Article

Behavior of pomegranate fruit borer, *Deudorix isocrates* (Fab.) (Lepidoptera: Lycaenidae)

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Abstract
Deudorix isocrates is one of the serious pests of pomegranate, *Punica granatum*. The present study was undertaken as behavioral understanding of pests has crucial significance in pest management. The present study deals with larval behavior like searching period, selection of site to enter in the fruit, increase in size of entrance hole, selection of sites for pupation and adult behavior with respect to oviposition and fecundity. It was found that the gravid female rarely lays egg on a flower, the twigs and the underside of leaves. The calyx end of fruit is the most preferred site of egg laying (68.65%) than other parts like the lower half (11.56%), the stalk base (8.31%), the upper half (7.49%) and the middle region (3.97%) of the fruit. After hatching, the most of the neonate larvae (32%) consumed 16 to 30 minutes of period to search suitable site to enter in the fruit. The searching period ranges between less than 15 minutes (6%) to more than 4 hours (2%) in the rare cases. The lower half of the fruit is the most suitable site for larval entry (57.77%) while the stalk base region of the fruit was rejected for larval entry (0%). The larvae cut the fruit rind to increase dimeter of entrance hole from the early to the late instar stages. It was found 0.84 mm in the first instar, 1.43 mm in the second instar, 2.68 mm in the third instar and 5.76 mm in the fourth instar stage of larva. While studying pupation sites it was revealed that maximum number of larvae (62.60%) pupated inside the fruit, least number (1.56%) of larvae pupated on the fruit surface. 2.43% of the larvae pupated inside the calyx while 33.39% of the larvae pupated somewhere else.

Keywords *Deudorix isocrates*; pomegranate; pest; behavior.

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1 Introduction

It is well known that larvae of butterflies and moths are serious pests of various crops and are responsible for a considerable economic loss. Hence, the lepidopteran pests have become “forefront object” of bioecological studies. In the pest ecology the behavioral study has a greater significance to understand adaptive features in lepidopteran pests and their host plant specificity (Chardonnet et al., 2014). Understanding of behavior of the

pest is also important to design Pest Management Strategies (PMS) and Insect Resistance Management (IRM) (Spangler and Calvin, 2001; Paula et al., 2012; Zhang and Zhang, 2018; Pannuti, 2019). Mostly behavioral studies on various species of lepidopteran pests have been carried out with respect to ovipositional behavior, feeding and searching behavior of larva (Li et al., 2015; Rajapakshe and Walter, 2007; Lakshmipathy, 2000; Dolek et al., 2013). *Deudorix* is one of the lepidopteran genera (formerly known '*Virachola*') which includes some pest species which attack wide range of host plants, mostly the fruit plants. *D. isocrates* is one of the polyphagous pest species of this genus. It attacks wide range of host plants like guava, aonla, ber, citrus, tamarind, apple, sapota apart from pomegranate (Singh and Singh, 2009; Balikai et al., 2011; Chhetry et al., 2015; Arya and Dubey, 2017; Gundappa et al., 2017; Muthiah and Indragandhi, 2021). However, it appeared as the most destructive pest of Pomegranate, *Punica granatum* (Gupta and Dubey,2005, Kumar, 2010) causing 65-70% yield loss worldwide (Kumar et al., 2017). Itis distributed all over India and common in Maharashtra (Riteshkumar, 2020). In India it causes 40-90% yield loss of pomegranate (Wadhi and Batra, 1969; Nair, 1978) that consequently results in to remarkable economic loss of the pomegranate growers. In Maharashtra and Karnataka, the incidence of *D. isocrates* on pomegranate has been reported throughout the year with varying degrees of intensity (Shevale and Khaire, 1999). A considerable research work has been carried out on bionomics, pest incidences on various host plants, population dynamics, and management of *D. isocrates* but its behavioral aspects are not studied extensively. The present study is an important contribution to the knowledge of behavioral understanding of this pest which will help to suggest and plan the pest management practices against its attack on pomegranate.

