Inhibition of fungal endophytes by camptothecine produced by their host plant, *Nothapodytes nimmoniana* (Grahm) Mabb. (Icacinaceae)

S. Shweta[1,2], M. B. Shivanna[2], B. R. Gurumurthy[3], R. Uma Shaanker[1,4], T. R. Santhosh Kumar[5] and G. Ravikanth[4,*]

[1]School of Ecology and Conservation and Department of Crop Physiology, University of Agricultural Sciences, GKVK, Bangalore 560 065, India
[2]Kuvempu University, Jnana Sahyadri, Shankaraghatta, Shimoga 577 451, India
[3]University of Agriculture and Horticulture Sciences, Shimoga 577 204, India
[4]Ashoka Trust for Research in Ecology and the Environment, Royal Enclave, Srirampura, Jakkur PO, Bangalore 560 064, India
[5]Apoptosis and Cell Signalling, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram 695 014, India

Camptothecine (CPT), a monoterpene indole alkaloid, is a potent inhibitor of eukaryotic topoisomerase I. It is produced by a number of plants, including *Nothapodytes nimmoniana* (Grahm) Mabb. (Icacinaceae), occurring naturally in the Western Ghats, India. The plant is inhabited by a number of endophytic fungi, many of which have been isolated and shown to produce CPT, in culture, independent of the host. In this article, we examine the sensitivity of endophytic fungi isolated from *N. nimmoniana* to CPT. Contrary to our hypothesis that these fungi should be resistant to CPT (as they are exposed to host CPT as well as that produced by themselves), we report that these fungi are sensitive and thus inhibited by CPT. We discuss these results in the context of the role of CPT in limiting endophytic fungal growth.

Keywords: Camptothecine, endophytic fungi, *Nothapodytes nimmoniana*, sensitivity and inhibition,

CAMPTOTHECINE (CPT), a monoterpene indole alkaloid, is a potent inhibitor of eukaryotic topoisomerase I, an enzyme responsible for the unwinding of DNA during replication and transcription[1]. Because of this property, CPT is one of the most cytotoxic compounds[1,2] and several derivatives of CPT are being used in the treatment of a wide variety of cancers, including ovarian, small lung and refractory ovarian cancers[2]. CPT is produced by several plant species as a secondary metabolite, presumably as a deterrent to plant pathogens and pests[3,4]. Among these, *Camptotheca acuminata* Decne.(Nyssaceae) and *Nothapodytes nimmoniana* (Grahm) Mabb. (Icacinaceae) are the major commercial sources of CPT[3]. Besides these plant sources, CPT has also been reported from a number of endophytic fungal[5–10] and more recently, bacterial associates of plants producing CPT[11].

*For correspondence. (e-mail: gravikanth@gmail.com)

An intriguing feature of the production of such cytotoxic compounds by plants and other organisms is how they themselves elude the toxicity of the compounds. With specific reference to CPT, Sirikantaramas *et al.*[12], showed that plants producing CPT possessed critical mutations at the catalytic and binding domain of their topoisomerase I, in contrast to plants that did not produce CPT. These mutations prevent CPT from binding to topoisomerase I. Similar mutations have also been reported in human cancer lines tolerant to CPT[13]. Thus it is evident that organisms producing cytotoxic compounds such as CPT may have evolved strategies to prevent self-toxicity[14].

In this study, we examine the sensitivity of endophytic fungi isolated from *N. nimmoniana* to CPT. A number of endophytes have been isolated from plants producing CPT[5,7–10,15]. Many of these have been demonstrated to produce CPT, in culture, independent of the host tissue[7–10,16]. However, little is known about the sensitivity of the isolated fungi to CPT. Reinscheid and Liu[17] examined the sensitivity of ten endophytic fungi isolated from *C. acuminata* to CPT. Two of the ten isolates showed almost no inhibition at 10 μg ml^{-1} CPT while being moderately inhibited at 100 μg ml^{-1}; the remaining eight isolates were completely inhibited. In a study involving pathogenic fungi[18], it was shown that CPT inhibited the growth of a number of fungal pathogens at concentrations ranging from 10 to 30 μg ml^{-1}; the fungi were completely inhibited at CPT concentrations between 75 and 125 μg ml^{-1}. In a more recent study, Kusari *et al.*[19] claimed that endophytic fungi isolated from *C. acuminata* were resistant to CPT; however the authors do not provide clear data to support this claim. Gurudatt *et al.*[5] isolated 26 endophytic fungi from *N. nimmoniana*, all of which produced CPT in culture. They showed that the fungal biomass was negatively related to the amount of CPT produced by the fungi and suggested that CPT produced by the fungus might be self-limiting its growth.

