African Journal of Biotechnology Vol. 11(104), pp. 16790-16799, 27 December, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3644
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Isolation, genetic diversity and identification of a virulent pathogen of eriophyid mite, *Aceria guerreronis* (Acari: Eriophyidae) by DNA marker in Karnataka, India

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Accepted 5 March, 2012

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 Karnataka area. Of the total collected nuts, 3.54% were infected by *H. thompsonii,* 2.46 and 0.29% by *B. bassiana* and *F. semitectum*, respectively. A lower number of nuts (0.03 to 0.79%) were infected by opportunistic pathogens. The incidence of pathogen infected coconuts in areas with lower temperature and higher humidity were ranged from 4.37 to 19.52% whereas with higher temperature and lower humidity it was 0 to 4.54%. Occurrence of *B. bassiana* and *F. semitectum* on *A. guerreronis* are new records. Among isolates of *H. thompsonii* collected from different places, the isolate Bangalore was more virulent followed by Mysore, Mandya, Kanakapura, Arsikere and Hiriyur isolates, as it recorded maximum infection (HTCMBAN- 88.63%). The *B. bassiana* isolates caused mortality ranging from 72.87 to 86.97%. The virulent isolate *H. thompsonii* (HTCMBAN) was tested at different concentrations, with increase in the concentration mortality rate. The genetic diversity of isolates of *H. thompsonii* by random amplification of polymorphic DNA (RAPD) revealed that grouping of the isolates was in accordance with geographic location. DNA fragments of 850 and 950 bp (OPA-20) were specific to the virulent isolate HTCMBAN.**

Key words: Fungal pathogens, eriophyid mites, genetic diversity, DNA marker.

INTRODUCTION

Mites (Acari) are the most diverse and abundant organisms of all arachnids. They are one of the oldest terrestrial animals with fossils known from the early Devonian period (nearly 400 million years ago) (Norton et al., 1988). Mites are ubiquitous in nature, they have successfully colonized most of the terrestrial, marine, fresh water habitats and most of them have complex symbiotic associations with larger organisms like plants and animals. Many mites found on agricultural crops are economically important pests (for example, spider mites, eriophyid mites, tarsonemid mites and false spider mites) and few of them are useful as biocontrol agents (for example, phytoseiid mites). Spider mites are considered to be one of the most economically important pests in agriculture. Eriophyoid mites also play a significant role as pests of agricultural crops and chemical control is often required to reduce their damage below economic levels (Van Leewen et al., 2010).

In recent years, the eriophyid mite, *Aceria guerreronis* Keifer has become a serious pest of coconut in peninsular India, reducing the yield up to 30% (Kumar,

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2002). The pest first appeared in huge proportions in coconut plantations in Ernakulam, Kerala in 1998 and later spread to the rest of the coconut growing regions of India.

Increase in pest problems has forced indiscriminate use of pesticides causing resurgence, resistance and residue problems. Several eco-friendly approaches such as use of botanical products and pathogens are available for the management of phytophagous mites, which are safer to environment and human beings. Biological control by means of natural enemies offers a long-lasting and eco-friendly solution to the problem. Pathogens, due to their versatility and ease of formulating into products, are considered as the best alternatives for effective management of the mites. Pathogens include fungi, viruses, bacteria, rickettsia, etc., some of which have been employed against different mite pest species throughout the world (Geest et al., 2000). Among these, fungi are one of the important bioagents in which the class Deuteromycetes appears to be important pathogens of mites. The fungal species recorded so far on mites belong to the genera*, Beauveria, Lecanicillium, Paecilomyces, Sporothrix, Fusarium* and *Aspergillus* (Odindo, 1992). Fungal entomopathogens such as *Hirsutella thompsonii* (mitosporic fungus) and *Neozygites floridana* (Entomophthorales) are specific to Acari, whereas other species of fungi kill both mites and insects.

Except few reports on the role of natural enemies against eriophyid mite populations and attempts to use *H. thompsonii* on *A. guerreronis* (Kumar, 2000; Beevi et al., 2000) in India, meagre research has been carried out on the role of entomopathogen fungi in management of coconut mite populations. However, there is need to identify and develop fungal biocontrol agents against a range of mite pests, since they fit very well in a viable, long lasting eco-friendly and economically integrated pest management strategy against these pests. In view of this, investigations were under taken to isolate and identify potent fungal pathogens which can be employed in the management of eriophyid mites in Karnataka.

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 growing districts of southern and northern Karnataka in 2004 and 2005. In each district, 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 Bi-MSZ). 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). The fungi were identified by Dr. Sanjay K. Singh, Scientist, Department of Mycology and Plant Pathology, Agharkar Research Institute Pune, Maharashtra.

Testing for pathogenicity by Koch's postulates

Ten millilitres of sterile distilled water with 0.05% Tween 80 was taken in sterilized micropipette and transferred to Petri plates containing fully grown fungal culture (10 to 13 days old) grown on potato dextrose agar (PDA) media. The surface of culture was disturbed with sterile brush to spread the spores in the solution. The solution was filtered through double-layered muslin cloth or 300-mesh sieve to exclude mycelia. The spore count was estimated using a haemocytometer and adjusted to the required level.

