PARASITISM OF *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnell ON COCONUT ERIOPHYID MITE *Aceria guerreronis* (Keifer)

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

DEPARTMENT OF PLANT PATHOLOGY

COLLEGE OF HORTICULTURE

VELLANIKKARA, THRISSUR-680 656

KERALA, INDIA

2002

DECLARATION

I hereby declare that this thesis entitled **"Parasitism of *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnellon coconut eriophyid mite *Aceria guerreronis* (Keifer)"** is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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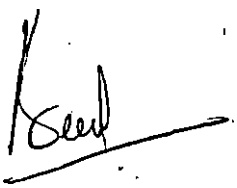
Certified that this thesis, entitled **"Parasitism of *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnellon coconut eriophyid mite *Aceria guerreronis* (Keifer)"** is a record of research work done independently by **Smt.Shabnaz Padiyath** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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ACKNOWLEDGEMENT

I express my sincere gratitude and indebtedness to ***Dr.S.Beena,*** *Assistant Professor (Sln. Grade), Department of Plant Pathology, College of Horticulture and Chairperson of the Advisory Committee and moreover my favourite teacher. She deserves a lot more of gratitude than words can express for her unfailing help, sustained interest, keenness for details and a loving and sincere attitude which make me feel lucky to be under her guidance.*

I extent my great sense of gratitude to ***Dr.Koshy Abraham,*** *Associate Professor and Head, Department of Plant Pathology, College of Horticulture, Vellanikkara and member of the Advisory Committee for his valuable suggestions and help during the preparation of the thesis.*

I am very much obliged to ***Dr.K.Anita Cherian,*** *Assistant Professor, Banana Research Station, Kannara and member of the Advisory Committee for her guidance, timely help, critical suggestions and for the valuable corrections suggested in the thesis preparation.*

My sincere gratitude is also due to ***Dr.S.Pathummal Beevi,*** *Associate Professor, BCCP, Department of Entomology, College of Horticulture, Vellanikkara and member of the Advisory Committee for the encouragement, guidance and suggestions in the conduct of the investigation and preparation of the thesis.*

I respectfully acknowledge Dr.A.Sukumara Varma, Associate Dean, College of Horticulture, Vellanikkara for the guidance, encouragement and help as the member of my Advisory Committee in the early stages of the investigation.

I am extremely grateful to Sri.S.Krishnan, Assistant Professor, Department of Agricultural Statistics, College of Horticulture, Vellanikkara for the valuable guidance and help rendered during the statistical analysis of the data and preparation of the thesis.

My sincere thanks are due to Dr.M.V.Rajendran Pillai, Associate Professor, Department of Plant Pathology, College of Horticulture, Vellanikkara for taking the photographs.

I also utilize this opportunity to thank each and every members of Department of Plant Pathology, especially Dr.Sally K.Mathew, Dr.T.Sheela Paul, Dr.Rehumath Niza T.J. and Dr.K. Surendra Gopal for extending all possible help in proper conduct of the programme. The assistance and co-operation rendered by Sri.Shaju, Sri.Varghese, Sri.Biju, Sri.Nandakumar, Smt.Nabeesa and Smt.Santha is also gratefully acknowledged.

I am thankful to the members of BCCP, Department of Entomology, College of Horticulture, Vellanikkara, who helped me in the collection of mite infested nuts.

I also thank Center for Plant biotechnology and Molecular Biology Lab, College of Horticulture, Vellanikkara for providing necessary lab facilities.

Thanks are due to Sri.K.A.Joy and his family, JMJ Computer Center, Thottappady for the prompt and neat typing of the manuscript, photo scanning and colour printout. I also express my thanks to Smt.Joyce for rendering help in statistical analysis.

Let me place on my record my sincere thanks to my friend, Ms.Deepa James for her sincere help during the preparation of the thesis. I also thank my friends, Saifunneesa, Vidya, Shylaja, Anila, R., Sheron Fernandez, Zahida and Saira Banu for their help and co-operation.

I am deeply indebted to my husband who was always with me in my efforts for completing this endeavor. I remember with gratitude the love and affection of my parents and in laws who supported me at all the stages.

The grand of KAU Research Fellowship is gratefully acknowledged.

*Above all, I bow my head before God, the **ALMIGHTY** whose blessings were always with me for the success of this venture.*

Shabnaz Padiyath

Dedicated

to my Husband

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Introduction

INTRODUCTION

The coconut tree, reverently called 'Kalpa Vriksha' is an important plantation crop of South India. All parts of the tree are useful and yield more products of use to mankind than any other tree.

Coconut is currently facing a serious threat from a newly reported mite pest, *Aceria guerreronis* Keifer, which was so far not known to exist in India. The agent causing the infestation was described as early as in 1965 from the Guerrero state, Mexico by Ortega *et al.* there by conferring the species name as *guerreronis.*

The colonies of the coconut mites live inside the white tender portion of nut covered by inner bracts of the perianth and suck sap from the tender coconut tissues. As the mites feed by injuring the tender portions the damage initially appears as a triangular yellowish patch at the level of the perianth. As the nuts grow, a number of yellowish brown triangular patches appear on the nut which ultimately leads to warting and longitudinal fissures on the nut.

The out break of coconut mite has threatened the very survival of the copra industry. Mite infestation in African and American coconut growing areas caused an increased nut fall resulting in the reduction of copra production upto 25 per cent (Mariau, 1977). Upto 31.5 per cent loss of potential yield was reported from St. Lucia (Moore *et al.*, 1989). Recently the coconut cultivators in many locations especially in Ernakulam district of Kerala experienced the production of extremely small sized coconuts due to the mite attack. The cultivators faced severe loss as these small nuts were discarded by the buyers in copra industry and almost 50 per cent of their produce failed to fetch the actual market price. Severe immature nut fall was also reported from these areas (Sathiamma *et al.*, 1998). Later it was reported from many parts of Kerala.

Various control measures mostly involving the aerial application and root feeding of chemical pesticides are being recommended at present. Most of these pesticides are also in the process of being banned owing to their extreme poisonous nature. Adoption of these modern agricultural practices which include usage of chemical pesticides to large extent have only resulted in the elimination of natural parasites and predators rather than controlling the eriophyid mite itself. The occurrence of the mites within the tightly pressed perianth affords protection from direct contact with pesticides that are applied on bunches which limits the scope of chemical control programmes. Furthermore for effective control by chemicals, pesticides should be applied six times in an year, which is uneconomic.

Hence, a need has arisen to look for alternative control measures which are ecofriendly and easy to adopt. A 'green' alternative to this in the form of biological control using an entomopathogenic fungus *Hirsutella thompsonii* var. *synnematosa* promises to be the answer for sustainable management of this minute pest.

Hall *et al.* (1980) were the first to study the natural mortality factors of coconut eriophyid mite and established the fungus *H. thompsonii* to be a naturally occurring control agent of *A. guerreronis*. From Kerala, Beevi *et al.* (1999) reported the isolation of a fungus from dead mites and was identified as *H. thompsonii* Fischer var. *synnematosa*, Samson, McCoy and O'Donnell (IMI No.382199).

The present study conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara was aimed at identifying the cultural, physiological, toxicological and compatibility characters of the fungus and also developing a method of mass multiplication of the fungus. The programme of research envisaged the following.

1. Isolation of the fungus *H. thompsonii* var. *synnematosa* from dead coconut mites.

2. Cultural and morphological studies of *H. thompsonii* var. *synnematosa.*
3. Physiological studies of the fungus.
4. Isolation of toxic metabolites from broth culture of the fungus and its efficiency on coconut mite.
5. *In vitro* studies on compatibility of the fungus with selected pesticides and botanicals commonly used in coconut.
6. Mass multiplication of the fungus using raw and powdered form of locally available substrates.

Review of Literature

2. REVIEW OF LITERATURE

The coconut eriophyid mite was first reported in 1960 from the state of Guerrero in Mexico and was named in Latin as *Aceria guerreronis* (K.) (Ortega *et al.*, 1965). Later eriophyid mite infestation on coconut was reported from Puerto Rico (Medina *et al.*, 1986). It was reported that the eriophyid mite attacked the nuts of different developmental stages and caused damage by producing necrotic lesions on the nuts. Heavily attacked green nuts dropped off and older nuts failed to develop fully. The weight and the quantity of pulp were reduced in the infested nuts.

2.1 Infestation of eriophyid mite on coconut in India

A severe out break of eriophyid mites in coconut was reported from Ernakulam district of Kerala and caused a heavy yield reduction (Sathiamma *et al.*, 1998). Haq (1999a) reported mite attack from the coconut plantations of Kerala, Tamil Nadu and Karnataka. He also reported an annual loss of 2000-2500 million rupees due to mite infestation in Kerala (Haq 1999b). Muthiah and Bhaskaran (2000) reported that within a period of two years the damage had spread to almost all the major coconut growing districts in Tamil Nadu and up to 70 per cent damage was in coconut palms of 15-25 years of age.

2.2 *Hirsutella thompsonii* as a biocontrol agent against mites

The fungus *Hirsutella thompsonii* was found parasitising citrus rust mite, *Phyllocoptruta oleivora* (Ashm.) from Rio Grande Valley, Texas. Pathogenicity was demonstrated and it was found to be promising for control of *P. oleivora* (Villalon and Dean, 1974). Gerson *et al.* (1979) reported that *H. thompsonii* was highly pathogenic to *Tetranychus cinnabarinus* (Bolsd.) and *Eutetranychus orientalis* (Klein) and the fungus could be used for mite control in the field, particularly in tropical and humid subtropical areas.

In the Ivory coast and New Guinea, *H. thompsonii* was considered to be the most promising biocontrol agent of coconut mite (Hall *et al.*, 1980). Samson *et al.* (1980) also reported *H. thompsonii* as an important pathogen of various eriophyid mites. Three morphologically distinguished groups and three varieties of *Hirsutella sp.* were proposed viz., *H. thompsonii* var. *thompsonii, H. thompsonii* var. *vinacea* and *H. thomposnii* var. *synnematosa.* McCoy (1981) also reported *H. thompsonii* as a biocontrol agent against citrus rust mite, *P. oleivora* and had been used commercially with some success.

The control of *Eriophyes guerreronis* through the applications of *H. thompsonii* was studied in coconut plantations in Guerrero, Mexico where a mortality of 25 to 75 per cent was reported (Espinosa and Carrillo, 1986). Cabreba and Dominguez (1987) reported *H. nodulosa* as a biocontrol agent of *E. guerreronis* on coconut in Cuba under natural conditions. The pathogenicity on *E. guerreronis* was done with seven strains of *H. thompsonii* (Lampedro and Rosas 1989). All the strains were pathogenic, but the maximum mortality of 88.36 per cent was recorded by the strain Ht MOR. Natural and artificial biological control of citrus mites, *Panonychus citri* and *P. oleivora* using pathogens were reported by Rath (1991). A non-occluded virus and *H. thompsonii* (as Mycar) were used for the control of these mites.

2.3 Isolation of the fungus

McCoy *et al.* (1978) isolated the fungal pathogen *H. thompsonii* from citrus rust mite, *P. oleivora* (Ashm). Minter *et al.* (1983) reported that the fungus *H. kirchneri* was initially isolated from the mite *Stenotarsonemus sp.* found on cultivated oats. The fungus was subsequently recollected from the cereal rust mite *Abacarus hystrix* infesting leaves of Lolinum perenne L. in England. McInnis and Jaffee (1989) isolated the nematophagous fungus *H. rhossiliensis* from the infested soils containing the nematode *Criconemella xenoplax* from peach orchards. Brownbridge *et al* (1993) reported the isolation of *Metarhizium anisopliae,*

Beauveria bassiana and *Verticillium lecanii* from major green house pests such as western flower thrips and green peach aphid. They identified these fungi as effective biocontrol agents. Shimazu *et al.* (1996) reported the isolation of the entomogenous fungus, *B. bassiana* from soil samples. Beevi *et al.* (1999) isolated *H. thompsonii* var. *synnematosa* from the mite infested coconuts for the first time in India.

2.4 Cultural and morphological characters of *H. thompsonii* var. *synnematosa*

Considerable variations in the morphological characters of *H. thompsonii* grown on different solid media were reported by Fisher in 1950. Sporulation of *H. thompsonii* var. *synnematosa* in submerged culture was reported by van-Winkelhoff and McCoy in 1984. It produced phialidic like conidiophores and under submerged conditions, the conidia had smooth but rugose conidial walls, whereas aerially formed conidia were distinctly verrucose.

The cultural and morphological characters of the fungus were studied in detail by Beevi *et al.* (1999). They observed mycelial growth on the second day of isolation and attained a colony diameter of 2 to 2.5 cm within 15 days and were grey coloured, raised and produced pale pinkish synnemata. They also took the measurements of the fungal structures and reported that the hyphae were hyaline, septate, wide (1.7 to 2.7 µm), smooth and highly branched and from the vegetative hyphae large number of conical to flask shaped phialides of size 6.5 to 8.7 µm length x 2.3 to 2.8 µm width arose singly at regular intervals. They observed spherical, verrucose and hyaline conidia measuring 2.5 to 3.8 µm in diameter produced at the tip of the phialide.

2.5 Selection of suitable synthetic media for entomopathogens

2.5.1 Solid media

H. thompsonii grown on potato dextrose agar medium was pathogenic to mites, *T. cinnabarinus* (Boisd) and *E. orientalis* (Klein) (Gerson *et al.*, 1979). Latge and Sanglier (1985) reported that the nutritional concentration and quality that favour sporogenesis of the fungi were usually different from those controlling the mycelial growth, the requirements sometimes being highly specific. The effect of nutrients on growth and sporulation of *H. strigosa* and *Hirsutella* sp. was reported by Im *et al.* (1988). It was studied in various solid and liquid media using four carbon and six nitrogen sources. They observed the growth of *H. strigosa* on all media tested and the highest in media containing dextrose. They reported the importance of yeast extract for the mycelial growth of the fungi. Hareendranathan (1989) conducted studies to select a suitable medium for the growth of the fungus *Fusarium pallidoroseum*, an effective biocontrol agent against pea aphid *Aphis craccivora*. He observed maximum growth, sporulation and virulence of the fungus in Sabouraud's medium followed by potato dextrose agar medium and also noticed maximum sporulation in six days old culture and highest virulence was in six days and seven days old cultures. Patel *et al.* (1990) reported that Czapeck's dox medium was the most suitable for growth, sporulation and biomass production of *Metarhizium anisopliae* var. *anisopliae*. A new selective medium was developed for the isolation of the entomogeneous fungus *B. bassiana* by integrating copper oxychloride, low sugar and either crystal violet or brilliant green in agar media (Shimazu *et al* 1996). Culturing of *H. thompsonii* var. *synnematosa* was reported on three different types of media-semisolid, solid and liquid by Maimala *et al.* (1999). Among the solid media tried the maximum number of spores was reported in soyabean agar (9.15×10^7 spores/ml). Malt extract agar produced significantly higher number of colony forming units (CFUs), 4.78×10^9 cfu/ml. Cherian (2000) observed the level of sporulation of *B. bassiana* and *V. lecanii* at different positions of the fungal growth in petridishes. She reported that in both the fungi,

maximum sporulation was recorded at the center of the fungal colony irrespective of the difference in the type of the medium.

2.5.2 Liquid media

van-Winkelhoff and McCoy (1984) reported the sporulation of *H. thompsonii* var. *synnematosa* in submerged culture. Out of 14 monosporal isolates of *H. thompsonii* grown in various liquid media, *H. thompsonii* var. *synnematosa* from Ivory coast was the only pathotype that produced true conidia after three days of incubation. A fermenter method for producing conidiospores of *H. thompsonii* was developed by Latge *et al.* in 1988 by using a strain capable of producing microcycle conidiation in submerged culture. The growth and sporulation pattern of the fungus were examined by them and an average of 2.5 x 10^8 spores/ml was obtained after three days of growth. Moore *et al.* (1989) mass multiplied the seed cultures of the Mexican strain *H. thompsonii* in liquid medium. They got a total dry weight yield of 302 g from 1.5 lit. of the medium containing glucose, yeast extract and peptone .

The liquid fermentation of *M. anisopliae* for dry mycelium production was investigated by Magalhaes *et al. in* 1994. Several culture media based on sucrose, dextrose, yeast water and yeast extract were tested in agitation cultures (150 rpm). The best medium composition contained sucrose (4 per cent) and yeast extract (1 per cent). They also reported that the dry mass varied according to the concentration of conidia. They recorded the highest dry mass at 10^8 conidia/ml per flask. Liu *et al.* (1995) studied the culturing of *H. thompsonii* on potato dextrose yeast broth. The spores of fungus were observed on potato dextrose yeast broth at 25°C with agitation at 250 rpm for three days.

Sabouraud's maltose + yeast extract medium was reported as the best liquid medium for the mycelial weight of *B. bassiana* and Richard's medium for

V. lecanii and the maximum sporulation was recorded in malt extract for *B. bassiana* and in Hawkins and Aasthana 'A' for *V. lecanii* (U.A.S.,1996).

2.6 Physiological characteristics of entomopathogens

2.6.1 Influence of temperature on fungal growth

Host pathogen interactions of *H. thompsonii* on mites were studied by Gerson *et al.* (1979). They reported that the fungus killed most mites and most quickly at 25, 27 and 30°C and least at 13 and 35°C. Sporulation of the fungus on mites was best at 24, 27 and 30°C. They observed maximum sporulation at 27°C.

Chen *et al.* (1981) noticed 93.1 to 98.4 per cent reduction in citrus rust mite population when *H. thompsonii* was sprayed during fine weather with a temperature of more than 25°C. The fermenter method for the production of the fungal culture of *H. thompsonii* was reported by Moore *et al.* (1989). Here the fermenter was maintained at an optimum temperature of 27 ± 0.1°C, corresponding to the optimum temperature required for the growth of the fungus and under that condition the fermentation completed within 72 hours.

Fargues *et al.* (1993) analysed the influence of temperature on the *in vitro* growth of entomopathogens. The effect of temperatures ranging from 8 to 37°C on the *in vitro* growth of 31 isolates of six entomogenous hyphomycetes, viz., *B. bassiana, B. bronguiartii, M. anisopliae, M. flavoviride, Nomuraea rileyi* and *Paecilomyces fumosoroseus* was studied. They observed maximum growth at 25°C for 26 isolates.

Effects of temperature on conidial viability and virulence of *B. bassiana* against chickpea borer, *Helicoverpa armigera* was studied by Sandhu *et al.* in 1993. They noticed the maximum survival of the conidia at lower temperature of 0 to 20°C and the conidia did not survive at higher temperature of 30 to 40°C.

Yaninek *et al.* (1996) reported the seasonal and habitat variability of fungal pathogens, *Neozygites* cf. *floridana* and *H. thompsonii* on cassava mites in West Africa and they concluded that the frequency of sites with infected mites during the wet season was 3.5 times greater than that seen during the dry season.

Influence of temperature on the growth of entomopathogen *V. lecanii* (Beijing strain) was reported by Li *et al.* (1997). They noticed that the optimum temperature for mycelial growth was 23 to 28°C and 23 to 25°C recorded maximum sporulation of 6.32 to 6.94 x 10^9 spores/petridish. Germination of spores was maximum at 15 to 32°C and germination rate within 24 hours was 81.36 to 96.60 per cent.

In a study on the biology of the acaro pathogenic fungus *H. kirchneri*, Sztejnberg *et al.* (1997) reported that the maximum growth on artificial media was at 25°C and conidial germination was high within a wide range of temperatures of 10 to 35°C. They also reported the maximum sporulation of 80 per cent at 25 and 30°C.