In this article, we show that endophytic fungi isolated from *N. nimmoniana* are inhibited by CPT produced by the host tissue. Furthermore, these fungi are also inhibited in culture, when supplemented with CPT. We discuss these results in the light of the role of CPT in the host plant as a possible deterrence to pathogens and pests.

Materials and methods

Isolation of endophytic fungi

Endophytic fungi were isolated from leaves of *N. nimmoniana* (Grahm) Mabb. (Icacinaceae) plants maintained at the School of Ecology and Conservation, University of Agricultural Sciences, GKVK, Bangalore, India. The leaves were first washed in running tap water and then cut into segments. The segments were surface-sterilized by consecutive immersion for 1 min in 70% ethanol, 1–2 min in 4% sodium hypochlorite and 30 sec in 70% ethanol. They were then rinsed thrice with sterile distilled water and dried with sterilized tissue paper. The leaf imprint assay was made to evaluate the effectiveness of surface sterilization[20]. The surface-sterilized segments were placed on plain agar media (HiMedia, Mumbai) and maintained at 28° ± 2°C. As and when the hyphae emerged and grew to cover approximately 2 cm^2, single hyphal tips were isolated and sub-cultured on potato dextrose agar (PDA; HiMedia, Bangalore), incubated at 28° ± 2°C and brought to pure culture by serial sub-culturing.

Identification of endophytic fungi using spore morphology and ITS rDNA sequencing

The identity of the purified fungi was established both by spore morphology[9] and ITS rDNA sequencing[21,22]. Fungal preparations were stained using lacto phenol cotton blue and observed under a light microscope (Olympus, USA). Photomicrographs of the spores were taken using Olympus microscope (1X 81) and pore characteristics were compared and assigned using appropriate keys[21,23] (Supplementary information, Figure S1, see online). Their identity was further confirmed by amplifying the internal transcribed regions using ITS1 and ITS4 primers[24]. Culturing of the fungus, extraction of DNA and amplification reactions were performed following Shweta *et al.*[9]. The full-length ITS sequences were submitted to the NCBI and BLAST analysis was performed; the fungi were identified by examining the closest match in the GenBank database. The sequences were matched with type strain sequences and/or the reference sequences obtained from NCBI (http://ncbi.nlm.nih.gov/), ITS2 database (http://its2-6old.bioapps.biozentrum.uni-wuerzburg.de), UNITE (http://unite.ut.ee/) and staininfo (http://www.staininfo.net). The sequence with the highest homology, maximum query coverage and maximum score was used as a reference to assign the identity of the endophytic fungus[25,26].

Extraction and determination of CPT in endophytic fungi

All the fungal isolates were cultured in liquid media following Amna *et al.*[6] and CPT extracted from the fungal mycelia as described by Shweta *et al.*[9]. The presence of CPT was determined using LC-ESI-MS (LCMS-2020, Shimadzu, Japan). All LC-MS analyses were done following Shweta *et al.*[11].

Chromatin condensation analysis of extract of endophytic fungi producing CPT

For chromatin condensation analysis, breast cancer cell line MCF-7 and colon cancer cell line HCT-116 (DTP, NCI, USA) maintained in RPMI medium supplemented with 10% foetal bovine serum were used. Chromatin condensation analysis was done by subjecting the cells to Hoechst 33342 staining. The cells were grown on 96-well plates after treating them with different concentrations of mycelial extracts (as indicated in Figure 2). Mycelial extract was prepared in ethanol. Cells were stained with Hoechst 33342 (0.5 μg ml^{-1} for 10 min) and observed under Nikon Epifluorescent microscope (TE2000E) fitted with UV filters. The number of cells with apoptotic-condensed nuclei was scored and expressed as percentage of cell mortality.

Sensitivity of endophytic fungi to CPT

Experiment 1: Effect of host and non-host tissue extract on fungal growth: In this experiment, we examined the effect of host and non-host tissue extract on the growth of the endophytic fungus, *Phomopsis* sp. (MTCC 10178). PDA was supplemented with leaf extract of the host plant (*N. nimmoniana*) or non-host plants, *Azadirachta indica* A. Juss (Meliaceae) and *Simarouba glauca* Linn. (Simaroubaceae). *A. indica* and *S. glauca* were chosen to serve as negative controls as these species do not produce CPT. Fifteen grams of fresh leaves was extracted in 61% ethanol. The filtrate thus obtained was scaled up to 100 ml by adding distilled water and this was used to prepare the PDA (15 ml/plate). The isolate (MTCC 10178) was inoculated on plates containing PDA prepared in various plant species extracts along with a PDA control plate. All treatments were maintained in quadruplets and the radial growth of the fungi was recorded using digital vernier calipers on alternative days. The significance of the results was tested using a two-way ANOVA.

