kept in a climate room until they reached a specific age (4 h) needed for the experimentation.

INTRASPECIFIC COMPETITION

To investigate the intraspecific competition between the individuals of same *Trichogramma* species, the ACB and RM eggs (aged 0-4 h) were parasitized separately under controlled laboratory conditions (*i.e.* 65 ± 5% RH, 25 ± 2 °C and a photoperiod of L14: D10 h). For this purpose, one or two newly emerged (12 h old) mated adult females of *T. dendrolimi* were introduced separately into two glass tubes (10 × 3 cm, length × diameter; Shanghai Allcan Medical Co., Ltd, China) comprising ACB egg cards (90 eggs per card). Earlier studies by Schöller et al. (2006) and Wang et al. (2022) suggested that a single *Trichogramma* female wasp can parasitize up to 40–50 host eggs during her adult life span of 3–14 days. Consequently, 90 ACB eggs are deemed adequate to satisfy the parasitization requirement of two wasps for 24 h.

Each test tube contained a 20% honey solution on a cotton wick as sustenance for adult parasitoids. Furthermore, in two separate glass tubes containing RM egg cards (200 eggs per card), one and two newly emerged (12 h old) mated mature females of *T. dendrolimi* were introduced, respectively. The same arena was established for the *T. ostriniae*. The *Trichogramma* was removed from each glass tube after 24 h of parasitism, and the parasitized egg cards were transferred to MLR-351H (Sanyo Corporation, Osaka, Japan) for development under stable conditions (*i.e.* 70 ± 5% RH, 25 ± 2 °C, photoperiod of L14: D10 h). After five days, the quantity of parasitized eggs (*i.e*. those distinguished by a dark color) was recorded after the egg masses of each treatment were observed under a stereomicroscope (Leica S6, Leica Microsystems, Wetzlar, Germany). After examination, the parasitized egg cards were returned to the incubator. The emergence of adult parasitoids was monitored daily, and the quantity was recorded until complete emergence. The number of dead parasitoids was determined by dissecting the unhatched eggs. All treatments were replicated fifteen times. The expected number of parasitized eggs were compared to the actual number of parasitized eggs based on the following formula;

$$\text{Expected number of eggs parasitized by a specific parasitoid} = 2 \times \text{actual number of eggs parasitized by the parasitoid alone}$$

INTERSPECIFIC COMPETITION

To investigate the interspecific competition between *T. dendrolimi* and *T. ostriniae*, the ACB and RM eggs (aged 0–4 h) were parasitized under controlled conditions (*i.e.* 65 ± 5% RH, 25 ± 2 °C, and a photoperiod of L14: D10 h). For single parasitism, one newly emerged (12 h old) mated adult female of *T. dendrolimi* was dropped into a glass tube (10 × 3 cm, length × diameter; Shanghai Allcan Medical Co., Ltd., China), which contained an ACB egg card (90 eggs). Similarly, one female from *T. ostriniae* was placed in a separate glass tube with another ACB egg card (90 eggs). Furthermore, for co-parasitism, a newly emerged (12 h old) mated adult female wasp from both species (*T. dendrolimi* and *T. ostriniae*) was placed together in the same glass tube (10 × 3 cm, length × diameter; Shanghai Allcan Medical Co., LTD), with one ACB egg card containing 90 eggs.

The similar above-described arena was also established for both *Trichogramma* species to parasitize RM eggs (200 eggs per card). Adult parasitoids were fed with a honey solution (20%) on a cotton wick in each test tube. Both *Trichogramma* species were removed from each glass tube after 24 h of parasitism, and the parasitized egg cards were transferred to MLR-351H (Sanyo Corporation, Osaka, Japan) for development under stable conditions (*i.e.* 70 ± 5% RH, 25 ± 2 °C and photoperiod of L14: D10).

After five days, the quantity of parasitized eggs (those differentiated by black color) was recorded after the egg masses of each treatment were inspected under a stereomicroscope (Leica S6, Leica Microsystems, Wetzlar, Germany). The parasitized egg cards were returned to the incubator after they were examined. The emergence of adult parasitoids was monitored daily, and the number was recorded until complete emergence. The *T. dendrolimi* and *T. ostriniae* were identified with the help of morphological description provided by Myint et al. (2021).

