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Studies on little leaf of brinjal and morphotaxonomy of the leafhopper species associated from Bengal

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Abstract

Little Leaf of Brinjal (LLB) is one of the major potential threats of brinjal cultivation in West Bengal as well as in India. The present study, carried out at Kalyani, West Bengal, revealed the occurrence of the two insect vectors of LLB viz. *Hishimonus phycitis* (Distant) and *Amrasca biguttula* (Ishida) in the brinjal ecosystem from this region. This is the first report of *H. phycitis* in brinjal ecosystem from West Bengal. The details of morphotaxonomical studies of these two species of leafhoppers were carried out and compared with recently available literature. Disease incidence percentage of LLB ranged between 12-14% in the experimental field and 7-9 % in the farmers' field during the period of the study. Among the two vectors, *A. biguttula* was dominantly prevalent in brinjal throughout the growth stages. The transmission status of the two vectors in these areas can be assessed for the better and eco-friendly management of the disease.

Keywords: Little leaf of brinjal, insect vector, morphotaxonomy

1. Introduction

Brinjal or eggplant (*Solanum melongena* L.) is an important vegetable crop of tropics and subtropics. India is the second-largest producer of brinjal in the world after China. In India, West Bengal has largest share of area (162.93 thousand ha) and production (3019 thousand MT) of brinjal (Horticulture Database, 2017) [19]. Cultivation of brinjal is threatened by a good number of biotic and abiotic constraints both in India as well as in West Bengal. Among the major biotic constraints, Little Leaf of Brinjal (LLB) is a noteworthy one causing considerable economic loss to the cultivators (Mitra, 1993) [30]. In India, the disease was first reported from Coimbatore (Thomas and Krishnaswami, 1939) [38]. Later, it was reported from several states of the country viz. Delhi (Vasudeva, 1956) [41], Maharashtra (Verma *et al.*, 1965) [39], Punjab (Bindra *et al.*, 1972) [4], Madhya Pradesh (Mall and Sheikh, 1977) [27], Orissa (Kar *et al.*, 1982) [21], Kerala (Anjaneyulu and Ramakrishnan, 1973) [1], Tamil Nadu (Srinivasan and Chelliah, 1977) [37] and West Bengal (Chakrabarty and Choudhury, 1972) [5] posing threat to brinjal cultivation with moderate to severe infestation. The disease, LLB is caused by one phytoplasma, a wall-less mollicutes (Varma *et al.*, 1969) [40] which is currently at Candidatus status, the term commonly used for bacteria that cannot be cultured *in vitro*. There are six groups of this phytoplasma so far reported from the world; out of them only one group, 16SrVI-D is known to be dominant in India (Kumar *et al.*, 2017) [26]. The symptoms of the disease include smalling of leaves, proliferation of axillary shoots and buds, stunting of shoot and root growth, virescence, and floral phyllody.

The transmission of LLB is so far reported by two species of leafhopper viz. *Hishimonus phycitis* (Distant) and *Amrasca (Sundapteryx) biguttula* (Ishida) [*A. biguttula biguttula* (Ishida)] (Thomas and Krishnaswami, 1939; Hill, 1943; Raychaudhuri, 1974; Bindra and Singh, 1969) [38, 18, 32, 3]. However, both of these vectors may not be equally efficient in transmitting the disease. Based on the results of transmission, Thomas and Krishnaswami (1939) [38] evidently reported that *H. phycitis* could incite symptom of little leaf in only 14 out of total 27 plants tested, while, in case of *A. biguttula*, only 1 plant showed symptom out of 6 plants tested. The need for detailed morphotaxonomical study for correct identification of the concerned leafhopper species cannot be ignored before exploitation of their transmission efficiency. In West Bengal, detailed studies on the various aspects of the disease as well as of its vectors is really scanty. Additionally, there is no well-documented report of the occurrence

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of both the insect vectors on brinjal in West Bengal. The present experiment has been undertaken to study the incidence and symptomatology of LLB along with morphotaxonomy and species composition of leafhoppers occurring on brinjal which are associated with the disease at Kalyani, Nadia, West Bengal.