Experiment 2: Effect of host tissue extract, with and without CPT fraction, on fungal growth: Bark tissue of *N. nimmoniana* was collected and ground to fine powder.

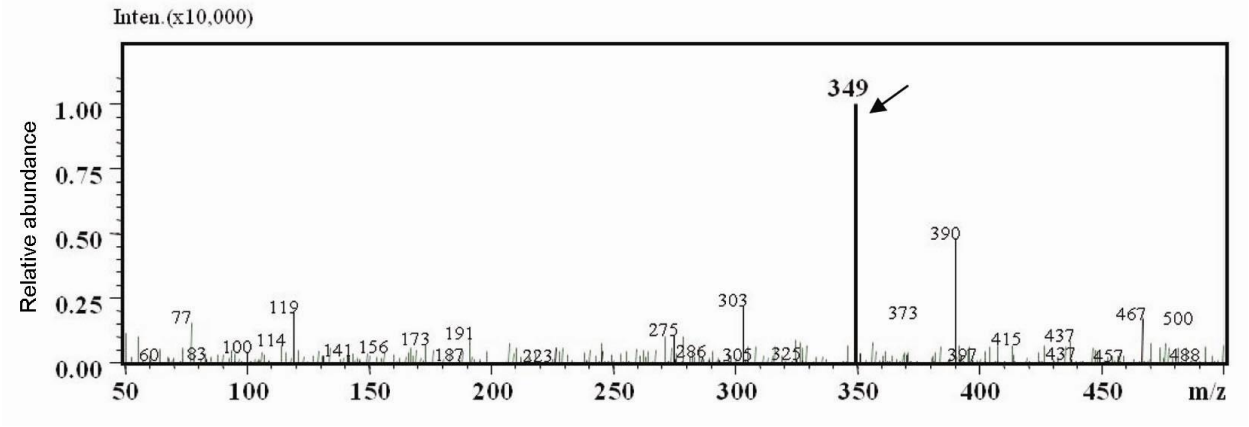

Figure 1. LC-MS spectra of endophytic fungus, *Phomopsis* sp. (MTCC 10178) isolated from *N. nimmoniana* showing the presence of CPT (*m*/*z* 349).

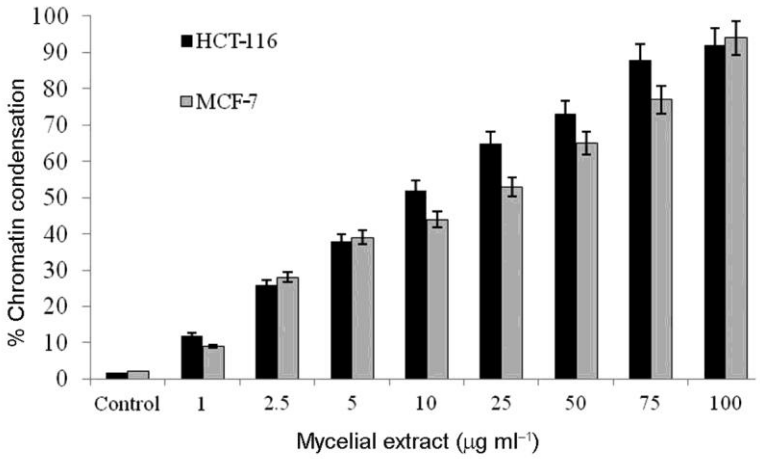

Figure 2. Percentage of chromatin condensation of breast cancer cell line (MCF-7) and colon cancer cell line (HCT-116) by extract of endophytic fungus, *Phomopsis* sp. Vertical bars indicate the standard deviation.

