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Research Article

ISOLATION AND IDENTIFICATION OF ENDOPHYTIC FUNGI FROM *RICINUS COMMUNIS* LINN. AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The aim of this study was to identify the endophytic fungi from medicinal plant *Ricinus communis* and to investigate their potential antibacterial activity. 10 fungal species of endophytic fungi were successfully isolated from *Ricinus communis* including *Aspergillus fumigates, Aspergillus japonicas, Aspergillus niger, Fusarium semitectum, Curvularia pallescens, Phoma hedericola, Alternaria tenuissima, Fusarium solani, Drechslera australien* and *Aspergillus repens*. The fungal extracts were assessed for antibacterial activity against six human pathogenic bacterial strains: *Bacillus subtilis*, *Enterococcous* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus*. Most of the extracts showed *in vitro* inhibition of bacterial growth. The phytochemical screening revealed the existence of a diverse group of secondary metabolites in the crude extracts of the endophytic fungi that resemble those in the host plant extracts.

Keywords: Antibacterial Activity, Endophytes, Bioactive Compounds, pharmaceutical products.

INTRODUCTION

Endophytes are microorganisms that are present in living tissue of various plants (root, fruit, stem, seed, leaf etc.) establishing mutual relationship without apparently any symptom of diseases.[1, 2] These endophytes protect their hosts from infectious agents and adverse conditions by secreting bioactive secondary metabolites.[3,4] The endophytic fungi play important physiological and ecological roles in their host life. Recent investigations have been intensified by the potentialities of endophytic fungal strains in production of bioactive metabolites like taxol, pestaloside, torreyanic acid and enzymes, i.e: Xylanase**,** Isoflavonoids**,** Asparaginase.[5,6,7,8]

Endophytic fungi are a good source of antibiotics. Natural products from entophytic microbes have been observed to inhabit or kill a wide variety of harmful disease causing agents but not limited to phytopathogens, as well as bacteria, fungi, viruses and protozoan that affect humans and animals. Endophytic fungi are also capable to produce antimicrobial metabolites. The production of Hypericin ($C_{30}H_{16}O_8$), a naphthodianthrone derivative and Emodin ($C_{15}H_{10}O_5$) believed to be the main precursor of hypericin, by the endophytic fungus isolated from an Indian medicinal plant, having an antimicrobial activity against several bacteria and fungi, including *Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella enteric and Escherichia coli, and fungal organisms Aspergillus niger and Candida albicans*.[9] A compound polyketide citrinin produced by endophytic fungus *Penicillium janthinellum* from fruits of *Melia azedarach*, presented 100% antibacterial activity against *Leishmania* sp.[10]

Medicinal plants are known to harbor endophytic fungi that are believed to be associated with the production of pharmaceutical products.[11] Therefore, it is important to explore endophytic mycoflora in the medicinal plants. The present study was carried out to isolate, identify and test antibacterial activity of endophytic fungi which

are isolated from *Ricinus communis* (Aurundi) against a number of pathogenic bacteria. It is an indigenous medicinal plant in India and USA. *Ricinus communis* is an evergreen plant of the tropical and temperate climates of the world belonging to the family Euphorbiaceae. It is widely used in India traditional medicine for various therapeutic purposes as well as the source of agrochemical and castor oil from many centuries. Petroleum ether extracts of this plant roots posses anti inflammatory in formaldehyde and adjuvant induced rat's paw arthritis and also effective against skin diseases such as burns, ulcers etc.

MATERIALS AND METHODS

Plant Material

In the present study fungal species were isolated from different parts of the *Ricinus communis* Linn. medicinal plant commonly called Aurundi was collected from different sites of Priyadarshni colony Dumna road Jabalpur, Madhya Pradesh (India). Healthy and mature plant was carefully chosen for sampling. The plant parts were brought to the laboratory in sterilized bags and processed within a few hours after sampling.

Isolation of Endophytic fungi

Isolation of endophytic fungi from plant parts was done according to the method described by Petrini (1986). [12]First the plant material was rinsed in tap water to remove the dust and debris then cut into small pieces by a sterilized blade under aseptic conditions. Each sample was surface sterilized by 70% ethanol for 1 minute and after that immersed the plant parts in sodium hypochloride (NaOCl) solution for 30 seconds to 1 minute. The samples were rinsed in sterile distilled water for 1 minute and then allowed to surface dry on filter paper. After proper drying 4 pieces of plant parts were inoculated in PDA plate supplemented with antibiotic (Tetracycline) and incubated at 28 $\pm 1^0$C for 5 to 7 days. Pure colonies were transferred on PDA slant. The fungal strains in the pure culture were preserved on potato dextrose agar (PDA) slant at 4 to 5^{o}C with proper labeling and were sub-cultured from time to time.

