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Investigation on optimization parameters of tannase influencing Gallic acid production by fungi

Rajeswari Anburaj

Department of Microbiology, Bharathidasan University, Tiruchirapalli, Tamil Nadu, India

ABSTRACT

Gallic acid, a product of tannin degradation, finds application in various fields including biological activities such as antibacterial, antiviral and analgesic. The work was undertaken to produce gallic acid from banana flower stalk and coir pith using fungal system isolated from soil of different places. Optimization of incubation period for maximum fungal biomass was found to be 6 days and 9 days in banana flower stalk and coir pith amended broth respectively. Five fungal strains were chosen for Gallic acid production and the results reveal that banana flower stalk was found to be the better substrate than coir pith in producing gallic acid and fungal strains C_1 and I_2 were found to be better producers than I_3 , T_1 and T_3 . Therefore it is suggested that agricultural wastes containing tannin could be considered as alternative substrates for Gallic acid production.

Keywords: Tannin, Gallic acid, Plant source, Biomass, Fungi

INTRODUCTION

Agricultural wastes and agro industrial wastes are generally considered as the efficient and cost effective substrates for production of industrially important biological products. Therefore attempts have been made to find out some of the suitable agroresidues for the enzymatic conversion of their lignocellulosic fractions to value added products [1]. There has been an increasing trend toward efficient use of plant residues and helps to solve pollution problem, in turn their disposal would be minimized.

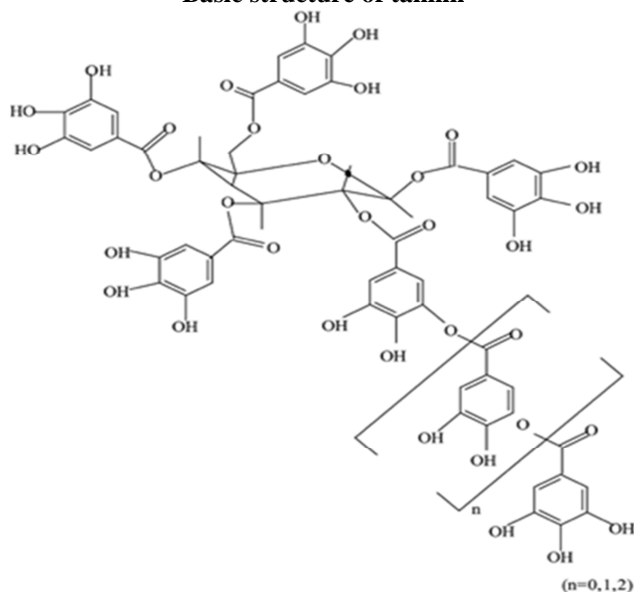
Tannins

The presence of a large number of phenolic hydroxyl groups in tannin enables it to form large complexes, mainly with protein and a lesser extent with other macromolecules like cellulose and pectin [2].

The group of phenolic compound known as tannins is clearly distinguished from other plant secondary phenolics in their chemical reactivities and biological activities [3]. Tannins are the polyphenolic secondary metabolites of plants, found in a large array of herbaceous and woody plants. On the basis of structural features, tannins are segregated into four major groups namely, gallotannins, ellagitannins, complex tannins and condensed tannins [4,5]. Hydrolysable tannins are soluble in water and their solution produces blue colour with ferric chloride [6].

Tannins are readily hydrolyzed chemically by acidification or biologically by an enzyme known as tannase. Tannase catalyses hydrolysable tannins like tannic acid releasing glucose and Gallic acid [7]. Gallic acid is a phenolic compound and finds application in various fields. Gallic acid production has been reported from myrobalan, tara, sumac and chinese tannins [8].

Basic structure of tannin



Ellagitannins is an anti-mutagenic and anti-carcinogenic starter. EA decreased the abnormal cell growth in human colon, prevents the development of cells infected with human papilloma virus which is related to cervical cancer and promotes the apoptotic growth of cancerous cells of the prostate [9]. Complex tannins are characterized by a C-C condensed structure of C- glycosidic tannins with flavan-3-ol catechin. Complex tannins are reported in Combretaceae and Myrtaceae members. An attempt has been made to develop a HPTLC method for quantitative estimation of Ellagic acid and Gallic acid in laboratory preparation of Triphala churnam [10].

Flower stalks of *Musa paradisiaca* are dumped off after harvesting the bunch. Coir pith from *Cocos nucifera* though utilized for several purposes, yet remains near the industrial site without complete utilization [11]. Research over past years resulted in the discovery of a great variety of tannin degraders including bacteria, fungi and yeast.

