less, the trapped pollen and spores from analysed spider webs depict in general the existing vegetation in Lucknow City and its outskirts.

Ground vegetation is honestly portrayed in a majority of the analysed webs where grasses range from 13 to 33%. Associated recorded major nonarboreal taxa show moderate values – Tubuliflorae (2–13%), Solanaceae (9–12%). *Brassica* dominated in web sample no. 2 (11.8%). Other regularly met with pollen taxa belong to Acanthaceae, Lemiaceae and Malvaceae though in low values in almost all the samples.

The present studies based on limited number of web samples have demonstrated that the commonly growing arboreal taxa – chiefly on the roadsides are quite honestly portrayed in the spectrum of the area. Apparently the trapped pollen and spores looking to their frequencies are chiefly those which remain suspended in the air close to the ground depending upon the exposure of the webs to the flowing air currents or wind. Further studies are needed to throw more light on various aspects of the webs as pollen/spore trappers and their relevance to the extant vegetation while evaluating the modern pollen/spore rain in a region. Analysis of spider webs collected from inside the residential houses may bring out much sought-for data on the aerial suspension of various allergens causing asthma, dermitites and similar ailments. Furthermore, pollen analysis of spider web can also be taken as an important additional parameter for preparing the modern pollen-spore depositional model of a region, supplementary to the conventional study of moss cushions, surface soil samples and aerospora to construct the regional pollen spectra.

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ACKNOWLEDGEMENTS. We thank Prof. A. K. Sinha, Director of the Institute for prompting us to undertake this type of study. Mrs Indra Goel, Technical Officer is acknowledged for the analysis of web samples.

Received 27 June 2002; revised accepted 18 October 2002

Efficacy of *Chrysosporium lobatum* against larvae of malaria vector, *Anopheles stephensi* in the laboratory

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***Chrysosporium lobatum*, a deuteromycetous (Deuteromycotina: Moniliales) fungus isolated from soil at Dayalbagh, Agra caused high mortality of *Anopheles stephensi* larvae in the laboratory. The effectiveness of conidia of *C. lobatum* against *An. stephensi* has been evaluated at six different concentrations. The conidia were used for the control of 1st-, 2nd- and 3rd-instar larvae of *An. stephensi*. The LC_{50} values were calculated by probit analysis. Larvae of 3rd-instar *An. stephensi* were more susceptible to *C. lobatum* (0.13×10^3 conidia/ml) than the other instars. It could be a successful tool for controlling malaria vector in tropical climates with more field trials.**

IN India, *Anopheles stephensi* is one of the most important human biting mosquitoes. As a vector it transmits the protozoa that causes malaria. The drawbacks associated with synthetic insecticides have initiated the search for alternative vector control methods, including biological control[1]. About 750 species of fungi have been recorded as pathogenic for insects. However, many of these records are based on single observations[2]. Among the entomopathogenic fungi, *Lagenidium giganteum*, *Tolypocladium cylindrosporum*, *Culicinomyces clavosporus* and *Metarrhizium anisopliae* have been widely studied against the larvae of mosquitoes and have generated some interest as potential biocontrol agents of mosquito larvae[3–7]. More recently, *Leucothecium emdenii* and some *Penicillium* species have been observed to be pathogenic against mosquito larvae[8,9].

Chrysosporium lobatum Scharapov is a deuteromycetous fungus belonging to the order Moniliales and the family Moniliaceae. It is not a natural pathogen of *An. stephensi* larvae. Many deuteromycetes including *C. lobatum* are saprophytic and keratinophilic in nature and have recently been screened and found effective against the larvae of mosquitoes[10,11]. The absence of studies on *C. lobatum* and its apparent effectiveness in causing mortality in mosquito larvae and its potential use as a vector control agent prompted us to undertake the study. The effect of conidia of *C. lobatum* was evaluated against *An. stephensi*.

In this study, *C. lobatum* was isolated from soil near the Institute in Dayalbagh with a hair-baiting technique using Indian peacock (*Pavo cristatus* Linn) feather as

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bait[12]. For the preparation of a pure culture, a piece of all fungal colonies grown on the feathers was individually transferred with inoculation needle to petri plates containing Sabouraud dextrose agar (SDA). Chloramphenicol, 50 µg/ml, was added to SDA medium as a bacteriostatic agent[4]. Petri plates (containing SDA) with more than one fungal colony were recultured as needed to isolate a pure colony. After fifteen days of growth, *C. lobatum* colonies were tested for toxicity against *An. stephensi*. The isolates of fungi were identified according to the following authorities: *Chrysosporium* spp. according to Van Oorschot, *Aspergillus* spp. according to Raper and Fennell and other fungi according to Barnett[13–15]. *C. lobatum* was identified by the Institute's Mycology Division of the Botany Department.

