Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Isolation and molecular characterization of *Cordyceps* sp. from *Bemisia tabaci* (Hemiptera: Aleyrodidae) and pathogenic to *Glycaspis brimblecombei* (Hemiptera: Aphalaridae)

Isolamento e caracterização molecular de *Cordyceps* sp. de *Bemisia tabaci* e patogênico para *Glycaspis brimblecombei* (Hemiptera: Aphalaridae)

M. M. Domingues[a]*, P. L. Santos[a], B. C. C. Gêa[a], V. R. Carvalho[a], F. N. Oliveira[a], E. P. Soliman[b], W. M. Silva[c], J. C. Zanuncio[d], V. C. Santos Junior[d] and C. F. Wilcken[a]

[a]Universidade Estadual Paulista – UNESP, Faculdade de Ciências Agronômicas, Botucatu, SP, Brasil

[b]Suzano Papel e Celulose/Tecnologia Florestal, Itapetininga, SP, Brasil

[c]Universidade Federal de Viçosa – UFV, Departamento de Engenharia Florestal, Viçosa, MG, Brasil

[d]Universidade Federal de Viçosa – UFV, Instituto de Biotecnologia Aplicada à Agropecuária – BIOAGRO, Departamento de Entomologia, Viçosa, MG, Brasil

Abstract

The Brazilian forestry sector stands out for its technology, forestry management practices, social and environmental responsibility and, mainly, for its high productivity and exotic pests can reduce it. The red gum lerp psyllid *Glycaspis brimblecombei* (Moore, 1964) (Hemiptera: Aphalaridae) is an important pest in *Eucalyptus* plantations. The parasitoid *Psyllaephagus bliteus* (Riek, 1962) (Hymenoptera: Encyrtidae), predatory bugs and entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* are the natural enemies and used in the biological control of the red gum lerp psyllid. The use of entomopathogenic fungi against exotic pests is increasing in the forestry sector and the prospecting and identification of fungus isolates is important for integrated pest management. The objective of this work was the isolation and molecular identification of *Cordyceps* spp. And to evaluate the pathogenicity of isolates, obtained from *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae) adults, against to the red gum lerp psyllid *G. brimblecombei*. The fungi were isolated from *B. tabaci* adults found in soybean and tomato crops and molecularly identified. The conidia obtained were suspended in solution with Tween 80 (0.1%) at a concentration of 1.0×10^8 conidia/mL and sprayed on ten *G. brimblecombei* nymphs per *Eucalyptus* leaf cut and placed on a hydroretentive gel inside per Petri dishes as a replication. The number of dead insects was quantified, daily, for seven days, and transferred to humid chambers. *Cordyceps javanica* (LCBPF 11) and *C. fumosorosea* (LCBPF 12 and LCBPF 63) were identified with a molecular analysis and all isolates were pathogenic to the insects and indicates that they could be used to manage *G. brimblecombei* and adds to reports that, normally, fungi cause greater mortality on insects of the same order as that from which they were isolated.

Keywords: biological control, *Cordyceps fumosorosea*, *Cordyceps javanica*, entomopathogenic fungi, *Eucalyptus*.