At present, gallic acid is produced industrially by acid hydrolysis of naturally occurring gallotannins. Due to high cost, low yield of desired product and production of large toxic effluent by acid hydrolysis, an enzyme based eco-friendly technology for gallic acid production is urgently required. The present study was attempted to study the characteristics of plant wastes, screen the fungal strains for biomass, optimize the incubation period and to investigate Gallic acid production from plant wastes.

MATERIALS AND METHODS

Collection of plant sources:

Plant wastes such as flower stalks of *Musa paradisiaca* (Plate 1) and coir pith from *Cocos nucifera* (Plate 2) were used as tannin sources. Banana flower stalks and coir pith were collected from Agriculture field and Coir pith industry, Solavandhan, respectively. They were dried under sunlight and processed by means of powdering and stored for further use.

Quality of tannin:

The plant sources were analyzed for the presence of tannin. Ten mg of dry powdered Flower stalks of banana and coir pith from coconut were treated separately with 3ml of Butanol-HCl reagent (95ml of n-Butanol and 5ml of concentrated HCl) were added and heated at 70°C on water bath for an hour. Appearance of pink colour was considered as the presence of tannin [12].

Quantity of tannin:

Preparation of Aqueous tannin Solution:

Five gram of powdered plant sources such as flower stalks of banana and coir pith from coconut were dissolved separately in 100 ml of distilled water and sterilized at 121 °C for 15 min. The solution was filtered using sterile cloth.

BUFFER A:

Glacial Acetic Acid -11.4 ml
Sodium Chloride - 9.86g

These were dissolved in 800ml of distilled water and then pH was adjusted to 4.9 using sodium hydroxide solution. The final volume was made upto 1000ml using distilled water.

FeCl₃

0.01M FeCl₃ in 0.01M HCl.

Two ml of BSA was taken into a centrifuge tubes to which 1ml of aqueous tannin solution was added. After mixing, the mixture was allowed to stand for 24 hrs at 4°C and the contents were centrifuged at 3000rpm for 15 minutes. The pellet was collected and redissolved in 4ml of SDS/TEA. After adding 1ml of FeCl₃, the mixture was subjected to vortex immediately. After 15 min, the absorbance was read at 510nm. Amount of tannin present in the plant sources was calculated using tannic acid as standard [13, 14].

Estimation of coloring matter in powdered plant sources

Whatman filter paper was heated at 105 °C for 1 hour. Then the filter paper was cooled and weighed. Three gram of sample was accurately weighed in the filter paper and was placed in soxhlet extraction unit. The coloring matter was extracted in the Ethanol-Benzene mixture at 80°C till no colour was appeared in the solvent mixture in the extractor. The filter paper was removed from the extractor and the solvent mixture was allowed to evaporate at room temperature. The filter paper was dried in a hot air oven at 105°C to a constant weight [15].

Coloring matter was estimated using the given formula

Weight of the empty filter paper = A

Weight of the filter paper + oven dry sample = B

Weight of the filter paper + oven dry sample after extraction = C

Coloring matter in the Dry Sample (%) = $\frac{B-C}{B-A} \times 100$

Fungal strains used in the study:

Fungi isolated from soil of tannery waste water disposal site (I₁, I₂, I₃, I₄ and I₅) coffee pulp disposal site (C₁, C₂, C₃, C₄ and C₅) and Tea leaves disposal site (T₁, T₂, T₃, T₄ and T₅) were maintained as slants in the culture collection of Department of Botany and Microbiology, Lady Doak College, Madurai. These 15 isolates were procured and used as strains for this study. Sub cultures were maintained in Potato Dextrose Agar slants [16].

| Chemicals | Gram/Litre |
|------------------------|------------|
| Infusion from potatoes | - 300g |
| Dextrose | - 20g |
| Agar | - 20 g |
| pH | - 5.5 |

Staining of fungal cultures:

Fungal cultures were stained using Lactophenol cotton blue, [17] and observed under microscope (COS LAB).

Screening of fungal isolates

The medium consists of the following Components:

| Chemicals | Gram/ Litre |
|---------------------------------------|-------------|
| KH ₂ PO ₄ | - 4.38 |
| NaNO ₃ | - 8.79 |
| MgSO ₄ .7H ₂ O | - 0.88 |
| CaCl ₂ .2H ₂ O | - 0.088 |
| MnCl ₂ .6H ₂ O | - 0.018 |
| NaMoO ₄ .2H ₂ O | - 0.0088 |
| FeSO ₄ .7H ₂ O | - 0.012 |
| pH | - 5.5 |

Fungal culture broth was prepared and impregnated with 0.5% tannic acid, powdered flower stalk of banana and pith of coconut husk separately. They were sterilized at 15 lb for 30 minutes and cooled. The flasks were incubated at 37°C for 12 days and at every 3 days interval, dry biomass was estimated [18,19]. The fungal mat was filtered from the broth in a pre-weighed filter paper and dried for 2hr at 70°C. Thereafter, it was weighed again and dry biomass was calculated. Fungal isolates with high biomass with short incubation period were chosen for further studies.

