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Occurrence of fungi and other microorganisms in association with the coconut eriophyid mite, *Aceria guerreronis*, in Kerala

V. S. AMRITHA[1] and S. PATHUMMAL BEEVI[2]

[1]College of Agriculture, Padannakkad, Kasaragod 671 328, Kerala, India.
E-mail: amritvs@gmail.com

[2]College of Horticulture, Vellanikkara, Thrissur 680 656, Kerala, India.
E-mail: pathummalbeevi@yahoo.com

ABSTRACT: The occurrence of *Hirsutella thompsonii*, the mite specific pathogen was confirmed and it was isolated from ten out of the twelve locations surveyed in Thrissur district of Kerala. Apart from the mite-specific pathogen, *Hirsutella* spp., other fungal pathogens such as *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. were isolated from the eriophyid mite infested coconut samples in the surveys conducted in Thrissur district, Kerala, during the period from July 2003 to June 2004. Natural occurrence of *H. thompsonii* contributed to 4.93 per cent. Acarifungal pathogens belonging to the genera *Hirsutella*, *Fusarium*, *Acremonium* and *Paecilomyces* were mostly isolated during the monsoon season and the actinomycetes during summer period in the entire four panchayaths. Pathogenicity to coconut eriophyid mite was proved for two species of *Hirsutella*, *H. thompsonii* and *H. kirchnerii.* The other non-specific fungi found to be pathogenic to mites were *Acremonium strictum, A. incoloratum, Fusarium lateritium, F. verticillioides, Paecilomyces fumosoroseus* and *P. lilacinus.* The wide spectrum of fungal pathogens associated with *A. guerreronis* indicates the significant role played by them in the natural suppression of the pest.

KEY WORDS: *Aceria guerreronis*, coconut eriophyid mite, fungal pathogens, *Hirsutella* spp.

INTRODUCTION

In recent years, the eriophyid mite, *Aceria guerreronis*, has become a serious pest of coconut in India and has threatened the very survival of copra and coir industry in Southern India. Beneath the shelter of the tightly pressed bracts, the mite is well protected from pesticide application. Alternative control measures involving ecofriendly biocontrol agents such as the entomopathogenic fungus specific to mites, *Hirsutella thompsonii* (Hall *et al*., 1980; Sampedro and Rosas, 1989) within the array of Integrated Pest Management principles have become imperative. The biological agents are well adapted to spread within the perianth and can be effectively used for mite control. Beevi *et al*. (1999) isolated the fungus *H. thompsonii* var. *synnematosa* from dead coconut mites in India. For any biocontrol programme to be successful, the first and foremost step is the identification of locally adapted strains of various potential biocontrol agents. Though *H. thompsonii* is reported to be the specific fungal pathogen associated with the mite, the association of other fungi should not be ruled out. The information generated in the present study on the association of other fungal pathogens will be helpful in identifying their utility for the biocontrol of coconut mite.

MATERIALS AND METHODS

The present study was conducted in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, during 2002-05. A survey was conducted in Thrissur district of Kerala State (10°31' N latitude, 76°13' E longitude) for one year during July 2003 – June 2004. Four panchayaths, *viz*., Pananchery, Madakkathara, Koorkenchery and Pariyaram of Thrissur district, were selected for the survey. From each panchayath, three locations/plots with a minimum of 100 full bearing coconut trees of uniform age (15-20 years) with heavy eriophyid mite infestation on nuts were selected randomly. Two mite infested nuts were taken from four randomly selected trees of each plot, one nut each from the 3rd and 4th bunches labelled from the top (approximately three and four months old with yellow triangular patches) which were reported to have the active live mite colony. The buttons excised were packed in polythene covers, tied well and brought to the laboratory.

Dead mite colonies were observed under a stereo zoom binocular microscope for different types of mortality symptoms. Isolation of the pathogen was done separately for the individual nut samples collected from individual palms of each location. Dead patches of mites showing mycoses were selected for fungus isolation in Potato Dextrose Agar (PDA) medium. The tepals (outer and inner) and the excised meristematic tissue of the nut surface containing mycosed mites were sterilized by dipping in sodium hypochlorite (0.5%) for five to ten seconds followed by rinsing in sterile water for three times. Tepals and nut surface were kept for drying on a sterile blotting paper in a sterile Petri plate. Dead mite patches were carefully lifted with a sterile micro-needle from the bracts and nut surface while observing under a stereo zoom binocular microscope and were placed directly on PDA within the marked circles. Petri dishes were labelled properly with the details of location, nut samples and date of inoculation. They were kept for incubation at 25°C and observed for fungal growth for up to two weeks.

