Research Article

BIOLOGY AND BIOASSAY OF SPOTTED BEETLE IN BITTER GOURD IN CHITWAN, NEPAL

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ABSTRACT

Biological study of *Epilachna dodecastigma* revealed that a female laid average of 283.25 ± 25.88 eggs in different clusters; each cluster contained 25-65 eggs on the surface of the leaves and the ovi-position period was 10.13 ± 3.33 days. The eggs of *E. dodecastigma* were bright yellow, elongate, oval and usually lay on the undersurface of the leaves with size of 54.8 ± 0.37µm length and 15.26 ± 0.6 µm in breadth. The larva of *E. dodecastigma* was yellow in color, elongate and elliptical in shape with moderately long legs and a well-developed head and mandibles. The length and breadth of first, second, third and four instars were 1.41 ± 0.24 mm and 0.56 ± 0.06 mm; 4.2 ± 0.2 mm and 1.9 ± 0.1 mm; 6.2 ± 0.2 mm and 3.2 ± 0.2 mm and 7.8 ± 0.2 mm and 3.4 ± 0.24 mm, respectively. Total duration of larvae was 11.54 ± 0.43 days. The average length and breadth of pupae was measured 7 ± 0.3 mm and 5.4 ± 0.24 mm in the field condition with pupal duration of 5.50 ± 0.24 days. The average length and breadth of the male adult measured 7.2 ± 0.24 mm and 5.8±0.2 mmand average length and breadth of female adult 7.6±0.24 mm and 6 ± 0.0 mm respectively in the field condition. The results of the bioassay experiments showed the highest larval mortality with chloropyriphos & cypermethrin followed by spinosad, derisom, margosom, *B. bassiana* and water spray (control).

Keywords : *Epilachna dodecastigma, oviposition, spotted beetle.*

INTRODUCTION

Epilachna beetle, *Epilachna dodecastigma* (Wied.) is one of the major important vegetable pests in South-East Asia and also most common pest in Nepal. They attack especially the leaves and feed on the chlorophillous green portion of leaves and thereby preventing the synthesis of carbohydrate by the host plants due to lack of sufficient chlorophyll even though the sunlight is present (Endo *et al.,* 2004). Grubs and adults both cause great damage to the host plants. Infestation primarily begins just after hatching of egg mass (Murata *et al*., 1994). The nature of damage by the larvae is somewhat distinct from that of the adults (Pradhan *et al*., 1990). Adult epilachna beetle, are spherical, pale yellowish brown elytra mottled with 12 black spots, male genitalia with median lobe dentulate dorsally, sipho

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nearly straight at the apex which has an eyelet, female genital plate with rounded notch on the inner margin. The males are slightly smaller than the females. Males can be distinguished from females by having a small notch on the ventral side of the last abdominal segment.

The Epilachna beetles are polyphagous mostly feeding on vegetables such as bitter gourd, brinjal, potato, tomato, etc. The pest, Epilachna beetle prefers cucurbits vegetables, while *E. vigintioctopuntata* (Fab.) mostly feeds on solanacious vegetables (Singh, 2004). The grubs feed on the epidermal layer of leaves and the adults feed irregularly upon the upper surface of leaves and its grubs also feed on the lower surface of leaves by scraping, causing net like appearance of the host plants leaves that turn brown in color, entirely dry up due to extensive infestation by the growing population and finally defoliate (Pradhan *et al*., 1990).

Adults and grubs both fed on the leaves voraciously (Shrivastava, 2004). Singh (2004) found average larval development periods ranging from 11-16 days at about 27.5 and larval survival rates ranging from 70-97%, both depending on the cucurbits. Singh (2004) reported that adults survived for 4 weeks to 6 months and there were 7-8 generations of the epilachna beetle in plains and 1-2 generations in hills.

In some pocket area of Nepal, farmers spray chemical pesticides frequently without considering economic threshold of the pests and at higher doses than it is required Over-use, mis-use and haphazard use of pesticide has harmed the natural enemies, non-target organisms, human beings, natural environment. Application of pesticides to this crop causes several unfavorable effects, such as pest resistance, pest resurgence, pesticide residues in the field .Majority of the farmers are unaware of pesticide types, level of poisoning, safety precautions and potential hazards on health and environment. The trend of pesticide use is increasing in Nepal by about 10-20% per year and expenses on pesticide in market- oriented vegetables and fruit production has been a major cost factor. Hence this research tends to understand the biology of Epilachna beetle in relation to host- pest association. And to evaluate the efficacy of botanicals, microbial and chemical insecticides for management of Epilachna beetle on bitter gourd.

