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Temperature-Dependent Development and Degree-day Model of *Epilachna vigintioctopunctata* (Coleoptera: Coccinellidae) on Ashwagandha (*Withania somnifera*)

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The spotted or Hadda beetle, *Epilachna vigintioctopunctata* is a polyphagous pest and mainly attacking the crops of the family Solanaceae (potato, egg plant, tomato), Cucurbitaceae (gourds, melon, cucumber etc.) and Fabaceae (soya and haricot beans) (Ghosh and Senapati, 2001; Venkatesh, 2006; Varma and Anandhi, 2008). It also attacks several wild solanaceous medicinal plants such as *Physalis* spp. (Mohanasundaram and Uthamaswamy, 1973), *Datura* spp., *Solanum* spp. and wild ashwagandha (*Withania somnifera*) etc. (Mathur and Srivastava, 1964; and Ramanna *et al*., 2010). It is widely distributed in South East Asia, Australia, Sri Lanka, China, Japan including India (Kapoor, 1950 and Richards, 1983).

The spotted beetle is a defoliating pest and attacks the leaves of ashwagandha, both grubs and adults skeletonize the leaves by eating the chlorophyll and leaving the veins in a characteristic manner. In severe cases, leaves dry and fall down, and plant growth is stunted. If the infestation is at early stage, the plants get dried. (Mitra and Biswas, 2002; Tripathi *et al*., 2005; Venkatesh, 2006; Ravikumar *et al*., 2008; Ramanna *et al*., 2010). Seasonal occurrence and biology of spotted beetle has been studied on several wild and cultivated solonaceous crops including ashwagandha (Manjoo and Swaminathan, 2007; Varma and Anandhi, 2008; Venkatesh, 2006). Such information can not draw any relationship between developmental rate and temperature. Understanding the physiological relationship between temperature and development is important for the prediction of pests on crops and its seasonal emergence (Jervis and Copland, 1996). This information could be useful in developing sampling protocols, timing of insecticide application or implementing a biological control strategy targeting susceptible life stages of this insect in ashwagandha and other Solanaceous crops. Therefore, through present study an attempt has been made to determine the relationship between temperature and development of the immature stages of *E. vigintioctopunctata* on ashwagandha under controlled temperature conditions using linear regression model.

Temperature-dependent development of different immature stages of hadda beetle was studied under laboratory conditions. Insects from laboratory colony were reared on leaves of ashwagandha to study the developmental rates and thermal requirements for development.

Grubs of *E. vigintioctopunctata* were collected from the field of Ashwagandha variety JA-134 grown in Farm, Directorate of Medicinal and Aromatic Plants, Anand, Gujarat. These grubs were transferred to the laboratory and reared on the leaves of same host in transparent plastic containers of 25 x 10 cm lined with moistened blotting paper until they reached the pupal stage. The pupae were then separated and kept in a single layer in small transparent plastic containers having perforated lid and lined with moistened blotting paper. The emerged adults were collected, sexed and cultured on the leaves of same host in transparent plastic containers lined with moistened blotting paper to prevent desiccation of leaves and life stages. Fresh leaves were offered to the adults daily. Adults copulated and laid eggs in batches on the leaves. The egg masses were carefully removed along with a small bit of leaf on which they were attached and placed in transparent plastic Petri dish (60 mm) lined with moistened blotting paper to avoid desiccation of eggs. The Petri dishes were placed into BOD incubators set at 20, 25, 30 and 35 ^{o}C. Twenty five sets of egg masses of 20-30 eggs each were used for respective temperature regime used in the study. Egg masses kept at each temperature regime were monitored daily for recording their hatching period. Since, all the eggs in an egg mass at a particular temperature hatched at more or less same time, therefore for recording hatching period egg mass as whole was considered rather than the individual egg. The newly hatched larvae were separated by using soft camel's hair brush and transferred in a transparent plastic Petri dish lined with moistened blotting paper. For each regime of temperature 25 individual Ist instar larvae were used. They were regularly fed on fresh ashwagandha leaves. Each individual was monitored regularly and moulting was confirmed by the presence of cast head capsule (exuviae).

The duration of each instar of grubs, pre-pupa and pupa (up to adult emergence to determine pupal duration) were recorded.