The isolates pathogenic to mites were identified, collected from cadavers and re-inoculated, confirmation of their pathogenicity was carried out and these were used for further studies. Isolates failing to cause any mortality were rejected.

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 (1×10^8 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).

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 was 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).

Genetic diversity of fungal pathogens by random amplified polymorphic DNA (RAPD)

DNA extraction protocol

0.3 g of air dried fungal hyphae was taken from a 4 ml extraction buffer, transferred to pestle and mortar with liquid nitrogen and was then ground afterwards. 500 µl of the extract was transferred to a 1.5 ml Eppendorff tube, and 300 µl of cetyl trimethyl ammonium bromide (CTAB) solution was added to each tube. The mixture was incubated at 65°C for 15 min, 6 µl of RNase (100 ug/ml) was added and kept in water bath at 35°C for 30 min. Later, 200 µl of chilled phenol: chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 10,000 rpm for 10 min. Top supernatant layer was transferred to fresh tube; 200 µl of chilled chloroform: Isoamyl alcohol (24:12) was added and again, centrifuged at 10,000 rpm for 10 min. Later, the supernatant was transferred to a fresh 200 µl tube with an addition of 100% chilled alcohol and was kept in a deep freezer overnight for precipitation. Again, the tubes were centrifuged overnight and were kept at 10000 rpm for 10 min. The supernatant was removed, the pellet was washed with 200 µl of 70% ethanol and the tubes were centrifuged at 10,000 rpm for 10 min. Once more, supernatant was removed and pellet was air dried for 1 to 2 h. The pellet was redissolved in 30 to 40 µl of Tris-EDTA (TE) buffer. Samples were stored in deep freezer and working samples were prepared by dilution with sterile distilled water.

Polymerase chain reaction (PCR)

Amplification was achieved by following the procedure outlined by Williams et al. (1990) with slight modifications. A single decamer primer was used in each reaction. PCR conditions were optimized to achieve informative and reproducible fingerprinting profiles using different levels of template DNA (10 to 15, 25 to 35 and 40 to 50 ng), $MgCl_2$ (1.0, 1.5, 2.0 and 2.5 mM) and dNTPs (150, 200, 215 and 225 mM). The PCR reactions were carried out in a final volume of 15 µl, reaction mixture containing 25 ng of template DNA, dNTPs (3 µl of 1 mM), Taq DNA polymerase [0.3 µl (1 unit)], MgCl2 {0.5 µl (30 mM)}, random primer (1 µl of 10 pico moles), 2 µl of Taq assay buffer 10× (50 mM KCl, 10 mM Tris-HCl pH 9.0 ,1.5 mM $MgCl_2$ 0.1% gelatin, 0.05% Triton -×100 and 0.05% NP40) and finally, sterile distilled water (6.3 µl). Amplification was achieved by the following Touchdown programme: 94°C for 5.00 min (initial denaturation), 94°C for 1.00 min, 40°C for 1.10 min (annealing) and -0.5°C for 2.00 min, 72°C for 2.00 min. This cycle was repeated 10 times. This was followed by 30 cycles of 94°C for 0.30 s, 35°C for 1.20 min, and 72°C for 2.00 min (extension). A final extension of 72°C for 15.00 min was added. The PCR reactions were repeated three times using the same conditions to check the repeatability of amplification products both within and between reactions.

Primer selection

To select primers that can amplify reproducible RAPD fragments, PCR was carried out to screen 30 random primers of arbitrary Sequence (Operon Technologies Inc.). Finally, seven primers producing strong, intense and unambiguous bands were selected for characterizing the isolates (primers OPB-10, OPB-01, OPC-19, OPA-03, OPA-20, OPC-06 and OPA-04).

Agarose gel electrophoresis and statistical analysis

Amplification products were resolved by electrophoresing on a 1.4% agarose gel containing ethidium bromide (0.5 µg/ml) using 1 X Tris-borate-EDTA (TBE) (Sambrook et al., 1989); 15 µl of PCR products were mixed with 5 µl of loading buffer and applied on the agarose gel. Electrophoresis was carried out at a constant voltage of 60 V for 4 to 5 h. The gels were visualized under UV light and documented using Hero Lab Gel Documentation Unit.

The data from each study were analyzed using the "STATISTICA" package. The dissimilarity matrix was developed using squared Euclidean distance (SED) that estimated all pair wise differences in the amplified product (Sokal and Sneath, 1973).

Identification of isolate specific DNA marker for virulent pathogens

Genomic DNA of *H. thompsonii* strains of the fungal pathogens were extracted and screened with RAPD primers to identify the isolate-specific RAPD markers.

RESULTS

Fungal pathogens associated with eriophyid mites

The following fungal pathogens of eriophyid mites were detected during the survey, *H. thompsonii* (six isolates), *Beauveria bassiana* (eight isolates), *Fusarium moniliforme* (many), *Fusarium semitectum* (five isolates), *Cladosporium tennuissimum*, *Aspergillus niger*, *Pencillium* sp. and *Mucor* sp.

