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Influence of host egg age on parasitic potential of the entomophagous, *Telenomus remus* Nixon (Hymenoptera: Scelionidae) against the Fall armyworm, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) and investigations on the developmental biology and ultrastructure of egg parasitoid immature stages

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Abstract

Background Biological control through augmentative release of egg parasitoids is a potential tool in integrated management of field crop pests. The egg parasitoid, *Telenomus remus* Nixon (1937) (Hymenoptera: Scelionidae), is found promising against the fall armyworm, *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) in different parts of the world. However, fundamental information on the parasitic capabilities with reference to the host eggs of progressing ages is very limited. The investigations reported herein focused on the influence of host egg age on the parasitic potential, developmental biology and morphological features of the sexes of *T. remus* that could aid in decisions on their field release. The laboratory experiments were carried out with FAW egg masses of different ages (24, 48 and 72 h old). Host eggs glued to paper strips (3 × 5 cm) were offered to *T. remus* for parasitization at the parasitoid: host ratio 1:40 under standard laboratory conditions. Developmental biology of immature stages of *T. remus* and the adult sexual dimorphism were studied through stereomicroscopic and scanning electron microscopic examinations on 24 h old FAW egg masses parasitized by *T. remus*.

Result Parasitic potential of *T. remus* on FAW eggs of different ages indicated the highest level of parasitization on 24 h old eggs with 98.2% parasitization, followed by 48 h old eggs and 72 h old eggs of the host were avoided by the parasitoid female and hence no parasitism occurred. The developmental duration of *T. remus* on 24 h old FAW eggs was recorded as 9.61 days and 48 h old eggs as 9.52 days, respectively. Microscopic examinations revealed the presence of six immature bio-stages viz., egg (1 day), two larval instars (each 1 day), pre-pupal (1 day), pupal (5 days) and adult stage with a total developmental duration of 9 days. *T. remus* adults can be differentiated by the morphology,

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type and number of antennal segments. The scape or the basal antennal segment was slender and longer in males, whereas shorter in females. *T. remus* female has a club typed antenna with 11 segments, while male has geniculate antenna with 12 segments.

Conclusions The parasitoid exhibited selective preference for fresh stages of eggs than the matured ones indicating requirement of early field interventions coinciding with fresh brood emergence of FAW. Among the six stages of development, the pupal duration was found to be the longest in the study indicating that in augmentative biocontrol, host eggs with fully developed parasitoid stages, especially the pupal stage could be the appropriate one for field deployment.

Keywords *Spodoptera frugiperda*, Biological stages, *Telenomus remus*, Parasitization, Immature stages, Sexual dimorphism

Background

Invasive insect pests are serious threats to agriculture and are responsible for reduced crop production and productivity. Their impact is observed widely and attributed as principal causes for low economic returns. Due to increased transboundary movement of agricultural commodities, anthropogenic activities, climate change, etc., there has been large scale spread of exotic pests which is a serious concern on global scale through wide range of commodities (Evans et al. 2016). The Fall armyworm (FAW), *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) native to the tropical and subtropical regions of America is one among the devastating crop pests reported to cause extensive crop losses, especially on maize (Lungbill 1928). The results of literature reviews and surveys in the Americas reported that there were 353 FAW larval hosts plant records belonging to 76 plant families, principally Poaceae, Asteraceae, and Fabaceae (Montezano et al. 2018). Till 2015, this pest was not reported in any other part of the globe beyond its native ranges in Americas. In 2016, it was recorded in African countries causing serious damage on maize crop (Goergen et al. 2016). It has been declared as an invasive crop pest in Africa, Asia and Australia (Maino et al. 2021) and a risk to food security. In India, its occurrence was reported during 2018 in maize fields in the southern state of Karnataka (Sharanabasappa et al. 2018). After invasion, the pest has spread across the geographical locations of India causing major crop losses, especially to maize production (Prasanna et al. 2018).

Emergency responses were geared up with the use of chemical insecticides for the management of FAW on a large scale as a principal component in countries where FAW became yield limiting factor. Frequent application of insecticides as a selective management measure is unsustainable in the longer run due to the possibilities of resistance development and associated residues in the produces (Song and Swinton 2009). Biological control-based pest management offers ecologically sustainable and economically viable option against crop pests

in general. Among the biological control agents, parasitoids of crop pests play major role in maintaining the host population in a density dependant manner.

Telenomus remus Nixon (1937) (Hymenoptera: Scelionidae) an egg parasitoid of lepidopterous pests is native to peninsular Malaysia and Papua New Guinea (Wengrat et al. 2021). It has been utilized successfully in biological control programmes in several countries and especially against the genus *Spodoptera* (Ferrer 2021). *T. remus* was introduced for classical biological control of *Spodoptera* spp. in 1963 in India (Sankaran 1974). It is also reported as efficient against FAW (Fernandes et al. 2015). The parasitoid has been recorded associated with FAW in initial field surveys carried out in India (Shylesha et al. 2018). *T. remus* females have high fecundity rates, host searching ability, dispersal capacity and the parasitoids are also amenable for mass multiplication (Cave 2000). They can parasitize more than 250 eggs during their entire lifespan (Pomari et al. 2013). These characters make *T. remus* as an attractive biological control agent in the management of fall armyworm.

In applied biological control programs involving entomophagous, studies on biological parameters and amenability for mass rearing are prerequisites. In this context, knowledge on the host age preference and biology of immature stages is important to determine the laboratory or pilot scale production and timing of field release of parasitoids synchronizing with host broods for successful pest management. Limited information on the behavior of *T. remus* towards host eggs at different stages of embryonic development was reported by Ventura et al. (2001). Our investigations at the Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University were focussed on the studies involving the factors viz., age of the host eggs, parasitic potential of *T. remus* and developmental biology of immature stages of *T. remus* within the parasitized eggs of FAW through microscopic examinations.

Methods

Laboratory culture of *S. frugiperda*

The insects for the experiments were obtained from disease-free colony of FAW maintained at the Insect Culture Laboratory, Department of Agricultural Entomology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, India. The larval stages of FAW were originally collected from infested maize fields of University Research Farm during 2019 (11° 07' 33" N and 76° 59' 39"). The population of FAW was continuously maintained under laboratory conditions of 27 ± 2 °C with 12 h photoperiod and $70 \pm 5\%$ relative humidity for all experimental purposes.

Batches of eggs of FAW for the experiments were received from the parent stock and allowed for eclosion in rectangular plastic containers ($18 \times 7 \times 7$ cm) containing 10 days old maize seedlings (cultivar: CoHM 8) raised in hydroponics at the rearing facility. A day after, neonates belonging to the same batch along with the maize seedlings were transferred in groups to separate rectangular plastic boxes ($18 \times 7 \times 7$ cm) containing artificial diet CIMMYT (International Maize and Wheat Improvement Center) as larval feed (Tefera et al. 2010) and maintained up to early third instar stage in groups. Larvae showing uniform growth were selected from the group culture and transferred individually to sterile plastic containers ($3 \times 4 \times 4$ cm) with ventilated lids. These larvae were fed ad libitum with fresh CIMMYT artificial diet cakes. Matured larvae showing uniform growth based on visual examination were selected from the culture and allowed for pupation within the diet mass. The pupae were collected individually 5–6 days later, sexed based on the genital characters (Angulo and Jans 1982) and transferred to adult emergence cages ($30 \times 30 \times 30$ cm). The adults emerging on the same day were released into oviposition cages for mating at 1:1 sex ratio. Fifteen days old maize seedlings grown in hydroponics or oleander shoots were provided as oviposition substrate. The adults were fed with sugar and honey solution (1:1) supplemented with 100 µl of commercial grade multivitamin in cotton swabs. The eggs patches from the seedlings were collected again and the rearing cycle was continued.