Resumo

O setor florestal brasileiro se destaca pela tecnologia, práticas de manejo florestal, responsabilidade social e ambiental e, principalmente, alta produtividade, mas pragas exóticas podem reduzir isto. O parasitoide *Psyllaephagus bliteus* (Riek, 1962) (Hymenoptera: Encyrtidae), percevejos predadores e fungos entomopatogênicos como *Beauveria bassiana* e *Metarhizium anisopliae* são inimigos naturais usados no controle biológico do psilídeo-de-concha do eucalipto *Glycaspis brimblecombei* (Moore, 1964) (Hemiptera: Aphalaridae), praga em plantios de eucalipto. A utilização de fungos entomopatogênicos contra pragas exóticas vem aumentando no setor florestal e a prospecção e identificação de isolados fúngicos é importante para o manejo integrado de pragas. O objetivo deste estudo foi o isolamento e identificação molecular de *Cordyceps* spp. e avaliar a patogenicidade dos isolados de *Cordyceps javanica* (LCBPF 11) e *Cordyceps fumosorosea* (LCBPF 12 e LCBPF 63), obtidos de adultos de *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae), ao psilídeo-de-concha *G. brimblecombei*. Os fungos foram isolados de adultos de *B. tabaci* encontrados em culturas de soja e tomate e identificados molecularmente. Os conídios foram suspensos em solução com Tween 80 (0,1%) na concentração de 1,0 x 10^8 conídios/mL e pulverizados sobre dez ninfas de *G. brimblecombei* por folha de *Eucalyptus* cortada e colocada sobre gel hidrorretentor no interior de placas de Petri por repetição. O número de insetos mortos foi quantificado, diariamente, por sete dias e transferidos para câmaras úmidas. *Cordyceps javanica* (LCBPF 11) e *C. fumosorosea* (LCBPF 12 e LCBPF 63) foram identificados por análise molecular e todos os isolados foram patogênicos para os insetos. Isto indica serem importantes para o manejo de

*e-mail: mauricio.m.domingues@unesp.br
Received: June 8, 2021 – Accepted: April 5, 2022

G. brimblecombei e confirma relatos de que, normalmente, fungos causam maiores mortalidades em insetos da mesma ordem daquela da qual foram isolados.

Palavras-chave: controle biológico, *Cordyceps fumosorosea*, *Cordyceps javanica*, fungos entomopatogênicos, *Eucalyptus*.

1. Introduction

The forest sector represents 1.2% of Brazil Gross Domestic Product (GDP) with sustainability, technology, forest management practices, environmental and social responsibility and, above all, high productivity. The *Eucalyptus* genus, with 6.97 million hectares of cultivated area, represents 77% of the total trees planted in Brazil (IBÁ, 2020). Exotic pests reduce the productivity of these plants. The red gum lerp psyllid *Glycaspis brimblecombei* (Moore, 1964) (Hemiptera: Aphalaridae) is a sucking insect of Australian origin (Kolar, et al., 2021). The reduction and deformation of the leaf blade, of the photosynthetic area, premature leaf fall, overgrowth, pointer drought and, in cases of continuous attacks, plant death, characterize its damage (Dal-Pogetto et al., 2022). *Glycaspis brimblecombei* attacks on *E. camaldulensis* can cause 15% mortality in the first year and up to 40% in the second year of infestation (Wilcken et al., 2015).

The biological control of *G. brimblecombei* is carried out with the parasitoid *Psyllaephagus bliteus* (Riek, 1962) (Hymenoptera: Encyrtidae) (Santos et al., 2021; Favoreto et al., 2021), predatory bugs such as *Atopozelus opsimus* (Elkins, 1954) (Hemiptera: Reduviidae) (Dias et al., 2012), and entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* (Dal-Pogetto, et al., 2011). The use of entomopathogenic fungi against exotic pests are increasing in the forest sector, and the prospecting and identification of fungus isolates is important for integrated pest management (Singh et al., 2017).

The objective of this study was the isolation and molecular identification of *Cordyceps* spp. and to evaluate whether isolates of *Cordyceps javanica* (LCBPF 11) and *C. fumosorosea* (LCBPF 12 and LCBPF 63) isolates, obtained from *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) adults, can be an alternative to manage the *Eucalyptus* red gum lerp psyllid *G. brimblecombei*.

2. Material and Methods

2.1. Isolation and identification of the fungi

Bemisia tabaci adults randomly collected in the field, with symptoms, dead individuals attached to the leaves, and signs of fungi infection and presence of fungus growth, were collected in soybean and tomato crops, disinfected with 0.1% sodium hypochlorite solution, washed with autoclaved distilled water, and kept in a humid chamber (plastic pot with lid and a cotton "roller", previously autoclaved and moistened with sterile water), until fungi sporulation (Figure 1). The fungus isolates were seeded in PDA medium and their genera pre-identified based on their morphology. The fungus mycelia were collected with a platinum loop, transferred to a PDA nutrient medium (potato-dextrose-agar), placed in an incubator chamber and, after seven days, fungus structures were prepared for molecular identification of this microorganism.