2. Materials and Methods

The present investigation was carried out at the University Experimental Field, Kalyani (23 ° N latitude, 89 ° E longitude, 9.75m above MSL), Nadia, from March, 2018 to February, 2019. The laboratory works were conducted in the Medicinal and Aromatic Plant Research Laboratory at Kalyani of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. The study of symptomatology for LLB and occurrence of leafhoppers were studied in a brinjal field (Var. Goria) transplanted in the experimental plots of size 20m×10m with spacing 90cm×60cm, following all the recommended agronomical practices. Transplanting was done in two seasons during the investigation - once at 2nd week of March, 2018 and another at 3rd week of September, 2018. During the entire period of crop growth the overall experimental field was kept free from all kinds of pesticides. Data for disease incidence percentage of LLB were taken from the brinjal field at the end of both the seasons. Three Farmer's fields at each of the two locations near Kalyani (Gayeshpur and Fatepur) were selected and visited in both the seasons. Disease incidence percentages for LLB were recorded accordingly at the end of each season.

2.1 Collection, preservation, and morphometric study of leafhoppers:

The leafhoppers were collected by sweep net from the experimental field and the collected leafhoppers were transferred to an insect-killing bottle for a few minutes and preserved properly in a desiccator containing fused calcium chloride for further studies.

Dissection of male genitalia and preparation of slides were carried out following the techniques given by Knight (1965) [23]. Measurements of various external morphological characters of different species were taken from the dried specimens under Zeiss Stereoscopic Trinocular Microscope using ocular micrometer and photographs were taken using Zeiss Axio CamERc 5s. Morphological terminology follows Dai *et al.* (2013) [7], Viraktamath and Anantha Murthy (2014) [43] for *H. phycitis* and Ye *et al.* (2017) [44] for *A. biguttula*. Measurements were made based on the observations from 5 female and 5 male specimens of each of the species. Mean and SD values of each of the characters were taken. The unit of all measurements used in the description is millimeter (mm).

2.2 Study of species composition of leafhoppers on brinjal:

In order to study the species composition of leafhoppers occurring on brinjal, 10 sweeps were made on randomly selected brinjal plants grown in the experimental field with the help of a sweep net. The samplings were done at a regular interval of 10days starting from 30 DAT to 110 DAT (DAT-Days After Transplanting). The process was followed in both the seasons.

3. Results and Discussion

3.1 Incidence and Symptomatology of Little Leaf of Brinjal (LLB):

During the experiment, percentages of LLB incidence were recorded from the brinjal plants grown in the University Experimental Field, Kalyani in two seasons (March transplanted and September transplanted). Regular observation for disease incidence was done carefully throughout the growth period and the incidence percentage was calculated at the end of each season. In the present investigation incidence of LLB was found to be 13.54% for March- transplanted brinjal while in the case of September-transplanted brinjal it was only 12%. Survey at farmers' fields near Kalyani revealed that the disease incidence percentage for LLB in the area ranged between 7-9% during the entire period of the present experiment.

Thorough observations were taken to understand the levels of manifestation and symptoms at different plant growth stages. In March and September transplanted brinjal first visual symptoms of little leaf were noticed at about 60 and 65 DAT respectively. Significant infestation was not found at the early growth stages of plants; however, there was a higher level of infestation during the latter stages. An array of symptoms for phytoplasmal little leaf of brinjal that was noted, can be summarized under two broad categories -

- When infestation took place at early growth stages of plants, leaves first showed curling or cupping type of symptom, gradually there was a drastic reduction in the size of the leaves with modification in the texture and leaves tending to sessile or subsessile in nature. Heights of infested plants were greatly reduced followed by general stunting. Plant bore no fruit and became sterile. (Fig-1, A-D)
- Infestation at later stages of plant growth stimulated the proliferation of axillary buds followed by shortening of internodes along with a reduction in leaf size. Subsequently, there have been crowding of small leaves at the axils of plants giving them bushy appearance followed by phyllody and virescence. Alternation of floral structure leads to nonbearing of fruits and when formed fruits were deformed. (Fig-1, E-H).





Fig 1(A-H): A-D Symptom of early infestation, E-H Symptom of late infestation

3.2 Identification and taxonomy of insect vectors of little leaf of brinjal

In India two species of leafhoppers are reported as vectors of brinjal little leaf - *Amrasca biguttula* and *Hishimonus phycitis* (Thomas and Krishnaswami, 1939; Hill, 1943; Raychaudhuri, 1974; Bindra and Singh, 1969) [38, 18, 32, 31]; among them, *H. phycitis* is a well-recognized one (Azadvar and Baranwal, 2012; Srinivasan and Chelliah, 1977) [2, 37]. During the present investigation, these two species of leafhopper could also be traced from the experimental field and the identifications were confirmed following detailed morphotaxonomical study.

