RESEARCH ARTICLE

Influence of Adult Diet and Cold Storage on the Biological Performance of *Telenomus remus* Nixon (Hymenoptera: Scelionidae) Against *Spodoptera frugiperda* (J.E. Smith)

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith), is a globally invasive pest posing serious threat~~s~~ to maize production. Augmentative biological control using the egg parasitoid *Telenomus remus* Nixon has emerged as a sustainable management strategy. The present study evaluated the influence of different adult feeding diets and cold storage duration on the biological performance of *T. remus* under laboratory conditions (25 ± 2 °C, 70 ± 5% RH, 14 h photoperiod). Four adult diets-honey, honey + water (1:1), probiotic diet, and a standard fall armyworm adult diet-were assessed for their effects on longevity, parasitism, adult emergence, and sex ratio of *T. remus* parasitoids. Honey and probiotic diets significantly enhanced parental parasitism (80.70 and 79.20 eggs, respectively), female longevity (6.70 and 6.40 days), and F_1 total parasitism (115.60 and 108.40 eggs), compared to other treatments. Adult emergence remained high (>96%) across diets, but the female-biased sex ratio was greatest in honey-fed parasitoids (0.78). Cold storage studies revealed that parasitized eggs stored at 4 °C for up to five days maintained high adult emergence (96.67-99.33%), ~~comparable to the control~~. However, emergence declined sharply beyond five days, dropping below 55% after seven days. The results demonstrate that carbohydrate-rich diets, particularly honey, significantly improve the immediate and transgenerational fitness of *T. remus*, while short-term cold storage (≤5 days) preserves parasitoid quality. These findings provide practical insights for mass rearing, storage, and field-release programs aimed at the sustainable management of *S. frugiperda* using *T. remus* egg parasitoids.

Keywords: *Fall Armyworm, Artificial diet, Egg parasitoid, Telenomus remus, Mass rearing, Refrigerated storage*

INTRODUCTION

The fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is a highly destructive pest that damages several crops and is considered a major threat to maize cultivation worldwide. More

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recently, maize production has been seriously threatened by the invasion of this pest, which has rapidly emerged as a dominant species across maize-growing regions. (Borgohain *et al.,* 2024). Being native to tropical and subtropical regions of the Americas (Sparks, 1979), *S. frugiperda* was first reported outside its native range in West Africa in 2016 (Goergen *et al.,* 2016). Since then, it has spread rapidly across Asia and other parts of the world, infesting over 353 plant species belonging to 76 botanical families, with a strong preference for maize (Suby *et al.,* 2020). The pest attacks a wide range of crops, including cereals, millets, legumes, oilseeds, sugarcane, cotton, vegetables, and ornamentals (FAO, 2018).

In India, the fall armyworm was first detected in maize fields at the College of Agriculture, Shivamogga, Karnataka, between early May and June 2018 (Sharanabasappa *et al.,* 2018). The larval stage is the most destructive phase of the pest, as larvae feed voraciously on all parts of the maize plant, *viz.,* leaves, stems, tassels, and cobs throughout the crop's phenological stages, causing extensive damage (Shylesha *et al.,* 2018). The economic and yield impacts of *S. frugiperda* are severe if left unmanaged. The pest is expected to cause an annual maize yield loss of 8.3–20.6 million tonnes, corresponding to 21–53% of total production, with an estimated economic loss of USD 2.5–6.2 billion (Day *et al.,* 2017). Its rapid spread across two continents within just three to four years highlights its potential to threaten global food and nutritional security (FAO, 2019).

The management of the fall armyworm remains a major challenge due to its high fecundity, lack of diapause, strong migratory ability, and rapid multiplication under favorable climatic conditions, which facilitate population build-up (Borgohain *et al.,* 2024). A wide range of strategies have been employed for its management in maize, including agronomic practices such as early sowing, intercropping, trap cropping, and conservation tillage; technological interventions such as pheromone traps and host plant resistance; and chemical control using selective insecticides (Kumar *et al.,* 2022). Although insecticides provide rapid suppression, their excessive use has raised serious concerns regarding the development of resistance, non-target effects, and environmental risks (FAO, 2018).

