BIOLOGICAL CONTROL OF THE TOBACCO CUTWORM *Spodoptera litura* (FABRICIUS) (LEPIDOPTERA: NOCTUIDAE) USING *Sycanus collaris* (FABRICIUS) (HEMIPTERA: REDUVIIDAE)

by

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(2018-11-120)

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY
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KERALA, INDIA
2021

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THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

VELLANIKKARA, THRISSUR-680656

KERALA, INDIA

2021

DECLARATION

I hereby declare that the thesis entitled **“Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae)”** is a bona fide record of research work done by me during the course of research and the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

G.Sth

Vellanikkara
09/04/2021

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CERTIFICATE

Certified that the thesis entitled **"Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae)"** is a bona fide record of research work done independently by **Ms. Gadikota Sravanthi (2018-11-120)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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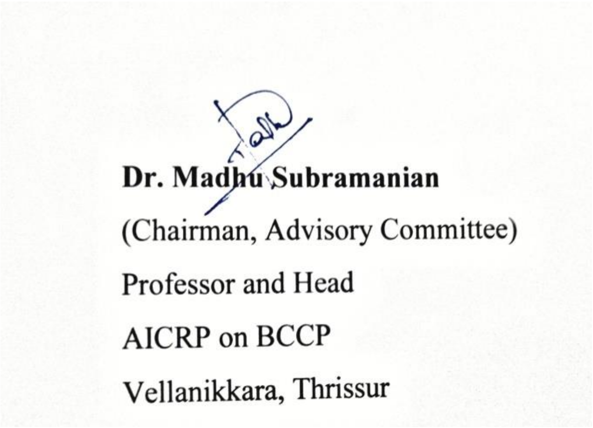
09/04/2021



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CERTIFICATE

We, the undersigned members of the advisory committee of **Ms. Gadikota Sravanthi (2018-11-120),** a candidate for the degree of **Master of Science in Agriculture** with major field in **Agricultural Entomology**, agree that this thesis entitled **"Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae)"** may be submitted by **Ms. Gadikota Sravanthi** in partial fulfilment of the requirement for the degree.

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ACKNOWLEDGEMENT

With the blessings of almighty, as I complete this venture, I express my heartfelt gratitude towards all the people who helped, guided and inspired me to complete this endeavor successfully.

I feel short of words to express my deep sense of reverence and gratitude for my major advisor, esteemed teacher, a brilliant academician and an eminent scholar **Dr. Madhu Subramanian,** professor and Head, AICRP on BCCP, Vellanikkara and Director of Research, KAU. I owe a substantial depth of sincere regard and gratitude for his endless inspiration, ample guidance, optimistic criticism, immense help, affectionate advice and unceasing encouragement throughout this study. It has been a proud privilege to have had an opportunity to work under his guidance. This is my turn to express heartfelt thanks to him for having shaped my work culture towards the attainment of perfection.

I am extremely thankful to **Dr. Mani Chellappan,** Professor and Head, Department of Agricultural Entomology and member of my advisory committee for regular support, unstinted co-operation, valuable suggestions and immense help rendered during the study. My sincere thanks to **Dr. Smitha M. S,** Assistant Professor, Cashew Research Station, Madakkathara, for her support, valuable suggestions and co-operation throughout the research work and during the formulation of thesis.

I am highly indebted to **Dr. C. Narayanankutty**, Professor (Horticulture), Agriculture Research Station, Mannuthy, for his affectionate advice and generous guidance rendered during the whole venture.

Whole hearted thanks to my teachers **Dr. Haseena Bhaskar**, **Dr. Berin Pathrose, Dr. Deepthy K. B., Dr. Vidya C. V., Smitha Revi and Dr. Ranjith, M. T.** for their expert teaching, moral support and co-operation throughout the period of study.

My special thanks to the non teaching staffs, especially ***Rema chechi****, Surya chechi, for their motherly affection and encouragement throughout my study.*

I owe my sincere thanks to ***Anieta chechi****, Jisna chechi, Akhila chechi, Gokul chettan, Nabeesatha, Bindhu chechi, Devu chechi, Latha chechi, Sarathettan and Saran of AICRP on BCCP.*

I also use this opportunity to thank my batchmates ***Sachin G. Pai, Pavithrakumar, Abinsha Ashraf, Laya, A. C.,*** *and* ***Beegam Salma M. P.,*** *whose helping hands, love and affection fetched a remarkable place in my heart. I take this opportunity to extend my gratitude to my seniors* ***Anna chechi,*** *Jhansi chechi, Anusree chechi, Vineetha chechi, Anju chechi and Nimisha chechi and juniors Srilakshmi, Rahul, Sireesha, Shika and Abishek for their support in different stages of the study.*

I wish to extend my thanks to my friends ***Anusha, Harshapradha,*** *Apeksha, Pranali, Aswini, Nivethitha, Ayesha, Aswathi, Sivadarshanapriya and Anju jayachandran for their support and affection which rendered mental strength to me during the research work.*

I can never thank enough to my beloved father ***Raghunatha Reddy****, my mother,* ***Hemalatha*** *and my sister* ***Sowmya*** *for their selfless love, personal sacrifices and prayers which helped me to move forward with a smile, inspite of all the hardships in research.*

The award of ***ICAR-NTS fellowship*** *is thankfully acknowledged.*

Gadikota Sravanthi

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Introduction

1. INTRODUCTION

India is the second largest producer of vegetables in the world, next only to China with 185.88 million tonnes per year or 11.2 per cent of global vegetable production (National Horticulture Board, 2019). Cucumber (*Cucumis sativus* L.) is one of the most popular vegetables grown in the country. As per the data published by National Horticulture Board, India produced 1.26 million tonnes of cucumber from an area of 82,000 hectares during 2018.

Cucumber is an important vegetable grown in polyhouses across Kerala and has been gaining significance in the state in recent years. The warm, humid conditions, abundance of food and the relatively low presence of natural enemies, however, encourage pest build up in polyhouse cultivation. Nearly twenty insect pest species have been recorded on cucumber under protected cultivation (Sood *et al.*, 2006), the most important being the tobacco cutworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae).

The tobacco cutworm is one of the most destructive polyhouse pests, especially in vegetables like cabbage, cauliflower, cucumber, cowpea *etc.* The damage potential of the pest in cucumber was only second to that in cabbage (Vashisth *et al.*, 2012). Considering the short duration of crop as well as the consumption of fruits in raw form, insecticide based management strategies are hardly advisable in cucumber. Moreover, it could result in resurgence of pests like mites and thrips as well (FAO, 2007). An eco-friendly strategy like biological control could be a safer and acceptable alternative in such a context.

Augmentative release of assassin bugs (Hemiptera: Reduviidae) is increasingly being viewed as a viable biocontrol option for the management of *S. litura* in protected cultivation. Reduviidae is a cosmopolitan megadiverse family of the suborder Heteroptera under Hemiptera. This large group of predatory fauna includes 7000 described species under 25 sub families (Maldonado, 1990). As many as 464 species, belonging to 144 genera occur in India (Sahayaraj, 2004). The prey record of reduviids includes insect families of Lepidoptera, Orthoptera, Hemiptera

(Ambrose, 1999; Sahayaraj, 2003; 2006), Coleoptera (Denlinger, 1994), Isoptera (Sahayaraj, 1991) and Hymenoptera (Grossi *et al*., 2012; Hwang and Weirauch, 2012). Though reduviids are generalized predators, they demonstrate a distinct preference towards lepidopteran caterpillars like *S. litura* (Ambrose, 2001).

Reduviid predators like *Rhynocoris marginatus* have been evaluated for the biological control of tobacco caterpillar with varied results (Ambrose, 2003). Their tendency for dispersal often limits their utility in open fields but is not a constraint under protected cultivation. The members of the relatively less studied genus *Sycanus* are also potential predators of several insect pest species. Their broad host range, which includes common pests of cucumber such as *S. litura*, aphids, leafhoppers *etc.*, render them ideal bioagents for the management of arthropod pests in cucumber. *Sycanus collaris* (Fab.) is an indigenous reduviid considered as a potential candidate for biocontrol of *S. litura* in polyhouses. However, very few investigations pertaining to biology and efficacy of *S. collaris* has been carried out in the country.

The present study, titled "Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae)" was therefore proposed to evaluate the feasibility of utilising the reduviid predator *S. collaris* for the management of the tobacco cutworm, *S. litura*. The objectives of the study were as follows:

1. To study the biology of reduviid bug *S. collaris*
2. To study functional response of *S. collaris* against *S. litura*
3. To study prey stage preference of *S. collaris* on *S. litura*
4. To evaluate field efficacy of *S. collaris* against *S. litura* on cucumber in polyhouse

Review of literature

2. REVIEW OF LITERATURE

The effectiveness of a biocontrol agent depends, to a great extent, on the functional and numerical responses of the organism towards the population densities of the target pest. The above parameters are in turn, embedded in characteristics such as biology, predatory potential and stage preference of the natural enemy. Hence, a comprehensive knowledge on these aspects is essential to evaluate the potential of a natural enemy as an agent of biocontrol. In this backdrop, the available literature on biology and predatory potential of *Sycanus collaris* is reviewed below. As the works on biology, functional response, prey stage preference and field efficacy of *S. collaris* on tobacco caterpillar *Spodoptera litura* were limited, studies on similar aspects of other reduviid species have been included.

2.1. BIOLOGY OF THE REDUVIID BUG *Sycanus collaris*

2.1.1. *Sycanus collaris*

Sahayaraj (2012) studied the biology of *Sycanus collaris* along with that of *Rhynocoris kumarii* Ambrose and Livingstone and *Panthous bimaculatus* on meat based artificial media. The eggs hatched 15, 20 and 21 days after oviposition in case of *S. collaris*, *R. kumarii* and *P. bimaculatus*, respectively. The developmental duration of first generation *S. collaris* was 75.67 ± 9.06 days while in case of *R. kumarii* it was 88.30 ± 3.60 days and for *P. bimaculatus* it lasted for 101.12 ± 2.30 days. Female biased sex ratio was observed for *S. collaris* (0.67:1), *P. bimaculatus* (0.60:1) and *R. kumarii* (0.57:1).

Rajan *et al.* (2017) compared the biology of *S. collaris* reared on two lepidopteran species, namely, rice meal moth *Corcyra cephalonica* (Stainton) and tobacco caterpillar *S. litura*. The bug was shown to have comparable development duration on both the hosts. Eggs hatched after 12.45 and 11.56 days when reared on *C. cephalonica* and *S. litura*, respectively. Five nymphal stages were recorded. The duration of each nymphal instar ranged from 11-12, 12-14, 12-14, 14-16 and 15-16 days on *C. cephalonica* while the corresponding values on *S. litura* were 12-13, 12-

13, 13-14, 14-15 and 16-18 days, respectively. Males and females had comparatively shorter life spans of 73.58 and 80.64 days when offered with *C. cephalonica* than 75.82 and 85.48 days on *S. litura*. However, *S. collaris* laid a significantly higher number of 340.82 eggs on *S. litura* as against 256.58 eggs on *C. cephalonica*. Sex ratio of the bug, at 0.85:1 on *C. cephalonica* and 0.82:1 on *S. litura* was female biased. A specific prey-predator interaction in which life stages of *S. collaris* handled larvae of *S. litura* more easily than *C. cephalonica* was also observed.

Srikumar *et al.* (2017) studied the biology of two reduviid predators *S. collaris* and *Epidaus bicolor* Distant maintained on *C. cephalonica* larvae at 60-70 per cent R.H. and 22-26°C. *Sycanus collaris* laid yellowish oval egg clutches, whereas, *E. bicolor* egg clutches were broadly oval with orange-yellow colour. Total developmental duration as well as mean fecundity of *S. collaris* was relatively higher at 71.7 days and 145.2 eggs/female, respectively as against the corresponding values of 66.8 days and 69.3 eggs/female for *E. bicolor*. The sex ratio of 0.5:1.0 was female biased in case of *S. collaris* but *E. bicolor* had a male-biased sex ratio of 1.0:0.6. As no cannibalism was observed during rearing, *S. collaris* was reported as more amenable for mass rearing than *E. bicolor*.

Biology of the reduviid bug, *S. collaris* on rice meal moth *C. cephalonica* and tea looper *Hyposidra talaca* Walker was studied by Sarkar *et al.* (2019). Mean incubation period of 13.6 and 11.8 days and mean nymphal period of 58.4 and 64.8 days, along with mean fecundity of 280 and 320 eggs were recorded on rice moth and tea looper larvae, respectively. Longevity of adult female (108.6 days) and male (99.8 days) bugs were not significantly different when fed with two hosts.

2.1.2. Other reduviid predators

Ambrose and Kumaraswami (1992) mass reared *Sycanus versicolor* Dohrn on *Heliothis armigera* (Hubner) and *Earias insulana* (Boisd.) and observed that the former was more preferred as prey. The female bug, on an average, laid eggs in three batches and eggs hatched after 9-10 days with 76.66 per cent survival. The stadial period of five instars lasted for 7-17, 7-12, 10-20, 10-21 and 12-30 days, respectively.

Total developmental period from first instar nymph to adult ranged from 53-69 days. Mortality of 6.06, 3.33 and 30 per cent was observed for the first, second and third instar nymphs, respectively. The mortality of nymphs was highest in case of fourth instar nymphs (42.8%) while no mortality occurred during the fifth instar. Mean longevity of adult males was 23.5 days and that of females was 44 days. A male biased sex ratio of 1.0:0.6 was also noticed.

