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Formulation of a biocontrol product for the coconut eriophyid mite using entomogenous fungus *Hirsutella thompsonii* fisher

Sandeep Garg[1], Lal Sahab Yadav[2*]
[1] Assistant Professor, Department of Zoology, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar, Mumbai University, Maharashtra, India
[2] Assistant Professor, Department of Botany, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar, Mumbai University, Maharashtra, India

Abstract
Coconut mites (CM), *Aceria guerreronis* Keifer, are the most notorious and an important pest of coconut crops widespread in almost all coconut producing countries. Although CM has been reported worldwide and has been damaging coconuts, which causes huge losses in coconut production in India, Asian countries and in Europe. The biological control of CM using active entomopathogenic fungi is a good sustainable alternative over the chemical pesticides. The objective of this study were to assess the pathogenicity of an Entomogenous fungus *Hirsutella thompsonii against Aceria guerreronis* under laboratory conditions, optimization of media for mass production, formulation of product and *in-vitro* application. Application of formulated product (1×10^7 spores per gram) applied by three different modes *viz*. Dry (dust), wet (liquid) and wet+ dry. The mite mortality was assessed after four and eight days of inoculation. Among three mode of application wet+ dry mode showed excellent result over others. The mortality percentage after 4 days of incubation was 62% while after 8 days of incubation percentage of mortality rises up to 81 %. This study demonstrates that *Hirsutella thompsonii* is a promising candidate for the development of biological control agent against coconut Eriophyid Mite.

Keywords: *cocos nucifera, eriophyid mite, hirsutella thompsonii* fisher, biocontrol, coconut mites

Introduction

The coconut palm (*Cocos nucifera* L.) hailed as a "Tree of Heaven "is a long-growing palm and Nature's gift to human civilization from time immemorial. This tree has got many quotes in various religious inscriptions and literatures including "Ramayana". Coconut is one of the important cash crops in tropical & sub-tropical countries. Coconut is grown in more than 93 countries of the world and Indonesia, Philippines, India are the major coconut producing countries of the world. Traditional areas of coconut cultivation in India are the states of Kerala, Tamilnadu, Karnataka, Andhra Pradesh, Orissa, Goa, West Bengal, Pondicherry, Maharashtra and Islands of Lakshadweep and Andaman and Nicobar. However, several states like Assam, Gujarat, Madhya Pradesh, Bihar, Tripura, Manipur, Nagaland and Arunachal Pradesh have emerged as non-traditional areas for the cultivation of coconut (Coconut Development Board of India 2010). Coconut palm has now a wide pantropical distribution (Chan and Elevitch 2006).

Every part of coconut fruit is useful to get important products to human use. Currently, hundreds of millions of people consume everyday both coconut water, kernel derivatives and several products derived from fruit (Foale 2003). Probably due to the wide dissemination of coconuts around the globe, many coconut pests have also became widely distributed. Coconut palms are attacked by nearly 200 types of insects and pests (Rabindra, 2000). Among them, rhinoceros mites, rats, Rhinoceros beetle, red palm weevil, black head caterpillar, and *scarabaeidaeis* are the major pests found on coconut trees. However, some new invasive pests associated with coconut trees have been a cause of concern during the last decades. These including the coconut leaf beetle, *Brontispa longissima Gestro* (Aquino 2008); the palm red mite, *Raoiella indica Hirst* (Pena *et al*. 2006; Welbourn 2009), and the coconut mite (CM), *Aceria guerreronis Keifer*.

