Research Article

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Occurrence of different varieties and types of *Hirsutella* spp. on coconut eriophyid mite

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Abstract

Nine *Hirsutella* isolates varying in colour and growth pattern were isolated from the coconut eriophyid mite (*Aceria gurerreronis* Keifer) during the survey conducted from July 2003 to June 2004 on mite infested nut samples from twelve locations of Thrissur district of Kerala state. Based on the presence or absence of synnemata, two different varieties namely, *H. thompsonii* var. *thompsonii* and *H. thompsonii* var. *synnematosa* of *Hirsutella* were found to occur. Apart from *H. thompsonii*, a different species *H. kirchnerii* was also isolated during the study. *H. kirchnerii* and *H. thompsonii* var. *synnematosa* (Madakkathara-I isolate) varied morphologically from the other *Hirsutella* isolates, where it produced off white coloured mycelia throughout the growth. All the other *Hirsutella* isolates produced a greyish white coloured growth. Cultural and morphological observations revealed that among the nine isolates coming under the two varieties, *H. thompsonii* var. *synnematosa* Madakkathara-I isolate recorded maximal fungal growth (4.167 cm) and biomass (2.660 g) while the *H. thompsonii* var. *thompsonii* Chirakkekodu-I isolate possessed maximum mean sporulation (3.33×10^6 spores ml^{-1}). Thus, in the present study, the *Hirsutella* isolate with maximum biomass and growth rate did not possess maximum spore count. Micrometry studies revealed that the *H. thompsonii* isolates possessed a hyphal width, phialide width and spore diameter of 3.44 µm.

Keywords: Coconut eriophyid mite, *Hirsutella spp.*, varieties and types

Introduction

The mite specific fungal pathogen, *Hirsutella thompsonii* has already been reported as the most promising and potential biocontrol agent against coconut eriophyid mite (CEM) in the Ivory Coast and Mexico (Julia and Mariau, 1979; Hall *et al.*, 1980; Berril and Sanchez, 1986; Sampedro and Rosas, 1989). Beevi *et al.* (1999) isolated the fungus *H. thompsonii* var. *synnematosa* from the dead coconut mites from Kerala in India. In the present investigation, different strains/ isolates of mite specific pathogen, *H. thompsonii* associated with mites were studied. At the time of isolation, *H. thompsonii* showed wide variations in colour and growth pattern. Apart from that, changes could be noticed in some of the isolates during its subsequent subculturing. Samson *et al.* (1980) described and illustrated the cultural features and morphology of 11 isolates of the hyphomycete, *H. thompsonii*. They proposed three varieties, namely, *H. thompsonii* var. *thompsonii*, *H. thompsonii* var. *vinacea* and *H. thompsonii* var. *synnematosa*. In the present study, fungus isolation was carried out intensively from the nut samples collected from 12 different locations for one year in Thrissur district of Kerala state to study their occurrence and cultural and morphological features.

Materials and Methods

The present study was conducted in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, during the period from October 2002 to December 2005. A survey was conducted in four panchayaths, viz., Pananchery, Madakkathara, Koorkenchery and Pariyaram of Thrissur district of Kerala state (10°31' N latitude, 76°13' E longitude) for one year during July 2003 to June 2004.

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From each panchayath, three locations/plots with a minimum of 100 full bearing coconut trees (variety West Coast Tall) of uniform age (15-20 years) with heavy eriophyid mite infestation on nuts was 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 was reported to have the active live mite colony. The sampling was done at monthly intervals. The buttons excised were packed in polythene covers and tied well. 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.

Different *Hirsutella* isolates from different locations which varied in morphological characters were maintained to study the variation among the isolates. Cultural and morphological characteristics of *Hirsutella* isolates were studied in the medium, Sabouraud's Maltose Yeast + Agar (SMA + Y) having a pH of 6.0. Various growth aspects of *Hirsutella viz.*, growth rate, colony characters, sporulation, biomass studies and micrometry were studied. Fungal disc of one cm diameter (cut by cork borer) was taken from 12 day old fungal culture. The disc was placed at the centre of the dish. Three replications were maintained for each treatment. The petri dishes were incubated in BOD incubator at a temperature of 27°C. Measurement on radial growth of the culture was taken from second day of incubation to ten days. The characteristics like colour, growth pattern and the reaction of fungal culture in the solid media (SMA+Y) were recorded. Presence of honeydew and number of synnemata were also noted on tenth day after the growth initiation.

For the spore count, fungal disc of one cm was cut from the 15 day old culture of *Hirsutella* sp. and transferred to 10 ml of sterilized water in a test tube. A drop of Tween 80 was added and shaken well for the uniform distribution of spores. The spore count was calculated per ml of spore suspension by using the Haemocytometer.

