Biolife
AN INTERNATIONAL QUARTERLY JOURNAL OF BIOLOGY & LIFE SCIENCES
3(3):688-693
ISSN (online): 2320-4257
www.biolifejournals.com
BIOLIFE RESEARCH ARTICLE

# Triungulin occurrence and its load carrying capacity by *Xylocopa letipes* Drury (Hymenoptera: Xylocopidae)

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## ABSTRACT

**Triungulins are young instar larvae of meloid (blister) beetles. Blister beetles are pest insects feeding on flowering bodies of several agricultural and non agricultural plants. On the contrary *Xylocopa letipes* Drury is very potential plant pollinator but triungulins of *Nemognatha plazata* Fab. act as limiting factor of *X. letipes* population by parasitism. Therefore, any advance knowledge on host parasite association of this model will add great relevance in protecting *X. letipes*. Triungulins of blister beetle *N. plazata* ascended on *X. letipes* body during the visits to flowers of *Peltophorum pterocarpum* L. and later brought to the *Xylocopa* nesting. Triungulins used grubs of *X. letipes* for food. The triungulins *N. plazata* were different from others by having 1 or 2 stemmata on each side of head and was not parallel sided but pointed and with two short bristles to posterior side of the body. Triungulin aggregations responded as bridge between to substrates, contraction and expansion of biomass for external stimuli and as attractant for *N. plazata*. The per cent parasitism recorded on *X. Letipes* was 36.84% and 37. 14% in the years 2014 and 2015 respectively and Xylocopa comfortably carried the load of 23 triungulins on its body.**

**Key words- Xylocopa, parasitism, Triungulins, Host - parasite interaction, pollination**

## Introduction

Triungulins are young larval forms of meloid or blister beetle (Coleoptera: Meloidae). The adult beetle feeds on flowering bodies of several plants including Peltophorum, Tamarind, cassia, cucurbits and other agricultural and forest plants. They lay eggs on the flower and the eggs hatch into triungulins. The body of Triungulin may be navicular boat shaped or a slender flat and heavily sclerotized and with pattern of setations with transverse head and 1-2 stemmata on each side, 3 antennal segments, abdomen with two bristles or without bristles and narrow. They are specially characterized by having 3 claws (tri-ungulae), highly adapted for grasping their hosts like bees and Xylocopa.

***How to Site This Article:***
Anna Gophane, T. V. Sathe, Pallavi Khairmode, Sheetal Londhe and Shilpa Kurne (2015). Triungulin occurrence and its load carrying capacity by *Xylocopa letipes* Drury (Hymenoptera: Xylocopidae). *Biolife*, 3(3), pp 688-693.

The life cycle of parasites often involve complex behavioural, physiological and morphological adaptations in order to find a suitable host. Such type of complex adaptions also seen in bees and blister beetle-triungulin model (Saul- Gershenz & Millar, 2006).

The carpenter bees *Xylocopa* spp. are characterised by making tunnels for nesting in solid wood or in stumps, logs or dead branches of trees. *Xylocopa* spp. occurs throughout the year and forage on wide array of flowers during the day or some time even during moonlit nights. They are also very potential pollinators of several agricultural plants and play an important role in increasing the yield of crops through their pollinating services (Sathe & Gophane, 2015). However, triungulins are acting limiting factor for *Xylocopa* spp. for pollinating services by parasitizing them. Therefore, the present study was undertaken to enlighten the host parasite relationship and parasite load carrying capacity of Xylocopa.

Review of literature indicates that Wheeler (1928), Enns (1956), Selander & Bouseman (1960), Askew (1971), Wilson (1971), Adams & Selander (1979), Borg & Karlson (1990), Pinto (1991), Costa & Pierce (1997), Hafernik & Saul-Gershenz (2000), Selander & Fasulo (2000), Luckman & Scharf (2004), Saul- Gershenz & Millar (2006) etc. worked on triungulins and their hosts.

## MATERIALS AND METHODS

Occurrence of triungulins on *X. letipes* was studied by collecting Xylocopa with the help of insect net at 1 week interval from morning hours 8.00 AM to 10.00 AM on host plant *P. pterocarpum*, *Tamarindus indica* during the dry and clear days of the year 2014-2015. The collected Xylocopa were examined for noting the presence of triungulins on their body. The number of infected *X. letipes* has been noted and per cent parasitism was calculated. The number of triungulins was noted on each Xylocopa infected and further morphological features of triungulins were taken into account for their description.

Triungulin load carrying capacity was noted by counting number of triungulins present on Xylocopa and noting the comfort of flight of Xylocopa. Observations were also made on feeding behaviour of triungulins on grubs of *X. letipes* and nesting material and aggregation of triungulins and movement for any specific activity or against the host activities like mating. The triungulin number present on male and female was observed by spot observations especially females were observed before and after mating for presence of triungulins on their bodies. Morphological features of triungulin were noted under compound microscope and photographed with microscopic unit. All measurements were taken in milimeters.