Among the various pests, coconut eriophyid mite, *Aceria guerreronis Kiefer* is a serious mite pest and these mites live by sucking the sap from tender meristematic tissues of nuts. The damage initially appears as a triangular patch at the level of the perianth, when the nut grows this injury on the nuts leads to warting and longitudinal fissures on the nut surface. This phytophagous mite spread to most coconut production areas worldwide and has been considered as one of the most notorious and important pests of coconut fruits in many countries. In recent years, severe infestations of *Aceria guerreronis* Keifer (Acari: Eriophyidae) in many countries have had a negative impact on production and resulted in severe economic losses. (Fernando *et al*. 2002; Haq 2011) Due to the very large plant and coconut fruits at huge height and the size of pest are very small it is difficult for growers to detect it on crops before the development of symptoms on fruits. *Aceria guerreronis* populations develop on the meristematic zone of the fruits covered by the perianth (bracts). Feeding in this zone causes physical damage that leads to necrosis of perianth (Moore and Howard 1996). Mites also infested to inflorescences during the dispersion process. Infection of CM creating a havoc among coconut growing farmers, due to its mode of infection & ability to damage the fruit (Murali Gopal; Alaka Gupta 2003) [12]. Mite infestation significantly reduced fiber quality and reduced length by 26–53% in moderately to heavily infested nuts (Naseema *et al*., 2003). An outbreak of

Eriophyid mites on coconuts in Maharashtra affected about 84% of palm trees

There are several cultural practices and nutrients management has been recommended to cultivate the coconut with recommended doses of fertilizers to prevent from diseases and pest. Some botanicals like Fenazaquin 10 EC, Propargite 57 EC, Monocrotophos 36 SL, Dicofol 18.5 EC, Wettable sulphur 80 WP, Triazophos 40 EC etc. are used to prevent coconut from Eriophyid mites. Herbal products also have been used for prevention and control time to time by coconut grower.

The application of entomopathogenic fungi to control Eriophyid mites are a more sustainable alternative to chemical pesticides (Wekesa *et al*., 2015). Entomogenous fungi are used in classical and augmentative biological control strategies because they are environment friendly and cost effective over chemical pesticides (Lacey *et al*., 2001; Shah *et al*., 2003). Several Entomogenous fungi *viz*. *Hirsutella thompsonii* var synematosa, *Verticillium lecani*, *Verticillium chlamydosporium*, *Entomophtora* sp., *Beaveria bassiana*, *Paecilomyces lilacinus, Sporothrix fungorum* and P. *gracilis* are efficient biological control agents of a wide range of mites (Amritha & Pathummal, 2009, Camille Minguely *et al*., 2021) [1].

The objective of this study is to standardization of culture media for cultivation of Entomogenous fungi *Hirsutella thompsonii*, their mass multiplication (product formulation), effective modes of application and assess the pathogenicity against the Eriophyoid mite, *Aceria guerreronis* under laboratory conditions.

Materials and Methods

Procurement of Fungal Culture

Fungal culture of *Hirsutella thompsonii* Fisher procured from MTCC, Chandigarh (Microbial Type Culture Collection, Accession No. 2339) and sub-cultured on PDA media. Pure culture preserved in mineral oil, 15% glycerol and in the form of slant for further work.

A. Characterization of Media for growth of *Hirsutella thompsoni* Fisher

To get prominent growth in submerged culture, four different media were used, namely Sabourds Dextrose broth (SDB), Potato Dextrose broth (PDB), Rice Dextrose Broth (RDB), Czapek Dox Broth (CDB). The composition of media as Sabourds Dextrose Broth (SDB): Peptone (10 g/l), Dextrose (40g/l), pH (5.5)., Potato Dextrose broth (PDB): Starch (20 g/l), Sugar (15 g/l) pH (5.5). Rice Dextrose broth (RDB): Rice Starch (200 ml/l); Yeast Extract (3 g/l); Sugar (10 g/l), pH (5.5). Czapek Dox broth (CDB): $NaNO_3$ (2 g/l), KCl (0.5g/l), $MgSO_4$ (0.5 g/l), K_2HPO_4 (1 g/ l), FeSO4 (0.01 g/l), Sugar (20g/l) pH (5.5).

A loopful culture of *Hirsutella thompsonii* grown on the PDA medium, inoculated in flask containing 500 ml broth of SD, PD, RD, & CD respectively. Flasks were kept on rotatory shaker at 200 rpm, for 8 days at room temperature. After 8 days, conidial growth observed in the flasks. This broth culture was used for the formulation process.

