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Biology and predation of assassin bug (*Sycanus dichotomus*) on fall armyworm (*Spodoptera frugiperda*) larvae

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ABSTRACT

The experiment was conducted during 2023 and 2024 at the Sungai Burung, Tanjung Karang, Selangor, Malaysia to investigate the life cycle and predation ability of assassin bug (*S. dichotomus*) on fall armyworm (*S. frugiperda*) larvae. Results revealed that *S. dichotomus* laid eggs in a cluster of 34–116 eggs, and the mean egg incubation period was 18.50 ± 0.27 days with a 12–25 days range. The nymph passed through five instars, and the respective mean nymphal durations of females and males were 107.16 ± 0.94 days and 106.13 ± 1.07 days. Adult female longevity was 55.18 ± 0.41 days, and adult male longevity was 55.12 ± 0.51 days. The predation ability of the nymphal and adult stages of *S. dichotomus* varied with the different larval categories of *S. frugiperda*. Where all the nymphal and adult stages of *S. dichotomus* have the ability to attack 1st category larvae of *S. frugiperda*, conversely, the 1st instar nymph of *S. dichotomus* was unable to prey on the 2nd category larvae of *S. frugiperda*. Additionally, the 1st, 2nd, and 3rd nymphs of *S. dichotomus* were unable to attack the 3rd larval category of *S. frugiperda*. Results on the predation potentiality of *S. dichotomus* revealed the adult females and males showed significantly higher ($p<0.05$) performances than their nymphal stages, with the adult females the most voracious. Thus the adults of *S. dichotomus* could be an efficient biocontrol for *S. frugiperda*.

Keywords: Life cycle, Maize, Predation, *Spodoptera frugiperda*, *Sycanus dichotomus*

Assassin bugs are diverse predatory insect family, with around 7,000 recognized species worldwide (Gil-Santana *et al*. 2015). *Sycanus dichotomus* (Stal.) is a widespread predator species belonging to the assassin bugs (Hemiptera: Reduviidae) attacking bagworms (*Metisa plana* Walker) in oil palm plantations in Malaysia (Halil *et al*. 2021). It is considered an efficient biological control agent due to its wide prey spectrum and longer rostrum than other hemipteran predators (Zulkefli 1996). Further, it is reported in Indonesia as an efficient predator species of the invasive *Spodoptera frugiperda* (J E Smith) (Pebriansyah 2023).

S. frugiperda (Lepidoptera: Noctuidae) is a newly invaded lepidopteran pest species in Asia, and it has rapidly dispersed from South Asia to Southeast Asia (Lamsal *et al*. 2020). It is a severe agricultural pest species for the family Poaceae, and maize is the most preferred host (Cock *et al*. 2017). This pest has been reported in over 109 countries worldwide, including Malaysia (Kenis *et al*. 2022). This notorious pest species was first detected in Malaysia in early 2019 (IPPC 2019) in the northern state and reported in the whole of Peninsular Malaysia in 2019 (Jamil *et al*. 2021). It was assessed that the total area affected by *S. frugiperda* was 246.35 ha by 2019 in Malaysia in maize plantations, with damage severity of 50–100% for immature stages of maize plants of less than 40 days (DOA 2019).

In Malaysia, biological control has not been attempted on *S. frugiperda*, and research is needed for the identification of natural enemies that are locally available (Jamil *et al*. 2021). As *S. frugiperda* has recently invaded the country, very limited information is available on its natural enemies and their biology in Malaysia (Jamil *et al*. 2021). Therefore, information on the biology and predatory efficiency of *S. dichotomus* on *S. frugiperda* larvae has yet to be gathered. This is because the information is crucial for implementing a biological control programme for *S. frugiperda* in Malaysia. Hence, this study aimed to investigate the life cycle of *S. dichotomus* and its predation ability on *S. frugiperda* larvae.

MATERIALS AND METHODS

The experiment was conducted during 2023 and 2024 at the Sungai Burung, Tanjung Karang, Selangor, Malaysia. Insect rearing and experiments were conducted in standard laboratory conditions with a room temperature of 26–28°C,

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a photoperiod of 12:12 (L:D) h, and relative humidity of 60–70% monitored with a hygrothermograph.