Calculation of colonizing frequency

Colonization frequency (CF %) was calculated as described by Suryanarayanan et al. (2003) and Photita (2001). [13,14] Briefly, proper time of incubation was given for colonizing frequency counting. Colonization frequency (%) of an endophyte species was equal to the number of segments colonized by a single endophyte divided by the total number of segments observed x 100. (Table 2 & figure 1)

$$\text{Colonizing frequency \%} = \frac{\text{Number of segment colonized by fungi}}{\text{Total number of segment observed}} \times 100$$

Morphological Identification of Endophytic fungi

The fungi were identified on the basis of morphological characteristics according to Domsch (1980) and Aggarwal and Hasija (1980).[15,16] The colonies appearing on petri-plates were sub-cultured into the tube containing potato dextrose agar medium for identification. Fungi were again cultured from slant to petri-plates containing potato dextrose agar medium without antibiotic (Tetracycline) for 7 days. Morphological identification was done according to the standard taxonomic key included colony diameter, texture, color and the dimensions and morphology of hyphae and conidia.

Mass Production of Antibacterial Metabolites

150 ml of Potato Dextrose Broth was prepared in 250 ml flasks and autoclaved at 15 lbs psi for 20 min. The medium was inoculated with various fungi culture and incubated at 28±1°C in the incubator. After 7, 14 and 21 days of incubation the crude culture broth was filtered and tested for antibacterial activity against the six pathogenic bacteria by using agar well diffusion methods.

Antibacterial Activity

Test organisms

There were six strains of pathogenic bacteria such as *Bacillus subtilis*, *Enterococcous* sp., *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhimurium* and *Staphylococcus aureus* were used to evaluate the antibacterial activity of fungi. These bacterial strains were taken from Microbial Type Culture Collection (MTCC) Chandigarh (India).

Antibacterial Activity

The agar well diffusion assay method was used to assess the antibacterial activity of the fungi. In this method wells were aseptically made in the seeded media using sterile cork borer and appropriate amount of the bioactive metabolite

was dropped in the prepared wells and incubated at 37°C in bacteriological incubator for 24 hrs. Finally plates were observed for zones of inhibition and their diameter was measured with the help of Hi-Antibiotic zone scale, Hi Media Laboratories Mumbai. [17]

RESULTS

Isolation of endophytic fungi

In the present study, fungal strains were isolated from the different parts of *Ricinus communis*. In this, we taken total 46 segments (16 leaves, 16 stem and 14 segment of root) were processed for the isolation of fungi. A total 10 fungi were isolated out of which five fungal strains belong to Ascmycetes, one belong to Ulovophycetes and two belongs to class Hypomycetes and one belong to Ascomycota which showed in table 1.

Identification of Endophytic Fungi

Identification of these fungal strains was done by using standard protocol of Barnett and Hunter (1998) and Aggarwal and Hasija (1980) on the basis of their cultural and microscopic properties these fungi show different characteristics. [18,16] These fungi were successfully identified as *Aspergillus fumigates, Aspergillus japonicas, Aspergillus niger, Fusarium semitectum, Curvularia pallescens, Phoma hedericola, Alternaria tenuissima, Fusarium solani, Drechslera australien* and *Aspergillus repens*.

Screening of endophytic fungi for antibacterial activity

Screening of endophytic fungi to determine the antibacterial activity was done by agar well diffusion method against 6 pathogenic human bacterial strains after 7, 14 and 21 days old metabolites of fungi. These fungi provided maximum zone of inhibition after 14 days of incubation except 7 and 21 days are shown in (Table 3 and figure 2, 3). *Phoma hedericola* showed maximum zone of inhibition against *B. subtilis* (25mm), *K. pneumoniae* (25mm), *S. aureus* (24mm), *E. coli* (22mm), *S. typhimurium* (20mm) and *Enterococcous* sp. (19mm). Similarly, *Alternaria tenuissima* showed utmost zone of inhibition against *B. subtilis* (22mm), *E. coli* (20mm), *K. pneumoniae* (25mm), *S. aureus* (23mm), *Enterococcous* sp. (22mm) but it showed least activity against *S. typhimurium* (18mm). Likewise, *Fusarium semitectum* showed maximum activity against *S. typhi* (19mm), *Enterococcous* (16mm), *K. pneumoniae* (16mm), *S. aureus* (15mm) and minimum activity against *B. subtilis* (13mm) and *E. coli* (8mm) respectively. *Curvularia pallescens* give maximum zone of inhibition against *S. typhimurium* (20mm), *B. subtilis* (18mm) and *Enterococcous* sp. (15mm) and least activity against *S. aureus* (15mm), *E. coli* (12mm) and *K. pneumoniae* (13mm). The other fungi *Drechslera australiensis* show antibacterial activity against *B. subtilis* (15mm), *S. aureus* (19mm) *E. coli* (10mm), *K. pneumoniae* (12mm), *Enterococcous* sp. (22mm), *S. typhimurium* (10mm).