Biocontrol Product Formulation

Under the aseptic conditions *Hirsutella thornpsonii* culture grown in Czapek Dox broth mixed in an inert carrier material (sterile talc powder grade-3) and blended properly so that inoculums homogenously spread in talcum powder. Formulation was carried out in the proportion of 1: 3 (1-part culture and 3-part carrier material). Formulated mixture kept for drying in sterile container for 24 hrs. After the moisture escapes out, powder mixture filled in plastic bags under the aseptic conditions & sealed it properly.

Quality Testing

Total Viability Count (TVC) was performed to know the initial CFU (colony forming units) of product by serial dilution method taking 1 gram of product and product was sampled for analysis to know shelf life at room temperature. For shelf life of product TVC was performed after two months of interval up to one year. For TVC, 1 gm of biocontrol product was taken in 9 ml of sterile water and serial diluted up to 10^9. Then 1 ml of fungal suspension was added to sterile plates and molten cooled media was poured in the respective plates. Suspension was properly mixed with media by rotation of plates and plates were allowed to solidify in incubation at RT for 24-48 Hrs. After 48 hrs, number of colonies of microorganisms were counted and CFU / ml/gm was noted down.

B. Bioassay of *Hirsutella thompsonii* against *Aceria guerreronis*:

For *in-vitro* bio assay, Coconut fruits of about 2-3 months old which were heavily infested by *Aceria guerreronis* (Eriophyid mite) obtained from 'Regional Coconut Research Centre (Ratnagiri). *Aceria guerreronis* Mites were reared on tender coconut fruits in a moist chamber at room temperature with a relative humidity of 70% and a photoperiod of 10 h. for *in-vitro* bioassay. After successful rearing of mite's underside, the perianth of tender coconut fruit, perianth were removed and formulated product was applied on known population of mites (50 number) in the form of wet-solution (1g in 5ml D.W) and dry (dust) and Wet +Dry. The death of mites was confirmed by method describe by Omoto *et al*., 1994 [13]. The mortality percentage was calculated after 4 and 8 days of application and data were analysed statistically (Lefort *et al*., 2014) [8] Application of formulated product was also tested on infested coconut directly by following three different modes.

1. Wet Application: 5 gm of formulated mixture mixed in 50 ml of distilled water, few drops of Tween 80 (a surfactant) added in the solution and sprayed over the perianth of infected fruits using hand sprayer.
2. Dry Application: 5 gm of formulated mixture dusted over the perianth of infected fruits.
3. Wet + Dry Application: First infested coconut was moistened with diluted Tween 80 using sprayer and then after 5 gm of formulated powder was dusted over the fruits, so that it sticked to the surface of nut.

A control set was maintained to compare the result using 5 ml distilled water as product. After the application, all nuts were kept separately in polvthene bags with cotton plug on it so as to maintain the humid condition. After 10 days, observations were recorded.

Observations and Results

Hirsutella thompsonii Fisheris is a slow growing fungus which forms very scanty whitish mycelium on culture media. Four different culture media (broth) were used to test for good mycelial mass and spore production. Among four selected media Czapek Dox broth was found more suitable

over other three in terms of amount of mycelial mass formation and spore count in liquid media. Sporulation in *Hirsutella thompsonii recorded* in CDB after 4 days of incubation where sporulation obtained in RDB after 7 days, and SDB after 9 days, no sporulation in PDB (Table-1, Photo Plate-1).

