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ASSEMBLAGE OF FUNGAL ENDOPHYTES IN LEAF AND STEM OF *Aegle marmelos* (L.) Correa AND THEIR EFFICACY IN THE PRODUCTION OF ENZYMES AND IN SHOWING ANTIBACTERIAL ACTIVITY

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***Abstract*:** Leaf and stem segments of *Aegle marmelos* (L.) Correa were employed for isolation of fungal endophytes and determination of their colonization frequency, production of enzymes like Amylase, Cx cellulase, C1 cellulase and antagonistic activity. A total of five endophytic fungi were isolated, viz. *Penicillium Sp*.1, *Penicillium Sp*.2 and *Fusarium redolens* from leaf discs and *Penicillium Sp*.3 and *Fusarium redolens* from stem bits. Highest colonization frequency was noted in *Penicillium Sp*.2 followed by *Penicillium Sp*.1 from leaf and *Penicillium Sp*.3 from stem. The dominant colonizers of leaf and stem were tested for their efficiency in the production of Amylase, Cx cellulase and C1 cellulase and in inhibiting the Gram positive and Gram negative bacteria. Highest production of Amylase by *Penicillium Sp*.3, Cx cellulase by *Penicillium Sp*.3 and C1 cellulase by *Penicillium Sp*.1 were noted. Highest antagonistic activity against Gram positive bacterium was found in *Penicillium Sp*.3 and against Gram negative bacterium in *Penicillium Sp*.1.

Key Words: *Aegle marmelos*, *Penicillium*, *Fusarium redolens*, Amylase, Cx cellulase, C1 cellulase

INTRODUCTION

Medicinal plants, also called medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesise hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions, if any, of many plants having medicinal potential remain unassessed by rigorous scientific research to define efficacy and safety. In the United States over the period 1999 to 2012, despite several hundred applications for new drug status, only two botanical drug candidates had sufficient evidence of medicinal value to be approved by the Food and Drug Administration. Different parts of medicinal plants are traditionally claimed to be used for the treatment of antifungal, antitumour, antihelminthic, antidiuretic, antiulcerative, diseases of heart, rheumatic pains, chest pain, dyspepsia, fever, diabetes, burning of liver and kidney diseases (Verma *et al*., 2011).

All plants produce chemical compounds which give them an evolutionary advantage, such as defending against herbivores in the example of salicylic acid, as a hormone in plant defenses. These phytochemicals have potential for use as drugs, and the content and known pharmacological activity of these substances in medicinal plants is the scientific basis for their use in modern medicine, if scientifically confirmed. Phytochemical is a natural bioactive compound found in plants, such as vegetables, fruits, medicinal plants, flower, leaves, roots that are rich in nutrient and fibres to protect against diseases. Phytochemicals are divided into two groups, primary and secondary, based on their function in plant metabolism. Primary phytochemicals comprise common sugar, aminoacids, protein and chlorophyll while secondary phytochemicals consists of alkaloid, terpenoid, phenolic compounds, flavonoids, tannins, coumarin, anthroquinone etc.

Endophytes are microorganisms that live within a plant intracellularly or intercellularly for whole or at least a part of their life cycle establishing symbiotic relationship with the host without causing any harm to the host (Petrini, 1991; Bacon and White, 2000). Endophytic microorganism may be fungi and/or bacteria are grow asymptomatically within plant parts like stems, leaves etc. (Bills, 1996).

