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**RESEARCH ARTICLE**

# LIPID PROFILE OF PLANTS INFLUENCE HOST SELECTION BY GUSTATORY SENSILLA IN THE LARVAE OF *ANTHERAEA ASSAMA* WESTWOOD (LEPIDOPTERA: SATURNIIDAE)

**Dipsikha Bora, Bhabesh Deka, Urbbi Devi and Amrita Mech**

Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India

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**ABSTRACT**

*A. assama* Westwood, the producer of golden silk, is a lepidopteran insect endemic to northeastern India. They are polyphagous, but thrive primarily on two host plants, *Persea bombycina* Kost. and *Litsea monopetala Roxb*.. Food choice test of larvae of *A. assama* retaining specific peripheral gustatory sensilla, carried out using lipid fractions of host plants, *Persea bombycina* and *Litsea monopetala* and non-host plants namely *Litsea grandifolia* Teschner (Laurales: Lauraceae) and *Ziziphus jujuba* Miller (Rosales: Rhamnaceae) revealed that non-polar and medially polar lipids of non-host plants play key role in food rejection/selection and the galeal and labrum epipharyngeal sensilla are involved in the process.



## INTRODUCTION

Food selection of many phytophagous lepidopteran larvae is largely influenced by phytochemicals detected by peripheral sense organs around the buccal cavity [de Boer, 2006]. Presumably, each plant species has a unique phytochemical profile that is encoded by chemoreceptor cells into a 'sensory profile', containing information about the quality and quantity of the plant chemicals [Dethier, 1973; Schoonhoven *et al*., 1998]. On the basis of this sensory information, insect interacts with the plant, be it for nutrition or reproduction. For example, *Pierid* butterflies use glucosinolates present in the plant cuticle to recognize cruciferous host plants for ovipositions [van Loon *et al*., 1992, Chew and Renwick, 1995].

*A. assama* Westwood, the producer of golden silk, is a lepidopteran insect endemic to northeastern India . They are polyphagous, but thrive primarily on two host plants, *Persea bombycina* Kost. and *Litsea monopetala* Roxb. Restrictive feeding on a few plants might be the reason for its confinement to northeastern India only. No artificial diet has been possible to be developed for indoor rearing of the insect although several attempts have been in progress [Barman and Rajan,2011]. Very few studies have been carried out so far regarding the feeding behavior of *A. assama* with respect to the influence of host plant chemical content. While Hazarika *et al*. [1994] categorized preference to *Machilus* on the basis of dodecanal and caryophyllene, Neog *et al*. [2011] showed a mixture of caryophyllene, decyl aldehyde, and dodecylaldehyde to be attractive for biting behavior of *A. assama* larvae.

However, no work has been carried out to probe into the chemosensory basis of the restricted diet-breadth in *A. assama*. Acceptance or rejection of the food by a herbivorous insect is based on the balance between sensory inputs that invoke behavioral responses [Dethier, 1982].

According to Schoonhoven [1987], the chemoreceptor cells involved in food selection by *M. sexta* larvae are located within five different peripheral sensory organs. Each of these chemosensory organs by itself mediates a particular feeding response to a certain plant species [De Boer and Hanson, 1987; De Boer, 1992]. Hazarika and Bordoloi [1998] worked on antennal and mouthparts sensilla of the Muga Silkworm, *A. assama* and identified six types of sensilla on antennae and the different mouthparts of the larvae. Dey *et al*. [2011] also worked in details about distribution of different sensilla on the body of surface of *A. assama.*

Through the present study on role of chemosensory organ in food selection by this extremely host sensitive insect, the fundamental question of chemosensory basis of its restricted diet breadth is addressed.

**Corresponding author:* **Dipsikha Bora**
Department of Life Sciences, Dibrugarh University, Dibrugarh-786004, Assam, India

## MATERIALS AND METHODS

### *Insects*

### *Antheraea assama Westwood*

The larvae of *A. assama* that hatched from disease-free eggs obtained from the Government Sericulture Farms of Assam were cultured on leaves of its primary host plant, *Persea bombycina*, grown in the botanical garden within the campus of the Department of Life Sciences, Dibrugarh University, Assam, India.

### *Plants*

For carrying out different experiments, two host plants of *A. assama* larvae, *Persea bombycina* King ex Hook.f. (Laurales: Lauraceae) and *Litsea monopetala* (Roxb.) Pers. (Laurales: Lauraceae) and two nonhosts, *Litsea grandifolia* Teschner (Laurales: Lauraceae) and *Ziziphus jujuba* Miller (Rosales: Rhamnaceae), were selected. All the plants were about four years old and grown in the botanical garden of Department of Life Sciences, Dibrugarh University, Assam.

