Enhancement of vincristine under in vitro culture of Catharanthus roseus supplemented with Alternaria sesami endophytic fungal extract as a biotic elicitor

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Abstract
Vincristine, one of the major vinca alkaloid of Catharanthus roseus (L.) G. Don. (Apocynaceae), was enhanced under in vitro callus culture of C. roseus using fungal extract of an endophyte Alternaria sesami isolated from the surface-sterilized root cuttings of C. roseus. Vindoline, a precursor molecule for vincristine production, was detected for the first time in the fungal endophyte A. sesami which was used as a biotic elicitor in this study to enhance vincristine content in the C. roseus callus. It was identified using high-performance liquid chromatography and mass spectroscopy techniques by matching retention time and mass data with reference molecule. Supplementing the heat sterilized A. sesami endophytic fungal culture extract into the callus culture medium of C. roseus resulted in the enhancement of vincristine content in C. roseus callus by 21.717% after 105-day culture.

Keywords Vindoline · Vincristine · Catharanthus roseus · Alternaria sesami · Endophyte

Introduction
Catharanthus roseus (L.) G. Don. (Apocynaceae), commonly known as Madagascar periwinkle or rosy periwinkle, has been a centre of attraction for the vinca alkaloids. Dimeric vinca alkaloids (vinblastine and vincristine) are anticancer drugs that have been originally extracted from the plant Vinca rosea. These indole alkaloids are highly potent anticancer drugs and find application in treating various types of cancers. The biological property of these anticancer drugs depends on their affinity to bind with tubulin protein which counters the microtubule formation and mitotic spindle dynamics thus inhibiting the mitosis process. Vinca alkaloids may also act as angiogenesis inhibitor thereby disrupting the intracellular transport of essential products and blood flow to the tumour (Bates and Eastman 2017). Vindoline, a monomeric vinca alkaloid constitutes the lower half of dimeric vinca alkaloid vinblastine. Vindoline along with catharanthine serves as a natural precursor for the biosynthesis of vincristine and vinblastine. Vindoline also acts as a synthetic precursor for semi-synthetic analogue of vinca alkaloids such as vindesine (Eldesine®), vinorelbine (Navelbine®) and vinflunine (Javor®). These semi-synthetic molecules exhibit superior efficacy in the treatment of breast, bladder and lung cancer (Brown et al. 2008).
have been carried out using in vitro culture methods like callus culture, cell suspension and hairy root (Goldhaber- Pasillas et al. 2014). Although semi-synthetic analogue of *vinca* alkaloids (vindesine, vinorelbine and vinflunine) are superior to natural *vinca* alkaloids (vinblastine and vincristine), their clinical supply depends on the availability of their precursor molecules, i.e. monomeric natural *vinca* alkaloids (vindoline and catharanthine) (Zhu et al. 2014; Lee et al. 2015). Therefore, there is a need to explore other sources (non-plant) for an uninterrupted supply of precursor molecules (monomeric *vinca* alkaloids such as vindoline) and enhancement of natural vinca alkaloids in the plant.

Fungal endophytes are well-known resources of functional molecules and have been reported to produce a plethora of bioactive compounds both inside and outside of their host plant. Endophytic fungi are currently explored as an alternative source for plant bioactive compounds. There are pieces of evidence of a variety of bioactive compounds producing endophytes isolated from various medicinal plants such as taxol producing *Fusarium redolens* and *Cladosporium* species from *Taxus baccata*, podophyllotoxin producing *Chaetomium globosum* from *Sinopodophyllum hexandrum* (Uzma et al. 2018). Some endophytic fungi are reported to produce deoxypodophyllotoxin, camptothecin, hypercin, silybin A and B, etc. (Pandey et al. 2016; Rai et al. 2021).