Growth Pattern and Bioactive Metabolite production analysis of potent Fungi

Time duration required for growth and metabolite production by different fungi varies significantly.[19] In the present investigation, the extract of *Phoma hedericola* isolated from *Ricinus communis* was showed maximum antibacterial activity against pathogenic bacteria. It produced the maximum inhibition of zone against *B. subtillis, S. aureus, K. pneumoniae* and *E. coli.* Production of bioactive metabolite was observed from 1st to 21 days of incubation, whereas maximum production was seen on 12th day of incubation in relation to the growth pattern studies, it was inferred that when fungi enter to the stationary phase of growth, it secreted desire metabolite. Further in the study, the antibacterial activity against test bacteria from 11th to 12th day of incubation was approximately equal; therefore, 12th day of incubation was taken as optimum period of incubation for the further study. Effect of incubation period on antibacterial metabolite production by *Phoma hedericola* are given in (Table 4 & fig 3)

DISCUSSION

The numerous species of fungal endophytes made an ecological niche in the inner space of plants. These ubiquitous fungi interact positively with their environment. In addition, they are the group of organism with very good potential for application in plant improvement and disease control. Isolation of endophytic fungi from medicinal and other plant results to produce bioactive compound which has greater activity against various pathogenic microbes. Hence, large scale production of these bioactive compounds must be necessary to fulfill the needs of agriculture and pharmaceutical industries. Sukanyanee et al. (2006) studied the antimicrobial potential of endophytic fungi *Phomopsis Alternaria*, *Colletotrichum*, *Nigrospora* and sterile mycelia isolated from the leaf tissues of *Tectona grandis* and *Samanea*

saman. [20] Phongpaichit et al. (2006) isolated fungal endophytes from five medicinal *Garcinia* plants and verified that the metabolites produced by 70 fungal isolates and extracted with ethyl acetate showed antimicrobial activity by agar well diffusion method against: *Staphylococcus aureus, Candida albicans, Cryptococcus neoformans* and *Microsporum gypseu*. [21] They also identified the genera *Aspergillus, Botryosphaeria, Eutypella, Fusarium, Guignardia, Penicillium, Phomopsis* and *Xylaria* as the ones which showed the greatest results for the inhibition of microbial growth. Among these endophytes, three strains showed most active for production of secondary metabolites were *Phomopsis* sp., *Botryo* sp, *haeria* sp. and one non- identified fungus. In other research work Sandhu et al. (2014b) studied the antibacterial activity of endophytic fungi isolated from plant *Calotropis procera* Linn. against six human pathogenic bacteria.[22]

CONCLUSION

In the present study a total 10 endophytic fungi were isolated from the *Ricinus communis* show a great antibacterial activity against 6 human pathogenic bacteria. *Phoma hedericola* showed maximum zone of inhibition against *B. subtilis*, *K. pneumoniae*, *S. aureus*, *E. coli*, *S. typhimurium* and *Entercoccous* sp. Therefore, there is a need of further in depth studies of these isolated endophytes. Further growing those on large scale, modifying culture conditions and supplying some stimulants might help in getting better production of particular bioactive compound.

