EVALUATION AND CHARACTERISATION OF EFFECTIVE FUNGAL PATHOGENS ASSOCIATED WITH THE COCONUT ERIOPHYID MITE (*Aceria guerreronis* Keifer)

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled **"Evaluation and characterisation of effective fungal pathogens associated with the coconut eriophyid mite (*Aceria guerreronis* Keifer)"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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CERTIFICATE

Certified that this thesis entitled **"Evaluation and characterisation of effective fungal pathogens associated with the coconut eriophyid mite (*Aceria guerreronis* Keifer)"** is a record of research work done independently by Ms. Amritha. V. S (2002-21-09) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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MY ACHAN AND AMMA

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INTRODUCTION

INTRODUCTION

The coconut, *Cocos nucifera* L. is popular as one of the remunerative plantation crops in Kerala. Among the coconut growing states in India, Kerala is the leading one which contributes to 53.46 per cent of the area (1.10 mha). The total production of coconut in Kerala is 5479 million nuts per year with an average yield of 6049 nuts per hectare (Anonymous, 2005).

Coconut is an integral part of every day life of almost every Keralite. The crop is affected by several pests and diseases. Coconut palms in and around Cochin, the port city of Kerala were seen affected by an unknown pest during the later part of 1997. The pest in question was identified as an eriophyid mite *Aceria guerreronis* Keifer (Sathiamma *et al.*, 1998). This is known as coconut eriophyid mite (CEM) and also known as coconut perianth mite.

The preliminary studies indicated that there is a production fall of 30 to 40 per cent in the most affected districts of the State. Actual loss due to mite infestation alone is estimated between Rs.100-150 crores (Nair *et al.*, 2000). The market of coconut related products such as copra, coconut oil, coir industry, food processing and soap manufacturing was seriously affected. Low prices for the produce coupled with mite infestation have resulted in an unprecedented economic crisis for the coconut farming community.

Based on the trials conducted by the Kerala Agricultural University to manage the mite menace, an adhoc recommendation to spray coconut bunches with an eco-friendly plant based pesticide namely neem oil garlic emulsion (2.0%) with soap solution (0.5%) alternatively with organic chemical acaricide dicofol (0.1%) was suggested (Saradamma *et al.*, 2000a). However, chemical methods of control will have only temporary effect on the mite menace.

CEM live beneath the shelter of the bract and is well protected from pesticide application. So biological agents have to be utilized which will give a permanent and sustainable way to manage the pest. *Hirsutella thompsonii* Fisher, the only pathogen that has so far been extensively used all over the world to manage mite havoc is present in India on the mites. Beevi *et al.* (1999) isolated *H. thompsonii* var. *synnematosa* from the coconut eriophyid mite for the first time in India, from Thrissur district.

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 natural field occurrence of the specific pathogen *H. thompsonii*, the association of other species of fungal pathogen and studies on their comparative bioefficacy will be helpful in identifying potential fungal pathogen to be utilized for the biocontrol of coconut mite. The results of the present study would help in formulating non-hazardous specific products of bio-control agents for managing this mite.

In this context, the present investigation was undertaken with the following objectives:

- Isolation and identification of the fungal pathogens associated with coconut eriophyid mite (CEM) from Thrissur district
- Confirmation of the pathogenicity of the fungal spp. isolated from CEM
- Identification of the potential acarifungal pathogens
- Molecular characterisation of the selected fungal isolates

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

The coconut eriophyid mite *Aceria guerreronis* Keifer is a serious coconut pest in several states of India particularly Kerala, Tamil Nadu and Karnataka. Although chemical control of the coconut mite is possible, it is economically unfeasible, environmentally hazardous and unacceptable. In this context, biological control of the eriophyid mite is one of the promising lines of research that could lead to management of the pest below economic threshold levels. The literature pertaining to the seasonal abundance of mite population, natural mortality, acarifungal pathogens, cultural and morphological characters, bio-efficacy studies and their characterization are briefly reviewed here.

2.1 COCONUT ERIOPHYID MITE *Aceria guerreronis* KEIFER

2.1.1 Pest status

The CEM is the only eriophyid mite species causing potential loss to the coconut palm.

The CEM was reported for the first time in 1960 in the West Coast of Mexico in the State of Guerrero (Cartujano, 1963; Ortega *et al.* 1965) and was described by Keifer (1965). It was also reported from Africa (Mariau, 1969) then in Togo, Nigeria, Cameroon and Ivory Coast (Mariau, 1977) where it appeared simultaneously in several coconut plantations far from each other.

Hall *et al.* (1980) and Kang (1981) reported CEM as a pest of coconut from the coconut belt of Americas and West Africa. CEM has been a serious pest in West Africa, Asia and Oceania (Moore, 2000); Cuba (Cabrera, 2000); mainland Tanzania and in the islands of Mafia, Zanzibar and Pembar (Seguni, 2000).

In Asia, CEM was reported first from Ernakulam district of Kerala State (Sathiamma *et al.*, 1998). Within a short span of time the pest spread to most of the districts of Kerala (Saradamma *et al.*, 2000a) and to the neighbouring States like Tamil Nadu, Karnataka (Ramaraju *et al.* 2000) and Andhra Pradesh (Reddy and Naik, 2000).

In the same period, occurrence of CEM was also reported from Goa and Islands of Lakshadweep (Haq, 1999), Sri Lanka (Fernando *et al.*, 2000) and Andamans (Prasad and Ranganath, 2000).

Incidence of CEM was reported from Coastal Orissa, India especially in districts of Ganjan, Puri, Cuttack, Khurda and Jagat Singh Pure surveyed during September 2000 to August 2001 (Rao *et al.*, 2001). Roving surveys conducted in the Southern districts of Tamil Nadu, India during 1999-2000 showed the heavy occurrence of *A. guerreronis* (5 grade injury score) on coconuts (Varadarajan and David, 2003).

Desai *et al.* (2003) reported the incidence of mite (84% palms affected) at Gadat village of Navsari district of Gujarat with a mean population of 51.25 mites in 2 x 2 mm^2 of the nut surface. As per the intensity of damage, 79.80 per cent marketed nuts were damaged by mites.

2.1.2 Biology

Studies conducted by Mohanasundaram *et al.* (1999) and Haq (2000) on the biology of mite revealed that a female mite laid about 20-100 eggs during its life time. Eggs are round, glossy, transparent and have a diameter of about 35 microns. Eggs hatch in about 3-3.5 days. The first instar nymph moults after two days and second instar nymph moults as adult in 2-3 days. Nymphal stages are usually sedentary. CEM completed life cycle in 10-12 days. Ramarethinam and Loganathan (2000) also reported an average of 10.50±1.27 days required by CEM to complete one generation. The adult CEM lived upto 25 days (Haq, 2000).

2.1.3 Damage

Draining of the sap from young buttons result in poor development of the nut, reduction in nut size, kernel content and poor quality husk (Sathiamma *et al.*, 1998) leads to reduction in copra yield by 25 per cent (Mariau, 1977) and even upto 40 per cent (Muthiah and Bhaskaran, 2000).

Reduction in albumin content, weight and quality of pulp was also reported by Mariau (1977). At maturity the husk of infested nut is very tight and shrunken causing difficulty in dehusking (Nadarajan *et al.*, 2000).

Ranjith (2001) observed that nuts with surface damage 5 and 15 per cent possess maximum mite population. He opined that development of surface damage is not an indicative of population but the reverse is true.

2.1.4 Yield loss

In Kerala, crop loss due to CEM infestation ranged between 30-40 per cent and severe infestation resulted in more than 50 per cent loss in weight of kernel (Nair *et al.*, 2000a). Reddy and Naik (2000) reported that the CEM damage caused about 25 per cent yield loss in copra content.

CEM infestation caused extensive premature dropping of nuts (Mohanasundaram *et al.*, 1999). Haq (1999) indicated a tentative estimate of 200 to 250 crores of rupees annual loss in the State of Kerala alone due to the CEM attack.

2.1.5 Spread

The principal way in which coconut mites spread and colonize new plant, particularly over long distance, is probably through aerial dispersal of inseminated female mites. Moore and Howard (1996) opined that some dispersal of mites may take place by phoresy, either on animals directly attracted to the inflorescence (eg. pollinating agents such as bees, rodents which feed on fruits), or on those attracted by such animals (eg. predatory lizards, birds, predaceous insects). Rapid spread of the mite occurred during humid warm weather in the premonsoon season (Nair *et al.*, 2000b).

2.1.6 Seasonal abundance

Population dynamics of adult mites, nymphs and eggs in young bunches of increasing age studied in West Coast Tall palms of Instructional Farm, Vellayani revealed that maximum population was found in the young colony under the overlapping tepal number four or five. Fifth bunch with 90 to 110 days old nuts was the most critical stage having highest population. Monthly variations in total population observed in the three bunches (fourth to sixth bunch) showed a distinct peak in February-March (>12000/3 nuts) with a sharp declining trend in the subsequent months indicating a negative correlation with rainfall (Mathew *et al.*, 2000).

A survey conducted by Nair *et al.* (2000b) revealed heavy incidence of the mite in Kerala, Karnataka and Tamil Nadu. Observations on the population dynamics showed that the mite is persistent on coconut with peak incidence during summer months.

Muthiah and Bhaskaran (2000) opined that the coconut palms of all ages are infested, among them plants at 40 years of age are more severely affected (96%) compared to 15 years of age (81%). The severity of mite incidence was more in five month old bunches (84%) compared to three months old bunches (14%).

Observations on the seasonal abundance of the mite showed the persistent nature of the pest with the population peak in summer months (April-May). The extent of damage varied from 20-60 per cent (Nair and Koshy, 2000).

A preliminary survey conducted in a few villages of Coimbatore district on the incidence of the mite indicated that the mite infestation ranged from 5.0 to 48.0 per cent in the nuts on the trees and 12 to 52 per cent in the harvested nuts. The population was very high in 2 to 6 month old buttons. As many as 2 to 140 mites along with eggs were found in an area of 4 mm^2 (Ramaraju *et al.*, 2000).

Studies by Yaligar (2004) on seasonal incidence of eriophyid mite on coconut palms in Dharwad taluk indicated that the mite population occurred throughout the year with variation during different seasons of the year. The mite population was high during January and May, 2003. It started declining with the onset of rains during second fortnight of June, 2003 and increased again during November when rainfall reduced. No clear-cut relationship observed when egg and mite population correlated with weather parameters. However, mites on nut surface were positively correlated with temperature and negatively correlated with evening humidity.

Results of the field experiment at Millet Breeding Station of TNAU about the build up of eriophyid mite and the predatory mites revealed that the population trend is almost constant throughout the year. The population was high during the second fortnight of May (32.88/4 mm^2) and November (34.26/4 mm^2)

and declined during the second fortnight of December and February, 2001 (0.83-3.60/4 mm²). Correlation and regression analysis of the data for the year 1998-1999 revealed no significant relationship between live mite population and weather parameters (Kannaiyan *et al.*, 2002).

According to Haq (1999), the population density of CEM at Puthukkod in Thrissur district increased slightly from July to August and then declined upto October. A slow increase of the population occurred from October to December which accelerated from December to March-April, then declined from May to July. A positive correlation between mite population and dry climate during summer months and negative relationship with the rainfall during the monsoon was observed.

Vidhyasagar (2000) noticed the incidence of the mite in the gardens throughout the year with a peak in the population during summer or dry periods in Kerala. Similar observations have been reported from Chittoor district of Andhra Pradesh (Reddy and Naik, 2000). While Varadarajan (2000) found that the population of mites and their eggs had no significant relationship with any of the weather factors.

Studies conducted in the three agro-ecological zones of Kerala showed a significant difference in mite population according to variation in locations. In comparison of three zones, live mite count on inner perianth and nut surface was significantly high in central zone (mean number 33.486 and 104.094/5 mm²) followed by Northern and Southern zone which were on par. Similarly Central zone recorded a significantly high dead mite population on inner perianth (18.728/5 mm²) as compared to Northern and Southern zones which were on par. Dead mite population on nut surface was also significantly high in Central zone (9.304/5 mm^2). The seasonal variation on coconut mite population showed more or less same trend in all the six districts. The peak live and dead mite population was during summer months (February to April) in most of the districts and was very low or almost steady during rainy season (Anonymous, 2005).

2.1.7 Management

2.1.7.1 Water management

Mariau (1986) observed that a hybrid plantation after a period of water shortage aggravated mites due to drought. This was because the nuts developed more slowly and thus remain susceptible to the mites for a longer period. While Alencar *et al.* (1999) suggested avoidance of excessive irrigation as a cultural control method.

2.1.7.2 Nutritional management

According to Mandal (1991) indiscriminate usage of nitrogen fertilizers decreased the resistance of plants, whereas potassium is reported to impart resistance potential in coconut towards insects and non-insect pests like mites.

Ramarethinam and Marimuthu (1998) demonstrated that lack of potassium lead to poor water retention which leads to poor insect and mite resistance. This was similar with that of the findings by Kannaiyan *et al.* (2000) where they found that increased application of potassium @ 3.5 kg per palm per year recorded the least mite population. Application of neem cake @ 2 kg + bone meal 0.5 kg + mill ash 4 kg (per tree per year) also recorded least mite damage (29%).

Varadarajan (2000) observed that the mite population was lowered by 40 to 80 per cent on the nuts of palms that received boron nutrition either as root feeding with two per cent borax solution @ 250 ml per palm or as soil application of borax @ 400 to 600 g per palm.

2.1.7.3 Crown cleaning

Removal of infested plant parts was recommended as cultural control method against CEM (Alencar *et al.*, 1999).

Studies conducted by Ranjith (2003) revealed that coconut crown cannot sustain mite population in areas other than the fertilized coconut buttons. Eventhough the crown cleaning may not reduce the population of perianth mite in coconut; it results in less harbour of saprophytes which can reduce phoresy of mites.

2.1.7.4 Innovative farmer practices

According to Mariau (1977) monthly treatment of bunches with seawater reduced the mite attack probably by half. Chezhiyan and Ramar (2000) suggested that crown cleaning with water spray could lead to low mite infestation.

Nair *et al.* (2000a) reported the innovative practices adopted by farmers of Kerala State viz., application of neem cake powder, garlic powder, turmeric powder etc. on the crown, generating smoke from farm waste, garbage waste, camphor etc. in the garden, hanging sticky traps on the crown, spraying rice water and other sticky materials on the bunches.

2.1.7.5 Natural products

Application of neem cake @ 2 kg along with bone meal 0.5 kg and mill ash 4 kg on the crown resulted in significant reduction in CEM damage (Muthiah and Bhaskaran, 2000). But no significant reduction in CEM was obtained by application of neem cake on the crown @ one kg per palm at 45 days interval (Saradamma *et al.*, 2000b).

Field evaluation studies of oil cakes, wood ash, kaoline and innovative farmer practices against CEM showed that maximum mite population suppression was obtained with starch solution five per cent (90.67%) closely followed by kaoline one kg per palm (89.85%), neem cake one kg per palm (88.33%) and salt solution five per cent (84.94%) after three rounds of application at one month interval (Amritha, 2001).

2.1.7.6 Botanicals

Neem is known to control plant mites (Ramarethinam and Marimuthu, 1998).

Acaricidal property of hedge plant, *Lantana camera* extract on coconut palms infested by CEM was identified and confirmed under bioassay and field trials for the first time by Edwin *et al.* (2000).

Saradamma *et al.* (2000a) recommended neem oil-garlic soap emulsion (2%) an eco-friendly plant based pesticide as an adhoc measure. The recommendation was found to be effective in India (in the States of Kerala, Tamil Nadu and Karnataka) and Sri Lanka (Fernando *et al.*, 2000). Further field trials

conducted by the same group indicated the effectiveness of a neem based formulation viz., Neemazal T/S one per cent @ 3 ml per litre against CEM (Saradamma *et al.*, 2001).

Balaji and Hariprasad (2003) evaluated the efficacy of aqueous suspension of five plant extracts namely phytopalm (3% and 5%), NSKE (5%), Neem oil (3%), Nochi leaf extract (3%), calotropis leaf extract (5%) and commercial neem formulations viz., Neemazal 1000 ppm (1%) and Fortuneaza 3000 ppm. Among the different plant products, phytopalm gave significantly higher per cent reduction of mite population at early stage of nut development. Neem formulations were on par with each other in which Nochi leaf extract (3%) was least effective.

Thirumalaithevan *et al.* (2003) reported that NSKE (10%), garlic extract (10%), neem oil (6%) and sweet flag (10%) were significantly effective in reducing the mite population and nut damage.

Studies conducted by Amritha (2001) on laboratory screening of various oils and botanicals observed that the mite population was reduced in neem oil three per cent (60.07%) followed by Neemazal (Azadirachtin 1%) 0.4 per cent (57.77%) and castor oil three per cent (57.41%).

2.1.7.7 Chemical control

Mohanasundaram *et al.* (1999) reported that spraying of triazophos 5 ml or methyldemeton 4 ml or phosalone 3 ml per litre of water, root feeding of Triazophos 20 ml + 20 ml water per palm were effective against CEM.

Anonymous (1999) reported that 0.05 per cent of Triazophos, carbosulfan and endosulfan applied as spray on the affected bunches controlled the CEM infestation. They also reported that wettable sulphur 0.4 per cent and Azadirachtin 0.004 per cent gave results comparable to that of chemical pesticides.

An experiment conducted at Krishnapuram, Kerala showed that the application of 0.4 per cent wettable sulphur, 0.004 per cent azadirachtin and 0.05 per cent endosulfan were found effective in the management of CEM (Chandrikamohan and Nair, 2000).

Based on the field trials conducted, Nair *et al.* (2000a) and Saradamma *et al.* (2000b) reported that neem oil-garlic mixture two per cent alternatively with Dicofol 0.1 per cent, Monocrotophos 0.1 per cent and wettable sulphur 0.4 per cent were effective in managing CEM.

Pesticide trials conducted at Kasargod indicated that root feeding with Monocrotophos @ 10 ml per palm with equal quantity of water at monthly intervals proved effective in containing the mite infestation (Vidhyasagar, 2000).

Amritha (2001) reported that Fenazaquin 0.05 per cent (96.49%) was effective against CEM followed by sulphur WP 0.4 per cent (95.42%) among the promising synthetic acaricides in the laboratory studies.

2.1.7.8 Biological control

Biocontrol with natural enemies (pathogens and predators) offers an effective, cheap, eco-friendly and long lasting solution to the pest problems.

The biological control agents can be effectively utilized to suppress CEM (Julia and Mariau, 1979). According to them, microbes and predators attack CEM, but under natural circumstances their effects are minor.

2.2. SURVEY OF FUNGAL PATHOGENS

2.2.1 *Hirsutella thompsonii* (Fisher)

The genus *Hirsutella* infects a number of different types of insects as well as mites and nematodes (Mc Coy and Samson, 1988; Jaffee, 2000). The acarine parasite, *H. thompsonii* Fisher has been reported to be associated with an array of mites and has been used as a biocontrol agent in different ecosystems all over the world with considerable success.

The fungus *H. thompsonii* has already been reported as the most promising and potential biological control agent against CEM in the Ivory Coast and Mexico (Julia and Mariau, 1979; Hall *et al.*, 1980; Berril and Sanchez, 1986; Sampedro and Rosas, 1989).

H. thompsonii has been isolated from the samples of CEM from tropical America and West Africa and from samples of *Colomerus novahebridensis* from New Hebrides, New Guinea and Sri Lanka (Hall *et al.*,

1980). Espinosa and Carrillo (1986) reported 75 per cent mortality of CEM in Mexico but no success was obtained in St. Lucia (Moore *et al.*, 1989).

Survey on the fungal isolates from CEM for one year under three different agro-climatic situations in Kerala resulted in maximum *Hirsutella* isolation (19 nut samples) from the Thrissur district. About 30 per cent of the nuts were infected with *Hirsutella* spp. (Anonymous, 2005). But the pathogen was not encountered during the collaborative studies conducted by Gopal *et al.* (2003) at Thrissur district during the year, 2001.

Kumar *et al.* (2001b) developed *H. thompsonii* based mycoacaricide namely 'Mycohit' which has a potency of 2.5 x 10^8 CFU/g with moisture content of 12 per cent. Survey conducted by them revealed that *H. thompsonii* was found to be wide spread in three districts of Karnataka (Bangalore Rural, Mandya and Kolar) and one in Tamil Nadu (Coimbatore) with an average incidence of 6.85 per cent mite infested coconut samples yielded the fungus.

2.2.2 Varieties of *Hirsutella thompsonii*

The fungus seems to be restricted mostly to the tropics (Samson *et al.*, 1980). *H. thompsonii* var. *synnematosa* was isolated by Mietkiewski *et al.* (2000) from infested phytophagous mites during the survey conducted in Poland.

Beevi *et al.* (1999) isolated *H. thompsonii* var. *synnematosa* from CEM.

2.2.3 Other species of *Hirsutella*

Another species, *H. nodulosa* has also been reported from CEM from Cuba (Cabrera and Dominguez, 1987).

Mietkiewski *et al.* (2000) isolated the *Hirsutella* species namely, *H. nodulosa and H. kirchinerii* during the survey of entomopathogenic fungi conducted from 1995 to 1998 in Siedlce, Poland from infested phytophagous mites. They also reported that most of the fungi are representatives of the genus *Hirsutella*, found both on phytophagous, parasitic or predaceous mites.

Two more species of *Hirsutella* other than *H. thompsonii*, namely, *H. nodulosa* and *H. kirchnerii* were isolated from CEM, (Anonymous, 2005) during the survey conducted in the three agro-ecological zones in Kerala.

2.2.4. Other fungal species and microorganisms

Mietkiewski *et al.* (2000) isolated the fungal species *Neozygites floridana, N. abacaridis, Conidiobolus* spp. and *Verticillium lecanii* during the survey of entomopathogenic fungi conducted from 1995 to 1998 in Siedlce, Poland from infected phytophagous mites.

The bacteria viz., *Serratia marcescens* and *Pseudomonas* spp. and seven fungal isolates were obtained from CEM affected nuts. The fungal isolates were identified as *Rhizopus* spp., *Fusarium* spp., *Aspergillus niger, A. flavus, A. terreus, Trichoderma* spp. and *Penicillium* spp. (Kumar *et al.*, 2000).

A collaborative study conducted at Thrissur district by Gopal *et al.* (2003) found that the Actinomycetes constituted the predominant microflora (seven isolates) followed by yeasts (four isolates), fungi (three isolates) and bacteria (two isolates) from the different locations.

Kumar *et al.* (2004) reported that the hyphomycetous fungus, *Sporothrix fungorum* de Hoog and Vries was affecting the CEM in Karnataka and Tamil Nadu in India during 1999-2000 upto 15 per cent of coconut samples.

2.3. SEASONAL OCCURRENCE

2.3.1 *Hirsutella* spp.

In hot, humid weather, *H. thompsonii* can cause spectacular natural epizootics among mite populations (citrus, blue berry, coconut, tomato mites etc.) and is considered to be one of the key natural enemies of various mites pests (Chandler *et al.*, 2000).

Mietkiewski *et al.* (2000) reported that the per cent infection by *H. thompsonii* in populations of some tarsonemid and eriophyid mites increased slowly from the end of spring reaching a maximum of 30-60 per cent in August-September.

2.4 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

2.4.1 *H. thompsonii*

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*. The strains accommodated in the *H. thompsonii* var. *synnematosa* were isolated from *Eriophyes* spp. and related genera. In fresh cultures, they produced cream coloured cylindrical synnemata bearing two kinds of phialides. Single phialides with solitary conidia and densely crowded phialides with catenulate conidia. In all three varieties, two different conidiogenous structures were formed-solitary often proliferating phialides producing one or more globose, verrucose conidia and polyblastic conidiogenous cells with smooth-walled subglobose to ellipsoidal conidia. The variety *vinacea* is characterized by vinaceous colonies.

The fungus displays a simple growth cycle in *in vitro* studies where the conidia germinate and produce the mycelial phase that gives rise to conidiophores and/or chlamydospores. According to Liu *et al.* (1995), Mazet and Vey (1995) and Vey *et al.* (1993), two strains of *H. thompsonii* var. *thompsonii* have been reported to produce Hirsutellin A (Ht A), a protein that has potent insecticidal and cytotoxic activities. *In vivo*, Ht A displays specific activity to certain insect hosts and insect cell lines (Liu *et al.*, 1996; Vey *et al.*, 1993), suggesting that this protein may play a role in the pathogenic process.

Ht A, produced and readily isolated from cultural filtrates of *H. thompsonii*, displays contact/residual activity against the adult citrus mite *Phyllocoptruta oleivora*, the natural host of this mycopathogen (Omoto and Mc Coy, 1999) and to neonate mosquito *Aedes aegyptii* larvae. Significant reduction in egg production was also reported when the mites were topically treated with the mycopathogen.

The effects of environmental factors on *H. thompsonii*, a moniliaceous fungus were studied in the culture medium, PDA. The fungus was mesothermophilic where the growth, sporulation and conidial germination were found best at 25 to 30°C. Almost all conidial germ tubes survived an 8 h exposure to 3-5 per cent relative humidity (RH) and to 60 per cent RH; but subsequently the former grew poorly at 100 per cent RH. Light has no influence, *H. thompsonii* sporulated equally well in continuous darkness or light, and produced typical chlorinous to light olive-green mycelium and conidia under all conditions. A two

hour exposure of naked mycelium and conidia (have melanised walls) to U.V. irradiation failed to kill the fungus (Kenneth *et al.*, 1979). Similar was the results obtained by Gerson *et al.* (1979) where he opined that use of the fungus for mite control would be suitable particularly in tropical and humid subtropical areas.

Studies conducted by Maimala *et al.* (2002) revealed that *H. thompsonii* isolates developed as mycelial cultures, and certain isolates produced chalmydospore - type structures on the mycelia after four days of incubation. In general, *H. thompsonii* displayed as much heterogeneity in broth as it did on solid agar plates.

Certain strains acidified the broth, whereas other isolates caused an increase in pH of the media. At four days, the colour of broth cultures ranged from yellow to burgundy red. While microscopic examination using lacto-phenol blue confirmed that all isolates produced mononematous conidiophores, characteristic of the genus. Maimala *et al.* (1999) also conducted studies on the growth of *Hirsutella* in three different types of media; semi-solid, solid and liquid media. Colonies on soya bean agar produced the highest number of spores (9.15 x 10^7 spores/ml) whereas those on malt extract agar provided a significantly higher number of colony forming units (CFUs) (4.78 x 10^9 CFU/ml). Semi-solid agar is not recommended because of its high cost. Suitable liquid medium comprised 8 per cent unpolished rice, 2 per cent molasses and mineral salts. Dried mycelium harvested from liquid medium contained 7.92 x 10^6 CFU/g. The fungus failed to produce spores in the broth, but many chlamydospores were observed within the hyphae.

Rosas *et al.* (1995) developed a large-scale method for producing entomogenous fungi, *H. thompsonii* and *H. nodulosa* in two-phase culture (liquid and solid). The vegetative growth obtained in liquid media was inoculated on to eight solid media. For most strains, the greatest conidiogenesis was obtained on rice, barley and bran. The maximum production of conidia was obtained with the Ht M2, Ht M4481 and Ht C59 strains of *H. thompsonii* being 334.75, 269.68 and 137.12 x 10^7 conidia per g respectively.