### *Ablation of the sensory organs*

Early 5$^{th}$ instar larvae of *A. assama* were immobilized on ice for 15-30 minutes and the peripheral sense organs, namely the maxillary palp, maxillary galea, and labrum epipharynx were removed selectively by microsurgery, keeping only the organ considered for study. Extirpations were performed on the two-day old 5$^{th}$ instar larvae under a dissecting binocular microscope (Olympus, www.olympus.co.uk).

After recovery, the larvae were allowed to feed normally on leaves of their primary host plant. Insects having unsuccessful operations were not considered further. Larvae retaining only maxillary palpi were designated as MAX, larvae retaining only galeal sensilla styloconica as GAL, larvae retaining only labrum-epipharynx as LAB, larvae retaining all chemosensory organs (both olfactory and gustatory) as ALL, larvae retaining none of the chemosensory organs (both olfactory and gustatory) as NONE and larvae retaining all organs (both olfactory and gustatory) unilaterally as UNI. As all the chemosensory organs are bilaterally represented, the UNI group was used as control larvae and all comparisons were made with the response of UNI.

### *Bioassay of Food choice test*

Bioassay was done through a food choice test carried out in two ways following the method of de Boer and Hanson (1984). The food choice test was carried out between an extract of host or non-host plant and water in order to evaluate the degree of preference for different food plants by comparing the response to the plant extracts with that of solvent. In order to assay larval food preferences, four leaf discs (14 mm in diameter) of each plant species (A or B) arranged alternately were placed on the floor around the circumference of a transparent plastic container (10 cm diameter). Leaf disks were prepared by soaking a whatman fibre disc (GF/A, 14 mm in diameter) in leaf extracts or only with the solvent. The leaf discs were fitted to the distal end of bamboo sticks, whose proximal ends were fixed on hard cardboard kept at 1 cm above the bottom of the container. The bamboo sticks were used to hold the leaf disc like a stem of a plant and to provide crawling space for the larvae. All the larvae were not fed for 2–4 hours before being subjected to the food choice test, and then were placed in the center of the floor of the container. When the larva had eaten about 50% of the area of one of the two plant species (A or B), the test was stopped. The amount of time it took, called T50, varied from 2 minutes to 1 hour from the start of the test. Tests were repeated with a minimum of 10 larvae. The 50% food consumption in T50 time was expressed in terms of percent consumption per minute using the unitary method of mathematical calculation. The percentage of choosing larvae was based on the number of larvae in one group opting for a particular food.

### *Fractionations of crude extracts of host and non-host Plants*

The leaves (2 kg) were shade dried and crushed to powder. Solvent extracts were prepared in petroleum ether, diethyl ether and ethanol by soaking at room temperature for 24 hrs.

The single choice food choice test was carried out using diethyl ether, petroleum ether and ethanol extracts of both plants. Based on the response in terms of the mean percent consumption per minute, the diethyl ether extract of both host and non-host plants were further fractionated into different groups of lipids viz. polar lipids, non polar lipids and moderately polar lipids following the method of Harborne [1998]. The constituents of the different types of lipids were identified by thin layer chromatography. The solvent systems used for polar lipids were acetone: water (17:3), for moderately polar lipid was acetic acid: chloroform (3:2 & 3:5) and for non polar lipid were hexane: diethyl ether: acetic acid (70:30:1)

### Statistical analysis

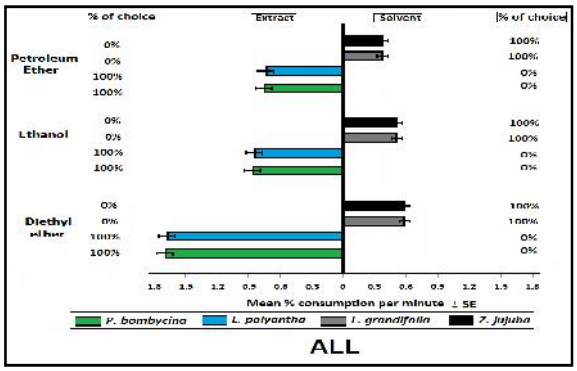
All analysis was done by SPSS 17. Differences in food choice between groups of larvae having different complements of chemosensory organs remaining were evaluated statistically by comparing the response of individual group of larvae in one ablation group with those of UNI group. Preference based on mean percent consumption per minute was analyzed by Mann-Whitney test at 0.025 and 0.05 level of significance. Wilcoxon sign ranked test was done to assign degree of competence to sensory organ in host chemical preference.