H. thompsonii was isolated from Kanakapura, Mandya, Mysore, Bangalore, Arsikere and Hiriyur from *A. guerreronis* infesting coconut. All the isolates were *H. thompsonii* var. *thompsonii,* whereas the isolate from Mysore was *H. thompsonii* var. *synnematosa.* Isolates of *B. bassiana* were collected from *A. guerreronis* infesting coconuts in Hassan, Mandya, Mysore, Chitradurga, Davangere, Gadag, Dharwad, and Bangalore areas. This is the first report of *Beauveria* sp. on coconut mite. *F. moniliforme* was also collected from coconut eriophyid mite in Shimoga, Hassan, Mandya, Gadag, Belgaum, Davangere, Bangalore and Haveri and from *Phyllocoptruta oleivora* on citrus in Bangalore and Davangere. *F. semitectum* was found on coconut mite in Mysore, Arsikere, Hiriyur, Gadag and Hubli. This is a new record of this pathogen on coconut mite (Table 1). Among the total collected nuts, 3.54, 2.46 and 0.29% nuts were infected with *H. thompsonii, B. bassiana* and *F. semitectum* respectively (Figure 1). Other nuts (0.03 to 0.79%) were infected with opportunistic pathogens namely, *F. moniliforme*, *F. semitectum*, *C. tennuissimum*, *A. niger*, *Pencillium* sp. and *Mucor* sp. (Figure 1).

Nuts collected from different places revealed that the

Table 1. Incidence of mycosis in eriophyid mite population on different host plants in Karnataka India.

Pathogen	Place of collection	Mite species	Host plant	Date of collection	Designation of the isolate
H. thompsonii var. *synnematosa*.	Mysore	*Aceria guerreronis*	coconut	20-09-2004	HTCMMYS
Hirsutella thompsonii thompsonii	Kanakapura	*A. guerreronis*	Coconut	08-09-2004	HTCMKAN
	Hiriyur	*A. guerreronis*	Coconut	08-01-2004	HTCMHIR
	Mandya	*A. guerreronis*	Coconut	21-09-2004	HTCMMAN
	Bangalore	*A. guerreronis*	Coconut	06-06-2004	HTCMBAN
	Arsikere	*A. guerreronis*	Coconut	06-01-2004	HTCMARA
Beauveria bassiana	Hassan	*A. guerreronis*	Coconut	15-06-2004	BBCMHAS
	Mandya	*A. guerreronis*	Coconut	21-09-2004	BBCMMAN
	Mysore	*A. guerreronis*	Coconut	20-09-2004	BBCMMYS
	Chikkamagalur	*A. guerreronis*	Coconut	01-10-2004	BBCMCHI
	Davangere	*A. guerreronis*	Coconut	08-07-2004	BBCM DAV
	Gadag	*A. guerreronis*	Coconut	10-07-2004	BBCMGAD
	Dharwad	*A. guerreronis*	Coconut	11-07-2004	BBCMDHA
	Bangalore	*A. guerreronis*	Coconut	05-10-2004	BBCMBAN
Fusarium moniliforme	Hassan	*A. guerreronis*	Coconut	15-06-2004	FMCMHAS
	Shimoga	*A. guerreronis*	Coconut	20-12-2004	FMCMSHI
	Haveri	*A. guerreronis*	Coconut	13-07-2004	FMCMHAV
	Mangalore	*A. guerreronis*	Coconut	15-11-2004	FMCMMAG
	Gadag	*A. guerreronis*	Coconut	10-07-2004	FMCMGAD
	Hubli	*A. guerreronis*	Coconut	11-07-2004	FMCMHUB
	Belgaum	*A. guerreronis*	Coconut	04-01-2005	FMCMBELG
	Davangere	*Phyllocoptruta oleivora*	Citrus	08-07-2004	FMCMDAV
	Bangalore	*P. oleivora*	Citrus	05-10-2004	FMCMBAN
	Mysore	*A. guerreronis*	Coconut	20-09-2004	FMCMMYS
Fusarium semitectum	Arsikere	*A. guerreronis*	Coconut	06-01-2005	FMCMARA
	Hiriyur	*A. guerreronis*	Coconut	08-01-2005	FMCMHIR
	Gadag	*A. guerreronis*	Coconut	10-02-2005	FMCMGAD
	Hubli	*A. guerreronis*	Coconut	11-02-2005	FMCMHUB
Cladosporium tennuissimum	Kolar	*A. guerreronis*	Coconut	05-04-2005	CTCMKOL
	Mangalore	*A. guerreronis*	Coconut	15-04-2005	CTCMMAN
	Kanakapura	*A. guerreronis*	Coconut	20-09-2004	CTCMKAK
	Hiriyur	*A. guerreronis*	Coconut	08-01-2004	CTCMHIR
	Mysore	*A. guerreronis*	Coconut	20-09-2004	CTCMMYS
	Mandya	*A. guerreronis*	Coconut	21-09-2004	CTCMMAN
	Bangalore	*A. guerreronis*	Coconut	06-06-2004	CTCMBAN
	Davanagere	*A. guerreronis*	Coconut	08-07-2004	CTCMDAV
	Gadag	*A. guerreronis*	Coconut	10-07-2004	CTCMGAD
	Shimoga	*A. guerreronis*	Coconut	20-12-2004	CTCMSHI
	Dharwad	*A. guerreronis*	Coconut	11-07-2004	CTCMDWD
Aspergillus niger	Hiriyur	*A. guerreronis*	Coconut	08-01-2005	ANCMHIR
	Arasikere	*A. guerreronis*	Coconut	06-01-2005	ANCMARS
	Haveri	*A. guerreronis*	Coconut	13-07-2004	ANCMHAV

Table 1. Continued.

