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RESEARCH ARTICLE

Antibacterial potential of fungal endophytes isolated from medicinal weed *Physalis angulata* L.

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Abstract

Endophytes are microorganisms, typically fungi and bacteria that live between the living cells of plants. The current research work has been carried out on isolation and morphological characterization of fungal endophytes from medicinal plant *Physalis angulata* L. (Syn. *Physalis minima* L.) of Solanaceae along with their respective antibacterial efficacy. A total of 90 endophytic fungal isolates consigned to 10 representative morphotypes belonging to five different genera *viz., Curvularia, Paecilomyces, Penicillium, Phoma, Fusarium* and five sterile mycelial forms were isolated. Leaf samples (95%) compared to stem (37.5) and roots (92.5%) have higher colonization percentage. The agar disc diffusion method was employed to measure antibacterial activity of these fungal mycelial extracts against both gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria. The inhibition zone ranges from 5.11±0.22 mm to 17.05±2.37 mm. From the present study, it is evident that the different plant parts of *P. angulata* are colonized by different endophytic fungi and all the fungal isolates have antibacterial efficacy. Future research may be directed towards isolation of novel compounds associated with these fungal endophytes by using modern scientific tools to unknot the actual mechanistic approach behind their effectiveness against the bacteria.

Keywords: *Physalis angulata;* Medicinal Plant; Antibacterial Activity; Fungal Extracts*;* Endophytes

1. Introduction

Endophytes are microorganisms inhabiting in the core plant tissues exclusive of any disease of host plants but there are chances that they may be harmful to the host once their respective latency period is over (Petrini, 1991). These micro-organisms are attributable to the improvement of host plant resistance against disease and biotic stress, as well as the amelioration of growth of crop plants (Frank et al., 2005). These fungi are also associated with the production of bioactive substances (Lin et al., 2010; Selim et al., 2011). Antibacterial potential of such fungi has also been endorsed (Kharwar et al., 2010). The distribution and association between fungi and plants along with the environmental gradient have also been proclaimed (Redman and Rodriguez, 2007). They also persuade host plant tolerance by regulating plant physiological responses against phytopathogenic organisms (Gimenez et al., 2007). Endophytic fungi through the production of bioactive compounds play a critical role in the amelioration of host plant tolerance against various environmental stresses (Firakova et al., 2007).

Physalis angulata L. belonging to Solanaceae (Syn. *Physalis minima* L.) (Sosef, 2020), commonly known as wild cape-gooseberry is a small annual herbaceous weed plant that flourish in crop fields and the truits are reported to be a curative agent for spleen disorders (Shariff et al., 2006). Leaves with alcoholic extract and plant callus exhibit significant antimicrobial activity (Sudhakaran et al., 1999). The demand for this fruit has amplified owing to anticancer and antioxidant potential (Pietro et al., 2000; Jualang et al., 2002; Shariff et al., 2006). The fruit has also been reported to contain high amount of vitamin A and C (El-Sheikha et al., 2010). The aerial plant parts have also been reported to possess alkaloids, saponins, tannins, alkenyl phenols, glycoalkaloids, lactones, flavonoids, terpenoids, and sesquiterpenes (Karpagasundari and Kulothunga 2014).

Existing literature survey piercing out the fact that reports on endophytic fungi along with their antibacterial proficiency associated with *Physalis angulata* are lacking although reports are available from other species of *Physalis* (Diaz et al., 2019; Palupi et al., 2021) which promoted us to carry out this present research work to assess the endophytic fungal assemblages along with their antibacterial potentiality.

2. Materials and methods

2.1. Plant sample collection

Leaf, stem and root samples were collected from apparently healthy and disease-free plants of *Physalis angulata* from Madhupur, Tripura (Lat 23.763269° Long 91.260286°). The plant samples were harvested carefully and kept in marked sterilized plastic bags. The samples were then taken to the Mycology and Plant Pathology Laboratory at Tripura University for further study (Figure 1). A voucher specimen (Coll. No. 4433) of the plant sample was prepared and submitted to the Herbarium Centre Department of Botany, Tripura University for future record.