MATERIALS AND METHODS

Experiments were carried out to study the biology and bioassay of *E. dodecastigna*.

Biology study

Five mating pairs were collected and placed in the insect zoo separately one pair in each plant screened with net. Earthen pots were used for the preparation of insect zoo with bitter gourd plants placed near the research field. Daily observations were made on the Epilachna beetle and time to complete its different stages. Life cycle was studied on the caged bitter gourd plants. Incubation period, percent hatchability, larval and pupal period and longevity of male and female were recorded.

Observation parameters

The following observations were recorded for the study of the biology of the Epilachna beetle:

- Incubation period
- Number of batches of eggs laid and number of eggs/batch.
- Larval period
- Pupal period
- Longevity of the adult

Bioassay

Bioassays were performed at the Entomology Laboratory of Agriculture and Forestry University (AFU), Rampur, chitwan, Nepal. The mean temperature, photoperiod and relative humidity of the laboratory were 26 ± 30C, 12 hours, 80 ± 5% RH respectively. The bitter gourd leaves used in the bioassays were collected from untreated plants cultivated in experimental field. The experiments were carried out in a completely randomized design with 4 replication and 6 treatment (spinosad 0.5 ml/lit, chloropyriphous & cypermethrin 2 ml/lit, control (water spraying), margosom 4 ml/lit, derisom 3 ml/lit, *Beauveria bassiana* 3 gm/lit of water). The standard technique used in toxicity bioassays was leaf immersion. This technique allows the product to be evenly distributed on the leaf surface and makes it possible to check whether or not the field doses are effective for the pest control. Bitter gourd leaves of equal size were dipped for ten seconds into the insecticide solution and water (control). After drying at room temperature, bitter gourd leaves were transferred into 1 liter PET bottles. Ten number of second instar larvae from mass colony rearing were released into each PET bottle and covered with fine muslin cloth and made tight. Insect mortality in PET bottles was observed and recorded. The insects were considered dead if they were unable to show response when prodded with a fine paint brush. Bitter gourd leaves were replaced each day with fresh leaf after treating with same dose of insecticide solution. Percentage mortality data was analyzed by square root transformation in MS Excel and R Studio software package.

REULTS AND DISCUSSION

Biology of Spotted beetle *E. dodecastigma*

Egg length was measured with an average of 54.8 ± 0.37 µm (n = 3) in the field condition. Similarly, measurement of breadth in the field condition was found 15.26 ± 0.6 µm. The female laid average of 283.25 ± 25.88 (n = 3) eggs in different clusters; each cluster contained 25-65 eggs on the surface of the leaves. Singh (2004) reported that the female laid about 250 eggs in cluster; each cluster contained 15-50 eggs on the under surface of the leaves. The eggs of Epilachna were bright yellow, elongate, oval and usually laid on the undersurface of a leaf. Hossain *et al*. (2009) reported similar type of finding that eggs of Epilachna beetle were yellow in color and spindle shaped appearing at the micropolar

region. Srivastava (2004) also mentioned that the eggs were laid in cluster on the undersurface of the leaves. cypermethrin was found to reduce egg numbers laid in the mustard beetle (Hajjar and Ford, 1989).