In this context, biological control offers an ecologically sustainable and long-term alternative. Among the natural enemies reported against *S. frugiperda*, parasitoids are particularly important owing to their host specificity, efficiency, and suitability for mass rearing and field release. More than 100 parasitoid species have been recorded worldwide, with several already evaluated in India, including *Telenomus remus* Nixon, *Trichogramma pretiosum* Riley, *Cotesia marginiventris* (Cresson), *Coccygidium melleum* (Roman), *Chelonus insularis* (Cresson), *Glyptapanteles creatanoti* (Viereck), *Exorista sorbillans* (Wiedemann), and *Tachina sobria* (Walker) (Shylesha *et al.,* 2018).

Egg parasitoids play a crucial role in augmentative biological control programs, as they attack the pest at an early developmental stage, thereby preventing large-scale crop damage (Parra *et al.,* 2019). Among the various egg parasitoids, *T. remus* (Hymenoptera: Scelionidae), native to Peninsular Malaysia and Papua New Guinea (Wengrat *et al.,* 2021), stands out for its remarkable efficacy against several lepidopteran pests, particularly those in the genus *Spodoptera* (Colmenarez *et al.,* 2022). Field studies have reported parasitism rates of *S. frugiperda* egg masses ranging from 33% to 100% (Liao *et al.,* 2019; Agboyi *et al.,* 2021). Moreover, *T. remus* exhibits strong dispersal ability, successfully parasitizing egg masses up to 150-400 m from release sites (Agboyi et al., 2021), and is capable of parasitizing eggs in multiple layers, including those within the inner portions of egg masses (Bueno *et al.,* 2008).

Owing to its high fecundity, strong host-finding behavior, and adaptability, *T. remus* has emerged as a promising agent in the sustainable management of FAW. However, to enhance its field performance and facilitate large-scale use, it is essential to improve its efficacy and assess its storability for use in integrated pest management (IPM) programs. Keeping this in view, the present study was undertaken to evaluate the influence of different diets on the longevity, fecundity, and parasitism efficiency of *T. remus* and to assess the storage potential of *T. remus* under refrigerated conditions (4 °C) for varying durations.

MATERIALS AND METHODS

The ~~present~~ was carried out at the Fall armyworm Laboratory, Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore. The experiments were conducted under controlled laboratory conditions at 25 ± 2 °C, 70 ± 5 % relative humidity, and a 14 h photoperiod.

Mass culturing of fall armyworm, *S. frugiperda*

Mass culturing of *S. frugiperda* was done in the Fall armyworm Laboratory, Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore. To initiate the culture, field-collected larvae were individually reared in plastic containers measuring 30 × 40 × 40 ~~mm~~ following the method described by Bueno *et al.* (2008). The lids of the containers were perforated with 12-mm-diameter holes and covered with fine synthetic mesh to allow adequate ventilation. The larvae were fed with an artificial diet until pupation. Upon pupation, the pupae were transferred to adult emergence cages (30 × 30 × 30 cm).

Emerging adults were sexed and released in pairs into oviposition cages for mating. Sugar solution was provided as a food source for adults, and *Nerium* leaves were used as an oviposition substrate because they remain green and intact for about 5-6 days without losing turbidity when their cut ends are placed in water, offering a longer shelf life than maize leaves. Eggs laid on the leaves were carefully collected and transferred into transparent plastic boxes (17 × 11 × 5 cm) containing artificial diet cut into small pieces. Late second- or early third-instar larvae were transferred to individual containers to avoid cannibalism (Shu *et al.,* 2024) and fed an artificial diet and reared until pupation. This culture was maintained continuously and served as the source for subsequent experimental studies

Mass culturing of egg parasitoid, *Telenomus remus*

The culture of *T. remus* is being maintained at the Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore. Adult parasitoids were provided with honey using cotton swabs and allowed to mate for 24 h. Freshly laid egg masses (< 24 h) of the fall armyworm were affixed to paper strips (3 × 5 cm) using a thin layer of gum *Acacia*, with 5-6 egg masses per strip. These strips were then shade-dried at room temperature for 10 to 15 minutes. The prepared egg cards were placed inside plastic containers (18 × 7 × 7 cm) for parasitization by mated *T. remus* females, maintaining a host-parasitoid ratio of 40:1, as described by Pomari *et al.* (2013). Fresh egg cards were introduced every 24 hours until the death of all adult parasitoids. The parasitized egg cards were kept separately to facilitate parasitoid emergence. This method ensured the continuous maintenance of the parasitoid culture for experimental use (Priyanka *et al.,* 2023).