	Kolar	*A. guerreronis*	Coconut	05-04-2005	ANCMKOL
A. wentii	Gadag	*A. guerreronis*	Coconut	10-02-2005	AWCMGAD
Aspergillus sp.	Gadag	*A. guerreronis*	Coconut	10-02-2005	ACMGAD
Rhizopus sp.	Hiriyur	*A. guerreronis*	Coconut	08-01-2005	RCMHIR
Pencillium sp.	Hiriyur	*A. guerreronis*	Coconut	08-01-2005	PCMHIR
Mucor sp.	Gadag	*A. guerreronis*	Coconut	10-02-2005	MCMGAD
-	Bijapur	*A. guerreronis*	Coconut	24-05-2005	
-	Sindhanur	*A. guerreronis*	Coconut	22-05-2005	
-	Gajendragad	*A. guerreronis*	Coconut	21-05-2005	
	Annigere	*A. guerreronis*	Coconut	28-05-2005	
-	Shirahatii	*A. guerreronis*	Coconut	27-05-2005	
-	Lakshmeshwar	*A. guerreronis*	Coconut	28-05-2005	
	Ron	*A. guerreronis*	Coconut	20-05-2005	

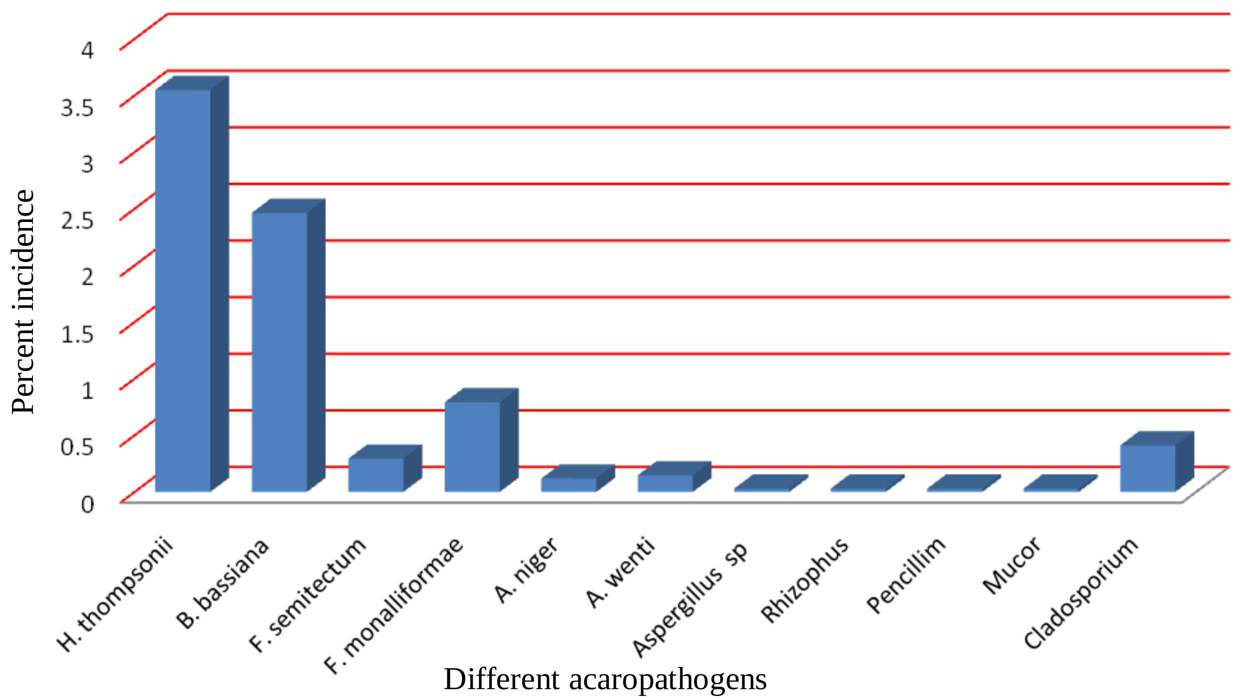

Figure 1. Incidence of acaropathogens on coconuts in Karnataka.

incidence of pathogens was higher (4.37 to 19.52%) in areas with higher rainfall and lower temperature. Incidence of pathogens was lower (0 to 5.45%) in areas with less rainfall and higher temperature (Figure 2).

Pathogenicity of fungi against coconut mite

The pathogens collected were used for pathogenicity test against eriophyid mites at 1×10^8 spores/ml. The isolate HTCMBAN was significantly superior to other treatments, causing 88.63% mortality. Isolates HTCMYS, HTCMMAN, HTCMKAN and HTCMHIR were less effective. Mortality of 80.96% was caused by HTCMARS isolate, while the remaining *H. thompsonii* isolates were associated mortalities ranging from 80.96 to 86.3% (Table 2). The *Beauveria* isolate BBDAV-1 caused significantly higher mortality (86.97%) which was on par with BBHAS (85.30%), BBMAN-1 (84.57%) and BBDHA (83.40%) isolates. Mortality in remaining *Beauveria* isolates ranged from 72.87 to 79.93%. *F. semitectum* caused 64.76% mortality.