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Table 1: Endophytic fungi isolated from different parts of *Ricinus communis*

S. No.	Plant parts	Name of endophytic fungi	Class
1.	Leaf	*Aspergillus fumigates*	Ascomycetes
2.	Leaf	*Aspergillus japonicas*	Ascomycetes
3.	Stem	*Aspergillus niger*	Ascomycetes
4.	Stem	*Fusarium semitectum*	Hyphomycetes
5.	Leaf	*Curvularia pallescens*	Ascomycetes
6.	Leaf	*Phoma hedericola*	Ascomycota
7.	Leaf	*Alternaria tenuissima*	Ascomycetes
8.	Root	*Fusarium solani,*	Hyphomycetes
9.	Root	*Drechslera australien*	Ulvophycetes
10.	Leaf	*Aspergillus repens*	Ascomycetes

Table 2: Name and colonizing frequency of Endophytic fungi isolate from *Ricinus communis*

S. No.	Name of Endophytic Fungi	Isolate from	% Frequency of colonization	No. of isolates
1.	*Aspergillus fumigates*	Leaf	12.50%	2
2.	*Aspergillus japonicas*	Leaf	18.75%	3
3.	*Aspergillus niger*	Stem	25.00%	4
4.	*Fusarium semitectum*	Stem	14.28%	2
5.	*Curvularia pallescens*	Leaf	18.75%	3
6.	*Phoma hedericola*	Leaf	12.50%	2
7.	*Alternaria tenuissima*	Leaf	25.00%	4
8.	*Fusarium solani*	Root	14.28%	2
9.	*Drechslera australien*	Root	07.14%	1
10.	*Aspergillus repens*	Leaf	12.50%	2

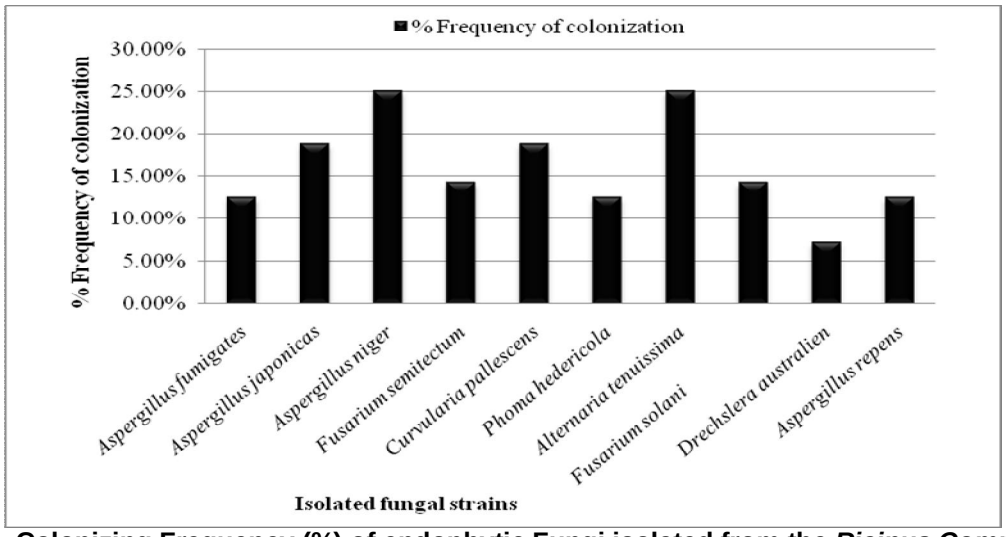


Fig. 1: Colonizing Frequency (%) of endophytic Fungi isolated from the *Ricinus Communis*

Table 3: Antibacterial activity of endophytic fungal isolates against pathogenic bacteria strain

S. No.	Name of endophytic fungi	Zone of inhibition (in mm)					
		B. subtilis	*S. aureus*	*E. coli*	*K. pneumoniae*	*Enterococcous* sp.	*S. typhi*
1.	*Fusarium semitectum*	13	15	08	16	16	19
2.	*Curvularia pallescens*	18	10	12	13	15	20
3.	*Drechslera australiensis*	15	19	10	12	22	10
4.	*Alternaria tenuissima*	22	23	20	25	22	18
5.	*Phoma hedericola*	25	24	22	25	19	20