The unhatched eggs were dissected to ascertain the number of parasitoids that had died. All treatments were replicated fifteen times. The expected number of parasitized eggs were compared to the actual number of parasitized eggs based on the following formula;

$$\begin{aligned}\text{Expected number of eggs parasitized by } & T.\,dendrolimi \text{ and } T.\,ostriniae \\ &= \text{actual number of eggs parasitized by } T.\,dendrolimi \\ &+ \text{actual number of eggs parasitized by } T.\,ostriniae\end{aligned}$$

STATISTICAL ANALYSIS

Data were analyzed by the statistical program Statistix 8.1® (Analytical Software, Tallahassee, FL, USA). The data regarding the number of ACB and RM eggs parasitized by two *Trichogramma* species, the number of emerged offspring of both species, and the number of dead offspring were analyzed using factorial analysis of variance (ANOVA), and treatment means were further compared by Tukey's honestly significant difference (HSD) test at a 95% confidence interval. Furthermore, the figures were plotted using SigmaPlot 12.5 software.

RESULTS

IMPACT OF INTRASPECIFIC COMPETITION ON PARASITIZATION

By comparing the expected and actual numbers of the parasitized eggs of different hosts (ACB and RM eggs) by two *Trichogramma* species, it was found that there was no significant difference between ACB eggs ($F_{1,28} = 0.545$, $p = 0.4662$) and RM eggs ($F_{1,28} = 1.095$, $p = 0.3041$), parasitized by *T. dendrolimi* (Fig. 1a). Therefore, no significant intraspecific competition was existed between the individuals of *T. dendrolimi* on the two host eggs.

Similarly, comparing the expected and actual number of ACB and RM eggs parasitized by *T. ostriniae*, no significant difference was observed between ACB eggs ($F_{1,28} = 0.205$, $p = 0.6538$) and RM eggs ($F_{1,28} = 0.306$, $p = 0.5841$) parasitized by *T. ostriniae* (Fig. 1b). Therefore, no significant intraspecific competition was existed between the individuals of *T. ostriniae* on the two host eggs. As a result, we did not analyze this competition's impact on progeny's emergence and death.

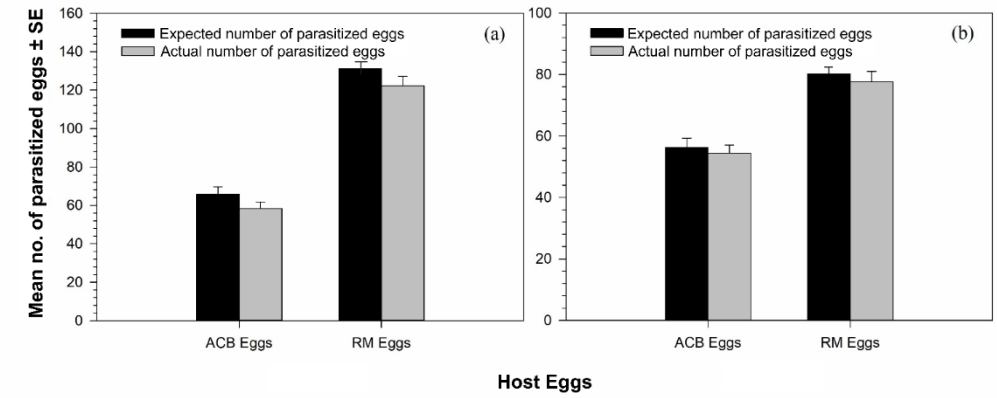


Figure 1. Determination of the expected and actual number of two host eggs ± SE parasitized by **(a)** *T. dendrolimi* and **(b)** *T. ostriniae* separately (the paired bars with * indicate significant difference in means ($P < 0.05$, Tukey's HSD test), and no * indicates no difference in means).

INTERSPECIFIC COMPETITION AMONG *T. DENDROLIMI* AND *T. OSTRINIAE* PARASITOIDS

IMPACT OF INTERSPECIFIC COMPETITION ON PARASITIZATION

According to the study results, the actual number of ACB eggs parasitized by both *Trichogramma* species was lower than the expected number of parasitized eggs. However, a significant difference was found between the expected and actual number of parasitized eggs ($F_{1,28} = 69.96$, $p < 0.0001$) (Fig. 2). Consequently, it was revealed that interspecific competition occurred between the individuals of *T. dendrolimi* and the *T. ostriniae* when parasitizing ACB eggs. Nevertheless, the subsequent section describes the extent to which the influence of one *Trichogramma* species on the other occurs during interspecific competition. Additionally, the expected and actual number of RM parasitized eggs by both *Trichogramma* species were not significantly different ($F_{1,28} = 1.074$, $p = 0.3087$) (Fig. 2).