It is recognized that endophytes are of great importance for plants, protecting them against pathogenic fungi, bacteria, insect and nematodes (Azevedo *et al*., 2000; Strobel, 2003; Gimenez *et al*., 2007). They also include physiological modification in their hosts, making them more resistance against biotic and antibiotic stresses (Tan and Zou, 2001; Musetti *et al*., 2007). There is a strong need for new drugs especially antibiotic, anticancer agents and agrochemicals that are highly effective but have low toxicity and a minor adverse environmental impact. Ever increasing problem of the development of multidrug resistance in bacterial pathogens, demands the necessity for obtaining newer antibiotics. Studies on endophytes during the past thirty years indicate that they may be the "Treasure Trove' for new pharmaceutical agents and agrochemical compounds. As only a few of the 300000 plant species have been surveyed so far, there is tremendous possibility for discovering noble endophytic microbes and isolating novel bioactive molecules from them (Tan and Zou, 2001; Strobel, 2003; El-hawary *et al*.,2020; Bhattacharya and Mitra,2022) and many of the bioactive compounds, has been suggested, to have potential for the treatment of human diseases (Onifade, 2007). The bioactive compounds, particularly those which are useful in pharmaceutical industries, are mainly the secondary metabolites of the endophytes (Strobel, 2002; Naik *et al*., 2008; and Khan *et al*., 2010; El-hawary *et al*.,2020; Bhattacharya and Mitra,2022) colonizing plants. Dreyfuss and Chapela (1994) estimated that there may be one million species of endophytic fungi alone. Investigation have demonstrated that a number of ecological factors such as geographic location (Fisher *et al*., 1994; Collado *et al*., 1999), differences in site (Okane *et al*., 1998), seasonal variation (Gangadevi and Muthumary 2008), microclimate (Johnson and Whitney, 1989), age and specificity of the colonized tissue (Bills and Polishook, 1991; Sahashi *et al*., 2000) could greatly influence endophytic assemblages as well as their metabolic activities. Since the ancient past it could be observed that the plants having pharmaceutical importance are largely exploited for healing different diseases. Large scale harvesting of medicinal plants have become a major threat to biodiversity. Some plants have literally been threatened with extinction as a result of enormous pressure brought upon them by virtue of their disease curative properties, for example, various new species having their taxol content. As an alternative it could be suggested that the endophytic microorganisms that may have tremendous potential source of therapeutic compounds may be used to get rid of this crisis. At present times emphasis is given not only on the secondary metabolites but also on the primary metabolites of the endophytes like enzymes. It has been put forwarded that horizontally transmitted endophytes can live as saprobes and cause leaf on plant litter degradation after shedding of leaves twigs with the help of their wide range of extracellular enzyme (Osono and Hirose, 2009; Voriskova and Baldrian, 2012; Cline et al.,2017 and Guerreir *et al*.,2018). In recent years, extensive researches have been focused on the

bioactive compounds of fungal endophytes, such as, antitumour agent (Taxol), antibacterial and antifungal agents (Quercine), plant growth factors, and enzymes (Arora *et al*., 2016; Diana Victoria,2015; Okezie *et al*., 2020; Gupta *et al*., 2023). Therefore, the present studies have concentrated to the endophyte mycoflora of a well-known medicinal plant *Aegle marmelos* L. Correa belonging to the family Rutaceae

along the following lines:-1)Isolation of fungal endophytes from stem and leaves.2) Characterization and identification of the endophytic fungi. 3) Determination of colonization frequency (CF%). 4) Estimation of total Cellulase enzyme. 5) Estimation of total Amylase enzyme. 6) Determination of antibacterial activity.

MATERIALS AND METHODS

ISOLATION AND IDENTIFICATION OF ENDOPHYTES

SAMPLE COLLECTION:-

Fresh middle aged healthy leaves of *Aegle marmelos* were collected from Hooghly Mohsin College garden and immediately placed in sterile plastic bags and brought to the laboratory.

ISOLATION OF FUNGI :-

The collected materials were washed with running tap water for 30 minutes and then washed with sterilized distilled water for 2-3 minutes, surface sterilised of the materials was done by 90% ethanol (for 1 minute), followed by 3% NaOCl (for 8 minutes) and finally by 90% ethanol (30 sec) and then washed with sterilized distilled water (2-3times). After surface sterilization processes, leaf discs (0.5 cm) were prepared by using sterilized cork borer. Three leaf discs were then transferred to the petri plates containing sterilized potato dextrose agar (PDA) medium with Streptomycin (100units/ml) for isolation of endophytic fungi. The petri plates were rotated clockwise, anti-clock wise and moved to and fro for proper mixing. Three replicates were maintained. Same process was followed in the case of stem (stem was cut into pieces of 1cm). The plates were then incubated at 30°C for a period of 10 days. Interim inspection was done after 3 days and 5 days for appearance of the fungal colonies. The organism that come out of the plant materials were isolated and subculture in slants.

IDENTIFICATION

The fungal endophytes were identified based on cultural characteristic and available reproductive structure following Burnett and Hunter (1998), Uddin *et al*.,2016, Gilman (1967), Whatnabe (2010), and internet information.

