Predation efficiency and Life cycle of Pentatomid bug, *Eocanthecona furcellata* (Wolff.) on the Brinjal pest, *Henosepilachna vigintioctopunctata*.

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ABSTRACT

Henosepilachna vigintioctopunctata is one of the most serious pests of brinjal crop. A predatory stink bug, *Eocanthecona furcellata* were identified in the field and evaluated against different immature stages of the pest population under laboratory condition. The predatory potential of thenymphs was tested which revealed that among all the life stages, the *E. furcellata*nymphs prefer the larval stage followed by pupal and egg stages. In addition, the life cycle duration of the nymphs reared on four different larval stages of*H.vigintioctopunctata* showed that, predation rate occurs based on the prey size. The nymphs that were fed with third and fourth instars had lowest predation rate but showed similar developmental duration as in control than first and second instars. A significant difference has also been observed between all the treatments ($p \leq 0.05$). Moreover, a nymph can feed on an average of 127.33 first instars during its nymphal period. From this study, we conclude that *E. furcellata* had high predatory potential against the pest, *H. vigintioctopunctata* to be mass reared and utilized as a bio control agent.

Keywords: Biocontrol agent; Brinjal crop; *Eocanthecona furcellata*; *Henosepilachna vigintioctopunctata*; Natural enemy; Predatory stink bug.

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INTRODUCTION

In the tropics, *Solanum melongena* (Brinjal) production is severely affected by several insect pests which are listed as many as 26 pests (Vevai, 1970). Among them Shoot borer, fruit borer, stem borer, Hadda/Epilachna beetles are considered as major pests since it is responsible for extreme reduction of yield and quality of brinjal production. Epilachna beetle or *Henosepilachna vigintioctopunctata* (Coleoptera: Coccinellidae) is considered as one of the most serious pests causing severe damage to brinjal (Anam*et al*., 2006; Rahaman*et al*., 2008). Epilachna beetle are polyphagous, and feed predominantly on cucurbits, tomato, potato, kidney bean and brinjal. Both the grub and adult have chewing mouthparts. They scrape the chlorophyll content from the epidermal layer of the leavesresulting in formation of a typical ladder-like window making the plant to dry and wither, leading to skeletonization of the leaf (Imuraand Ninomiya, 1978; Srivastava and Butani, 1998).

The frequent and indiscriminate usage of pesticides lead to widespread development of resistance, harmful effects on non-target organisms, presence of toxic residues on food, environmental and health hazards (Subramanyam and Hagstrum, 1995; Kranthi*et al*., 2002).These problems furthermore insist the need for development of new, safer and eco-friendly pest control measures (Sharma and Saxena, 2012). Biological control has been actively practiced for more than hundred years and the history of biocontrol, its failure and success has been extensively studied. Interest on biological control has increased over recent decades for many reasons (Bailey *et al*., 2009).

For many decades now, biological control has been an increasingly popular alternative method to traditionalinsect control, especially considering the current growing concern for environmental protection and food safety (De Clercq*et al*., 2003; Zanuncio*et al*., 2006). *Eocanthecona furcellata* is a common predatory stink bug distributed throughout South east Asian countries including India, China, Indonesia, Thailand, Taiwan, and parts of Japan and has often been observed in cotton, chickpea, and vegetable fields (De Clercq, 2000; Chang and Hsieh, 2001; Nyunt, 2008). It is most active at a temperature between 25-30^{o}C. Morphologically, adults are diagnosed by its black or dark-grey colour with white and grey spots all over their prothorax and hemelytra. Nymphs are black with red markings on their abdomen and prothorax and have a reddish proboscis. Since 1996, *E. furcellata* has been mass reared and used as biocontrol agent in Taiwan against a few pests including *S. litura*and *P. xylostella* (Chang, 2002). There is no research involved in *E. furcellata* against *H.vigintioctopunctata* on brinjal.

Accordingly, the main aim of our study was therefore to investigate the predatory bug, *Eocanthecona furcellata* on each life stages of *H. vigintioctopunctata* to determine the prey stage preference and prey consumption rate. During the course of this study, the life cycle was also noted for *E. furcellata*. Furthermore, our second aim was to know how far the relationship occurs between the developmental period and prey consumption behaviour of *Eocanthecona furcellata* while feeding on individually with different stages of the pest *Henosepilachna vigintioctopunctata*.

