variation in vertical eddy diffusion which is being carried out and the results will be published elsewhere.


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# Watermolds: Potential biological control agents of malaria vector *Anopheles culicifacies*

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***Leptolegnia caudata* and *Aphanomyces laevis* (Saprolegniales: Oomycetes) were found for the first time as naturally occurring parasites of mosquito larvae, causing high mortality in *Anopheles culicifacies* larvae. Artificial inoculation under laboratory condition revealed *L. caudata* more pathogenic, caused 100% mortality within 7 days of inoculation as compared to *A. laevis* (70% mortality after 10 days). None of these is pathogenic to any crop and have no toxic effect on aquatic fauna, and thus, can be proposed as mosquito control agents, alternative to chemical insecticides. Mass culture of these pathogens, preferably *L. caudata* can be applied in major larval habitats for long term, non-hazardous, economically sustainable control of malarial vector *A. culicifacies*, thereby malarial transmission.**

MALARIA, caused by *Plasmodium* spp. (Protozoa) is the most important insect-transmitted human disease of the tropics and subtropics. Two to three million people die from malaria each year[1]. In India, more than two million clinical cases are reported annually[2]. Of the four recognized human parasites that cause malaria, three (*P. falciparum*, *P. malariae* and *P. vivax*) are found in India, and *P. falciparum* as elsewhere[1] is most pernicious[2], whereas *Anopheles culicifacies* (Diptera: Culicidae) is the most important vector and accounts for about 70% malaria cases in the country[2].

The super-resistant strains of *Plasmodium* spp. which appeared in Colombia and Thailand in the seventies are spreading. Because of these drug-resistant strains of parasites and insecticide resistance of the vectors[1–5], malaria has posed a renewed threat, and thus has warranted research for novel and cost-effective, non-hazardous control measures[2,6]. Besides larvivorous fishes[1,7,8] and nematodes, several species of viruses, protozoa and fungi[9] are known to parasitize mosquito larvae, but none of these proved efficient as well as economical for malaria control[9]. However, *Lagenidium gigantum*, a zoosporic fungus, has been registered for its use as a biocontrol agent of mosquitoes belonging to genera *Anopheles*, *Aedes*, *Culex*, *Culiseta* and *Psorophora*[10]. In view of the malaria problem, a study was conducted to explore pathogenic association of watermolds with mosquito larvae in a malaria-prone zone of UP hills, in order to exploit it for malaria control.

Sampling of *Anopheles* larvae was done during the rainy season from irrigation channels, paddy fields and from river Gaula, between Haldwani and Santipuri in Nainital district, UP (29°55′N and 70°40′E; 400 msl), with the technical assistance of Malaria Research Centre, Haldwani. Invisible disease symptoms were observed with a powerful hand lens and compound microscope. Symptomatic/dead *A. culicifacies* larvae were immediately brought to the laboratory and the associated fungal species were isolated by placing boiled hemp seed (*Cannabis sativa* L.) halves in close contact with larvae, treated with 0.01% potassium tellurite solution (v/v) and duly rinsed with sterilized water. Fungal species from water were isolated by baiting with boiled hemp seed halves. Treated larvae were also inoculated onto potato dextrose agar media (PDA). The inoculated PDA plates and baited petri plates were incubated at 20 ± 1°C and the unifungal culture of the pathogen(s) that colonized on the baits and on PDA was prepared; purified following Raper[11]. Stock cultures were maintained on PDA for further investigation.