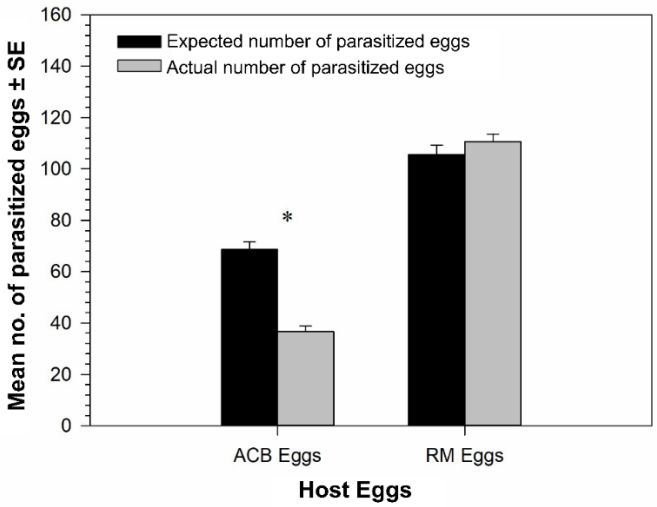


Figure 2. Determination of expected and actual number of a host eggs parasitized by both *T. dendrolimi* and *T. ostriniae* (the paired bars with * indicate significant difference in means ($p < 0.05$, Tukey's HSD test), and no * indicates no difference in means).

IMPACT OF INTERSPECIFIC COMPETITION ON OFFSPRING EMERGENCE OF PARASITOIDS

For offspring emergence of *T. dendrolimi* from ACB eggs, a significant difference was observed in single- and co-parasitism. When ACB eggs were parasitized by a *T. dendrolimi* (single parasitism), the number of emerged offsprings was significantly higher compared to offspring that emerged from the ACB eggs parasitized by both *T. dendrolimi* and *T. ostriniae* (co-parasitism) ($F_{1,28} = 46.15$, $p < 0.0001$) (Fig. 3a). In contrast, the *T. ostriniae* offspring from ACB eggs did not exhibit a

significant difference ($F_{1,28}$ = 0.395, p < 0.5385) between single- and co-parasitism (Fig. 3a). Comparing the two treatments of single- and co-parasitism on RM eggs, there was no significant variation between the emerged offspring of *T. dendrolimi* ($F_{1,28}$ = 1.659, p < 0.2086), and *T. ostriniae* ($F_{1,28}$ = 1.920, p < 0.1769) (Fig. 3b). Consequently, the results suggested that the interspecific competition between *T. ostriniae* and *T. dendrolimi* on ACB eggs substantially impacts the *T. dendrolimi* by reducing the emergence of its progeny.

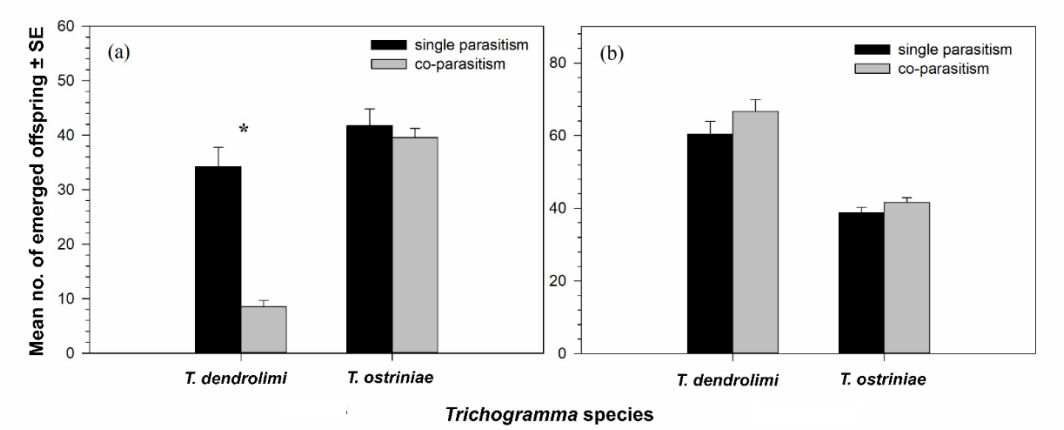


Figure 3. The emergence of offspring ± SE from two *Trichogramma* species on **(a)** ACB eggs and **(b)** RM eggs under single and co-parasitism (the paired bars with * indicate significant difference in means (p < 0.05, Tukey's HSD test), and no * indicates no difference in means).