The levels of sporulation at different radial distances of the fungal colony were also assessed. For this, one cm mycelial discs were taken from the centre; half of the radial distance and from periphery of the colony of 15 days old culture and the spore count was estimated using Haemocytometer.

To study the growth of *Hirsutella* isolates in liquid medium, broth of SM + Y was prepared. Using a cork borer, one cm disc was cut from 15 days old fungal culture of the respective isolates. The disc was transferred to 100 ml of the autoclaved SM + Y broth in 250 ml conical flasks under sterilized conditions. The flasks were agitated in a shaker adjusted to 180 rpm for six days and kept for incubation at a temperature of 27° C for the fungal growth. After four weeks the fungal growth was filtered through a pre-weighed filter paper. After taking fresh weight it was kept for oven drying at 60° C and dry weight was taken on successive days till it became constant.

Slide culture of the fungal isolates was prepared for studying the micrometry. Fungal culture disc of one cm (15 days old) was placed on a sterile glass slide supported by two glass rods within a Petri dish under sterile conditions. A cover slip was placed carefully over the fungal disc. Two blotting papers moistened with sterile water were also kept at the top and bottom of the Petri dish. After 48 h cover slip was taken carefully from the mycelial bit. Slide was mounted with the cover slip and observed under the calibrated microscope. Measurement on width of hyphae, width and length of phialide, length of hyphae between phialides and diameter of spores were noted. The microscope was calibrated using ocular and stage micrometer.

The laboratory studies were designed in a Completely Randomized Design (CRD). The treatments were compared using Duncan's Multiple Range Test (DMRT).

