**Chilodonella uncinata** – a protozoa pathogenic to mosquito larvae

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During August 2001, repeated high mortalities observed in *Culex triaeniorhynchus* and *Cx. pseudovishnui* mosquito larvae collected from paddy fields prompted me to detect the causative organism. A ciliated protozoan, *Chilodonella uncinata*, was found to cause chronic and fatal infection in the natural population of mosquitoes in and around Delhi, North India. Anopheline larvae were less (14.13%) susceptible to *Chilodonella* infection than culicine larvae (75.21%). Body cavities of dead and transparent larval were found packed with thousands of motile endoparasitic stage of the ciliate. Numerous cuticular cysts were observed on cadaver of larvae and pupae. The use of cuticular cyst in differentiating ciliate genera pathogenic to mosquitoes is discussed. The limited study points to the biological control potential of *Ch. uncinata* against vectors of Japanese encephalitis.

Natural population of mosquitoes is kept under check by the activities of parasites and predators. Several species of viruses, bacteria, fungi, protozoa and nematodes are known to cause infection in mosquito larvae. Endoparasitic ciliated protozoa have been known to infect mosquito larvae since 1921, when Lamborn first reported the occurrence of *Lambornella stegomyiae* infection in the larvae of *Aedes albopictus* in a sample collected from an earthen pot in Kuala Lumpur. After a gap of fifty years, a second species of *Lambornella* (*L. clarkii*) was isolated from tree-hole-breeding mosquito larvae, *Aedes sirensis* in California, USA. In India, studies on endoparasitic ciliates of mosquito larvae started in recent years, wherein anopheline larvae (*Anopheles barbirostris*, *An. hyrcanus* group and *An. philippinensis*) breeding in peridomestic ditches were found infected with a *Lambornella* sp. in Northeast India. The third ciliate species belonging to another genus, *Tetrahymena pyriformis*, was observed in the body cavity and anal gills of mosquito larvae of bamboo-breeding species *Arimigeres* (*Leicesteria*) dolichocephalus, *Ar. (L.) dentatus* and *Ar. (L.) digitatus* collected from a bamboo forest near Kuala Lumpur.

The present communication reports the fourth endoparasitic ciliate, belonging to an entirely different group, *Chilodonella uncinata*, in the head capsule, antenae, body cavity, saddle, anal gills and siphon of culicine and anopheline larvae (*Culex triaeniorhynchus*, *Cx. pseudovishnui*, *Cx. (Lutzii)* sp., *An. stephensi mysorensis* and *An. hyrcanus* group) breeding in paddy fields, irrigation channels, marshy areas, wells, ponds and pools in North India. *Ch. uncinata* was found to cause 25–100% mortalities in Japanese encephalitis (JE) vector larvae in marshy areas to paddy fields, and has the facility for trans-ovarian transmission through its mosquito host. The infectious stage of *Ch. uncinata* was found to be resistant to desiccation and can be produced asexually in a variety of media. It is hoped that the present communication would stimulate further work in biological control potential of *Ch. uncinata*.

Mosquito larvae used in this study were collected from surface-water habitats supporting scanty to profuse aquatic vegetation in and around Delhi (North India) from August to December 2001 following conventional sampling methods. Mosquito-breeding sources studied include (a) paddy fields, burrow pits, ponds and wells in a village on Delhi–Haryana border where paddy is grown during monsoon season (July to October), and (b) vast marshy areas, irrigation canals, ponds and pools covered with prolific growth of water hyacinth round the year in northern, eastern and southern parts of Delhi. The other flora nearly common in both the study areas consisted of mainly algae, Lemna and elephant grass.

Mosquito larvae collected from different breeding sites were kept separately and brought to the laboratory for examination. The sick/dead larvae were washed in distilled water and examined under a compound microscope for detection of pathogenic organisms. Live mosquito larvae in the collected sample were kept under laboratory conditions for a period of 5–10 days. The pathogenic organisms were isolated from the infected larvae, colonized under laboratory conditions and sent to experts for species identification following the protargol impregnation method. Based on the available literature and observation of the present study, keys to pathogenic ciliate genera of mosquito larvae (*Lambornella, Tetrahymena* and *Chilodonella*) and four Indian species of *Chilodonella* (*Ch. cucullatus*, *Ch. rhesus*, *Ch. spiralidontis* and *Ch. uncinata*) are provided for species identification in (Figures 1 and 2), respectively.

