plantation crops and other agricultural residues. Establishment and enhanced activities of *A. magna* in organically-managed arecapalm farms or mixed plantations indicate the rejuvenation of soil-fertility status. These millipedes are sensitive to water-deficit and fail to overcome the limitation of even a single edaphic factor (particularly the soil texture and litter thickness); thus enhancement of their population in any farmyard denotes the restoration of suitable conditions for their natural breeding, establishment and activities. Their sensitive nature may also serve as an authentic measure as indicators of pollution-free status of soil in agricultural, plantation and horticultural farms. Hence, there is an urgent need to study the distribution, conservation strategies, lifecycle, rearing and compost production by *A. magna* found in the Western Ghats and the west coast of India.

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Study of the microflora associated with coconut eriophyid mite as a preliminary step towards pathogen isolation

The coconut eriophyid mite *Aceria guerreronis* Keifer is a serious coconut pest in several states of India. Hidden habitat under the nut bracts, high reproductive rate and a short lifecycle of 10–11 days pose difficulties to control this acarine pest by insecticides, and thorough investigations on parasites, predators and pathogens are essential[1]. Among the pathogens, the targeted group is the fungi, as theoretically, the soft-bodied nature of acari and their inhabit environment with humid microclimates make them good hosts of fungal pathogens[2,3]. However, finding the microflora associated with the insects and their significance may lead to isolation of possible pathogen(s)[4]. The work done on microorganisms associated with the eriophyid mite from the coconuts collected from five districts of Kerala, with the aim to isolate putative pathogen(s) is presented here.

Three to nine-month-old coconuts with different degrees of mite-induced damage stages were collected from Alappuzha, Ernakulam, Kottayam, Thrissur and Kollam districts of Kerala during 1999 September–2000 November from the coconut gardens managed agronomically with chemical fertilizers and pesticides, organics only, chemicals and organics, and unattended (neglected) gardens.

The nuts were brought from the gardens to the laboratory in an open container, as it was observed that bringing them in plastic bags had suffocated the mites, resulting in their mass migration out of their habitat. A total of 286 nuts were screened during this study. For the observation of the mite colonies and any microbial growth, the upper whorl of bracts of larger nuts (more than 5 months old) was carefully removed using a blunt forceps. In case of the younger nuts (3–4 months old), if removal of the upper whorl of bracts was not done carefully, usually the top of the nut broke spilling the contents. Once the upper whorls were removed, the lower whorls could be pried open easily. Yet, during the removal of the bracts, injury was caused to the mites because of the entry of the forceps in the mite colony, many a times resulting in crushing and death of the host. Casualty of the pest because of such handling had to be clearly discerned from other modes of death. Smaller nuts were observed directly using a stereo microscope (Leica Wild M-10) for dead patches of the mite colonies. Slices of the meristematic portion of the larger nuts were used for similar observations. The microorganisms associated were isolated by plating the unsterilized dead, surface-sterilized (with 2.5% $NaOCl_2$, 2 min) dead, unsterilized live, surface-sterilized live eriophyid mites and other mites/insects too from the same habitat on Sabourauds, potato dextrose and Richards Synthetic (for fungi), nutrient (for bacteria) and Kenknights and Munaiers (for actinomycetes) media, incubated at $28 \pm 2°C$ and obser-

ved every 24 h. The microorganisms were purified and tested in the laboratory for pathogenicity against the mites.

Under the microscope, it was observed that the mites move away swiftly from the field of observation, trying to escape the incident light. Colonies of dead mites with shrunken body without any external fungal outgrowths were observed towards the outer edge inside the lower whorls of the bracts. Rarely, colonies with brown discolouration, with or without the association of any fungal growth, as mentioned by Hall and coworkers[5] or with outgrowth of silvery white strands typical of *Hirsutella* infection[6] were observed.

From the unsterilized dead mites, *Fusarium moniliforme* and other *Fusarium* spp (with a frequency of 50–58%) followed by various species of *Aspergillus* (30–40%), *Penicillium* (20–30%) and *Mucor* (7–10%) were isolated. *F. moniliforme* from eriophyid mite[5], *Aspergillus* sp. from cassava green mite, *Mononychellus tanajoa*[7] and *Penicillium insectivorum* from acarine host *Ixodus ricinus* from erstwhile USSR[8] have already been reported. In addition to the fungal flora, bacterial isolates of *Pseudomonas* spp (≤ 5%) and *Bacillus* spp (≤ 5%), an actinomycetes (≤ 5%) and a yeast (10–15%) were also obtained from the unsterilized mite bodies (Table 1). In their pathogen isolations, Hall *et al.*[5] also obtained an actinomycetes from this mite. From surface-sterilized mites, *Scopulariopsis brevicaulis* (earlier *Scopulariopsis flava*) (Sopp.) Morton & Smith and *Cladophialophora* sp. De Hoog were obtained (Table 1). Association of *S. brevicaulis* from two mite species, *Acotyledon krameri* and *Tyrophagus putrescentiae* has been reported earlier[9]. *Scopulariopsis* has also been reported to be a pathogen of mole crickets, a minor pest of tea in India[10].