2.6.2 Influence of relative humidity on fungal growth

Regarding the humidity suitable for the entomogenous fungus *H. thompsonii*, Gerson *et al.* (1979) reported that the fungus germinated, penetrated the mites and sporulated very poorly below cent per cent relative humidity. When the relative humidity of cent percent was reduced the fungus associated mortality dropped greatly. Thus they concluded that the fungus could be used for mite control in the field particularly in tropical and humid subtropical areas.

Maximum reduction in citrus rust mite population was resulted at a relative humidity of more than 80 per cent due to spraying of *H. thompsonii* (Chen *et al.*, 1981). Sandhu *et al.* (1993) reported that the survival of conidia of *B. bassiana* which is effective against *H. armigera* was maximum at lower relative

humidity level of 0 to 53 per cent. A study regarding the effect of humidity on the acaropathogenic fungus *H. kirchneri* was reported by Sztejnberg *et al.* (1997). They observed rapid mortality of mites when they were held under saturated conditions.

2.6.3 Influence of pH on fungal growth

Im *et al.* (1988) reported the effect of pH on growth and sporulation of four important entomogeneous fungi namely *H. strigosa, Hirsutella sp., M. flavoviridae* var. *minus* and *N. rileyi*. They observed maximum growth in media with pH 5 to 8. The highest dry biomass yield of the fungi *V. lecanii, P. farinosus, B. bassiana* and *Aegertia webberi* were obtained when they were cultured in liquid media with pH ranging between 5 and 8.5 (Galani, 1988). A study was conducted on the growth of *B. bassiana* at various pHs at 25°C (Shimazu *et al.*, 1996). The fungus was found to be able to grow well at high pHs of more than 10.

2.7 Extraction of toxins from Entomopathogens

Vey *et al.* (1993) reported the toxicity effect of secretions of *H. thompsonii* subsp. *thompsonii* in broth culture on the larvae of *Galleria mellonella*, adults of *Drosophila melanogaster* and a cell line of *Bombyx mori*. They observed a positive relationship between mycelium production and toxin production. Cytotoxic effect was detected in cells of all the three insects.

Gindin *et al.*(1994) purified endotoxic compounds from *V. lecanii* and reported that the toxic products were phospholipids.

The first biologically active compound of low molecular weight was isolated from the genus *Hirsutella* by Krasnoff *et al.* (1994) and was identified as an antibiotic, phomalactone. Dichloromethane extracts of culture broth from three strains of the entomopathogenic fungus *H. thompsonii* var. *synnematosa* were

found toxic to the insect *Rhagoletis pomonella* and inhibitory to conidial germination in *B. bassiana.*

The toxin, hirsutellin-A was purified from the culture filtrates of *H. thompsonii* var. *thompsonii* JAB-OH by Liu *et al.*(1995). They reported that Hirsutellin-A had insecticidal activity against the wax moth (*Galleria mellonella*) and peak production of hirsutellin-A (13-14 µg/ml) occurred during the late exponential phase of growth after 39-45 hours.

Mazet and Vey (1995) reported the purification of toxic protein, hirsutellin A from the mite fungal pathogen, *H. thompsonii.* They found that this toxin was distinct from other known proteins as it was not glycosylated, did not show proteolytic activity, antigenic, thermostable and not inactivated by treatments with proteolytic enzymes. Injection of larvae of *G. mellonella* with hirsutellin A at 1 µg/g body weight caused a high mortality rate.

Mode of action of hirsutellin A on eukaryotic cells was studied by Jiu *et al.* (1996). The toxicity of hirsutellin A (extract of *H. thompsonii*) against sf-9 cells was examined using *in vitro* assay and determined the ability of HtA to inhibit protein synthesis. They observed the cytopathic effects of toxin on sf-9 cells within 2-4 hr and completely inhibited the growth.

Chernin *et al.* (1997) investigated the chitinolytic activity of *H. thompsonii* and *H. necatrix* and observed that the two isolates of *H. thompsonii* (255 and 414) and *H. necatrix* were able to produce and excrete chitinolytic enzymes like chitobiase and endochitinase. They reported that in addition to chitinolytic enzymes, the *H. thompsonii* isolates excreted proteolytic enzymes including elastase, alpha-esterase and alpha-amylase.

2.8 Compatibility of Entomopathogens with pesticides and botanicals

2.8.1 Compatibility with insecticides

Influence of pesticides like chlorphyrifos, malathion, parathion and oil were tested on three isolates of *H. thompsonii* Fischer, in *in vitro* conditions (Gomez *et al.*, 1987). They observed the decreasing order of toxicity of the test compounds to the fungus, and it was malathion, chlorpyrifos, parathion and oil. They also compared the effects of these chemicals on conidia formation and mycelial growth of the fungus.

Aguda *et al.* (1988) investigated the effect of the insecticide carbaryl on growth and germination of three fungi of rice insects, *B. bassiana*, *M. anisopliae* and *H. citriformis*. They reported that all the five concentrations of the insecticide tried inhibited the germination of conidia of all fungi.

In vitro study was conducted to know the effect of different pesticides on the entomogenous fungi, M. *anisopliae, B. bassiana, P. fumosoroseus* and *P. farinosus* by Vanninen and Hokkanen (1988). Among the pesticides tested, the insecticides diazinon, pirimicarb, cypermethrin and oxanyl did not affect the growth or sporulation of any of the fungi.

Laboratory tests were conducted by Duarte *et al.*(1992)to determine the effect of various types of pesticides on the entomopathogenic fungus, *M. anisopliae* strain Niha Bonita. It was observed that the pesticides in the form of a concentrated emulsion and systemic pesticides greatly inhibited the development of the fungus. The contact pesticides had a certain degree of compatibility with the fungus. They noticed a synergistic effect on the fungal growth with insecticides in the form of wettable powder, the best being dipterex 80.

Influence of pesticides on the entomogenous fungus *M. anisopliae* was reported by Moorhouse *et al.* in 1992. The insecticides dichlorvos and Hostathion

(triazophos) at 10 times the recommended rate was found preventing the growth of the fungus.

Li and Holdon (1994) evaluated the effect of commercial formulations of pesticides on the growth of *M. anisopliae* in the laboratory. Among 14 formulations tried, they found that the fungus was compatible only with the insecticides carbofuran, aldicarb and 2,4-D amine.

Wright and Kennedy (1996) reported that *B. bassiana*, a biocontrol agent of white flies, thrips and mites could be tank mixed with most pesticides and fertilizers. It did not show any phytotoxicity effects and adverse effects on beneficial insects and mammals.

2.8.2 Compatibility with fungicides

Tedders (1981) and Machowicz-Stefaniac (1983) conducted *in vitro* studies on compatibility of fungicides with the entomopathogenic fungus *B. bassiana.* They reported that the fungicides maneb, benomyl, methomyl, fentin hydroxide, zineb, mancozeb, dodine, copper oxy chloride, thiram, captafol, triforine, tenarimol, carbendazim and triademetone were exerting either toxic or fungistatic action on the fungus.

Effect of fungicides on growth and germination of endomopathogenic fungi of rice insects like *B. bassiana, M. anisopliae* and *H. citriformis* were analysed in the laboratory by Aguda *et al.* (1988). All the concentrations of fungicides used like benomyl and edifenphos were found inhibitory on the insects. *H. citriformis* was more susceptible to benomyl at concentrations of 10 and 100 ppm.

Duarte and Menendez (1989) reported the compatibility of seven commercial fungicides with entomopathogenic fungus *M. anisopliae* on potato dextrose agar. The systemic fungicides, benomyl, isoprothiolane and kitazine

(iprobenfos) inhibited the growth of the fungus, whereas contact fungicides copper oxychloride, zineb, triazine and tetra methyl thiuram disulfide (thiram) were to some extent compatible with the fungus.

Compatibility of the fungicide carbendazim with the nematophagous fungus *H. rhossiliensis* was tested (Jaffee and McInnis, 1990) and found inhibition of sporulation and infectivity of the fungus due to carbendazim treatments. Pullen *et al.* (1990) tested the influence of seven fungicides commonly used in South Carolina Peach orchards on *H. rhossiliensis*, a fungal parasite of *Criconemella xenoplax*. Among the fungicides tried they found that benomyl at 1, 5 and 10 µg a.i./ml completely inhibited hyphal growth of the fungus whereas chlorothalonil, dichloran, iprodione and triforine significantly suppressed hyphal growth at 20 µg a.i./ml, but captan inhibited the growth only at 40 µg a.i./ml. Sulphur had no effect at the concentrations studied. Moorhouse *et al.* (1992) investigated the influence of fungicides on the entomogenous fungus *M. anisopliae*. They reported that the fungicides chlorothalonin and zineb prevented the germination of conidia of the fungus when incorporated into Sabouraud dextrose agar at the commercial concentration. Two fungicides viz., benomyl and carbendazim totally inhibited the mycelial growth at 0.1 times the recommended rate. Growth was also completely prevented by the fungicides etridiazole, triforine and zineb.

Majchrowicz and Poprawski (1993) in their study found that the dithiocarbamate derivations, zineb + copper oxychloride and mancozeb completely inhibited the germination of *Conidiobolus coronatus, B. bassiana, P. farinosus, M. anisopliae* and *V. lecanii*.

Effect of copper sprays on the population dynamics of the citrus rust mite, *P. oleivora* and its fungal pathogen *H. thompsonii* was studied by McCoy *et al. in* 1995. They reported that the copper sprays applied before, during or after mite populations peaked in summer caused a reduction in both citrus rust mite populations and the incidence of infected mites.

2.8.3 Compatibility with botanicals

Vyas *et al.* (1992) studied the effect of some natural pesticides on entomogenous muscardine fungus. From the lab trials conducted by them, it was observed that *in vitro* application of nicotine sulphate, RD-9 replin (extracts of *Azadirachta indica, Pongamia glabra (Pongamia pinnata)* and *Madhuca indica* (*Madhuca longifolia*) and Indiara (diallyl disulfide and allyl propyl disulphide) inhibited the growth of *B. bassiana* and *M. anisopliae.* But Neemark (azadirachtin) did not inhibit the growth of either fungus.

Devi and Prasad (1996) in their study conducted a compatibility test of seed kernel extracts from *A. indica, Melia azedarach* and *P. pinnata*, whole plant extracts from *Tephrosia purpurea, Parthenium hysterophorus* and *Cleome viscosa* and vegetable oils from sunflower, safflower, groundnut, rape seed, sesame, coconut and cotton seed with the entomogeneous fungus *N. rileyi.* They reported that none of the oils were detrimental to the fungus.

2.9 Mass multiplication of entomopathogens using different substrates

Samsinakova *et al.* (1981) reported a two phase mass production technique for the spores of the entomopathogenic fungi. In this method they produced the fungal inoculum or hyphal bodies in liquid culture in flasks and transferred to solid substrates for the production of conidia.

Hareendranathan (1989) conducted the mass production study of the fungus *F. pallidoroseum,* an effective biocontrol agent against pea aphid. In different substrates tried by him, broken maize grain was appeared to be the most suitable media for the mass production of the fungus.

Mathai *et al.* (1992) used different locally available substrates for mass multiplication of *F. pallidoroseum.* The substrates tried were rice bran, wheat bran,

paddy straw bits, tapioca bits, tapioca stem peelings, vegetable wastes and their different combinations. Of the substrates tried wheat bran and rice bran plus tapioca bits gave the maximum mean spore count of 110 per microscopic field.

Quintela (1994) described a method for production of the entomogenous fungus *M. anisopliae* on coarse grain rice. He concluded that the substitution of whole grains with coarse grain reduced the cost of production by four times and increased the production of conidia by 30 per cent.

A new solid culture substrate based on barley was used for growing entomopathogenic fungi including *Hirsutella* sp. (Bradley *et al.*, 1995). A solid state fermentation culture comprising a fungal inoculum on the new barley culture substrate was also reported. They developed a packaged solid culture substrate comprising barley to grow entomopathogenic fungi.

Mazumder *et al.* (1995) reported the technique for mass production of *B. bassiana* and its potentiality on rice hispa, *Dicladispa armigera*. They found that rice husk supplemented with two per cent dextrose was the most suitable medium for the mass production of the fungus and it yielded 5.80 x 10^7 conidia/ml of water with a potentiality of 91.83 per cent on rice hispa adults when the conidial density was maintained at 10^7 conidia/ml of water.

A large scale method was developed for producing the entomogenous fungi *H. thompsonii* and *H. nodulosa* in two phase culture, liquid and solid (Rosas-Acevedo *et al.*, 1995). They reported that for most strains, the greatest conidiogenesis was obtained on rice, barley and bran and the maximum production of conidia was recorded with Ht M2, Ht M4481 and Ht C59 strains of *H. thompsonii*, being 334.75, 269.68 and 137.12 x 10^7 conidia/g respectively.

Dorta *et al.* (1996) described the growth and sporulation characteristics of *M. anisopliae* in solid substrate fermentation. They used a medium based on a

mixture of rice bran and rice husk for the growth of the fungus and they recorded a spore yield of 2.3 x 10^{10} spores/g of rice bran. This was as high as 80.4 per cent of the maximum theoretical value.

Myeong *et al.* (1996) observed better mycelial growth of *B. bassiana* GY1-17, *B. felina* GHI and *M. anisopliae* CN 14 on rice bran, barley or corn extracts than on SMA+Y medium. Spore production of *M. anisopliae*, CN 14 was better on a rice bran medium than on others. They also found that mass production by automatic processing resulted in the greatest number of spore production by *M. anisopliae* CN 14, 28 days after inoculation. They got better growth of *B. bassiana* GY1-17 and *M. anisopliae* CN 14 in saw dust + rice bran medium than in an organic fertilizer + rice bran medium.

Jenkins *et al* (1998) reported LUBILOSA mass production system for aerial conidia of *M. flavoviride.* The fungal inoculum was produced in liquid culture in flasks kept in shaker. When they transferred it to solid substrates for mass multiplication conidia production was observed in the substrates.

Cherry *et al* (1999) explained a system for the production of aerial conidia of *M. anisopliae* which is efficient for the biological control of locusts and grasshoppers. They used a standard two stage mass production system. The substrate used was rice and the yield of conidia obtained was about 8x10^{8} conidia/g of rice.

Preliminary testing of mass production of *H. thompsonii* var. *synnematosa* was done on various substrates (Maimala *et al.*, 1999). It was cultured on semisolid, solid and liquid media. Among these ground corn (maize) coated with molasses produced 3.07 x 10^{7} spores/ml and 2.64 x 10^{7} cfu/ml and was reported as the best solid medium for mass multiplication of the fungus.

Materials and Methods

3. MATERIALS AND METHODS

The present study on 'Parasitism of *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnell on coconut eriophyid mite *Aceria guerreronis* (Keifer)' was conducted in the Department of Plant Pathology, College of Horticulture, Vellanikkara, Thrissur during the period from 2000 May to 2001 August. The details of the materials used and the techniques adopted for the investigation are described below.

3.1 Isolation of the fungus from coconut mite

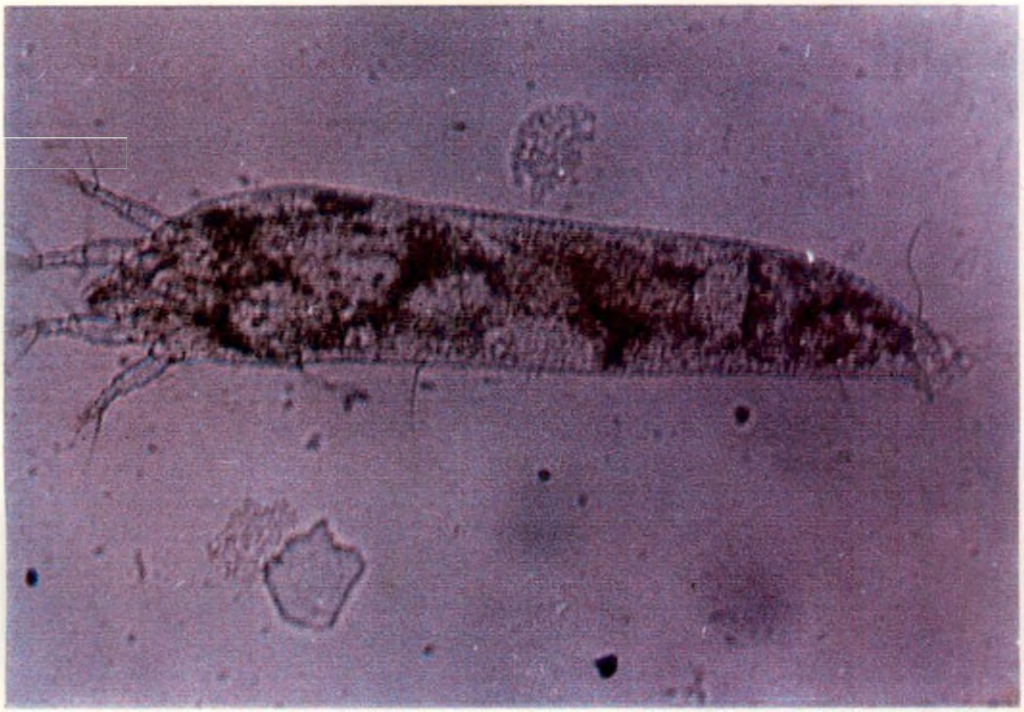
The mite infested nuts of approximately two months of age were collected from 23 year old T x D hybrid palms from the coconut garden of College of Horticulture, Vellanikkara, Thrissur district (Plate 1). The perianth was separated from the nuts and observed both perianth and nuts under stereomicroscope. The colonies of dead mites on the perianth were identified and marked with a pen. The mites along with the perianth were surface sterilized with 0.1 per cent sodium hypochlorite solution for 2-3 minutes and then washed three times with sterile distilled water. After drying, the dead mites were carefully picked up with needle and kept in Potato dextrose agar plates. The petridishes were incubated at room temperature (25±1°C) and examined daily for the growth of the fungus. The pure culture of the fungus was maintained on PDA slants.

3.1.1 Pathogenicity test

For testing the pathogenicity of the fungi isolated on healthy mites, the perianth was separated from two months old nuts and spore solution of the fungus was sprayed using an atomizer on the inner side of the perianth where healthy mites were present. Spore concentration was estimated using a haemocytometer and adjusted to $8x10^5$ colony forming units/ml of the spray fluid. While spraying utmost care was taken to spray uniformly so that equal

Plate 1. Coconut mite and infested nuts

Coconut mite - *Aceria guerreronis* Keifer



Mite infested nuts

quantity of inoculum hits on the mites. High humidity was maintained by covering the perianth with bell jar coated innerside with wet cotton. After five days of incubation dead mites were collected and the fungus was reisolated from the dead mites. The fungus so obtained was compared with the original culture.

3.2 Cultural and morphological characters of *H. thompsonii* var. *synnematosa* on different media

The cultural and morphological characters of the fungus were evaluated on different media. Solid and liquid media of seven types were selected for the study. The composition of the media used is given below and were prepared by the procedure given by Rangaswamy *et al.* (1968), Tuite (1969) and Lomer and Lomer (1995).