## RESULTS

### *Petroleum Ether, Ethanol and Diethyl Ether Extracts of both host and non-host plants vs solvents*

When the larvae with different complements of peripheral sense organs were given food choice between the diethyl ether (DEE), petroleum ether(PET.E) and ethanol (ETH) extracts of *P. bombycina* or *L. monopetala* or *L. grandifolia* or *Z. jujuba* and the respective solvent, variations in preference to different solvent extracts were observed as given below.

***P. bombycina* vs. solvent*:*** Larvae were given a choice between a diethyl ether (DEE), petroleum ether (PET.E) and ethanol (ETH) extracts of *P. bombycina* and solvent. 100% of ALL, UNI, LAB and GAL larvae opted for the DEE, PET.E and ETH extracts of *P. bombycina* [Fig1]. 40% of NONE opted for DEE, PET.E and 60% ETH extracts of *P. bombycina*. The mean percent consumption per minute in ALL, UNI, LAB and GAL was high in case of DEE extract (DEE=1.72±0.0011, 1.47±0.0013, 1.28±0.0012, 1.28±0.0015, 1.28±0.0011) and the variation in the mean percent consumption with the other solvent extract was statistically significant ($p<0.05$)[Fig1]. The variation in the mean percent consumption per minute in case of NONE in comparison to UNI with respect to DEE vs solvent (DEE=1.29±0.0018, solvent=1.27±0.0017; Mann-Whitney ANOVA U=44.255, Z=-1.9235, $p>0.05$), PET.E. vs solvent (PET.E=0.62±0.0013, solvent=0.61±0.0016; Mann-Whitney ANOVA U=46.219, Z=-1.568, $p>0.05$) and ETH vs solvent (ETH=0.72±0.0016, solvent=0.71±0.0016; Mann-Whitney ANOVA U=47.541, Z=-1.874, $p>0.05$) was not significant[Fig 1F].

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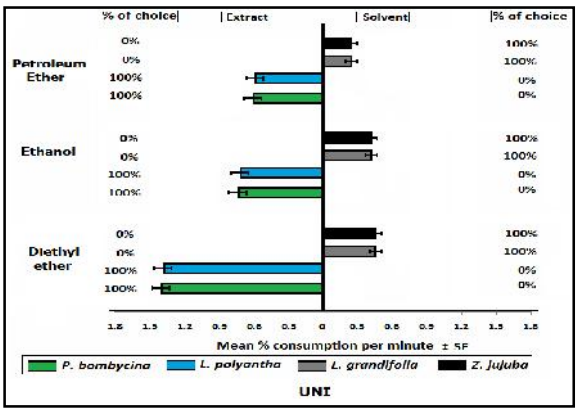


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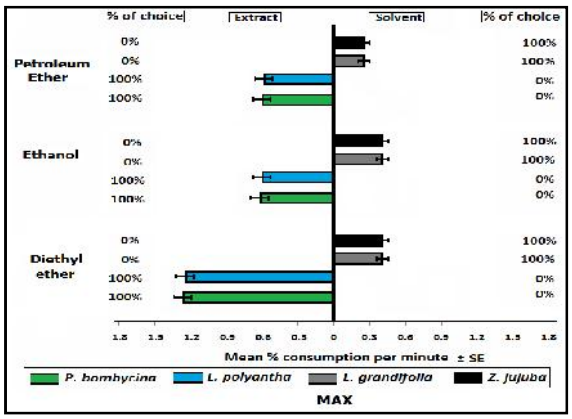


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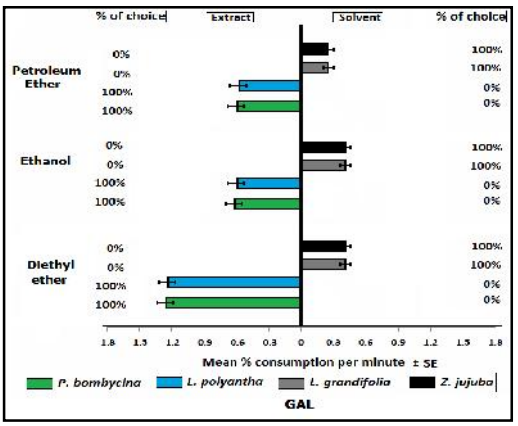