## RESULTS

Results recorded in table 1 & 2 and figs.1 to 5 indicated that triungulins climbed on the body of Xylocopa when Xylocopa visited the flowers of *P. pterocarpum* for feeding on pollen & nectors. The meloid beetle (blister beetle) *N.*

**Table-1. Tringulin load carring capacity of *X. letipes***

| Number X. letipes | Date | Tringulin number on body | Flying behaviour |
|---|---|---|---|
| A | 4/4/2014 | 20 | comfortable |
| B | 11/4/2014 | 18 | comfortable |
| C | 27/4/2014 | 25 | uncomfortable |
| D | 8/4/2015 | 07 | comfortable |
| E | 15/4/2015 | 19 | comfortable |
| F | 22/4/2015 | 23 | comfortable |
| G | 29/4/2015 | 32 | uncomfortable |
| H | 5/5/2015 | 18 | comfortable |

**Table-2. *X. letipes* parasitism by triungulins**

| Sr. No. | Number of X. letipes collected | Number of X. letipes parasitized | year | Per cent parasitism |
|---|---|---|---|---|
| 1 | 19 | 07 | 2014 | 36.84 |
| 2 | 35 | 13 | 2015 | 37.14 |

*plazata* deposited eggs on the flowers of *P. pterocarpum*. The blister beetle eggs hatched into the triungulins, were immediately climbed on the body of Xylocopa.

**Figure-1. 1. *X. letipes*: Adult**

**Figure-2. *X. letipes* with triungulins on body**

*X. letipes* carried the triungulins on their body and deposited into its nesting. Wherein the triungulins consumed the grubs of Xylocopa and became matured, pupated and became adult blister beetle. Thus, triungulins were frequently brought to the nesting by Xylocopa. On the male Xylocopa triungulins were attached to the underside of the body. But after mating and courtship they were transferred on the dorsal side of females. The females carried such triungulins to the nesting wherein triungulins consumed the grubs and pupae of Xylocopa. Triungulins responded to vegetation or nesting substrate by collective aggregations and by waving their front legs and making bridge between two holding objects and also responded to external stimuli by either collectively expanding or contracting the aggregation mass. The aggregations were lasted in the field for 10.5 days (range 3-13 days). Triungulin aggregation may be seen either on twig of the host plant or Xylocopa body.

**Figure- 3. *N. plazata* triungulin dorsal view**

**Figure- 4. *N. plazata* triungulin Ventral view**

A single *Xylocopa* carried as much as 23 triungulins on its body. However, easy carrying triungulin load was ranged from 7-23 individuals for comfortable flight. The triungulin aggregation attracted the host insect *X. letipes* and Xylocopa was parasitized by triungulins with 36.84% and 37.14% during the year 2014 and 2015 respectively.

**Figure-5. *N. plazata* triungulins**

**Morphological features of triungulin:**

Triungulins of *N. plazata* (Figure.1) were brownish with 1 or 2 stemmata on each side of the head and not parallel sided but pointed and with short two bristles to posterior side. Triungulins measured 1.5 mm and 0.2 mm in length and width respectively. Abdomen was with 10 segments and posteriorly tapered with two fine short bristles on tip of abdomen. Antenna was pale brown with 3 segments. Legs were pale brown, with 3 ungulins at the tip.

## DISCUSSION

Most adults of meloid beetle eat only floral parts but some, particularly those of *Epicauta* spp. eat leaves as well. A few adults were nocturnal but most diurnal or showed no distinct diel cycle. Adults were gregarious and highly coloured and associated with flowering bodies. The larvae of meloid beetle showed hyper metamorphosis. Therefore, first instars were different from others by having triungulae on terminal end of the legs and well suited for clinging and holding the body of adult honey bee.

Hafernik & Saul – Gershenz (2000) reported a remarkable mode of host finding by the blister beetle *Maleo franciscans*, in which young larvae (triungulins) aggregated together on vegetation to mimic the appearance of a female bee, luring male bees to land on them and collect the aggregation as an unit for transmitting to female during real matings.

As like *M. franciscans* some mites parasitized their hosts by venereal transmission. However, such type of parasitic association was not recorded in other *Meloe* spp. According to Hafernik & Saul- Gershenz (2000) triungulins of *Maloe* larvae were highly adapted for grasping bees, structurally resembling lice rather than beetle larvae. The triungulins gathered on the body of bee drop off at the bee's nest and developed primarily the pollen provided by the bee. They also highlighted the interaction of bees and triungulin aggregations at the Kelso Dunes in California's Mojave Desert during April 1992 and April – May 1999.

