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Isolation and characterization of a *Lecanicillium psalliotae* isolate infecting cardamom thrips (*Sciothrips cardamomi*) in India

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Abstract Cardamom thrips (*Sciothrips cardamomi* Ramk.) (Thysanoptera: Thripidae) cause serious economic loss to cardamom, *Elettaria cardamomum* (L.) Maton (Zingiberaceae). A fungus was isolated from cadavers of cardamom thrips collected during a survey in cardamom plantations of Wayanad District, Kerala, India and identified as *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales) based on morphological and molecular studies. Bioassay studies with purified conidial suspension further confirmed the infectivity of the fungus to thrips. At the highest dose tested (1×10^7 conidia ml^{-1}), up to 62.9 % mortality was recorded in the test population, ten days post inoculation. The fungal pathogenesis on adult thrips was documented by scanning electron microscopic studies. This is the first record of *L. psalliotae* in India and also a first report of a fungus infecting cardamom thrips.

Keywords Biological control · *Elettaria cardamomum* · Electron microscopy · Entomopathogenic fungi · *Lecanicillium psalliotae* · *Sciothrips cardamomi*

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Introduction

Cardamom thrips (*Sciothrips cardamomi* Ramk.) (Thysanoptera: Thripidae) cause serious economic loss to cardamom (*Elettaria cardamomum* (L.) Maton) (Zingiberaceae) in India and Sri Lanka. The pest infested capsules shrivel and lack the usual aroma and fetch a low price in the market (Gopakumar and Chandrasekar 2002). The extent of capsule damage by thrips is 60–90 % and the estimated crop loss is 45–48 % (Gopakumar and Chandrasekar 2002; Dharmadasa et al. 2008). The control measures developed against the pest are based mainly on chemical insecticides (Gopakumar and Chandrasekar 2002; Dharmadasa et al. 2008). Farmers sometimes adopt up to 12 sprays per year for thrips control, more than

the recommended schedule of 4–9 sprays leading to environmental and pesticide residue issues. Cardamom ranks as the highest pesticide consuming rainfed crop in the world (Murugan et al. 2011). Many of the chemicals used for thrips control in cardamom are also highly toxic to honey bees which are major pollinators of the crop. Hence development of alternate strategies for the management of this pest is important. Until now no microbial biocontrol agent has been reported for management of cardamom thrips. We have isolated and characterized *Lecanicillium psalliotae* (Treschew) Zare & W. Gams (Ascomycota: Hypocreales), an entomopathogenic fungus from field collected cadavers of cardamom thrips, and tested its pathogenicity in the laboratory, which offers potential for the development of a biocontrol agent against the pest. This is the first record of the occurrence of *L. psalliotae* in India and also a first report of a fungus naturally infecting cardamom thrips.

Materials and methods

Fungus isolation and testing Koch's postulates

Cadavers of cardamom thrips found adhered to the inner side of leaf sheaths and shoots of cardamom plants were collected from a cardamom plantation in Vythiri Taluk (11°32′57.27″N, 76°01′20.26″E, altitude: 656 m) of Wayanad District in Kerala, India on June 12, 2012. The cadavers were incubated in glass Petri dishes of 100 × 17 mm (diameter × height) lined with a layer of moist cotton and Whatman No. 1 filter paper for sporulation of the fungi at 25 °C in dark for two days in a biochemical oxygen demand (BOD) incubator. Cadavers showing signs of fungal growth were transferred to plates of entomopathogenic fungi-specific Oat Meal Agar (OMA)—CTAB medium (Posadas et al. 2012). Single spore cultures of the entomopathogenic fungus were established on Potato Dextrose Agar (PDA) medium for further studies. The isolate was deposited in the Entomopathogenic Fungal Repository of Indian Institute of Spices Research (IISR) with accession number IISR-EPF-02.

To satisfy Koch's postulates, the pathogenicity of the isolated fungus was tested by two methods. In the first method, ten adult thrips were allowed to crawl for 30 s on the surface of fungal culture grown in a Petri dish and transferred to rearing chambers following the technique of Krishnamurthy et al. (1989) with slight modifications. Briefly, the technique included a glass Petri dish (100 × 17 mm) lined at the bottom with a layer of moist cotton beneath a circular filter paper of the same dimension of the Petri dish and an inverted glass funnel (75 mm diameter and 145 mm height) placed above the filter paper. The opening of the funnel stem was closed by a wet cotton plug to maintain the relative humidity (RH). Thrips were released into the Petri dish and 4–5 immature cardamom capsules placed inside the funnel provided food and shelter to the insects. Field collected thrips directly transferred to rearing chambers served as control. In the second method, the crude conidial suspension was prepared by washing a plate of fungal culture with 3 ml of sterile 0.05 % Triton-X 100. Ten adult thrips were immersed individually for 10 s in the conidial suspension and transferred to the rearing chambers described above. In control, ten adult thrips were dipped for 10 s in sterile 0.05 % Triton-X 100. Both the experiments were replicated four times. Thrips mortality was recorded at 24 h intervals in treatments and control. Dead thrips were surface sterilized with 0.2 % sodium hypochlorite solution for 3 s, washed twice in sterile distilled water and inoculated on OMA-CTAB medium for re-isolation of the pathogen.

