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Natural incidence of *Hirsutella thompsonii* Fisher on the coconut eriophyid mite, *Aceria guerreronis* Keifer in certain districts of Karnataka and Tamil Nadu in India

P. SREERAMA KUMAR, S. P. SINGH and T. S. GOPAL
Project Directorate of Biological Control (ICAR)
Post Bag No. 2491, H. A. Farm Post, Hebbal, Bellary Road
Bangalore 560 024, Karnataka, India
E-mail: pdblc@kar.nic.in

ABSTRACT: ***Hirsutella thompsoni*** **Fisher was found to be widespread in the three districts of Karnataka (Bangalore Rural, Mandya and Kolar) and one in Tamil Nadu (Coimbatore) during the surveys conducted. The incidence of the pathogen was the highest in Coimbatore where 17.19 per cent of mite-infested coconut samples yielded the fungus. The presence of the fungal pathogen was the lowest in Bangalore rural district, where only 1.37 per cent of the samples showed the occurrence. The average incidence of *H. thompsonii* in mite-infested coconuts was 6.85 per cent. In Karnataka, only Mandya district showed significant presence (6.22%) of the fungus. Overall, 7.03 per cent of the population of mites was infected with *H. thompsonii*. The nymphal stages (9.34%) were found to be more susceptible than the adults (4.72%). The consistent association of the fungus with the mite in the two states surveyed indicates that augmentation of the pathogen in the form of a mycoacaricide may result in artificial epizootics.**

KEY WORDS: *Aceria guerreronis*, coconut, eriophyid mite, *Hirsutella thompsonii*, natural incidence

The coconut mite, *Aceria guerreronis* Keifer, is the most serious recently introduced pest of coconut in India. This eriophyid mite was first noticed in Ernakulam district of Kerala (Sathiamma *et al.*, 1998). Since then, it has been reported from the entire southern region, including Goa and Lakshadweep islands, jeopardizing the future of coconut farming in India.

Aceria guerreronis inhabits the floral bracts and the tender meristematic portion of the nuts covered by the perianth. Initially cream-coloured triangular patches develop on the surface of the nut just below the edges of the bracts due to sucking of the sap and they turn into dark scars in course of time. The injury subsequently leads to warting and longitudinal fissures on the nut surface.

Biological control with natural enemies is not only an attractive alternative but also an effective, cheap, eco-friendly solution to the problem. The acarine parasite, *Hirsutella thompsonii* Fisher, has been reported to be associated with an array in of mites and has been used as a biocontrol agent in different ecosystems all over the world with considerable success.

Since January 1999, intensive surveys for pathogens associated with the coconut mite in the southern States had indicated the important role played by fungal parasites in the natural regulation of the pest. Since the presence of *H. thompsonii* was suspected to be the most dominant among all the pathogens of the mite, concerted surveys were planned in selected districts based on the

preliminary information. This paper reports the natural incidence of *H. thompsonii* on the coconut mite in four districts in Karnataka and Tamil Nadu.

MATERIALS AND METHODS

Surveys, collection and analysis of samples

A detailed study was undertaken in three districts of Karnataka, namely, Bangalore Rural, Mandya and Kolar and one district in Tamil Nadu, namely, Coimbatore. Ten locations in each district were randomly visited between October, 1999 and March, 2000. Samples were collected by harvesting mite infested young nuts (*ca.*10cm diam) randomly from trees spread over a particular locality. Surveys were made only in coconut groves where there had been no application of pesticides for at least two months.

Within 48 hours of collection of samples, bracts were removed from each coconut and scrutinized using a stereoscopic zoom microscope for the presence of mite and its pathogen. All the six bracts (tepals) were closely seen for the mites, concentrating more on the 4th, 5th and 6th bracts. The meristematic region immediately below the perianth was also sliced and examined for the mites.

Mites showing sporulation of *H. thompsonii* were considered to have died as a result of fungal infection. The infected mites were grouped into adults and nymphs for arriving at the proportions. To double-check the association of *H. thompsonii* with the dead mites, the cadavers that did not show any sporulation were stained with lactophenol-cotton blue and examined for the presence of hyphal bodies or external growth of the mycelium. At least 10 randomly picked up nymphs or adults were stained for each locality. Similar replicates were provided for naturally dead mites showing no external mycelium. Phase-contrast microscopy was done to study the fungal infection.