In the year 1998, Guo et al. first reported *Alternaria* sp. (a fungal endophyte) producing vinblastine obtained from *C. roseus* (Guo et al. 1998). In the year 2004, Xianzhi et al. found an unidentified endophytic fungus from the leaves of *C. roseus* capable of producing vincristine (Xianzhi et al. 2004). Numerous such studies depict those endophytic fungi inhabiting *C. roseus* could also be a potential source for the production of vindoline (the precursor molecules for the biosynthesis of dimeric *vinca* alkaloids).

In this work, we have reported for the first time the production of a monomeric natural *vinca* alkaloid, vindoline, in a fungal endophyte isolated from *Catharanthus roseus* and enhancement of vincristine content in *C. roseus* callus by treating it with sterilized cellular extract of vindoline producing endophytic fungus. The fungus was identified as *Alternaria sesami* (*Pleosporaceae*) using genomic techniques.

**Materials and methods**

**Isolation, maintenance and fermentation of the fungal endophyte** A young and healthy plant material of *Catharanthus roseus* (L.) G. Don (EC 120,837) was obtained from the herbal garden of Jamia Hamdard University, New Delhi, India. The leaves, stem and root of the plant were cut into small pieces (approximately 2-mm size) with the help of a surgical blade and washed in reverse osmosis water using cetrimide. Afterwards, the plant segments were surface sterilized (using the optimised method) by adding plant material in 70% ethanol for 1 min, rinsed twice with autoclaved water, and then, the plant material was added to 0.1% mercuric chloride for 90 s, finally washed thrice with sterile water. The effectiveness of surface sterilization was determined by observing no bacterial growth in the rinsed water on potato dextrose agar (PDA) media after 7 days of incubation at 28 °C. The segments were transferred onto Petri plates containing PDA media (HiMedia, Mumbai, India) supplemented with an antibiotic mixture of streptomycin sulphate 250 mg/l and penicillin G 250 mg/l in order to grow endophytic fungus without any contamination. Plates were sealed with parafilm and incubated at 28 °C until the fungal colonies started oozing out of the explants. All of the isolated endophytic fungal cultures were maintained in PDA slant and sub-cultured in every 30-day interval. Isolated endophytes were cultured in a liquid medium (20 g dextrose, 1 g yeast extract, 3 g potassium dihydrogen sulphate, 1.5 g magnesium sulphate.7H2O, pH 6.0) in 250-ml Erlenmeyer flasks at 28 °C, 110 rpm in a shaker incubator. After 6 days, the culture (fungal biomass) was separated and analysed for monomeric/dimeric *vinca* alkaloids by qualitative analysis using high-performance liquid chromatography (HPLC) and mass spectrometry separately.

**Extraction and analysis of vinca alkaloids** Various endophytes isolated from the explants were analysed for the presence of the secondary metabolites through qualitative analysis mode using the high-performance liquid chromatography (HPLC) technique. The fungal biomass was obtained by the filtration process using Whatman No 1 filter paper and dried at 50 °C for 4 h in a hot air oven. To the dried fungal biomass, methanol was added (25 ml per 5 g of dried biomass) and sonicated (pulse, 30 kHz; gap, 10 s; amplitude, 100%) for 10 min using a probe sonicator (VCX 130, Vibra Cell, Sonics, USA). After the sonication process, the supernatant was collected by centrifugation (1537 g for 5 min), evaporated to dryness in a vacuum evaporator at 60 °C. For HPLC–UV analysis, the dried extract was dissolved in an equal volume of methanol and filtered through a 0.45-μm membrane. Elution was carried out by the mobile phase consisting of methanol and water with 0.1% triethylamine at a flow rate of 1 ml/min under gradient mode (55% v/v methanol at 0 min, 65% v/v methanol at 5 min, 70% v/v methanol at 15 min, 80% v/v methanol at 18 min, 90% v/v methanol at 35 min, 55% v/v methanol at 40 min) (Kumar et al. 2013; Zhou et al. 2005). Detection was carried at 260 nm. The molecular weight of compounds was confirmed by the electrospray-mass spectrometry (ESI–MS) method in positive ion mode by using instrument XevoTQDS, Waters Pvt. Ltd. equipped with MassLynx4.1 software. The used MS
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parameter was source temperature 110 °C, capillary voltage 3.5 kV, cone voltage 45 V, desolvation temperature 350 °C, desolvation gas flow 800 l/h and cone gas 50 l/h.

