International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 08-08-2021, Accepted: 23-08-2021, Published: 08-09-2021
Volume 6, Issue 5, 2021, Page No. 342-345

Isolation and identification of *Hirsutella thompsonii* and management of *Aceria guerreronis* in Machenahalli, Shimoga district, Karnataka, India

Nafeesa Begum, Shaila M
Department of Botany, Sahyadri Science College, Kuvempu University, Shivamogga, Karnataka, India

Abstract
Aceria guerreronis is a serious pest of coconut in India. Investigations were carried out to investigate fungal pathogens infecting the eriophyid mites for their utilisation as biocontrol agents in Karnataka, India. The fungal pathogens namely, *Hirsutella thompsonii, Beauveria bassiana, Fusarium semitectum* and few opportunistic pathogens namely, *Fusarium moniliforme, Cladosporium tennuissimum, Aspergillus niger, Penicillium sp.* and Mucor sp. were collected from eriophyid mite populations in different parts of Machenahalli region. Of the total collected nuts, 3.54% were infected by *H. thompsonii*. The incidence of pathogen infected coconuts in areas with lower temperature and higher humidity. *H.thompsonii* was isolated from dead cadavers of coconut mite *Aceria gurreronis* on PDA and SDA media. For further field application the pure culture bought from NBAII, Banglore. The present work with the fungi *Hirsutella thompsonii* was mass cultured in SMB media weighed mat was tested as biopesticide in different formulations. Three replicates of a spore suspension of 0.5. 0.1, 1.5 and 2g/L sprays were carried out on the three youngest coconuts bunch. *H. thompsonii*, distilled water and Sabouraud Dextrose broth (2g+2mL+1L) showed reduction of mite population of about 6.29±0.17. When compared all the *H. thompsonii* treatments along with adjuvant glycerol showed effectiveness in reduction of *Aceria guerreronis* mite population.

Keywords: *Aceria gurreronis*, coconut, eriophyid mite, *Hirsutella thompsonii*, pathogens

Introduction
Hirsutella is one of the most abundant and important entomogenous fungi, and might play an important role in the control of pest insects in nature. *Hirsutella* includes three important species, *Hirsutella thompsonii, Hirsutella gigantea*, and *Hirsutella citriformis*. This genus has been one of the most difficult members for identification among all major genera of fungal entomopathogens largely because of the huge number of species and high variability among these species. *Hirsutella* sp has been found efficient against nymph and adults of red spider mite, adults. Varroa mite, (*Varroa destructor*) of Honey bees, Coconut eriophyid mite (*Aceria gueneronis*). (Narasa Reddy et al 2020) [11]. *Hirsutella thompsonii* is an important naturally occurring fungal pathogen of eriophyoid mites, especially citrus rust mite (*Phyllocoptrura oleivora*) which inhabits numerous host plants in subtropical and tropical regions. *H. thompsonii* is a parasitic fungus belonging to the phylum Deuteromycota (Fungi Imperfecti). Order Moniliales (McCoy Kanavel, 1969) and was first found in citrus orchards in Florida, USA (Speare and Youthers, 1924). The hyphomycete genus Hirsutella includes about 94 species; some of them are pathogenic to insects, mites and other invertebrates. Thus, they have been regarded as biological control of plant parasitic nematodes. *H. thompsonii* was also treated as a potential microbial control agent. (Kumar and Singh 2008) [16]. The genus HirsuteIla infects a number of different types of insects as well as mites and nematodes (Jaffee, 2000). In hot, humid weather, H. thompsonii can cause spectacular natural epizootics among mite populations (citrus rust, blueberry, coconut, tomato mites, etc.) and is considered to be one of the key natural enemies of various mite pests (Chandler et al., 2000). In vitro, this fungus displays, a simple growth cycle. Conidia germinate and produce the mycelia phase that gives rise to conidiophores and/or chlamydospores. To date, two strains of H. thompsonii var. thompsonii have been reported to produce and secrete Hirsutellin A (HtA), a Protein that has, potent insecticidal cytotoxic activities (Liu et al., 1995; Mazer and Vey 1995: Vey et al., 1993) suggesting that this protein may play a role in the pathogenic process.
The bio control agent is the important natural regulator of the coconut mite and this subsequently led to the development of Mycohit, a powder formulation of the fungus. The pathogen has been evaluated as a short-term to well as a long-term biocontrol agent since 2000. The fungus was found to be capable of bringing down the mite population up to 90%, resulting in considerable reduction in pre-harvest nut damage. In several trials, the fungal treatment was superior to azadirachtin. dicofol, triazophos, and/or wettable sulphur.