2.2. Surface sterilization of the samples

Surface sterilization of freshly harvested plant segments was done by adopting the methods described by Tan et al (2012) with requisite minor modifications. Briefly, all the selected samples were carefully washed under running tap water in order to remove the adhering dust particles followed by dipping the samples in 70% ethanol for 30 sec and 3% sodium hypochlorite (NaOCl) solution for 5 mins. After this all plant tissues were rinsed three times with sterile distilled water and surface-dried with sterile filter paper. All these surface sterilization steps were performed in laminar air flow.

2.3. Isolation and identification of fungal endophytes

The surface sterilized plant segments were excised into small pieces with a dimension of 0.5 cm × 0.5 cm by using a sterile blade and forceps. The fragments were aseptically transferred to previously prepared Petri dishes containing Malt extract agar (MEA) medium supplemented with streptomycin (50 µg/ml) to inhibit bacterial growth. The Petri dishes were incubated at 25°±2°C for 5 days at the BOD incubator. Colonies that emerged from the cultures were subcultured in a new MEA medium under same conditions for purification. The fungal tissues from respective fungal colonies were

Table 1. Colonization rate (CR) of endophytic fungi from *Physalis angulata* L.

Plant parts	Segments examined	Segments infected	Total CR (%)
Leaf	40	38	95
Stem	40	15	37.5
Root	40	37	92.5
Total	120	90	75

Table 2. Isolation rate (IR) of endophytic fungi isolated from *Physalis angulata* L.

Lab. Accession number	Plant tissue			Fungal isolates	IR (%)
	Leaf	Root	Stem		
S9L2A	+	+	-	*Curvularia* sp.	3.33
S9L3A	+	+	-	*Paecilomyces* sp.	1.66
S9L7A	+	+	-	*Penicillium* sp.	0.83
S9R1C	+	+	+	*Phoma* sp.	11.66
S9L4A	+	-	+	*Fusarium* sp.	3.33
S9L1A	+	+	+	White sterile1	28.33
S9L2B	+	+	+	White sterile 2	3.33
S9S1A	-	-	+	White sterile 3	0.83
S9L1B	+	+	-	Black sterile 1	18.33
S9R1A	-	+	-	Yellow sterile 1	4.16

Table 3. Antibacterial activity of crude extracts of endophytic fungi against four pathogenic bacteria

Fungal isolates	**Inhibition zone (mm)**			
	Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtilis*	*Staphylococcus aureus*
Curvularia sp.	6.61±0.48	5.77±0.50	9.38±0.85	14.33±1.08
Paecilomyces sp.	7.05±0.52	11.88±1.45	16.61±0.92	11.38±.05
Penicillium sp.	7.33±0.5	9.27±0.66	14.33±1.14	8.88±0.60
Phoma sp.	9.6±1.75	7.2±1.12	8.05±2.09	10.72±1.03
Fusarium sp.	10.72±0.66	9.55±1.74	13.77±0.66	11.27±0.56
White sterile1	9.72±0.97	11.38±0.38	10.11±1.96	17.05±2.37
White sterile 2	15.1±0.6	07.6±0.2	09.6±0.2	13.9±0.2
White sterile 3	07.05±0.52	16.61±0.92	11.88±1.45	11.38±.05
Black sterile 1	6.72±0.75	5.11±0.22	5.22±0.26	6.38±0.48
Yellow sterile 1	15.1±0.6	09.6±0.2	07.6±0.2	13.9±0.2
Positive control (Tetracyclin 0.001mg/ml)	26.93±3.81	32.27±2.48	22.38±0.99	21.61±0.92
Negative control	-	-	-	-

taken into the greese free slides supplemented with lacto phenol cotton blue and observed under light microscopes (OLYMPUS-CX21i). The morphological properties of fungal isolates were noted down and then compared to accessible standard text and literature (Ellis, 1993; Domsch et al., 1980; Watanabe, 2002).