2 Materials and Methods

The present research work was undertaken at the pomegranate farms nearby villages of Baramati, District Pune from Maharashtra state of India. The behavioral study of *D. isocrates* (Fig. 1) was undertaken with respect to site of oviposition, searching behavior of newly hatched larva for entry site, site of larval entry into the fruit, relative size of entrance hole in accordance with the larval growth and the site of pupation. Field observations were made to study the site of oviposition, site of larval entry into the fruit while studying the searching behavior of newly hatched larva for the entry site and the relative size of the entrance hole in accordance with the larval growth were undertaken in laboratory.

2.1 Site of oviposition

The plant parts of pomegranate such as leaves, twigs and fruits were screened to know oviposition sites during the field survey. When it came to notice that most of the eggs were deposited on the fruits, special attention was given to check the regions of the fruit surface preferred by the female to lay its the eggs. Five different regions of the fruit such as, inside of the calyx, the lower half portion, the middle portion, the upper half portion and base of the fruit were taken into consideration (Fig. 1). Total 1107 observations were recorded and analyzed in the form of specific region of the fruit and the number of eggs deposited them. The percentage of eggs deposited on each specific region of the fruit was calculated to find the most preferred site of oviposition

2.2 Searching behavior of larva for entry site

The fruits along with eggs were plucked and brought to the laboratory. The partial rind of the fruit bearing egg was cut carefully and placed in glass petri dish (9.5 cm dia.). The precaution was taken to avoid fungal growth on the fruit rind and the egg by keeping experimental set always clean. The eggs were observed regularly till hatching. The time of hatching was noted. The newly hatched larva was gently lifted a camel hairbrush and placed on a fresh pomegranate fruit so as to allow it to enter into the fruit. A separate fresh fruit was provided for each larva and observed critically till it started boring the fruit rind. The period between hatching and commencement of boring the rind was referred to as searching period. Total 50 observations were recorded for

further analysis.

Fig. 1 Pomegranate fruit portions. **IC:** Inside the calyx, **LH:** Lower half, **MP:** Middle Portion, **SB:** Stalk base, **UH:** Upper half portion

Fig. 2 Adult *D. isocrates* (Female).

2.3 Site of larval entry into the fruit

The larva entered into the fruit by making an entrance hole. The location of the entrance hole on a specific region of the fruit was taken as a clue of the larval entry site. Considering the entrance hole as the site of larval entry, total 1113 entrance holes were observed to note the site of the larval entry into the fruit from five different regions of the fruit such as inside of the calyx, the lower half portion, the middle portion, the upper half portion and the base of the fruit. The observations were analyzed in the form of specific region of the fruit and number of the entrance holes observed on it. The percentage of entrance holes corresponding to the specific regions was calculated so as to know the choice of larva of specific region to get entry into the fruit.

2.4 Relative size of entrance hole in accordance with larval growth

To find out the relative size of the entrance hole in accordance with the larval growth, fruits with live infestation were collected from the field and brought to the laboratory. The live infestation was identified by the fresh excreta oozing out from the entrance hole. From each infested fruit the diameter of the entrance hole was measured and the fruit was dissected to report the instar stage of the larva inside it. The same procedure was repeated till getting 50 observations of each larval instar stage. The observations were summarized in the form of a larval instar stage and an average dimeter of the entrance hole.

2.5 Site of pupation

The pest-infested fruits were marked in the field. The marked fruits were observed daily to check the larva pupated on the fruit surface and inside the calyx. The sites of pupation on the fruit surface and inside calyx were easy to locate and were recorded accordingly. During this survey it was noticed that the infested fruits with the dried excreta at the entrance hole were tied to the twig by the web of the silk thread to accommodate the pupa inside the fruit. Such fruits were collected from the field during the survey, brought to the laboratory and dissected open to confirm the presence of pupa inside the fruit. When the fruit was found without pupa inside it was treated that the pupation site is elsewhere from the fruit. Such 575 fruits were observed to record various pupation sites. The data was presented in the form number and percent of pupae were found on the particular site.