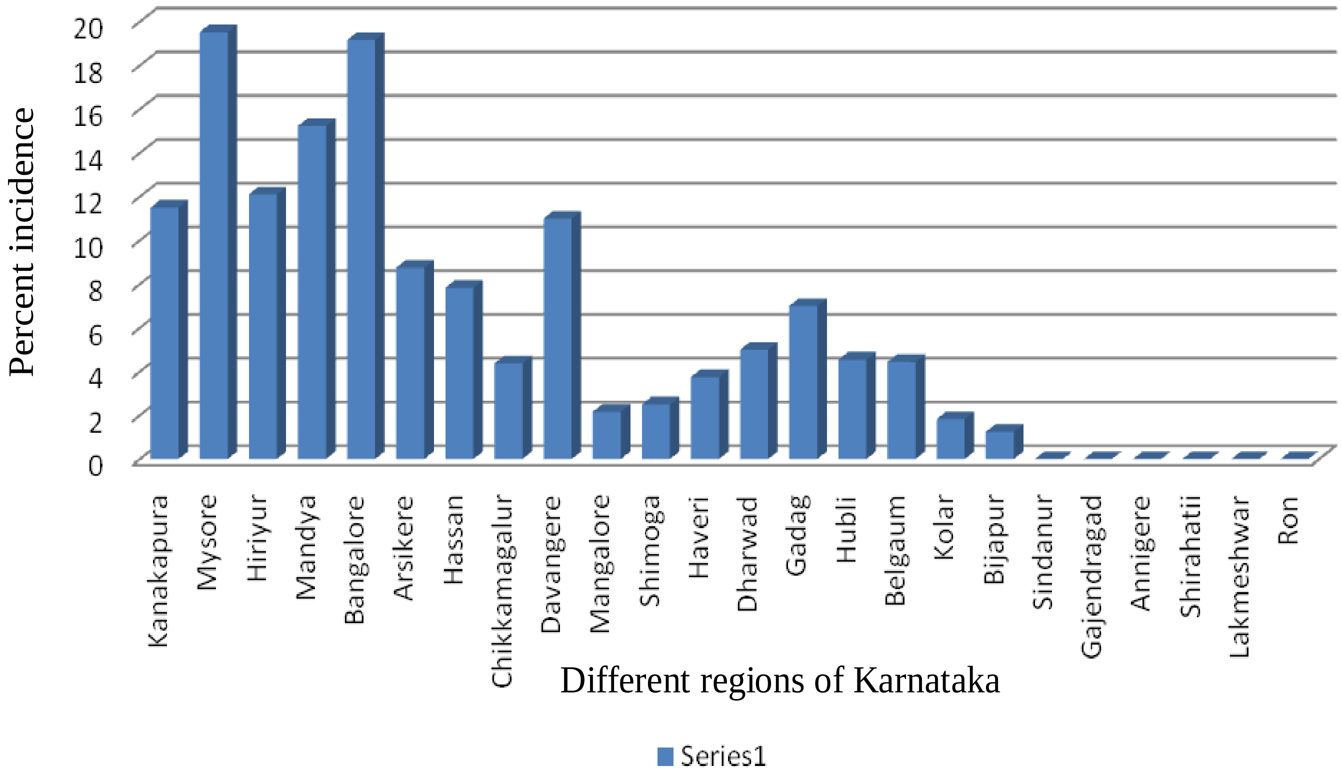

Figure 2. Incidence of acarpathogens on coconuts in different places in Karnataka.

Table 2. Bioefficacy of the fungal pathogens (1×10^8 conidia/ ml) against coconut mite under laboratory conditions.

Fungal pathogen	Percent mortality
	5DAT
HTCMMYS	88.63 (70.30)[a]
HTCMBAN	86.3(68.27)[ab]
HTCMHIR	84.34(66.69)[bcd]
HTCMMAN	81.63(64.64)[cdef]
HTCMKAN	85.96(68.00)[ab]
HTCMARS	80.96(64.33)[def]
BBHAS	85.30(67.46)[abcd]
BBMAN-1	84.57(66.87)[bcd]
BBMYS-1	79.93(63.40)[ef]
BBCHI-1	72.87(58.68)[h]
BBDAV-1	86.97(68.87)[ab]
BBGAD	78.23(62.19)[fg]
BBDHA	83.4(65.96)[bcde]
BBBAN	75.37(60.25)[gh]
Fusarium semitectum	64.76(53.59)[i]
Control (Water injection)	2.33(8.78)[j]
CD at 1% level of significance	5.322
CV	3.22

DAT, Days after treatment; # average of three replications; means followed by same alphabets within the column are not significantly different at p=0.05 by DMRT; figures in the parentheses are arcsine transformed values.

Table 3. Effect of different concentrations of HTCMBAN on coconut mite.