Evaluation of Optimal Adult Feeding Diet for *Telenomus remus*

The experiment was conducted using a Completely Randomized Design (CRD) comprising four adult feeding treatments (Table 1), with ten replications per treatment.

Treatments	Details
T1	Honey
T2	Honey + water (1:1 ratio)
T3	Probiotic diet (Fructose, yeast, honey, Protein X, water, vitamin E capsule at 1:1:1:1:1 ratio)
T4	Standard Fall Armyworm (FAW) adult diet containing ascorbic acid (0.5 g), sucrose (30 g), methyl *p*-hydroxy benzoate (0.5 g dissolved in 5 ml ethanol), honey (5 ml), and Zincovit (5 ml)

Approximately 100 parasitized FAW eggs [6 days after parasitization (DAP)] were affixed onto paper strips (1 × 5 cm) using a thin coating of gum *Acacia* and air-dried for 10-15 min. Each egg card was placed individually in a clean, labeled glass test tube (15 × 2.5 cm), and the opening was covered with nylon cloth secured with a rubber band. The respective diet for each treatment was smeared onto the nylon cloth to serve as an adult food source. Adult emergence occurred on the 10^{th} day, after which the emerging parasitoids were allowed to feed and mate within the same test tube for 24 h.

To assess the influence of the different diets on the parental generation, fresh (~24 h old) *S. frugiperda* eggs (~150) were pasted onto 1 × 5 cm paper strips using gum *Acacia* and air-dried for 10-15 min to prepare egg cards. A single mated *T. remus* female from each treatment was introduced into a single labeled test tube (10 cm × 1.5 cm) containing one FAW egg card. The test tube was covered with nylon cloth smeared with the respective diet and maintained inside a climatic chamber at 25 ± 2 °C, 70 ± 5 % RH, and a 14 h photoperiod. Parasitization was allowed for 24 h, after which the parasitized egg cards were removed and transferred to fresh test tubes. The parasitized egg cards were monitored daily for three days, and any hatched neonates from unparasitized eggs were carefully removed using a camel hair brush to prevent damage to neighboring parasitized eggs. On the 5th day after parasitization, the number of parasitized eggs was recorded by egg darkening, followed by observation of adult emergence, after which per cent adult emergence and sex ratio were also documented.

To assess the influence of the different diets on the offspring generation, the same protocol was followed. In this phase, fresh FAW egg cards were provided daily until each female's death to determine lifetime fecundity. Diets and egg cards were replaced every 24 h. Ten replications were maintained per treatment. Observations recorded from the progeny generation included female longevity, total parasitism, and per cent adult emergence.

Female longevity: Life span of the female parasitoid (in days)

Total parasitism: Total number of host eggs parasitized per female during her lifetime

Per cent adult emergence: (Number of adults emerged / Number of eggs parasitized) *100

Sex ratio: (Females/(Females+males))

Storability of parasitized eggs

The experiment was conducted to determine the maximum duration for which *T. remus*-parasitized *S frugiperda* eggs can be stored at low temperature (4 °C) without compromising adult emergence, thereby identifying the ideal storage period for field distribution. Ten storage durations (1-10 days) were evaluated, each representing a treatment, with three replications. For each replication, 50 parasitized FAW eggs (7 days old) were placed inside glass vials (8 × 1.6 cm), covered with nylon cloth, and stored in a refrigerator at 4 °C. On each designated storage day, vials corresponding to that treatment were removed from cold storage, brought to room temperature, and monitored for adult emergence. This procedure was followed for all storage durations up to 10 days. Per cent adult emergence was recorded for each treatment to assess the viability of stored parasitized eggs.

Statistical analysis

Experiments were conducted under controlled laboratory conditions following a completely randomized design (CRD). Percentage data obtained from different experiments were subjected to arcsine transformation, while numerical count data were normalized using square root transformation before analysis. Statistical analyses were performed using IBM SPSS Statistics, version 21.0. Treatment means were compared using Tukey's test at a significance level of $P = 0.05$.