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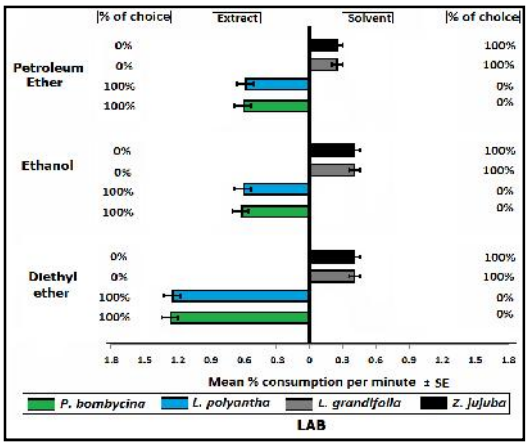


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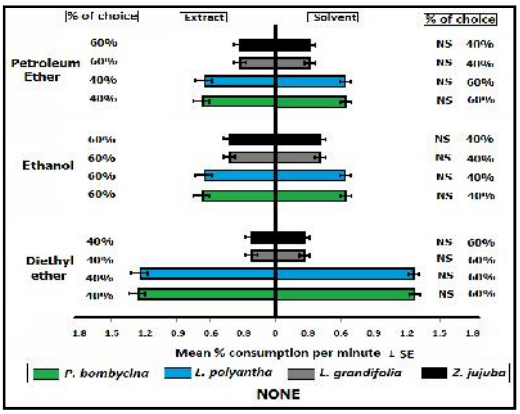


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For Mean % consumption per minute: Mann-Whitney ANOVA test: $p < 0.05$ (two tailed); **; $p < 0.025$ (one tailed); NS: Not significant

**Fig 1**Mean % consumption per minute and % of choosing larvae in food choice test (for PET. E., ETH & DEE Extracts of both host and non-host plants)

***L. polyantha* vs. solvent*:*** When the larvae were given a choice between DEE, PET.E and ETH extracts of *L. polyantha* and the solvent, 100% of ALL, UNI, LAB and GAL opted for the DEE, PET.E and ETH extracts of *L. polyantha* [Fig1]. 40% of NONE opted for DEE, PET.E and 60% ETH extracts of *L. polyantha*. The mean percent consumption per minute in ALL, UNI, LAB and GAL was high in case of DEE extract (1.71±0.0011, 1.46±0.0015, 1.28±0.0013, 1.27±0.0011, 1.27±0.0015) and the variation in the mean percent consumption with the other solvent extract was statistically significant ($p<0.05$).

**Table 1** Variations in mean percent consumption of polar lipid of host and non-host plants. PB: *Persea bombycina*, LM: *Litsea monopetala*, LG: *Litsea grandifolia* , ZJ : *Ziziphus jujuba*, Df : Degrees of freedom

| Larval Group | Mean percent consumption for polar lipids of hosts and non-Host-Nonhost plants | | | | One way ANOVA |
|---|---|---|---|---|---|
| | PB | LM | LG | ZJ | |
| ALL | 1.2±0.0013 | 1.49±0.0011 | 0.7±0.00112 | 0.7±0.00121 | Df: 3, F: 483.21, P: 0.0013 |
| UNI | 1.18±0.00112 | 1.38±0.0013 | 0.58±0.00115 | 0.59±0.0015 | Df: 3, F: 471.12, P: 0.001 |
| MAX | 1.17±0.00112 | 1.37±0.0013 | 0.55±0.00106 | 0.57±0.0013 | Df: 3, F: 401.19, P: 0.0015 |
| GAL | 1.17±0.00112 | 1.36±0.0011 | 0.49±0.00112 | 0.54±0.0016 | Df: 3, F: 419.12, P: 0.0021 |
| LAB | 1.17±0.0013 | 1.37±0.0016 | 0.52±0.00115 | 0.57±0.00121 | Df: 3, F: 438.11, P: 0.0019 |

**Table 2** Ranking of peripheral sensilla's involvement in feeding of non-polar and moderately polar fractions of host plants through Wilcoxon sign rank test

| Mean percent consumption per minute for non-polar lipid | | | Mean percent consumption per minute for moderately polar lipid | | |
|---|---|---|---|---|---|
| | Mean rank | | | Mean rank | |
| Max | 2.00 | Chi-square=8.000, df=2, p:0.018 | Max | 6.50 | Chi-square=8.000, df=2, p:0.018 |
| Gal | 5.00 | | Gal | 2.00 | |
| Lab | 8.00 | | Lab | 6.50 | |

The variation in the mean percent consumption per minute between the NONE and UNI with respect to DEE vs solvent (DEE=1.28±0.0015, solvent=1.27±0.0013; Mann-Whitney ANOVA U=46.154, Z=-1.974, p>0.05), PET.E. vs solvent (PET.E=0.58±0.0013, solvent=0.59±0.0011; Mann-Whitney ANOVA U=61.141, Z=-1.781, p>0.05) and ETH vs solvent (ETH=0.68±0.0011, solvent=0.68±0.0013; Mann-Whitney ANOVA U=54.211, Z=-1.973, p>0.05) was not significant [Fig 1F].