Mode of action
Hirsutella thompsonii acts through degradation of the mite's cuticle, with subsequent fungal growth in the haemolymph and tissues of the mites. Re-sporulation from dead mites leads to infection of epidemic proportions. Biology it is an entomopathogenic fungus specific to eriophyid mites that exerts its effect by invasion of the living mite. Spores adhere to the cuticle of the mite and under ideal conditions germinate producing a germ tube that penetrates the host mite's cuticle by physical and enzymic processes and subsequently invades the haemolymph and other tissues. The fungal hyphae develop in the mite and sporulation takes place through the living and dead mite's cuticle providing infectious spores to continue the epidemic.

Eriopyide mite pest, *Aceria guerreronis* which was so far not known to exist in India. This mite which is microscopic completes its life cycle hiding beneath the perianth of the coconut fruit. In the process, it sucks the sap from the tender nuts (two to seven months old) resulting in their malformations and ultimately 20-30% loss in terms of copra yield (Moore 2000). The protected habitat of *A. guerreronis* shields it from the effect of the chemicals, thus limiting their use in the on-going control programmes.

Hirsutella thompsonii can easily be cultivated on artificial media in contrast to most Entomophthorales. It grows on a variety of agar-based and liquid media (McCoy and Kanavel, 1969). The fungus *Hirsutella* is mesothermophilic. Growth, sporulation and conidial germination were best at 25 º – 30 º C. H. *thompsonii* has been cultured on agar media including potato dextrose, Modified Soil Fungus, Sabouraud-dextrose (McCoy and Kanavel, 1969). Dextrose and sucrose at an optimum concentration of 5 and 10 mg mL^{-1}, respectively, were the most effective sources of carbon among sugars tested for use in the large-scale production in submerged culture.

In view of this, investigations were under taken to isolate and identify potent fungal pathogen Hirsutella thompsonii, which was employed in the management of eriophyid mites in Machenahalli, Shimoga. In the present study the fungus *Hirsutella thompsonii* was isolated from *Aceria guerrerunis* and for field application the pure culture of *Hirsutella thompsonii* was bought from NBAII, Bangalore and which was mass multiplied and tested in both laboratory and field conditions.

Materials and Methods

Survey for fungi infecting the coconut eriophyid mite. A survey was carried out to investigate the occurrence of fungal pathogens of coconut eriophyid mite in the coconut farm at Machenhahalli near Shimoga district, Karnataka State. In this area, coconut growing regions were selected. Five palms were chosen randomly in each garden for collecting mite infested nut samples; two mite-infested nuts showing white or brown damage symptoms were removed from each of the fourth and fifth bunches (approximately four to five months old nuts) of the palm. Nuts from each palm were kept in separate paper bags; the bags were labelled with place of collection, date of collection and name of host plant. In total, 20 nuts were collected from each garden. The nuts collected were brought to the laboratory and kept in refrigerator at 4°C. In the laboratory, the bracts of the nuts were carefully removed to expose the colonies of the eriophyid mite present beneath the perianth. The dead cadavers of eriophyid mite were taken out and placed on media for isolation of fungi. The number of nuts infected with different fungal pathogens was recorded using a stereo zoom binocular microscope (Olympus BiMSZ). The percentage of infected nuts was calculated.