Five hundred grams of the bark tissue powder was subjected to solvent extraction using 61% ethanol. Fractionation of the crude extract was done using preparatory HPLC into CPT fractions and non-CPT fractions. The Prep HPLC separations were achieved using an RP-18 Merck (4.6 × 250 mm, 5 μm) column. The mobile phase consisted of a gradient of water and acetonitrile at a flow rate of 0.5 ml/min. The CPT fraction was eluted at a retention time of 20.1 min and the non-CPT fractions were eluted before and after elution of the CPT fraction. The total analysis run time was 50 min. These fractions were used to prepare PDA, with crude extracts containing CPT and without CPT respectively. The crude extracts were prepared by extracting 15 g of fresh tissue using 61% ethanol. The filtrate thus obtained was scaled up to 100 ml by adding distilled water and used to prepare the PDA (15 ml/plate). The isolate MTCC 10178 was inoculated on both these plates and with a PDA control. All treatments were maintained in quadruplets and the radial growth of the mycelia was measured using digital vernier calipers on alternative days. The significance of the results was tested using a two-way ANOVA.

Experiment 3: Effect of CPT on fungal growth: PDA amended with different concentrations of CPT (10, 25, 50, 75 and 100 μg ml^{-1}, Sigma-grade) was prepared and the fungus MTCC 10178 was inoculated. The different concentrations of CPT were prepared from a stock solution (1 mg ml^{-1} prepared in 61% ethanol). All treatments were maintained in quadruplets and the radial growth of mycelia was measured using digital vernier calipers on alternative days. The significance of the results was tested using a two-way ANOVA.

Results

Eleven endophytic fungi were isolated from *N. nimmoniana*. These endophytes were examined for their sensitivity to CPT. All the fungi were found to be inhibited by exogenously applied CPT (data not provided). Here we

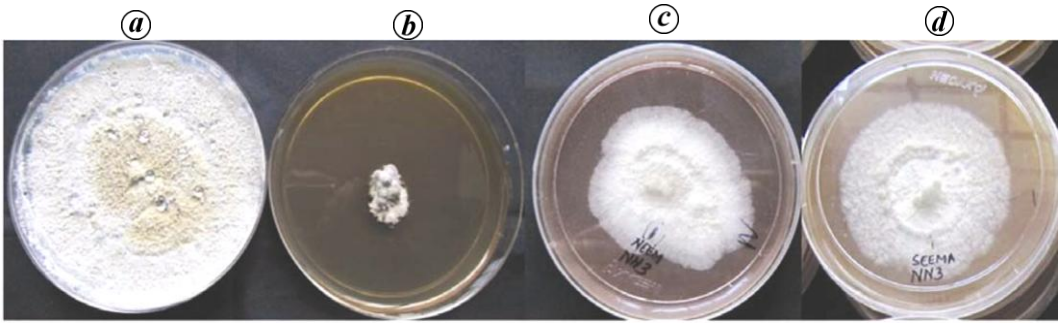

Figure 3. Inhibition of endophytic fungus, *Phomopsis* sp. by tissue extract of its host, *N. nimmoniana* and other non-hosts. ***a***, Control; ***b***, *N. nimmoniana* (host); ***c***, *A. indica* (non-host); ***d***, *S. glauca* (non-host).

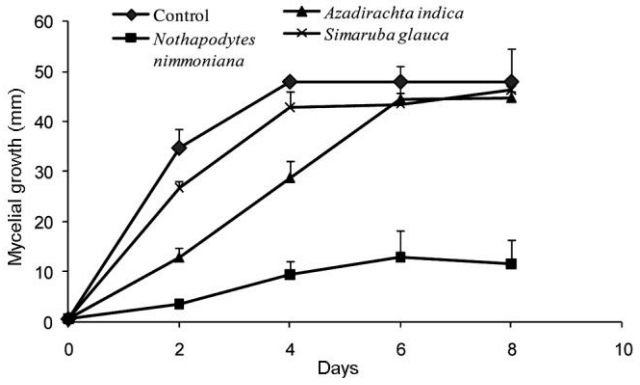

Figure 4. Inhibition of endophytic fungus *Phomopsis* sp. by tissue extract of its host *N.nimmoniana* and other non-host plants (*A. indica* and *S. glauca*). Values are mean of four replicates.

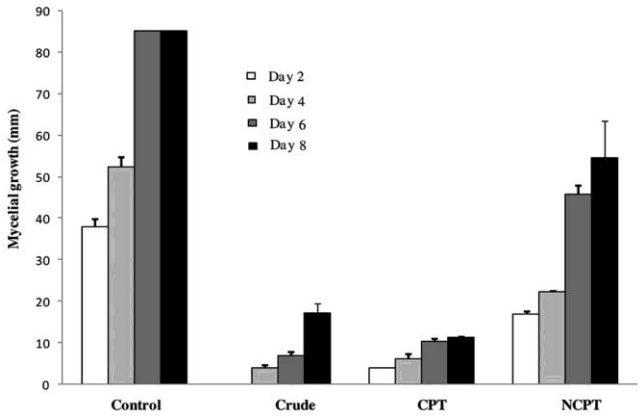

Figure 5. Inhibition of endophytic fungus, *Phomopsis* sp. by host tissue extract with and without endogenous camptothecine. Crude, Crude extract; CPT, Fraction containing endogenous camptothecine; NCPT, Fraction without endogenous camptothecine. Vertical bars indicate standard deviation.