Developmental rate (DR) for each developmental life stage was determined using the reciprocal of the mean development time (i.e. DR=1 / d) required to complete a particular life stage. The relationship between development rate (DR) and temperature was described by a linear regression model (Arnold, 1959) fit as, DR= a +bT (where a, b are the parameters of linear regression, 'T' is temperature) using the SPSS 13.0 and Excel (Microsoft corporation). The data generated for 35 ± 0.5 ^{o}C temperature regime was not used for estimating degree day requirement, as at this temperature very less number of eggs were hatched and complete mortality of grubs at earlier instars were observed. The lower developmental threshold (LDT) i.e. the temperatures when development ceases was determined as — a / b and the number of degree-days (DD) above LDT necessary for completion of development of each stage was calculated as 1/b of regression model (Arnold, 1959). For the purpose of estimating LDT and DD for pupae, the values of pre pupal stage was added to pupa, as the grubs already stopped feeding, attached to the substrate and started converting the larval tissues to pupal form.

The development of *E. vigintioctopunctata* completed in 36.8d from egg deposition to adult emergence stage at 20^{0}C. This temperature is lower than the optimum temperature of 28-30^{0}C required for development in field condition in this region. The eggs took 7.7d to hatch at this temperature, whereas, larvae and pupa took about a month time to complete their development.

At higher range of temperatures (25 and 30^{0}C) rate of development decreased and at 25^{0}C *E. vigintioctopunctata* completed its development (from egg deposition to adult emergence) in 27.5d only. Since, in the field condition optimum temperature for development is more, the beetle took more time in completing its development at 25^{0}C. The incubation period for eggs at this temperature was 6.1d, whereas, larval and pupal period lasted for 21.5d)

At 30^{0}C, which is in the range of optimum temperature (28-30^{0}C) in the field conditions, the *E. vigintioctopunctata* took least time (19.4d) in completing its development. It took only 4.4d for eggs to hatch at this temperature and larval and pupal stage lasted for 10.9d and 3.4d, respectively .

At higher temperature (35^{0}C), very less eggs were hatched and the grubs were died in early stage, therefore, total development at this temperature was assumed as zero.

The total development from egg deposition to adult emergence decreased from 36.8d to 27.5d to 19.4d, when temperature was 20, 25 and 30^{0}C, respectively. In the three thermal regimes, the development of larvae took longer time than pupae and that of pupae than egg. The detailed developmental periods of this insect on various crops has been also reported by several workers in India. According to their studies, development time normally ranged between 4.0 to 5.6 days for eggs, 11.0 to 15.1 days for grubs and 4 to 6 days for pupa at ambient room temperature which is nearer to the present study (Dhamdhere *et al.*,1990 and Ghosh and Senapati, 2001).

The lower threshold temperature for development varied with development stages. The rate of development increased linearly with temperature range 20-30^{0}C, as indicated by the high values of coefficient of determination (R^2=093-0.98). The linear regression model estimated that hadda beetle required 416.7 DD to complete the development from egg deposition to adult emergence on ashwagandha, with a lower development threshold of 9.2^{0}C. Similarly, the DD required for egg, larvae and pupa to complete their development was 105.3, 232.6 & 89.3 with a lower development threshold of 6.7, 8.6 and 10.9, respectively (Table 1).

The present study demonstrated that the developmental thresholds and degree day requirement were not similar between the life stages of the insect. However, the differences in developmental thresholds between life stages are not uncommon (Butler and Wardecker, 1971 and Hanula *et al.,* 1987).

The information on phenology, developmental threshold and thermal requirements generated in this study could be used to develop sampling protocols under varying temperature conditions.

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Table 1. Linear regression of development rate of *E. vigintioctopunctata*

Stage of development	Linear equation	R^2	Lower development threshold °C(LDT)	Degree-days (DD)
Egg	Y=0.0095x-0.06317	0.97	6.7	105.3
Grub	Y=0.0043x-0.03717	0.99	8.6	232.6
Pupa	Y=0.0112x-0.12167	0.93	10.9	89.3
Total development	Y=0.0024x-0.022	0.98	9.2	416.7

LDT = Lower development threshold calculated as a / b of regression model
DD = Degree- days calculated as 1 / b of the regression model

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