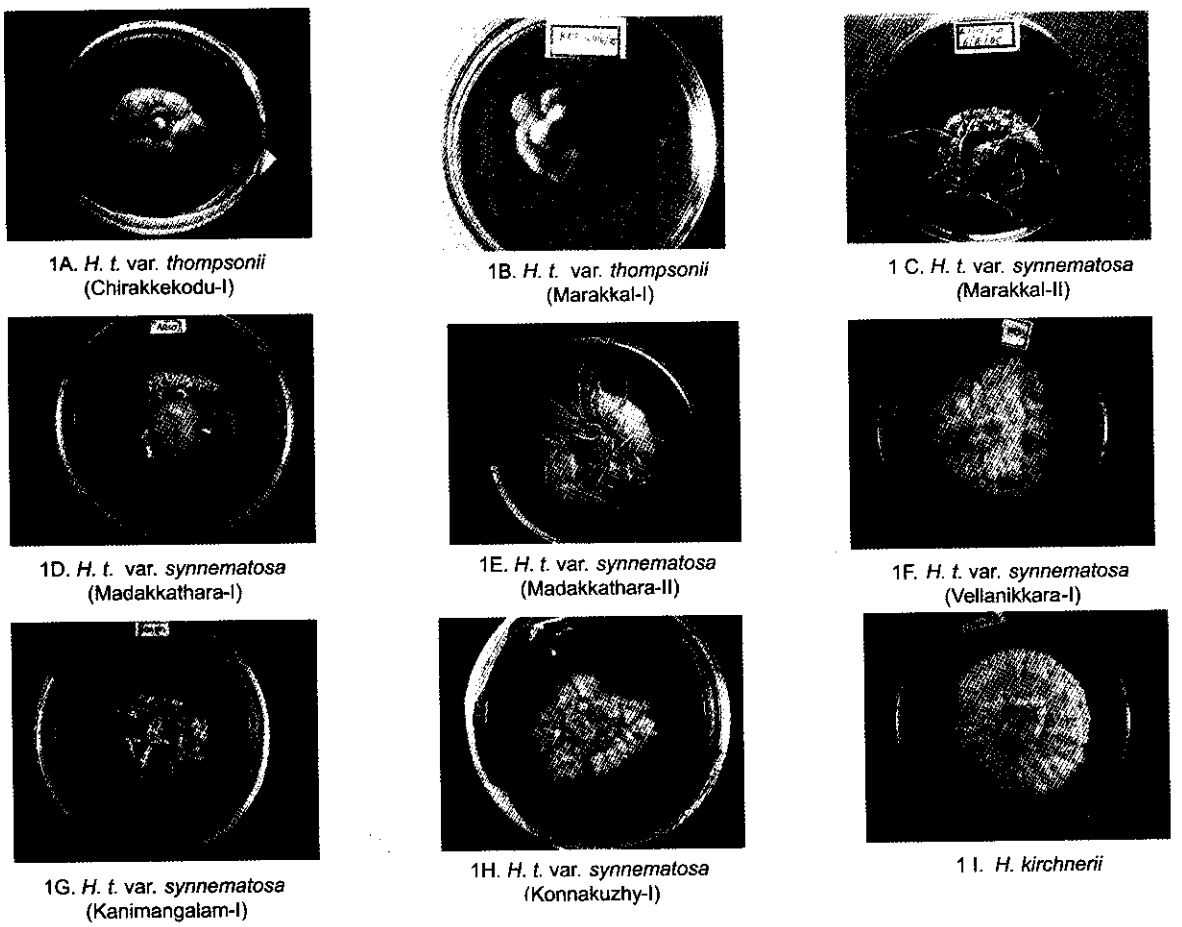
Results and Discussion

Based on the presence or absence of synnemata, two varieties of *H. thompsonii viz.*, *H. thompsonii* var. *thompsonii* with no synnemata and *H. thompsonii* var. *synnematosa* which possessed synnemata were found to occur with dead mites. Among the two varieties, the frequency of isolation was the highest for the variety, *H. thompsonii* var. *synnematosa*. The colony characters varied with different isolates (Table 1). All the *Hirsutella* isolates except one, Madakkathara-I produced a grey coloured initial growth which gradually changed to greyish white at the periphery of the colony (Fig 1). The *Hirsutella* isolate, Madakkathara-I produced off white coloured mycelial growth throughout the period. Different growth patterns *viz.*, uniform, raised, dome shaped and folded ones were observed. Honeydew was present in both the types of isolates.

Table 1. Colony characters of *Hirsutella* isolates

Sl. no.	*Hirsutella* sp.	*Hirsutella* isolates	Colour		Growth pattern	Reaction in media	Honey dew	Synnemata(15 DAI)
			Initial	Final (12 DAI)				
1	*H. thompsonii* var. *thompsonii*	Chirakkekodu-I	Greyish	Greyish	Uniform, slightly raised with foldings	No reaction	Along the foldings (63 nos.)	Nil
2	„	Marakkal-I	Ash coloured	Ash coloured	Raised and dome	No reaction, media shaped	5 to 9 nos. breaking was observed	Nil
3	*H. thompsonii* var. *synnematosa*	Marakkal-II	Greyish	Greyish	Uniform with foldings towards the periphery	No reaction	Nil	Cream coloured - 96 nos.
4	„	Madakkathara-I	Greyish off white	Off white	Uniform, slightly raised	No reaction	Nil	Off white 15 nos.
5	„	Madakkathara-II	Greyish	Greyish	Fluffy raised with striations at the periphery	No reaction	Nil	Cream coloured - 78 nos.
6	„	Vellanikkara-I	Greyish	Greyish with off white	Cloudy appearance with small foldings	No reaction	Nil	A few synnemata
7	„	Kanimangalam-I	Greyish	Greyish	Uniform, cottony appearance	No reaction	Nil	Cream coloured, Elongated ones - 30 nos.
8	„	Konnakuzhy-I	Light greyish	Greyish	Slightly fluffy textured	No reaction	Nil	10 nos.
9	H. *kirchnerii*	Kanjirampally-I	Light greyish	Off white	Slightly raised with radial foldings	Media has got a brown line, media breaking present	Nil	Cream coloured thick -12 nos.

DAI- Days after inoculation



1A. *H. t.* var. *thompsonii* (Chirakkekodu-I)
1B. *H. t.* var. *thompsonii* (Marakkal-I)
1 C. *H. t.* var. *synnematosa* (Marakkal-II)
1D. *H. t.* var. *synnematosa* (Madakkathara-I)
1E. *H. t.* var. *synnematosa* (Madakkathara-II)
1F. *H. t.* var. *synnematosa* (Vellanikkara-I)
1G. *H. t.* var. *synnematosa* (Kanimangalam-I)
1H. *H. t.* var. *synnematosa* (Konnakuzhy-I)
1 I. *H. kirchnerii*

Fig. 1. Colony characters of *Hirsutella thompsonii*

Observations of the colony diameter on the twelfth day (Table 2) recorded maximum fungal growth in the *Hirsutella* isolates, Madakkathara-I (4.167 cm) and Madakkathara -II (4.077 cm), which were on par with Vellanikkara-I (3.927 cm). Vellanikkara-I was closely followed by Chirakkekodu-I with a mean fungal growth of 3.763 cm. The observation on colony diameter taken on consecutive days revealed that the rate of growth was faster in Madakkathara -I, while, Marakkal-I recorded the slowest growth rate.

