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First record of occurrence of *Dervishiya cadambae* on grapevine, *Vitis vinifera*, along with its morphological and molecular identification and pathogenicity evaluation potential of *Metarhizium brunneum* as its biocontrol agent

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Abstract

Increasing demand for grapes (*Vitis vinifera* L.) worldwide makes it a high-value crop and also an important, valuable export commodity for India. In February 2016, a vineyard from Maharashtra, India was identified with entirely new insect damage symptoms from those observed earlier. After, further inspection, a new type of wood borer was noticed which was later identified as *Dervishiya cadambae* (Moore) (Lepidoptera Cossidae). *D. cadambae* is known as a major pest of *Tectona grandis* L.f. plantations in Kerala, Karnataka and Tamil Nadu states of India and causes extensive damage to the timber. Surveys were conducted in affected vineyards to assess the extent of infestation from 2016 to 2018. In 20 infested vineyards located in Sangli and Nashik districts of Maharashtra, 12-72% of grapevines were found to have active infestations. Young larvae fed under the bark and later instars bored inside and made galleries. *D. cadambae* caused extensive damage to the sapwood and heartwood of grapevine stem and reduced both vitality and productivity of the vines. Deoxyribonucleic acid (DNA) barcode for this new pest of grapevines was also provided. The DNA barcode grouped *D. cadambae* in the clade of Cossidae samples supported with a bootstrap value of 85% in phylogenetic analysis. To our knowledge, this is the first record of *D. cadambae* occurrence on *V. vinifera*. A green muscardine fungus was isolated from the field infected larvae of *D. cadambae*. The pathogenicity test confirmed Koch's postulates. The fungus was identified as *Metarhizium brunneum* (Petch), which proved to be an efficient antagonist of this pest in laboratory bioassays.

Key words: grapevine new pest, wood borer, Cossidae, DNA barcoding, entomopathogenic fungi, bioassay, India.

Introduction

Vitis vinifera L. vineyard is an important commercial fruit crop in temperate regions and has been adopted in Indian sub-tropical and tropical conditions (Mani *et al*., 2014). Grapevine cultivation requires high investments and also fetches high returns to the growers. Two species of wood borers, *Celosterna scabrator* (F.) and *Stromatium barbatum* (F.) (Coleoptera Cerambycidae) have been reported causing damage to grapevines (Ranga Rao *et al*., 1979; Salini and Yadav, 2011).

During a survey conducted in February 2016, 48% of grapevines were found infested with a new type of wood borer at Shivani, Sangali, Maharashtra, India. The symptoms of infestation were different from those observed previously for cerambycid wood borers infesting grapevines. Farmers at Vijayapura in the Karnataka state of India also reported similar infestation symptoms and presence of red coloured wood borer larvae during April, 2016. The wood borer was later identified as *Dervishiya cadambae* (Moore) (Lepidoptera Cossidae).

The genus *Dervishiya* Yakovlev was established for *Cossus cadambae* Moore (type locality- Calcutta) (Yakovlev, 2006). Later, two new species from Afghanistan and Pakistan were also described, namely, *Dervishiya vartianae* Yakovlev (type locality- Afghanistan, Nimla, 40 km SW v. Dschelalabad) and *Dervishiya sindhi* Yakovlev et Saldaitis (type locality- Pakistan, Sindh province, near Sanghar, Shahdadpur) (Yakovlev, 2011; Yakovlev and Saldaitis, 2016). *D. cadambae* is widespread in India and was also reported for the first time from Sri Lanka (=Ceylon) by Arora (1976). It has been earlier reported to infest *Ficus* L. (Moraceae), *Mangifera indica* L. (Anacardiaceae), *Diospyros melanoxylon* Roxburg (Ebenaceae), *Tectona grandis* L.f. (Verbenaceae), *Nauclea cadamba* Roxburg (Rubiaceae), *Grewia tiliaefolia* Vahl. (Tiliaceae), *Terminalia bellerica* Roxburg (Combretaceae), *Butea monosperma* Taub. (Fabaceae) (Gardner, 1945; Mathew *et al*., 1989; Robinson *et al*., 2001; Santosh and Kumar, 2003). *D. cadambae* has assumed major pest status in *T. grandis* plantations in Kerala, Tamil Nadu and Karnataka states of India and cause extensive damage to the timber (Mathew, 1990). *T. grandis* is naturally distributed in the peninsular region of India stretching along the Western Ghats which also has prominent Indian grape growing areas such as Pune and Nashik districts of Maharashtra and Vijayapura district of Karnataka states (Kaosa-ard, 1989; Ratan, 2013). It can be assumed that *D. cadambae* came from *T. grandis* and other forest host trees and started infesting nearby vineyards initially and later on increased its spread in other vineyards assuming a major pest status.

Systemic work on biology, ecology, and management of *D. cadambae* in *T. grandis* was carried out by Mathew (1990). He reported that *D. cadambae* was emerging as a serious pest of *T. grandis* in the Kerala

state of India. He also stated that the trees weakened as a result of mechanical injuries were more susceptible to the attack. The infestation resulted in girdling of side shoots which subsequently fell off leaving a scar on the wood through which the larva tunneled into the wood. Extensive larval feeding led to callus growth and distorted bark formation. The bark never recovered with was lost due to larval feeding leading to wood exposure which resulted in subsequent decay. The infested trees suffered repeated attacks during subsequent years which led to the riddling of the timber with several holes. He found that trunk injection of insecticides were not feasible to manage this pest due to the larval habit of boring into the heartwood where pesticide did not get translocated. He recorded six microbial pathogens and two bird species as natural enemies but found them to be not directly useful in developing management strategies. He used William's type light trap for monitoring of *D. cadambae* moths and Veeranna and Remadevi (2011) used Robinson light trap. Bhandari and Upadhyay (1986) reported that *D. cadambae* larva fed cambium and sapwood of stem, collar region and root of *D. melanoxylon* leading to girdling, reduced vigour as well as mortality of the plant. He reported annual lopping and fire damage to *D. melanoxylon* as factors for the borer attack. Mathew and Rugmini (1996) reported that it caused extensive bark injury and riddling of the trunk of *T. grandis* with numerous holes. The infested tree subsequently got attacked by various pathogenic or saprophytic fungi which resulted in dieback as well as decay of wood.