Key to the pathogenic ciliates of mosquito larvae (Figure 1):

1. Body flat, dorso-ventrally compressed (Figure 1 A); with distinct pre-oral beak (a), cilia absent on dorsal surface, present on most of ventral surface; oral region behind pre-oral suture (b), cytropharynx with well-developed rod apparatus (c). *Chilodonella*

2. Body round in transverse section, uniformly covered with cilia; no distinct pre-oral beak (Figure 1 B, C) 2

(a, Figure 1 B) small, drop-shaped; from 25 to 31 merid-
ian ciliary rows of which 1 or 2 are post-oral (b, Figure 1 B); oral polykinetids (a–c, Figure 1 C) straight, never sigmoid.

Tetrahymena

Body about 75 \( \mu \text{m} \times 35 \mu \text{m} \), spindle-shaped (Figure 1 D) to ovate (Figure 1 E), anterior end pointed to rounded; buccal apparatus small, pointed to more or less rounded; from 30 to 46 meridian ciliary rows, of which 3 to 7 are post-oral (a, Figure 1 D, E); only 2nd polykinetid sigmoid (b, Figure 1 D, E).

Lambornella

Contractile vacuole 1–3 in number; size less than 100 \( \mu \text{m} \).

2. Contractile vacuole single (a, Figure 2 B); cytopharynx short and straight (b, Figure 2 B); size (50–65 \( \mu \text{m} \)).

West Bengal: Kolkata. Ch. rhesus (Ghosh), 1929

Contractile vacuole two to three in number.

3. Contractile vacuole three in number, largest postero-terminal (a, Figure 2 C); cytopharynx spirally curved behind (b, Figure 2 C); size 97 \( \mu \text{m} \). Jammu & Kashmir: Srinagar. Ch. spiralidontis (Bhatia & Mullick), 1930

Contractile vacuole two in number (a, Figure 2 D); preoral kinety complete (b, Figure 2 D); cytopharynx long and curved, fairly indistinct in the middle (c, Figure 2 D); small size (30–50 \( \mu \text{m} \); macronucleus large and round (d, Figure 2 D). Delhi and Haryana. Sonipat (present study).

Ch. uncinata (Ehrbg.), 1838

A few species of lower invertebrates, viz. ciliates (Paramecium, Euplotes, Stylonychia), Thecate Amoeba and a species of Rotifer were observed in the same habitat wherein mosquito larvae were found to be infected with an endoparasitic ciliate of the genus Chilodonella. Besides, dead mosquito larvae many-a-times were found infested with Vorticella sp.

Cx. pseudovishnui and C. tritaeniorhynchus larvae collected from paddy fields during August 2001 died by the time they were brought to the laboratory. Thirty percent of the dead larvae, were transparent and on examination under microscope were found to be severely infected with endoparasitic ciliates, which were found moving inside the body: in the haemocoel, head capsule, siphon, saddle and anal gills (Figure 3 A, a, Figure 4 A).

These ciliates were isolated from the host larvae, reared in the laboratory and identified by experts in the field as Ch. uncinata (Ehrenberg), 1838 [Subphylum: Ciliophora: Cryptophorida: Chilodellidae] by observing the ciliates in wet mounts. In this study the genus Chilodonella is found to be pathogenic to mosquito larvae in nature. According to the available literature, three species of Chilodonella (Ch. cucullulus, Ch. rhesus and Ch. spiralidontis)\(^8\)–\(^10\) were reported earlier from India\(^11\). The present pathogenic ciliate Ch. uncinata\(^12\) has been found in and around Delhi.
In the present study, it was observed consistently that dead mosquito larvae were mainly older (4th stage). In contrast, live larvae of younger stages in the same sample, died after a gap of 2–5 days when they grew older and reached the 4th stage. A sizeable number of dead and discoloured larvae that were not transparent at the point of initial examination became transparent after a gap of few days with endoparasitic ciliates clearly visible, when kept under observation under room temperature. These dead transparent mosquito larvae were kept as such for a few days under laboratory conditions and observed under microscope using 100X magnification. Numerous dorso-ventrally flattened ciliated protozoa as shown in Figure 2D, and not noticed earlier, now appeared in the water containing the dead larvae indicating that these pathogenic microbes were capable of escaping dead host larva after increasing their number manifold at the expense of internal tissues of the host. At times, dead 2nd stage larvae collected from the field revealed the presence of numerous ‘cuticular cysts’ on the body on examination under the microscope (Figure 5), which are the invasion sites for the entrance of Ch. uncinata into the host haemocoel. Besides, innumerable cuticular cysts were observed in the dead 4th stage larvae of Cx. tritaeniorhynchus and Cx. pseudovishnui.