Insect rearing: A culture of *S. dichotomus* was established in the laboratory using specimens collected from maize (variety- sweet corn, F1 351 “The Hulk”) plantations in Sungai Burung, Tanjung Karang, Selangor, Malaysia. Confirmation of the *S. dichotomus* identity was made by Prof. Christiane Weirauch and Dr. Michael Lee from the Department of Entomology, University of California, USA. The adults were paired and caged in transparent cylindrical cages (30 cm in height and 11 cm in diameter) with ventilation lids. The adults were fed daily with field-collected *S. frugiperda* larvae. The rearing cages were examined daily, and egg masses were transferred to transparent cups (top diameter 95 mm, base diameter 55 mm, height 130 mm) with muslin cloth lids (Ahmad and Kamarudin 2016). The newly hatched of the 1st and up to the 5th nymphal stage and adult stages of the 1st generation, were used for this study.

Biology of S. dichotomus *on* S. frugiperda: The incubated egg masses of *S. dichotomus* were observed daily until the emergence of nymphs to determine the egg incubation period. A total of 192 individuals of *S. dichotomus* which consisted of 102 females and 90 males were used in this study. The nymphs were transferred individually to transparent rearing cups with muslin cloth lids, and each rearing cup was labelled to determine the date of moulting and the sex of the adult individually. Each nymph was fed throughout its lifespan with *S. frugiperda* larvae. Food requirement was detected when the larvae carcass dried after being sucked by *S. dichotomus*. The rearing cups were examined daily to find the presence of an exoskeleton after moulting. The interval between moultings was utilized to determine the developmental period of each nymphal instar. Sex was determined upon the emergence of adults. The gender of *S. dichotomus* can only be distinguished in the adult stage by observing the size and shape of the abdomen (Zulkefli *et al*. 2004). The female’s abdomen is V-shaped, while the male's abdomen is U-shaped (Syari *et al*. 2011). The longevity of the adults was determined by their emergence from the 5th nymphal stage up to death.

Predation efficiency of S dichotomus *on* S frugiperda: The predation ability of different nymphal instars and adult stages of *S. dichotomus* was tested on three larval instar categories (1st category, 1–2 instar; 2nd category, 3–4 instar; and 3rd category, 5-6 instar) of *S. frugiperda* according to their body morphological characters and size (CABI 2017) (Fig. 1).

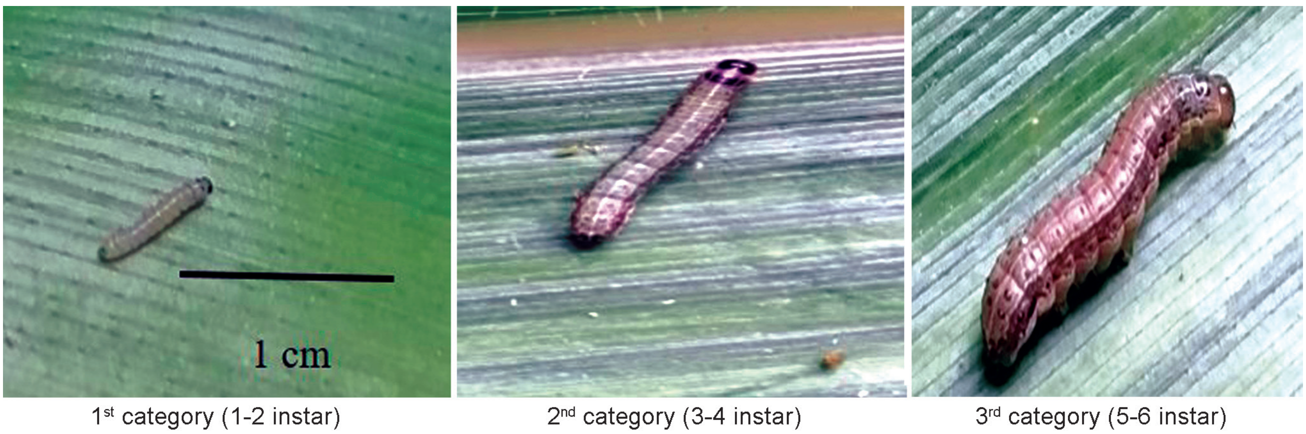
Each nymphal instar and adult *S. dichotomus* were left starved for 24 h before the experiment. The experiment was begun by providing 10 larvae of each *S. frugiperda* larval category to different nymphal instars and adult stages of *S. dichotomus* individually. Dead preys were replaced at 5 h intervals to maintain a steady prey density throughout 48 h. The number of larvae killed by the predators was determined after 48 h. Individual predators were conceded as replications, and 20 replicates were performed for each nymphal instar and adult male and female of *S. dichotomus* for each larval category of *S. frugiperda*.

