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Identification of fungal pathogens of the spider mites, *Tetranychus neocaledonicus* André and *Tetranychus urticae* Koch from natural associations and through artificial inoculations

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ABSTRACT: In an effort to identify fungal pathogens of two economically important phytophagous mites, *viz.* the vegetable spider mite, *Tetranychus neocaledonicus* André and the two-spotted spider mite, *T. urticae* Koch, the species naturally associated were studied before experimentally testing other known acaro- and entomofungal species for pathogenicity towards the two. A comprehensive search in and around Bangalore during 2001-2005 indicated the recurrent association of many fungal genera, which were classified as acaropathogens, non-specific entomopathogens and opportunistic or minor pathogens. The entomophthoralean fungus, *Neozygites floridana* (Weiser & Muma) Remaudière & Keller and several species of the hyphomycetous genera, *Acremonium*, *Aspergillus*, *Fusarium*, *Lecanicillium*, *Paecilomyces* and *Penicillium* were found on field-collected as well as greenhouse- and laboratory-reared tetranychids. Koch's postulates were proved wherever required to confirm pathogenicity. When artificially inoculated with nine pathogens from other mite and insect hosts, only *Hirsutella thompsonii* Fisher, *H. thompsonii* var. *synnematosa* Samson, McCoy & O'Donnell, *L. lecanii* (Zimm.) Zare & W. Gams and *L. psalliotae* (Treschew) Zare & W. Gams caused death by way of pathogenicity. *Acremonium* sp., *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metschn.) Sorokîn and *Sporothrix fungorum* de Hoog & de Vries, despite causing mortality, could not be recovered from dead mites, thus suggesting that mortality alone did not guarantee pathogenic host-fungus interaction. *Nomuraea rileyi* (Farlow) Samson was unable to cause mortality in either of the test mites.

KEYWORDS: Fungal pathogens, spider mites, *Tetranychus neocaledonicus*, *T. urticae*

INTRODUCTION

Spider mites (Acari: Tetranychidae) occur on many host plants (Pritchard and Baker, 1955), and are of more importance than mite species from other families such as Tenuipalpidae, Tarsonemidae, Acaridae and Oribatidae, which are considered problematic on only some crops and under special conditions (van de Vrie, 1985).

Chemical pesticides are the only option resorted to by vegetable farmers and commercial floriculturists to control spider mites in the field, greenhouses and polyhouses across India. However, resistance to acaricides is one of the concerns in the management of spider mites (Cranham and Helle, 1985).

The predatory mite, *Phytoseiulus persimilis* Athias-Henriot is one of the biocontrol options available, but in the case of the spider mites, rapid population development and heavy webbing characteristics hinder the efficacy of this predator. Microbial control remains an unexploited area, despite an expansion of literature on the use of pathogenic fungi for mite control in recent years (Chandler *et al.*, 2000; van der Geest *et al.*, 2000; van der Geest, 2004).

In addition to the regular observations for pathogens of mites since 2001, a specific research project was taken up from April 2003 at the Project Directorate of Biological Control (PDBC), Bangalore, to document fungal species that are pathogenic to various

phytophagous mites of economic importance.

This paper reports on the results from the investigations on the identification of fungal pathogens of two economically important phytophagous mites, *viz.* the vegetable spider mite, *Tetranychus neocaledonicus* André and the two-spotted spider mite/ red spider mite/ glasshouse spider mite, *T. urticae* Koch from natural associations and through artificial inoculations.

MATERIALS AND METHODS

Source and propagation of fungal cultures

Acremonium sp. [isolate MF(Ag)24], *Hirsutella thompsonii* Fisher [MF(Ag)5], *H. thompsonii* var. *synnematosa* Samson, McCoy & O'Donnell [MF(Ag)27], *Lecanicillium psalliotae* (Treschew) Zare & W. Gams [MF(Ag)22] and *Sporothrix fungorum* de Hoog & de Vries [MF(Ag)15], originally isolated from the coconut eriophyid mite (*Aceria guerreronis* Keifer), were used in the pathogenicity study. Additionally, entomopathogens, *viz. Beauveria bassiana* (Bals.) Vuill. [IF(Ne)40] (from *Neochetina eichhorniae* Warner), *Lecanicillium lecanii* (Zimm.) Zare & W. Gams [IF(Cv)44] [from *Coccus viridis* (Green)], *Metarhizium anisopliae* (Metschn.) Sorokîn [IF(Gm)50] [from *Galleria mellonella* (Linnaeus)] and *Nomuraea rileyi* (Farlow) Samson [IF(Sl)12] [from *Spodoptera litura* (Fabricius)], were also used.

All the test fungi were propagated on homemade potato dextrose agar (PDA), except *N. rileyi*, which was multiplied on Sabouraud maltose agar containing yeast extract powder. In general, actively growing Petri-dish (90-mm diam.) cultures were used as inoculum. Stock cultures were stored at 4-5 °C in the refrigerator on slants of the same medium.