***L.grandifolia* vs. solvent*:*** Larvae were given a choice between a diethyl ether (DEE), petroleum ether (PET.E) and ethanol (ETH) extracts of *L. grandifolia* and solvent. 100% of ALL, UNI, LAB and GAL rejected the leaf extracts and opted for the solvent only [Fig 1]. 40% of NONE opted for DEE and 60% PET.E and ETH extracts of *L.grandifolia.*

The variation in the mean percent consumption per minute in case of NONE in comparison to UNI with respect to DEE vs solvent (DEE=0.28±0.00115, solvent=0.3±0.00112; Mann-Whitney ANOVA U=63.142, Z=-1.786, p>0.05), PET.E. vs solvent (PET.E=0.29±0.00106, solvent=0.3±0.00112; Mann-Whitney ANOVA U=36.636, Z=-1.254, p>0.05) and ETH vs solvent (ETH=0.41±0.00121, solvent=0.42±0.00113; Mann-Whitney ANOVA U=63.214, Z=-1.563, p>0.05) was not significant [Fig 1F].

***Z. jujuba* vs. solvent*:*** Similarly when the larvae were given a choice between DEE, PET.E and ETH extracts of *Z. jujuba* and solvent 100% of ALL, UNI, LAB and GAL opted for the solvent only[Fig1]. 40% of NONE opted for DEE and 60% PET.E and ETH extracts of *Z. jujuba.*

The variation in the mean percent consumption per minute between the NONE and the UNI with respect to DEE vs solvent (DEE=0.28±0.0016, solvent=0.3±0.00121; Mann-Whitney ANOVA U=56.321, Z=--1.678, p>0.05), PET.E. vs solvent (PET.E=0.3±0.0016, solvent=0.28±0.00121; Mann-Whitney ANOVA U=49.147, Z=-1.964, p>0.05) and ETH vs solvent (ETH=0.42±0.0016, solvent=0.41±0.0016; Mann-Whitney ANOVA U=65.214, Z=-1.943, p>0.05) was not significant [Fig 1F].

**Food choice test using lipid fractions**

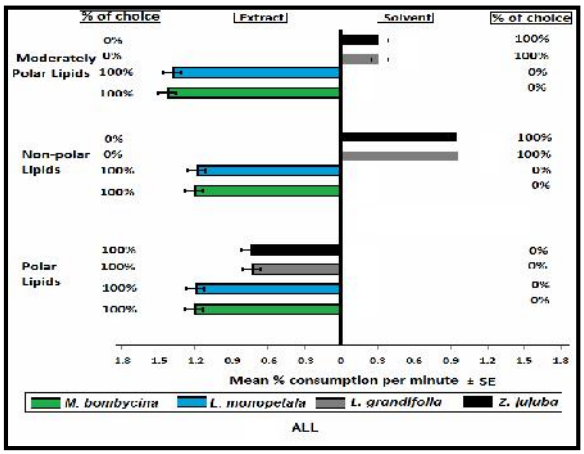
***Response to Polar lipids fractions***

Polar lipid contains phospholipid and glycolipid such as inositol and glycerol. When the larvae were given a choice between the polar lipids fraction of both host and non-host plants and the solvent, it was observed that 100% ALL, UNI, MAX, GAL and LAB opted for the polar lipids fractions of both host and non-host plants [Fig 2A-E]. Their mean percent consumption per minute was for *P. bombycina* 1.20±0.0013, 1.18±0.00112, 1.17±0.00112, 1.17±0.00112 and 1.17±0.0013 respectively, for *L. polyantha* 1.49±0.0011, 1.38±0.0013, 1.37±0.0013, 1.36±0.0011 and 1.37±0.0016 respectively, for *L. grandifolia* vs solvent 0.7±0.00112, 0.58±0.00115, 0.55±0.00106, 0.49±0.00112 and 0.52±0.00115 respectively and for *Z. jujuba* vs solvent 0.7±0.00121, 0.59±0.0015, 0.57±0.0013, 0.54±0.0016 and 0.57±0.00121 respectively. The variations in mean percent consumption of polar fraction of host and non-host was statistically significant (P<0.025) [ Table 1].