Table 1: Vegetative mycelial growth and sporulation of *Hirsutella thompsonii* on different media

Media	Fresh wt. of mycelium after 10 days of incubation	Spore count after 10 days of incubation (CFU)
Potato Dextrose Broth (PDB)	98gm/500ml	-
Sabourds Dextrose Broth (SDB)	85gm/500ml	1×10^6
Rice Dextrose Broth (RDB)	155gm/500ml	1×10^6
Czapek Dox broth (CDB)	166gm/500ml	2×10^8

Photo Plate-1: A. *Hirsutella thompsonii* culture on PDA. B and C Mycellium and Spores D. Biomass production in submerged culture

Formulation of Product using submerge culture of *Hirsutella thompsonii*

Based on growth performance of *Hirsutella thompsonii* in different media (table-1) Czapek Dox broth was selected for mass multiplication of fungus in submerged condition. The optimization of growth condition like pH of media, temperature and incubation condition were standardized and pure culture inoculated in sterilized Czapek Dox broth (500ml) media in conical flask. After 10 day fully grown culture (conical flask) 500ml mixed in sterilized carrier material (talcum powder grade-3) in ratio 1:3 and mixed it properly and physical condition of product was analysed (Table-2)

Table 2: Physical Condition of Product and their CFU

CFU/g	Texture	Moisture	Colour	odor
2.8×10^8	Fine powder	55%	Off white	Nil

C. Bioassay

The formulated product of *Hirsutella thomsoni* was undertaken for their efficacy testing *in-vitro*. The Perianth of treated nuts was removed with the help of knife and mite

colonies were observed under the sterio-zoom microscope. The mouth parts of the mites were gently pinched with a fine needle to see the stimulus response and the mites were considered dead if they did not react. At two measurement point (day after application) mortality of Eriophyte mites was highest in wet+ dust application in compare to other two modes (fig.1). Thin section of infected area was also taken and observed the internal mycellial growth in the insect body. The entomopathogenic action of *Hirsuella thopsoni* was confirmed by placing dead mites on PDA plate and pure fungal colony was obtained. Photo Plate-2.

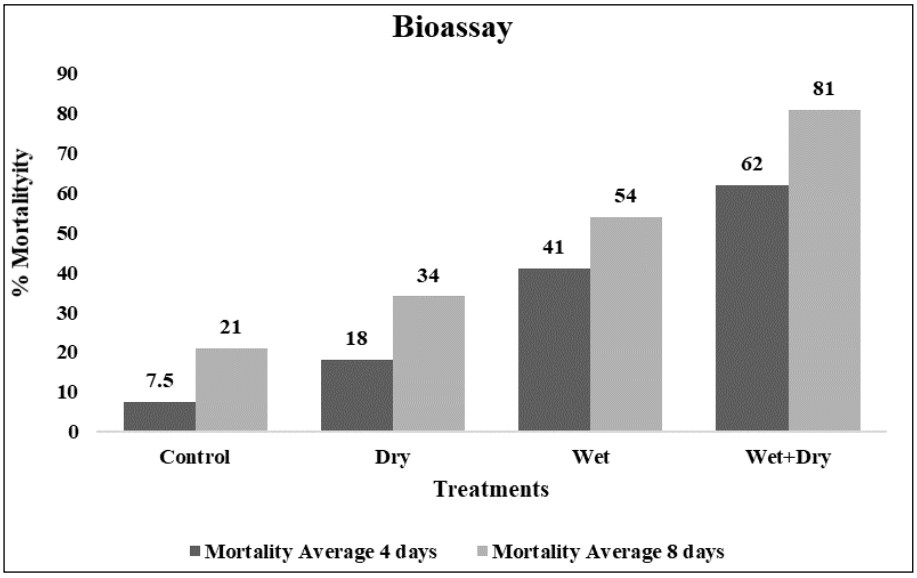

Fig 1: Percentage mortality of Eriophyid Mite by application of product in three different modes