Vennison and Ambrose (1992) reared *Sycanus reclinatus* Dohrn on nymphs and adults of grasshopper *Trilophidia* sp. and reported that brown coloured eggs were laid by female within 22 days after emergence. Eggs hatched after 14 to 23 days and nymphs changed from pale to deep ochreous colour in one hour. Longevity of female and male *S. reclinatus* varied from 5-54 days and 5-50 days, respectively. Sex ratio was slightly male biased (1:0.8). At 32° C, the stadial period (first instar nymph to adult) ranged from 61-90 days.

George (1999) compared the biology of *Rhynocoris marginatus* (Fab.) on three prey species such as S. *litura, Corcyra cephalonica* and *Earias vittella* (Fab). Mean generation length was shortest on *S. litura* (100.34 days) as compared to that on *C. cephalonica* (110.85 days) and *E. vittella* (112.15 days). The reproduction rate was higher when *S. litura* larvae (191.89 ± 39.69 eggs/female) were offered as prey instead of *C. cephalonica* (121.75 ± 25.15 eggs/female) or *E. vittella* (151.44 ± 30.69eggs/female). Fecundity was also higher with lower developmental time when *R. marginatus* was reared on *S. litura* larvae.

Sahayaraj and Paulraj (2001) carried out a similar study on biology of *R. marginatus* with *S. litura* as prey. Fecundity was 405.28 eggs/female with a mean incubation period of 6.81 days. On an average, nymphal stage lasted up to 46.71 days. The oviposition period lasted for 117.61 days which was 6.31 times longer than the pre-oviposition period of 18.64 days. A slightly female biased sex ratio of 0.91:1 was also recorded.

The biology of *Rhynocoris longifrons* (Stal.) on four different hosts such as *H. armigera*, *C. cephalonica*, *Clavigralla gibbosa* and *Odontotermes obesus* was studied by Ambrose *et al.* (2003). They reported that the shortest incubation period (7.8 days) and nymphal duration (58.4 days) were observed when reared on *H. armigera* larvae, whereas the longest incubation period (8.3 days) and nymphal developmental time (72.7 days) were observed on *O. obesus*. Mean fecundity was also lowest when offered with *O. obesus* (41.8 ± 20.9 eggs) and was significantly higher on larvae of *H. armigera* (159.3 ± 22.4 eggs). Adult longevity was markedly longer on *H. armigera* (111.49 ± 13.4 days) followed by *C. gibbosa* (104.9 ± 6.9 eggs), *O. obesus* (78.6 ± 12.0 days) and *C. cephalonica* (66.8 ± 10.4 days).

Zulkefli *et al.* (2004) documented life cycle of *Sycanus dichotomus* Stal. on *C. cephalonica* and *Plutella xylostella* under controlled environment. The predator laid eggs in a chevron pattern in three batches with 50 per cent survival rate. Each egg cluster had 15 to 119 eggs with a mean incubation period of 11 to 39 days. Mean longevity of each developmental stage was 16.72, 15.78, 14.88, 24.03 and 46.84 days when provided with *P. xylostella* and 24.35, 16.95, 20.35, 25.32 and 43.51 days when fed with *C. cephalonica*. The developmental time from egg to adult, when fed with *C. cephalonica* and *P. xylostella* were 193.44 and 203.91 days, respectively. Longevity of adult males and females were significantly higher when reared on *P. xylostella* (83-84 days) than on *C. cephalonica* (61-63 days).

The biology of reduviid predator, *Panthous bimaculatus* was investigated on S. *litura*, *C. cephalonica* and artificial diet by Muthupandi *et al.* (2014). Eggs hatched after 21, 21.3 and 23 days when fed with *S. litura*, *C. cephalonica* and artificial diet, respectively. The developmental duration of first, second, third, fourth and fifth instars nymphs on *S. litura* were 13.67 ± 0.55, 11.97 ± 0.60, 15.86 ± 1.04, 15.36 ± 1.47 and 16.25 ± 1.82 days, respectively. The corresponding values were 13.47 ± 0.44, 11.56 ± 0.34, 16.24 ± 0.77, 15.24 ± 0.96 and 18.57 ± 2.53 on *C. cephalonica* and 15.76 ± 1.36, 17.00 ± 3.27, 24.25 ± 5.08, 15.00 ± 0.00 and 17.20 ± 1.57 days on artificial diet. Survival rate of nymphs was greater on artificial diet (16.03%) than on live larvae (15% on *S. litura* and 12% on *C. cephalonica*). The sex ratio of *P. bimaculatus* on *S. litura*, *C. cephalnica* and artificial diet were observed to be 1:0.55, 1:0.65 and 1:0.71, respectively.

Srikumar *et al*. (2014) recorded biology of *Cydnocoris gilvus* Brum. on the larvae of wax moth, *Galleria mellonella* Linnaeus. A female, on an average laid 56.33 ± 7.88 oval yellowish orange coloured eggs in 8.67 batches. Egg clutches varied from 3.33 ± 0.33 to 11.33 ± 1.45 in number. The mean incubation period was 8.17 ± 0.31 days. The developmental interval of first, second, third, fourth and fifth nymphal instars were 9.50 ± 1.45, 6.33 ± 0.33, 4.50 ± 0.62, 6.00 ± 0.68 and 11.00 ± 1.88 days, respectively. Female and male predators lived for 42.00 ± 4.98 and 41.67 ± 4.69 days, respectively. Mean length of a generation varied from 45.5 to 52.26 days.

Pravallika (2015) reported that the nymphal period of *R. marginatus* was 72.9 days when mass reared on *Mythimna separata* (Walker). The duration of various instars ranged from 14.7 to 20.2 days with 60-100 per cent survival rate. The oviposition started 18.82 ± 0.37 days after adult emergence and a female laid 380.53 ± 11.90 eggs in its life time. The longevity of adult male and female bugs was 82.84 ± 11.09 and 128.04 ± 8.48 days, respectively.

The first instar nymphs of *Rhynocoris albopilosus* Signoret started feeding within 6-12 h after hatching and had a stadial period of 8.67 days when reared on larvae of eri silkworm, *Samia cynthia ricini*. The second and third instar nymphs took 8.00 and 6.67 days, respectively to complete their development (Sahayaraj *et al.,* 2015).

Eggs of *Sycanus aurantiacus* hatched after 12-14 days and had a mean nymphal period of 63.6 days when reared on larvae of *Tenebrio molitor*. Females laid eggs 8-14 days after copulation in 5-11 clusters containing 35-73 eggs each indicating the potential of the predator to spawn a large number of offsprings (Yuliadhi *et al.*, 2015).

Ahmad and Kamarudin (2017) reported that *S. dichotomus* took an average of 15.16, 12.09, 11.63, 14.25 and 18.53 days from first to fifth nymphal instars, respectively on *C. cephalonica* while the same required 15.21, 12.34, 12.64, 14.84 and 21.89 days, respectively on *T. molitor* larvae. The developmental time was 122.8 days on *C. cephalonica* which was significantly lower than the 156.5 days on larvae of *T. molitor*. Survival of adults, however, was greater (81%) when nymphs were reared on larvae of *T. molitor* than on *C. cephalonica* larvae (76%).

Mohasina (2017) studied various biological parameters of *R. marginatus* on *S. litura*. The incubation period of eggs was 6.80 ± 0.91 days. Nymphal development comprised of five instars and took 32.95 ± 1.81 days to complete. A female predator on an average laid 377.22 ± 45.52 eggs throughout the oviposition period of 66.12 ± 6.27 days.

Nitin *et al.* (2017) reared *Sycanus glabnus* Distant under laboratory conditions using larvae of greater wax moth *G. mellonella* as prey. Brownish yellow colour eggs were laid in clusters of 80-100 with a mean incubation period of 17.2 days. Nymphal stages lasted for 51.7 days. The mean longevity of adult female was 81.1 days while that of male was 74.0 days, thus demonstrating that the females survived for longer period.

Sahid *et al.* (2018) who studied the biology of *Sycanus annulicornis* on cabbage caterpillar *Crocidolomia pavonana* (F.) and *Tenebrio molitor* larvae, had observed that the biological parameters of *S. annulicornis* such as developmental time and adult longevity were influenced by nutritional composition of prey species. The predator population, which was mass reared on *C. pavonana* had shorter nymphal duration and prolonged adult longevity. The nymphal developmental period of *S. annulicornis* was approximately 74 days on *C. pavonana* and 80 days on *Tenebrio molitor* larvae. Adult longevity was 81 days for male and 64.8 days for female on *C. pavonana* while the corresponding values were 44 and 52.6 days, respectively on *T. molitor* larvae. There existed no significant difference between two preys with regard to number of eggs batches laid by a female.

2.2. PREDATORY POTENTIAL OF REDUVIID BUGS

2.2.1. ***Sycanus collaris***

Sarkar *et al.* (2019), in the only study on predatory potential of *S. collaris* reported so far, observed that the predatory efficiency of *S. collaris* on the lepidopteran larvae *Hyposidra talaca* Walker increased with advancement in life stages. The third, fourth and fifth nymphal instars as well as adult males and females consumed an average of 2, 3.6, 4, 4.6 and 5 larvae of *H. talaca* per day, respectively.

2.2.2. **Other reduviid predators**

Vennison and Ambrose (1989) assessed the feeding potential of final instar nymphs and adults of *Oncocephalus annulipes* on *Earias vitella*, *E. insulana*, *S. litura* and *Heliothis armigera*. The predator killed 2-3 larvae of both *Earias* spp. and 1-3 larvae each of *S. litura* and *H. armigera* per day.

A comparative study on predatory potential of *S. dichotomus* on oil palm bagworm, *Metisa plana* Walker was carried out under laboratory as well as caged conditions by Jamian *et al*. (2010). Results indicated that there was no significant difference in feeding potential of female (10.0 ± 0.8) and male (10.7 ± 0.7) *S. dichotomus* in the laboratory. A similar pattern was noticed in caged study as well with female (12.3 ± 0.5) and male (12.6 ± 0.8) predators showing identical feeding potential. However, adults of *S. dichotomus* consumed significantly higher number of 12.5 ± 0.5 larvae under caged conditions than 8.2 ± 0.6 larvae under laboratory conditions.

Sahayaraj and Asha (2010) studied consumption of *Aphis craccivora* (Koch) by immature stages of the reduviid *R. kumarii*. Rate of predation increased as the age of predator advanced. The first, second, third and fourth instar nymphs killed 24, 47, 59 and 75 aphids per day, respectively and were on par with predatory efficacy of 79 aphids/day in case of *Menochilus sexmaculatus* (F.). The authors concluded that aphid population in groundnut ecosystem could be controlled by release of fourth instar nymphs of the bug at 1:75 ratio.

The predatory potential of immature stages of *R. albopilosus* (F.) was evaluated on grubs and pupae of *Tribolium castaneum* by Kwadjo *et al.* (2013). Results revealed a significant increase in number of prey killed by successive nymphal stages of the predator. However, the most voracious among them was fifth instar nymph. The feeding efficacy from first to fifth instar nymph increased from 1.4 ± 0.1 to 6.3 ± 0.3 and from 1.5 ± 0.1 to 5.8 ± 0.2 on grubs and pupae of *T. castaneum,* respectively.

Mohasina (2017) evaluated predatory capacity of nymphal and adult life stages of *R. marginatus* on third instar *S. litura* larvae at various prey densities of 2, 4, 6, 8 and 10 larvae per predator. While the first instar nymphs were unable to prey on *S. litura* larvae, second instar nymphs killed 1.13 to 1.60 larvae at all prey densities. The third instar nymphs consumed 1.86, 3.67, 5.67, 5.20 and 5.67 larvae while fourth instar nymphs killed 2.00, 3.80, 6.67, 5.20 and 6.67 larvae, respectively at each of the prey densities. At 2, 4 and 6 larvae per predator, complete kill of prey was achieved by fifth instars nymphs, adult females and males. Kill by fifth instar nymphs, adult females and males increased from 7.13 to 7.60, 7.13 to 7.87 and 7.13 to 8.00 larvae, respectively when prey density increased from 8 to 10 prey per predator.

Sahid *et al.* (2018) compared predatory potential of *S. annulicornis* on two different hosts such as *C. pavonana* and *T. molitor*. A gradual increase in rate of consumption of *T. molitor* from first to fifth instar nymph was exhibited by the bug. The first and second instar nymphs fed on 1-4 larvae of *T. molitor* for up to 7 days, while third and fourth instars consumed up to 2-5 prey larvae. The fifth instar nymphs caused the highest mortality of 3-10 larvae. In contrast, a uniform consumption rate of 2-7 larvae per nymph was recorded for all nymphal stages on *C. pavonana*. No significant difference between number of prey consumed by fourth and fifth instar nymphs of *S. annulicornis* was observed on either of the prey species. However adult female bugs, consumed significantly higher number of prey (2.91 and 3.21 larvae of *C. pavonana* and *T. molitor*, respectively) than adult males (1.80 and 1.91 larvae of *C. pavonana* and *T. molitor*, respectively). Females of *S. annulicornis* consumed greater number of *T. molitor* than that of *C. pavonana* larvae while in males the rate of predation did not differ significantly on either of the prey species.

