Research Article

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Isolation of microbial pathogens of *Aceria guerreronis*, the coconut eriophyid mite

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Abstract

A multi-disciplinary team consisting of microbiologists, pathologists, entomologists and nematologists for the isolation of possible pathogens, specifically *Hirsutella thompsonii,* from the coconut eriophyid mite, *Aceria guerreronis,* conducted a collaborative study during the month of May 2001, when the mite population was at its peak in Kerala. One hundred and sixty mite-infested coconuts were collected from Thrissur District for this purpose. Isolation of microbes from these nuts was done by plating the mite-wash and its serial dilutions, surface-sterilizing the whole nut and then plating the mites and the perianth tissue and plating of the individual mites. It was observed that the actinomycetes constituted the predominant microflora (seven isolates) during this period followed by yeasts (four isolates); fungi (three isolates) and bacteria (two isolates). Pathogenicity trials in the laboratory revealed that fungi caused mortality to the mites in the range of 10-20%; actinomycetes, 15-20% and bacteria, less than 10%. Yeasts were non-pathogenic to the mites. The well-known mite pathogen *Hirsutella thompsonii* was not encountered/isolated during this study.

Key words: *Aceria guerreronis* (eriophyid mite), coconut, *Hirsutella thompsonii*, microbial pathogens

Introduction

Microbes are one of the most diverse groups of organisms inhabiting the earth. This diversity makes them an ideal choice to work with for improvement of agriculture. One of the important areas where microbes make an impact is the ecologically safe and specific management of pests. However, this involves obtaining virulent pathogen(s) for which extensive isolations and screening of a wide variety of the microorganisms is necessary (Gopal and Gupta, 2001). A sound understanding of the diversity and population dynamics of the microbes associated with the host and its habitat, becomes paramount for such studies. The eriophyid mite, *Aceria guerreronis*, has become an important pest of coconut since its observation in 1998. It has been reported that microorganisms, especially the fungus *Hirsu thompsonii*, is the long-term solution for managem this acarine pest. In order to isolate possible patho associated with this mite specifically *H.thompson* rapid-fire and multi- disciplinary effort invol microbiologists, pathologists, entomologists nematologists was undertaken during the month of 2001, when the mite population was at its peak in K Results of this effort are outlined in this rese communication.

Materials and Methods

Around 160 mite-infested nuts were collected KAU experimental farm, Vellanikkara and other of Thrissur District of Kerala. The nuts were mos 3-9 months age with varying degrees of mite da

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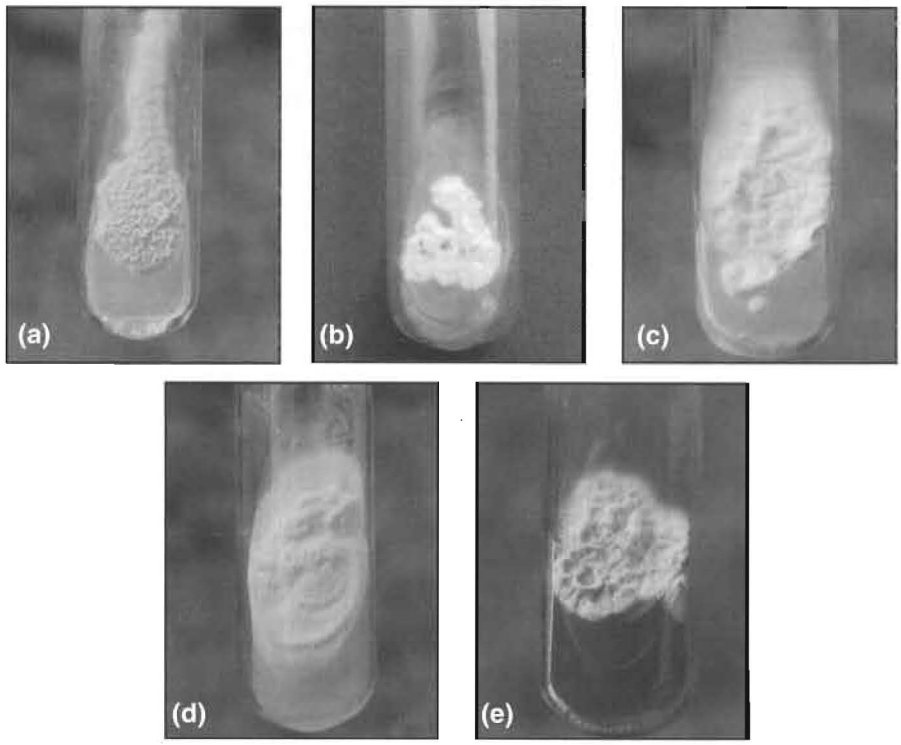
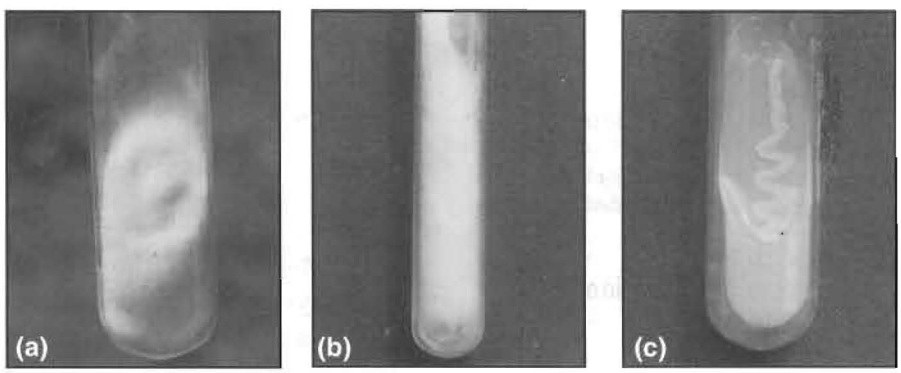
were brought to the laboratory and grouped in such that each group had nuts representative of the areas from where they were collected. These nuts were distributed to the different discipline ers. Isolation of the microorganisms was done by llowing methods : (a) plating of mite washings as (b) by serial dilution of mite washings, (c) surface izing the whole nut and then plating the mites and ith tissue and (d) plating of individual mites. ent agar, potato dextrose agar and Kuster's agar media were used for isolation of different groups of microorganisms (bacteria, fungi and actinomycetes). The plates were incubated at 30 °C and the resultant colonies were purified. Pathogenicity bioassays with the purified microbes were conducted on the mite colonies by spraying their cell suspensions, grown in their respective growth media, on the detached nuts. The control nuts were applied with sterilized broth only. The mortality of the mites was recorded by washing technique, 5 days after the application of the treatments.