1. Sabouraud's maltose agar + yeast (SMA+Y)

Maltose	- 40.00 g
Yeast extract	- 10.00 g
Bactopeptone	- 10.00 g
Agar	- 15.00 g
Distilled water	- 1 l

2. Potato dextrose agar (PDA)

Potato	- 200.00 g
Dextrose	- 20.00 g
Agar	- 20.00 g
Distilled water	- 1 l

3. Potato carrot agar (PCA)

Potato	- 20.00 g
Carrot	- 20.00 g
Agar	- 20.00 g
Distilled Water	- 1 l

4. Richard's medium (RM)

Sucrose	- 50.00 g
Potassium nitrate	- 10.00 g
Potassium dihydrogen phosphate	- 5.00 g
Magnesium sulphate	- 2.50 g
Ferric chloride	- 0.02 g
Agar	- 20.00 g
Distilled water	- 1 l

5. Leonolian medium (LM)

Maltose	- 6.25 g
Malt extract	- 6.25 g
Potassium dihydrogen phosphate	- 1.25 g
Magnesium sulphate	- 0.625 g
Peptone	- 0.625 g
Agar	- 20.00 g
Distilled water	- 1 l

6. Czapeck's medium (CM)

Sucrose	- 30.00 g
Sodium nitrate	- 3.00 g
Ferrous sulphate	- 0.11 g
Dipotassium hydrogen phosphate	- 1.00 g
Magnesium sulphate	- 0.50 g
Potassium chloride	- 0.50 g
Agar	- 20.00 g
Distilled water	- 1 l

7. Hawkins and Aasthana 'A' medium (H&A'A')

Glucose	- 5.00 g
Potassium nitrate	- 3.50 g
Potassium dihydrogen phosphate	- 0.75 g
Agar	- 20.00 g
Distilled Water	- 1 l

3.2.1 Cultural characters of *H. thompsonii* var. *synnematosa*

3.2.1.1 Growth of fungus in different solid media

The different sterile solid media were plated in sterile petridishes under aseptic conditions. Three replications were kept for each medium. Each plate was later inoculated with 1cm diameter disc cut from ten days old fungal culture. The inoculated petridishes were incubated at room temperature. The observations on the diameter of the fungal growth were recorded daily for ten days. Number of synnemata produced was noted after ten days.

3.2.1.2 Sporulation in different solid media

From the ten days old culture of the fungus grown in different solid media, 1 cm disc was taken and transferred to 10 ml of sterilised distilled water in test tube. The solution was shaken thoroughly and calculated the spore count per ml of spore solution 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 totally

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

10 = depth factor

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

D = Dilution factor

The levels of sporulation at different radial distance of the fungal colony were assessed in media which recorded maximum sporulation in the previous experiment. One cm mycelial discs were taken from the centre, half of the radial distance and periphery of the colony of 10 day old culture grown in the selected media and the spore count was estimated using haemocytometer.

3.2.1.3 Effect of solid media on the production of synnemata

Number of synnemata produced in different solid media after 10 days growth of the fungus was counted.

3.2.1.4 Germination percentage of the spores

Germination of spores produced in SMA+Y, which was found to be the best solid medium for the growth of the fungus was observed on 10 days after inoculation (DAI) and calculated the germination percentage of spores. From the spore solution prepared transferred a drop to a sterilised glass slide with micropipette. The slide was observed under microscope and noted the total number of spores in a microscopic field. Marked the microscopic field on the lower side of the slide with a glass marker pen. This slide was kept in a sterilised petriplate with moistened blotting paper on the inner side of the lid to maintain a humid condition in the petriplate. Observation on the number of spores germinated at one hour interval was recorded. The germination percentage of spores was calculated using the formula.

$$\text{Germination percentage} = \frac{\text{Number of spores germinated}}{\text{Total number of spores}} \times 100$$

3.2.2 Growth of *H. thompsonii* var. *synnematosa* in different liquid media

To study the growth of the fungus in liquid media, the broth of different media mentioned in 3.2 was prepared. Using a cork borer 1 cm disc was cut from 10 days old fungal culture and transferred to 100 ml of the liquid media in 250 ml conical flasks under sterilised condition. These flasks were incubated in a shaker adjusted to 250 rpm for 10 days. The growth of fungus after 10 days was filtered through a previously weighed muslin cloth. The filtrate was kept for taking spore count. The fresh weight of the fungal culture was noted. After taking fresh weight

it was kept in drying oven at 60°C for drying. The dry weight was taken on successive days till it became constant.

3.2.2.1 Sporulation of the fungus in different liquid media

Spore count was calculated using haemocytometer directly from the filtrate of the liquid media. Spore count/ml was calculated using the formula mentioned in 3.2.1.2.

3.2.2.2 Germination percentage of spores

In the liquid media the germination percentage was calculated in the medium SMA+Y which recorded the maximum dry weight. The procedure was same as in section 3.2.1.3.

3.2.3 Morphological characters of *H. thompsonii* var. *synnematosa* on solid media

3.2.3.1 Colony character

The colour, shape and reaction of fungal growth on different solid media were studied.

3.2.3.2 Micrometry of fungal structures

Measurements on width of hyphae, width and length of phialide, length of hyphae between phialides, number of phialides in a microscopic field and diameter of spore from each medium after 10 days growth were taken using a calibrated microscope. The microscope was calibrated using ocular and stage micrometer. Formula used was

$$\text{Value of one ocular division} = \frac{\text{Number of stage divisions}}{\text{Number of ocular division}} \times 10\mu$$

3.3 Physiological studies of *H. thompsonii* var. *synnematosa*

In this experiment the effect of different pH, temperature and humidity was evaluated on the growth of the fungus. The study was conducted in three separate experiments on SMA+Y which was selected as the best medium from Experiment No.2.

3.3.1 Effect of pH on the growth of the fungus

The different pH tested for the growth of the fungus were pH 5, 6, 7, 8 and 9. To convert the SMA+Y in to acidic and alkaline pH, glacial acetic acid and ammonia solution were used respectively. pH paper was used for testing the correct pH of the medium. Five replications were kept for each pH. To each medium with different pH in petridishes fungal growth of 1 cm disc was transferred at the centre and incubated the dishes at room temperature. Observations on the growth of fungus were taken by measuring the diameter of growth up to 10 consecutive days, from second day after inoculation.

3.3.2 Effect of temperature on the growth of the fungus

The different temperatures tested to evaluate the growth of the fungus were 10, 15, 20, 25, 30, 35 and 40°C. Using a cork borer 1 cm disc of ten days old fungal growth was cut and inoculated at the centre of the sterilised petridishes plated with SMA+Y and incubated at different temperatures maintained in BOD incubator. Five replications were kept for each treatment. The diameter of fungal growth was taken for ten consecutive days.

3.3.3 Effect of humidity on the growth of the fungus

Growth of the fungus was assessed under humidity conditions of 50, 60, 70, 80 and 90 per cent. The different levels of relative humidity were maintained inside dessicators by keeping various concentrations of sulphuric acid.

Sulphuric acid per cent	Relative humidity per cent
50	50
40	60
30	70
20	80
10	90

One cm disc of the fungal growth was inoculated at the centre of sterilised petridishes plated with SMA+Y medium and incubated at various levels of humidity inside the dessicator. Diameter of the fungal growth was recorded for ten consecutive days.

3.4 Isolation of toxin from broth culture of *H. thompsonii* var. *synnematosa*

The toxic metabolites, both endotoxin and exotoxin were isolated from the broth culture of *H. thompsonii* var. *synnematosa* in SM+Y medium. Hundred ml each of the medium was taken in five conical flasks and sterilised. All the flasks were inoculated with 1 cm disc of fungal growth of ten days old culture and shaken at 250 rpm at room temperature for ten days.

The broth culture was then filtered through a previously weighed Whatman No.1 filter paper. The filtrate and the mycelium obtained were collected separately. The culture filtrate was designated as exotoxin. The mycelium was then homogenised with five volumes of water, centrifuged at 1000 rpm for 15 minutes and the pellet was discarded. The supernatent solution was again centrifuged at 1000rpm for 15 minutes. The supernatent after second centrifugation was taken as endotoxin.

3.4.1 Evaluation of the toxin on coconut mite

Both exotoxin and endotoxin were sprayed separately on mite infested nuts of 2-2½ months age. Clean nuts with opened and unopened perianth were

sprayed with these toxins. In the case of nuts with opened perianth, the perianth was separated carefully from the nuts with a knife. Five replications each for the nuts with opened and unopened perianth were kept for both toxins. Control was maintained by spraying with water. After spraying, the detached perianths were kept as such on the nuts and fixed with cellotape. All the nuts were kept in separate beaker and covered with bell jar with wet cotton to maintain humidity. After five days, the counts of live and dead mites were taken under a stereomicroscope and percentage of mortality was calculated. In the case of nuts with unopened perianth, the toxins were sprayed on the nut surface and the nuts were kept in separate beakers and covered with bell jar. After five days, the perianths were separated carefully from the nuts and observed under the stereomicroscope. The count of live and dead mites were taken and the percentage mortality was calculated using the formula

$$\text{Percentage mortality} = \frac{\text{Number of dead mites}}{\text{Total number of mites}} \times 100$$

3.5 Compatibility of *H. thompsonii* var. *synnematosa* with pesticides and botanicals

In vitro studies were conducted to find out the compatibility of the fungus with selected insecticides, fungicides and botanicals which were commonly used for the pest and disease management in coconut. The medium used for the study was SMA+Y.

3.5.1 Compatibility of the fungus with insecticides/acaricides

Five insecticides were tested against the fungus by following the poison food technique (Falck, 1907). The recommended doses of the following insecticides/acaricides were used.

Sl.No.	Common name/ Scientific name	Trade name	Dose Percent
1	Dicofol	Kelthane 18.5 EC	0.60
2	Carbaryl	Sevin 50 WP	0.20
3	Endosulfan	Thiodan 35 EC	0.05
4	Wettable sulphur	Sulfex 80 WP	0.40
5	Triazophos	Hostathion 40EC	0.05

Hundred ml of the medium SMA+Y was prepared in 250 ml conical flasks and sterilised. The required quantities of the insecticides were added aseptically to 100 ml medium to get the required concentration. After adding the insecticides/acaricides to the medium, mixed them well and transferred to sterilised petridishes. For each insecticide/acaricide three replications were kept. Fungal growth of 1 cm disc taken from 10 days old culture was kept at the centre of the above petridishes using a sterilized needle. The dishes were incubated at room temperature and observed daily for the growth. Control was also maintained without adding the insecticides. The diameter of the fungal growth was taken on 10 consecutive days and the per cent inhibition was evaluated using the formula suggested by Vincent (1927).

$$I = \frac{C-T}{C} \times 100$$

Where

I = Per cent inhibition of growth

C = Diameter of fungal growth in control

T = Diameter of fungal growth in treatment

3.5.2 Compatibility of the fungus with fungicides

The following fungicides were used for the study.

Sl.No.	Common name/ Scientific name	Trade name	Dose Per cent
1	Bordeaux mixture	Bordeaux mixture	1.0
2	Copper oxychloride	Fytolan 50 WP	0.3
3	Mancozeb	Indofil M-45 75 WP	0.3
4	Tridemorph	Calixin 75 EC	0.1
5	Potassium phosphonate	Akomin 40	0.3

Here the procedure used was same as that described in section 3.5.1.

3.5.3 Compatibility of the fungus with botanicals

The botanicals recommended against the mite infestation in coconut were used for this study.

Sl.No.	Botanicals used	Concentration
1	Garlic	20 g/litre
2	Garlic + Neem oil	2%
3	Azadirachtin (Neemazal)	0.03%

Two grams of garlic was weighed after removing the outer scales and surface sterilised with alcohol for one minute followed by washing with three changes of sterile water. Then it was made into a paste by crushing in a sterilised pestle and mortar, and added to 100 ml of sterilised SMA+Y. To prepare the garlic + neem oil mixture, 2 g of surface sterilised garlic and 2 ml of the pure neem oil were taken and added into 100 ml of the sterilised medium to get 2 per cent concentration. After preparing the medium with the required concentration of the botanicals, it was transferred to petridishes. Actively growing ten days old fungal growth of 1 cm diameter disc was transferred aseptically to the centre of the dishes using a sterilised needle. The dishes were incubated at room temperature and observed daily for growth of the fungus. The diameter of fungal growth was taken

on ten consecutive days in five replications kept for each botanical. Control was also maintained without adding the botanicals.

3.6 Mass multiplication of *H. thompsonii* var. *synnematosa*

The growth and sporulation of the fungus were evaluated using raw and powdered forms of the following locally available substrates.

Sl. No.	Substrates used (Raw and powdered forms)
1	Sorghum
2	Rice bran
3	Rice
4	Wheat
5	Barley
6	Maize
7	Tapioca
8	Tea waste

3.6.1 Mass multiplication in the raw form of substrates

The mass production of *H. thompsonii* var. *synnematosa* was done as per the method standardized by Jenkins (1996) with slight modifications.

The mass multiplication was done in two stages. The first phase was the preparation and inoculation of liquid medium and the second stage was the preparation and inoculation of the substrates.

Stage 1: Preparation and inoculation of the liquid broth

The first phase was the preparation of an autoclaved liquid medium of 100 ml of SMA+Y in 250 ml conical flasks. These were inoculated with the ten

days old fungal culture. The inoculated flasks were kept in shaker at 250 rpm for ten days.

Stage 2: Preparation and inoculation of substrates

Hundred grams each of the various substrates except tea waste and rice bran were washed with tap water to remove dirt materials. Then the substrates were half boiled, drained excess water and transferred to 250 ml conical flasks. Hundred grams each of rice bran and tea waste were moistened with water to get 20 percent moisture and then transferred to 250 ml conical flasks. All the substrates were autoclaved at 121^0c, for two hours and after cooling, these were kept outside for one day. Three replications were kept for each substrate.

The substrates were inoculated with the inoculum prepared in stage 1. Fifteen ml of the medium was used for each 100g of the substrate. These were incubated at room temperature for three months to estimate the sporulation and growth of fungus. Observations were taken on time of initiation of fungal growth, 50 per cent and cent per cent growth, sporulation, germination percentage and formation of synnemata.

3.6.1.1 Estimation of spore count of the fungus in different substrates

The spore count of the fungus in all the substrates was taken using haemocytometer after preparing spore solution from each substrate separately. The fungal growth on the substrate was mixed thoroughly with a sterilized glass rod. One gram of the fungal growth with substrate was transferred aseptically from each replication to 50ml conical flasks containing 10 ml of sterilised water. It was shaken well to separate the spores from the mycelium and also to get a uniform distribution of spores in the solution. The number of spores present in the spore solution was estimated directly using haemocytometer. The spore count was

calculated by the formula mentioned in section 3.2.1.2. The spore count was estimated for three months at 15 days interval starting from 30 DAI.

3.6.1.2 Germination percentage of spores from different substrates

Germination of spores in different substrates was recorded and calculated the germination percentage. For this the spore solution used for estimation of spore count was used. The procedure was same as in section 3.2.1.3.

3.6.2 Mass multiplication of the fungus in the powdered form of the substrates

Hundred grams, each of the substrates was weighed, cleaned, washed properly and powdered in a mixer. The powdered substrates were moistened uniformly with water and transferred to 250 ml conical flasks separately. Sterilisation of the substrates and inoculation of the fungus into the substrates were carried out as described in raw form of substrates.

3.6.2.1 Estimation of fungal population in powdered form of substrates

The population of the fungus in various substrates was estimated by serial dilution plate technique. Thirty DAI one gram of substrate with fungal growth on it was transferred to 10 ml of sterilised water taken in a test tube, after proper mixing. Of the dilution prepared, 0.1 ml was pipetted to sterilised petridishes containing solidified SMA+Y. Colonies formed were counted after five days of growth. The fungal population in different substrates was expressed as number of colony forming units per gram of substrate.

3.7 Statistical analysis of the data

Experiments were designed in a completely randomised design and the data was analysed using the analysis of variance technique. The treatments were compared using Duncan's Multiple Range Test (DMRT).

Results

4. RESULTS

In the present investigation on the "Parasitism of *Hirsutella thompsonii* Fischer var. *synnematosa* Samson McCoy & O'Donnellon coconut eriophyid mite *Aceria guerreronis* (Keifer)" detailed studies were conducted on the cultural, morphological and physiological characters of the fungus, compatibility with pesticides and botanicals and mass multiplication of the fungus in different substrates. The results of the study are presented below.

4.1 Isolation of the fungus *H. thompsonii* var. *synnematosa*

The acaropathogenic fungus, *H. thompsonii* var. *synnematosa* was isolated in Potato dextrose agar (PDA) from the mite infested nuts collected from the coconut gardens of College of Horticulture, Vellanikkara. On the third day of isolation grey coloured fungal growth was observed on the medium. The radial growth of the fungus was very slow, but instead it showed a dome shaped growth (Plate 2a). The isolate of the fungus was purified by hyphal tip method and maintained on PDA slants by periodic subculturing.

After seven days of growth, the colour of the mycelium gradually changed from grey to greyish white. Numerous pinkish white coloured fruiting bodies called synnemata were produced from the greyish white fungal growth (Plate 2b). The length and number of synnemata after 10 days of incubation ranged from 4-6 cm and 4-8 respectively.

The fungus was identified as *Hirsutella thompsonii* var. *synnematosa* after comparing its morphological and cultural characters with that of already identified culture of *H. thompsonii* var. *synnematosa* isolated from mite infested coconuts collected from the same location (IMI No.382199) (Plate 3).