60% NONE opted for polar lipid fraction of both host and non-host plants and 40% opted for the solvent [Fig 2F]. Their mean percent consumption per minute were in case of *P. bombycina* vs solvent (for *P. bombycina* 1.17±0.0013 and for solvent 1.15±0.00112), in case of *L. polyantha* vs solvent (for *L. polyantha* 1.37±0.0011 and for solvent 1.33±0.0013), in case of *L. grandifolia* vs solvent (for *L. grandifolia* 0.51±0.00112 and for solvent 0.49±0.00121) and in case of *Z. jujuba* vs solvent (for *Z. jujuba* 0.58±0.00121 and for solvent 0.57±0.0012) respectively. The variations in mean percent consumption were not significant (p>0.05).

***Response to Non polar lipid fractions***

The non polar lipid contains fatty acids like linoleic, linolenic acid. When larvae were given a choice between the non- polar lipids fraction of host /non-host plants and the solvent, 100% ALL, UNI, MAX, GAL and LAB opted for the non-polar lipid fractions of host plants only and they rejected those of the non-host plants [Fig 3 A-E].

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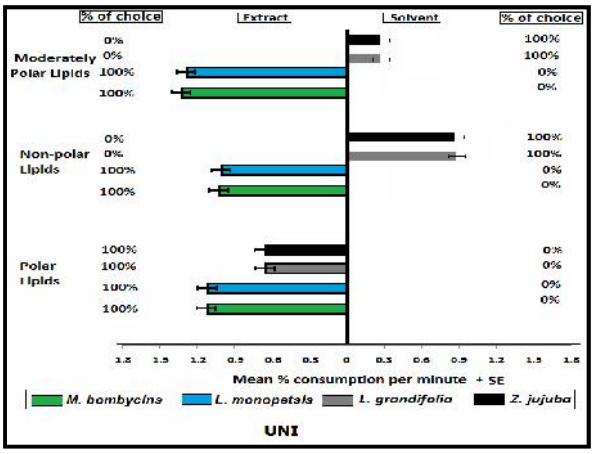


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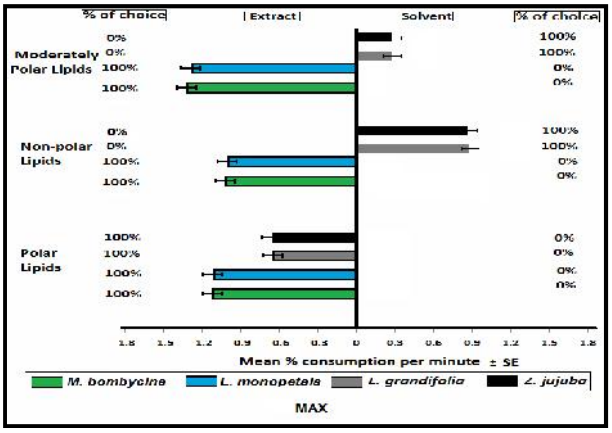


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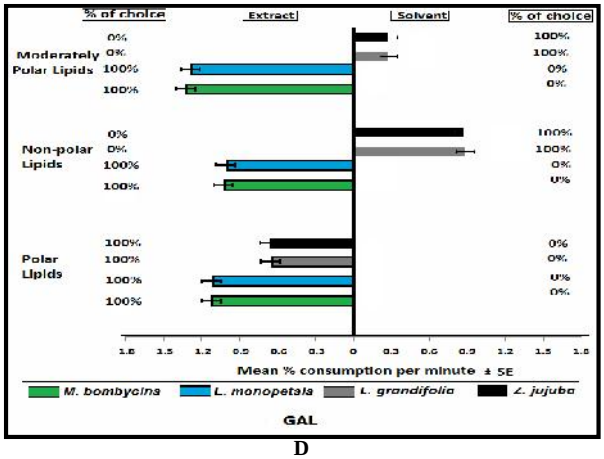