Source and maintenance of mite cultures

Healthy cultures of both the test mite species, *viz. T. neocaledonicus* and *T. urticae* were multiplied and maintained on pot-grown cowpea plants [*Vigna unguiculata* (L.) Walp.] in the greenhouse, and on detached cowpea leaves in Petri- dish bases lined with moist cotton or sponge in the laboratory.

Collection and diagnosis of diseased mites

Mite cadavers with external fungal growth and showing putative infections were collected from the field, greenhouse and the laboratory to document the fungal natural regulators as well as to isolate the associated fungal species, pathogenic or otherwise.

Neozygites floridana (Weiser & Muma) Remaudière & Keller was specifically studied to know its prevalence. The frequency of occurrence, expressed in percentage, was the number of times the pathogen was encountered out of the total number of times the collections were made. The collection period was divided into three sets and the mean frequency of occurrence was worked out.

Two methods were used to prepare mites for microscopic examination (French, 2003): (i) to screen for resting structures, internal hyphae, etc. specimens were mounted in Hoyer's fluid on glass slides and cleared at 70° C for 1 h, (ii) to detect other developmental stages of the associated fungi, mites were stained with 0.1% cotton blue in lactic acid and either heated at 80 °C for 2 h before observation or observed immediately.

Infected mites were used to retrieve the pathogens and grow or maintain the fungi in pure form using selective media. The associated fungi were isolated following the 'cavity-slide method' of Kumar *et al.* (2001). The pathogens were then identified up to the genus level and wherever possible, up to the species level.

Screening of entomofungal pathogens for pathogenicity

A homogeneous (uniform-age adults with a female majority) test material was used in the pathogenicity experiments. Two methods, *viz.* application of conidia on the leaf and caging of mites on culture, were followed.

Moist chambers were made with clean, pre-sterilized, glass Petri-dishes (90 x 15 mm) holding a sponge disc (90-mm diam.) and a layer of country filter paper disc of the same size on the top. Sterile deionized water was used to moisten the sponge. Cowpea leaf was placed on the damp filter paper with the under surface facing up. In the first method, fresh conidial suspension (2×10^8 conidia/ ml) was applied with a fine brush on the exposed leaf surface, air-dried, and mites were released individually.

In the second method, mites to be inoculated were caged (Gerson *et al.*, 1979) for 30-60 minutes on a mat of sporulating Petri-dish cultures. The inoculated mites were individually transferred to moist chambers as in the first method for incubation and observation for a week. Moisture was increased as and when required during the experiment period.

After releasing the inoculated mites, the plates were kept at ambient conditions (28 ± 2 °C day temperature)

Table 1. Fungi found in association with *Tetranychus* spp. during 2001-05

Source of samples	Associated fungi
Field	*Acremonium* spp. (several); *Aspergillus* spp. (several); *Fusarium* spp. (several); *Lecanicillium* spp. (*L. lecanii*, *L. psalliotae*, etc.); *Neozygites floridana*; *Paecilomyces* spp. (*P. fumosoroseus*, etc.); *Penicillium* spp. (several)
Greenhouse	All the above; many opportunistic or minor pathogens or generalists
Laboratory	*Hirsutella* sp. (*H. thompsonii* and others?); many opportunistic or minor pathogens or generalists

without lids. Ten replicates (10 mites/ replicate) were run for each test fungus. Similar replicates were provided for controls as well.

Fungi were reisolated from artificially-inoculated mites to prove Koch's postulates. A fungus was considered pathogenic only when Koch's postulates could be satisfied with at least one reisolate from each replicate plate.

RESULTS AND DISCUSSION

A comprehensive search in and around Bangalore during 2001-2005 indicated the recurrent association of many fungal genera, which were classified as acaropathogens, non-specific entomopathogens and opportunistic or minor pathogens (Table 1). Non-specific entomopathogenic hyphomycetous genera, *viz. Acremonium*, *Lecanicillium* and *Paecilomyces* were found on field-collected as well as greenhouse- and laboratory-reared tetranychids. However, in spite of their recurrent occurrence, several species of the generalists such as *Aspergillus*, *Fusarium*, and *Penicillium* turned out to be just opportunistic or minor pathogens. Previously, Rao *et al.* (1970) recorded several saprophytic fungi, but not any parasitic fungus, on the red spider mite, *Oligonychus coffeae* (Nietner), infesting tea in India.

The entomophthoralean fungus, *N. floridana* was the only proven acaropathogen that dominated the fungal species associated with the two target tetranychids during 2001-05 (Table 2).