3 Results

To lay eggs females *D. isocrates* (Fig. 2) preferred the fruit, leaves and twigs of pomegranate trees. The oviposition site on the fruit selected by the female was also not one and the same. Majority of the eggs (68.65%) were laid inside the calyx (Fig. 3) while least number (3.97%) of eggs were laid on the middle region of the fruit (Fig. 4). These results suggest that the preferable oviposition site is inside of the calyx; while the least preferable site is the middle region of the fruit.

Fig. 3 Egg of *D. isocrates* laid inside the Calyx. **EG:** Egg, **CA:** Calyx.

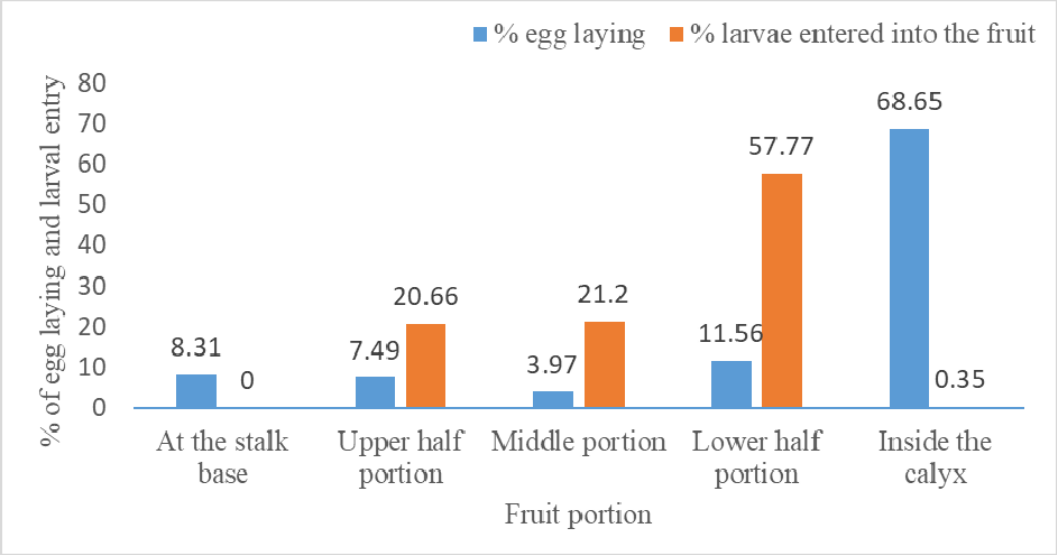

Fig. 4 Percent egg laying and larval entry sites of *D. isocrates*.

After hatching, the larvae spent varying time period in crawling on the fruit surface to search an entry site on the fruit rind. It is referred here as searching period. The searching period was reported from less than 15 minutes up to more than 4hrs.but maximum number (32%) of larvae spent 16 to 30 minutes to search an entry site (Fig. 5).

Majority of the larvae (57.77%) preferred the lower half of the fruit for entry, 0.35% of the larvae preferred calyx as an entry site even though a significant number of eggs were deposited inside the calyx. The stalk base was totally avoided for an entry into the fruit though a few eggs were laid on that region. The upper half and the mid region accounted for 20.66% and 21.20% respectively for the larval entry into the fruit (Fig. 4). This implies that larvae prefer these regions equally for the entry into the fruits.

In the present study it was observed that at the beginning the newly hatched larva entered the rind of the fruit by continuous chewing and cutting the rind till it entered in to the fruit completely (Fig. 6a). The larva made a narrow tunnel always heading towards inside of the fruit. In the later days of instar to instar, the larva turned the heading outwards it and cut the rind time to time. This activity resulted into gradual increase in size of the entrance hole. According to the present investigation an average size of entrance whole was found 0.84 mm in the first instar, 1.43 mm in the second instar, 2.68 mm in the third instar and 5.76 mm in the forth instar stage of the larva.

