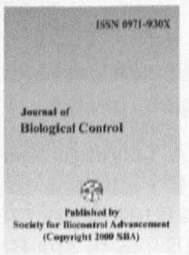

Journal of Biological Control, 25 (2): 114–117, 2011

Research Article

Fungal endophytes, *Phlebiopsis gigantea* (Fr.) Jülich and *Phanerochaete sordida* (P. Karst.) J. Erikss and Ryvarden: New aspirants in biopesticide scenario

C. P. BIJI, V. V. SUDHEENDRAKUMAR* and S. SHABNA
Forest Entomology Department, Kerala Forest Research Institute, Peechi 680 653, Kerala, India.
**Corresponding author E-mail*: bijicp@gmail.com; sudhi@kfri.org

ABSTRACT: Endophytes to which several roles of plant defense are ascribed have huge potential for development as biocontrol agents. Biocontrol prospects of two foliar endophytes, *Phlebiopsis gigantea* and *Phanerochaete sordida* isolated from *Adhatoda vasica* Nees and *Andrographis paniculata* Nees, respectively, are reported here. In *per os* treatment against third instar teak defoliator larvae [*Hyblaea puera* (Cramer)], *P. sordida* and *P. gigantea* showed considerable insecticidal activity in terms of median lethal doses ($6x10^5$ conidia and $3x10^8$ oidia per larva, respectively). In dip treatment using oidial suspension, *P. gigantea* at its median lethal dosage inflicted cent percent mortality in less than a minute's time. This result is extremely fascinating considering its knockdown action and its enormous potential as a biocontrol agent.

KEY WORDS: Biocontrol, endophytes, *Phlebiopsis gigantea, Phanerochaete sordida, Hyblaea puera*

(Article chronicle: Received: 29.01.2011; Sent for revision: 17.03.2011; Accepted: 02.04.2011)

INTRODUCTION

The market for bioinsecticides, even though they comprise only a small part of the insecticide field, is steadily increasing. There is a continuous search to cater to the ever growing need for new and more competitive biocontrol candidates. Endophytes, the microorganisms that reside in the tissues of living plants, are relatively unstudied and are potential sources of novel biocontrol agents.

Usually, there are many rationales for selecting plants for exploring endophytic fauna for a particular purpose. Plants that have an ethnobotanical history (use by indigenous people) and related to specific uses or applications of interest always happened to be a favorite source (Strobel and Daisy, 2003). Biologically active plant extracts have been well documented for evolving ecologically sound and environmentally acceptable insect control programmes from time immemorial. About 2000 species of terrestrial plants have been reported for their insecticidal properties (Feinstein, 1952). Plant products like azadirachtin, pyrethrins, nicotines and rotenones are well known insecticides used for pest control.

Our study focuses on *Andrographis paniculata* Nees and *Adhatoda vasica* Nees of the family Acanthaceae which have been known for their ethnobotanical importance as insect deterrents and insecticides over centuries in Asia. Here, we report the insecticidal prospects of two fungal endophytes isolated from the leaves of *A. paniculata* and *A. vasica.*

MATERIALS AND METHODS

Symptomless mature leaves of *A. paniculata* and *A. vasica* were collected from the medicinal garden maintained at Kerala Forest Research Institute, Peechi, India. In the laboratory, the plant surfaces were sterilized to remove all microbial epiphytes by soaking in 70% alcohol for 1 min followed by immersion in 2-5% sodium hypochlorite and again in 70% alcohol for 30 sec. and later were rinsed three times in sterile distilled water (Petrini, 1986; Kharwar *et al.*, 2008). In a laminar-flow chamber, the tissues of the samples were sliced with a sterile knife so as to expose the interior surface to Potato Dextrose Agar (PDA) medium amended with penicillin-G @ 100 units ml^{-1} and streptomycin @ 100µg ml^{-1}. The samples were processed within 2h of collection.

After 3-5 days of incubation at room temperature (30 ± 2° C), hyphal tips of fungi exuding from the plant sample were cut and transferred onto new PDA plates and repetitive re-plating of the microbial colonies was

continued until a pure culture was obtained. Based on the culture morphology, 15 morphotypes were initially identified and screened for insecticidal activity (data not shown). After initial screening, two efficient foliar endophytic fungi, namely, *Phanerochaete sordida* and *Phlebiopsis gigantea* (molecular characterization* at Agharkar Research Institute, Pune, India) (Fig. 1) were selected for further study.