MATERIALS AND METHODS

Collectionof Host plant

During the life cycle and predation study, brinjal leaves was used as a host plant for both the insect *Henosepilachna vigintioctopunctata* and *Eocanthecona furcellata*. The brinjal seedlings were bought from the local market place, and planted in a vegetable garden for doing further research work.

Insect - Prey Rearing

The insect prey species, *Henosepilachna vigintioctopunctata*adults and its egg stages were collected sufficiently from different places such as from the MCC farm, Tambaram, the local agricultural area located in Chengalpattu, and also from Urapakkam.

The collected *H. vigintioctopunctata* adults and egg masses were brought to the laboratory and egg masses were isolated and kept in clean petridishes for hatching. The adults were kept in a one litter capacity glass container for egg laying. The freshly laid eggs on the leaves were monitored regularly and taken out from the container and transferred into another petridishfor incubation of eggs under room temperature (31.1±1.35^{o}C). Newly emerged first instars were isolated from the other egg masses to avoid egg cannibalism (Chaudhary and Saravanan, 2011). Every morning, fresh, tender *S. melongena* leaves were picked up from the garden and brought in a closed, airtight plastic container and provided to the growing larvae and adults. Thus, the larvae of the pest were mass reared under laboratory condition from first instar stage to adult stage.

Insect Predator Rearing

The (n=18) number of third and fourth nymphal stages, *E. furcellata* were originally collected from the plant *Acalyphaindica*. These predatory nymphs were reared with eggs and immature stages of Coccinellid beetle, *H. vigintioctopunctata* under laboratory condition in the glass petridishes (100 x 15mm). When the nymph reached the fifth nymphal stage, it was transferred into a large glass container. For proper ventilation, the outlet was hidden with a blotting paper and tightly fixed using rubber band. On the first day of rearing, both the plant *A. indica* and *S. melongena* leaves were given as supplementary feed. Having observed that the food preference of the predatory nymphs, *A. indica* leaves were not given; instead *S.melongena* leaves were provided regularly as preferred by the host.

Life cycle Study

For egg laying, a pair of *Eocanthecona furcellata* bugs (i.e. one male and one female bug) were isolated to an oviposition chamber. Egg masses laid by the female bug were collected from the chamber and transferred to 500ml capacity glass container for incubation. The opening of the glass container was tightly secured with blotting paper. This experimental setup was kept in the laboratory condition (31.1±1.35ºC). The incubation period, total nymphal stage and total life cycle duration were noted. Each stage of the predatory bugs was identified by its size, colour variation and also exuviae (Khune and Ganvir, 2015). In total, around 150 *E. furcellata* nymphs were used for this study.

Predation Efficiency of *E. furcellata*

The experiment was conducted at the laboratory condition to investigate the predatory efficiency of all the stages of the predatory bug *E. furcellata* on three stages (eggs, grubs and pupae) of brinjal pest, *H. vigintioctopunctata*. The second nymphal stage was taken for conducting the experiments (Gupta *et al*., 2004). For the control experiment, the nymphal stages of the predatory species were fed on with all the life stages of the pest species. The predatory nymphs fed only with egg stages of the pest speciesas T1, with each larval stages of the pest species separately as T2 and with pupal stages of pest species as T3. Based on the larval stage, the number of prey (n=2, 3, 4….30 larvae/nymph/day) was administered to the predatory nymphs. The prey consumed everyday was noted down. The petridishes were cleaned and the predators were fed with fresh prey every day. This procedure was repeated for all the treatments and replicated six times.

The data were calculated with one-way Anova to know the overall mean difference between the variables. Tukey's Test was performed for multiple comparison between each variable. This statistical analysis was carried out using statistical software, SPSS version 24.

RESULTS

From the result, it was observed that a significant difference occurs in the feeding behaviour, prey consumption and developmental period of the predator due to the variation in the food source.

