Has *Hirsutella thompsonii* the wherewithal to counter coconut eriophyid mite scourge?

Murali Gopal and Alka Gupta*

Coconut, an important plantation crop, is currently facing a serious threat from a newly reported 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. The protected habitat of A. guerreronis shields it from the effect of the chemicals, thus limiting their use in the ongoing control programmes. A 'green' alternative to this, in the form of biological control, using an entomopathogenic fungus Hirsutella thompsonii, promises to be the answer for sustainable management of this minute pest. This article analyses the potential of this fungal candidate to tackle the pest in the light of the research work done in India and elsewhere.

The issue

The coconut growers of southern India would never have faced such a crunch situation before, for, on the one hand, with the Indian government lifting import restrictions on coconut and coconut products, the competition from neighbouring countries will be enormous and on the other hand, a recent scourge by a hitherto unreported coconut pest in India, is crippling this plantation crop and industry.

Genesis of the mite problem

The pest in question is a minute arthropod identified as an eriophyid mite, *Aceria guerreronis* Keifer. Though the symptoms of damage of this pest were described as early as 1949 from Columbia by Martyn[1], the agent causing this was described in 1965 from the Guerrero State, Mexico[2], thereby conferring the specific name as *guerreronis*. It was subsequently observed in coconut-growing regions of Latin America and West Africa[3]. Moore and Howard[4] raised serious concern about the spread of this mite to Asia and Oceania, where coconut has a much greater role in the daily life of individuals and suggested that the outcome in terms of losses will be far worse if the mite attacks coconut in these regions. Their fear did not seem to be unfounded, for ever since *A. guerreronis* was first reported from Ernakulam district of Kerala in 1998 (ref. 5), it has been marching rampantly to the nearby coconut-growing states like Tamil Nadu[6], Karnataka and Andhra Pradesh[7,8] and along the east and west coast towards the north of India. The intensity with which this pest has wreaked havoc to the coconut groves has stirred up the farmers, scientists, policy makers and media to be seized of the problem and to be vocal about it.

Biology of the mite and damage it causes

A. guerreronis is microscopic in dimension, the adults are of 35–50 µm width and 200–250 µm length[2,9] (Figure 1 *a* and *b*). They have a high reproductive rate and a very short life cycle of 10–11 days[9]. The meristematic zone of the coconuts, covered by the perianth (also referred as tepals or bracts) is the site for the mite development (Figure 2 *a* and *b*). The mites suck the sap from the tender tissues using their cheliceral stylets[10], resulting in whitish triangular patches at the base of the perianth which later turns brown, followed by warting and suberization (thickening) of the nut epidermis (Figure 3 *a–d*). This leads to (a) drying of young buttons; (b) premature nut dropping; (c) reduction in nut size; and most important of all (d) loss in copra yield to the extent of 20–30% (refs 11–13). Yield losses are also compounded because of compaction and toughening of the mesocarp (coir) fibres which increase the labour requirements for dehusking.

The dispersal of eriophyid mite has been hypothesized to take place by many methods; however, the exact mechanism is yet to be elucidated[4].

Control strategies

Chemicals

Management of *A. guerreronis* is very difficult because of its cryptic nature of breeding beneath the tightly

Murali Gopal and Alka Gupta are in the Central Plantation Crops Research Institute, Regional Station, Kayangulam, Krishnapuram 690 533, India.
*For correspondence. (e-mail: murali_g67@usa.net)

adpressed bracts, which afford protection from direct contact with pesticides that are applied. A host of chemicals have been tried for the control of *A. guerreronis* by spraying, stem injection and also by root feeding techniques. Appreciable control had been achieved by using dicrotophos, monocrotophos, chiromethionate, cyhexatin, methyl demeton and triazophos[9,11,14–18]. In addition to these, Endosulfan, Dicofol and Carbosulfan have also been proved to be effective for the management of the mite[19]. Use of wettable sulphur[16,19–21], apart from botanicals based on combination of neem oil (*Azadirachta indica*) 2% and garlic (*Allium cepa*) and Azadirachtin, 0.004% (refs 19 and 22) has also given good results. Depending solely on the chemicals for the control of this mite is questionable since frequent applications are necessary, owing to the very short life cycle and the very high rate of multiplication of this pest. Moreover, spraying of the pesticide should be done from the top of the bunches such that the perianth area and the nuts are effectively drenched. Despite taking a lot of care the actual quantity of the chemical reaching the target site is less compared to the loss by drift to non-target area. Ecocidal effect, residual toxicity in the matrix of the nut due to improper application and above all economics restrict the use of pesticides.