Thus in the present study, the *Hirsutella* isolate, *H. thompsonii* var. *synnematosa* isolate namely Madakkathara-I with maximum biomass and growth rate did not possess maximum spore count. This may be due to the variation among the different strains of *Hirsutella* spp. isolated from different locations. Results of the study by Padiyath (2002) revealed a significant correlation between the mycelial growth and sporulation of fungus whereas, it recorded a non-significant negative correlation between sporulation and synnematosa

Table 2. Growth rate and sporulation of *Hirsutella* isolates

Sl. No.	*Hirsutella* species	*Hirsutella* isolates	Growth rate (Colony diameter in cm12 DAI)*	No. of spores ($x10^6$)ml^{-1}			Biomass wt. (g/100 ml)
				Centre	Half the radial distance	Periphery	Mean	
1	*H.* thompsonii var. *thompsonii*	Chirakkekodu-I	3.763BC	2.25	5.92	1.50	3.22	1.560BC
2	,,	Marakkal-I	1.570G	1.17	0.33	1.08	0.86	1.663 BC
3	*H. thompsonii* var. *synnematosa*	Marakkal-II	2.503E	2.25	1.08	2.08	1.81	1.477C
4	,,	Madakkathara-I	4.167A	0.33	0.58	0.17	0.36	2.660A
5	,,	Madakkathara-II	4.077A	0.42	0.33	0.25	0.33	2.047 BC
6	,,	Vellanikkara-I	3.927AB	0.92	0.08	0.83	0.61	1.893 BC
7	,,	Kanimangalam-I	2.843D	0.25	0.67	0.75	0.56	1.737 BC
8	,,	Konnakuzhy-I	2.170F	1.08	1.25	0.33	0.89	1.640 BC
9	*H. kirchnerii*	Kanjirampally-I	3.603C	0.17	0.17	0.17	0.17	2.140AB

*Mean of three observations

The figures followed by the same letter do not differ significantly according to DMRT

Sporulation at various radial distances in solid media of SMA + Y (Table 2) revealed that Chirakkekodu-I isolate recorded an average spore count of 3.22 x 10^6 spores ml^{-1} with the maximum sporulation at half of the radial distance (5.92 x 10^6 spores ml^{-1}) followed by the centre (2.25 x 10^6 spores ml^{-1}) and periphery (1.50 x 10^6 spores ml^{-1}). The lowest mean sporulation was observed in the Kanjirampally-I isolate (0.17 x 10^6 spores ml^{-1}). Portions with maximum spore load varied with different *Hirsutella* isolates. Marakkal-I and II, Madakkathara-II and Vellanikkara-I isolates had maximum sporulation at the centre portion which ranged from 0.42 to 2.25 x 10^6 spores ml^{-1}. Kanjirampally-I isolate recorded uniform spore count of 0.17 x 10^6 spores ml^{-1} in all the three portions.

Weight of dry mycelium (biomass) of the *Hirsutella* isolates in SM+Y broth on 20 DAI (Table 2) indicated that the maximum dry weight was in the *Hirsutella* isolate, *H. thompsonii var. synnematosa* Madakkathara-I (2.660 g), which was on par with Kanjirampally-I (2.140 g). Marakkal-II recorded significantly low biomass with a value of 1.477 g. All the other isolates were on par with Kanjirampally-I where the dry weight of biomass ranged from 1.560 to 2.140 g.

production. This may be one of the reasons which support the low sporulation rate in the synnemata producing *Hirsutella* variety, even though it stands first in growth rate and biomass production.

Microscopic observations revealed that the hyphae produced in all the media were hyaline, septate, smooth and branched. Large number of conical to flask shaped phialides arose from the vegetative hyphae. Phialides vary with the isolates, most of them had broad base and a narrow neck bearing single spore. Conidia were spherical, verrucose and hyaline except in *H. kirchnerii* where the conidia were like segments of lemon.

