Send Orders for Reprints to reprints@benthamscience.ae

Current Biotechnology, **2016,** *5,* **000-000**

RESEARCH ARTICLE

Biological Control Through Fungal Endophytes: Gaps in Knowledge Hindering Success

Suryanarayanan, T.S.[1,*], Govinda Rajulu, M.B.[1] and Vidal, S.[2]

[1]*Vivekananda Institute of Tropical Mycology (VINSTROM), Ramakrishna Mission Vidyapith, Chennai 60004, India and* [2]*Georg-August-Universität Göttingen, Department of Crop Protection, Agricultural Entomology, Grisebachstrasse 6, 37077 Göttingen, Germany.*

Suryanarayanan, T.S.

Abstract:

Background: The asymptomatic fungal endosymbionts of plants called the endophytes are universal in their occurrence. The occurrence of some entomopathogenic fungi which infect and kill insect pests of crops as endophytes provides an excellent opportunity for using these fungi in biocontrol. A few studies substantiate this novel approach of pest management; however, since this facet of endophyte research is nascent, enquiries answering basic questions on various aspects of endophyte biology are essential to successfully implement endophyte-mediated biocontrol. In a collaborative research, we addressed a few of these questions including (i) to what extent do fungal genera reported to have entomopathogenic species colonize non-crops as endophytes? (ii) can an endophyte from a non-crop source and vested with anti-insect property survive as an endophyte when introduced into a crop? (iii) how does an anti-insect endophyte in a crop affect an insect pest? and (iv) can endophytes be used for controlling weeds? The results of this study show that endophytes have enormous potential as biocontrol agents and reiterate the need for concerted efforts to obtain more basic information about endophyte biology before they can be used effectively for biocontrol.

Methods: Non-entomopathogenic but anti-insect metabolites producing endophytes from plant and non-plant sources are shown to have potential biocontrol properties. Survival of an effective fungus from an alien source in a crop plant as an endophyte has been shown to be only for a limited period. Endophytes are shown to possess weedicidal activity.

Results: Entomopathogenic fungal genera rarely colonize leaves of forest trees as foliar endophytes. Non-entomogenous endophytes produce ant-insect compounds affecting pests of crops thus having the potential as biocontrol agents. Such fungi isolated from non-plant sources can be inoculated into crops to function as endophytes, for brief periods. Insect pests avoid crops with anti-insect endophytes for oviposition and feeding. Some endophytes elaborate weedicidal compounds.

Conclusion: Our basic study underscores the need to screen plants and non-plants of different habitats for entomopathogenic and anti-insect endosymbiotic fungi. Futhermore, it highlights the importance of screening endophytes for weedicidal compounds. We emphasise the need for focused studies for better understanding of the interaction between endophytes and their plant hosts and the biotic stressors of the host plants to enhance the success of using endophytes for biological control.

ARTICLE HISTORY

Received: November 11, 2015
Revised: April 27, 2016
Accepted: May 2, 2016

DOI:
10.2174/2211550105666160504130322

Keywords: Biocontrol, pest management, entomopathogens, weedicides.

INTRODUCTION

Crop losses at the global scale due to biotic stressors such as weeds, pests (e.g. insects, mites and nematodes) and pathogens (e.g. fungi, bacteria and viruses) are substantial [1] and are predicted to increase as a consequence of global warming [2]. The use of synthetic chemicals to control these biotic stressors has ensured that crop losses fall within economically viable levels. However, farmers of the developing world cannot afford the recently developed less toxic and more eco-friendly third-generation pesticides and would continue to use the more toxic older chemicals thus affecting the environment [2]. This is confounded by the evolution of resistance to pesticides among the pest and pathogen populations [3], specifically because the numbers of active ingredients available are limited due to political

*Address correspondence to this author at the Vivekananda Institute of Tropical Mycology (VINSTROM), Ramakrishna Mission Vidyapith, Chennai 60004, India; E-mail: t_sury2002@yahoo.com

2211-5501/16 $58.00+.00

prerequisites. Pests with different reproductive strategies such as aphids rapidly evolve to break the resistance of host plants conferred by resistance genes and the protection afforded by pesticides [4,5]. Therefore an integrated pest management aiming at establishing a holistic approach to reduce crop loss by incorporating cultural, biological and chemical methods is advised to rationalize the use of chemicals. Under biological control methods using natural enemies of pests and pathogens to control crop loss, fungi such as species of *Trichoderma* spp. have been employed successfully to control soil borne pathogens and phytophagous nematodes [6,7]. Entomopathogenic fungi such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Verticillium lecanii* and *Paecilomyces lilacinus* infecting and killing insect pests have also been shown to be successful in the biological control of crop pests[8-11]. Similarly, several fungal species have been shown to be effective against weeds which compete with crops for resources and reduce yields [12]. However, lack of information especially pertaining to the ecology of such fungi is hampering the effective use of in the control of crop pests [13].

Endophytes are an ecological group of fungi which infect plants and survive within their living tissues without causing disease symptoms. These symptomless endosymbionts (mostly Ascomycetes and their anamorphic states) are ubiquitous and have been isolated from all plants (including the algae, bryophyta, pteridophyta, gymnosperms and angiosperms) which have been screened for their presence. Although in the last couple of decades endophytes have been studied for their ability to produce a variety of bioactive compounds [14], enzymes of industrial importance [15-17] and to enhance the host plant's abiotic [18] and biotic stress tolerance [19], many aspects of their biology remain unknown [20]. Interestingly, some genera of fungi such as *Acremonium*, *Beauveria*, *Isaria*, *Paecilomyces* and *Verticillium* which encompass well established entomopathogenic species surviveas endophytes in the tissues of non-crop and crop plants [10, 21]. Being natural symbionts of plants and not causing diseases in them make entomopathogenic endophytes admirably suitable for bicontrol program. Furthermore, most of these fungal species can be easily cultured to obtain large quantities of spores which are their infective propagules. Despite these desirable features, there are only limited studies on entomopathogenic fungi which exist as endophytes in plants [22]. In a pioneering study, Vega *et al*. (2008) demonstrated that the entomopathogenic fungi occurring as endophytes in coffee plants are potentially effective in controlling the coffee berry borer although the exact mechanism of insect control by these fungi is not clear [22]. Artificial establishment of entomopathogenic fungi as endophytes in plants has also been investigated as a method of pest control [11,23,24]. Apart from studies focusing on such fungi in individual crop species, information on their ecology and interaction with host plants are needed to effectively utilize them for crop protection [24]. Here, we address the following apparently disjointed issues of endophyte-mediated biocontrol: (i) what is the contribution of entomopathogenic fungi to the foliar endophyte community of non-crop plants such as forest trees? (ii) can an endophyte possessing insecticidal properties (not necessarily entomopathogenic) isolated from a non-crop sourcesurvive as an endophyte when introduced into a crop plant? (iii) how does an anti-insect endophyte in a crop affect an insect pest? and (iv) do endophytes exhibit weedicide activity? Based on the results of these basic studies we discuss issues needing further investigations to enhance the value of endophytes as biocontrol agents allowing for a more consistent implementation in integrated pest management programs.

MATERIALS AND METHODS

Collection Site

Twenty-five woody tree species each from a tropical dry thorn forest, dry deciduous forest and a montane evergreen forest situated in the NBR (Latitude 11°32' and 11°43'N, Long 76°22' and 76°45'E) were studied for their leaf endophytes to assess the frequency of occurrence of genera of fungi which are known to have entomopathogenic species.

Isolation of Endophytes

From each leaf, three segments (0.5 cm^2) were cut from the midrib region (including the lamina portion)—one each from the apical, middle and the basal region of the leaf. The one hundred and fifty leaf segments thus obtained from 50 leaves for each host species were surface sterilized by dipping them in 70 % ethanol for 5 s, followed by immersion in sodium hypochlorite (4 % available chlorine) for 90 s and finally rinsed in sterile distilled water for 10 s [25]. Such segments were plated on chloramphenicol (150mg l^{-1}) amended Potato Dextrose Agar (PDA) medium contained in 9 cm diameter Petri dishes. The Petri dishes were incubated in a light chamber with a 12 h light:12 h dark cycle for 28 days at 26 ± 1°C to induce sporulation in the endophytes [26]. The light chamber had a bank of three 4 foot Philips day light fluorescent lamps. The tissue segments received about 2200 Lx of light through the Petri dish lid as measured by a Lutron (Germany) Lx-101 Lux meter. The leaf segments were inspected periodically and the fungi growing out of them were scored, isolated and cultured in PDA slants. They were identified on their cultural and spore morphology.