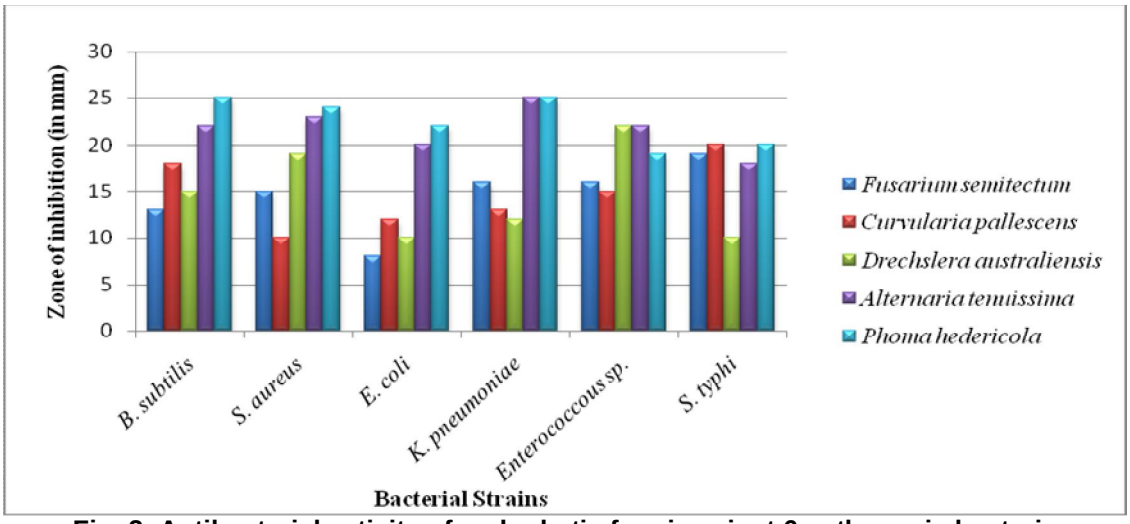
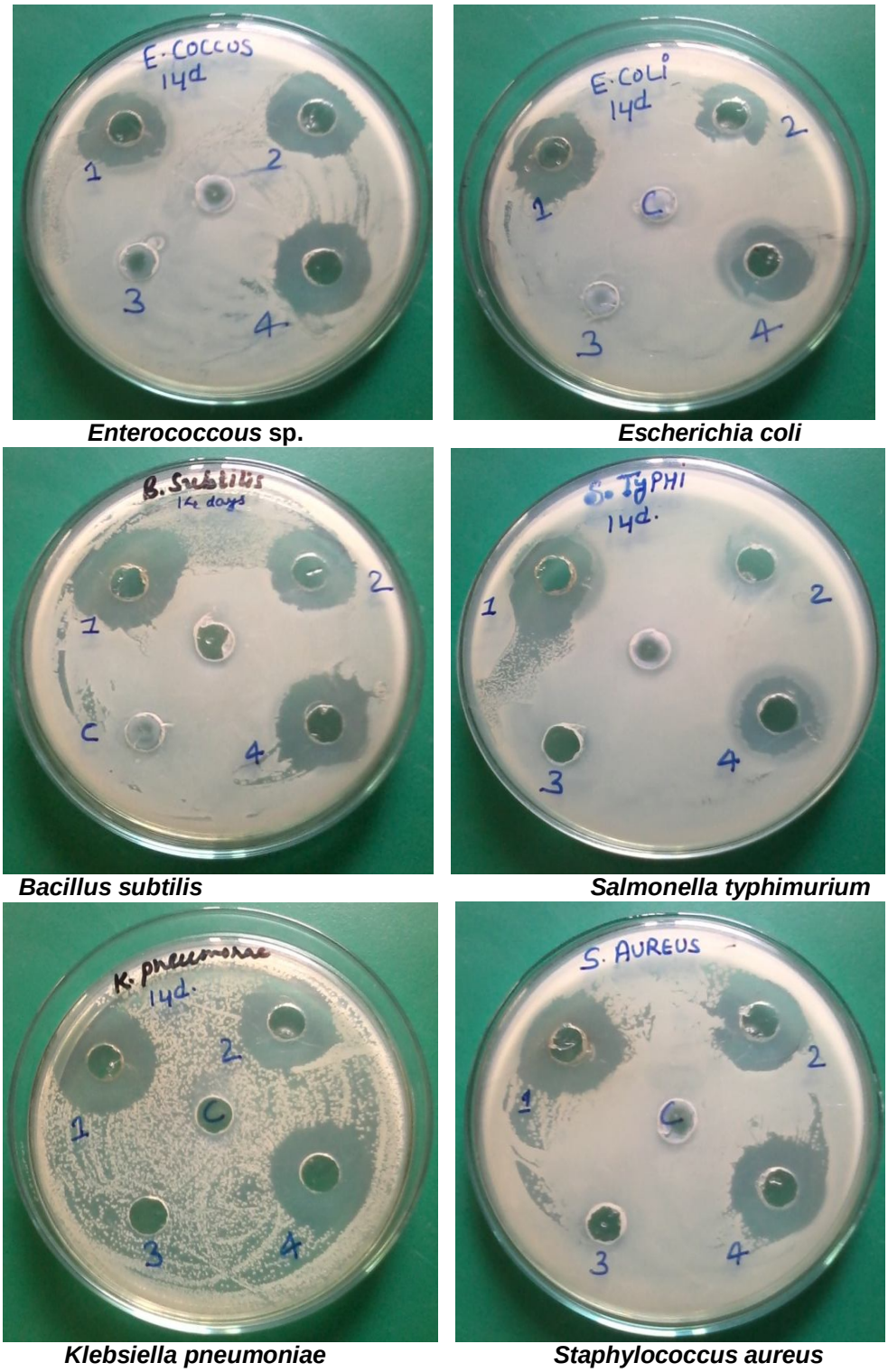