Isolation and purification of the pathogen

To isolate the fungus in pure form, diseased mites were carefully lifted with a micro needle from the inner side of the perianth or meristematic region while observing through a stereoscopic zoom microscope. They were surface sterilized in a solution of 2.5 per cent hypochlorite (McCoy and Kanavel, 1969) for 2 minutes and then rinsed in sterile water twice. For this, a simple cavity slide method was developed, in which a slide (75x25mm; 1.4mm thick) with three spherical cavities (16mm diam; 0.5mm deep) was used. The first cavity consisted of hypochlorite and the other two, sterile distilled water. Observing through a stereoscopic zoom microscope the mites were transferred from one cavity to another with the aid of two micro needles. The small quantity (*ca.* 100ml) of the liquids and the proximity of the cavities with one another aids in the easy lifting and serial transfer of mites from one cavity to another. The mites were then placed directly on freshly prepared potato dextrose agar (PDA) and incubated at 25°C with a 12h photoperiod. Surface sterilized specimens were placed directly on PDA slopes in 15ml screw-capped culture tubes. In less than 48 hours, strands of mycelia were seen growing from a number of mites and conidia formed on some phialides arising from mycelial strands. Phase contrast microscopy was followed again to study the fungus.

RESULTS AND DISCUSSION

Hirsutella thompsonii was widespread in the four districts of the coconut growing area in Karnataka and Tamil Nadu (Table1). As in the case of the citrus rust mite (McCoy and Kanavel, 1969), cadavers with advanced infection produced one or more silvery-white strands of mycelium growing in all directions. Phase contrast microscopy of the mycosed mites revealed the identity of the pathogen. The fungus produced conidia on solitary phialides arising from external mycelia emerging from the dead host. Mycelial strands were seen emerging from cadavers through the oral and anal openings, appendages and also laterally through the body wall. The presence of hyphae bearing solitary phialides and globose rough-walled conidia indicated the identity of *H. thompsonii* (Figure 1).

Table 1. Natural incidence of *H. thompsonii* in mite-affected coconuts

District (State)	Period of survey	Per cent nuts yielding *H. thompsonii*
Bangalore Rural (Karnataka)	October 1999-March 2000	1.37# (3.65)@
Mandya (Karnataka)	February-March 2000	6.22 (11.91)
Kolar (Karnataka)	December 1999-March 2000	2.63 (5.93)
Coimbatore (Tamil Nadu)	October 1999-February 2000	17.19 (1.91)
SEM± CD (P=0.05)		2.70 7.82