2.3. FUNCTIONAL RESPONSE OF REDUVIID BUGS

2.3.1. *Sycanus collaris*

Srikumar *et al.* (2017) analyzed functional response of two reduviid species *Sycanus collaris* and *Epidaus bicolor* on tea mosquito bug *Helopeltis theivora* at four different ratios of 1, 2, 4 and 8 prey per predator. Both predatory species exhibited Hollings type II functional response. In addition, significantly higher proportion of prey was consumed at lower prey densities for both species. As the density of prey increased from 1 to 8, searching time decreased from 4.1 to 0.03 days for *S. collaris* and from 4.1 to 0.01 days for *E. bicolor* suggesting a strong negative correlation between prey density and searching time.

2.3.2. Other reduviid predators

Sahayaraj and Ambrose (1994) obtained a type II functional response curve in case of adults of *Acanthaspis pedestris* Stal. on *Pectinophora gossypiella*. The number of prey consumed decreased from 3.6 to 3.4 and 3.9 to 3.8 for male and female predators, respectively as the density of *P. gossypiella* increased from 5 to 6. The number of prey killed reached peak at a density of 5 prey per predator and stabilised thereafter. The females of *A. pedestris* took significantly lesser mean handling time (0.034 days) than males (0.064 days).

A Hollings type II response curve was also observed by Ambrose and Claver (1996) while studying the functional response of reduviid predator *Rhynocoris marginatus* on *S. litura* larvae. Prey was provided at a rate of 1, 2, 4, 8 and 16 prey per predator and six replicates were maintained for each density. The attack ratio was highest at the ratio of one prey per predator and lowest at a density of 16 prey per predator.

Ambrose and Claver (1997) also reported a type II functional response of *Rhynocoris fuscipes* (Fab.) against tobacco caterpillar *S. litura.*

Functional response of adult female bugs of the reduviid *Coranus spiniscutis* Reuter was evaluated by Claver *et al.* (2004) on fourth instar larvae of *H. armigera*, *S.*

litura and *Annomis flava* (Fab.) at four different densities viz., 1, 2, 4 and 8 prey per predator. *C. spiniscutis* killed greater extent of prey at higher prey densities, and demonstrated a type II functional response. An inverse relation was obtained between prey density and handling time at all the prey densities tested. A perceptible decline in attack rate was also observed at higher densities of prey.

The functional response of *Acanthaspis pedestris* was assessed on three lepidopteran larvae, namely, *Achaea janata*, *Helicoverpa armigera* and *S. litura* by Ravichandran and Ambrose (2006). Fourth instar larvae of each prey species were provided at ratios of 1, 2, 4, 8 and 16 prey per predator. A decline in predation rate was observed at higher prey density, indicating a type II functional response. The searching time decreased with increased prey density at all densities of prey. The female predators reportedly responded to higher prey density more vigorously.

Ambrose *et al*. (2009) who studied the functional response of *Sphedanolestes variabilis* Distant under laboratory conditions on *C. cephalonica* revealed that the consumption rate was highest for the bug at the ratio of four larvae per predator. The predation rate showed a steep rise from 1 to 4 prey per predator and plateaued off thereafter. The number of prey killed by individual predator increased as a function of successive increase in prey density.

Muniyandi *et al.* (2011) reported that predation by *R. kumarii* on *S. litura* had resulted in a type II curvilinear functional response. The bug consumed most or all of the prey at lower densities with decline in consumption rate at higher prey densities. The rate of predation showed a steep rise from prey densities of 1 to 8 per predator but plateaued off beyond.

Functional response of the reduviid predator *Zelus longipes* on adults of citrus psyllid *Diaphorina citri* was analysed at various densities of prey viz., 4, 8, 12, 16, 20 and 24 prey by Navarette *et al*. (2014). Each prey density was replicated six times. One day old female predator was released in to each arena. The mortality of the prey increased with prey density. The highest consumption rate of 18.17 was recorded at

24 prey per predator. The predator exhibited type III functional response, suggesting that *Z. longipes* had a general predatory nature that varied from one prey to other.

Srikumar *et al.* (2014) evaluated the functional response of *Cydnocoris gilvus* against tea mosquito bug *Helopeltis antonii* at four prey ratios of 1, 2, 4 and 8 prey per predator and had observed that 24 h old starved adults of *C. gilvus* showed Hollings disc type II functional response by killing more number of *H. antonii* adults at higher densities. The prey density and attack ratio were indirectly proportional to each other, resulting in a discernable decline in attack rate as the prey density increased. The lowest and highest attack rates were observed at densities of eight and two prey per predator, respectively.

Sahayaraj *et al.* (2015) analysed functional response of *R. kumarii* life stages on mealybug *Phenacoccus solenopsis* Tinsley at population densities of 1, 2, 4, 6, 8 and 10 prey per predator. The nymphs as well as adult stages of the predator showed Hollings type II functional response. The number of mealybugs consumed by the five nymphal instars, adult males and females at 24 h interval was 0.8, 1.8, 5.0, 5.2, 5.5, 2.3 and 1.5 bugs, respectively at highest prey densities provided. The predation rate was higher for late instar nymphs than adults, with fifth instar nymphs consuming the highest number of prey. The handling time, at 3.75 h, was highest for first instar nymphs while fifth instar nymphs registered a handling time of 0.54 h. Adult males and female bugs recorded intermediate values of 1.3 h and 2.0 h, respectively.

Sarashimatun and Samsudin (2016) evaluated the functional response of fourth and fifth instar nymphs as well as adult females and males of *S. dichotomus* on third and fourth instar larvae of bagworm *Metisa plana* Walker at prey densities of 5, 10, 15, 20, 30 and 40 larvae per predator and reported that the bug showed type II functional response. The number of *M. plana* larvae killed by adult females of *S. dichotomus* increased significantly from the lowest density and reached a plateau at 30 prey per predator. Similarly, voracity of fifth instar nymphs and males increased significantly and attained satiation beyond 20 prey per predator. In contrast, response of fourth instar nymphs at various densities of bagworm did not vary significantly.

Different stages of *R. marginatus* such as second, third, fourth and fifth instar nymphs as well as adult female and male showed a type II curvilinear response when offered with third instar larvae of *S. litura* at densities of 2, 4, 6, 8 and 10 prey per predator. Both second and third instar nymphs attained satiation at lower prey densities. The late nymphal instars as well as adults had higher attack rate at lower densities of prey which diminished gradually at higher prey densities. The number of prey killed showed a positive correlation with prey density. The adult males recorded the highest predation rate of 8.00 ± 0.23 larvae at the ratio of 10 prey per predator as against second (1.60 ± 0.13), third (5.66 ± 0.49), fourth (6.66 ± 0.40) and fifth instar (7.60 ± 0.15) nymphs as well as adult females (7.85 ± 0.50) (Mohasina, 2017).

2.3. PREY STAGE PREFERENCE OF REDUVIID BUGS

It has been extensively demonstrated that a predator does not respond similarly to all stages of prey. Several factors such as size of prey *vis a vis* that of the predator, ease of kill and prey defences influence the choice of a prey stage by a predator, including reduviid bugs. For instance, Sahayaraj and Ambrose (1994) had demonstrated that adults of *Acanthaspis pedestris* Stal. preferred medium sized second and third instar larvae of *E. insulana* (78%), *S. litura* (66.66%) and *H. armigera* (83.33%) but only large sized larvae (fourth and fifth instar) of *P. gossypiella* (75%) when different larval instars of each prey were offered together. *A. pedestris* adults did not prefer *P. gossypiella* larvae smaller than its body size in contrast to relative preference towards medium sized larvae of other prey species

Sahayaraj and Ambrose (1995) also had noted the preference of fifth instar nymphs and adults of reduviid predator *Ectomocoris tibialis* Distant on various stages of *D. cingulatus.* They observed that adult predators preferred adult females (66.66%) of *D. cingulatus*, followed by male (25%) and fifth instar nymphs (8.33%). In contrast, greater number of fifth nymphal instar (60%) of *D. cingulatus* followed by female (28.86%) and male (13.33%) were consumed by fifth instar nymphs of *E. tibialis.*

Ambrose and Claver (1996) observed that *R. marginatus* preferred *S. litura* larvae over those of *Euproctis mollifera* Thunberg and *M. pustulata* due to their slower movement and softer cuticle. The first instar nymphs of the predator preferred the smaller first and second instar (0.1 - 1 cm) larvae of *S. litura*. The second, third and fourth instar nymphs, on the other hand, preferred first, second and third instar larvae (0.1 - 2 cm). The fifth instar nymphs, males and females killed large sized third to fifth instar larvae of *S. litura* (0.6 - 2.5 cm).

The above authors, in a later study, exposed various life stages of *R. kumarii* to larvae of three cotton pests, namely, leaf cutworm *S. litura*, American bollworm *H. armigera* and hairy caterpillar *Eupterote mollifera* Walker. First instar nymphs of *R. kumarii* preferred first instar larvae of *S. litura* to other larval instars. The second and third instar nymphs consumed more of first and second instar larvae whereas fourth and fifth instar nymphs as well as adult males preferred fourth and fifth instar larvae over other stages. The adult females evidently preferred fifth instar larvae of *S. litura*. Similar trend was also observed in case of *H. armigera* while more of fourth and fifth instar larvae of *E. mollifera* were consumed by *R. kumarii* adults (Ambrose and Claver, 2001).

Muniyandi *et al.* (2011) examined preference of *R. kumarii* adults to different life stages of *S. litura*. The predator preferred medium sized second and third instar larvae 44.64 per cent of times, followed by the first and fourth instar larvae with values of 26.95 and 18.16 per cent, respectively. The fifth instar larvae, at 10.23 per cent of times were preferred the least.

Stage preference studies of third, fourth, fifth instar nymphs and adults of *R. longifrons* on second, third and fourth instar nymphs of red cotton bug *Dysdercus cingulatus*, cotton mealybug *Phenacoccus solenopsis* and cotton aphid *Aphis gossyppi* were carried out by Sahayaraj *et al.* (2012). Nymphs of *R. longifrons* evidently preferred second instar nymphs of *D. cingulatus* while adults had a distinct preference towards third instar nymphs. Both nymphs and adults of predator consumed greater number of *P. solenopsis* and *A. gossyppi* adults than other stages.

2.4. FIELD EFFICACY OF REDUVIID BUGS

2.4.1. *Sycanus collaris*

Gafur *et al*. (2008) recorded predatory efficiency of *S. collaris* on tea mosquito bug *H. antonii* and leaf roller *Archips semiferanus* in a tea tree *Melaleuca* sp. plantation in Indonesia. The fourth and fifth instar nymphs of the predator as well as adults were released in a compartment at the rate of 260 numbers per 300 m^2. Damage by tea mosquito bug decreased from 30 to 10 per cent while that by leaf roller came down from 40 to 10 per cent. Mean population of *H. antonii* was also reduced from seven to less than one per tree within three months after the release of the predator.

2.4.2. Other reduviid bugs

Field cage evaluation of *Rhyncoris marginatus* against insect pests of cotton was conducted by Ambrose and Claver (1999). The pest infestation of *S. litura*, *M. pustulata* and *D. cingulatus* reduced by 45.7, 52.2 and 57.4 per cent, respectively and boll damage was reduced by 1.2 to 1.6 times relative to the plots without predator.

Field release of different stages of *R. marginatus* separately against *H. armigera* and *S. litura* in groundnut was attempted by Sahayaraj (1999a) at Tamil Nadu. The incidence of both *H. armigera* and *S. litura* were reduced to 0.77 and 0.88 per plant, respectively in treated plots as against corresponding values of 6.44 and 5.44 larvae per plant in control plots.

Sahayaraj (1999b) released different life stages of *R. marginatus* in 2500 numbers per hectare of groundnut plot. The pest fauna such as *H. armigera* and *S. litura* were reduced by 92.6 and 94.8 per cent, respectively. The predator treated plot yielded the highest yield of 1867.7 kg ha^{-1}, which was significantly superior to control plots with 1021.7 kg ha^{-1}. The cost benefit ratio and net returns were also greater in plots in which the predator was released.

Grundy (2000) investigated the prospects of using *Pristhesancus plagipennis* (Walker) as a biocontrol agent against *H. armigera* in cotton fields at University of Queensland, Australia. Third instar nymphs of *P. plagipennis* were released thrice at densities of three, six and twelve nymphs per metre row crop. Seven days after release, the population of *H. armigera* was significantly reduced to 0.6, 0.1 and 0 per m row crop respectively at the above three densities, compared to 1.2 larvae in control. The average number of retained bolls also increased markedly from 101.3 to 109.2 and to 116.5 following each release and were superior to control with 63.3 bolls per m row crop.

Sahayaraj (2002) examined effectiveness of *R. marginatus* and various leaf extracts combinations (three per cent each of calotropis, neem, pongamia and vitex) against *Aproaerema modicella* (Deventer) and *S. litura* in groundnut plot. Population of *A. modicella* was not affected by predator release. The neem leaf extracts integrated with *R. marginatus* was found effective among the different treatments.

Sahayaraj and Martin (2003) recorded that release of *R. marginatus* at the rate of 5000 per hectare in groundnut fields at 30, 50 and 70 days after sowing led to reduction in populations of *S. litura, H. armigera, Aphis craccivora* as well as that of the grasshoppers *Attractomorpha crenulata* and *Chrotogonus trachypterous* (Blanch) by 85.8, 67.65, 42.86 and 46.34 per cent, respectively though the predator had no effect on the population of the flower beetle *Mylabris* sp. population. Plots in which predator was released registered the highest productivity of 1480 kg ha^{-1} among different treatments including control plot which yielded 1104 kg ha^{-1}.