Egg predation and Developmental period

The nymphs that reared only on egg stages (T1) of Epilachna beetle could not able to complete whole of its life stage. It was reached up to second and third nymphal stages from first nymphal stages and then gets died. Meanwhile, there is prolonged longevity observed with an average of 4.41 days (ranged from 4-5) and 5.83 days (ranged from 5-6) for crossing second and third nymphal stage. This developmental duration was longest when compared to the control experiment which showed only 2.87 (2.5-3.0) and 2.95 days (ranged from 2.5-3.0) showed in Table 3. The significant difference between the longevity of control and egg predated second nymphs were (df=1, 22, MS=14.26, F=90.17, P<0.00), third nymphs (df=1, 22, MS=49.594, F=575.505, P<0.00).The total predation rate observed throughout all the nymphal stages were 130.66 eggs (Table 1).

Table 1. Prey consumption rate of *E. furcellata* on eggs and pupal stages (Mean±SD)

Stages	Egg(T1)	Pupa(T3)
II nymphs	58.33±6.11	2.33±0.57
III nymphs	72.33±12.05	3.33±0.57
IV nymphs	0.00	6.33±0.57
V nymphs	0.00	10.33±1.15
Total prey consumed	130.66±18.14	22.33±0.57

Table 2 Prey consumption rate of *E. furcellata* on different larval stages (T2)

Stages	Control	T2(I)	T2(II)	T2(III)	T2 (IV)
II nymphs	3.66±0.57a	7.00±1.00b	4.00±0.00a	2.00±0.00d	1.33±0.57c
III nymphs	10.66±1.52a	14.33±4.50a	7.00±1.00a	3.33±0.57c	3.00±1.00b
IV nymphs	26.66±1.52a	47.66±10.40c	16.33±7.50a	10.33±4.04d	5.00±1.00b
V nymphs	34.00±0.86a	58.33±5.13b	42.00±3.00c	26.33±2.51a	10.66±0.57d
Total prey consumed	75.00±0.86a	127.33±2.30b	69.33±8.50a	42.00±7.00c	20.00±1.00d

In the horizontal rows, the means followed by the same letters are not significantly different at 0.05 % level of probability (One- way ANOVA, Tukey HSD)

Table 3 Developmental period of *E. furcellata* on different stages of *H. vigintioctopunctata*

Stages	Control	T1	T2(I)	T2(II)	T2(III)	T2(IV)	T3
Incubation period	5.95 ±0.25a	5.95 ±0.25a	5.95 ±0.25a	5.95 ±0.25a	5.95 ±0.25	5.95 ±0.25a	5.95 ±0.25a
I nympal period	1.75±0.26a	1.75±0.26a	1.75±0.26a	1.75±0.26a	1.75±0.26	1.75±0.26a	1.75±0.26a
II nymphal period	2.87±0.22a	4.41±0.51b	2.83±0.24a	2.91±0.19a	3.95±0.25	4.00±0.30c	3.41±0.51e
III nymphalperiod	2.95±0.14a	5.83±0.38b	3.16±0.38a	2.95±0.25a	2.91±0.19	2.95±0.14a	3.50±0.52c
IV nymphal Period	3.16±0.38a	0.00	5.08±0.79b	3.83±0.38d	3.04±0.14	3.08±0.19a	4.87±0.22c
V nymphal period	5.12±0.22a	0.00	7.58±0.66b	5.50±0.52a	4.95±0.14	4.91±0.28a	7.05±0.36c
Total nymphal period	15.87±0.48a	12.00±0.63e	20.41±1.22c	16.95±0.65d	16.62±0.31	16.70±0.33a	20.60±1.26b
Total Develop. period	21.83±0.38a	17.95±0.54e	26.37±1.28c	22.91±0.51d	22.58±0.28	22.66±0.24a	26.55±1.28b

(n=12) for each treatment; Value (0) indicates that the nymph gets died; In the horizontal rows, the means followed by the same letters are not significantly different at 0.05 % level of probability (One- way ANOVA, TukeyHSD)

Pupal predation and Developmental period

The nymphs reared only on pupal stage (T3) showed similar prolonged duration at each life stage. But, most of these nymphs died at the last day before moulting into adult. If they moulted into adult, gets died in few days due to unsuccessful development. The total predation rate and total longevity was observed as 22.33 pupae and 26.55 days which is significantly different from control (df=1, 22, MS=133.954, F=149.473, P<0.00) shown in Table 1.