present results in detail of only one endophyte, *Phomopsis* sp. (GenBank No JQ406613; MTCC 10178). The species *Phomopsis* was consistently obtained in all the tissues and comprised 10% of all the isolates obtained. The isolate produced CPT in culture as evident by LC-MS analysis (Figure 1). The CPT produced by the endophyte was structurally similar to that produced by the host (data not shown). Mycelial extract caused significant chromatin condensation of both MCF-7 and HCT-116 (Figure 2), indicating that the CPT produced by the endophytes was functionally similar to that exhibited by the host CPT as also Sigma-grade CPT.

Effect of host and non-host tissue extract on fungal growth

Growth of the isolate MTCC 10178 was distinctly inhibited in PDA plates supplemented with extract of *N. nimmoniana* leaf tissue (Figure 3) compared to plates without the extract. The growth was inhibited by more than 82.4% (as estimated through the radial growth in PDA with leaf extract compared to control plates without leaf extract).

Interestingly, the growth of the fungi was not inhibited in PDA plates supplemented with extracts from non-host plants such as *A. indica* and *S. glauca* (Figures 3 and 4). In the latter, the growth through days of incubation was similar to control PDA plates. On the other hand, there was a significant inhibition of fungal growth in PDA plates with host tissue extract ($P < 0.01$).

Effect of host tissue extract with and without CPT fraction on fungal growth

Host tissue extracts were separated into fractions with and without CPT. PDA made in fractions with CPT significantly inhibited mycelial growth compared to PDA containing tissue extract without the CPT fractions ($P < 0.01$; Figure 5). The inhibition of the former was comparable to that obtained from the unseparated crude tissue fractions. These results indicate that the host tissue extract-induced inhibition of fungal growth is predominantly due to CPT.

Effect of CPT on fungal growth

There was a clear CPT dose-dependent inhibition of fungal growth. Compared to mycelial growth in control PDA

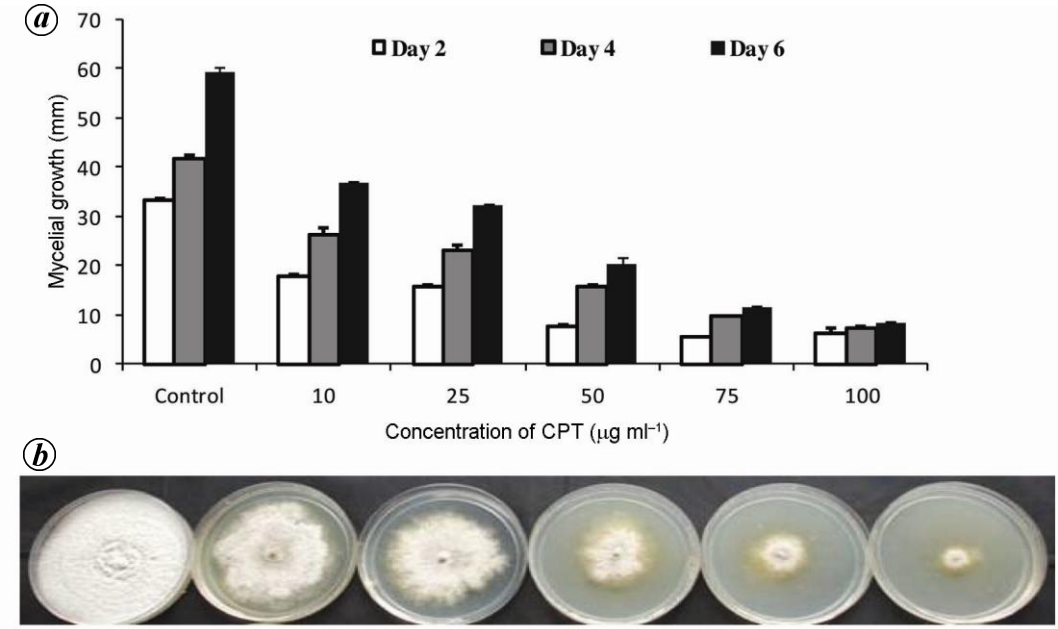

Figure 6. ***a***, Inhibition of endophytic fungus, *Phomopsis* sp. in PDA amended with different concentrations of CPT. Vertical bars indicate standard deviation. ***b***, Representative illustration of inhibition of *Phomopsis* sp. by different concentrations of CPT (as in the above graph).

on day 2, growth in 10 μg ml^{-1} CPT was significantly inhibited by 45% ($P < 0.01$; Figure 6).