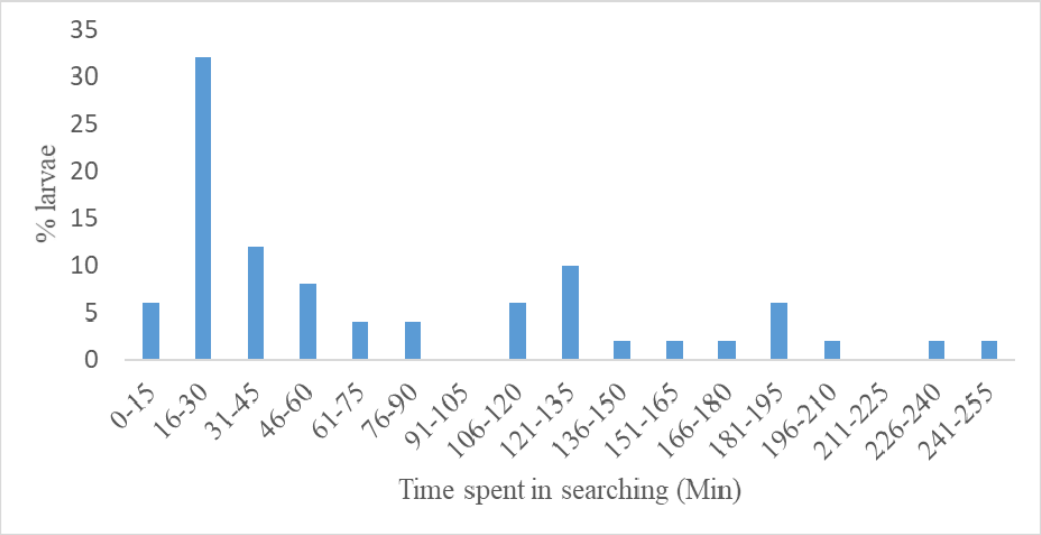

Fig. 5 Searching period (in minutes) of 1st instar larva of *D. isocrates*.