Plate 2. Culture of *H. thompsonii* var. *synnematosa*

2a. Dome shaped growth

2b. Synnemata

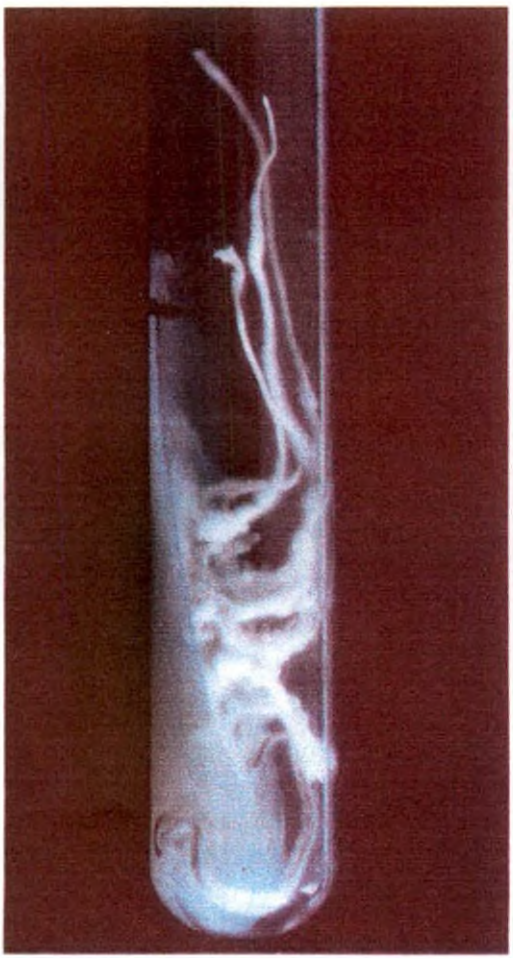
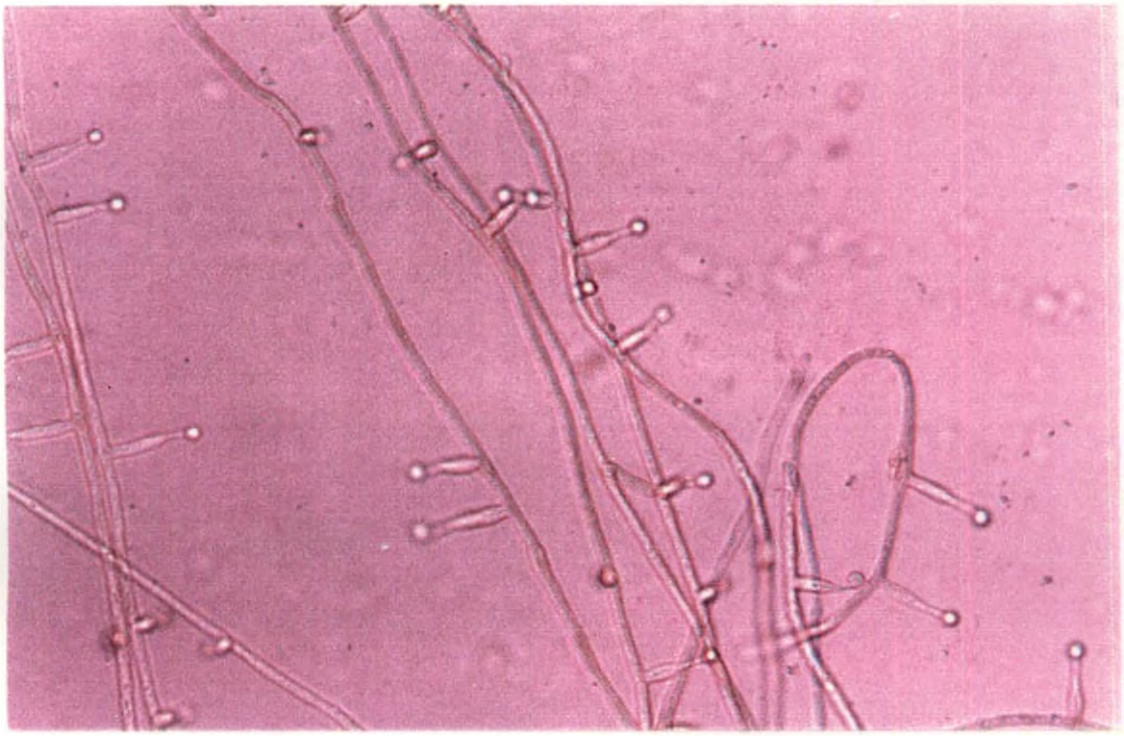
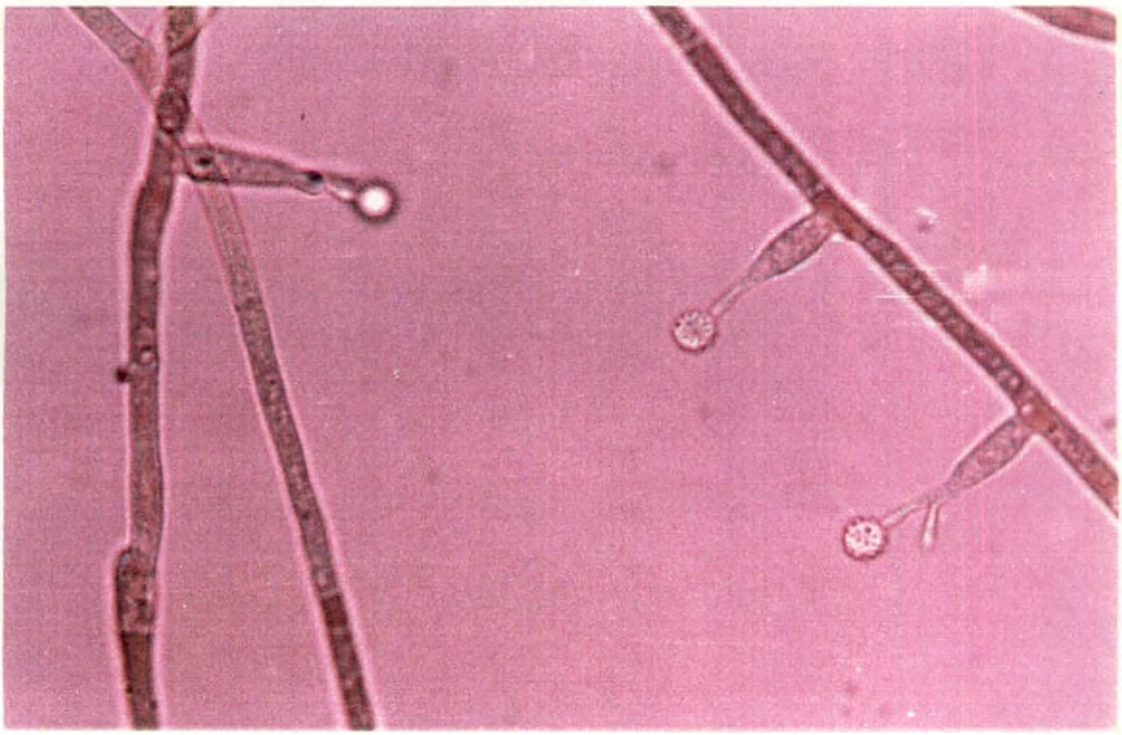


Plate 3. Microscopic view of fungal mycelium of *H. thompsonii* var. *synnematosa*

3a. Hyphae bearing phialides with conidia (x400)



3b. Magnified view of hyphae, phialide, branched neck and verrucose conidia (x1000)



4.1(a) Other microorganisms associated with dead mite

In addition to the acaropathogen, *H. thompsonii* var. *synnematosa,* other fungi were also isolated from dead mites. None of them were pathogenic to the mites. These included *Aspergillus niger.*, *Aspergillus flavus, Penicillium* sp. and *Fusarium* sp. (Table 1).. The pure cultures of these organisms were also maintained on PDA slants.

Table 1. Fungi associated with dead mite

Associated fungi
Aspergillus niger
Aspergillus flavus
Penicillum sp.
Fusarium sp.

4.1.1 Pathogenicity of *H. thompsonii* var. *synnematosa*

The pathogenicity of the fungus was proved in *in vitro*. On the fifth day of inoculation dead mites were observed on the inner side of the perianth. The pathogen was reisolated from these dead mites on PDA medium and compared with the original culture of the fungus and con.firmed its pathogenicity on mite.

4.2 Cultural and morphological characters of *Hirsutella thompsonii* var. *synnematosa*

4.2.1 Cultural characters

4.2.1.1 Effect of different solid media on the growth of the fungus

The colony diameter of the fungus for ten consecutive days after inoculation was recorded and the results are presented in Table 2. After 10 days of inoculation, maximum fungal growth was observed on the medium Sabouraud's maltose agar + yeast (SMA+Y)(4.25 cm). Statistically the growth of fungus on this

Table 2. Effect of different solid media on the growth of *H. thompsonii* var. *synnematosa*

Medium	Days after inoculation [*Mean colony diameter (cm)]									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
SMA+Y	1.20[a]	1.54[a]	1.73[a]	2.07[a]	2.42[a]	2.92[a]	3.22[a]	3.50[a]	4.12[a]	4.25[a]
PDA	1.13[ab]	1.18[bc]	1.27[b]	1.55[b]	1.91[b]	2.23[b]	2.41[b]	2.63[b]	2.79[b]	2.90[b]
PCA	1.00[b]	1.05[cd]	1.12[bc]	1.14[d]	1.15[d]	1.17[d]	1.27[d]	1.30[d]	1.37[d]	1.54[d]
RM	1.19[a]	1.25[b]	1.31[b]	1.43[bc]	1.61[bc]	1.77[c]	1.94[c]	2.08[c]	2.19[c]	2.29[c]
LM	1.00[b]	1.03[d]	1.10[bc]	1.25[cd]	1.36[cd]	1.42[d]	1.56[cd]	1.65[cd]	1.72[cd]	1.77[cd]
CM	1.00[b]	1.03[cd]	1.09[bc]	1.09[d]	1.13[d]	1.13[d]	1.20[d]	1.22[d]	1.32[d]	1.40[d]
H&A'A'	1.01[b]	1.02[d]	1.03[c]	1.16[d]	1.21[d]	1.21[d]	1.38[d]	1.44[d]	1.47[d]	1.65[d]

* Mean of five replications

In each column figures followed by the same letter do not differ significantly according to DMRT.

medium was significantly different from the growth on other media. The mean colony diameter on PDA (2.90 cm) was also significantly different from the growth on other media except SMA+Y. The minimum growth was recorded on CM (1.40 cm) which was on par with PCA (1.54 cm) and H&A'A' media (1.65 cm). The observation on colony diameter taken on consecutive days revealed that the rate of growth was faster in SMA+Y than in all other media. (Plate 4).

4.2.1.2 Effect of different solid media on the sporulation of the fungus

Number of spores produced by the fungus in different solid media were estimated using haemocytometer. From the results presented in Table 3 it was observed that the highest number of spores (7.9 x 10^6/ ml) was produced in SMA+Y. Statistically there was no significant difference in spore count noticed in all other media except SMA+Y.

4.2.1.2(a) Evaluation of sporulation of the fungus at different radial distances

Sporulation at various radial distances was taken in solid media of SMA+Y and PDA. The results are presented in Table 3a. In SMA+Y the maximum sporulation noted was at the centre of the fungal colony (7.9 x 10^6 spores/ml). In PDA also the sporulation at the centre of the colony was the highest ($1.52x10^6$/ml) followed by $1.40x10^6$/ml at half of the radial distance of the colony. In both media lowest sporulation was recorded in the periphery of the colony.

4.2.1.3 Effect of solid media on the production of synnemata

Number of synnemata produced in different solid media after 10 days growth of the fungus was counted. The results on the mean number of synnemata produced showed that the highest number of synnemata was produced in RM (8.6) (Table 3). This was on par with the number of synnemata produced in CM (6.2). But the average number of synnemata produced in CM (6.2), LM (4.8), H&A'A'M

Plate 4. Growth of *H. thompsonii* var. *synnematosa* in different solid media

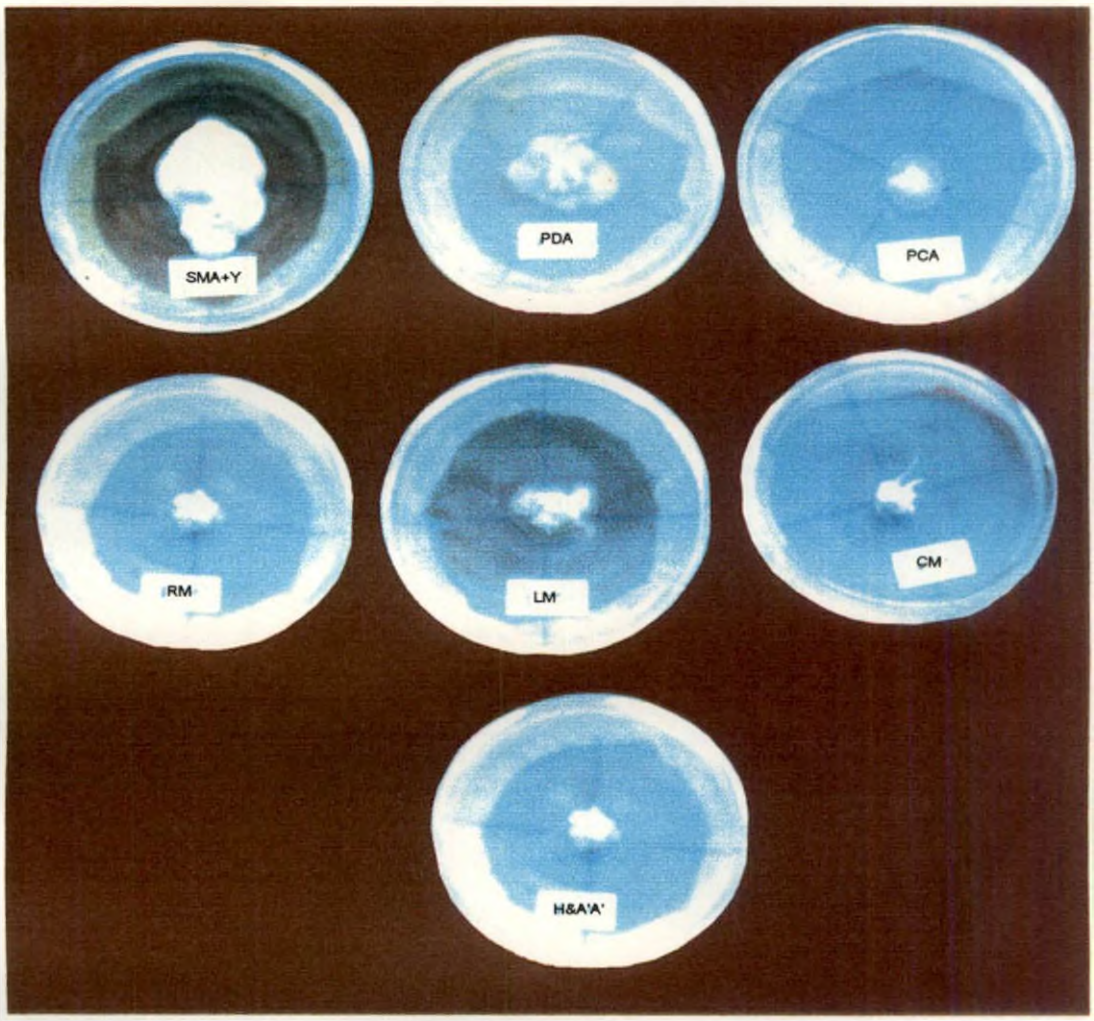


Table 3. Effect of different solid media on the sporulation and synnemata production

Media	* Number of spores (x 10^6/ml)	Number of synnemata
SMA+Y	7.90[a]	4.2 (2.121)[b]
PDA	1.52[b]	4.4 (2.167)[b]
PCA	1.20[b]	1.0 (1.225)[c]
RM	1.50[b]	8.6 (2.978)[a]
LM	1.62[b]	4.8 (2.274)[b]
CM	1.80[b]	6.2 (2.512)[ab]
H&A 'A'	0.50[b]	4.6 (2.246)[b]

* Mean of five replications

Figures in parenthesis are transformed values

In each column figures followed by the same letter do not differ significantly according to DMRT.

Table 3a. Spore count at various radial distances in SMA+Y and PDA

Media	Spore count (x10^6 spores/ml)		
	Centre	Half of the radial distance	Periphery
SMA+Y	7.90	5.70	3.50
PDA	1.52	1.40	1.20

(4.6), PDA (4.4) and SMA+Y (4.2) did not differ significantly. PCA recorded the lowest number of synnemata (1.0).

4.2.1.4 Germination percentage of spores

The percentage germination of spores produced in the solid medium SMA+Y was calculated hourly for eight hours and results are given in Table 4. Twenty five spores were noted in a microscopic field before incubating the spores for germination. After an hour of incubation none of the spores germinated. But later at each hour of interval the germination percentage increased, reaching a maximum of 96 per cent after eight hours of incubation.

4.2.1.5 Correlation between fungal growth, spore count and synnemata production by the fungus

Statistical analysis on the correlation between sporulation and synnemata production was carried out and the results are presented in Table 5. From the table it was observed that the fungal growth had a significant positive correlation (0.800) with the sporulation of the fungus in solid medium. The correlation was non significant between the fungal growth and number of synnemata. Non significant negative correlation (-0.029) was observed between number of synnemata and spore count of the fungus.

4.2.2 Effect of different liquid media on the growth of *H. thompsonii* var. *synnematosa.*

Weights of dry mycelium of the fungus in different liquid media 10 DAI were recorded to select the liquid medium most suitable for the fungal growth. Data in Table 6 indicated that the highest dry weight (490 mg) was in Sabouraud's maltose + yeast extract medium (SM+Y). This was significantly superior to the dry weight of fungal growth in other liquid media. The dry weight of fungal growth recorded on potato dextrose (PD) was 418 mg which was also significantly different from other liquid media except SM+Y. The statistical analysis of the data

Table 4. Germination percentage of spores grown in SMA+Y

Time (HAI)*	Number of spores germinated (out of 25)	Percentage of germination (%)
1	0	0
2	1	4
3	4	16
4	6	24
5	12	48
6	18	72
7	23	92
8	24	96

*- Hours after incubation

Table 5. Correlation between fungal growth, synnemata production and spore count in different solid media (Correlation coefficient)

	Number of synnemata	Spore count	Diameter of fungal growth
Number of synnemata	–	–	–
Spore count	-0.029^{NS}	–	–
Diameter of fungal growth	0.018^{NS}	0.800*	–

NS- Non significant

* - Significant

revealed that the dry weight obtained in broth of LM (154 mg) was on par with that on the broth of H&A 'A' (105 mg) and RM (96 mg). The lowest dry weight recorded was in potato carrot medium (PC) (28 mg) and was on par with that on liquid RM.

4.2.2.1 Effect of different liquid media on the sporulation of the fungus

From the different liquid media tried, the spore count of the fungus was assessed from the culture filtrate obtained after the growth of the fungus. The results are presented in Table 6. The highest spore count (3.5 x 10^5/ml) was recorded in the broth of H&A'A'. Analysis of the data showed that the different media did not differ significantly in the spore count. The lowest spore count (1.9 x 10^5/ml) was in potato carrot medium (PC).

4.2.2.2 Germination percentage of spores grown in liquid broth of SM+Y

Germination percentage was assessed from the spores taken from SM+Y. The results are presented in Table 7. Twelve spores were recorded in a microscopic field before incubating the spores for germination. Germination of spores started only after three hours of incubation with 8.33 per cent germination. After six hours of incubation germination percentage was 25 per cent. Even after eight hours the observation recorded was the same (25 per cent germination).

4.2.2.3 Comparison of sporulation in different solid and liquid media

On comparing the sporulation in different solid and liquid media it was clear that the efficiency of the fungus to produce spores in liquid media was lesser than that in solid media (Table 8). Among the seven solid media tried, SMA+Y recorded the maximum spore count (7.9x10^6/ml) and it was statistically superior to all other media where as in the case of liquid media the maximum spore count was recorded in SM+Y, but statistically there was no significant difference between the sporulation recorded in different liquid media.

Table 6. Effect of different liquid media on dry weight and spore count of the fungus

*Media	**Dry weight (mg)	**Spore count(10^5/ml)
SM+Y	490[a]	2.3 (1.67)[a]
PD	418[b]	2.6 (1.76)[a]
PC	28[e]	1.9 (1.46)[a]
RM	96[de]	2.8 (1.78)[a]
LM	154[d]	2.2 (1.64)[a]
CM	250[c]	3.1 (1.89)[a]
H&A'A'	105[d]	3.5 (1.96)[a]

* SM+Y-Sabouraud's Maltose + Yeast broth
PD -Potato Dextrose broth
PC -Potato carrot broth
RM- Richard's medium broth
LM- Leonolian medium broth
CM-Czapecks medium broth
H&A'A' – Hawkins and Aasthana 'A' Medium broth

** Mean of five replications
Figures in parenthesis are transformed values
In each column figures followed by the same letter do not differ significantly according to DMRT.

Table 7. Germination percentage of spores grown in broth of SM+Y

Time (HAI)*	Number of spores germinated (Out of 12)	Percentage of germination (%)
1	0	0
2	0	0
3	1	8.33
4	1	8.33
5	2	16.67
6	3	25.00
7	3	25.00
8	3	25.00

* - Hours after incubation

Table 8. Comparison of spore count in different solid and liquid media

Media	Solid media (x10^6 spores/ml)	Liquid media (x10^5 spores/ml)
SMA+Y	7.90	2.3
PDA	1.52	2.6
PCA	1.20	1.9
RM	1.50	2.8
LM	1.62	2.2
CM	1.80	3.1
H&A 'A'	0.50	3.5

4.2.3 Morphological characters of *H. thompsonii* var. *synnematosa* in different solid media

4.2.3.1 Colony characters

Observations on the colour and shape of the fungal growth and its reaction in the medium were taken in seven different media tried. There was no variation in the colony characters of the fungal growth in different media. In all the media the fungus produced a grey coloured growth at first and gradually the colour changed to greyish white on the periphery of the fungal colony. The fungus exhibited a characteristic dome shaped growth in all the media. No specific reaction was observed in any of the media tested due to the fungal growth.