McCoy and Kanavel (1969) isolated the fungus and studied its growth and sporulation in culture. Colonies on potato dextrose agar (PDA) were grey, raised, cottony with grey reverse; hyphae 1.5-2 μ wide, smooth, conidiogenous cells arising singly at intervals from the vegetative hyphae, mono or polyphialidic, unevenly verrucose, consisting of a broadly based inflated portion 5-8 μ high x 2-2.5 μ wide at the base, conical to flask-shaped, increasing more or less to 2-3.5 μ wide at the broadest part then decreasing abruptly to a narrow neck, 2-5 μ long by 0.5-0.75 μ wide. The neck is sometimes unbranched, often branched once, rarely two or more times, bearing enteroblastic conidia singly at the tip of each branch. Conidia spherical, strongly verrucose, 2.5-3.5 μ diameter perfect state unknown (Minter and Brady, 1980).

Phase contrast microscopy revealed that the hyphae were wide and smooth. According to Brady (1979) the conidia are spherical, strongly verrucose, up to 3.5 μm in diameter. 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.

Studies conducted by Padiyath (2002) revealed that Sabouraud's Maltose Agar + Yeast (SMA + Y) was the most suitable solid medium for growth, sporulation and germination of the *Hirsutella* fungus.

Beevi *et al.* (1999) isolated *H. thompsonii* from dead colonies of coconut eriophyid mite and brought to pure culture on PDA. Mycelial growth was observed on second day and the colonies attaining a diameter 2.0 to 5.0 cm within 15 days at 28°C. Grey raised and fast emerging with pale pinkish synnemata were noticed. Hyphal characters were same with 1.7-2.7 μm in width. Large number of conical to flask shaped phialides (6.5-8.7 μm high x 2.3-2.8 μ wide) arose singly at regular intervals. Phialides consists of broadly based inflated portion with a narrow neck (1.9-4.5 μm long x 0.5-0.8 μm wide) bearing single spore. Conidia spherical, verrucose and hyaline measuring 2.5-3.8 μm diameter.

2.4.2 *Hirsutella kirchnerii*

Culture of *H. kirchinerii* on SDA is slow growing achieving a diameter of less than 25 mm in one month, with a convoluted surface, aerial mycelium cream coloured sometimes exuding a brown pigment into the agar which is visible from above as a brown perimeter line 1-5 mm wide. On the reverse, brown colour and the agar split in places through which the aerial mycelium becomes visible sometimes. Mycelium in culture hyaline to brown, septate, thin-walled, smooth, branched, 2-4 µm diameter, with many branched chains of swollen hyphae, 4-14 µm diameter, with hyaline walls, these swollen hyphae easily disarticulating into their component cells. Sessile conidiogenous cells arising singly, more or less at right angles from vegetative hyphae. Conidiogenous cells phialides, hyaline, thin-walled, smooth, flask-shaped, 15-24 µm long, consisting of a more or less cylindrical venter, 7-14 x 2.5-4 µm, tapering gradually between one and two-thirds of the total height to a narrow, sometimes slightly roughened neck, 7-13 x 0.5-1 µm, which may bear up to three branches, 7-13 µm long, at the apices of which conidia are produced. Conidia hyaline, aseptate, thin-walled, smooth, more or less lemon-shaped, minutely truncate at the base, with no visible scar after recession, 4.5-6 x 2.5-3.5 µm (Minter *et al.*, 1983).

The acaropathogenic fungus *H. kirchnerii* (Rostrup) Minter, Brady and Hall grew best and produced most mycelia on a medium containing yeast extract, dextrose and agar. However, conidial production was maximum on potato dextrose agar (PDA). Best growth was at 25°C, while conidial germination ranged at temperature of 10 to 35°C. Eventhough, the yield was best under alternate dark and light regimes, maximal germination occurred under dark conditions. Under continuous light, the fungus produced synnemata which remained viable for 22 weeks (Sztejnberg *et al.*, 1997).

2.4.3 *Hirsutella nodulosa*

Mycelium sparse to abundant, immersed and superficial, hyaline to pale brown, septate, rarely branched, smooth or more frequently slightly rough, with hyphae 2-3 µm wide. Conidiophores reduced to sessile conidiogenous cells or occasionally conidiogenous cells borne on a single stalk, arising singly or in

pairs, more or less at right angles from the vegetative hyphae, 'sometimes in verticils. Conidiogenous cells monophialidic, also polyphialidic, hyaline, with tiny warts, 20-35 µm long, swollen to 4 µm wide towards the base, gradually tapering to 1 µm wide in a neck about 10 µm long which often twists in a helix towards the apex, or when polyphialidic, with up to three such necks each of which may be twisted. Conidia aseptate, hyaline, smooth, ellipsoid or the shape of an orange segment, 5-6 µm long, about 3 µm wide, arising in small groups embedded in a pigmented mucous sheath at the apex of each neck, the groups being limoniform 6-7 µm in their largest dimension (Minter and Brady, 1980).

2.4.4 Other fungal species

The fungus, *Sporothrix fungorum* was able to grow and conidiate profusely on PDA as well as SDA. 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. Enrichment medium (EM), best liquid medium 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 83.10^7/ml) (Kumar *et al.*, 2004).

2.5 PATHOGENICITY AND BIOASSAY STUDIES

2.5.1 *Hirsutella* sp

Ramarethinam *et al.* (2000) studied the potentials of *H. thompsonii* as entomopathogenic fungi in the control of CEM which has been observed to be a significant one. They suggested a neem oil based EC formulation Nimbecidine (Azadirachtin 0.03 per cent) of 500 ml in combination with the entomopathogenic fungi, *H. thompsonii* (1x10^7 CFU/g) 1000 g in 200 l of water for CEM control where he obtained 37 per cent and 42 per cent infectivity in the field and laboratory maintained coconut bunches.

Hall *et al.* (1980) were the first to study the natural mortality factors of coconut eriophyid mite and establish the fungus *H. thompsonii* to be a naturally occurring control agent of *A. guerreronis*. Pathogenicity tests conducted by spraying the fungal spores and mycelial fragments on the mite colonies after removing the bract and then replacing them proved all isolated strains of *H.*

thompsonii to be pathogenic to CEM killing the mites within 48 h. The favourable microclimate with high humidity was found to be conducive for fungal development and the spread of the fungus among the mite populations.

Single application of laboratory produced *H. thompsonii* mycelia at a dose of 0.5-1.0 g/l to citrus caused 90 per cent reduction in the population of mites in three days (Chiang and Huffaker, 1976). Efficacy of *H. thompsonii* to control citrus rust mite in the field in USA, Surinam, Israel and China has been recorded to be very promising (McCoy, 1978 and Gerson *et al.*, 1979).

Pathogenicity tests performed by Sampedro and Rosas, 1989 with seven strains of *H. thompsonii* at a conidial concentration of 7000±500, 14000±500 and 26000±500 c/ml against *A. guerreronis* resulted in the greatest mortality (88.36%) with the strain *H. thompsonii* MOR.

Saradamma *et al.* (2001) reported CEM mortality of 30-60 per cent in the laboratory evaluation of the pathogenicity of *H. thompsonii* fungus.

Field experiment conducted at the Instructional Farm, Vellanikkara revealed the effectiveness of *H. thompsonii* where three sprays of its formulated product at 14 days interval gave a mortality of 93 per cent (Anonymous, 2002).

Laboratory bio-efficacy studies of three formulations of entomogenous fungi (*Verticillium lecanii, Paecilomyces fumosoroseus* and *H. thompsonii*) at concentrations 10^6, 10^7 and 10^8 spores per ml against pink mite in tea showed that they were susceptible to all the three pathogens. Satisfactory control was obtained from 5th day after the application and its efficacy was 100 per cent on the 10th day after application (Selvasundaram *et al.*, 2001).

2.5.2 Other fungal species

Significant reduction in mite population by 51 and 55 per cent respectively were observed by Muthiah and Bhaskaran (2000) 15 days after spraying with Fish Oil Rosin Soap (FORS) alone and in combination with *Fusarium* spp.

Among the cell free filtrates of fungal isolates evaluated, *Fusarium* spp. was found to be more effective in reducing the population of CEM (Kumar *et al.*, 2000).

Cell free extracts and cell homogenates of nine isolates of Actinomycetes obtained from rhizosphere of soil of CEM infested coconut palms were observed for reduction in mite population at 4 h intervals from 2nd day onwards up to two days by destructive sampling. Results showed no mortality of mites due to the treatments (Prasad, 2000).

Studies conducted by Natarajan *et al.* (2000) on nutrients in comparison with standard recommended insecticides against CEM revealed that *Pseudomonas fluorescens* application in soil @ 100 g per tree recorded lowest incidence of mite population.

Saradamma *et al.* (2001) reported that among the various entomogenous fungi tested, *Verticillium suchlasporium* was found to infect CEM. They also reported a mortality of 30-60 per cent in the laboratory and field evaluation of the pathogenicity of fungus.

Pathogenicity trials in the laboratory revealed that fungi and Actinomycetes isolated during the survey at Thrissur district caused mortality to the mites in the range of 10-20 per cent, Actinomycetes 15-20 per cent and bacteria less than 10 per cent. Yeasts were found to be non pathogenic to the mite (Gopal *et al.*, 2003).

Laboratory evaluation of the fungus, *S. fungorum* at 2 x 10^5, 2 x 10^6 and 2 x 10^7 conidia per ml could bring about 47.03, 92.75 and 98.29 per cent moralities respectively, 96 h after inoculation (Kumar *et al.*, 2004).

2.6 MOLECULAR CHARACTERISATION

Molecular markers provide immense sources of data that can assist scientists in developing tools to monitor the genetic and environmental fate of these agents.

H. thompsonii is polymorphic in several biological characteristics, including its potency to serve as a biocontrol agent. Reliable identification of native isolates is crucial for the study of *Hirsutella* related control of mites.

Mozes *et al.* (1995) identified three *Hirsutella* species (*H. necatrix* and *H. kirchnerii* and six isolates of *H. thompsonii)* by an assay for random amplified polymorphic DNA (RAPD). RAPD markers correlated with the mite host of

specific fungus isolates. They also reported that RAPD was useful for identifying interspecific heterokaryons obtained by hyphal anastomosis. Correct RAPD identification was corroborated by comparing the alpha-esterase isoenzyme patterns in these isolates.

Maimala *et al.* (2002) found that most of the *Hirsutella* isolates (100 out of 162) isolated from infected mites world wide were shown to possess the toxin Hirsutellin A (Ht A). The presence of the gene coding for Ht A was determined by PCR amplification using gene-specific primers.

Hirsutella longicolla var. *longicolla* and *Hirsutella longicolla* var. *cornuata*, entomogenous fungi (19 isolates) isolated from tortricid *Choristoneura fumiferana*, were distinguished by Strongman and Mackay (1993) using RAPD DNA finger printing. Two banding patterns, one corresponding to isolates of the species and the other to the variety, were generated with 2 primers and DNA from 19 isolates. Further analysis using nine isolates (4 of the species and 5 of the variety) with three additional primers confirmed the distinction between the species and its variety. The work illustrated the usefulness of the RAPD technique in taxonomy.

2.7 PREDATORY MITES

2.7.1 Predacious mites

Hall *et al.* (1980) observed predation of adults of CEM and *Colomerus novahebridensis* by two species of *Lupotarsonemus*, but they caused only minor effect on population of either pest species. The predators *Bdella distincta*, three phytoseids *Amblyseius largoensis* Muma, *Neoseiulus mumai* Denmark and *N. paspalivorus* Deleon were reported by Julia *et al.* (1979) and Howard *et al.* (1990). A species of phytoseid predatory mite, *Amblyseius (Neoseiulus) parpalivorus* (Nair *et al.*, 2000a) a tarsonemid mite (Ramaraju *et al.*, 2000) and *Bdella* spp. (Fernando *et al.*, 2000) were found inhabiting the perianth region in very low population. Saradamma *et al.* (2001) also reported predatory mites from infested nut samples and they were identified as *Amblyseius* spp. (Phytoseidae), *Bdella* spp. (Bdellidae) and a tarsonemid mite.

Tydeidae mites have also been shown to feed on CEM and have significant impact on other species of eriophyid mites (Moraes, 2000).

Studies on the predatory mite population showed that two predatory mites, *Bdella* spp. *Amblyseius* spp. were found inside the perianth. Predatory mite population varied from 19.942 to 20.477 per cent and no significant difference was noted among the three zones (Anonymous, 2005).

Survey conducted by Beevi *et al.* (2004) revealed that three species of phytoseids viz., *Amblyseius largoensis* (Muma), *Amblyseius* (*Asperoseius*) *nuciferae* (Gupta) and *Amblyseius* (*Euseius*) *alstoniae* (Gupta) (Phytoseiidae: Acari) and other acarines *Hypoaspis krameri* (Laelapidae:Acari), *Pachygnathus* spp.) (Pachygnathidae:Acari), *Rhyzoglyphus echinops* (Fumuse & Robin) (Acaridae:Acari) and *Tyrophagous putrescentiae* (Schrank) (Acaridae: Acari) were associated with CEM collected from fourth/fifth bunch of coconut with high mite population.

2.7.2 Other predators

A coccinellid and a syrphid larva (Nair *et al.*, 2000a); thrips and anthocorid bugs (Ramaraju *et al.*, 2000) were also reported, but the feeding potential are yet to be assessed.

A species of syrphid maggot, coccinellid grub and predatory thrips were found in CEM infested colonies (Saradamma *et al.*, 2001).

MATERIALS AND METHODS

MATERIALS AND METHODS

The present study on "Evaluation and characterization of effective fungal pathogens associated with the coconut eriophyid mite *Aceria guerreronis* Keifer was conducted in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, during the period October 2002 to December 2005. The details of the materials used and the techniques adopted for the investigation are described below.

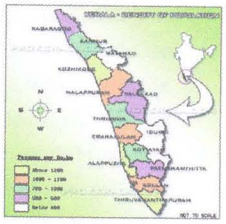
3.1 SURVEY

3.1.1 Plot Selection

The survey was conducted at Thrissur district (10°31' N latitude, 76°13' E longitude) during July 2003 to June 2004. Four panchayaths, viz., Pananchery, Madakkathara, Koorkenchery and Pariyaram of Thrissur district were selected for the survey (Fig. 1). From each panchayath, three plots with a minimum of 100 full bearing coconut trees of uniform age (15-20 years) with heavy eriophyid mite infestation on nuts was selected randomly. The details of the plots selected for the collection of sample nuts are presented below:

Table. 1 Plots selected in Thrissur district for sample nut collection

Sl. No.	Panchayath	Location / Plots
1	Pananchery	a) Banana Research Station (BRS), **Marakkal** b) Central Plantation Crops Research Institute (CPCRI), **Kannara** c) State Seed Farm (SSF), **Mudicode**
2	Madakkathara	a) Mr. Sathyan, Thekkevila veedu, **Chirakkekodu** b) Instructional Farm (IF), **Vellanikkara** c) Cashew Research Station (CRS), **Madakkathara**
3	Koorkenchery	a) Mr. Balachandran, Mankuzhy, **Kanimangalam** b) Mr. Sankaran, Ashok Bhavan, **Nedupuzha** c) Mr. K. C. Sundaran, Korottil House, **Panamukku**
4	Pariyaram	a) Cattle Breeding Farm (CBF), **Thumbermuzhy** b) Mr. P. K. Sadasivan, **Konnakuzhy** c) Mr. David M. P., Mecheryveedu, **Kanjirampally**



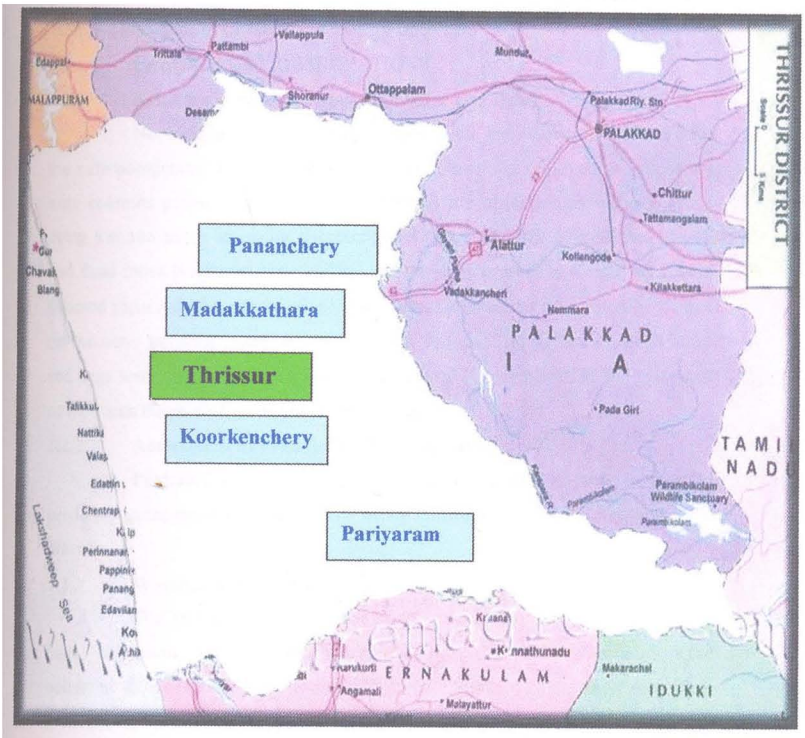


Fig. 1 Panchayaths selected from Thrissur district for sample nut collection

3.1.2 Sampling

Four trees were chosen randomly from each of the selected plots for collecting the mite infested nut samples. Two mite infested nuts were taken from the marked trees, one nut each from the 3rd and 4th bunches labelled from the top (approximately three and four months old) which was reported to have the active live mite colony (Ranjith *et al.*, 2001). Buttons showing external symptoms of mite damage viz., yellow triangular patches which have active CEM colonies were taken for the study (Plate.1). Fresh infested buttons excised were packed in polythene covers and tied well. Then they were brought to the laboratory. The sampling was done at monthly intervals for one year.

3.2 LABORATORY OBSERVATIONS

3.2.1 Assessment of Mite Population (Live and Dead)

Nut samples collected were brought to the laboratory and observed for the mite population. The bracts of the nuts were carefully removed to expose the mite colonies present beneath the perianth and the observations were recorded using a stereo zoom binocular microscope (Nikon SMZ800). The number of live and dead mites (scattered and patches) and the eggs present in 4 mm^2 area were counted separately from three mite infested areas with active mite colonies located on the outer perianth, inner perianth, and nut surface. The live mites, dead mites and eggs were counted from each 4 mm^2 area at 40 X magnification and presented as the mean number of mites per 4 mm^2 per nut.

3.2.2 Assessment of Predatory Mite Population

Predatory mite count was recorded as the total number of moving predatory mites present on the nut as a whole immediately after the removal of the bracts.

3.2.3 Weather Parameters

The monthly weather parameters such as rainfall, number of rainy days, maximum and minimum temperature and the relative humidity were collected from meteorological observatory from one of the three respective locations of the three panchayaths. Weather data was collected from the locations, Kannara of Pananchery panchayath, Chirakkekodu of Madakkathara panchayath

and Thumbermuzhy of Pariyaram panchayath (Appendix 1 to 3). Monthly mean counts of the predatory mites, active stages of the mite, dead mite and egg population of the respective locations were correlated with weather parameters to assess the influence of season on mite population. For the correlation analysis, the observations on mean number of live and dead mites and egg count recorded on perianth and nut surface were added and the total was estimated.

3.2.4 Standardisation for the selection of mycosed mites for fungus isolation

Dead mite colonies were observed under a phase contrast microscope (Nikon) for different types of mortality symptoms. Different types of dead mite colonies such as opaque/transparent and patch/scattered were selected for the fungal isolation.

3.3 FUNGUS ISOLATION

Mycosed mites were selected based on the mortality symptoms standardized earlier. Dead mites occurring in patch/scattered mainly on inner perianth were selected for isolation (Plate.2A & 2B). 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 with a pH of 6.0.

3.3.1 Composition of the Media

Potato Dextrose Agar (PDA)

Potato - 200 g

Dextrose - 20 g

Agar - 20 g

Water - 1 litre

Sterilised Petri dishes plated with PDA were used for fungal isolation. Antibiotic, Streptomycin was added to the medium before plating the Petri dishes @ 0.16 g per 200 ml to avoid bacterial contamination. Petri dishes were marked with four circles on the underside for inoculation of mites.

The tepals (outer and inner) and nut surface containing mycosed mites were sterilized by dipping in sodium hypochlorite 0.5 per cent 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 sterile micro-needle from the bracts and nut surface while observing under a stereo zoom microscope.

Surface sterilized mites 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 upto two weeks.

3.3.2.1 H. thompsonii isolates

H. thompsonii growth was identified based on the growth characters reported by Beevi *et al.*, 1999. An initial grey coloured with raised uniform growth was observed three days after inoculation. Microscopic observations revealed the presence of hyaline segmented mycelium with phialides. Phialides possessed swollen base and a narrow neck with corrugated spherical spores at the tip of the phialides (Plate.3).

3.3.2.2 Other entomofungal species

Preliminary identification of the acarifungal pathogen 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 the published records.

3.3.3 Maintenance of the Fungal Cultures

All the entomofungal pathogens obtained from the field-collected mites were maintained in pure culture and used for further pathogenicity tests after their identification.

3.3.4 Seasonal Variation of Entomofungal Pathogens

Observations were recorded on the number of samples yielding *Hirsutella* spp. and their occurrence during the survey. Number of other fungal species isolated during the survey was also recorded. Seasonal variation in occurrence of *H. thompsonii* and other entomofungal pathogens were also assessed.

3.4 IDENTIFICATION OF THE FUNGI

The final confirmation of species identity was done by Common Mycological Institute (CMI), Commonwealth Agricultural Bureau International (CABI), United Kingdom.

3.5 CONFIRMATION OF PATHOGENICITY

The fungi repeatedly isolated from the dead mite patches were subjected to pathogenicity tests. The experiment was conducted to confirm the pathogenicity of all fungal species isolated from CEM and proved the Koch's postulate. The following treatments were used.

Treatments : 11

Replications : 3 nuts per treatment

T_1 – *Acremonium strictum*

T_2 – *Acremonium implicatum*

T_3 – *Acremonium incoloratum*

T_4 – *Acremonium terricola*

T_5 - *Fusarium lateritium*

T_6 - *Fusarium verticillioides*

T_7 – *Paecilomyces fumosoroseus*

T_8 – *Paecilomyces lilacinus*

T_9 – *Hirsutella thompsonii* (all isolates)

T_{10} – *Hirsutella kirchnerii*

T_{11} – Untreated control

Two to three months old nuts exhibiting single triangular patches of damage which indicated the presence of an active colony of mites were selected. The respective fungal suspensions were prepared by mixing 1 cm fungal culture disc (ten days old taken from half the radial distance) with 10 ml of sterilized water. Tween 80 (0.2 per cent) prepared in sterile distilled water was added for uniform distribution of the spores. Tween 80, 0.2 per cent was used as control. Using a fine syringe (1 ml capacity), the prepared suspensions, 60 µl were injected separately into the space between the perianth and meristem on the nut

surface (Plate.4) exactly at the place of the mite infestation (Kumar and Anuroop, 2004).

Nuts in each replication (three per treatment) were kept in a polythene cover filled with air (Plate.5), so that the nuts remain fresh up to one week without reduction in turgor pressure. The treated nuts were incubated in BOD incubator at a temperature of 27°C. Counts of live; dead and mycosed mites as well as the microscopic observations 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 phase contrast microscope. The dead mites were collected and subjected to reisolation as per the procedure mentioned in 3.3.1. The fungal cultures thus obtained were compared with the original cultures.

3.6 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS OF FUNGI

The fungal cultures which proved positive in the pathogenicity tests were selected for studying their cultural and morphological characteristics.

3.6.1 *Hirsutella* spp.

In the case of *Hirsutella*, the cultural characteristics of each isolate were studied in Sabouraud's Maltose Yeast + Agar (SMA + Y) medium with a pH of 6.0 (Padiyath, 2002). The composition of SMA + Y is given below:-

Maltose - 40 g
Bactopeptone - 10 g
Yeast extract - 10 g
Agar - 15 g
Water - 1 l

3.6.1.1 Growth rate.

Various growth aspects of *Hirsutella* were studied. Fungal disc of 1 cm diameter (cut by cork borer) was taken from 15 day old fungal culture. The disc was placed at the centre of the media plated petri dish. Three replications were maintained for each treatment. The Petri dishes were incubated in BOD

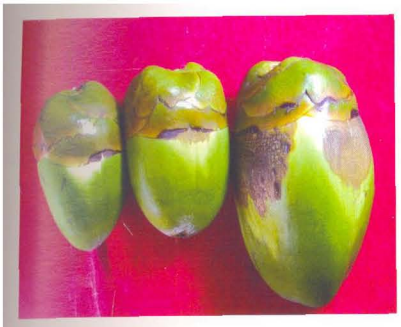


Plate 1. Mite infested nuts

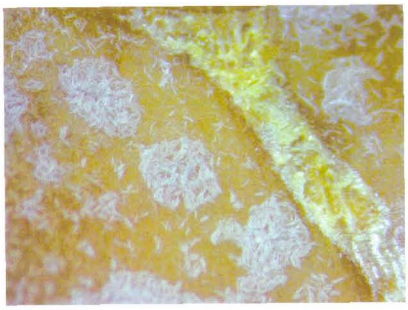


Plate 2A. Patch mortality of mites on inner perianth

Plate 2B. Scattered mortality of mites on inner perianth

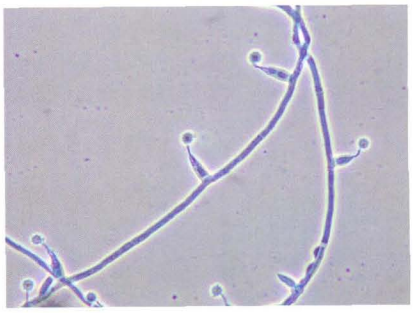


Plate 3. *H. thompsonii* (Phialide with conidia)-1000x

Plate 4. Injection of fungal suspension into perianth space

Plate 5. Treated nuts in air filled polythene cover

incubator at a temperature of 27°C. Measurement on radial growth (cm) of the culture was taken from second day of incubation upto ten days.

3.6.1.2 ***Colony character***

The characteristics like colour, growth pattern and the reaction of fungal culture in the solid media (SMA+Y) were recorded. Presence of honeydew, number of synnemata was also noted on tenth day after the growth initiation.

3.6.1.3 ***Sporulation of the Hirsutella spp.***

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 Haemocytometer (Lomer and Lomer, 1996) using the formula

$$\text{Number of spores/ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

where,

X = Number of spores counted from five small diagonal squares

Y = Number of smaller (1/400) squares checked

10 = Depth factor

1000 = Conversion factor from mm^3 to cm^3

D = Dilution factor

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

3.6.1.4 Growth of different isolates of Hirsutella in broth of SM + Y (Biomass studies)

To study the growth of *Hirsutella* isolates in liquid medium, broth of SM + Y was prepared. Using a cork borer, 1 cm disc was cut from 15 day 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 incubated in a shaker adjusted to 180 rpm for 6 days. The growth of the fungus after 4 weeks was filtered through a pre-weighed filter paper. The fresh weight of the fungal culture was noted. After taking fresh weight it was kept for oven drying at 60°C. The dry weight of the same fungal culture was taken on successive days till it become constant.

3.6.1.5 Micrometry of fungal structures

Slide culture of the fungal isolates was prepared for studying the micrometry. Fungal culture disc of 1 cm (15 day 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 hrs 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.

3.6.2 Cultural and morphological characters of other fungal isolates

The cultural (growth rate and sporulation) and morphological characteristics (colony characters) of other fungi which proved pathogenic were studied in the PDA medium. The growth aspects of fungal pathogens were studied similar to that of the procedure given in 3.6.1.1 and 3.6.1.2 and 3.6.1.3.