Larval predation and Developmental period

The first nymphal stage of *E. furcellata* were very tiny. When it hatched out from the egg, they were light reddish black in colour. After it started sucking on plant sap using its proboscis, their abdomen region become larger in size, appeared as bright red and black colour. They could be found as aggregations (Fig 2B-E). The first nymphal stage (N1) lasted for 1-2 days (avg. 1.75 days) and it prefers plant sap only (Fig 2C).

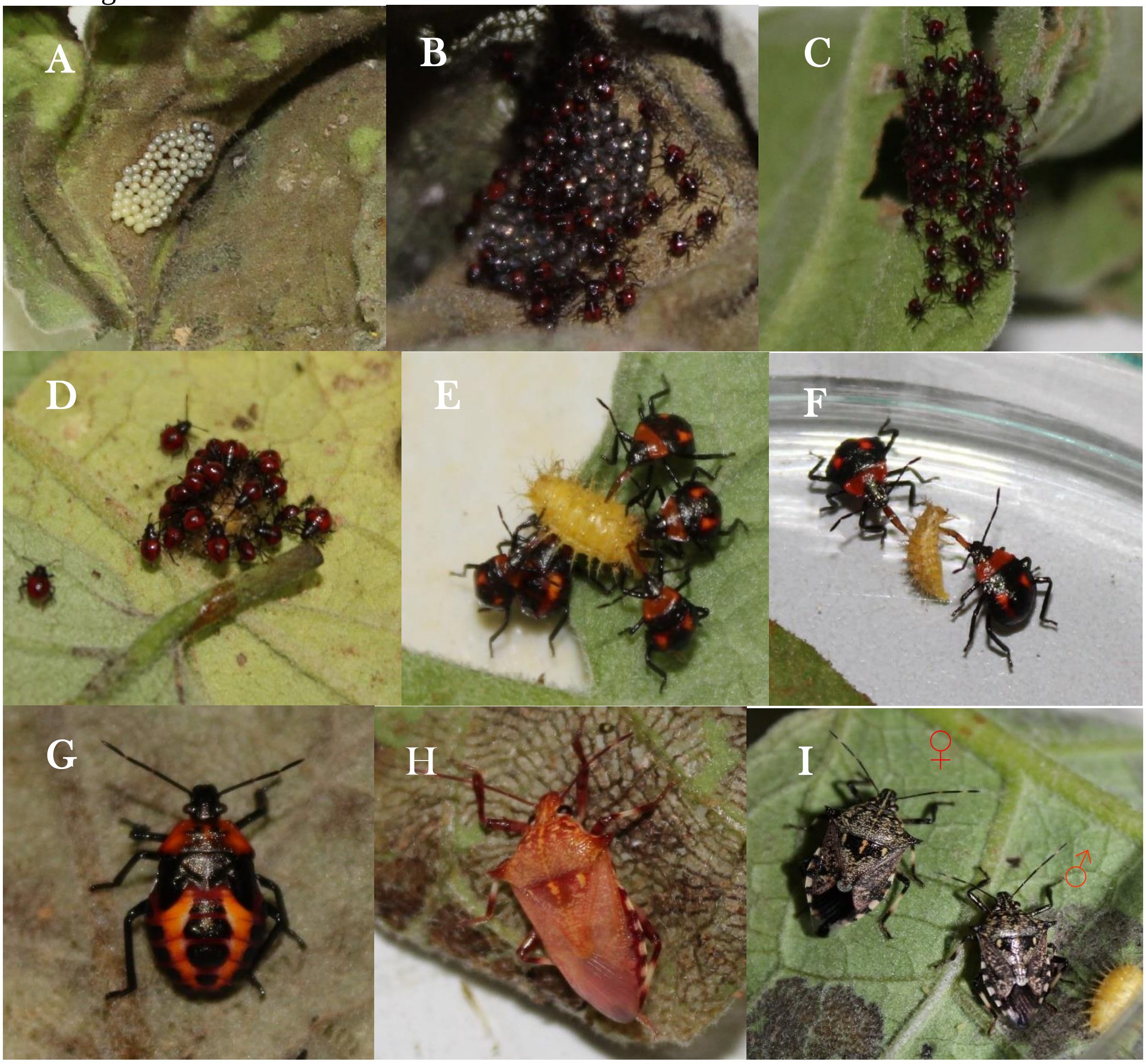
The predatory potential was checked upon all the nymphal stage, but from the second nymphal stage (N2), prey consumption behaviour was aroused. Generally, the predatory nymphs fed on haemolymph of the larval stages of the prey leaving an empty skeleton. The prey consumption was recognised by counting the empty shell or dead ones on each rearing petriplates. According to the result, the second nymphal stage (N2) of *E. furcellata* reared on first and second larval stages of *H. vigintioctopunctata*, started to feed from the first day of its moulting process. But when N2 was reared on third and fourth larval stage, it was observed that it did not consume any prey for the first day, later on in the next coming days it started to feed. This might be due to its Prey size.Thus, based on the prey size the number of predation rate occurs. Since, the first instar of *H. vigintioctopunctata* is smaller in size than other larval instars, highest number of predations was ever observed at every nymphal stage.Thus, a single nymph, *E. furcellata* could feed and kill around 120 to 150 first larval instars (avg. 127.33) within 20.41 days (Table 2 and 3).