Identification of the fungus

Macro- and micro-morphological features of fungal colonies and molecular tools were used in the identification of the fungus.

Morphological characterization

To study the morphological features, agar plugs (6 mm diameter) obtained from single monosporic, two week old cultures were inoculated in the centre of ten Petri dishes (100 × 17 mm) containing PDA medium and incubated at 25 °C in darkness. Macroscopic characteristics such as colony growth and appearance, pigmentation at the reverse of PDA plate and diffusion of pigmented metabolites into the Potato Dextrose Broth (PDB) medium were recorded. For micro-morphological features, size and shape of macro- and microconidia, phialide size and their arrangement were recorded ($n = 50$). The measurements were made using Leica DMRB research microscope fitted with a Moticam 2300 digital camera.

DNA extraction, PCR, sequencing and phylogenetic analysis

DNA sequencing and phylogenetic analysis were carried out on three conserved gene regions. DNA was extracted and purified from mycelia of the fungus grown on PDA medium for 21 days at 25 °C, using fungal genomic DNA extraction Kit (Chromous Biotech, India) following the manufacturer's instructions. PCRs were performed to amplify ITS rDNA with primers ITS4 and ITS5 (White et al. 1990), partial β-tubulin gene (TUB) with primers bt2a and bt2b (Glass and Donaldson 1995) and partial translation elongation factor 1α gene (TEF) with primers 983F and 2218R (Rehner and Buckley 2005). Amplification reactions were made in volumes of 25 μl with 10 ng of template DNA. PCR conditions for ITS rDNA, TUB and TEF were as described by Saito et al. (2012). Briefly, except for the annealing temperature of 55 °C for ITS rDNA, PCR conditions for ITS rDNA and TUB were: one cycle at 94 °C for 4 min, followed by 35 cycles at 94 °C for 1 min, 58 °C for 1 min, 72 °C for 2 min, and then one cycle at 72 °C for 7 min. Amplification of the TEF was made under the following conditions: one cycle at 94 °C for 4 min followed by 37 cycles at 94 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min, and then one cycle at 72 °C for 7 min. The amplified bands were excised, purified using Sigma GelElute^{TM} Gel Extraction Kit (Sigma, India) as per manufacturer's protocol and outsourced to Merck Millipore India Ltd., for sequencing. Sequencing was carried out with the same set of primers used for PCR amplification. DNA sequences were subjected to BLAST (Basic Local Alignment Search Tool) search to identify sequences deposited in GenBank that had significant homology (Saito et al. 2012). The sequences were deposited in GenBank and aligned with gene sequences of reference taxa retrieved from the GenBank (Table 1) using MUSCLE incorporated in MEGA 5 (Tamura et al. 2011). Gaps in alignment were treated as missing data and the phylogenetic trees were constructed by the neighbor-joining method with Kimura two-parameter model. Bootstrapping was performed with 1,000 replicates and the alignment was deposited in TreeBASE S16105. *Pochonia chlamydosporia* var. *chlamydosporia*, *P. chlamydosporia* var. *catenulatum* (Metacordycipitaceae) and *Verticillium dahliae* (Plectosphaerellaceae) were included as outgroups (Sung et al. 2007). Molecular data for neotype strain of *L. psalliotae* (CBS 505.48 = IMI 092016) was not available for comparison.

Bioassay

Preparation of spore suspension

Twenty-one day old single spore fungal cultures grown on PDA were used for preparation of conidial suspensions. The conidia were harvested by flooding the cultures with 3 ml of 0.05 % Triton-X 100, the suspension vortexed for 1 min and filtered through four layers of cheese cloth. The final volume of the filtrate was made up to 10 ml with 0.05 % Triton-X 100. The conidial concentration in the filtrate was adjusted to 1×10^7 conidia ml^{-1} using an improved Neubauer haemocytometer. The suspension was serially diluted further to get concentrations of 1×10^6 and 1×10^5 conidia ml^{-1}. In bioassay studies, each conidial suspension (1×10^7, 1×10^6 and 1×10^5 conidia ml^{-1}) constituted a treatment and sterile 0.05 % Triton-X 100 in distilled water served as control. The viability of conidia was assessed prior to use and it was more than 95 %.