Fungal genomic DNA was extracted from the material scraped from the culture medium in Petri dishes with 100 µl of Chelex 10% and 10 ul of proteinase K (20 mg/ml) incubated in a thermal block for 100ºC for five minutes. The PCR reaction for amplification of the ITS1-5.8S-ITS2 rDNA region was performed in a total volume of 50 µL using 1X Taq DNA polymerase buffer, 1.5 mM MgCl2; 0.4 µM of each primer ITS1 (5'–TCCGTAGGTGAACCTGCGG–3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'), 0.2 mM of dNTPs, and 0.2 U of Taq DNA polymerase and 25ng of DNA. This amplification was carried out in a thermocycler, programmed for an initial denaturation of 95°C for 5 min, followed by 30 cycles of denaturation at 95°C for 30s; annealing at 62°C for 1min; extension at 72°C for 2 min and final extension at 72°C for 5 min (Xiong et al., 2013). The products of DNA extractions and PCR reactions (50 µL) were visualized by electrophoresis in 1% agarose gel under UV light and photodocumentation, and those of amplification purified by magnetic beads and sent to IBTEC, UNESP/Botucatu for Sanger sequencing.

2.2. Obtaining Glycaspis brimblecombei

Adults of *Glycaspis brimblecombei* were collected from *Eucalyptus camaldulensis* plants at FCA/UNESP and taken to the laboratory. This insect was reared on seedlings of *E. camaldulensis* and the clone 3025 (*E. grandis* x *E. camaldulensis*), highly susceptible to this pest, in 1L pots or in tubes placed in standard cages (40 cm × 45 cm × 80 cm) under controlled conditions with temperature of 25 ± 2 ºC, 60 ± 10% RH and photophase of 13 hours. A total of 80 to 100 adults of *G. brimblecombei* was released per cage and the *Eucalyptus* seedlings were irrigated daily with 500 ml of water in a bottle (Wilcken et al., 2010).

2.3. Fungi production

The fungal colonies used in the bioassay was obtained by superficial scraping of the culture medium with microscope slide to remove conidia from fungal colonies developed for 14 days in the potato dextrose agar medium (PDA). The conidia obtained were suspended in Tween 80 (0.1%) counted in a hemocytometer with an optical microscope and calibrated to the concentration of 1.0×10^8 conidia/mL.

2.4. Pathogenicity assay with the fungus isolates to Glycaspis brimblecombei

Ten *G. brimblecombei* nymphs were used per Petri dish (90 x 15mm) containing a leaf piece of the clone 433 (*E. urophylla* var. *Platyphylla*) with approximately 5 cm^2, placed on water-repellent gel to reduce leaf dehydration, representing a parcel with 10 replications.

Figure 1. Adults of *Bemisia tabaci* (Hemiptera: Aleyrodidae) infected by *Cordyceps fumosorosea* (A) and *Cordyceps javanica* (B); healthy adult (C).

A total of 125 µL of the conidial suspension was sprayed with a DB134K airbrush (Fenghua Bida Machinery Manufacture Co., China) onto an acrylic cylinder tube, 25 cm apart and 10 PSI working pressure, over *G. brimblecombei* nymphs. The control had only Tween 80 (0.1%). The airbrush was washed with 70% alcohol and rinsed with autoclaved distilled water after each treatment.

After pulverization, Petri dishes were kept in incubators chambers (25.0 ± 1.0 °C, relative humidity of 83.0 ± 2.0% and a 12 h photophase). Insects were daily evaluated, for seven days, and the dead ones counted and transferred to humid chambers to confirm their mortality by the fungus. Ten replicates were used with ten insects per treatment. Mortality values were corrected using the Schneider-Orelli formula. Equation (r^2) was estimated by adjusted polynomial trendline.