l. Actinomycetes isolates giving more than 17.5% mortality of mite

EM Act 2 (isolated from Kodagara, Thrissur from an unattended coconut garden)
EM Act 4 (isolated from Aloor, Thrissur from an organically maintained coconut garden)
EM Act 5 (isolated from Aloor)
EM Act 6 (isolated from Instructional Farm plot, KAU, Thrissur)
EM Act 7 (isolated from Vattakad, Thrissur, from a garden which was sprayed with neem oil-garlic mixture)



Fungi and bacteria isolated from eriophyid mite

EM Fungi (*Fusarium* sp isolated from mite and perianth tissue of coconut from instructional Farm plot, KAU, Thrissur)
EM Fungi (*Fusarium* sp with pinkish pigmentation, giving pathogenicity of 18.5%, isolated from Kodagara, Thrissur)
EM Bacterium (unidentified, isolated from CPCRI, Kannara, Thrissur)

Results and Discussion

Bucher (1960) reported that screening of the micro flora associated with the insects and their significance may lead to isolation of possible pathogens. Isolations made from the coconut eriophyid mite during the peak infestation period i.e. April to May (Anon., 1999) revealed that actinomycetes were the predominant micro flora with 7 types of isolates obtained with an isolation frequency of 54%. Yeasts were the second dominant group of microbes with 4 types of isolates and an isolation frequency of 40%. The fungi could be isolated at a frequency slightly higher than 30%, and *Aspergillus*, *Penicillium* and *Fusarium* were the commonest fungi observed. The bacterial isolates were obtained at the least frequency (10- 20%) and *Serratia marcescens* was one of the bacteria identified from the isolations. Detailed results are presented in Table-1. The succession of the micro flora association was not acutely dependent on the infestation stages of the mite. In the early stages of the mite colony development, rarely any microbial association was observed. With the advancement in infestation, yeasts were the earliest to be isolated. As the infestation reached the middle stage with browning of the nuts visible externally, fungi, actinomycetes and bacteria started appearing during the isolations.