3.7 ASSESSMENT OF MITE MORTALITY

The different isolates of *Hirsutella* along with the other fungal pathogens isolated during the survey were tested at respective doses of their formulation against *A. guerreronis* and mortality percentage was calculated. The *Hirsutella* spp., *H. nodulosa* (Plate. 14 & Plate. 16F) was also included in the mortality assessment studies. The same procedure as that in pathogenicity studies was used for assessing the mortality percentage. The conidial concentration of the suspension was determined using an improved Neubauer Heamocytometer as given in 3.6.1.3. The following treatments were used

	Treatments	Spore count (x 10^6 spores per ml)
T_1	*H. thompsonii* var. *thompsonii* (Chirakkekodu-I)	5.92
T_2	*H. t.* var. *thompsonii* (Marakkal-I)	0.33
T_3	*H. t.* var. *synnematosa* (Marakkal-II)	1.08
T_4	*H. t.* var. *synnematosa* (Madakkathara-I)	0.58
T_5	*H. t.* var. *synnematosa* (Madakkathara-II)	0.33
T_6	*H. t.* var. *synnematosa* (Vellanikkara-I)	0.08
T_7	*H. t.* var. *synnematosa* (Kanimangalam-I)	0.67
T_8	*H. t.* var. *synnematosa* (Konnakuzhy-I)	1.25
T_9	*H. kirchnerii* (Kanjirampally-I)	0.17
T_{10}	*H. nodulosa*	0.33
T_{11}	*Acremonium strictum*	37.33
T_{12}	*Acremonium incoloratum*	56.33
T_{13}	*Fusarium lateritium*	3.84
T_{14}	*Fusarium verticillioides*	9.00
T_{15}	*Paecilomyces fumosoroseus*	3.50
T_{16}	*Paecilomyces lilacinus*	5.33
T_{17}	*Verticillium suchlasporium*	5.99
T_{18}	*Sporothrix fungorum*	2.33
T_{19}	*Pseudomonas fluorescens*	49.13
T_{20}	Neemazal – 0.4 per cent	
T_{21}	Wettable sulphur – 0.4 per cent	
T_{22}	Untreated control	

3.8 MOLECULAR CHARACTERIZATION OF ACAROFUNGAL PATHOGENS

Seven isolates of *H. thompsonii* differing in cultural, morphological and bio-efficacy characters were used for molecular studies. The study was intended to find out the variations in different species/strains by RAPD-PCR. Oven dried fungal cultures were utilized for characterization studies.

3.8.1 DNA Isolation

The DNA of the fungal culture was isolated by following the protocol of (Murray and Thompson, 1980) with necessary modifications.

Procedure

Two grams of the dried mycelia was ground to a fine powder with mortar and pestle (sterilized) using liquid nitrogen. Powdered mycelia were then suspended in 10 ml of prewarmed (50°C) extraction buffer [10 pM Tris Hcl (pH 8), 1.4 ml NaCl, 20 µm EDTA, 4% SDS and 0.02% BME] and was subjected to:

Kept on water bath at 37°C for one hour with gentle stirring

↓

1.5 ml of 5 M NaCl was added and mixed and kept for incubation for twenty minutes at 65°C

↓

The mixture was emulsified with an equal volume of phenol:chloroform:isoamylalcohol (25:24:1)

↓

Mixed well and spun at 10,000 rpm for 10 minutes at 10°C (step was repeated till there was no inter-phase)

↓

Aqueous phase was collected and DNA precipitated by adding half the volume of chilled isopropanol

↓

DNA was spooled out, washed thrice in 70% cold ethanol and dried in air

↓

Pellet was dissolved in 1 ml of TE (Tris 10 mM, EDTA 1 mM) buffer

3.8.2 Analysis of Isolated DNA

The quality of DNA isolated was analysed on 0.9 per cent agarose gels. 3 µl of the DNA sample was mixed with 2 µl of gel loading dye and loaded for electrophoresis.

3.8.2.1 Agarose gel electrophoresis-preparation of agarose gel

Gel casting tray was sealed with cellotape to form a mould and set on a horizontal section of the bench after checking the level

↓

Solution boiled and allowed to cool to 42 to 45°C

↓

Ethidium bromide added to a final concentration of 0.5 µg/ml of the agarose solution

↓

Gel poured to a height of 3 to 5 mm into the casting tray with comb in position and the comb was previously adjusted in such a way that the teeth were 0.5 to 1 mm above the plate

↓

Gel was allowed to solidify for 15 to 20 minutes, tape and comb removed; tray was mounted in electrophoresis tank

↓

Electrophoresis buffer (1 x TAE) added to the tank to cover the gel to a depth of 1 mm

↓

DNA samples prepared by mixing DNA with gel loading dye in the ratio 3:2.5 µl was loaded into the slots of gel using a micropipette near the cathode

↓

Gel tank was closed, the cathode and anode of the electrophoresis unit were attached to the power supply and a constant voltage of 110 volts was used for the run

↓

Power supply turned off when the loading dye moved about 2/3rd of the gel

The gel was taken from electrophoresis unit and viewed under UV light in a UV transilluminator. Then the gel was documented using Alpha Imager 1200 (Alpha Innotech Corporation, USA).

3.8.3 RAPD-PCR

Genomic variability can be easily assessed based on length and sequence differences in PCR-amplified segments generated with primers of arbitrary nucleotide sequence. RAPD is used as a molecular marker with which the different strains can be characterized. In this technique, a single short oligo nucleotide primer which binds to many different loci is used to amplify random sequences from a template DNA. The amplified DNA samples were electrophoresed as described earlier.

Procedure

The isolated DNA was subjected to RAPD analysis. The procedure of Williams *et al.* (1990) was modified and used for the amplification of fungal DNA. One cycle included

a) initial denaturation of 1 min at 94°C

b) samples were subjected to 35 cycles of denaturation (94°C, 1 min)

c) primer annealing (35°C, 2 min)

d) primer extension (72°C, 2 min) and

e) final extension of 6 min at 72°C.

The reaction mixture (25µl) consisted of

1. 10x Assay buffer with $MgCl_2$ - 2.5 µl
2. dNTP mix - 10 mM
3. Primer - 25pM
4. Taq DNA polymerase - 1 µl (0.3 units)
5. Template DNA - 25 ng/µl
6. Sterile milli Q water to make up to 25 µl

A master mix without primer and template was prepared using the reaction mixture for the required number of reactions. From the master mix, 20.5 µl was pipetted into each PCR tube, 1.5 µl of primer and 3 µl of template DNA were added. PCR tubes were loaded in the thermal cycles. The programme was run. The programme took 3 hours and 45 minutes for completion.

The amplified products were electrophoresed /size fractionated on 1.2 per cent agarose gel containing ethidium bromide using 1X TAE buffer. DNA fragments were viewed under UV light in transilluminator and then documented using Alphaimager.

3.8.4 Screening of Random Primers for RAPD

Primers under different Operon series viz., OPE (10 Nos.) and OPAH (15 Nos.) were screened for amplification of genomic DNA extracted from the isolate Madakkathara-I using the thermal cycle mentioned under 3.7.4. From these five primers which gave good amplification (≥ five bands) were selected and utilized for further characterisation of the seven *Hirsutella* isolates. The total

number of bands along with the number of polymorphic bands obtained in all the isolates with each of seven primers tried were recorded.

3.8.5 Analysis of RAPD Profiles

The amplification profiles for all the primers were compared with each other and the bands of DNA fragments were scored as present (1) or absent (0) generating the 0, 1 matrices.

The genetic similarity was estimated by computing DICE co-efficient using NTSYS PC-2.0 software programme (Dice, 1945; Nei and Li, 1979). The clustering was done and dendrograms were drawn by following unweighted pair group with metic mean (UPGMA) routine, using the above programme.

3.9 STATISTICAL ANALYSIS

Data on mite population was analysed using three factorial Randomised Block Design (RBD). Bio-efficacy studies in the laboratory were designed in a Completely Randomized Design CRD) and the data on mite population was analysed using the analysis of variance technique. The treatments were compared using Duncan's Multiple Range Test (DMRT). Necessary transformation of the data was done before the analysis of the data.

RESULTS

4. RESULTS

The studies were conducted during October 2002 to December 2005 at College of Horticulture, Vellanikkara, Thrissur. The results of the study are presented below:

4.1. SURVEY ON THE ERIOPHYID AND PREDATORY MITE POPULATION (LOCATION WISE)

Statistical analysis of the overall mean data of 12 months observations on the eriophyid mite population and egg count per 4 mm^2 on the outer perianth (OP), inner perianth (IP) and nut surface (NS) and predatory mite count per nut from four panchayaths of the Thrissur district are given in the Tables.2 to 5. In the present survey, mainly predatory mites of two genus, *Amblyseius* spp. and *Bdella* spp. were found inhabiting the perianth region (Plate. 6).

4.1.1 Pananchery panchayath

Among the three locations of Pananchery panchayath, Mudicode recorded significantly high live mite population on OP (2.438 / 4 mm^2). It was followed by the other two locations, Marakkal and Kannara, which were on par. No significant difference in the live mite population on IP and NS was observed among the three locations. The live mite population per 4 mm^2 ranged from 6.878 to 11.319 on IP and 18.245 to 32.917 on the NS, respectively (Table.2).

Significantly low dead mite population was recorded on the IP (13.811 / 4 mm^2) at Kannara, while the other two locations were on par and recorded a maximum population of 16.174 and 17.785. Live mite population was comparatively high on NS (18.245 to 32.917) as compared to that of perianth (2.302 to 11.319), whereas dead mite population was highest in IP than that of NS. Dead mite population on the NS of the three locations was on par with a value ranging from 7.257 to 8.587.

In the case of egg count, Marakkal location showed significantly high egg count (2.039/4 mm^2) on the OP. The IP and NS had no significant variation in the egg count among the three locations. Egg count ranged from 7.069 to 11.479/4 mm^2 on the IP and 18.031 to 21.445/4 mm^2 on the NS.

While considering the mean predatory mite population per nut, Marakkal location had significantly high population of 13.979 when compared to the other two locations, Kannara (12.448) and Mudicode (7.021) which were on par.

4.1.2 Madakkathara panchayath

On comparing the three locations of the Madakkathara panchayath (Table. 3), the live mite population on OP was significantly high in the Madakkathara location (2.642/4 mm^2). All the locations were on par when considering the live mite population on the IP, which ranged from 6.681 to 8.222 per 4 mm^2. While taking into account of the population of the NS, Vellanikkara recorded significantly low value (18.635/4 mm^2), whereas other two locations were on par with the highest population of 33.826/4 mm^2 in Chirakkekodu and 26.243 in Madakkathara.

Significant difference among the locations was observed in the case of dead mite population of IP. Chirakkekodu had significantly high population of 19.802 per 4 mm^2 on the IP whereas the other two locations were on par (12.944 and 15.493). The dead mite population on the OP and NS did not differ significantly, which ranged from 4.399 to 4.854 and 6.514 to 10.444 per 4 mm^2 respectively.

In the case of egg count, on OP and NS significant difference among the locations was observed. The location, Vellanikkara possessed a significant high egg count of 1.423 per 4 mm^2 on OP. No significant difference was obtained in the egg count on the IP, values ranged from 9.490 to 11.965. Highest egg count was recorded on NS in Madakkathara (31.958) and lowest in Vellanikkara location (16.170).

While taking into consideration of the predatory mite count, significantly high population was obtained from Vellanikkara (9.625/nut) while the other two locations were on par.

Table. 2 Mite population and egg count in different locations of Pananchery panchayath

Locations	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
Marakkal	2.302^{b} (1.149)	11.319^{a} (1.565)	32.917^{a} (2.095)	3.729^{a} (1.245)	16.174^{a} (1.822)	8.587^{a} (1.548)	2.039^{a} (1.100)	11.479^{a} (1.572)	21.445^{a} (1.836)	13.979^{a} (1.702)
Kannara	2.743^{b} (1.121)	6.878^{a} (1.437)	18.245^{b} (1.797)	2.099^{b} (1.129)	13.811^{b} (1.665)	7.257^{a} (1.419)	1.937^{b} (0.997)	7.069^{b} (1.386)	18.938^{a} (1.723)	12.448^{b} (1.523)
Mudicode	2.438^{a} (1.264)	8.653^{a} (1.475)	25.966^{ab} (1.933)	3.847^{ab} (1.200)	17.785^{a} (1.838)	7.761^{a} (1.482)	1.628^{b} (1.024)	8.084^{ab} (1.460)	18.031^{a} (1.789)	7.021^{b} (1.390)

* Mean of eight nuts over 12 months

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

Table. 3 Mite population and egg count in different locations of Madakkathara panchayath

Locations	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
Chirakkekodu	1.951^{b} (1.067)	8.222^{a} (1.511)	33.826^{a} (1.982)	4.854^{a} (1.241)	19.802^{a} (1.903)	10.444^{a} (1.558)	0.531^{b} (0.927)	9.490^{a} (1.472)	28.413^{ab} (1.838)	4.469^{b} (1.237)
Vellanikkara	1.850^{b} (1.085)	6.681^{a} (1.471)	18.635^{b} (1.768)	4.399^{a} (1.240)	12.944^{b} (1.732)	6.514^{a} (1.453)	1.423^{a} (1.024)	9.604^{a} (1.474)	16.170^{b} (1.760)	9.625^{a} (1.418)
Madakkathara	2.642^{a} (1.243)	7.233^{a} (1.435)	26.243^{a} (1.977)	4.722^{a} (1.171)	15.493^{b} (1.719)	9.521^{a} (1.577)	0.583^{b} (0.935)	11.965^{a} (1.520)	31.958^{a} (2.015)	8.021^{b} (1.276)

* Mean of eight nuts over 12 months

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

4.1.3 Koorkenchery panchayath

Mean data (12 months) of the mite population per 4 mm^2 and predatory mite count per nut of three locations of Koorkenchery is depicted in the Table. 4.

In the case of live mite population, Kanimangalam possessed significantly highest population on the OP (1.937/4 mm^2) and on NS (41.437/4 mm^2). Mite population in Nedupuzha was on par with Panamukku on the OP and NS. In the case of IP, Panamukku possessed lowest live mite population of 5.414/4 mm^2 while the other locations had no significant difference with a maximum population of 9.452/4 mm^2 in the Kanimangalam.

While considering the dead mite population, Kanimangalam recorded significantly high mean count on the OP (6.857), IP (19.650) and NS (11.362) per 4 mm^2, whereas the other two locations were on par.

A similar trend was observed in the egg population on the OP, with a significantly high count of 2.132 at the Kanimangalam location. It was lowest (0.656) in Panamukku. Panamukku recorded significantly lowest egg count (9.323) on IP. The other locations were on par with a significantly high count of 12.059 and 15.504. Egg count on the NS did not differ significantly among the locations which ranged from 19.521 to 29.420 per 4 mm^2.

Mean predatory mite count of 15.719 per nut was obtained from Kanimangalam, which was significantly higher than other two locations Nedupuzha (8.875) and Panamukku (8.853) which were on par.

4.1.4 Pariyaram panchayath

Table. 5 illustrates the 12 months mean data on mite, egg counts per 4 mm^2 and predatory mite population per nut of the three locations of the Pariyaram panchayath.

The live mite population showed significant variation among the locations only on the OP. The highest population was from Konnakuzhy (1.792) which was closely followed by Kanjirampally (1.301) and the least population from Thumbermuzhy (0.808). Three locations were on par while considering the

Table. 4 Mite population and egg count in different locations of Koorkenchery panchayath

Locations	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
Kanimangalam	1.937[a] (1.114)	9.452[a] (1.541)	41.437[a] (2.189)	6.857[a] (1.346)	19.650[a] (1.901)	11.362[a] (1.546)	2.132[a] (1.122)	15.504[a] (1.681)	29.420[a] (1.969)	15.719[a] (1.655)
Nedupuzha	1.017[b] (1.026)	6.962[ab] (1.425)	20.364[b] (1.785)	2.652[b] (1.170)	10.524[b] (1.598)	6.823[b] (1.353)	1.010[b] (0.999)	12.059[a] (1.612)	19.521[a] (1.803)	8.875[b] (1.389)
Panamukku	1.066[b] (1.011)	5.414[b] (1.372)	17.556[b] (1.754)	2.798[b] (1.133)	11.330[b] (1.609)	6.531[b] (1.360)	0.656[b] (0.962)	9.323[b] (1.422)	20.691[a] (1.766)	8.853[b] (1.449)

* Mean of eight nuts over 12 months

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

Table. 5 Mite population and egg count in different locations of Pariyaram panchayath

Locations	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
Thumbermuzhy	0.868[b] (1.007)	5.854[a] (1.357)	21.990[a] (1.781)	2.688[b] (1.167)	13.747[a] (1.684)	8.076[a] (1.403)	1.069[a] (0.998)	10.684[a] (1.501)	21.976[a] (1.819)	5.948[b] (1.326)
Konnakuzhy	1.792[a] (1.096)	6.741[a] (1.404)	29.476[a] (1.915)	4.968[a] (1.356)	13.629[a] (1.745)	7.535[a] (1.421)	1.260[a] (1.014)	9.493[a] (1.429)	22.286[a] (1.763)	7.594[a] (1.377)
Kanjirapmpally	1.301[ab] (1.061)	8.590[a] (1.465)	28.076[a] (1.909)	3.302[b] (1.201)	14.135[a] (1.752)	8.337[a] (1.404)	1.118[a] (0.999)	13.618[a] (1.585)	25.073[a] (1.863)	12.802[a] (1.479)

* Mean of eight nuts over 12 months

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

live mite population on the IP and NS which ranged from 5.854 to 8.590 and 21.990 to 29.476 per 4 mm^2 respectively.

A similar trend was noticed in the dead mite population. Konnakuzhy recorded significantly high dead mite count on the OP (4.968) while the other two locations were on par. No significant difference in dead mite count was observed among the locations on the IP and NS which ranged from 13.629 to 14.135 and 7.535 to 8.337 respectively.

The egg count among the locations had no significant difference in the perianth and NS. Egg count ranged from 1.069 to 1.260 on the OP, 9.493 to 13.618 on the IP and 21.976 to 26.073 on the NS per 4 mm^2.

In the case of mean predatory mite per nut, Kanjirampally recorded maximum count of 12.802 followed by Konnakuzhy (7.594) and the lowest in Thumbermuzhy (5.948) in the Pariyaram panchayath of Thrissur district.

4.1.5 Eriophyid mite and predatory mite population (panchayath wise)

Table. 6 illustrates the data on the mean live and dead mite population, egg count per 4 mm^2 and predatory mite count on the whole nut over the four panchayaths of Thrissur district irrespective of the locations.

In the case of live mite population, Pananchery panchayath recorded significantly high population on the OP (2.494/4 mm^2) followed by Madakkathara panchayath (2.148/4 mm^2) while Koorkenchery and Pariyaram panchayaths recorded significantly low values of 1.320 and 1.340, respectively which were on par. While considering the live mite population of the IP and NS, all the panchayaths were on par with values ranging from 7.062 to 8.950 per 4 mm^2 on the IP and 25.709 to 26.514 per 4 mm^2 on the NS.

No significant difference in dead mite population on the perianth and NS was observed among the four panchayaths. The dead mite population ranged from 3.225 to 4.658; 13.835 to 16.080 and 7.868 to 8.826 per 4 mm^2 on the OP, IP and NS respectively.

Significantly low egg count on the OP was obtained from the Pariyaram panchayath (1.149/4mm^2) while all the other panchayaths were on par ranging from 0.846 to 1.868 per 4 mm^2. Egg count on the IP and NS had no

Table. 6 Population of live and dead mites; egg count and predatory mite in four panchayaths

Panchayaths	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
Pananchery	2.494[a] (1.178)	8.950[a] (1.493)	25.709[a] (1.942)	3.225[a] (1.191)	15.923[a] (1.755)	7.868[a] (1.483)	1.868[a] (1.040)	8.877[a] (1.473)	19.471[a] (1.783)	11.149[a] (1.538)
Madakkathara	2.148[b] (1.132)	7.379[ab] (1.473)	26.235[a] (1.909)	4.658[a] (1.217)	16.080[a] (1.785)	8.826[ab] (1.529)	0.846[a] (0.962)	10.353[ab] (1.489)	25.514[a] (1.871)	7.372[c] (1.310)
Koorkenchery	1.340[c] (1.050)	7.276[ab] (1.466)	26.452[a] (1.909)	4.103[a] (1.216)	13.835[a] (1.703)	8.239[b] (1.419)	1.266[a] (1.028)	12.295[ab] (1.572)	23.211[a] (1.846)	11.059[a] (1.514)
Pariyaram	1.320[c] (1.055)	7.062[b] (1.409)	26.514[a] (1.868)	3.653[a] (1.241)	13.837[a] (1.727)	7.983[b] (1.409)	1.146[b] (1.004)	11.265[b] (1.505)	23.446[a] (1.815)	8.781[b] (1.394)

* Mean of three locations

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

significant difference among the four panchayaths. The egg count varied from 8.877 to 12.295 and 19.471 to 25.514 per 4 mm^2 on the IP and NS respectively.

Mean predatory mite population of 11.149 and 11.059 per nut was recorded in Pananchery and Madakkathara panchayaths which were on par. They were followed by Pariyaram (8.781) and Madakkathara (7.372) panchayaths.

4.1.6 Distribution of mite population in the perianth and nut surface

Fig. 2 illustrates the distribution of live mites over perianth and soft tender portion of nut in four panchayaths. Maximum live mite population was recorded on the NS (27 mites/4mm^2) from the four panchayaths. This was followed by the IP and the OP where the population was below 10 mites per 4 mm^2. Same trend was obtained from all the four panchayaths.

Fig. 2 Distribution of live mite population on the perianth and nut surface

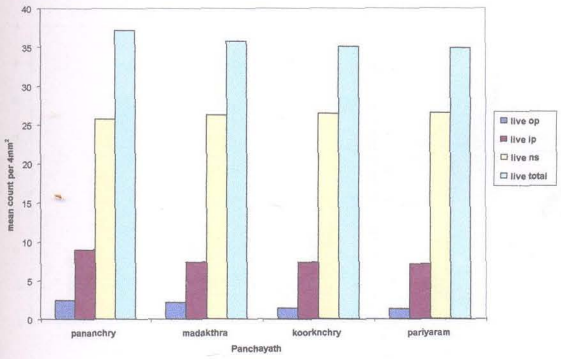
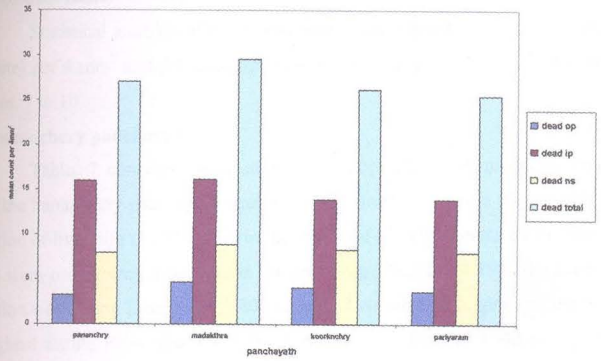


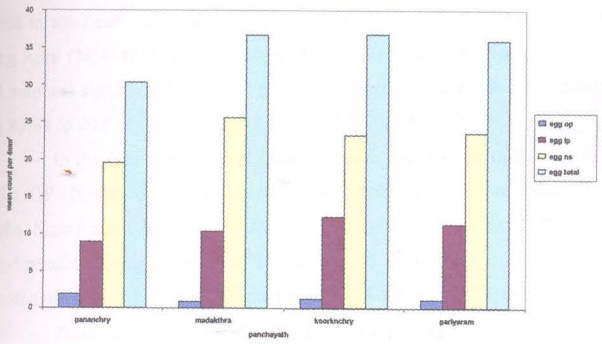
Fig. 3 depicts the dead mite distribution in the perianth and NS over the four panchayaths. Dead mite count was highest on the IP which ranged from 10 to 15 mites per 4 mm^2 throughout the four panchayaths. Least count was recorded from the OP (below 5 mites/4mm^2) while the NS recorded a count between 5 and 10. The same trend was recorded from the four panchayaths.

Fig. 3 Distribution of dead mite population on the perianth and nut surface



A similar trend to that of the live mite population was observed in the distribution of egg count in the perianth and NS over the four panchayaths (Fig. 4).

Fig. 4 Distribution of egg count on the perianth and nut surface



Maximum egg count was recorded on NS which ranged from 19.471 to 25.514 per 4mm^2 followed by the IP whereas the OP possessed least egg count (below 5 mites/4mm^2).

4.2 SEASONAL VARIATION OF ERIOPHYID AND PREDATORY MITE POPULATION

Statistical analysis of the month wise mean population data (live and dead mite) per 4 mm^2 and predatory mite per nut of four panchayaths are shown in Tables. 7 to 10.

4.2.1 Pananchery panchayath

Table. 7 contains the monthly mean population and predatory mite data of the Pananchery panchayath irrespective of locations. On the IP, maximum population of live mite (15.610) was in the month of March whereas all the other months were on par with a population ranging from 3.805 to 14.250. The lowest population was during December (3.805) month. Live mite population on the NS was highest during November (50.556). A population range of 9.542 to 38.556 was observed among other months which were on par.

While considering the dead mite population on the OP maximum population of 6.465 was recorded during March. It was closely followed by April (5.069), May (5.736) and August (5.208). February month had the lowest population of 1.028, which was on par with other months. A similar trend was noticed in the dead mite count on IP and on the NS with maximum population during April (34.444) on the IP and March (16.679) on the NS. The population of dead mite was significantly low and on par among the other months, which ranged from 8.084 to 29.834 on the IP and 3.348 to 11.653 on NS.

In the case of egg count, August month had maximum count of 6.375 on the OP. It was closely followed by September (5.223) and May (2.681) months. Significantly high egg count was observed during March (22.223) on the IP and maximum egg count of 34.264 on the NS. During the other periods egg count ranged from 2.166 to 12.736 on the IP and 3.875 to 33.153 on NS.

Predatory mite count was significantly high during March (24.583) and November (23.458) whereas all the other months were on par. The population of predatory mite ranged from 3.292 to 11.417.