Pathogenicity of the isolates was tested under

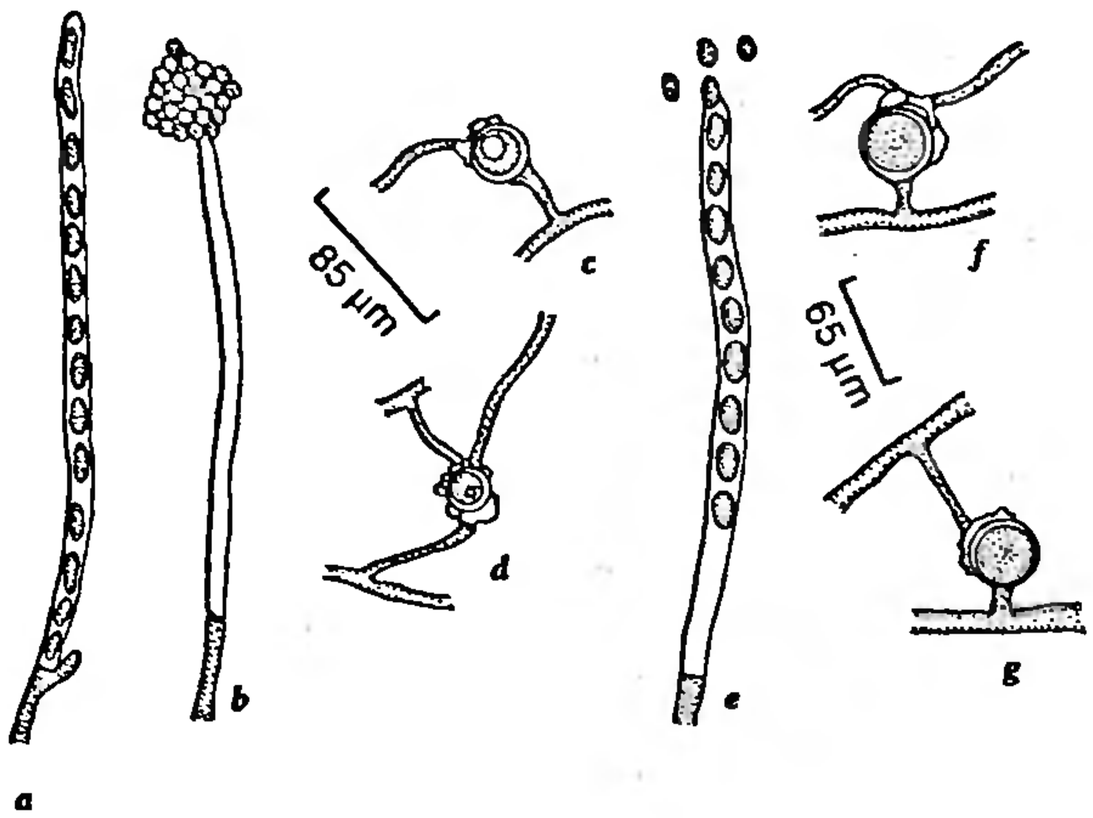


**Figure 1.** *Aphanomyces laevis*: *a, b*, Zoosporangium-releasing zoospores; *c, d*, Mature oogonia with antheridial attachment containing oospores. *Leptolegnia caudata*: *e*, Zoosporangium-releasing zoospores; *f, g*, Oogonia containing oospores.

laboratory conditions on *A. culicifacies* larvae. Two pure 2-day-old colonies on hemp seed halves (inoculum) of the isolates were kept separately in 250 ml beakers, containing 100 ml sterilized water and left till 4 days for zoosporogenesis. The zoospore concentration of the test isolates was maintained at about $7 \times 10^3\ l^{-1}$, as described by Tiffney[12], before 20 II instar larvae of the test vector from laboratory culture stock were released in each beaker. A control without the inoculum was also run simultaneously for comparison. Three replicates were prepared for each isolate. The larvae were fed on a mixture of dog biscuits and yeast tablets (4 : 1). Mortality was counted after 24 h up to the emergence of adult. The pathogen(s) associated with the dead larvae was reisolated and compared with the original inoculum.

Larval mortality was recorded higher in the paddy fields than in the other habitats investigated. This can be attributed to the high larval density coupled with inoculum (zoospores) accumulation in the field (zoospore density $5–15 \times 10^3\ l^{-1}$). Mycelial growth was apparently visible on a few larvae.