Test insect

The test insect used in the study was *Hyblaea puera* (Cramer) (Lepidoptera: Hyblaeaidae), a serious pest of teak (Nair *et al.,* 1985). The larvae required for the study were obtained from the Bio-control Laboratory at Kerala Forest Research Institute (KFRI) Sub Center, Nilambur, India, and had been reared over 50 disease-free cycles.

Per os treatment

Variation in insecticidal activity was based on median lethal dosage with respect to the samples. Based on preliminary experiments five doses bracketing 5% to 95% mortality were selected. Aliquots of conidia/oidia were prepared by serial dilution from a stock solution using distilled water. Twenty-five third instar larvae weighing 9 to 13 mg were used against each dose per replicate for the study. Prior to the bioassay, test larvae were washed in 0.2% sodium hypochlorite solution for 2 min, rinsed thrice in sterile distilled water and dried in a container lined with sterile paper towels so as to eliminate any fungi present on the larval surface. Leaf disc method was opted for inoculating test larvae (Biji *et al.,* 2006). Inoculated larvae were transferred on to artificial diet for the rest of the experimental time. Larval mortality was assessed on a daily basis and tabulated till complete death or pupation of the test larvae. The whole experiment was set up at room temperature (30 ± 2° C) and replicated thrice with distilled water as control.

Dip treatment

Based on the lethal values obtained after *per os* treatment, the larvae were subjected to dip treatment. The surface sterilized third instar *H. puera* larvae assigned to the treatments were dipped in a 5ml spore suspension with LD_{50} dose for respective endophytic fungi; control insects were dipped in sterile distilled water. The larvae were shaken gently for 1 min in their respective treatments and then placed in a sterile container from which they were taken individually with a sterile paint brush and placed in rearing tubes containing semi-synthetic diet. All treatments were incubated at room temperature (30 ± 2°C) and replicated thrice with 25 larvae each. In the control treatment, distilled water was used instead of spore suspension. Insect mortality was observed on a daily basis.

Data analysis

The data obtained from insecticidal bioassays were subjected to probit analysis (Finney, 1971) and median lethal dosage (LD_{50}) for the test insect was estimated using a computer specific software package: Polo Plus (Version 2, ©2002–2009, LeOra Software). Arcsine transformed values of percentage mortality at LD_{50} dose were subjected to LSD to investigate variation with respect to lethality of endophytes. LSD analysis was performed using a computer specific software package: SPSS for windows (Standard Version 11) (©SPSS Inc., 1989-2001).

RESULTS AND DISCUSSION

Per os treatment

The percentage mortality due to fungal disease was related to the test dose of the endophytes. Third instar larvae were significantly more susceptible to *P. sordida*

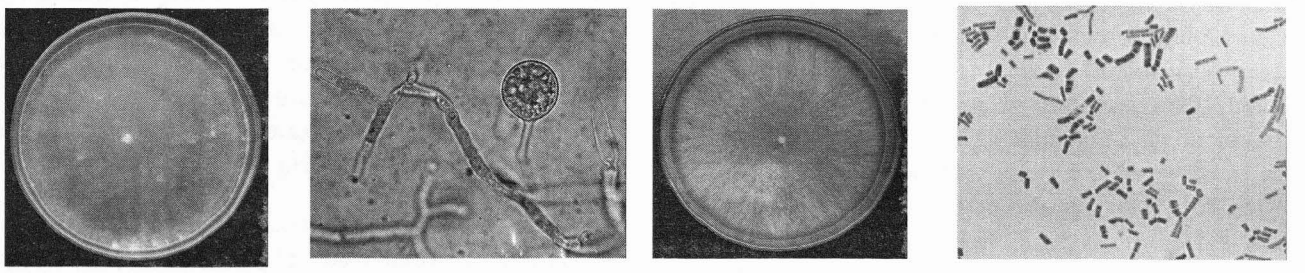

Fig. 1. Macroscopic and microscopic morphology of *Phanerochaete sordida* (left two photos) and *Phlebiopsis gigantea* (right two photos)

*Molecular identification of the endophytes is based on rDNA sequence analysis. It was found that one of the endophytes showed 94% sequence similarity with *Phlebiopsis gigantea* (Fr.) Julich 1978 and the other endophyte showed 98% sequence similarity with *Phanerochaete sordida* (P. Karst.) J. Erikss & Ryvarden.