RESULTS AND DISCUSSION

Effect of Adult Feeding Diet on Parental Performance

Adult nutrition significantly influenced the biological performance of *T. remus* under laboratory conditions. Among the diets evaluated, honey (T1) resulted in the highest parasitism within 24 h (80.70 eggs), which was not statistically different from the probiotic diet (T3; 79.20 eggs). The honey + water diet (T2) recorded moderate parasitism (66.10 eggs), whereas the FAW adult diet (T4) resulted in significantly lower parasitization (37.90 eggs). Adult emergence followed a similar pattern, with maximum emergence observed in the honey diet (99.01%), followed by the probiotic diet (98.11%) and the honey + water diet (97.88%), which were statistically comparable. Although the FAW adult diet recorded a slightly lower emergence (96.83%), it remained above 95% across all treatments. The female-biased sex ratio was highest in honey (0.78), on par with the probiotic diet (0.76) and honey + water (0.72), whereas the FAW adult diet resulted in the lowest female proportion (0.70). The higher female ratio under carbohydrate-rich diets indicates improved reproductive allocation towards daughters, a desirable trait in augmentative biological control (Table 1) (Figure 1).

Our findings clearly demonstrate that carbohydrate-rich diets, particularly honey and probiotics, enhance parasitization efficiency and progeny quality. These results are in agreement with Lü *et al.* (2024), who reported that honey feeding significantly increased longevity (11.37 days) and lifetime parasitism (117.91 eggs) in *T. remus* compared to unfed adults. Similarly, Sutil *et al.* (2025) demonstrated improved survival and effective release performance with honey-based diets. Comparable trends have been documented in *Trichogramma* spp., in which honey- and sugar-based diets significantly improved longevity and fecundity (Leatemia *et al.,* 1995; Gurr & Nicol, 2000; Tunçbilek *et al.,* 2012; Bari *et al.,* 2016; Wang *et al.,* 2022). Thus, our results are consistent with previous studies highlighting the importance of readily available

Table 1: Effect of different artificial diets on the parental generation

Treatments	Eggs parasitized in 24h (no.)*	%Adult emergence#	Sex ratio*
T1 (Honey)	80.7 ± 0.06 (9.01)[a]	99.01±1.06 (85.27)[a]	0.78±0.01 (1.44)[a]
T2 (Honey + Water)	66.1±0.11 (8.15)[b]	97.88±1.65 (83.18)[a]	0.72±0.01 (1.11)[ab]
T3 (Probiotics)	79.2±0.09 (8.92)[a]	98.11±1.02 (82.74)[a]	0.76±0.01 (1.13)[ab]
T4 (FAW adult diet)	37.9±0.22 (6.16)[c]	96.83±2.03 (81.29)[a]	0.70±0.01 (1.10)[b]
SE.d	0.20	2.33	0.01
CD (5%)	0.42	4.79	0.02

All values are the mean ± SE of ten replications

Values in parentheses are square root transformed values*

Values in parentheses are arcsine-transformed values#

Means followed by a common alphabet do not differ significantly by Tukey's test, P = 0.05