Culturing of egg parasitoid, *T. remus*

The nucleus culture of *T. remus* for investigations was provided by the National Repository of National Bureau of Agricultural Insect Resources (NBAIR), Indian Council of Agricultural Research (ICAR), Bengaluru, India during 2020. A single consignment of parasitized egg cards of *T. remus* received through material transfer agreement methods served as source for experiments and the progeny population was maintained under laboratory conditions continuously at the Insect Rearing Laboratory

of the Department of Agricultural Entomology. The nucleus stock was kept in transparent plastic containers ($18 \times 7 \times 7$ cm) for parasitoid emergence. Adult feed on honey solution (50%) in the form of droplets on waxed paper strips (144gsm) was provided to the emerging adults. Groups of *T. remus* adults were allowed to mate randomly for a day, following emergence. A day later, they were given FAW eggs for oviposition. Day old eggs of FAW numbering 200 approximately in patches of 2 to 4 masses were glued to 144 gsm paper strips (3×5 cm) with thin layer of gum arabic/nontoxic glue and air dried for 5 to 10 min at room temperature. These egg cards were introduced into plastic containers for parasitization by mated females of *T. remus* at parasitoid: host ratio of 1: 40 (Pomari et al. 2013). The parasitized egg cards were replaced with fresh ones at 24 h interval for obtaining sufficient population of *T. remus* and discontinued when all *T. remus* adults died. The parasitized egg cards were maintained separately for parasitoid emergence and the culture was maintained continuously for experimental purpose under controlled conditions ($28 \pm$ °C, $65 \pm 10\%$ RH and 12:12 h (L: D) photoperiod).

Influence of host age eggs and parasitism by *T. remus*

The preference of *T. remus* for fresh and other age classes (<24 h, <48 and <72 h old) of FAW was determined under laboratory conditions in a completely randomized design. The treatments were replicated 13 times and each replicate in the test had 240 eggs of appropriate age class with six mated *T. remus* females at the host parasitoid ratio of 40:1.

Host eggs

The insects for the experiment were derived from the primary culture of FAW maintained during the studies. Pupae were sexed based on the morphological features under stereomicroscope (Leica™), segregated and transferred to adult emergence cages ($30 \times 30 \times 30$ cm). Each cage was stocked with 50 healthy pupae of similar age and same sex over wad of cotton wool held in Petri plates. This arrangement ensured that adults emerging overnight were of similar biological age without mating experience in sufficient numbers. Adult feed was provided in the cages daily to prevent starvation of emerging adults. Healthy adults from the population emerging during the scotophase were carefully collected the next day morning and transferred at the rate 25 pairs to mating and oviposition cages ($30 \times 30 \times 30$ cm) for egg laying. Maize seedlings arranged in bouquets and placed in a receptacle with potable water were provided as oviposition substrate and replaced with fresh ones at 12 h interval. Adult feed was provided daily ad libitum and replenished with fresh stock.

Eggs laid in masses during the night hours of third day were utilized in the experiment, while the first two days egg laying was utilized for host culture. FAW eggs stacked in layers and covered with tuft of scales were harvested from the oviposition substrate and the scales were removed with fine camel hair artist brush to expose naked eggs and counted under stereozoom-microscope (Leica™). Batches of forty eggs with general physical characteristics were selected, glued to egg card (144gsm) of size 3 × 5 cm, air dried and transferred to plastic containers (18 × 7 × 7 cm). Each replication had six egg laden cards in separate plastic containers vials for parasitization by *T. remus*. To assess the biological fitness of the FAW eggs used in the experiment, each replicate of the experiment had 40 eggs without exposure to parasitoids and hatchability was observed.

***T. remus* parasitoids**

FAW egg cards exposed previously to *T. remus* females at host: parasitoid ratio of 40: 1 were transferred to clear plastic cylindrical containers (1L) with aerated lid and allowed to emerge in groups. Honey droplets on waxed paper strips were provided as feed for emerging adults. Upon emergence of adults in sufficient numbers, the parasitized egg cards with left over imago were removed and this population was used for subsequent bioassay carried under controlled conditions (26 ± 2 °C, 70 ± 5% RH and 12L: 12 D photoperiod).

Laboratory bioassay

The freshly emerged *T. remus* adults were allowed to mate at random for a day within the same container and in the following day females were aspirated individually with simple pooters and transferred to clear plastic containers with FAW egg cards of different age classes. A day after parasitism under non-choice condition, the females were removed from the individual containers and the egg cards were examined at 12 h interval for shrivelled host eggs and FAW eclosion. Whenever host neonates were observed to emerge, they were removed appropriately. The host eggs were observed under stereozoom microscope for number of parasitized eggs on the succeeding days for working out the cumulative parasitism in treatments involving *T. remus*. The parasitoids in the treatments were allowed to emerge up to 10th day and the adults were collected for observation on the parameters viz., adult emergence and proportion of the sexes. In control the number of eggs hatched was counted at 12 h interval and the observation were completed on the 4th day to assess the fitness of the host eggs in the experiment. The observation in control indicated that the hatchability ranged between 97.5 and 100% in the

treatments with an average of 98.85%. The following calculations were made:

Per cent parasitization by *T. remus* = (No. of FAW eggs parasitized ÷ No. of FAW eggs exposed) × 100.

Per cent parasitoid adult emergence = (No. of adults emerged ÷ Mean No. of eggs exposed) × 100.

Morphology and ultrastructure of *T. remus*

Immature development stages

The development features of *T. remus* parasitic on FAW eggs and morphology of immature stages were studied by visual observation, stereo microscopy and scanning electron microscopy. Host eggs of age class < 24 h were allowed for parasitization at the host: parasitoid ratio of 40:1 as it was observed significantly the suitable stage in the laboratory bioassay. The numbers of FAW eggs within the egg patch were assessed based on procedure of Leuck and Perkins (1972) and the parasitoid females required were maintained based on the density of host eggs arrived in the present investigation. *T. remus* adults emerging freshly from the egg cards were allowed to mate for 24 h and the eggs were exposed in clear cylindrical plastic containers (18 × 7 × 7 cm) for a short period 4 h to obtain homogenous population of parasitoid stages for further evaluation. The FAW eggs at the end of exposure were transferred to spoutless glass beakers (50 ml), covered with food wrapping cling films and incubated under controlled conditions. Batches of FAW eggs allowed for parasitization were removed from this stock and dissected to expose the life stages of the parasitoids for further studies. The host eggs were examined 1, 2, 3, 4, 5, 6, 7 and 8 days after exposure at 24 h interval.

Stereomicroscopy

The FAW eggs were carefully removed from the patch and individual eggs were dissected at different time intervals in physiological saline for exposing the biostages of *T. remus*. The dissected specimens were placed on glass microscopy slides with mounting medium. The images of the life stages of *T. remus* were observed regularly on daily basis (for every 24 h) and acquired with stereozoom-microscope (Leica: M205C, Software LAS V4.12). Adults of the parasitoid were also photographed. A minimum of 50 host eggs were dissected in each case and specimens were utilized for photography.