It was observed that mites plated from the nuts sprayed with wettable sulphur (0.4%) on the bract portion, failed to produce any fungal growth even after 3–4 days of incubation. However, subsequently the plated perianth tissues produced few colonies of *Fusarium* and *Aspergillus* species. This observation of wettable sulphur being fungicidal/fungistatic is corroborated by earlier workers[11].

Microfloral diversity (Table 2) and population were high (65–70%) in the mites on the nuts collected from organically amended and unattended gardens than those from gardens managed with inorganic fertilizers and pesticides (30–40%). These observations indicate that several fungi and bacteria besides some actinomycetes and yeasts are associated with the acarine pest.

Pathogenicity of the isolated microbes was assayed in the laboratory on detached nuts with the cut-end of the rachis wrapped in sterile water-soaked cotton. Homogenized spores and hyphal mass/cell suspensions of bacteria (with 0.05% Tween-20) were sprayed on the nuts. Three replications per treatment were maintained, with ten nuts per replication. Five days after the microbial spray the nuts were observed for the total live and dead mite population, using the washing technique. The results indicated that *S. brevicaulis* gave a maximum of 27% mortality of the mites followed by *Cladophialophora* sp., 14%. *F. moniliforme*, *A. niger* and *A. flavus* gave around 10%, whereas bacteria gave less than 5% mortality. The control treatments also showed 5–6% dead mites (Table 3). By plating these experimental dead mites without surface-sterilization, *Fusarium* spp at 60–70%, *Aspergillus* spp at 45–50%, and yeast at less than 10% frequency could be

Table 1. Frequency of isolation of different microorganisms from the coconut eriophyid mite

Source	Isolated microorganism	Frequency of isolation (%)
Unsterilized dead eriophyid mite	*Fusarium moniliforme* *F. solani* *Fusarium* spp	50–60
	Aspergillus niger *A. flavus* *Aspergillus* spp	30–40
	Penicillium spp	20–30
	Mucor spp	7–10
	Scopulariopsis brevicaulis	< 5
	Cladophialophora sp.	< 5
	Pseudomonas spp	< 5
	Bacillus spp	< 5
	Unidentified bacteria	< 5
	Actinomycetes (unidentified)	< 5
	Yeast (unidentified)	10–15
Surface-sterilized dead eriophyid mite	*Scopulariopsis brevicaulis*	5
	Cladophialophora sp.	< 5
Unsterilized live eriophyid mite	*Fusarium* spp	10
	Yeast (unidentified)	< 5
Surface-sterilized live eriophyid mite	None	0
Other insects*	*Fusarium* spp	45–50
	Aspergillus spp	40
	Penicillium spp	10
	Yeast	15
	Actinomycetes	< 5

*Associated mites and insects (unidentified).

Table 2. Microfloral diversity of mites of coconut collected from different gardens

Source of nuts	Microflora
Organically amended or unattended garden	*Fusarium*, *Aspergillus*, *Penicillium*, *Mucor*, *Scopulariopsis* sp., *Cladophialophora* sp., *Pseudomonas* spp, *Bacillus* spp, yeast and actinomycete
Inorganic fertilizers amended garden	*Aspergillus*, *Fusarium*, *Scopulariopsis* sp., *Pseudomonas* spp, *Bacillus* spp, yeasts and actinomycete
Pesticides amended garden	*Aspergillus*, *Fusarium*, actinomycete, yeasts

Table 3. Pathogenicity trials of microbial isolates on detached eriophyid mite-affected coconuts

Microorganism	Mortality of mites (%)
F. moniliforme	9.6
A. niger	7.4
Penicillium spp	4.7
A. flavus	8.0
S. brevicaulis	27.0
Cladophialophora sp.	14.0
Pseudomonas sp.	4.9
Bacillus sp.	4.1
Control	5.7

Results are an average of three replications with each replication having 10 nuts (3–4 months old, with distinct mite-infestation symptoms).

re-isolated, irrespective of the kind of the treatment that the nuts were subjected to. Surface-sterilized mites failed to produce fungal/bacterial growth, except in the case of *S. brevicaulis*-treated nuts, where the same fungi could be re-isolated. This indicates that *S. brevicaulis* has the ability to be a true pathogen; however, its capacity to cause 25–27% mortality may not be very useful to suppress the mite.

The work broadly gives an idea of the common microorganisms that are associated with the mite body. Pathogenicity assay conducted indicates the possibility of a pathogen establishment against the mite in development. However, a thorough investigation is necessary to screen more microbes from different mite-infested areas in order to reveal the significance and potency of the microorganisms isolated for the management of this pest.

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