Statistical analysis: The development periods from the egg to the adult stages were analysed using an independent t-test. The predation abilities of different nymphal instars and adult stages on three categories of *S. frugiperda* larvae were analysed using one-way ANOVA, and interaction between the development stages of *S. dichotomus* and different larval categories of *S. frugiperda* was analysed using two-way ANOVA, where the F test indicated a significant difference among means. Tukey's test (p<0.05) was used to examine the significance of differences between means.

RESULTS AND DISCUSSION

Biology of S. dichotomus *on* S. frugiperda: This study demonstrated the life cycle of *S. dichotomus* on *S. frugiperda* larvae. The prey species is an important aspect of understanding the biology of predators. Hence, investigation of the biology (life cycle) of *S. dichotomus* on invasive *S. frugiperda* larvae is imperative.

Egg stage: The eggs were laid as brown, hexagonal clusters with about 34–116 eggs and fastened inside the surface of the rearing cage by the females (Fig. 2). The mean egg incubation period was recorded as 18.50 ± 0.27 days. The maximum incubation period was recorded as 25



1st category (1-2 instar) 2nd category (3-4 instar) 3rd category (5-6 instar)

Fig. 1 Different larval categories of *S. frugiperda*.

Fig. 2 Emergence of *S. dichotomus* nymphs from egg mass.

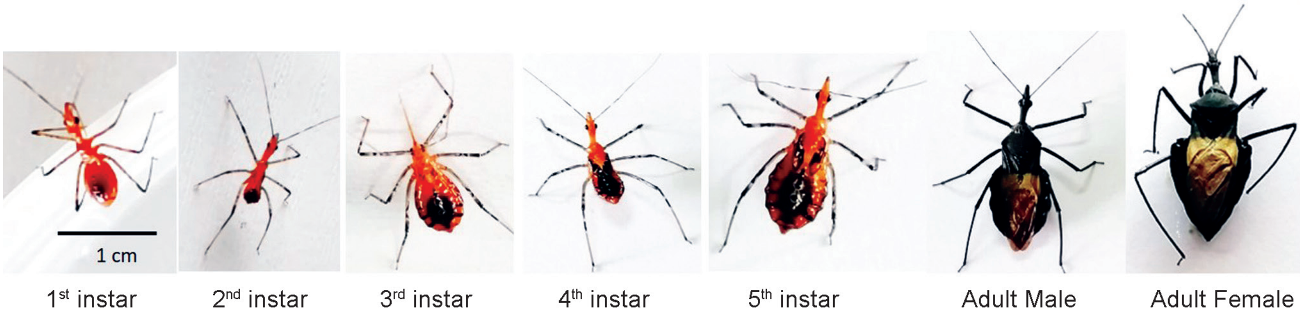


Fig. 3 Nymphal and adult stages of *S. dichotomus.*

days, and the minimum incubation period was recorded as 12 days, which was supported by Ibrahim and Othman (2011) and Jamian and Adam (2018), who reported that the *S. dichotomus* egg incubation period was 11–25 days and 19 days, respectively.

Nymphal stage: Nymphs were moulted five times to become adults (Fig. 3). The first instar nymph stayed stationary as a cluster for the first two days after hatching. All nymphal instars and newly emerged adults were brownish orange in colour with a darkened femur and tibia of legs, and adults were black bodies with bronze-coloured wings after 2–4 h of moulting.