COLONIZATION FREQUENCY FOR ENDOPHYTE :-

The colonization frequency (CF %) of a single endophyte in leaf and stem tissue was determined by followings (Hata and Futai, 1995).

$$CF\ \% = \frac{\text{The no. of segments colonized by endophytes species}}{\text{Total no. of segments}} \times 100$$

PREPARATION OF FUNGAL EXTRACTS:-

The test fungi were then separately in 250ml Erlenmeyer containing 150ml of Czapek Dox Broth (CDB). The flask were then intermittent sacking on a rotary shaker for 2 hours at 2 days intervals for maintaining more or less uniform inoculums potency and incubated at 30°C for 12 days. 3 replica were maintain for each set. The culture filtrates were then obtained by filtering through whatman No.1 filter paper placed on Buchner funnel. After extraction, the culture filtrates were used for assay of enzymes (Amylase and cellulase) and antibiotics tests.

ASSAY OF AMYLASE

For this experiment culture filtrate was obtained by passing the culture broth primarily through Whatman No. 1 filter paper followed by its centrifugation at 1000 rpm. for 10 minutes. After centrifugation the supernatant was used for Amylase assay.

Assay of Amylase was conducted following the methods describe by Thimmaiah, 1999a after certain modifications.

1ml of culture filtrate taken in a test tube was added with 1ml of starch solution (1%),prepared by dissolving 1g. starch in 100 ml of 0.2M sodium acetate buffer, pH 4.6. The reaction mixture was subjected to incubation at 27°C for 20 minutes. The reaction was stopped by adding 2 ml. of Dinitro Salicylic acid (DNS) reagent. The solution was then kept in boiling water bath for 10 minutes. Rochelle salt solution (40%) was then added, while the tubes remained warm. The temperature within the tube was cooled down by keeping them in running tap water. The volume of the reaction mixture within the tube was made up to 10 ml. by adding distilled water and the absorbance was measured at 560 nm. setting control as zero. Production of Maltose in the above reaction was determined by calibrating the absorbance with a standard curve prepared using 10- 250 µg Maltose solution.

ASSAY OF CELLULASE

For this experiment culture filtrate was obtained by passing the culture broth primarily through fine glass wool, followed by its centrifugation at 1000 rpm for 10 minutes. After centrifugation the supernatant was used for cellulase assay.

Assay of C1 cellulase was carried out mainly by the methods of Mandel (1976) and assay of Cx cellulase was carried out following the methods describe by Thimmaiah (1999b) with some modifications.

ASSAY OF CMCase (Cx CELLULASE ENZYME):

1ml of culture filtrate taken in a test tube was added with 1ml of CMC solution (1%), prepared by dissolving 1g. CMC in 100 ml of 0.2M sodium acetate buffer, pH 4.6. The reaction mixture was subjected to incubation at 27°C for 30 minutes. The reaction was stopped by adding 2 ml. of Dinitro salicylic acid (DNS) reagent. The solution was then kept in boiling water bath for 10 minutes. Rochelle salt solution (40%) was then added, while the tubes remained warm. The temperature within the tube was cooled down by keeping them in running tap water. The volume of the reaction mixture within the tube was made up to 10 ml. by adding distilled water and the absorbance was measured at 510 nm. setting control as zero. Production of Glucose in the above reaction was determined by calibrating the absorbance with a standard curve prepared using 40- 300 ug glucose solution.

ASSAY OF C1 CELLULASE ENZYME:

For determination of cellulase enzyme (C1) activity through filter paper disc degradation, filter paper discs (20mg) are to be used in place of CMC. The reactions mixture thus by taking filter paper disc, 1ml distilled water, 1ml buffer, 2ml culture filtrate, is to be kept in an incubator for one hour at 37°C temperature. The subsequent steps are however similar to the determination of CMCase activity.

ANTAGONISTIC ACTIVITY:-

Antagonistic activity of the fungal endophytes were determined by antibacterial assay against both Gram positive and Gram negative bacteria using disc diffusion method. The Gram positive bacteria tested was Bacillus subtilis and Gram negative bacteria tasted was *Escherichia coli*.