Isolation of fungi

Mycosed mites were placed on glass slides containing water agar and incubated at 25°C±1 for 24 to 48 h. The fungi were isolated following the procedure described by Lomer and Lomer (1995). The cadavers were surface sterilized with 0.1% sodium hypochlorite solution for 2 to 3 min in cavity blocks, and immediately rinsed with sterile distilled water three times, by transferring to cavity blocks containing 10 ml sterile distilled water to remove the traces of sodium hypochlorite to prevent toxicity to the fungus. Treated specimens were then placed on 20 ml water agar plates (agar: 20 g, water: 1000 ml, chloramphenicol: 80 mg) and incubated at 25±1°C. The developed fungi were subcultured and purified by hyphal tip method (Tuite, 1969).

Mass culturing of Fungi

The isolated *Hirsutella thompsonii* fungus was cultured over PDA medium. For further work, the pure culture of *Hirsutella thompsonii* was bought from NBAII, Banglore. The pure culture bought was mass cultured in different selected liquid media like SMB (Sabouraud Maltose Broth), SDB (Sabouraud Dextrose Broth), PDB (Potato Dextrose Broth) (Winkelhoff and McCoy 1984). The high yield given liquid media was further used for mass multiplication process for field application.

Pathogenicity of fungal pathogens against *A. guerreronis*

Four to five months old nuts with white or brown triangular patches, indicating the presence of active colonies of mites, were selected. On each nut, one triangular patch was selected, marked, and used for injection of spore suspension. The fungal suspensions were prepared as aforementioned. Using a syringe (1 ml capacity) with fine needle, 40 µl spore suspension (1×108 spores/ml) was injected into the space between the perianth and the nut surface where the white triangular patch was present. The point of injection was sealed using parafilm to prevent secondary infection. Three replications were maintained with each nut representing one replication. Nuts were then kept in polythene cover and retained in good condition up to one week. The treated nuts were incubated in a biological oxygen demand (BOD) incubator at 25±1°C (Kumar and Anuroop, 2004) [6]. The live and dead mites were observed and recorded five days after treatment. Mortality was calculated from the population of mites counted under a microscope at 5 randomly selected spots (4 mm diameter) on inner bracts and on nut surface. The dead mites were collected, subjected to re-isolation and were then used in identifying the fungus.

Efficacy of virulent fungal pathogen at different concentrations against coconut mite

Different concentrations of spore suspension of the pathogens were prepared as aforementioned. About 40 µl of spore suspension injected between the bracts and nut surface, where the white or triangular patch was present; each nut represented one replication and as such, five replications were maintained. The infected part of the nut was covered by parafilm to prevent secondary infection by making the bracts intact with nut surface (Kumar and Anuroop, 2004) [6].

Result and Discussion

In the present work the *Hirsutella thompsonii* isolated from dead cadavers of coconut mite *Aceria guerreronis* on Potato Dextrose Agar (PDA) and Sabradose Dextrose Agar (SDA) media (McCoy and Kanavel. 1969).

For further field application the pure culture bought from NBAII. Bangalore was grown on PDA media and which was further cultured in three different liquid broth media like SMB (Sabouraud Maltose Broth). SDB (Sabouraud Dextrose Broth). PDB (Potato Dextrose Broth). In that we obtained high yield of mycelial mat dry weight from SMB media of about 2.43g/150mL (Table: 1).

Maximum vegetative growth occurs on media high in carbon, and maximum condition on nutrient-deficient agar

(McCoy and Kanavel, 1969). Dextrose and sucrose at an optimum concentration of 5 and 10 mg mL $^{-1}$ respectively were the most effective sources of carbon among sugars tested for use in the large-scale production in submerged culture. In the present work the maximum yield of mycelia mat was obtained by SMB media (Fig: I) so further mass multiplication of *Hirsutella thompsoni* was carried with the SMB media.