Table. 7 Month wise eriophyid and predatory mite population of Pananchery panchayath

Months	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
July 2003	1.701 ^{bc} (1.115)	11.973 ^{bc} (1.411)	24.403 ^{bc} (1.826)	1.834 ^{abc} (1.130)	15.736 ^{bcd} (1.829)	7.070 ^{bc} (1.402)	1.181 ^{b} (1.045)	10.736 ^{b} (1.760)	21.403 ^{abc} (1.760)	3.292 ^{d} (1.191)
August	4.890 ^{ab} (1.283)	11.347 ^{ab} (1.605)	38.556 ^{ab} (2.095)	5.208 ^{a} (1.341)	17.736 ^{bc} (1.874)	8.556 ^{b} (1.554)	6.375 ^{a} (1.213)	12.736 ^{b} (1.557)	15.848 ^{bc} (1.666)	10.625 ^{bcd} (1.473)
September	3.507 ^{abc} (1.222)	6.958 ^{bc} (1.404)	36.340 ^{ab} (2.166)	3.903 ^{ab} (1.272)	10.035 ^{cd} (1.640)	3.348 ^{c} (1.270)	5.223 ^{a} (1.231)	8.736 ^{b} (1.485)	19.111 ^{ab} (1.895)	10.250 ^{bcd} (1.482)
October	1.355 ^{c} (1.088)	6.236 ^{bc} (1.413)	19.028 ^{bc} (1.901)	1.694 ^{abc} (1.117)	8.084 ^{d} (1.514)	4.722 ^{bc} (1.377)	1.569 ^{b} (1.015)	4.597 ^{bc} (1.360)	17.431 ^{ab} (1.890)	6.625 ^{cd} (1.408)
November	2.202 ^{abc} (1.713)	8.653 ^{bc} (1.452)	50.556 ^{a} (2.350)	2.125 ^{abc} (1.141)	15.680 ^{cd} (1.763)	8.361 ^{b} (1.597)	1.319 ^{b} (1.048)	10.709 ^{b} (1.529)	33.153 ^{ab} (2.102)	23.458 ^{a} (2.079)
December	1.589 ^{abc} (1.138)	3.805 ^{c} (1.276)	14.763 ^{bc} (1.829)	1.820 ^{abc} (1.146)	8.765 ^{cd} (1.585)	5.348 ^{bc} (1.422)	0.527 ^{bc} (0.957)	6.000 ^{bc} (1.421)	14.805 ^{abc} (1.763)	9.875 ^{bc} (1.576)
January 2004	1.333 ^{c} (1.073)	7.208 ^{abc} (1.522)	19.305 ^{abc} (1.965)	1.250 ^{bc} (1.030)	13.111 ^{bcd} (1.822)	6.612 ^{bc} (1.508)	0.000 ^{c} (0.841)	3.958 ^{bc} (1.306)	18.139 ^{abc} (1.764)	11.417 ^{bcd} (1.515)
February	1.778 ^{abc} (1.145)	8.875 ^{bc} (1.508)	14.847 ^{c} (1.536)	1.028 ^{c} (1.013)	13.750 ^{cd} (1.566)	11.653 ^{bc} (1.470)	0.514 ^{bc} (0.930)	2.166 ^{c} (1.127)	3.875 ^{d} (1.156)	11.167 ^{bc} (1.612)
March	1.631 ^{bc} (1.131)	15.610 ^{a} (1.808)	30.612 ^{ab} (2.182)	6.465 ^{a} (1.311)	29.834 ^{ab} (2.103)	16.679 ^{a} (1.871)	0.874 ^{bc} (1.000)	22.223 ^{a} (1.978)	34.264 ^{a} (2.793)	24.583 ^{a} (1.762)
April	2.048 ^{abc} (1.94)	5.403 ^{bc} (1.354)	21.889 ^{abc} (1.960)	5.069 ^{a} (1.325)	34.444 ^{a} (2.243)	9.264 ^{b} (1.522)	0.667 ^{bc} (0.957)	8.000 ^{bc} (1.390)	20.042 ^{abc} (1.759)	8.583 ^{bcd} (1.506)
May	3.105 ^{a} (1.300)	14.250 ^{ab} (1.638)	27.666 ^{abc} (1.915)	5.736 ^{a} (1.347)	14.458 ^{cd} (1.693)	6.653 ^{bc} (1.413)	2.681 ^{b} (1.212)	9.291 ^{b} (1.579)	26.638 ^{ab} (2.041)	8.583 ^{bcd} (1.475)
June	4.791 ^{ab} (1.275)	7.083 ^{abc} (1.520)	9.542 ^{c} (1.574)	2.570 ^{abc} (1.119)	9.444 ^{cd} (1.666)	6.152 ^{bc} (1.391)	1.486 ^{b} (1.032)	7.375 ^{bc} (1.414)	8.945 ^{cd} (1.402)	5.333 ^{cc} (1.381)

* Mean of the 24 observations

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

4.2.2 Madakkathara panchayath

Month wise analysed data of mite population and predatory mite of Madakkathara panchayath is represented in Table. 8.

The live mite population was significantly high during August (8.145) on the OP. It was followed by April (4.354) and May (2.916). There was no significant difference amongst other months and the population ranged from 0.673 to 2.513. April had maximum population on the IP (12.196) and on NS (51.971). The live mite population ranged from 3.333 to 11.222 and 6.569 to 49.541 on the IP and NS during other months which were on par.

In the case of dead mite population, significantly high population was recorded during April month on the OP (12.694), IP (34.028) and NS (18.639). The population did not differ significantly among other months, where it ranged from 0.597 to 11.125 on the OP, 7.862 to 27.138 on the IP and 2.944 to 16.986 on the NS.

A different trend was observed in the egg count where the maximum count was observed during April month on the OP (2.569) and it was on par with August (1.624). On the IP, highest population was in August (26.347), while the lowest population was in July (1.777). Similarly on NS also egg count was highest in August (46.013) and December (51.569) and the lowest in July (7.111).

Predatory mite count also showed the same trend as that of the egg count. Significantly high predatory mite count was obtained during August month (36.375). The remaining months were on par with minimum population during September (1.000) and October (0.917).

4.2.3 Koorkenchery panchayath

Table. 9 depicts the month wise mean data of live and dead mite population, egg count and predatory mite population of Koorkenchery panchayath.

The data revealed a significantly high live mite population during May (2.763) on the OP and during January on the IP (16.973) and NS (49.431). The live mite population during the remaining months did not differ significantly in

Table. 8 Month wise eriophyid and predatory mite population of Madakkathara panchayath

Months	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
July 2003	0.889 ^{d} (1.036)	4.278 ^{b} (1.244)	6.569 ^{e} (1.428)	6.278 ^{ab} (1.384)	10.305 ^{cde} (1.680)	3.194 ^{e} (1.246)	0.194 ^{c} (0.900)	1.777 ^{d} (1.379)	7.111 ^{e} (1.379)	2.333 ^{cde} (1.168)
August	8.145 ^{a} (1.583)	11.222 ^{ab} (1.575)	49.541 ^{a} (2.371)	3.667 ^{bc} (1.283)	7.862 ^{e} (1.483)	9.833 ^{bcd} (1.541)	1.624 ^{a} (1.134)	26.347 ^{a} (1.941)	46.013 ^{ab} (2.258)	36.375 ^{a} (2.129)
September	0.673 ^{d} (0.973)	7.264 ^{ab} (1.488)	22.805 ^{abc} (2.032)	0.597 ^{d} (0.972)	11.430 ^{bcde} (1.735)	11.277 ^{abc} (1.721)	0.292 ^{c} (0.888)	8.153 ^{bc} (1.449)	21.515 ^{abc} (2.000)	1.000 ^{e} (0.990)
October	0.757 ^{d} (1.013)	4.542 ^{b} (1.350)	22.167 ^{bcd} (1.936)	0.916 ^{d} (1.013)	15.333 ^{bc} (1.870)	6.458 ^{cde} (1.467)	0.111 ^{c} (0.867)	7.527 ^{bc} (1.473)	18.667 ^{cde} (1.786)	0.917 ^{e} (0.990)
November	1.166 ^{d} (1.067)	6.069 ^{ab} (1.419)	13.695 ^{cde} (1.718)	2.764 ^{bcd} (1.183)	10.431 ^{cde} (1.622)	4.680 ^{de} (1.405)	0.139 ^{c} (0.864)	8.625 ^{bc} (1.469)	12.750 ^{cde} (1.650)	9.375 ^{b} (1.515)
December	1.041 ^{d} (1.068)	8.917 ^{ab} (1.398)	50.167 ^{ab} (2.334)	6.028 ^{bc} (1.275)	16.958 ^{bcd} (1.823)	9.917 ^{abcd} (1.650)	1.180 ^{abc} (0.984)	20.083 ^{bc} (1.576)	51.569 ^{a} (2.304)	10.917 ^{bc} (1.432)
January 2004	1.409 ^{cd} (1.100)	8.027 ^{ab} (1.586)	22.222 ^{cde} (1.710)	3.333 ^{bcd} (1.155)	23.750 ^{abc} (1.941)	6.972 ^{bcde} (1.483)	0.542 ^{bc} (0.945)	8.056 ^{bc} (1.490)	30.125 ^{abc} (2.096)	6.542 ^{bc} (1.425)
February	2.513 ^{cd} (1.115)	8.028 ^{ab} (1.567)	32.472 ^{abc} (2.055)	11.125 ^{bc} (1.286)	27.138 ^{ab} (2.035)	10.528 ^{abcd} (1.605)	0.611 ^{bc} (0.926)	7.222 ^{bcd} (1.383)	26.875 ^{bcd} (1.849)	6.083 ^{bcd} (1.316)
March	0.930 ^{d} (1.029)	9.125 ^{ab} (1.516)	22.944 ^{cde} (1.819)	3.083 ^{cd} (1.128)	17.750 ^{bcde} (1.753)	16.986 ^{ab} (1.768)	1.194 ^{abc} (0.984)	13.264 ^{abc} (1.621)	23.750 ^{cde} (1.778)	3.000 ^{cde} (1.120)
April	4.354 ^{b} (1.299)	12.196 ^{a} (1.677)	51.971 ^{a} (2.275)	12.694 ^{a} (1.567)	34.028 ^{a} (2.202)	18.639 ^{a} (1.838)	2.569 ^{ab} (1.080)	9.125 ^{bc} (1.441)	38.111 ^{abcd} (1.962)	5.500 ^{bcde} (1.287)
May	2.916 ^{bc} (1.228)	3.333 ^{b} (1.281)	9.195 ^{de} (1.513)	4.112 ^{bc} (1.306)	6.875 ^{de} (1.512)	2.944 ^{e} (1.215)	1.042 ^{abc} (1.008)	4.514 ^{cd} (1.283)	10.000 ^{de} (1.534)	4.667 ^{bcde} (1.267)
June	0.978 ^{d} (1.068)	5.542 ^{ab} (1.469)	11.069 ^{cde} (1.719)	1.305 ^{cd} (1.057)	10.916 ^{bcde} (1.759)	4.487 ^{de} (1.413)	0.652 ^{bc} (0.967)	9.542 ^{ab} (1.655)	19.681 ^{bcd} (1.857)	1.750 ^{de} (1.084)

* Mean of the 24 observations

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

Table. 9 Month wise eriophyid and predatory mite population of Koorkenchery panchayath

Months	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
July 2003	1.527 ^{b} (1.101)	4.223 ^{b} (1.329)	19.542 ^{bc} (1.686)	2.375 ^{cd} (1.151)	11.417 ^{bcde} (1.642)	3.959 ^{d} (1.168)	1.945 ^{b} (1.126)	16.125 ^{abcd} (1.592)	17.222 ^{bc} (1.686)	15.417 ^{bc} (1.636)
August	1.292 ^{bcd} (1.040)	10.042 ^{b} (1.514)	23.305 ^{b} (1.959)	2.625 ^{cd} (1.157)	16.084 ^{ab} (1.874)	4.195 ^{cd} (1.320)	2.139 ^{bc} (1.104)	17.820 ^{ab} (1.744)	27.361 ^{ab} (2.017)	24.125 ^{a} (1.837)
September	2.223 ^{bc} (1.077)	5.583 ^{b} (1.326)	32.125 ^{bc} (1.876)	2.486 ^{bcd} (1.213)	9.570 ^{bcde} (1.615)	5.472 ^{cd} (1.385)	1.527 ^{bcd} (1.079)	8.305 ^{bcd} (1.549)	12.695 ^{bc} (1.647)	5.292 ^{cde} (1.314)
October	1.222 ^{b} (1.094)	8.806 ^{b} (1.464)	28.944 ^{ab} (2.112)	2.860 ^{abcd} (1.248)	10.485 ^{abcde} (1.684)	12.250 ^{ab} (1.726)	2.180 ^{ab} (1.166)	15.583 ^{abc} (1.711)	28.722 ^{a} (2.151)	6.000 ^{cde} (1.437)
November	1.458 ^{bc} (1.073)	6.750 ^{b} (1.452)	22.208 ^{bc} (1.912)	4.166 ^{abc} (1.313)	7.750 ^{de} (1.471)	5.306 ^{cd} (1.380)	0.458 ^{de} (0.941)	5.960 ^{bcd} (1.439)	12.373 ^{bc} (1.683)	5.083 ^{cde} (1.328)
December	0.194 ^{d} (0.899)	6.625 ^{b} (1.397)	14.708 ^{bc} (1.702)	1.542 ^{d} (1.052)	17.250 ^{abc} (1.847)	9.958 ^{bc} (1.532)	0.486 ^{de} (0.933)	7.611 ^{d} (1.325)	19.958 ^{abc} (1.824)	11.667 ^{bcd} (1.594)
January 2004	2.000 ^{bcd} (1.038)	16.973 ^{a} (1.804)	49.431 ^{a} (2.459)	10.361 ^{a} (1.465)	25.890 ^{a} (1.999)	5.223 ^{d} (1.161)	0.000 ^{e} (0.841)	8.653 ^{cd} (1.355)	45.486 ^{a} (2.166)	2.833 ^{e} (1.152)
February	0.625 ^{bcd} (0.981)	6.321 ^{b} (1.476)	29.166 ^{b} (2.009)	1.610 ^{d} (1.003)	14.736 ^{abcd} (1.742)	18.626 ^{ab} (1.682)	0.889 ^{de} (0.939)	11.278 ^{abcd} (1.572)	29.111 ^{ab} (1.984)	18.250 ^{ab} (1.785)
March	0.292 ^{cd} (0.910)	7.153 ^{b} (1.472)	39.667 ^{b} (2.003)	7.449 ^{cd} (1.144)	22.306 ^{ab} (1.922)	21.737 ^{a} (1.836)	0.833 ^{de} (0.934)	15.930 ^{ab} (1.732)	33.555 ^{abc} (1.893)	10.875 ^{cde} (1.438)
April	1.250 ^{bc} (1.069)	5.320 ^{b} (1.422)	15.625 ^{bc} (1.714)	7.875 ^{ab} (1.440)	17.014 ^{abcde} (1.692)	5.278 ^{cd} (1.321)	1.333 ^{bcd} (1.027)	8.723 ^{bcd} (1.494)	20.847 ^{abc} (1.754)	4.750 ^{de} (1.298)
May	2.763 ^{a} (1.253)	6.431 ^{b} (1.430)	33.375 ^{b} (1.995)	2.666 ^{bcd} (1.204)	5.389 ^{e} (1.411)	3.014 ^{d} (1.218)	2.833 ^{a} (1.281)	24.639 ^{a} (1.913)	21.500 ^{abc} (1.868)	21.708 ^{a} (1.980)
June	1.236 ^{bc} (1.070)	3.084 ^{b} (1.268)	9.333 ^{c} (1.485)	3.167 ^{bcd} (1.205)	8.125 ^{cde} (1.534)	3.846 ^{cd} (1.305)	0.570 ^{cde} (0.965)	6.917 ^{bcd} (1.436)	9.695 ^{c} (1.482)	6.708 ^{cde} (1.374)

* Mean of the 24 observations

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

the perianth. The population ranged from 0.194 to 2.223 on the OP, 3.084 to 10.042 on the IP and 9.333 to 39.667 on the NS.

The dead mite population was maximum in January on OP (10.361) and IP (25.890) while it was in March on the NS (21.737). Population during other months were on par and it ranged from 1.610 to 7.875 on OP, 5.389 to 22.306 on IP and 3.014 to 18.626 on NS.

Maximum egg count was observed during May month on the OP (2.833) and IP (24.639). Egg count was found to increase towards October and then declined during subsequent months up to January on the OP and IP. Whereas egg count on the NS was in the peak during January (45.486), closely followed by October (28.722).

Predatory mite had its peak population during August (24.125) and May (21.708). The population decreased towards January with a mean number of 2.833.

4.2.4 Pariyaram panchayath

Analysis of the monthly mean population data of mites, eggs and predatory mites of Pariyaram panchayath showed maximum population of live mites during August (3.417) on the OP (Table. 10). It was closely followed by May (1.944). Live mite count on OP was lowest in the month of January (0.195). February month recorded maximum population of live mites 16.042 on the IP, whereas it was highest in January (71.222) in the case of NS.

In the case of dead mite population, maximum population was recorded during February on the OP (6.208), IP (33.708) and significantly high population on the NS (37.485). The population trend in the perianth and NS were same during the months July to December. It increased during January and February, further it declined and remained the same during March to June.

Significantly high egg count was recorded during May on OP (3.847) and on IP (31.680) and NS (48.139). The egg count was on par during other months with a range from 0.180 to 2.319 on the OP; 2.556 to 27.584 and 10.612 to 45.849 on the IP and NS.

Table. 10 Month wise eriophyid and predatory mite population of Pariyaram panchayath

Months	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
July 2003	0.625 ^{bc} (0.956)	4.070 ^{d} (1.226)	16.861 ^{cd} (1.655)	4.598 ^{abc} (1.253)	9.445 ^{c} (1.471)	2.680 ^{d} (1.112)	0.236 ^{c} (0.875)	6.014 ^{f} (1.188)	15.014 ^{e} (1.518)	3.417 ^{de} (1.132)
August	3.417 ^{a} (1.157)	4.348 ^{bcd} (1.307)	9.334 ^{d} (1.444)	6.375 ^{a} (1.388)	10.542 ^{bc} (1.618)	4.638 ^{cd} (1.233)	0.487 ^{c} (0.922)	4.291 ^{ef} (1.218)	14.555 ^{e} (1.462)	1.500 ^{e} (1.028)
September	0.862 ^{abc} (1.026)	8.764 ^{bcd} (1.396)	27.889 ^{cd} (1.777)	2.139 ^{abc} (1.164)	8.152 ^{bc} (1.516)	3.584 ^{cd} (1.273)	2.319 ^{b} (1.158)	9.917 ^{cd} (1.635)	26.680 ^{abc} (2.064)	20.042 ^{a} (1.779)
October	1.040 ^{abc} (1.050)	2.695 ^{d} (1.166)	11.181 ^{cd} (1.567)	3.667 ^{abc} (1.220)	7.736 ^{bc} (1.549)	5.000 ^{cd} (1.367)	0.180 ^{c} (0.877)	6.125 ^{def} (1.345)	10.612 ^{e} (1.504)	5.042 ^{de} (1.233)
November	0.652 ^{bc} (0.982)	4.805 ^{bcd} (1.347)	12.861 ^{cd} (1.552)	3.250 ^{ab} (1.249)	10.889 ^{bc} (1.68)	2.361 ^{c} (1.128)	1.235 ^{bc} (1.018)	6.958 ^{def} (1.394)	11.320 ^{e} (1.480)	11.375 ^{bcd} (1.388)
December	1.013 ^{ab} (1.068)	8.888 ^{abc} (1.560)	20.777 ^{bc} (1.942)	3.667 ^{ab} (1.316)	15.20 ^{b} (1.842)	2.458 ^{cd} (1.144)	0.723 ^{c} (0.960)	11.611 ^{cde} (1.555)	21.597 ^{bcde} (1.784)	9.292 ^{cde} (1.322)
January 2004	0.195 ^{c} (0.895)	10.625 ^{bcd} (1.392)	71.222 ^{a} (2.612)	1.361 ^{c} (1.040)	29.236 ^{a} (2.205)	14.500 ^{b} (1.856)	0.528 ^{c} (0.893)	4.417 ^{f} (1.130)	45.849 ^{a} (2.279)	4.500 ^{de} (1.257)
February	1.875 ^{ab} (1.088)	16.042 ^{a} (1.765)	44.500 ^{ab} (2.273)	6.208 ^{a} (1.353)	33.708 ^{a} (2.229)	37.485 ^{a} (2.189)	1.417 ^{bc} (1.010)	27.584 ^{ab} (2.007)	37.278 ^{ab} (2.134)	6.708 ^{bcde} (1.350)
March	1.485 ^{ab} (1.088)	3.236 ^{cd} (1.273)	15.681 ^{cd} (1.722)	3.694 ^{abc} (1.264)	10.320 ^{bc} (1.584)	2.236 ^{d} (1.102)	0.208 ^{c} (0.886)	2.556 ^{f} (1.085)	15.944 ^{de} (1.622)	11.333 ^{abc} (1.619)
April	1.222 ^{abc} (1.052)	6.707 ^{abc} (1.554)	16.028 ^{cd} (1.835)	3.305 ^{abc} (1.248)	11.264 ^{bc} (1.766)	11.293 ^{b} (1.760)	0.542 ^{c} (0.931)	14.708 ^{bc} (1.775)	21.055 ^{abcd} (1.956)	7.042 ^{bcde} (1.339)
May	1.944 ^{a} (1.185)	0.605 ^{ab} (1.597)	60.083 ^{a} (2.349)	1.931 ^{bc} (1.068)	7.361 ^{bc} (1.528)	5.680 ^{c} (1.428)	3.847 ^{a} (1.368)	31.680 ^{a} (2.177)	48.139 ^{a} (2.306)	13.458 ^{ab} (1.653)
June	1.514 ^{ab} (1.107)	3.959 ^{bcd} (1.320)	11.750 ^{cd} (1.690)	3.639 ^{abc} (1.284)	12.181 ^{bc} (1.733)	3.875 ^{cd} (1.320)	2.069 ^{b} (1.147)	9.319 ^{cde} (1.549)	13.305 ^{cde} (1.672)	11.667 ^{abc} (1.629)

* Mean of the 24 observations

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

Predatory mites count stands apart from that of egg count with maximum population during September (20.042).

4.2.5 Eriophyid mite and predatory mite population (monthwise)

Results of the statistical analysis of the month wise mean data of mite population (live and dead) and egg count per 4 mm^2 and predatory mite per nut over the four panchayaths of Thrissur district is depicted in Table. 11.

Maximum live mite population on the OP was obtained during August month (4.436) and May (2.682) which were on par. The population decreased towards September to December with the least during December (0.959). Then the population increased from January till May. On the IP, the peak population was recorded during January (10.708) which was closely followed by February (9.816) and August (9.240). While taking into account of the population on the NS, significantly high population was obtained during January (40.545) and the lowest during June (10.423) which was on par with July (17.094). All the other months were on par with a value ranging from 20.330 to 35.280.

In the case of dead mite population, maximum dead mite population on the OP was found during April (7.236). Populations during other months were on par, the lowest population was observed during September (2.281) and October (2.284). Maximum dead mite population was recorded during April (24.232), January (22.997) and February (22.333) on IP whereas no significant difference was observed among other months with the lowest count during May (8.521). The dead mite population on the NS was at the peak during February (19.573) and was closely followed by March (14.410) and April (11.118) months. A significantly low and on par population of dead mite on NS was recorded during May-June to December-January with a mean value below 10.

Maximum egg count was recorded in the month of August (2.656), September (2.340) and May (2.601) on the OP. During the other periods, the egg count was very low and the values ranged from 0.267 to 1.194. On the IP maximum egg count was observed during May (17.531). The egg count declined further and increased during August (15.299) and decreased towards subsequent months with the least count during January (6.271). While on the NS January

Table. 11 Months wise eriophyid and predatory mite population in Thrissur district

Months	Mean number per 4 mm² per nut*									Pred. mites (Mean no/nut)
	Live mite			Dead mite			Egg count			
	OP	IP	NS	OP	IP	NS	OP	IP	NS	
July 2003	1.185 ^{cd} (1.052)	6.136 ^{d} (1.328)	17.094 ^{c} (1.649)	3.771 ^{bc} (1.230)	11.726 ^{ef} (1.655)	4.225 ^{e} (1.232)	0.889 ^{c} (0.987)	8.663 ^{cd} (1.347)	15.187 ^{c} (1.586)	6.115 ^{d} (1.282)
August	4.436 ^{a} (1.266)	9.240 ^{abc} (1.500)	30.184 ^{b} (1.968)	4.469 ^{ab} (1.292)	13.056 ^{de} (1.713)	6.806 ^{cd} (1.412)	2.656 ^{b} (1.093)	15.299 ^{ab} (1.615)	25.944 ^{b} (1.851)	18.156 ^{a} (1.617)
September	1.816 ^{cd} (1.074)	7.142 ^{bcd} (1.403)	29.790 ^{b} (1.963)	2.281 ^{c} (1.155)	9.797 ^{ef} (1.626)	5.920 ^{cd} (1.412)	2.340 ^{b} (1.089)	8.778 ^{b} (1.529)	20.000 ^{ab} (1.901)	9.146 ^{bcd} (1.392)
October	1.094 ^{cd} (1.061)	5.570 ^{cd} (1.348)	20.330 ^{b} (1.879)	2.284 ^{c} (1.150)	10.410 ^{ef} (1.654)	7.108 ^{bc} (1.484)	1.010 ^{c} (0.981)	8.458 ^{bcd} (1.472)	18.858 ^{b} (1.833)	4.646 ^{d} (1.267)
November	1.369 ^{cd} (1.074)	6.569 ^{bcd} (1.417)	24.830 ^{b} (1.883)	3.076 ^{bc} (1.234)	11.188 ^{ef} (1.634)	5.177 ^{cd} (1.377)	0.788 ^{c} (0.968)	8.063 ^{bcd} (1.457)	17.399 ^{bc} (1.729)	12.323 ^{a} (1.578)
December	0.959 ^{d} (1.043)	7.059 ^{bcd} (1.408)	24.104 ^{b} (1.952)	3.264 ^{bc} (1.197)	14.545 ^{cde} (1.774)	6.920 ^{cd} (1.437)	0.729 ^{c} (0.959)	11.326 ^{bcd} (1.469)	26.982 ^{ab} (1.919)	10.438 ^{abc} (1.481)
January 2004	1.234 ^{d} (1.027)	10.708 ^{a} (1.576)	40.545 ^{a} (2.187)	4.076 ^{bc} (1.173)	22.997 ^{a} (1.992)	8.327 ^{bc} (1.502)	0.267 ^{d} (0.880)	6.271 ^{d} (1.320)	34.900 ^{a} (2.076)	6.323 ^{cd} (1.337)
February	1.698 ^{bcd} (1.082)	9.816 ^{a} (1.579)	30.246 ^{b} (1.968)	4.993 ^{bc} (1.164)	22.333 ^{abc} (1.893)	19.573 ^{a} (1.736)	0.858 ^{cd} (0.951)	12.063 ^{b} (1.522)	24.285 ^{bc} (1.781)	10.552 ^{ab} (1.516)
March	1.085 ^{d} (1.039)	8.781 ^{ab} (1.517)	27.226 ^{b} (1.932)	5.185 ^{bc} (1.212)	20.052 ^{bcd} (1.840)	14.410 ^{a} (1.644)	0.778 ^{cd} (0.951)	13.493 ^{ab} (1.604)	26.878 ^{ab} (1.871)	12.448 ^{abc} (1.484)
April	2.219 ^{b} (1.154)	7.407 ^{abc} (1.502)	26.378 ^{b} (1.946)	7.236 ^{a} (1.395)	24.232 ^{ab} (1.976)	11.118 ^{ab} (1.610)	1.278 ^{c} (0.999)	10.139 ^{b} (1.525)	24.014 ^{ab} (1.858)	6.469 ^{bcd} (1.358)
May	2.682 ^{a} (1.241)	8.655 ^{abc} (1.486)	35.280 ^{b} (1.943)	3.611 ^{bc} (1.231)	8.521 ^{f} (1.536)	4.573 ^{de} (1.319)	2.601 ^{a} (1.217)	17.531 ^{a} (1.738)	26.569 ^{ab} (1.937)	12.104 ^{a} (1.594)
June	2.130 ^{bc} (1.130)	4.917 ^{bcd} (1.394)	10.423 ^{c} (1.617)	2.670 ^{bc} (1.166)	10.167 ^{ef} (1.673)	4.590 ^{cde} (1.357)	1.194 ^{bc} (1.028)	8.288 ^{bc} (1.514)	12.906 ^{c} (1.603)	6.365 ^{bcd} (1.367)

* Mean of the four panchayaths

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are power transformed ones $(x+0.5)^{0.25}$

month had got maximum egg count of 34.900. Lowest was recorded in June (12.906) and July (15.187). All the other months were on par with an egg count ranging from 17.399 to 26.982.