In order to determine the effect of dryness on the stability of the pathogen, a number of plastic cups containing infected and dead larvae of Cx. tritaeniorhynchus were allowed to dry at room temperature, re-flooded with distilled water after a varying period of time and examined for revival of the pathogen using 100X magnification. Ch. uncinata reappeared in the wet plastic cups in a span of 2–5 days, thereby indicating its capability to stand desiccation.

Various typical JE vector-breeding habitats, viz. paddy fields, irrigation channels, marshy areas, wells, ponds and pools in the study area were surveyed from September to December 2001 to determine the distribution, infectivity rate and host range of Ch. uncinata.

It has been observed that Ch. uncinata is a facultative parasite of mosquito larvae, particularly vectors of JE. Its
distribution as a free-swimming (trophont) stage is scanty. After paddy is harvested, JE vector-breeding habitats are limited and confined to wells, permanent ponds, etc. with restricted distribution of *Ch. uncinata* in host mosquito larvae. *Cx. vishnui* group of mosquitoes over winters in adult stage during extreme winter months (January–February) in the study area and larval population literally gets extinct at this time of the year. With the result, scanty distribution of *Ch. uncinata* (as free-swimming form) was observed in some natural surface water habitats (wells and permanent ponds). As the weather condition improves from March onwards, the JE vector population starts building up and host larvae are now available in the natural habitat for the propagation, multiplication and dispersal of parasitic ciliate to new habitat in the area. However, wide distribution and maximum infection rate of *Ch. uncinata* in host mosquito larvae were observed only after paddy transplantations coupled with monsoon rains under high vector density situation.

A total of 4828 fourth-stage larvae belonging to six mosquito species collected from paddy fields, irrigation channels, marshy areas, wells, ponds, etc in the study area during September–December 2001 were examined for *Chilodonella uncinata* infection. This total comprised of 92.58% *Cx. tritaeniorhynchus*, 3.14% *Anopheles stephensi mysoriensis*, 1.76% *Cx. (Lutzia)* sp., 1.22% *Cx. pseudovishnui*, 0.67% *An. hyrcanus* group and 0.63% *Cx. (Culex)* sp. All these mosquito larvae were found to foster *Ch. uncinata* infection, but there were wide variations in the degree of parasitism in different species of mosquitoes. Out of a total of 4644 fourth-stage culicine mosquito larvae collected and examined, 3493 (75.21%) were found to be positive for *Chilodonella* infection. The infectivity rate (percentage of larvae found to be infected with *Ch. uncinata*) in *Cx. tritaeniorhynchus* was found to vary from 24.79 to 88.66, followed by 79.66, 72.94 and 63.33 in *Cx. pseudovishnui*, *Cx. (Lutzia)* sp. and *Cx. (Culex)* sp., respectively (Table 1). During the entire period of study, among the 184 anopheline larvae collected and examined only 26 (14.13%) were found to harbour *Ch. uncinata*, indicating relatively low susceptibility of anopheline larvae to ciliate infection.

Host range of *Ch. uncinata* includes all species of mosquitoes breeding in paddy fields, viz. *Cx. tritaeniorhynchus*, *Cx. pseudovishnui*, *Cx. vishnui*, *Cx. (Lutzia)* sp., *An. stephensi var. mysoriensis* (identified based on egg characters, 12–13 ridges on float, obtained from adult *An. stephensi* reared from larvae collected in rice fields) and *An. hyrcanus* group. Natural infections of immature stages of mosquitoes by pathogenic ciliates indicating host species and stage, location of host body wherein ciliate infection was detected, degree of infectivity and geographical source so far reported from India, including the present study are given in Table 2.

### Table 1.

<table>
<thead>
<tr>
<th>Type of habitat searched</th>
<th>No. of habitats examined</th>
<th>Number of mosquito larvae found infected/ numbers examined for infection</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>*</td>
</tr>
<tr>
<td>Nursery plots (paddy)</td>
<td>14</td>
<td>–</td>
</tr>
<tr>
<td>Paddy fields</td>
<td>34</td>
<td>2174/2703</td>
</tr>
<tr>
<td>Wells</td>
<td>5</td>
<td>86/97</td>
</tr>
<tr>
<td>Irrigation canals</td>
<td>4</td>
<td>30/121</td>
</tr>
<tr>
<td>Temporary ponds</td>
<td>6</td>
<td>257/426</td>
</tr>
<tr>
<td>Barrow pits</td>
<td>18</td>
<td>751/895</td>
</tr>
<tr>
<td>Water-hyacinth ponds</td>
<td>15</td>
<td>45/156</td>
</tr>
<tr>
<td>Marshy areas</td>
<td>8</td>
<td>22/72</td>
</tr>
<tr>
<td>Total</td>
<td>104</td>
<td>3365/4470</td>
</tr>
</tbody>
</table>