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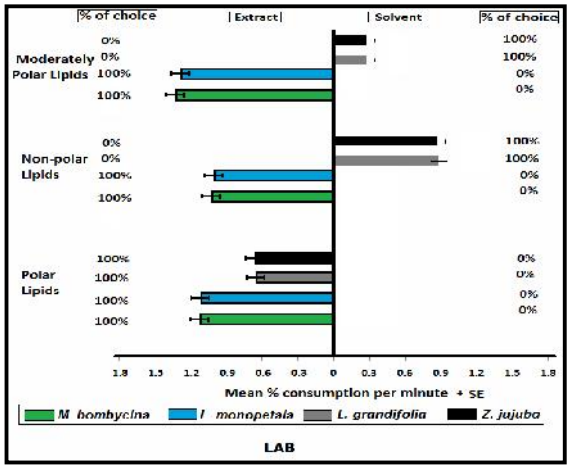


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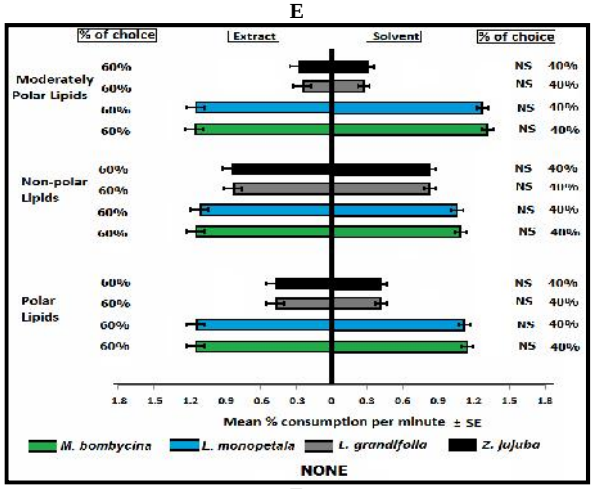


**F**

For Mean % consumption per minute: Mann-Whitney ANOVA test: *: p < 0.05 ; **: p < 0.025 ; ns: no significance

**Fig 2** Mean % consumption per minute and % of choosing larvae in food choice test (for polar, non-polar and moderately polar lipid extracts of both host and non-host plants.

The mean percent consumption for ALL, UNI, MAX, GAL and LAB in case of *P. bombycina* non-polar lipid vs solvent was 1.2±0.00112, 1.18±0.00112, 1.15±0.00112, 1.16±0.00112 and 1.17±0.0016 respectively and for *L. polyantha* non-polar lipid vs solvent was 1.38±0.0015, 1.16±0.0011, 1.15±0.0015, 1.13±0.0015 and 1.16±0.0011 respectively.

60% NONE opted for Non Polar lipids fraction of both host and non-host plants and 40% opted for the solvent [Fig 2F]. The mean percent consumption were in case of *P. bombycina* vs solvent (for *P. bombycina* 1.17±0.00112 and for solvent 1.15±0.0016), in case of *L. polyantha* vs solvent (for *L. polyantha* 1.15±0.0011 and for solvent 1.13±0.0015), in case of *L. grandifolia* vs solvent (for *L. grandifolia* 0.88±0.00121 and for solvent 0.86±0.00113) and in case of *Z. jujuba* vs solvent (for *Z. jujuba* 0.92±0.0016 and for solvent 0.89±0.0016) respectively The variations in mean percent consumption were not significant (p>0.05).

The Wilcoxon sign rank test for mean food consumption assigned higher rank to galea and labrum epipharynx in consumption of non-polar lipid fraction [ Table-2].

***Response to moderately polar lipids fractions***

The moderately polar lipid fraction of host plants contain β-sitosterol, phenols like gallic acid, chlorogenic acid , salicylic acid , quercitin and terpenoids like caryophyllene , eugenol. When larvae were given a choice between the moderately polar lipids fraction of host plants and the solvent, 100% ALL, UNI, MAX, GAL and LAB opted for the moderately polar lipids fractions of host plants only. They rejected the moderately polar lipids fractions of the non-host plants and 100% ALL, UNI, MAX, GAL and LAB opted for solvent only [Fig 2 A-E]. The mean percent consumption for ALL, UNI, MAX, GAL and LAB in case of *P. bombycina* moderately polar lipid vs solvent was 1.49±0.0016, 1.38±0.00112, 1.38±0.0011, 1.37±0.0016 and 1.38±0.0013 respectively, for *L. polyantha* vs solvent 1.2±0.0013, 1.18±0.0011, 1.17±0.0015, 1.15±0.0011 and 1.17±0.0013 respectively. 60% NONE opted for moderately polar lipids fraction of both host and non-host plants and 40% opted for the solvent [Fig 3F]. The mean percent consumption were in case of *P. bombycina* vs solvent (for *P. bombycina* 1.3±0.00112 and for solvent 1.28±0.0013), in case of *L. polyantha* vs solvent (for *L. polyantha* 1.18±0.0013 and for solvent 1.17±0.0011), in case of *L. grandifolia* vs solvent (for *L. grandifolia* 0.21±0.00121 and for solvent 0.24±0.00115) and in case of *Z. jujuba* vs solvent (for *Z. jujuba* 0.25±0.0012 and for solvent 0.27±0.00121) respectively. The variations in mean percent consumption were not significant ($p>0.05$).