Scanning electron microscopy

The studies were carried out at the Department of Nano Science and Technology at Directorate of Natural Resources Management, Tamil Nadu Agricultural University, Coimbatore, India. A separate batch of parasitized eggs was used as described previously. The dissected specimens of FAW eggs with the stages of *T. remus*

at different periods lifecycle were mounted on aluminium stubs with adhesive double side sticking carbon tapes and sputter coated with gold for 20 s in automated sputter coater (Model: 119 EMITEC—SC 7620). The sputter coated samples were then lyophilized to remove the moisture and to retain the shape and contour. The processed samples were examined with high resolution scanning electron microscope (HR—SEM: Hitachi S—4800, Japan) and the elemental composition was carried out using energy dispersive X-ray analysis (EDAX, Oxford Instruments). The biological stages were measured throughout the mid length and mid width of the whole body from the SEM micrographs (Kannan et al. 2021).

Results

Influence of host eggs age and parasitism by *T. remus*

The non-choice bioassay to determine the suitability of FAW eggs of three age classes for parasitization by *T. remus* females showed significantly the highest proportion of young host eggs (<24 h) being parasitized (98.20%), followed by <48 h old egg class (49.78%). *T. remus* females avoided <72 h old egg class and there was no parasitization but for the hatching of host larvae. Correspondingly, the emergence of parasitoids was found to be the highest from <24 h old eggs (97.42%), followed by <48 h old eggs class (45.92%). Considering the proportion of the sexes of adults that emerged, it was found that host eggs of age class <24 h could significantly give rise to more females (66.54%), followed by <48 h old eggs (64.86) with sex ratio of 1.99:1 and 1.85:1, respectively (Table 1). The results of the laboratory trial showed the significant interaction between the host egg age and acceptance by the biocontrol agent.

Examination of *T. remus* morphological and biological stages

Based on the above results, 24 h old eggs exhibiting the highest parasitic potential were dissected to present in

depth information about the biological stages of *T. remus* through Stereo zoom microscopic and Scanning Electron Microscopic examinations. Results of the Stereo zoom microscopic (Leica: M205C, Software LAS V4.12) and Scanning electron microscope examination of *T. remus* are summarised below. The development of *T. remus* began with the oviposition/parasitization by mated adult female of *T. remus* within the host egg of *S. frugiperda*. The total developmental duration was nine days with six developmental stages. The egg stage lasted for one day, first and second instars' larvae lasted one day for each, followed by pre-pupal period of one day and pupal period of five days.

Phenotypic characterization of immature stages

Stereo zoom microscopic and scanning electron microscopic examination of *T. remus* biological stages

Egg At one day after parasitization (1 DAP), the egg stage of *T. remus* was evident in the microscopic examination. The results revealed the egg period lasted for one day. The egg of *T. remus* was creamy white in colour. It was smooth surfaced, translucent, oval in shape and wider than long. A central opaque area was visible within the egg revealing the development of first instar larva as shown in (Fig. 3A). Mean egg length and width were recorded as 310.6 μm and 281.5 μm , respectively (Fig. 3B, Table 2).

Larva *Telenomus remus* comprised of two larval instars. The difference in larval instars was determined by the change in shape and colour and overall measurements of the larval body. The second instar larva was different from the first instar by the transparency. The second instar larva showed protrusion and elongated mass of cells indicating metamorphosis.

First instar Two days after parasitization (2 DAP), development of first instar larva of *T. remus* was evident in the microscopic examination. The first instar larva appeared

Table 1 Biological parameters of *T. remus* emerging from FAW eggs of different age classes

Host Egg age (h)	Parasitization (%)*	Parasitoid Emergence (%)*	Male parasitoids (%)*	Female parasitoids (%)*	Sex ratio Female:male
< 24	98.2 \pm 0.49 ^a (82.28)	97.42 \pm 0.76 ^a (80.79)	33.46 \pm 0.49 ^b (35.34)	66.54 \pm 0.42 ^a (54.66)	1.99: 1
< 48	49.78 \pm 0.49 ^b (44.88)	45.92 \pm 0.49 ^b (42.66)	35.14 \pm 0.40 ^a (36.36)	64.86 \pm 0.32 ^b (53.64)	1.85:1
< 72	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)	0.00 (0.33)
SE(d)	1.17	1.43	0.37	0.31	
CD (0.05)	2.43	2.95	0.78	0.65	
CV%	4.69	5.85	2.70	1.49	

Values represent means of thirteen replications

*Values in parenthesis are arcsine transformed values and in columns, means (\pm S.E.) followed by similar alphabets do not differ statistically by LSD ($p=0.05$)

Table 2 Stereomicroscopic examinations of biological stages of *Telenomus remus*

Biological Stage of <i>Telenomus remus</i>	Duration/Longevity (days)	Body length (µm)	Body width (µm)	Head capsule*	
				Length (µm)	Width (µm)
Egg	1	310.6	281.5	–	–
I Instar larva	2	305.0	283.3	–	–
II Instar larva	3	327.2	326.4	–	–
Pre-pupa	4	70.06	86.21	–	–
Pupal phase I	5	115.0	177.8	–	–
Pupal phase II	6	432.2	171.3	161.3	236.7
Pupal phase III	7	539.1	196.0	69.21	231.7
Pupal phase IV	8	505.5	209.9	64.96	258.7
Pupal phase V	9	473.7	304.9	170.7	220.1
Female adult	10	626.6	254.1	115.4	237.8
Male adult	10	634.5	193.3	118.2	240.2

*Upto pupal phase I clear demarcation of body segments was not observed

to be an undifferentiated mass of tissues. It was the most transparent among all the biological stages. The development of first instar was apparent by the bigger size than the egg of *T. remus* and sparse distribution of the cellular contents (Fig. 4A). Mean larval length was 305.0 µm and width was 283.3 µm (Fig. 4B, Table 2). The first instar larva existed for one day and was spherical in shape. Thorax was fused with abdomen such that it seems to have cephalothorax and abdomen. Respiration was cuticular and there was no segmentation in first instar.

Second instar At three days after parasitization (3 DAP), the second instar larva of *T. remus* was observed in both Stereo and Scanning Electron Microscopic images. The second instar larva seemed to have a change in colour and form. It was opaque white in colour and there was slight protrusion of body appendages with development of reddish—brown eye spot (Fig. 5A). Mean larval length was 327.2 µm and width was 326.4 µm (Fig. 5B, Table 2). The second instar larval period also lasted for one day.

Pre-pupa At four days after parasitization (4 DAP) pre-pupal of *T. remus* was observed. It was creamy white in colour, translucent and was oval in shape, obtect with concealed appendages. The pre-pupal duration lasted one day (Fig. 6A). Mean pre-pupal length was 70.06 µm and width was 86.21 µm (Fig. 6B, Table 2).

Pupa The pupa of *T. remus* was exarate type with clearly visible appendages. There were four pupal phases revealing the stage wise development towards adult *T. remus*. The initial pupal phases (pupal phase I, pupal phase II and pupal phase III) were whitish in colour with reddish brown eyes and antennal sockets. Later pupal phases

(pupal phase IV and pupal phase V) showed a complete tanning of body parts with well—defined head, thorax and abdominal regions. The pupal duration lasted for five days.

Pupal phase I At five days after parasitization (5 DAP), the pupal phase I was observed. The first pupal phase of *T. remus* was indicated by black colouration of abdominal portion (Fig. 7A). Mean length of the pupal phase I was 115.0 µm and width was 177.8 µm (Fig. 7B, Table 2).

Pupal phase II The pupal phase was recorded on six days after parasitization (6 DAP). Pupal phase II of *T. remus* was opaque white in colour with partial development of cephalic region showing reddish—brown eyes and antennal socket, thoracic, abdominal regions with visible appendages (Fig. 8A). Mean length of the pupal phase II was 432.2 µm and mean width was 171.3 µm. Head capsule length was 161.3 µm and width was 236.7 µm (Fig. 8B, Table 2).