Table 1. LD_{50} values and associated statistics for *Phanerochaete sordida* and *Phlebiopsis gigantea* against third instar larvae of *Hyblaea puera*

Endophytes	LD_{50} value (conidia/ oidia per larva)	95% limits		Slope	$\div^2$	% Mortality @ LD_{50} dose in dip treatment*
		Lower	Upper			
Phanerochaete sordida	6.04×10^5	2.32×10^5	5.75×10^6	0.766 ± 0.110	6.66	50^a (0.52 ± 0.1)
Phlebiopsis gigantea	2.30×10^8	6.85×10^7	1.27×10^9	0.345 ± 0.047	4.01	100^b (1.57 ± 0.2)

*Means followed by the same letter do not differ significantly by LSD ($P = 0.05$); values in parentheses are arcsine transformed mean values ± S.E.

than *P. gigantea* (6×10^5 against 2.9×10^8 conidia per larva) as evident from the lack of overlap of median lethal values at 95% fiducial limits (Table 1). The residuals plotted against predicted values remained within a horizontal band between –2 and 2 indicating good fit of data to the assumptions of the probit model.

Dip treatment

Topical application of the endophytes resulted in mortality of the test larvae (Table 1). Dipping the larvae in a conidial suspension of *P. sordida* having LD_{50} dose (6×10^5 conidia per larva) resulted in 50% mortality by 72h post-treatment. At the same time, dipping the larvae in a conidial suspension of *P. gigantea* at its median lethal dose (2.9×10^8 oidia per larva) resulted in 100% mortality. This occurred within the treatment time, *i.e.*, within 1 min and the larval carcasses showed stiffness.

The prime objective of our study was to bio-prospect endophytic fungi, *Phanerochaete sordida* and *Phlebiopsis gigantea* associated with *A. paniculata* and *A. vasica* for biocontrol properties.

Evaluation of *P. sordida* and *P. gigantea* against third instar *H. puera* larvae under *per os* conditions showed that they have insecticidal potential but it was not significant when compared to that of *Hyblaea puera* Nucleopolyhedrovirus (HpNPV) (1.2×10^2 OBs / larva) – the current biocontrol agent. On the other hand, *P. gigantea* inflicted 100% mortality in a minute's time during dip treatment at its LD_{50} dose (2.30×10^8 oidia ml^{-1}). This result is of extreme novelty and fascinating as HpNPV requires 72h post-inoculation to ensure 95% mortality against a dose of 8×10^4 OBs per third instar larva.

The endophyte, *P. gigantea* which showed knock down effect on *H. puera* larvae was isolated from the leaf of *A. vasica*. It is already known that the leaves of this plant contain several alkaloids and the chief principle is a quinazolin alkaloid, vasicine. Ware and Whitacre (2004) observed that the quinazoline containing insecticides act as contact and stomach poisons and also give rapid knockdown effect on the insects. Hence, the immediate kill caused by the conidial suspension of *P. gigantea* upon dip treatment may be due to a bioactive metabolite having similar attributes to that of vasicine. By identifying paclitaxel producing endophyte from yew tree, Stierle *et al.* (1993) showed that bioactive plants have associated endophytes that share analogous virtues with that of the host for the first time. This finding was further supported by the studies of various other researchers (Strobel *et al.*, 1999; Castillo *et al.*, 2002; Strobel, 2003; Gangadevi and Muthumary, 2007). Further, Liu *et al.* (2009) developed nine polymorphic microsatellite markers for making predictions about the impact of *P. gigantea* application in conifer forests.

Both *P. gigantea* and *P. sordida* have been known to be used as biological agents in different contexts. The possibilities of *P. gigantea* as a repellent against large pine weevil (*Hylobius abietis)* females have been probed and it was found that this fungus could inhibit the attractiveness of pine branches to this insect (Skrzecz and Moore, 1997). In Europe, for nearly 40 years *P. gigantea* has been used against conifer root and butt caused by *Heterobasidion annosum*. *P. gigantea* competes with *H. annosum* for the woody resource within conifer stump and is applied to stump surfaces at felling (Pratt *et al.*, 2000). The fungus *P. sordida* is used largely as a bioremedial agent rather than a biocontrol agent because of the presence of lignin-modifying enzymes (LMEs) such as lignin peroxidase, manganese dependent peroxidase, and laccase (Mester and Tien, 2000; Bucke, 1998).

In conclusion, the endophytes *P. sordida* and *P. gigantea* isolated from *A. paniculata* and *A. vasica* respectively could be potential aspirants to be developed as biocontrol agents. Isolation and identification of bioactive metabolites having insecticidal potential from *P. gigantea* is worth exploring. Our study also demonstrates that a plethora of endophytes with immense bioactive potential are waiting to be explored.