Field efficacy of fifth instar nymphs of *R. marginatus* against red cotton bug *D. cingulatus* in an open cotton field was evaluated by Ravichandran (2004). Pest population declined by two fold compared to control within three weeks after release of the predator. The predator did not affect population of natural enemies in field conditions. The yield of good quality cotton also increased by 63.1 per cent as compared to control plot.

Sahayaraj and Ravi (2007) evaluated *R. kumarii* along with botanicals such as hot water extract of *Ipomea carnea* (300g L^{-1}) and *Vitex negundo* (300g L^{-1}) as well as pesticides like endosulfan for the management of selected pests of groundnut in Tamil Nadu. The mean incidence of *S. litura* (0.76/plant), grasshoppers (0.12/plant), *H.*

armigera (0.30/plant) and *A. craccivora* (42.96/plant) were the highest in untreated plots. A significant reduction in mean infestation of leaves was recorded in plots treated with a combination of both the botanicals compared to pest population in plots treated with either of the botanicals alone. Augmentative release of sixty numbers each of *R. kumarii* nymphs and adults at 40th, 55th and 65th day after seedling emergence resulted in significant reduction in populations of *S. litura* (0.28/plant), *H. armigera* (0.12/plant) and aphids (34.31/plant) over control plots. However, predator release had no effect on grasshopper population. Plots in which *R. kumarii* was released also recorded significantly higher groundnut yield of 1655.5 kg ha^{-1} than control plots with mean yield of 1042.1 kg ha^{-1}.

The first, second, third and fourth instar nymphs of *R. kumarii*, when released at prey predator ratios of 1:24, 1:47, 1:59 and 1:75 effectively suppressed the population of cowpea aphid *A. craccivora* in groundnut ecosystem. The fourth instar nymphs were found to be more efficient when released at the ratio of 1:75, according to Sahayaraj and Asha (2010).

Periodic release of *Rhynocoris fuscipes* against cotton pests at 40, 55 and 70 days after sowing had resulted in a significant reduction in population of *Aphis gossyppi* by 28 per cent as against 23 per cent reduction in plots treated with monocrotophos (0.3%) following the first release (Sahayaraj *et al*., 2017). Plots in which *R. fuscipes* was released recorded the lowest aphid count after second and third release as well. Similarly, a 70 per cent reduction in *Phenacoccus solenopsis* population was reported in *R. fuscipes* treated plots as against 35 per cent reduction in pesticide treated plots. Likewise, the counts of *H. armigera* larvae (43%) and red cotton bugs (29%) were also significantly lower in plots where the predator was released as against pesticide treated plots (50 and 43 per cent, respectively). The results were indicative of the biocontrol potential of *R. marginatus* as a part of integrated pest management strategy in cotton ecosystem.

Materials & Methods

3. MATERIALS AND METHODS

The present study on the "Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae)" was carried out at Department of Agricultural Entomology, College of Horticulture, Vellanikkara during 2018-2020 period. Facilities at All India Coordinated Research Project on Biological Control of Crop Pests (AICRP on BCCP) were utilised for this purpose. Biology, functional response and prey stage preference of *S. collaris* on leaf feeding caterpillar *Spodoptera litura* and field efficacy of *S. collaris* against *S. litura* on cucumber in polyhouse were studied. The materials used and the methods followed in laboratory and field experiments are presented in this chapter.

3.1. BIOLOGY OF *Sycanus collaris*

3.1.1. Mass culturing of host insects

3.1.1.1. Mass culturing of Corcyra cephalonica

A continuous culture of the rice meal moth, *C. cephalonica* was maintained to rear *S. collaris*. Plastic basins of 90 cm diameter and 15 cm height, which were thoroughly washed with 0.5 per cent detergent solution, rinsed in water and shade dried were utilised for rearing *C. cephalonica* larvae. Each basin was filled up to a quarter with 2.5 kg of fortified bajra medium, the composition of which is given in Table 1.

Broken bajra grains were taken in a plastic basin and were mixed with broken groundnut kernel and yeast. Aqueous solution of streptomycin sulphate (0.1%) was added to the above mixture to prevent microbial contamination. The contents were hand mixed thoroughly. Wettable sulphur was added along the sides of each basin to prevent infestation by storage mites. The basins were then sprinkled with 0.25 cc each of 0-24 h old rice meal moth eggs. After securing the mouths of the basins with mull cloth, the basins were left undisturbed in culture rooms at 28 ± 2 ^{0}C and 70 per cent RH. The adult moths which started emerging after 35 to 40 days were collected daily using modified vacuum cleaner and released in to cages for mating and oviposition. Honey (50 per cent diluted) along with vitamin E solution was offered to adult moths. Eggs were collected daily and were used to continue *C. cephalonica* culture.

Table 1. Composition of fortified bajra medium

Sl. no	**Ingredient**	**Quantity**
1	Bajra	2.5 kg
2	Groundnut	100 g
3	Yeast	5 g
4	Streptomycin sulphate 0.1%	40 ml
5	Sulphur powder	5 g

3.1.1.2. Mass culturing of Galleria mellonella

Larvae of *G. mellonella* were utilised to study the biology of *S. collaris*. For this purpose, nucleus culture of *G. mellonella* was obtained from All India Coordinated Research Project on Biological Control of Crop Pests (AICRP on BCCP), College of Horticulture, Vellanikkara. The culture was maintained on a standard diet (Table 2).

Cornflakes were ground in to fine powder. All the ingredients except honey and glycerol were individually weighed and mixed together in a dry and clean container. Honey was dissolved in glycerol and stirred to form a homogenized mixture, which was then added to other ingredients. The above diet was transferred to breeding boxes of 30 cm diameter and 5 cm height and egg masses of the wax moth were placed in it. The diet was replenished with honey once every three days to prevent dehydration. The moths, which started emerging after 35 to 40 days were collected and transferred to plastic jars of 40 cm diameter and 15 cm height on a daily basis. These jars were covered with muslin cloth. Paper strips folded in zigzag manner were kept in jars for egg laying. The moths were provided with honey (10%) and vitamin E solution to enhance oviposition.

Rearing of *Corcyra cephalonica*

Collection of adult moths

Plate 1. Production of larvae of *Corcyra cephalonica*

Table 2. Composition of diet of greater wax moth *Galleria mellonella*

Sl. no	Ingredient	Quantity
1	Cornflake	200 g
2	Wheat bran	100 g
3	Wheat flour	100 g
4	Milk powder	100 g
5	Yeast	30 g
6	Honey	100 ml
7	Glycerol	50 ml

3.1.1.3. Mass culturing of Spodoptera litura

Nucleus culture of tobacco cutworm *S. litura* was obtained from National Bureau of Agricultural Insect Resources, Bengaluru. They were established on cleaned castor leaves placed in plastic boxes of 45 cm diameter and 5 cm height, lined with tissue paper. The lid of each box was provided with a square window (5 cm x 5 cm) covered with muslin cloth for proper aeration. The petioles of castor leaves were covered with moistened cotton to prevent drying up of leaves. The containers were cleaned and provided with fresh castor leaves every day until larvae reached pre pupal stage. Pupae were collected and introduced in to a plastic container for adult emergence. Adult moths were collected daily in to cylindrical containers of 40 cm diameter and 15 cm height. Fresh castor leaves and paper strips were provided inside the container along with cotton soaked in honey and vitamin E solution for mating and oviposition. After three days, egg masses were collected and placed in containers of 30 cm diameter and 5 cm height along with fresh castor leaves. After hatching, neonate larvae were transferred in to another container of similar dimension and fed with castor leaves. The larvae thus obtained were used for studies on functional response, prey-stage preference and field evaluation.

3.1.2. Mass culturing of *Sycanus collaris*

Initial culture of the reduviid bug *S. collaris* was obtained from National Institute of Plant Health Management, Hyderabad. These bugs were reared under laboratory conditions (28 ± 2°C and 70% RH) in plastic boxes of 45 cm diameter and 5 cm height lined with tissue paper at the base. Larvae of *C. cephalonica* were provided on daily basis as food for the predator. Egg masses laid were collected and were transferred to another container for hatching. The emerged nymphs were fed with *C. cephalonica* larvae of appropriate size. Freshly moulted adults were introduced in to another container lined with tissue paper for mating and egg laying.

3.1. Biology of *Sycanus collaris*

Studies on the biology of *S. collaris* was carried out at AICRP on BCCP, College of Horticulture, Vellanikkara at 28°C temperature and 70 per cent relative humidity from September, 2019 to February, 2020.

Adult male and female bugs of uniform age were formed in to pairs and were allowed to mate and oviposit. Ten such pairs were maintained in well ventilated plastic boxes lined with tissue paper and were provided with *G. mellonella* larvae as prey. Egg masses of 0-24 h age were collected and kept for hatching in plastic boxes of 30 cm diameter and 5 cm height. Upon hatching, a total of one hundred nymphs from the same egg mass were transferred individually using fine camel brush in to plastic cups of 22 cm diameter and 3 cm height lined with tissue paper and were covered with muslin cloth. Each nymph was provided with *G. mellonella* larvae of appropriate size. The prey was replenished as and when required. Newly moulted 0-24 h old female and male bugs were formed in to pairs and were maintained as already described. The paired bugs were observed daily for oviposition and the egg mass laid were collected. Eggs in each egg mass were counted under stereo microscope (Leica 10 X). Data pertaining to incubation period, number and duration of each nymphal instar, pre oviposition period, fecundity, oviposition period, post oviposition period, sex ratio and adult longevity were recorded.

Plate 2. Rearing of *Galleria mellonella*

Plate 3. Rearing of *Spodoptera litura*

Plate 4. Rearing of *Sycanus collaris*

Plate 5. Rearing nymphs of *Sycanus collaris* for studies on biology

Plate 6. Nymph of *Sycanus collaris* feeding on wax moth larva

Plate 7. Rearing adults of *Sycanus collaris* for studies on biology

3.2. FUNCTIONAL RESPONSE OF *Sycanus collaris*

The functional response of *S. collaris* was studied in plastic containers of 35 cm diameter and 5 cm height and lined with tissue paper. Third instar larvae of *S. litura* of uniform size were transferred in to the container using a fine camel hair brush at five different densities of 1, 2, 4, 8 and 16 larvae per container. They were provided with fresh castor leaves as food. Thereafter, 0-24 h old fifth instar nymphs of *S. collaris*, pre-starved for 24 h were introduced individually into each container. Six replicates of each density were maintained. The number of larvae killed by each nymph in 24 h was recorded.

Functional response parameters were studied using regression analysis and linear correlation. The proportion of larvae killed to the number of larvae offered appraise fitting of response curve and was determined by logistic regression (Sinha *et al.*, 1982; Trexler *et al.*, 1988). The following polynomial function was used to fit the above data on proportion of larvae killed as a function of initial prey density (Juliano, 1993).

$$\frac{Ne}{N0} = \frac{\exp(L_0 + L_1N_0 + L_2 N_0^2 + L_3 N_0^3)}{1 + \exp(L_0 + L_1N_0 + L_2 N_0^2 + L_3 N_0^3)}$$

where, N_0 was initial density of prey offered, Ne represented the number of larvae killed and Ne/N_0 was the probability of larva being killed by predator. The parameters L_o, L_1, L_2 and L_3 represented intercept, linear, quadratic and cubic coefficients, respectively. The maximum likelihood method was used to estimate coefficients. Depending on negative and positive signs of coefficients, type of functional response was determined. Type I functional response was characterised by linear terms not differing significantly from zero ($L_1 = 0$). A negative linear term ($L_1 < 0$) significantly suggested a type II functional response, whereas a positive linear term ($L_1 > 0$) significantly suggested a type III functional response (Juliano, 2001).

Non-linear least square regression (NLIN in SAS) was carried out to assess associated parameters after evaluation of type of functional response (Holling, 1966; Houk and Strauss, 1985; Williams and Juliano, 1985). Analysis of variance

(ANOVA) was carried out to compare average number of larvae killed at each of the prey density. During present study, the number of larvae consumed showed an accelerating relation with successive increase in prey densities. The functional response equation of type II predator is mentioned below:

$$Ne = N_0 \, [1\text{-}\exp \{a \, (T_h Ne - T)\}]$$

where, a is the predator attack coefficient, T_h is handling time and T is the entire time for which predators were exposed to larvae (Rogers, 1972).

3.3. PREY STAGE PREFERENCE OF *Sycanus collaris*

The experimental arena of prey stage preference studies also comprised of plastic containers of 35 cm diameter and 5 cm height lined with tissue papers. In to each container was placed a castor leaf with moistened absorbent cotton wrapped around its petiole. Two larvae of *S. litura* from each of the five instars were transferred from stock culture and transferred to the plastic containers using a fine camel hair brush. The larvae were allowed to settle. Freshly moulted fifth instar nymphs (0-24 h) of *S. collaris* were starved for 24 h to standardise response and were released individually in to each of the test arenas. Ten replications were maintained. The number of *S. litura* larvae of each stage killed after 24 h was recorded. Test for proportions was utilised to analyse the results obtained.

3.4. FIELD EFFICACY OF *Sycanus collaris* AGAINST TOBACCO CATERPILLAR *Spodoptera litura* ON CUCUMBER IN POLYHOUSE

Evaluation of the field efficacy of *S. collaris* against *S. litura* on cucumber in polyhouse was conducted at College of Horticulture, Vellanikkara during September-November, 2020.