Compared to the control, a significant difference observed on the prey consumption efficiency (df=4, 10, MS=14.567, F=43.70, P<0.00), and longevity (df=6, 77, MS=4.963, F=41.503, P<0.00) of second nymphal stage between all the treatments. In particularly, the difference noted on the third and fourth instars showed the lower predation rate as 2.00 and 1.33 instars, while had longest developmental duration with an average of 3.95 and 4 days respectively. The highest predation recorded on the first instars (avg.7 ins.). A significant difference was observed in the third nymphal stage from all the treatments on the prey consumption efficiency as (df=4, 10, MS=70.833, F=14.167, P<0.00), and in

Fig.1 Predatory action of*E. furcellata* on different immature stages of *H. vigintioctopunctata*. **A.** Egg mass predation; **B.** Larval predation; **C.** Pupal stage predation; **D.** Pupal stage predation by a gravid female; **E.** Larval stages fed by the predatory nymphs (per day); **F.** Rearing predator species in the petridishes.

longevity (df=6, 77, MS=13.540, F=131.346, P<0.00). At this stage also the difference noted on the third and fourth instar showed the lower predation rate as 3.33 and 3.00 instars, but had similar developmental duration as in control (avg. 2.95 days) about 2.91 and 2.95 days respectively. Among fourth nymphal stage, the differences difference between the prey consumption efficiency (df=4, 10, MS=850.933, F=23.081, P<0.00), and between the longevity (df=6, 77, MS=33.934, F=228.033, P<0.00) of all the treatments were observed. The significant difference noted on the fourth instars showed the lowest predation rate as 5.00 instars compared to control (avg. 26.66 ins.) but not significant differences in

Fig.2 Life cycle of *E. furcellata* on the pest insect, *H. vigintioctopunctata*. **A.** freshly laid egg masses; **B.** nymphs gets hatching out from the eggs; **C.** first nymphal instars sucking the leaf sap (Brinjal); **D.** second nymphal instars feeding an Epilachna larva; **E.** third nymphal stage; **F.** Fourth nymphal stage; **G.** fifth nymphal stage; **H.** a youngest adult bug; **I.** an oldest female and male bug.



developmental duration (avg. 3.08 days) with control (3.16). The highest predation (47.66). For fifth nymphal stage, a significant difference observed between the prey consumption efficiency (df=4, 10, MS=944.233, F=110.437, P<0.00), and between longevity (df=6, 77, MS=72.364, F=503.153, P<0.00) of all the treatments. Similarly, the significant difference noted on the fourth instars showed the lowest predation rate as 10.66 instars compared to control (avg. 34.00 ins.) but not significant differences seen in developmental duration (avg.4.91 days) with control (5.12 days).

and longest developmental duration (5.08 days) both recorded on the first instar While introducing the larval stage of Epilachna beetle into the predator petridish, it was found that they readily started to predate by its powerful olfactory behaviour which showed suitability of the prey. Thus, when there was insufficient larval stage, the predatory nymphs started to go for other stages such as egg predation and pupal predation. Among all these stages, the nymphs prefer the larval stage, then pupal stage and finally the egg stage which directly affects its developmental cycle.