3. Results

Three isolates of the *Cordyceps* genus were identified with a molecular analysis (Table 1). *Cordyceps fumosorosea* and *C. javanica* isolates, obtained from *B. tabaci* adults, infected and sporulated on *G. brimblecombei* nymphs. The sporulation of the fungi *C. fumosorosea* and *C. javanica* on *G. brimblecombei* presents whitish color and purple pink nuances (Figure 2).

Mortality in the control was low with an average of two nymphs dead per replication, starting on the third day after application, without evidence of fungus infection. *Cordyceps fumosorosea* and *C. javanica* isolates caused mortality of this insect mainly from the third day after their application (Figure 3). All *G. brimblecombei* nymphs died within seven days after application of the fungus isolates (Figure 3).

4. Discussion

Infection and sporulation of *C. javanica* and *C. fumosorosea* isolates on red gum lerp psyllid nymphs may be related to their origin, from *B. tabaci*, of the order Hemiptera, the same as *G. brimblecombei*. This is similar to that observed for *B. bassiana* isolates from *Odoiporus longicollis* (Olivier, 1807) (Coleoptera: Curculionidae) and *Cosmopolites sordidus* (Germar, 1824) (Coleoptera:

Table 1. Molecular identification code (Code), species, culture, coverage, identity and GenBank access code (Access Code) of entomopathogenic isolates of the fungi *Cordyceps javanica* and *Cordyceps fumosorosea* obtained from infected *Bemisia tabaci* adults in soybean (SO) and tomato (TO), respectively, in Botucatu, São Paulo, Brazil.

Code	Species	Culture	Coverage	Identity	Access Code
LCBPF 11	*Cordyceps javanica*	SO	100%	100%	MW138089
LCBPF 12	*Cordyceps fumosorosea*	TO	100%	92%	MW138059
LCBPF 63	*Cordyceps fumosorosea*	SO	100%	100%	MW137998

Figure 2. Nymphs of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) infected by *Cordyceps fumosorosea* (A) and *Cordyceps javanica* (B); healthy nymph (C).

Curculionidae) and pathogenic to *Basilepta subcostata* (Jacoby, 1889) (Coleoptera: Chrysomelidae) with 91.7% mortality eight days after application (Viswakethu et al., 2021). The pathogenicity of fungus isolates, of the same