3.2.1 Taxonomy of *Hishimonus phycitis* (Distant)

Eutettix phycitis Distant, 1908 [8]

Hishimonus phycitis (Distant) Nielson, 1968 [31]

Eutettix lugubris Distant, 1918 [9], synonymised with by Knight, 1970 [24].

Hishimonus orientalis Emeljanov, 1969 [14], synonymised by Knight, 1970 [24].

Cestius phycitis (Distant) Singh, 1971 [35]

Hishimonus phycitis (Distant) [Knight (1973) reinstated] [25]

Note. Distant (1908) [8] first described *Eutettix phycitis* from several locations in India and Sri Lanka. But Nielson (1968) [31] transferred *E. phycitis* to the genus *Hishimonus* Ishihara. Singh (1971) [35] considered *Hishimonus* as junior synonym of the genus *Cestius* Distant. Later Knight (1973) [25] reinstated *Hishimonus* as a valid and separate genus and distinguished it from *Cestius* based on external features.

3.2.1.1 Description: (Fig-2, Fig-3):

Length of Body (up to wing tip) $3.544 \pm 0.12\text{mm}$ (in female) and $3.328 \pm 0.08\text{mm}$ (in male).

Head

Head is pale yellowish to green, sub-acute with distinct black compound eyes, ocelli on anterior margin, contiguous with eyes. Anterior margin of head is devoid of coronal suture which is present medially on the vertex. Width of vertex is almost twice its length. Head including eyes and pronotum are almost of equal width. Antenna setaceous and are situated at or slightly below the middle margin of compound eyes at facial view.

Frontoclypeal region is light green without any characteristic marking, lateral frontal suture distinct. Clypeal suture is easily distinguishable. Clypeus is longer than wide. Anteclypeus is elongate with sides parallel for the basal two-third region and slightly expanded apically. Lora or mandibular plates on either side of the clypeus are distinct. Length of clypellus is about one-third the length of head along the ventral side. Maximum width of clypellus found to be greater in females than that of males.

Pronotum

Pronotum is greenish to yellowish-green, almost equal in width to head (including eyes), slightly expanded beyond the compound eyes on either side. Anterior margin of pronotum is broadly rounded, posterior margin shallowly concave; surface smooth, feeble striation along the anterior margin. Pronotum is wider than long.

Scutellum

Scutellum is triangular and wider than long, yellowish-brown in color with variable coloring pattern, depressed with a distinct transverse suture almost in the middle. The portion below the depression is flattened while the upper part is slightly raised and convex. Length of scutellum is almost one-third the width of head.

Forewing

Tegmen is silvery-white with dark brown mottling spots all over the wing surface but more densely along the apical margin. Series of dark spots are also found along inside of the costal margin (Fig.2 C). Distinct brown semicircular spot visible against midlength of commissural margin of each tegmen and form a conspicuous median circular spot with that of an opposite wing when wings are at rest (Fig.2 A). Appendix is well developed. Claval veins are separate, joined by a cross vein near the median length. Length of tegmen $2.88\text{mm} \pm 0.114$ and $2.0696\text{mm} \pm 0.059$ in female and male respectively.

Leg

Legs are ochraceous brown, femur with two apical claw, rows of setae along lateral sides of the tibia. Bases of setae on hind tibia are brown to black; tarsus 3 segmented, pretarsus with apical claw. Length of hind tibia is at least half the length of the body.

Abdomen

Abdomen consists of eleven segments out of which eight are visible externally. Venter of abdominal segments are greenish with brownish-black tinge on some of the lower abdominal segments. Dorsum of abdominal segments are with characteristics black to brown color patch covering more than 90% area. Length of ovipositor is $1.132\text{mm} \pm 0.084$.

Male genitalia

Pygophore is acutely rounded with stout setae along the posterior half, processes are absent (Fig.3 A). Subgenital plates are broadly rounded at the base and abruptly tapering to posterior finger-like lobes. Rows of setae and hair like processes are present on the ventrolateral sides of the plates. (Fig.3 B). Parameres or style with apical paraphyses are elongated, somewhat blunt, slightly curved at the posterior end and almost equal with opposing paramere (Fig.3 E).

