JEB®

Journal of Environmental Biology

p-ISSN: 0254-8704 • e-ISSN: 2394-0379 • CODEN: JEBIDP

Journal website : www.jeb.co.in ★ **E-mail :** editor@jeb.co.in

Triveni Enterprises (Educational Services Set-up)

Original Research

DOI : http://doi.org/10.22438/jeb/44/3(SI)/JEB-04

Host selection behavior of tasar silkworm, *Antheraea mylitta* (Drury) for oviposition

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Received: 10.12.2022 *Revised*: 14.02.2023 *Accepted*: 01.04.2023

Abstract

Aim: Seed production is the backbone of sericulture in India. In order to cater for the increased demand for seed (egg), the already prevailing seed production techniques need to replace with new and robust techniques. Therefore, the present study was designed to understand the oviposition preference of *Antheraea mylitta* and selected suitable host plants for future improvement of seed production.

Methodology: TChoice (nylon net cage) and no-choice (earthen cup) experiments were conducted to study the oviposition preference of *A. mylitta* on *Shorea robusta, Terminalia tomentosa, T. arjuna, Ziziphus mauritiana, Careya arborea, Syzygium cumini* and *Buchanania lanzan.* Mated female moths were released freely into the nylon cage to observe their oviposition preference in the choice test. Whereas, half of both fore wings and hind wings of mated female moths were excised and individually placed in an earthen cup for oviposition. The total number of eggs laid on the host plants in both experiments was recorded and analysed.

Results: Mated females released freely within the cage have laid the majority of their eggs on the floor and net instead of their host plants. Among the host plants, the highest number of oviposition was recorded on the *S. robusta, T. tomentosa* and *T. arjuna* compared to other host plants. The highest number of eggs per moth was observed on *T. tomentosa* and followed by *S. robusta, T. arjuna* and *Z. mauritiana,* and exceeded the numbers of eggs in the control in the no-choice experiment.

Interpretation: The study revealed that both *S. robusta* and *T. tomentosa* were highly preferred host plants of *A. mylitta* for oviposition. Therefore, the volatiles or feeding supplements of *S. robusta* and *T. tomentosa* may be identified and applied to *A. mylitta* during rearing and grainage to enhance seed production.

Study the host plant preference of *A. mylitta*

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Choice and no-choice oviposition preference studies designed

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In the choice study, host plants kept within the nylon cage and mated females released for oviposition

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In the no-choice study, half of the fore and hind wings of the mated moths were excised and kept in earthen cup for oviposition

↓

S. robusta and *T. tomentosa* recorded as the most preferred host plants of *A. mylitta*

Key words: Host plants, Oviposition preference, Sericulture, Tasar silkworm, *Terminalia tomentosa*

How to cite : Reddy, B.Thirupam, M. Chandrashekharaiah, S.M. Mazumdar, K. Sathyanarayana, N.B. Chowdary, D.M. Bawaskar, C. Selvaraj and M.S. Rathore: Host selection behavior of tasar silkworm, *Antheraea mylitta* (Drury) for oviposition. *J. Environ. Biol.*, **44**, 452-457 (2023).

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Introduction

Antheraea mylitta Drury (Lepidoptera: Saturniidae) is a semi-domesticated polyphagous silk-producing insect and endemic to the tropical part of India (Jolly *et al.,* 1974). Tribes living in and around the vicinity of the forest patches in Central India practise Tasar sericulture as a subsidiary livelihood practice, along with their regular farming activities (Vishaka *et al.,* 2020). In India, natural forest occupies about 44.50 million ha in the tropical tasar sericulture practising states. Out of this, about 11.16 million ha are available for Tasar culture. Approximately, 3.5 lakh tribal families are engaged in tasar silkworm rearing, and an equal number are also engaged in the processing of silk such as reeling, spinning, weaving, dyeing and manufacturing of finished silk commodities (CTR and TI, 2010). Forty-four eco-races of *A. mylitta* were recorded in different ecological regions of India, ranging from 12-31°N latitude and 72-96°E longitude in India (Jolly *et al.,* 1974). The DABA [both Bivotine (BV) and Trivotine (TV) type] eco-race is the only ruling variety of *A. mylitta*, which is currently being utilised for commercial culture in India.