Concentration (conidia/ml)	Per cent mortality five days after treatment#
3.2×10^4	37.07(37.34)[c]
3.2×10^5	42.73(40.82)[c]
3.2×10^6	70.9(57.46)[b]
3.2×10^7	82.43(65.22)[a]
3.2×10^8	87.53(69.33)[a]
Water injection	3.5(10.77)[d]
Without water injection	0.67 (2.71)[e]

#Average of three replications; means followed by same alphabets within the column are not significantly different at p=0.05 by DMRT; figures in the parentheses are arcsine transformed values.

Table 4. List of primers and their products generated through amplification with six isolates of *H. Thompsonii.*

Primer	No of polymorphic band	Number of monomorphic band	Total number of band
OPB-10	1	4	5
OPB-01	2	3	5
OPC-19	4	3	7
OPA-03	1	5	6
OPA-20	2	2	4
OPC-06	3	4	7
OPA-04	1	3	4
Total	14	24	38

Evaluation of HTCMBAN on coconut mite at different spore concentrations

The isolate HTCMBAN was selected and further evaluated at different spore concentrations (3.2×10^4, 3.2×10^5, 3.2×10^6, 3.2×10^7 and 3.2×10^8 conidia/ml) against the coconut mite (Table 3). The concentration of 3.2×10^8 conidia/ml caused significantly higher mortality of 87.53%. This was followed by spore concentrations of 3.2×10^7 conidia/ml (82.43%) and 3.2×10^6 conidia/ml (70.9%). The treatment with water injection caused 3.5% mortality. The mortality was observed in control.

Genetic diversity of *H. thompsonii* isolates

Genomic DNA of six isolates of *H. thompsonii* were extracted and quantified by agarose gel, electrophoresis. Preliminary screening of the genomic DNA was done with randomly chosen RAPD decamer primers from OPA, OPB and OPC series (Operon technologies, USA) resulting to seven primers only and yielding clear and reproducible amplification. The summary of the total number of scorable bands amplified and the number of polymorphic bands revealed by each primer are presented in Table 4.

Dendrogram constructed based on RAPD data revealed that, there are two main clusters (Figure 3). Cluster A includes isolates from Mandya (HTCMMAN), Mysore (HTCMMYS), Bangalore (HTCMBAN) and Kanakapura (HTCMKAN). The isolate HTCMBAN and HTCMKAN were clustered in group A with dissimilarity of 14%. HTCMMAN was grouped in the cluster A with dissimilarity of 36% (Table 5). However, the isolate HTCMMYS is *H. thompsonii var synnematosa,* which clustered at 48% dissimilarity. The group B included the isolates from Arsikere (HTCMARS) and Hiriyur (HTCMHIR). The isolates HTCMARS and HTCMHIR were clustered with a dissimilarity of 38.00% (Table 5).

Identification of isolate specific DNA marker for virulent pathogens

The RAPD primer OPA-20 amplified 850 and 950 bp DNA fragments, which were specific to the isolate *H. thompsonii* HTCMBAN (Figure 4).

DISCUSSION

A survey was conducted on eriophyid mites for fungal pathogens in major districts of Karnataka India. Occurrence of *H. thompsonii* was noticed on coconut