The average incubation period of egg of the Epilachna beetle was recorded 4.04 ± 0.04 days in the field condition. Hossain *et al*. (2009) reported similar types of finding that the incubation period was 4.19 ± 0.23 days in bittergourd. The result was similar to the finding of (Singh, 2004) that the eggs hatch within 2-7 days (2-3 days in summer and 4-7 days in winter). Almeida and Marileusa (2004) reported incubation period were 7.14 days when reared on *Lycopersicum esculentum* Mill (Solanaceae). In this research, Epilachna beetle had four instars having spines all over the body and yellowish in color. Srivastava (2004) supported that the Epilachna larva possessed yellowish bearing spines all over the body. Hossain *et al*. (2009) reported that Epilachna beetle, had four instars, immediately after hatching neonate grubs were light yellow in color with branching body hairs. The length and breadth of final instar grubs were the highest feeding on leaves of sponge gourd (7.90 ± 0.27 mm, 6.95 ± 0.43 mm), which were statistically identical to teasel gourd (7.82 ± 0.38 mm, 6.93 ± 0.09 mm) and bittergourd (9.71 ± 0.09 mm, 6.83 ± 0.38 mm). The length and breadth of first, second, third and four instars were 1.41 ± 0.24 mm and 0.56 ± 0.06; 4.2 ± 0.2 mm and 1.9 ± 0.1 mm; 6.2 ± 0.2 mm and 3.2 ± 0.2 mm and 7.8 ± 0.2 mm and 3.4 ± 0.24 mm respectively. The final instar larva is about 6 mm long and 2.8 mm wide across the third abdominal segment (CABI, 2005). The full grown larva was about 8mm long and 4mm in width (Singh, 2004) the average larval period was recorded 11.54 ± 0.43 days in the field condition. In laboratory feeding, Singh (2004) found average larval development periods ranging from 11-16 days at about 27.5 ºC and larval survival rates ranging from 70-97%, both depending on cucurbit host. Almeida and Marileusa (2004) reported the average of the larval development was 26.19 days when reared on *L. esculentum*.The pupae were whitish yellow in color almost with flat ventral body surface and slightly raised dorsal portion with two black spots on the surface. Anterior part was covered with white hairs and posterior part was round in shape. Pupa was yellow orange and color having brown white margins on the dorsum. Its anterior portion was smooth while posterior region is spinous (Singh, 2004). The average length of pupae was measured 7 ± 0.31 mm and average breadth of 5.4 ± 0.24 mm in the field condition. The length and breadth of pupae developed from teasel gourd were highest (7.52 ± 0.24 mm, 6.24 ± 0.47 mm), which was statistically similar to bitter gourd and sponge gourd and significantly lowest measurement of the pupa was observed on yard long bean (Hossain *et al.*, 2009). The average pupal period was recorded 5.50 ± 0.24 days in the field condition. The pupal period lasts about 4 days (CABI, 2005). Almeida and Marileusa (2004) reported the average the pupal 8.19 days when reared on *L. esculentum*.The newly emerged adults were entirely yellow, but as the body hardens, the dark spots developed over a period of 6-12 hours. Just after emergence, the color of the beetle was bright yellow and distinct spots were not clearly visible on the elytra. Adult Epilachna beetle were typically ladybird shaped and 6 black spots on each elytron. The upper surface was covered with fine and short hairs. The newly emerged adults were

entirely yellow but as the body hardens, the dark spots developed gradually (CABI, 2005). The average length of the male adult was measured 7.2 ± 0.24 mm with an average breadth of 5.8 ± 0.2 mm in the field condition. The average length of female adult was measured 7.6 ± 0.24 mm with average breadth 6 ± 0.0 mm in the field condition.

Adults were alive till the end of research period. It was 3 months old till the end. (Almeida and Marileusa, 2004) the average of longevity of male and female was 81.44 and 97.89 days, respectively when reared on *L. esculentum*. The total life cycle (from egg to egg) was recorded an average of 27.19±0.50 days in the field condition. Khadka and Shah (1979) reported that the duration of adult emergence from egg was found to be 31.06 days in the field and 23.3 days in the laboratory condition. Yoshida (1974) reported this duration to be 20 to 30 days depending the season and availability of food. Mandal (1957) recorded this time for about 17-21 days in hot season and as long as 50 days in cold season. Pradhan (1966) recorded this time to be inversely related to the temperature. Shorter time period to complete its life cycle was found within 25-30 ^{0}C. Almeida and Marileusa (2004) reported the average of life cycle was 41.44 days in the laboratory when reared on *L. esculentum.* Oviposition period was found to be of 10.13 ± 3.33 days. The average fecundity per female was found an average of 283.25 ± 25.88 eggs/female in the field condition. Almeida and Marileusa (2004) reported the average eggs per oviposition were 24.45 and period of pre-oviposition, oviposition and post-oviposition were 23.57, 17.86 and 53.86 days respectively and the fecundity of the females was 59.78 eggs when reared on *L. esculentum*. Araujo and Almeida (2004) kept adults of *E. vigintioctopunctata* under ± 24 °C, >53% RH, at 12 hour photoperiod, and fed with leaves of *L. esculentum*. The average eggs were 24.45 per oviposition and the incubation period was 7.14 days.

Table 1. Measurement of different stages of *E. dodecastigma* at Rampur, Chitwan, Nepal 2015

Stages	Field condition	
	Length (mm) (Mean ± SE)	**Breadth (mm) (Mean ± SE)**
Egg	54.8±0.37µm	15.26±0.6µm
Larvae		
1st instar	1.41 ±0.24	0.56±0.06
2nd instar	4.2±0.2	1.9±0.1
3rd instar	6.2±0.2mm	3.2±0.2mm
4th instar	8±0.2mm	3.4±0.24mm
Pupa	7±0.31mm	5.4±0.24
Adult		
Male	7.2±0.24 mm	5.8±0.2 mm
Female	7.6±0.24 mm	6±0.0 mm