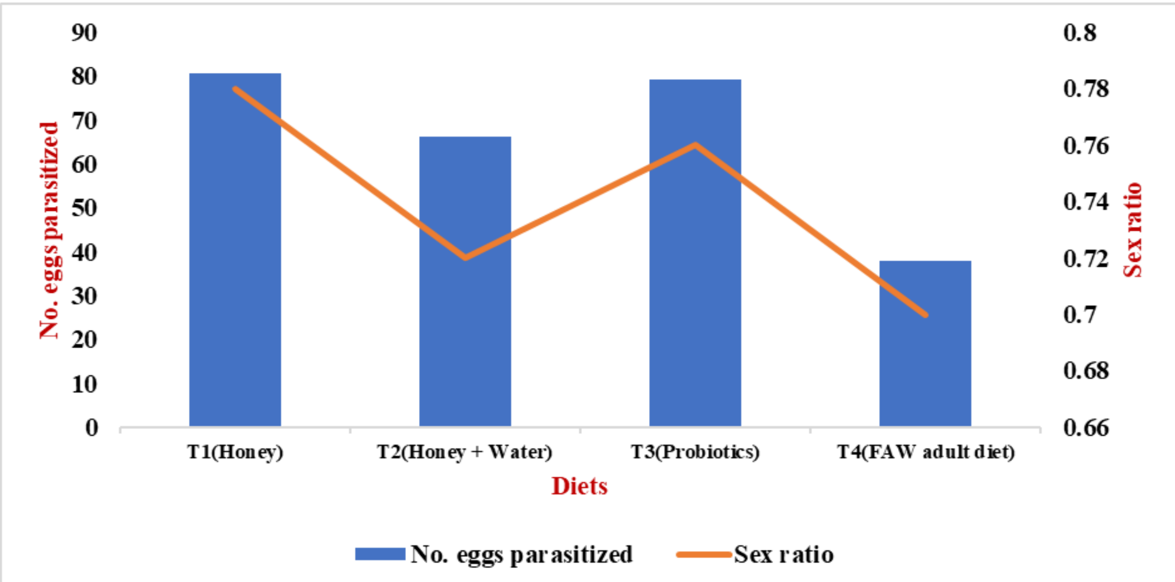

Figure 1: Effect of different artificial diets on the parental generation

carbohydrates in sustaining parasitoid energy metabolism and egg maturation.

Effect of Adult Feeding Diet on F_1 Generation

Provision of superior diets to the parental generation had a pronounced transgenerational effect on F_1 performance. Female longevity was highest in honey-fed adults (6.70 days), which was statistically on par with probiotics (6.40 days), followed by honey + water (4.70 days). The FAW diet resulted in significantly shorter longevity (2.60 days). Total parasitism in the F_1 generation was also highest in honey (115.60 eggs) and probiotics (108.40 eggs), which were on par with each other, followed by honey + water (90.10 eggs). The FAW diet recorded the lowest parasitization (34.60 eggs). Adult emergence remained high across treatments, with honey (99.04%) and probiotics (98.15%) performing significantly better than the FAW diet (96.24%) (Table 2) (Figure 2).

These findings indicate that parental nutrition directly influences progeny fitness, likely through enhanced maternal resource allocation. Similar enhancements in fecundity and population parameters following honey feeding have been reported in *Trichogramma* spp. (Leatemia *et al.,* 1995; Wang *et al.,* 2022). Therefore, our study confirms that carbohydrate-rich diets not only improve immediate adult performance but also enhance subsequent generational fitness, strengthening the overall biological control potential of *T. remus*.

Evaluation of Storability of Parasitized Eggs

Cold storage significantly influenced adult emergence from parasitized eggs. Eggs stored for 1-5 days maintained high adult emergence (96.67-99.33%), which was statistically on par with the control (99.63%), indicating that short-term storage does not adversely affect parasitoid viability. However, a significant decline was observed beyond five days, with emergence decreasing to 78.00% (6 days), 54.67% (7 days), 28.67% (8 days), 4.67% (9 days), and 3.33% (10 days) (Table 3) (Figure 3).

Table 2: Effect of different artificial diets on the offspring generation (F1)

Treatments	Female longevity (Days)*	Total parasitism (F1) (no.)*	% Adult emergence#
T1 (Honey)	6.70 ± 0.07 (2.67)[a]	115.60 ± 0.13 (10.77)[a]	99.04 ± 1.11 (85.52)[a]
T2 (Honey + Water)	4.70 ± 0.11 (2.25)[b]	90.10 ± 0.12 (9.51)[b]	97.78 ± 1.00 (81.90)b
T3 (Probiotics)	6.40 ± 0.13 (2.60)[ab]	108.40 ± 0.10 (10.43)[a]	98.15 ± 0.51 (82.35)[ab]
T4 (FAW diet)	2.60 ± 0.08 (1.74)[c]	34.60 ± 0.15 (5.91)[c]	96.24 ± 0.80 (78.98)[b]
SE.d	0.14	0.19	1.25
CD (5%)	0.28	0.38	2.57

All values are the mean ± SE of ten replications

Values in parentheses are square root transformed values*

Values in parentheses are arcsine-transformed values#

Means followed by a common alphabet do not differ significantly by Tukey's test, P = 0.05