4.2.3.2 Micrometry of fungal structures

Microscopic observations were taken on the hyphal width, phialide width, phialide length, spore diameter, distance between the phialides and number of phialides in a microscopic field. The hyphae produced in all the media were hyaline, septate, smooth and highly branched. Large number of conical to flask shaped phialides arose from the vegetative hyphae. These phialides had broad base and a narrow neck bearing single spore. The neck was branched and often branched once. Conidia was spherical, verrucose and hyaline. But the measurements of different fungal structures varied slightly in different media tried. The details of microscopic measurements are given in Table 9.

a) Sabouraud's maltose agar + yeast (SMA+Y)

The fungi in SMA+Y recorded a hyphal width, phialide width and spore diameter of 3.33 µm which was the lowest recorded value of these observations. Phialide length was also the lowest in this medium (7.57 µm). The subsequent phialides were produced at the shortest distance of 25.03 µm. In this medium the maximum number of phialides (24 numbers) in a microscopic field was recorded.

Table 9. Microscopic measurement of fungal structures of *H. thompsonii* var. *synnematosa* from different solid media

Media	Hyphal width (µm)	Phialide width (µm)	Phialide length (µm)	Spore diameter (µm)	Distance between phialides(µm)	Number of phialides in a field
SMA+Y	3.33	3.33	7.57	3.33	35.03	24
PDA	3.47	3.47	10.09	3.47	51.26	4
PCA	3.76	3.76	9.24	3.76	63.42	6
RM	3.62	3.62	9.75	3.62	41.68	7
LM	3.76	3.76	9.62	3.76	77.26	6
CM	3.76	3.76	9.91	3.76	52.35	11
H&A 'A'	3.33	3.33	8.83	3.33	62.61	6

b) Potato dextrose agar medium (PDA)

In this medium the hyphal width, phialide width and spore diameter recorded a medium value of 3.47 µm. The length of the phialide recorded was the highest, 10.09 µm. The number of phialides in a microscopic field was least in this medium (4) which recorded 51.26 µm distance between the phialides.

c) Potato carrot agar medium (PCA)

Observations recorded on hyphal width, phialide width and spore diameter resulted in a maximum value of 3.76 µm. The length of phialide recorded was 9.24 µm. Observations on number of phialides in a microscopic field also showed a lower value of six at an interval of 63.42 µm.

d) Richard's medium (RM)

This medium recorded a hyphal width, phialide width and spore diameter of 3.62 µm. Phialide length of 9.75 µm was observed. A distance of 41.68 µm between the phialides resulted in the production of seven phialides in a microscopic field.

e) Leonolian medium (LM)

In this medium the fungus produced the same hyphal width, phialide width and spore diameter (3.76 µm) as in PCA. A medium phialide length of 9.62 µm was recorded. The distance between two phialides was highest in this medium (77.26 µm) and produced six phialides in a microscopic field.

f) Czapeck's medium (CM)

Observations on hyphal width, phialide width and spore diameter recorded the maximum value of 3.76 µm as in LM and PCA. Phialides having an average length of 9.91 µm were produced. The number of phialides produced in a microscopic field (11) was comparatively higher which resulted in a distance of 52.35 µm between the phialides.

g) Hawkins and Aasthana 'A' medium (H&A'A')

The lowest hyphal width, phialide width and spore diameter of 3.33 µm was recorded in this medium as in the case of SMA+Y. Phialide length recorded was also comparatively lower (8.83 µm). The distance between the phialides was 62.61 µm, which recorded six phialides in a microscopic field similar to that in LM and PCA.

4.3 Physiological studies of *H. thompsonii* var.*synnematosa*

The effect of temperature, pH and relative humidity on the growth of the fungus, *Hirsutella thompsonii* var. *synnematosa* was studied.

4.3.1 Effect of pH on the growth of fungus

The fungal growth was recorded as mean colony diameter in five different pH conditions of 5, 6, 7, 8 and 9 on ten consecutive DAI in SMA+Y medium (Table 10). Maximum growth was observed in pH of 9 (3.08 cm). Statistical analysis of the data revealed that the growth at pH 9 was on par with the growth at pH 6 (2.80 cm) and pH 7 (2.84 cm). Minimum growth was recorded at pH 5 (2.67 cm) which was on par with the growth at pH 6,7 and 8 (plate 5).

4.3.2 Effect of temperature on the growth of the fungus

The mean colony diameter on 10 consecutive days was recorded at temperatures of 10, 15, 20, 25, 30, 35 and 40°C. No growth was observed in all temperatures except 25 and 30°C. It was found that the temperatures 10, 15, 20, 35 and 40°C were not favourable for the growth of *H. thompsonii* var. *synnematosa.* Statistical analysis was conducted for the comparison of the fungal growth at 25 and 30°C (Table 11). After 10 days of growth there was significant difference between the growth measured at 25 and 30°C. Growth at 25°C (2.95 cm) was found to be superior to that at 30°C (2.47cm) (Plate 6).

Table 10. Effect of pH on the growth of *H. thompsonii* var. *synnematosa*

pH	Days after inoculation [*Mean colony diameter (cm)]									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
5	1	1.11[a]	1.21[a]	1.40[a]	1.80[a]	2.06[a]	2.26[a]	2.42[a]	2.53[b]	2.67[b]
6	1	1.01[b]	1.19[a]	1.35[a]	1.85[a]	2.14[a]	2.30[a]	2.51[a]	2.63[ab]	2.80[ab]
7	1	1.09[a]	1.23[a]	1.45[a]	1.96[a]	2.16[a]	2.38[a]	2.50[a]	2.66[ab]	2.84[ab]
8	1	1.09[a]	1.27[a]	1.56[a]	1.95[a]	2.13[a]	2.29[a]	2.50[a]	2.58[ab]	2.72[b]
9	1	1.01[b]	1.19[a]	1.51[a]	2.11[a]	2.34[a]	2.55[a]	2.75[a]	2.91[a]	3.08[a]

* Mean of five replications

In each column figures followed by the same letter do not differ significantly according to DMRT.

Table11. Effect of temperature on the growth of *H. thompsonii* var. *synnematosa*

Temperature	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
25° C	1	1.23	1.40	1.54	1.66	2.08	2.26	2.51	2.76	2.95
30° C	1	1.14	1.26	1.41	1.59	1.73	1.90	2.05	2.24	2.47
t statistic	-	NS	2.86	2.89	NS	5.23	5.62	6.97	5.03	5.76

4.3.3 Effect of humidity on the growth of the fungus

The statistical analysis of the data on the effect of humidity levels of 50, 60, 70, 80 and 90 per cent on the fungal growth revealed that different humidity levels had no significant effect on the growth of fungus (Table 12 and Plate 7). However the maximum growth (2.20 cm) was recorded at 90 per cent humidity and minimum at 50 and 70 per cent humidity levels (2.06 cm).

4.4 Isolation of toxin and evaluation of the toxin on coconut mite

The toxins extracted from *H. thompsonii* var. *synnematosa* was sprayed on nuts with closed and opened perianth and per cent mortality of mites was recorded in both cases. Observations are given in Table 13. In the case of closed perianth, maximum per cent mortality was observed on spraying with the exotoxin (88.03 percent) and it was on par with the per cent mortality recorded on spraying with the endotoxin (83.79 per cent). The per cent mortality of mite due to spraying with both the toxins was found to be superior to that in control (30.99 per cent). When the perianth was opened the endotoxin spray resulted in maximum percentage mortality of 94.81 per cent while spraying with exotoxin and water (control) resulted in per cent mortality of 77.50 and 57.14 per cent respectively. Further there was no significant difference between the per cent mortality of mite due to spraying of exotoxin, endotoxin and water (control).

Table 13. Effect of toxin produced by *H. thompsonii* var. synnematosa on mortality of Eriophyid mite

I. Closed perianth	Percent mortality (%)
Exotoxin	88.03[a]
Endotoxin	83.79[a]
Control	30.99[b]
II Opened perianth	
Exotoxin	77.50[a]
Endotoxin	94.81[a]
Control	57.14[a]

Table 12. Effect of humidity on the growth of *H. thompsonii* var. *synnematosa*

Humidity (%)	Days after inoculation [*Mean colony diameter (cm)]									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
50	1	1	1.13[a]	1.19[a]	1.20[a]	1.27[c]	1.46[b]	1.65[a]	1.83[a]	2.06[a]
60	1	1	1.08[a]	1.17[a]	1.23[a]	1.30[bc]	1.51[ab]	1.61[a]	1.96[a]	2.11[a]
70	1	1	1.08[a]	1.20[a]	1.31[a]	1.38[abc]	1.57[ab]	1.76[a]	1.93[a]	2.06[a]
80	1	1	1.10[a]	1.19[a]	1.31[a]	1.42[ab]	1.58[ab]	1.72[a]	1.89[a]	2.11[a]
90	1	1	1.07[a]	1.19[a]	1.34[a]	1.49[a]	1.68[a]	1.91[a]	2.03[a]	2.20[a]

* Mean of five replications

In each column figures followed by the same letter do not differ significantly according to DMRT.

Plate 5. Growth of *H. thompsonii* var. *synnematosa* at different pH

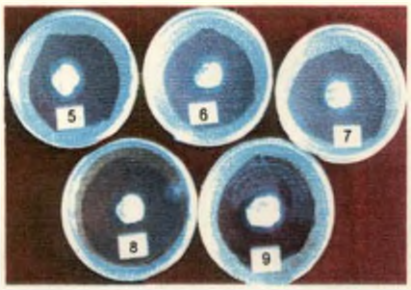


Plate 6. Growth of *H. thompsonii* var. *synnematosa* at different temperatures

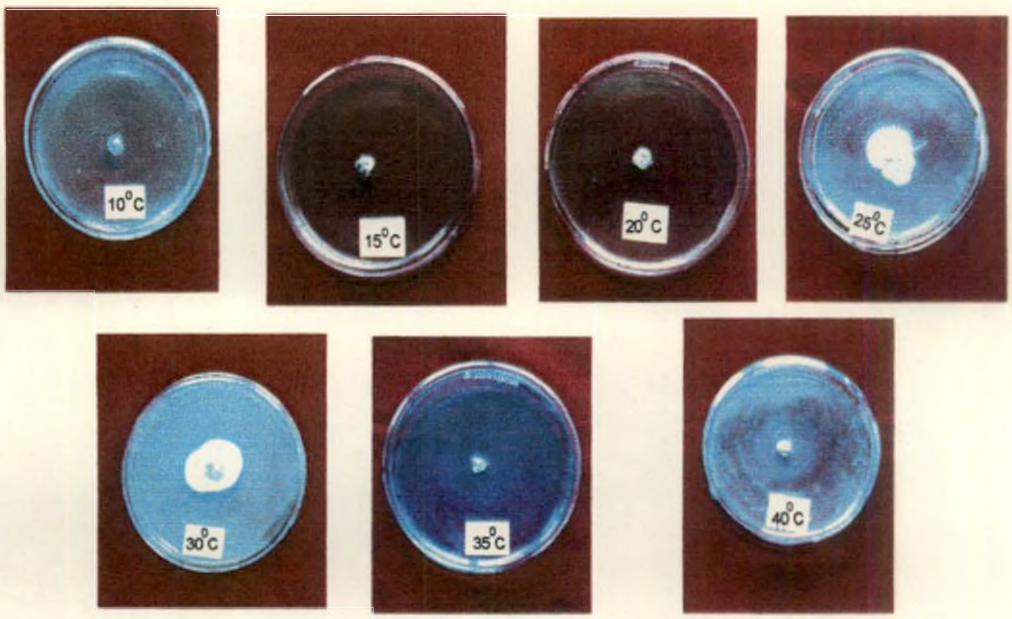
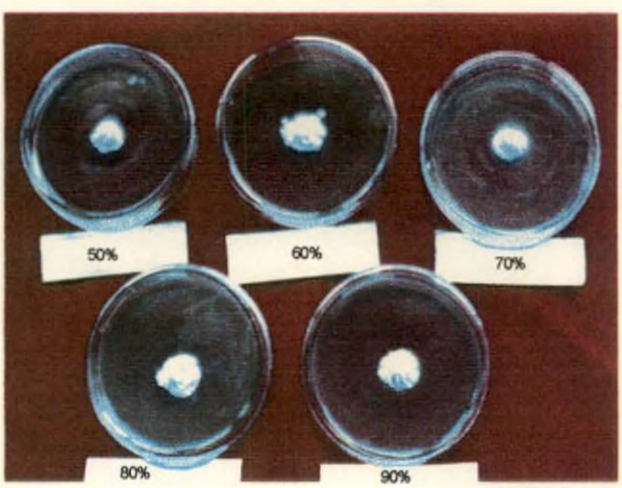


Plate 7. Growth of *H. thompsonii* var. *synnematosa* at different humidity



4.5 Compatibility of *H. thompsonii* var. *synnematosa* with pesticides

In vitro evaluation was carried out to know the compatibility of the fungus with selected insecticides, fungicides and botanicals. Per cent inhibition on fungal growth due to the effect of these pesticides and botanicals was recorded.

4.5.1 Compatibility of the fungus with insecticides/acaricides

Among the insecticides/acaricides tested, endosulfan recorded the maximum compatibility with the fungus (Table 14, Plate 8a). Here the fungal growth started at 6 DAI and recorded a colony diameter of 19.14 mm on 10 DAI which was on par with the growth recorded in control (21.90 mm). In this case the inhibition of growth over control was 11.34 per cent (Table 17a). The radial growth of the fungus in medium incorporated with carbaryl, wettable sulphur and triazophos was also found statistically on par with endosulfan at 10 DAI. In triazophos, the fungal growth started at 4 DAI whereas in wettable sulphur and carbaryl on 7 DAI and 8 DAI respectively. The least radial growth was recorded in dicofol (12.20 mm) which recorded the maximum per cent inhibition of growth (43.84) over control. The decreasing order of compatibility of the fungus with selected insecticides/acaricides was endosulfan, wettable sulfur, triazophos, carbaryl and dicofol.

4.5.2 Compatibility of *H. thompsonii* var. *synnematosa* with fungicides

The fungicides viz., Bordeaux mixture 1 per cent and copper oxychloride (Fytolan) 0.3 per cent were found to completely inhibit the growth of the fungus (Table 15, Plate 8b). There was no growth of the fungus in the media treated with these fungicides throughout the incubation period and resulted in cent per cent inhibition over the growth in control (36.80 mm) (Table 17b). The percentage inhibition by mancozeb (62.09) was on par with that by tridemorph (62.15), which resulted in a mean colony diameter of 13.86 mm and 13.92 mm respectively at 10 DAI (Table 15). The mean colony diameter in potassium

Table 14. *In vitro* evaluation on compatibility of *H. thompsonii* var. *synnematosa* with insecticides/acaricides

Insecticides	Mean colony diameter (mm)** (DAI)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Dicofol - 0.6 %	10.00^{a}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{c}	10.00^{c}	10.00^{c}	10.90^{c}	11.70^{c}	12.20^{c}
Carbaryl - 0.2%	10.00^{a}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{c}	10.00^{c}	10.00^{c}	10.90^{c}	12.86^{cd}	16.40^{b}
Endosulfan - 0.05%	10.00^{a}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{c}	11.00^{bc}	12.80^{b}	14.36^{b}	16.30^{b}	19.14^{ab}
Wettable sulphur (Sulfex - 0.4%)	10.00^{a}	10.00^{b}	10.00^{b}	10.00^{b}	10.00^{c}	10.00^{c}	10.60^{c}	11.40^{c}	15.60^{bc}	17.30^{b}
Triazophos - 0.05%	10.00^{a}	10.00^{b}	10.00^{b}	10.50^{b}	11.50^{b}	11.76^{b}	12.46^{b}	14.50^{b}	15.80^{b}	16.90^{b}
Control	10.00^{a}	11.30^{a}	12.30^{a}	12.80^{a}	14.00^{a}	15.00^{a}	16.00^{a}	18.90^{a}	21.00^{a}	21.90^{a}

*Mean of five replications

**Days after inoculation

In each column figures followed by the same letter do not differ significantly according to DMRT.

Table 15. *In vitro* evaluation on compatibility of *H. thompsonii* var. *synnematosa* with fungicides

Fungicides	*Mean colony diameter (mm) (DAI)									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Bordeaux mixture (1%)	10.00[a]	10.00[c]	10.00[b]	10.00[b]	10.00[c]	10.00[c]	10.00[c]	10.00[c]	10.00[c]	10.00[d]
Copper oxychloride (fytolan) 0.3%	10.00[a]	10.00[c]	10.00[b]	10.00[b]	10.00[c]	10.00[c]	10.00[c]	10.00[c]	10.00[c]	10.00[d]
Mancozeb 0.3%	10.00[a]	10.00[c]	10.20[b]	10.30[b]	11.00[b]	11.80[b]	12.50[b]	13.20[b]	13.58[b]	13.86[c]
Tridemorph 0.1%	10.00[a]	10.00[c]	10.00[b]	10.00[b]	10.70[b]	11.90[b]	12.20[b]	12.78[b]	13.30[b]	13.92[c]
Potassium phosphonate (Akomin)0.3%	10.00[a]	11.60[a]	13.30[a]	15.00[a]	17.00[a]	19.00[a]	24.80[a]	28.50[a]	30.10[a]	31.50[b]
Control	10.00[a]	10.82[b]	13.82[a]	15.30[a]	17.78[a]	21.50[a]	25.40[a]	29.50[a]	31.80[a]	36.80[a]

* Mean of five replications

In each column figures followed by the same letter do not differ significantly according to DMRT.

phosphonate at 10 DAI was 31.50 mm which was superior to the growth noted in other fungicides except control. It recorded the lowest per cent of inhibition over control (13.76).

The growth of the fungus in the medium incorporated with potassium phosphonate initiated on second DAI and was statistically superior than the growth in other treatments. From the third day onwards the growth in this treatment and control were on par upto ninth DAI. On 10 DAI, eventhough there was increase in the growth of fungus (31.50 mm) in Akomin incorporated medium, it was not statistically on par with that in control.

4.5.3 Compatibility of the fungus with botanicals

The results given in Table 16 revealed that the medium incorporated with azadirachtin recorded the maximum growth of fungus (22.00 mm) among botanicals at 10 DAI, which recorded only 9.12 per cent inhibition of growth over control (Table 17c and Plate 8c). It was on par with that of control. The percentage inhibition of growth recorded in garlic and garlic + neem oil treatments were 21.76 and 36.57 respectively. So among botanicals, garlic + neem oil recorded the maximum per cent inhibition of growth over control. In azadirachtin throughout the period of incubation, except in third and sixth DAI the fungal growth was on par with the growth on control.

4.6 Mass multiplication of *H. thompsonii* var. *synnematosa*

Raw and powdered forms of eight different substrates were used to find out the most suitable substrate for the mass multiplication of the fungus.