Extraction of Secondary Metabolites from the Sceretome [27]

The endophytes were cultured in potato dextrose broth (300ml) containing XAD- amberlite (Rohm and Hass, Philadelphia, PA, USA) resin beads. XAD is a polystyrene resin that adsorbs small molecular metabolites from the secretome of fungi and facilitates their easy extraction. The XAD beads were collected, suspended in 30 ml of methanol for 20 min, filtered and the methanol extract was concentrated to 1.5 ml in a Rotavapor (Buchi, Postfach, Switzerland) apparatus. This concentrated extract was used in the following assays.

Anti-Feedant Assay

20µl methanol extract of the secretome of an endophyte was placed on a leaf disc (10cm dia) of *Abelmoschus esculentus* and air dried. Four such leaf discs were provided as food for one larva of *Helicoverpa armigera*. For control, 20µl methanol extract of non-inoculated culture medium

placed on leaf discs was provided as food. Five replicates were maintained and the area of the leaf eaten by the larvae was determined after 24 hours of incubation by placing the leaf discs on graph paper and measuring the uneaten area. For this assay, the secretomes of a few endophytes isolated from marine algae were also included since endophytes of macroalgae have been reported to be a promising source of novel bioactive compounds [28].

Survival of Effective Endophyte in Alien Plant Crops [29]

The endophyte *Trichoderma harzianum* isolated from the brown marine alga *Sargassum wightii* (which was most effective as revealed by the above anti-feedant assay) was grown on PDA medium for one week under NUV light to induce sporulation. Although the brown algae are not plants, we refer to this endosymbiont as 'endophyte' for the sake of convenience. The spores were collected in sterile distilled water and the concentration of spore suspension was adjusted to 1 x 10^7 spores ml^{-1}. This spore suspension was sprayed on the mature leaves of ten 30- day old plants of *Abelmoschus esculantus, Capsicum annum* and *Solanum melongena* maintained in an open field at the rate of 10 ml per plant. Plants sprayed with water served as control. The leaves from sprayed and control plants were screened for the presence of this fungus as foliar endophyteon every 7^{th} day till the 42^{nd} day.The method stated under 'isolation of endophytes' was used to reisolate the endophyte from the leaves.

Assay for the Insecticidal Activity of Endophytic Strains of Trichoderma Harzianum

Since a *T. harzianum* surviving as an endosymbiont in non-plant host (brown alga) showed anti-insect effect, it was decided to study the established endophytic forms of this fungus for anti-insect activity. *Trichoderma harzianum* strains T39 (Makhteshim-AganLtD, Tel Aviv, Israel) and Tu (Uniseeds Co, Ltd, Thailand) were used in this study. To prepare the fungal suspensions, fungal conidia were harvested from 3-week-old PDA (potato dextrose agar) cultures with a camel hairbrush into a sterile beaker which contained 500ml sterile 0.1% Tween 20 solution. The suspensions were then homogenized by using a magnetic stirrer for 5 min and adjusted to 2×10^7 conidia ml^{-1} using a haemocytometer. The germination rates were examined by PDA medium using the method described in Posada and Vega (2005) to confirm the viability of the spores [30]. Cabbage plants (*Brassica oleracea* var. *capitata* L.) were used as host for the endophytes. Two weeks after the transplanting, the seedlings were gently removed from the pots and the soil was carefully washed off under tap water. The inoculation was performed by immersing the root of the seedlings into $2x10^7$ conidia/ml spore suspension or sterile water (control treatment) for 30min, then the seedlings were transplanted into a new pot, irrigated regularly and fertilized once a week. Inoculated plants were kept in greenhouse chamber for another three weeks, and then they were used for feeding experiments, oviposition choice assays and for fungal re-isolation, qPCR detection (data for fungal re-isolation and qPCR not shown). To test the insecticidal activity of inoculated cabbage plants on the diamondback moth the following experimental set-up was followed: Cabbage plants were grown from seeds in a greenhouse chamber with a 16-hours photophase. 10-days-old seedlings were transplanted into 11 cm diameter plastic pots with soil (Standard 25) and sand mixture (3:1 volume). A colony of the diamondback moth (DBM; *Plutella xylostella*) was maintained in a cage (90cm×50cm×50cm) in an insect rearing room (18 ℃, L16:D8) on cabbage plants until needed. Dual-choice experiments were performed to evaluate the impact of the *T. harzianum* endophytic root inoculation on DBM preference and performance.

Cages (size: 90cm×50cm×50cm) were used to compare the oviposition behavior of DBM females on control and endophyte inoculated plants. Fresh pupae from the lab culture were carefully collected into a plastic box (18cm×15cm×5cm). After approximately 5 days, 5 pairs of newly emerged DBM were carefully transferred to vials. Two cabbage plants were placed into each cage on opposite sides, one plant root-inoculated with one of the *Trichoderma* strains mentioned above, while the other plant was treated with sterile water. The vials containing the DBM adults were placed between the two plants. To avoid any influence of light, the cages were kept in darkness for 48 hours. Thereafter the cabbage plants were removed from the cages and the number of the eggs on each plant (including the pots, stems, and leaves, respectively) was counted. The experiment was set up in were 10 replicates.

In the no-choice feeding experiment, second instar DBM larvae were carefully collected from the laboratory cultures. Clip cages were used to confine the larvae to the new fully-expanded leaves (3 weeks after inoculation) until the larvae pupated approximate 8 days).Thereafter, pupae were carefully removed from the clip cages and weighed. Meanwhile, the leaf area consumed was calculated by using the software Digi-trace. Dual-choice clip cages (diameter: 6cm) were used in the DMB feeding choice assay, consisting of three perspex cylinders; two of them closed on one end with fine mesh. In order to protect the leaves from artificial damage, a sponge rime was glued to the edges of the open ends of the cylinders. The feeding choice assays were processed on non-detached leaves of whole plants in the same greenhouse chamber as previously mentioned.

Weedicide Assay [31]

Each endophyte was grown in PD medium (300ml) containing XAD for 20 days. The XAD was collected and washed in methanol and acetone (1:1). The concentrated extract was taken (100, 200 or 500 µl) in vials and the solvent was evaporated overnight. 20ml of E-medium (KH_2PO_4 - KNO_3 - 1515mg Ca $(No_3)_2$ - 1180 mg, $MgSO_4.7H_20$ - 492 mg, H_3BO_3 - 2.86 mg, $MnCl_2$ - 3.62 mg, $FeCl_3.6H_2O$ - 5.4 mg, $ZnSO_4.7H_2O$ - 0.22 mg, $CuSO_4.5H_2O$ – 0.08 mg, EDTA – 11.2 mg, distilled water 1l) was added to the vials containing the 100, 200, 500 µl dried extracts. A *Lemna minor* plant having a rosette of three fronds was placed in each vial. The vials were placed in a glass dish filled with water and the dish was sealed with Para film. The dish with vials was incubated for seven days at 26˚C under fluorescent lamps. Three replicates were maintained for each concentration.

Table 1. Colonization Frequency (%) of putative entomopathogenic endophytes isolated from the leaves of tree species growing in there different forest types