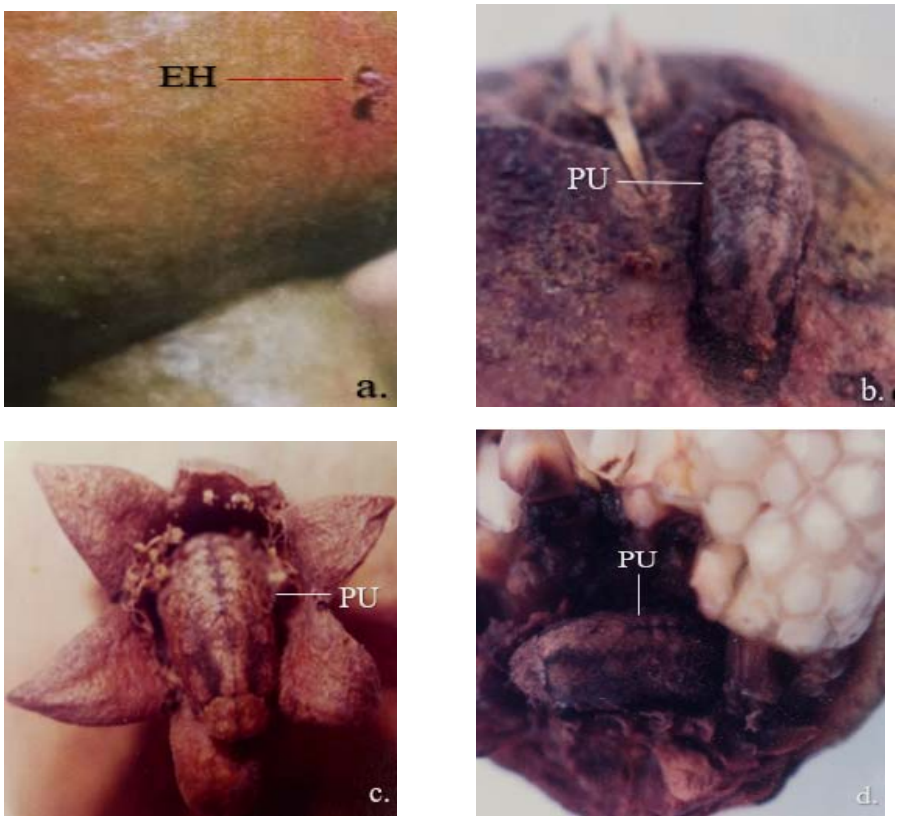

Fig. 6 a. 1st instar larva of *D. isocrates* cutting fruit rind to make entrance hole. **EH:** 1st instar larva making entrance hole by cutting fruit rind. **b.** Pupa of *D. isocrates* on fruit surface. **PU:** Pupa. **c.** Pupa of *D. isocrates* inside the calyx of fruit. **PU:** Pupa. **d.** Pupa of *D. isocrates* inside the fruit of pomegranate. **PU:** Pupa.

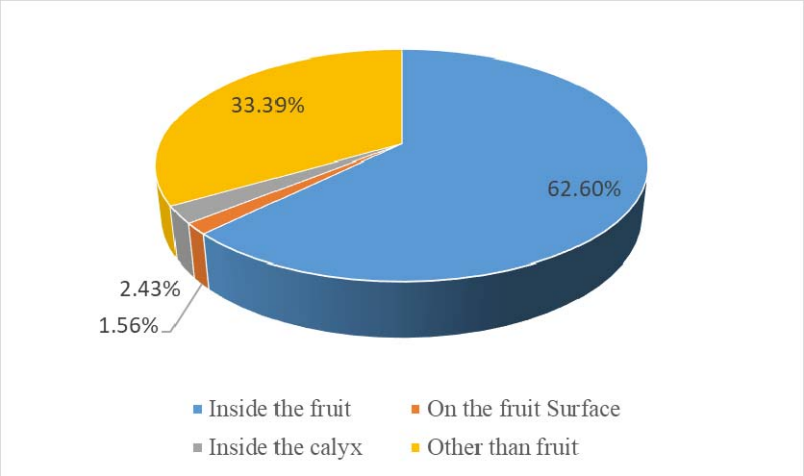

Fig. 7 Pupation sites of *D. isocrates*.

The field studies on pupation sites of *D. isocrates* revealed that with a varying number the larvae of *D. isocrates* pupated on the fruit surface, inside the calyx and inside the fruit (Fig. 6b, c, d). The maximum number of the larvae (62.60%) pupated inside the fruit, a least number (1.56%) of larvae pupated on the fruit surface. 2.43% of the larvae pupated inside the calyx while 33.39% of the larvae pupated somewhere else from the fruit. These places were not known and hence were not recorded. These observations imply that most preferable site of pupation for the larvae of *D. isocrates* is the inner portion of the pomegranate fruit (Fig. 7).

4 Discussion

A *D.isocrates* female lays eggs singly, some times in a pair and rarely in the small clutch on the flowers, leaves and young fruits surface including calyx cup. This general ovipositional behavior has been reported by earlier researchers (Balikai et al., 2011; Bhut et al., 2013; Kumar et al., 2017; Bharti et al., 2021; Kaushal et al., 2021) also. In the present investigation it was found that leaves and flowers were rarely preferred for oviposition and mostly the fruits of varying stages were used to deposit eggs. The calyx end of fruit is a highly preferred site of egg laying than the other parts like the lower half, the stalk base, the upper half and the middle region of the fruit. Similar ovipositional behavior of a female *D. isocrates* has been reported earlier (Shevale, 1997) by comparing egg laying on different portions of the fruit surface including the calyx end. An appropriate site of egg laying is the part of parental care (Mani, 1971). Deposition of eggs on the most protected site is a strategy for escaping egg predation (Baguette and Schtickzelle, 2003). Accordingly in the case of *D. isocrates*, an "appropriate" site of oviposition is found to be inside of the calyx where the eggs get protected from predation as a parental care. This finding is helpful to monitor pest attack during the early stage of egg laying before the fruit damage is caused by the early instar larva. The results also suggest paper bagging of fruits as a preventive measure against the pest attack through which we can avoid egg laying on the fruits. Paper bagging against this infestation is recommended by earlier work (Kumar and Kamala Jayanthi, 2018; Riteshkumar, 2020) so as to minimize pest attack. In case of *D. isocrates*, this control method reduces pest attack up to 10% against 90% in open fruits (Bagle, 2011).