Identification of the endophytic fungal strain The vindoline producing endophytic fungus was identified and characterized by using molecular biology techniques as per the manufacturer protocol (Bhat Biotech, Bangalore, India). Fungal genomic DNA was extracted from fungus using a genomic DNA extraction kit from a fresh culture of MPBL 105 (desired endophytic fungus). Amplification of the nuclear internal transcribed spacers (ITS) region was performed using the universal primers ITS1 (5′-TCCGTAAGTG GAACCTGCGG-3′) and ITS4 (5′-TCCTCGCTATTGAT TATGC-3′). The amplification was carried out in a Master cycler® Thermocycler (Eppendorf, Germany) using the following program. Polymerase chain reaction (PCR) was performed with initial denaturation of 94 °C for 2 min followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 55.5 °C for 1 min and extension at 72 °C for 30 s. The final extension was carried out at 72 °C for 10 min. Amplified PCR products were separated in 1% agarose gel containing ethidium bromide in 0.5 μg/ml concentration (for detection of bands under UV) in Tris–Borate-EDTA (TBE) buffer. The purified PCR product was used for sequencing. Sequencing was carried by an automated DNA sequence machine – 3037xl DNA analyzer from Applied Biosystems using BigDye® Terminator v3.1 cycle sequencing kit (Applied Biosystems). Sequence data were aligned and dendrograms were generated using sequence analysis software version 5.2 from Applied Biosystems. Sequences were compared to the non-redundant NCBI database by using BLASTN, with the default settings used to find the most similar sequence and were sorted by the E score. A representative sequence of the 10 most similar neighbours was aligned using CLUSTAL W2 for multiple alignments with the default settings. The multiple-alignment file was then used to create a phylogram using Molecular Evolutionary Genetic Analysis (MEGA 5) software. On the basis of the

Fig. 1 Isolated endophytic fungus Alternaria sesami in PDA Petri plate

Fig. 2 HPLC graph for vindoline in fungal extract (RT = 18.392)
results of the BLAST search, the isolated endophytic fungal strain MPBL 105 was identified as *Alternaria sesami* (*Pleosporaceae*).

**Establishment of C. roseus callus culture** Healthy and immature leaves of *Catharanthus roseus* (L.) G. Don. were collected from the herbal garden, Jamia Hamdard University, New Delhi, and used as explants for callus culture. The explants were washed in running reverse osmosis water for 10 min using cetrimide and were subjected to surface sterilization using 70% ethanol and 0.1% mercuric chloride. For *C. roseus* callus culture MS (Murashige and Skoog) media was used as basal media with 3% sugar, 1.1% agar, 0.01% myoinositol and a combination of different growth hormones (naphthalene acetic acid (NAA), indole acetic acid (IAA), kinetin, 2,4-dichlorophenoxyacetic acid (2,4-D), 6-benzyladenine (6-BA)) (Murashige and Skoog 1962). The incubation temperature was 25 °C and light intensity of 1600 lx with 16-h photoperiods per day. The callus was sub-cultured after every 21 days and grown and maintained for 90 days. After 90 days, *C. roseus* callus was treated with methanolic (dried and aqueous suspension) or aqueous extract of fungal endophyte (*A. sesami*) at a concentration of 25 μl/g of media, 50 μl/g of media and 100 μl/g of media and grown for another 15 days under same culture condition. After a total of 105 days of growth, each callus was collected and fresh weight was noted. Furthermore, the callus was dried at 60 °C. The drying was carried out up to the constant weight of the callus. To the dried callus, methanol was added (5% v/w) and sonicated (pulse, 30 kHz; gap, 10 s; amplitude, 100%) for 15 min using a probe sonicator (VCX 130, Vibra Cell, Sonics, USA). After the sonication, biomass was centrifuged for 5 min at 1537 g and the supernatant was collected and evaporated to dryness in a vacuum evaporator at 60 °C. The dried extract was dissolved in an equal volume of methanol and filtered through a 0.45-μm membrane and analysed by HPLC–UV using the same procedures as described in the above section.
Isolation, identification and characterization of isolated endophytic fungus