The total developmental duration of the predatory bug, *E. furcellata* differs according to the size of the prey. The nymphs which are reared on first and second larval stages of *H. vigintioctopunctata* showed the total life cycle duration of 26.37 days and 22.91 days which is significantly different from the control (21.83 days) (Table 3). While, on third and fourth larval stages showed similar developmental duration from control treatments about 22.58 and 22.66 days respectively. This result confirmed that the third and fourth larval stages of Epilachna beetle would be the favourable and preferable food stage for growing nymphs of the predator, *E. furcellata*. Most probably, *E. furcellata* responded to (E) - phytol, which is produced by larvae when feeding on the chlorophyll in their food plants. In addition to this, *E. furcellata* preferred to feed on larvae fed with a chlorophyll-rich diet rather than with a chlorophyll-poor diet as described by Yasuda, 1997, 1998a, 1998b.

DISCUSSION

Conservation of biological control seeks to understand human influences on resident natural enemies in a system, then manipulate those influences to enhance the ability of natural enemies to suppress the pests. De Bach (1964) considered conservation biological control to be an environmental modification to protect and enhance natural enemies. These activities range from modification of pesticide use practices to manipulation of beneficial insect habitat within an agroecosystem (Barbosa, 1998; Pimentel, 2008). In the recent years, a number of studies have been done by many researchers throughout the world on this broad area.The agricultural pest, *Henosepilachna vigintioctopunctata* has broad range of natural enemies in the field as parasitoids, predators and pathogens. Still when their favourable environmental condition occurs, the pest population would be very high and bring the idea to the farmers for judicious usage of pesticides. Therefore, to bring the pest in its controlled state, there is an urge to find out the natural predatory enemies for every pest species. In an agro ecosystem, parasitoids play a greatest role in controlling the Epilachna beetle in its egg stages itself but it is not applicable for all larval stages.

Parasitoids commonly attack immature life stages of *H. vigintioctopunctata* on *Solanum melongena* in India where parasitism rates vary over the year but can lead to substantial reductions as high as 38 % of eggs and 68% of larvae (Raju and Uma Maheswari, 2005; Hussain *et al*., 2006). Parasitoids may not provide economic control, in spite of high parasititism rates of phytophagous coccinellids. In Ethiopia, although the egg parasitoid*Ooencyrtusepulus* and the pupal parasitoids, *P. foveolatus* and *Mesopolobus* sp. were key mortality factors of *Chnootribasimilis*, the parasitoids did not reduce pest populations below the economically damaging levels (Beyene*et al*., 2007).Among the predators, *E. furcellata* has much attention because of most of the soft bodied, immature stages of coleopteran, lepidopteran and heteropteran insect species that were controlled by this bug (De Clercq, 2000).

In earlier studies, the predation efficiency of the bug, *E. furcellata* carried out against some pest species, *Hyblaeapuera* (Tubtimand Sakchowong, 2003), *Spodopteralitura* (Kobayashi, 2004), *Helicoverpa armigera* (Nyunt, 2008) and reported as suitable prey for mass rearing. However, *E. furcellata* feed some extent on plants and the frequency of plant feeding is higher at older age and during prey scarcity (Senrayan, 1988) which has also been observed in this experiment.

This study confirmed that, the developmental time and predation of *E. furcellata* nymphs completely correlate with the size of the prey. When the size of the prey is larger, the number of prey consumption rate is low, but the developmental period would be shorter, similar to the result of the nymphs reared in the control. When the prey size is smaller, the number of prey consumption rate is high, at the same time due to insufficiency of healthy prey, the developmental period would be longer. In this case, the nymphsfaced difficulties during moulting time, which led to death and in some cases, they were not able to complete all the stages properly when they had only smaller sized prey. Law in 1979 described that predators are always selective in the choice of their prey, from broad levels of preference for particular prey species to fine levels of preferred shapes, sizes, colours and palatability. More recently it was found that, the predatory bugs, *E. furcellata* and *R. fuscipes* both displayed a higher preference for volatiles produced by early instar *M. vitrata* larvae as compared to late instar larvae or prepupal stage of *M. vitrata* (Chakravarthy *et al*., 2017).

It can be concluded that, the predatory bug *E. furcellata* has efficient prey consumption ability and prey preference against *H. vigintioctopunctata* and could be utilized for mass rearing as efficiently and economically. The mass multiplication of this biocontrol agent would be a better solution for effective pest control in the field besides it will reduce the great risk of pesticide usage.

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