After hatching the 1st instar larva spent some time on fruit surface (Kaushal et al., 2021) to find a suitable

site to enter into the fruit. This period is treated here as a 'searching period' during which the larva crawls on the fruit rind. On getting a suitable site of entry, it cuts the rind (Kumar et al., 2017) with its mandibular mouth parts and makes a fine hole; the entrance hole. The larva continues to cut the fruit rind till it reaches inside the fruit. The present study revealed that the maximum number of larvae (32%) consumed 16 to 30 minutes as a searching period. The searching period of *D. isocrates* larva has been previously reported as 16 to 20 minutes (Shevale, 1997). A finding of the present study is nearly similar to the previous report. This result of the present study reveals that for a very short time period the larvae remain outside before entering into the fruit. Therefore, efficient control can be achieved by proper timing sprays of pesticides when neonate larvae are still on the fruit (Kaushal et al., 2021)

The present study on larval entry sites revealed that the most preferred site for the larval entry into the fruit is the lower half of the fruit. The larvae rejected stalk base of the fruit to enter into the fruit while under heavy infestation rarely the larval entry was found through calyx cup. According to the previous reports on *D. livia* (Awadallah et al., 1970) and *D. isocrates* larvae (Shevale, 1997) show the same behavior. Their observations are the same as that of the present study. It appears that the larva usually rejects stalk base and calyx since the rind of the fruit in these regions is comparatively thicker than the general surface of the fruit. The selection of the lower half for the larval entry may be the adaptation of the growing larva to drop out its excreta on the ground instead on the fruit surface. Due to which the larva tries to keep the infested fruit clean inside as well as outside. Furthermore, the present results also show that the larval entry site is different than oviposition site.

Necessarily, the larva increases the diameter of the entrance hole by cutting the fruit rind throughout the larval period. The size of the entrance hole increases from 0.4 mm up to 7.5 mm. This behavior of *D. isocrates* larva is also reported from the aonla fruit, *Emblica officinalis* (Srivastava and Jain, 1973) and noted the dimensions of the larval tunnel. The size of entrance hole was proportionate to the larval size and growth. According to the earlier report from pomegranate an increase in the size of the entrance hole is in the range of 0.41 mm, on the first day of the 1^{st} larval instar to 7.11 mm on the fifteenth day of the larval period, at the prepupation (Shevale, 1997). This result supports our findings. The present study also shows that on an average the dimeter of the entrance whole is 0.84 mm, 1.43 mm, 2.68 mm and 5.76 mm during the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar stages of the larva, respectively. There is no report available on the size of the entrance hole in accordance with the larval instar stage. The increased size of the entrance hole helps the sequential larval instar stage to drop out their excreta outside the fruit and also help the imago to come out of the fruit, when it ecloses. The pupation takes place preferably within the infested fruit of pomegranate. However, the outer surface, the calyx end and elsewhere from the fruit are other pupation sites selected rarely. The observations of the present study are supported by reports of the earlier research works on pupation of *D. isocrates* (Kumar et al., 2017; Bharti et al., 2021; Riteshkumar, 2020; Kaushal et al., 2021). This behavior appears common in other species of *Deudorix* (Mould, 1976). While studying the pupation site, one more interesting behavior of the last instar larva was found: Before prepupation the larva comes out from the fruit and spins a silken web on the stalk base portion of the infested fruit to tie it with the twig. It prevents fruit fall and protects the pupa inside it. These findings suggest the characteristic adaptation of the pest to protect pupa against predation by concealing it inside the infested fruit and avoiding fruit fall as it is tied to the twig by the silken web.

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