The measurements of different fungal structures varied slightly in different isolates. The details of microscopic measurements are depicted in Table 3. All the isolates of *Hirsutella* recorded a hyphal width, phialide width and spore diameter of 3.44 µm which was the lowest recorded value of these observations. The Vellanikkara-I isolate recorded a spore diameter of 5.16 µm which stands apart from other isolates. Length of hyphal cells among the isolates ranged from 12.73 to 26.14 µm. The maximum phialide length was recorded by the Vellanikkara-I isolate (20.30 µm) followed by the Madakkathara-II isolate (16.17 µm) while the other

Table 3. Micrometry of *Hirsutella* isolates (mean length in µm)

Hirsutella species	Isolate name	Width of hyphae*	Diam. of spore	Phialides Width of phialide	Length of swollen part	Length of neck	Total phialide length	Distance between phialides	Length of hyphal cell
H. t. var. *thompsonii*	Chirakkekodu-I	3.44	3.44	3.44	6.88	4.13	11.01	58.48	23.05
"	Marakkal-I	3.44	3.44	3.44	5.85	3.44	9.29	47.13	20.30
"	Marakkal-II	3.44	3.44	3.44	6.88	3.44	10.32	50.56	14.80
H. t. var. *synnematosa*	Madakkathara-I	3.44	3.44	3.44	6.88	3.44	10.32	27.18	17.89
"	Madakkathara-II	3.44	3.44	3.44	9.63	6.54	16.17	24.77	22.70
"	Vellanikkara-I	3.44	5.16	3.44	11.70	6.88	20.30	26.83	26.14
"	Kanimangalam-I	3.44	3.44	3.44	6.88	3.44	12.04	48.16	23.05
"	Konnakuzhy-I	3.44	3.44	3.44	8.60	3.44	12.04	48.16	22.70
H. kirchnerii	Kanjirampally-I	3.44	3.44	3.44	6.19	3.44	9.29	12.38	12.73

*Mean of ten observations

isolates possessed a phialide length within the range of 9.29 and 12.04 µm.

The length of the swollen part of the phialide was 11.70 µm for the Vellanikkara-I isolate and 9.63 µm for the Madakkathara-II isolate while the length of the neck was 6.88 and 6.54 µm, respectively. In rest of the isolates, length of swollen base ranged from 5.85 to 8.60 µm and length of neck from 3.44 to 4.13 µ. Distance between two consecutive phialides was the highest for the Chirakkekodu-I isolate (58.48 µm) followed by the Marakkal-II (50.56 µm). The Kanimangalam-I and Konnakuzhy-I isolates possessed phialides at a distance of 48.16 µm while Marakkal-I at 47.13 µm. Both the isolates of Madakkathara-I and II and Vellanikkara-I have the consecutive phialides at a distance ranging from 24.77 to 27.18 µm. Maximum number of phialides per field area was observed in the Kanjirampally-I isolate (*H. kirchnerii*) where the distance between the consecutive phialides was 12.38 µm.

Micrometry studies of *H. thompsonii* isolates were in accordance with that of Padiyath (2002) where it ranged between 3.33 to 3.76 µm. Samson *et al.* (1980) and Beevi *et al.* (1999) reported the colony characters and microscopic studies of *H. thompsonii.* var. *synnematosa* and the present results are in conformity with their observations. The observations are also in conformity with the characters of fungus described by International Mycological Institute.

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References

Beevi,S.P., Beena, S., Lyla, K.R., Varma, A.S., Mathew, M.P. and Nadarajan, L. 1999. *Hirsutella thompsoni* var. *synnematosa* Samson, Mc.Coy and O'Donnell on coconut mite *Aceria (Eriophyes) guerreronis* (Keifer) – a new report from India. *J. Trop. Agric.* **37**: 91-93.

Berril, E.A. and Sanchez, C.J.L. 1986. The fungus *Hirsutella thompsonii* Fisher for the control of the eriophyid *Eriophyes guerreronis. Agricultura-Tecnica-en-Mexico* **12**: 319-323.

Julia, J.F. and Mariau, D. 1979. Nouvelles recherches en Cote-d'Ivoire sur *Eriophyes guerreronis* K., acarien ravageur des noix du cocotier. *Oleagineux* **34**: 181-189.

Hall, R.A., Hussey, N.W. and Mariau, D. 1980. Results of the survey of biological control agents of the coconut mite, *Eriophyes guerreronis. Oleagineux* **35**: 359-398.

Padiyath, S. 2002. Parasitism of *Hirsutella thompsonii* Fisher var. *synnematosa* Samson, McCoy & O'Donnell on coconut eriophyid mite *Aceria guerreronis* (Keifer) M.Sc. (Ag.) thesis, Kerala Agricultural University, Thrissur, 77 p.

Sampedro, L. and Rosas, J.L. 1989. Seleccion de cepas de *Hirsutella thompsoni* Fisher para combatir al acaro del cocotero, *Eriophyes guerreronis* Keifer. L Bioensayos de Patogenicidad. *Revista Mexicana de Micologia* **5**: 225-232.

Samson, R.A., Mc Coy, C.W. and O'Donnell, K.L. 1980. Taxonomy of the acarine parasite *Hirsutella thompsonii. Mycologia* **72**: 359-377.