Connective is forked, Y shaped, with arms almost equally long with stem (Fig.3 C). Aedeagus is more or less V-shaped without any basal processes and broadly rounded at apex, aedeagal shaft broad, widely divergent, no concavity on the lateral margin of the shaft with an apical finger-like posteromedian lobe on each shaft (Fig.3 C, D).

3.2.1.2 Comments

The genus *Hishimonus* can be distinguished by the forewing with a medial spot attaining the posterior apex of the scutellum, and the aedeagus atrium not extending ventral of the shaft (Knight, 1970) [24]. *H. phycitis* can be recognized by its aedeagus without basal processes and broadly rounded at

apex (Dai *et al.*, 2013) [7] and by the male genital structure, especially the aedeagus without the concavity on the lateral margin of the shaft and absence of folding on the aedeagal shaft and apical finger-like process on each shaft respectively (Hassan and Zhang, 2018) [17]. Viraktamath and Anantha Murthy (2014) [43] have also provided morphological descriptions for *H. phycitis* and other related species from the Indian subcontinent.

Male genital structures of the present specimen resemble the figures and descriptions provided by Dai *et al.* (2013) [7], Viraktamath and Anantha Murthy (2014) [43] and Hassan and Zhang (2018) [17]. Morphology of other body parts was also found similar to the descriptions given by the early workers.

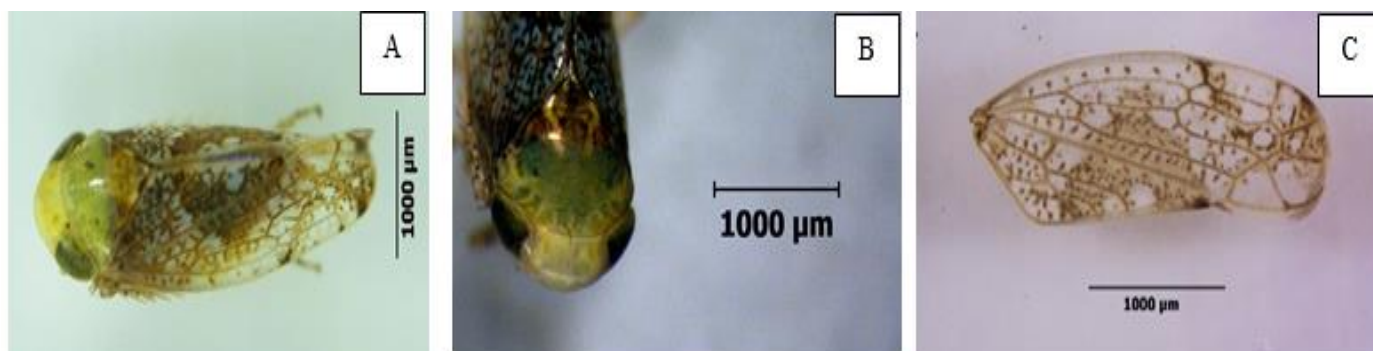


Fig 2(A-C): External Morphology of *Hishimonus phycitis*; A- Adult female, B- Head with Pronotum, C- Forewing.



Fig 3(A-E): Male genitalia of *Hishimonus phycitis*; A- Pygofer, lateral view, B- Subgenital plate ventral view, C- Aedeagus with connective, ventral view, D- Aedeagus dorsal view E- Style, dorsal view.

3.2.2 Taxonomy of *Amrasca (Sundapteryx) biguttula* (Ishida):

Chlorita biguttula Ishida, 1913 [20]

Empoasca biguttula Shiraki, 1913 [34]

Zygina punctata Melichar, 1914 [29], synonymized by Ye *et al.*, 2017 [44]

Empoasca bipunctata Schumacher, 1915 [33], synonymised by Dworakowska, 1970 [11]

Chlorita bimaculata Matsumura, 1916 [28], synonymised by Dworakowska, 1970 [11]

Empoasca devastans Distant, 1918 [9], synonymised by Dworakowska, 1970 [11]

Empoasca quadrinotatissima Dlabola, 1957 [10], synonymised by Dworakowska, 1970 [11]