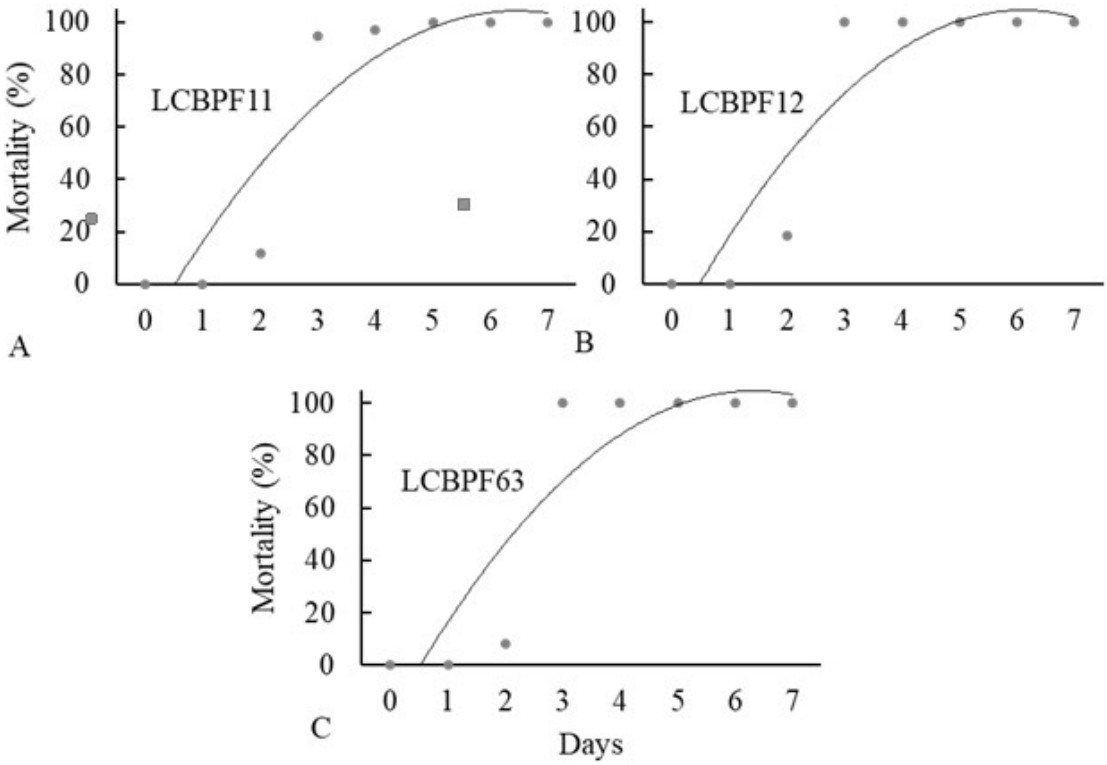

Figure 3. Accumulated corrected mortality of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) nymphs, over the days after application of *Cordyceps* sp. isolates, LCBPF 11 (A), LCBPF 12 (B) and LCBPF 17 (C), with trendline.

species, can vary between different insect groups (Roy and Pell, 2000). The results obtained confirm that fungus isolates can cause high mortality of insects of the same order as those from which they were isolated (De-La-Rosa et al., 2002) and the taxonomic proximity between insects must be taken into account to select fungus isolates for pest management.

The mortality of *G. brimblecombei* nymphs, up to seven days after the application of the fungus isolates is similar to that observed for three *Cordyceps* sp. isolates causing mortality up to 88.6% (Boaventura et al., 2021) and *C. javanica* with 81% for the third instar *B. tabaci* nymphs (Sain et al., 2021). The mortality of insects by the fungi is related to the production of bioactive compounds, such as trichodermin, 5-methylmellein, brevianamide F, enniatin and beauvericin produced by *C. fumosorosea* (Wu et al., 2021) and the secondary metabolites emericellin and fumosorinone by *C. javanica* (Lin et al., 2019) with insecticide properties.

This scientific note is the first step in the production of a mycoinsecticide, the results demonstrate the methodology of molecular identification of the genus *Cordyceps*, poorly studied in the forest area, and indicate the potential of its species in the management of *G. brimblecombei*. The LCBPF 11, LCBPF 12 and LCBPF 63 isolates, from *C. javanica* and *C. fumosorosea*, obtained from *B. tabaci* adults, have potential for using in the management of *G. brimblecombei* and confirms reports that usually, fungus isolates are more pathogenic to insects of the same order as that from which they were isolated, results that directly contribute to bioprospecting studies of entomopathogenic fungi, in the pre-selection for mass production.

Acknowledgements

To the Brazilian institutions "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES- Finance Code 001), "Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)" and "Programa Cooperativo sobre Proteção Florestal (PROTEF) do Instituto de Pesquisas e Estudos Florestais (IPEF)" for financial support.

References

BOAVENTURA, H.A., QUINTELA, E.D., SANTOS, E.N., SILVA, J.F.A. and HUMBER, R.A., 2021. Susceptibility of all nymphal stages of *Bemisia tabaci* Biotype B (Hemiptera: Aleyrodidae) to three Brazilian isolates of *Cordyceps* sp. (Hypocreales: Cordycipitaceae) in a screenhouse under variable temperature and moisture conditions. *Neotropical Entomology*, vol. 50, no. 1, pp. 100-113. https://doi.org/10.1007/s13744-020-00823-5.