INTERSPECIFIC COMPETITION CAUSED DEATH OF OFFSPRING OF BOTH PARASITOIDS

Results regarding the death of *T. dendrolimi* offsprings from ACB eggs showed a significant difference in single- and co-parasitism. When ACB eggs were parasitized by a *T. dendrolimi* wasp (single parasitism) alone, the number of dead parasitoids in the offspring was significantly higher than those observed in the ACB eggs when parasitized by both *T. dendrolimi* and *T. ostriniae* (co-parasitism) ($F_{1,28}$ = 18.20, p = 0.0002) (Fig. 4a). Conversely, the number of dead wasps in the progeny of *T. ostriniae* from ACB eggs did not demonstrate a statistically substantial disparity ($F_{1,28}$ = 0.0122, p = 0.9127) between single- and co-parasitism (Fig. 4a). The number of dead progenies of *T. dendrolimi* ($F_{1,28}$ = 1.320, p = 0.2602) and *T. ostriniae* ($F_{1,28}$ = 0.1027, p = 0.7509) was not significantly different between the two treatments of single- and co-parasitism on RM eggs (Fig. 4b). Consequently, the findings indicated that the death of *T. dendrolimi* progeny was significantly reduced by the interspecific competition between *T. ostriniae* and *T. dendrolimi* on ACB eggs.

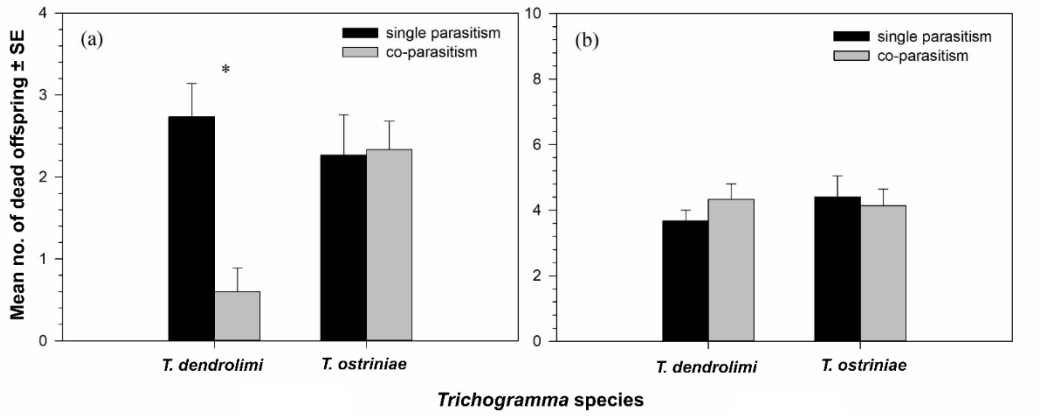


Figure 4. The number of dead offspring ± SE of two *Trichogramma* species from (a) ACB eggs and (b) RM eggs under single and co-parasitism (the paired bars with * indicate significant difference in means ($p < 0.05$, Tukey's HSD test), and no * indicates no difference in means).

DISCUSSION

This research work revealed the intraspecific and interspecific competition between *T. dendrolimi* and *T. ostriniae* on the eggs of two hosts *i.e.* ACB and RM. Comparison of the expected and actual numbers of the parasitized eggs of both host eggs by two *Trichogramma* species revealed that no significant impact of intraspecific competition between the individuals of *T. dendrolimi* and *T. ostriniae* on the parasitization. Our findings are consistent with those of Martel & Boivin (2004) who explored intraspecific competition among *Trichogramma minutum* Riley and *Trichogramma pinto* Voegele, and parasitization level and demonstrated a high male to female sex ratio of progeny by *T. pinto* in case of co-parasitism.

Second experiment was conducted using *T. dendrolimi* and *T. ostriniae* alone and in combination to estimate the influence of interspecific competition on parasitization, parasitoids' offspring emergence, and their death on ACB and RM eggs. The findings suggested that the interspecific competition between *T. ostriniae* and *T. dendrolimi* on ACB eggs significantly affected *T. dendrolimi* by reducing the emergence of its progeny. *Trichogramma* species are susceptible to interspecific competition because parasitoid larvae compete for host resources (Luo et al. 2014, Menail et al. 2020). Shi et al. (2004) investigated the interspecific competition between two larval parasitoids, *Diadegma semiclausum* Hellen and *Costesia plutellae* Kurdjumov by exposing them to the 3rd instar larvae ofdiamondback moth, *Plutella xylostella* Linnaeus. The host larvae were analyzed after multiple parasitism, and the 1st instar larvae of *C. plutellae* were observed physically attacking the larvae of *D. semiclausum*. Subsequently, death increased and the emergence of *D.*

semiclausum was diminished. In contrast, in our study, when ACB eggs were parasitized solely by *T. dendrolimi* (single parasitism), the number of dead parasitoids in the offspring was significantly higher than when ACB eggs were parasitized by both *T. dendrolimi* and *T. ostriniae* (co-parasitism), indicating that co-parasitism reduced the death of *T. dendrolimi* offspring. It suggests that *T. ostriniae* may not physically attack a competitor species, which increases the survival of *T. dendrolimi*.