DISC DIFFUSION METHOD:-

Partially purified antibiotics was used in this method. The culture filtrate showing the formation of zone of inhibition was used for solvent extraction and purification of the antibiotic present in it. Before solvent extraction of the antibiotics, the culture filtrate was acidified (with ortho phosphoric acid) to pH 2.0-2.5. The filtrate was then added with equal amount of ethyl acetate and thoroughly shaken for 7 minutes in a separating flask to extract the antibiotics into the organic solvent. The antibiotic was back extracted into an aqueous phase at pH 7.5-8 by adding NaOH solution. The process of extraction in organic solvent and back extraction into NaOH solution was repeated once again for purification. Finally the antibiotics was extracted in ethyl acetate which was then evaporated to dryness in a rotary vacuum evaporator at 43°C. The residue was weighted and dissolved in methanol. The partially purified antibiotic in methanol (0.04ml). was soaked in sterilised filter paper discs with the help of 0.1ml sterile pipette in front of a laminar air-flow. The discs were then allowed to dry by the evaporation of methanol and used for antibiotic bio assay against the Gram positive and Gram negative bacteria pre-inoculated in nutrient agar plates. The disc soaked with methanol only were used as negative control. Standard ampicillin disc was used for comparison. The zone of inhibition (cm^2) was measured after 72 hours.

RESULTS AND DISCUSSION

The leaf discs and stem bits of *Aegle marmelos* were subjected to plate culture on Potato Dextrose Agar media (PDA) and showed emergence of fungal endophytes from the plant segments in the media in most of the cases. A total of five fungal endophytes were isolated from both leaf discs and stem bits. Out of five isolates three organisms (*Penicillium sp* l, *Penicillium sp*2 and *Fusarium redolens*) were obtained from leaf discs and two (*Penicillium sp*3 and *Fusarium redolens*) were isolated from stem bits.

Morphological features of the isolated fungal endophytes were as follows:-

FUNGAL ENDOPHYTES FROM LEAF DISCS:-

***Penicillium sp*1 (Fig:3):-**

Growth moderate, diameter 4.8 cm. after 7 days in Czapek Dox Agar media (CDA media).Colony, centrally elevated, white with hyaline margin, reverse white, margin serrated, diffusion of pigment absent.

Conidiophore (72.09 x 3.08µm) smooth, branched or unbranched. Branched conidiophores terminated into a whorl of metula (three in no.), each metulae (18.12 x 3.78 µm) bears a whorl of sterigmata (4-5 in no.). Each sterigma (16.38 x 3.22 µm) bears a chain of conidia. Conidia (3.95 µm) round, smooth walled.

***Penicillium sp*2 (Fig:4):-**

Growth show 2.6 cm. in diameter after 7 days in CDA media. Obverse centrally elevated, velvety, grey-green at the centre followed by rings of pinkish grey, greenish yellow and light yellow, margin-hyaline. Reverse orange for most part of the colony followed by yellow orange at the periphery, No. of diffusion of pigment found.

Conidiophore (57.91-63 µm. x 4.69-7.30 µm.) unbranched bearing a whorl of 4-7 sterigmata. Sterigmata flask shaped, each sterigma (19.99 x 3.76 µm .) produced a chain of conidia. Conidia round (5.59 µm.) or oval (7.74 x 5.31 µm .) smooth walled.

***Fusarium redolens* Wollenweber (Fig:5):-**

Growth moderately rapid 6.4 cm. after 7 days on CDA media, colony floccose, white, yellowish, white at the central part. Reverse reddish yellow or orange.

Microconidia one celled (9-11 µm. x 4.5-5 µm.) or two celled (16 - 20 µm x5 µm.) elongated or oval or elliptical. Microconidia usually three or four celled, sparingly five celled, sickle shaped, curved, growing in sporodochia. Surrounding spores often pionnotes present. Size of the macroconidia: single celled 23.41 x 5.06 µm., two celled- 37.52 x 5.63 µm., three celled- 49.21 µm. x 5.58-6.5 µm., four celled-41-55 µm. x5 - 6.69 µm ., five celled-64.30 µm. x 6.63-7.2 µm..Chlamydospores- terminal or intercalary single or two in number- round (13.80- 15.12 µm), smooth or rough walled.

FUNGAL ENDOPHYTES FROM STEM BITS:-

***Penicillium sp*.3 (Fig:6):-**

Growth restricted, diameter 1.5 cm. after 7 days in CDA media. Upper surface centrally greyish brown followed by wide white periphery. Appearance velvety to floccose, slightly elevated from the media. Reverse light orange or white. Diffusion of any pigment absent.

Conidiophore (132.92 - 145.95 µm x 3.95-4.17 µm) branched to give rise to a whorl of 4 metula, each metulae (20.86 x 2.99 µm) terminated into a whorl of 3-7 sterigmata. Sterigma (19.25 - 21.57 µm x3.56-4.39 µm.) flask shaped and terminate into a chain of conidia. Conidia oval (5.63 x 4.42 µm) or round (5.53 µm), rough surface.

