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Original Research Article

Laboratory Rearing Protocol for Pomegranate Fruit Borer (*Deudorix isocrates*)

M. H. Mallikarjun* and R. K. Pal

ICAR-National Research Center on Pomegranate, Solapur-413 255, Maharashtra, India
**Corresponding author*

A B S T R A C T

Study was conducted to standardize the laboratory rearing protocol for rearing of pomegranate bore (*Deudorix isocrates*). The rearing was carried out on pomegranate fruits (*Punica granatum*) and on semi synthetic diet under ambient room conditions (T = 27 ± 1°C, RH = 70 ± 10%; 12L: 12D). Among the three different artificial diets of lepidoptera, the diet on which the larvae initiated feeding was taken for assessing the biological parameters insect along with pomegranate. The female lay round creamy white eggs singly but also in pair either on the fruits or on the side cloth walls of rearing cases and sometimes on the upper or lower surface the cage. Hatching of eggs was observed from the insects which are reared on both the substrates, they accomplished their total imago developmental in 45.3 ± 1.82 days on pomegranate and in 35.3 ± 1.88 days on artificial diet. Pre-oviposition periods were, (1.2 ±0.42 and 1.8 ±0.31) days, and ovipositional periods (3.6±1.07 and 5.8±1.52) respectively. The longevity of females was (12.05 ± 1.52) days and their fecundity on pomegranate was 27.4 ± 3.30 as compared to 13.06 ± 1.62 days and 28.5 ± 4.20 daysin artificial diet reared female respectively. The average sex ratio of female to male was (1.80:1±0.07 and 1.90:1±0.9) respectively. *Tridax procumbens* used as the pollen host for the adult male and female.

Keywords

Deuodorix isocrates, Semisynthetic diet, Life cycle, Laboratory rearing

Introduction

Pomegranate (*Punica granatum* L.) is one of the important fruit crops in India and is being cultivated in Gujarat, Maharashtra, Karnataka, Uttar Pradesh, Andhra Pradesh and Tamil Nadu. Thorough scanning of literature revealed a total of 91 insects, 6 mites and 1 snail pest feeding on pomegranate crop in India (R.A. Balikai, 2013). The most obnoxious enemy is pomegranate butterfly, *Deudorix* (=*Deuodorix*) *isocrates* Fab. which may destroy more than 50% of fruits due to the release and wide spread cultivation of commercial high yielding varieties; there is a dramatic increase in the pest infestation over the years. *Deudorix isocrates* Fab. (Lycaenidae: Lepidoptera), it has gained the status of major pest of pomegranate and causes severe losses.

The penetration feeding of the caterpillar in the pomegranate fruit is also accompanied with an invasion by saprobe fungi and bacteria making the fruit unmarketable. As there exists very little work on biology of *Deudorix isocrates*, the present study was undertaken to record the biological properties of the fruit borer on pomegranate fruits and on artificial diet under laboratory conditions

Materials and Methods

Study on the biology of *Deudorix isocrates,* was carried out in the laboratory of the National Bureau of Agricultural Important insects and at NRC on pomegranate solapur during the year 2015-2016, with ambient room temperature (27±1) and relative humidity of (70±10) Infested fruits having Eggs/larvae/pupae were collected from pomegranate plants/orchards from four different localities viz., National Research centre on Pomegranate Solapur, Maharashtra, Mandya (Sadenahalli), Hoskote (Attibele), Hosduraga (Farmer Field) Karnataka. Among the three different artificial diets of lepidoptera, the diet on which the larvae initiated feeding was taken for assessing the biological parameters insect along with pomegranate. First instar larvae were identified and were separately reared in the laboratory on the same food substrates from which they were collected and on artificial diet. Rearing was carried out in rectangular one foot cages under ambient temperature and relative humidity of (T = 27 ± 1°C, HR = 70 ± 10%, 12L: 12D).

20 pupae were classified as male and female and transferred into two cylindrical Plexiglas cages. The boxes were maintained under the same rearing conditions until adult emergence. Freshly emerged male and female insects were differentiated, kept in pairs into separate cages containing pomegranate fruits as substrate for egg laying water and a 5% honey solution soaked in cotton swabs kept as water and food source to study adult behaviour and. The *Tridax procumbens* used as the pollen host for the adult male and female. Observations were recorded on duration of (pre-mating, mating was recorded under laboratory conditions) pre-oviposition, oviposition, post-oviposition behaviour fecundity and longevity of adult male and female. Detailed observations on eggs and larvae of each instar, pre-pupae, pupa and adults were also made from the from laboratory collected eggs. The data on morphometrics, *viz.,* the size of egg, different larval instars, pre-pupa, pupa male and female adults and body length were measured with the help of computer mounted stereo zoom binocular microscope in insect ecology laboratory NBAII.