Major tasar silkworm host plants like Arjun (*Terminalia arjuna*), Asan (*Terminalia tomentos*a) and Sal (*Shorea robusta*) are extensively used for rearing silkworm. Other primary food plants are Sidha (*Lagerstroemia parviflora*), Jarul (*Lagerstroemia speciosa*). Saoni (*Lagerstroemia indica*), Ber (*Zizyphus mauritiana*) and Anjan (*Hardwickia binata*). The important secondary food plants are Haritaki (Terminalia chebulal), Bahera (*Terminalia belerica*), Jamun (*Syzygium cuminii*), Mahua (*Madhuca indica*), Kinjal (*Terminalia paniculata*) etc. (Suryanarayana *et al.,* 2005). Studies have also shown better cocoon yield with enviable economic characters when silkworms reared on *S. robusta, T. tomentosa, T. arjuna* and *Z. jujube* (Suryanarayana and Srivastava, 2005; Rath *et al.,* 2008; Deka, 2016; Jena, 2016). Currently, the major tasar sericulture activities are concentrated on *T. arjuna* and *T. tomentosa* in India. Host selection by gravid moths for their half-springs involves a sequence of behaviour and discrimination events like habitat selection, host selection and host acceptance for oviposition (Carriere and Roitberg, 1995). Host selection also depends on the right-choice of ovipositing female (Singer, 1986).

Studies have also demonstrated a strong relationship between female preference and off spring performance (Singer *et al.,* 1994; Berdegue *et al.,* 1998). Host choice and larval performance in certain insects also depend on the ecological conditions and selection pressures within the ecosystem (Thompson, 1988). Highly mobile larvae of some lepidopteran species search for a suitable host to fulfil their nutritional benefits (White and Singer, 1974; Bernays and Chapman, 1994). Ovipositing females also prefer good quality plants for oviposition which is demonstrated in oligophagous insects than in polyphagous insects (Gripenberg *et al.,* 2010). Generally insects tend to oviposit on sites that are not always optimal for larval survival (Cunningham, 2012). However, polyphagous insects have a complex host recognition system based on innate or adaptive host plant choices through behavioural plasticity and a host volatile recognition system (Galizia and Rossler 2010; Anderson and Anton, 2014; Andersson *et al.,* 2015). Host selection by ovipositing females is a fundamental step for the fitness of offspring in polyphagous insects like *A. mylitta*. The DABA BV & TV stocks currently being used for commercial rearing are derived way back to 1960s from the wild population through selection and mass multiplication. Since then, the selected stocks have been maintained and mass multiplied on *T. arjuna* and *T. tomentosa* under outdoor conditions. An understanding of the oviposition preference of *A. mylitta* stocks helps in devising suitable measures to increase the production of seeds (egg), which is presently facing various lacunas for self-sufficiency in India.

Materials and Methods

Choice oviposition preference: The Daba bivoltine (BV) stock maintained at Basic Seed Multiplication and Training Centre (BSM&TC), Kharsawan and Kathikund were used for the oviposition preference studies. The stock was replenished bi-annually with fresh stocks of high vigour and disease free and further maintained on the mixed plantation of *T. arjuna* and *T. tomentosa* at BSM&TC, Kharsawan as per the package of practices (Sahay, 2018). The cocoons harvested were brought to indoor conditions and maintained as per the standard procedure (Chandrashekharaiah *et al.,* 2018) for disease-free laying (DFL) production (seed). A cage was constructed using a nylon net (1.7 m × 1.7 m × 1.7 m) to study the oviposition choice of free-flying female moths on different host plants. Fresh branches of *Shorea robusta, T. tomentosa, T. arjuna, Ziziphus mauritiana, Careya arborea, Syzygium cumini* and *Buchanania lanzan* host plants were collected and placed randomly within the nylon net at an equal distance. Ten mated female moths were released into the nylon cage to observe their oviposition preference. The experiment was repeated three times. The total number of eggs laid on the host plants, floor and nylon net during each experiment were also recorded.

No-choice oviposition preference: Mated female moths, immediately after decoupling were induced for urination and half of both forewings and hindwings were cut off by passing through mid-costa to mid-hind-margin, moths were then individually placed in an earthen cup to monitor oviposition (Mohanraj *et al.,* 2021). The earthen cups were each provided with a fresh leaf of the respective host plants. Earthen cups without leaves served as a control and ten such replications were maintained for each treatment. After 24 hr, the leaves were removed from the earthen cups and replaced with a fresh leaf of each of the respective plants. This process was repeated for three days and each day the number of eggs laid on each leaf was recorded.

Statistical analyses: Data of egg counts were pooled for each treatment and data were Log (x+1) transformed. The oviposition preference of *A. mylitta* on different host plants (treatments) was analysed using Generalized Linear Models, followed by Tukey's test for multiple comparisons (SPSS 13.0).