Pathogenicity study

The pathogenicity of the isolated fungus was tested against field collected adult thrips that were free of entomopathogens and insecticide applications. The thrips were directly used for the study because laboratory breeding of cardamom thrips was difficult. Ten adult thrips were used in each treatment and the treatments were replicated four times. In a treatment, 2.5 ml of each conidial suspension was directly sprayed on each replicated Petri dish with the thrips, completely wetting them and the filter paper lining inside the dish using a hand held bottle sprayer. Similarly, thrips in each control dish were sprayed with 2.5 ml of 0.05 % Triton-X 100 solution. The rearing setup (as described for testing Koch's postulates) was maintained at 26 °C. Observations on thrips mortality were recorded daily and the experiment was terminated after ten days. The experiment was repeated three times to confirm the results.

Scanning electron microscopy (SEM) studies

Thrips that died 72 h to ten days post-inoculation (p.i.) during bioassay studies were used for scanning

Table 1 Fungal isolates used for phylogenetic analyses

Fungal species	Isolate	Origin	Host	GenBank accession number		
				ITS rDNA	β-tubulin	TEF 1α
L. psalliotae	IISR-EPF-02	India	*Sciothrips cardamomi*	KF358373	KF358374	KF358375
L. psalliotae	QNCSE0810	China	*Meloidogyne incognita*	JN797793	–	–
L. psalliotae	XSD08038	China	–	EU918702	–	–
L. psalliotae	ARSEF 2234	Unknown	*Rhizoecus* sp.	EF513016	–	–
L. psalliotae	KYK 00031	Japan	Sternorrhyncha on leaf of *Aucuba japonica*	AB378519	–	–
L. psalliotae	ARSEF2332	USA	*Sitobion avenae*	EF513018	–	–
L. psalliotae	CBS 117544	Spain	Date palm and red scale insect	DQ131172	–	–
L. psalliotae	CBS 100172	USA	Nymph of *Ixodes* sp.	AJ292390	–	–
L. psalliotae	CBS 532.81	Unknown	Soil	–	EF469145	EF469067
L. psalliotae	CBS 101270	Unknown	Soil	–	EF469146	EF469066
L. saksenae	IMI 179841	India	Forest soil	AJ292432	–	–
L. lecanii	IMI 255033	Sri Lanka	Scale insect	EF513001	–	–
L. muscarium	IMI 268316	UK	*Thrips tabaci*	EF513002	–	–
L. muscarium	IMI 246427	UK	Peat		EU000248	–
L. longisporum	IMI 021167	Sri Lanka	*Icerya purchasi*	AJ292385	–	–
L. nodulosum	IMI 338014R	Mexico	Scale insect	EF513012	–	–
L. attenuatum	CBS 170.76	Poland	Caterpillar of *Carpocapsa pomonella*	EF679164	EF679176	–
L. attenuatum	CBS 402.78	Unknown	Leaf litter of *Acer saccharum*	–	–	EF468782
L. dimorphum	CBS 363.86	China	*Agaricus bisporus*	AJ292429	–	EF468784
L. aphanocladii	CBS 376.77	Netherlands	*Agaricus bitorquis*	AJ292431	–	–
L. tenuipes	CBS 309.85	Spain	Araneae	AJ292391	–	–
L. aranearum	CBS 726.73	Ghana	Araneae	AJ292464	–	EF468781
L. antillanum	CBS 350.85	Cuba	Agaric	AJ292392	DQ522514	DQ522350
L. fusisporum	CBS 164.70	Netherlands	*Coltricia perennis*	AJ292428	–	EF468783
L. acerosum	CBS 418.81	Brazil	*Crinipellis perniciosa*	EF641893	–	–
P. chlamydosporia var. *chlamydosporia*	CBS 103.65	Germany	Soil	AJ292397	–	–
P. chlamydosporia var. *catenulatum*	CBS 504.66		Nematode	AJ292398	EF469149	EF469069
Verticillium dahliae	MD 80	Germany	*Brassica napus*	AF364004	–	–
V. dahliae	ATCC 16535		*Crataegus crus-galli*	–	DQ522531	AY489632

electron microscopic studies. The cadavers were stored in a Petri dish lined with moist cotton and Whatman No. 1 filter paper in a BOD incubator at 25 °C for fungal sporulation. The cadavers were dehydrated for five days at 25 °C in an incubator. Dried specimens were mounted on carbon stubs, gold sputtered for 20 s and directly viewed under a scanning electron microscope (Hitachi SU6600) to examine the growth and development of *L. psalliotae* on thrips.

Statistical analysis

Data on colony growth were subjected to one-way ANOVA followed by least significant difference (LSD) test ($\alpha = 0.05$). Percent mortality of thrips

during bioassay was subjected to Abbott's correction (Abbott 1925). Significance among the treatments was analyzed using Generalized Linear Mixed Model (Binomial distribution with logit link function) (PROC GLIMMIX) by Tukey–Kramer grouping for Least Squares Means ($\alpha = 0.05$) using SAS® 9.3 software for statistical analysis (SAS 2011).