Results and Discussion

Hatching of eggs was observed from both the pomegranate and artificial diet reared individuals at temperature range of (T = 27 ± 1°C; RH = 70 ± 10%; 12L: 12D). An adult female laid eggs either in the morning or in the evening with pre-ovipositional period of (1.2 ± 0.42 and 1.8±.31) days respectively. The average fecundity was (27.4 ± 3.3 and 28.6±4.72) eggs, with an ovipositional period of (3.6 ± 1.07 and 5.8±1.52) days respectively for artificial diet and pomegranate reared adults. The post-ovipositional period lasted for (5.2 ± 1.22 and 7.2± 1.61) days (Table 1).These results were almost similar to the observations made by Bisht *et al.,* (2001).

The female lay round creamy white eggs singly but also in pair either on the fruits or on the side walls cloth of rearing cases and sometimes on the upper or lower surface the cage. The freshly laid eggs on an average measured 0.50 ± 0.05 mm in diameter (Table 3).

Freshly emerged larva were cylindrical and creamy white in colour except head and last abdominal segments being black. The body of larva with scattered white hairs and measured 1.50 ± 0.23 mm in length and 0.88 ± 0.09 mm in width (Table 3). This instar lasted (6.6 ± 1.12 and 7.1±1.13.

The colour of second instar larva varied from creamy white to greenish brown with scattered hairs and yellowish spots on the body. Slant and flat last abdominal segment formed a shield over the anus in the full-grown larva (Mishra *et al.,* 2001). It measured 6.80 ± 0.83 mm in length and 2.40 ± 0.45 mm in width. This instar lasted 7.1 ± 0.87 and 8.1±0.89) days (T = 27 ± 1°C; RH = 70 ± 10%; 12L: 12D). Third instar larva was completely different from second instar and light brown in colour and measured 12.00 ± 2.18 mm in length and 3.80 ± 0.50 mm in width. These instar lasted for (8.5 ± 0.59 and 9.2± 0.61) days (T = 27 ± 1°C; RH = 70 ± 10%; 12L: 12D). The colour of fourth instar larva was dark brown or black in colour and with pale yellow patches on head and tail region and creamy white patches are seen on the abdominal region. It measured 17.50 ± 1.27 mm in length and 4.25 ± 0.50 mm in width (Table 3), and lasted for (7.5 ± 0.52 and 8.5±0.72) days at (T = 27 ± 1°C; RH = 70 ± 10%; 12L: 12D). Full grown larva was dark brown with pale yellowish patches and short hairs on the body as also reported by Sharma *et al.,* (2003). Shevale (2003) reported similar characters of the larvae with dense covering of hairs. It measured 21.00 ± 1.12 mm in length and 6.00 ± 0.50 mm in width which were smaller but wider than the larvae reported by Vijaychander and Arivudainambi (2007) and lasted 5.5 ± 0.46 days at (T = 27 ± 1°C; RH = 70 ± 10%; 12L: 12D). Total larval period was 35.3 ± 1.88 and 38± 2.1) days. The larvae of *Deuodorix isocrate* passed through five instars to reach pre-pupal stage (Shevale, 2003). Before pupal stage, the colour of larvae changed from dark brown to dark bluish on dorsal side and dirty white on ventral side, shrunk in size, stopped feeding and turned into pre-pupal stage. It measured 16.50 ± 1.69 mm in length and 7.10 ± 0.61 mm in width (Table 3). This stage lasted for 3.4 ± 0.45 and 3.8±.56) days. Moreover, on artificial diet and during larval development, the insect gains about 10 days in comparison with the caterpillars developing on pomegranate fruits. The larval developmental time accounted for 48% of the total developmental time on artificial diet as compared to 59% on pomegranate fruits. The pupa was obtect, adecticous with light brown in colour, measured 13.00 ± 0.77 mm in length and 6.25 ± 0.70 mm in width (Table 3). Pupation occurred inside the damaged fruit hole. Pupal period lasted for 14.5 ± 2.70 and 15.5 ± 2.9) days at temperature range of (T = 27 ± 1°C; RH = 70 ± 10%; 12L: 12D). The viability of pupa was (81.6 ± 2.6% and 841±3.2). The adult butterflies were dull brown in the case of male and dark brown in the case of female with an orange patch on each fore wing.