A colony of *An. stephensi* was maintained throughout the year in the laboratory at a temperature of 25 ± 2°C, relative humidity of 70 ± 5% and photoperiod of 14 : 10 h (L : D). All the larval instars of *An. stephensi* were maintained in separate containers. Larvae were placed in double-distilled sterile water at pH 7.0. To counteract evaporation, water was added daily using sterilized glassware. Larvae were fed 0.4 ml/beaker of a 5% (w/v) autoclaved suspension of freeze-dried yeast in distilled water on day-1 and day-2.

The colonies of *C. lobatum* grown on Sabouraud dextrose agar for a period of fifteen days were used for the separation of conidia and mycelia[16,17]. The total yield of conidia from each plate was estimated using Neubaur haemocytometer. Serial dilutions were made of the conidial concentrate. Various dilutions of conidia were made so that the mortality will be between 20% and 95%.

Twenty *An. stephensi* larvae of 1st-, 2nd- and 3rd-instar were used in all the bioassays. Each instar was exposed separately to conidia of *C. lobatum*. Larvae were added to 750 ml beaker containing 500 ml test concentrations. Each of the instars were run at six different concentrations chosen to produce larval mortalities between 20% and 95% for calculating LC_{50} values. Every 12 h, all dead larvae were counted and removed from the test medium. Dead larvae were examined individually, under a microscope with a Pixera digital video (Model No-PSS-30142) for fungal infection. Assays were terminated at 72 h when mortality counts were made based on the number of live larvae remaining in each beaker. The moribund and dead larvae were plated in Sabouraud dextrose agar to culture the fungi grown on them. After fifteen days of growth, fungi were identified to prove the Koch's postulate.

Each assay was repeated five times on separate days. In controls, twenty larvae were put in beakers with 500 ml sterile double distilled water. Each instar was maintained separately. Larvae were fed the same food, which was given to experimental larvae. In all the cases controls were run with the experiments simultaneously. *Trichophyton ajelloi* was used as a positive control and the LC_{50} values were obtained within the fiducial limits[10]. Dosage-mortality responses were subjected to the probit analysis method of Finney[18] and the LC_{50} with 95% fiducial limits, slope and the regression lines were then computed using software SPSS and M. S. Excel.

A total of eleven fungal species in three genera were isolated from soil samples screened for the presence of entomopathogenic fungi using feathers of *Pavo cristatus* as bait. *Aspergillus* was the most frequently isolated genus and comprised five out of eleven species. Three species each of *Chrysosporium* and *Microsporum* were isolated. Colonies of *C. lobatum* attained a diameter of 30 mm after fifteen days of growth (Figure 1). Initially the colonies were white and later became pale gray with a powdery form. Terminal and lateral conidia developed simultaneously and were sessile. Initially, conidia were transparent, smooth and thin walled but later become reddish brown and thick walled (Figure 2).

In a preliminary efficacy test against larvae of *An. stephensi,* the mortalities of *Aspergillus* spp. were recorded below 20% at a concentration of 10^5 conidia/ml, whereas the mortalities of *Microsporum* spp. were observed bet-

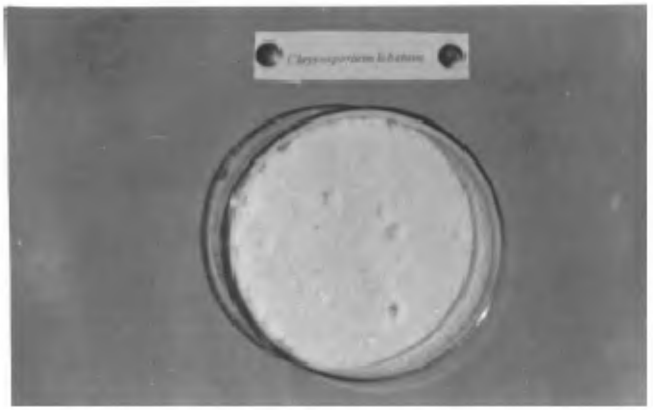

Figure 1. Front view of *C. lobatum* colonies after a growth of fifteen days.

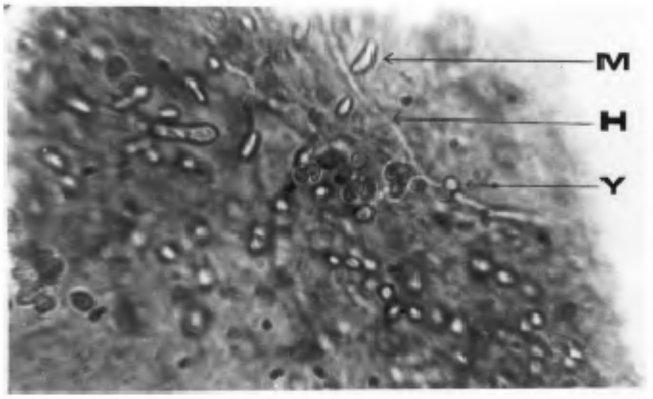

Figure 2. Colonies of *C. lobatum* with conidia and hyphae. M, Mature conidia; Y, Young conidia; H, Hyphae after staining with cotton blue (450 ×).

ween 24 and 30%. *C. pseudomerdarium* and *C. verruculatum* caused 40 and 32% mortality, respectively. However *C. lobatum* caused 100% mortality. Therefore in the present investigation we have targeted a detailed study on *C. lobatum*.