Pupal phase III The third pupal phase of *T. remus* was noticed at seven days after parasitization (7 DAP). Third pupal phase was with free appendages and opaque white in colour. This phase manifested the complete development of head region showing reddish—brown eyes and antennal socket, thoracic, abdominal regions, wing buds and leg musculature (Fig. 9A). Mean length of pupal phase III was 539.1 µm and width was 196.0 µm. Head capsule length was 69.21 µm and width was 231.7 µm (Fig. 9B, Table 2).

Pupal phase IV The fourth pupal phase was observed in eight days after parasitization (8 DAP). The head

and thoracic regions were opaque white and abdominal region was black in colour showing distinct segmentation and initiation of tanning (Fig. 10A). Mean length was 505.5 μm and width was 209.9 μm . Head capsule length was 64.96 μm and width was 258.7 μm (Fig. 10B, Table 2).

Pupal phase V At nine days after parasitization (9 DAP) revealed the fifth pupal phase of *T. remus*. Complete tanning, development of abdomen, thorax and wing pads were observed at this stage. However, the fifth pupal phase lacked developed antenna, wings, legs and sexual characters discriminating between male and female adults of *T. remus* (Fig. 11A). Mean length was 473.7 μm and width was 304.9 μm . Head length was 170.7 μm and width was 220.1 μm (Fig. 11B, Table 2). On 10th day after parasitization (10 DAP), there was emergence of fully developed female and male adults of *T. remus*.

Adult Fully developed adult *T. remus* female measured about 626.6 μm in length and 254.1 μm in width (Fig. 12A, B). Fully developed adult *T. remus* male was 634.5 μm longer and 193.3 μm wider (Fig. 13A, B) with a clearly demarked head capsule of length 118.2 μm and width

240.2 μm (Table 2). Both forewings and hindwings of *T. remus* were veinless.

Ovipositor of *T. remus* females resembles like a pincer (Fig. 14A, B). The ovipositor measured about 36.31 μm in length and 11.15 μm in width (Table 3). In males, the copulatory organ, aedeagus measured about 103.5 μm longer and 10.97 μm wider (Table 3). The aedeagus was longer than ovipositor and was curved (Fig. 15A, B). Apart from this, *T. remus* adults can also be differentiated by the morphology, type and number of antennal segments. The scape or the basal antennal segment was slender and longer in males (123.6 μm) and shorter (122.1 μm) in females. *T. remus* female has a club typed antenna with 11 segments (Fig. 16A, B), while male has geniculate antenna with 12 segments (Fig. 17A, B). The total length of antenna measured about 383.8 μm and 365.5 μm in both males and females with a width of 20.23 μm and 24.37 μm , respectively.

The biological stages of *T. remus* through stereo zoom microscope (Leica: M205C, Software LAS V4.12) and scanning electron microscope are furnished in Figs. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 and 17.

Discussion

There is limited information on the behavior of *T. remus* towards host eggs at different stages of embryonic development as reported by Ventura et al. (2001). This study aimed to assess the preference of *T. remus* on *S. frugiperda* eggs of different ages. Host age is a limiting factor that decides on the parasitic potential of an entomophagous. Knowledge on the suitability of host eggs at different stages of embryonic development for parasitism is mandatory, as under field conditions host eggs may exist in different stages of development (Queiroz et al.

Table 3 Sexual dimorphism in *Telenomus remus* adults

Character	Female		Male	
	Length (μm)	Width (μm)	Length (μm)	Width (μm)
Ovipositor	36.31	11.15	–	–
Aedeagus	–	–	103.5	10.97
Antenna	Clubbed		Geniculate	
Male	–	–	383.8	20.23
Female	365.5	24.37	–	–



Fig. 1 A, B Freshly laid FAW egg mass

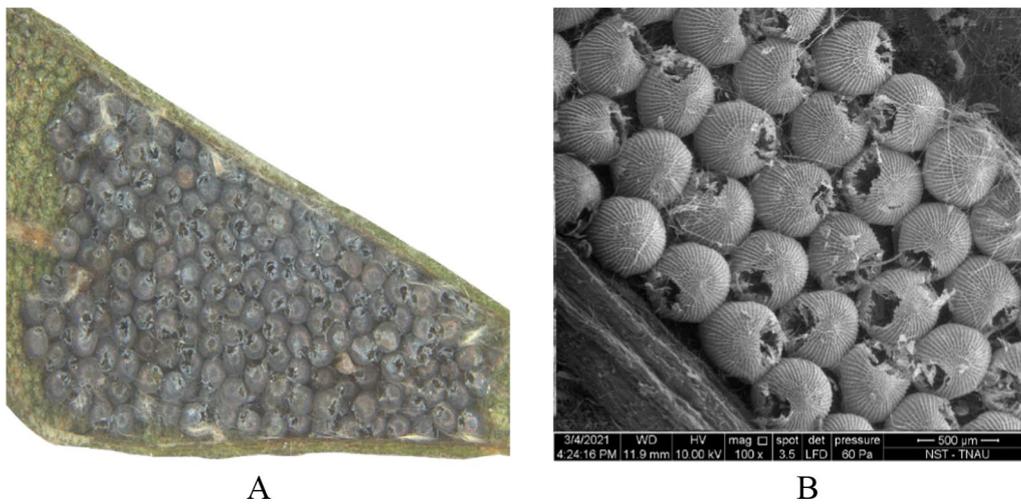


Fig. 2 A, B Parasitized egg mass of FAW showing emergence holes of *T. remus*

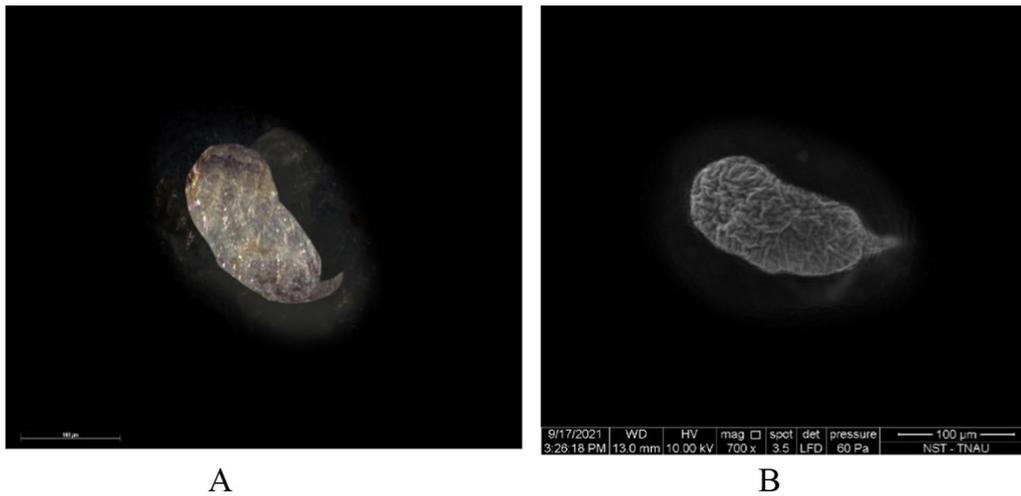


Fig. 3 A, B Egg stage of *T. remus*

2019). Parasitization of host egg by *T. remus* indicated the highest parasitization and parasitoid adult emergence on < 24 h old eggs, followed by < 48 h old eggs. This corroborates with the findings of Queiroz et al. (2019) who reported a higher parasitization on 24 h and 48 h old eggs, followed by 72 h old eggs of FAW. Penaflor et al. (2012) documented similar findings and reported that the parasitism percentage and parasitoid adult emergence was inversely proportional to the age of the host egg. Thus, from the present study, it is evident that lesser the age of the host egg, higher will be the parasitoid preference and its parasitic potential.