Table.1 Duration of different life stage of *Deuodorix isocrates* on pomegranate and artificial diet under laboratory condition

Sl. No	Particulars/Stage Duration/No. of	Durations/ No. of (Pomegranate)		Durations/No. of (Artificial diet)	
		Range	Average	Range	Average
1	Pre-oviposition (Days)	1-2	1.2±0.42	1-3	1.8±.31
2	Oviposition (Days)	2-5	3.6±1.07	2-6	5.8±.52
3	Post-oviposition (Days)	4-7	5.22±1.22	5-8	7.2±1.61
4	Fecundity (Numbers)	21-30	27.4±3.30	25-32	28.2±4.72
6	Larval period (Days)	33-38	35.3±1.88	43-48	45.3±1.82
7	Viability of pupae (%)	-	81.6±2.6	-	83.1±3.1

Table.2 Artificial diet ingredients for rearing of pomegranate fruit borer

Kabuligram flour	105g
Yeast tablets	25 No. s
Water	400 ml
Water	400 ml
Agar agar	12.75 g
Water	400 ml
Ascorbic acid	3.25g
Methyl para-hydroxy-benzoate	2g
Sorbic acid	1g
Streptomycin sulphate	0.25
Multivetaplex	2 capsules
10 % formaldehyde solution	2 ml
Pomegranate rind powder	5g

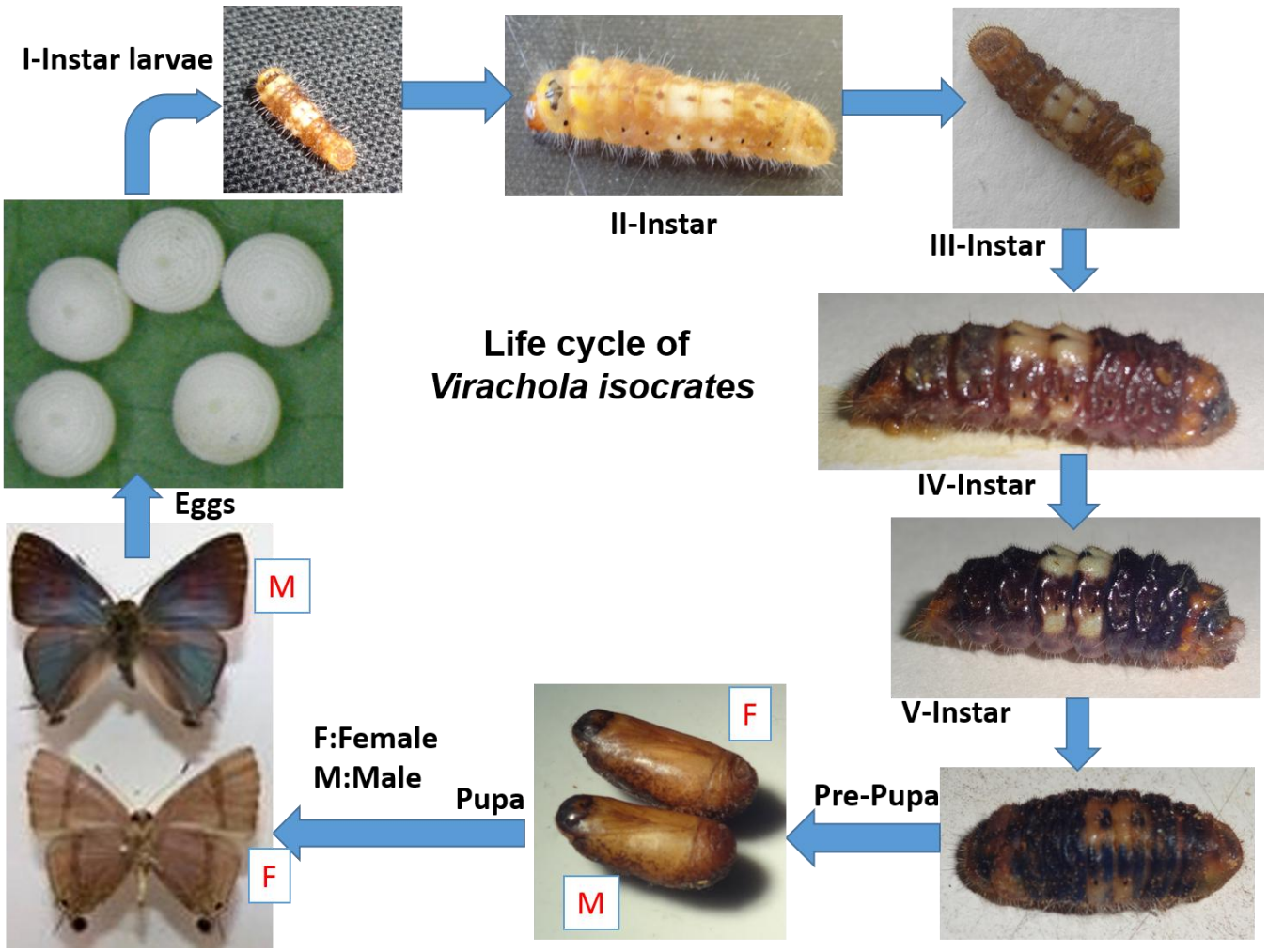
Table.3 Morphometric and duration of stages of *Deuodorix isocrates*