With regard to predatory mite count, maximum population was observed during August (18.156). It was closely followed by November (12.323), May (12.104) and March (12.448). In between these months, a declining trend in the population was obtained with the lowest count during October (4.646).

4.3 CORRELATION OF MITE POPULATION WITH WEATHER PARAMETERS

The monthly weather parameters viz., rainfall, rainy days, maximum and minimum temperature and relative humidity were collected from respective weather stations of three locations of three panchayaths selected for the study. The mean mite population (live and dead), egg count per 4 mm^2 on the perianth and NS and predatory mite population were correlated with the weather data of the respective locations of three panchayaths.

4.3.1 Pananchery panchayath-Kannara

Results of the correlation analysis of mite population and weather parameters at Kannara did not show a significant relationship (Table. 12). However, a positive correlation was found to exist in the case of maximum temperature. Positive correlation was also observed for the relative humidity on egg population and minimum temperature on live mite population. A negative correlation was observed in the case of rainfall and number of rainy days on all the population parameters.

Table. 12 Correlation co-efficient of mite population with weather parameters at CPCRI, Kannara

Mite population/ weather parameters	Rainfall	Rainy days	Max. temp.	Min. temp.	Relative humidity
Predatory mite	-0.305	-0.330	0.361	-0.303	-0.458
Live	-0.205	-0.170	0.067	0.076	-0.178
Dead	-0.411	-0.457	0.475	-0.205	-0.342
Egg	-0.159	-0.038	0.234	-0.056	0.054

4.3.2 Madakkathara panchayath-Chirakkekodu

Table. 13 represent the correlation analysis between population data of mite and weather parameters of Chirakkekodu location of Madakkathara panchayath. Though not significant, the mite population parameters had a positive correlation with the weather parameters, maximum and minimum temperature. While the mite population, egg count and predatory mite count decreased with increase in rainfall, number of rainy days and relative humidity.

Table. 13 Correlation co-efficient of mite population with weather parameters at Chirakkekodu, Madakkathara

Mite population/ weather parameters	Rainfall	Rainy days	Max. temp.	Min. temp.	Relative humidity
Predatory mite	-0.317	-0.390	0.268	0.097	-0.379
Live total	-0.215	-0.205	0.066	0.316	-0.272
Dead total	-0.392	-0.345	0.139	0.431	-0.382
Egg total	-0.460	-0.490	0.241	0.100	-0.483

4.3.3 Pariyaram panchayath-Thumbermuzhy

The relationship between the mite population and weather parameters of Thumbermuzhy of Pariyaram panchayath is illustrated in the Table. 14.

A similar trend as that of the other two locations was observed at Thumbermuzhy, where predatory mite, live and dead mite and the egg count had a positive correlation with the weather parameter, maximum temperature. Predatory mite count also possessed a positive correlation with minimum temperature. While for the live and dead mite and for egg count, minimum temperature had a significantly high negative correlation.

Table. 14 Correlation co-efficient of mite population with weather parameters at Thumbermuzhy, Pariyaram

Mite population/ weather parameters	Rainfall	Rainy days	Max. temp.	Min. temp.	Relative humidity
Predatory mite	0.108	-0.042	0.335	0.041	-0.327
Live total	-0.273	-0.415	0.504	-0.702**	-0.070
Dead total	-0.380	-0.521	0.477	-0.603*	-0.126
Egg total	-0.205	-0.355	0.387	-0.635*	-0.585*

Predatory mite also had a positive correlation with rainfall, where as the eriophyid mite population decreased with increase in rainfall. All the population parameters showed a negative correlation with rainy days. Eggs count showed a significantly high negative correlation with relative humidity.

4.4 ISOLATION OF *H. thompsonii*

Fungus isolation was done intensively from dead mites (Plate. 7) at monthly intervals from July 2003 to June 2004. Out of the 12 locations from four panchayaths *Hirsutella* could be isolated in pure form from ten locations only (Table. 15).

Table. 15 Isolation of *H. thompsonii* in pure form from different locations

Panchayaths	Locations	Total no. of nut samples	Total no. of samples isolated	No. of samples yielding *H. thompsonii*	Per cent incidence of *H. thompsonii*
Pananchery	Marakkal	96	87	2	2.30
	Kannara	96	70	0	0.00
	Mudicode	96	81	0	0.00
Madakkathara	Chirakkekodu	96	74	5	6.76
	Vellanikkara	96	75	3	4.00
	Madakkathara	96	73	4	5.48
Koorkenchery	Kanimangalam	96	86	7	8.14
	Nedupuzha	96	63	4	6.35
	Panamukku	96	71	5	7.04
Pariyaram	Thumbermuzhy	96	72	5	6.94
	Konnakuzhy	96	89	4	4.49
	Kanjirampally	96	78	6	7.69
Total		1152	919	45	4.93

Of the 919 nut samples isolated over the entire four panchayaths, only 45 samples yielded *H. thompsonii*. Maximum number of *Hirsutella* isolates was obtained from the Koorkenchery panchayath (16 nos.) which was closely followed by Pariyaram panchayath (15 nos.) and Madakkathara panchayath (12 nos.). Only two samples yielded *H. thompsonii* from Pananchery panchayath which was the lowest value.

The frequency of isolation of *H. thompsonii* varied according to the locations within a panchayath. Kanimangalam location of Koorkenchery panchayath recorded seven number of *Hirsutella* isolates out of the 86 samples isolated. It accounts maximum percentage of isolation (8.14) among the locations

of four panchayath. It was followed by Kanjirampally at Pariyaram panchayath which recorded 7.69 per cent of isolation (6 isolates obtained from 78 samples isolated). The per cent of isolation ranged from 4.00 to 7.04 per cent in the remaining locations of three panchayaths other than that of Pananchery panchayath. Least number of isolates was obtained from the Marakkal location of Pananchery panchayath (2 samples out of the total 87 samples isolated) with 2.30 per cent of isolation. No *Hirsutella* isolates were obtained from the other two locations, Kannara and Mudicode of the Pananchery panchayath.

Considering the number of samples taken for isolation, maximum number of samples (89 nos.) was isolated from Konnakuzhy of Pariyaram panchayath. The Nedupuzha location at Koorkenchery panchayath recorded lowest incidence (6.35 per cent).

4.5 SEASONAL VARIATION OF *Hirsutella* spp.

Table. 16 illustrates the seasonal variation in occurrence of H*irsutella* spp. The entire period of survey was divided into four seasons. They included monsoon (June-September), post monsoon (October-November), winter (December-February) and summer (March-May).

Table. 16 *H. thompsonii* isolated in pure form during different seasons

Sl. No.	Panchayath	Locations	Monsoon (Jun.-Sep.)	Post monsoon (Oct.-Nov.)	Winter (Dec.-Feb.)	Summer (Mar.-May)
			H. thompsonii			
1	Pananchery	Marakkal	-	-	1	1
		Kannara	-	-	-	-
		Mudicode	-	-	-	-
2	Madakkathara	Chirakkekodu	2	1	-	2
		Vellanikkara	-	1	1	1
		Madakkathara	1	2	1	-
3	Koorkenchery	Kanimangalam	3	1	3	-
		Nedupuzha	2	-	1	1
		Panamukku	4	-	1	-
4	Pariyaram	Thumbermuzhy	1	-	2	2
		Konnakuzhy	1	1	-	2
		Kanjirampally	-	-	6	-
		Total	14	6	16	9

In the study on incidence of *H. thompsonii* conducted for one year, maximum number of samples with *Hirsutella* infection was obtained during winter season (16 nos.) followed by monsoon season (14 nos.). Only samples

infected with *Hirsutella* were obtained during summer season with the least number during post monsoon period (6 nos.).

4.6 OTHER FUNGAL PATHOGENS AND MICRO-ORGANISMS ASSOCIATED WITH COCONUT ERIOPHYID MITE

In addition to the commonly occurring acaropathogen, *Hirsutella thompsonii* a few other fungi coming under the Hyphomycetes were also isolated consistently from the dead coconut mite. Fungal species coming under three genera *viz. Fusarium, Acremonium, Paecilomyces* and bacteria with mycelial growth, *Actinomycetes* (Table. 17) were also isolated from various locations of the four panchayaths.

A total of 98 nut samples yielded *Fusarium* coming under three species, *F. lateritium* (69 isolates), *F. verticillioides* (20 isolates) and *F. solani* (9 isolates) were isolated over the entire four panchayaths of Thrissur district. Four species of *Acremonium*, *A. zeylanicus* (97 isolates), *A. incoloratum* (25 isolates), *A. strictum* (11 isolates) and *Acremonium* spp. (15 isolates) were isolated which accounts to the maximum number of isolates (148 nos.) under this genus. Least number of isolates were obtained under the genus *Paecilomyces* (15 nos.) which included two species *P. fumosoroseus* (13 nos.) and *P. lilacinus* (2 nos.). Apart from the above genus, 95 isolates of *Actinomycetes* were frequently isolated.

Among the four panchayaths, Madakkathara yielded 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.).

Apart from the above fungi, bacteria *Serratia* spp. (105 nut samples) and the commonly occurring fungi like *Aspergillus* spp. (31 nut samples), *Penicillium* spp. (11 samples) were also isolated from the dead mites over the four panchayaths (Table. 18).

Table. 17 Number of nut samples infected by different fungal pathogens and actinomycetes obtained from different locations

Sl. No.	Panchayath	Location	*Fusarium* sp.			*Acremonium* sp.				*Paecilomyces* sp.		Actino-mycetes
			F. lateritium	*F. verticillioides*	*F. solani*	*A. zeylanicus*	*A. incoloratum*	*A. strictum*	*Acremonium* sp.	*P. fumosoroseus*	*P. lilacinus*	
1	Pananchery	Marakkal	8	-	-	19	1	-	-	1	-	15
		Kannara	4	1	-	5	1	-	-	2	-	4
		Mudicode	6	6	1	7	1	1	-	-	-	8
2	Madakkathara	Chirakkekodu	9	-	-	8	5	-	1	2	-	1
		Vellanikkara	5	2	2	8	-	-	1	3	-	14
		Madakkathara	9	2	2	2	3	-	2	3	2	14
3	Koorkenchery	Kanimangalam	6	1	-	15	-	2	3	-	-	16
		Nedupuzha	3	1	-	5	2	-	4	1	-	5
		Panamukku	3	1	1	10	3	-	1	1	-	5
4	Pariyaram	Thumbermuzhy	3	1	1	7	1	1	2	-	-	4
		Konnakuzhy	10	4	-	5	5	2	-	-	-	6
		Kanjirampally	3	1	2	6	3	5	1	-	-	3
		Total	69	20	9	97	25	11	15	13	2	95

Table. 18 Frequency of isolation of bacteria and other commonly occurring fungi

Sl. No.	Panchayath	Location	*Aspergillus* spp.	*Penicillium* spp.	Bacteria (*Serratia* spp.)
	Pananchery	Marakkal	3	1	16
		Kannara	0	0	11
		Mudicode	1	0	10
2	Madakkathara	Chirakkekodu	4	2	13
		Vellanikkara	7	0	7
		Madakkathara	3	1	8
3	Koorkenchery	Kanimangalam	4	2	8
		Nedupuzha	1	3	4
		Panamukku	4	1	3
4	Pariyaram	Thumbermuzhy	0	0	7
		Konnakuzhy	3	1	9
		Kanjirampally	1	0	9
		Total	31	11	105

4.7 OTHER FUNGI ISOLATED FROM COCONUT MITE IN DIFFERENT SEASONS

Isolation of fungal cultures from the nut samples varied with seasons with maximum number during the monsoon period (Table. 19).

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

Least number of *Acremonium* spp. (23 nos.) and *Paecilomyces* spp. (1 no.) were isolated during the post monsoon season while the *Fusarium* spp. (15 nos.) were isolated during winter season and the *Actinomycetes* (15 nos.) during the monsoon season.

4.8 IDENTIFICATION OF FUNGI

In addition to the acaropathogen, *H. thompsonii* other fungal species were also isolated from the dead mites (Table.20). The fungal cultures were identified upto the species level from various institutions namely Commonwealth Agricultural Bioscience (CABI), UK; Kerala Forest Research Institute (KFRI),

Table. 19 Seasonal variation on the occurrence of other fungi

Panchayath	Locations	Numbers isolated															
		Monsoon (Jun.-Sep.)				Post monsoon (Oct.-Nov.)				Winter (Dec.-Feb.)				Summer (Mar.-May.)			
		A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
Pananchery	Marakkal	4	5	-	3	-	6	-	2	1	8	-	-	3	1	1	10
	Kannara	-	2	-	-	1	-	-	-	2	-	2	1	2	4	-	3
	Mudicode	5	3	-	1	5	-	-	2	3	1	-	2	-	5	-	3
Madakkathara	Chirakkekodu	4	5	1	-	2	5	-	1	1	3	-	-	2	1	1	-
	Vellanikkara	6	5	3	1	2	-	-	3	-	2	-	9	1	2	-	1
	Madakkathara	3	2	2	3	5	1	1	3	3	1	1	2	2	3	1	6
Koorkenchery	Kanimangalam	1	13	-	1	-	5	-	-	-	-	-	7	6	2	-	8
	Nedupuzha	-	6	-	-	4	1	-	2	-	-	-	2	-	4	1	1
	Panamukku	4	7	-	1	-	-	-	1	-	5	-	3	1	2	1	-
Pariyaram	Thumbermuzhy	3	4	-	-	1	1	-	1	-	3	-	1	1	3	-	2
	Konnakuzhy	6	7	-	3	5	1	-	1	3	3	-	1	-	1	-	1
	Kanjirampally	1	2	-	2	1	3	-	1	2	5	-	-	2	5	-	-
Total		37	61	6	15	26	23	1	17	15	31	3	28	20	33	5	35

A – *Fusarium* spp. C – *Paecilomyces* spp.

B- *Acremonium* spp D – Actinomycetes

Peechi; College of Horticulture (COH), Vellanikkara. The pure cultures of the organisms were maintained on PDA slants.

Table. 20 Fungal species isolated and identified from *A. guerreronis*

Sl. No.	Fungal species	Identified by
1	*Acremonium strictum* W. Gams (IMI 392485)	CABI Bioscience, UK
2	*Acremonium implicatum* (J. Gilman & E.V. Abott) W. Gams (IMI 392486)	"
3	*Fusarium lateritium* Nees (IMI 392487)	"
4	*Fusarium verticillioides* (Sacc.) Nirenberg (IMI 392488)	"
5	*Acremonium incoloratum* (Sukapure & Thirum) W. Gams (IMI 392489)	"
6	*Paecilomyces fumosoroseus* (Wize) A.H.S.Br. & G.S.m (IMI 392490)	"
7	*Acremonium terricola* (J.H.Mill; Giddens & A.A.Foster) W. Gams (IMI 392663)	"
8	*Ramichloridium subulatum* de Hoog (IMI 392664)	"
9	*Acremonium* spp. (IMI 392665)	"
10	*Acremonium* spp. (IMI 392665)	"
11	*Acremonium* spp. (IMI 392666)	"
12	*Paecilomyces lilacinus*	KFRI, Peechi
13	*Actinomycetes*	COH, Vellanikkara
14	*Aspergillus* spp.	CMI descriptions of Pathogenic Fungi and Bacteria
15	*Penicillium* spp.	"
16	Bacteria (*Serratia* spp.)	"
17	*H. thompsonii* var. *synnematosa*	CABI, Bioscience, UK
18	*H. thompsonii* var. *thompsonii*	CMI descriptions
19	*H. kirchnerii*	"

4.9 CONFIRMATION OF PATHOGENICITY

A total of 17 fungal isolates identified were subjected to *in vitro* pathogenicity tests on coconut mite, *A. guerreronis* as per the methodology described in 3.5. Dead mites were observed on the inner side of the perianth on the third day of inoculation of the respective fungal cultures. The pathogen was reisolated from those dead mites on PDA medium and compared with the original culture of the fungus and confirmed its pathogenicity on mites (Plate. 8). Fungal species which proved the Koch postulates are shown in Table. 21.

Table. 21 Fungal species pathogenic to *A. guerreronis*

Sl. No.	Fungal species
1	*Hirsutella thompsonii*
2	*Hirsutella kirchnerii*
3	*Acremonium strictum*
4	*Acremonium incoloratum*
5	*Fusarium lateritium*
6	*Fusarium verticillioides*
7	*Paecilomyces fumosoroseus*
8	*Paecilomyces lilacinus*

Mycelial growth with characteristic phialides was observed on the mites inoculated with *H. thompsonii* (Plate. 9) and *H. kirchnerii*. Other fungal cultures could not be identified based on mycelial outgrowth from the infected mites and their eggs. Hyaline hyphae of the other fungi emerged in large numbers from the coconut mites. No spores were produced from such hyphae which were the distinguishing characteristics for the identification. So after reisolation the fungi were identified and confirmed based on the cultural and morphological characters.

Plate 6A. *Amblyseius* spp.

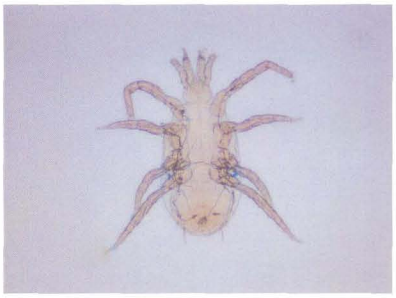


Plate 6B. *Bdella* spp.

Plate 6. Predatory mites

Plate 7A & 7B. Isolation of *Hirsutella thompsonii*

Plate 8A. *Fusarium* spp.

Plate 8B. *Paecilomyces* spp.

Plate 8. Reisolation of fungal pathogens from dead mites on PDA

4.10 CULTURAL AND MORPHOLOGICAL CHARACTERISTICS

4.10.1 *H. thompsonii* isolates

4.10.1.1Growth rate

The colony diameter of the *Hirsutella* isolates in SMA+Y for ten consecutive days after the initiation of growth was recorded and the results are presented in Table. 22.

Observations on the twelfth day 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 another isolate, Chirakkekodu-I with a mean fungal growth of 3.763 cm. The mean colony diameter of *Hirsutella* isolates Kanjirampally-I (3.603 cm), Kanimangalam-I (2.843 cm), Marakkal-II (2.503 cm), Konnakuzhy-I (2.170 cm) were significantly different with the least growth in Marakkal-I (1.570 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 (Plate. 10).

4.10.1.2 Colony characters of Hirsutella isolates- varieties and other species

Observations on colour (initial and final), shape (growth pattern) of the *Hirsutella* isolates, its reaction with the medium (SMA+Y), presence or absence of synnemmata and secretions (honeydew like) were taken. The colony characters varied with different isolates (Table. 23). 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. The *Hirsutella* isolate, Madakkathara-I produced off white coloured mycelial growth throughout the period. Different growth patterns - uniform, raised, dome shaped and folded ones were observed.

Based on the presence or absence of synnemata, two types of *H. thompsonii* varieties were observed. *H. thompsonii* var. *thompsonii* with no synnemata and *H. thompsonii* var. *synnematosa* which possessed synnemata. Honeydew was present in both the types of isolates.

Table. 22 Growth rate of *Hirsutella* isolates

Sl. No.	*Hirsutella* spp.	*Hirsutella* isolates	Days after inoculation [*mean colony diameter (cm)]									
			3rd	4th	5th	6th	7th	8th	9th	10th	11th	12th
1	*H. thompsonii* var. *thompsonii*	Chirakkekodu-I	*1.527^{AB}	1.557^{B}	1.653^{ABC}	1.853^{BC}	2.093^{BCD}	2.277^{C}	2.827^{CD}	3.130^{B}	3.387^{B}	3.763^{BC}
2	,,	Marakkal-I	1.053^{C}	1.093^{C}	1.230^{D}	1.247^{D}	1.280^{E}	1.437^{E}	1.520^{G}	1.530^{E}	1.547^{E}	1.570^{G}
3	*H. thompsonii* var. *synnematosa*	Marakkal-II	1.070^{C}	1.213^{C}	1.303^{CD}	1.437^{D}	1.740^{D}	1.887^{D}	2.027^{F}	2.160^{D}	2.277^{D}	2.503^{E}
4	,,	Madakkathara-I	1.603^{A}	1.693^{AB}	1.893^{AB}	2.047^{AB}	2.770^{A}	3.233^{A}	3.480^{A}	3.850^{A}	4.047^{A}	4.167^{A}
5	,,	Madakkathara-II	1.587^{AB}	1.820^{A}	1.910^{AB}	2.127^{A}	2.203^{BC}	2.587^{BC}	3.020^{BC}	3.280^{B}	3.310^{B}	4.077^{A}
6	,,	Vellanikkara-I	1.733^{A}	1.787^{A}	1.963^{A}	2.133^{A}	2.277^{B}	2.537^{BC}	3.093^{B}	3.387^{B}	3.547^{B}	3.927^{AB}
7	,,	Kanimangalam-I	1.567^{AB}	1.667^{AB}	1.777^{AB}	2.067^{AB}	2.133^{BC}	2.293^{C}	2.377^{E}	2.637^{C}	2.747^{C}	2.843^{D}
8	,,	Konnakuzhy-I	1.313^{BC}	1.500^{B}	1.570^{BCD}	1.703^{C}	1.843^{CD}	1.910^{D}	1.987^{F}	2.137^{D}	2.287^{D}	2.170^{F}
9	*H. kirchnerii*	Kanjirampally-I	1.760^{A}	1.907^{A}	2.027^{A}	2.243^{A}	2.377^{B}	2.627^{B}	2.643^{D}	3.193^{B}	3.453^{B}	3.603^{C}

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are square root transformed ones

Table. 23 Colony characters of *Hirsutella* isolates

Sl. No.	*Hirsutella* spp.	*Hirsutella* isolates	Colour		Growth pattern	Reaction in media	Honey dew	Synnemata (15 DAI)
			Initial	Final				
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 shaped	No reaction, media breaking was observed	5 to 9 nos.	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.

H. thompsonii* var. *thompsonii

The isolates from Chirakkekodu and Marakkal were identified as *H. thompsonii var. thompsonii.*

Chirakkekodu-I

Greyish coloured uniform mycelial growth, slightly raised with foldings. Honey dew (49 to 63 nos.) were present along the foldings. No reaction with the medium and no medium break was noticed (Plate. 11A).

Marakkal-I

Ash coloured growth with black colour on the underside, raised and dome shaped growth with honey dew (5 to 9 nos.). No foldings were present. Media breakage was noticed (Plate. 11B).

H. thompsonii* var. *synnematosa

Six isolates were identified under this group - Marakkal-II, Madakkathara-I and II, Vellanikkara-I, Kanimangalam-I, and Konnakuzhy-I.

Marakkal-II

Grey coloured uniform growth with numerous foldings towards the periphery. Numerous slender cream-coloured synnemata (84 to 96 nos.) were produced as sprouts. No honey dew and media breakage was there (Plate. 12A).

Madakkathara-I

Initially the isolate was grey coloured which turned to off white later. Synnemata present (4 to 15 nos.), no honey dew (Plate. 12B).

Madakkathara-II

Fluffy raised greyish colony with striations at the periphery. Synnemata (60 to 78 nos.) arose as numerous sprouts which was cream coloured (Plate. 12C).

Vellanikkara-I

Off white colouration with greyish initial growth, colony has got a cloudy appearance with small foldings over the entire growth. A very few synnemata but no honey dew (Plate. 12D).

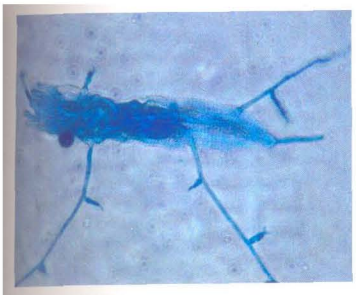


Plate 9. Mycelial outgrowth of *Hirsutella* spp. from CEM

PLate 10. Growth rate of *Hirsutella* isolates

Plate 11A. *H. thompsonii* var. *thompsonii* (Chirakkekodu-I)

Plate 11B. *H. thompsonii* var. *thompsonii* (Marakkal-I)

Plate 12A. *H. thompsonii* var. *synnematosa* (Marakkal-II)

Plate 12B. *H. thompsonii* var. *synnematosa* (Madakkathara-I)

Kanimangalam-I

This isolate has got a similarity with that of Madakkathara-II. Greyish coloured one throughout the growth. Synnemata numerous (24 to 30 nos.); cream coloured and not much elongated. Honey dew absent (Plate. 12E) .

Konnakuzhy-I

Light greyish coloured growth, slightly fluffy textured one. Synnemata 5 to 10 numbers. No honey dew (Plate. 12F).

H. kirchnerii

Kanjirampally-I

Light greyish coloured mycelial growth which later turned to off white colour. Slightly raised growth with radial foldings. Media has got a brown line as perimeter and medium break was also observed. Synnemata (3 to 12 nos.) were cream coloured and thick. No honey dew was present (Plate. 13).

4.10.1.3 Evaluation of the sporulation

Number of spores produced by the *Hirsutella* isolates was estimated using haemocytometer. Sporulation at various radial distances in solid media of SMA + Y is presented in Table. 24.

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}).

Marakkal-II isolate possessed an average spore count of 1.81 x 10^6 spores $ml^{-1}$where as the other isolates ranged from 0.33 to 0.89 x 10^6 spores ml^{-1}. Lowest sporulation was observed in the Kanjirampally-II 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}. Maximum sporulation was recorded at half the radial distance in the Chirakkekodu-I, and Konnakuzhy-I isolates while at peripheral region in the Kanimangalam-I isolates.

Plate 12C. *H. thompsonii* var. *synnematosa* (Madakkathara-II)

Plate 12D. *H. thompsonii* var. *synnematosa* (Vellanikkara-I)

Plate 12E. *H. thompsonii* var. *synnematosa* (Kanimangalam-I)

Plate 12F. *H. thompsonii* var. *synnematosa* (Konnakuzhy-I)

Plate 13. Colony character of *H. kirchnerii*

Plate 14. Colony character of *H. nodulosa*

Table. 24. Sporulation of *Hirsutella* isolates

Sl. No.	*Hirsutella* species	*Hirsutella* isolates	No. of spores ($x10^6$)ml^{-1}			
			Centre	Half the radial distance	Periphery	Mean
1	*H. thompsonii* var. *thompsonii*	Chirakkekodu-I	2.25	5.92	1.50	3.22
2	,,	Marakkal-I	1.17	0.33	1.08	0.86
3	*H. thompsonii* var. *synnematosa*	Marakkal-II	2.25	1.08	2.08	1.81
4	,,	Madakkathara-I	0.33	0.58	0.17	0.36
5	,,	Madakkathara-II	0.42	0.33	0.25	0.33
6	,,	Vellanikkara-I	0.92	0.08	0.83	0.61
7	,,	Kanimangalam-I	0.25	0.67	0.75	0.56
8	,,	Konnakuzhy-I	1.08	1.25	0.33	0.89
9	*H. kirchnerii*	Kanjirampally-I	0.17	0.17	0.17	0.17

Kanjirampally-I isolate recorded uniform spore count of 0.17 x 10^6 spores ml^{-1} in all the three portions.

4.10.1.4 Biomass studies

Weight of dry mycelium (biomass) of the *Hirsutella* isolates in SM+Y broth on 20 DAI was recorded to select the best isolate. Data in Table. 25 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 1.640 g to 2.140 g (Plate. 15 A to E).