DAL-POGETTO, M.H.F.A., WILCKEN, C.F., CHRISTOVAM, R.S., PRADO, E.P. and GIMENES, M.J., 2011. Effect of formulated entomopathogenic fungi on red gum lerp psyllid *Glycaspis*

brimblecombei. *Research Journal of Forestry*, vol. 5, no. 2, pp. 99-106. http://dx.doi.org/10.3923/rjf.2011.99.106.

DAL-POGETTO, M.H.F.A., TAVARES, W.S., ZANUNCIO, J.C., SILVA, W.M., MASSON, M.V., FERREIRA-FILHO, P.J., BARBOSA, L.R. and WILCKEN, C.F., 2022. High population levels lead *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) to unrecorded feeding and oviposition behaviors on *Eucalyptus urograndis* plants. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, pp. e250931. http://dx.doi.org/10.1590/1519-6984.250931. PMid:35137846.

DE-LA-ROSA, W., LÓPEZ, F.L. and LIEDO, P., 2002. *Beauveria bassiana* as a pathogen of the Mexican fruit fly (Diptera: Tephritidae) under laboratory conditions. *Journal of Economic Entomology*, vol. 95, no. 1, pp. 36-43. http://dx.doi.org/10.1603/0022-0493-95.1.36. PMid:11942762.

DIAS, T.K.R., WILCKEN, C.F., SOLIMAN, E.P., GIL-SANTANA, H.R. and ZACHÉ, B., 2012. Occurrence of *Atopozelus opsimus* preying on nymphs and adults of *Glycaspis brimblecombe*i. *Phytoparasitica*, vol. 40, no. 2, pp. 137-141. http://dx.doi.org/10.1007/s12600-011-0213-1.

FAVORETO, A.L., DOMINGUES, M.M., SERRÃO, J.E., RIBEIRO, M.F., SILVA, C.A.D., ZANUNCIO, J.C. and WILCKEN, C.F., 2021. Courtship, mating behavior, and ovary histology of the nymph parasitoid *Psyllaephagus bliteus* (Hymenoptera: encyrtidae). *Journal of Insect Science*, vol. 21, no. 2, pp. 16. http://dx.doi.org/10.1093/jisesa/ieab020. PMid:33861348.

INDUSTRIA BRASILEIRA DE ÁRVORES – IBÁ, 2020 [viewed 12 May 2021]. *Relatório Anual 2020* [online]. Brasília: IBÁ. Available from: https://iba.org/datafiles/publicacoes/relatorios/relatorio-iba-2020.pdf

KOLAR, A., HLEBEC, D., DOLINA, K., FRANJEVIĆ, M. and KUČINIĆ, M., 2021. First DNA barcoding of a new alien species *Glycaspis brimblecombei* Moore, 1964 (Hemiptera: Aphalaridae) in Croatia with a distribution note. *Ecologica Montenegrina*, vol. 43, pp. 59-68. http://dx.doi.org/10.37828/em.2021.43.9.

LIN, R., ZHANG, X., XIN, B., ZOU, M., GAO, Y., QIN, F., HU, Q., XIE, B. and CHENG, X., 2019. Genome sequence of *Isaria javanica* and comparative genome analysis insights into family S53 peptidase evolution in fungal entomopathogens. *Applied Microbiology and Biotechnology*, vol. 103, no. 17, pp. 7111-7128. http://dx.doi.org/10.1007/s00253-019-09997-4. PMid:31273397.

ROY, H.E. and PELL, J.K., 2000. Interactions between entomopathogenic fungi and other natural enemies: implications for biological control. *Biocontrol Science and Technology*, vol. 10, no. 6, pp. 737-752. http://dx.doi.org/10.1080/09583150020011708.