Hirsutella thompsonii growth was identified based on the growth characters reported by Samson *et al*. (1980). Preliminary identification of the other acari fungal pathogens at the genus and species level was done by comparing the already available fungus collection maintained at the Department of Agricultural Entomology, College of Horticulture and from published records. The species identity was confirmed by International Mycological Institute (IMI), CABI Bioscience, United Kingdom. All the entomofungal pathogens obtained from the field collected mites were maintained in pure culture and were subjected to pathogenicity tests after their identification. Observations were also recorded on the number of samples yielding fungal species and their frequency of occurrence during the survey. Seasonal variation in occurrence of these entomofungal pathogens was also assessed.

The fungi repeatedly isolated from the dead mite patches were subjected to pathogenicity tests confirm their pathogenicity to coconut eriophyid mite and to prove Koch's postulate. For this, two to three months old nuts with single scar or triangular patch of damage which indicated the presence of an active colony of mites were selected. The respective fungal suspensions were prepared by mixing 1cm fungal culture disc (ten days old' taken from half the radial distance) with 10ml of sterilized water. Tween 80 (0.2%) prepared in sterile distilled water was added for uniform distribution of the spores. Tween 80, (0.2%) in distilled water was used as control. Using a fine syringe (1ml capacity), the fungal suspensions @ 60µl were injected separately into the space between the perianth and meristem on the nut surface exactly at the place of the mite infestation (Sreeramakumar and Anuroop, 2004). Nuts in each replication (three per treatment) were kept in a polythene cover filled with air and incubated in a BOD incubator at a temperature of 27°C. Microscopic observations on dead mites were taken 72 hrs after inoculation by removing the perianth and exposing the meristematic region of the nuts. Mycosed mites were mounted on slides, observed for fungal outgrowth under a phase contrast microscope. The dead mites were collected and subjected to reisolation. The fungal cultures thus obtained were compared with the original cultures.

Table 1. Fungi and other microorganisms isolated and identified from coconut eriophyid mite

Sl. No.	Fungal species	Accession No. IMI, CABI Bioscience, UK
1	*H. thompsonii* var. *synnematosa*	(IMI 3382199)
2	*Acremonium strictum* W. Gams	(IMI 392485)
3	*Acremonium implicatum* (J. Gilman & E.V. Abott) W. Gams	(IMI 392486)
4	*Fusarium lateritium* Nees	(IMI 392487)
5	*Fusarium verticillioides* (Sacc.) Nirenberg	(IMI 392488)
6	*Acremonium incoloratum* (Sukapure & Thirum) W. Gams	(IMI 392489)
7	*Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G.S.m	(IMI 392490)
8	*Acremonium terricola* (J.H. Mill; Giddens & A.A. Foster) W. Gams	(IMI 392663)
9	*Ramichloridium subulatum* de Hoog	(IMI 392664)
10	*Acremonium* spp.	(IMI 392665)
11	*Acremonium* spp.	(IMI 392666)
12	*Acremonium* spp.	(IMI 392667)

The fungal cultures were identified (Table 1) up to the species level from various institutions namely Centre for Agriculture and Biosciences International (CABI), UK; Kerala Forest Research Institute (KFRI), Peechi; College of Horticulture (COH), Vellanikkara. The pure cultures of the organisms were maintained on PDA slants.