*, *Cx. tritaeniorhynchus*; †, *Cx. pseudovishnui*; ‡, *Cx. (Lutzia)* sp.; §, *Cx. (Culex)* sp.; ||, *Anopheles stephensi mysoriensis*; ¶, *An. hyrcanus* group.

Figures in parenthesis indicate percentage of mosquito larvae found to be infected (infectivity rate).
mosquito larvae. However, under special circumstances, Vortecellids are not pathogenic to mosquito larvae. Similarly, Vortecellids are not pathogenic to Stylonychia, thecate microscopic endoparasitic stage visible in side the host infected larva turns transparent, with thousands of its microorganisms involved gut epithelium, fat bodies, muscles, malpighian tubules, etc. (b, Figure 3; c, Figure 4). The study revealed that Ch. uncinata induces chronic infection, causing high mortality in susceptible host mosquito larvae. They attack young mosquito larvae and invade the host haemocoel by dissolving the host cuticle and forming cuticular cysts. It appears that after reaching the host haemocoel, the ciliate allows the host larva to grow in size and reach the 4th stage. Simultaneously, the ciliate also multiplies and increases its number immensely at the expense of host tissues. It is only at this point of time that the ciliate kills the host and the larva turns discoloured and opaque, with perhaps commensal bacterial movement visible at some points of the dead larva. After the death of the host larva, Ch. uncinata continues to reproduce for some more time and fill almost the entire body cavity of the susceptible host. At this stage, the infected larva turns transparent, with thousands of its motile microscopic endoparasitic stage visible inside the host body cavity. However, further controlled laboratory studies are required to confirm the above hypothesis.

This mode of entry of Ch. uncinata from outside through cuticular cysts is similar to that reported in the mosquito larva, the host larva is drowned and dead due to increased weight.

**Table 2.** Endoparasitic ciliates naturally infecting mosquitoes in India

<table>
<thead>
<tr>
<th>Host, host stage; location of infection</th>
<th>Ciliate species</th>
<th>Infectivity rate (%)</th>
<th>Geographical source of material</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anopheles barbirostris,</td>
<td>Lambornella sp.</td>
<td>65.1</td>
<td>Peridomestic ditches in Northeast India</td>
<td>Narain et al.3</td>
</tr>
<tr>
<td>An. hyrcanus, Larvae; Body cavity, head capsule; cuticular invasion cyst</td>
<td>Chilodonella uncinita</td>
<td>24.8–88.6</td>
<td>Paddy fields, irrigation canals, wells, ponds, burrow pits, pools, marshy lands in North India</td>
<td>Present study</td>
</tr>
<tr>
<td>Cx. tritaeniorhynchus; larvae, pupae (rarely); live ciliate in the head capsule, antennae, body cavity, saddle, siphon (Figure 3); cuticular invasion cysts: dead 2nd stage larvae (Figure 5)</td>
<td>Ch. uncinita</td>
<td>79.66</td>
<td>Paddy fields in North India</td>
<td>Present study</td>
</tr>
<tr>
<td>An. hyrcanus group; larvae; ciliates found trapped between cuticle and epidermis of the host</td>
<td>Ch. uncinita</td>
<td>31.25</td>
<td>Paddy fields, temporary ponds, burrow pits in North India</td>
<td>Present study</td>
</tr>
<tr>
<td>Culex (Lutzia) sp.; larvae; body cavity</td>
<td>Ch. uncinita</td>
<td>72.94</td>
<td>Wells in North India</td>
<td>Present study</td>
</tr>
<tr>
<td>An. stephensi mysoriensis; larvae; body cavity</td>
<td>Ch. uncinita</td>
<td>10.52</td>
<td>Nursery plots (paddy) in North India</td>
<td>Present study</td>
</tr>
<tr>
<td>Culex (Culex) sp.; larvae; body cavity</td>
<td>Ch. uncinita</td>
<td>63.33</td>
<td>Pools, marshy areas in North India</td>
<td>Present study</td>
</tr>
</tbody>
</table>