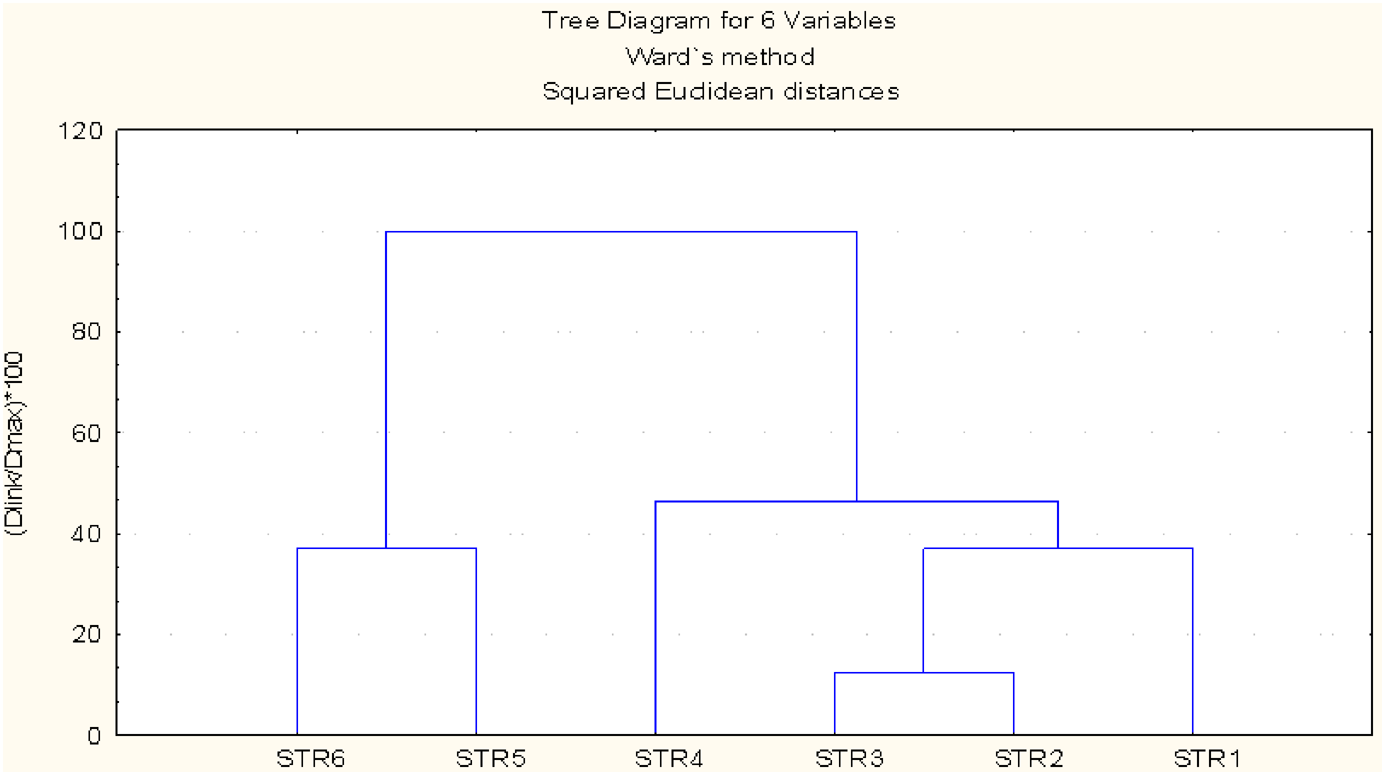

Figure 3. Dendrogram showing the genetic distance between the six isolates of *H. thompsonii*; ST1, HTCMMAN; ST2, HTCMBAN; ST3, HTCMKAN; ST4, HTCMMYS; ST5, HTCMARS; ST6:HTCMHI.

Table 5. Dissimilarity matrix table for *H. thompsonii* isolates.

Isolate	St1	St2	St3	St4	St5	St6
St1	1E-35	6	9	16	24	33
St2	6	1E-35	3	10	18	27
St3	9	3	1E-35	7	13	22
St4	16	10	7	1E-35	8	15
St5	24	18	13	8	1E-35	7
St6	33	27	15	15	7	1E-35

eriophyid mite, *Aceria guerreronis* in Kanakapura, while other *H. thompsonii* strains were also collected from Hiriyur, Mandya, Bangalore and Arsikere. *H. thompsonii synnematosa* was collected from Mysore*. B. bassiana* isolates were also collected from eriophyid mites*.* This is a new record for this pathogen on eriophyid mites. Another pathogen, *F. semitectum*, was also recorded on eriophyid mite *A. guerreronis* species. The following pathogens, *F. moniliforme*, *C. tennuissimum*, *A. niger*, *A. wentii*, *Pencillium* sp. and *Mucor* sp., were also isolated from eriophyid mites. Among the collected nuts, 3.54, 2.46 and 0.79% were infected with *H. Thompsonii, B. bassiana* and *F. semitectum* respectively. The results are in line with reports of Kumar and Singh, (2001) who found that *H. thompsonii* was high in Coimbatore (India) with 17.19% infection, while it was lowest in Bangalore (India) (0.37%). Gopal et al. (2002) reported the incidence of *F. moniliforme*, *F. solani*, *Cladosporium* sp., *A. niger*, *Aspergillus* sp., *Pencillium* sp., *Mucor* sp., actinomycetes, yeast and *Pseudomonas* sp. on coconut mite. Also, they reported that *F. moniliforme* (50 to 60%) had the maximum infection. Results are in agreement with reports of Beevi et al. (2000) who also reported for the first time, the infection of *H. thompsonii var.* synnematosa on *A. guerreronis* at Vellanikkara, Kerala, India. The infection of coconut mite *B. bassiana* and *F. semitectum* observed in the present study is a new record. Earlier, *F. semitectum* was reported from yellow mite on chilli crop by Mikunthan (2004).