***Fusarium redolens* Wollenweber (Fig: 5) : -**

As described earlier.

As revealed from Table-1 the colonization frequency (CF%) of the fungal endophytes isolated from leaf discs varied within the range 16.66%-58.33% and the highest C.F.% was noted in *Penicillium sp*.2 (58.33%) was followed by

Penicillium sp.1 (25%) and *Fusarium redolens* (16.66%) in descending order. In case of stem bits highest colonization frequency was recorded in *Penicillium sp*.3 (46.66%), followed by *Fusarium redolens* (6.66%).

Existence of *Penicillium spp* and *Fusarium sp*. As fungal endophytes were earlier reported in a no. of publications (Pancher *et al*., 2012; Nagda *et al*., 2017; Roychowdhury *et al*.,2018). Garyali *et al*., (2013) reported the existence of *Fusarium redolens* as fungal endophyte in Himalayan Yew. Gond *et al*., (2007) investigated on endophytic fungal community from *Aegle marmelos* and they recorded *Fusarium spp* as having highest colonization frequency.

Dominant fungal endophytes from leaf discs, as revealed from the above statement were found to be *Penicillium sp*.2 (58.33%) and *Penicillium sp*.1 (25%) and from stem bits was *Penicillium sp*.3 (46.66%). The dominant colonizers were used for further investigation in the production of enzymes (Amylase, C1 cellulase, Cx cellulase) and antibiotic activity against Gram positive and Gram negative bacteria.

It is evident from Fig:8 that out of the three dominant fungal isolates (two from leaf discs and one from stem bits) highest extent of Amylase production was noted in *Penicillium sp*.3 (616.66 µg/ml) followed by *Penicillium sp*.2 (475 µg/ml) and *Penicillium sp*.1 (221.66 µg/ml) in descending order.

Regarding production of C1 cellulase by the dominant isolates it is, however transpired from Fig:9 that *Penicillium sp*.1 proved to be the most potent (179 µg/ml) producer. This was followed by *Penicillium sp*.2 (125.33 µg/ml) and *Penicillium sp.3* (96.66 µg/ml).

Production of Cx cellulase from the Fig:10 was found to be highest by *Penicillium sp*.3 (306 µg/ml), closely followed by *Penicillium sp*.1 (287.66 µg/ml) and *Penicillium sp*.2 (261.66 µg/ml).

Production of extracellular enzymes like- Amylase and Cellulases by fungal endophytes was earlier recorded by Fouda *et al*.,(2015), Mani *et al*., (2015), D'Souza and Hiremath (2015), Uzma *et al*., (2016). Mani *et al*. (2018) carried out enzymatic investigations (including Amylase and Cellulase) on fungal endophytes isolated from *Aegle marmelos*.

The dominant isolates were tested for their relative efficacy in showing antagonistic activity against *Bacillus subtilis* (Gram positive bacterium) and *Escherichia coli* (Gram negative bacterium), employing disc diffusion method on nutrient agar plates containing bacterial suspension.

Table-2 reveals that all the organisms tested were capable in inhibiting Gram negative bacterium. *Penicillium sp*.1 as well as *Penicillium sp*.3 were found to be capable in inhibiting both the gram positive and gram negative bacteria, while *Penicillium sp*.2 failed to be show that property against the gram positive bacterium. Highest extent of inhibition against gram positive bacteria was noted in *Penicillium sp*.3 (zone of inhibition 0.354 cm^2) followed by *Penicillium sp*.1 (zone of inhibition 0.188 cm^2). Inhibiting Gram negative bacterium *Penicillium sp*. 1, however showed highest potency (zone of inhibition 0.439 cm^2 followed by *Penicillium sp*.3 (zone of inhibition 0.304 cm^2 and *Penicillium sp*.2 (zone of inhibition 0.125 cm^2).

A parallel set of standard Ampicillin disc was run to compare fungal isolates with the Ampicillin in showing antagonistic activity against the tested bacteria. The lower extent of inhibitory capability of the antimicrobial compounds isolated from culture filtrate from the fungal isolates due to their lower extent of purification i.e. the compounds were semi-purified preparation while the Ampicillin discs used represented the content of absolutely pure compound.