Discussion

Endophytic fungi are an important group of plant symbionts that live asymptomatically within plant tissues. They have been shown to aid the performance of plants against abiotic and biotic stresses[27,28]. They have also been reported to produce a number of secondary metabolites, many of which closely mimic those produced by their respective host plants[8,29–31]. Stierle and co-workers[32] showed that an endophytic fungus, *Taxomyces andreanae* isolated from the yew plant, *Taxus brevifolia* produced paclitaxol, the multi-billion dollar anti-cancer compound, just as it is produced by the yew plant. Following this report, endophytic fungi producing plant secondary metabolites, including CPT, podophyllotoxin, vinblastine, hypercin, diosgenin, azadiracthin and rohitukine have been reported[8–10,29,30,33].

Many of these metabolites are cytotoxic, inhibiting key metabolic steps involved in cell division[31]. Obviously plants producing these cytotoxic compounds may have evolved strategies that circumvent the implied self-toxicity. For example, CPT-producing plants have been shown to possess critical mutations in their topoisomerase-I, which prevents them from being inhibited by CPT[12].

Little is however known about the fate of endophytic fungi and other organisms that are associated with these plants. The pathogenic fungus *Aspergillus fumigatus* that produced gliotoxin was shown to be itself resistant to the toxin[14]. More recently, Soliman *et al.*[34] showed that endophytic fungus producing taxol are themselves tolerant to it. Ramesha *et al.*[35] reported a chrysomelid beetle (*Kanarella unicolor* Jacobby) feeding on the leaves of *N. nimmoniana*, without any apparent adverse effect. They showed that over 60% of the ingested CPT is sequestered in metabolically inactive tissues such as wings and suggested that this could be one of the strategies of the beetles to reduce their cytotoxic burden. Kusari *et al.*[19] showed that endophytic fungi isolated from *C. accuminata* producing CPT may have certain intrinsic mechanisms to tolerate CPT, but the authors failed to show conclusive evidence of the resistance of the fungus to CPT. On the other hand, Reinscheid and Liu[17] showed that eight of the ten endophytic fungi isolated from *C. accuminata* were significantly inhibited by CPT. Li *et al.*[18] showed that CPT significantly inhibited fungal pathogens. Against this background, our study of a CPT-producing endophytic fungus MTCC 10178, showed that CPT did indeed inhibit the growth of the fungus. Aqueous host tissue extract was able to significantly subdue the growth of the endophytic fungus. Most of this inhibition was found to be due to the cytotoxicity of CPT. Host tissue extracts free from CPT were less inhibitory. These results are challenging to explain, especially in the context that the fungi were isolated from host tissues producing CPT and furthermore, the fungi themselves also produce CPT, independent of the host tissue.

While we have not elucidated the topoisomerase I sequences for these endophytic fungi, the fact that they are inhibited by CPT in a dose-dependent manner, indicates that they may not share the point mutations in their topoisomerase I, as reported for some CPT-producing plants[12]. These results appear consistent considering the fact that endophytic fungi are not necessarily symbiotic with their host[31,34] and thus may not be under selection to mutate their topoisomerase I. A large number of diverse endophytic fungi have been recovered from *N. nimmoniana;* many of these have been reported to produce CPT in culture. It is highly unlikely that selection would have favoured all of these fungi to evolve mutations to survive in a CPT environment. In summary, in the absence of a strict co-evolutionary interaction between the host and the endophytic fungi, it is conceivable that the latter may not have developed tolerance to host secondary metabolites. In this context, the production of secondary metabolites such as CPT by plants may be viewed as a host defence strategy aimed at reducing the pathogen load on the plant. By corollary, this could also lead to a subdued growth of the endophyte.