Gallic acid production from plant sources using screened fungal strains:**Estimation of fungal Biomass:**

Fungal culture broth was prepared and impregnated with 0.5% flower stalk of banana and pith of coconut husk separately. They were sterilized at 15 lb for 30 minutes. After cooling the broth was inoculated with screened fungal strains such as C₁, I₂, I₃, T₁, T₃ and incubated at 37°C for five days. At every 24 hours interval fungal biomass was estimated.

Determination of pH:

pH was determined for the culture broth at every 24 hours interval for 5 days using Elico pH meter.

Estimation of Gallic acid:

At 24 hours interval, 0.5 ml of fermented broth was taken and 0.3 ml of methanolic rhodanine was added followed by 0.5M KOH (0.2 ml). Gallic acid content was estimated by measuring the absorbance at 520nm. The gallic acid content in the broth was calculated using standard graph prepared with gallic acid (10µg - 100 µg) [14].

Detection of Gallic acid by TLC:

TLC plates were prepared by using Silica gel. The plates were dried under hot air oven at 60°C for 20 min. At 24 hours interval, the culture broth was spotted on TLC plates using gallic acid as standard. Butanol(40): Acetone(10): Water(10) mixture which was used as a solvent. TLC plate was placed on solvent chamber. The solvent was allowed to run for 1 hour. The plates were dried under hot air oven at 60°C for 15 min and was sprayed using Methanol(30) : Water(70) : FeCl₃ (0.1g) mixture [20]. The plates were dried under hot air oven at 60°C for 10 min. The R_f value was calculated by

$$R_f \text{ value} = \frac{\text{Distance moved by solute}}{\text{Distance moved by solvent}}$$

RESULTS AND DISCUSSION

Plant wastes are defined as an unusable or unwanted substance or material produced during growth of plant cellulose, hemicellulose, pectin, starch etc from bulk of plant wastes. As they are organic in nature, these wastes can be recycled using microorganisms through various biological process into products. All plants contain tannin with varying quality and quantity, which could be utilized through several biotechnological processes for Tannase and Gallic acid, the product of tannin hydrolysis [21]. Therefore, an attempt has been made to find out gallic acid production from agriculture residues like banana flower stalk and coir pith.

Banana is a major cash crop. After the harvest of the fruits completely, the plant leaves, rhizome and stem are left in the field without proper utilization [22]. On the other hand the inflorescence of banana bears numerous flower stalks (Plate 1). These flower stalks are yet another waste material which are also disposed of without proper utilization which could be utilized for industrially important products. Coir pith (Plate 2) is an agrowaste from coir industry. It is a light weight spongy material found between the coir fibres of a coconut. It generally accounts for 50-60% of total weight of the husk. Total generation of coir pith in India is estimated to be around 0.5 million tonnes / year. In India, many innovative practices are followed to utilize this coir pith as a renewable resource [11]. Such unutilized materials could be utilized for product recovery through microbial degradation.

For this study, 4 random samples (fresh flowers and husk of banana tree and coconut tree respectively) were collected and weighed. Flower stalks were separated and weighed before and after drying. Pith particles were separated manually and weighed before and after drying. The results tabulated are presented in (Table 1). It is evident that dry biomass of coconut coir pith has tenfold increase over that of flower stalk.

The plant wastes were pulverized and used as tannin source. They were brown in colour and the extracts recovered after solvent extractions were yellow and pink in banana. Flower stalk and coir pith respectively (Table 2). Colouring matter was found to be low (4.4%) in banana flower stalk powder and high (7.1%) in coir pith. Presence of tannin was confirmed by qualitative test (Plate 3). Quantity of tannin was high (1.44 mg/ml) in banana flower stalk and low (0.59 mg/ml) in coir pith. pH of the plant powders was found to be alkaline (Table 2).

These plant biomass were utilized as carbon source supplemented with simple salts for the growth of fungal strains. Isolates from soil of tannery waste water disposal site (Plate 4) soil of coffee pulp disposal site (Plate 5) and soil of tea leaves disposal site (Plate 6) were mentioned in figure. These cultures were procured and purified in potato dextrose agar medium. The characteristics of the 15 fungal strains were studied and presented in Table 3, 4 and 5. The fungal strains varied in colour and colony morphology.