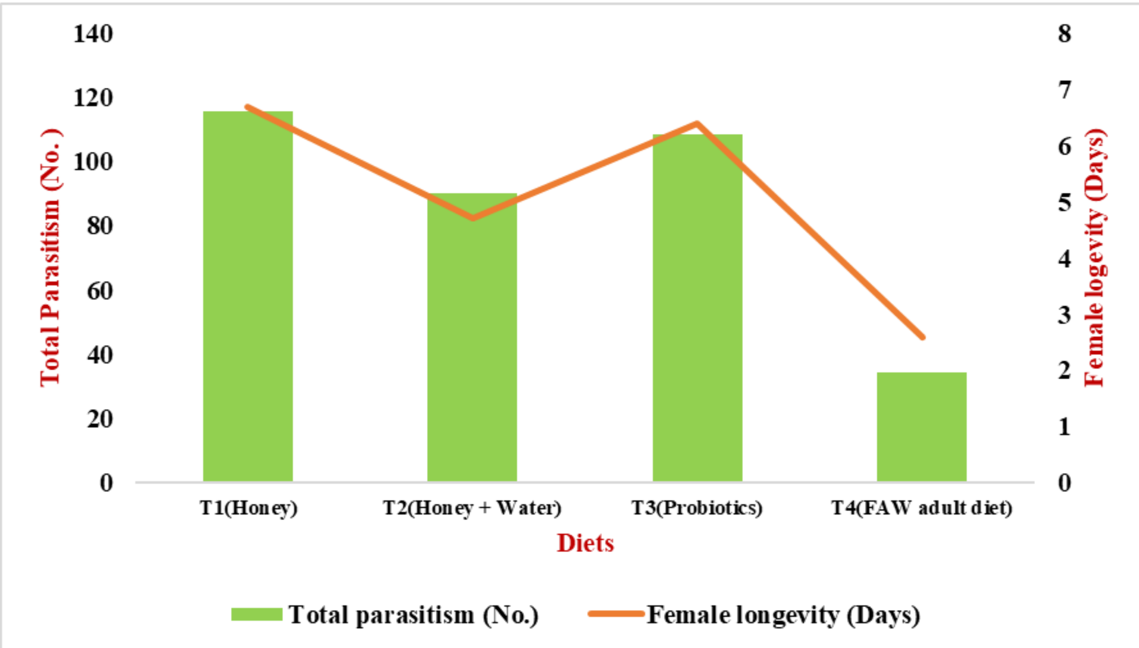

Figure 2: Effect of different artificial diets on the offspring generation (F1)

Table 3: Effect of storage duration of parasitized eggs on adult emergence of *T. remus*

Storage duration of parasitized eggs (days)	%Adult emergence#
1 day	99.33± 2.71 (87.29)[a]
2 days	98.67 ±2.71 (84.58)[a]
3 days	98.00 ±3.42 (83.44)[a]
4 days	98.00 ±3.42 (83.44)[a]
5 days	96.67 ±1.13 (79.60)[a]
6 days	78.00±1.60 (62.09)[b]
7 days	54.67±5.82 (47.75)[b]
8 days	28.67±2.27 (32.28)[c]
9 days	4.67 ±2.01 (12.16)[d]
10 days	3.33±2.01 (10.15)[d]
Control	99.63±1.13 (87.53)[a]
SE.d	4.42
CD (5%)	9.28

All values are the mean ± SE of three replications
Values in parentheses are arcsine-transformed values#
Means followed by a common alphabet do not differ significantly by Tukey's test, P = 0.05