1. Hsiang, C. Y., Ho, T. Y., Lin, C. H., Wu, K. and Chang, T. J., Analysis of upregulated cellular genes in pseudorabies virus infection: use of mRNA differential display. *J. Virol. Methods*, 1996, **62**, 11–19.
2. Pommier, Y., Topoisomerase I inhibitors: camptothecines and beyond. *Nature Rev. Cancer.*, 2006, **6**, 789–802.
3. Uma Shaanker, R., Ramesha, B. T., Ravikanth, G., Gunaga, R., Vasudeva, R. and Ganeshaiah, K. N., Chemical profiling of *Nothapodytes nimmoniana* for camptothecin, an important anticancer alkaloid: toward the development of a sustainable production system. In *Bioactive Molecules and Medicinal Plants* (eds Ramawat, K. G. and Merillon, J. M.), Springer, UK, 2008, pp. 197–213.
4. Ramesha, B. T. *et al.*, New plant sources of the anti-cancer alkaloid, camptothecin from the Icacinaceae taxa, India. *Phytomedicine*, 2013, **20**, 521–527.
5. Gurudatt, P. S. *et al.*, Attenuation of camptothecin production and negative relation between hyphal biomass and camptothecin content in endophytic fungal strains isolated from *Nothapodytes nimmoniana* Grahm (Icacinaceae). *Curr. Sci*., 2010, **98**, 1006–1009.
6. Amna, T. *et al.*, Bioreactor studies on the endophytic fungus *Entrophospora infrequens* for the production of an anticancer alkaloid camptothecin. *Can. J. Microbiol*., 2006, **52**, 189–196.
7. Puri, S. C., Verma, V., Amna, T., Qazi, G. N. and Spiteller, M., An endophytic fungus from *Nothapodytes foetida* that produces camptothecin. *J. Nat. Prod*., 2005, **68**, 1717–1719.
8. Kusari, S., Zuhlke, S. and Spiteller, M., An endophytic fungus from *Camptotheca acuminata* that produces camptothecin and analogues. *J. Nat. Prod*., 2009, **72**, 2–7.
9. Shweta, S. *et al.*, Endophytic fungal strains of *Fusarium solani*, from *Apodytes dimidiata* E. Mey.exArn (Icacinaceae) produce camptothecin, 10-hydroxycamptothecin and 9-methoxycamptothecin. *Phytochemistry*, 2010, **71**, 117–122.
10. Shweta, S., Gurumurthy, B. R., Ravikanth, G., Uma Shaanker, R. and Shivanna, M. B., Endophytic fungi from *Miquelia dentata* Bedd., produce the anti-cancer alkaloid, Camptothecine. *Phytomedicine*, 2013, **20**, 337–342.
11. Shweta, S. *et al*., Isolation of endophytic bacteria producing the anti-cancer alkaloid camptothecine from *Miquelia dentata* Bedd., (Icacinaceae). *Phytomedicine*, 2013, **20**, 913–917.
12. Sirikantaramas, S., Yamazaki, M. and Saito, K., Mutations in topoisomerase I as a self-resistance mechanism coevolved with the production of the anticancer alkaloid camptothecin in plants. *Proc. Natl. Acad. Sci. USA*, 2008, **105**, 6782–6786.
13. Yoshikazu, S., Satomi, T., Tomoko, O., Leroy, F. L. and Takashi, T., Elevated expression of DNA topoisomerase II in camptothecin-resistant human tumor cell lines. *Cancer Res*., 1990, **50**, 5919–5924.
14. Schrettl, M. *et al*., Self-Protection against Gliotoxin – a component of the gliotoxin biosynthetic cluster, GliT, completely protects *Aspergillus fumigatus* against exogenous gliotoxin. *PLoS Pathog*., 2010, **6**(6), e1000952; doi:10.1371/journal.ppat.1000952.
15. Min, C. and Wang, X., Isolation and identification of the 10-hydroxycamptothecin-producing endophytic fungi from *Camptotheca acuminata* Decne. *Acta Bot. Boreali–Occidential Sin*., 2009, **29**, 0614–0617.
16. Rehman, S. *et al.*, An endophytic *Neurospora* sp. from *Nothapodytes foetida* producing camptothecin. *Appl. Biochem. Microbiol*., 2008, **44**, 203–209.
17. Reinscheid, U. M. and Liu, W., Camptothecin-resistant fungal endophytes of *Camptotheca acuminata. Mycol. Prog*., 2004, **3**, 189–192.
18. Li, S., Zhang, Z., Cain, A., Wang, B., Long, M. and Taylor, J., Antifungal activity of camptothecin, trifolin, and hyperoside isolated from *Camptotheca acuminata*. *J. Agric. Food Chem*., 2005, **53**(1), 32–37; DOI:10.1021/jf0484780.
19. Kusari, S., Sebastian, Z. and Spiteller, M., Effect of artificial reconstitution of the interaction between the plant *Camptotheca acuminata* and the fungal endophyte *Fusarium solani* on camptothecin biosynthesis. *J. Nat. Prod*., 2011, **74**, 764–775.
20. Schulz, B. and Boyle, C., The endophytic continuum. *Mycol. Res*., 2005, **109**, 661–686.
21. Sutton, B. C., The Coelomycetes. Fungi imperfecti with pycnidia, acervuli and stromata. Commonwealth Mycological Institute, Kew, Surrey, England, 1980, p. 696.
22. Arx, V. J. A., The genera of fungi sporulating in pure culture. A. R. Gartner Verlag Kommanditgesellschaft, FL-9490 Vaduz Germany, 1981, p. 424.
23. Simmons, E. G. and Roberts, R. G., *Alternaria* themes and variations [Sporulation patterns]. *Mycotaxon*, 1993, **48**, 109–140.
24. White, T. J., Bruns, T., Lee, S. and Taylor, J. W., Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: A Guide to Methods and Applications* (eds Innis, M. A. *et al.*), Academic Press, San Diego, 1990, pp. 315–322.
25. Higgins, K. L., Arnold, A. E., Miadlikowska, J., Sarvate, S. D. and Lutzoni, F., Phylogenetic relationships, host affinity, and geographic structure of boreal and arctic endophytes from three major plant lineages. *Mol. Phylogenet. Evol*., 2007, **42**, 543–555.
26. Marquez, S. S., Bills, G. F. and Zabalgogeazcoa, I., The endophytic mycobiota of the grass *Dactylisglomerata*. *Fungal. Divers.*, 2007, **27**, 171–195.
27. Azevedo, J. L., Maccheroni Jr, W., Pereira, J. O. and Araújo, W. L., Endophytic microorganisms: a review on insect control and recent advances in tropical plants. *Electron. J. Biotechnol*., 2000, **3**, 40–65.
28. Rahman, M. H. and Saiga, S., Endophytic fungi (*Neotyphodium coenophialum*) affect the growth and mineral uptake, transport and efficiency ratios in tall fescue (*Festuca arundinacea*). *Plant Soil*, 2005, **272**, 163–171.
29. Zhang, B. *et al.*, Discovery of small molecule insulin mimetic with anti-diabetic activity in mice. *Science*, 1999, **284**, 974–981.
30. MohanaKumara, P. *et al.*, *Fusarium proliferatum*, an endophytic fungus from *Dysoxylum binectariferum* Hook. f, produces