Particulars/Stage		**Measurement in mm**		**Duration in days (Artificial diet)**	**Duration in days (Pomegranate)**
		Length	**Width**		
Egg		-	0.50±0.05 (0.46-0.56)	-	-
Larva	1st Instar	1.50±0.23 (1.30-1.85)	0.88±0.09 (0.70-1.00)	6.6±1.12 (5-8)	7.1±1.13 (6-9)
	2nd Instar	6.80±0.83 (5.50-8.0)	2.40±0.45 (1.80-3.0)	7.1±0.87 (6-8)	8.1±0.89 (7-10)
	3rd Instar	12.0±2.18 (10.0-15.0)	3.80±0.50 (3.20-4.50)	8.5±0.59 (8-9)	9.2±0.61 (9-11)
	4th Instar	17.50±1.27 (15.50-18.50)	4.25±0.50 (3.90-5.0)	7.5±0.52 (7-8)	8.5±0.72 (8-9)
	5th Instar	21.00±1.12 (20.0-23.0)	6.0±0.50 (5.50-6.50)	5.5±0.46 (5-6)	5.5±0.46 (5-6)
Pre-pupa		16.50±1.69 (14.0-18.50)	7.10±0.61 (5.80-7.50)	3.4±0.45 (3-4)	3.4±0.45 (3-4)
Pupa		13.0±0.77 (11.50-14.50)	6.25±0.70 (5.0-7.0)	14.5±2.7 (10-18)	15.5±2.9 (11-19)
Adult	Male	22.0±1.58 (20.0-24.0) *	42.0±2.50 (39.0-47.0)	6.7±1.15 (5-8)	6.7±1.15 (5-8)
	Female	24.0±1.22 (22.0-25.0) *	46.0±2.34 (44.0-50.0)	10.5±1.08 (8-12)	12.5±1.12 (10-14)

*Width with expanded wings **diameter
Figure in parentheses indicate range.

Table.4 Female sex ratio of *Deudorix isocrates* reared on pomegranate and artificial diet under laboratory conditions

Food substrate	**Female Sex ratio**
Pomegranate	57.14
Artificial diet	66.67



However, male butterfly had three bluish spots on each hind wing. The hind wings of both sexes had 3-6 mm long tail like structures on its anal margin. Female butterfly was bigger than the male butterfly.

The male butterfly was measured 22.00 ± 1.58 mm in length and 42.00 ± 2.50 mm in width across the wings, while female butterfly was measured 24.00 1.22 mm in length and 46.00 ± 2.34 mm in width across the wings (Table 3).

The longevity of male and female lasted for 6.7 ± 1.15 and 12.5± 1.12 days, respectively at temperature range of 20.3 to 33.3 °C and RH of 87.1%. Almost similar observations were made by Pathak and Rizvi (2002). The sex ratio of female to male was 1.80:1 ± 0.07. As shown in Table 1, the insect developmental time varied according to the host plant.

On artificial diet, development was faster than on pomegranate. The results indicate that semisynthetic diets can also be utilized effectively to rear different stages of *Deudorix isocrates.*

Consistent observations showed that males emerged earlier than females. This protandrous phenomenon is typical for Lepidoptera. Males usually emerged in the morning and females later in the day. Data

shows that the sex ratio is not independent of the food source. The sex-ratio of the emerging adults of *D. Isocrates* was near to 1 (Table 4). On both food sources, oviposition began on the second day after female's emergence. On artificial diet reared unfertilized females laid 25 to 32 eggs (28.5.5 ± 4.20 eggs), while on pomegranate fruits laid between 21 and 30 eggs (27.4 ± 3.30 eggs). Thus, artificial diet is more favorable for oviposition than pomegranate fruits. The fecundity, noted on the artificial diet and on pomegranate fruits, was significantly different. Females lived for 13.06 ±. 62 days reared on artificial diet and for 10.5 ± 1.08 days on pomegranate fruits. Female longevities were identical and practically independent from the type of food substrate.

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