Dry Thorn Forest

Family	Host plant	*Cladosporiumcladosporioides*	*Cladosporium*sp. 1	*Fusarium*sp. 1	*Fusarium*sp. 2	*Fusarium*sp. 3	*Fusarium*sp. 4	*Fusarium*sp. 5	*Fusarium*sp. 6	*Nodulisporiumgregarium*	*Nodulisporium*sp. 1	*Paecilomyces*sp. 1	*Paecilomyces*sp. 2	*Paecilomyces*sp. 3
Bignoniaceae	*Sterospermum angustifolium*	-	-	-	-	-	-	-	-	-	-	-	-	-
Boraginaceae	*Cordiawallichii*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Ehretia canarensis	-	-	1.3	-	-	-	-	-	-	-	-	-	-
Celastraceae	*Elaeodendron glaucum*	1.3	-	0.7	-	-	-	-	-	0.7	-	4	-	-
	Maytenus emarginatus	-	6	-	-	-	-	-	-	-	0.7	-	-	-
Combretaceae	*Anogeissus latifolia*	1.3	-	-	-	-	-	-	-	2	-	-	-	-
	Terminalia chebula	-	-	-	-	-	-	-	-	-	1.3	-	0.7	0.7
Ebenaceae	*Diospyros montana*	-	-	2.7	-	0.7	-	-	-	-	-	4.7	-	-
Erythroxylaceae	*Erythroxylon monogynum*	0.7	-	-	-	-	-	-	-	6	-	-	-	-
Euphorbiaceae	*Brideliaretusa*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Givotiarottleriformis	-	-	2	-	-	-	-	-	-	-	-	-	-
Fabaceae	*Bauhinia racemosa*	1.3	-	-	-	-	-	-	-	-	-	5.3	-	-
	Buteamonosperma	-	-	1.3	-	-	-	-	-	-	-	3.3	-	-
	Cassia fistula	-	-	-	-	-	-	-	-	-	-	-	-	-
	Dalbergialanceolaria	-	-	-	1.3	-	-	-	-	-	-	2	-	-
	Pongamiapinnata	-	-	2	-	-	-	-	-	-	-	-	-	-
	Pterocarpusmarsupium	-	-	-	-	-	-	-	-	-	-	-	-	-
Loganiaceae	*Strychnospotatorum*	0.7	-	3.3	-	-	-	-	-	-	-	-	-	-
Mimosaceae	*Acacia ferruginea*	-	-	-	-	-	-	-	-	-	2.7	-	-	-
Rhamnaceae	*Ziziphusjujuba*	-	1.3	-	-	-	-	-	-	-	-	-	-	-
	Zizyphusxylopyrus	-	-	-	-	-	-	-	-	-	-	-	-	-
Rubiaceae	*Ixoranigricans*	5.3	-	-	0.7	-	-	-	-	-	-	-	-	-
	Randiadumetorum	-	-	-	-	-	-	-	-	-	-	-	-	-
Verbenaceae	*Gmelinaasiatica*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Premnatomentosa	-	-	2	-	-	-	-	-	-	-	-	-	-
	Mean (%)	0.40	0.30	0.60	0.10	0.03	0.00	0.00	0.00	0.30	0.20	0.80	0.03	0.03

Dry deciduous Forest

Family	Host plant	*Cladosporiumcladosporioides*	*Cladosporium* sp. 1	*Fusariumsp. 1*	*Fusarium* sp. 2	*Fusariumsp. 3*	*Fusariumsp. 4*	*Fusariumsp. 5*	*Fusarium* sp. 6	*Nodulisporiumgregarium*	*Nodulisporium* sp. 1	*Paecilomycessp. 1*	*Paecilomycessp. 2*	*Paecilomycessp. 3*
Barringtoniaceae	*Careyaarborea*	-	-	-	2	-	-	-	-	-	-	-	-	-
Bignoniaceae	*Stereospermumpersonatum*	-	-	-	-	-	-	-	-	-	-	-	-	-
Boraginaceae	*Cordiaobliqua*	-	-	-	4	-	-	-	-	-	-	-	-	-
	Cordiawallichii	-	-	-	-	-	-	-	-	-	-	-	-	-
Combretaceae	*Anogeissuslatifolia*	-	-	-	2.7	1.3	-	-	-	-	-	-	-	-
	Terminaliaalata	-	-	-	-	1.3	-	-	-	-	-	-	-	-
	Terminaliacrenulata	-	-	-	-	-	-	-	-	-	-	-	-	-
Dipterocarpaceae	*Shorearoxburghii*	-	-	-	5.3	1.3	-	-	-	-	-	-	-	-
Euphorbiaceae	*Phyllanthusemblica*	-	1.3	-	1.3	-	-	-	-	0.7	-	-	-	-
Fabaceae	*Cassia fistula*	-	-	2.7	2	-	-	-	-	1.3	-	-	-	-
	Ougeniaoojeinensis	-	-	-	-	-	-	-	-	-	-	-	-	-
Lythraceae	*Lagerstroemia microcarpa*	-	-	-	-	-	-	1.3	12.7	-	-	0.7	-	-
	Lagerstroemia parviflora	-	-	-	-	-	-	-	-	-	-	-	-	-
Malvaceae	*Kydiacalycina*	-	-	-	0.7	-	-	-	-	-	-	-	-	-
Myrtaceae	*Syzygiumcummini*	-	0.7	-	2	-	1.3	0.7	-	-	-	-	-	-
Oliaceae	*Schreberaswietenioides*	0.7	-	-	-	-	-	-	-	-	-	-	-	-
Pedaliaceae	*Radermacheraxylocarpa*	-	1.3	-	-	-	-	-	-	-	-	-	-	-
Rubiaceae	*Catunaregamspinosa*	-	-	-	-	-	-	-	-	-	-	-	-	-
Samydaceae	*Casearіaesculenta*	-	-	-	-	-	-	-	-	-	-	-	-	-
Sterculiaceae	*Helicteresisora*	-	-	-	-	-	-	-	-	-	-	-	-	-
Tiliaceae	*Grewiatiliifolia*	2	-	-	-	-	-	-	-	-	-	-	-	-
Verbenaceae	*Gmelinaarborea*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Premnatomentosa	-	-	-	-	-	-	-	-	-	-	-	-	-
	Tectonagrandis	-	-	-	-	-	-	-	-	-	-	-	-	-
	Vitexaltissima	-	-	-	-	-	-	-	0.7	-	-	-	-	-
	Mean (%)	0.10	0.10	0.10	0.80	0.20	0.10	0.10	0.50	0.10	0.00	0.03	0.00	0.00

MontaneEvergreen Forest

Family	Host plant	*Cladosporiumcladosporioides*	*Cladosporium* sp. 1	*Fusarium* sp. 1	*Fusarium* sp. 2	*Fusarium* sp. 3	*Fusarium* sp. 4	*Fusarium* sp. 5	*Fusarium* sp. 6	*Nodulisporiumgregarium*	*Nodulisporium* sp. 1	*Paecilomyces* sp. 1	*Paecilomyces* sp. 2	*Paecilomyces* sp. 3
Aquifoliaceae	*Ilex denticulata*	1.3	-	-	-	-	-	-	-	-	5.3	0.7	-	-
	Ilex wightiana	6	-	-	-	-	-	-	-	-	0.7	0.7	-	-
Celastraceae	*Euonymus angulatus*	2	-	-	-	-	-	-	-	-	6	0.7	-	-
Euphorbiaceae	*Daphniphylliumneilgherrense*	-	-	-	-	-	-	-	-	-	-	-	-	-
	Glochidionzeylanicum	2	-	-	-	-	-	-	-	-	-	-	-	-
Lauraceae	*Cinnamomummalabatrum*	-	-	-	-	-	3.3	-	-	-	-	0.7	-	-
	Cryptocaryabourdillonii	-	-	-	-	-	-	-	-	-	3.3	-	4	0.7
	Litsea floribunda	-	-	-	-	-	-	-	-	-	-	-	-	-
	Litseastocksii	-	-	-	-	-	-	-	-	-	1.3	-	-	-
	Neolitseazeylanica	0.7	-	-	-	-	-	-	-	-	12.7	2.7	-	-
	Phoebe lanceolata	-	-	-	-	-	-	-	-	-	0.7	-	-	-
Magnoliaceae	*Michelianilagirica*	-	-	-	8	-	-	-	-	-	-	1.3	-	-
Melastomaceae	*Memecylonmalabaricum*	-	-	-	-	-	-	-	-	0.7	2	0.7	-	-
Myrtaceae	*Rhodomyrtustomentosa*	-	-	-	1.3	-	-	-	-	-	-	2	-	-
	Syzigiumdensiflorum	4.7	-	-	-	-	-	-	-	-	1.3	4	-	-
Oleaceae	*Ligustrumroxburghii*	0.7	-	-	-	-	-	-	-	-	8.7	1.3	-	-
Rubiaceae	*Lasianthusvenulosus*	-	-	-	-	-	-	-	-	-	-	0.7	-	-
	Psychotriabisulcata	1.3	-	-	-	-	-	-	-	-	-	-	-	-
Rutaceae	*Verpisbilocularis*	32.7	-	-	-	-	-	-	-	-	-	-	-	-
Sabiaceae	*Meliosmasimplicifolia*	-	-	0.7	-	-	-	-	-	-	-	-	-	-
Sapotaceae	*Isonandracandolleana*	1.3	-	-	-	-	-	-	-	-	6.7	2.7	-	-
Staphylaceae	*Turpinianepalensis*	-	-	-	-	-	-	-	-	-	-	-	-	-
Symplocaceae	*Symplocoscochinchinensis*	1.3	-	-	-	-	-	-	-	-	6.7	1.3	-	-
	Symplocosobtusa	0.7	-	-	-	-	-	-	-	-	1.3	0.7	-	-
Ternstromiaceae	*Euryanitida*	-	-	-	-	-	-	-	-	-	9.3	4	-	-
	Mean (%)	2.20	0.00	0.03	0.40	0.00	0.10	0.00	0.00	0.03	2.60	1.00	0.20	0.03

On the third day, the status of the plant (browning and death) was recorded. An extract obtained from an uninoculated medium and treated similarly served as control.