Results

Identification of fungus

The entomopathogenic fungus was identified as *L. psalliotae* based on morphological and molecular data.

Morphological characteristics

Monosporic colonies of the fungus grown on PDA medium were white, cottony and reached an average diameter of 5.0 cm (range 4.5–5.8 cm) in ten days at 25 °C (Table 2). The undersurface of the colonies were cream colored initially, but became reddish with secretion of colored pigments from the 3rd day of inoculation. When inoculated into PDB, the fungus secreted purple colored pigments into the media on the 3rd day of inoculation and the medium turned deep red in 10–12 days. The fungus produced two types of conidia: macro- and microconidia. The macroconidia measured (n = 50) on average 7.19 ± 0.11 µm in length (range 6.0–9.0 µm) and 1.39 ± 0.02 µm (range 1.1–1.8 µm) in width. The microconidia (n = 50) were with a mean length of 3.36 ± 0.06 µm (range 2.40–4.30 µm) and mean width of 1.24 ± 0.02 µm (range 0.9–1.6 µm). The length/width ratios were 5.22 ± 0.11 and 2.75 ± 0.07 for macro- and microconidia, respectively. The phialides measured 19.54 ± 0.88 µm (range 5.2–30.7 µm) in length and 0.86 ± 0.03 µm (range 0.6–1.3 µm) in width (Table 3). The macroconidia were falcate, pointed at both ends, typically solitary and a few transversely positioned on phialides. Microconidia were oval to ellipsoidal with blunt ends. Phialides, solitary or in whorls of 2–3 arising from prostrate conidiophores. The present isolate was morphologically identical to the description of the neotype strain of *L. psalliotae* (CBS 505.48 = IMI 092016) of Zare and Gams (2001) and with isolates of *L. psalliotae* reported elsewhere (Table 3).

Table 2 Mean (±SE) colony diameter of *L. psalliotae* isolated from cardamom thrips grown on PDA at 25 °C

Days after inoculation	Mean diameter (cm ± SE)*
3	1.96 ± 0.02^{c}
7	4.02 ± 0.24^{b}
10	4.99 ± 0.23^{a}

* Mean of five replicates; $F = 63.0$; $P < 0.0001$; df = 2, 12

Values with different letters are significantly different from each other by LSD ($\alpha = 0.05$)

Molecular analysis

The sequence data generated for the three conserved regions of the present isolate were deposited in GenBank with accession numbers listed in Table 1. A BLAST search for the ITS rDNA sequence of the present isolate (IISR-EPF-02) indicated that it was 99 % similar to a Chinese isolate of *L. psalliotae*

Table 3 Morphological characters of *L. psalliotae* isolates

Strain/isolate	Host	Origin	Macroconidia (µm) (range)		Microconidia (µm) (range)		Phialides (µm) (range)		References
			Length	Width	Length	Width	Length	Width	
CBS 505.48	Mushroom	England	5.0–10.0	1.2–1.7	2.7–3.7	1.0–1.5	25.0–35.0	1.0–1.5	Zare and Gams (2001)
BT5	*Bemisia tabaci*	Denmark	8.8–15.2	1.6–3.2	3.6–5.6	2.4–2.8	25.6–36.0	1.6–2.4	Steenberg and Humber (1999)
CGMCC5329	*Meloidogyne incognita*	China	4.15–7.53	1.43–3.76	2.7–3.7	1.0–1.5	13.7–32.0	1.16–2.4	(Zheng et al. 2012)
IISR-EPF-02	*Sciothrips cardamomi*	India	6.0–9.0	1.1–1.8	2.4–4.3	0.9–1.6	5.2–30.7	0.6–1.3	Present study