4.6.1 Mass multiplication of the fungus in the raw form of substrates

The growth medium was prepared, inoculated with the fungus and incubated at room temperature. Observations on the period of initiation of fungal

Table 16. *In vitro* evaluation on compatibility of *H. thompsonii* var. *synnematosa* with botanicals

Botanicals	*Mean colony diameter (mm) [DAI]									
	1st	2nd	3rd	4th	5th	6th	7th	8th	9th	10th
Garlic 2%	10.00[a]	10.00[a]	10.00[b]	11.00[b]	12.44[a]	13.50[c]	14.70[b]	16.80[b]	17.80[b]	18.70[b]
Garlic + Neem oil (2%)	10.00[a]	10.00[a]	10.00[b]	10.00[b]	10.00[b]	10.56[d]	11.80[b]	12.90[c]	14.00[c]	15.10[c]
Azadirachtin (0.03%) (Neemazal)	10.00[a]	10.00[a]	10.00[b]	12.20[a]	14.40[a]	16.52[b]	19.50[a]	20.50[a]	21.24[a]	22.00[a]
Control	10.00[a]	10.00[a]	11.50[a]	12.58[a]	14.24[a]	19.20[a]	21.20[a]	22.10[a]	23.20[a]	24.00[a]

* Mean of five replications

In each column figures followed by the same letter do not differ significantly according to DMRT.

Table 17. Per cent inhibition of growth of *H. thompsonii* var. *synnematosa* by pesticides and botanicals

Table 17 (a)

Insecticides/Acaricides	Per cent inhibition of mycelial growth over control
Dicofol 0.6%	43.838(0.723)[a]
Carbaryl 0.2%	25.446(0.499)[ab]
Endosulfan 0.05%	11.344(0.327)[b]
Wettable sulphur 0.4%	18.566(0.374)[b]
Triazophos 0.05%	21.506(0.462)[ab]

Table 17(b)

Fungicides	Per cent inhibition of mycelial growth over control
Bordeaux mixture 1%	100.000(1.521)[a]
Copper oxy chloride (Fytolan) 0.3%	100.000(1.521)[a]
Mancozeb 0.3%	62.090(0.908)[b]
Tridemorph 0.1%	62.150(0.908)[b]
Potassium phosphonate(Akomin)0.3%	13.764(0.357)[c]

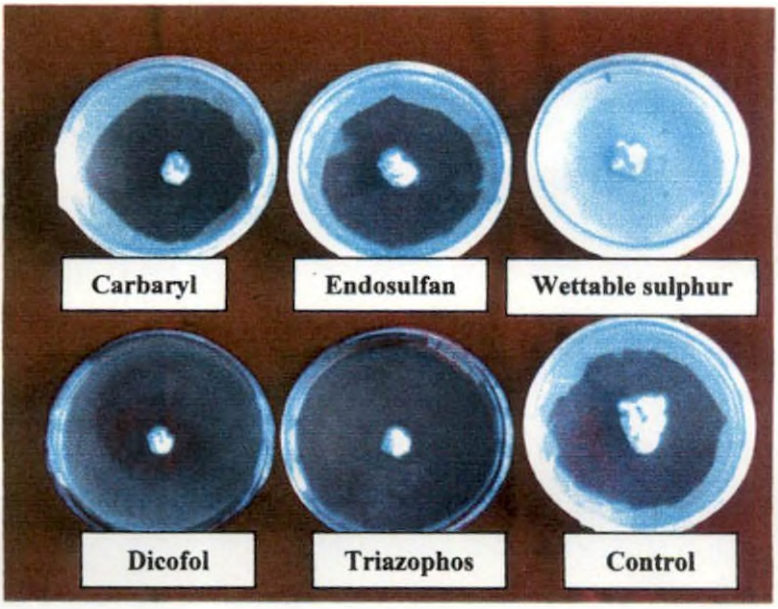
Table 17(c)

Botanicals	Per cent inhibition of mycelial growth over control
Garlic 2%	21.76 (0.480)[a]
Garlic+ Neem oil 2%	36.57 (0.640)[a]
Azadirachtin 0.03%	9.12 (27. 40)[b]

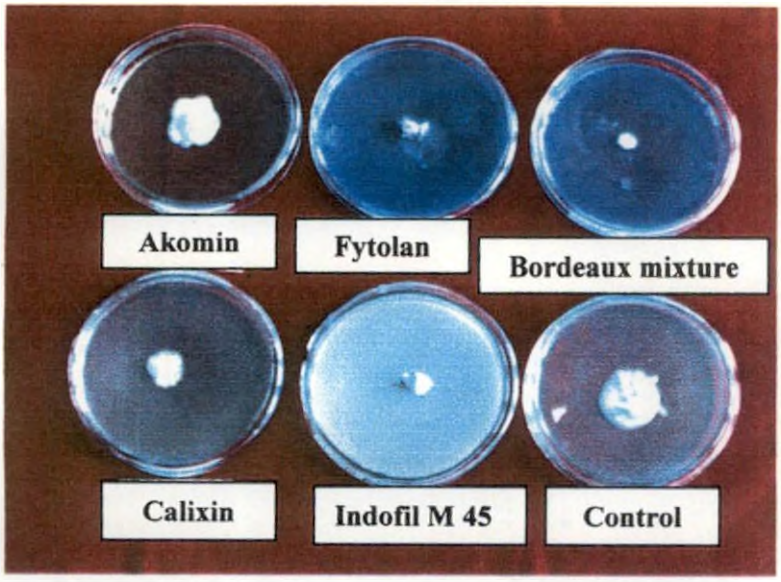
In each column figures followed by the same letter do not differ significantly according to DMRT

Plate 8. *In vitro* evaluation on compatibility of *H. thompsonii* var. *synnematosa* with pesticides and botanicals

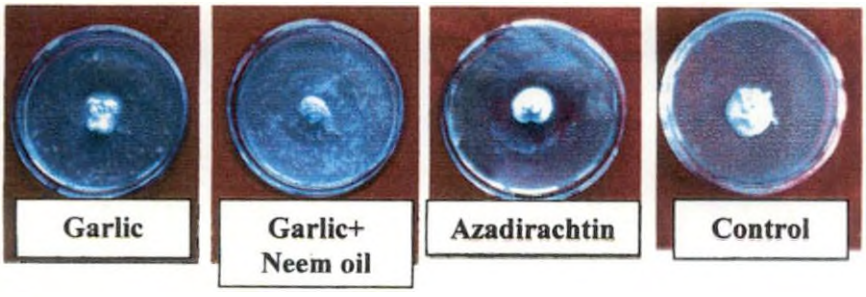
8a. Insecticides/Acaricides



8b. Fungicides



8c. Botanicals



growth, 50 per cent growth, 100 per cent growth, formation of synnemata were taken and are presented in Table 18 (Plate 9). The fastest initiation of fungal growth was recorded in substrates like barley and rice. Maize, wheat, sorghum and tapioca also recorded comparatively faster growth rate of the fungus than in substrates like tea waste and rice bran. Hundred per cent growth was recorded at around 30 to 35 days of growth in maize, rice, wheat and sorghum whereas substrates like tapioca, rice bran and barley took 45, 50, and 55 days respectively for the completion of fungal growth. Cent per cent growth was not observed in tea waste. Synnemata were produced only in substrates like barley, rice and wheat.

4.6.1.1 Evaluation of raw form of substrates on the sporulation of *H. thompsonii* var. *synnematosa*

The substrates were incubated for three months after inoculation for recording the spore count at 15 days interval starting from 30 DAI. The results are presented in Table 19. After 30 days of inoculation the highest sporulation was recorded in maize (12.7 x 10^6 spores/g). Statistically it showed significant difference from the spore count recorded at 30 DAI in other substrates. It was immediately followed by barley (8.3 x 10^6 spores/g) which was also significantly different from others. The substrates sorghum and rice bran recorded the same level of sporulation at 30 DAI. The lowest spore count observed was in tapioca (1.6 x 10^6 spores/g). The other substrates viz. rice, wheat and tea waste recorded a comparatively higher spore count of 6.6 x 10^6, 5.5 x 10^6 and 4 x 10^6 spores/g respectively compared to sorghum and rice bran.

Fourty five DAI, the sporulation in barley was increased to 9.3 x 10^6 spores/g which was statistically superior to other substrates. In maize, it was reduced to 8.4 x 10^6 spores/g. There was also reduction in the sporulation recorded in the substrates like sorghum (2.6x10^6 spores/g), rice (5.2x10^6 spores/g), wheat (5.0x10^6 spores/g) and teawaste (3.5x10^6 spores/g). But rice bran (3.2 x 10^6

Plate 9. Mass multiplication of *H. thompsonii* var. *synnematosa* in different substrates

Table 18. Observations on the growth of fungus and formation of synnemata in different substrates

Substrate	Initiation of fungal growth (DAI)	50 per cent growth (DAI)	Cent per cent growth (DAI)	Formation of synnemata (DAI)
Sorghum	7	20	35	-
Rice bran	12	35	50	-
Rice	5	17	30	35
Wheat	6	25	32	30
Barley	3	25	55	30
Maize	6	15	35	-
Tapioca	6	25	45	-
Tea waste	10	25	-	-

Table 19. Effect of different substrates (raw form) on the sporulation of *H. thompsonii* var. *synnematosa*

Substrates	Spore count - x 10^6/g of substrates				
	*30 DAI	45 DAI	60 DAI	75 DAI	90 DAI
Sorghum	2.8^{f}	2.6^{g}	2.0^{f}	2.2^{f}	1.2^{ef}
Rice bran	2.8^{f}	3.2^{f}	4.9^{c}	3.7^{c}	2.8^{c}
Rice	6.6^{c}	5.2^{c}	2.3^{e}	2.5^{e}	2.3^{d}
Wheat	5.5^{d}	5.0^{d}	5.3^{b}	5.4^{b}	5.1^{a}
Barley	8.3^{b}	9.3^{a}	12.3^{a}	7.8^{a}	3.6^{b}
Maize	12.7^{a}	8.4^{b}	4.1^{d}	3.2^{d}	2.1^{d}
Tapioca	1.6^{g}	1.7^{h}	1.3^{g}	1.4^{h}	1.1^{f}
Tea waste	4.0^{e}	3.5^{e}	2.2^{e}	1.8^{e}	1.4^{e}

* DAI - Days after inoculation

In each column figures followed by the same letter do not differ significantly according to DMRT.

spores/g) and tapioca (1.7 x 10^6 spores/g) showed a slight increase in sporulation. However sporulation in tapioca (1.7 x 10^6 spores/g) was the lowest at 45 DAI.

Sixty DAI, the highest spore count of 12.3 x 10^6 spores/g was recorded in barley which was statistically superior to that in other substrates. Similarly an increase in the number of spores per gram of substrate was increased in rice bran (4.9 x 10^6 spores/g) and wheat (5.3 x 10^6 spores/g). The sporulation in rice (2.3 x 10^6 spores/g) was statistically on par with that in tea waste (2.2 x 10^6 spores/g). The lowest sporulation was observed in tapioca (1.3 x 10^6 spores/g).

Seventy five DAI, spore count in barley was reduced from 12.3 x 10^6 to 7.8 x 10^6 spores/g of the substrates. But at that period it was the highest and was statistically superior to the sporulation recorded in other substrates. Sporulation in rice (2.5 x 10^6 spores/g) was statistically on par with that in rice bran (3.7x 10^6 spores/g). The lowest spore count was recorded in tapioca (1.4 x 10^6 spores/g).

On completion of 90 days of incubation it was observed that in all substrates the sporulation was very much reduced. The highest value was recorded in wheat (5.1 x 10^6 spores/g) which was statistically superior to that in other substrates. It was followed by barley (3.6 x 10^6 spores/g) and rice bran (2.8 x 10^6 spores/g). The spore count in maize was very much reduced at 90 DAI and was recorded as 2.1 x 10^6 spores/g of the substrate. This was also on par with that in rice (2.3 x 10^6 spores/g). Sporulation in tapioca recorded the lowest value of 1.1 x 10^6 spores/g which was on par with that in sorghum (1.2 x 10^6 spores/g).

4.6.1.2 Germination percentage of spores in different substrates

The germination percentage of the spores collected from different substrates was calculated at 30,60 and 90 DAI. The results are presented in Table 20. At 30 DAI maximum germination percentage was recorded by the spores obtained from barley (86.67 per cent). The lowest germination percentage of spores was recorded in tapioca (75 per cent). At 60 DAI the highest germination

Table 20. Effect of different substrates (raw form) on the germination of spores

Name of the substrates	Germination percentage		
	30 DAI*	60 DAI	90 DAI
Sorghum	77.78	57.14	37.50
Rice bran	83.33	55.56	28.57
Rice	83.33	57.14	28.57
Wheat	81.81	60.00	40.00
Barley	86.67	53.85	33.33
Maize	85.00	50.00	33.33
Tapioca	75.00	33.33	16.67
Tea waste	80.00	50.00	25.00

*-Days after inoculation

percentage recorded was in wheat (60 percent). Here also the lowest germination percentage was recorded in tapioca (33.33 per cent). After 90 days of inoculation the highest germination percentage observed was in wheat (40 per cent). In maize and barley the germination percentage recorded was the same (33.33 per cent). In tapioca the germination percentage recorded was the lowest (16.67 per cent).

4.6.2 Mass multiplication of *H. thompsonii* var.*synnematosa* in the powdered form of substrates

Hundred grams of powdered substrates were sterilized in 250 ml conical flasks and inoculated with the fungus and incubated for 30 days for the estimation of fungal population.

4.6.2.1 Estimation of viable fungal propagules in substrates

Serial dilution plate technique was conducted to estimate the colony forming units produced per gram of the substrates. Growth of the fungus was noticed only in lesser compact media like rice bran and tea waste. Among these rice bran recorded the maximum cfu of 3.78×10^2 cfu/g of the substrate. Tea waste recorded 2.57×10^2 cfu/g of the substrate. In other substrates there was no fungal growth and fungal colonies were not produced, even after 30 days of incubation.

Discussion

5. DISCUSSION

Recently coconut mite has emerged as an important pest of coconut particularly in Kerala. This very small microscopic mite completes its life cycle hiding beneath the perianth of the coconut. When buttons of three to four months old are affected, yield loss in terms of copra output is as high as 40 per cent. Many attempts have already been made to control the mite with acaricides, polybutene (Moore *et al.*, 1989) and with neem based pesticides. But the management of coconut mite with these pesticides spraying is very difficult because of its cryptic nature of breeding beneath the tightly pressed bracts. Hence attention has been given to find out the prevalence of biotic agents for the management of mites. Information is available on the occurrence of natural enemies including predacious mites and a pathogenic fungus *Hirsutella thompsonii* (Hall *et al*, 1980; Lampedro and Rosas, 1989).

In India Beevi *et al.* (1999) reported that the fungus *H. thompsonii* var. *synnematosa* could be isolated from dead coconut mites. The efficiency of this fungus against coconut mite as an acaropathogen was already established, therefore, the study was undertaken to evaluate the different media and locally available substrates for the mass production of this entomopathogenic fungus. The physiological characteristics of the fungus, efficiency of the toxin extracted from the fungus on coconut mite and compatibility of the fungus with pesticides and botanicals commonly used in coconut were also studied.

Initially the aim of the study was to isolate the fungus *H. thompsonii* from mites collected from the infested young nuts. The fungus was isolated in PDA and proved the pathogenicity. During isolation, the association of many fungi viz. *Aspergillus niger, A. flavus, Penicillium* sp. and *Fusarium* sp. was observed, but they were not pathogenic to eriophyid mite. All these microflora associated with coconut mites might not be the primary pathogens but certainly could impose

some limitations on the survival of coconut mite population. Similarly the association of other fungi along with true entomopathogen was reported in broad mites and whitefly by Rao and Reddy (1992) and Pena *et al.* (1996).

Further studies were limited to *H. thompsonii* var. *synnematosa* which was proved as pathogen of coconut mite.

Selection of suitable media for an entomopathogenic fungus is essential to obtain maximum growth and sporulation or the production of infective propagules which ultimately decides the success of a mycoinsecticide. In the present study, the influence of different media were studied on the growth and sporulation of the fungus. The results of the study showed that maximum mycelial growth in terms of colony diameter of *H. thompsonii* var. *synnematosa* was recorded in the medium SMA+Y (4.25 cm) (Fig.1). Minimum growth was recorded in CM and PCA (1.40 cm and 1.54 cm). Maximum sporulation of the fungus was also in SMA+Y (7.9×10^6 spores/ml). Other media tried were not efficient as that of SMA+Y. Thus SMA+Y was selected as the best medium for the growth and sporulation of the fungus. In the composition of SMA+Y, maltose and yeast extract are included which served mainly as carbon and nitrogen sources respectively. Yeast extract was absent in all other media tried, where as maltose was included in LM. So this emphasizes the importance of yeast extract on mycelial growth and sporulation of the fungus. Im *et al.* (1988) got the same result when they studied the effect of nutrients on growth and sporulation of *Hirsutella* sp. and reported the importance of yeast extract for the mycelial growth of the fungus. Sztejnberg *et al.* (1997) also got best growth of an acaropathogenic fungus, *H. kirchneri* on a medium containing yeast extract and dextrose. Jenkins *et al.* (1998) reported that working cultures of mitosporic fungi could be grown on standard mycological agar such as Sabouraud's dextrose agar and malt extract agar.

The levels of sporulation at different positions of the 10 day old colonies were compared on a petridish containing SMA+Y and PDA medium. In both media maximum sporulation was occurred at the centre of the colony. As the radial distance from the centre of the colony was increased, the sporulation was reduced and minimum sporulation was noticed in the periphery of the colony. Cherian (2000) also reported the same observations in *Beauvaria bassiana* and *Verticillium lecanii* grown on different media.

The solid medium, SMA+Y recorded the maximum mycelial growth as well as the sporulation (Fig.1). But it recorded a comparatively lesser number of synnemata (4.2). The maximum number of synnemata was noticed in RM (8.6) where the mycelial growth and sporulation were comparatively lesser. Similarly the CM also produced comparatively higher number of synnemata (6.2) which recorded lowest mycelial growth and medium sporulation. This showed the absence of any relation between the mycelial growth and synnemata production. The correlation study conducted between growth, sporulation and synnemata production showed a significant positive correlation between the growth of fungus and sporulation (0.8). But it recorded a non significant negative correlation between sporulation and synnemata production (-0.029) and the correlation was not significant between number of synnemata and fungal growth (0.018). Synnemata are compact group of erect conidiophores bearing conidia (Hawksworth *et al.*, 1995) which grow towards the light source. Usually it is produced by clustering of mycelium, become thick, elongated and produce spores on its surface (Plate 3). Under insufficient nutrient conditions the fungus produces synnemata for its survival. From the data it was concluded that the fungus produced its fruiting body. (synnemata) for its survival under unfavourable conditions. Sztejnberg *et al.* (1997) in their study on the biology of the acaropathogenic fungus, *H. kirchneri* suggested that the synnemata produced by the fungus could be used to promote its survival under dry conditions.

Germination percentage of the spores of fungus was recorded in the best solid medium SMA+Y. It was found that the germination of the spores was started only after two hours of incubation and 96 per cent germination was recorded after eight hours of incubation. Magalhaes *et al.* (1994) reported 90 per cent germination of spores of *Metarhizium anisopliae* produced in liquid medium.

The growth and sporulation of the fungus were recorded in liquid broth also. The maximum dry mycelial weight was recorded in SM+Y (490 mg) whereas the maximum sporulation was in H&A'A' (3.5×10^5 spores/ml) (Fig. 2). This might be due to the difference in the nutritional concentration and quality that favour sporogenesis and mycelial growth. Sometimes the requirements might be highly specific as reported by Latge and Sanglier (1985). Eventhough SM+Y recorded a lower level of sporulation than H&A'A' statistically there was no significant difference between these two media in sporulation. So SM+Y was selected as the best liquid medium for the growth and sporulation of *H. thompsonii* var. *synnematosa*. UAS (1996) reported SM+Y to be the best liquid medium for dry mycelial weight of *B. bassiana* and RM for *V. lecanii*. The sporulation was found best in malt extract and H&A'A' for *B. bassiana* and *V. lecanii* respectively. Jenkins *et al.* (1998) reported the importance of nitrogen and carbon source in the liquid media for the growth of *M. flavoviride*.