The application of entomopathogens for pest control is important to reduce the harmful side effects of chemical pesticides. Species belonging to the genus *Metarhizium* Sorokin are largely reported as biocontrol agents and play a very important role in structuring the IPM program (Chandler *et al*., 2011). Its wide geographical distribution and infectivity make them an important biological control agent (Zimmermann, 2007). They degrade the insect cuticle and produce mycolytic enzymes to kill the pest (Wang *et al*., 2004).

Here, in this paper, we provide the first report of the occurrence of *D. cadambae* in grapevine, *V. vinifera*. Various life stages of *D. cadambae*, its damage symptoms on vines and its effect on vitality and productivity of grapevines are also described along with its deoxyribonucleic acid (DNA) barcode. We also report isolation and identification of the entomopathogenic fungi *Metarhizium brunneum* (Petch) from field infected larvae of *D. cadambae* and its use in laboratory bioassay against larvae.

Materials and methods

Symptoms and infestation level

Surveys were conducted from June 2016 to February 2018 in 20 *D. cadambae* infested vineyards located in Sangli and Nashik districts of Maharashtra state of India. For each vineyard, location, variety, year of the planting of the vineyard and sampling date were recorded. Visual observations on symptoms were noted during the surveys. In each vineyard, the number of infested grapevines and the total number of grapevines were counted, and then the percentage of infested grapevines was calculated. The correlation coefficient was calculated between year of the planting of the vineyard and per cent infestation to determine the effect of vineyard age with the infestation level. Effect of variety on the per cent infestation was calculated between Sharad Seedless and its clones versus Thompson Seedless and its clones using two-sample t-test. We also collected *D. cadambae* larval specimens during surveys and paired them in the laboratory on an artificial diet of wet blotting paper for knowing the time of moth emergence. An infested vineyard (variety Sharad Seedless, year of planting 15 years) was surveyed at Sayyad Pimpri (20°02'48.9"N 73°55'43.4"E) in Nashik district during February 2020 to find out whether *D. cadambae* could infest grapevine roots or not. Twenty vines were uprooted completely from the ground and infestation on roots was observed. Further, five infested vineyards were surveyed in Nashik district from January to February, 2020 for estimation of the effect of *D. cadambae* infestation on vitality and production of grapevines.

Taxonomy and morphological description of insect

The distinctive external features of the genus *Dervishiya* were analysed and described. Male and female genitalia studies were carried out as described by Robinson (1976). The abdomen was treated in 10% KOH for 10 to 20 min at 90 °C using a Dry Block Heizgerat 2800. Genitalia was cleaned and stored in glycerol. For pictures, genitalia was placed on a slide in glycerol with a coverslip. Photographs were taken with a Leica DFC425C digital camera mounted on a Leica M205FA stereozoom microscope and processed with Automontage© software.

Deoxyribonucleic acid (DNA) barcoding

DNA was extracted from the skin tissue of *D. cadambae* larva and adult using universal and rapid salt extraction method (Aljanabi and Martinez, 1997). For amplification of COI gene primers LCO1490:5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' were used (Folmer *et al*., 1994). Polymerase chain reaction (PCR) reaction was performed as described earlier (Jadhav *et al*., 2016).

Sequencing and phylogenetic analysis

The resultant forward and reverse sequences were aligned using BioEdit Sequence Alignment Editor to generate a consensus sequence. The resultant FASTA format of these sequences was used for species identification using BLAST search at the National Centre for Biotechnology Information (NCBI) and using identification request on Barcode of Life Database (BOLD). For phylogenetic analysis, sequences with maximum hits were retrieved from GenBank and analysis was carried out using the MEGA 6 program. A neighbour-joining tree was constructed using the Kimura-2-parameter distance model. A 1000 bootstrap replications were used for consensus tree generation.

Fungus isolation and identification

The entomopathogenic fungus used in the study was isolated from field infected larvae as per the procedure described by Jaber *et al.*, (2016). Pathogenicity was confirmed as per Koch's postulate. Single spore fungal culture was maintained on potato dextrose agar (PDA) medium. Growth, appearance, and coloration of the fungus on the PDA plate was used for morphological identification. Conidia were identified using lacto phenol-cotton blue stain. For molecular identification DNA was extracted using DNeasy Plant Mini Kit (Qiagen, GMBH, Germany). The fungus was identified based on four different genes: Exon region of the translation elongation factor 1-alpha (*EF*-1α exon), 5' intron-rich region of the translation elongation factor 1-alpha (5'*EF*-1α intron), beta-tubulin, RNA polymerase second largest subunit (*RPB2*) (White *et al.*, 1990; O'Donnell and Cigelnik, 1997; Rehner and Buckley, 2005).

PCR amplification was performed in 50 µl reaction mixture containing 2 U *Taq* polymerase, 5 µl of 10X *Taq* buffer, dNTP at 200 µM, 10 pmol of gene primers and 50 ng DNA. PCR cycling conditions for amplification of the *EF-1α* exon were, an initial denaturation for 4 min at 94 °C, followed by 37 cycles of denaturation for 30 s at 94 °C, annealing for 1 min at 60 °C, extension for 1 min at 72 °C, and a final extension for 10 min at 72 °C while, for *b-tubulin* annealing temp was used as 58 °C. Reaction mixture for amplification of 5'*EF*-1α intron region was similar to *EF-1α* exon except for dNTP which was used at 250 µM. PCR cycling parameters were an initial denaturation for 4 min at 94 °C, followed by 35 cycles of denaturation for 30 s at 94 °C, annealing for 40 s at 56 °C, extension for 30 s at 72 °C, and a final extension for 10 min at 72 °C. Amplification of *RPB2* was performed as described earlier (Sawant *et al*., 2017). The PCR products were sequenced in both direction and the obtained sequences were compared with type cultures at NCBI using BLAST search. Multigene analysis of *EF-1α* exon + *5'EF-1α* intron region + *beta-tubulin* + *RPB2* gene sequences was performed using the authentic sequences as described earlier (Bischoff *et al*., 2009).

Fungal inoculum and laboratory bioassay

Roux bottle containing 200 ml sterile potato dextrose broth (PDB) medium (Hi-Media) was inoculated with one disc of 10 mm from a 4-day old culture on PDA plate. Fungal mat was blended and filtered through a muslin cloth and diluted with sterile distilled water (SDW) after 15 days of incubation. Two-three drops of 0.1% Tween 80 was added. The conidia count was adjusted using a haemocytometer. Five different treatments, which included four conidia concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 conidia/ml) and control (without conidia), were compared. Treatment consisted of three replications and each replication consisted of five larvae. Healthy *D. cadambae* larvae were collected from vineyards and placed individually in glass Petri plates containing a moistened filter paper. As the instar of the field-collected larvae could not be ascertained, the larvae which ranged from 18 to 25 mm in length, 1.5 to 2.5 mm in width and 0.08 to 0.15 gram in weight were selected for the experiment. With the help of a hand atomizer, the conidial suspension and control treatments were sprayed on the larvae. The larvae were monitored daily and mortality was recorded up to 15 days.