An attempt was made to test the trans-ovarian transmission potential of the candidate pathogen through its mosquito host. During September 2002, twenty-five fed Cx. tritaeniorhynchus were collected from the study area having extensive paddy cultivation and were kept separately in glass specimen tubes for egg-laying. Water in experimental tubes was examined using 100X magnification for the presence of candidate pathogen for a period of seven days. Ch. uncinita was detected within a period of 2–5 days in water in 80% of the specimen tubes used for egg-laying of wild caught Cx. tritaeniorhynchus, confirming the trans-ovarian transmission potential of Ch. uncinita through its mosquito host. Eggs were laid in 55% and larvae were hatched in 30% of the experimental tubes, respectively. However, all the larvae died before reaching the late 3rd instar and subsequently, all of them were found infected with Ch. uncinita.

JE is a mosquito-borne zoonotic disease and is caused by a group-B arbovirus. It is a disease of public-health importance because of its epidemic potential and high case fatality rate. Majority of vectors of the JE virus predominantly use paddy fields as larval habitats.

According to the experts in the field (pers. commun.), none of the micro-organisms, viz. Paramecium, Euplotes, Stylonychia, Thecate Ameoba and Rotifers found in the study area, has been recorded to be pathogenic to mosquito larvae. Similarly, Vortecellids are not pathogenic to mosquito larvae. However, under special circumstances, when many individuals of Vortecella get attached to one bulk of mosquito larvae, the host larva is drowned and dead due to increased weight.

Ch. uncinita was found to be a free-living facultative parasite of mosquito larvae. The parasite appeared to be virulent in highly susceptible host (Cx. tritaeniorhynchus and Cx. pseudovishnui, Table 2). Host tissues affected involved gut epithelium, fat bodies, muscles, malpighian tubules, etc. (b, Figure 3; b, Figure 4A).
Irrigation canals, ponds and marshy areas with prolific growth of floating and emergent vegetations, predominantly of water hyacinth contribute immensely to the adult mosquito population of Cx. tritaeniorynchus. Analysis of data from these habitats in the present study (Table 1) indicates that Cx. tritaeniorynchus has a comparatively low level of susceptibility to Ch. uncinata infection (with infectivity rate ranging from 24.8 to 30.5) compared to other habitats, viz. seasonal ponds, paddy fields, burrow pits and wells having aquatic vegetation, excluding water hyacinth (infectivity rate ranging from 60.3 to 88.6). However, further studies are required to correlate vegetation cover of breeding habitats, the incidence of Ch. uncinata infection and the population dynamics of the mosquito host.

Because of the extreme vastness of paddy fields as larval habitat of JE vectors in developing countries and the limitations of resources in manpower and materials required for larval control, a microbial control agent will not only have to be efficacious against target mosquitoes and safe to non-target organisms, but it should also replicate, persist and disperse within the environment 17.

The present study reveals that some of the infected host larvae survive, continue to develop in the same natural habitat and become adults but still remain infected, reproduce and disperse the parasitic ciliate to new habitat in the environment through trans-ovarian transmission. Moreover, they are highly robust against all aggressive environmental conditions like temperature, dryness, vagaries of agricultural practices, including pesticide application, etc. Since Ch. uncinata has the facility to increase tremendously, the parasitic load inside the body of susceptible host larvae and cause fatal infection, it can be postulated that as a natural enemy it can effectively eliminate larval population and reduce adult population of JE vectors in an area.

It can be concluded from the above that Ch. uncinata is a free-living facultative, ciliated, protozoan parasite with many attributes of a good microbial pathogen, widely distributed in typical JE vector-breeding habitats in and around Delhi. It causes low to very high (25–100) per cent mortalities in mosquito larvae, particularly Cx. tritaeniorynchus and Cx. pseudovishnui, important JE vectors in India. Like a pathogen it is highly virulent, desiccation resistant, can be cultured in vitro and has a high reproductive potential with facility to disperse in the environment by way of trans-ovarian transmission through its mosquito hosts. Investigations are currently in progress on mass cultivation and easy formulation of Ch. uncinata for its eventual use in laboratory and field evaluation. The present investigation points to desirability of further studies on the distribution and host range of Ch. uncinata in an area and the role played by Ch. uncinata in the control of host mosquito species, vectors of human diseases.
Endophytic fungal communities in leaves of tropical forest trees: Diversity and distribution patterns