Table 1: Dry mycelial weight (g/150 mL) of *H. thompsonii* on the three different liquid media (after 20 days of inoculation).

Isolate media	*Hirsutella thompsonii* g/150mL
SMB	2.43±0.09
SDB	1.98±0.01
PDB	1.43±0.09

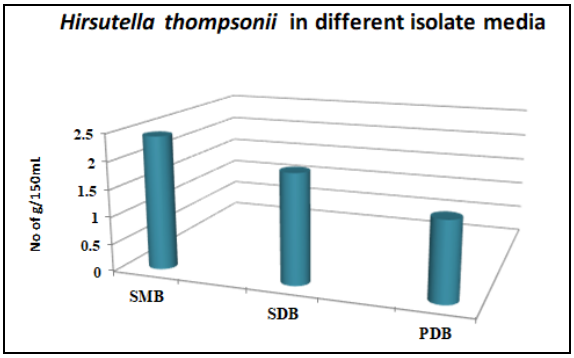

Fig 1: Pattern of mycelia yield on various media

Kalmath et al., 2012 reported that, the pathogens collected were includes *Hirsutella thompsoni*, *Beauveria bassiana*, *Fusarium semitectum*, *Fusarium moniliforme*, *Cladosporium tennuissimum*, *Aspergillus niger*, *Penicillium* sp. and *Mucor* sp. Were used for pathogenicity test against eriophyid mites at 1x108 spores/ml, in that the isolate HTCMBAN was significantly superior to other treatments, causing 88.63% mortality. The virulent isolate *Hirsutella thompsonii* (HTCMBAN) was tested at different concentrations, which was showed with increase in the concentration mortality rate. The present work with the fungi *Hirsutella thompsonii*, was mass cultured in SMB media and after 11-20 days of incubation, the mycelia were harvested from the liquid medium by filtering the liquid through a filter paper (Watman No. 1) which had been dried for 24 h at 60°C and weighed. The weighed mat was tested as biopesticide in different formulations. Three replicates of a spore suspension of 0.5. 0.1, 1.5 and 2g/L sprays were carried out on the three youngest coconuts bunch. The treatments carried out for four weeks in different interval period in coconut mite-infested coconut field. The population of mite reduced in decreasing order with the different formulations of treatments (Fig: 2).

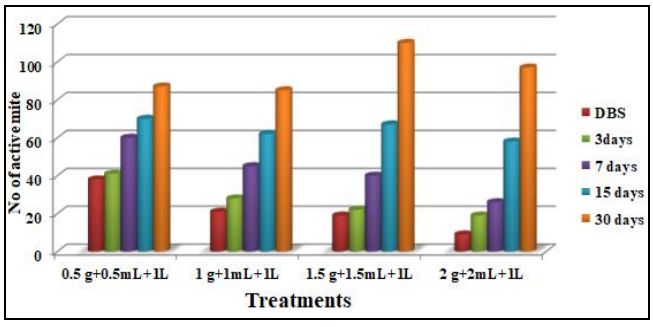

Fig 2: The *Hirsutella thompsonii* with Distilled water (g/L) treatment showing reduced *Aceria guerreronis* mite population.

DBS-Day before spraying, DAS- Day after spraying.

Kumar and singh (2008) [16] carried out work over enabling mycelia application of *H. thompsonii* in management of coconut mite where they carried out a series of experiments with different substance as adjuvant in enabling mycelia application. Glycerol, yeast extract powder and dehydrated malt extract broth were found to be the best among nine substances investigated as possible adjuvants for use on coconut palms in the field along with *H.thompsonii* mycelia. Glycerol boosted the pathogenicity of *H. thompsonii* by 16.5% over control. Application of the fungus in combination with glycerol resulted in a tolerable mean nut damage grade of 2.0 during the pre-harvest stage, compared with an acute score of 4.0 in control palms. In the present work *Hirsutella thompsoni* dried powder and distilled water of different formulations were tested in three replicates *H. thompsonii* and distilled water (2g+1 L) showed mite population reduced about 9.69±0.57/4 mm diameter (Fig:2). Next the two adjuvants were selected and pathogenicity of *H. thompsonii* along with adjuvants were tested in laboratory condition and then applied for field application. After four week of application The *H. thompsonii*, Glycerol and distilled water (2g+2mL+1L) showed reduction of mite population of about 5.61±0.49 (Fig: 3) and *H. thompsonii*, distilled water and Sabouraud Dextrose broth (2g+2mL+1L) showed reduction of mite population of about 6.29±0.17 (Fig: 4).