The mortality rate was calculated using corrected Abbott's formula (Abbott, 1925). Data were analysed in CRD with an analysis of variance (ANOVA). Means were compared using Tukey's test using SAS (ver. 9.3; SAS Institute Inc., Cary, North Carolina, USA).

Results and discussion

Symptoms and infestation level

Eggs of *D. cadambae* are yellowish-white and round in shape (figure 1A). Mathew (1990) and Veeranna and Remadevi (2011) also reported the eggs to be spherical. They mentioned that the egg chorion had a reticulate sculptured pattern resembling the bark of teak (*T. grandis*) which made the eggs difficult to detect. The eggs are mainly laid in groups in cracks and crevices or under the loose bark of the main trunk and cordons of grapevines (figure 2A). Similarly, the eggs were laid in small cracks and crevices or depression on the bark of trees of *T. grandis* (Mathew, 1990; Veeranna and Remadevi, 2011). However, in *D. melanoxylon,* the eggs were laid on pruning snags of wounds caused by dying back of branches, fire or coppicing (Bhandari and Upadhyay, 1986).

The young larvae remain under the bark and feed on sapwood in grapevines. As larvae feed under the bark, their presence goes undetected. Careful observation allows to detect, excreta entangled with webbings protruding outside bark (figure 2B). After the removal of loose bark, the larva becomes visible feeding on softwood (figure 2C). The later instar larva bore inside and make galleries in the direction along the length of the main trunk and cordons (figure 2D). The average length of the gallery was 71.94 cm (range 8.4 to 184 cm) and the average diameter was 3.43 cm (range 1.56 to 4.7 cm). The galleries were mostly irregular elongated round or oval-shaped, however, few galleries were also round and dumbbell-shaped (figure 2E). The entry hole made by later instar larva is closed with excreta and webbings (figure 1F). Similarly, when *D. cadambae* infests *T. grandis*, the young larva remain concealed under a web of silken fibres and feed on the bark including outer sapwood as reported by Mathew (1990). He also reported that the larval feeding resulted in girdling of the side shoots leading to die-back in *T. grandis*. However, the die-back symptoms were not observed in grapes due to *D. cadambae* infestation. Bhandari and Upadhyay (1986) also reported that newly hatched *D. cadambae* larva feeds on the cambium and sapwood of the stem of *D. melanoxylon*. *D. cadambae* caused numerous borer holes on the stem causing extensive bark damage to *B. monosperma* (Santosh and Kumar, 2003). Bhandari and Upadhyay (1986) also reported damage to *D. melanoxylon* roots by *D. cadambae* larva. Similarly, when the vines were uprooted during February 2020, 60% of the infested grapevines also had infestations in

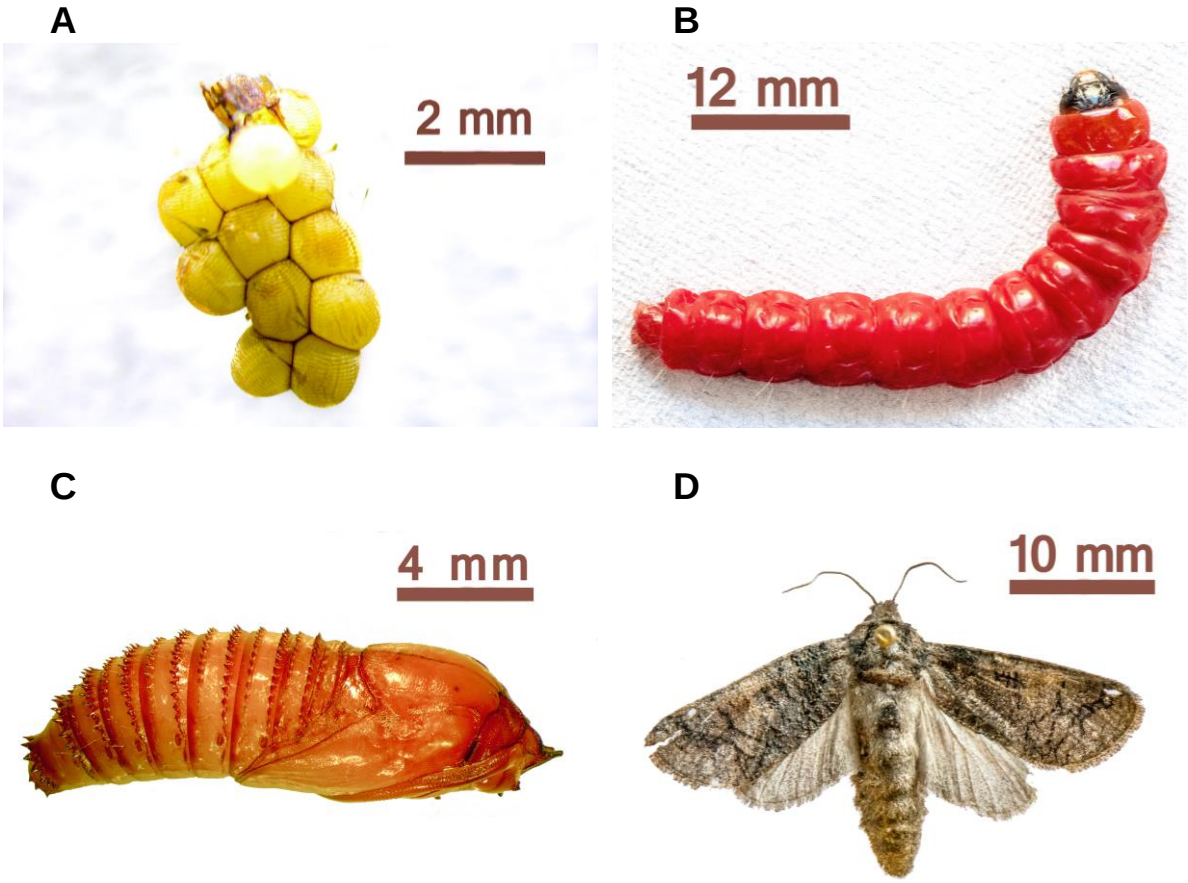

Figure 1. *D. cadambae*: (**A**) eggs; (**B**) seventh instar larva; (**C**) pupa; (**D**) female.

the roots as well (figure 2G). The galleries were filled with mud, however, no larva or pupa was observed in any of the uprooted vines indicating the old infestation in the roots. Veeranna and Remadevi (2011) reported that the first and second instar larva were located just under the bark and by the third instar, the larva had moved into the heartwood of *T. grandis*.