Moreover, the competitive capacity of mymarid egg parasitoids, *Gonatocerus fasciatus* Girault and *Gonatocerus ashmeadi* Girault was estimated by Irvin & Hoddle (2005) for a glassy-winged sharpshooter, *Homalodisca coagulate* Say. The results showed that the parasitism rate for *G. fasciatus* was less (10%) than that of *G. ashmeadi* (68%), indicating low parasitism rate and weak competitive ability of *G. fasciatus* larvae. Pedata et al. (2002) described the mechanism of the interspecific host competition between two endoparasitoids. For the purpose, they employed the *Encarsia formosa* Gahan and *Encarsia pergandiella* Howard alone and in combination for oviposition of the nymphs of whitefly, *Trialeurodes vaporariorum* Westwood under laboratory conditions. After dissecting the host nymph, the authors observed a larva of *E. formosa* attacking an egg of *E. pergandiella*. Because *E. formosa* embryos hatch before *E. pergandiella* eggs (necessitating an additional half day for hatching), and hence their emergence was ultimately affected. In this investigation, the emergence of *T. dendrolimi* was also affected by *T. ostriniae*. However, the assaulting behavior of these two *Trichogramma* species necessitates a future perspective of this investigation. The superiority of parasitoids also varies at their life stages; for instance, Mahmoud & Lim (2008) investigated the interspecific competition between *Trissolcus nigripedius* Nakagawa and *Telenomus gifuensis* Ashmead by exposing them to the eggs of seed sucking bug, *Dolycoris baccarum* Linnaeus. This study demonstrated that *Te. gifuensis* exhibited superiority in embryonic competition, likely due to a shortened egg incubation period following oviposition. Consequently, the progeny of *Te. gifuensis* reached the 1st instar at a quick rate than that of *Tr. nigripendius*. Whereas, *Tr. nigripendius* is a better competitor because of its natural behavior of guarding the host eggs even after oviposition against *Te. gifuensis*.

In addition to the detrimental impact of interspecific competition on the ability of parasitoids to be parasitized, ChaoRan et al. (2016) found that this competition had a positive effect on parasitism. Their findings indicated that the combined release of *Encarsia sophia* and *E. formosa*, compared to the release of *E. sophia* alone parasitized a higher number of the *T. vaporariorum*, indicating that interspecies competition is beneficial for enhancing the parasitism effect of *E. sophia*. Previous

evidence revealed that interspecific competition exists between various parasitoids, and in this experiment, *T. dendrolimi* was adversely affected by *T. ostriniae* during interspecific competition. However, further investigations are needed to assess the competitive ability of *T. ostriniae* over *T. dendrolimi* under natural conditions.

CONCLUSIONS

Based on the overall study results, it is concluded that single parasitism by *T. dendrolimi* on ACB eggs yielded better results than co-parasitism with *T. ostriniae*, likely due to reduced competition. However, the higher number of dead *T. dendrolimi* offspring in single parasitism raises questions about its fitness under different parasitism scenarios. In contrast, RM eggs showed no significant differences in parasitization or offspring survival between single and co-parasitism, indicating their reliability as a factitious host for mass rearing. These results emphasize the importance of selecting the appropriate *Trichogramma* species and host eggs for optimizing biological control programs. *T. ostriniae* appears more effective in competitive environments, making it a strong candidate for field applications against ACB. Further research should validate these findings under real-world conditions to refine integrated pest management strategies.

AUTHOR CONTRIBUTIONS

Conceptualization, Y.W., and C.Z.; Resources (Parasitoids), C.Z.; Methodology, Y.W., and C.Z.; Investigation, Y.W., and C.Z.; Data Curation, Y.W., C.Z., and K.S.A.; Formal Analysis, Y.W., C.Z., A.I. and K.S.A.; Visualization, Y.W., C.Z., K.S.A. and M.Z.M; Writing – Original Draft Preparation, Y.W., A.I., M.Z.M., K.S.A, and C.Z.; Writing – Review & Editing, C.Z., and K.S.A.; Supervision, Y.W; Funding Acquisition, Y.W., and C.Z. All authors have read and agreed to the published version of the manuscript.

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DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors upon request.

CONFLICTS OF INTEREST

The authors declare that they have no known competing interest or personal relationships that could have appeared to influence the work reported in this paper.

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