The germination percentage of spores in liquid medium SM+Y was recorded and observed a lower rate of germination compared to that recorded in solid medium. Here the spores were started germination only after three hours of incubation and only 25 per cent spores were germinated even after eight hours of incubation. Van Winkelhoff and McCoy (1984) also reported the lower rate of germination (5.2-12.9 per cent) of conidia produced in submerged culture of *H. thompsonii*.

On comparing the sporulation in solid and liquid media, it was observed that the sporulation was lesser in broth culture than in solid media (Fig. 3). As

Fig. 1. Effect of different solid media on growth and sporulation of *H. thompsonii* var. *synnematosa*

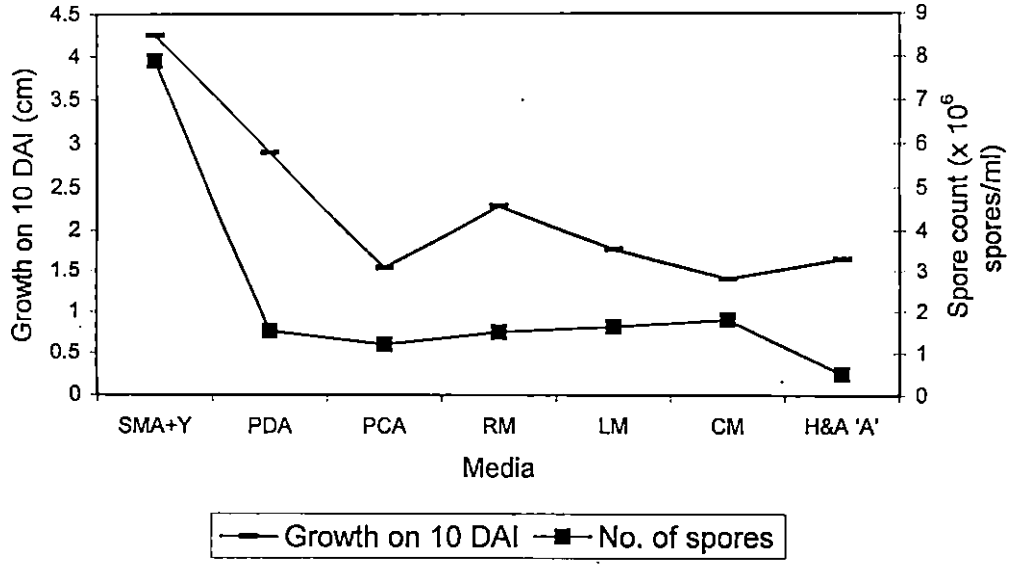
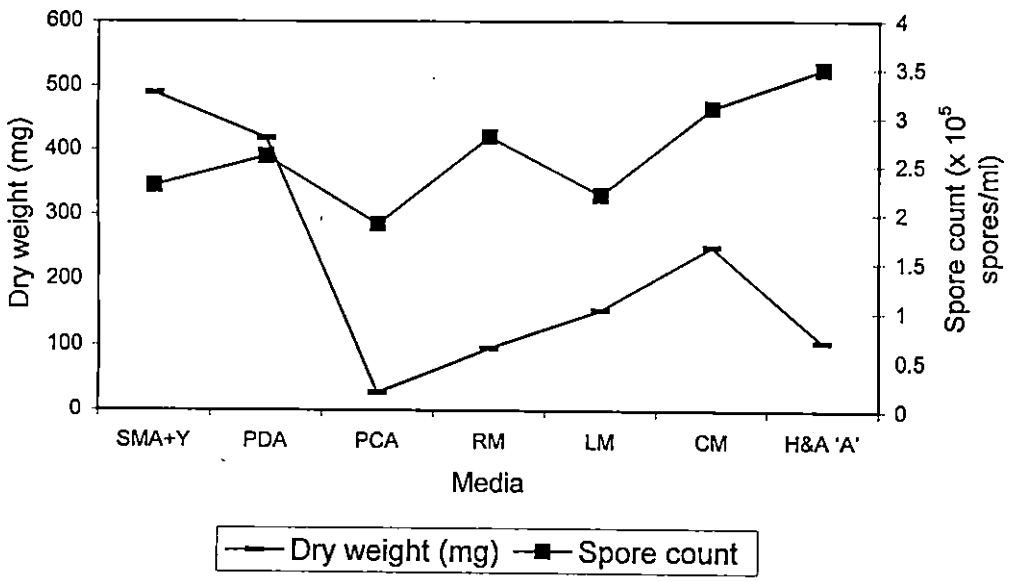


Fig. 2. Effect of different liquid media on growth and sporulation of *H. thompsonii* var. *synnematosa*



reported by Moore *et al.* (1989) *H. thompsonii* develop filamentously in liquid media and it does not 'willingly' sporulate in liquid culture. In general, mitosporic fungi grow readily as mycelium or hyphal bodies (also referred to as blastospores) in liquid media (Jenkins *et al.*, 1998). But in the present study production of spores by the fungus was observed in liquid media. There were reports on the sporulation of *Hirsutella* sp. in submerged cultures. van-Winkelhoff and Mc Coy (1984) noticed true conidia production only in *H. thompsonii* var. *synnematosa* grown in different liquid media among the 14 isolates of *H. thompsonii* tried. Liu *et al.* (1995) also observed the spores of *H. thompsonii* on potato dextrose yeast browth with agitation at 250 rpm for three days.

Morphological characters of *H. thompsonii* var. *synnematosa* grown in different solid media were recorded using microscope. In all the media the fungus produced greyish white fungal colony with dome shaped growth and without any specific reaction on the media. So there was no difference in the external appearance of the fungus in different media. The microscopic observation of the fungal structures produced in different media showed that in all the media 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 branched often branched once. The spores produced in all the media were spherical, verrucose and hyaline. These observations are in conformity with the characters of the fungus described by International Mycological Institute.

No change was observed in the basic characters of the fungus grown in different media. But the microscopic measurements recorded on different fungal structures showed some variations. The results showed that the hyphal width, the phialide width and the spore diameter ranged between 3.33 µm-3.76 µm. The maximum of these observations was in media like, LM, CM and PCA and the minimum was observed in SMA+Y and H&A'A'. The length of the phialides was maximum in PDA (10.09 µm) and minimum in SMA+Y (7.57 µm). The phialides

were at maximum distance in LM (77.26 µm) which produced six phialides in a microscopic field. Minimum distance between the phialides was observed in SMA+Y (35.03 µm) which recorded the maximum number of phialides in a microscopic field. The most important characteristics of a fungus to be rated as a good mycopesticide were their high growth and sporulation rate and fast germination. In the present study, the hyphae grown in SMA+Y produced the phialides and spores at shortest interval eventhough hyphal width, phialide width, spore diameter and length of phialides were minimum. So the number of phialides and thus the spores produced on a unit length of hypha were more in the case of SMA+Y. This indicated the vigorous growth and high sporulation of fungus in SMA+Y. This observation once more confirmed the selection of SMA+Y as the best medium for the growth of the fungus. Considerable variations in the morphological characters of *H. thompsonii* grown on different solid media were reported earlier by Fisher (1950). Samson *et al.* (1980) and Beevi *et al.* (1999) reported the colony characters of *H. thompsonii* var. *synnematosa* on malt extract agar and PDA respectively. Their observations confirmed the present results.

It is very essential to know the optimum pH of medium during culture process to get maximum growth of the fungus. In the present study, the fungus preferred pH 9 for better growth (3.08 cm) (Fig. 4). Eventhough it recorded maximum growth in pH 9 it was statistically on par with the growth in pH 6 and 7. But the growth recorded in pH 6 and 7 was on par with that in pH 5 and 8. So it was concluded that eventhough the fungus preferred an alkaline pH of 9 it could grow on a wide range of pH from 5 to 9. This showed the wide adaptability of the fungus to survive under adverse conditions from acidic to extreme alkaline conditions. Same result was reported in *Hirsutella* sp. (Im *et al.*, 1988) and in *V. lecanii*, *Paecilomyces farinosus, B. bassiana* and *Aegartia webberi* (Gelani, 1988) grown in liquid media with a pH range of 5 to 8.

The necessity to maintain optimum temperature during culture process, for better efficiency of the entomopathogenic fungus was well documented in literature. In the present study, the maximum radial growth of *H. thompsonii* var. *synnematosa* was recorded at 25°C (2.95 cm) (Fig. 4). But it could also grow at 30°C. Statistically the growth at 25°C was found superior to the growth at 30°C. Same result was reported by Moore *et al.* (1989) and found that maximum growth of *H. thompsonii* during fermentation was at a temperature of 27±0.1°C. Fargues *et al.* (1993) also observed maximum growth of six entomogenous fungus at 25°C and he concluded that the optimum temperature for fungal growth under *in vitro* conditions was not necessarily the same as that for growth on insects. But the establishment of temperature ranges according to *in vitro* experiments might be used for selecting fungal candidates for microbial control. The maximum growth of the acaropathogen of *H. kirchneri* on artificial media was at 25°C and considerable mite mortality occurred at 25°C and 30°C (Sztejnberg *et al.*, 1997). All the reports are similar to the results of present investigation.

On analyzing the most suitable humidity level for the fungal growth under *in vitro* conditions, it was observed that the maximum growth of the fungus was at 90 per cent humidity (2.20 cm) (Fig 4). But there was no significant difference in the fungal growth at different humidity levels ranged from 50 per cent to 90 per cent. The search of literature revealed the reports of requirement of high relative humidity (>80 per cent) for the infection of mites by *H. thompsonii* (Chen *et al.*, 1981) and *H. kirchneri* and *H. thompsonii* (Sztejnberg *et al.*, 1997). These reports confirm the results that the fungus is efficient in the field for mite control under high humid conditions eventhough there was not much difference in growth at different humidity levels.

In the present investigation, the exotoxin and endotoxin were extracted from the liquid culture of the fungus by centrifugation and its efficiency on coconut mite was evaluated by observing the percent mortality of mites after

Fig. 3. Comparison of spore count in different solid and liquid media

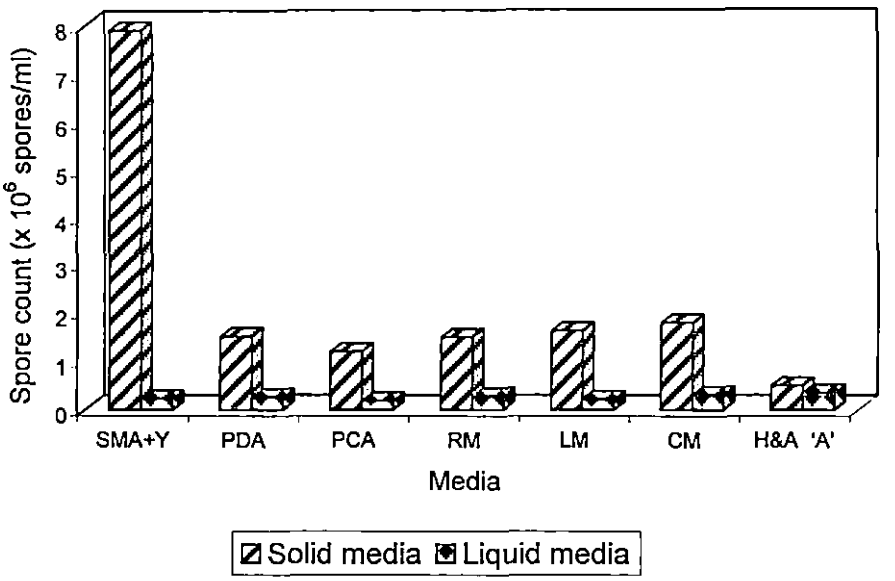
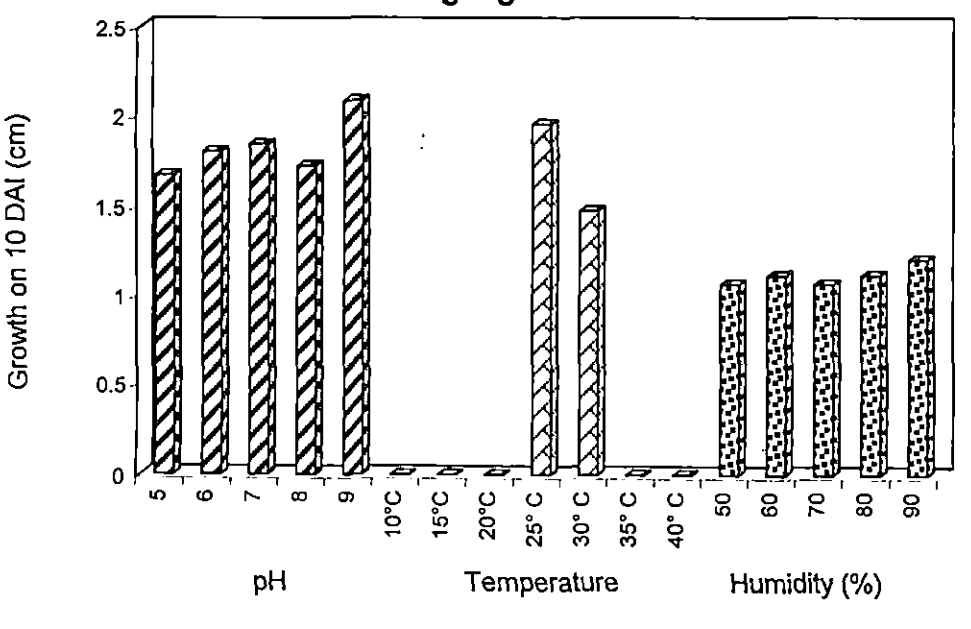


Fig. 4. Effect of pH, temperature and humidity on fungal growth



spraying. Under closed perianth situations the maximum percent mortality was recorded from nuts sprayed with exotoxin (88.03 per cent) whereas the endotoxin spray resulted in maximum percent mortality in the case of opened perianth (94.81 per cent). The percent mortality due to spraying of both the toxins and water (control) was statistically on par under open perianth conditions. But under closed perianth condition the mortality due to spraying with both the toxins was statistically superior to that with water spray. This reveals that when perianths are opened the mite mortality may occur under natural conditions itself. Moreover toxin spray under opened perianth conditions will not be successful in practical situations. In the present study all the observations were taken under lab conditions. Vey *et al.* (1993) reported toxin production in *H. thompsonii* sub sp. *thompsonii*. Insecticidal property was reported by Liu *et al.* (1995) to the toxin, hirsutellin A purified from the culture filtrates of *H. thompsonii* var. *thompsonii* JAB-OH against the wax moth. Chernin *et al.* (1997) reported that the two isolates 255 and 414 of *H. thompsonii* and the isolate of *H. necatrix* were able to produce and excrete chitinolytic enzyme and caused mite mortality of 80, 85 and 115 per cent respectively. These reports reveal the presence of toxin in Hirsutella genus which causes the mortality of coconut mite.

The application of entomopathogenic fungi needs to be integrated into the management system. So the information on the sensitivity of different isolates of the fungal species to insecticides/acaricides, fungicides and botanicals is very important. In the present study the fungicides, insecticides/acaricides and botanicals at the recommended doses for various diseases and pests of coconut were selected for the *in vitro* evaluation against *H. thompsonii* var. *synnematosa*. Among the insecticides/acaricides, the maximum inhibition of fungal growth was brought about by dicofol (43.84 per cent) (Fig.5). The percentage of inhibition brought about by wettable sulphur, triazophos and endosulfan was below 25 per cent and among these, endosulfan recorded the least percentage of inhibition (11.34 per cent). Statistically carbaryl and triazophos were on par with dicofol.

Dicofol, endosulfan, triazophos and wettable sulphur have been proved to be effective for the management of coconut eriophyid mite (Mohanasundaram, 2000; Fernando *et al.*, 2000). Among these dicofol @ 6 ml/l and wettable sulphur @ 4 ml/l were widely used in Kerala for spraying against the mite. In this study, it was found that under laboratory conditions, the insecticide dicofol @ 6 ml/l was least compatible with the acaropathogen *H. thompsonii* var. *synnematosa*. It was followed by carbaryl, triazophos, wettable sulphur and endosulfan. Inhibition on the germination of conidia of *H. citriformis* by carbaryl was observed by Aguda *et al.* (1988). Triazophos at 10 times the recommended dose was found preventing the growth of *M. anisopliae* (Moorhouse *et al.*, 1992).

In the case of fungicides, Bordeaux mixture and copper oxychloride showed cent per cent inhibition of fungal growth (Fig.6). The fungicides like mancozeb and calixin showed about 62 per cent inhibition of growth over control. But potassium phosphonate (akomin) which is commonly considered as a fungicide as well as a plant tonic showed a minimum inhibition of 13.76 per cent on the growth of the fungus. From the third day of incubation the growth of fungus in Akomin incorporated medium and control plates were statistically on par with each other except on 10 DAI. Toxic or fungistatic effect of copper oxychloride, mancozeb and zineb in *B. bassiana* (Tedders, 1981, Machowicz-Stefaniak, 1983), copper oxychloride in *M. anisoplia* (Duarte and Menendez, 1989) and copper oxychloride in *H. thompsonii* (McCoy *et al.*, 1995) have been reported earlier.

In vitro evaluation was also conducted to know the compatibility of the fungus with botanicals (Fig.7). Among botanicals maximum inhibition of fungal growth was recorded in garlic + neem oil @ 2 per cent (36.57 per cent). Other botanicals showed a less than 25 per cent inhibition on the fungal growth over control. Azadirachtin was found least inhibitory to the fungus *H. thompsonii* var.