There was an appreciable difference in mortality among the instars of *An. stephensi* against *C. lobatum* observed. A comparison of the LC_{50} values between different instars of *An. stephensi* (Table 1) showed that LC_{50} of 3rd-instar larvae (0.13×10^3 conidia/ml) depicted about 10^3-fold and 70-fold less than that of 1st-(1×10^5 conidia/ml) and 2nd-instar larvae (9.37×10^3 conidia/ml) respectively.

The development of a biocontrol agent includes assessing its efficacy under laboratory conditions. Larval stages are usually more susceptible to fungi than adults due to a different nutritional state of the host[19]. Cooper and Sweeney[5] tested the 1st-instar larvae of *An. hilli* and *Culex quinquefasciatus* against *Culicinomyces clavosporus*[20] and reported their LC_{50} as 5.01×10^3 and 1.66×10^3 conidia/ml, respectively. Whereas the LC_{50} of conidia of *C. lobatum* against the 1st-instar larvae of *An. stephensi* was 500-fold higher than *Culicinomyces clavosporus*. However the 3rd-instar larvae of *An. stephensi* were most susceptible to *C. lobatum* as 1st-instar in case of *Culicinomyces clavosporus*.

Serit and Yap[21] have observed the efficacy of *Tolypocladium cylindrosporum* against 3rd-instar larvae of four mosquito species and reported that *Tolypocladium cylindrosporum* was most effective against *Mansonia uniformis* and least in *Aedes aegypti* in laboratory. The 3rd-instar larvae of *An. balabacensis* were 40-fold susceptible compared to *Aedes aegypti* against *Tolypocladium cylindrosporum*. However, the LC_{50} of *C. lobatum* against the 3rd-instar larvae of *An. stephensi* (0.13×10^3 conidia/ml) was recorded 3000-fold less than that of *Tolypocladium cylindrosporum* (4.76×10^5 conidia/ml) against *An. balabacensis*.

Balaraman *et al.*[6] extensively studied the efficacy of three fungi, viz. *Metarrhizium anisopliae*, *Beauveria tenella* and *Fusarium oxysporum* against three mosquito species, *An. stephensi*, *Culex fatigans* and *Aedes aegypti*. The LC_{50} values of *Metarrhizium anisopliae* (40×10^4 spores/ml), *Beauveria tenella* (125×10^4 spores/ml) and *Fusarium oxysporum* (400×10^4 spores/ml) against the 2nd-instar larvae of *An. stephensi* were 40-fold, 130-fold and 460-fold higher than that of *C. lobatum* (9.37×10^3 conidia/ml) against 2nd-instar larvae of *An. stephensi*. However, the 3rd-instar larvae of *An. stephensi* against *C. lobatum* were more susceptible than the 1st- and 2nd-instar larvae. This was contradictory to the result recorded by Balaraman *et al.*[6]. Kramer[22] evaluated the efficacy of *Lagenidium giganteum* against 3rd-instar larvae of *An. punctipennis* and scored more than 90% mortality at a concentration of 3×10^6 spores/ml. It is hard to compare the efficacy of *Lagenidium giganteum* with the present study because the LC_{50} values were not recorded. The fungus *C. lobatum* was recovered from both the moribund and dead larvae, which confirm the postulates of Koch.

Table 1. Probit equations and susceptibilities of different instars of *Anopheles stephensi* against conidia of *Chrysosporium lobatum* (x is the log concentration of conidia of *C. lobatum* in conidia/ml)

	First-instar	Second-instar	Third-instar
Probit equation	$3.559 + 0.288\,x$	$3.729 + 0.320\,x$	$4.335 + 0.315\,x$
LC_{50} (conidia/ml)	1×10^5	9.37×10^3	0.13×10^3
Fiducial limits (95%)	0.9×10^5– 1.2×10^5	9.10×10^3– 9.65×10^3	0.05×10^3– 0.55×10^3