Plate 15A. *H. thompsonii* var. *thompsonii* (Chirakkekodu-I)

Plate 15B. *H. thompsonii* var. *thompsonii* (Marakkal-II)

Plate 15C. *H. thompsonii* var. *synnematosa* (Vellanikkara-I)

Plate 15D. *H. thompsonii* var. *synnematosa* (Kanimangalam-I)

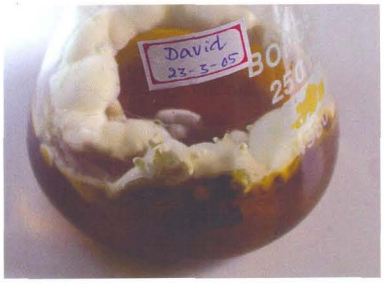


Plate 15E. *H. kirchnerii*

Plate 15. Growth of *Hirsutella* isolates in SM+Y broth (20 DAI)

Table. 25 Biomass of *Hirsutella* isolates

Sl. No.	*Hirsutella* isolates		Biomass (weight in g/100 ml)
1	*H. thompsonii* var. *thompsonii*	Chirakkekodu-I	1.560^{bc}
2	,,	Marakkal-I	1.663^{bc}
3	*H. thompsonii* var. *synnematosa*	Marakkal-II	1.477^{c}
4	,,	Madakkathara-I	2.660^{a}
5	,,	Madakkathara-II	2.047^{bc}
6	,,	Vellanikkara-I	1.893^{bc}
7	,,	Kanimangalam-I	1.737^{bc}
8	,,	Konnakuzhy-I	1.640^{bc}
9	*H. kirchnerii*	Kanjirampally-I	2.140^{ab}

4.10.1.5 Micrometry studies

Microscopic observations were taken on the width of hyphae, spore size, phialide width, length of the swollen part and neck, distance between phialides and length of hyphal cell. 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. 26.

All 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. The distance between cells among the isolates ranged from 12.73 to 26.14 µm (Plate. 16A to F)

Maximum phialide length was recorded by the Vellanikkara-I isolate (20.30 µm) followed by Madakkathara-II isolate (16.17 µm) while the others isolates possessed a phialide length within the range of 9.29 and 12.04 µm.

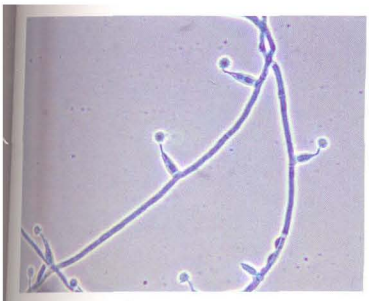


Plate 16A. *H. thompsonii* var. *thompsonii* (Chirakkekodu-I)

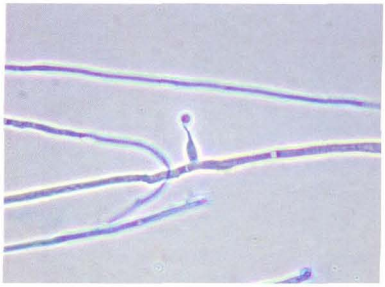


Plate 16B. *H. thompsonii* var. *synnematosa* (Marakkal-II)

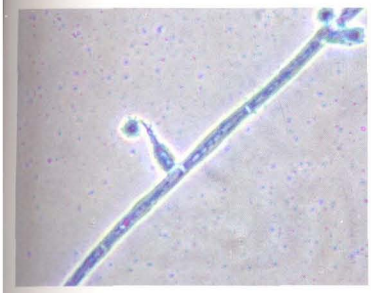


Plate 16C. *H. thompsonii* var. *synnematosa* (Madakkathara-I)

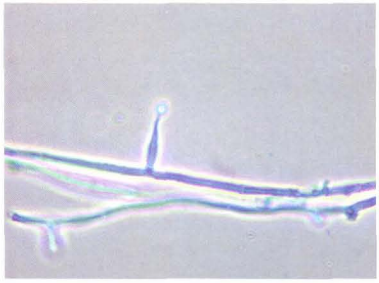


Plate 16D. *H. thompsonii* var. *synnematosa* (Kanimangalam-I)

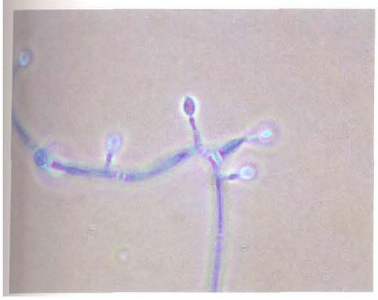


Plate 16E. *H. kirchnerii*

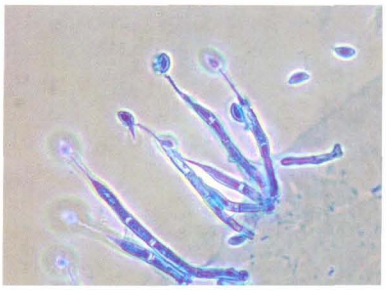


Plate 16F. *H. nodulosa*

Plate 16. Comparison of *Hirsutella* isolates (Phialide and conidia)-1000x

Table. 26 Micrometry of *Hirsutella* isolates (mean length in µm)

Hirsutella species	Isolate name	Width of hyphe	Diam. of spore	Phialides				Distance between phialides	Length of hyphal cell
				Width of phialide	Length of swollen part	Length of neck	Total phialide length		
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
H. t. var. *synnematosa*	Marakkal-II	3.44	3.44	3.44	6.88	3.44	10.32	50.56	14.80
,,	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

The length of the swollen part of the phialide was 11.70 µm for the Vellanikkara-I isolate and 9.63 µm for Madakkathara-II isolate while the length of the neck was 6.88 and 6.54 µm respectively. 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 highest for the Chirakkekodu-I isolate (58.48 µm) followed by Marakkal-II (50.56 µm). Kanimangalam-I and Konnakuzhy-I 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.

4.10.2 Other fungal pathogens

4.10.2.1 Growth rate

The colony diameter of the six fungal cultures was recorded for ten consecutive days after inoculation and the results are presented in Table. 27. At

10th day of inoculation, maximum growth was observed on *A. strictum* (7.99 cm) which was closely followed by *F. verticillioides* (7.77 cm). Both the cultures were statistically on par. The mean colony diameter of *F. lateritium* recorded 6.96 cm which was on par with the *P. lilacinus* (6.94 cm). *A. incoloratum* showed significantly different growth (5.02 cm). The minimum growth was recorded on *P. fumosoroseus* (4.30 cm) which was significantly different from all the other fungal cultures.

Table. 27 Growth rate of other fungal pathogens

Fungal isolates	Mean colony diameter of three observations (cm)*									
	Days After Inoculation (DAI)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
A. strictum	1.98a*	2.97b	4.04b	4.51b	4.94b	6.25b	6.57b	7.19ab	7.87a	7.99a
A. incoloratum	1.75ab	1.93c	2.33d	2.75d	3.07d	3.75d	4.36c	4.62d	4.76c	5.02c
F. lateritium	1.60b	3.10b	3.89b	4.58b	4.84b	5.87b	6.28b	6.62bc	6.82b	6.96b
F. verticillioides	2.03a	3.88a	4.99a	5.92a	6.94a	7.29a	7.50a	7.56a	7.65a	7.77a
P. fumosoroseus	1.46b	1.98c	2.21d	2.52d	2.90d	3.13d	3.49d	3.74e	3.75d	4.30d
P. lilacinus	1.44b	2.17c	2.80c	3.50c	3.96c	4.71c	5.87b	6.38c	6.68b	6.94b

In each column figures followed by the same letter do not differ significantly according to DMRT; values in the parentheses are square root transformed ones

Observations on colony diameter taken on consecutive days revealed that the rate of growth was faster for the fungal culture, *F. verticillioides* during the initial days which declined in the subsequent days.

4.10.2.2 Colony characters

Observations on colour, growth pattern and reaction with the media PDA upto ten days after inoculation are shown in the Table. 28. The colony characters of other fungal pathogens, *Acremonium*, *Fusarium* and *Paecilomyces* varied with the species (Plate. 17A to F). The fungal cultures were of pure white or off white during the initial growth which later changed to pink, lilac, violet or

remains as the same. Microscopic observations on the mycelial and conidial characteristics were also taken and depicted in the Table. 29.

Table. 28 Colony characters of other fungal pathogens

Sl. No.	Fungal species	Colour of the mycelium		Growth pattern	Reaction in media
		Initial	Final		
1.	*Acremonium strictum*	Off white	Off white	Uniform & spreading	Light golden yellow
2	*A. incoloratum*	Pure white	Pure white	Raised & dome shaped	Light brown
3	*Fusarium lateritium*	Off white	Light pink	Uniform & fast growing	Peach coloured pigment
4	*F. verticillioides*	Off white	Light purple	Uniform & fast growing	Dark purple
5	*Paecilomyces fumosoroseus*	White	White with light pink tinge	Uniform, slightly fluffy	Golden yellow with brown patch
6	*P. lilacinus*	Off white	Lilac	Uniform, fluffy & raised growth	No reaction

***Acremonium* spp.**

Possessed hyaline and septate hyphae which were typically very fine and narrow. Vegetative hyphae often formed hyphal ropes. Unbranched, solitary, erect phialides are formed directly on the hyphal tips. The phialides are separated from hyphae by a septum and tapers towards the apices. The apices of the phialides bear the hyaline conidia which usually appeared in clusters, in balls or rarely as fragile chains. Conidia are bound by a gelatinous material. They were of single or multi cellular, fusiform with slight curve or resembled a shallow crescent. These structural properties of conidia varied depending on the species.

Acremonium strictum

Colour of the colony was off white throughout the growth on the surface. Powdery texture with an uniform and spreading growth pattern was observed. The reverse side was light golden yellow coloured. Thin walled, one-celled, colourless, smooth rather cylindrical conidia arose from the apex of the conidiogenous cells (phialides) (Plate. 17A).

Acremonium incoloratum

Pure white coloured mycelial growth, texture of the colony was compact, raised, dome shaped and velvetty during the entire growth. Light brown

colour was observed on the reverse side. This species produced its conidia in slimy masses, it differed from *A. strictum* in having more globose conidia (Plate. 17B).

Table. 29 Microscopic observations of other fungal pathogens

Sl. No.'	Fungal species	Characteristics of the genus	Identifying features of the species
1	*Acremonium strictum*	Hyaline and septate hyphae, often form hyphal ropes. Phialides, unbranched, solitary and erect. Conidia, hyaline bound by gelatinous material	Cylindrical conidia
2	*A. incoloratum*		Globose conidia produced in slimy mass
3	*Fusarium lateritium*	Hyaline and septate hyphae, conidiophores, aggregated, simple or branched with phialides - short, cylindrical to much elongated, awl-like. Two types of conidia - macro conidia septate (canoe to curve shaped) in the foot cell and micro conidia - aseptate, small, ovoid to cylindrical.	Macro and micro conidia are present
4	*F. verticillioides*		Microconidia produced in chains
5	*Paecilomyces fumosoroseus*	Hyaline and septate hyphae, conidiophores branched with phialides at the tip. Phialides swollen at base and taper towards apices, grouped in pairs or clusters. Conidia unicellular, hyaline to darkly coloured, smooth or rough, oval to fusoid, form long chains	Conidia cylindrical to fusiform, smooth walled and hyaline
6	*P. lilacinus*		Conidia in divergent chains, ellipsoid to fusiform, smooth-walled and hyaline

***Fusarium* spp.**

Hyaline and septate hyphae, conidiophores solitary or aggregated, simple or branched bearing apical conidiogenous cells (phialides). Phialides were short, cylindrical to much elongated, awl-like, produced two types of conidia - macroconidia - curved to canoe-shaped with prominent foot like appendage on base cell, with one or more transverse septa; microconidia - aseptate, small, ovoid to cylindrical.

Fusarium lateritium

This species showed an off white colour during the initial stage which turned to light pink later. By ageing the surface of the colony became cottony due to the overgrowth of loose hyphae. Peach coloured pigmentation was observed on the reverse side. Macro and micro conidia were produced (Plate. 17C).

Fusarium verticillioides

Colour of the colony turned to light purple from the initial off white colour on the surface. Cottony texture with a dark purple pigmentation in the media. Macro and micro conidia were present. Microconidia were produced in chains (Plate. 17D). Both the *Fusarium* spp. were fast growing with an uniform growth pattern.

***Paecilomyces* spp.**

Microscopic features revealed septate and hyaline hyphae; conidiophores often branched with phialides at their tip. Phialides swollen at their bases and taper towards the apices. They were usually grouped in pairs or clusters. Conidia unicellular, hyaline to darkly coloured, smooth or rough, oval to fusoid, form long chains.

Paecilomyces fumosoroseus

White colony with a light pink tinge; uniform and fluffy growth. Golden yellow with brown patch was observed on the reverse side. Conidia cylindrical to fusiform, smooth-walled and hyaline (Plate. 17E).

Paecilomyces lilacinus

Colour of the colony changed to lilac from the off white initial colour on the surface. Uniform and fluffy raised growth was observed. Conidia in divergent chains, ellipsoid to fusiform, smooth-walled and hyaline (Plate. 17F).

4.10.2.3 Evaluation of sporulation of fungal cultures

Sporulation at three radial distances of the different fungi was taken in solid medium of PDA. Results presented in the Table. 30 revealed that *A. incoloratum* recorded the highest sporulation at the three radial distances with a mean value of 43.11 x 10^6 spores ml^{-1}. Maximum spore load was obtained from half the radial distance (56.33 x 10^6) followed by the periphery (38.00 x 11^6 spores ml^{-1}) and centre portion (35 x 10^6 spores ml^{-1}). Other species of *Acremonium*, *A. strictum* also recorded maximum sporulation at half the radial distance of the colony (37.33 x 10^6 spores ml^{-1}).

Plate 17A. *Acremonium strictum*

Plate 17B . *A. incoloratum*

Plate 17C. *Fusarium lateritium*

Plate 17D. *F. verticilloides*

Plate 17E. *Paecilomyces fumosoroseus*

Plate 17F . *P. lilacinus*

Plate 17. Colony characters of other fungal pathogens

Among the *Fusarium* spp. *F. verticillioides* showed highest spore count at the centre (17.50 x 10^6 spores ml^{-1}) followed by portion at the middle (9.00 x 10^6 spores ml^{-1}) and periphery (3.17 x 16^6 spores ml^{-1}).

Lowest sporulation was noticed in the *Paecilomyces* spp. A different trend with maximum sporulation at the periphery (17.34 x 10^6 spores ml^{-1}) was recorded in the case of *P. fumosoroseus* while *P. lilacinus* sporulated well at the centre (7.17 x 10^6 spores ml^{-1}).

Table. 30 Sporulation of other fungal pathogens

Fungus	Number of spores (x 10^6 ml^{-1})			Mean
	Centre	Half of radial distance	Periphery	
A. strictum	24.16	37.33	9.34	23.61
A. incoloratum	35.00	56.33	38.00	43.11
F. lateritium	16.84	3.84	0.84	7.17
F. verticillioides	17.50	9.00	3.17	9.89
P. fumosoroseus	2.34	3.50	17.34	7.73
P. lilacinus	7.17	5.33	1.84	4.78

On considering the mean sporulation, highest sporulation was recorded by *A. incoloratum* (43.11 x 10^6 spores ml^{-1}) and the least by *P. lilacinus* (4.78 x 10^6 spores ml^{-1}).

4.11 ASSESSMENT OF MITE MORTALITY

Different isolates of *H. thompsonii* which differed in their cultural and morphological characters along with other fungal species which proved the pathogenicity were tested at respective doses of their sporulation against *A. guerreronis*. Statistical analysis of the data on mortality percentage of mites on the perianth and nut surface of the treated nuts after 72 hours is presented in Table. 31.

Table. 31 Per cent mortality of mites by fungal pathogens at respective dose of sporulation (after 72 hours)

Sl. No.	Treatments	Concentration Spore count (x 10^6 spores ml^{-1})	Mortality percentage
1	*H. thompsonii* var. *thompsonii* (Chirakkekodu-I)	5.92	80.63[a]
2	*H. thompsonii* var. *thompsonii* (Marakkal-I)	0.33	58.30[abc]
3	*H. thompsonii* var. *synnematosa* (Marakkal-II)	1.08	58.53[abc]
4	*H. thompsonii* var. *synnematosa* (Madakkathara-I)	0.58	60.43[abc]
5	*H. thompsonii* var. *synnematosa* (Madakkathara-II)	0.33	49.41[bc]
6	*H. thompsonii* var. *synnematosa* (Vellanikkara-I)	0.08	79.40[a]
7	*H. thompsonii* var. *synnematosa* (Kanimangalam-I)	0.67	43.42[cd]
8	*H. thompsonii* var. *synnematosa* (Konnakuzhy-I)	1.25	50.19[ab]
9	*H. kirchnerii* (Kanjirampally-I)	0.17	77.40[a]
10	*H. nodulosa*	0.33	75.33[ab]
11	*Acremonium strictum*	37.33	66.28[abc]
12	*Acremonium incoloratum*	56.33	67.21[abc]
13	*Fusarium lateritum*	3.84	72.08[ab]
14	*Fusarium verticillioides*	9.00	69.66[ab]
15	*Paecilomyces fumosoroseus*	3.50	83.65[a]
16	*Paecilomyces lilacinus*	5.33	60.89[abc]
17	*Verticillium suchlasporium*	5.99	59.87[abc]
18	*Sporothrix fungorum*	2.33	68.35[abc]
19	*Pseudomonas fluorescens*	49.13	57.99[abc]
20	Neemazal	0.4 per cent	60.36[abc]
21	Wettable sulphur	0.4 per cent	74.08[ab]
22	Control		26.34[d]

The fungal pathogen, *P. fumosoroseus* recorded maximum mortality percentage of 83.65. It was closely followed by *H. thompsonii* var. *thompsonii* (Chirakkekodu-I), *H. thompsonii* var. *synnematosa* (Vellanikkara-I) and *H. kirchnerii* with a mortality percentage of 80.63, 79.40 and 77.40 respectively which were on par with each other. All the other treatments recorded a significantly low mortality and were on par with a mortality percentage ranging from 26.34 to 75.33 the lowest being the untreated control. The bacterium, *Pseudomonas fluorescens*, recorded mortality of 57.99 per cent while the botanical insecticide, Neemazal and the acaricide, Wettable sulphur had a maximum mortality of 60.36 and 74.08 per cent respectively.

4.12 MOLECULAR CHARACTERIZATION

Based on the cultural, morphological and bioassay studies, seven different *Hirsutella* isolates were selected for the characterization studies.

4.12.1 DNA isolation

The *Hirsutella* isolate, Madakkathara-I was subjected to DNA isolation for the primer screening. A discrete and intact DNA was obtained during the gel documentation.

4.12.2 Screening of primers

Out of the 25 decamer primers of OPE and OPAH (Table. 32) series screened in RAPD analysis, amplification was observed for 17 primers. The number of bands per primer ranged from nil (OPE-3, 4, 5, 6, 8, 9, 10 and OPAH-7) to nine (OPAH-13). Primers with more than five bands included OPE-7 (8), OPE-12 (7), OPAH-3 (7), OPAH-6 (6), OPAH-9 (7), OPAH-10 (6), OPAH-13 (9) and OPAH-15 (6).

The following five primers were used for RAPD-PCR, OPE-12, OPAH-5, OPAH-9, OPAH-13 and OPAH-15. Based on the RAPD profile with the various primers, primer OPAH-13 was identified as the most promising one for characterizing the *Hirsutella* isolates.

Table. 32 Amplification bands obtained in screening of primers with *H. thompsonii* var. *thompsonii* isolate (Madakkathara-I)

Sl. No.	Series	Sequence of primers	No. of amplification bands
1	OPE- 3	CCAGATGCAC	0
2	4	GTGACATGCC	0
3	5	TCAGGGAGGT	0
4	6	AAGACCCCTC	0
5	7	AGATGCAGCC	8
6	8	TCACCACGGT	0
7	9	CTTCACCCGA	0
8	10	CACCAGGTGA	0
9	11	GAGTCTCAGG	2
10	12	TTATCGCCCC	7
11	OPAH-1	TCCGCAACCA	4
12	2	CACTTCCGCT	5
13	3	GGTTACTGCC	7
14	4	CTCCCCAGAC	1
15	5	TTGCAGGCAG	5
16	6	GTAAGCCCCT	6
17	7	CCCTACGGAG	0
18	8	TTCCCGTGCC	5
19	9	AGAACCGAGG	7
20	10	GGGATGACCA	6
21	11	TCCGCTGAGA	1
22	12	TCCAACGGCT	3
23	13	TGAGTCCGCA	9
24	14	TGTGGCCGAA	2
25	15	CTACAGCGAC	6

4.12.3 RAPD-PCR of *Hirsutella* isolates

The following *Hirsutella* isolates were selected for molecular characterization (Table. 33).

Table. 33 *Hirsutella* isolates selected for molecular characterization

Sl. No.	*Hirsutella* species	*Hirsutella* isolates
1	*H. thompsonii* var. *thompsonii*	Chirakkekodu-I (*H.t.t.*Cdu)
2	*H. thompsonii* var. *thompsonii*	Marakkal-I (*H.t.t.*Mal)
3	*H. thompsonii* var. *synnematosa*	Vellanikkara-I (*H.t.s.*Vra)
4	*H. thompsonii* var. *synnematosa*	Madakkathara-I (*H.t.s.*Mra)
5	*H. thompsonii* var. *synnematosa*	Kanimangalam-I (*H.t.s.*Kam)
6	*H. thompsonii* var. *synnematosa*	Konnakuzhy-I (*H.t.s.*Khy)
7	*H. kirchnerii*	Kanjirampally-I (*H.k.*Kly)

The quality of the DNA isolated from the above *Hirsutella* isolates was assessed using agarose gel electrophoresis (Plate. 18). The bands of the DNA were intact.

Seven isolates of *Hirsutella* showing cultural and morphological variations were subjected to RAPD-PCR using five selected random primers belonging to two different operon primer kits (Plate. 19A to 19E).

Table. 34 illustrates the number of amplification products obtained for seven isolates of *Hirsutella* with each of the five selected primers.

Table. 34 Amplification pattern of *Hirsutella* isolates using five selected random primers

Primer/isolates	H- 1	H-2	H-3	H-4	H-5	H-6	H-7
OPE-12	1	2	2	1	4	0	1
OPAH-5	0	3	1	0	1	1	0
OPAH-9	4	5	1	5	3	4	0
OPAH-13	1	2	0	1	3	0	3
OPAH-15	3	1	1	0	1	1	1

Hirsutella isolates

H-1 - Chirakkekodu-I
H-2 - Marakkal-I
H-3 - Vellanikkara-I
H-4 - Madakkathara-I
H-5 - Kanimangalam-I
H-6 - Konnakuzhy-I
H-7 - Kanjirampally-I

On the whole, the five selected operon primers generated a total of 82 bands with an average number of 11 bands per primer.

4.12.4 Genetic analysis

The RAPD data was used to generate a similarity matrix using the SIMQUAL of the NTSYS programme. Based on estimated Genetic similarity Matrix the highest (74 per cent) genetic similarities were noticed between the Hirsutella isolates, Madakkathara-I and Konnakuzhy-I.

The phenetic representation of similarity co-efficients among seven Hirsutella isolates are presented in Fig. 5. In the dendrogram, all the seven *Hirsutella* isolates were divided into two distinct major clusters. '1' and '2' at 50 per cent similarity. The subcluster-2 has got only a single *Hirsutella* isolate, Marakkal-I. The first cluster with six isolates was again divided into three sub

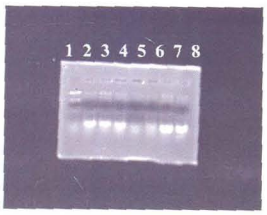


Plate 18. DNA bands of *Hirsutella* isolates

Plate 19A. Primer OPAH-5

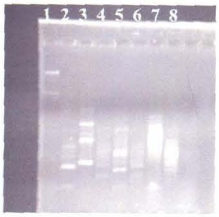


Plate 19B . Primer OPAH-9

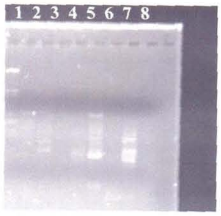


Plate 19C. Primer OPAH-13

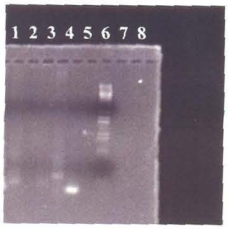


Plate 19D. Primer OPAH-15

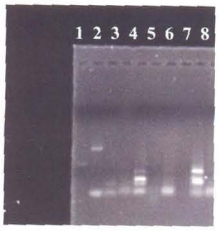


Plate 19E . Primer OPE-12

1 - Molecular marker, 2 to 8 - *Hirsutella* isolates:
2-*H.t.t.* Cdy, 3-*H.t.t.* Mal, 4- *H.t.s.* Vra, 5-*H.t.s.*Mra, 6. *H.t.s.*Kam, 7. *H.t.s.* Khy, 8. *H.k.* Kly

Plate 19. Amplification pattern of DNA - *Hirsutella* isolates using selected primers

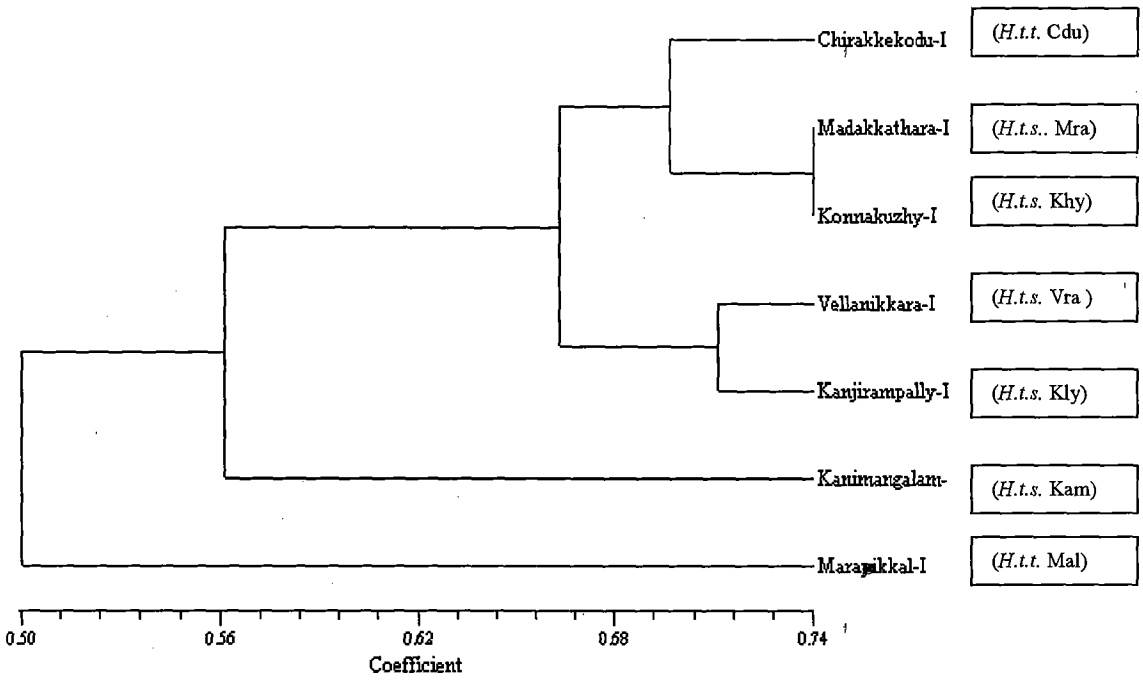


Fig. 5 Dendrogram from the analysis of seven *Hirsutella* isolates using five random primers

clusters. The first among this cluster 1A, had three isolates, Chirakkekodu-I, Madakkathara-I and Konnakuzhy-I with the later two having maximum similarity of 74 per cent. In the second major subcluster (1B), Vellanikkara-I and Kanjirampally-I are grouped together in one group. In the third subcluster (1C), a single isolate, Kanimangalam-I was present. The isolates of cluster 1A and 1B together had 56 per cent similarity with cluster 1C.