Similar search antimicrobial property of the fungal endophytes were took forwarded by several researchers (Tejesvi *et al*., 2007; Tejesvi *et al*., 2008; Fouda *et al*., 2015; Kandasamy *et al*., 2015; D'Souza and Hiremath, 2015; Yadav.2014; Khadka *et al*., 2024).

Fungal endophytes are considered as treasure trove of several bioactive compounds which may be categorised under either primary metabolites or secondary metabolites (Bhadra *et al*., 2022). Enzymes that are produced by the endophytes in present primary metabolites while the antibiotics they produced represent the secondary metabolites. Enzymes in fungal endophytes (Cellulase, Pectinase, Amylase etc.) play important role in the establishment of the endophytes within the host tissue as well as in recycling of plants remain in soil often the death of plant or shedding of plant parts. While the primary metabolites in endophytes are directly related with the growth of the organisms, the secondary metabolites are indirectly related for the existence of endophytes Since the industrial, social and agricultural impact of both such categories of metabolites of endophytes are immense and we are on the stepping stones in unravelling the endophytic association with the vast number of plants, extensive researches for obtaining information about the mystery of endophytic association with the various plants and to get efficient producers of such metabolites are essential in subsequent years of decades. However, conducting a thorough review of the literature and documentation regarding host plants, biosynthetic machineries, and their mechanism of action can yield valuable insights for potential

explorations and bioprospecting endeavours. This comprehensive understanding offers opportunities to harness endophytic fungi as a sustainable and renewable source of bioactive compounds, contributing to human health and addressing the challenges of antibiotic resistance (Gupta *et al*., 2023)

FIGURE:1

A) Leaves of Aegle marmelos B) Cork borer C) Leaf disc preparation



FIGURE:2

A)Growth of fungal endophytes from leaf dics B) Growth of fungal endophytes from stem bits

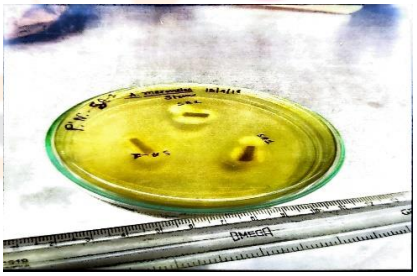



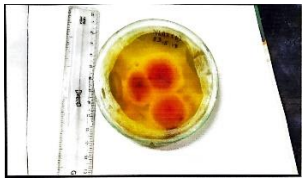
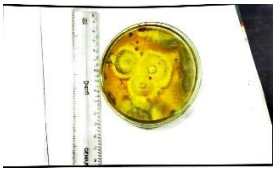
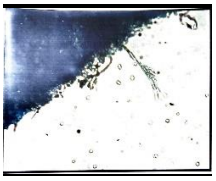
FIGURE:3 Penicillium sp.1

A)Lower surface of the colony B) Upper surface of the colony C) Conidiophore with matulae and Sterigmata

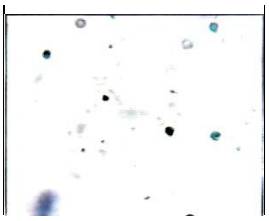


FIGURE:4 Penicillium sp2

A) Lower surface of the colony B) Upper surface of the colony C) Unbranched conidiophores

D) Enlarged conidia



E) Conidiophore with sterigmata and chain of conidia

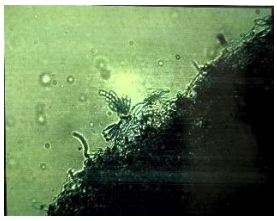
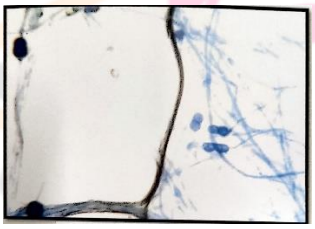


FIGURE:5 Fusarium redolens

A)Lower surface of the colony

B)Upper surface of the colony

C) Terminal and intercalary single and paired chlamydospores

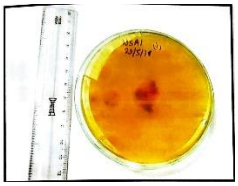


D)Macro conidia

E)Macro conidia

FIGURE:6 Penicillium sp3

A) Lower surface of the colony



B) Upper surface of the colony

C) Conidiophore with sterigmata and chain of conidia

D)Conidia

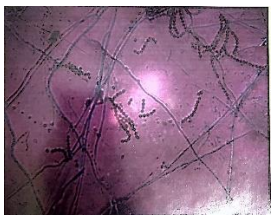
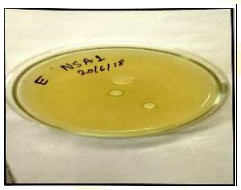