Fig. 5. Per cent inhibition of fungal growth by insecticides/acaricides over control

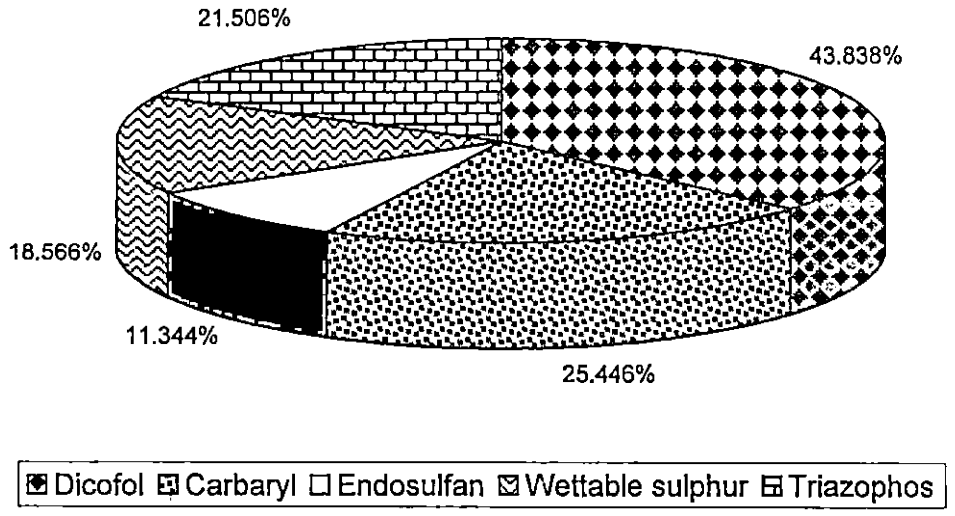
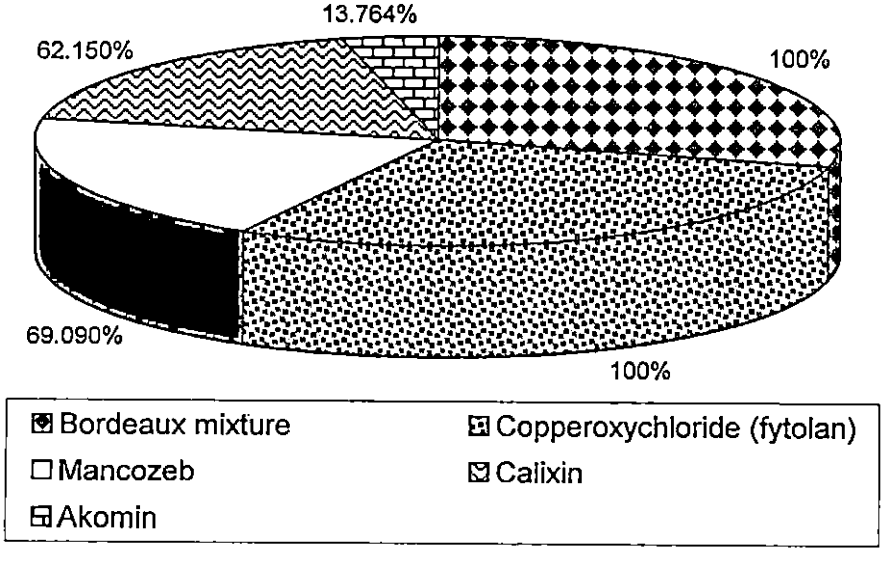


Fig. 6. Per cent inhibition of fungal growth by fungicides over control



synnematosa (9.12 per cent). In the lab trials conducted by Vyas *et al.* (1992) it was reported that azadirachtin did not inhibit the growth of entomogeneous fungus *B. bassiana* and *M. anisoplia.* Devi and Prasad (1996) also found the non detrimental effect of plant extracts to the entomogeneous fungus *Nomuraea rileyi.* These reports confirm the results of the present investigation. This study indicated that care should be taken when integrating mycopesticide with the chemical pesticides especially fungicides. In the light of the current recommendation of wettable sulphur, care should be taken when it is sprayed. Its application should not lead to the loss of natural *Hirsutella* population. In general, a fungicide could only be used upto three days before application of mycopesticides or more than three days after the application of mycopesticides. Since most of the entomopathogenic fungi occur naturally, the application of pesticides should be done carefully if there is a natural outbreak of fungal epizootics.

Raw and powdered form of different locally available materials like barley, maize, rice, wheat, sorghum, tapioca, tea waste and rice bran were compared for the mass production of the fungus *H. thompsonii* var. *synnematosa.* The observations on growth of the fungus showed that the fastest initiation of the fungal growth was in barley (3 DAI). The growth was very slow in tea waste and rice bran (10 and 12 DAI respectively). The fastest completion of cent percent growth of the fungus was in rice (30 DAI). In tea waste cent percent fungal growth was not produced. Synnemata was produced only in barley, rice and wheat where as no synnemata was developed in other substrates.

Observation on sporulation was taken in different substrates for three months at 15 days interval starting from 30 DAI (Fig.8). All the substrates yielded above 10 lakhs (1×10^6) conidia per gram of the substrate. At first the maximum sporulation was recorded in maize (12.7×10^6 spores/ml) followed by barley and rice (8.3×10^6 and 6.6×10^6 spores/ml respectively). In wheat a medium sporulation of 5.5×10^6 spores/gram of the substrate was observed. On completion

Fig. 7. Per cent inhibition of fungal growth by botanicals over control

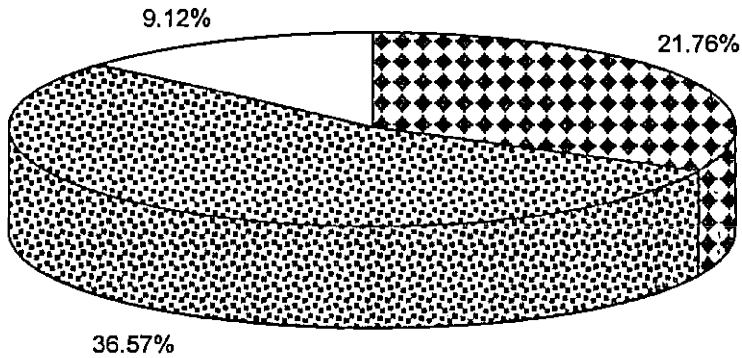
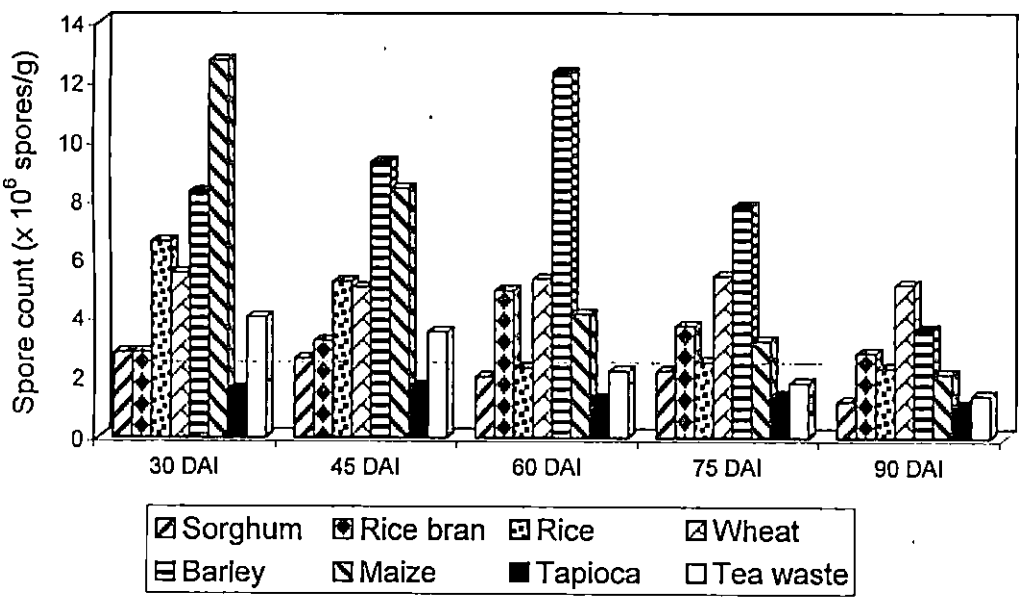


Fig. 8. Effect of different substrates on sporulation of *H. thompsonii* var. *synnematosa*



of three months after inoculation the sporulation in maize, barley and rice reduced considerably whereas the sporulation in wheat was maintained (5.1 x 10^6 spores/ml) without much variation up to the end of 90 DAI. Thus wheat was selected as the most suitable substrate for the mass multiplication and sporulation of the fungus *H. thompsonii* var. *synnematosa*. Bradley *et al.* (1995) reported a new solid culture substrate based on barley for growing the entomopathogenic fungi including *Hirsutella* sp. In the present study it was clear that eventhough barley was a suitable substrate for initial growth and sporulation it was not so efficient in maintaining the sporulation. For most strains of the fungus *H. thompsonii* and *H. nodulosa* greatest conidiogenesis was obtained on rice, barley and bran (Rosas-Acevedo *et al.*, 1995). In the present study also the initial growth of *H. thompsonii* var. *synnematosa* was good in rice and barley. But the spore count was reduced gradually in both the substrates.

In the preliminary testing of mass production of *H. thompsonii* var. *synnematosa* Maimala *et al.* (1999) identified ground corn coated with molasses as the best solid medium.

Germination percentage of the spores was observed in different substrates at 30, 60 and 90 DAI. On 30 DAI the maximum germination percentage was recorded in barley (86.67 per cent). But on reaching 90 DAI barley showed lesser germination percentage (33.33 per cent). In wheat the initial germination percentage of spores was higher (81.81 per cent). At 90 DAI, wheat recorded the highest germination percentage (40.00 per cent). Throughout the period the lowest germination percentage was recorded in the substrate tapioca. Thus in the present study wheat was emerged as the most suitable substrate for sporulation and also in maintaining the viability of the spores.

Powdered forms of the substrates were also evaluated for the fungal growth of *H. thompsonii* var. *synnematosa*. Colony forming units per gram of the substrates was measured. The results were obtained only in loose substrates like

rice bran and tea waste. Among these two, rice bran recorded the maximum colony count (3.78×10^2). In all other substrates, it became hard and compact after sterilization and they were not suitable for the growth of the fungus. Estimation of spore count was not possible in the case of powdered substrates as it was difficult to distinguish the spores and the substrate particles in counting the number of spores.

A two phase production of conidia had been used for the mass production of spores of the fungus in different substrates. In this method, fungal inoculum or hyphal bodies were produced in liquid culture in shake flasks and transferred to solid substrates for production of conidia. This method was standardised by many workers (Samsinakova *et al.*, 1981, Guillon, 1997 and Jenkins *et al.*, 1998). According to Jenkins *et al.* (1998) the advantages of this method were to enhance the competitiveness of the fungus by reducing contamination of microorganisms, to reduce the incubation time due to rapid colonization and conidiation and also to ensure even coverage of the solid substrate particles resulting in homogeneous growth throughout the substrates. The method used in the present investigation is comparable with Cherry *et al.* (1999) who reported the two phase production of conidia of *M. anisopliae* yielding about 8×10^8 conidia per gram of rice.

Summary

6. SUMMARY

Coconut eriophyid mite, *Aceria guerreronis* was emerged recently as an important pest of coconut particularly in Kerala, which causes an yield loss of about 40 per cent in terms of copra output. Many attempts were made to control the mite with acaricides and with neem based pesticides. The management of mite with pesticide spraying is very difficult because of its specific nature of breeding beneath the tightly packed bracts. In India, Beevi *et al.* (1999) isolated an acaropathogen, *H. thompsonii* var. *synnematosa* from dead mites collected from the mite infested coconut and proved the pathogenicity of the fungus on the mite. The present study entitled "Parasitism of *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnell on coconut eriophyid mite *Aceria guerreronis* (Keifer)" was undertaken to search for the evaluation of different media and locally available substrates for the mass production of this entomopathogenic fungus, physiological characters of the fungus, efficiency of toxin extracted from the fungus on mite and compatibility of the fungus with pesticides and botanicals commonly used in coconut.

The fungus *H. thompsonii* var. *synnematosa* was isolated from the infested young nuts collected from the coconut garden of College of Horticulture, Vellanikkara and proved the pathogenicity of the fungus on coconut mite. Many other fungi like *Aspergillus niger, A. flavus, Penicillium* sp. and *Fusarium* sp. were found to be associated with the mite, but they were not primary pathogens of eriophyid mite.

The results on the evaluation of seven different solid media on the growth and sporulation of the fungus showed that the maximum colony diameter and sporulation of the fungus, *H. thompsonii* var. *synnematosa* were recorded in the solid medium, SMA+Y. The maximum number of fruiting bodies of the fungus called synnemata were produced in the medium RM where the mycelial growth

and sporulation of the fungus were comparatively lesser. The results of the correlation study revealed a significant positive correlation between the mycelial growth and sporulation of the fungus whereas it recorded a non significant negative correlation between sporulation and synnemata production. But no correlation was noticed between mycelial growth and synnemata production. The germination percentage of the spores was observed on the best solid medium, SMA+Y which recorded 96 per cent germination of spores after eight hours of incubation. Sporulation at various radial distances in SMA+Y and PDA revealed that the maximum sporulation was at the centre of the colony and minimum at the periphery.

The evaluation of seven different liquid media on the growth and sporulation of the fungus showed that maximum dry mycelial weight of *H. thompsonii* var. *synnematosa* in the broth, SM+Y. The maximum sporulation was in broth of H&A'A' medium. But statistically there was no significant difference between SM+Y and H&A'A' in sporulation. So SM+Y was selected as the best liquid medium for growth and sporulation of the fungus.

Observations on the germination percentage of spores on SM+Y revealed only 25 per cent germination of spores after eight hours of incubation. The sporulation of fungus and germination percentage of spores on seven different solid and liquid media were compared. It was observed that the sporulation of fungus and germination percentage of spores were lesser in liquid cultures than in solid media.

The microscopic observations of the fungal structures grown in different solid media revealed that in all the media the hyphae were hyaline, septate, smooth and branched producing conical to flask shaped phialides, with narrow neck. The neck was branched often branched once. The spores produced were spherical, verrucose and hyaline. The hyphal width, phialide width and spore diameter ranged between 3.33 µm to 3.76 µm. Length of the phialides was maximum in PDA and

minimum in SMA+Y. The phialides were at maximum distance in LM and at minimum distance in SMA+Y. So the number of phialides and thus the spores produced in a unit length of hypha were more in the case of SMA+Y. This indicated the vigorous growth and high sporulation of fungus in SMA+Y.

In vitro evaluation on suitable pH, temperature and relative humidity for the growth of the fungus was conducted. The optimum pH recorded for the maximum growth of the fungus was pH 9. Eventhough the fungus preferred an alkaline pH of 9, it gave a satisfactory growth on a wide range of pH from 5 to 9.

The maximum growth of the fungus was observed at a temperature of 25°C. From the data, it was found that the fungus could grow only in a temperature range of 25 to 30°C and it could not grow below 25 and above 30°C.

There was no significant difference in the fungal growth at different humidity levels ranged from 50 to 90 per cent. Maximum fungal growth was observed at 90 per cent humidity under *in vitro* condition.

Using centrifugation, both exotoxin and endotoxin were extracted from the liquid culture of *H. thompsonii* var. *synnematosa*. To know the efficiency of these toxins on coconut mite, the per cent mortality of mite was recorded after spraying with these toxins separately under lab condition. Exotoxin spray resulted in maximum percent mortality under closed perianth condition whereas the endotoxin showed the maximum per cent mortality when the perianth was opened. Significant difference was recorded in the per cent mortality of mites due to spraying of toxins and water (control) in closed perianth condition. In opened perianth condition, there was no significant difference in per cent mortality due to spraying of toxins and water.

In vitro evaluation of insecticides/acaricides on the growth of the fungus showed different levels of inhibition. Among them, maximum percentage of

inhibition was brought about by dicofol @ 6 ml/l. It was followed by carbaryl, triazophos, wettable sulphur and endosulfan. The insecticide endosulfan recorded the least percentage of inhibition of fungal growth over control.

Among the fungicides tested, bordeaux mixture and copper oxychloride showed cent per cent inhibition on the growth of the fungus *H. thompsonii* var. *synnematosa*. Mancozeb and Calixin showed about 62 per cent inhibition of growth over control. But potassium phosphonate (akomin) which is commonly considered as a fungicide as well as a plant tonic recorded the least per cent inhibition of fungal growth over control.

Evaluation of compatibility of *H. thompsonii* var. *synnematosa* with botanicals showed that maximum inhibition of fungal growth was with garlic + neem oil @ 2 per cent. Azadirachtin was found least inhibitory to the fungus.

Two phase mass production study was conducted for the production of conidia of *H. thompsonii* var. *synnematosa*. Among the raw form of the substrates tried, fastest initiation of fungal growth was in barley and cent per cent growth was produced fastest in rice. Synnemata was produced only in barley, rice and wheat. On recording the sporulation in different substrates, it was found that initially the maximum sporulation was recorded in maize followed by barley and rice. But sporulation was reduced considerably on reaching 90 DAI in these substrates. In wheat eventhough the initial sporulation was not so high it was identified as the most suitable substrate since it maintained the sporulation even at 90 DAI. Germination percentage of spores recorded was also the highest in wheat at 90 DAI. Initial germination percentage recorded also showed a comparatively higher value in wheat. Thus wheat was identified as the most promising raw form of the substrate for the mass production of the fungus *H. thompsonii* var. *synnematosa*.

In the case of powdered form of the substrates estimation of sporulation was not possible because of the small particle size of the substrates. Observations

on cfu/gram of the substrates was possible only in loose substrates like rice bran and tea waste. Among these two, rice bran recorded the maximum colony count. All other substrates became hard and compact after sterilization and no fungal growth was developed in these substrates.

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* Originals not seen

PARASITISM OF *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnell ON COCONUT ERIOPHYID MITE *Aceria guerreronis* (Keifer)

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ABSTRACT OF THE THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Science in Agriculture

Faculty of Agriculture
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2002

ABSTRACT

An investigation on "Parasitism of *Hirsutella thompsonii* Fischer var. *synnematosa* Samson, McCoy & O'Donnell on coconut eriophyid mite *Aceria guerreronis* (Keifer)" was carried out at the Department of Plant Pathology, College of Horticulture, Vellanikkara. The main objectives of the study were to explore the cultural, physiological, toxicological characters of the fungus, compatibility of the fungus with pesticides and botanicals and mass multiplication characteristics of the fungus, which could be used as an efficient biocontrol agent on coconut mite. The results of this investigation revealed the following conclusions.

From the mite infested young nuts collected from the coconut garden of College of Horticulture, Vellanikkara, the acaropathogen *H. thompsonii* var. *synnematosa* was isolated and its pathogenicity on mite was proved.

Sabouraud's maltose agar + yeast was selected as the most suitable solid medium for the growth and sporulation of the fungus. Maximum number of fruiting bodies called synnemata were produced in Richard's medium. Correlation study revealed a significant positive correlation between mycelial growth and sporulation. Ninety six per cent germination of spores of the fungus grown in solid medium was recorded after eight hours of incubation.

In the different liquid media tried, Sabouraud's maltose+yeast was selected as the best for maximum dry mycelial weight and sporulation. Only twenty five per cent germination was recorded after eight hours of incubation of spores of the fungus grown in liquid medium. Maximum sporulation and germination percentage of spores of the fungus were recorded in solid media compared to liquid media.

Microscopic observations showed that in all the solid media the hyphae were hyaline, septate, smooth and branched producing conical to flask shaped phialides with narrow neck. Phialides were branched often once. Spores were spherical, verrucose and hyaline. Hyphal width, phialide width and spore diameter ranged between 3.33 μm to 3.76 μm in different solid media. Maximum number of phialides in a unit length of hypha was in Sabouraud's maltose agar + yeast.

The fungus preferred an alkaline pH of 9. But it gave satisfactory growth on a wide range of pH from 5 to 9. Growth of the fungus was recorded only in temperatures of 25 and 30°C. No significant difference was recorded in the fungal growth at humidity levels of 50 to 90 per cent.

Both exotoxin and endotoxin were extracted from the liquid culture of the fungus. In closed perianth condition there was no significant difference in the per cent mortality of mites due to spraying with both the toxins, but it was superior than spraying with water (control). In open perianth condition there was no significant difference in the percentage mortality of mites on spraying with both the toxins and water.

The decreasing order of the inhibition of fungal growth by insecticides/acaricides was dicofol, carbaryl, triazophos, wettable sulphur and endosulfan. The fungicides bordeaux mixture and copper oxychloride showed cent per cent inhibition of fungal growth whereas potassium phosphonate (akomin) recorded minimum inhibition over control. Among the botanicals, maximum inhibition of fungal growth was with garlic + neem oil and azadirachtin showed least inhibition.

In the two phase mass production study, wheat was identified as the most promising raw substrate for sporulation and germination percentage of spores of the fungus. In the powdered substrates tried, growth was present only in loose substrates like rice bran and tea waste.