DISCUSSION

5. DISCUSSION

In recent years, the eriophyid mite *Aceria guerreronis* has become a serious pest in 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. The biological agents are well adapted to spread within the perianth, thus can be effectively used for mite control. The control measures currently employed, including the crown spraying and root feeding of chemical pesticides have proved to be a partial success only. Besides it causes environmental pollution and health hazards through the residues of the chemicals in the kernels and in the tender coconut water. Alternative control measures involving ecofriendly biocontrol agents such as entomopathogenic fungi specific to mites, *Hirsutella thompsonii* (Hall *et al.*, 1980; Sampedro and Rosas, 1989) within the array of Integrated Pest Management principles have become imperative.

Beevi *et al.* (1999) isolated the fungus *H. thompsonii* var. *synnematosa* from the dead coconut mites in India. Though *H. thompsonii* is reported to be specific fungal pathogen associated with the mite, association of other fungi should not be ruled out. In the present investigation, different strains/isolates of mite specific pathogen, *H. thompsonii* and other species of fungal pathogens associated with mites were studied. The population dynamics of eriophyid mite and predatory mite was studied in selected locations of Thrissur district. Based on the cultural, morphological and comparative bioefficacy studies, potential isolate of *H. thompsonii* was identified which can be utilized for the biocontrol of coconut mite. The results generated in the present studies are discussed below.

5.1 SURVEY ON MITE POPULATION

A study for one year (July 2003 to June 2004) was conducted at four randomly selected panchayaths of Thrissur district to assess the predatory mite population, live and dead mite population separately on the outer perianth (OP), inner perianth (IP) and nut surface (NS). Apart from this investigations were

carried out on the naturally occurring biocontrol agents associated with eriophyid mite.

5.1.1 Live mite population

The location wise data on live mite population on the OP, IP and NS for the four different panchayaths are presented in Tables 2 to 5.

A comparison of the live mite population on different parts of a single nut viz., OP, IP and NS was done. The results revealed that the live mites were more on the NS with a proportionate low numbers on the OP and IP (Fig. 2). So the feeding and feeding injury by mites was restricted to the tender meristematic region of NS. The trend in the distribution of the live mite population on NS and perianth followed an uniform pattern throughout the three locations surveyed in each of the four panchayaths. Irrespective of the locations and seasons, 74 per cent of the total mites were found on the NS while the remaining was found distributed on the OP and IP (Fig. 6). This was in accordance with the study conducted by Anonymous (2005) where 60.57 per cent of the live mite among the total population was on the nut surface. As the maximum population was on the NS, the results of the data on population dynamics is discussed based on the NS population.

In the present study, among the four panchayaths selected, significant variation was found among locations of two panchayaths, Pananchery and Pariyaram panchayaths. Live mite population ranged from 18.245 to 32.917 and 21.990 to 29.476 per 4 mm^2 respectively in the Pananchery and Pariyaram panchayath. Significantly low live mite population was recorded from the location, Vellanikkara (18.635/4 mm^2) of Madakkathara panchayath, whereas Kanimangalam of Koorkenchery panchayath recorded significantly high live mite population of 41.437 per 4 mm^2 on the nut surface.

Though the location wise variation was significant, the mite population did not vary significantly when the panchayath wise population of the same district was considered and the live mite population ranged from 25.709 to 26.514 per 4 mm^2. While considering the locations of the entire four panchayaths, the live mite population on the NS ranged from 18.245 to 41.437 per 4 mm^2. This

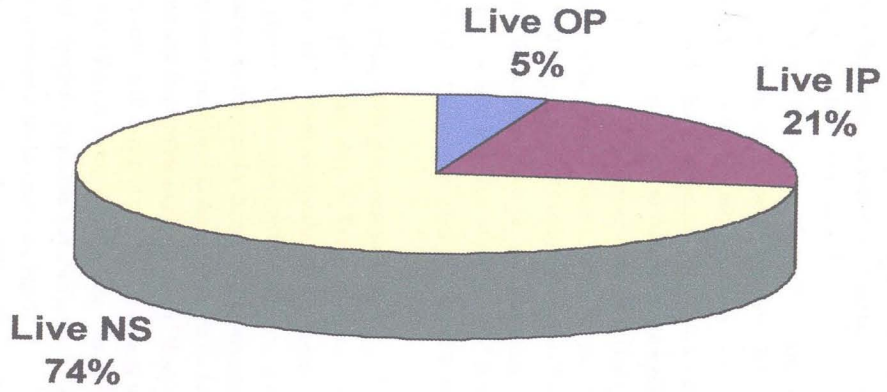


Fig. 6 Distribution of live mites on perianth and nut surface

observation confirmed the results with that of population data of survey undertaken by Umapathy *et al.* (2001) during June 2000 to October 2000. According to them, the live mite population ranged from 7.20 to 57.18 per 4 mm^2 in nut. Another study by Vidhya (2001) on the population dynamics was also agreeable with the present study where she reported a mite population of 1400 per cm^2 (corresponding value of 56 per 4 mm^2).

A different trend was observed in the studies carried out by Anonymous (2005) during the period December 2002 to November 2003 at Thrissur district, where the live mite population on the nut surface among the three locations varied from a minimum of 90.139 to a maximum of 131.665 per 5 mm^2. This variation may be due to the change in the study period or due to the variation in the locations of Thrissur district. Population dynamics studies of mites at Instructional Farm, Vellayani during the year 1999 by Mathew *et al.* (2000) recorded a population (> 12,000/3 nuts) in the 4^{th} to 6^{th} bunch. Thus it was confirmed that the population level i.e., the mean number of live mites present in the unit area of the infested surface of nut (3^{rd} to 4^{th} bunch) was found to vary, based on the data generated over years from different parts of Kerala and from other states.

5.1.1.1 Seasonal variation of live mite population

Monthly mean data of live mite population on OP, IP and NS irrespective of the locations and panchayaths are presented in Tables 7 to 10. A moderate to heavy population was seen throughout the year. Population was found to be fluctuating in three peaks during the late monsoon, winter and summer months. Maximum population was during August in the late monsoon, April/May in the summer and January/February in the winter. Similar trend was observed in all the locations as well as that of panchayaths. Among the three peaks, highest population was observed during winter months. During the heavy rainy season (June to July), live mite population was comparatively lowest and a population increase was observed in the late monsoon (Fig. 7).

Studies conducted by Haq (1999) in Thrissur district showed a similar trend to that of the present study where the population density of CEM increased

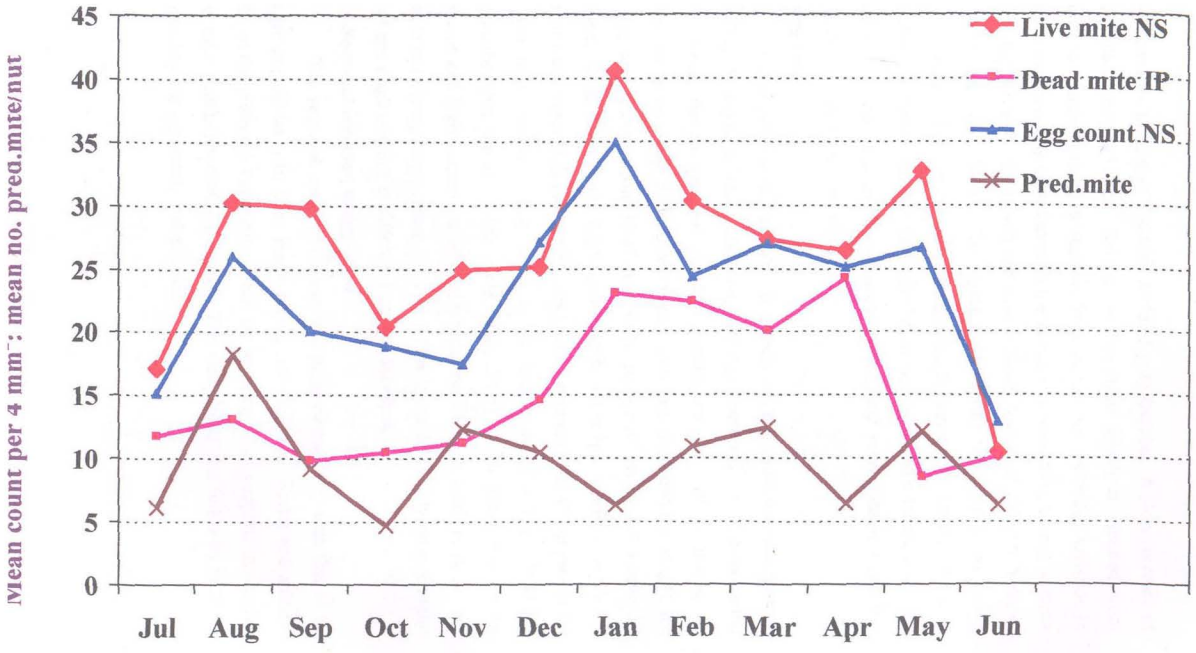


Fig. 7 Seasonal variation of eriophyid and predatory mite population

slightly from July to August and then declined upto October. A slow increase of the population occurred from October to December which accelerated from December to March-April, then declined from May to July. Monthly variation in the mite population was observed with the maximum population during summer months (Mathew *et al.*, 2000; Nair and Koshy, 2000; Nair *et al.*, 2000 b; Yaligar, 2004 and Anonymous, 2005). The reason for relatively high population during summer months and low population during rainy season can be attributed due to the positive correlation of live mite population with maximum temperature and negative correlation with minimum temperature during rainy season which has been established in the present study.

5.1.2 Egg count

Corresponding to the high mite population, egg count was also high on the NS (Fig. 4). No significant difference in the egg count was recorded on the nut surface among the locations of the four panchayaths as well as among the panchayaths. In an active mite colony mixed with live, dead and egg stages, the mean egg count ranged from 19.471 to 25.514 per 4 mm^2 on the NS among the four panchayaths. Though the egg stages were found at low intensity on the OP and IP, it was always high on the NS (65%), which accounted to 47 per cent of the total live mite colony. Egg count is an indication of a high fecundity accompanied with the short life cycle (Haq, 2000) which paves way to the continuous and high occurrence of mite population. Thus it leads to heavy and moderate nut damage throughout the year. This is the first study on population level of egg stage of CEM in different locations surveyed.

5.1.2.1 Seasonal variation in egg count

The seasonal count of the egg was proportional (1:1) with that of the live mite population with three peaks during late monsoon, winter and summer months in the present investigation. Thus the low egg count supports the finding of low mite population during monsoon. The same trend was followed in all the four panchayaths separately and as a whole.

5.1.3 Dead mite

Unlike that of the live mite population and egg count, the distribution of dead mites was highest in the perianth especially on the IP than on the NS (Fig. 8). Since the dead mite population varied among perianth and nut surface, data recorded on the IP was taken into consideration for easy comparison of the data.

In general, the natural mortality was found to be 28 per cent which varied from 13.84 to 16.08 on the IP. In the earlier studies by Anonymous (2005) a natural mortality of 15 to 19 per cent on the IP and NS was observed in the natural field conditions. While considering the location wise dead mite population on the IP of four panchayaths, significant variation was observed in three panchayaths except Pariyaram. The dead mite population ranged from 13.629 to 14.135 (Table. 6) in all the three locations of Pariyaram panchayath. Chirakkekodu (19.802) of the Madakkathara panchayath and Kanimangalam (19.650) of Koorkenchery panchayath recorded significantly high dead mite population. However, no significant difference in the dead mite population on IP was observed among the four panchayaths irrespective of the locations which ranged from 13.835 to 16.080 per 4 mm^2. The results of the present study is agreeable with that of Anonymous (2005), where the dead mite population did not differ significantly among the locations of Thrissur district, mean dead mite population ranged from 17.053 to 25.108.

The above studies revealed that the percentage of live mite and egg count contributed to 36 per cent each while the dead mite form 28 per cent of the total mite population (Fig.9).

5.1.3.1 Seasonal variation of dead mite

In the case of dead mite, the population was found to be fluctuating throughout the year. Peaks of the dead mite population were obtained in the winter and summer months irrespective of the locations in all the four panchayaths. January and February of the winter and March to April of the summer months had the peak dead mite population (Fig. 7). This was in accordance with the findings of Anonymous (2005), where the highest count of dead mite was during March and April. The exact cause of mortality cannot be

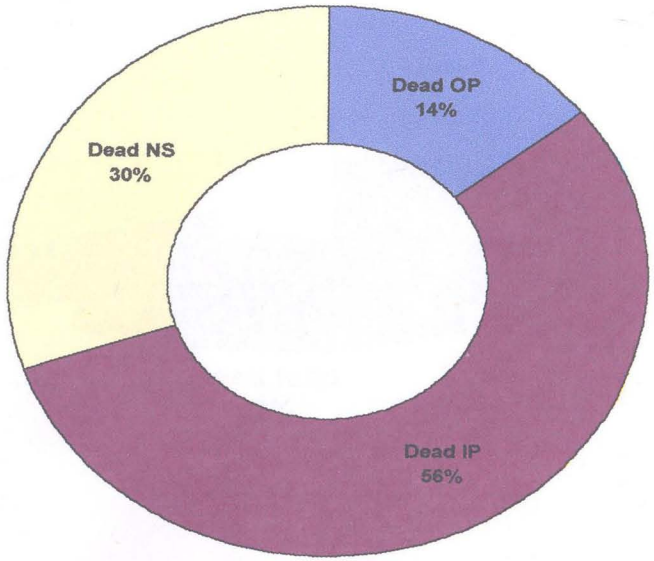


Fig. 8 Distribution of dead mites on perianth and nut surface

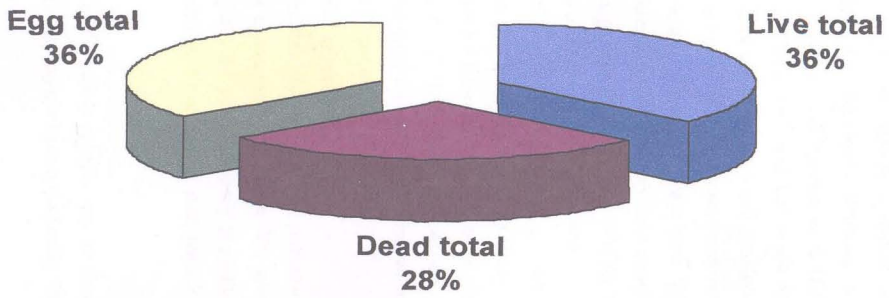


Fig. 9 Distribution of live mites, dead mites and egg count per nut

ascertained in the present study. It may be due to ageing and other biotic stress factors. Literature pertaining to the dead mite population was negligible, since most of the researchers have not adequately concentrated on quantification of dead mite population.

5.1.4 Predatory mite population

In the present survey, mainly predatory mites of two genus, *Amblyseius* spp. and *Bdella* spp. were found inhabiting the perianth region. This was reported earlier by Julia *et al.* (1979), Nair *et al.* (2000 b), Ramaraju *et al.* (2000), Saradamma *et al.* (2001), Beevi *et al.* (2004) and Anonymous (2005). The population of predatory mite was found to be well established in the mite infested nuts, where the percentage nuts infested with predatory mites ranged from 12.50 to 100.00. Maximum number of predatory mites present per nut was 94. There was no significant difference in the predatory mite population among the three locations of Pariyaram panchayath. While considering the four panchayaths, irrespective of locations of Thrissur district, they were on par where the predatory mite population ranged from 19.471 to 25.514 per nut. A similar trend was observed by Anonymous (2005) where the mean predatory mite population varied from 13.433 to 23.408 per nut where they could not find any significant difference among the three locations of Thrissur district. According to Ramaraju *et al.* (2000), the predatory potential was not encouraging where they recorded a low mean population of 5 to 10 per nut.

5.1.4.1 Seasonal variation of predatory mite

Fig. 7 illustrates the month wise variation of predatory mites irrespective of the four panchayaths as a whole. The predatory mite count was also fluctuating throughout the year with peak population during August and September of late monsoon, November of post monsoon and March/May of summer season.

5.1.5 Correlation

Correlation of the mite population with weather data was done in one location each from three panchayaths having the facility for recording the weather data (Tables.12 to 14).

In the present study, results of the correlation at the three locations showed that a significant relationship was obtained only in the Thumbermuzhy location of Pariyaram panchayath where a negative correlation was observed with weather parameter, minimum temperature. However, a positive correlation was found to exist between the population parameters (total live, dead and egg count) and maximum temperature in all the three locations, though it was not significant.

The results of the earlier studies also could not reveal a significant relationship on mite population with weather parameters. The findings for similar studies are:-

During the earlier studies, a positive correlation between the mite population and dry climate during the summer months and a negative relationship with that of the rainfall during the monsoon was observed by Haq (1999). In another investigation by Vidhya (2001), mite population was found to vary with weather parameters, where minimum and maximum temperature, morning humidity and wind speed were observed to be the important weather factors influencing population of eriophyid mite; wind speed possessed a negative correlation with mite population. Rainfall as such did not influence the population of mites. Low population during rainy season was due to the minimum temperature prevalent during the period. Yaligar, 2004 also reported that the mite population on nut surface were positively correlated with temperature and negatively correlated with evening humidity.

5.1.6 Frequency of isolation of fungal pathogens

***5.1.6.1 Hirsutella* spp.**

Isolation of fungal pathogens associated with dead mite was carried out continuously for 12 months period from the three locations each of four panchayaths. Even after repeated sampling and isolation, *Hirsutella* spp. could not be obtained from two locations, Kannara and Mudicode of Pananchery panchayath. From all the other ten locations, the per cent isolation of *Hirsutella* from the total nut samples collected during the entire period of study ranged from 2.30 to 8.14 per cent (Table 15), which forms a mean of 4.93 per cent over the entire four panchayaths.

The actual recovery of pathogen in the pure form from the 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. In a study conducted by Anonymous, 2005 at three locations of Thrissur district, about 5.16 per cent of mite infested nut samples had *Hirsutella*. While only 6.85 per cent of the mite infested nut samples yielded *H. thompsonii* in the survey conducted by Kumar *et al.* (2001) at the three districts of Karnataka (Bangalore Rural, Mandya and Kolar) and one in Tamil Nadu (Coimbatore). These observations support the views of Hall *et al.* (1980) which revealed 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. This might explain the spread of the fungus among the mite populations.

A separate study was conducted for assessing the natural infection of *H. thompsonii* on coconut mite. It revealed a natural infection ranging from 20 to 30 per cent. This has been confirmed by microscopic observation of mite colonies by the presence of mycelium and phialides emerging out from mycosed mites. In a similar study conducted by Kumar *et al.* (2002) at Karnataka also showed that the extent of natural infection was 20 per cent.

5.1.6.2 Other fungi

In addition to *Hirsutella* spp. the mite specific pathogen such as *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. could be isolated from all the four panchayaths of Thrissur district (Table. 17). 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* spp. and its mean isolation per cent during one year was 16.10 per

cent. Out of the 919 nut samples isolated, *Fusarium* spp. accounts to 10.66 per cent and *Paecilomyces* spp. (1.63%). Actinomycetes were another important microorganism encountered during isolation which contributed to 10.34 per cent.

In the earlier reports, association of many fungal species like *Paecilomyces, Beauveria, Sporothrix, Verticillium, Acremonium* were reported which were frequently isolated from the coconut eriophyid mite from the coconut growing tracts of South Indian States (Kumar *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. The earlier studies by Hall *et al.* (1980), Anonymous (2005) were also supportive to the present study where the isolation of dead eriophyid mite has repeatedly yielded to several fungi like *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. The spectrum of fungal pathogens associated with the coconut mite indicates the significant role played by them in the natural suppression of the pest.

5.1.7 Seasonal occurrence

5.1.7.1 Hirsutella spp.

Mite mortality and *Hirsutella* isolation was found to be coincidental with the live mite population. Of the total *Hirsutella* isolations obtained, over a period of one year from 12 locations, 35.56 per cent of total isolation was during winter season. Relatively higher isolation was during winter and monsoon seasons (Table. 16) thus indicates that the high dead mite population is coincided with the increase in the live mite population since one of the three peaks with maximum live mite population was during the winter season. Lowest occurrence of *Hirsutella* spp. was at the post monsoon (October - November) which accounts to 13.33 per cent, the live mite population was also very low during this season. It was found that the natural occurrence and spread of *H. thompsonii* and the resultant mortality is very much limited. The slow growing nature of the fungus may be the reason for this.

Research work related to the seasonal incidence of *H. thompsonii* of coconut eriophyid mite is negligible. While studies conducted in 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 the 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 the temperature, 25 to 30°C with a 60 per cent relative humidity. This suggests that the fungus survive diverse and variable environmental conditions. However, good results could only be expected during warm season as given in the report.

5.2 DIFFERENT VARIETIES OF *Hirsutella spp.*

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 subsequent subculturing. Based on the presence or absence of synnemata, two different varieties were found to occur, namely, *H. thompsonii* var. *thompsonii* and *H. thompsonii* var. *synnematosa*. Among the different isolates, the latter variety, *H. thompsonii* var. *synnematosa* was more frequently isolated during the study. Based on the colour and growth pattern variations, the *H. thompsonii* were classified into nine isolates, two isolates Marakkal-I and Chirakkekodu-I coming under *H. t.* var. *thompsonii* and six isolates, Marakkal-II, Madakkathara-I and II, Vellanikkara-I, Kanimangalam-I and Konnakuzhy-I under *H.t.* var. *synnematosa.* One more species could be isolated in the present study and it was tentatively identified as *H. kirchnerii*. However, this species could be isolated in pure form from the dead mite colony only once. It was isolated from the Kanjirampally location of the Pariyaram panchayath.

The genus *Hirsutella* infects a number of different types of insects as well as mites and nematodes (Mc Coy *et al.*, 1988; Jaffee, 2000). *H. thompsonii* has been isolated from the samples of eriophyid mite from tropical America and West Africa and from samples of *Colomerus novahebridensis* from New

Hebrides, New Guinea and Sri Lanka (Hall *et al.*, 1980). Liu *et al.* (1995); Mazet and Vey (1995); Vey *et al.* (1993) isolated two strains of *H. thompsonii* var. *thompsonii* from the tarsonemid mites which have been reported to produce and secrete Hirsutellin A (Ht A), a protein that has potent insecticidal and cytotoxic activities. The fungus *H. thompsonii* has already been reported as the most promising and potential biological control agent against CEM in the Ivory Coast and Mexico (Julia and Mariau, 1979; Hall *et al.*, 1980; Berril and Sanchez, 1986; Sampedro and Rosas, 1989). The *H. thompsonii* variety, *H. t. var. synnematosa* seems to be restricted to the tropics (Samson *et al.*, 1980). Beevi *et al.* (1999) isolated a local strain of *Hirsutella* spp. from CEM at Thrissur district which was identified as *H. thompsonii* var. *synnematosa*. Mietkiewski *et al.* (2000) isolated the *Hirsutella* spp. *H. thompsonii, H. thompsonii* var. *synnematosa, H. nodulosa* and *H. kirchnerii* during the survey of entomopathogenic fungi conducted from 1995 to 1998 in Siedlce, Poland from infested phytophagous mites. Two new species *H. kirchnerii, H. nodulosa* were also isolated from eriophyid mite by Anonymous (2005) during the survey conducted in the three agro-ecological zones in Kerala. *H. kirchnerii* was isolated from eriophyid mites, which act as vector of Ryegrass mosaic virus. Kirchner (1904) described a case of mycosis in *Tarsonemus spirifex* by a hyphomycete fungus, *H. kirchnerii*. Thus the present study on isolation of different *H. thompsonii* isolates and other *Hirsutella* spp. like *H. kirchnerii* from the mites is in confirmation with that of the earlier reports.

5.2.2 OTHER FUNGI AND MICROORGANISMS

In the present investigation, a number of non-specific fungal species and other microorganisms were isolated from the dead eriophyid mites (Table 20). They included fungi belonging to the genus *Acremonium, Fusarium, Paecilomyces, Ramichloridium, Aspergillus* and *Penicillium*. Other microorganism like actinomycetes and a bacterium, *Serratia* sp, also could be isolated from dead mites. Earlier studies reported that two bacterial isolates (*Serratia marcescens* and *Pseudomonas* spp.) and seven fungal isolates were obtained from eriophyid mite infested nuts. The fungal isolates were identified as *Rhizopus* spp., *Fusarium* spp., *Aspergillus niger, A. flavus, A. terreus,*

Trichoderma spp. and *Penicillium* spp. (Kumar *et al.*, 2000). Studies by Padiyath (2002) found that other fungi like *Aspergillus niger, A. flavus, Penicillium* spp. and *Fusarium* spp. were found to be associated with the mite. A collaborative study conducted by Gopal *et al.* (2003) in the months of May (2001) at Thrissur district observed that the Actinomycetes constituted the predominant microflora (seven isolates) followed by yeasts (four isolates); fungi (three isolates) and bacteria (two isolates). Kumar *et al.* (2001c) also reported that non specific fungi like *Paecilomyces* spp., *Beauveria, Metarhizium, Sporothrix, Verticillium, Acremonium* and other fungi like *Aspergillus, Penicillium* and *Fusarium* could be frequently isolated from the mites.

5.3 CONFIRMATION OF PATHOGENICITY

All the fungi isolated during the survey in the present study were subjected to pathogenicity studies. They included the *Acremonium* spp., *Fusarium* spp., *Paecilomyces* spp., *Ramichloridium* spp., *Aspergillus* spp. and *Penicillium* spp. and other microorganisms like bacteria and Actinomycetes. Among the different fungi and other microorganisms tested, two fungal species each coming under three genus, *Acremonium* (*A. strictum* and *A. incoloratum*), *Fusarium* (*F. lateritium* and *F. verticillioides*) and *Paecilomyces* (*P. fumosoroseus* and *P. lateritium*) proved the Koch postulates by the repeated reisolation of these pathogens from eriophyid mites. Assessment of mortality using these fungal pathogens also produced considerable effect in the range of 60.89 to 83.65 per cent which supports the pathogenicity of these microorganisms. Pathogenicity trials conducted by Gopal *et al.* (2003) with cell free filtrates of fungal isolates; *Fusarium* spp. gave a mortality range of 15 to 25 per cent. Observations in the present study are in confirmation with the results of Anonymous (2005) where the pathogenicity of *F. lateritium* and *A. incoloratum* has already been proved. But their exact role like *Hirsutella* spp. as a primary pathogen of eriophyid mite has to be established further. Microscopic observations by *Hirsutella* spp. showed distinct mycelial outgrowth with phialides and conidia from the affected mites, whereas other fungal cultures could not be identified based on mycelial growth. The hyphae of these fungi emerging out from the mites do not possess phialides

with conidia which form the distinguishing character for identification. However, from the present study and from the earlier reports the role of these microorganisms as a specific and primary pathogen as that of *Hirsutella* has not proved beyond doubt in any of these studies.