The mean nymphal duration of the first instar female nymph was 18.18 ± 0.29 days, and the male nymph was 18.11 ± 0.27 days. The mean nymphal durations of the 2nd instar female and male nymphs were 14.88 ± 0.32 days and 14.97 ± 0.34 days, respectively. The third instar female and male nymphs took about 19.05 ± 0.29 days and 18.89 ± 0.34 days, respectively before they moulted into the next instar. The mean durations of female and male nymphs for the fourth and 5th instar were 23.43 ± 0.38 days, 23.07 ± 0.47 days, and 31.59 ± 0.39 days, 31.07 ± 0.42 days, respectively. The mean nymphal durations have increased gradually after the 2nd nymphal stage. The highest mean nymphal duration was recorded in the 5th instar, whereas the lowest mean nymphal duration was recorded in the 2nd instar (Table 1). An earlier study by Ahmad and Kamarudin (2016) also observed a similar trend in nymphal durations

Table 1 Nymphal and adult longevity of *S. dichotomus* reared on *S. frugiperda* larvae

	Female *S. dichotomus* (days)			Male *S. dichotomus* (days)			P value
Nymph/Adult	n	Mean ± SD	Range	n	Mean ± SD	Range	
1st	102	18.18 ± 2.94	9 – 25	90	18.11 ± 2.64	12 – 24	0.853
2nd	102	14.88 ± 3.27	8 – 23	90	14.97 ± 3.28	7 – 23	0.841
3rd	102	19.05 ± 3.01	11 – 28	90	18.89 ± 3.23	10 – 26	0.707
4th	102	23.43 ± 3.85	15 – 35	90	23.07 ± 4.51	13 – 34	0.559
5th	102	31.59 ± 3.94	22 – 40	90	31.07 ± 4.05	22 – 41	0.369
Adult	102	55.18 ± 4.15	46 – 65	90	55.12 ± 4.89	42 – 65	0.922
Total life cycle	102	162.34 ± 10.88	129 – 188	90	161.26 ± 11.19	126 – 184	0.496

N, Number of sample; SD, Standard deviation.

of *S. dichotomus* when fed *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) larvae and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae. The female and male nymphal durations were not significantly different with different nymphal instars, and no significant difference ($p>$ 0.05) was observed between the total mean female nymphal duration (107.16 ± 0.94 days) and the total male mean nymphal duration (106.13 ± 1.07 days). This occurrence was supported by Zulkefli *et al*. (2004), who reported that the total female nymphal durations and total male nymphal durations of *S. dichotomus* were not significantly different when fed on *Plutella xylostella* L. Further, previous research revealed that prey species has encounter a direct impact on the nymphal duration of Reduviids as reported by Ahmad and Kamarudin (2016). The mean nymphal duration of *S. dichotomus* was 98 days when fed *C. cephalonica*, 115 days when fed *T. molitor*, and 95 days when fed both species (*C. cephalonica* + *T. molitor*) larvae. *Sycanus annulicornis* Dohrn (Hemiptera: Reduviidae) had exhibited a mean nymphal duration of 80.1 ± 5.3 days when fed *T. molitor* and 74.0 ± 7.3 days with *C. cephalonica* larvae (Sahid *et al*. 2018). The result revealed that the species of prey affects the nymphal duration of the insect predator.This phenomenon is supported by Morales-Ramos *et al*. (2023) who stated that the nutritional compositions of the body content among arthropod species may differ, particularly in protein, lipid, and carbohydrate proportions, which can influence the growth and development of their insect predators.

Adult stage: Adult female and adult male mean longevity showed 55.18 ± 0.41 days and 55.12 ± 0.51 days, respectively (Table 1). There was no significant difference between female and male longevity when fed with *S. frugiperda* larvae. In addition, the total life span of female *S. dichotomus* was not significantly different ($p>0.05$) from the total lifespan of males. The mean female *S. dichotomus* life span was 162.34 ± 1.08 days, while the mean male life span was 161.26 ± 1.18 days. Ahmad and Kamarudin (2016) stated that the total mean life span of *S. dichotomus* when fed with the combination of *T. molitor* and *C. cephalonica* larvae was 164.30 ± 6.83 days, but it was 153.70 ± 2.31 days when fed alone with *C. cephalonica* larvae. However, the mean longevity of adult *S. dichotomus* has prolonged significantly as 180.6 ± 8.31 days when fed with *T. molitor*. According to this result, the longevity of adult *S. dichotomus* has varied with their prey species. Bong *et al*. (2014) reported that the nutritional composition of prey species influences the growth and longevity of predatory insects.