Parasitoids

Amongst the natural control agents, as with other mites, coconut eriophyid mites are also not attacked by para

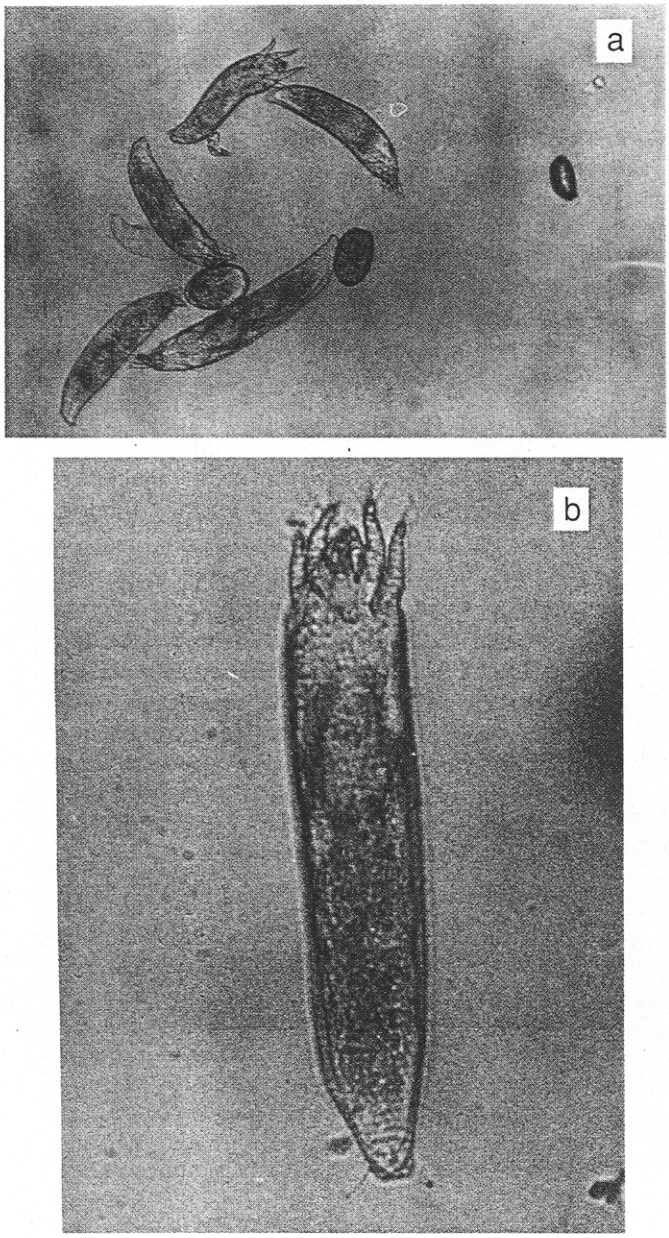

Figure 1. *a*, A cluster of coconut eriophyid mites in different stages of growth and their eggs; *b*, Fully grown adult mite with characteristic anteriorly placed two pairs of legs.