The Wilcoxon sign rank test for mean food consumption assigned the same higher rank to maxillary palp sensilla and labrum epipharynx in consumption of moderately polar lipid fraction and the lowest rank was assigned to the galeal sensilla [ Table-2].

## DISCUSSIONS

The study was aimed to identify the lipid types responsible for restricted diet breadth of A. *assama*. When the fifth instar larvae of *A. assama* were given a choice for polar, non-polar and moderately polar lipid fractions, the larvae preferred food disk having polar lipid extracts of both host and nonhost plants. The polar lipid extracts were detected to contain sucrose, inositol and glycerol in thin layer chromatographic examination and therefore both sucrose and inositol are considered to act as phagostimulant for the larvae of *A. assama*. Sucrose and inositol have been shown to stimulate feeding in other lepidopteran insects like tobacco hornworm [Stadler and Hanson, 1975]. Similarly Sumida *et al.* [2007] described the role of dietary sucrose as an effecter molecule to midgut cells in *B. mori* larvae. Consideration of the fact that, the larvae of *A. assama* opted for the polar lipid fraction of non-hosts also clearly revealed that sugar, glycerol or inositol are not detected by the deterrent cells irrespective of their origin in the plant source. On the other hand the larvae of *A. assama* although preferred food disk having both non-polar and moderately polar lipid extracts of host plants , they rejected both the fractions of the nonhost plants . The non polar lipid extracts contains mainly fats and wax and moderately polar lipid extracts of plants contain terpenoid and phenolics as major components. Different types of wax components (alkanes) are reported to act as feeding stimulants present in host plants in case of a number of insects [Thompson, 1963; Klingauf *et al*., 1971]. Probably the fat and wax of epicuticle of non-host plants contain certain feeding deterrent components for the larvae of *A. assama*. Additionally , linoleic acid present in the non-polar fraction is also known to act as phagostimulant in a number of insects. De Boer *et al*. [1992] also reported non polar lipids as some feeding stimulatory in *V. sinensis* . Therefore the non-polar fraction of the host plants may have phagostimulating action on the larvae of *A. assama*. Terpenoids are one of the many classes of allelochemicals known to play an important role in insect-plant interactions [Kessler and Baldwin, 2002]. When some plant derived terpenoids and phenolics act as phagostimulant, others act as deterrents for different insects [Isman, 2002]. Terpenoids of *Ziziphus jujube* are reported to have insect growth regulatory properties [Lingampally *et al*.2012]. The plants having growth regulatory properties are likely to be rejected as they exert adverse effect on growth and development of the insect. On the otherhand mixtures of phenolic compounds such as myrcetin, 7,2',4' trimethoxy dihydroxy flavone with sterol compound, β-sitosterol found in host plants are reported to elicit the greatest biting behavior in *A. assama* [Neog *et al*., 2011]. Low molecular weight phenols are reported to act as attractant for aphids [Jordens-Rottger, 1979]. Probably, similarly with the non-polar lipids of non-host plants, moderately polar lipids of non-host plants also contain certain feeding deterrent components for the larvae of *A. assama* while those of the host plants have phagostimulating actions and are perceived by the gustatory sensilla.

In the food choice tests performed using specific sensilla, ALL, UNI, MAX, GAL and LAB opted for the solvent extract of only the host plants [Fig.1 A-E]. But NONE opted for both the extracts of the host/ non-host plant as well as the solvent which indicated the necessity of presence of the gustatory sense organs. The maxillary palp contains both gustatory and olfactory sensilla and hence its role cannot be determined for gustation alone . As the GAL and LAB behaved similarly with UNI in choice tests, the gustatory sensilla present in galea and labrum epipharynx were competent in acceptance of polar lipids of both host and non-host, non-polar and moderately polar lipid of host and rejection of non-polar and moderately polar lipid of non-host. Based on sign-rank test, Labrum epipharyngeal sensilla with higher ranking can be said to be more competent that the galeal sensilla in the rejection of non-host for *A. assama* larvae.

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