Fig. 2: Antibacterial activity of endophytic fungi against 6 pathogenic bacteria



Enterococcous* sp.** ***Escherichia coli

Bacillus subtilis ***Salmonella typhimurium***

Klebsiella pneumoniae ***Staphylococcus aureus***

Fig. 3: Zone of inhibition of fungal bioactive compounds against pathogenic bacteria strains by Agar well diffusion method

Table 4: Effect of incubation period on antibacterial metabolite production by *Phoma hedericola*

Days of incubation	*E. coli*	*S. typhi*	*K. pneumoniae*	*Enterococcous* sp.	*S. aureus*	*B. subtilis*
1	-	-	-	-	-	-
2	-	-	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	-	-	-	-	-	-
6	-	-	7	9	-	12
7	-	10	11	12	10	15
8	8	12	13	13	13	17
9	11	12	17	13.78	15	20
10	15	13	20	15	15	23
11	19	14	23	18	21	24.90
12	23	20	28	20	26	25
13	23	19	26	19	25	24
14	21	19.25	25	19	24	23
15	13	10	16	14	19	19
16	12	7	12	11	13	15.25
17	10	5	9	9	10	10
18	7.33	-	7	-	9.30	8
19	-	-	-	-	7	-
20	-	-		-	-	-
21	-	-	-	-	-	-

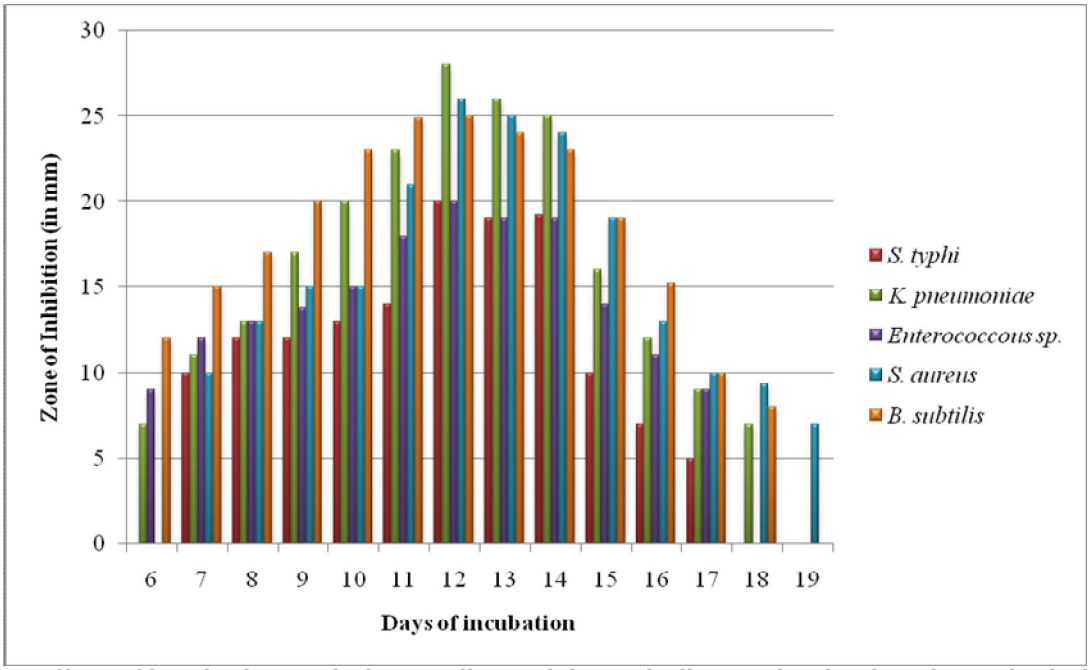


Fig. 3: Effect of incubation period on antibacterial metabolite production by *Phoma hedericola*

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