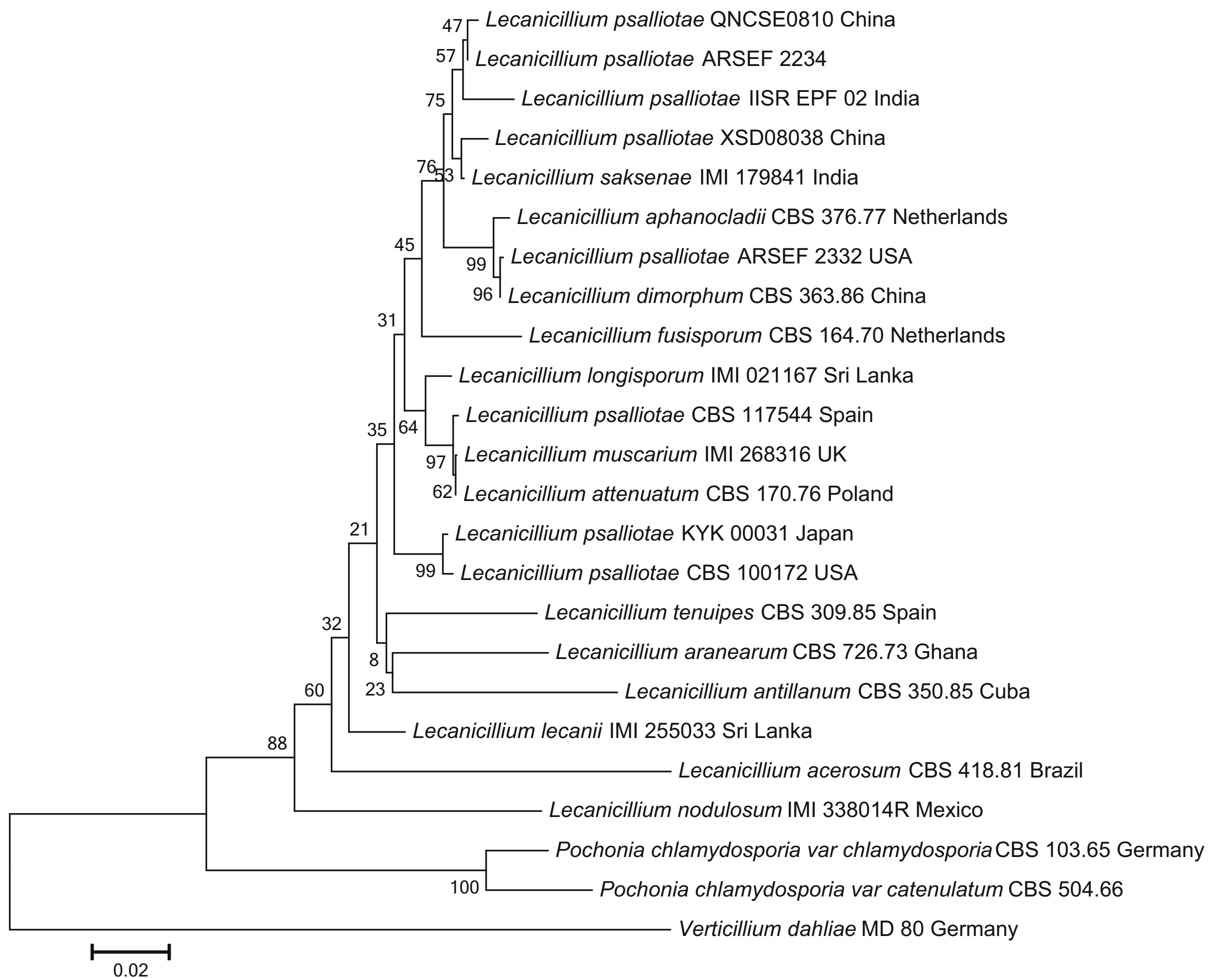


Fig. 1 Neighbor-joining tree based on analysis of partial ITS rDNA gene sequences of *L. psalliotae* and other *Lecanicillium* spp. Numbers above or below the nodes indicate bootstrap values generated after 1,000 replications. Fungal species and sequences obtained from GenBank are shown with their accession numbers in the figure. *P. chlamydosporia* var. *chlamydosporia*, *P. chlamydosporia* var. *catenulatum* and *V. dahliae* were used as outgroups. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura two-parameter method and are in the units of the number of base substitutions per site

(QNCSE0810) (Table 1). The present isolate along with nine other isolates of *L. psalliotae* and another 16 taxa were subjected to a phylogenetic analysis using available ITS rDNA, TUB and TEF sequence data downloaded from GenBank (Table 1). The ITS rDNA phylogram showed that our isolate clustered with *L. psalliotae* isolates reported elsewhere (Fig. 1). The Indian isolate of *L. saksenae* which was considered as a synonym of *L. psalliotae* by Zare and Gams (2001) did not cluster with the *L. psalliotae* isolate reported in this study. However, it shared a close relationship with the isolate, XSD08038 (Fig. 1). The TUB sequence of our isolate showed 94 % similarity with a *Lecanicillium* sp. reported from Canada. In the TUB phylogram, the present isolate shared a close relationship with *L. muscarium* and *L. attenuatum* (Figure not shown). The partial TEF sequence of our isolate had 97 % sequence similarity with the *L. psalliotae* isolate, CBS 532.81. The TEF phylogram supported the inclusion of the present isolate in a clade with *L. psalliotae*, *L. dimorphum*, *L. attenuatum* and *L. fusisporum* (Figure not shown). The molecular and phylogenetic analyses of the sequenced data supported the morphological identity of our isolate as *L. psalliotae*.