Large numbers of black cadavers of spider mites were noticed in a sporadic form on the foliage of certain crops and several weeds. The major crops that harboured infected mites were brinjal (*Solanum melongena* L.), french bean (*Phaseolus vulgaris* L.), okra (*Abelmoschus esculentus* Moench) and tomato (*Lycopersicon esculentum* Mill.) in and around Bangalore. In the greenhouse, apart from brinjal, cowpea, okra and tomato, diseased mites were found on the foliage of rose (*Rosa* sp.) and mile-a-minute weed (*Mikania micrantha* Kunth. ex H.B.K.). Laboratory populations were also decimated by the fungus on several occasions. Critical microscopic examination of collected specimens revealed that mite bodies were crowded with dark brown fungal spores. These were subsequently identified as resting spores of the fungus *N. floridana* with the help of the description given by French (2003), which matched the observations made during the current study. Sub-spherical (ca. 20 µm in diam.) resting spore, having a smooth three-layered wall, was the most common stage of *N. floridana* in diseased mites. In adult mites, resting spores filled the body cavity as well as the legs and head capsule (French, 2003).

Brown, striated and club-shaped (up to 20 µm long) anadhesive spores, which are the primary infective stage of the fungus, were found attached to the setae on the body or legs of the mite. In many specimens, germination of those spores resulted in the entry and spread of hyphae within the body, and led to the filling-up of the haemoceol with hyphal bodies. After the death of the mite, the fungus produced conidiophores bearing primary conidia externally. From initial infection of the mite to the production of primary conidia *N. floridana* required up to a week. According to Selhime and Muma (1966), this cycle took an average of six days in the laboratory at 26 °C and 100 per cent relative humidity for the Texas citrus mite, *Eutetranychus banksi* McGregor.

As observed by Mietkiewski *et al.* (1993), dead mites were frequently overgrown by dematiaceous hyphomycetes, such as *Alternaria* spp. and *Cladosporium* spp. Both *T. neocaledonicus* and *T. urticae* showed the maximum frequency of occurrence of *N. floridana* in the samples collected from different host plants in the field (Table 2). Under laboratory

Table 2. Occurrence of *N. floridana* in association with *Tetranychus* spp. during 2001-05

Mite species	Host plants	Source of samples	Period	Frequency of occurrence (%)	Maximum incidence (%)
Tetranychus neocaledonicus	Brinjal, okra, tomato, several weeds	Field	April 2003-January 2004	14.0	34.7
	Brinjal, cowpea, mikania, okra, rose, tomato	Greenhouse	April 2003-March 2004	8.7	25.0
	Cowpea, rose	Laboratory	April 2003-March 2004	2.7	22.3
T. urticae	Brinjal, french bean, okra, tomato, several weeds	Field	May-June 2001; April 2003-January 2004	22.0	42.0
	Brinjal, cowpea, mikania, okra, rose, tomato	Greenhouse	May-June 2001; April 2003-December 2005	10.7	30.0
	Cowpea, rose	Laboratory	May-June 2001; April 2003-March 2004	3.3	10.3

conditions, however, this frequency of occurrence was only 2.7 and 3.3 per cent, respectively. Almost the same trend was observed in the incidence of *N. floridana*, with the maximum of 34.7 and 42 per cent for *T. neocaledonicus* and *T. urticae*, respectively, in the field situation. In the laboratory, the incidence of this pathogen was the lowest. The only previous publication (Ramaseshiah, 1971) from India reported *N. floridana* incidence in *T. telarius* (= *T. urticae*) and other unconfirmed species, *viz. T. ludeni* and *T. cinnabarinus* or *T. neocaledonicus*. However, in the current study, *T. neocaledonicus* has been confirmed as a host for *N. floridana*. Through the preliminary investigations, cross infectivity of different known acaro- and entomopathogens to the two *Tetranychus* spp. was proved (Table 3). Only *H. thompsonii* (Fig. 1), *H. thompsonii* var. *synnematosa*, *L. lecanii* and *L. psalliotae* caused death by way of pathogenicity. The caging method was better in terms of bringing out pathogenicity and mortality.

Although *H. thompsonii* is known to be associated mainly with eriophyid mites in nature, several tetranychid mites have been proved to be susceptible to the fungus through artificial inoculations (van der Geest *et al.*, 2000; Chandler *et al.*, 2000). Gardner *et al.* (1982) was the first to determine the susceptibility of *T. urticae* to *H. thompsonii*. Earlier, Gerson *et al.* (1979) stated that *H. thompsonii* was highly pathogenic to *T. cinnabarinus* Boisduval. However, the present paper is the first report on the pathogenicity of *H. thompsonii* to *T. neocaledonicus*.

L. lecanii is known to infect *T. urticae* (Gams, 1971), but prior to the current study, there was no report on its pathogenicity to *T. neocaledonicus*. This is also the first report on the pathogenicity of *L. psalliotae* to both *T. neocaledonicus* and *T. urticae*.