From a variety of endophytic fungi isolated from *C. roseus*, only one fungus (Fig. 1) was showing a positive result for the presence of vindoline in the fungal extract. The presence of vindoline in the fungal extract was evident by the HPLC and mass spectrometry data. RT recorded for HPLC peak of vindoline standard was recorded to be 18.465 min (Supp Fig S4) and that of vindoline in the fungal extract was recorded to be 18.392 min at 260 nm (Fig. 2). Regression via area for vindoline was found to be 0.997 (Supp. Fig S5), and for vincristine, it was 0.983 (Supp. Fig S6). Mass spectrum of vindoline in fungal extract detected the molecular weight of vindoline to be 457.2969 m/z under +ve scan mode (Fig. 3) which matched the mass spectrum of vindoline standard which was 456.5 g/mol in the PubChem database. Hence, the mass spectrum and HPLC results both confirm the presence of vindoline in the fungal extract.

The band for PCR product was recorded to be around 600 bp (Fig. 4). The fungal strain was confirmed by

#### Table 1

| S. no | Total cell biomass of fungus (g) | Concentration of vindoline produced by total cell biomass (μg) | Concentration of vindoline produced per gram of cell biomass (μg/g) |
|-------|-------------------------------|---------------------------------------------------------------|---------------------------------------------------------------|
| 1     | 31.482                        | 46.1557 μg                                                   | 1.47 μg/g                                                     |

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**Results**

**Isolation, identification and characterization of isolated endophytic fungus** From a variety of endophytic fungi isolated from *C. roseus*, only one fungus (Fig. 1) was showing a positive result for the presence of vindoline in the fungal extract. The presence of vindoline in the fungal extract was evident by the HPLC and mass spectrometry data. RT recorded for HPLC peak of vindoline standard was recorded to be 18.465 min (Supp Fig S4) and that of vindoline in the fungal extract was recorded to be 18.392 min at 260 nm (Fig. 2). Regression via area for vindoline was found to be 0.997 (Supp Fig S5), and for vincristine, it was 0.983 (Supp. Fig S6). Mass spectrum of vindoline in fungal extract detected the molecular weight of vindoline to be 457.2969 m/z under +ve scan mode (Fig. 3) which matched the mass spectrum of vindoline standard which was 456.5 g/mol in the PubChem database. Hence, the mass spectrum and HPLC results both confirm the presence of vindoline in the fungal extract.

The band for PCR product was recorded to be around 600 bp (Fig. 4). The fungal strain was confirmed by
sequence analysis (Supp Data S1) and the formation of the phylogenetic tree (Fig. 5). The evolutionary history inferred using the neighbour-joining method shows the optimal tree with the sum of branch length $= 0.0040000$ (next to the branches). The evolutionary distances were computed using the $p$-distance method and are in the units of the number of base differences per site. The analysis involved 11 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 500 positions in the final dataset. Sequence analysis of the ITS region of the rRNA gene showed that the sample MPBL 103 is *Alternaria sesami*.

The quantitative analysis of fungal extracts showed that the isolated fungus *Alternaria sesami* produces vindoline in the concentration of 1.47 μg/g of cell biomass but it is not found to be producing vincristine (Table 1).

