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Investigations on *Sporothrix fungorum* de Hoog & de Vries, a newly recorded pathogen of *Aceria guerreronis* Keifer, the coconut eriophyid mite

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ABSTRACT: **The hyphomycetous fungus, *Sporothrix fungorum* de Hoog & de Vries was discovered affecting the coconut eriophyid mite, *Aceria guerreronis* Keifer in Karnataka and Tamil Nadu in India during 1999-2000 in up to 15 per cent of coconut samples. At $2x10^5$, $2x10^6$ and $2x10^7$ conidia per ml the fungus could bring about 47.03, 92.75 and 98.29 per cent mortalities, respectively, 96h after inoculation. It was able to grow and conidiate profusely on potato dextrose agar (PDA) as well as Sabouraud dextrose agar (SDA). The diameter of the colony increased significantly between 10 and 21 days of inoculation in both the media, reaching up to 35.83 and 36.00 mm on PDA and SDA, respectively. Among three different liquid media, *viz.*, potato dextrose broth (PDB), Sabouraud dextrose broth (SDB) and an enrichment medium (EM), EM was the best in terms of wet and dry weights (per 100 ml) as well as conidia production in both stationary (6450.3 mg, 1602.3 mg and 71.7 x 10^6/ ml) and shake cultures (13416.7 mg, 3402.7 mg and 83.0 x 10^7/ ml). The present report gives new details about *S. fungorum* as well as its association with the coconut mite.**

KEY WORDS: *Aceria guerreronis*, biological control, coconut, eriophyid mite, *Sporothrix fungorum*

INTRODUCTION

Aceria guerreronis Keifer (Acari: Eriophyidae) is one of the serious pests of coconut (Moore and Howard, 1996). Since its first occurrence in Kerala (Sathiamma *et al.*, 1998), *A. guerreronis* has been creating an unprecedented concern in south Indian States because of its extensive infestation and significant yield losses in coconuts.

The eriophyid mite feeds on the soft meristematic tissues beneath the floral bracts of the coconut in different stages of development and causes damage that develops into necrotic lesions. Severely infested green fruits may drop-off and older fruits fail to develop into full nuts. They are usually smaller and distorted, with cracks near the perianth. Other sucking pests like mealy bugs (*Pseudococcus* spp.) and coreid bug (*Paradasynus rostratus* Dist.) can invade the cracks to form a pest complex. Both quality and weight of the kernel are affected when the severity is very high.

Beneath the shelter of the bract, the mite is well protected from pesticide application so that biological agents well adapted to spread are

favoured for control (Hall *et al.*, 1980). *H. thompsonii* is the most widely recorded pathogen of the mite (Hall *et al.*, 1980; Kumar *et al.*, 2001; Kumar, 2002).

Periodic surveys since the later months of 1999 for the association of potential pathogens with the mite in the affected States in south India revealed the association of a fungal pathogen with the mite. The purpose of this study is to describe the newly recorded fungal pathogen by use of light microscopy and report the characterization of mycelial and conidia production on different standard media.

MATERIALS AND METHODS

Surveys and collection of samples

Unsprayed Coconut groves infested with *A. guerreronis* were identified in south India during 1999-2000. Young nut samples collected randomly from these trees produced numerous mite cadavers with apparent fungal disease. All the samples were placed individually in paper bags to avoid the spread of the disease from one nut to another.

Isolation and purification of the fungus

In less than 48 hours after the collection, the infected mites were transferred to microscope slides with a fine brush, mounted in lactophenol cotton blue and observed under 40x and 100x (oil-immersion) objectives in the microscope.

For isolation and detailed studies of the associated fungus, the tepals or meristematic tissue pieces harbouring diseased mites were placed separately in moist chambers devised with Petri-dishes (90 mm diam) lined with three layers of wet filter paper discs. The dishes were covered totally with black paper to prevent light from affecting the normal nature of the live mites, if any, along with the dead mites. Individual mites exhibiting mycelial strands on their bodies were carefully lifted with a micro-needle from the inner side of the perianth or the nut surface while observing through a stereoscopic zoom microscope (Nikon, SMZ800).

They were surface sterilized by placing them in a solution of 2.5 per cent hypochlorite (McCoy and Kanavel, 1969) for approximately 2 minutes and then rinsed in sterile deionized water twice. A three-cavity slide method, in which one cavity was used to keep the sterilant and the other two, sterile deionized water, was used for this purpose (Kumar *et al.*, 2001). Surface sterilized individual mites were placed directly on potato dextrose agar (PDA) slopes in 15ml screw-capped culture tubes and incubated at 25 °C with a 12 h photoperiod. Pure cultures of the fungus were obtained after 3 transfers at 72 h interval from the original dish onto fresh dishes.