T. S. Suryanarayanan*, G. Venkatesan and T. S. Murali

Endophytic fungi cause symptomless infections in healthy tissues of plants. This cryptic guild of fungi is regarded as a benchmark for estimating fungal biodiversity. We studied endophyte distribution, diversity and host recurrence in 24 tree hosts (belonging to 17 plant families) of two dry tropical forests of the Nilgiri Biosphere Reserve. A total of 81 endophyte taxa were isolated from 3600 tissue segments. Fifty-six species were isolated from more than one host. We discerned two groups of fungi in both forests, one group consisting of the ubiquitous forms that dominated the endophyte assemblage of many hosts and the second represented by the less frequent forms. Host density influenced the composition and distribution of endophytes in one of the forests. The existence of ubiquitous forms reduced the diversity of the endophytes in the plant communities. Our results suggest that dry tropical forests are not hyperdiverse with reference to endophytes and that the generalists among endophytes be identified before extrapolating data to calculate global fungal diversity.

Fungi are one of the most diverse life forms on this planet and predicting the number of fungal species is considered important among mycologists1. Hawksworth2 predicted that there are 1.5 million species of fungi; of these, about 74,000 are currently known4. Recent studies from tropical forests2–6 suggest that fungal diversity is greater in the tropics than in the temperate regions, and many tropical mycologists view 1.5 million as a conservative figure5. Some researchers however, feel that the figure of 1.5 million is too high13–19. Endophytes of tropical plants are among the groups of fungi that have been studied to arrive at the predicted figure of 1.5 million13. Based on their studies on nine neotropical trees, Arnold et al.10 concluded that fungal endophytes are hyperdiverse in the tropics and that the figure of 1.5 million may markedly underestimate fungal diversity. More recently, studies on a forest in Guyana11 and four forests in Mudumalai Wildlife Sanctuary, southern India12 revealed that certain tropical forests are not hyperdiverse with reference to fungal endophytes. In order to get a clear picture of the diversity of endophytes in tropical forest communities, we studied hosts of different abundance classes.

We collected leaves from 24 tree hosts found in the dry thorn and dry deciduous forests of Mudumalai Wildlife Sanctuary (11°32’ and 11°43’N lat and 76°22’ and 76°43’E long; area 312 km²), which is situated to the northwest of the Nilgiri Mountains in Tamil Nadu, and forms a part of the Nilgiri Biosphere Reserve. The tropical dry thorn forest is in the rain shadow of the Nilgiri Mountains and the mean annual rainfall is 800 ± 65 mm (ref. 13). The dry deciduous forest occupies the major part of the sanctuary and receives an annual rainfall of 1200 mm (ref. 13).

For each forest type, four hosts with higher abundance were grouped in abundance class I, four with intermediate abundance in class II and four with lower abundance in class III (Table 1). For each host, three individuals were selected and 20 healthy leaves were collected from each individual.

Leaves from each individual were processed separately within 48 h of collection. The leaves were washed thoroughly in running water and three segments of 0.5 cm² were cut from the midrib portion of each leaf and surface sterilized by immersing in 70% ethanol for 5 s, followed by 4% NaOCl for 90 s, and finally washed in sterile water for 10 s (ref. 14).

Fifty segments from each individual (150 segments for each host species) were randomly chosen and placed in Petri dishes containing potato dextrose agar (with chloramphenicol 150 mg l⁻¹). Ten leaf segments were plated in each Petri dish, the dishes were sealed with parafilm and incubated in a light chamber at 26 ± 1°C for 21 days. The light regimen provided was 12 h light: 12 h darkness from cool white, daylight fluorescent lamps14,15. The efficacy of the sterilization procedure was ascertained with the method of Schulz et al.16.

The fungi that grew out from the segments were periodically isolated and identified. Those fungi that failed to

ACKNOWLEDGEMENTS. I thank P. R. Arbani, World Health Organization, New Delhi, R. Rajagopal (retd.), Division of Medical Entomology and Vector Control, National Institute of Communicable Diseases (NICD) and D. Chattopadhyaya, NICD for reviewing an earlier draft of the manuscript; G. R. Sapra and Renu Gupta, Delhi University for providing technical advice and photographic facilities; Jotna Sokhey, K. K. Datta, Usha K. Baveja, Shiv Lal, past and present Directors, NICD for support; Dibyendu Das for assisting in the preparation of figures; and the technical staff of Bioassay Laboratory, NICD for their assistance during field studies.

Some of the figures in this paper have been redrawn from the following sources: Figure 1A, ref. 11; Figure 1B, ref. 7; Figure 1D, E, ref. 2; Figure 2A–C, ref. 11.

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