The fungal strains were stained using Lactophenol cotton blue and observed under microscope (Plate 7, 8 and 9). Fungal strains 1, 3, 4 and 5 from tannery waste water disposal sites could be species of *Aspergillus* and strain 2 could be *Fusarium* species. Fungi was isolated from tannery effluent and were identified them as *Aspergillus flavus*, *Fusarium sp.* and *Trichoderma sp.* [23].

Fungal strains isolated from coffee pulp disposal site were identified and strain 1 could be *Thamnidium sp.*, strain 2 could be *Aspergillus sp.* and strains 3, 4 and 5 could be *Rhizopus sp.* (Plate 8). Lactophenol cotton blue stained fungal strains of Tea leaves disposal site (Plate 9) were microscopically identified and strains 1 and 4 could be *Fusarium sp.* and strains 3 and 5 could be species of *Aspergillus*. *Aspergillus niger* and *Candida utilis* was effective in converting valonea tannins (*Quarceps aegilops*) into ellagic acid. [24]. Various groups of researchers and scientists isolated and screened microorganisms from forest soil and plant samples [25].

In the present study, the fungal strains procured were screened for their efficient growth and maximum biomass production (Plate 10). They were inoculated in broth supplemented with 0.5% of banana flower stalk and coir pith. Dry biomass were estimated at 3 days interval for a period of 12 days. Regardless of the plant wastes used, it is observed that as the incubation period proceeded there was an increase in fungal biomass. But, the fungal biomass was found to be maximum on 6th day in banana flower stalk amended broth (Figure 1, 2 and 3) and it was maximum on 9th day in coir pith amended broth (Figure 4, 5 and 6). The fungal biomass was comparatively higher in coir pith than banana flower stalk amended broth. It is also noted that, banana flower stalk was preferred by fungal strains isolated from coffee pulp waste and coir pith was preferred by fungal strains isolated from tannery waste disposal site. *Aspergillus tamarii* IMI388810 (B), a tannic acid degrading fungus, was isolated from soil inundated by effluent of a tannery [26].

From this primary screening study, fungal strains C₁, I₂, I₃, T₁ and T₃ were selected for gallic acid production. Various authors elaborated the structural features of Gallic acid [27]. Regarding the optimization of growth period, the fungal biomass was found to be maximum on 6th day and 9th day in banana flower stalk and coir pith amended broth respectively. In coir pith, the increase in fungal biomass was very meagre between 6 and 9 days. Most of the tannase and gallic acid production using agro-wastes were carried out for a period of 4-7 days [1, 25, 28]. So, further study on gallic acid production from plant wastes such as banana flower stalk and coir pith using, screened fungal strains (C₁, I₂, I₃, T₁ and T₃) was carried out for a period of 5 days (Plate 11).

pH profile of the culture broth is presented in Figure 7 and 8. The initial pH (0 hour) was found to be 5.5 and it decreased during 24 hours of incubation followed by an increase towards 48 hours (2 days). Thereafter the pH increased in broth supplemented with banana flower stalk. It is also seen that all the fungal strains exhibited almost similar trend (Figure 7). Production of gallic acid was maximum in medium containing 3% tannin and at pH 5.5 after 36 hours [13]. But in coir pith supplemented broth, the fungal strains showed a varying pH profile (Figure 8). This change in pH profile could be due to the production of acids by the fungal systems using plant substrates and further conversion of acids to other metabolites [25].

The fungal strains utilized the plant wastes present in the broth for their growth and regardless of the wastes used, the fungal biomass increased with an increase in incubation period (Figure 9 and 10). Belur and Mugeraya (2011) reported the microorganisms involved in tannin degradation and suggested that the optimum fermentation conditions and regulatory mechanism of tannase enzyme production seems to vary from one organism to other [29]. The dry fungal biomass was maximum (0.17g/l) in broth inoculated with fungal strains I₂ and minimum (0.07g/l) in banana flower stalk amended broth inoculated with fungal strain C₁. It is also evident from Figure 9, that fungal strains C₁ and T₁ were found to be slow growers. Belmares *et al* reported that during the fermentation of "tarbus", *A. niger* PSH degraded 35% of tannin content and the total protein was significantly less than the values obtained with creosote bush.

An increase in fungal biomass on par with incubation period was observed in coir pith supplemented broth (Figure 10). Over a period of 5 days, fungal strains T₁ and T₃ contributed more biomass than other strains. Agro residues and forest products are generally considered the best source of tannin rich substrate [30]. Generally tannins are accumulated as secondary metabolites in the bark and heart wood of plants. Although these substances have negligible value for plant growth, some microorganisms use these compounds (tannins) as a nutrient for their growth by hydrolyzing them into products. Tannase is the enzyme responsible for tannin degradation and Gallic acid is a hydrolytic product of tannin. This acid has different industrial uses [21].