Amrasca devastans (Distant) Ghauri, 1967 [16]

Sundapteryx biguttula biguttula (Ishida) Dworakowska, 1970 [11]

Sundapteryx biguttula punctata (Melichar) Dworakowska, 1970 [11]

Amrasca biguttula biguttula (Ishida) [Kapoor and Sohi, 1972 [22]; Dworakowska and Viraktamath, 1975 [13]]

Amrasca biguttula (Ishida) Chopra, 1973 [6]

Amrasca biguttula biguttula (Ishida), Dworakowska, 1977 [12]

Amrasca (Sundapteryx) biguttula (Ishida), Ye *et al.*, 2017 [44]

Notes. The genus *Amrasca* Ghauri is somewhat heterogeneous in the form and chaetotaxy of the subgenital plate (Ye *et al.*, 2017) [44]. There were two subgenera: *A. (Quartasca)* Dworakowska and *Amrasca (Amrasca)* Ghauri. While reviewing the Chinese species of *Amrasca*, Ye *et al.* (2017) [44] reinstated *Sundapteryx* Dworakowska from synonymy as a subgenus of *Amrasca* for a single well-distributed species, *A. biguttula biguttula* (Ishida). *A. (Sundapteryx)* was originally described as a separate genus by Dworakowska (1970) [11] based on type species *Chlorita biguttula* Ishida but was subsequently treated as a junior synonym of *Amrasca* by Dworakowska & Viraktamath (1975) [13]. This subgenus is characterized by the presence of macrosetae only in the basal half of the subgenital plate and by the presence of apodemes and other modifications to the male pregenital tergites. Species of the other two currently recognized subgenera have macrosetae extended from the base to or near the apex of the subgenital plate and lack apodemes and other modifications to pregenital abdominal tergites VI-VIII. Thus, the correct scientific name of this leafhopper should be *Amrasca biguttula* (Ishida) or, more

specifically, *Amrasca (Sundapteryx) biguttula* (Ishida) (Ye *et al.*, 2017) [44].

3.2.2.1 Description (Fig-4, Fig-5):

Length of Body (up to wing tip) 2.976 ± 0.054 mm (in female) and 2.816 ± 0.128 mm (in male)

Head

Head including compound eyes is as broad as pronotum. Compound eyes are black, ocelli distinct and located on anterior margin of vertex and away from the compound eyes. Antenna setaceous. Vertex is yellowish-white with two distinct apical black spots, surrounded by characteristic whitish patches, pointed to sub-acute with distinct coronal suture. Clypellus is broader at the base and narrower towards the apex, slightly extended beyond the margin of genae. Clypeal suture is not distinct.

Pronotum

Pronotum is wider than long, width almost twice its length; pronotal width almost equal to the width of the head. Anterior margin is narrowly rounded while the posterior margin somewhat flattened, often with characteristic white patches along the margin.

Scutellum

Scutellum is greenish with a median transverse suture. Distinctive patches of white color bands are often found along the sides of the posterior linear extension; width of scutellum more than one-third the width of head.

Wings

Forewings are light greenish, transparent with a distinct black spot in the apex of each forewing (Fig.4 B); MP'' fused with Cu A'; veins MP and MP'' + Cu A' arise from cell m and RP from cell r. There are four apical cells, no anteapical cell and appendix present. Length of forewing is sub-equal to the length of the body. Hind wings are hyaline white, typically of Empoascini type with submarginal veins reaching wing apex, fused with r+m and Cu unbranched preapically.

Leg

Legs are green with hind tibia having rows of spines along the lateral sides; hind tibia half the length of the body.

Abdomen

Abdomen is with eight visible segments. Pregenital

abdominal segments show uniform modification; tergum VII with a pair of large apodeme extending anterolaterally into segment VI and tergum VIII with a pair of conspicuous arched internal ridges (Fig.4 C).

Male genitalia

Sub genital plates are elongated, gradually narrowed towards the apex with stout macrosetae restricted only to the basal half while the distal half with numerous hair-like long fine setae and hair like processes (Fig.5 C); anal tube with a pair of slender, curved hooks (Fig.5 B); pygofer lobe broader at the base, narrower and very much elongated distally with a pair of elongated processes. Style or parameres are slender, basally broadened and narrowed apically with teeth like serration at the tip of the apex (Fig.5 A). Connective is fused with aedeagus with short arms. Aedeagus is very short, without aedeagal processes, aedeagal shaft tube-like, pointed and curved ventrally at apex (Fig.5 E).