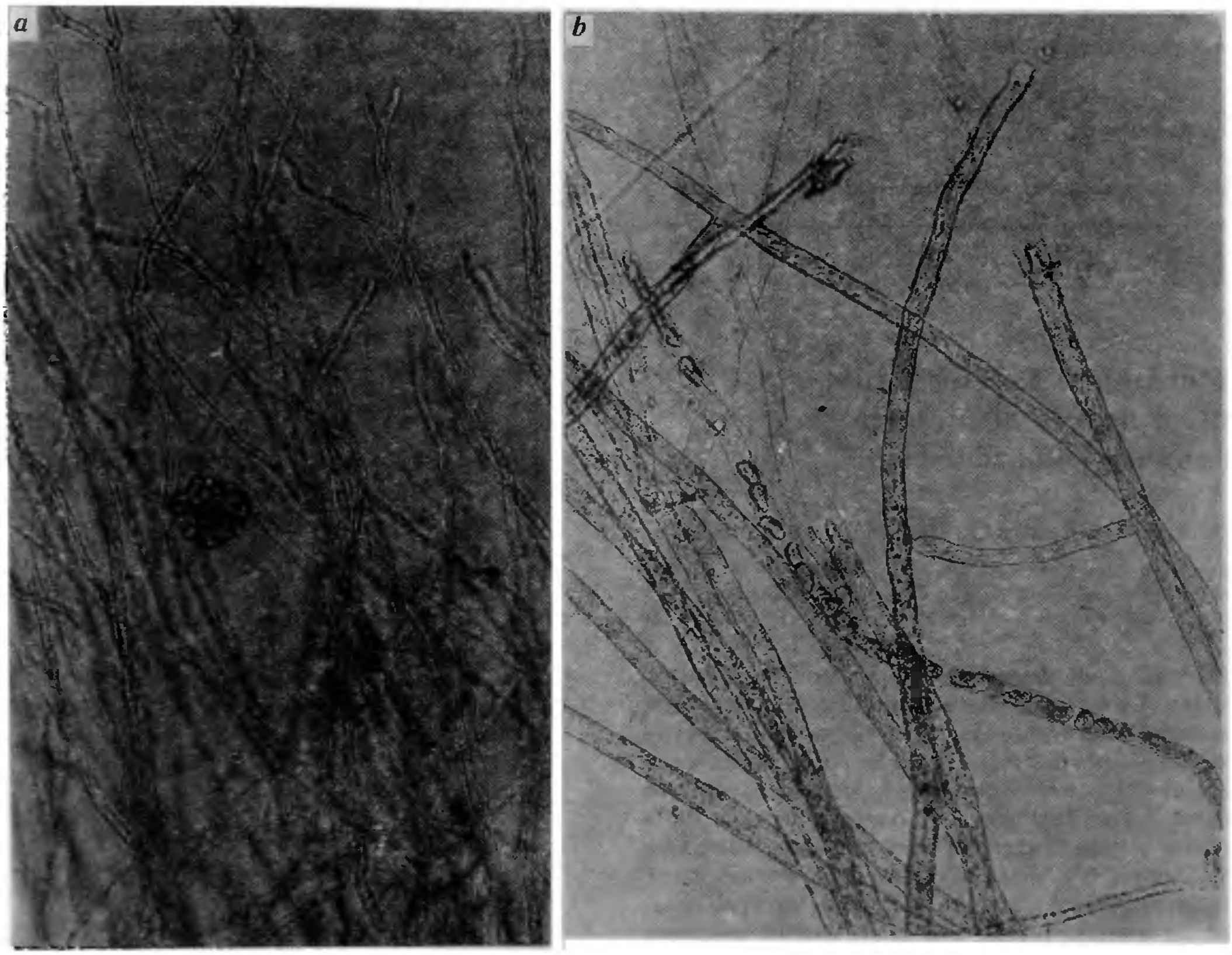


**Figure 2.** Mycelium- and zoosporangium-releasing zoospores; *a*, *Aphanomyces laevis* and *b*, *Leptolegnia caudata*.

**Table 1.** Pathogenicity of *Leptolegnia caudata* and *Aphanomyces laevis* on *Anopheles culicifacies* larvae

| Fungal species | Percentage of mortality after days | | | | | | | | | | Pupal emergence (%) | Adult emergence (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | |
| *L. caudata* | 32 | 53 | 60 | 72 | 85 | 87 | 100 | — | — | — | — | — |
| *A. laevis* | 00 | 25 | 37 | 45 | 50 | 63 | 63 | 70 | 70 | 70 | 30 | 17 |
| Control | 00 | 00 | 00 | 00 | 07 | 07 | 07 | 07 | 07 | 07 | 93 | 93 |

*Leptolegnia caudata* de Bary, *Aphanomyces laevis* de Bary (Figures 1 and 2) and *Saprolegnia* sp. were isolated from dead larvae. Of these, *L. caudata* was constantly isolated from dead larvae in the region. These are common species, frequently found in both tropical and temperate climate in different habitats[12]. However, the present communication reports for the first time of their pathogenic role in mosquito larvae. Pathogenicity test revealed that *L. caudata* and *A. laevis* parasitized *A. culicifacies* larvae; the former being more virulent (100% mortality) than the latter (70%) (Table 1). The infection established itself within 24–36 h of larval introduction into the pots. Infection occurs when zoospores encyst on the larval cuticle and form a germ tube which subsequently develops and penetrates into the host, leading to the destruction of tissues. Besides, formation of toxic substances and dilution of body fluid resulting from destruction of protective larval cuticle through enzyme action, may result in mortality[14,15]. Once fungi invade the hemocoel, the host may be killed by some combination of mechanical damage produced by fungal growth, nutrient exhaustion and toxicosis[16]. However, higher mortality in laboratory inoculation than in nature may be attributed to the composition of sterol in mosquito larvae which promote zoosporogenesis[17]. As reported in the present study, several species of zoosporic fungi, including *Coelomomyces* spp.[9,17–20], *Lagenidium giganteum*[10,14], and *Leptolegnia chapmanii*[21] have been reported to parasitize mosquito larvae.

It has been suggested that malaria cannot be dealt with as a single and uniform broom being a worldwide problem, susceptible to one global control strategy[6]. Since *A. culicifacies* takes 7–8 days to produce adult from egg stage, even under optimum condition, and *L. caudata* and *A. laevis* caused up to 100 and 70% mortality, respectively, within this period, thus, they hold potential to suppress population of *Anopheles* larvae. Further, unlike most of the zoosporic fungi, neither of these is reported pathogenic to paddy and other crops[13] and can be cultured and stocked with ease, besides, possessing wide adaptability from aquatic to semiaquatic habitats and ability to perennate. Therefore, mass culture reared *in vitro*, of these pathogens, preferably *L. caudata* can be applied to check mosquito population in breeding sites, thereby transmission of malaria, without hazard. Two types of spores can be applied simultaneously in the habitats harbouring high larval density; application of both zoospores (asexual) and oospores (sexual) of the pathogen(s) will be helpful in both immediate and long-term control of *A. culicifacies* larvae under varying environmental conditions[22]. Although reducing population density of malaria vector by means of larviciding is generally an inefficient way of affecting transmission, however, when a large proportion of larval habitats can be properly identified and targeted, control can be effective.


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