REFERENCES

Biji, C. P., Sudheendrakumar, V. V. and Sajeev, T. V. 2006. Influence of virus inoculation method and host larval age on productivity of the nucleopolyhedrovirus of the teak defoliator, *Hyblaea puera* (Cramer). *Journal of Virological Methods,* **133**: 100–104.

Bucke, C. 1998. Biochemistry of bioremediation of fungi. *Journal of Chemical Technology and Biotechnology*, **71**: 356–357.

Castillo, U. F., Strobel, G. A., Ford, E. J., Hess, W. M., Porter, H., Jensen, J. B., Albert, H., Robison, R., Condron, M. A., Teplow, D. B., Stevens, D. and Yaver, D. 2002. Munumbicins, wide-spectrum antibiotics produced by *Streptomyces* NRRL 30562, endophytic on *Kennedia nigriscans*. *Microbiology,* **148**: 2675–2685.

Feinstein, L. 1952. Insecticides from plants, pp. 222–229. In: Insects, The yearbook of agriculture, USDA, Washington, DC, USA.

Finney, D. J. 1971. *Probit Analysis*. Cambridge University Press, London, UK.

Gangadevi, V. and Muthumary, J. 2007. Endophytic fungal diversity from young, mature and senescent leaves of *Ocimum basilicum* L. with special reference to taxol production. *Indian Journal of Science and Technology,* **1**: 1–12.

Kharwar, R.N., Verma, V.C., Strobel, G. and Ezra, D., 2008. The endophytic fungal complex of *Catheran-thus roseus* (L.) G. Don., *Current Science*, **95**: 228–233.

Liu, A. Z., Samils, N., Higgins, B., Stenlid, J., Slippers, B., Nairn, C. J. and Covert, S. F. 2009. Microsatellite markers for the wood decay fungus *Phlebiopsis gigantea*. *Conservation Genetics*, **10**: 1529–1532.

Mester, T., Swarts, H.J., Sole, S., de Bont, J.A. and Field, J.A, 1997. Stimulation of aryl metabolite production in the basidiomycete *Bjerkandera* sp. strain BOS55 with biosynthetic precursors and lignin degradation products, *Applied and Environmental Microbiology,* **63**: 1987–1994.

Nair, K. S. S., Sudheendrakumar, V. V., Varma, R. V. and Chacko, K. C. 1985. Studies on the seasonal incidence of defoliators and the effect of defoliation on volume increment of teak. KFRI Research Report, No. 30, Kerala Forest Research Institute, Peechi, Kerala, India, 78 pp.

Petrini, O, 1986. Taxonomy of endophytic fungi of aerial plant tissues, pp.175–187. In: *Microbiology of the phyllosphere* (ed.): Fokkema, N. J. and J. Van den Heuvel. Cambridge University Press, Cambridge, UK.

Pratt, J. E., Niemi, M. and Sierota, Z. H. 2000. Comparison of three products based on *Phlebiopsis gigantea* for the control of *Heterobasidion annosum* in Europe. *Biocontrol Science and Technology,* **10**: 467–477.

Skrzecz, I. and Moore, R. 1997. The attractiveness of pine branches infected with selected wood colonizing fungi to the large pine weevil (*Hylobius abietis*), pp. 146–152. In: Grégoire, J. C., Liebhold, A. M., Stephen, F. M., Day, K. R. and Salom, S. M. (Ed.). *Integrating cultural tactics into the management of bark beetle and reforestation pests*. USDA Forest Service General Technical Report NE–236.

Stierle, A., Strobel, G.A. and Stierle, D. 1993. Taxol and taxane production by *Taxomyces andreane,* an endophytic fungus of Pacific yew. *Science*, **260**: 214–216.

Strobel, G. A. 2003. Endophytes as sources of bioactive products. *Microbes and Infection*, **5**: 535–544.

Strobel, G. A., Miller, R. V., Miller, C. M., Condron, D. B., Teplow and Hess, W. M. 1999. Cryptocandin, a potent antimycotic from the endophytic fungus *Cryptosporiopsis cf. quercina. Microbiology,* **145**: 1919–1926.

Strobel, G. A. and Daisy, B. 2003. Bioprospecting for microbial endophytes and their natural products. *Microbiology and Molecular Biology Reviews*, **67**: 491–502.

Ware, G. W. and Whitacre, D. M. 2004. An introduction to insecticides. *In:* Radcliffe, E. B., Hutchison, W. D. and Cancelado, R. E. (Eds.). *Radcliffe's IPM World Textbook*, URL: http://ipmworld.umn.edu, University of Minnesota, St. Paul, MN, USA.