RESULTS

Prevelance of Entomopathogenic Genera as Endophytes in Non-Crop Plants

Twenty five tree species from each of the three forests *viz*. dry thorn (DT), dry deciduous (DD) and montane evergreen (EG) forest belonging to 13, 17 and 15 families respectively were sampled for the presence of entomopathogenous fungal genera such as *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys*, *Fusarium*, *Paecilomyces* and *Verticillium* [22,32] as foliar endophytes. Irrespective of the family to which a tree species belonged or the forest type where it grew, the colonization frequency of genera of endophytes which could possibly have entomopathogenous species was very low (Table **1**). In DT forest, the highest mean colonization frequency % was observed for *Paecilomyces* sp. 1 (0.80); in DD, it was *Fusarium* sp. 2 (0.80) and in MD it was *Nodulisporium* (2.60). Entomopathogenic genera including *Acremonium*,

Beauveria, *Clonostachys*, and *Verticillium* were not present as endophyte in any of the tree species screened.

Production of Ant-Insect Metabolites by Non-Entomopathogenic Endophytes

In order to know if non-entomogenous endophytes produce anti-insect metabolites, we screened 90 endophytes isolated and maintained by VINSTROM from forest trees, marine algae and seagrasses for the production of anti-insect metabolites using *Helicoverpa armigera* larva. The anti-feedant assay revealed that 9 the 90 fungi produced some metabolites exhibiting anti-feedant activity. Of these, a *Trichoderma harzianum* endophytic in the brown seaweed *Sargassum wightii* was most effective (Table **2**). Inan earlier study, we had confirmed the identity of this fungus by sequencing the ITS1-5.8S-ITS2 region of its DNA [33].

Table 2. Leaf feeding activity of *H. armigera* larva as affected by methanol extract of the secretomes of endophytes.

Endophyte	Host plant	% of leaf area consumed after 24 h
Trichoderma harzianum	*Sargassum wightii*	36
Pithomyces sp.	*Sargassum* sp.	40
Aspergillus sp.	*Gracillaria edulism*	43
Stemphylium sp.	sponge 2	53
Fusarium sp.	sponge 2	69
Curvularia sp.	*Sargassum wightii*	80
Colletotrichum sp.	*Terminalia bellerica*	80
Corynespora sp.	*Entada rheedi*	91
Nigrospora sp.	*Turbinaria* sp.	98

Survival of An Effective Anti-Insect Fungus as Endophyte in Non-Host Crops

Since this *T. harzianum* was isolated from a seaweed, it was of interest to know if it could survive as an endophyte in a crop. This fungus, when sprayed as a spore suspension on the leaves, was able to infect crop plants such as *A. esculantus, C.annum* and *S. Melongena* and survive as a foliar endophyte in them (Fig. **1**). However, reisolation experiments showed that the fungus could not remain as endophyte in these plants for prolonged periods (Fig. **1**). Its % recovery as anendophyte from the sprayed plant fell more rapidly in *A. esculantus* and *C. annuam* and was almost nil on the 28th day after the spray treatment; in *S. melongena*, the recovery % fell at a slower rate and reached 0 on the 42nd day after spray (Fig. **1**). These experiments conducted in VINSTROM indicated that endophytic *T. harzianum* could be an effective insect antagonist. To confirm this observation, two endophyytic strains of *T. harzianum*, T39 and Tu, were studied in Georg-August-Universität laboratory for some more anti-insect properties. The results of these experiments showed that when presented with a choice of endophyte-inoculated and control cabbage plants for oviposition, DBM laid significantly more eggs on control plants compared to *Trichoderma*-inoculated plants, irrespective of the *Trichoderma* isolate used (Fig. **2**). Although the plants were placed close to each other, DBM females were able to discriminate between the treatments and did not randomly place the eggs on the plants. Similarly, the dual-choice clip cage feeding assay showed that DBM larvae fed significantly more on the control plants compared to the inoculated plants (Wilcoxon's signed–rank test of Tu: p = 0.22; T39: p = 0,03), irrespective of the *Trichoderma* isolate used (Fig. **3**).

Endophytes Produce Weedicidal Compounds

We observed that the secretome of some endophytes contain weedicidal compounds. Of the 28 species of endophytes from different plants, the secretomes of 9, 14 and 17 endophytes caused browning and death of *L. minor* at 100, 200 and 500 µl concentrations (Table **3** and Plate **1**). Several endophytes

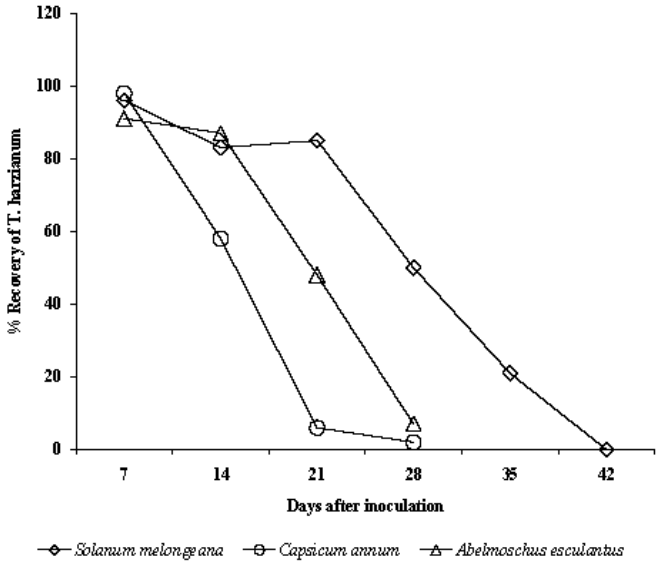

Fig. (1). Recovery of algal endophyte*Trichoderma harzianum* from leaves of *Abelmoschus esculantus, Capsicum annum* and *Solanum melongena* after inoculation with spores.

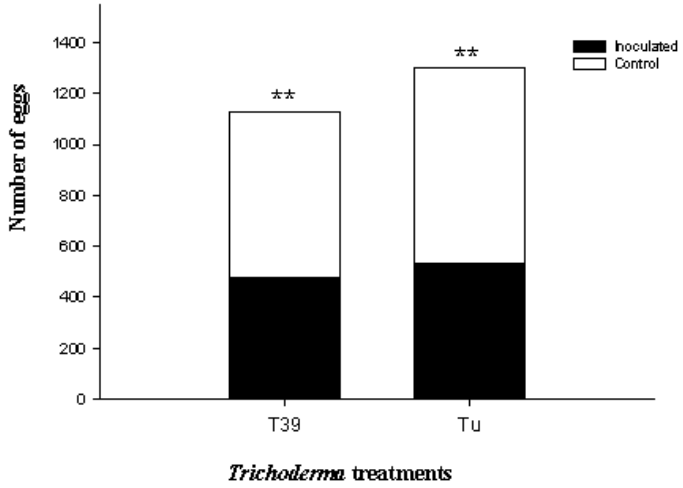

Fig. (2). Number of eggs found on control and *Trichoderma harzianum* root inoculated plants in a dual-choice oviposition assay. Five pairs of adult *DBM* were used in a single experiment; 10 experiments were performed in each treatment. Stars indicate significant differences ($p<0.01$) by goodness-of-fit test expecting a random distribution of the eggs between plants.