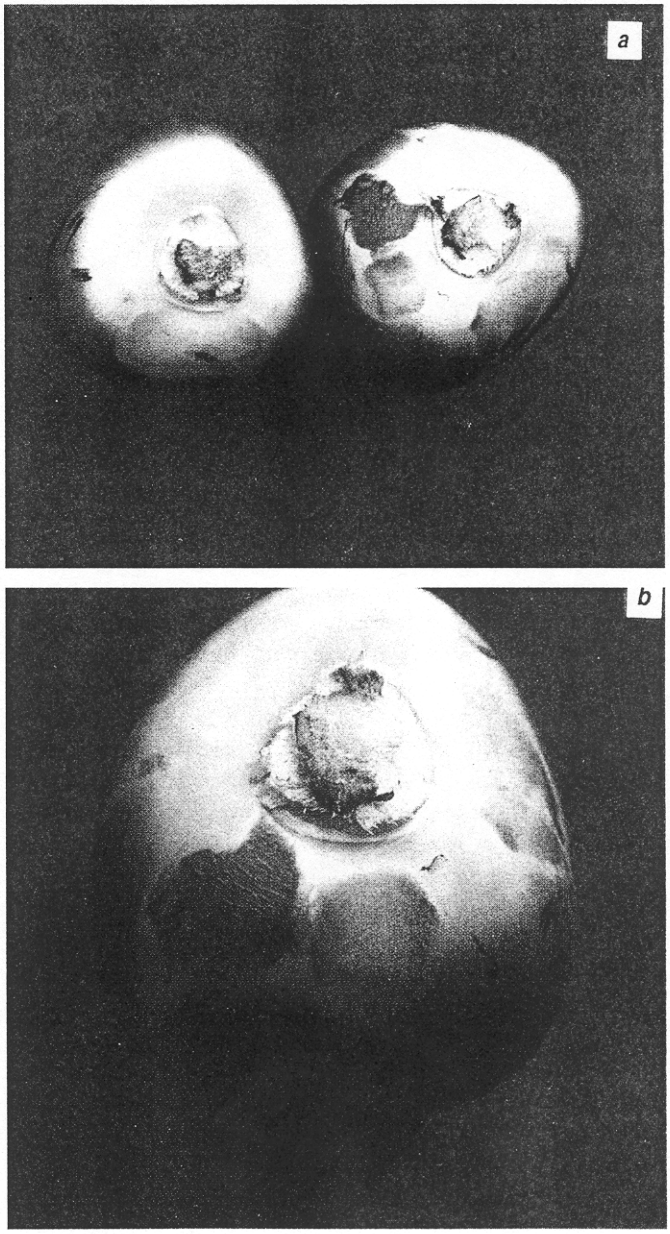

Figure 2. *a*, Feeding areas of mites on the meristematic zone of coconut exposed after removal of perianth; *b*, Closer view to show the white powdery patches of mite colonies.

sitoids because of their bunkered habitat. Some predators like *Bdella distincta*, *Amblyseius largoensis*, *A. mumai* and *A. paspalivorus*[17,23] and two unidentified phytoseids and a tarsenomid[24] have been implicated as biocontrol agents. The phytoseidae mites, which avoid exposure to direct sunlight and hide in protected areas of plants, could be of much interest in the control of *A. guerreronis*[25]. Even though few predacious mites have been observed on infested coconuts, their potential as control agents is yet to be proved[23], and in all probability, are unlikely to give the desired results[10].

Pathogens

Tackling such a difficult pest brightens the prospect of exploitation of micro-organisms, especially the fungi which are capable of reaching the prey through the minutest crevices present in the adpressed perianth lobes. *Hirsutella thompsonii* Fisher is a well known fungal pathogen which is commonly associated with the acarines. This parasitic fungus was originally described by Fisher[26], who isolated it from the citrus rust mite, *Phyllocoptruta oleivora*, in Florida.

H. thompsonii – An overview

The appearance of *H. thompsonii* colonies when grown on Sabouraud's dextrose, potato dextrose, corn meal and modified soil fungus agar media, are characteristically flat, but slightly elevated above the level of the medium, with grey, loose, fluffy mycelial growth and a brownish to greyish-green substratum colour[27]. The hyphae are 1.5–2.0 µm wide and smooth. The conidiogenous cells arise singly at intervals from the vegetative hyphae, mono- or polyphialide, unevenly verrucose, with conical to flask-shaped base and a narrow neck. The neck may be unbranched or branched, bearing enteroblastic conidia singly at the tip of the branch[26] (Figure 4 *a* and *b*). However, the gross morphology of *H. thompsonii* grown on various solid media varies considerably[26]. The geographical distribution of the fungus is widespread[28]. Enormous amount of research has been done with this fungus right from its isolation, mass multiplication, genetic improvement and formulation for the control of citrus rust mite[27,29–34]. The efficacy of *H. thompsonii* to control citrus rust mite in the field in USA, Surinam, Israel and China has been recorded to be very promising[35–37]. In China, a single application of laboratory-produced *H. thompsonii* mycelia at a dose of 0.5–1.0 g/l to citrus caused 90% reduction in the population of mites in three days[38].