The pathogenicity test against coconut mite at

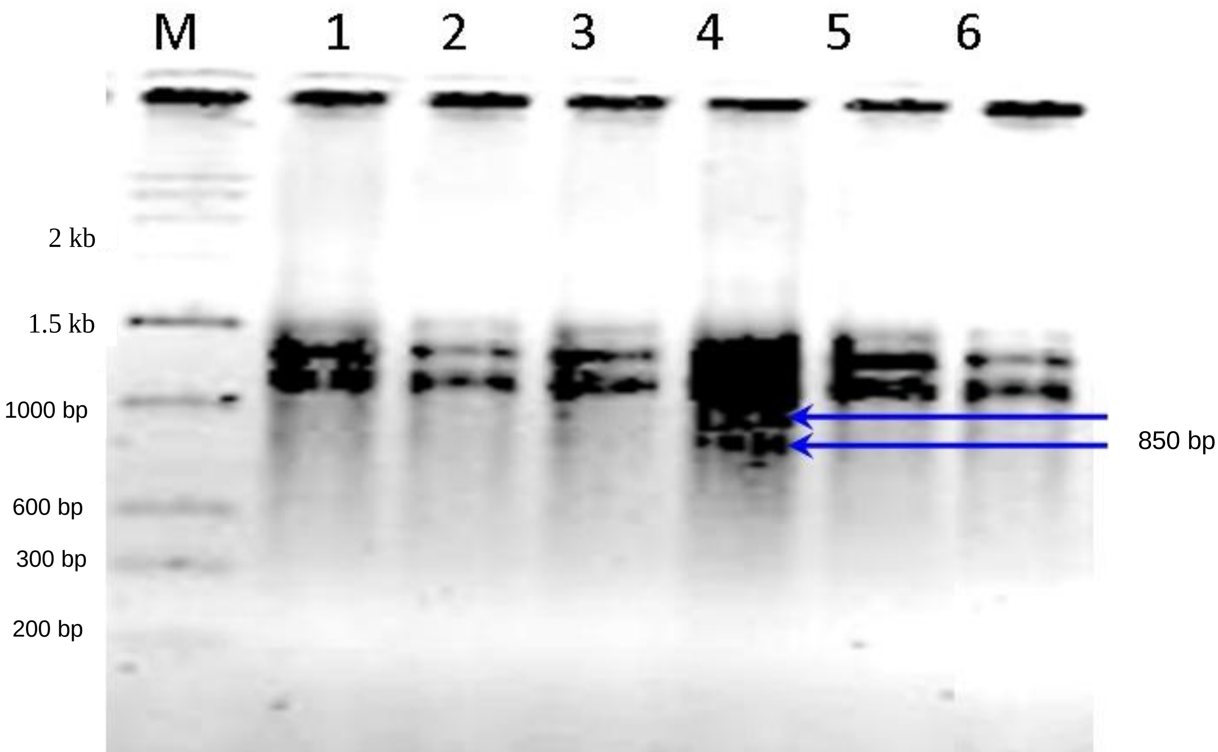

Figure 4. RAPD gel picture profile of *H. thompsoni* isolates amplified with primer OPA-20. M, Marker; Line 1, HTCMKAN; Line 2, HTCMHIR; Line 3, HTCMMAN; Line 4, HTCMBAN; Line 5, HTCMARS; Line 6, HTCMMYS. RAPD, Random amplification of polymorphic DNA.

concentration of 1×10^8 conidia/ml revealed that *H. thompsonii* isolate HTCMBAN caused significantly higher mortality (88.63%). *B. bassiana* isolates caused mortality ranging from 72.87 to 85.3%. The least mortality was observed in the isolate *F. semitectum* which caused 64.76% mortality. These results are likely related to the specificity of *H. thompsonii* for mites. Probably, host specificity may be the reason for the higher mortality observed. The fungi *P. fumosoroseus* and *F. semitectum* caused 45.1 and 64.76% mortality, respectively. The results corroborate the reports of Kumar and Anuroop (2004) who recorded 90.00% mortality of mites at 1×10^5 and 1×10^8 conidia/ml 96 h after treatment. The isolate BBDAV-1 of *B. bassiana*, which caused higher mortality, was selected to investigate mortality response at different concentrations (3.2×10^4, 3.2×10^5, 3.2×10^6, 3.2×10^7 and 3.2×10^8 conidia/ml). Five days after treatment, the highest mortality of 83.60% was observed at 3.2×10^8 conidia/ml and the least mortality (41.37%) was observed at a concentration of 3.2×10^4 conidia/ml. As the concentration decreased, percent mortality also decreased since the number of spores adhering to the body or legs decreases. Tamai et al., (1999) evaluated *B. bassiana* against *T. urticae* at concentrations of 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 and 1×10^9 conidia/ml. Mortality of mite was 10, 20, 20, 25, 28 and 48% at 5×10^6, 1×10^7, 5×10^7, 1×10^8, 5×10^8 and 1×10^9 conidia/ml, respectively.

H. thompsonii isolates were screened for 30 random primers, out of this, seven primers gave reproducible and unambiguous bands. The dendrogram showed three clusters. Cluster A includes isolates from Mandya (HTCMMAN), Mysore (HTCMMYS), Bangalore (HTCMBAN) and Kanakapura (HTCMKAN). Cluster B includes isolates from Arsikere(HTCMARS) and Hiriyur (HTCMHIR). From the dendrogram, it is clear that the isolates collected from different geographical regions formed different clusters. Arsikere and Hiriyur isolates clustered in the same group indicate that they are genetically more similar. Whereas Bangalore, Kanakapura and Mandya isolates clustering in group A are genetically more similar. However, the strain HTCMMYS is grouped in cluster A with higher dissimilarity of 48.00%, since this is *H. thompsonii* var. *synnematosa*. This is in line with report of Boucias et al. (1982), who opined that differentiation at sub cellular level may not be immediately associated with morphological differences.

Efforts were made to identify virulent pathogen-specific DNA marker. The DNA fragments of 850 and 950 bp were specific to the isolate of *H. thompsonii*, HTCMBAN (OPA-20). The non-amplification of the aforementioned specific sized DNA fragments in the other isolates of the fungal pathogens could be due to the absence or modification (insertion/deletion) of nucleotides in the primer binding site. These isolate-specific DNA markers will help in the identification of the respective pathogen.

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