FIGURE:7

A) Zone of inhibition against Escherichia coli



B) Zone of inhibition against Bacillus subtilis

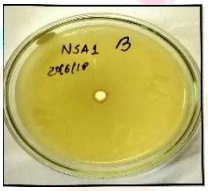


FIGURE:8 TOTAL AMYLASE CONTENT IN THE CULTURE FILTRATE OF THE DOMINANT FUNGAL ENDOPHYTES

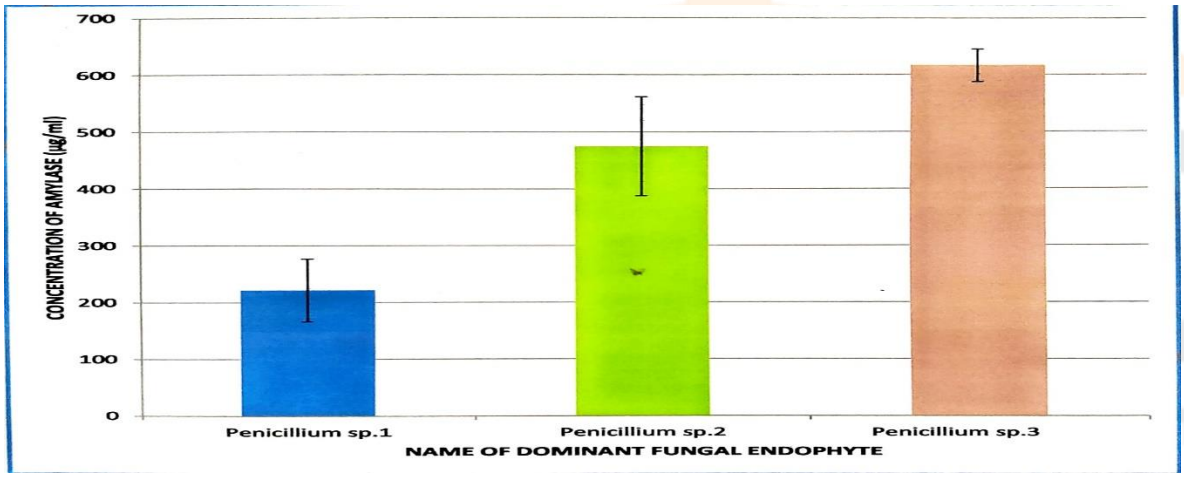


FIGURE:9 TOTAL C1 CELLULASE CONTENT IN THE CULTURE FILTRATE OF THE DOMINANT FUNGAL ENDOPHYTES

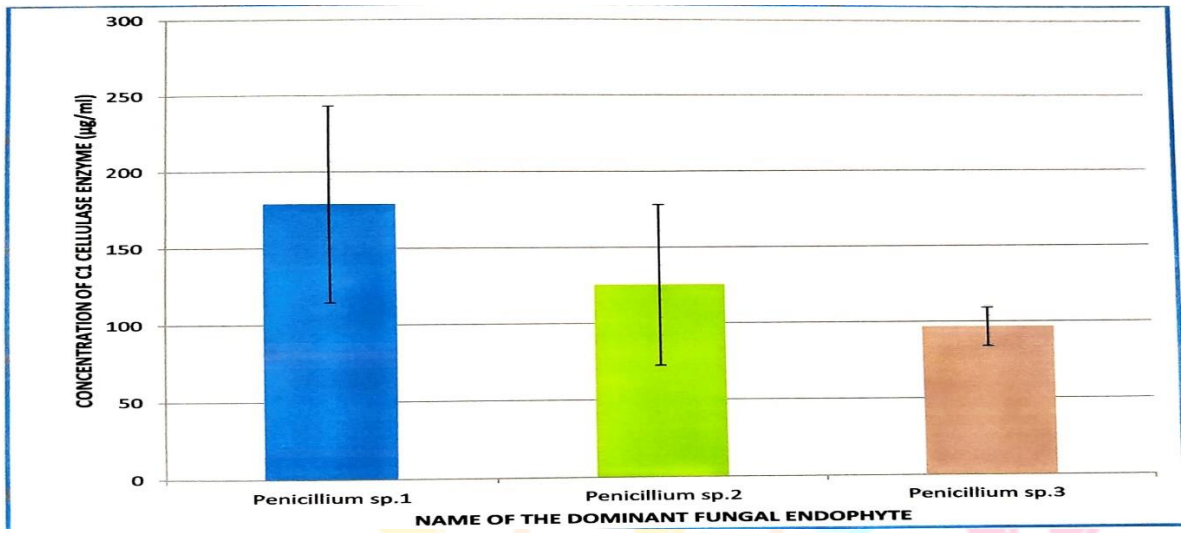


FIGURE:10 TOTAL Cx CELLULASE CONTENT IN THE CULTURE FILTRATE OF THE DOMINANT FUNGAL ENDOPHYTES

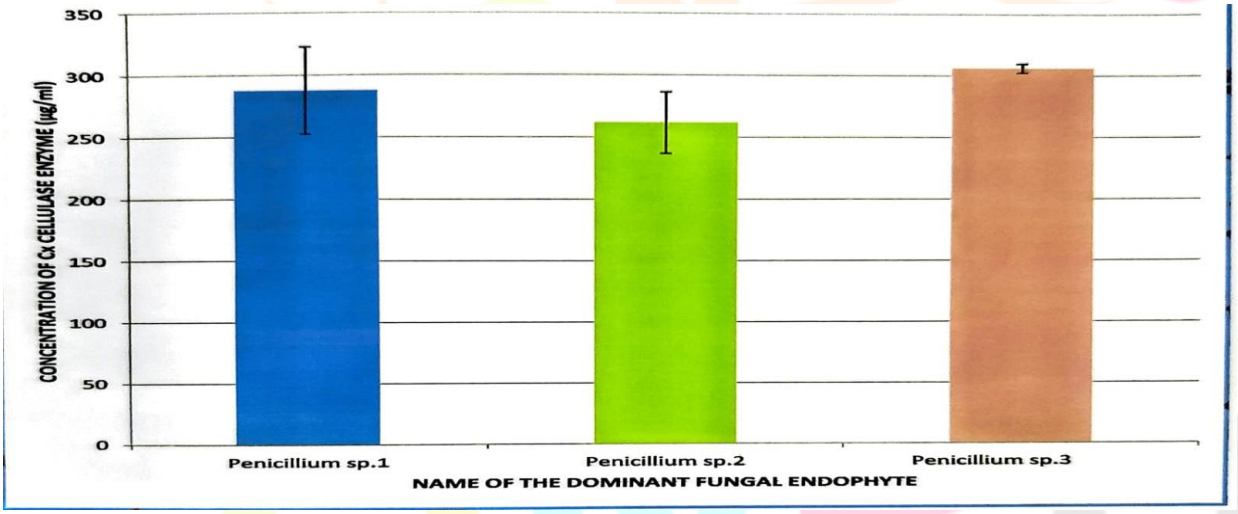


TABLE 1: DETERMINATION OF COLONIZATION FRQUENCY (C.F.%) OF THE FUNGAL ENDOPHYTES

Sl No.	Name of The Fungal Organism Isolated From The Plant	Tpe of Plant Material Used	Number Of discs or Bits Used	Number Of discs or Bits Showing Fungal Growth	Colonization Frequency(%)
1.	*Fusarium redolens*	LEAF DISC	12	2	16.66%
2.	*Penicillium sp.1*			3	58.33%
3.	*Penicillium sp.2*			7	46.66%
1.	Penicillium sp.3	STEM BITS	15	7	6.66%
2.	Fusarium redolens			1	25.00%

TABLE 2: ANTIBIOTIC ACTIVITY OF SOME DOMINANT ENDOPHYTES TESTED AGAINST GRAM POSITIVE AND GRAM NEGATIVE BACTERIA

Name of the Fungal Endophytes Tested	Zone of Inhibition(Cm2)By Dics Diffusion Method Against	
	Bacillus subtilis	*Eschrichia coli*
Ampicillin	3.603 cm^2	2.347 cm^2
Penicillium sp1	0.188 cm^2	0.439 cm^2
Paecilomyces sp.	--	0.125 cm^2
Penicillium sp2	0.354 cm^2	0.304 cm^2

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CONFLICTS OF INTEREST

The authors express no conflict of interest to carry forward this research finding to publish in this journal.

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