Micromorphological studies

Micromorphological characters of the isolated fungus on PDA were studied through phase contrast microscopy after 10 days' of incubation at 25 °C with a 12 h photoperiod.

Pathogenicity testing

For proving Koch's postulates as well as to study the mortality of the mite population, young nuts exhibiting a single triangular patch of damage, which indicated the presence of an active colony of mites were selected. Using a fine syringe (1ml capacity), a suspension (30μl) of conidia [three concentrations *viz.*, A: 2×10^5, B: 2×10^6 and C: 2×10^7 conidia per ml of sterile deionized water] prepared from 15-day-old actively growing PDA culture was injected between the perianth and the nut surface exactly at the place of the symptom. Each treatment including the check had 12 nuts.

All the treated nuts were kept in upright position and incubated at room temperature (26 ± 2 °C). Nuts were analysed at 24 h interval up to 96 h of incubation by taking three nuts randomly each time. The per cent mortality of the mites was worked out based on the total population counted at 10 randomly selected spots (4 mm diameter) on the inner surface of the six bracts as well as the nut surface enclosed by the perianth. Mortality percentages were corrected (Henderson and Tilton, 1955) before subjecting to arcsine transformation

for statistical analysis. Re-isolation percentage was also worked out.

Growth studies

Solid media

The radial growth was studied on PDA as well as Sabouraud dextrose agar (modified Emmons) (SDA) at a pH of 6.5. Discs of 6-mm diameter from an actively growing PDA culture of the fungus were used to inoculate 20ml of media in 90-mm diameter Petri-dishes. Nine replicate dishes were incubated at 25 °C (12 h photoperiod) in an incubation chamber. Radial growth was recorded as the mean of two perpendicular diameters including the diameter of the inoculum disc after 10 and 21 days of incubation. For estimating conidia production, 6-mm discs were cut from 3 different places away from the centre and put in 3ml of distilled water containing Tween-20 (0.05%), using a haemocytometer to get conidia number per disc. The first 3 replicates were used for 10 days' data and the next 3 for 21 days' data. The other 3 replicates were used to observe further growth behaviour at the end of 30 days.

Liquid media

Three different liquid media were evaluated for their suitability in enabling its profuse growth and sporulation. Potato dextrose broth (PDB), Sabouraud dextrose broth (SDB) and an enrichment medium (EM) (Formula I) originally developed for *H. thompsonii* by McCoy *et al.* (1975) were evaluated.

For both stationary and shake cultures, 100 ml of medium was prepared in 250-ml Erlenmeyer flasks and the final pH was adjusted to 6.5 before autoclaving at 1.05 kg/cm^2 pressure and 121°C for 15 minutes. Conidial suspension (7.5x10^6 conidia/ml) prepared from an actively growing PDA culture was used as inoculum (1 ml/flask) for stationary as well as shake cultures. The treatments were replicated thrice. A table-top laboratory orbital shaker was used for shake (25mm throw) culture and was run for 7 days at room temperature (26±2 °C) at 200 rpm (variation±2 rpm). Incubation for both stationary and shake cultures was done at room temperature and day light from west and south directions was the only light source during incubation. After every 24 hours, the shaker speed was increased to 300 rpm for a minute to dislodge the mycelium sticking to the surface of the flask so as to prevent conidia formation and re-inoculation of the medium. Conidia production in stationary cultures was estimated after adding Tween-20 (0.05%) in the flasks and shaking them vigorously on a shaker at 300 rpm for 15 minutes to dislodge conidia from the mycelial mat. The biomass was analysed directly for conidia in the case of shake cultures. For studying the wet and dry weight of the fungus in liquid culture, the biomass was separated from the spent medium by filtration after the incubation period. The retained biomass was dried at 60 °C for 24 h before weighing.

RESULTS AND DISCUSSION

A fungal pathogen was observed infecting the adults (both male and female), nymphs and eggs of the mite in Karnataka (Bangalore Urban and Chitradurga districts) and Tamil Nadu (Coimbatore district) during 1999-2000. The infected mites appeared in moribund condition and were found entangled in fine silvery white mycelial strands. Virtually no live mite was observed either on the inner surface of the bracts or on the nut surface in up to 15 per cent of samples, indicating the development of an epizootic of the fungus in the mite population.