The selection of a substrate for industrially important fermented product generation depends upon its availability and cost. Various groups [31, 1, 25] have reported Gallic acid production from several plant parts. Sawant *et al* (2010) determined gallic acid in *Phyllanthus emblica* Linn. dried fruit powder by HPTLC [32]. But there are no reports on

the utilization of banana wastes and coir pith for Gallic acid production. Moreover the availability of these substrates are huge in Madurai District. Gallic acid was optimized and produced by bio- transformation of plant tannin under solid- liquid fermentation [33].

So, in the present work, gallic acid production from plant wastes such as banana flower stalk and coir pith was attempted using fungal strains. Gallic acid content was estimated at 24 hours interval using the chromogen rhodanine (Plate 12). A method for assay of microbial tannase based on the formation of chromogen between gallic acid and rhodanine [34]. The trend in Gallic acid production varied between banana flower stalk (Figure 11) and coir pith (Figure 12). There was no acid production by the fungal strains during 48 hours (2 days) of incubation. Except fungal strains C_1 and I_3 other strains have not produced Gallic acid, even on 4 days of incubation in broth having banana flower stalk (Figure 11). Thereafter, all the strains produced Gallic acid and it was maximum ($2500\mu\text{g/ml}$) and minimum ($260\mu\text{g/ml}$) in broth inoculated with fungal strains I_2 and C_1 respectively.

An HPTLC method was developed for the quantitative estimation of gallic acid, rutin and quercetin from aqueous and other ethanolic extract of eight medicinal plants [35]. There was an increase in acid production after 48 hours (3days) followed by a gradual decrease in Gallic acid production in coir pith amended broth by the fungal strains, except strain C_1 which produced more Gallic acid even after 96 hours(4 days) of incubation(Figure 12). It is evident from the earlier studies that suitability of substrate is important for the growth of microbes as well as for the product release [1]. Gallic acid is one of the major products of tannin degradation which is readily utilized as substrate by oxidative break down to simple aliphatic acids which enter citric acid cycle [36].

Plate 1: Flower stalks of banana used as tannin source



Plate 2: Coir pith of coconut husk used as tannin source



The ability of microorganisms to degrade tannins has been attributed to production of tannase, an important enzyme capable of catalysing Gallotannins to Gallic acid and Glucose [20]. Interestingly, analysis of the culture broth using TLC method reveals the presence of Gallic acid. There was no Gallic acid production upto 2nd day. Thereafter it was noted in fungal strains inoculated broth having plant wastes. R_f value of the separated Gallic acid was calculated and presented in Table.6. The results are in coincidence with the trend in Gallic acid content of broth (Figure 11 and 12).i.e., no acid production during early period of incubation and acid production in later period of incubation.

HPTLC analysis of six varieties of *Eleusine coracana*, was carried out by Singh *et al.*, 2008 [37]. Gallic acid is a substrate for the chemical and enzymatic synthesis of propyl gallate, used as antioxidants in fats and oils as well as in beverage industry [38].

Table 1: Mean* weight of flower stalks of banana and coir pith of coconut

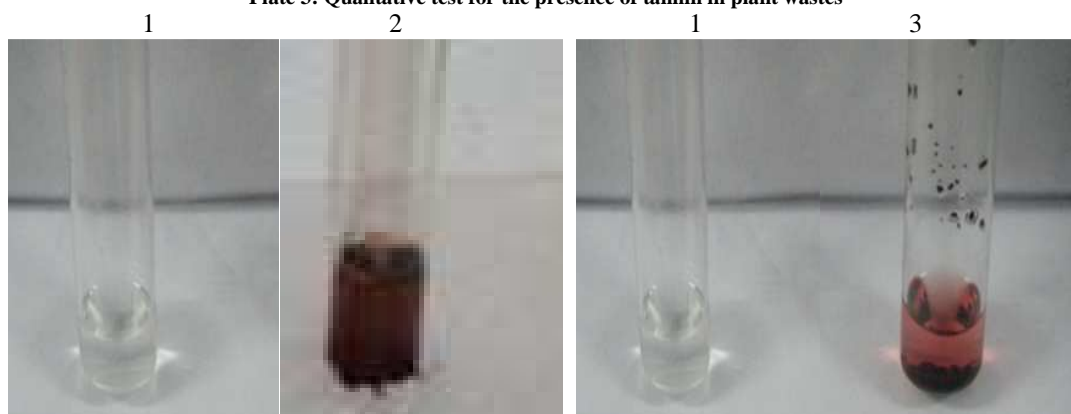
| S.No | Description | Weight (gm) | |
|------|-------------------------|---------------------|-------------------|
| | | Banana flower stalk | Coconut coir pith |
| 1. | Whole flower/husk | 435 | 334 |
| 2. | Fresh flower stalk/pith | 148 | 200 |
| 3. | Dry flower stalk/pith | 13.0 | 140 |