5.4. CULTURAL AND MORPHOLOGICAL STUDIES

5.4.1 Hirsutella spp.

Among all the isolates obtained, eight isolates of *H. thompsonii* coming under two varieties were found to be different in their growth rate, sporulation, biomass, micrometry, colour and pattern of growth. The *H. thompsonii* var. *synnematosa* isolate namely Madakkathara-I (Table. 22) had the maximum growth rate (4.167 cm) whereas it also possessed a significantly high biomass of 2.660 g (Table. 25). While in the case of spore count, Chirakkekodu-I, *H. thompsonii* var. *thompsonii* recorded a maximum mean spore count of 3.22 x 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. 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 production. This may be one of the reason which supports the low sporulation rate in the synnemata producing *Hirsutella* variety, even though it stands first in growth rate and biomass production.

Colony characters of *H. kirchnerii* and two varieties of *H. thompsonii* were observed. *H. kirchnerii* and *H. t.* var. *synnematosa* (Madakkathara-I) varied morphologically from the other *Hirsutella* isolates, where it produced an off white colour mycelia throughout the growth. Microscopic observations of the fungal structures showed that the hyphae were hyaline, septate, smooth and branched. From the vegetative hyphae conical to flask shaped phialides arose with broad base and narrow neck. The neck was often branched ones. The spores produced were spherical, verrucose and hyaline while that of *H. kirchnerii* like the segments

of lemon. The observations are in conformity with the characters of fungus described by International Mycological Institute.

Micrometry studies revealed that the *H. thompsonii* isolates possessed a hyphal width, phialide width and spore diameter of 3.44 µm which was in accordance with that of Padiyath (2002) where it ranged between 3.33 µm to 3.76 µm. Samson *et al.* (1980) and Beevi *et al.* (1999) reported the colony characters and microscopic studies of *H. t.* var. *synnematosa*, their observations confirmed the present results.

5.4.2 Other fungi

Among the other fungal pathogens, maximum growth and sporulation was observed on two species belonging to *Acremonium*. *A. strictum* recorded maximum growth of 7.99 cm while *A. incoloratum* recorded a mean sporulation of 43.11 x 10^6 spores ml^{-1}. The rate of growth was faster for the fungal pathogen, *F. verticillioides*. Morphological studies showed that all the fungal pathogens were of pure white or off white coloured during the initial growth which later changed to pink or lilac or violet or remains the same. Growth pattern was uniform with fluffy raised/dome shaped ones and fast growing. The fungal pathogen *P. lilacinus* only possessed reaction with the media, where the media has got variation in colour (Table. 29). All the fungal pathogens possessed hyaline and septate hyphae; conidiophores - aggregated, simple or branched with phialides branched or unbranched swollen at the base and tapered towards apices, grouped in pairs or clusters. Conidia - hyaline to darkly coloured, unicellular, smooth or rough, oval to fusoid, sometimes bound by gelatinous material. These observations on the cultural characters and morphological characters are in confirmation with that of Anonymous (2005) and International Mycological Institute.

5.5 ASSESSMENT OF MITE MORTALITY

In vitro evaluation of the eight isolates of *H. thompsonii, H. kirchnerii,* two species each of *Acremonium, Fusarium* and *Paecilomyces; Sporothrix fungorum, Verticillium lecanii* and *Pseudomonas fluorescens* were done along with the botanical insecticide, Neemazal and acaricide, wettable sulphur.

Maximum mortality of 83.65 per cent was obtained after 72 hrs by the fungal pathogen *P. fumosoroseus.* It was closely followed by *H. t.* var. *thompsonii* isolate (Chirakkekodu-I) which performed well with a mortality of 80.63 per cent. In the case of *Hirsutella* sporulation was a determining factor in the evaluation study of fungi, where Chirakkekodu-I isolate possessed maximum sporulation. Other treatments had a mortality range below 80 per cent only. Laboratory bio-efficacy studies of three formulations of entomogenous fungi (*Verticillium lecanii, Paecilomyces fumosoroseus* and *Hirsutella thompsonii*) at concentrations 10^6, 10^7 and 10^8 spores ml^{-1}against pink mite in tea showed that they were susceptible to all the three pathogens. Satisfactory control was obtained from 5^{th} day after the application and its efficacy was 100 per cent on the 10^{th} day after application (Selvasundaram *et al.*, 2001). Thus the sporulation and mortality percentage caused by the fungi are in confirmation with that of the earlier studies.

Wettable sulphur (WS) recorded a mortality of 74.08 per cent in the present study. It has already been proved effective for the management of coconut mite (Mohanasundaram, 2000; Fernando *et al.*, 2000). But information on compatibility studies by Padiyath (2002) revealed that WS is less compatible to *H. t.* var. *synnematosa.*

Mozes *et al.* (1995) identified three *Hirsutella* species (*H. necatrix* and *H. kirchnerii*) and six isolates of *H. thompsonii* by an assay for random amplified polymorphic DNA (RAPD). RAPD markers correlated with the mite host of specific fungus isolates. In the present study also, molecular characterization enabled that there was genetic variation among the native isolates of the *H. thompsonii* and *H. kirchnerii* using RAPD-PCR. Genetic analysis (Fig. 4) exhibited a fifty per cent similarity between Marakkal-I, *H. t. thompsonii* in one cluster and all the other isolates coming under *H. t.* var. *synnematosa, H. t.* var. *thompsonii* and *H. kirchnerii* in the other cluster. The isolates coming under two varieties of *H. thompsonii,* Chirakkekodu–I with maximum sporulation and Madakkathara-I with maximum growth rate and biomass came under the same subcluster.

After the cultural, morphological, mortality assessment and molecular studies of the seven *Hirsutella* isolates (Table. 35), Chirakkekodu I coming under the *H. thompsonii* var. *thompsonii* were discovered to be the potential isolate interms of mortality and percentage mortality. The growth rate, spore count and biomass of the isolate were 3.763 cm, 5.92 x 10^6 spores ml^{-1} and 1.560 g ml^{-1} respectively. Molecular studies have to be continued with more primers for getting accurate variation between the isolates.

While considering the frequency of isolation of the two *Hirsutella* varieties, the predominant naturally occurring one was *H. thompsonii* var. *synnematosa* (Table. 36).

Table. 36 Frequency of isolation of the *Hirsutella* varieties

Panchayath	Locations	*H. thompsonii* var. *thompsonii*	*H. thompsonii* var. *synnematosa*
Pananchery	Marakkal	2	0
	Kannara	0	0
	Mudicode	0	0
Madakkathara	Chirakkekodu	4	1
	Vellanikkara	0	3
	Madakkathara	0	4
Koorkenchery	Kanimangalam	0	7
	Nedupuzha	0	4
	Panamukku	0	5
Pariyaram	Thumbermuzhy	0	5
	Konnakuzhy	0	4
	Kanjirampally	0	6
Total		6	39

The factor behind the mortality caused by *H. thompsonii* var. *synnematosa* in the natural infection, whether its toxin or not has to be studied. An augmentative release of the potential isolate, *H. thompsonii* var. *thompsonii* is recommended since it is limited to restricted areas.

Table. 35 Comparative evaluation of the *Hirsutella* isolates in terms of the cultural and characterization studies

Sl. No.	*Hirsutella* spp.	*Hirsutella* isolates	Growth rate (cm)	Spore count (x 10^6 spores ml^{-1})	Biomass (g/100 ml)	% mortality at respective dose of sporulation	Molecular studies (clusters)
1	*H. thompsonii* var. *thompsonii*	Chirakkekodu-I	3.763^{BC}	3.22	1.560^{bc}	80.63^{a}	1A
2	,,	Marakkal-I	1.570^{G}	0.86	1.663^{bc}	58.30^{abc}	2
3	*H. thompsonii* var. *synnematosa*	Marakkal-II	2.503^{E}	1.81	1.477^{c}	58.53^{abc}	Not characterized
4	,,	Madakkathara-I	4.167^{A}	0.36	2.660^{a}	60.43^{abc}	1A
5	,,	Madakkathara-II	4.077^{A}	0.33	2.047^{bc}	49.41^{bc}	Not characterized
6	,,	Vellanikkara-I	3.927^{AB}	0.61	1.893^{bc}	79.40^{a}	1B
7	,,	Kanimangalam-I	2.843^{D}	0.56	1.737^{bc}	43.42^{cd}	1C
8	,,	Konnakuzhy-I	2.170^{F}	0.89	1.640^{bc}	50.19^{ab}	1A
9	*H. kirchnerii*	Kanjirampally-I	3.603^{C}	0.17	2.140^{ab}	77.40^{a}	1B

SUMMARY

SUMMARY

The study entitled 'Evaluation and characterisation of effective fungal pathogens against coconut eriophyid mite (*Aceria guerreronis* Keifer)' has been carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara and in four panchayaths of Thrissur district. The period of study was from October 2002 to December 2005. The main objective of the study was to isolate, identify and confirm the pathogenicity of fungi associated with the eriophyid mite, identification of the potential acarifungal pathogens and the molecular characterisation of selected isolates.

The following experiments were carried out.

i) Assessment of mite population (live and dead) from the randomly selected four panchayaths of Thrissur district

ii) Isolation and identification of the fungal species associated with coconut eriophyid mite

iii) Confirmation of pathogenicity of the fungal species

iv) Cultural and morphological characteristics of the fungal pathogens

v) Identification of the potential pathogen

vi) Molecular characterization of the selected fungal isolates

The survey was conducted from three randomly selected locations each from four random panchayaths viz., Pananchery, Madakkathara, Koorkenchery and Pariyaram of Thrissur district. There was no significant difference in mite population among the four panchayaths surveyed. The live mite population and egg count on the nut surface ranged from 25.709 to 26.514 and 19.471 to 25.514 respectively per 4 mm^2 per nut. However, there was a wide variation in the population when the different locations within each panchayath was considered where it ranged from 17.556 to 41.437 and 16.170 to 31.958 respectively per 4 mm^2 per nut. The percentage of live mite and egg count contributed to 36 per cent each while the dead mite form 28 per cent of the total mite population.

Among the four panchayaths irrespective of the locations, no significant difference was observed in the dead mite population on IP. The dead mite population on the inner perianth ranged from 13.835 to 16.080 per 4 mm^2 per nut

over the four panchayaths. While the dead mite population within the locations of the population varied significantly which ranged from 10.524 to 19.802 per 4 mm^2 per nut.

The predatory mite population was found to be well established on mite infested nuts. Maximum number of predatory mites at a time was 94 per nut. Significant variation was observed in the predatory mite population among the three different locations of four panchayaths as well as among the four panchayaths. The predatory mite population ranged from 4.469 to 15.719. Two types of predatory mites, *Amblyseius* spp. and *Bdella* spp. were mainly observed during the population assessment studies.

The distribution of the mite population in the outer perianth, inner perianth and nut surface followed an uniform pattern in the three different locations of each panchayath as well as in all the four panchayaths included in the present study. It was revealed that the distribution of live mite and egg count was high on the nut surface, while the dead mite population on the inner perianth.

A moderate to heavy mite population was seen throughout the year. Population was found to be fluctuating with three peaks during the late monsoon, winter and summer months. Maximum population was during August in the late monsoon, April/May in the summer and January/February in the winter. During heavy rainfall (June to July), population was comparatively lowest and an increase in population was observed in the post monsoon. The same trend as that of the live mite population was observed in the egg count over all the four panchayaths. In the case of dead mite population, a fluctuating population with two peaks during winter (January to February) and summer (March to April) months were obtained.

Predatory mite count also showed peaks during the year with a fluctuating maximum population during August and September months of post monsoon, March/May of summer season. To a certain degree, population of predatory mite increased with increase in the live mite population. But there was no proportionate increase in the predatory mite population as that of the live mite and egg count.

Correlation analysis of the population data with weather parameters at one location each of three panchayaths showed a significant negative relationship between mite population (predatory mite, live, dead and egg count of CEM) and the minimum temperature. Though not significant, a positive correlation was observed between mite population and maximum temperature in all the four panchayaths.

Out of the twelve locations surveyed in four panchayaths, *Hirsutella* spp. was isolated in pure form from ten locations. Only 45 nut samples yielded *Hirsutella* spp. from a total of 919 samples collected at monthly intervals for 12 months, over the entire four panchayaths, which accounts to about 4.90 per cent. Maximum number of *Hirsutella* isolates were obtained from the Koorkenchery panchayath (16 nos.); Pananchery panchayath recorded the lowest value where only two nut samples yielded *Hirsutella* spp. While considering the locations separately, Kanimangalam of Koorkenchery panchayath possessed highest isolation percentage (8.14%) where *Hirsutella* spp. was isolated from seven nut samples. The isolation of *Hirsutella* in pure form from the field collected infested mites is a challenge.

In addition to the commonly occurring acaropathogen, other fungi coming under the genus *Fusarium* spp. (98 nut samples), *Acremonium* spp. (148 nos.), *Paecilomyces* spp. (15 nos.) and Actinomycetes (95 nos.) were frequently isolated during the survey. Among the four panchayaths, Madakkathara had maximum number of nut samples containing *Fusarium* spp. (31 nos.), *Paecilomyces* spp. (10 nos.) and Actinomycetes (29 nos.) whereas Koorkenchery yielded maximum number of *Acremonium* spp. (45 nos.)

Studies on seasonal variation revealed that maximum number of *Hirsutella* isolates were obtained during the winter season (16 nos.) followed by monsoon season (14 nos.). Isolates belonging to the genus *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. were mostly isolated during the monsoon season which accounted to about 37, 61 and 6 nos., while the isolates of actinomycetes (35 nos.) during summer period over the entire four panchayaths.

Among the various fungal microorganism isolated in the present study, pathogenicity to CEM was proved for three 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.*

Based on the presence or absence of synnemata, two different varieties were found to occur, namely, *H. thompsonii* var. *thompsonii* and *H. thompsonii* var. *synnematosa.* Among the different isolates, the latter variety was more frequently isolated during the study. Based on the colour and growth pattern variations, the *H. thompsonii* were classified into eight isolates, two isolates Marakkal-I and Chirakkekodu-I coming under *H. t.* var. *thompsonii* and six isolates, Marakkal-II, Madakkathara-I and II, Vellanikkara-I, Kanimangalam-I and Konnakuzhy-I under *H.t.* var. *synnematosa.* One more species could be isolated in the present study and it was tentatively identified as *H. kirchnerii.*

Observations on the colony diameter of *Hirsutella* isolates in SMA+Y for ten consecutive days after the initiation of growth recorded maximal fungal growth in *H. thompsonii* var. *synnematosa* isolate, Madakkathara-I (4.167 cm) and Madakkathara-II (4.077 cm) which were on par with Vellanikkara-I (3.927 cm) on the twelfth day. Thus it was revealed that the growth rate was faster in Madakkathara-I isolate, while *H. thompsonii* var. *thompsonii* isolate, Marakkal-I recorded the lowest growth rate (1.570 cm).

The *H. thompsonii* var. *thompsonii* isolate, Chirakkekodu-I recorded an average spore count of 3.22×10^6 spores ml^{-1} with the maximum sporulation at half the radial distance (5.92×10^6 spores ml^{-1}) followed by the centre (2.25×10^6 spores ml^{-1}) and periphery (1.50×10^6 spores ml^{-1}). Thus the isolate, Chirakkekodu-I was the best one in terms of sporulation. *H. kirchnerii* recorded the lowest spore count of 0.17×10^6 spores ml^{-1} in all the three portions.

H. kirchnerii and *H. thompsonii* var. *synnematosa* isolate, Madakkathara-I, varied morphologically from the other *Hirsutella* isolates, where it produced off white coloured mycelia throughout the growth. All the other *Hirsutella* isolates produced a grey colcured initial growth which gradually

changed to greyish white at the periphery of the colony. Different types of growth pattern - uniform raised with foldings, fluffy/cloudy with striations/small foldings. With regard to the reaction in media, *H. kirchnerii* has got a brown line in the media. Media breakage was observed in *H. kirchnerii* and also in the *H. thompsonii* vaf. *thompsonii* (Marakkal-I). Honey dew was observed in both the varieties of *H. thompsonii,* but was absent in *H. kirchnerii*. Synnemata produced by *H. thompsonii* var. *synnematosa* were cream coloured which varied from a few to 96 numbers.

Micrometry studies revealed that the *Hirsutella* isolates produced hyaline, septate, smooth and branched hyphae. They recorded a hyphal width, phialide width and spore diameter of 3.44 µm. Phialides varied with the isolates most of them had broad base and narrow neck bearing single spore. Conidia spherical, verrucose and hyaline while that of *H. kirchnerii* the conidia resembled to the segments of lemon.

Madakkathara-I, *H. thompsonii* var. *synnematosa* possessed the maximum biomass of 2.660 g which was on par with *H. kirchnerii,* Kanjirampally-I (2.140 g).

Among the other fungal pathogens, maximum growth was observed on *Acremonium* spp., *A. strictum* (7.99 cm) which was closely followed by *Fusarium* spp., *F. verticillioides* (7.77 cm). Minimal growth was recorded on *Paecilomyces* spp., *P. fumosoroseus* (4.30 cm) which was significantly different from all other fungal pathogens. The rate of growth was faster for the fungal pathogen, *F. verticillioides*.

Acremonium incoloratum recorded maximum sporulation at the three radial distance with a mean value of 43.11 x 10^6 spores ml^{-1}. Maximum spore load was obtained from half the radial distance (56.33 x 10^6 spores per ml) followed by periphery (38 x 10^6 spores ml^{-1}) and central portion (35 x 10^6 spores ml^{-1}). Lowest sporulation was obtained from the fungal pathogen *Paecilomyces* spp., *P. lilacinus* (4.78 x 10^6 spores ml^{-1}).

All the fungal pathogens were of pure white or off white coloured during the initial growth which later changed to pink or lilac or violet or remains the same. Growth pattern was uniform and fast growing or fluffy raised or dome

shaped ones. The fungal pathogen, *P. lilacinus* only possessed reaction with the growth medium. The fungi under all the genera possessed hyaline and septate hyphae; conidiophores-aggregated, simple or branched with phialides branched or unbranched swollen at the base and taper towards apices, grouped in pairs or clusters. Conidia were hyaline to darkly coloured, unicellular smooth or rough, oval to fusoid, sometimes bound by gelatinous material.

The *Hirsutella* isolates which differed in the cultural and morphological characteristics and the other fungal pathogens which proved the Koch's postulates along with Wettable sulphur and Neemazal were subjected to mortality studies. Results revealed that the fungal pathogen, *P. fumosoroseus* recorded the maximum mortality of 83.65 per cent which was closely followed by *H. thompsonii* var. *thompsonii* (Chirakkekodu-I) with a mortality of 80.63 per cent. All the other treatments were on par which ranged from 26.34 to 79.40 per cent with the lowest value for the untreated control.

Five isolates of *H. thompsonii* var. *synnematosa* which differed in the cultural, morphological and bioefficacy studies, one isolate of *H. thompsonii* var. *thompsonii* and *H. kirchnerii* were subjected to characterization studies. Of the 25 decamer primers of OPE and OPAH series, screened in RAPD analysis, amplification bands was observed for 17 primers. The number of amplification products per primer ranged from nil to nine (OPAH-13). Five primers with good amplification, OPE-12, OPAH-5, OPAH-9, OPAH-13 and OPAH-15 were used for the RAPD-PCR of the seven isolates of *Hirsutella* spp. Analysis of the RAPD data using NTSYS programme exhibited a maximum genetic similarity of 74 per cent between the *Hirsutella* isolates, Madakkathara-I and Konnakuzhy-I. Fifty per cent genetic similarity was obtained between two clusters with the *Hirsutella* isolate, Marakkal-I in one cluster and all the other *Hirsutella* isolates in the other cluster. The first cluster has got three subclusters with Chirakkekodu-I, Madakkathara-I and Konnakuzhy-I under subsluster 1A, while Vellanikkara-I and *H. kirchnerii* (Kanjirampally-I) in the subcluster 1B. Third subcluster (1C) has got only one isolate, Kanimangalam-I. Subclusters 1A and 1B showed 56 per cent similarly with subcluster 1C.

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APPENDIX

Appendix 1. Weather data from Pananchery - CPCRI, Kannara

Period 2003-04	Rainfall (mm)	Rainy days	Max. Temp. (°C)	Min. Temp. (°C)	Humidity (%)
Jul - 2003	493.8	27	31.9	24.0	84
Aug - ,,	384.4	19	32.0	25.0	84
Sep. - ,,	115.0	12	33.3	24.5	82
Oct. - ,,	235.4	14	31.1	22.9	88
Nov.- ,,	19.0	3	31.5	23.6	68
Dec.- ,,	0	0	33.0	20.6	60
Jan - 2004	0	0	35.0	21.0	57
Feb.- ,,	0	0	35.0	21.0	57
Mar.- ,,	41.6	4	36.6	21.15	62
Apr. - ,,	37.6	2	34.9	23.7	72
May - ,,	507.1	23	31.6	23.6	84
Jun. - ,,	788.8	26	23.2	29.9	84
Jul. - ,,	788.8	26	23.2	29.9	84

Appendix 2. Weather data from Madakkathara - College of Horticulture, Vellanikkara

Period 2003-04	Rainfall (mm)	Rainy days	Max. Temp. (°C)	Min. Temp. (°C)	Humidity (%)
Jul - 2003	492.6	22	30.0	22.2	84
Aug - ,,	490.1	19	31.0	23.4	83
Sep. - ,,	53.7	7	30.8	22.7	79
Oct. - ,,	276.6	14	31.5	23.1	81
Nov.- ,,	18.2	1	32.2	23.9	66
Dec.- ,,	0	0	33.4	21.9	61
Jan - 2004	0	0	35.2	22.3	58
Feb.- ,,	0	0	36.5	22.5	50
Mar.- ,,	8.6	1	34.8	24.2	61
Apr. - ,,	60.2	6	30.4	25.2	69
May - ,,	678.3	21	29.6	23.6	84
Jun. - ,,	578.3	21	29.6	23.6	84
Jul. - ,,	786.0	24.	29.3	22.3	85

Appendix 3. Weather data from Pariyaram - Agronomic Research Station, Chalakudy

Period 2003-04	Rainfall (mm)	Rainy days	Max. Temp. (°C)	Min. Temp. (°C)	Humidity (%)
Jul - 2003	562.20	28	29.12	22.85	86.99
Aug - ,,	360.70	25	30.18	22.98	83.81
Sep. - ,,	121.30	12	30.85	23.72	82.24
Oct. - ,,	439.60	15	28.22	24.00	80.20
Nov.- ,,	55.30	7	32.80	21.04	78.55
Dec.- ,,	3.20	1	32.70	18.75	75.43
Jan - 2004	0	0	34.80	19.20	75.26
Feb.- ,,	0	0	35.20	20.22	70.42
Mar.- ,,	12.00	2	36.15	23.22	70.54
Apr. - ,,	61.00	3	35.02	23.67	70.75
May - ,,	657.10	29	31.48	22.10	53.33
Jun. - ,,	711.30	24	29.27	21.20	84.80

EVALUATION AND CHARACTERISATION OF EFFECTIVE FUNGAL PATHOGENS ASSOCIATED WITH THE COCONUT ERIOPHYID MITE (*Aceria guerreronis* Keifer)

By

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ABSTRACT OF THE THESIS

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ABSTRACT

Outbreak of coconut eriophyid mite has become a serious menace by inflicting heavy damage to coconut plantation both in terms of yield reduction and economic return. The increased awareness of the general public about the repercussions of extensive use of chemical pesticides and also because of the apprehension being expressed by scientists and policy makers on various side effects of chemicals, biocontrol is getting more attention in the management of mite.

The present study on "Evaluation and characterisation of effective fungal pathogens associated with coconut eriophyid mite (*Aceria guerreronis* Keifer)" was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara. The main objectives of the study were to isolate, identify and confirm the pathogenicity of fungi associated with the eriophyid mite, identification of the potential acarifungal pathogens and molecular characterisation of the selected isolates.

Survey conducted at three different locations each from four panchayaths of Thrissur district for one year revealed that there was no significant difference in mite population (live mite, dead mite and egg) among the four panchayaths, but it varied significantly within the three locations of the four panchayaths. A uniform distribution of mite population was followed in the outer perianth, inner perianth and nut surface with maximum live mites and egg count on the nut surface and dead mites on the inner perianth. Live mite and egg count contributed to about 36 per cent while the dead mite formed 28 per cent of the total mite population. Predatory mite population varied significantly both among the four panchayaths as well as among the locations within the four panchayaths. Two types of predatory mites, *Amblyseius* spp. and *Bdella* spp. were mainly observed during the study with an average population range from 4.469 to 15.719 per nut.

Seasonal variation showed three peaks of live mite population and egg count during the late monsoon (August), winter (January/February) and summer (April/May) months; where as two peaks during winter (January to February) and

summer (March to April) for the dead mite population. Predatory mite population followed a normal range which increased with increase in mite population to a certain degree within the limited range of predatory mite population.

Correlation analysis showed a positive relationship between population parameters of mite (live mite, dead mite, egg count) and predatory mite population and maximum temperature. A non-uniform correlation was observed between mite population and other weather parameters.

Natural occurrence of the mite specific pathogen, *Hirsutella thompsonii* isolates at monthly intervals for 12 months contributed to 4.90 per cent. Maximum number of *Hirsutella* isolates was obtained during winter season followed by the monsoon season. Isolates belonging to the genus *Fusarium* spp., *Acremonium* spp. and *Paecilomyces* spp. were mostly isolated during the monsoon season while the isolates of actinomycetes during summer period over the entire four panchayaths.

The fungal species which proved pathogenicity to coconut eriophyid mite were two species coming under the genus *Hirsutella, viz., H. thompsonii and H. kirchnerii* and the other fungal species coming under the genus *Acremonium viz., A. strictum* and *A. incoloratum, Fusarium viz., F. lateritium* and *F. verticillioides, Paecilomyces, P. fumosoroseus* and *P. lilacinus.*

Based on the presence or absence of synnemata, two different varieties of *Hirsutella* were found to occur, namely, *H. thompsonii* var. *thompsonii* and *H. thompsonii* var. *synnematosa.* Cultural and morphological observations revealed that among the eight isolates coming under the two varieties *H. thompsonii* var. *synnematosa* isolate, Madakkathara-I recorded maximal fungal growth (4.167 cm) and biomass (2.660 g) while the *H. thompsonii* var. *thompsonii* isolate, Chirakkekodu-I possessed maximum mean sporulation (3.33×10^6 spores ml^{-1}). Among the other fungal pathogens, maximum growth and sporulation was observed on *Acremonium* spp., *A. strictum* (7.99 cm) and *A. incoloratum* with a mean value of 43.11×10^6 spores ml^{-1}.

Mortality assessment of the *Hirsutella* isolates along with other fungi at their respective doses of sporulation revealed that the fungal pathogen, *P.*

fumosoroseus recorded the maximum mortality of 83.65 per cent which was closely followed by *H. thompsonii* var. *thompsonii* (Chirakkekodu-I) with a mortality of 80.63 per cent.

Molecular characterisation of the seven isolates of *Hirsutella* using the five primers comprising of the OPE and OPAH series exhibited a maximum genetic similarity of 74 per cent between the *Hirsutella* isolates, Madakkathara-I and Konnakuzhy-I. Fifty per cent genetic similarity was obtained between two clusters with the *Hirsutella* isolate, Marakkal-I in one cluster and all the other *Hirsutella* isolates and *H. kirchnerii* in the other cluster. The isolates coming under two varieties of *H. thompsonii*, *H. t.* var. *thompsonii*, Chirakkekodu–I with maximum sporulation and *H. t.* var. *synnematosa* Madakkathara-I with maximum growth rate and biomass came under the same subcluster.