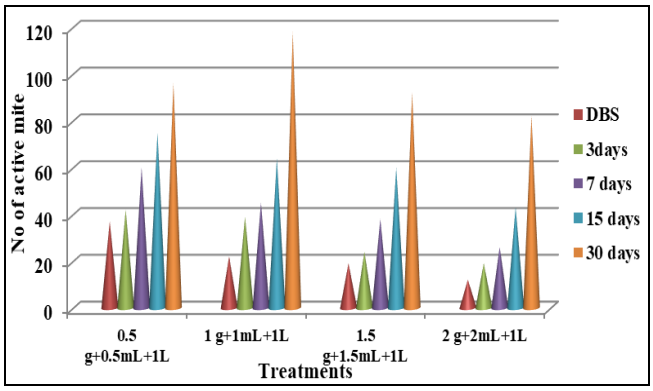

Fig 3: The *Hirsutella thompsonii* mixed with Glycerol and Distilled water (g/mL/L) treatment showing reduced mite population.

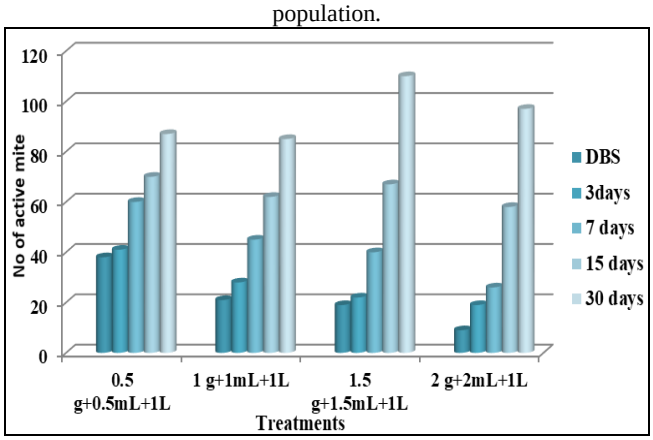

Fig 4: Bio control agent extracted from SDB. Growth and treated ou suite showing reduced population.

Analysis of variance (ANOVA) showed a significant difference ($p<0.01$) in efficacy between Tested formulations of *H. thompsonii*, and between the coconut eriophyide mite with respect to their sensitivities to the *H. thompsonii*.

Conclusion

Natural or augmented biological control of the coconut mite is here to stay. There has been a steady build-up in the diversity of natural enemy species, as well as their populations over the past few years in the regions infested with *A. guerreronis*. Although a suite of biological control agents remains desirable, the single currently available agent of *H. thompsonii* needs further thrust in the near future. Through a systematic research and development programme, adequate knowledge has been generated on the utility of *H. thompsonii* as a mycoacaricide for the coconut mite in India.