*Mean of four random samples

Table 2: Physico – Chemical characteristics of plant wastes used as tannin sources

| S.NO | CHARACTERISTICS | FLOWER STALKS | COIR PITH |
|------|-------------------------------|---------------|---------------|
| 1. | Colour of the powder | Dark brown | Sandy brown |
| 2. | Colour of the solvent extract | Yellow | Pink |
| 3. | Coloring matter | 4.4% | 7.1% |
| 4. | Quality | Brown | Moderate pink |
| 5. | Quantity of tannin (mg/ml) | 1.44 | 0.593 |
| 6. | pH | 7.80 | 7.14 |

Plate 3: Qualitative test for the presence of tannin in plant wastes



1. Butanol+Hcl reagent
2. Butanol + Hcl reagent + banana flower stalk powder
3. Butanol+Hcl reagent + coir pith

Plate 4: Pure cultures of fungi isolated from soil of tannery waste disposal site (I Strains)

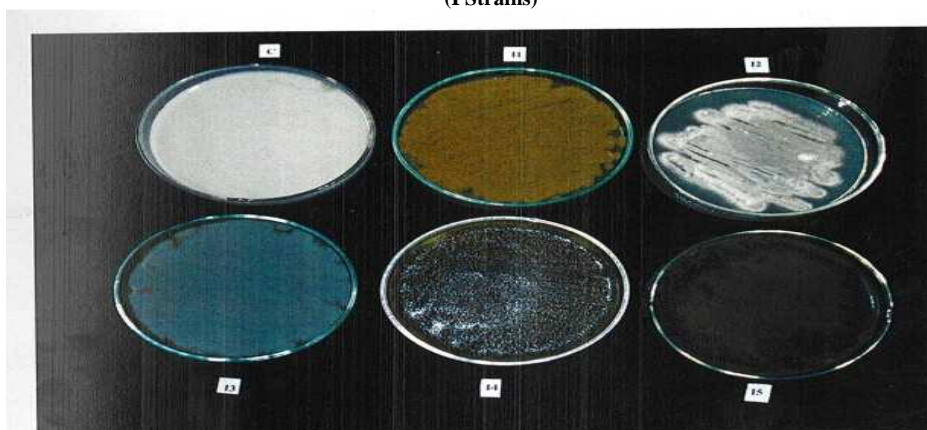


Plate 5: Pure cultures of fungi isolated from soil of Coffee pulp disposal site (C Strains)



Plate 6: Pure cultures of fungi isolated from tea leaves disposal site (T Strains)



Table 3: Colour and colony characteristics of I strains

| S.No | FUNGAL STRAINS | COLOUR | COLONY CHARACTERISTICS |
|------|----------------|-----------------|--|
| 1. | I ₁ | Greenish yellow | Light green changed to greenish yellow |
| 2. | I ₂ | Dark Green | White colonies changed to dark green |
| 3. | I ₃ | Dark Green | Green colony changed to dark green |
| 4. | I ₄ | Black | White colonies produced black spores |
| 5. | I ₅ | Black | White colonies produced black spores |

Table 4: Colour and colony characteristics of C strains

| S.No | FUNGAL STRAINS | COLOUR | COLONY CHARACTERISTICS |
|------|----------------|--------------|--------------------------------------|
| 1. | C ₁ | White spores | White cottony colonies |
| 2. | C ₂ | Dark Green | White colonies changed to dark green |
| 3. | C ₃ | Dark Green | White colonies changed to dark green |
| 4. | C ₄ | Dark Green | White colonies changed to dark green |
| 5. | C ₅ | Dark Green | White colonies changed to dark green |

Table 5: Colour and colony characteristics of T strains

| S.No | FUNGAL STRAINS | COLOUR | COLONY CHARACTERISTICS |
|------|----------------|-------------|---|
| 1. | T ₁ | Dark Green | White cottony colony produced dark green spores |
| 2. | T ₂ | Light Green | Greenish cottony colony produced greenish yellow spores |
| 3. | T ₃ | Light Brown | White cottony growth produced light brown spores |
| 4. | T ₄ | Dark Green | White cottony growth produced dark green spores |
| 5. | T ₅ | Black | White cottony growth produced black coloured spores |