3.2.2.2 Comments

The tribe Empoascini can be recognized by hindwing with submarginal vein reaching wing apex and fused with R or R + M or M_{1+2} (Sohi and Dworakowska, 1983^[36]; Viraktamath, 2005) ^[36, 42]. Ghauri (1967) ^[16] first defined the genus *Amrasca* Ghauri as the forewing with first and second apical veins arising from cell M, third apical vein arising from radial cell; hind wings typically empoascini type. Ganeswaran *et al.* (2008) ^[15] from Sri Lanka described the distinguishing characters of *A. biguttula* as green or light yellow body color with paired black spots on vertex and single black spot on the apical area of forewing, and subgenital plate long and finger-like with hair-like setae.

Recently Ye *et al.* (2017) ^[44] added two more fine distinguishing characters for identification of *A. biguttula* - i) Pregenital abdominal tergum VII with a pair of large lateral apodemes extended anterolateral into segment VI, tergum VIII with a pair of conspicuous arched internal ridges.

ii) Sub genital plate with macrosetae restricted to basal half, distal half with numerous conspicuous long, fine setae.

The present specimens showed all the distinguishing characters of the concerned species and coincide with the descriptions as provided by the previous workers. Modification of the pregenital abdominal segments and setal pattern of subgenital plates which are the two taxonomically distinguishing characters as found in the most recent literature available (Ye *et al.*, 2017) ^[44] has also been encountered in these specimens.

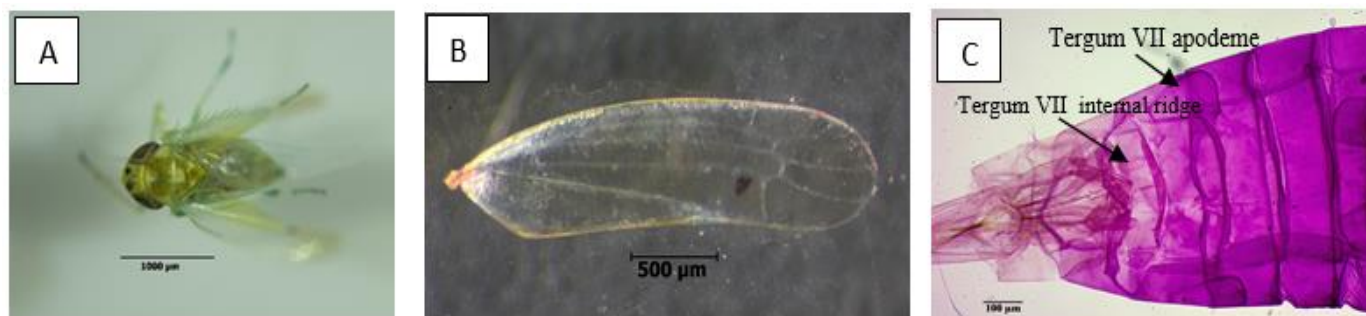


Fig 4(A-C): External morphology of *Amrasca biguttula*; A-Adult female, B- Fore wing, C- Modification of pregenital abdominal segments.