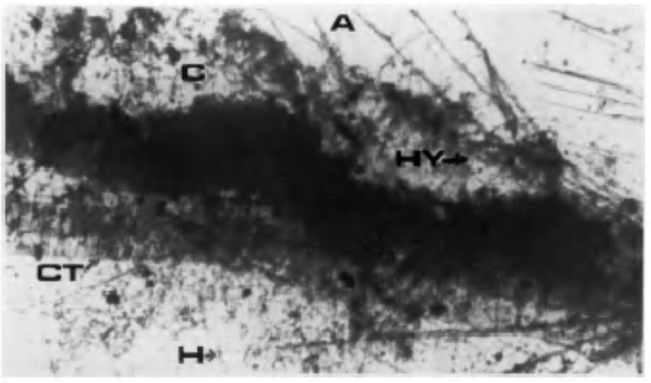

Figure 3. The 3rd-instar larva of *An. stephensi* infected with conidia of *C. lobatum*. CT, Cuticle; HY, Haemolymph; C, Conidia; A, Abdominal region after staining with cotton blue (450 ×).

The cuticle of the abdominal region was densely observed with conidia and hyphae (Figure 3). The conidia entered the haemolymph through the cuticle layers of the abdominal region. The ventral brush and anal papillae of the larvae were infected with conidia and hyphae. This is similar with the infection of deuteromycetous fungus *Tolypocladium cylindrosporum* against *Culex pipens*[23]. However, the anal papillae of the *An. gambiae* were less infected with zoospores of *Lagenidium giganteum*[24]. The differences in infection from one fungus to other occurred due to the affinity of fungi to cuticle which varied from one region to other. Thus we can conclude here on the basis of the LC_{50} values of *C. lobatum* that the 3rd-instar larvae were far better equipped to control vectors than the previously reported fungi such as *Tolypocladium cylindrosporum* and *Lagenidium giganteum*. Apart from this, it is further required to validate these findings during field trials in different geoclimatic zones.

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ACKNOWLEDGEMENTS. We thank the Director, Dayalbagh Educational Institute and also to the All India Council for Technical Education for the financial support to S.P. (212&245/Biotechnology/1998&1999/Soam Prakash) to conduct the research. S.S.M. acknowledges Council of Scientific and Industrial Research, New Delhi for awarding Senior Research Fellowship (9/607). We acknowledge Dr J. N. Srivastava and Mr Manish Mathur of Mycology Division, Department of Botany for their help in identification of fungi. We thank the reviewer for the constructive comments.

Received 3 June 2002; revised accepted 18 October 2002

Possible mechanism and implications of phenolics-mediated reduction of XTT (sodium, 3′-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-*bis*(4-methoxy-6-nitro) benzene-sulfonic acid hydrate)

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Sodium, 3′-[1-[phenylamino-carbonyl]-3,4-tetrazolium]-*bis*(4-methoxy-6-nitro) benzene-sulfonic acid hydrate (XTT)-based procedure is a simple method to estimate perhydroxyl/superoxide radical acid–base pair ($HO_2^{\cdot-}/O_2^{\cdot-}$) owing to the solubility of the reduced formazan. We show here that phenolic compounds, catechins and catechols, reduced XTT. Addition of superoxide dismutase in the medium enhanced the rate of oxygen consumption as well as XTT reduction. Based upon the data on XTT reduction and oxygen consumption, the mechanism of phenolics-mediated reduction has been proposed. Data on the limitation of adoption of the XTT-based procedure to estimate superoxide radicals in plant tissues is presented. Possible implications of the data have been discussed in wider perspectives.

ASSESSMENT of superoxide radical ($O_2^{\cdot-}$) in a living system is challenging due to rapid self-dismutation of the moiety and ubiquitous presence of an exceptionally proficient enzyme, superoxide dismutase, that catalyses dismutation of $O_2^{\cdot-}$ too efficiently to allow its accumulation and hence measurement (rate constant for the self-dismutation reaction = $< 2 \times 10^5\ M^{-1}\ s^{-1}$ as described by the reaction $O_2^{\cdot-} + O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$; rate constant for the enzyme catalysed reaction is $2 \times 10^9\ M^{-1}\ s^{-1}$, a value near the diffusion limit[1,2]). The presence of an unpaired electron in the outer orbital of $O_2^{\cdot-}$, makes the moiety reactive, and this upon reacting with hydrogen peroxide gives rise to another highly reactive hydroxyl radical ($^{\cdot}OH$) through Haber–Weiss reaction[3]. These reactive species of oxygen react with proteins, nucleic acids, lipids and virtually with all the biomolecules to cause severe damage to the cell[4,5]. Under stressful environment, $O_2^{\cdot-}$ generation exceeds beyond the dismutation capability of the cell to force the system to undergo oxidative stress[6]. Therefore, it becomes essential to quantify $O_2^{\cdot-}$ in a living system.

Various available procedures for the purpose include chemiluminescence[7], spin trapping/electron paramagnetic resonance[8], electron spin resonance[9], and the reduction of

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