Results and Discussion

Mated females released freely within the cage had laid majority of their eggs on the floor and net instead of their host plants (F=30.24; df=8,18; P<0.01). Among the host plants, the highest number of oviposition was recorded on *T. tomentosa, S. robusta and T. arjuna* compared to other host plants (Fig. 1). The number of eggs (Mean±SD) laid on the leaves of different host plants provided within the earthen cups varied significantly compared to the control (F=63.44; df=7,72; P<0.01). The highest number of eggs per moth was observed on the leaves of *T. tomentosa*, followed by *Z. mauritiana, S. robusta* and *T. arjuna* and exceeded the numbers of eggs in the control. The moths laid fewer eggs on the leaves of *S. cumini, B. lanzan* and *C. arborea* compared to the control (Fig. 2). Further observation revealed remarkable differences in the mean number of eggs per moth in the earthen cup (193.60±38.46) and cage (104.70±14.07) experiments. *A. mylitta* is a polyphagous insect and thrives on many host plants under natural conditions. However, the desirable commercial characters in terms of silk recovery were observed only when the silkworms were reared on the *T. tomentosa* and *T. arjuna* (Shrivastav *et al.,* 1994).

No-choice oviposition preference study revealed that *T. tomentosa* most preferred host for *A. mylitta* for oviposition. The leaves of *T. tomentosa* are physiological (leaf moisture content and net photosynthesis rate) and biochemical (Chlorophyll, protein, carbohydrate and crude fibre contents) superior over the other host plants in terms of nutritional status for growth and reproduction (Deka, 2016). Studies have also confirmed higher level of protein, ascorbic acid and reduced glutathione in *T. tomentosa* and *S. robusta*, and lowest level in *T. arjuna*. Whereas, lipid peroxidation was highest in *T. tomentosa* compared to *S. robusta* (Majhi *et al.,* 2018). Similarly, *T. tomentosa* also posessed highest percentage of moisture, chlorophyll and proteins than *T. arjuna*, whereas relatively higher percentage of sugar and phenol in the leaves of *T. arjuna* (Mukherjee *et al.,* 2016; Deshmukh *et al.,* 2019). Total flavonoid and tannin contents were much higher in the leaves of *T. tomentosa* and *S. robusta*, respectively (Sahu *et al.,* 2018). Rath *et al.* (2008) reported that the performance of *A. mylitta* on *T. tomentosa* superior than *T. arjuna* and *Z. jujuba*, in terms of fecundity and commercial characters. He also opined that *T. tomentosa* provides balanced amount of nutrients for *A. mylitta* to enhance their reproductive capacity as well as commercial characters.

The above nutritional superiority in *T. tomentosa* might have favoured *A. mylitta* host selection for oviposition. Under forest plantation, the final instar tasar silkworms feed all the host plants voraciously, which come in their way during rearing. However, the present prevailing situation in the Jharkhand and other states is that *A. mylitta* seed are being reared on the plantations dominated by *T. arjuna*. Within plantation, *T. tomentosa* and other host plants of *A. mylitta* are also raised at lower density. Therefore, identification of most suitable host plants for *A. mylitta* is essential to popularise tasar sericulture among the farming community to improve their income. *A. mylitta* prefers *T. tomentosa* due to previous learning, larval performance