Polyhouse of approximately 100 sq.m area was partitioned in to two plots. Following land preparation, the polyhouse was sterilised by formaldehyde (1:19). Cucumber (variety KPCH-1) seedlings were raised in a medium composed of vermicompost, perlite and vermiculite in 3:1:1 ratio. The seedlings were watered twice a day. Fertiliser mixture of 19:19:19 was applied three times a week as foliar spray. At three leaf stage (10 DAS), they were transplanted at 1m x 0.5m spacing. A total of 190 cucumber plants were maintained. Recommended fertiliser regime of cucumber was 70:25:25 kg NPK/ha as per Package of Practices Recommendations (KAU, 2016). The nitrogenous fertilisers were applied in split pattern. Half dose of nitrogen and full dose of phosphorous and potassium was applied at planting. The remaining dose of nitrogen were split in two halves and applied at vining and full blooming stage.

Cucumber plants at vining stage were vertically trailed on to nylon nets. Irrigation of crop was carried out daily. Third instar larvae of *S. litura* reared in the laboratory were released on to cucumber plants in both plots at a rate of five larvae per plant, 30 days after transplanting, by using fine camel hair brush. Fifth instar nymphs of *S. collaris* were released randomly in one plot at a rate of 100 nymphs/50 m^2 two days after establishment of pest on crop. Plot without *S. collaris* served as the untreated control. Twenty plants randomly selected from each plot were tagged for recording observations. The number of *S. litura* larvae was visually assessed at 1, 3 and 5 days after release of the predator. The results were analysed by using paired t test.

Plate 8. Experimental arena for study on functional response of *Sycanus collaris*

Plate 9. Experimental arena for study on prey stage preference of *Sycanus collaris*

Plate 10. Polyhouse at All India Coordinated Research Project on Biological Control of Crop Pests

Plate 11. Layout of experiment for evaluating efficacy of *Sycanus collaris* against *Spodoptera litura* on cucumber in polyhouse

Plate 12. Treatment and control separated using garden net

Table 13. Release of *Spodoptera litura* larva on cucumber plant

Larva of *Spodoptera litura* feeding on cucumber leaf

Larvae of *Spodoptera litura* feeding on cucumber fruit

Plate 14. Tobacco caterpillar damage on cucumber in polyhouse

Plate 15. Release of *Sycanus collaris* on cucumber plant

Results

4. RESULTS

The results of study on “Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae),” conducted at the AICRP on BCCP, College of Horticulture, Vellanikkara are presented hereunder.

4.1. BIOLOGY OF THE REDUVIID BUG *Sycanus collaris*

The studies on the biology of *Sycanus collaris* on *Galleria mellonella* were carried out in the laboratory at 28 ± 2 ^{0}C and 70 per cent RH during the period from September-February, 2019-2020. The results pertaining to the incubation period, duration of each developmental stage, fecundity, sex ratio and adult longevity are presented in Table 3.

4.1.1. Duration of development of *Sycanus collaris* on *Galleria mellonella*

Life cycle of *S. collaris* comprised of three different stages, namely, egg, nymph and adult. The nymphal stage consisted of five instars.

4.1.1.1. Egg

The eggs were laid in clusters which were cemented to the substratum. They were broadly oval, yellowish brown in colour and were arranged in oblique rows in a chevron pattern. They were glued to each other and were covered with a frothy secretion.

The operculum of shiny and smooth egg was milky white with exolateral parts of chorion being brown and endolateral parts being yellow in colour. Prior to hatching, the fertilized egg turned bright red, with the black eye spots of nymphs being visible through chorion, while unfertilized turned black and shrunk. The mean incubation period was 13.21 ± 0.04 days. The average egg hatchability was 96 per cent. The nymphs emerged through the operculum.

Table 3. Duration of life stages of *Sycanus collaris* on *Galleria mellonella*

Life stage	**Mean days ± SE**	**Range**
Egg*	13.21 ± 0.04	13-14
Nymph**		
First instar	12.55 ± 0.18	10-18
Second instar	9.12 ± 0.16	6-13
Third instar	8.88 ± 0.12	8-13
Fourth instar	10.95 ± 0.13	7-13
Fifth instar	15.93 ± 0.17	11-20
Total developmental period	57.37 ± 0.29	50-64
Adults***		
Male	66.29 ± 2.86	40-107
Female	78.95 ± 1.88	51-110
Total male longevity	123.66 ± 3.15	90-171
Total female longevity	136.32 ± 3.44	101-174

* Mean of 106 observations

**Mean of 100 observations

***Mean of 44 observations

4.1.1.2. First instar nymph

The first instar nymph was fragile and gregarious with body colour varying from light yellow to brown with brown to black colouration on abdominal tip. Head was longer than pronotum and ocelli were absent. The last antennal segment was red, while the remaining annuli were brown in colour. Tibia was longer than femur. The

Eggs laid in a mass

Loosely laid eggs

Plate 16. Oviposition by *Sycanus collaris*

Freshly laid eggs

Eggs just before eclosion

Plate 17. Eggs of *Sycanus collaris*

nymph became tanned 3-4 h after emergence and started feeding thereafter. The mean duration of first instar nymphs was 12.55 ± 0.18 days with 98 per cent survival rate.

4.1.1.3. Second instar nymph

The bright yellow second instar nymph, with head being similar to adult had two black spots on the dorsal surface of abdomen adjoining thorax. A black oval patch was observed at the tip of the abdomen. Ocelli were absent. The basal segments of antenna were brown and last antennal segment was red. The second instar nymphs lived for an average of 9.12 ± 0.16 days with 97 per cent nymphs moulting to next instar.

4.1.1.4. Third instar nymph

Third instar nymph had a deep yellow colour as compared to the second instar nymph. Abdomen was short, erect and was characterized by two black oval spots and a black patch at distal end of abdomen. Shape of head was similar to that of the adult. Ocelli were not present at this developmental stage either. The mean nymphal period was 8.88 ± 0.12 days with a 95 per cent survival rate.

4.1.1.5. Fourth instar nymph

The body was dark yellow in colour and abdomen was elongate with a prominent black patch and two black spots on dorsal surface of abdomen near the thorax. Head shape resembled that of adult. Ocelli were absent at this stage also. External wing pads were visible but were not fully developed. Connexivum was blunt and round. The mean duration of nymphal instar was 10.95 ± 0.13 days and 93 per cent nymphs survived to next moulting.

4.1.1.6. Fifth instar nymph

Fifth instar nymph showed a marked increase in size. Body was erect and long. Colour varied from bright yellow to orange with a large black patch covering abdomen entirely except at the borders. Presence of ocelli as well as well developed, black coloured wing pads separated this instar from preceding ones. Female and male nymphs could be distinguished based on the broader abdomen that the females possessed. Nymphs had protruding connexivum. The fifth instars took a longer mean duration of 15.93 ± 0.17 days to develop in to adults. No mortality of nymphs was observed.

4.1.1.7. Adults

Adults of *S. collaris* were comparable to fifth instar nymphs in dimensions and exhibited distinct sexual dimorphism. Abdomen and wings of both sexes were bright yellow with black patches. Males of *S. collaris* were slender and small with pointed abdominal base while females were relatively larger with round abdomen. Connexivum was black with yellow coloured stripes. The adult female was the largest among the different life stages of the predator and lived, on an average, for 78.95 ± 1.88 days, while adult male lived for a mean 66.29 ± 2.86 days. The total female and male longevity extended to 136.32 ± 3.44 and 123.66 ± 3.15 days, respectively.

4.1.2. Reproductive biology of *S. collaris*

4.1.2.1. Mating

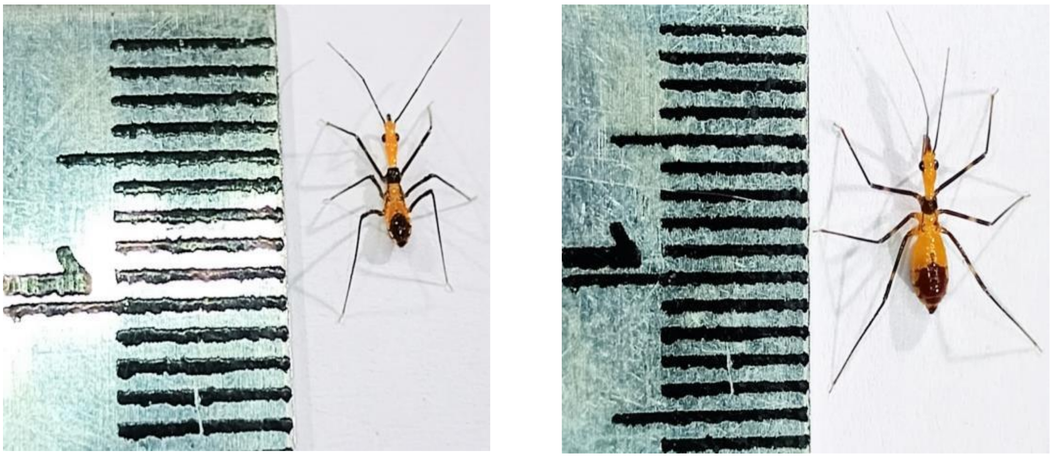
Mating was a sequential process and includes arousal, approach, riding over and copulation.

4.1.2.1.1. Arousal and approach

The sexually mature adult bugs were excited upon encountering each other. When a male reduviid was introduced in to container with a receptive female, it was aroused and approached the female and touched her with extended rostrum and antennae.

4.1.2.1.2. Riding over

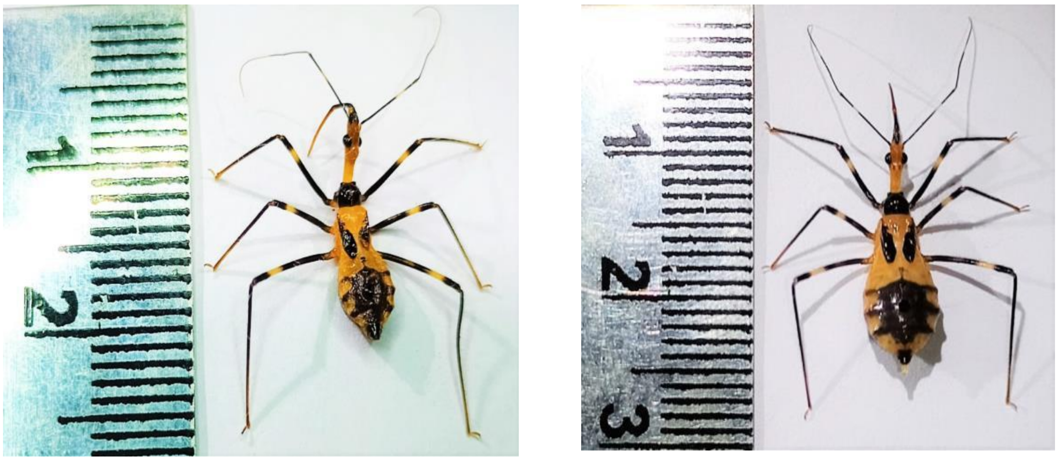
The male then positioned itself over the dorso-ventral surface of females and clasped the later with forelegs. The male held abdomen of female with its hindlegs. Rostrum was placed over pronotum and neck region of female.



First instar

Second instar

Third instar



Fourth instar

Fifth instar

Plate 18. Nymphal instars of *Sycanus collaris*

Female

Male

Plate 19. Adults of *Sycanus collaris*

4.1.2.1.3. Copulation

The riding was followed by extension of genitalia by male and intromission. During copulation, contact of each other's antenna and tibia was noticed. Copulation persisted from few minutes to an hour. The completion of copulation was marked by drooping of the antenna in both the sexes and subsequent ejaculation of spermatophore capsule by the female.

4.1.2.2. Pre-oviposition, oviposition and post-oviposition period

Female bug, on an average oviposited for 51.86 ± 1.03 days after a mean pre oviposition period of 12.6 ± 0.32 days. It died 13.43 ± 2.21 days after laying the last batch of eggs (Table 4).

Table 4. Reproductive parameters of *Sycanus collaris* on *Galleria mellonella*

Reproductive parameter	**Mean ± SE**	**Range**
Pre-oviposition period (days)	12.60 ± 0.32	8-17
Oviposition period (days)	51.86 ± 1.03	19-77
Post-oviposition period (days)	13.43 ± 2.21	5-20
Fecundity (eggs/female)	451.56 ± 7.92	344-538
Total number of egg clutches/female	9.45 ± 0.28	5-13
Sex ratio (male: female)	1: 1.39	

*Figures are mean of 44 observations

4.1.2.3. Fecundity and sex ratio

A gravid female, on an average, laid 451.56 eggs in 5 to 13 clusters during its life time. The average number of egg clusters was 9.45 per female. Each cluster consisted of approximately 12-80 eggs. The total number of eggs laid varied from 344-538. A slightly female biased sex ratio of 1:1.39 was observed for *S. collaris*.

4.2. FUNCTIONAL RESPONSE OF THE REDUVIID BUG *Sycanus collaris*

Functional response of fifth instar nymphs of *Sycanus collaris* was assessed using third instar larvae of *Spodoptera litura* at five different densities of 1, 2, 4, 8 and 16 prey per predator, replicated six times. The number of prey killed by the predator in 24h was recorded. The results are presented below (Table 5).