Mean of 10 locations

@ Arcsine-transformed values

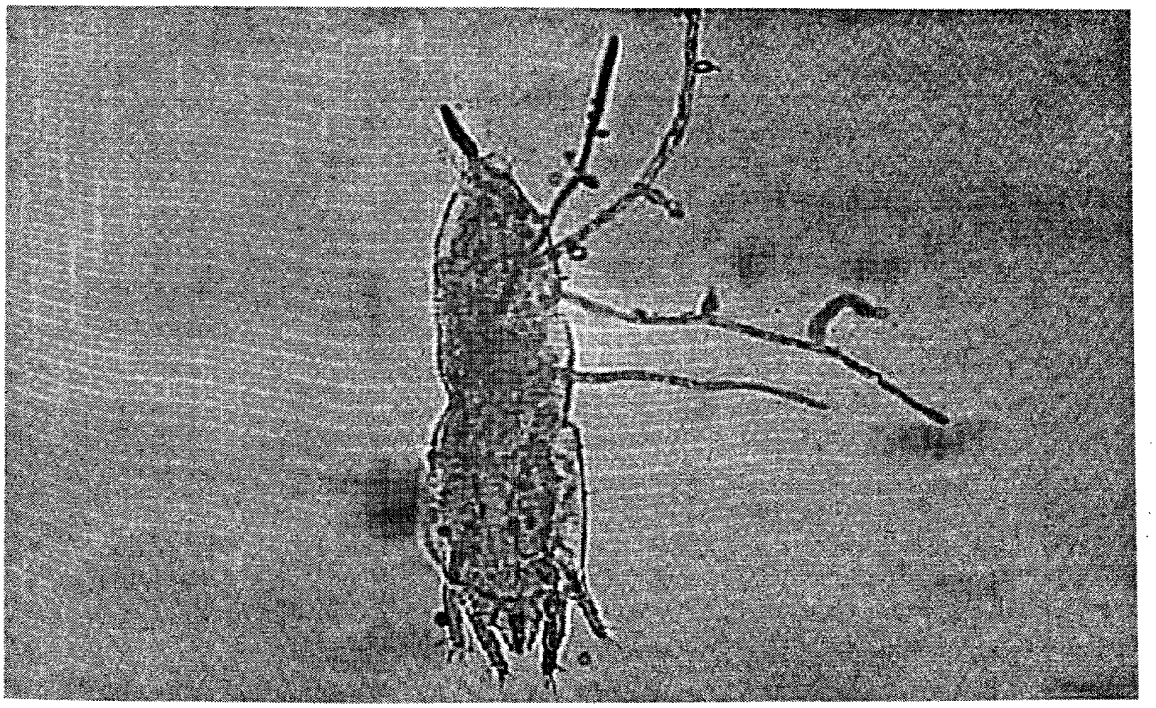

Figure 1. Mycelial strands of *H. thompsonii* emerging from a dead adult coconut mite

The pathogen was present in the largest scale in Coimbatore, Tamil Nadu. From the samples collected and examined, 17.19 per cent yielded the fungus. The presence of the pathogen was the lowest in Bangalore rural district (1.37 %). Significant differences were observed among the districts in terms of the samples yielding *H. thompsonii*. In Karnataka, only Mandya district showed significant presence (6.22%) of the fungus. The average incidence of *H. thompsonii* in mite-infested coconuts was 6.85 per cent.

Significant differences were observed between nymphal and adult infection within Mandya and Coimbatore, where the nymphs were found to be more susceptible to *H. thompsonii*. Overall, the incidence of the pathogen in the nymphs (9.34%) was significantly higher than that was observed in the adults (4.72%).

Overall, 7.03 per cent of the population of mites was found infected with *H. thompsonii*. Coimbatore was significantly different from all the three Karnataka districts *viz.*, Bangalore Rural ($t = 8.0$), Mandya ($t = 14.87$) and Kolar ($t = 8.29$), in terms of infection of the coconut mites (Figure 2).

Isolations yielded a slow-growing fungus, whose colonies on PDA after about 15 days were gray, raised, and cottony with grayish brown substratum. The aerial mycelium was white and powdery. Phase-contrast microscopy revealed that the hyphae were wide and smooth. Conidia were spherical, strongly verrucose, up to 3.5m in diameter (Brady, 1979). Conidiogenous cells arising singly at intervals from the vegetative hyphae and were monophialidic. Phialides were bowling pin-shaped (Villalon and Dean, 1974) with a broadly based inflated portion, wide at the base, conical to flask-shaped, wide at the broadest part then decreasing abruptly to a narrow neck.

Hirsutella thompsonii is considered a specific pathogen of mites, infesting a variety of plants the world over (Samson *et al.*, 1980). The present observations demonstrated that the pathogen is commonly associated with the coconut mite and