The mode of penetration of *H. thompsonii* into the mites is mainly through the legs, which later on forms hyphal bodies in chains in the haemolymph. Hyphae, on which spores are produced, emerge through the mouth as well as genital and anal apertures first and then from all over the body. This has been very well demonstrated in

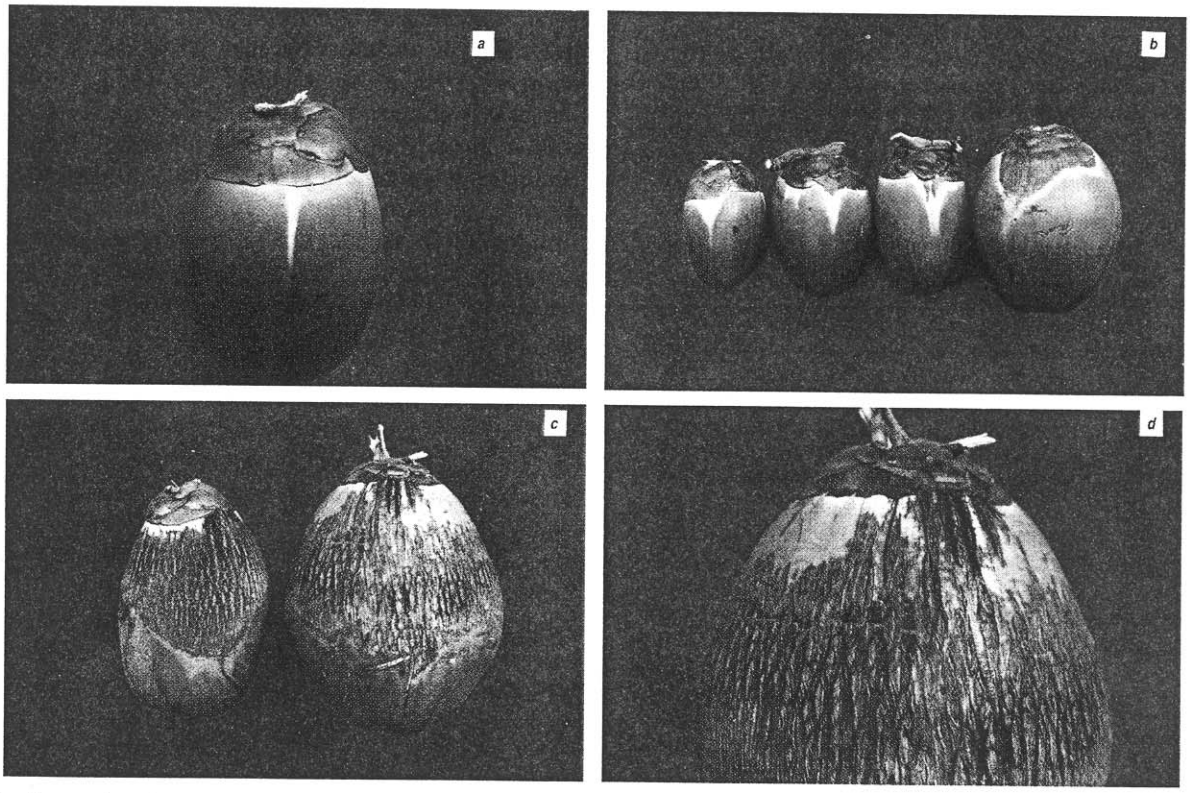

Figure 3. Succession of damage symptoms on coconut resulting from mite feeding. ***a***, White triangular streak extending from the base of the perianth of young nut is seen as the initial symptom of mite attack; ***b***, White triangular patch widens to cover more area and slowly turns brown (extreme right); ***c***, Nuts with severely warted epidermis; and ***d***, Closer view of the warting.

the case of carmine spider mite, *Tetranychus cinnabarinus*, and the oriental spider mite, *Eutetranychus orientalis*[37]. From the safety point of view, *H. thompsonii* has been found to be safe to mammals[39] and there is no report of researchers handling this fungus experiencing any ill-effects[30].