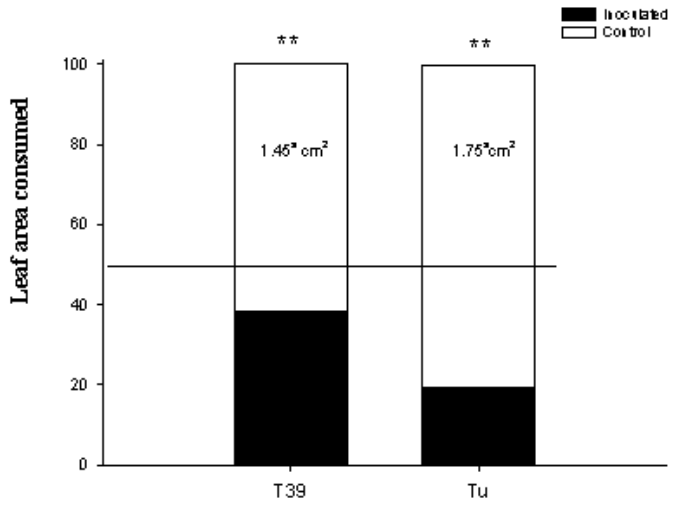

Fig. (3). Mean leaf area removed per DBM larvae during the dual-choice feeding assays on control and *Trichoderma harzianum* (strains T39 and Tu) root inoculated cabbage plants.

including species of *Botrytis, Colletotrichum, Curvularia, Periconia, Phomopsis, Trichoderma, Xylaria* and Sterile form 6 showed weedicide effect at the minimum concentration tried (Table **3**).

DISCUSSION

Endophytes contribute to the ecological success of their host plants enhancing their abiotic stress tolerance and defend them from pests and pathogens. It is therefore hypothesized that they played a seminal role in the evolution of plants [34]. Their non-disease causing nature and universal occurrence in plants along with their anti-pest/pathogen traits make endophytes ideal candidates for use in biocontrol [35]. Although several studies reporting on fungal endophytes in crop plants substantiate this view [10, 11, 36-38], there are many gaps in our knowledge regarding endophyte-plant host-pest/pathogen tripartite interactions limiting the effectiveness and the implementation of this strategy. Vidal and Jaber (2015) have highlighted many facets of endophyte-mediated pest control which need detailed study for the successful implementation of this ecological group of fungi in biocontrol [24]. Endophytism being a successful life strategy has culminated in the wide spread occurrence of fungi as plant endosymbionts [20]. Endophytes have broadened their host range by evolving many generalist species resulting in their loose host taxonomic affiliation and wide geographic distribution [39-41]. For instance, the beneficial endophytes isolated from a monocot plant upon transfer to eudicot plants survive and continue to function as endophytes [42]. Furthermore, endophytes ward off insect pests [19, 43] and reduce damage caused to crops by pathogenic fungi and bacteria, and phytophagous nematodes [44]. Such a broad host range and anti-pest properties of endophytes should motivate the screening of non-crop plants of different taxonomic and habitat category for identifying effective biocontrol agents including entomopathogenic and anti-insect endophytes. Fungi which are non–entomopathogenic but are opportunistic pathogens of pests are being increasingly used in biocontrol [45, 46]. The import of

such a screening is underscored by our finding that a *T. harzianum* endophyte from a non-plant host (brown seaweed) was effective against the devastating crop pest *H. armigera* andwas able to survive as an endophyte in some crop plants. Although we show that an endophyte from an alien host could colonize crops, its efficacy in warding off pests in the field is yet to be demonstrated. Furthermore, the introduced endophyte is eliminated by the crop with time necessitating periodic re-inoculation to maintain effective density of the endophyte. Successful colonization and survival of such anti-insect fungi in a crop as endophytes depends on many factor such as the method of inoculation, crop plant species and its growing conditions, and last but not least the fungal strain used [47]. The native endophyte community existing in a plant tissue could also inhibit the establishment alien form as endophytes; Mohandoss and Suryanarayanan (2009) showed that the non-native fungi could establish themselves as endophytes in mango leaves only after some native endophytes are eliminated by a systemic fungicide treatment [48]. In the present study, we found that DBM do not prefer *T. harzianum*-inoculated plants for oviposition or as feed. The type of interaction between endophyte and phytophagous insects could vary. While leaf-cutter ants avoid leaves supporting heavy load of endophytes [49], painted grasshoppers feed equally on *Calotropis* leaves with and without a heavy load of foliar endophytes [50]. These results warrant detailed investigations on the basics of interactions of endophyte-plant host –insect pest success in endophyte-mediated biocontrol.

Vega *et al*. (2008) reported that many entomopathogenic

Table 3. Weedicidal activity of methanol:acetone (1:1) extract of the secretomes of endophytes.

Endophyte	Concentration of extract		
	100 µl	**200 µl**	**500 µl**
Alternaria sp.	+	+	+
Arthrinium sp.	-	+	+
Aspergillus niger	-	-	+
*Bartalinia*sp.	-	-	-
Botrytis sp.	+	+	+
Chaetomium sp.	-	+	+
*Cladosporium*sp.	-	-	-
*Colletotrichum*sp.	+	+	+
*Corynespora*sp.	-	-	-
Curvularia sp.	+	+	+
Drechslera sp.	-	-	-
Fusarium sp.	-	+	+
Humicola sp.	-	-	-
Lasiodiplodia sp.	-	-	+
Nigrospora sp.	-	-	-
Nodulisporium sp.	-	-	-
Periconia sp.	+	+	+
Pestalotiopsis sp.	-	+	+
Phomopsis sp.	+	+	+
Phyllosticta sp.	-	-	-
Pithomyces sp.	-	-	-
Sporormiella sp.	-	-	-
Sterile form 1	-	-	+
Sterile form 6	+	+	+
Sterile form 10	-	-	-
Torulomyces sp.	-	+	+
Trichoderma sp.	+	+	+
Xylaria sp.	+	+	+
Total	9	14	17

+ = activity observed - = no activity

Plate (1). *Lemna minor* plant showing weedicidal activity of the extract from an endophyte secretome.

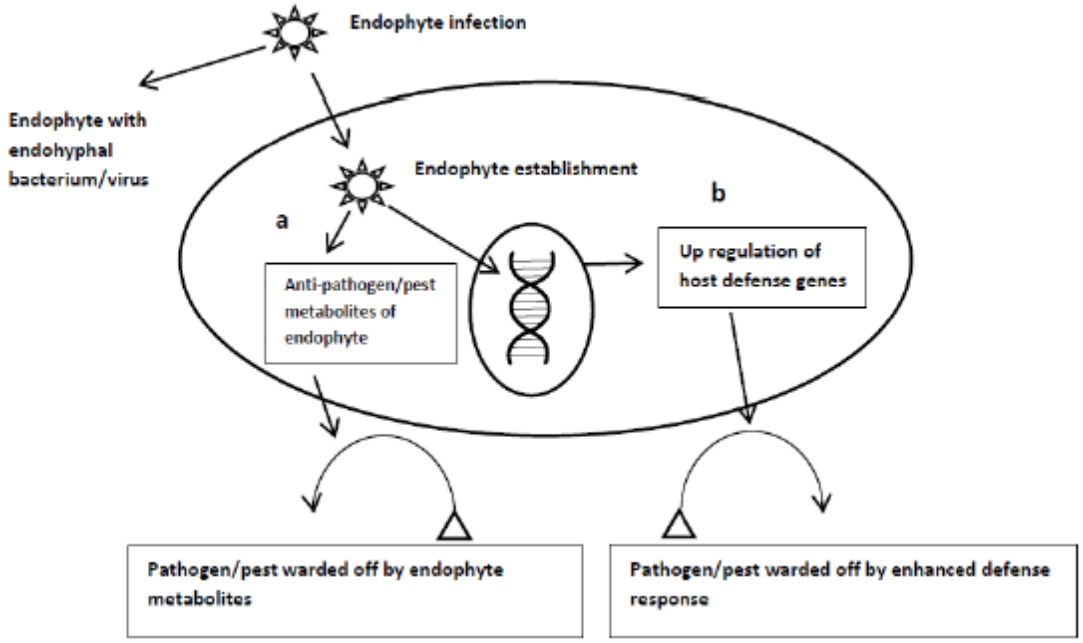

Fig. (4). a) Endophyte elaborates anti-pathogen/pest metabolites which ward off the pathogen; **b)** Endophyte infection activates several defence genes of the host resulting in heightened resistance.

fungal genera, including *B. bassiana*, are able to survive as endophytes in tissues of coffee [22]. Gazis and Chaverri (2015) observed the occurrence of many entomopathogenic fungi as endophytes in plants of the Amazonian region [38]. There are hardly any studies on the occurrence of entomopathogenic fungal genera as endophytes in non-crops such as forest trees. In the present study on the trees species of relatively dry tropical forests of the Western Ghats we found that entomopathogenic endophytes are not common. It is likely that the native community of endophytes in the leaves of these tree species inhibit the establishment of such endophytes [29, 48]. It is suggested that insects disseminate entomopathogenic fungi among crops [51] and endophyte density in crop plants is positively correlated with insect visitations [43]. In the light of these observations the question why crop plant species support more entomopathogenic genera as endophytes when compared to tree species needs to be addressed.