Reference

1. Basavaraj Kalmath, Mallik B, Onkarappa S, Girish R, Srinivasa N. "Isolation, genetic diversity and identification of a virulent pathogen of eriophyid mite, *Aceria guerreronis* (Acari: Eriophyidae) by DNA marker in Karnataka, India", African Journal of Biotechnology,2012:11(104):16790-16799.
2. Beevi P, Beena S, Varma SA. Report of pathogen, *Hirsutella thompsonii* var. synnematosa Samson, McCoy and Dnnell on coconut mite, Aceria guerreronis (Keifer). Insect. Environ,2000:5:90-191.
3. Girija VK, Umamaheswaran K, Paul A. Cellotape embedding technique for assessment of population of coconut eriophyid mite, *Aceria (Eriophyes) guerreronis* Keifer. Ins. Environ,2001:7(1):35.
4. Gopal M, Saleena A, McCoy CW. Study of the microflora associated with coconut eriophyid mite as a preliminary step towards pathogen isolation. Curr. Sci,2002:82(1):22-24
5. Kannaiyan S, Rabindra RJ, Ramaraju K, Doraiswamy S. Integrated management of eriophyid mite on coconut. Tamil Nadu Agricultural University, Coimbatore, India, 2002, 82.
6. Kumar PS, Anuroop CP. A method to test the pathogenicity of fungi to Aceria guerreronis, with particular reference to Hirsutella thompsonii. Syst. Appl. Acarol,2004:9:11-14.
7. Kumar PS, Singh SP. Development of 'Mycohit' the first mycoacaricide base exclusively on H. thompsonii, for suppressing the coconut mite in India. Bioinoculants for sustainable agriculture and forestry: Proceedings of the National Symposium,2001:16-18:209-213.
8. Mikunthan. Utilization of fungal pathogens for biological control of thrips (Scirtothrips dorsalis) and mite (Polypahagotersnemus latus) on chilli. Ph.D Thesis. University of Agricultural Science, Bangalore, 2004, 1-200.
9. Muralidharan K, Jacob Mathew, Thampan C, Amarnath CH, Anithakumari P, Chandrika Mohan et al. Pilot sample survey on the incidence and yield loss due to eriophyid mite in coconut in Alappuzha district. Indian Coconut Journal,2001:32:28-32.
10. Muthiah C, Bhaskaran R. Major outbreak of eriophyid mite of coconut in India. Planter,2000:76(889):243-246.
11. Narasa Reddy, Mahesh G, Priya M, Shailendra Singh RU, Manjunatha L. "Benificial Microbes in Agroecology, Chapter 43: Hirsutella, Academic Press, 2020, 817-831.
12. Ramaraju K, Rabindra RJ. Palmyrah, Borossus falbellifer Linn. (Palmae: a host of coconut eriophyid mite Aceria guerreronis Keifer. Pest Management in Horticultural Ecosystems,2001:7:149-151.
13. Sathiamma B, Nair CPR, Koshy PK. Outbreak of a nut infesting eriophyid mite, Eriophyes guerreronis (K.) in coconut plantations in India. Indian Coconut Journal,1998:29(2):1-3.
14. Sirous A. Characterisation and mass culturing of some isolates of *Hirsutella thompsonii* Fisher and their bio efficacy against citrus rust mite *Phyllocoptruta oleivora* Ashmead (Acari: Eriophyidae). Ph.D Thesis, University of Agric. Sci. Bangalore, 2002, 1-154.
15. Sreerama Kumar P. "*Hirsutella thompsonii* as a mycoacaricide for *Aceria guerreronis* on coconut in India: research, development, and other aspects", 2011, 441-443.
16. Sreerama Kumar P, Leena Singh. "Enabling mycelial application of Hirsutella thompsonii for managing the coconut mite", Journal Experimental and Applied Acarology,2008:46:1-4:69-182.
17. Sujatha A, Chalapathi Rao NBV. Studies on coconut eriophyid mite in Andra Pradesh. Indian Cocon. J,2004:32(12):8-11.
18. Thalakkattil Raghavan SOBH, Mohamed Abdul HAQ "Population dynamics of the coconut mite, *Aceria guerreronis* Keifer in Kerala, India", Acarological Studies,2019:1(2):165-173.
19. Thomas RJ, Nair RV Nair, Nampoothiri CPR, Mathews CKC. Growth and vigour of coconut seedlings raised from eriophyid mite (Aceria guerreronis Keifer) infested seed nuts. Journal of Plantation Crops,2004:32:25-29.