Colonization rates (CR %) of fungal isolates were calculated by employing the formula postulated by Tan et al (2018):

CR% = (Nsc/Nss) × 100
Where, Nsc = Number of segments infected by fungi, Nss = Total number of segments investigated.

Isolation rates (IR) of fungal isolates were calculated as follows (Tan et al., 2018):

IR% = (Ni/Nt) × 100,
Where, Ni = number of segments from which fungal species were isolated, Nt= total number of segments incubated.

2.4. Preparation of fungal mycelia extracts for antibacterial assay

Selected fungal isolates were grown on malt extract broth medium by inoculating them with mycelial plugs (3 mm diameter) from actively growing pure culture. The agar plugs were placed in 250 ml conical

Figure 1A. Adult plant of *Physalis angulata* L. (B) Habitat of the plant, (C) Voucher specimen

flasks containing 100 ml malt extract broth medium. The flasks were incubated for 3 weeks at 25±2° C. The mycelial mat and culture filtrate was separated by Whatman No. 1 filter paper and mat were dried at 50±2° C. Dried mycelia were crushed and dissolved in 100 ml methanol and incubated for 24 hours. The sample was then evaporated under reduced pressure using a rotary vacuum evaporator (ROTAVAP model number: PBV-7D) for removal of solvent and production of dried powder (Abd El Hady et al., 2016). The dried fungal extracts were dissolved in Dimethyl sulfoxide (1 mg/ml) and evaluated their antimicrobial activity by the agar disc diffusion method (Heatley et al., 1944). The plates were kept for incubation under suitable conditions. Tetracyclin (0.001 mg/ml) was used for positive control and Dimethyl sulfoxide (DMSO) for negative control. At the end of incubation, inhibition zones formed around the disc were measured. The bacterial species used for antimicrobial assay of endophytic fungal extract were the gram-negative *Escherichia coli* (MTCC-40), *Pseudomonas aeruginosa* (MTCC-424) and gram-positive *Bacillus subtilis* (MTCC-619), *Staphylococcus aureus* (MTCC-96). The bacterial strains were provided by the Institute of Microbial Technology (IMTech), Chandigarh, India. The bacterial cultures were subculturing every weeks on fresh nutrient agar (NA) and incubated at 37°C.

2.5. Statistical analysis

All results presented are the means of three independent replicates. Data were subjected to statistical analysis by Microsoft Excel. The results were expressed as mean values ± standard deviations (SD).

3. Results

3.1. Isolation of endophytic fungi

In the present study, endophytic fungi were isolated from different plant tissues (leaf, stem and roots) of *P. angulata*. A total 90 fungal colonies were obtained from 120 surface sterilized segments, 40 segments from each leaf, stem and root sample. The colonization and

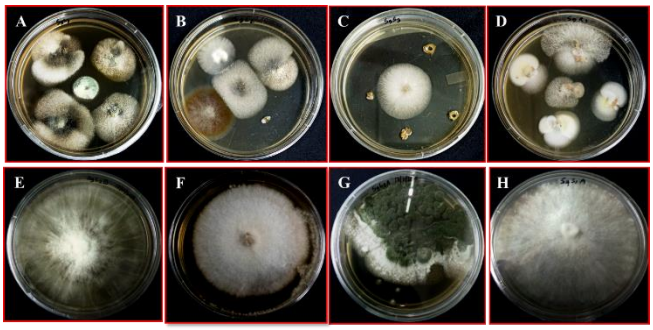

Figure 2 A-D. Plates showing mix cultures of fungal endophytes isolated from different plant part of *Physalis angulata*; pure culture of some endophytic fungal isolates E. *Curvularia* sp., F. *Fusarium* sp., G. *Penicillium* sp., H. a sterile species.