In the present study, no parasitization was recorded on < 72 h old eggs, which is similar to the findings of Oliveira et al. (2003). Development of host embryo

depletes the nutrients making the older eggs low quality host for the development of parasitoid which was evident from the present study and also the development of host embryo within the egg gives no room for the parasitoid development (Tuncbilek and Ayvaz 2003). Hence, the female parasitoid rejected the older eggs as there is no space for the development of her progeny. If at all parasitization occurs, older eggs may lead to the formation of malformed adults due to the deficit of nutrients (Pizzol et al. 2012). Also, as the age of the host advances, chemical properties of the host egg will change in the form of either hardening of the chorion or nutrients depletion in the host eggs. The parasitoid larva is incapable of digesting the developing larva found within the host egg (Bai et al. 1992). Calvin et al. (1997) described that the

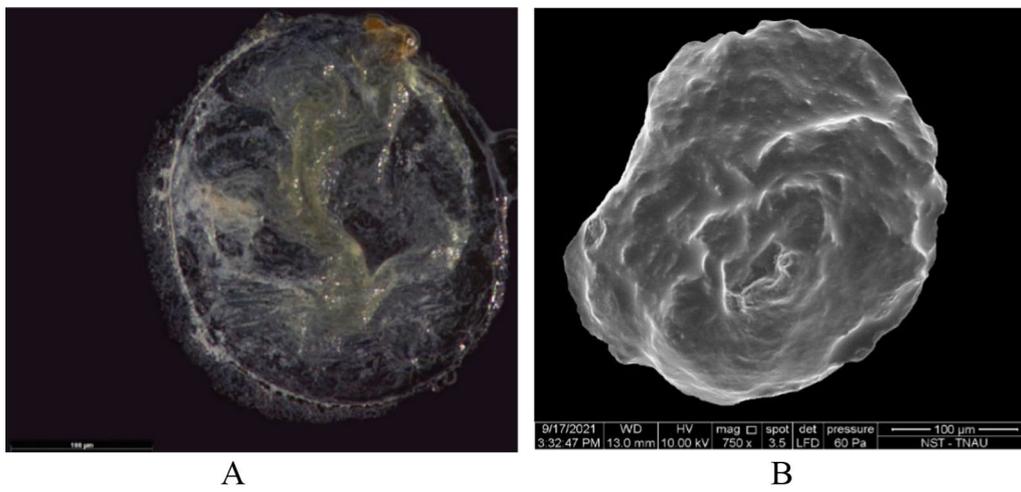


Fig. 4 A, B First instar larva of *T. remus*

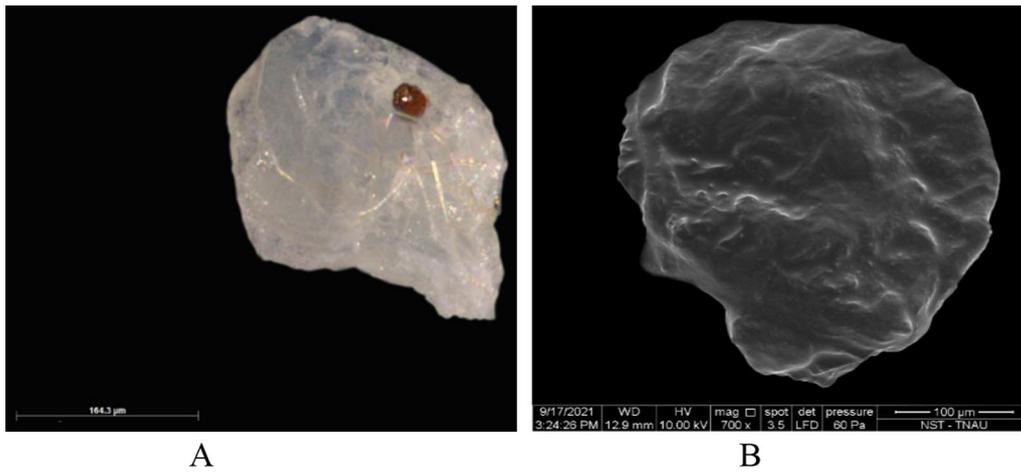


Fig. 5 A, B Second instar larva of *T. remus*

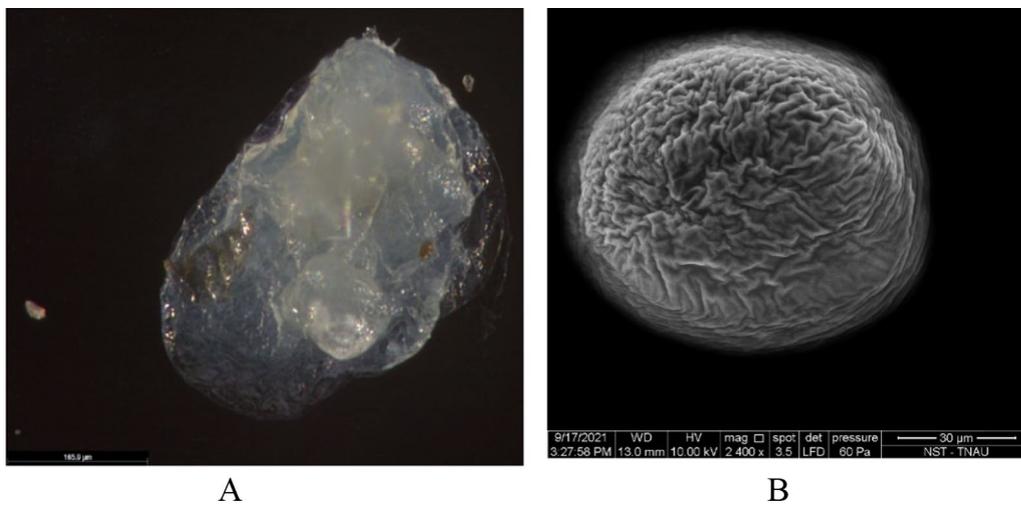


Fig. 6 A, B Pre-pupal stage of *T. remus*

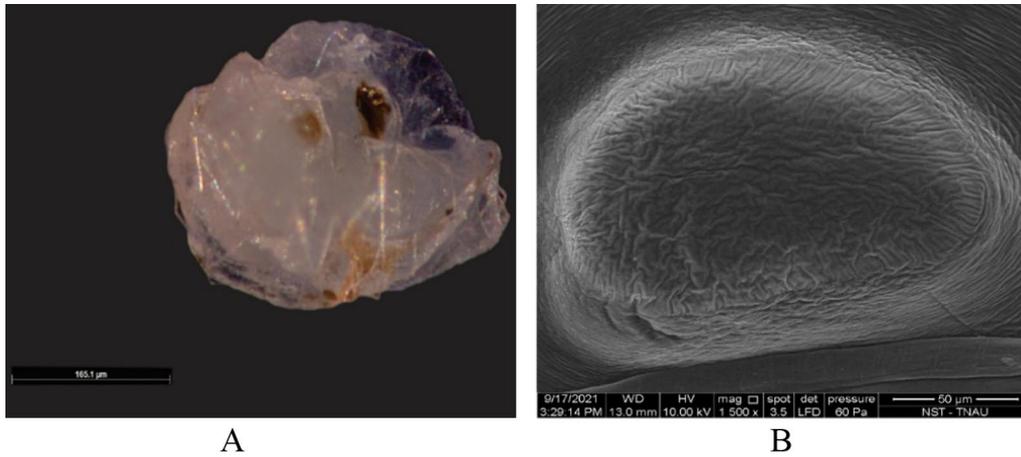


Fig. 7 A, B First pupal phase of *T. remus*

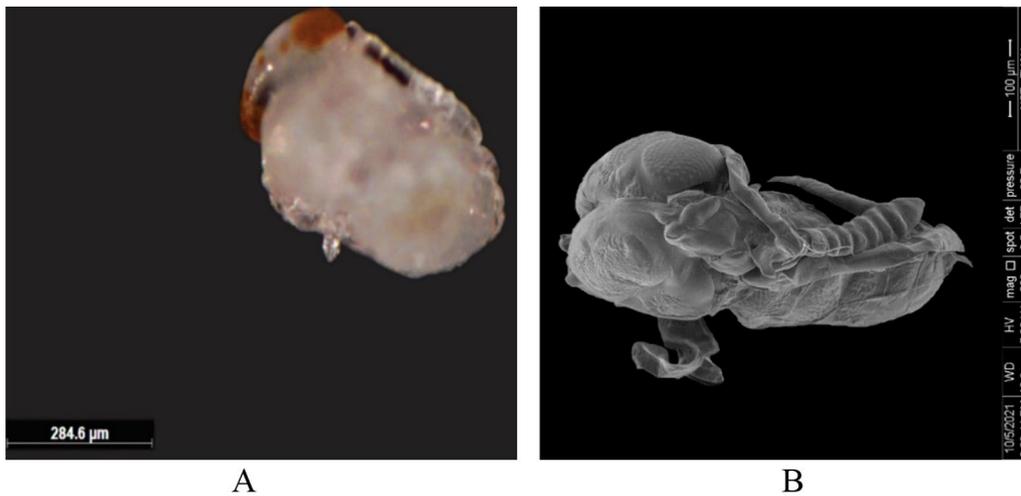


Fig. 8 A, B Second pupal phase of *T. remus*

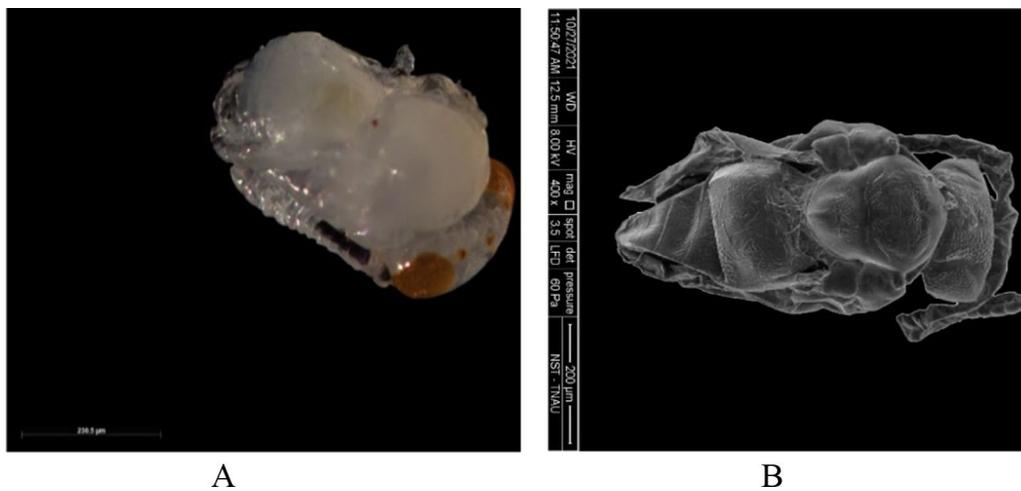


Fig. 9 A, B Third pupal phase of *T. remus*

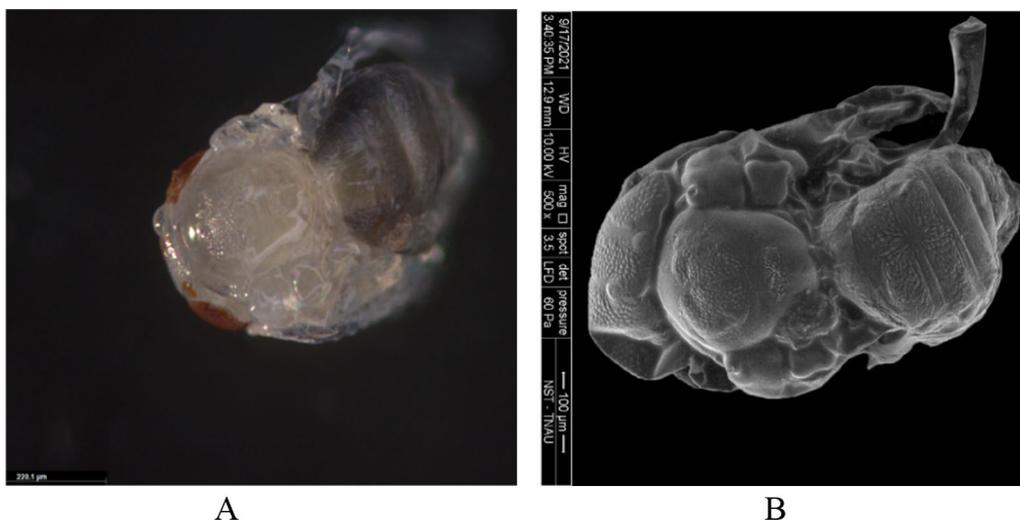


Fig. 10 A, B Fourth pupal phase of *T. remus*