Acremonium sp., *B. bassiana*, *M. anisopliae* and *S. fungorum*, despite causing mortality, could not be recovered from dead mites, thus suggesting that mortality alone did not guarantee pathogenic host-fungus interaction. The lepidopteran pathogen, *N. rileyi* was unable to cause mortality in either of the test mites. Natural occurrence of highly virulent anamorphic fungi with biocontrol potential is infrequent in spider mites (van der Geest, 1985; Chandler *et al.*, 2000; van der Geest *et al.*, 2000; van der Geest, 2004). Attempts are, therefore, being at the possible use of already available commercial biopesticides (based on broad-spectrum entomopathogenic fungi) against spider mites (Chandler *et al.*, 2005). Although in the present study, *B. bassiana* and *M. anisopliae* caused mortality in both *Tetranychus* spp., neither internal ramification of these test fungi could be observed nor the species could be isolated from the

Table 3. Effect of different acaropathogens and non-specific entomopathogens on *Tetranychus* spp.

Fungal species	Effect
Acremonium sp.	M-P
Beauveria bassiana	M-P
Hirsutella thompsonii	M+P
H. thompsonii var. *synnematosa*	M+P
Lecanicillium lecanii	M+P
L. psalliotae	M+P
Metarhizium anisopliae	M-P
Nomuraea rileyi	NM-P
Sporothrix fungorum	NM-P

M+P: Mortality with pathogenicity; M-P: Mortality without pathogenicity; NM-P: No mortality, no pathogenicity

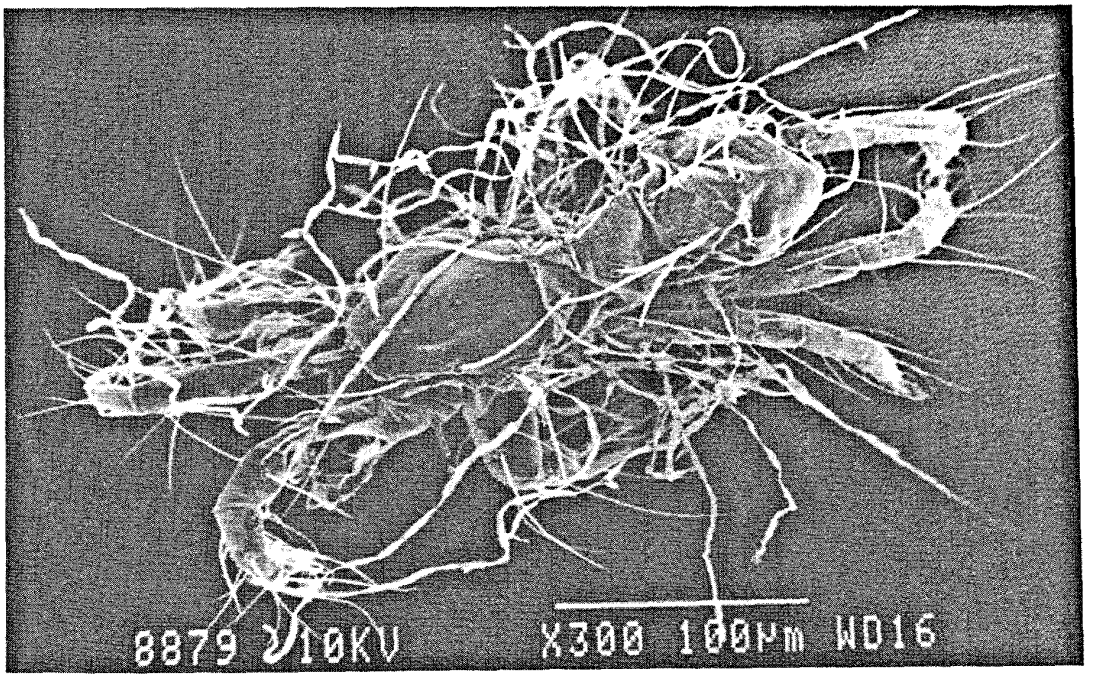

Fig. 1. *T. urticae* infected with *H. thompsonii* through artificial inoculation

dead mites. Attachment and sporulation of the fungi was seen only at the leg joints. This suggested the involvement of toxins produced by the test fungi in causing mortality. Recently, Wekesa *et al.* (2005) proved pathogenicity of 17 isolates of *M. anisopliae* and 2 isolates of *B. bassiana* towards adult females of the tobacco spider mite *T. evansi* Baker & Pritchard. This suggests the need for testing a range of isolates of the same species of the fungus derived from different hosts to identify the most pathogenic as well as the most potent isolate for use in the control of spider mites.

There is an urgent requirement for the integration of biocontrol measures into the management package

for spider mites in various ecosystems in India. Sustained research should result in evolving a package consisting of biocontrol measures.

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