isolation rate of endophytic fungi are depicted in Table.1 and 2. The colonization rate was higher in the leaves of *P. angulata* as compared to the stems and root segments. The present investigation revealed the fact that a total of 10 endophytic fungal morphotypes were identified based on their microscopic observation (morphology of vegetative and reproductive structures). The 90 colonies from

different plant parts were assigned to 10 representative morphotypes, including five genera i.e. *Curvularia, Paecilomyces, Penicillium, Phoma, Fusarium* and five sterile mycelium forms (Figure 3).
Ten different fungal strains, including five non-sporulating mycelia sterile forms were isolated from *P. angulata*. Of these, eight endophytic strains were isolated from leaf and stem segments, and five endophytic strains were discovered in leaves (Table. 2). Whereas, the maximum isolation rate of isolated fungal strains was recorded White sterile-1. (28.33%), followed by Black sterile-1 (18.33%), *Phoma* sp. (11.66%), Mycelia Sterile-Yellow (4.16%), *Curvularian* sp. (3.33%), *Fusarium* sp. (3.33%), White sterile-2 (3.33%), *Paecilomyces* sp. (1.66%), *Penicillium* sp. (0.83%) and White sterile -3 (0.83%).

3.2. *Antimicrobial assay*

The antimicrobial activity of the endophytic fungal extracts against pathogenic bacterial strains was determined by agar disc diffusion method. Mycelial extracts prepared from endophytic fungi exhibit antimicrobial activity against the tested organisms i.e. gram-negative stains *E. coli*, *P. aeruginosa* and gram-positive *B. subtilis*, *S. aureus* with inhibition zone diameter ranging from 5.11±0.22 mm to 17.05±2.37. *Curvularia* sp. showed the highest activity against *S. aureus* with an inhibition zone of 14.33±1.08 mm and least against *B. subtilis* with inhibition zone of 5.77±0.5 mm whilst *Penicillium* sp. showed maximum inhibition zone against *P. aeruginosa* with inhibition zone of 16.61±0.92 mm and minimum activity against *E .coli* with inhibition zone 7.05±0.52 mm. Maximum and minimum inhibition zones of *Paecilomyces* sp. was noted against *P. aeruginosa* with inhibition zone 14.33±1.1 mm *E. coli* with inhibition zone diameters of 7.33±0.5 mm, respectively. *Phoma* sp. displayed maximum and minimum antibacterial activity against *S. aureus* (inhibition zone diameter of 10.72±1.03mm) and *B. subtilis* (inhibition zone diameter of 7.2±1.12 mm). *Fusarium* sp. exhibited the highest and least activity against *B. subtilis* (13.77±0.66 mm) and *P. aeruginosa,* respectively. White sterile 1 showed the highest activity against *S. aureus* with an inhibition zone of 17.05±2.37 mm and lowest against *E. coli* with inhibition zone diameters of 9.72±0.97 mm. Black sterile-1 showed its maximum and minimum antibacterial activity against *E. coli* with inhibition zone 6.72±0.75 mm and *B. subtilis* with inhibition zone diameter 5.11±0.22 mm, respectively (Table 3 and Figure 4).

4. Discussion

Endophytic fungi, one of the most unexplored microorganisms are the treasurer of bioactive chemicals. Several studies have been carried