Fig. 11 A, B Fifth pupal phase of *T. remus*

lepidopteran eggs that have completed more than 75% of their embryonic development are not suitable for the development of egg parasitoids.

The developmental duration from egg to adult was recorded as 9.61 and 9.52 days on 24 and 48 h old eggs, which were statistically on par with each other and it corroborates with the findings of Chen et al. (2021). Obtained results are in conformity with the findings of Oliveira and Silva (2011) who reported that differences in the age of host egg did not have any influence in the developmental period of the parasitoid. The present results are in disagreement with the findings of Oktaviani et al. (2021) who reported that the egg to adult

developmental period of *T. remus* was 10.29 days on *S. frugiperda* eggs.

T. remus is an idiobiont endoparasitoid with cosmopolitan distribution. It parasitizes lepidopterans with hairy egg masses. The morphological characteristics of immature stages play an important role in the recognition, taxonomy, classification of parasitoids at different stages of development and understanding of host—parasitoid relations. Most morphological descriptions of Scelionidae are available for the adult stages, while information on the biology and morphology of immature stages are still lacking. Hence, the objective of this study was to describe the immature forms of *T. remus* in order to provide a



Fig. 12 A, B Female *T. remus*

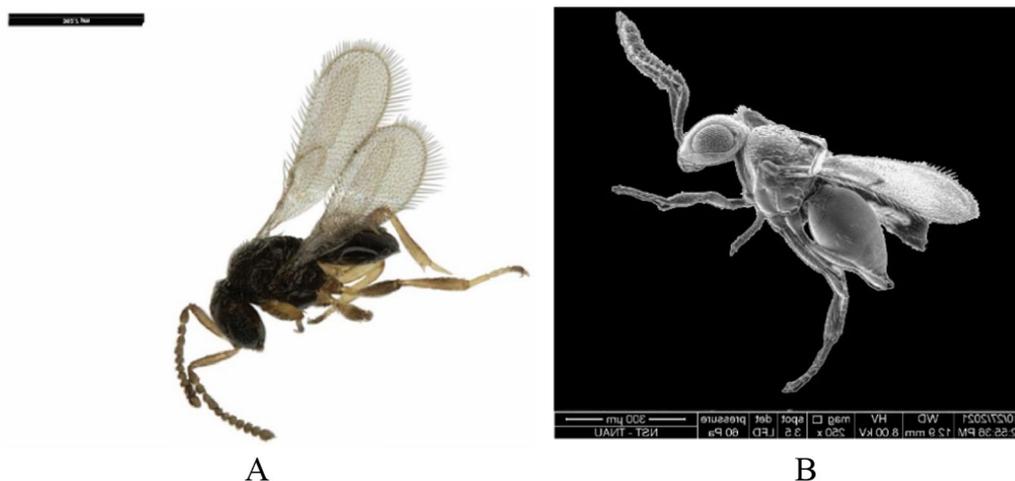


Fig. 13 A, B Male *T. remus*

detailed description on the phenotypic characters of the immature stages.