Table 5. Functional response of *Sycanus collaris* to different prey densities of *Spodoptera litura*

Treatments	**Mean number of prey killed**	**Proportion of prey killed**
T1 (1:1)	1.00 (1.00)^{a}	1.00
T2 (1:2)	1.67 (1.27)^{a}	0.83
T3 (1:4)	2.83 (1.67)^{b}	0.70
T4 (1:8)	4.50 (2.10)^{c}	0.56
T5 (1:16)	5.50 (2.33)^{d}	0.34
CD (P=0.05)	0.24	

Values in the parentheses were square root transformed; figures followed by similar alphabets did not differ significantly

The number of prey consumed increased gradually from one to 16 prey per predator but at a decelerating rate. The highest mean number of 5.50 larvae was killed at prey predator ratio of 1:16 which was significantly higher than the mortality recorded at lower prey densities. This was followed by the mean mortality of 4.50 and 2.83 at predator prey ratios of 1:8 and 1:4 respectively. Both the values were significantly different from each other but superior to the mortality values of 1.67 and

1.0 at prey densities of 1:2 and 1:1 respectively, which, however, were on par with each other. The proportion of prey killed was negatively correlated to initial prey densities, with values of 1, 0.83, 0.70, 0.56 and 0.34 at densities of 1, 2, 4, 8 and 16 respectively.

Table 6. Maximum likelihood estimates of proportion of *Spodoptera litura* killed by *Sycanus collaris* from logistic regression as a function of initial prey density

Parameter	**Estimate**	**Standard Error**	**Chi-square**	**Pr>Chi sq**
Intercept	0.9691	0.4219	5.28	0.0216
Linear (P1)	-0.0997	0.0330	9.15	0.0025

Linear parameters from logistic regression (Daniel, 1987) were negative ($P_1 < 0$) and significant ($P_1 < 0.05$) as mentioned above.

Table 7. Mean values of attack rate (a) and handling time (T_h) for *Sycanus collaris* on *Spodoptera litura*

Parameter	Estimate	Approximate standard error	Approximate 95% confidence limits	
a	0.0198	0.00953	-0.00032	0.0399
T_h	-0.3284	2.3394	-5.2641	4.6073

Holling's disc model evaluates vital parameters of attack rate and handling time by non-linear least square regression (Table 7). The handling time (T_h) of predator was time lag between feeding completion and resume attack again. Random predator equation for type II response was used to estimate attack rate of 0.0198 h^{-1} and handling time of -0.3284 h. The attack rate was greatest at one prey per predator and lowest at 16 prey per predator. At 95 per cent confidence limits, attack rate and handling time varied from -0.00032 to 0.0399 h^{-1} and -5.2641 to 4.6073 h, respectively. As handling time (T_h) cannot be negative, it was presumed to lie between 0 and 4.6073; i.e. $0 < T_h < 4.6073$ h.

4.3. PREY STAGE PREFERENCE OF THE REDUVIID BUG *Sycanus collaris*

The prey stage preference of *S. collaris* was studied by offering all five instars of *S. litura* at a rate derived from functional response study, to individual 0-24 h old final instar nymphs of *S. collaris*. The experiment was replicated ten times.

When all the five instars of *S. litura* were provided at an equal proportion of 2:2:2:2:2, fifth instar nymphs of *S. collaris* on an average killed 1.40 first instar, 1.50 second instar, 1.70 third instar, 0.60 fourth instar and 0.30 fifth instar larvae of *S. litura* in 24h which was equivalent to 70, 50, 85, 30 and 15 per cent, respectively. The data, when subjected to test for proportions, revealed significant preference for the first, second and third instar larvae of *S. litura* over the fifth instar ones. The predator also showed significantly higher preference to third instar larvae as compared to fourth instar larvae. No significant preference by the predator was observed between first three larval instars as well as between fourth and fifth instar larvae.

Table 8. Prey stage preference of fifth instar nymph of *Sycanus collaris* on different larval instars of *Spodoptera litura*

Larval instars of *S. litura* (2:2:2:2:2)	Predation	
	Mean ± SE	Per cent
First instar	1.4 ± 0.20	70
Second instar	1.5 ± 0.21	75
Third instar	1.7 ± 0.14	85
Fourth instar	0.6 ± 0.20	30
Fifth instar	0.3 ± 0.14	15

*Mean of ten observations

Table 9. Test for significance between proportions

Proportions	Z value
$p_1 - p_2$	0.25
$p_1 - p_3$	0.86
$p_1 - p_4$	1.60
$p_1 - p_5$	2.20*
$p_2 - p_3$	0.62
$p_2 - p_4$	1.80
$p_2 - p_5$	2.42*
$p_3 - p_4$	2.45*
$p_3 - p_5$	2.80*
$p_4 - p_5$	0.86

*Significant at 5% level

4.4. FIELD EFFICACY OF *Sycanus collaris* AGAINST *Spodoptera litura* IN POLYHOUSE ON CUCUMBER

The efficacy of *S. collaris* in managing tobacco caterpillar *S. litura* on cucumber was carried out in polyhouse conditions. Third instar *S. litura* larvae were released at a rate of 5 per plant which was followed by the release of the predator at a rate of 100 nymphs per 50 m^2. The number of live *S. litura* larvae was recorded at 1, 3 and 5 days after the release of the predator.

Table 10. Field efficacy of *Sycanus collaris* against *Spodoptera litura* on cucumber

Treatment	**Mean number of larvae per plant**		
	1 DAR	**3 DAR**	**5 DAR**
S. collaris@100 nymphs/50 m^2	1.83	0.53	0
Untreated control	5	5	5
P value	0.016*		

DAR- Days after release *****Significant at 5% level

One day after release of the predator, a mean number of 1.83 larvae/plant was observed in treated plot. The mean number of larvae recorded was 0.53/plant three days after application of treatment and no larvae were observed five days after release of the bug. The population of *S. litura* declined by 63.4 and 89.4 per cent, respectively after one day and three days after release of the predator. However, there was no reduction in the larval population in control plots throughout the period of observations.

A paired t test was conducted to analyse the results obtained. There was a significant difference between the treatment (M=0.78, SD=0.94) and control plots (M=5, SD=0) with regard to number of *S. litura* larvae [t (2) = -7.75, p = 0.016].

Plate 20. Final instar nymph of *Sycanus collaris* feeding on *Spodoptera litura* larva in polyhouse

Treated plot

Control plot

Plate 21. View of the experimental plot five days after release of predator

Discussion

5. DISCUSSION

The biology, functional response and prey stage preference of the reduviid predator *Sycanus collaris* as well as the efficacy of *S. collaris* against *Spodoptera litura* on cucumber under polyhouse condition were studied at College of Horticulture, Vellanikkara. The findings of above experiments are discussed in this chapter.

5.1. BIOLOGY OF THE REDUVIID BUG *Sycanus collaris*

Studies on biology of *S. collaris* were carried out using the greater wax moth *Galleria mellonella* larvae as prey in laboratory, at 28 ± 2 ^{0}C and 70 per cent RH.

5.1.1. Life cycle of *Sycanus collaris* on *Galleria mellonella* larvae

Life cycle of *S. collaris* consisted of three different developmental stages in the form of egg, nymph and adult.

5.1.1.1. Duration of development of life stages of Sycanus collaris

5.1.1.1.1. *Egg*

Sycanus collaris laid 12-80 oval yellowish brown eggs in clusters coated with white frothy secretion. Eggs in each cluster were arranged in oblique rows of chevron pattern and glued to the muslin cloth. Few eggs were also laid loosely on the sides of container as well as the cloth covering the container as recorded for other reduviid species, such as *S. pyrrhomelas* Walker (Ambrose and Paniadima, 2000), *S. auranticus* (Ishiwaka *et al.*, 2007) and *Coranus spiniscutis* (Claver and Reegan, 2010). Prior to hatching, eggs turned reddish brown with black eyespot of nymphs visible through chorion. The above observations were in accordance with the findings of Rajan *et al.* (2017) and Sarkar *et al.* (2019) who reported that *S. collaris* laid brownish egg clusters glued vertically to the substratum. Srikumar *et al.* (2017) also had observed that yellowish brown oval egg clutches of *S. collaris* were deposited on muslin cloth cover or on the sides of rearing bottles.

The eggs hatched after a mean incubation period of 13.21 ± 0.04 days with a range of 13-14 days. The hatchability of eggs was 96 per cent with 75 per cent of eggs hatching in 12-13 days and the remaining 21 per cent hatching within 13-14 days. Unfertilised eggs became shrivelled within few days. The high hatchability was a diagnostic feature of harpactocorine eggs (Das, 1996; Das *et al.*, 2007). George *et al.* (1998) who reared *S. collaris* on *S. litura* at 30°C and 75-80 per cent RH had recorded a lower mean incubation period of 7.17 days. The incubation period recorded in the study is, however, in close proximity with the findings of Rajan *et al.* (2017) who noticed an average incubation period of 12.42 and 11.56 days when *S. collaris* was reared on *C. cephalonica* and *S. litura*, respectively at 32 °C and 75 per cent RH. Srikumar *et al.* (2017) recorded a much longer incubation period of 22 days for *S. collaris* at 22-26 °C and 60-70 per cent RH on *C. cephalonica*. Sarkar *et al.* (2019) also reported a mean incubation period of 13.6 days on *C. cephalonica* and 11.8 days on *Hyposidra talaca* at 27 °C and 70-80 per cent RH which again broadly agrees with the findings of the present study.

5.1.1.1.2. *Nymph*

5.1.1.1.2.1. First instar

The mean duration of development of first instar nymphs was 12.55 ± 0.18 days, within a range of 10-18 days. Nymphs initiated feeding within 8-12 h after emergence. The above observation is in conformity with those of Rajan *et al.* (2017) who reported that the first instar nymphs lived for 11.38 and 12.54 days on an average, when fed with *C. cephalonica* and *S. litura*, respectively. However, several authors have recorded much lower stadia for first instar nymphs of *S. collaris*. Thus, George *et al.* (1998) had observed the average duration of first instar nymphs to be 6.6 days on *S. litura* while Srikumar *et al.* (2017) had recorded the mean duration of first instar nymphs as 9.9 days, when reared on *C. cephalonica.* Comparable values of 9.6 days on *C. cephalonica* and 10.4 days on *H. talaca* were also recorded by Sarkar *et al.* (2019).

5.1.1.1.2.2. Second instar

Second instar nymphs of *S. collaris* lived for 6 to 13 days with an average of 9.12 ± 0.16 days, which concurs with the results reported in a number of studies. George *et al.* (1998) recorded a mean duration of 6.3 days for second instar nymphs on *S. litura*. Similarly, Srikumar *et al.* (2017) observed 9.9 days duration on *C. cephalonica.* Rajan *et al.* (2017), however, reported a higher average developmental time of 12.24 days for second instar nymphs on both *C. cephalonica* and *S. litura*. Sarkar *et al.* (2019) also had documented higher mean values of 11.26 and 12.8 days on *C. cephalonica* and *H. talaca*, respectively.

5.1.1.1.2.3. Third instar

The duration of third instar nymphs ranged from 8 to 13 days, with an average of 8.88 ± 0.12 days. The above results are in broad agreement with the findings of George *et al.* (1998) who observed that third instar nymphs of the predator had a mean development duration of 6.90 days on *S. litura*. Rajan *et al.* (2017) had reported a longer duration for third instar nymphs on *C. cephalonica* (12.58 days) and *S. litura* (13.26 days), respectively. Srikumar *et al.* (2017) also reported an identical mean duration of 11.8 days when offered with *C. cephalonica* larvae.

5.1.1.1.2.4. Fourth instar

Fourth instar nymphs had a mean development duration of 10.95 ± 0.13 days ranging from 7 to 13 days, which agrees with the duration of 13.1 days reported by George *et al.* (1998), when reared on *S. litura.* Similar values of 12.1 and 12.6 days were also reported by Srikumar *et al.* (2017) and Sarkar *et al.* (2019), when the predator nymph was reared on *C. cephalonica*. Rajan *et al.,* (2017) on the other hand, discerned average duration of fourth instar nymphs to be 14.62 days and 12.1 days on *C. cephalonica* and *S. litura,* respectively.

5.1.1.1.2.5. Fifth instar

Among all nymphal stages, fifth instar nymphs had the longest duration of development of 15.93 ± 0.17 days with a range of 11 to 20 days. George *et al.* (1998)

had observed the nymphal duration of fifth instar to be 16.4 days. Rajan *et al.* (2017) also had noticed that development of fifth instar nymphs took 15.42 and 16.58 days when fed on *C. cephalonica* and *S. litura*, respectively. Srikumar *et al.* (2017), however, recorded a protracted nymphal period of 21.4 days on *C. cephalonica.* The final instar nymphs, according to Sarkar *et al*. (2019) lived for an average of 13.4 and 15 days on *C. cephalonica* and *H. talaca*, respectively.