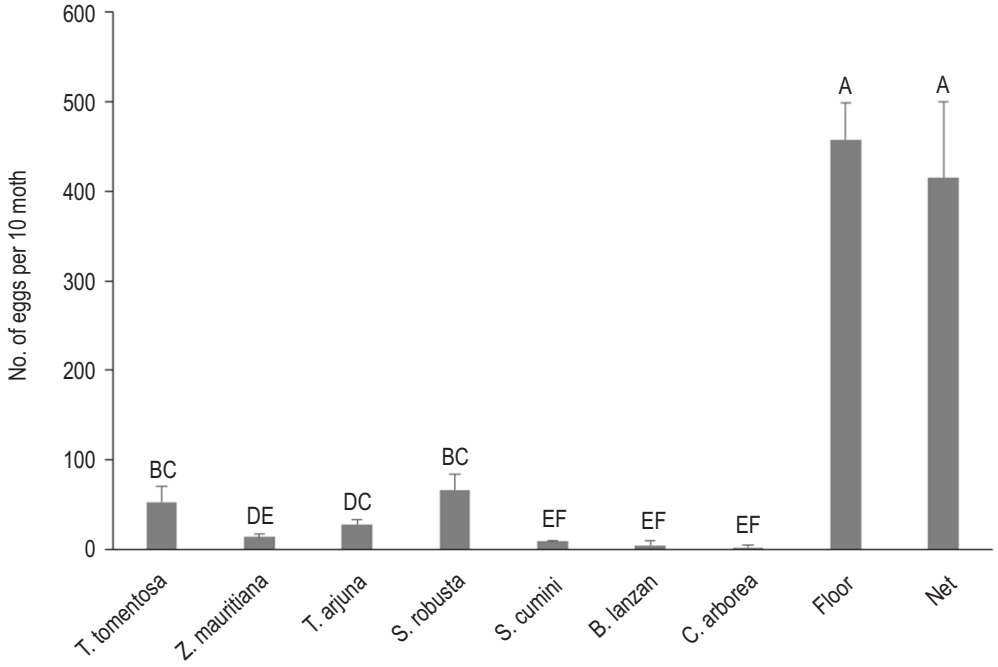

Fig. 1: Number of eggs laid on different host plants including net and floor surface when mated silkworm female moths (*A. mylitta*) were released freely within the cage. Value are mean ±S.D.

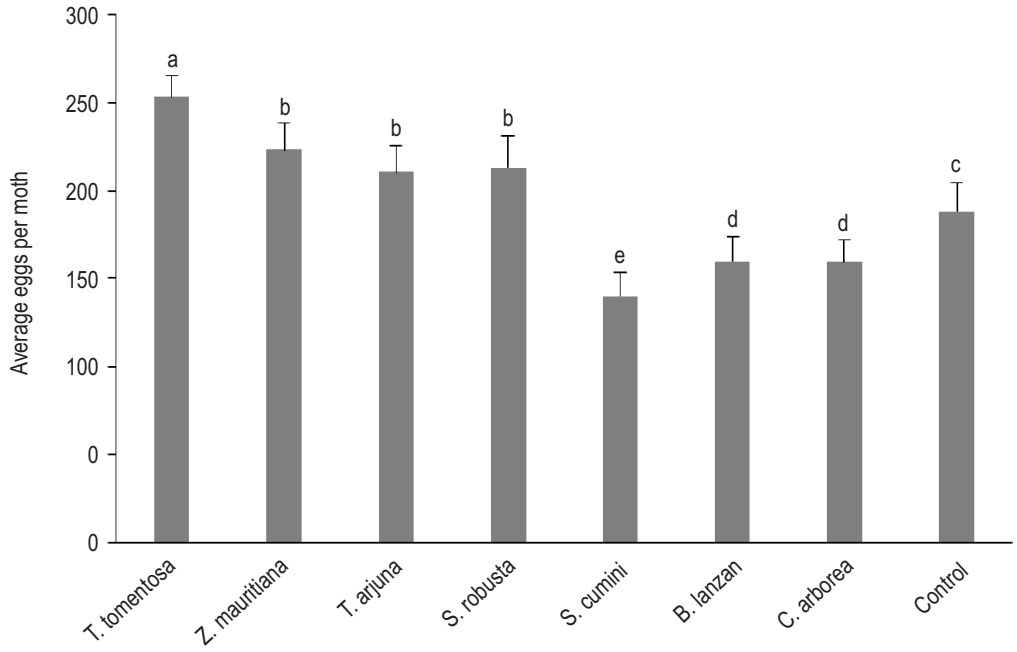

Fig. 2: Average eggs per moth (*A. mylitta*) recorded on the leaves of different host plants provided as a substrate within the oviposition device, *i.e.,* earthen cup. Value are mean ±S.D.

and higher fitness-related factors. Shifting of larvae reared on *T. arjuna* and *T. tomentosa* to *S. robusta* during rearing may cause negative effect on their growth and development. A confirmative study on host shift experiments by Sahu *et al.,* (2018) and Barbehenn (2002) indicated that silkworms reared on *S. robustahave* shown inferior performance due to oxidative stress in the epithelial tissues of midgutcomparison to *T. arjuna* and *T. tomentosa*. Therefore, mortality of silkworm is relatively high when they are reared on *S. robusta* compared to *T. tomentosa*. Redox active metals (iron and copper) and selenium were also high in *S. robusta* compared *T. tomentosa* and *T. arjuna* and deteriorate the nutritional quality of food plants by oxidization (Sahu *et al*., 2018).

Similarly, phenol and tannin contents were also relatively more in *S. robusta* and *T. arjuna* compared to *T. tomentosa*. As they are the most common defensive allelochemicals against herbivores (Bravo, 1998; Barbehenn and Peter Constabel, 2011), might have affected silkworm growth and reproduction. Flavonoids are more dominated allelochemicals in *T. tomentosa* which acts as plant defensive compound against many herbivores and pathogens. Alternatively, these compounds also reported as oviposition and feeding stimulants in *Papilio polyxenes, Luehdorfia japonica* and *Danaus plexippus* (Ohsugi *et al.,* 1985; Feeny *et al.,* 1988; Nishida, 1994; Tabashnik, 1987; War *et al.,* 2012). Biochemical profiles of *T. tomentosa* favoured the mother choice for oviposition and also larval performance.