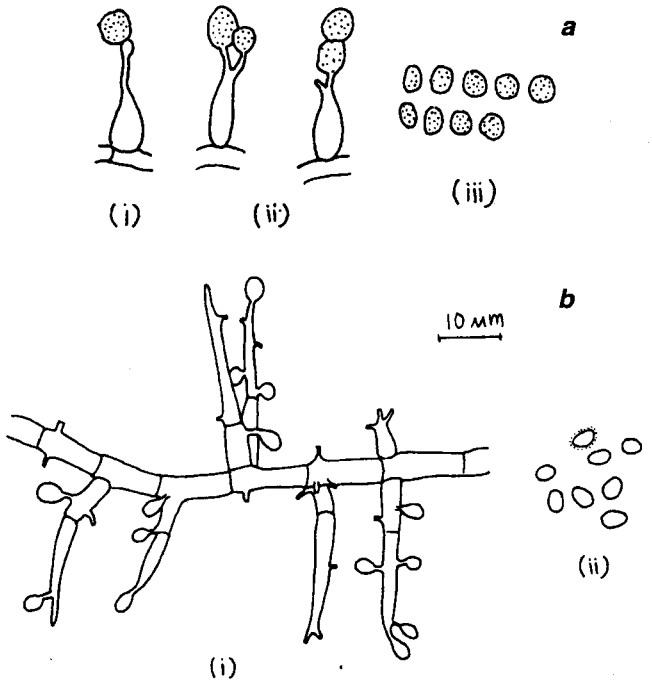

Figure 4. Characteristic cellular structures of *Hirsutella thompsonii*. *a*, Typical flask-shaped proliferating phialides with single (i) and multiple (ii) necks bearing primary verrucose conidia (iii) in 14 day-old-colonies; *b*, Erect and complexly branched conidiophores harbouring conidia laterally (i) and subglobose to ellipsoid conidia, sometimes with gelatinous covering (ii) in older colonies.

H. thompsonii against *A. guerreronis*

Hall *et al.*[40] were the first to study the natural mortality factors of coconut eriophyid mite and establish the fungus *H. thompsonii* to be a naturally occurring control agent of *A. guerreronis*. The pathogenicity tests conducted by spraying the fungal spores and mycelial fragments on the mite colonies after removing the bract and then replacing them, proved all isolated strains of *H. thompsonii* (Table 1) to be pathogenic to *A. guerreronis*, killing the mites within 48 h. Though the natural incidence of *H. thompsonii* was low, it assumed epizootic proportions upon reaching the regions below the perianth, perhaps due to the favourable micro-climate with high humidity, which is particularly conducive for fungal development and might explain the spread of this fungus among the mite populations. The field application of *H. thompsonii* is expected to increase the fungal disease incidence in coconut mites, perhaps by spread of spores from nuts, where reservoirs of sporulating mycelium have been established, to the bracts of other nuts.

Subsequent to this piece of work by Hall *et al.*, *H. thompsonii* was isolated from mite-infested coconut gardens in Mexico[41]. Another species, *H. nodulosa*, has also been obtained from this mite in Cuba[42]. Among *H. thompsonii* also, three separate varieties have been taxonomically defined, viz. *H. thompsonii* var. *thompsonii*, *H. thompsonii* var. *vinacea* and *H. thompsonii* var. *synnematosa*, based on the ultrastructural analysis of the conidiogenous structures of various *H. thompsonii* isolates[43]. The last variety is found to be somewhat specific to the tropics on *Aceria* spp. or related genera.

Field trials with *H. thompsonii* have given mixed results in the past. The field application of this fungus against coconut mite in Mexico resulted in mortality up to