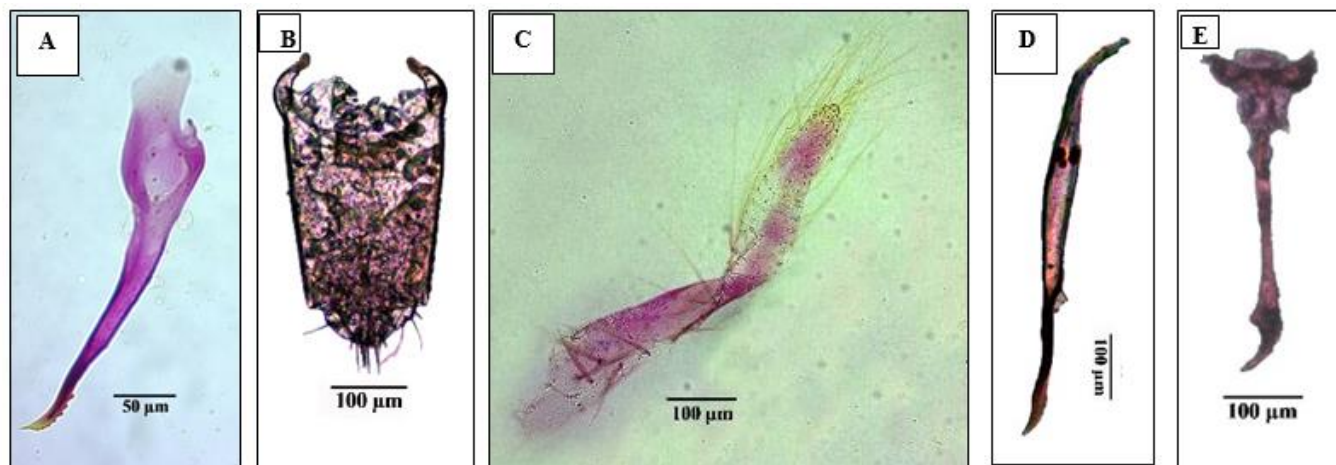


Fig 5: Male genitalia of *Amrasca biguttula*; A- Style dorsal view, B- Anal tube dorsal view, C- Subgenital plate, ventral view, D- Aedeagal spine, ventral view, E- Aedeagus with connective ventral view.

3.3 Composition of leafhopper species at different growth stages of brinjal:

To study the species composition of leafhoppers occurring on brinjal and associated with the disease LLB, 10 sweeps were

made on randomly selected brinjal plants grown in the experimental field with the help of a sweep net starting from 30 DAT to 110 DAT at a regular interval of 10 days in both the seasons. The detail of the study is shown in Table 1.

Table 1: Composition of leafhopper species in brinjal field at Kalyani:

Growth Stages	March transplanted brinjal (nos. per 10 sweeps)		September transplanted brinjal (nos. per 10 sweeps)	
	<i>A. biguttula</i>	<i>H. phycitis</i>	<i>A. biguttula</i>	<i>H. phycitis</i>
30 DAT	7	0	6	0
40 DAT	12	0	10	0
50 DAT	17	0	13	0
60 DAT	27	3	28	0
70 DAT	32	5	30	2
80 DAT	34	6	24	4
90 DAT	23	8	20	5
100 DAT	18	9	16	6
110 DAT	20	11	14	8

Of the two species, *A. biguttula* was dominantly prevalent in brinjal throughout the growth stages in both the seasons. Population of this species always outnumbered *H. phycitis* at all the plant growth stages. However, *H. phycitis* was absent or rarely present at early periods of plant growth, but its number tended to increase towards the end of the season. It would be worthy to note that an increase in the level of infestation of LLB later in the growth stages as found in the experimental field, may be particularly attributed to the abundance of *H. phycitis* at that time.

4. Conclusion

Severe infestations of LLB were previously recorded from different states of India. Occurrence of only two vectors of this disease is reported in India, out of which transmission ability of *H. phycitis* is well established but the transmission ability of *A. biguttula* is supported by less number of evidences. In the present investigation, moderate to severe infestations of LLB, ranging between 12-14%, have been reported in the experimental field, Kalyani. At farmers' fields near Kalyani low incidence of LLB (7-9%) was recorded compared to the experimental field during the period of study. Farmers generally rogue out the infected plants as soon as the appearance of visual symptoms to avoid further spread of the disease. Therefore the actual disease incidence percentage in the farmers' fields might be higher than the data recorded during the survey. Instead of several physical and chemical

precautionary measures taken, disease incidence percentage for LLB recorded at farmers' field during the period of investigation is still concerning. Both the vectors of LLB viz. *H. phycitis* and *A. biguttula* could be collected from the brinjal ecosystem at Kalyani, W.B. and they were identified critically with morphotaxonomical study. Present specimens of the two species of leafhopper resemble with the respective descriptions and figures provided by the previous workers. However, relative abundance of *H. phycitis*, the comparatively potent vector of the disease, was lesser than that of *A. biguttula* at every growth stage of brinjal plants. Therefore, the transmission status of the vectors in the area of investigation needs to be established. Data on transmission parameters of both the vector species are to be generated for epidemiological study as well as for the management of the disease. It is also felt that the rigorous study of the population behavior of important insect vectors of this disease is the urgent need of the time.

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