Isolations yielded a white fungus. From the front and the reverse, the colour was white initially and became cream to dark brown in time. The fungus produced conidia holoblastically in apical clusters at the top of each conidiogenous cell, a feature typical of *Sporothrix*, and to some extent further conidia were produced in chains. Conidiophores arose mostly at right angles from the thin septate hyphae and were hypha-like, hyaline, sympodial, solitary, erect and tapered towards the apex. Conidia were ovoid or elongated, 2.5-5 x 2-3 mm in size (n =100), hyaline, one-celled and smooth-walled. In some isolates, solitary, darkly pigmented, thick-walled, obovate to angular conidia were also observed. As the culture aged, conidia were

subsequently formed singly along the sides of both conidiophores and undifferentiated hyphae. Some isolates produced short aerial hyphae. Spherical or oval budding yeast cells were also occasionally seen in culture.

Koch's postulates were satisfied by artificially inoculating and reisolating the fungus from infected mites. Among the doses, C was the best as it could bring about 35.78 per cent corrected mortality of the mites by the end of 24 h (Fig. 1). The difference in corrected mortalities was significant till the end, at which the percentage mortalities were 98.29, 92.75 and 47.73 for C, B and A, respectively. The maximum control mortality observed was 21.47 per cent at 96 h. Whereas the fungus was isolated consistently from diseased mites from treated nuts, with 88 per cent frequency of reisolation, mites from uninoculated controls neither showed symptoms nor yielded the pathogen.

The virulent isolate, MF (Ag) 15 (ex Pollachi, Coimbatore district, Tamil Nadu, India; 19 February 2000) was identified as *Sporothrix fungorum* de Hoog & de Vries (IMI 384502) by the mycologists at CABI Bioscience, UK.

About 58 species of fungi are reported to be infective to at least 73 species of Acari, either naturally or in experiments (Chandler *et al.*, 2000). Four fungal genera, *Hirsutella*, *Verticillium*, *Paecilomyces* and *Sporothrix* have sofar been reported to be containing species infectious to the eriophyids (McCoy, 1996). However, only *Sporothrix schenckii* has sofar been recorded as a pathogen of an eriophyid mite, *Aculus fockeui* Nalepa & Trouessart, in Germany (Schliesske, 1992).

The fungus was able to grow and conidiate profusely on PDA as well as SDA (Table 1). On both the media, colonies were slow growing, moist, wrinkled, leathery to velvety, glabrous, white to brownish white and yeast-like, with irregular margins. The diameter of the colony increased significantly between the 10th and 21st days of inoculation in both the media. Nevertheless, there was no significant difference in the growth rate and conidial production between the media. The colonies, which were at first white, became cream to light brown. Colonies turned lilac-coloured on PDA with age. The foldings/ wrinkles increased gradually and the centre of the colonies were raised. The pigmentation varied from white to cream to dark tan. On SDA, pigmentation was slightly darker than on PDA. The fungus could attain a maximum colony diameter of 40 mm at the end of 30 days. It stopped growing further once the medium in the entire Petri dish turned dark brown to tan.

Stationary cultures showed the same kind of wrinkling that was observed on solid media. The characteristics of the fungus were same as those studied on the solid media. However, in shake