rohitukine, a chromane alkaloid possessing anti-cancer activity *Antonie van Leeuwenhoek*, 2012, **101**(2), 323–329; DOI: 10.1007/s10482-011-9638-2.

31. MohanaKumara, P. *et al.*, Endophytes and plant secondary metabolite synthesis: molecular and evolutionary perspectives. In *Advances in Endophytic Research* (eds Verma, V. and Gange, A. C.), Springer, India, 2013, pp. 177–190; DOI: 10.1007/978-81-322-1575-2_9; http://www.springer.com/life+sciences/microbiology/book/978-81-322-1574-5.
32. Stierle, A., Strobel, G. A. and Stierle, D., Taxol and taxane production by *Taxomyces andreanae*. *Science*, 1993, **260**, 214–216.
33. MohanaKumara, P., Soujanya, K. N., Ravikanth, G., Vasudeva, R., Ganeshaiah, K. N. and Uma Shaanker, R., Production of the chromane alkaloid, rohitukine and its attenuation in endophytic fungi isolated from *Dysoxylum binectariferum* Hook.f and *Amoora rohituka* (Roxb). Wight & Arn. *Phytomedicine*, 2013; http://dx.doi.org/10.1016/j.phymed.2013.09.019.
34. Soliman, S. S. M., Trobacher, P. C., Tsao, R., Greenwood, S. J. and Raizada, M. N., A fungal endophyte induces transcription of genes encoding a redundant fungicide pathway in its host plant. *BMC Plant Biol.*, 2013, **13**, 93.
35. Ramesha, B. T. *et al.*, Sequestration of camptothecin, an anticancer alkaloid, by chrysomelid beetles. *J. Chem. Ecol.*, 2011, **37**(5), 533–536.

ACKNOWLEDGEMENTS. This work was supported by grants from the Department of Biotechnology (No. BT/PR/8825/NDB/52/53/2007), Government of India to R.U.S. The permission given by the Karnataka Forest Department to visit and collect samples is gratefully acknowledged. U. Senthil Kumar assisted in collecting the plant material.

Received 18 March 2014; revised accepted 2 July 2014