Table 1. *Hirsutella thompsonii* isolated from various mites

Mite				
Family	Species	Location	Host plant	Reference
Eriophyidae	*Phyllocoptruta oleivora*	Florida, USA	Citrus	54
	Phyllocoptruta oleivora	Texas, USA	Citrus	55
	Phyllocoptruta oleivora	China	Citrus	56
	Phyllocoptruta oleivora	Surinam	Citrus	36
	Phyllocoptruta oleivora	Cuba	Citrus	57
	Acalitus vaccinii	North Carolina, USA	Blueberry	58
	Eriophyes sheldonii	Rhodesia	Citrus	31
	Eriophyes sp.	Florida, USA	Poison ivy	31
	Aceria guerreronis	Jamaica	Coconut	40
	Aceria guerreronis	Ivory Coast	Coconut	40
	Colomerus novahebridensis	New Guinea	Coconut	40
	Aceria guerreronis	India	Coconut	47
Tetranychidae	*Eutetranychus banksi*	Florida, USA	Citrus	31
	Eutetranychus sexmaculatus	Florida, USA	Citrus	31
	Panonychus citri	Florida, USA	Citrus	31
Phytoseiidae	*Typhlodromalus peregrinus*	Florida, USA	Citrus	31
Tydeidae	*Tydeus gloveri*	Florida, USA	Citrus	31

75% (ref. 41), whereas in certain other field trials in West Africa[44] and St. Lucia, West Indies[45], this pathogen was not effective. It has been suggested that *H. thompsonii* isolated from *A. guerreronis* is more effective, bringing about 88% mortality compared to 35% mortality inflicted by the isolate from *P. oleivora* (citrus rust mite)[46], thus elucidating the need for isolating a pest-specific and pest-associated pathogen.

Indian scenario

With the mite currently on a devastation trail in India, studies for the isolation of indigenous *H. thompsonii* from coconut mite are being seriously pursued. The first report in this regard is by Ramarethinam *et al.*[47] who have isolated *H. thompsoni* from coconut eriophyid mite. Their observation is that application of *H. thompsonii* alone at the rate of 10 g per tree brings about 22–25% reduction in mite damage. However, when combined with *Verticillium lecanii*, *Paecilomyces* sp. and nimbecidine (an azadirachtin containing neem derivative), suppression is effected to the tune of 30–40%. Subsequently, a formulation of *H. thompsonii* named 'Mycohit' has been developed by the Project Directorate of Biological Control, Bangalore. Kerala Agricultural University, Thrissur has also isolated *H. thompsonii* var. *synnematosa* which is specific to eriophyids, especially *A. guerreronis* (Pathummal Beevi, pers. commun.).

The grim reality is that merely obtaining a few isolates of *Hirsutella* may not be the end point for pest management. Efforts should be made to isolate numerous ecotypes/strains and their pathogenic potential should be ascertained. Since the current method of testing pathogenicity on detached nuts is found inadequate, a sound rearing technique developed[48,49] could go a long way in improving the efficacy of such studies. Furthermore, multi-locational field trials should be conducted, cheap formulation developed, safety to non-target organisms be tested and an user-friendly application technology be developed for the ultimate success. *H. thompsonii* use has to be integrated with other measures after checking its compatibility with the chemicals, especially when insecticides like dicofol, dichlorvos, Omite and sulphur at recommended rates have been found to cause moderate inhibition of *H. thompsonii* under laboratory conditions[50,51]. Sulphur compounds have shown higher antagonism to *H. thompsonii* than other miticides. Moreover, neem also has been reported to have a wide spectrum antifungal activities. In the light of the current recommendations of wettable sulphur and botanicals for the control of this mite, their application should not become a cause of loss of natural *Hirsutella* population.

Besides this, focusing attention only on *Hirsutella* may also be a highly conservative approach. The association of this pathogen with eriophyid mite has been noticed wherever the pest had occurred for a long period. In India, as this pest had established itself in the last couple of years, it deems but necessary to screen for other possible entomopathogens also. Though it is understood that bacteria and viruses need to be ingested orally for causing diseases and the mite may escape them because of their desapping feeding character[10,52], these microbes which offer a vast potential for possible suppression, also merit our close attention. This approach may gain pertinence considering the fact that *H. thompsonii* spores when fed to worker honey bees caused 29% mortality[53]. The effect of this fungal pathogen on honey bees should therefore be ascertained before its field application, as the bees are one of the major pollinators of the coconut crop.

Conclusions

Yet on viewing the overall situation and as mentioned by the leading workers in this field[4,40], the most promising agent with the wherewithal to effect long-term control of *A. guerreronis* is *H. thompsonii* at the moment. With its diverse ecological zones and a vast amount of, yet to be probed, equally diverse microflora, India stands every chance of yielding *H. thompsonii* strains with desirable traits, which could counter this mite scourge in the coconut groves of India.

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