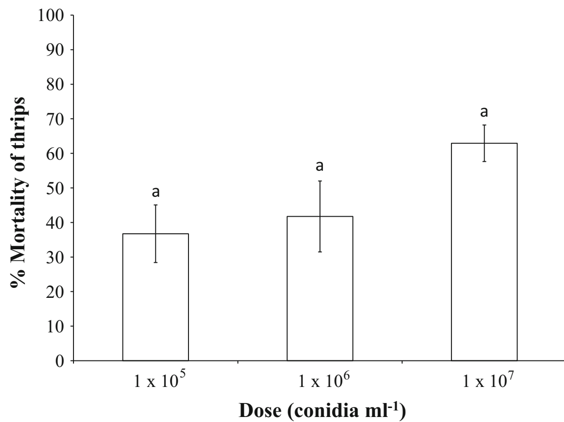


Fig. 2 Mean (±SE) corrected mortality of cardamom thrips caused by various concentrations of *L. psalliotae* at ten days p.i. Error bar represent SE error of four replicates. Bars represented by the same letter are not significantly different by Tukey–Kramer grouping for least squares means ($\alpha = 0.05$)

Bioassay studies

The isolated fungus killed the thrips and was re-isolated in pure culture satisfying Koch's postulates. In our bioassay studies, mortality was observed in treated thrips within 72 h p.i. The dead thrips showed signs of mycosis in treatments. However, thrips that died in control, when plated on PDA did not show fungal growth and the average control mortality was only 7.5 %. Mortality up to 62.9 % was recorded at 1×10^7 conidia ml^{-1} at ten days p.i. (Fig. 2). A higher thrips mortality was observed in fungal treatments compared to 0.05 % Triton-X 100 in distilled water (control treatment) ($F = 7.04$; $P = 0.006$; df = 3, 12). However, the multiple comparison test (Tukey's HSD, $\alpha = 0.05$) among the different levels of doses showed that only control treatment had significantly lower mortality.