In the 20 infested vineyards which were surveyed, 12-72% of grapevines were found having active infestations in Sangli and Nashik districts of Maharashtra state of India (table 1). Initially, only grape variety Sharad Seedless and its clones were found infested, however, later, Thompson Seedless and its clones also showed similar symptoms on the grapevines. The year of the planting of the vineyard and per cent infestation were found moderately correlated (correlation coefficient = 0.50). This increase might be due to the re-infestation of the same vineyard with new healthy vines being infested over the years. Mathew (1990) also reported the positive correlation between *D. cadambae* infestation level and age of *T. grandis* plantation due to the spread of infestation on healthy trees in the same plantation over the years. The mean infested vines were 33.33 and 31.37 % on Sharad Seedless and its clones and Thompson Seedless and its clones, respectively. The varietal difference in *D. cadambae* infestation was non-significant (Difference = 1.96, F = 1.17, Pr>F = 0.7840, Pr>ItI = 0.8186). During surveys, different sizes of larva were observed on the grapevines suggesting overlapping generations. Smaller larvae were pale pinkish in colour and larger larvae were pink to dark pink (figure 1B). Veeranna and Remadevi (2011) also found different larval instars throughout the year and Mathew (1990) reported moth emergence during the whole year supporting the presence of overlapping generations. Veeranna and Remadevi (2011) reported seven larval instars of *D. cadambae* with the total larval duration of 218 days in the field. They also mentioned that all cossids studied so far by various workers show that the larval period was very long and there was mostly one generation per year. Similarly, Mathew (1990) reported egg, larval, pupal and adult average duration to be 20, 213, 11 and 5 days, respectively with the total life cycle of average 249 days. The pupa was brownish in colour and had rows of spine-like processes on the dorsum of abdominal segments (figure 1C). During surveys, neither pupal case nor pupa was found either under the bark or inside the gallery in grapevines. Mathew (1990) reported that the pupation took place in ground inside a pupal chamber lined with silken fibres. On the contrary, Beeson (1941) reported that the pupation took place in the tunnel where larva was feeding on *T. grandis*.

All the five *D. cadambae* infested vineyards which were surveyed for estimating the effect of the infestation on vitality and production of grapevines during January-February 2020, showed significant reduction on all these

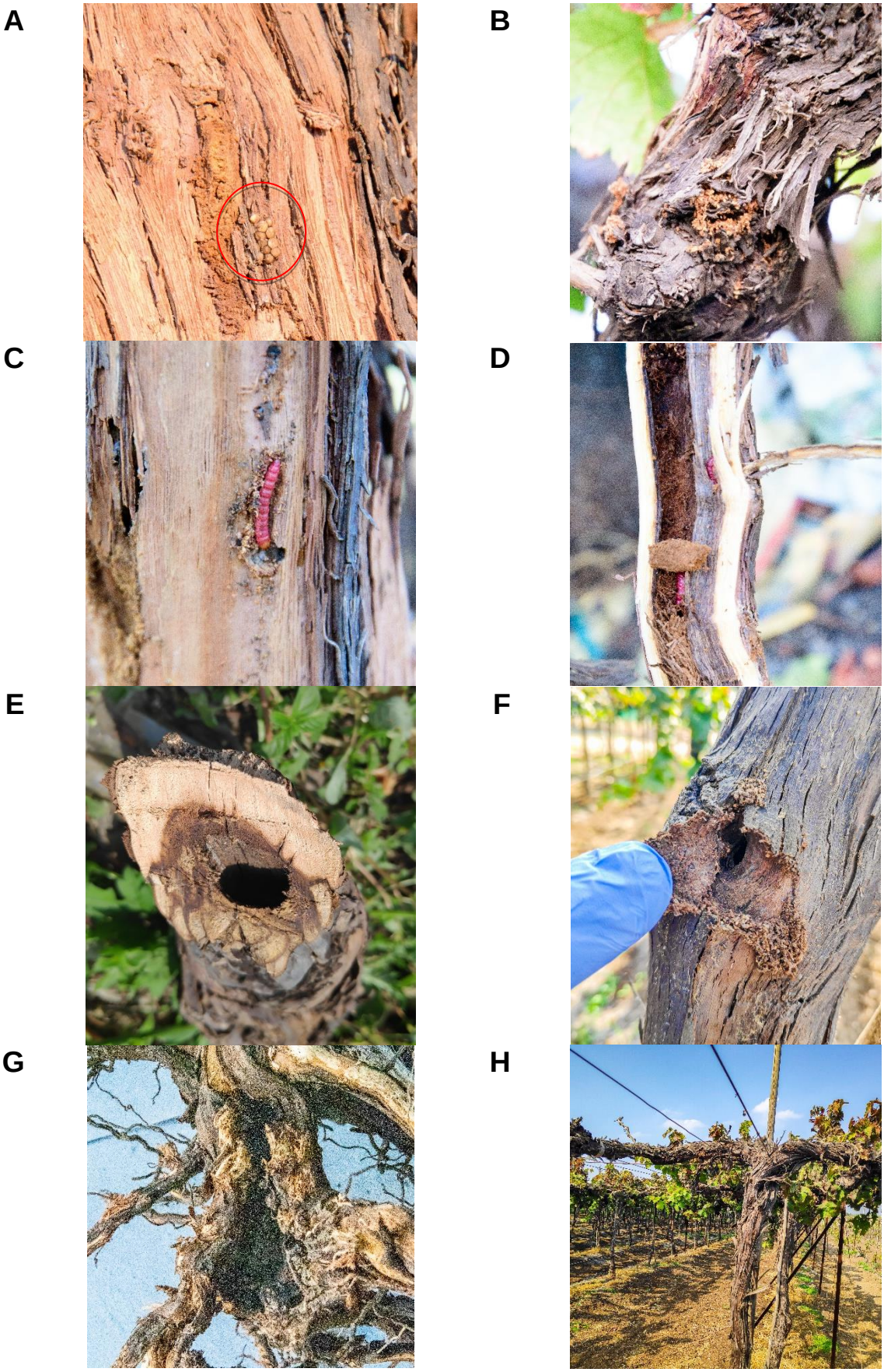

Figure 2. Symptoms of infestation of *D. cadambae* in grapevine: (**A**) eggs; (**B**) excreta mixed with webbings protruding over loose bark; (**C**) larva feeding on sapwood; (**D**) larval gallery (**E**); larval gallery shape (**F**); entry hole covered with excreta and webbings; (**G**) root infestation; (**H**) dead vine due to infestation.