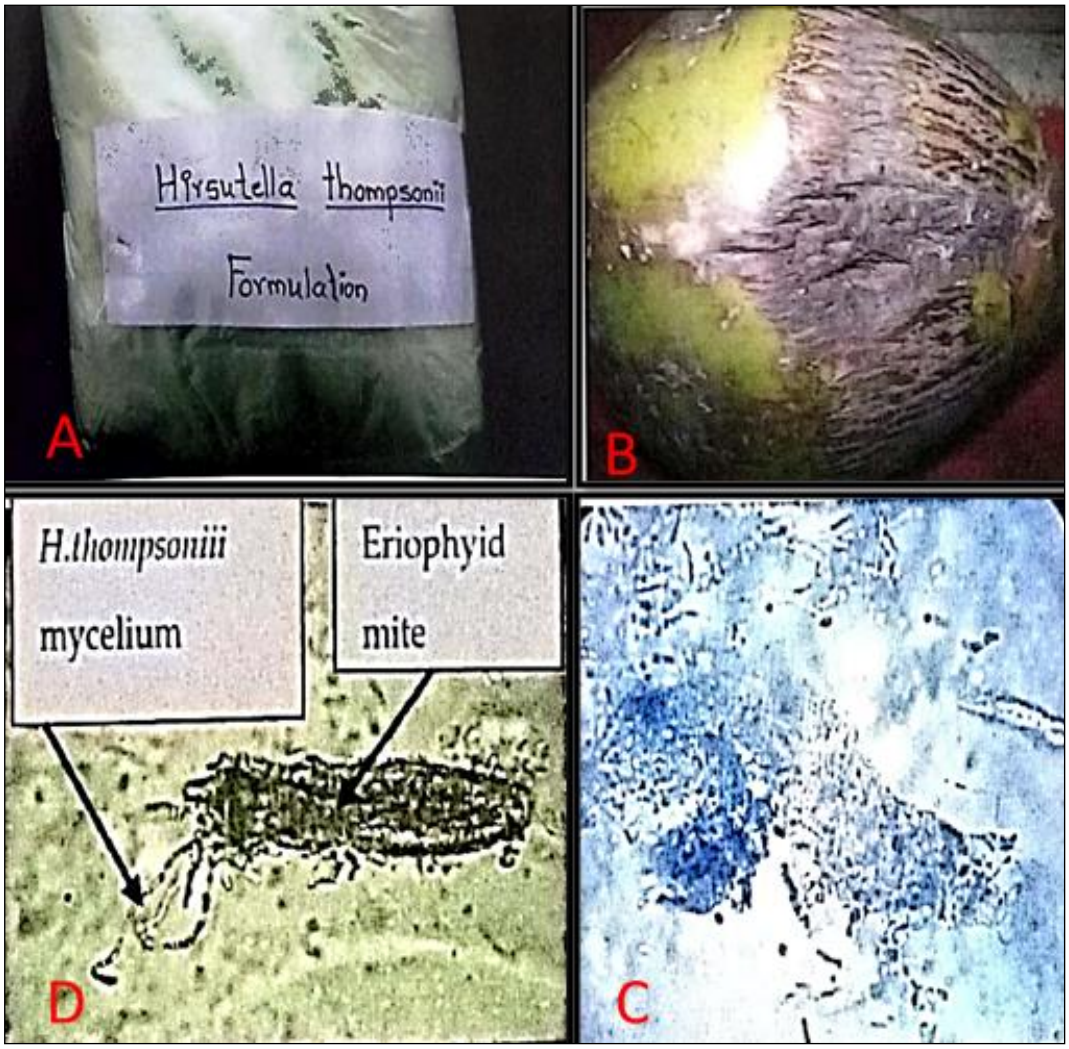

Photo Plate-2: A. Product of *Hirsutella thompsonii* B. Infected Coconut. C. Dead mite colonized by fungal mycellia D. Section of infected part

Discussion and Conclusion

It has been observed that *Hirsutella thompsonii* Fisher shows rapid conidia formation in Czapek Dox media, other tested media showed scanty sporulation in submerge culture. it might be due to inorganic salts and some macro and micro elements were available in the said media. The other tested media contains only carbon sources like starch and glucose. Analysis of formulated product showed excellent CFU count 2 x 10^8 in CDB media, followed by 10^6 on RDB and SDB media. This suggests that Czapek Dox media can be used as mass culture media for *Hirsuiella thompsonii*,