Plate 7: Microscopic view of stained I strains

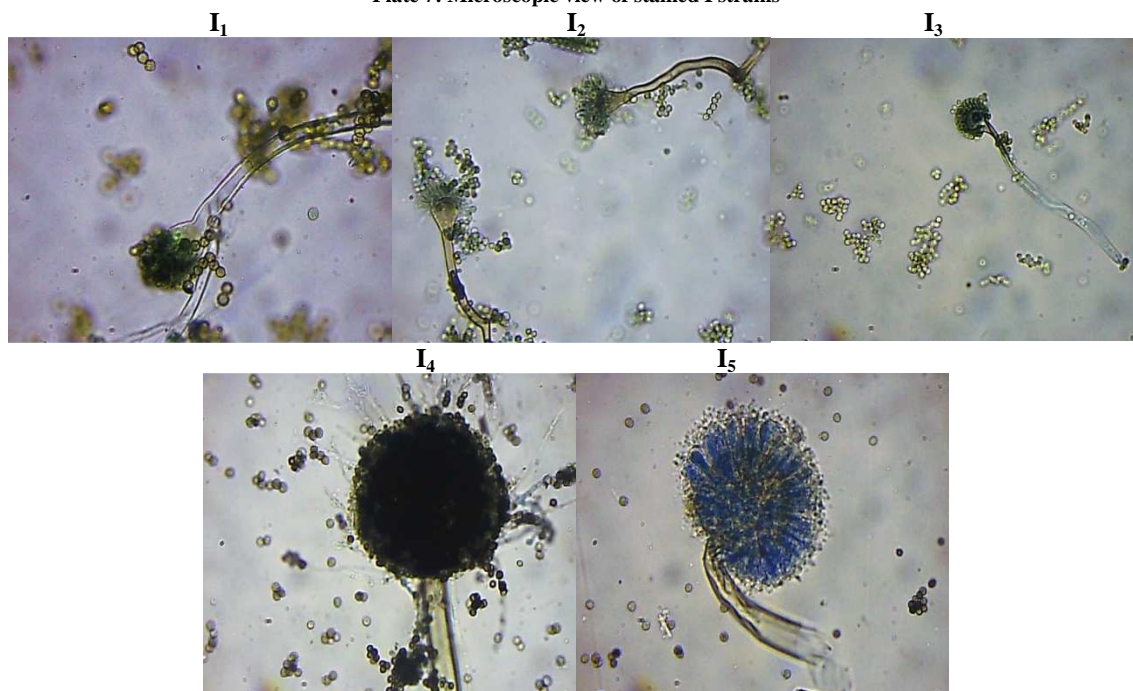


Plate 8 : Microscopic view of stained C strains

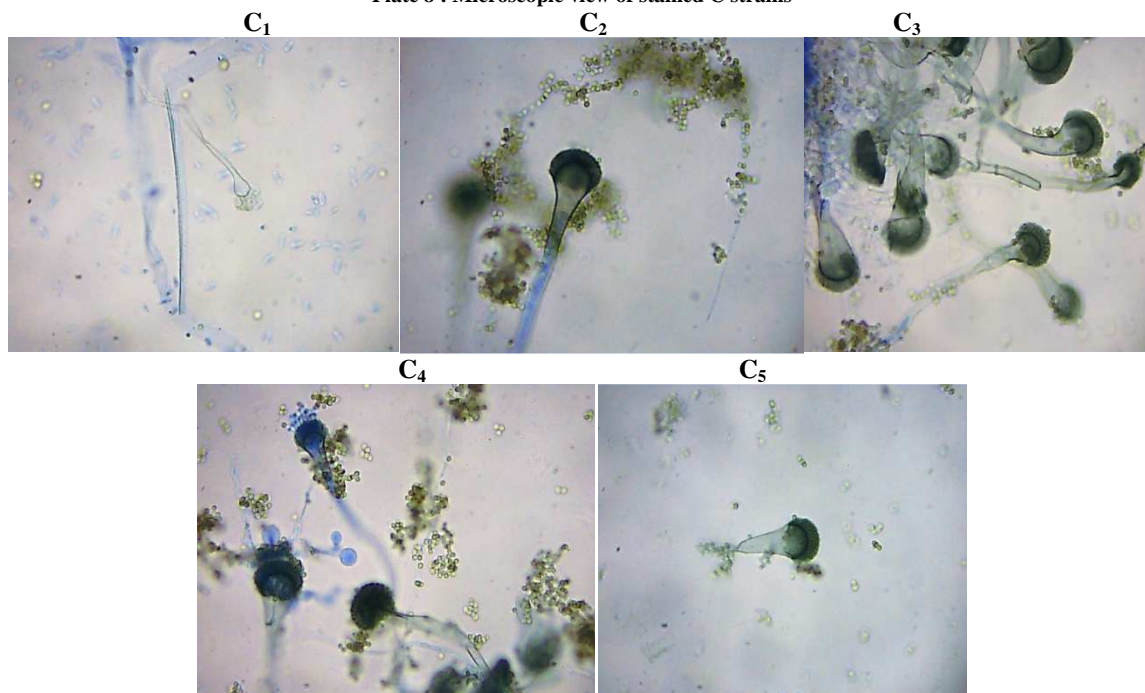


Plate 9: Microscopic view of stained T strains

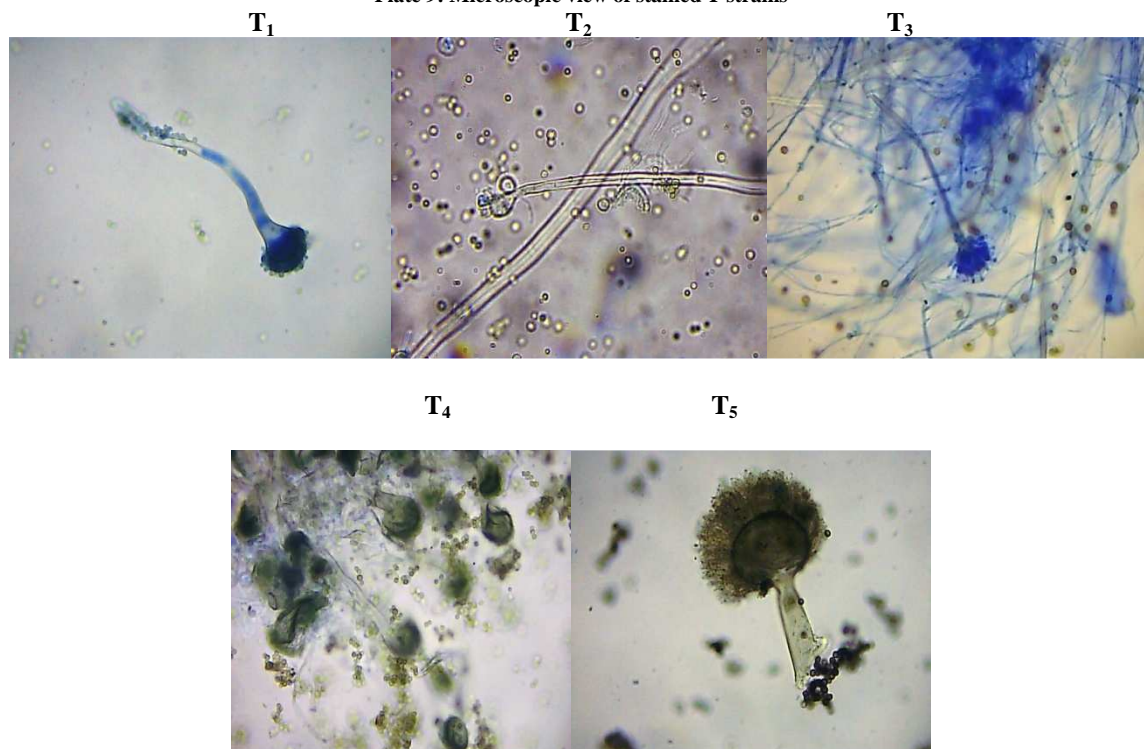