Table 1. Infestation level by *D. cadambae* in vineyards of Maharashtra state.

Variety	Years of planting of vineyard	Infested vines (%)	Month of survey	Location	Latitude Longitude
Sharad Seedless or its clone					
Jumbo Seedless	4	26	June, 2017	Bhavaninagar, Pune	18005'43.6"N 74040'45.4"E
Sharad Seedless	6	30	June, 2017	Bhavaninagar, Pune	18005'45.4"N 74040'45.5"E
Sharad Seedless	7	12	July, 2017	Pingalwade, Nashik	20043'44.0"N 74007'32.0"E
Sharad Seedless	8	36	June, 2017	Nandurdi, Nashik	20008'00.3"N 74006'03.8"E
Sharad Seedless	8	32	July, 2017	Sayyad Pimpri, Nashik	20002'49.2"N 73055'43.3"E
Sharad Seedless	8	22	July, 2017	Sayyad Pimpri, Nashik	20002'50.8"N 73055'43.3"E
Sarita Seedless	8	38	September, 2017	Morale, Sangali	17007'08.3"N 74030'53.3"E
Sharad Seedless	9	72	November, 2017	Mahadeopur, Nashik	20003'17.6"N 73041'49.7"E
Sharad Seedless	10	18	July, 2017	Pingalwade, Nashik	20043'45.2"N 74007'34.0"E
Krishna Seedless	10	22	December, 2016	Walwa, Sangali	16001'27.3"N 74021'52.2"E
Krishna Seedless	11	64	February, 2018	Malangaon, Sangali	17002'47.0"N 74048'03.4"E
Krishna Seedless	12	28	September, 2016	Walwa, Sangali	17001'59.7"N 74022'18.3"E
Thompson Seedless or its clone					
Tas A Ganesh	5	21	July, 2017	Bhavaninagar, Pune	18005'48.1"N 74040'39.8"E
Tas A Ganesh	5	18	July, 2017	Bhavaninagar, Pune	18005'49.8"N 74040'43.7"E
Tas A Ganesh	6	15	July, 2017	Katewadi, Pune	18007'39.5"N 74039'13.5"E
Thompson Seedless	7	15	February, 2018	Nandurdi, Nashik	20007'56.0"N 74006'06.2"E
Sonaka	12	68	February, 2018	Malangaon, Sangali	17002'46.9"N 74048'04.3"E
Thompson Seedless	12	42	June, 2016	Bedag, Sangali	16046'33.3"N 74043'17.1"E
Thompson Seedless	12	24	June, 2016	Vaddi, Sangali	16047'58.5"N 74041'12.9"E
Thompson Seedless	12	48	July, 2017	Sayyad Pimpri, Nashik	20002'59.6"N 73055'24.6"E

parameters due to the infestation (table 2). On the scale of 0-10 (rating 0 depicting dead vine, 10 means completely healthy vine), the average vitality ratings ranged from 3.2 to 5.84 and 8.1 to 8.94 in *D. cadambae* infested and healthy vineyards, respectively. Out of total 250 infested vines observed for vitality, 20 vines were found dead as a result of *D. cadambae* infestation. *D. cadambae* infested vines had lower number of bunches per vine which ranged from 5.14 to 9.76 as compared to 20.46 to 34.16 in healthy vines. The average yield per vine ranged from 2.39 to 4.12 and 7.01 to 12.87 kg in *D. cadambae* infested and healthy vines (table 2).

Bhandari and Upadhyay (1986) also reported the adult emergence primary period as after pre-monsoon rains in May-June months to August in Jabalpur, Madhya Pradesh, India. Mathew (1990) found adult emergence throughout the year with two distinct peaks during May-June and September-October in Kerala, India. Moore (1965) also reported that according to Mr. Atkinson, the moth of *D. cadambae* appeared at intervals from the end of February till November. When we collected *D. cadambae* larvae during surveys and reared them in the laboratory, the moths emerged during last week of February to third week of March during 2017 and 2018.

The surveys for *D. cadambae* infestation were not conducted in other important grape growing states of India which are Tamil Nadu, Karnataka, Andhra Pradesh, Telangana and Mizoram. Among these states,

Table 2. Effect of *D. cadambae* infestation on production and vitality of grapevines.

Vineyard details	Grapevine status	Number of bunches per vine	Yield per vine (Kg)	Vitality ratings (0-10)
Vineyard 1 (Pimpri Sayyad, Nashik, 20°01'38.6"N 73°55'47.7"E, Sharad Seedless, 11 years old)	Infested vines	7.60	2.39	3.98
	Healthy vines	34.16	11.50	8.94
	Difference	−26.5600	−9.111	−4.96
	F Value	5.43	5.8	2.68
	Pr> F	< 0.0001	< 0.0001	0.0008
Vineyard 2 (Thergaon, Niphad, Nashik, 20°06'32.9"N 73°58'36.4"E, Sharad Seedless, 12 years old)	Infested vines	6.68	3.40	5.32
	Healthy vines	29.32	12.87	8.56
	Difference	−22.6400	−9.4781	−3.24
	F Value	5.64	7.35	3.65
	Pr> F	< 0.0001	< 0.0001	< 0.0001
Vineyard 3 (Pimpri Sayyad, Nashik, 20°02'48.9"N 73°55'43.4"E, Sharad Seedless, 15 years old)	Infested vines	5.14	2.41	3.92
	Healthy vines	20.46	9.01	8.1
	Difference	−15.3200	−6.5956	−4.18
	F Value	3.10	2.09	3.53
	Pr> F	< 0.0001	< 0.0001	< 0.0001
Vineyard 4 (Jaulake Vani, Dindori, Nashik, 20°12'38.2"N 73°57'14.2"E, Sharad Seedless, 10 years old)	Infested vines	9.76	4.12	5.84
	Healthy vines	26.24	10.96	8.66
	Difference	−16.4800	−6.8316	−2.82
	F Value	2.40	2.16	4.24
	Pr> F	0.0027	0.0081	< 0.0001
Vineyard 5 (Pimpri Sayyad, Nashik, 20°03'53.9"N 73°55'33.0"E, Sharad Seedless, 11 years old)	Infested vines	8.30	3.73	4.88
	Healthy vines	24.58	7.01	8.48
	Difference	−16.2800	−3.274	−3.6
	F Value	4.85	1.9	1.77
	Pr> F	< 0.0001	0.0272	0.0472

D. cadambae has already established as a major pest of *T. grandis* in Karnataka and Tamil Nadu states of India (Mathew, 1990). Therefore, it has potential to cause economic damages to grapes being grown in Karnataka and Tamil Nadu states as well.