**Callus culture and hormone treatment** Callus initiation was optimized using different hormone combinations.

### Table 2: Treatment of the callus of the 7th hormone combination (2, 4-D + IAA + 6-BA)

| S. no | Type of callus | Initial fresh weight (g) | Fresh weight after 20 days (g) | Initial colour | Colour after 20 days | Concentration of vindoline (μg/mg of dry callus) | Concentration of vincristine (μg/mg of dry callus) |
|-------|----------------|--------------------------|-------------------------------|----------------|----------------------|-----------------------------------------------|--------------------------------------------------|
| 1     | Untreated      | 0.356                    | 2.00                          | Yellowish white | Yellowish white      | 0.7894                                       | 5.765                                           |
|       | Treated with fungal water extract |                 |                               |                |                      |                                               |                                                  |
| 2a    | 25 μl/g        | 0.367                    | 2.39                          | Yellowish white | Dark yellow          | 1.559                                        | -                                               |
| 2b    | 50 μl/g        | 0.400                    | 2.17                          | Yellowish white | Dark yellow          | 2.495                                        | -                                               |
| 2c    | 100 μl/g       | 0.346                    | 2.228                         | Yellowish white | Dark yellow          | 4.675                                        | -                                               |

| Treated with fungal methanolic extract |                                           |                          |                      |                |                        |                                              |                                                 |
| 3a    | 25 μl/g        | 0.398                    | 2.188                        | Yellowish white     | Dark yellow      | 7.867                                        | -                                               |
| 3b    | 50 μl/g        | 0.347                    | 0.532                        | Yellowish white     | Dark yellow      | 2.610                                        | -                                               |
| 3c    | 100 μl/g       | 0.410                    | 0.733                        | Yellowish white     | Dark yellow      | 1.739                                        | -                                               |

### Table 3: Treatment of the callus of the 10th hormone combination (2.4-D + IAA + Kinetin + 6-BA)

| S. no | Type of callus | Initial fresh weight (g) | Fresh weight after 20 days (g) | Initial colour | Colour after 20 days | Concentration of vindoline (μg/mg of dry callus) | Concentration of vincristine (μg/mg of dry callus) |
|-------|----------------|--------------------------|-------------------------------|----------------|----------------------|-----------------------------------------------|--------------------------------------------------|
| 1     | Untreated      | 0.350                    | 1.209                         | Yellowish white | Yellowish white      | 4.43                                         | -                                               |
|       | Treated with fungal water extract |                 |                               |                |                      |                                               |                                                  |
| 2a    | 25 μl/g        | 0.347                    | 4.180                         | Yellowish white | Dark yellow          | 2.558                                        | 0.444                                           |
| 2b    | 50 μl/g        | 0.346                    | 2.333                         | Yellowish white | Dark yellow          | 0.60                                         | -                                               |
| 2c    | 100 μl/g       | 0.356                    | 0.967                         | Yellowish white | Dark yellow          | 1.38                                         | -                                               |

| Treated with fungal methanolic extract |                                           |                          |                      |                |                        |                                              |                                                 |
| 3a    | 25 μl/g        | 2.188                    | 1.100                        | Yellowish white     | Dark yellow      | 4.77                                         | -                                               |
| 3b    | 50 μl/g        | 0.532                    | 0.830                        | Yellowish white     | Dark yellow      | 3.28                                         | 21.717                                          |
| 3c    | 100 μl/g       | 0.733                    | 0.500                         | Yellowish white     | Dark yellow      | 0.699                                        | 9.209                                           |
Best hormone combinations and their concentration for callus growth were observed (Supp Table S1 & S2; Supp Fig S1 & S2). With the 7th hormone combination (2,4-D + IAA + 6-BA) (Supp Fig. 1C) and the 10th hormone combination (2,4-D + IAA + Kinetin + 6-BA) (Supp Fig S1E), good callus growth was observed after 15 days. Therefore, these hormone combinations were used for the optimum growth initiation and sub-culturing. A healthy callus was observed using the 7th hormone combination (Fig. 6a) and the 10th hormone combination (Fig. 6b). For callus development and good growth in the 7th hormone combination (Fig. 7a) and the 10th hormone combination (Fig. 7b), 1 ppm (each) was considered as suitable hormone concentration. The growth pattern and production of vindoline and vincristine were observed in untreated callus, callus treated with fungal water extract and callus treated with fungal methanol extract with the 7th hormone combinations (Table 2) and the 10th hormone combinations (Table 3).