Predation efficiency of S. dichotomus *on* S. frugiperda: This study demonstrated that the effect of the development stages of *S. dichotomus* (nymphal instars and adults) on predation ability was significantly different (F = 258.84;

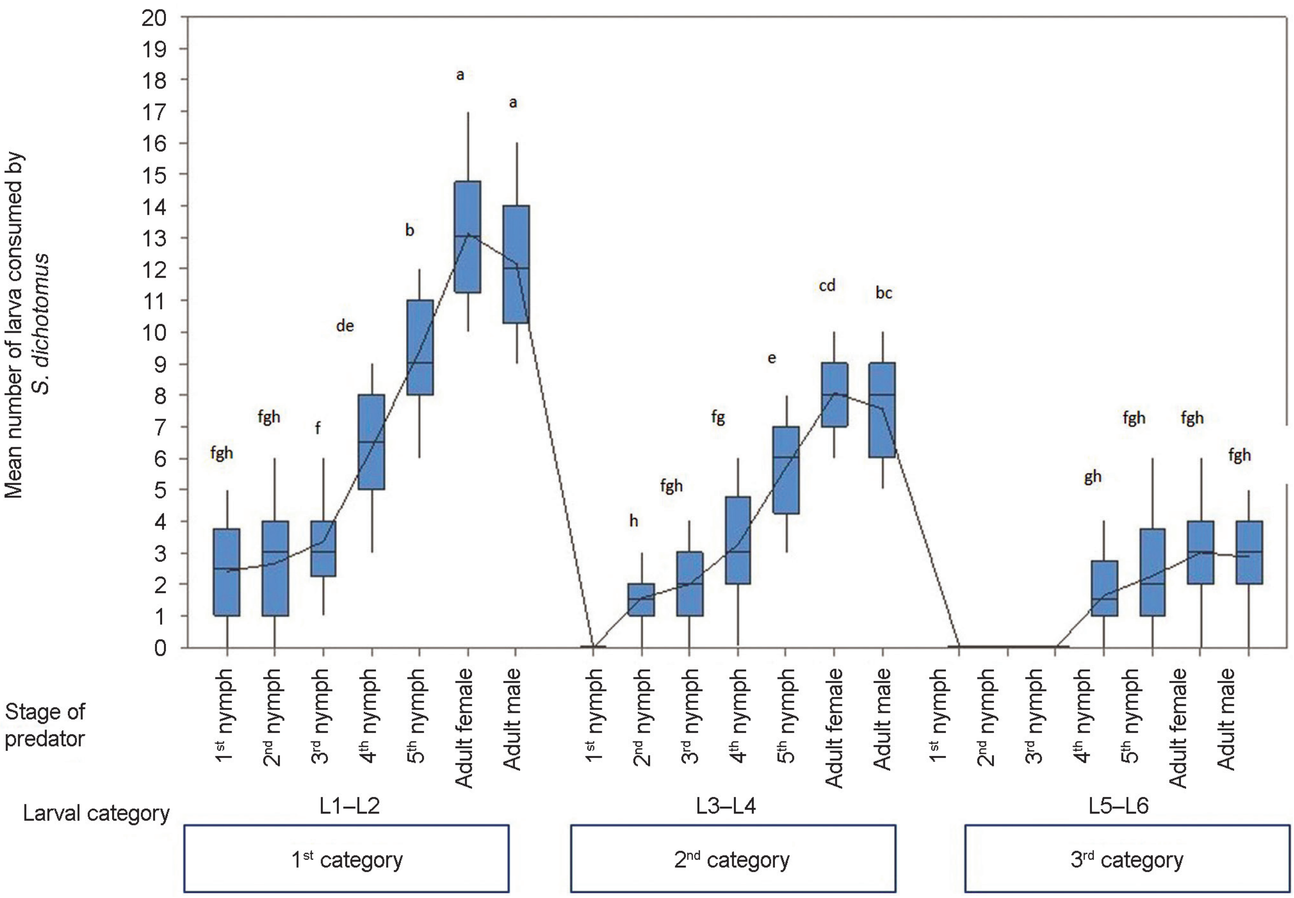


Fig. 4 Predation efficiency of different stages of *S. dichotomus* on various larval category of *S. frugiperda.*
*, The same letter represents not significantly different.