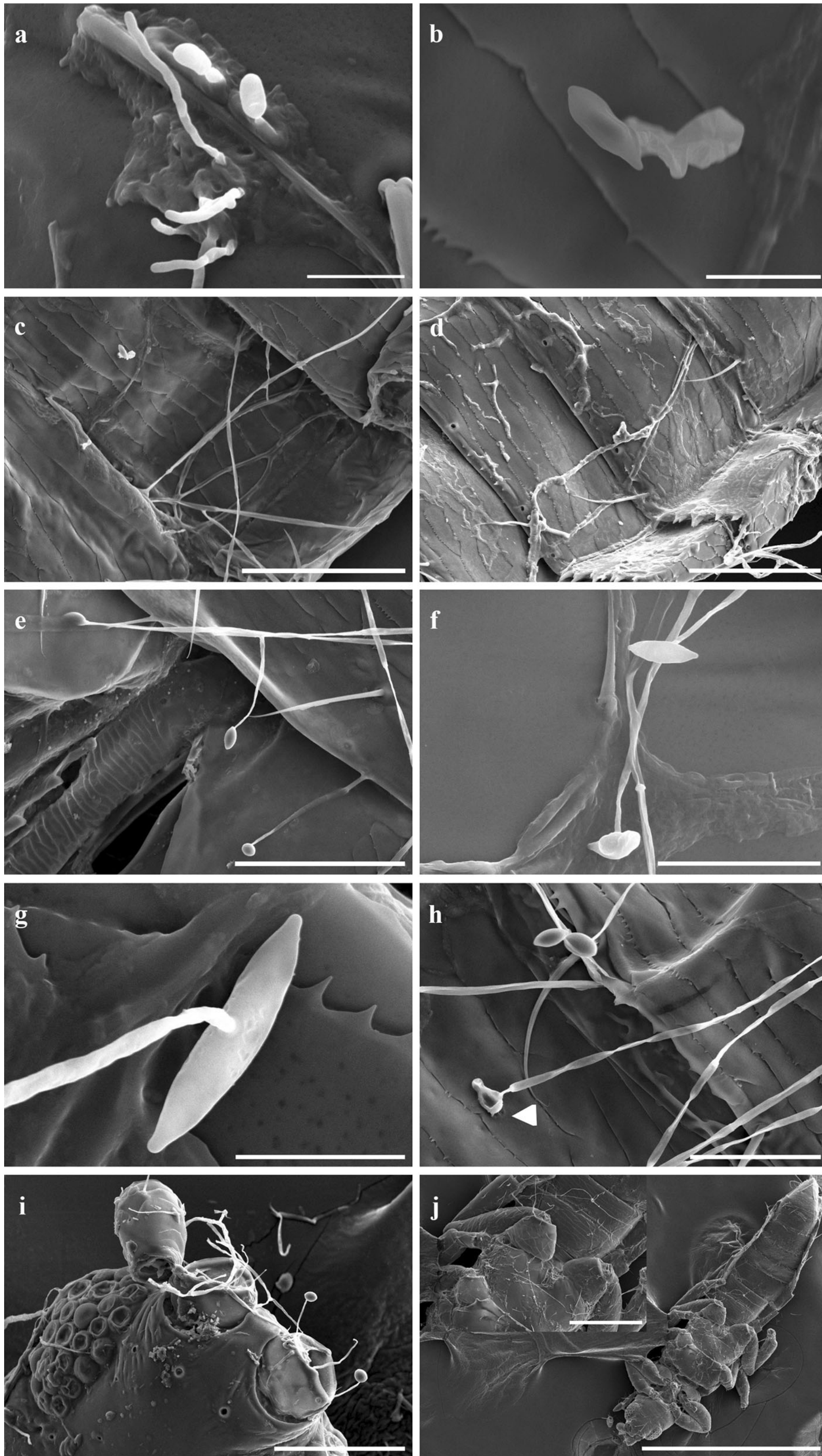
SEM studies

SEM studies showed the fungus adhering to and penetrating the cuticle of the insect. The conidia adhered and germinated throughout the body surface of thrips. Conidia were also found adhering to the body setae (Fig. 3a). Appresoria was directly produced from the conidia (Fig. 3b) and the infective pegs were found to penetrate through the intersegmental membranes (Fig. 3c). Also, the hyphae emerged out through the intersegmental membranes of the body (Fig. 3d). The hyphae differentiated into phialides in single or whorls of 2–3 bearing conidia (Fig. 3e, f). Conidia dimorphic, both macroconidia (Fig. 3g) and microconidia (Fig. 3h) were observed. An obvious sign of cuticle degradation was found at the site of conidial germination, which could be due to secretion of cuticle degrading enzymes or may be due to mechanical pressure (Fig. 3h). Conidiation was observed throughout the body surface, including the antennae (Fig. 3i). The dead thrips presented a mummified appearance (Fig. 3j) with mycelial growth all over the body.

Discussion

Fungi are currently believed to be the commonest insect pathogens, and are well suited for being developed as contact biopesticides (Thomas and Read 2007). We have isolated an entomopathogenic fungus from cadavers of cardamom thrips and identified as *L. psalliotae* based on morphological, molecular and SEM studies. To the best of our knowledge, this is the first record of a fungus infecting cardamom thrips and also a first report of *L. psalliotae* from India. Elsewhere, the fungus has been recorded in USA, Cuba, Ghana, Spain, UK (Zare and Gams 2001), Brazil (Arevalo et al. 2009), Iran (Asad et al. 2008), Indonesia, Japan (Sukarno et al. 2009) and China (Zheng et al. 2012).

Macro- and micro-morphological features of the fungus grown on PDA agreed with the features reported by Zare and Gams (2001) for *L. psalliotae*. We observed slight differences in the minimum and maximum measurements of macro- and microconidia and phialides. Similar variations in dimensions have been reported earlier (Steenberg and Humber 1999). From India, the earlier report of *L. saksenae* (Kushwaha 1980) was considered as a synonym of *L. psalliotae* (Zare and Gams 2001). In further studies based on colony characteristics and phylogenetic analyses using ITS regions, *L. psalliotae* was considered as a separate species from *L. saksenae* (Sukarno et al. 2009). Another taxonomically important characteristic feature of *L. psalliotae* is the presence of octahedral crystals in media and production of a diffuse red pigment into the agar media. These characters were used to differentiate *L. psalliotae* from *L. saksenae* which are otherwise very closely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◀**Fig. 3** Scanning electron microscopy (SEM) of pathogenesis by *L. psalliotae* on *S. cardamomi*. **a** Adhesion, germination of spores and development of infective pegs. **b** Close-up of conidum with appresorium on body surface. Bars 5 μm in **a** and **b**. **c, d** Penetration and emergence of hyphae through inter-segmental regions. Bars 50 μm. **e, f** Phialides bearing conidia. Bars 40 μm in **e** and 10 μm in **f**. **g** Macroconidia. Bar 40 μm. **h** Microconidia (arrow shows signs of cuticle degradation). Bar 20 μm. **i** Mycelial ramification on head region. Bar 50 μm. **j** Mummified thrips with mycelial growth and (inset) close up of hyphal growth on the ventral surface of thoracic region. Bar 500 μm and inset bar 100 μm

related (Sukarno et al. 2009; Zare and Gams 2001). These diffusing pigments were normally produced after ten days of growth of the fungus in the medium (Zare and Gams 2001). Our isolate produced the characteristic pigments within 72 h of inoculation into PDB at 25 °C and the medium turned deep red after 10–12 days. Early pigmentation and its relation to virulence need to be further studied. These findings along with the observations on variations in morphological measurements suggested the possibility of the present fungus belonging to a new strain/isolate of *L. psalliotae*, not reported so far.