Taxonomy and morphological description of the insect

The genus *Dervishiya* belongs to the nominative subfamily Cossinae and has several significant distinctive morphological features. It is medium-sized and dark-coloured moth. Male antenna are bipectinate with long comb processes, while the female antenna are of filiform type. Fore wing with rounded apex, thin undulated pattern of fine transverse lines, more developed in proximal half of wing. Hind wing with poorly developed reticulated pattern.

The male genital apparatus has a long uncus rounded at the apex (figure 3). Tegumen large. Gnathos small with arms relatively short. Transtilla processes almost fully reduced, like small lobate outgrowths. Valves with expressed sacculus and large process on costal edge. Juxta large, with big leaf-like lateral processes equal in size to half of valve length, saccus very big, positioned backwards. Aedeagus big, strongly sclerotized, slightly longer than valve, almost straight. Strongly widened in distal part, with two tooth-like processes on edges. Distal aperture in dorso-apical position, about 3/5 of aedeagus in length. On lower edge of aedeagus, two small spinous processes directed abdominally.

Female genitalia is with papillae anales oval and posterior apophyses three times longer than anterior apophyses. Ostium aperture is covered with large sclerotized plate. Ductus is short, strongly sclerotized. Bursa copulatrix is long, sack-like, with two parallel ribbon-like signa on lateral surface and the ovipositor is long.

The voucher material (Reference number: V. no. 1312/17; Sex: Male; Identified by: R. V. Yakovlev and P. R. Shashank) has been deposited at the National Pusa Collection (NPC), Division of Entomology, Indian Agricultural Research Institute, New Delhi, India (figure 3A).

DNA barcoding

Sequence of more than 630 base pairs was used for sequence similarity search. BLAST analysis of both sequence showed 98% sequence similarity with cytochrome oxidase-1 (CO1) sequences from Cossidae (figure 4). While in BOLD sequence showed 98.75% sequence similarity with CO1 sequences from Cossidae. In phylogenetic analysis too *D. cadambae* grouped in clade of Cossidae samples supported with bootstrap value of 85% with internal node supported with bootstrap value of 100%. CO1 region sequences of *D. cadambae* larva and adult have been deposited in NCBI GenBank under the accession numbers MG279391 and MG746603, respectively.

Fungus identification

Green coloured colony with radial growth was observed on PDA plate. The fungus was named R1. Microscopic observation revealed elongated rounded

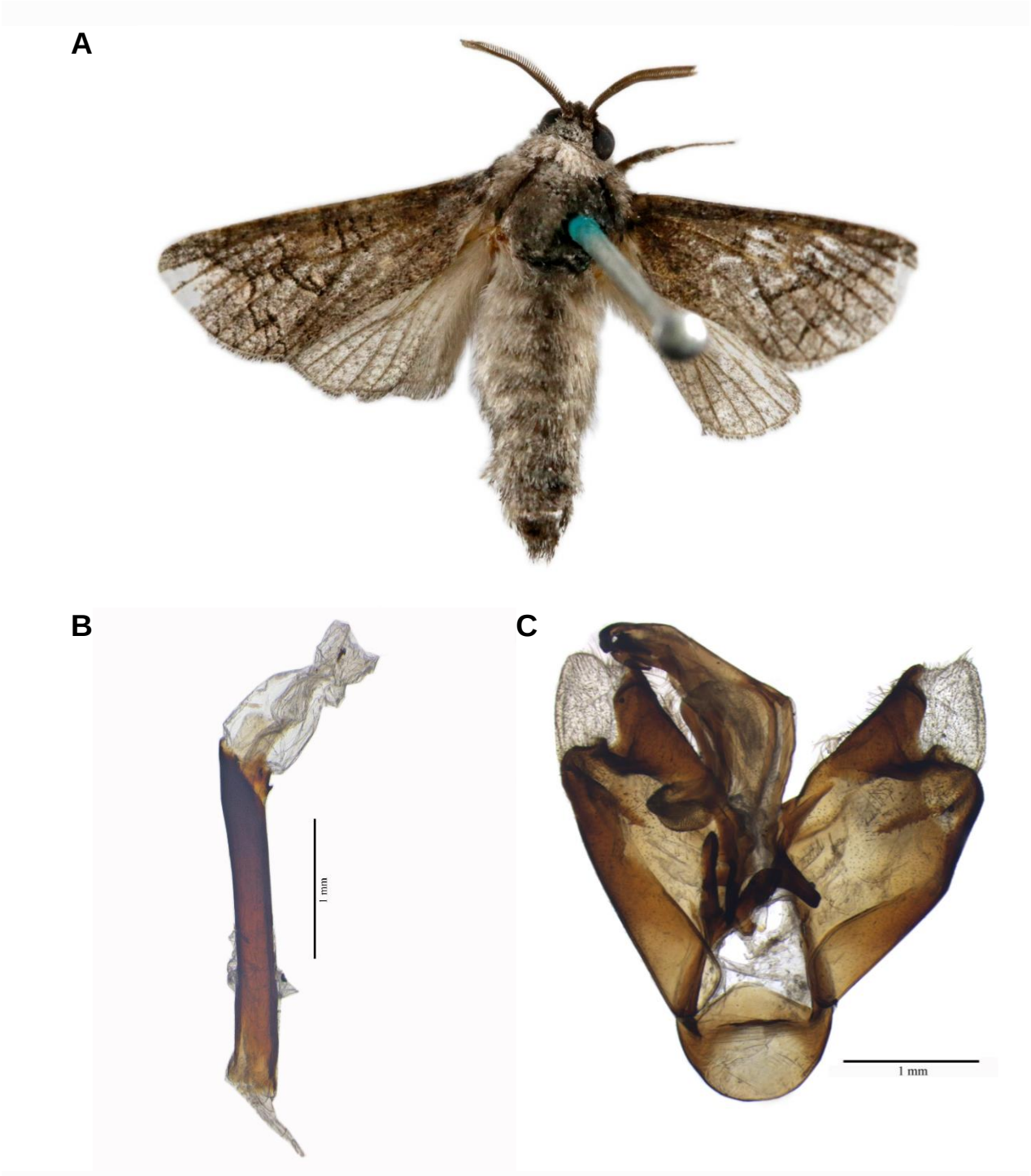

Figure 3. *D. cadambae* male: (**A**) voucher specimen; (**B**) aedeagus; (**C**) genitalia.