After 90 days of callus growth in the 7th hormone combination (2, 4-D+IAA+6-BA) (1 ppm each) and the 10th hormone combination (2, 4-D+IAA+Kinetin+6-BA) (1 ppm each), a good growth pattern was observed (Supp Table S3; Supp Fig S3A & S3B).

In the 7th hormone combination, the growth was more in the callus treated with water extract than in the untreated callus. However, the growth was less in methanol extract treated callus. The change in colour from yellowish white to dark yellow was observed in all treated callus while no change in colour was observed in untreated callus. The concentration of vindoline was lowest in the untreated callus which was found to be 0.7894 μg/mg of dry callus and highest in callus treated with 25 μl/g of methanol extract which was 7.867 μg/mg of dry callus; however, vincristine was found present in a concentration of 5.765 μg/mg of dry callus in untreated callus but absent in all the treated callus.

In the 10th hormone combination, the growth of callus was more in the callus treated with water extract than in the untreated callus. However, the growth was less in methanol extract treated callus. The change in colour was the same in all treated callus, i.e. from yellowish white to dark yellow while no change in colour was observed in untreated callus. The concentration of vindoline was found to be lowest in the water extract-treated callus (50 μl/g) which was noted to be 0.60 μg/mg of dry callus. The concentration of vindoline was highest in methanol extract treated callus (25 μl/g) which was noted to be 4.77 μg/mg of dry callus. The concentration of vindoline decreases as the concentration of fungal methanol extract increases. Vincristine was produced in the treated callus and the highest conversion from vindoline to vincristine, i.e. 3.28 μg/mg of dry callus to 21.717 μg/mg of dry callus, took place in the callus treated with 50 μl/g methanol extract. The presence of vincristine in the treated callus was confirmed through ESI–MS analysis and the data was matched with the reference molecule (Figs. 8 and 9) (Yang et al. 2013; Zhou et al. 2005).

The HPLC data for the callus cultures containing fungal water extract and fungal methanol extract showed the presence of vindoline and vincristine (Table 4; Fig. 8a, b, c and d). The standard plot data for vindoline and vincristine are presented in supplementary Table S4 & S5.
Discussion

Vincristine and vinblastine are of great importance in cancer treatment and vindoline is a precursor molecule for their synthesis. Through various biosynthetic pathways and enzymes, the coupling of monomeric alkaloids catharanthine and vindoline helps in the production of these dimeric alkaloids. The product of the coupling reaction is 3',4'-anhydrovinblastine which gets converted into vinblastine and further converts into vincristine (Supp Fig S7). These processes may speed up if there is an increased supply of monomeric precursor molecules like vindoline and catharanthine in the biosynthetic pathway. Ellicitor–receptor interaction is an important step in the mechanism of elicitation to trigger a rapid array of biochemical responses in plants (El-Sayed and Verpoorte 2007).

The endophytes may benefit either by producing host secondary metabolites or by augmenting plant defences against pathogens or pest invasions to effectively prevent the other invading pathogens. This can happen only if the endophyte is resistant to the metabolite and the metabolite is transported across the mycelial cell membrane and cell wall into the intercellular spaces of plant tissue (Naik et al. 2019).