We sequenced the amplicons of the ITS rDNA, TUB and TEF of our *L. psalliotae* isolate and performed phylogenetic analyses with corresponding gene sequence data retrieved from GenBank. Some of the *L. psalliotae* isolates used for comparison in the ITS phylogeny showed a close relationship with *L. aphanocladii, L. dimorphum, L. attenuatum* and *L. muscarium* which were also reported by earlier workers (Sukarno et al. 2009; Zare and Gams 2001; Kouvelis et al. 2008). Interestingly, our isolate clustered together with an isolate from China showing signs of geographical association among the isolates. Unfortunately, the TUB and TEF sequence data of this Chinese isolate was not available for comparison with our isolate. Clustering of entomopathogenic fungi strongly associated with different habitats and to a lesser extent with insect hosts were observed in *Metarhizium anisopliae* and *Beauveria* spp. (Aquino de Muro et al. 2005; Bidochka et al. 2001) whereas, it appears the converse associations are more common with *Lecanicillium* spp. studied so far (Kouvelis et al. 2008). However, more studies are required with a large collection of different isolates from various geographical locations to confirm this observation. The ITS rDNA phylogeny further demonstrated that the Indian isolate of *L. saksenae*, which was considered as a synonym of *L. psalliotae*, did not cluster with our isolate proving the distinctness of the latter. Earlier, Sukarno et al. (2009) also made a similar observation that none of their *L. psalliotae* isolates from Indonesia and Japan clustered with the ex-type strain of *L. saksenae* from India.

The bioassay studies conducted with purified conidial suspension of our isolate confirmed the infectivity of the fungus to thrips. At the highest dose tested (1×10^7 conidia ml^{-1}), up to 62 % mortality was recorded ten days post inoculation. Earlier studies have demonstrated the utility of entomopathogenic fungi such as *L. lecanii, L. muscarium, M. anisopliae* and *B. bassiana* for the control of different thrips species under laboratory and field conditions (Gouli et al. 2008; Maniania et al. 2003; North et al. 2006). To the best of our knowledge, there were no reports on the isolation of *L. psalliotae* or its evaluation against thrips as a biocontrol agent, though its entomogenous activity was proved in other insects (Asensio et al. 2005). An isolate of *L. psalliotae* from Iran was equally virulent against eggs and larvae of *Rhipicephalus annulatus* (Say). This strain when tested under laboratory conditions caused 56.6 % mortality in female ticks within 6–11 days at 1×10^7 conidia ml^{-1} and decreased the reproductive efficiency of females by 78.15 % (Kheirabadi et al. 2007).

SEM is an effective tool to evaluate the infection process of various entomopathogenic fungi against their insect hosts (Askary et al. 1999; Asensio et al. 2005; Vestergaard et al. 1999). In our studies, we found conidia adhering throughout the body surface. The initial attachment of fungal conidia to the host surface is based on hydrophobic interactions (Boucias and Pendland 1991). Usually, conidia of *Lecanicillium* spp. are less hydrophobic or hydrophilic than *B. bassiana* (Jeffs et al. 1999) and are coated with a thin layer of mucilage that mediates the permanent attachment of the fungal conidia to the host cuticle (Boucias and Pendland 1991). Appresoria of *L. psalliotae* were formed directly from the conidia that are well-known penetrating structures of fungal invertebrate pathogens (Asensio et al. 2005; Vestergaard et al. 1999). We observed depression at the site of germination of the conidia confirming signs of secretion of enzymes/mechanical pressure. The penetration sites were often seen as dark melanotic lesions in the epicuticle, which could be due to cuticle degrading enzymes and mechanical pressure (Vestergaard et al. 1999). The

infective pegs were found to penetrate through the intersegmental membranes of the thrips. Towards later stages of infection, the fungus emerged out through the inter-segmental regions of thrips and the hyphae differentiated into phialides bearing conidia and spread throughout the body presenting a mummified appearance. This indicated the completion of the life cycle of the fungus in the insect host and confirmed the full infection process.

The diseases so far reported in Thysanoptera were only caused by entomopathogenic fungi. The virulence of *L. psalliotae* as demonstrated in the present bioassay and SEM studies proved that the fungus could be developed as a candidate biological control agent against cardamom thrips reducing over dependence on currently used insecticides harmful to the environment. Earlier studies with *L. psalliotae* demonstrated its nematophagous (Yang et al. 2005), entomopathogenic (Asensio et al. 2005; Steenberg and Humber 1999) and antagonistic activity against plant pathogens (Nagaoka et al. 2004), proving its potential for development as a single commercial biocontrol agent against insect pests and diseases. Our future studies are aimed at field testing of this pathogen and development of a suitable formulation for inclusion in IPM schedules against cardamom thrips.

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