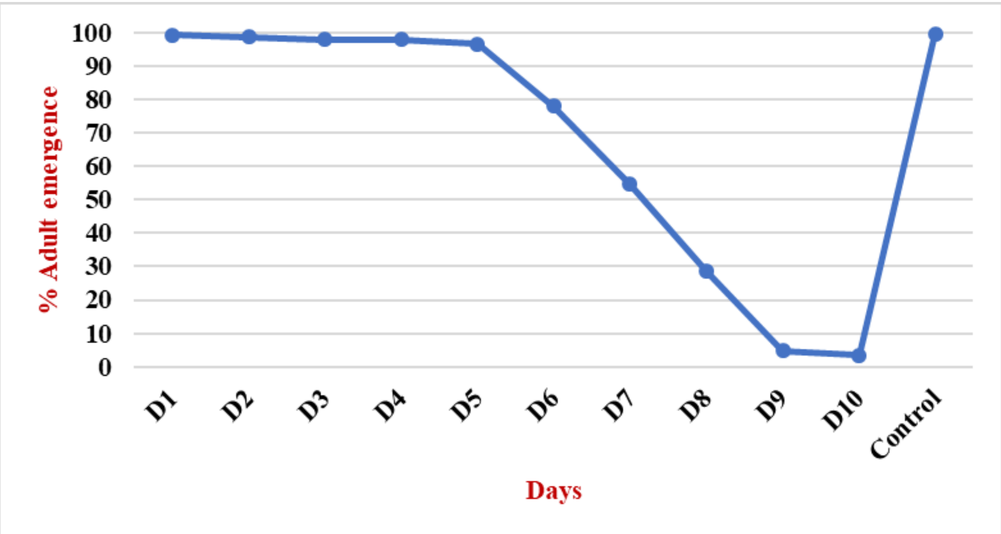

Figure 3: Evaluation of the storability of parasitized eggs

The sharp reduction in emergence after extended storage suggests increased physiological stress and potential disruption of parasitoid development. Our findings are consistent with previous reports indicating that prolonged cold storage negatively affects parasitoid emergence and parasitism capacity (Queiroz *et al.,* 2017; Chen *et al.,* 2022; Chen *et al.,* 2023; Qiu *et al.,* 2024). For instance, Queiroz *et al.* (2017) recommended limiting storage of *T. remus* pupae to 7 days at 10 °C to maintain quality, while Qiu *et al.* (2024) reported reduced reproductive capacity when host eggs were stored beyond optimal durations. Similar reductions in parasitism and emergence with extended storage have been documented in related species such as *Telenomus busseolae* (Bayram *et al.,* 2005) and *Telenomus podisi* (Silva *et al.,* 2019).

Overall, our results indicate that parasitized eggs of *T. remus* can be safely stored for up to five days without compromising emergence, providing operational flexibility for the synchronization of mass production and field release schedules. However, storage beyond this threshold significantly compromises parasitoid quality.

CONCLUSION

The present study demonstrates that adult nutrition and storage duration play a crucial role in determining the biological efficiency of *T. remus*. Carbohydrate-rich diets, particularly honey, significantly enhanced parasitism, female longevity, progeny performance, and female-biased sex ratio. The probiotic diet also performed comparably to honey, indicating its potential as an alternative ~~formulation~~. However, it is important to note that the probiotic diet was evaluated only for two generations in the present study. Extended evaluation across multiple generations may be necessary to determine whether continuous feeding leads to improved acceptance, physiological acclimatization, or further enhancement in reproductive performance. Such long-term adaptation studies could provide deeper insights into the suitability of probiotics for sustained mass rearing.

Cold storage studies further revealed that parasitized eggs can be safely stored at 4 °C for up to five days without compromising adult emergence. Beyond this period, a marked decline in emergence was observed, indicating increased physiological stress and deterioration in parasitoid quality. These findings suggest that short-term refrigerated storage is operationally feasible for synchronizing mass production with field release schedules, while prolonged storage should be avoided.

Overall, the study provides practical insights for improving the efficiency of mass rearing and the storability of *T. remus*, thereby strengthening its application in sustainable management programs against *S. frugiperda*. Further validation under field conditions and long-term nutritional studies would support its large-scale integration into integrated pest management systems.

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This study did not require any ethical approval

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The authors confirm that the submitted manuscript is an original work and has not been published or submitted elsewhere. All relevant sources have been properly cited.

Consent for Publication:

All authors express their consent to publish the manuscript

Competing Interests:

The authors have no relevant financial or non-financial interests to disclose

Data Availability:

The data supporting the findings of this study are included within the manuscript. Additional data related to this study are available from the corresponding author upon reasonable request.

Author Contributions:

NMS drafted the manuscript and guided the conduct of the experiments. SM, GM, and MP performed the experiments. TS and PSS conceived the research concept and contributed to manuscript editing. ARR, SRS, and PM assisted in fine-tuning and correcting the manuscript. All authors read and approved the final version of the manuscript

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