In the biosynthetic pathway of terpenoid indole alkaloids inside the plant C. roseus, various enzymes including deacetylvinodoline 4-O-acetyltransferase, strictosidine synthase, tabersonine 16-hydroxylase, S-adenosyl-l-methionine, 16-hydroxytabersonine-O-methyltransferase, 2,3-dihydro-3-hydroxytabersonine-N-methyltransferase, deacetoxyvindoline 4-hydroxylase, etc. are responsible for the production of the final product vincristine. When C. roseus is subjected to tissue culture, some of these enzymes are not expressed in the callus which ultimately

Table 4  HPLC data for simultaneous analysis of vindoline and vincristine in untreated and treated callus

| S. no | Type of callus          | Hormone combination | Concentration of fungal extract | Compound detected | RT (minutes) at 260 nm | Figure   |
|-------|-------------------------|---------------------|---------------------------------|-------------------|------------------------|----------|
| 1     | Untreated               | 7th                 | –                               | Vindoline         | 15.547                 | Figure 8a |
| 2     | Untreated               | 7th                 | –                               | Vincristine       | 20.414                 | Figure 8a |
| 3     | Treated with fungal methanol extract | 7th             | 25 μl/g                         | Vindoline         | 15.173                 | Figure 8b |
| 4     | Treated with fungal methanol extract | 10th            | 25 μl/g                         | Vindoline         | 15.173                 | Figure 8c |
| 5     | Treated with fungal methanol extract | 10th            | 50 μl/g                         | Vindoline         | 15.436                 | Figure 8d |
| 6     | Treated with fungal methanol extract | 10th            | 50 μl/g                         | Vincristine       | 19.123                 | Figure 8d |
leads to a deficiency of vindoline in the callus (St-Pierre et al. 1999). Its deficiency leads to limited or no production of the dimeric compound vincristine in such cases. Vindoline being one of the essential monomer units for the production of vincristine is required to be added in the medium. Therefore, supplementing the medium with natural vindoline would help boosting the production of vincristine to many folds. The deficiency of vindoline in callus culture indicates further exploration of the abovementioned enzymes in C. roseus and also for obtaining such enzymes through synthetic production.

Hormones are a necessity for the induction of growth in any callus. MS medium supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (6-BA) might give 100% callus induction (Liu et al. 2018). The presence of kinetin in the 10th hormone combination in the media might have helped in the conversion process of vindoline to vincristine in the callus with the help of vindoline present in fungal methanol extract (Almagro et al. 2015).

Alternaria specie is found as endophyte in various medicinal plants such as Catharanthus roseus, Rauvolfia serpentina, Ziziphus jujube and Salvadora persica (Elgorban et al. 2019; Ghosh et al. 2018; Orfali et al. 2017; Sreekanth et al. 2017). However, it produces a variety of host-specific products such as antimicrobial, anticancer and antioxidant compounds which are beneficial for the medical industry.

Stress conditions may induce the production of secondary metabolite synthesis in plants. Abiotic and biotic elicitors are used for obtaining medicinally important bioactive secondary metabolites from the plants in higher concentrations (Halder et al. 2019). Hence, elicitation using Alternaria sesami fungal extracts in optimum concentration may be used as a biotic elicitor for enhanced production of the anticancer bioactive metabolite vincristine under in vitro culture of C. roseus.

**Conclusion**

Catharanthus roseus is a well-known plant to have high medicinal value and is home to a variety of endophytic fungi. It produces secondary metabolites known to have anticancer properties. This study shows that Alternaria sesami isolated from C. roseus is a producer of vindoline, which is a precursor molecule in vincristine biosynthesis and helps in the enhancement of vincristine content in the callus culture of C. roseus. Alternaria sesami fungal dried methanol extract can be used as a biotic elicitor for the large-scale production of vincristine in the callus of C. roseus cultured in laboratory condition which might help in increment of the yield of the bioactive secondary metabolite vincristine with the help of vindoline produced in the fungal culture. As vincristine is used in various cancer treatments, this method might reduce the high cost of such products and may prove to be revolutionary in the medical industry. However, further study may be required where in plant concentrations of vincristine may also be increased using A. sesami or any other vindoline producing fungal extract.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s10123-021-00213-w.

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**Declarations**

**Competing interests** The authors declare no competing interests.

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