RESULTS AND DISCUSSION

Of the 919 nut samples collected for fungus isolation from the four panchayaths, only 45 samples yielded *H. thompsonii*. Maximum number of *Hirsutella* isolates was obtained from the Koorkenchery panchayath (16 nos.) where as only two nut samples of the Pananchery panchayath yielded *H. thompsonii* (Table 2). In a study conducted by Sreeramakumar *et al*. (2001b) in three districts of Karnataka (Bangalore, Rural Mandya and Kolar) and one in Tamil Nadu (Coimbatore), 6.85 per cent of the mite infested nut samples yielded *H. thompsonii.* Mite mortality and *Hirsutella* isolation was found to be coincidental with the live mite population. In addition to the commonly occurring acaropathogen, *H. thompsonii*, a few other fungi coming under the Hyphomycetes and bacteria were also isolated consistently from dead coconut mites. Fungal species belonging to *Fusarium, Acremonium* and *Paecilomyces* and bacteria with mycelial growth, *Actinomycetes* were also isolated from various locations of the four panchayaths (Table 2). A total of 98 nut samples yielded *Fusarium* indicating three species, *F. lateritium* (69 nos.), *F. verticillioides* (20 nos.) and *F. solani* (9 nos.), isolated from all the four panchayaths of Thrissur district during the one year period. Four species of *Acremonium*, *A. zeylanicus* (97 nos.), *A. incoloratum* (25 nos.), *A. strictum* (11 nos.) and *Acremonium* spp. (15 nos.) were isolated, which accounted for the maximum number (148 nos.) under this genus. The frequency of isolation was very low in the case of *Paecilomyces* (15 nos.), which included two species, *P. fumosoroseus* (13 nos.) and *P. lilacinus* (2 nos.). Apart from the above, *Actinomycetes* were frequently isolated (95 nos.). Among the four panchayaths, Madakkathara recorded the maximum number of nut samples containing *Fusarium* spp. (31 nos.), *Paecilomyces* spp. (10 nos.) and Actinomycetes (29 nos.) whereas the Koorkenchery panchayath yielded maximum number of *Acremonium* spp. (45 nos.). All the fungal species except *Paecilomyces* spp. were isolated from all the three locations of four panchayaths. The most predominant genus isolated from dead mites was *Acremonium*. Apart from the above fungi, commonly occurring fungi like *Aspergillus* spp. (31 nut samples) and *Penicillium* spp. (11 samples) were also isolated from dead mites in the four panchayaths.

In earlier reports, association of many fungal species like *Paecilomyces, Beauveria, Sporothrix, Verticillium* and *Acremonium* was reported which were frequently isolated from the coconut eriophyid mite from the coconut growing tracts of South Indian States (Sreeramakumar *et al*. 2001a). Isolation of microbes from 160 mite infested samples during the year 2001 from Thrissur district by Gopal *et al*. (2003) revealed that actinomycetes constituted the predominant microflora with an isolation frequency of 54 per cent, while the other fungi contributed a frequency slightly higher than 30 per cent. In earlier studies by Hall *et al*. (1980) and NATP (2005) also reported that dead eriophyid mites repeatedly yielded several fungi like *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. Studies by Padiyath (2002) found that other fungi like *Aspergillus niger, A. flavus, Penicillium* spp. and *Fusarium* spp. were associated with the mite. The spectrum of fungal pathogens associated with the coconut mite indicates the significant role played by them in the natural suppression of the pest.

The actual recovery of a pathogen in the pure form from field collected mite infested nuts is a challenge. Frequent isolation of other entomofungal pathogens like *Acremonium* spp., *Fusarium* spp. and *Paecilomyces* spp. and their interaction might have affected the easy recovery of *Hirsutella* spp. in pure form. Moreover, the mycosed mites obtained from natural field population may be in the late stages of mycoses or infection. This may result in the emergence of other fast growing and commonly occurring entomopathogenic fungi. These observations support the views of Hall *et al*. (1980) that even though the natural incidence of *H. thompsonii* was low, it assumed epizootic proportions upon reaching the region below the perianth, perhaps due to the favourable micro-climate with high humidity, which is particularly conducive for fungal development.

The isolation of fungal pathogens from the coconut mite varied with seasons. Table 3 illustrates the influence of seasonal variation on the occurrence of fungal pathogens. The entire period of survey was divided into four seasons, *viz*., monsoon (June-September), post monsoon (October-November), winter (December-February) and summer (March-May). Maximum number of *Hirsutella* isolations was obtained during winter season (16 nos.) followed by monsoon season (14 nos.). Lowest occurrence of *Hirsutella* spp. was in the post monsoon season (October - November). Of the total *Hirsutella* isolations obtained over a period of one year from 12 locations, 35.56 per cent was during winter season. It was found that the natural occurrence and spread of *H. thompsonii* and the resultant mortality were very much limited. The slow growing nature of the fungus may be the reason for this.