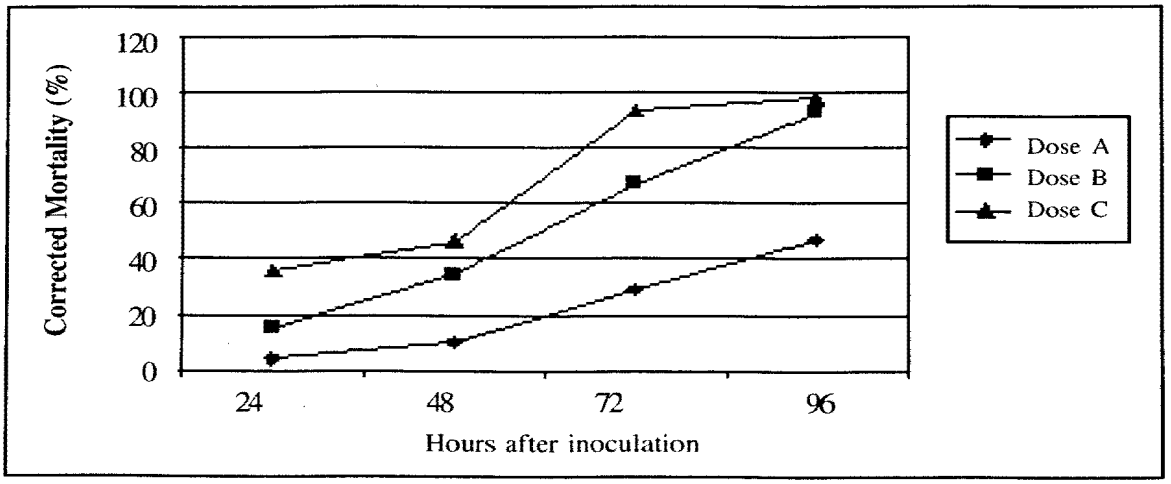

Figure 1. Percentage corrected mortality of *A. guerreronis* treated with three doses (Dose A: 2 x 10^5, Dose B: 2 x 10^6 and Dose C: 2 x 10^7 conidia/ml) of *S. fungorum*

Table 1. Growth, conidiation and pigmentation of *S. fungorum* on two agar media

Medium	Colony diameter (mm)[a] (± SEM) after		't' value	Conidia production[b] (x 10^7) (± SEM) after		't' value	Pigmentation in the medium
	10 days	21 days		10 days	21 days		
Potato dextrose agar	28.5±0.29	35.83±1.64	4.40*	1.83±0.09	2.37±0.03	5.66**	Light tan
Sabouraud dextrose agar	29.5±1.32	36.0±0.29	4.80**	1.99±0.05	2.50±0.06	6.52**	Dark tan
't' value	0.74[@]	0.10[@]	-	1.56[@]	2.00[@]	-	-

[a] Including the original 6-mm inoculum disc

[b] Conidia number per 6-mm diameter disc

*Significant ($t = 0.05$); **Significant ($t = 0.01$)

[@] Not significant

cultures, the viscosity of the medium increased and reached the maximum on the seventh day.

Among the three media, EM was the best in terms of wet and dry weights as well as conidia production in both stationary and shake cultures (Table 2). The wet and dry weights of the fungal biomass obtained in EM in stationary culture were more than five and three times, respectively, of that obtained in the next best medium, SDB. There was significant difference between SDB and PDB in performance in shake culture, although the latter was found to be inferior to the former in stationary culture.

Table 2. Wet weight, dry weight and conidia production of *S. fungorum* in three different liquid media in stationary and shake cultures

Medium	Stationary culture			Shake culture		
	Wet wt[a]	Dry wt[b]	Conidia production[c]	Wet wt	Dry wt	Conidia production[d]
Potato dextrose broth	711.7	229.0	44.3	4223.3	678.7	53.8
Sabouraud dextrose broth	1303.3	509.0	53.7	4716.7	784.3	63.0
Enrichment medium	6450.3	1602.3	71.7	13416.7	3402.7	83.0
SEM ±	29.7	7.1	1.4	232.3	51.3	2.2
CD (P=0.01)	193.1	45.9	9.2	1512.3	333.7	14.2

[a] Wet weight (mg/100 ml) was recorded immediately after harvest.

[b] Dry weight (mg/100 ml) is the constant weight checked three times on subsequent days.

[c] Number (x 10^6)/ml.

[d] Number (x 10^7)/ml.

Species of *Sporothrix* are thermally dimorphic and have been isolated from soil, decaying plant material, other fungi, insects and air (de Hoog, 1974). Several species of *Sporothrix* are known entomopathogens and they have been evaluated as biocontrol agents (Fan *et al.*, 1988; Huang *et al.*, 1997).

The present report gives new details about *S. fungorum* as such as well as its association with the coconut mite. The association of the fungus with the egg, nymphal and adult stages of the coconut mite in several places indicates that viable fungal inoculum is naturally present in the coconut environment.

Mitosporic fungi tend to be favoured as mycopesticides as they can be produced more readily in bulk than entomophthoralean fungi (Chandler *et al.*, 2000). Therefore, being a mitosporic fungus, *S. fungorum* can be investigated for its potential as one more effective mycoacaricide in addition to the already proven mite pathogen *H. thompsonii*. Though, Schliesske (1990) speculated the possible use of *S. schenckii* against the coconut mite in Costa Rica, no serious thought was given to that idea.

Further observational and experimental studies on the role of *S. fungorum* in the natural regulation of *A. guerreronis*, its host range and efficacy as a biocontrol agent are envisaged before embarking on the development of a mycoacaricide based on the same.

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