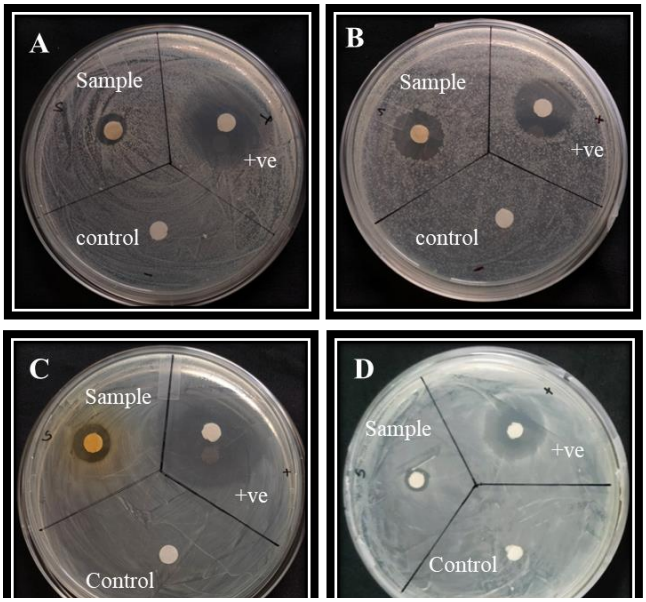

Figure 4. Disc diffusion assay (Antibacterial activity) of endophytic fungi isolated from *Physalis angulata*: A. Methanolic extract of *Penicillium* sp. against *E Coli*, B. *Penicillium* sp. against *B. subtilis*. C. *Fusarium* sp. against *P. aeruginosa,* D. Black sterile sp. against *S. aureus*.

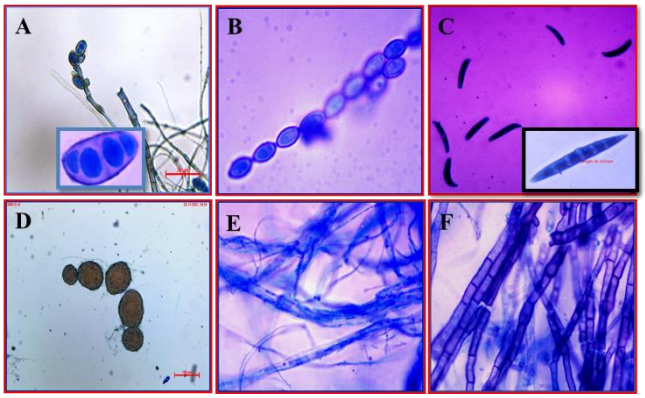

Figure 3. Microscopic study of some endophytic fungal isolates isolated from *Physalis angulata*. A. *Curvularia* sp., B. *Paecilomyces* sp., C. *Fusarium* sp., D. *Phoma* sp. E and F. Sterile hyphae.

out regarding biodiversity, taxonomy, ecology and the symbiotic relationship with the host (Clay and Schardl, 2002). This present study revealed that *P. angulata* exhibit a good diversity of endophytic fungi. Endophytic fungi are belonging to Ascomycetes, Deuteromycetes and Basidiomycetes (Clay and Schardl, 2002). Leaf samples possess maximum colonization percentage compared to stem and root which is in accordance of the previous findings (Gamboa and Bayman, 2001; Weber et al., 2006). The endophytic fungal isolates which do not produce any reproductive structures are assigned to the group sterile mycelia which is in line of earlier findings (Gamboa and Bayman, 2001; Weber and Anke, 2006). In the present study, sterile sp. were found to be the most dominant endophytic fungal species which is not organ-specific, this results came in accordance with many endophytic studies (Froehlich and Petrini, 2000; Promputtha et al., 2005).
Microorganisms produce many bioactive compounds as secondary metabolites including antibiotics and cytotoxic compounds (Anzai et al., 2008). Antibacterial activity of endophytic fungi isolated from different host plants may be due to the active components present in the fungal extracts (Joel and Bhimba 2013). Endophytic organisms especially the fungal endophytes that existed within the medicinal plant tissues have the ability of biosynthesizing pharmacologically active secondary metabolites similar to those produced by the host plants (Venieraki et al., 2017).

5. Conclusion

From the current observation it is evident that, endophytic fungi may serve as sustainable sources of novel natural bioactive compounds. Endophytic fungal assemblages was noted more on leaves as compared to stem and roots, and endophytic fungi exhibited significant antimicrobial potentiality against selected gram negative and gram positive bacteria. Further studies may be directed towards the isolation of bioactive components from the endophytic fungi having antibacterial potentiality which can be a narrative approach towards the discovery of novel drugs through the involvement of pharmaceutical industry.

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Authors' contributions

UD and ST conducted the work and photographic documentation. UD and KC analyzed the data. AKS and PD designed the work. UD, KC and AKS wrote the manuscript. All authors read and approved the final manuscript.

Conflicts of interests

Authors have no conflict of interests.

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