A plant organ such as a leaf harbours a high diversity of endophyte species in different densities [52, 53]. The interactions in the plant tissue milieu could be between the endophyte species supported by it, between the plant tissue and

its endophytes, as well as between the endophytes and pests and pathogens dependant on that tissues. The details of these possible interactions are not known [20, 24, 54]. We envisage a few possible scenarios of these interactions here with a view to design better methodologies to improve the performance of endophytes as biocontrol agents. An endophyte could ward off insect an pest or pathogen by elaborating specific secondary metabolites *in planta* [55] (Fig. **4a**); in cocoa, endophyte infection upregulates numerous defense genes of the host plant resulting in enhanced resistance of the plant [56]; metabolomic analyses revealed that the inoculation of *Cirsium arvense* leaves with spores of an endophytic *Chaetomium cochlioides* induces the production of metabolites which are normally produced by the plant as a response to herbivory or pathogen invasion [57], (Fig.**4b**). Thus, inoculating a crop with an endophyte which upregulates most defence-related genes or elaborates insect-deterring metabolites could be an effective strategy. Alternatively, an endophyte could exclude a pathogen if its nutritional niche overlaps that of the pathogen [54] (Fig. **5a**). Employing phenotypemicroarrayand a niche overlap index, Blumenstein *et al*. (2015) demonstrated that the nutritional niche of endophytes of *Ulmus* sp. overlaps widely with that of its fungal pathogen *Ophiostoma novo-ulmi* [54]. Schulz *et al*. (2015) hypothesise that since a plant tissue hosts many endophyte species, a successful endophyte could be one which secretes antifungal and antibacterial metabolites *in planta* thus inhibiting its competitors [58]. Recent findings of Yan *et al*. (2015) that endophytes in a herb secrete metabolites which are detrimental to co-colonizers lend credence to this [59]. Mohandoss and Suryanarayanan (2009) showed that exclusion of some endophytes from the leaves of *Mangifera indica* by a fungicide treatment resulted in the colonization of the leaf by non-native endophytes suggestive of an *in vivo* inter-specific competition among endophytes [48]. Thus, a situation similar to antibiosis in soils could operate in tissues harbouring a suit of endophytes where an antibiotic produced by an endophyte as a result of interspecific competition could ward off a pathogen (Fig. **5b**).

Endophytes can biotransform natural products, especially of their host origin [60-62]. Given this ability, it can be conceived that an endophyte biotransforms an 'ineffective' host or co-occurring endophyte's metabolite in to an active form which inhibits a pest/pathogen attacking the plant (Fig. **5c**).

The inside of a plant tissue is a complex microhabitat for an endophyte as it has to establish an infection, colonize the tissue, survive the interspecific competition as well as counter the host defence reactions. In this context, the work of Estrada *et al*. (2012) is instructive [55]. They showed that when *Fusarium verticillioides* is present as an endophyte in *Zea mays*, it reduces the aggressiveness of the plant's pathogen *Ustilago maydis*; the endophyte also degrades the defensive chemicals produced by the plant against the *U. maydis* pathogen. Therefore it is critical to know the mode of action of the fungus in question for an effective and sustained endophyte-mediated biocontrol.

Once an effective endophyte and its mode of action are known, gene silencing and promoting methods could be used to improve its performance as a biocontrol agent. For instance, endophytes carrying endoviruses which induce the expression specific fungal genes [63] could be chosen for action against specific herbivorous pests. It is imperative that an effective biocontrol endophyte does not produce toxic secondary metabolites such as mycotoxins. Induction of small interfering RNAs (siRNAs) in the host plant could be an effective mechanism to silence specific fungal genes [64] such as those coding for mycotoxins [65]. Masci *et al*. (2014) induced RNAi expression in a plant pathogenic fungus by direct transfection with plant virus vector [66]. Considering the advantages of this technique over the conventional gene knockout approaches [66], it could be

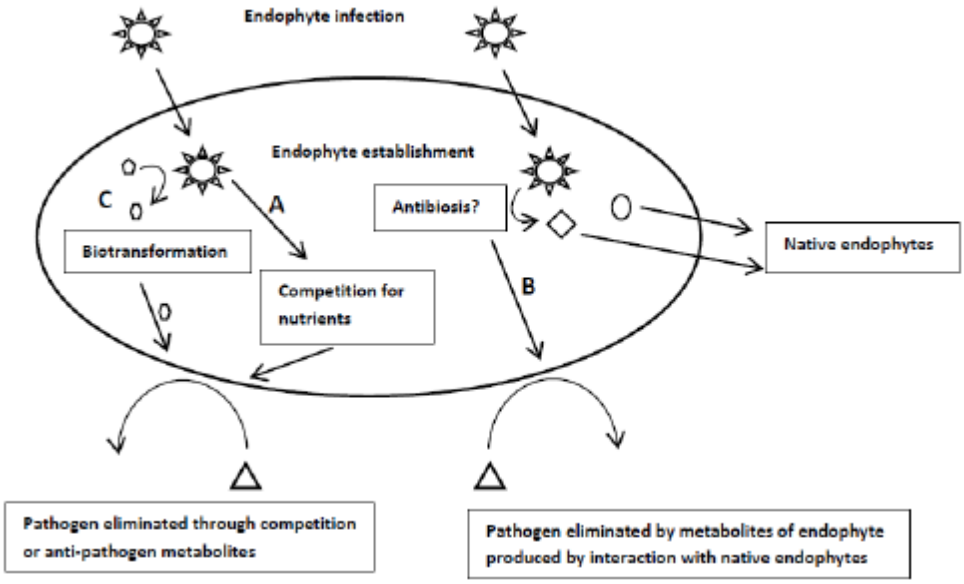

Fig. (5) a) Pathogen/pest gets eliminated or subdued due to overlapping nutrient requirements with resident endophytes; **b)** Introduced endophyte competes with native endophytes through antibiosis and the resultanat antibiotic eliminated pathogen; **c)** Endophyte biotransforms host/native endophyte metabolite which inhibits pathogenesis.

used to express or silence specific genes of an endophyte to enhance its biocontrol potential.

Yet another facet of biocontrol is the possible use of chitinase enzymes in managing insect pests and fungal pathogens of plants [67, 68]. Here again, it would be worthwhile to screen endophytes for variants of chitinase, chitosanase and chitin deacetylase enzyme, as our studies on endophytes of land plants [69,70] and marine algae and seagrasses [71] show that they elaborate a high diversity of such chitin modifying enzymes.

Many endophytes are closely related to phytopathogenic fungi [72-74]. The causal organism of pepper spot disease of peanut in many peanut growing areas of the world exists as symptomless endophyte in the leaves of many varieties of peanut cultivated in the state of Tamilnadu, India [75]. It is now known that the infection by endophytes upregulates many defense genes in plants [56]. Therefore, it is reasonable to argue that inoculation of crop cultivars by permanently avirulent endophyte strains should increase their resistance to pathogenic strains of the fungus - a situation akin to systemic acquired resistance in plants induced due to infection by avirulent pathogens [76].

There are very few studies on the possible use of endophytes to control weeds. In a recent work, Bao *et al*. (2015) reported that aqueous extracts of the hemiparasitic weed *Pedicularis kansuensis* inhibited more the seed germination and seedling growth of grasses devoid of their systemic fungal endophyte compared to those harbouring the endophyte [77]. We demonstrate for the first time that some of the non-systemic fungal endophytes do produce metabolites inimical to weeds. It is not clear what ecological benefit would accrue to the plant symbiont by the production of metabolites which are lethal to its host. Identification of such bioactive compounds of endophyes specific to weeds may lead to the development of novel weedicide formulations.

The use of entomopathogenic and anti-insect fungi or anti-pathogen metabolite(s) producing fungi as endophytes in crop plants along with other prescribed control practices could reduce considerably the dependence on synthetic chemicals for pest and disease management of crops. However, knowledge regarding the successful route of inoculation, inoculum dose, period of survival of an introduced endophyte in a crop plant, its interactions with the resident endophytes and the crop cultivar, the loss suffered by the crop versus the benefit it gains in supporting the endophtye for every effective endophyte-crop plant combination is essential for the successful implementation of this novel method of biological control [24, 47].