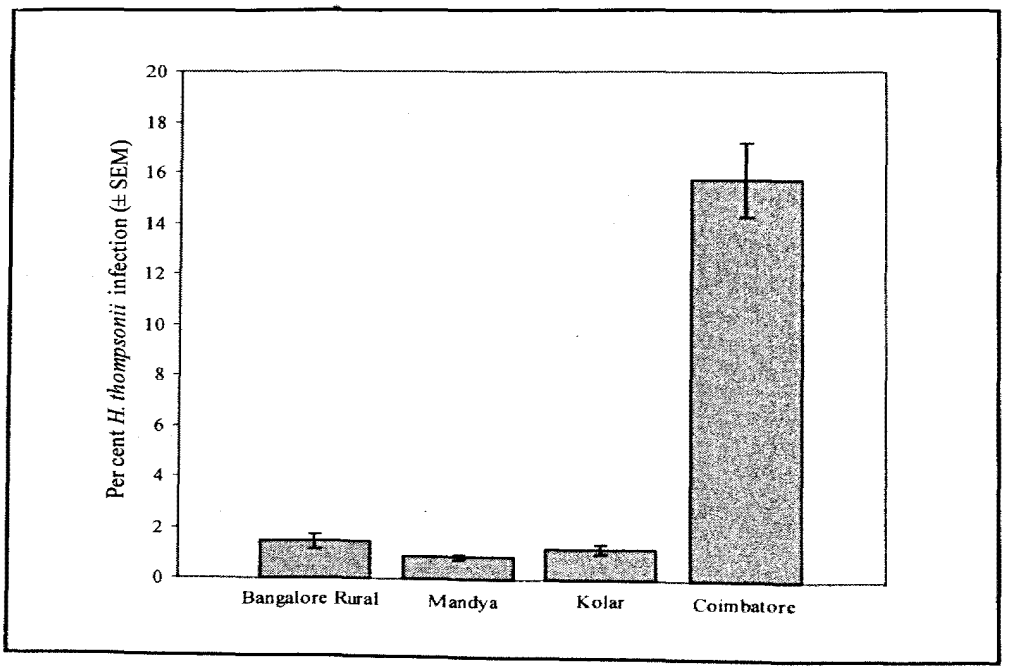

Figure 2. Natural incidence of *H. thompsonii* in overall population of coconut mite

Table 2. *Hirsutella thompsonii* infection in adults and nymphs of coconut mite

District (State)	Per cent *H. thompsonii* infection (± SEM)		
	Adult	Nymph	't' value
Bangalore Rural (Karnataka)	0.94 ± 0.30 (4.96 ± 1.05)@	1.92 ± 0.44 (7.64 ± 0.94)	1.94
Mandya (Karnataka)	0.63 ± 0.10 (4.08 ± 0.45)	1.12 ± 0.15 (5.58 ± 0.54)	2.15*
Kolar (Karnataka)	1.05 ± 0.38 (5.13 ± 1.19)	1.34 ± 0.20 (6.54 ± 0.49)	1.09
Coimbatore (Tamil Nadu)	10.47 ± 1.12 (18.45 ± 0.93)	21.03 ± 2.24 (26.69 ± 1.52)	4.62**
Overall	4.72 ± 0.77 (10.15 ± 1.01)	9.34 ± 1.56 (14.53 ± 1.48)	2.45*

@Arcsine-transformed values

*Significant (P = 0.05); ** Significant (P = 0.01) (Student's *t*-test)

that it might have a significant role in the suppression of the pest in India. *H. thompsonii* was considered as the main regulator of the coconut mite in Central America and the Caribbean (Cabrera, 1977; Hall and Becerril, 1981). Similarly, *H. thompsonii* is a key factor in natural control of citrus rust mite (McCoy, 1981). In India, the mite is presumed to have first spread from Kerala to Tamil Nadu and then to the southern districts of Karnataka. The association of the pathogen with the mite in the southern Karnataka districts indicates the spread of the pathogen along with the pest.

In the present study although there were variations in the number of mites that actually showed infection, there would have been every chance of the spread of the pathogen within the entire population of the mite had the nuts been left untouched for a longer time. The observations supported the views of Hall *et al.* (1980) that the natural incidence of *H. thompsonii* was low in coconut samples collected from several countries, but when present on a nut, the fungus had assumed epizootic proportions beneath the bracts of coconuts.

In the field, use of this fungus for mite control would be suitable in tropical and humid subtropical areas (Gerson *et al.*, 1979). The consistent association of the fungus with the mite in the two states surveyed indicates that augmentation of the pathogen may result in artificial epizootics and spread of the fungus in areas where there is no natural presence. Thick-walled chlamydospores formed within the host probably provide the seasonal carry-over (Samson *et al.*, 1988).

The collections of the fungus showed variation in morphology and other cultural characteristics, suggesting the presence of more than a variety of the fungus species. Efforts are on to distinguish and classify the various isolates in our collection into distinct varieties as suggested by Samson *et al.* (1980). Studies with 15 local isolates of the fungus, specifically isolated from the coconut mite, have shown promise against the

pest. The isolate, MF(Ag)5 (ITCC 4962; IMI 385470) has been taken up for multiplication and formulation as a mycoacaricide (Kumar and Singh, 2000).

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