5.1.1.1.3. Adult

The adult females of *S. collaris* lived for 78.95 ± 1.88 days on an average within a range of 51-110 days while adult males had a mean life span of 66.29 ± 2.86 days within a range of 40-107 days. The total life cycle of male bugs lasted for 123.66 ± 3.15 days and that of female bugs lasted for 136.32 ± 3.44 days. The longevity recorded in case of females are identical with the mean longevity of 80.64 and 85.48 days while male bugs had a duration of 73.58 and 75.82 days, respectively on *C. cephalonica* and *S. litura* (Rajan *et al.,* 2017). The variations in the current findings might have been due to difference in the host and weather conditions under which bugs were reared. Srikumar *et al.* (2017) observed females survived for 113.1days and males for 60.2 days when fed with *C. cephalonica*. In contrast to findings obtained from present study, adult females and males lived for 30 to 45 days on *S. litura* larvae (George *et al.*, 1998). Likewise, Sarkar *et al.* (2019) also reported that mean adult longevity of females and males was 58.2 and 41.6 days, respectively on *C. cephalonica.*

5.1.1.1.4. Total nymphal developmental period and their survivability

The total nymphal developmental duration of *S. collaris* took 57.37 ± 0.29 days and ranged from 50-64 days on *G. mellonella*. Similar findings with varied results by Rajan *et al.* (2017) revealed nymphal duration of 66.24 days on lepidopterans like *C. cephalonica* and 69.04 days on *S. litura*. A recent study by Sarkar *et al.* (2019) recorded a mean nymphal duration of 58.4 and 64.8 days on *C. cephalonica* and *H. talaca,* respectively which was close to 57.37 days found in the current study.

Ninety three per cent of *S. collaris* immature stages survived to adulthood. Total mortality induced was seven per cent. Mortality was comparatively higher in early instars with subsequent reduction as the age advanced. Nymphal mortality was due to abnormalities pronounced at hatching, moulting, battling against prey, starvation resulting from inability to combat larger prey or cannibalistic behaviour (Vennison and Ambrose, 1990; Sahayaraj, 2007; Das and Ambrose, 2008). These studies were in line with the findings of Rajan *et al.*, (2017) who stated 92.11 per cent survival of nymphs of *S. collaris* when reared on *S. litura* larvae. Reduviids, including *R. marginatus* on *S. litura* (George, 1999), *R. kumarii* on larvae of *C. cephalonica, S. litura* and *Earias vitella* (George, 2000) as well as *S. dichotomus* on larvae of *C. cephalonica* (Zulkefli *et al.,* 2004) also had exhibited higher survival rate. Nymphal mortality may differ between reduviid species and those with minimum mortality can be used for mass production (Sahayaraj, 2007)

5.1.1.2. Reproductive biology of Sycanus collaris

5.1.1.2.1. Pre-oviposition, oviposition and post-oviposition period

Freshly moulted adults mated with in one day after their emergence. The recipient female laid eggs after an average pre oviposition period of 12.60 ± 0.32 days with a range of 8-17 days. Rajan *et al.* (2017) recorded a mean pre oviposition period of 8.48 days on *C. cephalonica* and 7.86 days on *S. litura*. However, Sarkar *et al.* (2019) observed S. *collaris* laid eggs after 9.2 days when reared on *C. cephalonica*. These studies concur with above reports.

Egg laying continued for 51.86 ± 1.03 days ranging from 19-77 days. The findings were quite similar to studies by Rajan *et al.* (2017) who noticed ovipositional period of 48.52 and 53.48 days on *C. cephalonica* and *S. litura*, respectively. Sarkar *et al.* (2019) however, reported a much lower 39.6 days as oviposition period when *C. cephalonica* was provided as prey.

The ovipositional period was followed by an average post ovipositional period of 13.43 ± 2.21 days varying from 5-20 days. This was comparable with the post ovipositional period of 15.18 days on *C. cephalonica* and 12.56 days on *S. litura* recorded by Rajan *et al.* (2017) but differed from the 9.4 days on *C. cephalonica* as observed by Sarkar *et al.* (2019). These divergent values of pre oviposition, oviposition and post oviposition periods of *S. collaris* could be due to disparity in population utilized for study or nutritional composition of prey species.

5.1.1.2.2. Fecundity and sex ratio

The mean fecundity of *S. collaris* was 451.56 ± 7.92 eggs with a range of 344-538 eggs during its lifetime. This was at variance with the fecundity of 105.67 eggs on *S. litura* reported by George *et al.* (1998) as well as 256.58 and 340.82 eggs on *C. cephalonica* and *S. litura*, respectively reported by Rajan *et al.* (2017). A similar figure of 282 eggs was reported on *C. cephalonica* by Sarkar *et al.* (2019). Considerable variation can be observed among the different reports on the fecundity of *S. collaris*. Srikumar *et al.* (2017), for instance, recorded highest fecundity of 720 eggs when the bug was reared on *C. cephalonica* at 22-26 °C.

A single female in its life span laid 5 to 13 egg clutches each consisting of 12 to 80 eggs. Srikumar *et al.* (2017) revealed *S. collaris* laid 8 to 9 egg masses whereas, Sarkar *et al*. (2019) revealed egg clutches were 3 to 5 in number containing 55-63 eggs per clutch.

S. collaris showed a female biased sex ratio of 1:1.39. Studies by Srikumar *et al.* (2017) recorded similar male: female ratio of 0.85:1 on *C. cephalonica* and 0.82:1 on *S. litura* while sex ratio of 0.5:1 on *C. cephalonica* was noticed by Sarkar *et al.* (2019).

The results of the present study broadly agree with earlier reports on the biology of *Sycanus collaris.* However, it is noteworthy that variations in development and reproduction can be observed among the aforementioned studies themselves. The influence that the type of the prey species and experimental conditions exert on the biology of natural enemies is well documented (Mendes *et al*., 2002; Chandral and Sinazer, 2011; Syari *et al*., 2011). While previous studies employed species like *S. litura* and *C. cephalonica* as prey, larvae of the wax moth *Galleria mellonella* were used as prey in the present study. The above fact coupled with differences in the experimental conditions could have contributed to the variations reported in the biology of *S. collaris* in the present study.

5.2. FUNCTIONAL RESPONSE OF *Sycanus collaris*

The functional response of fifth instar nymphs of *S. collaris* was studied by offering third instar larvae of *S. litura* at densities of 1, 2, 4, 8 and 16 prey per predator under ambient conditions.

The successful attack of the prey by the predator as a function of initial prey densities or response of the predator to altered prey densities in a particular time frame is defined as functional response (Solomon, 1949). Hollings model commonly called as disc equation is a core feature in determining functional response. Number of prey killed plotted against initial prey density is used to assess whether data fits any of the three types of functional response curves (Hollings, 1959; 1959a).

In type I functional response, number of prey killed is independent of prey density and proportion of prey killed increases linearly to maximum beyond which it remains constant. In type II, response is negatively density dependent where prey killed increases but at a declining rate towards maximum and proportion of prey killed decreases as the density of prey offered is increased. Type III functional response is positively density dependent as the number of prey killed increases corresponding to an increase in proportion of prey killed up to inflection point followed by reduction in proportion of prey killed yielding a sigmoidal curve (Holling, 1965, 1966; Hassell *et al.,* 1977; Trexler *et al.,* 1988).

S. collaris killed greater number of *S. litura* larvae at higher prey densities than at lower densities. As the initial prey density increased from one to 16 in the test arena, the number of *S. litura* larvae killed by the individual predator also increased gradually, from one to 5.50 though at a decreasing rate. The proportion of larvae killed, however, was negatively correlated to initial prey densities, with values of 1.0 and 0.34 at prey densities of 1 and 16, respectively. The above observations are typical of type II functional response. The linear parameters were also negative ($P_1 <$

0) and significant ($P_1 < 0.05$) which further support the above conclusion. Attack rate of *S. collaris* was 0.0198 h^{-1} while the handling time was between 0 to 0.460 h.

At higher densities, the number of *S. litura* larvae killed continued to increase, though at decreasing proportions. This was along expected lines for a predator with type II functional response. At lower densities, the predator needs to kill more number of prey and inter alia, a greater proportion of prey to satiate itself. At higher densities of prey, the predator encounters prey more often, leading to enhanced number of kills till satiation is attained (Akhtaruzzaman and Ahmed, 1998; Claver and Ambrose, 2003). In addition, aggregation of prey in test arena could incite the predator to feed till satiation or even beyond (Neil, 1988; Jeschke, 2002).

Type II functional response has been reported for a number of important reduviid predators. Sahayaraj and Ambrose (1994), for instance, had recorded a type II functional response by *Acanthaspis pedestris* when offered with *Pectinophora gossypiella* larvae. The handling time reported for females and males of *A. pedestris* was 0.91 and 1.53 h, respectively. Ravichandran and Ambrose (2006) subsequently confirmed the earlier reported type II response by adult females and males of *Acanthaspis pedestris* on prey such as *Achaea Janata*, *Helicoverpa armigera* and *S. litura*. The results of yet another experiment by Muniyandi *et al*. (2011), show a remarkable agreement with the findings of the present study. The number of *S. litura* larvae consumed by *R. kumari* significantly increased with prey density following a declining trend, reaching a peak value of 4.53 at 16 larvae per predator. Handling time was found to be 1.10 h.

The findings of Srikumar *et al.* (2017) who had studied functional response of *S. collaris* also support the results of the present study. The mean number of prey killed reportedly increased as the density of the prey *Helopeltis theivora* was increased. The handling time was estimated as 1.3 h. In a more recent study, Kumar *et al.* (2019) also found that late nymphal instars and adults of *R. kumari* showed type II response when offered with *Odontotermes brunneu*.

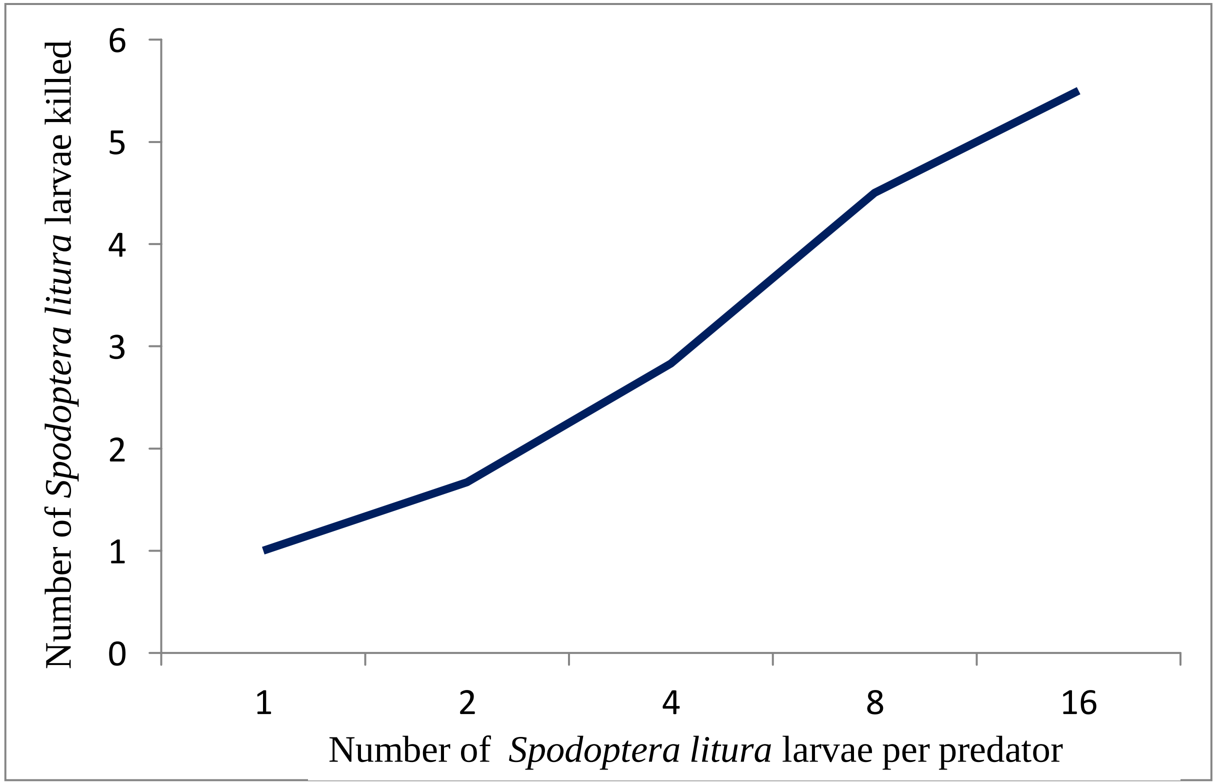


Fig 1. Number of *Spodoptera litura* larvae killed at varying densities

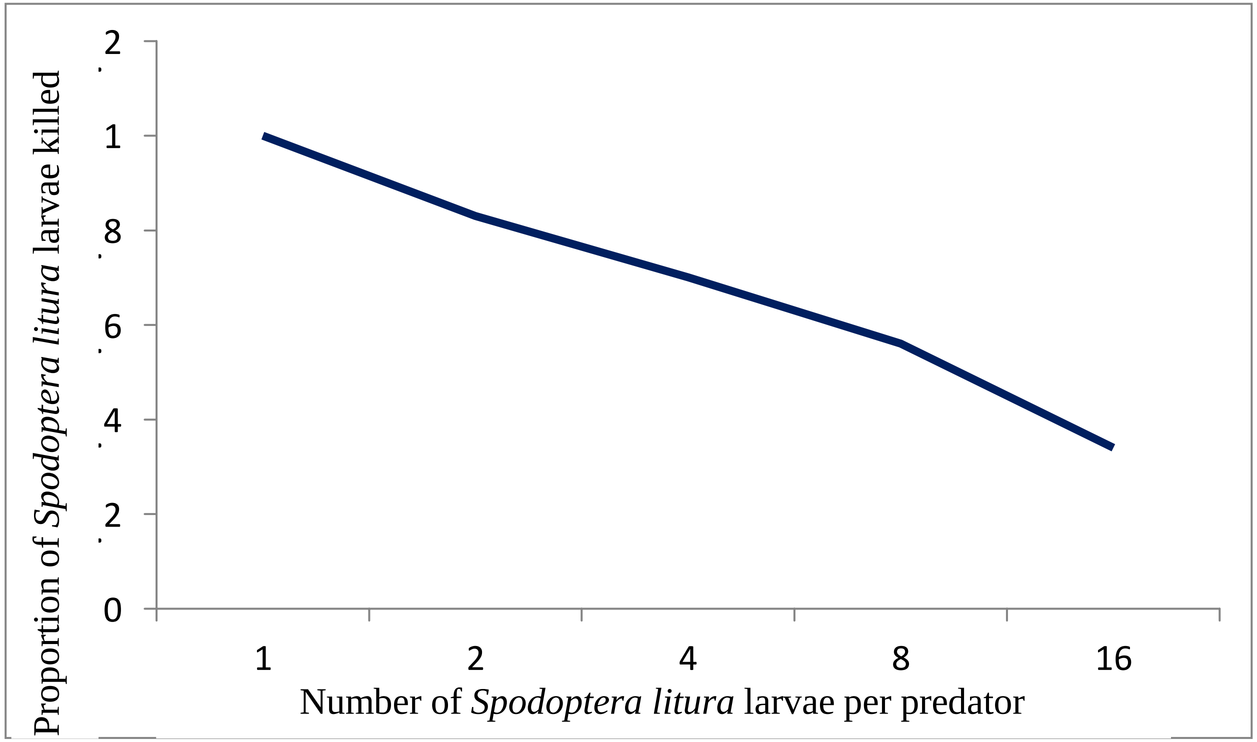


Fig 2. Proportion of *Spodoptera litura* larvae killed at varying densities

It can be seen that, heteropteran predators have been attributed with a type II response by majority of studies reported (Cohen and Byrne, 1992; Cohen, 2000), though other types of responses, such as the type III response of *Zelus longipes* on adults of citrus psyllid *Diaphorina citri* (Navarette *et al*., 2014) have also been reported.