df = 6,399; p<0.001). A similar result was found by Ahmad *et al*. (2020) when *S. dichotomus* fed on *Pteroma pendula* de Joannis larvae. Moreover, the predation ability of *S. dichotomus* was significantly different (F = 540.14; df = 2,399; p<0.001) among the three larval categories of *S. frugiperda* (Fig. 4). In addition, the interaction of the development stages of *S. dichotomus* and different larval categories of *S. frugiperda* was also significantly different (F=25.54; df = 12,399; p<0.001). Predation capacity of *S. dichotomus* dropped when *S. frugiperda* larvae proceeded from the first to the third category, with higher predation in the first category and the lowest predation in the third category (Fig. 4). All nymphal instars and adults of *S. dichotomus* preyed on *S. frugiperda* larvae of the first category, whereas the first nymph of *S. dichotomus* was unable to attack *S. frugiperda* larvae of the second and third categories. Furthermore, second and third nymphs of *S. dichotomus* were unable to kill third-category larvae of *S. frugiperda* (Fig. 4). Adult *S. dichotomus* had the highest predation ability for all three *S. frugiperda* larval categories when compared to their nymphal instars, with adult females being the most voracious.

The predation ability of different nymphal instars and adults of *S. dichotomus* was significantly different in the first category (1–2 instar group) larvae of *S. frugiperda* (F=131.08; df = 6,133; p<0.001). The mean predations of the 1^{st}, 2^{nd}, 3^{rd}, 4^{th}, and 5^{th} nymphal instars of *S. dichotomus* on the 1^{st} category larvae (1–2 instar group) of *S. frugiperda* were 2.40 ± 0.32, 2.65 ± 0.35, 3.35 ± 0.30, 6.30 ± 0.42, and 9.40 ± 0.39 larvae, respectively. The mean predations of adult female and male *S. dichotomus* on the first category larvae *of S. frugiperda* were 13.10 ± 0.50 and 12.15 ± 0.46 larvae, respectively. There was also no significant difference (p>0.05) in the predation ability among adult females and males of *S. dichotomus* on the first category (1–2 instar group) larvae of *S. frugiperda*. Although the predation ability of adults was significantly (p<0.05) higher than the nymphal stages. This was supported by Ahmad *et al*. (2020) who also found that the maximum predation rate in both adult genders of *S. dichotomus* was in *P. pendula* larvae. There was no significant difference (p>0.05) in the predation ability among the 1^{st}, 2^{nd}, and 3^{rd} nymphal stages of *S. dichotomus* on the first category (1–2 instar group) larvae of *S. frugiperda*. However, the predation abilities of 3^{rd}, 4^{th}, and 5^{th} nymphal instars on first-category larvae were significantly different (p<0.05) from each other (Fig. 4). The lowest number of prey was consumed by 1^{st} instar nymph, while the highest number of prey was consumed by the adult female of *S. dichotomus*. The higher prey requirement of the female insect has been explained by Shingleton (2015). According to him, females require higher nutrients to produce eggs and oviposition, therefore, females are more voracious.