Ultrastructure examinations on the parasitoid development revealed that *T. remus* is a holometabolous insect consisting of egg, two larval instars, pre-pupa, pupa (5 phases) and adult. Chen et al. (2021) also reported stages of *T. remus* in *Spodoptera litura* eggs. However, he reported two pre-pupal periods which may be due to the difference in host.

Microscopic images observed at one day after parasitization showed the presence of single egg of the parasitoid within each host egg indicating simple parasitism as described by (Carneiro and Fernandes (2012).

Our findings are in conformity with Pickford (1964) who observed two larval instars of *Scelio calopteni* (Hymenoptera: Scelionidae) on grasshopper eggs. However, Rothschild (1970) reported three larval instars,

while Kotchetova (1966) indicated three or four larval instars in egg parasitoids. In the present study, the instars were differentiated based on variation in the measurements and also colouration. Among the larval instars, the first instar larvae of *T. remus* was teleaform with unsegmented body as reported by Navasero and Oatman (1989).

Second instar larvae were hymenopteriform with a cylindrical body, opaque creamy white and oval with segmented body comprising of head, mesosoma and metasoma as documented by Navasero and Oatman (1989). The second instar grows in size and attains the prepupal by shedding the meconium as reported by Gerling (1974) on *T. remus* in *Spodoptera littoralis* (Boisduval).

After the mature larvae entering the prepupal phase, they become well defined and enter the pupal stage at five days after parasitization. The pupal phases could



Fig. 14 A, B Female *T. remus* showing ovipositor



Fig. 15 A, B Male *T. remus* showing aedeagus

be differentiated based on the development of external organs viz., blackening of abdominal region (I phase), partial development of head, thoracic and abdominal regions (II phase), complete development of head region showing antennal socket, eye region, thoracic and abdominal regions (III phase), complete development and blackening of abdominal region showing distinct segments and initiation of tanning (IV phase) and completion of tanning and complete development of abdomen, thorax and wing pads (V phase). On the first day, the pupa is opaque white and later it turns to black colour as described by Oktaviani et al. (2021) on *T. remus* in FAW eggs which was in conformity with our

findings. Then, sequential changes take place aiding the development and emergence of *T. remus* adults. Developmental periods of the pupa was the longest due to different phases of development which is in conformity with the findings of Pezzini et al. (2017).

The present findings are in agreement with the findings of Paladino et al. (2010) who reported that the pupa of *T. remus* was excrete showing the development of ocelli, thorax, wing buds and unsclerotized body appendages and lies on its venter side within the host egg. The formation of adult stage is characterized by the change of skin, wrinkled body shape and appearance of the body parts such as head, thorax and



Fig. 16 A, B Club shaped antenna of female *T. remus*



Fig. 17 A, B Geniculate antenna of male *T. remus*

abdomen as described by Oktaviani et al. (2021) on *T. remus* in FAW eggs.

The parasitoid adults emerged ten days after parasitization and were found to have veinless wings, males with aedeagus and females with ovipositor. Our results are in affirmation with the findings Cave (2000) who reported that the parasitoids belonging to the family Platygasteridae have veinless wings. Also, the wing-span of forewings is larger than hindwings as reported by Oktaviani et al. (2021) on *T. remus* in FAW eggs. Besides, the males could be differentiated based on the possession of 12 segmented geniculate antenna and females with 11 segmented clubbed antenna which is in line with (Oktaviani et al. 2021).

Conclusions

Present investigation provides basic information for future taxonomic studies on immature stages of parasitoid *T. remus*. Also, the developmental biology that may help to improve culturing of *T. remus* and predict the appropriate time for its field release. Also, *Spodoptera litura* (Fabricius) eggs can be used as an alternate host for culturing *T. remus* because of the progeny fitness and prolonged emergence of *T. remus* progenies from *S. litura* eggs which lasts for 6 days on comparison with *S. frugiperda* eggs which was only 4 days.

Abbreviations

<i>T. remus</i>	<i>Telenomus remus</i>
FAW	Fall armyworm
DAP	Days after parasitization
DAS	Days after sowing

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Author contributions

SJ, NS, SM and SN provided the technical guidance. SLP carried out the research. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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References