Table 2. Developmental periods of at *E. dodecastigma* at Rampur, Chitwan, Nepal 2015

S.N.	Stages	In the field condition (Mean ± SE)
1	Egg incubation period (days)	4.04±0.04
2	Larval period (days)	10.75±0.16
3	Pupal period (days)	5.30±0.20
4	Total life cycle (days)	27.19±0.50
5	Preoviposition period (days)	7.10±0.40
6.	Number of egg batches/pair	6.00±0.41
7.	Number of eggs/batch/female	44.86±10.28
8.	Fecundity (Total egg laid/ female)	283.25±25.88
9.	Oviposition period (days)	10.13±3.33

Bioassay on efficacy of biorational and chemical pesticide

The efficacy of the treatments was significantly different among different treatments. At the end of the study Epilachna larvae killed were significantly higher by chloropyriphous & cypermethrin followed by spinosad, derisom, margosom, *B. bassiana* and water spray. However, number of Epilachna larvae insects killed by the margosom, *B. bassiana* were not significantly different ($p = 0.001$, $F = 251.1$). Based upon the LT_{50} values, chlorpyrifos & cypermethrin has highest test insect killing speed followed by spinosad, derisom, *B. bassiana*, margosom, respectively.

Table 3. Efficacy of different treatment on test insect under laboratory conditions (26 ± 3°C, RH 80 ± 5%), 2015

Concentrations	No. of dead grubs (mean ± SE)[a]	LT_{50} (Hours)[b]
Dermisom@3ml/lit	6.25± 1.18^{c}	24.11
Margosom@4ml/lit	5.25± 25^{d}	46.43
Beuveria bassiana@3gm/lit	5.25± 25^{d}	45.88
Chloropyriphous+cypermethrin@2ml/lit	10± 0^{a}	6.52
Spinosad@0.5ml/lit	8.5± 0.29^{b}	15.19
Control (water spray)	0± 0^{e}	------

[a] Means within the same column followed by the same letter are not significantly different ($p \leq 0.05$) using SNK test of SAS (2016).

[b]LT_{50} values (in days) were determined by probit analysis (StatPlus, 2015).

Microbial were found slow in their action and kills the larvae gradually because fungi require several days of high humidity for sporulation and infection and generally infects the insect through the integument (Roberts and Yendol, 1971). Microbial generally take 7-10

days affecting the insect pests when environment is favorable (GC, 2009). Jotwani *et al*. (1962) determined relative toxicity of some important insecticides against the grubs and adults of *E. vigintioctopunctata* (F). According to Rajendran and Gopalan (1999), they reported direct spraying of *B. bassiana* killed 58.1 per cent first instar larvae and 35.2 percent pre-pupal stage larvae. The adults were not susceptible to *B. bassiana*, though the maximum mortality being 10.3 percent in the case of newly emerged adults.Swaminathan *et al.* (2010) reported that the antifeedant activity of *Azadirachta indica* seed kernel extract against *Henosepilachna vigintioctopunctata* (F.) showed 60, 40 and 20 per cent mortality at 5, 2.5 and 1.25 per cent concentrations respectively. Reddy *et al.* (1990) reported that petroleum ether (1%) extracts of *A. indica* A.Juss and *Annona squamosa* L., reduced the number of *H. vigintioctopunctata* larvae infesting brinjal by 88.0 and 92.99; 85.98 and 91.02 percent 24 hours and 3 days after spraying, respectively. The effect of rotenone may remain up to 5-10 days (Neupane, 2005). Chattopadhyay (1991) reported that *Derris elliptica* root extract is active against a wide range of insects and leaves no toxic residues. Similarly, Neupane (2000) reported that Derris plant, *D. elliptica* (Lamk) extract with rotenone insecticidal property is effective in controlling wide range of insect pests.

CONCLUSION

Eggs were laid on masses 25-65 eggs per batches. Range of incubation period of 2-7 days, larval period of 11-16 days, pupal period of 4-6 days and total life period of male 80-90 days, and female 90 -110 days was observed. In the bioassay experiment, the highest larval mortality was recorded in chloropyriphous & cypermethrin followed by spinosad and botanicals (derisom and margosom), *B. bassiana* and control (water spray). The chemical insecticide (chloropyrifos & cypermethrin) was found the quickest of all for killing the larvae of Epilachna beetles and losing its effectiveness as well. The botanicals (derisom and margosom) were found equally effective to cause mortality of the larvae and was found little slower than the chemical but their effectiveness was found to be more durable than chemical pesticide as they have insecticidal as well as antifeedant and repellant properties.

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