Functional response can display wide range of patterns and inference of functional response models on predatory capacity cannot be made reliably without considering factors such as constant handling time (Okuyama, 2012). Factors such as the prey and predator developmental synchronization (Thompson, 1975; Lawton and Warren, 1987; Donnelly and Phillips, 2001), search pattern of predators (Arke and Johnson, 1979), refugee availability (Hildrew and Townsend, 1980; Stewart *et al*., 2002) and prevailing environmental conditions (Thompson, 1978; Gresens *et al.*, 1982; Allahyari *et al*., 2004) also influence the magnitude of functional response.

Predators showing type II functional response have been utilised successfully in biocontrol programmes (Pervez and Omkar, 2005) as they are capable of efficiently regulating prey even at lower densities.

5.3. PREY STAGE PREFERENCE OF *Sycanus collaris*

The preference of *S. collaris* fifth instar nymphs to different larval instars of *S. litura* was studied using free choice feeding test. The results showed that *S. collaris* final nymphal instars had a distinct preference towards first, second and third instar of *S. litura* larvae, with consumption of 70, 75 and 85 per cent larvae, respectively. Fourth and fifth instar larvae, with 30 and 15 per cent kill, respectively, were preferred least.

Sahayaraj and Sivakumar (1995) had observed that first and second instar nymphs of *Rhynocoris kumarii* preferred the smaller first and second instar larvae of *S. litura*, while the third instar nymphs preferred second and third instar larvae. The fourth and fifth instar nymphs as well as adults fed on the larger third to fifth instar larvae. The prey choice was consistent with the general observation of large sized predators preferring larger prey and small sized predators preferring small prey.

However, Muniyandi *et al.* (2011) observed that adults of *R. kumarii* preferred medium sized second and third instar *S. litura* larvae over other instars, which is consistent with the findings of the present study. The greater speed of movement and the relatively harder cuticle of late instar *S. litura* larvae reportedly deterred attack by the bugs. Similar choices have also been demonstrated by other reduviids like *R. fuscipes* (Claver and Ambrose, 2002), *Agriosphodrus dohrni* (Luo *et al.*, 2010) and *R. marginatus* (Sahayaraj, 2012).

The prey stage preference, an important component of the prey-predator dynamics, is governed principally by prey size, mobility, thickness of cuticle and nutrient requirement of predator (Richman *et al.*, 1980; Dolling, 1991; Ambrose, 1999). The preference for early instars could be attributed to cuticle softness and relatively slow mobility. Besides, prey consumption to acquire high nutrients per effort orient predators preference for medium sized prey (Fuller, 1988; Clum *et al*., 1996).

In the light of the above considerations, fifth instar nymphs of *S. collaris* can be regarded as promising biocontrol agent, particularly against early instar larvae of *S. litura.*

5.5. FIELD EFFICIENCY OF *Sycanus collaris* AGAINST *Spodoptera litura* IN POLYHOUSE ON CUCUMBER

Field efficacy of the reduviid predator *S. collaris* against *S. litura* was assessed under polyhouse conditions on KPCH1 variety of cucumber.

The mean number of *S. litura* larvae declined by 63.4 per cent in the treated plot one day after release of the predator. Three days after release, the treated plot again had a significantly lower population of 0.53 larvae per plot, equivalent to 89.4 per cent reduction. The predator was effective in supressing the pest population completely five days after the release. No mortality of the released larvae was observed in control plot. The treated plot was significantly superior to the control plot in terms of *S. litura* population throughout the study.

The ability of *S. collaris* in managing pest population has also been documented by Gafur *et al.* (2008), who released a mixed population of *S. collaris* at the rate of 260/300 m^2 in a tea tree oil plantation at Indonesia. The *Helopeltis antonii* population was reduced significantly from seven to less than one per tree within three months after release. Consequently, damage severity was also reduced from 30 to 10 per cent.

Similar instances of suppression of pests by related reduviid species also have been documented. Ambrose and Claver (1999) had recorded that *R. marginatus* suppressed *S. litura* infestation by 57.4 per cent on cotton. Sahayaraj (1999a) reported that release of *R. marginatus* in groundnut had resulted in reduction of *S. litura* larvae to 0.88/plant and that of *H. armigera* larvae to 0.77 per plant in contrast to control with 5.44 and 6.44 larvae of *S. litura* and *H. armigera* per plant, respectively. Sahayaraj (1999b) had also observed that *R. marginatus,* released at the rate of 5000 per hectare in groundnut plots lowered *S. litura* and *H. armigera* numbers from 6.55 to 0.76 and 5.66 to 0.87 per plant, respectively. Predator released plots also recorded a significantly higher pod yield of 1867 kg/ha as against 1023 kg/ha in control plots.

The reported efficacy of augmentative release of *R. marginatus* for pest management in groundnut has been confirmed by Sahayaraj and Martin (2003) as well as Sahayaraj and Ravi (2007) in subsequent studies. Mohasina (2017) reported that third instar *R. marginatus* nymphs released at the rate of one nymph per caged cowpea plant caused reduction in *S. litura* to 1.50, 1.33, 0.17 and 0 larvae, respectively during first four consecutive days following predator release. The mean number of 4.05 damaged leaves per plant in caged cowpea plants that received the predator was significantly superior to the 7.27 leaves per plant in control.

The results are indicative of the potential of *S. collaris* in managing the population of *S. litura* on cucumber under polyhouse conditions, though a comparison with similar studies could not be undertaken for want of previous reports. The recovery of the predator following field releases also augurs well for augmentative release of the bug as a viable strategy for the management of tobacco caterpillar.

Summary

6. SUMMARY

The research study entitled "Biological control of the tobacco cutworm *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) using *Sycanus collaris* (Fabricius) (Hemiptera: Reduviidae)" was undertaken at the All India Coordinated Research Project on Biological Control of Crop Pests (AICRP on BCCP), Department of Agricultural Entomology, College of Horticulture, Kerala Agricultural University, Vellanikkara during 2018-2020 period. The objectives were to study the biology, functional response, prey stage preference and field efficacy of *S. collaris* against the tobacco cutworm *Spodoptera litura* on cucumber in polyhouse.

The salient research findings of the present study are presented below.

- Life cycle of *S. collaris* consisted of three different stages in the form of egg, nymph and adult.
- *S. collaris* laid yellowish brown oval egg masses**.** The eggs hatched after a mean incubation period of 13.21 ± 0.04 days with a range of 13-14 days. The hatchability of eggs was 96 per cent.
- The nymphal stage comprised of five instars, with the mean stadial period of the first to fifth instar nymphs being 12.55, 9.12, 8.88, 10.95 and 15.93 days, respectively
- The total nymphal duration ranged from 50 to 64 days with a mean of 57.37 days and 93 per cent of nymphs survived to adulthood.
- The mean pre oviposition period of female bugs was 12.60 days. During the mean oviposition period of 51.86 days, a female on an average laid 451.56 eggs in 5 to 13 clusters. Subsequently, females died after the post oviposition period of 13.43 days.
- The average longevity of adult male and female bugs was 66.29 and 78.95 days, respectively.
- The total life span of male bug was 123.66 days and that of females was 136.32 days.

- The sex ratio, at 1:1.39 was female biased.
- Type II response was exhibited by fifth instar nymphs of *S. collaris* when *S. litura* larvae were offered as prey at different predator prey ratios of 1:1, 1:2, 1:4, 1:8 and 1:16.
- Attack rate was 0.0198 h^{-1} and handling time varied from 0 and 4.607 h.
- An individual fifth instar nymph killed up to 5.50 *S. litura* larvae in a day.
- *S. collaris* preferred first, second and third instar *S. litura* larvae over other instars.
- Plot in which fifth instar nymphs were released at the rate of 100 nymphs/50 m^2 recorded significantly lower number of *S. litura* larvae as compared to control plot.
- Mean number of *S. litura* larvae were 1.83, 0.53 and 0 at 1, 3 and 5 days after predator release.
- The study indicated that *S. collaris* can be considered as a potential biocontrol agent for the management of *S. litura* in polyhouse conditions, thereby reducing insecticide based management strategies to a great extent.

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BIOLOGICAL CONTROL OF THE TOBACCO CUTWORM *Spodoptera litura* (FABRICIUS) (LEPIDOPTERA: NOCTUIDAE) USING *Sycanus collaris* (FABRICIUS) (HEMIPTERA: REDUVIIDAE)

by

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(2018-11-120)

ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture

Kerala Agricultural University

DEPARTMENT OF AGRICULTURAL ENTOMOLOGY

COLLEGE OF AGRICULTURE

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KERALA, INDIA

2021

ABSTRACT

Salad cucumber (*Cucumis sativus* L.) is among the most popular vegetables grown in polyhouses across Kerala, which has been gaining significance in recent years. However, the warm, humid conditions, abundance of food and the relatively low presence of natural enemies encourage build up of arthropod pests in polyhouse cultivation. Among these, the tobacco cutworm *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is one of the destructive pest. Management of the tobacco cutworm using insecticide based management strategies is not advisable as cucumber fruit is consumed in raw form. Biocontrol could be a more ecologically sound and economically feasible alternative. The reduviid bug, *Sycanus collaris* (Fab.) (Hemiptera: Reduviidae) is one such potential candidate for biocontrol of *S. litura* in polyhouses. However, *S. collaris* remains to be evaluated as a biocontrol agent under Kerala conditions. Hence a study was conducted to investigate the biology, functional response and prey stage preference of the reduviid predator *S. collaris* as well as to evaluate its potential for the management of *S. litura* on cucumber under polyhouse cultivation.

Biology of *S. collaris* was studied on larvae of *Galleria mellonella*. Eggs were hatched after a mean incubation period of 13.21 days. Development of nymphs, normally with five instars, with a mean duration of 12.55, 9.12, 8.88, 10.95 and 15.93 days respectively. After the pre oviposition period of 12.60 days, egg laying continued for 51.86 days. Females died in 13.43 days after laying last batch of eggs. Average fecundity of the bug was 451.56 eggs. Mean longevity of 78.95 days was longer for females than 66.29 days for adult males.

Functional response of fifth instar nymphs of *S. collaris* on third instar larvae of *S. litura* at different prey densities of 1, 2, 4, 8 and 16 larvae/bug was assessed in laboratory. Nymphs of *S. collaris* killed up to 5.50 larvae in 24 h at a prey density of 16 larvae per bug. Logistic regression analysis revealed a type II curvilinear functional response with an attack rate of 0.0198 h^{-1} while the handling time was between 0 to 0.460 h.

The prey stage preference of the fifth instar nymphs of *S. collaris* on *S. litura* was studied by offering all the five different larval instars larval instars in equal proportions of 2:2:2:2:2 ratio. The predator showed marked preference towards first, second and third instar larvae of *S. litura* larvae over fifth instar larvae. The predator also showed significantly higher preference to third instar *S. litura* larvae as compared to fourth instar larvae.

Biocontrol potential of *S. collaris* against tobacco caterpillar, *S. litura* on salad cucumber in polyhouse conditions was evaluated on KPCH 1 variety of cucumber. The plot in which predator was released recorded significantly lower number of *S. litura* compared to untreated control. The population of *S. litura* declined by 63.4 and 89.4 per cent, respectively on the first and third day after release of the predator and no larvae were observed on the five days after bug release. However, the larval population of five per plant showed no reduction after five days, in control plots. The mean yield per plant in which predator was released @ 100 nymphs/50 m^2 recorded significantly higher yield than control plot.

The study demonstrated that the reduviid predator *S. collaris* can be used for inoculative release for the management of *S. litura* in protected cultivation.