There was a significant difference (F = 109.45; df = 6,133; p<0.001) in the mortality of *S. frugiperda* 2^{nd} category (3–4 instar) larvae, consumed by different nymphal instars and adult stages of *S. dichotomus*. There was also no significant difference (p>0.05) in the predation ability of the 3^{rd} instar nymph of *S. dichotomus* with the 2^{nd} and 4^{th} instar nymphs of *S. dichotomus* (Fig. 4). However, the predation ability of *S. dichotomus* on 2^{nd} category larvae of *S. frugiperda* was significantly different (p<0.05) among the 4^{th}, 5^{th} nymphal instars, and adult stages. The 1^{st} instar nymph of *S. dichotomus* was unable to prey on the 2^{nd} category larvae of *S. frugiperda*. The mean predations of the 2^{nd} instar nymph up to the 5^{th} instar nymph of *S. dichotomus* on 2^{nd} category larvae of *S. frugiperda* were 01.55 ± 0.25, 02.00 ± 0.26, 03.25 ± 0.36, and 05.70 ± 0.38 larvae, respectively. The mean predations of adult female and male of *S. dichotomus* on the 2^{nd} category larvae of *S. frugiperda* were 08.05 ± 0.27, and 7.55 ± 0.37 larvae, respectively and there was no significant difference (p>0.05) among the predation ability of adult males and females. The lowest mean consumption of 2^{nd} category larvae (3–4 instars) of *S. frugiperda* was recorded in the 2^{nd} instar nymph, while the highest consumption was recorded in adult females. Halil *et al*. (2020) also reported a higher predation rate of adult female *S. dichotomus* on *Metisa plana*.

The mean 3^{rd} category (5–6 instars) of *S. frugiperda* consumed by *S. dichotomus* was significantly different (F = 28.72; df = 6,133; p<0.001) among nymphal stages (4^{th} and 5^{th} nymph) and adult stages (male and female). However, 1^{st}, 2^{nd}, and 3^{rd} nymphs of *S. dichotomus* were unable to attack the 3^{rd} larval category (5–6 instars) of *S. frugiperda* because of the larger body size of the 3^{rd} category larvae of *S. frugiperda* compared to the 1^{st}, 2^{nd}, and 3^{rd} nymphs of *S. dichotomus*. This finding has been confirmed by McClure and Despland (2011) who stated that the capture success of predators decreases with increasing size of the prey. The mean predations of the 4^{th} instar nymph and the 5^{th} instar nymph of *S. dichotomus* on 3^{rd} category larvae of *S. frugiperda* were 01.65 ± 0.28, and 2.25 ± 0.39 larvae, respectively. The mean predations of adult female and male of *S. dichotomus* on the 3^{rd} category larvae of *S. frugiperda* were 03.00 ± 0.36 and 2.85 ± 0.30 larvae and there was no significant difference (p>0.05) among the predation ability of adult male and adult female of *S. dichotomus* on 3^{rd} category larvae of *S. frugiperda*. There was also no significant difference (p>0.05) in the predation ability of the 5^{th} instar nymph of *S. dichotomus* with the 4^{th} instar nymph and adult stages of *S. dichotomus*. The highest mean predation was recorded in adult females, and the lowest predation was recorded in the 4^{th} nymphal stage (Fig. 4). Females with higher prey requirements have been observed not only in *S. dichotomus* but also in other Reduviidae species such as *S. annulicornis* (Hemiptera: Reduviidae) (Sahid *et al*. 2018) and *Rhynocoris marginatus* Fab. (Heteroptera: Reduviidae) (Saharayaj *et al*. 2016). This is supported by Duran Prieto *et al*. (2016), who found that female predatory insects require more prey for reproduction.

This study shown that the predation ability increased with the increasing body size of the predator, which is also supported by Balakrishnan *et al*. (2011). The body content of the first-category larvae is significantly lesser than that of the late-instar larvae of *S. frugiperda*. Therefore, a greater number of small larvae are required to meet the dietary requirements of the predator. The experiment demonstrated that extremely juvenile instars of *S. dichotomus* were unable to handle the larger larvae of *S. frugiperda* because of their smaller body size and inability to manipulate larger larvae.

The present results demonstrated that the adults *S. dichotomus* were voraciously consuming different categories of *S. frugiperda* larvae compared to their nymphal instars. In addition, *S. dichotomus* adults have long lifespans, flying abilities, and are easy to mass produce in laboratory conditions. This indicates the capability and potential of *S. dichotomus* adults as biocontrol agents on *S. frugiperda* larvae compared to their nymphal instars. Nevertheless, this first discovery on the predation ability of *S. dichtomus* on *S. frugiperda* in Malaysia warrants further investigations in order for them to be used and to verify their performance in the field.

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