SAIN, S.K., MONGA, D., HIREMANI, N.S., NAGRALE, D.T., KRANTHI, S., KUMAR, R., KRANTHI, K.R., TUTEJA, O.P. and WAGHMARE, V.N., 2021. Evaluation of bioefficacy potential of entomopathogenic fungi against the whitefly (*Bemisia tabaci* Genn.) on cotton under polyhouse and field conditions. *Journal of Invertebrate Pathology*, vol. 183, pp. 107618. http://dx.doi.org/10.1016/j.jip.2021.107618. PMid:33992641.

SANTOS, F.A., CORREA, A.S., NANINI, F., NASCIMENTO, D.A., JUNQUEIRA, L.R. and WILCKEN, C.F., 2021. Genetic diversity of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) and its parasitoid *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) in Brazil. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 81, no. 3, pp. 838-841. http://dx.doi.org/10.1590/1519-6984.230486. PMid:32876174.

SINGH, D., RAINA, T.K. and SINGH, J., 2017. [viewed 20 November 2021]. Entomopathogenic fungi: an effective biocontrol agent for management of insect populations naturally. *Journal of Pharmaceutical Sciences and Research* [online], vol. 9, no. 6, pp. 833. Available from: https://www.jpsr.pharmainfo.in/Documents/Volumes/vol9Issue06/jpsr09061715.pdf

VISWAKETHU, V., BALAKRISHANAN, P., MURUGAN, L., NARAYANA SWAMY, B. and SUBBARAYA, U., 2021. Entomopathogenic fungi as a promising biological control agent against banana fruit scarring beetle, *Basilepta subcostata* (Jac.) (Chrysomelidae: coleoptera). *Egyptian Journal of Biological Pest Control*, vol. 31, no. 1, pp. 1-7. http://dx.doi.org/10.1186/s41938-021-00382-w.

WILCKEN, C.F., FIRMINO-WILCKLER, D.C., DAL-POGETTO, M.H.F.A., DIAS, T.K.R., LIMA, A.C.V., DE-SÁ, L.A.N. and FERREIRA-FILHO, P.J., 2015 [viewed 12 May 2021]. Psilídeo-de-concha-do-eucalipto *Glycaspis brimblecombei* Moore. In: E. F. VILELA and R. A. ZUCCHI. *Pragas introduzidas no Brasil - insetos e ácaros* [online]. Piracicaba: FEALQ, pp. 883-897. Available from: https://www.researchgate.net/profile/Pj-Filho/publication/280807903_Red_Gum_Lerp_Psyllid_Glycaspis_brimblecombei_Moore/links/55d3a15908ae7fb244f58d41/Red-Gum-Lerp-Psyllid-Glycaspis-brimblecombei-Moore.pdf

WILCKEN, C.F., SÁ, L.A.N., DAL-POGETTO, M.H.F.A., COUTO, E.B., FERREIRA-FILHO, P.J. and FIRMINO-WINCKLER, D.C., 2010 [viewed 12 March 2021]. Sistema de criação do psilídeo-de-concha Glycaspis brimblecombei e de seu parasitóide Psyllaephagus bliteus. *Documentos Técnicos IPEF* [online], vol. 2, no. 2, pp. 1-23. Available from: http://www.bibliotecaflorestal.ufv.br/bitstream/handle/123456789/14461/ipef-documentos-tecnicos-2010-volume-2-n-2.pdf?sequence=1&isAllowed=y

WU, J., YANG, B., XU, J., CUTHBERTSON, A.G. and ALI, S., 2021. Characterization and toxicity of crude toxins produced by *Cordyceps fumosorosea* against *Bemisia tabaci* (Gennadius) and *Aphis craccivora* (Koch). *Toxins*, vol. 13, no. 3, pp. 220. http://dx.doi.org/10.3390/toxins13030220. PMid:33803611.

XIONG, Z.Q., YANG, Y.Y., ZHAO, N. and WANG, Y., 2013. Diversity of endophytic fungi and screening of fungal paclitaxel producer from Anglojap yew, *Taxus x media*. *BMC Microbiology*, vol. 13, pp. 71. http://dx.doi.org/10.1186/1471-2180-13-71. PMid:23537181.