conidia on conidiophores. Isolate showed similarity with *M. brunneum*. Phylogenetic analysis using EF-1α exon + 5'EF-1α intron region + beta-tubulin+ RPB2 gene datasets, showed that the isolate formed the clade with authentic sequences of *M. brunneum* supported with 97% bootstrap values (figure 5). Thus this isolate was identified as *M. brunneum.* Combine gene sequence of EF (EF-1α exon + 5'EF-1α intron region), beta-tubulin and RPB2 gene were submitted to NCBI Genebank with accession number MH711929, MH711930 and MH711931 respectively.

Laboratory bioassay

The fungus isolated from the dead field infected larvae was responsible for mortality of the insect under laboratory condition. The fungus was re-isolated from the laboratory infected dead larvae and fulfilled Koch's postulate. The whitish mycelial growth started appearing on the body of the infected larvae from 4th day post inoculation even when the larvae was alive, however, the body movements slowed down. Two-three days post inoculation the intersegmental membranes showed brown coloration which gradually turned black and

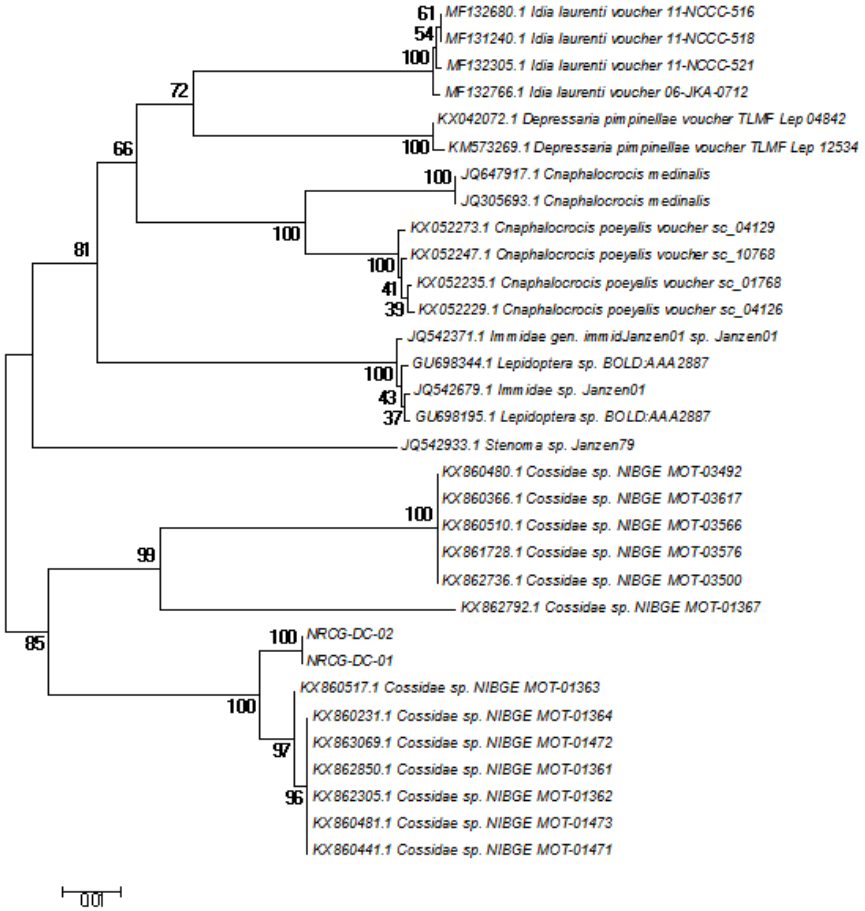

Figure 4. Phylogenetic position of *D. cadambae* inferred by analysis of COI sequences.

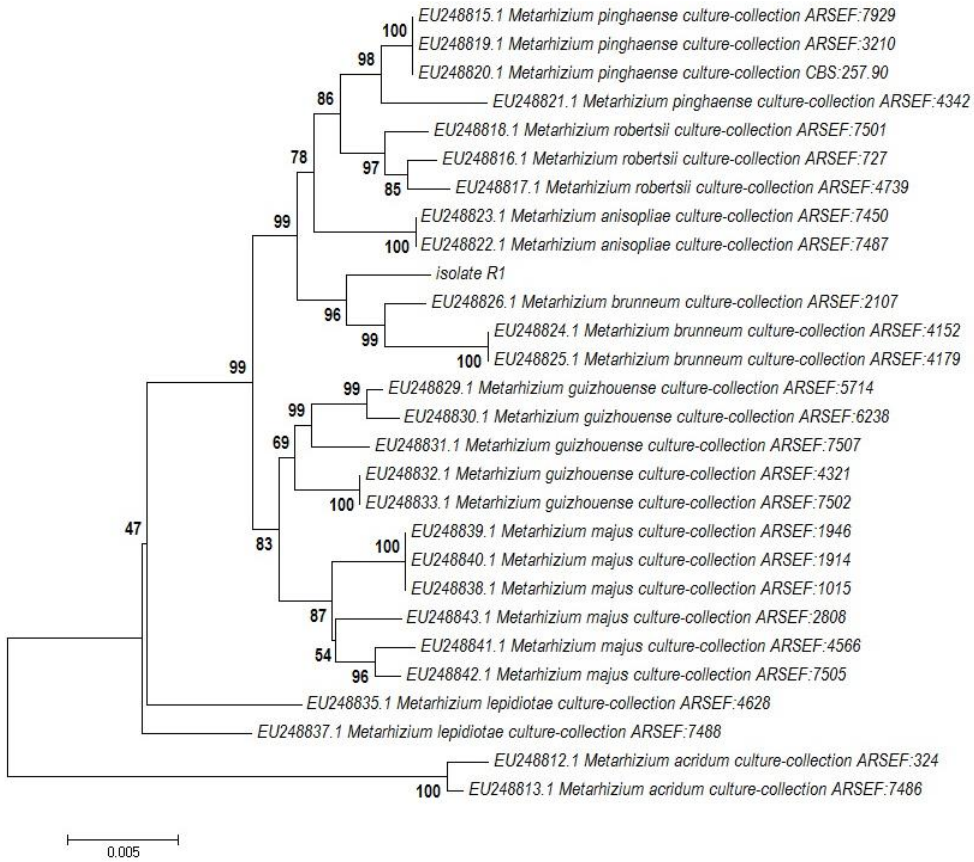

Figure 5. Phylogenetic tree based on multigene sequencing of isolate R1.