Field experiments have also shown that the performance of *A. mylitta* in terms of economic characteristics and fecundity was better on *T. tomentosa,* followed by *T. arjuna*, *S. robusta* and *Z. jujube* (Suryanarayana and Srivastava, 2005; Rath *et al.,* 2008; Deka, 2016; Jena, 2016). The preference-performance hypothesis (PPH) has also suggested a positive relationship between female-oviposition preference and offspring performance (Gripenberg *et al.,* 2010). Lepidopterans tend to locate host species with previous experience, *i.e.* learning through oviposition stimulus (Traynier, 1984; Papaj, 1986; Cunningham *et al.,* 1999). Therefore, the gravid moths exhibited a preference among potential hosts including environmental effects on behaviour (Cunningham *et al.,* 2012). Certain lepidopterans like Spodoptera exigua (Hübner) (Lepidoptera, Noctuidae), *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) have also exhibited an innate and learning experience during host selection (Berdegue *et al.,* 1998; Jallow and Zalucki, 1995). Innate behaviour helps to predict the expected environment and has a strong influence on floral choice in newly emerged adults. Learning shapes, the insect's response to odours in its local environment, and increasing the response to odour cues leads to successful foraging (Cunningham *et al.,* 1998a, 1998b, 2008).

Presently, tasar silkworm are being reared predominantly on *T. arjuna* in India. The biochemical and behavioural effect of *T. arjuna* on *A. mylitta* is still known during largescale rearing programme. Therefore, promotion of *T. tomentosa* planation for rearing *A. mylitta* is most essential to obtain better performance. Female moths released freely within the cage, without imposing any damage to moths had selected the floor and net surfaces for

oviposition at a maximum rate. A very small number of eggs were also laid on the leaves of *S. robusta*, *T. tomentosa* and *T. arjuna*. Female moths are habitually heavy-bodied and weigh two times as much as do conspecific males at the time of eclosion, with a capacity to produce more than 200 eggs and weighing 2 mg each. The initial egg load usually constitutes half of her initial body weight, *i.e.,* mated female lays nearly 40-50 per cent of total capacity eggs within 24 hr after mating. During the cage experiment, a maximum number of eggs were laid on the floor and the net might be due to the heavy body weight of female moths making them unable to fly initially for the selection of host plant until the initial heavy load of eggs dislodge wherever they are located. Studies have also suggested that the saturniid moths attain flight status when thoracic muscles achieve a temperature of 35°C due to muscular shivering (Blest, 1960). The actual cause for such disparity in the oviposition of female moths as observed in the cage experiment has to be studied for valid reasons. The average eggs per moth were high in the earthen cup experiment compared to the cage experiment. The action of the wing cut employed on the moths in the earthen cup experiment might have induced the release of stress-related neuro-hormone-inducing oviposition in mother moths (Morohoshi, 2001). The host plant selection behaviour for oviposition in *A. mylitta* may be related to the nutrient status of host plants.

Further, studies are required to identify the host plant specific volatile compounds as well as nutritional supplements to enhance the growth and reproductive capacity of *A. mylitta* (oviposition stimulation). Since, there is a huge demand for more than 50,000,000 commercial DFLs annually in India and production of such a huge number is difficult to achieve with the existing technology. Therefore, host plant based ovipositional stimulants and nutritional supplements may be developed for improvement of Tasar sericulture in India.

Acknowledgments

We are thankful to Dr. R. B. Sinha former Director of BTSSO, Bilaspur for his continuous support during the experiment and we are grateful to Director BTSSO, Central Silk Board, Ministry of Textiles, GoI for his continuous support and we are also thankful to The Director, CTR&TI, Ranchi, Central Silk Board, Ministry of Textiles, GoI for permitting and helping to carry out the study.

Authors' contribution: B.T. Reddy: Data collections interpretation, study MS writing; **M. Chandrashekharaiah:** Guidance and MS corrections; **S.M. Mazumdar:** Guidance & MS review; **K. Sathyanarayana:** Proof reading and MS editing; **N.B. Chowdary:** Revised MS; **D.M. Bawaskar:** Revised MS; **C. Selvaraj:** Revised MS; **M.S. Rathore:** Revised MS.

Funding: No funding has been received for this study.

Research content: The research content of manuscript is original and has not been published elsewhere.

Ethical approval: No animals were harmed during the study. It was an observational approach.

Conflict of interest: The authors declare that there is no conflict of interest.

Data availability: Data not obtained from other sources.

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology.*

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