Table 1 Types and frequency of microorganisms isolated from coconut eriophyid mite during its peak infestation season (May, 2001)

Type of microorganism	Frequency of individual isolation (%)	Average frequency (%)
Actinomycetes		
EM*-Act1	45.00	
EM-Act2	50.00	
EM-Act3	70.00	54.00
EM-Act4	30.00	
EM-Act5	55.00	
EM-Act6	60.00	
EM-Act7	70.00	
Fungi		
Fusarium sp	30.00	
Aspergillus sp	45.00	32.00
Penicillium sp	20.00	
Yeasts		
EM-Yeast1	30.00	
EM-Yeast2	45.00	
EM-Yeast3	35.00	40.00
EM-Yeast4	50.00	
Bacteria		
Serratia marcescens	20.00	17.50
Unidentified bacterium	15.00	

* EM stands for eriophyid mite

It was noted that while plating the mites, peri portion or nut tissue, the actinomycetes and fungi growing in abundance. However, when washings of mites were plated, *Serratia marcescens* dominated plates (Table 2). Yeasts were seen to grow with ease in both the plating techniques. Our obervations supportive of earlier reports of isolation of *Fusarium* actinomycetes from coconut eriophyid mite (Hall 1980). *Aspergillus* sp. and *Penicillium insectivorum* been reported to be pathogenic to cassava green *Mononychellus tanejoa* (Bartowski *et al.*, 198 *Ixodus ricinus* (Kalsbeek *et al.*, 1995). Isolation bacteria - *Pseudomonas* and *Bacillus*, yeasts actinomycetes were also reported in our earlier st (Gopal *et al.*, 2002), but *Serratia marcescens* is observed to be associated with the mites for the firs Another important observation is the preponderan the actinomycetes association with the mite duri summer period and when the mite infestation is peak. Actinomycetes are known to flourish in temperature and low moisture habitats. These condi could be prevailing in the nut microenvironment d the summers. However, there is enough moistu sustain some of the common genera of fungi th reported in this study. Notwithstanding the actinom domination during the summer months as reported paper, fungi are the overall preponderant microorga associated with this mite when observed throug year (Gopal *et al.*, 2002).

Table 2 Growth of microorganisms as per the isolation technique

Isolation technique	Microorganism
Simple mite plating	Actinomycetes, Fungi and yea
Plating mite from surface sterilized nut	Fungi, Actinomycetes and yeas
Plating of perianth pieces with mite colonies	Fungi, actinomycetes, yeasts
Plating of perianth pieces with mite colonies from surface sterilized nut	Actinomycetes, fungi, yeasts a
Plating of mite colony wash	*S. marcescens*, Unidentified bacteria, yeasts
Plating of mite colony wash after serial dilution	*S.marcescens*, Unidentified b yeasts
Plating of nut tissue with mite colony	Actinomycetes, fungi, yeasts
Streaking of mite(s) on agar plates	Actinomycetes, fungi, yeasts

Mortality of the mites in the pathog bioassays conducted with the actinomycetes bacteria and yeasts is outlined in Table actinomycetes gave a maximum mortality of 15 followed by the fungi with 10 to 20%. *S marcescens* gave a maximum of 10% mortality the 4 yeasts were found to be non-pathogenic.

organism to be designated as a potential pathogen, ecessary that it kills at least 50% of the target host. study, a maximum of 25% death of the mites could ained by the application of actinomycetes and the the microbes gave still lesser mortality. The ion is that besides the fungus *Scopulariopsis aulis* which gave 27% mortality of coconut yid mite (Gopal *et al.*, 2002), the actinomycetes also bring about the same as seen in this study.

Results of pathogenicity assay with isolated microbes on detached eriophyid mite affected coconuts

microorganism	Mortality of mites (%)
cetes	
Act1	17.5
Act2	23.5
Act3	14.5
Act4	25.0
Act5	22.0
Act6	18.5
Act7	23.5
rium sp	18.5
gillus sp	15.0
illium sp	9.0
Yeast1	2.0
Yeast2	2.5
Yeast3	0.0
Yeast4	1.5
ia marcescens	7.5
dentified bacterium	8.5
ent broth	5.0
dextrose broth	7.5
's broth	7.5
roth	2.5

an average of three replications with 5 nuts in each replication

The difference in the extent of mortality caused different category of the microbes is attributed to mode of action and their preponderance. The mycetes are reported to produce toxins like ctins, melbectimycin and spinosad, which have icidal/acaricidal properties. The bacterium *S. marcescens* kills the insects only on ingestion, however they also produce proteolytic enzymes capable of killing insects. Fungi as mentioned earlier are best suited for mite suppression.

Isolation of the well-known acarine pathogenic fungus *Hirsutella thompsonii* from the coconut mite collected from Thrissur area had been reported by Beevi *et al.*, (1999), and from Pollachi by Sreerama Kumar and Singh (2000). However, it was not encountered/ isolated in this study or during our earlier work (Gopal *et al.*, 2002). More thorough and regular isolation studies carried out through different weather periods and on coconuts collected from different mite-infested locations will definitely yield potential pathogen(s) for the long-term management of this important coconut pest.

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