eventually larvae died starting from 8th day post inoculation. The insect body became hard and within 10 days post inoculation white mycelium covered the whole larva and started forming conidia making a green colour to the larval cuticle (figure 6). Similar symptoms of *M. anisopliae* infection were reported on *Tenebrio molitor* L. (yellow mealworm) larvae studied by Chen *et al.* (2014). Mean mortality percentage ranged from 6.67 to 76.78% (figure 7). Highest concentration of conidia (1×10^8 conidia/ml) showed maximum mortality (f = 30.68, df = 4, $p < 0.0001$). Similar results were shown by Dragnova *et al*. (2013) for *Lymantria dispar* (L.) (Lepidoptera Lymantriidae). Larvae in control treatment survived till the end of the bioassay. *Metarhizium* sp. are known to affect lepidopteran insects. Earlier, effect of *Metarhizium* sp. was studied on larvae of *Hyphantria cunea* (Drury) (Aker and Tuncer, 2016), *Plutella xylostella* (L.) (Loc and Chi, 2007) and *Spilarctia obliqua* (Walker) (Sapna *et al*., 2010). Mathew (1990) isolated *Aspergillus flavus*, *Paecilomyces fumosoroseus* and *Serratia marcescens* from infected *D. cadambae* larvae collected from *T. grandis* plantation and *Penicillium citrinum*, *Fusarium solani* and *Pseudomonas* sp. from laboratory reared larvae. He recorded maximum larval mortality in case of *S. marcescens* under laboratory experiments. Yu (1979) reported *S. marcescens* to be human pathogenic and therefore, it cannot be used for developing management strategy. Mathew (1990) concluded that microbial pathogens were not feasible for developing management strategy due to difficulties in their application as the larva had habit of boring into the heartwood where infection of the larva was difficult. However, when *D. cadambae* larva infest grapevines, they remain under the loose bark of the plant for a considerable period of time. Removal of loose bark and trunk wash with the isolated *M. brunneum* has the potential to develop into management strategy.

Figure 6. Larvae of *D. cadambae* infected with *M. brunneum*.

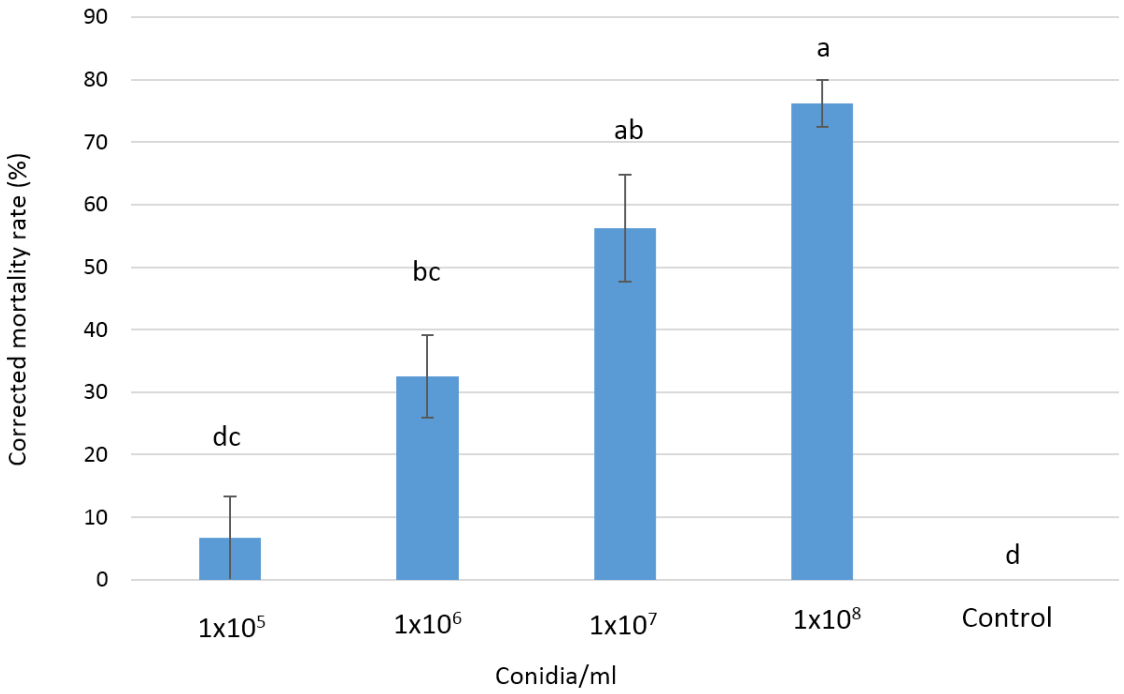

Figure 7. Mortality rate of larvae of *D. cadambae* at different conidia concentration of *M. brunneum*. Means followed by same letter do not differ significantly from each other (P = 0.05). Bar indicate ± standard error.

Conclusion

In conclusion, we report first finding of *D. cadambae* infestation on grape (*V. vinifera*) and virulence of *M. brunneum* to the larvae of *D. cadambae* which suggest its potential as a biocontrol agent in laboratory bioassays. *D. cadambae* succeeded in spreading to new host species *V. vinifera*. It emerged as a new pest threat by not only reducing vitality and productivity but also causing mortality of the grapevines in Maharashtra, India. It has potential to spread to grapes in Karnataka and Tamil Nadu states of India where the pest is already reported as a major pest of *T. grandis*. There are no effective management strategies developed so far for this pest. In grapevines, younger larva remains under bark before boring inside main trunk or cordons. Regular monitoring, removal of loose bark and application of *M. brunneum* on main stem and cordons when the young larvae are feeding under loose bark may be an effective management strategy for *D. cadambae.* Field research is required to find out the peak periods of adult emergence and oviposition in vineyards and effectiveness of this management strategy against *D. cadambae*.

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