Triungulins cooperated in forming aggregation and in holding onto vegetation. They collectively responded to outside stimuli, such as nearby movement by waving their front legs or by contracting as a unit. They also collectively formed bridge between adjacent grass blades by a unit movement. Long lasting aggregations were noted ranging from 1 day to 15 days on grass and twigs but very interestingly no aggregation of triungulins was noted on or near flowers.

According to Hafernik and Saul – Gershenz (2000) all male bees sampled during the year 1992 have carried triungulins. Most (26 of 27) carried them on their underside, and transferred to dorsal surface of female during matings. Thus, newly mated females showed large number of triungulin on the dorsal surface of body. The phenomenon was mediated in a way similar to pseudopopulation in orchids (Borg-Karlson, 1990).

Female of *Meloe* species produced upto 3000 larvae per clutch. Such a high reproductive rate was associated with high larval mortality and low individual success in host finding (Pinto & Salander, 1970). According to Hafernik & Saul-Gershenz (2000) the mimicry practiced by *Meloe franciscans* larvae together with their subsequent venereal transmission, enhanced their chance of finding a bee's nest.

Saul – Gershenz and Millar (2006) reported that larval aggregations of the blister beetle *M. franciscanus* which parasitized nest of the solitary bee *Habropoda pallida*, cooperated to exploit the sexual communication system of their hosts by producing a chemical clue that mimics the sex pheromone of female bee. Male bees were lured to larval aggregations and upon contact (pseudocopulation) the beetle larvae attached to the male bees. The larvae transferred to female bees during mating and subsequently were transported to the nest of their host. To mimic the chemical and visual signals of female bees effectively, the parasite larvae must cooperate, emphasizing the adaptive value of cooperation between them. The aggressive chemical mimicry by the beetle larvae and their subsequent transport to their host's nest by the hosts themselves provide an effective solution to the problem of harsh environment (Saul-Gershenz & Millar, 2000).

The phoretic triungulins, have evolved a complex four-step mechanism for ensuring their survival, which resulted in the host species transporting the triungulin back to their nests. Saul – Gershenz & Millar (2006) documented cooperative aggressive chemical mimicry in *M. franciscanus* larvae wherein the larvae have evolved a suite of complementary semio-chemical and behavioural characters in response to the challenge of locating their host's nest. In evolution of larval sociality, phoresy and aggressive chemical mimicry in *M. franciscans* several factor were involved (Saul - Gershenr & Millar, 2006; Sathe & Margaj, 2001; Sathe & Chougule, 2014; Sathe, 2014; Sathe *et al.* 2015). Alkenes were most important factor found in aggregative and attractive mechanisms. The alkene blend from female bees and the triungulins blend were equally attractive to males. Analysis of hexane extract of heads of bees and whole - body extract of triungulin revealed remarkable correspondence in the profile of chemical extracted. Extract of both the host and the parasitic species were dominated by $C_{23}$ and $C_{25}$ straight-chain alkanes and alkenes in, with lesser amount of homologs.

According to Saul – Gershenz & Millar (2006) triungulins attracted male bees by mimicking the sex pheromones of female bees, exhibiting cooperative aggressive chemical mimicry. In the present study triungulin aggregation attracted the males but the type of chemicals involved are yet be identified.

Parasite load carrying capacity plays an important role in survival of both parasites and hosts (Sathe & Margaj, 2001; Vinson, 1976). The weight of parasite and flying capacity has also direct relationship and may be stage, age, sex and species specific (Sathe, 2014).

*Habropoda pallida* Timber lake male bees were carrying large numbers (42) of triungulins. In the present study *X. letipes* carried 20 triungulins successfully. However, exceeding the number 25, the *Xylocopa* settled down on the ground within a very short-time. Therefore, appropriate and comfortable parasitic load is essential factor for easy and successful transportation of triungulins.

The triungulins of *Meloe* were Campodeiform, elongated, parallel sided and with two bristles like longe processes. While the triungulins of *N. plazata* were with 1 or 2 stemmata on each side of the head and was non parallel sided but pointed towards posteriorly and with two short bristles on terminal portion of abdomen.

Since *Xylocopa* is very potential pollinator of various crop plants (Sathe & Gophane, 2015); the studies related to triungulins occurrence and load carrying capacity of Xylocopa will add great relevance in solving the problems of survival of pollinators including honey bees megachile bees and carpenter bees.

## Acknowledgement

Authors are thankful to Shivaji University for providing the facilities to this work.

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*DOI: https://dx.doi.org/10.5281/zenodo.7294460*
*Received: 4 July 2015;*
*Accepted; 22 August 2015;*
*Available online : 6 September 2015*