- Angulo AO, Jans SC (1982) Pupae of the genus *Spodoptera* Guenee 1852, from the north of Chile (Lepidoptera: Noctuidae). *Agricultura Tecnica* 42(4):347–349
- Bai B, Luck RF, Forster L, Stephens B, Janssen JAM (1992) The effect of host size on quality attributes of the egg parasitoid *Trichogramma pretiosum* (Lepidoptera: Trichogrammatidae). *Entomol Exp Appl* 64(1):37–48. <https://doi.org/10.1111/j.1570-7458.1992.tb01592.x>
- Calvin DD, Losey JE, Knapp MC, Poston FL (1997) Oviposition and development of *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) in three age classes of southwestern corn borer eggs. *Environ Entomol* 26:385–390. <https://doi.org/10.1093/ee/26.2.385>
- Carneiro TR, Fernandes OA (2012) Interspecific interaction between *Telenomus remus* (Hymenoptera: Platygastridae) and *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs. *Ann Braz Acad Sci* 84(4):1127–1135. <https://doi.org/10.1590/S0001-37652012000400027>
- Cave RD (2000) Biology, ecology and use in pest management of *Telenomus remus*. *Biocontrol News Inf* 21(1):21–26
- Chen W, Li Y, Wang M, Mao J, Zhang L (2021) Evaluating the potential of using *Spodoptera litura* eggs for mass rearing *Telenomus remus*, a promising egg parasitoid of *Spodoptera frugiperda*. *InSects* 12(5):1–12. <https://doi.org/10.3390/insects12050384>
- Evans T, Kumschick S, Blackburn TM (2016) Application of the environmental impact classification for alien taxa (EICAT) to a global assessment of alien bird impacts. *Divers Distrib* 22(9):919–931. <https://doi.org/10.1111/ddi.12464>
- Fernandes AP, Queiroz ADP, Bueno ADF, Sanzovo AW, Bortoli SDA (2015) The importance of relative humidity for *Telenomus remus* (Hymenoptera: Platygastridae) parasitism and development on *Coryca cephalonica* (Lepidoptera: Pyralidae) and *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs. *Ann Entomol Soc Am* 108(1):11–17. <https://doi.org/10.1093/aesa/sau002>
- Ferrer F (2021) Biological control of agricultural pests in Venezuela: historical achievements of Servicio Biológico (SERVIBIO). *Rev Ambient* 55(1):327–344. <https://doi.org/10.15359/rca.55-1.16>
- Gerling D, Schwartz A (1974) Host selection by *Telenomus remus*, a parasite of *Spodoptera littoralis* eggs. *Ent Exp Appl* 17(3):391–396. <https://doi.org/10.1111/j.1570-7458.1974.tb00357.x>
- Goergen G, Kumar PL, Sankung SB, Togola A, Tamo M (2016) First report of outbreaks of the fall armyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), a new alien invasive pest in West and Central Africa. *PLoS ONE* 11(10):1–9. <https://doi.org/10.1371/journal.pone.0165632>
- Kannan M, Elango K, Kalyanasundaram M, Govindaraju K (2021) Ultra-structural and physico-chemical characterization of eggs and egg hairs (setae) of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J. E. Smith) in India: a first report. *Microsc Res Tech*. <https://doi.org/10.1002/jemt.23698>
- Kotchetova NI (1966) Development of *Asolcus semistriatus* Nees (Hymenoptera: Scelionidae), egg parasites of the little tortoise bug and other Pentatomoidea (Hemiptera). *Zool Zhurnal* 45:558–567
- Leuck DB, Perkins WD (1972) A method of estimating fall armyworm progeny reduction when evaluating control achieved host: plant resistance. *J Econ Entomol* 65:482–483
- Lungbill P (1928) The Fall Armyworm. *Tech. Bull.* 34, United States Department of Agriculture, Washington DC., United States of America, pp. 93. <https://naldc.nal.usda.gov/download/CAT90913266/PDF>
- Maino JL, Schouten R, Overton K, Day R, Ekese S, Bett B, Barton M, Gregg PC, Umina PA, Olivia L, Reynolds OL (2021) Regional and seasonal activity predictions for fall armyworm in Australia. *Curr Res Insect Sci* 1:1–11. <https://doi.org/10.1016/j.cris.2021.100010>
- Montezano DG, Specht A, Gomez DRS, Specht VFR, Silva JCS, Moraes SVP, Peterson JA, Hunt TE (2018) Host plants of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in the Americas. *Afr Entomol* 26(2):286–300. <https://doi.org/10.4001/003.026.0286>
- Navasero RC, Oatman ER (1989) Life history, immature morphology and adult behaviour of *Telenomus solitus* [Hymenoptera: Scelionidae]. *Entomophaga* 34:165–177. <https://doi.org/10.1007/BF02372665>
- Oktaviani MN (2021) Biology and life tables of *Telenomus remus* (Hymenoptera: Scelionidae) as parasitoid of *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae). *IOP Conf Ser Earth Environ Sci* 948:1–8
- Oliveira HN, Pratisoli D, Colombi CA, Espindula MC (2003) Biology of *Trichogramma exigum* Pinto and Platner on eggs of *Coryca cephalonica* Stainton. *Cruz Das Almas* 15(1):103–105
- Oliveira HN, Silva CJ (2011) Beneficial arthropods in *Jatropha* culture in Mato Grosso do Sul. *Embrapa Agropecuária Oeste, Dourados. Brazil Embrapa* 1:1–4
- Paladino LZC, Papeschi AG, Cladera JL (2010) Immature stages of development in the parasitoid wasp. *Diachasmimorpha longicaudata* J Insect Sci 10:56. <https://doi.org/10.1673/031.010.5601>
- Penafior MFGV, Sarmiento MMDM, Silva CSBD, Werneburg AG, Bento JMS (2012) Effect of host egg age on preference, development and arrestment of *Telenomus remus* (Hymenoptera: Scelionidae). *Eur J Entomol* 109:15–20
- Pezzini C, Jahnke SM, Kohler A (2017) Morphological characterization of immature stages of *Habrobracon hebetor* (Hymenoptera: Braconidae) ectoparasitoid of *Ephesia kuehniella* (Lepidoptera: Pyralidae). *J Hymenopt Res* 60:157–171. <https://doi.org/10.3897/jhr.60.20104>
- Pickford R (1964) Life history and behaviour of *Scelio calopteni* Riley (Hymenoptera: Scelionidae), a parasite of grasshopper eggs. *Can Entomol* 96:1167–1172. <https://doi.org/10.4039/Ent961167-9>

- Pizzol J, Desneux N, Wajnberg E, Thiery D (2012) Parasitoid and host egg ages have independent impact on various biological traits in a *Trichogramma* species. *J Pest Sci* 85(1):489–496. <https://doi.org/10.1007/s10340-012-0434-1>
- Pomari AF, Bueno AF, Bueno RCOF, Menezes AO (2013) *Telenomus remus* Nixon Egg Parasitization of Three Species of Spodoptera under different temperatures. *Neotrop Entomol* 42(4):399–406. <https://doi.org/10.1007/s13744-013-0138-0>
- Prasanna BM, Huesing JE, Eddy R, Peschke VM (2018) Fall Armyworm in Africa: a guide for integrated pest management, 1st edn. CIMMYT, Mexico, CDMX
- Queiroz APD, Favetti BM, Luski PGG, Goncalves J, Neves PMOJ, Bueno ADF (2019) *Telenomus remus* (Hymenoptera: Platygasteridae) parasitism on *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs: different parasitoid and host egg ages. *Semina Cienc Agrar* 40(6):2933–2946. <https://doi.org/10.5433/1679-0359.2019v40n6Supl2p2933>
- Rothschild GHL (1970) Parasites of rice stemborers in Sarawak (Malaysian Borneo). *Entomophaga* 15:21–51. <https://doi.org/10.1007/BF02371624>
- Sankaran T (1974) Natural enemies introduced in recent years for biological control of agricultural pests in India. *Indian J Agric Sci* 44:425–433
- Sharanabassapa KCM, Asokan R, Swamy HMM, Maruthi MS, Pavithra HB, Hedge K, Navi S, Prabhu ST, Goergen GE (2018) First report of the Fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. *Pest Manage Horticult Ecsyst* 24(1):23–29
- Shylesha A, Jalali S, Gupta A, Varshney R, Venkatesan T, Shetty P, Ojha R, Ganiger PC, Navik O, Subaharan K (2018) Studies on new invasive pest *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) and its natural enemies. *J Biol Control* 32(3):1–7. <https://doi.org/10.18311/jbc/2018/21707>
- Song F, Swinton SM (2009) Returns to integrated pest management research and outreach for soybean aphid. *J Econ Entomol* 102:2116–2125. <https://doi.org/10.1603/029.102.0615>
- Tefera T, Mugo S, Tende R and Likhayo P (2010) Mass Rearing of Stem Borers, Maize Weevil, and Larger Grain Borer Insect Pests of Maize. CIMMYT: Nairobi, Kenya
- Tuncbilek AS, Ayvaz A (2003) Influences of host age, sex ratio, population density, and photoperiod on parasitism by *Trichogramma evanescens* Westw. (Hymenoptera: Trichogrammatidae). *J Pest Sci* 76:176–180. <https://doi.org/10.1007/s10340-003-0018-1>
- Ventura GPE, Weinberg ML, Menlo O, Aviaris J (2001) Is the parasitization capacity of *Trichogramma crudeness* influenced by the age of the females? *Entomol Exp Appl* 98(2):219–224
- Wengrat APGS, Coelho-Junior A, Parra JRP, Takahashi TA, Foerster LA, Correa AS, Polaszek A, Johnson NF, Costa VA, Zucchi RA (2021) Integrative taxonomy and phylogeography of *Telenomus remus* (Scelionidae), with the first record of natural parasitism of *Spodoptera* spp. *Brazil Sci Rep* 11(1):1–9. <https://doi.org/10.1038/s41598-021-93510-3>

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