Bioassay results showed that, as long as humid conditions are maintained *HirsuteIla thompsonii* grows well under the perianth and causes death to Eriophyid mite. As far as mode of application is concerned wet + dry application gave better results, 62% Mortality obtained after 4 days of application and after 8 days percentage rises up to 81 % which suggesting the excellent efficiency of the product and mode of applicability. As dry powder stick on the body part of mites and to the surface of nut and spores are germinated in humid condition, which infect directly to the mites resulting high mortality. Lowest mortality percentage was observed by dry mode of application, which was 34% after 8 days. The application of product solution showed 41 and 54 percent mortality respectively after 4 and 8 days of application. Durinnf course of application and observation it was observed that infection of *Hirsutella tlioinpsonii* in mite population increases when there is an increment in the moisture and humidity. So dusting of formulated product at the time of humid condition will be recommended. As this fungus is very much host specific in action so there is no any environmental threats. The described method in this research to make product of Entomogenous *Hirsutella thompsonii* is very simple which can be materialized in less expenses in small setup. Coconut grower can use this bio-control agent in the form of dusting on plants as a precautionary measure against this infection.

References

1. Minguely C, Norgrove L, Burren A, Christ B. Biological Control of the Raspberry Eriophyoid Mite Phyllocoptes gracilis Using Entomopathogenic Fungi. Horticulture,2021:1(7):54.
2. Chandler, *et al*. Biocontrol Science and Technology. Taylor And Francis Ltd.,2000:10(4):357- 384.
3. Chandler D, Davidson G, Jacobson RJ. Biocontrol Science and Technology. Taylor and Francis Ltd.,2005:15:37-54.
4. Donald W Roberts, Richerd A. Humber. Biology of Conidial Fungi. Academic Press Inc.,1981:2(21):219-217.
5. Hill DS, Waller JM. Pests and Diseases of Tropical Crops, Field Hand Book. Longman Scientific and Technology Publication., 1990, 115-117.
6. Fisher FE. Journal Econ. Entomol.,1950:43:305-309.
7. Keifer HH. Eriophyid Studies B-14. California Dept. of Agriculture. Bur. Entomol., 1965, 20.
8. Lefort F, Fleury D, Fleury I, Coutant C, Kuske S, Kehrli P, *et al*. Pathogenicity of entomopathogenic fungi to the green peach aphid Myzus persicae Sulzer (Aphididae) and the European tarnished bug Lygus rugulipennis Poppius (Miridae). Egypt. J. Biol. Pest Control,2014:24:379-386.
9. McCoy CW, Kanavel RF. Isolation of Hirsutella thompsonii from Citrus Rust mite, Phyllocoptruta oleivora and its cultivation on various synthetic media. Journal of Invertebrate Pathology.,1969:14:386-390.
10. Mains EB. Entomogenous species of Hirsutella, Tilachlidium, and Synematium. Mycologia., 43, 691-718.
11. Mathew J. Central Plantation Crops Research Institute, Regional Station, Kayangulam, Pers. Commun.
12. Murali Gopal, Alka Gupta. Has Hirsutella thompsonii wherewithal to counter coconut eriophyid mite Scourage? Current Science.,2003:80(7):831-836.
13. Omoto C, Dennehy TJ, McCoy CW, Crane SE, Long JW. Detection and characterization of the interpopulation variation of citrus rust mite (Acari: Eriophyidae) resistance to dicofol in Florida citrus. J. Econ. Entomol.,1994:87:566–572. [CrossRef]
14. Sreerama Kumar P, Singh SP. Coconut Mite in India: Biopesticide Breakthrough. Biocontrol News and Information.,2000:22:4.
15. Vimala Devi PS, Prasad RD. Hands on Training on Microbial Agents of Major Insect Pests and Diseases of Crops, Manual, Directorate of Oilseeds Research, Hyderabad., 53-54.
16. Shivprasad Desai. Infection of Eriophyid Mite in Konkan Area. Agrowon. Sakal Press. 14-7-07., pp 1- 4.
17. Maimala S, Tarter A, Boucias D, Chandrapatya A. Detection of Toxin Hirsutellin A from Hirsutella thonipsonii. Journal of Invertebrate Pathology. Academic Press.,2002:80:112-126.