In conclusion, our basic study underscores the need to screen plants and non-plants of different habitats for entomopathogenic and anti-insect endosymbiotic fungi. Futhermore, it highlights the importance of screening endophytes for weedicidal compounds. We emphasise the need for focused studies for better understanding of the interaction between endophytes and their plant hosts and the biotic stressors of the host plants to enhance the success of using endophytes for biological control.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

TSS thanks Swami Shukadevananda, Secretary, RKM Vidyapith for facilities and the Department of Biotechnology, Govt. of India, New Delhi for funding the Indo-German collaborative project (BT/IN/German/11/TSS/2010).

REFERENCES

[1] Oerke E-C. Crop losses to pests.J Agri Sci 2006; 144: 31 - 43.
[2] Maxmen, A. Crop pests: Under attack. Nature 2013; 501: S15 - 17.
[3] Denholm I, Cahill M, Dennehy TJ, Horowitz AR. Challenges with managing insecticide resistance in agricultural pests, exemplified by the white fly *Bemisia tabaci*. Phil Trans R SocLond B 1998; 353: 1757 - 67.
[4] Dogimont C, Bendahmane A, Chovelon V, Biossot N. Host plant resistance to aphids in cultivated crops: genetic and molecular bases, and interactions with aphid populations. C R Biol 2010; 333, 566 - 73.
[5] Birch AEN, Begg GS, Squire GR. How agro-ecological research helps to address food security issues under new IPM and pesticidereduction policies for global crop production systems. J Exp Bot 2011; 1 - 11.
[6] Howell CR. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: The history and evolution of current concepts. Plant Disease 2003; 87: 4-10.
[7] Knudsen GR, Dandurand L-MC. Ecological complexity and the success of fungal biological control agents. AdvAgric 2014; 2014: Article ID 542703.
[8] Goettel MS, Eilenberg J, Glare TR. In: Entomopathogenic fungi and their role in regulation of insect populations. Gilbert L, Iatrou K, Gill D. Eds. Comprehensive Molecular Insect Science, Elsevier Boston 2005; pp. 361- 406.
[9] Vincent C, Goettel MS, Lazarovits G. Biological Control: a global Perspective. CAB International-AFFC: Wallingford, UK2007.
[10] Vega FE. Insect pathology and fungal endophytes. J Inv Pathol 2008; 98: 277-9.
[11] Lopez CD, Zhu-Salzman K, Ek-Ramos MJ, Sword GA. The entomopathogenic fungal endophytes *Purpureocillium lilacinum* (Formerly *Paecilomyces lilacinus*) and *Beauveria bassiana* negatively affect cotton aphid reproduction under both greenhouse and field conditions. PLoS ONE2014; 9: e103891.
[12] Rinaudo V, Bàrberi P, Giovannetti M, van der Heijden MGA. Mutualistic fungi suppress aggressive agricultural weeds. Plant and Soil 2010; 333: 7-20.
[13] Vega FE, Goettel MS, Blackwell M, *et al.* Fungal entomopathogens: new insights on their ecology. Fungal Ecol 2009; 2:149-59.
[14] Suryanarayanan TS, Thirunavukkarasu N, Govindarajulu MB, Sasse F, Jansen R,Murali TS.Fungal endophytes and bioprospecting. Fungal Biol Rev 2009; 23: 9 - 19.
[15] Suryanarayanan TS, Thirunavukkarasu N, GovindaRajulu MB, Gopalan V. Fungal endophytes: an untapped source of biocatalysts. Fungal Divers 2012; 54: 19 - 30.
[16] Nagarajan A, Thirunavukkarasu N, Suryanarayanan TS,Gummadi SN. Screening and isolation of novel glutaminase free l-asparaginase from fungal endophytes. Res J Microbiol 2014; 9: 163 - 76.
[17] Thirunavukkarasu N, Jahnes B, Broadstock A, *et al.* Screening marine-derived endophytic fungi for xylan-degrading enzymes. Curr Sci 2015; 109: 112-20.
[18] Redman RS, Sheehan KB, Stout RG, Rodriguez RJ, Henson JM. Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. Science 2002; 298: 1581.
[19] Estrada C, Degner EC, Rojas EI, Wcislo WT, Van Bael SA. The role of endophyte diversity in protecting plants from defoliation by leaf-cutting ants. Curr Sci 2015; 109: 55 - 61.

[20] Suryanarayanan TS. Endophyte research: going beyond isolation and metabolite documentation. Fungal Ecol 2013; 6: 561 - 8.

[21] Murali TS, Suryanarayanan TS, Venkatesan G. Fungal endophyte communities in two tropical forests of southern India: diversity and host affiliation. Mycol Prog 2007; 6: 191 - 9.

[22] Vega FE, Posada F, Aime MC, Peterson SW, Rehner SA. Fungal endophytes in green coffee seeds. Mycosystema 2008; 27: 75-84.

[23] Posada F, Vega FE. Inoculation and colonization of coffee seedlings (*Coffeaarabica* L.) with the fungal entomopathogen *Beauvria bassiana* (Ascomycota:Hypocreales), Mycoscience 2006; 47:284 - 9.

[24] Vidal S, Jaber LR. Entomopathogenic fungi as endophytes: plant-endophyte-herbivore interactions and prospects for use in biological control. Curr Sci 2015; 109: 46 - 54.

[25] Suryanarayanan TS, Kumaresan V, Johnson JA. 1998. Foliar fungal endophytes from two species of the mangrove *Rhizophora*. Can J Microbiol 1998; 44: 1003 - 6.

[26] Suryanarayanan TS. 1992. Light-incubation: a neglected procedure in mycology. The Mycologist 1992; 6: 144.

[27] Suryanarayanan TS, Venkatachalam A, Thirunavukkarasu N, Ravishankar JP, Doble M, Geetha, V. Internal mycobiota of marine macroalgae from the Tamilnadu coast: distribution, diversity and biotechnological potential. Bot Mar 2010; 53: 456 - 68.

[28] Suryanarayanan TS, Johnson JA. Fungal endosymbionts of macroalgae: Need for enquiries into diversity and technological potential. Oceanography 2014; 2: 1

[29] Posada F,Aime MC, Peterson SW, Rehner SA, Vega FE. Inoculation of coffee plants with the fungal entomopathogen *Beauveria bassiana* (Ascomycota:Hypocreales). Mycol Res 2007; 111: 748 - 57.

[30] Posada F, Vega FE. Establishment of the fungal entomopathogen *Beauveria bassiana* (Ascomycota: Hypocreales) as an endophyte in cocoa seedlings (*Theobroma cacao*). Mycologia 2005; 97: 1195 - 200.

[31] Rahman A, Choudhary MI, Thomsen WJ. Bioassay techniques for drug development. Harwood Academic Publishers: Singapore 2001.

[32] Teetor-Barsch GH, Roberts DW. Entomogenous *Fusarium* species. Mycopathologia 1983; 84: 3- 16.

[33] Kaushik NK, Murali TS, Sahal D, Suryanarayanan TS. A search for antiplasmodial metabolites among fungal endophytes of terrestrial and marine plants of southern India. Acta Parasitol 2014; 59: 745 - 57.

[34] White Jr JF, Torres MS, Johnson H, Irizarry I, Tadych M. In: Advances in Endophytic Research, Verma VC,Gange AC, Eds. Springer India 2014; pp. 425 - 39.

[35] Backman PA, Sikora RA. Endophytes: An emerging tool for biological control. Biol Cont 2008; 46: 1 - 3.

[36] Arnold AE, Lewis LC. In: Ecological and evolutionary advances in insect-fungus associations; Vega F, BlackwellM, Eds. Oxford University Press: Oxford.2005; pp. 74-96.

[37] Zhang HW, Ying C, Bai XL. Advancement in endophytic microbes from medicinal plants. Int J Pharm Sci Res 2014; 5: 1589-600.

[38] Gazis R, Chaverri P. Wild trees in the Amazon basin harbor a great diversity of beneficial endosymbiotic fungi: Is this evidence of protective mutualism? Fungal Ecol 2015; 17: 18 - 29.

[39] Davis EC, Franklin JB, Shaw AJ, Vilgalys R. Endophytic *Xylaria* (Xylariaceae) among liverworts and angiosperms: phylogenetics, distribution and symbiosis. American J Bot 2003; 90: 1661- 7.