Among the different fungal pathogens, *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. were isolated for the maximum number of times during monsoon period (37, 61 and 6 numbers, respectively). The isolates of actinomycetes (35 nos.) were high during the summer

Table 2. Frequency of isolation of different fungal pathogens, other commonly occurring fungi and actinomycetes from four panchayaths (Total of three locations in each panchayat)

Panchayath/ Locations	NS	NSI	*H. t*	*Fusarium* spp.			*Acremonium* spp.				*Paecilomyces* spp.		*Act.*	*Asp.*	*Penc.*
				F. l.	*F. v.*	*F. s.*	*A. z.*	*A. i.*	*A. s.*	*A.* spp.	*P. f.*	*P. l.*			
Pananchery	288	238	2	18	7	1	31	3	1	0	3	0	27	4	1
Madakkathara	288	222	12	23	4	4	18	8	0	4	8	2	29	14	3
Koorkenchery	288	220	16	12	3	1	30	5	2	8	2	0	26	9	6
Pariyaram	288	239	15	16	6	3	18	9	8	3	0	0	13	4	1
Total	1152	919	45	69	20	9	97	25	11	15	13	2	95	31	11

NS- Total no. of nut samples; NSI- Total no. of nut samples subjected for fungus isolation; *H. t. –Hirsutella thompsonii*; *A. z. – A. zeylanicus*; *P. f. – P. fumosoroseus*; A. sp. – *Aspergillus* spp; *F. l. – F. lateritium*; *A. i. – A. incoloratum*; *P. l. – P. lilacinus*; *Penc. – Pencillium* spp.; *F. v. – F. verticillioides*; *A. s. –A. strictum*; *Act.* – Actinomycetes; *F. s. – F. solani*; *A.* spp.*–Acremonium* spp.

Table 3. Seasonal variation on the occurrence of fungal pathogens (Total of three locations in each panchayat)

Seasons	Numbers isolated																								
	Hirsutella spp.					*Fusarium* spp.					*Acremonium* spp.					*Paecilomyces* spp.					Actinomycetes				
	A	B	C	D	T	A	B	C	D	T	A	B	C	D	T	A	B	C	D	T	A	B	C	D	T
Monsoon (Jun.-Sep.)	0	3	9	2	14	9	13	5	10	37	10	12	26	13	61	0	6	0	0	6	4	4	2	5	15
Post monsoon (Oct.-Nov.)	0	4	1	1	6	6	9	4	7	26	6	6	6	5	23	0	1	0	0	1	4	7	3	3	17
Winter (Dec.-Feb.)	1	2	5	8	16	6	4	0	5	15	9	6	5	11	31	2	1	0	0	3	3	11	12	2	28
Summer (Mar.-May.)	1	3	1	4	9	5	5	7	3	20	10	6	8	9	33	1	2	2	0	5	16	7	9	3	35
Total	2	12	16	15	45	26	31	16	25	98	35	30	45	38	148	3	10	2	0	15	27	29	26	13	95

A- Panancherry; B- Madakkathara; C- Koorkenchery; D- Pariyaram; T- Total

period. Research work related to the seasonal incidence of *H. thompsonii* of coconut eriophyid mite is negligible. Studies conducted on the populations of some tarsonemid and eriophyid mites showed that the percentage infection by *H. thompsonii* increases slowly from the end of spring reaching a maximum of 30-60 per cent in August-September (Mietkiewski *et al*., 2000). Another study by Chandler *et al*. (2000) revealed that *H. thompsonii* can cause spectacular natural epizootics among mite populations in hot, humid weather. Effect of environmental factors on *H. thompsonii* studied by Kenneth *et al*. (1979) revealed that growth, sporulation and conidial germination were best at 25° to 30°C with a 60 per cent relative humidity. This suggests that the fungus can survive diverse and variable environmental conditions. However, good results could only be expected during warm season as given in the report.

Among the different fungi and other microorganisms tested for two fungal species each belonging to *Acremonium* (*A. strictum* and *A. incoloratum*), *Fusarium* (*F. lateritium* and *F. verticillioides*) and *Paecilomyces* (*P. fumosoroseus* and *P. lateritium*) Koch's postulates were proved by repeated reisolation of these pathogens from eriophyid mites. Observations in the present study are in conformity with the results of NATP (2005) where the pathogenicity of *F. lateritium* and *A. incoloratum* was proved. But their exact role as primary pathogens of eriophyid mite has to be established further. Mycelial growth with characteristic phialides was observed on the mites inoculated with *H. thompsonii* and *H. kirchnerii*. Other fungal cultures from the infected mites and their eggs could not be identified as they possessed only hyaline hyphae without spores which were the distinguishing characteristics for identification.

From the present study and from the earlier reports, the role of these microorganisms as specific and primary pathogens as *Hirsutella* has not been proved beyond doubt. The factor behind the mortality caused by *H. thompsonii* var. *synnematosa* in natural infection, whether it is toxin or not, has to be studied. Future studies have to be conducted to evolve suitable techniques to utilize the fungus as a successful biological control agent against coconut eriophyid mite.

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