[40] Murali TS, Suryanarayanan TS, Geeta R. Endophytic *Phomopsis* species: host range and implications for diversity estimates. Can J Microbiol 2006; 52: 673 - 80.

[41] Suryanarayanan TS. In: Endophytes of Forest Trees: Biology and Applications; Pirttilä AM, Frank AC, eds. Forestry sciences series 2011; 80: pp 67-80.

[42] Rodriguez RJ, Redman RS, Henson JM. In: The Fungal Community: Its Organization and Role in the Ecosystem; DightonJ, White JrJF, Oudemans P, Eds. CRC Press, Boca Raton: FL, 2004; pp. 683 - 95.

[43] Suryanarayanan TS, Venkatachalam A, Govinda Rajulu MB. A comparison of endophyte assemblages in transgenic and non-transgenic cotton plant tissues. Curr Sci 2011; 101:1472 - 4.

[43] Weber J. A natural control of Dutch elm disease. Nature 1981; 292: 449 - 51.

[44] Lacava PT, Azevedo JL. In: Biological Control of Insect-Pest and Diseases by Endophytes. Verma VC, Gange AC. Eds. Advances in Endophytic Research, Elsevier 2014; pp. 231-256.

[45] Brodeur J. Host specificity in biological control: insights from opportunistic pathogens. Evol Appl 2012; 5: 470 - 80.

[46] Mukherjee AK, Sampath Kumar A, Kranthi S, Mukherjee PK. Biocontrol potential of three novel *Trichoderma* strains: isolation, evaluation and formulation. 3 Biotech 2014; 4: 275 - 81.

[47] Parsa S, Ortiz V, Vega FE. Establishing fungal entomopathogens as endophytes: Towards endophytic biological control. J Vis Exp 2013; 74: e50360.

[48] Mohandoss J, Suryanarayanan TS. Effect of fungicide treatment on foliar fungal endophyte diversity in mango. Sydowia 2009; 61: 11 - 24.

[49] Estrada, C, Wesilo WT, Van Bael SA. Symbiotic fungi alter plant chemistry that discourages leaf-cutting ants. New Phytol 2013; 198: 241 - 51.

[50] Devarajan PT, Suryanarayanan TS. Evidence for the role of phytophagous insects in dispersal of non-grass fungal endophytes. Fungal Divers 2006; 23: 111 - 9.

[51] Maniania NK, Ouna E, Ahuya P, *et al.* Dissemination of entomopathogenic fungi using *Busseola fusca* male as vector. Biol Cont 201; 58: 374 - 8.

[52] Arnold AE, Maynard Z, Gilbert GS, Coley PD, Kursar TA. Are tropical fungal endophytes hyperdiverse? Ecol Lett 2000; 3: 267-74.

[53] Suryanarayanan TS, Murali TS, Venkatesan G. Occurrence and distribution of fungal endophytes in tropical forests across a rainfall gradient. Can J Bot 2002; 80: 818 - 26.

[54] Blumenstein K, Macaya-Sanz D, Martín JA, Albrectsen BR,Witzell J. Phenotype Micro Arrays as a complementary tool to next generation sequencing for characterization of tree endophytes. Front Microbiol 2015; 6: 1033.

[55] Estrada AER, Jonkers W, Kistler HC, May G. Interactions between *Fusarium verticillioides*, *Ustilago maydis*, and *Zea mays*: An endophyte, a pathogen, and their shared plant host. Fungal Gen Biol 2012; 49: 578 - 87.

[56] Mejía LC, Herre EA, Sparks JP, *et al.* Pervasive effects of a dominant foliar endophytic fungus on host genetic and phenotypic expression in a tropical tree. Front Microbiol 2014; 5: 479.

[57] Hartley SE, Eschen R, Horwood JM, Gange AC, Hill EM. Infection by a foliar endophyte elicits novel arabidopside-based plant defence reactions in its host, *Cirsium arvense*. New Phytol 2015; 205: 816 - 27.

[58] Schulz B, Haas S, Junker C, Andrée N, Schobert M. Fungal endophytes are involved in multiple balanced antagonisms. Curr Sci 2015; 109: 39-45.

[59] Yan JF, Broughton SJ, Yang SL,Gange AC. Do endophytic fungi grow through their hosts systemically? Fungal Ecol 2015; 13:53-9.

[60] Pimentel MR, Molina G, Dionisio AP, Junior MRM, Pastore GM. The use of endophytes to obtain bioactive compounds and their application in biotransformation process. Biotech Res Intl 2011; 1:11.

[61] Luo S-L, Dang L-Z, Li J-F, Zou C-G, Zhang K-Q, Li G-H. Biotransformation of saponins by endophytes isolated from Panaxnoto ginseng. ChemBiodivers 2013;10: 2021 - 31.

[62] Ying YM, Shan WG, Zhan ZJ. Biotransformation of Huperzine A by a fungal endophyte of *Huperzia serrata* furnished sesquiterpenoid–alkaloid hybrids. J Nat Prod 2014, 77: 2054 - 9.

[63] Márquez LM, Redman RS, Rodriguez RJ, Roossinck MJ. A virus in a fungus in a plant: three-way symbiosis required for thermal tolerance. Science 2007; 315: 513 - 15.

[64] Tinoco MLP, Dias BB, Dall'Astta RC, Pamphile JA, Aragão FJ. *In vivo* trans-specific gene silencing in fungal cells by *in planta* expression of a double-stranded RNA. BMC Biology 2010; 8: 27.

[65] Strasser H, Vey A, Butt, TM.Are there any risks in using entomopathogenic fungi for pest control, with particular reference to the bioactive metabolites of *Metarhizium*, *Tolypocladium* and *Beauveria* species? Biocontrol Sci Technol 2000; 10: 717 - 35.

[66] Masci T, Nigro F, Abdallah A, *et al.* Gene silencing and gene expression in phytopathogenic fungi using a plant virus vector. PNAS 2014 ; 111: 4291- 6

[67] Zhang Z, Yuen GY, Sarath G, Penheiter AR. Chitinases from the plant disease biocontrol agent, *Stenotrophomonas maltophilia* C3. Phytopathology 2001; 91: 204 - 11.

[68] Sharp RG. A review of the applications of chitin and its derivatives in agriculture to modify plant-microbial interactions and improve crop yields. Agronomy 2013; 3: 757 - 93.

[69] Govinda Rajulu MB, Thirunavukkarasu N, Suryanarayanan TS, Ravishankar JP, El Gueddari NE, Moerschbacher BM. Chitinolytic enzymes from endophytic fungi. Fungal Divers 2011; 47: 43-53.

[70] Nagaraju D, GovindaRajulu MB, El Gueddari NE, Suryanarayanan TS, Moerschbacher BM. Identification and characterization of chitinolytic enzymes from endophytic fungi. `Sugars in Norwich` – Royal Soc. Chemistry, Carbohydrate Meeting; 2009 Sep 15-16; London, UK 2009; pp. 23.

[71] Venkatachalam A, Govinda Rajulu MB, Thirunavukkarasu N, Suryanarayanan TS. Endophytic fungi of marine algae and seagrasses: a novel source of chitin modifying enzymes. Mycosphere 2015; 6, 345 - 55.

[72] Hyde KD, Cai L, McKenzie EHC, Yang YL, Zhang JZ, Prihastuti H. *Colletotrichum*: a catalogue of confusion. Fungal Divers 2009; 39: 1-17.

[73] Zhang Y, Schoch CL, Fournier J, *et al*. 2009. Multi-Locus Phylogeny of Pleosporales: A Taxonomic, Ecological and Evolutionary Re-Evaluation. Stud Mycol 2009; 64:85 -102.

[74] Slippers B, Boissin E, Phillips AJL, *et al.* Phylogenetic lineages in the Botryosphaeriales: a systematic and evolutionary framework. Stud Mycol 2013; 76: 31- 49.

[75] Suryanarayanan TS, Murali TS. Incidence of *Leptosphaerulina crassiasca* in symptomless leaves of peanut in southern India.J Basic Microbiol 2006; 46: 305 - 9.

[76] Cao H, Bowling SA, Gordon AS, Dong X. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance.The Plant Cell 1994; 6: 1583 - 92.

[77] Bao G, Saikkonen K, Wang H, *et al*.Does endophyte symbiosis resist allelopathic effects of an invasive plant in degraded grassland? Fungal Ecol 2015; 17: 114-25.