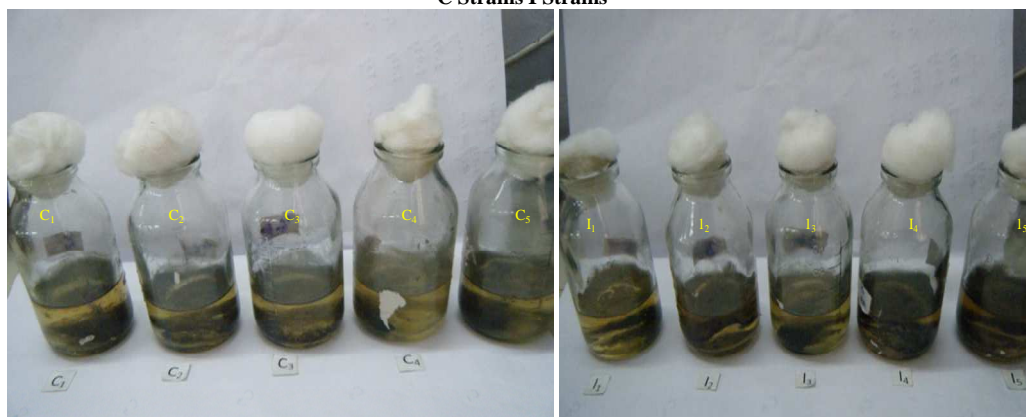


Plate 10: Primary screening of fungal strains grown in broth for 12 days

C Strains I Strains



T Strains



Figure 1: Dry biomass (g/l) of fungal strains inoculated broth having banana flower stalk (0.5%) C strains

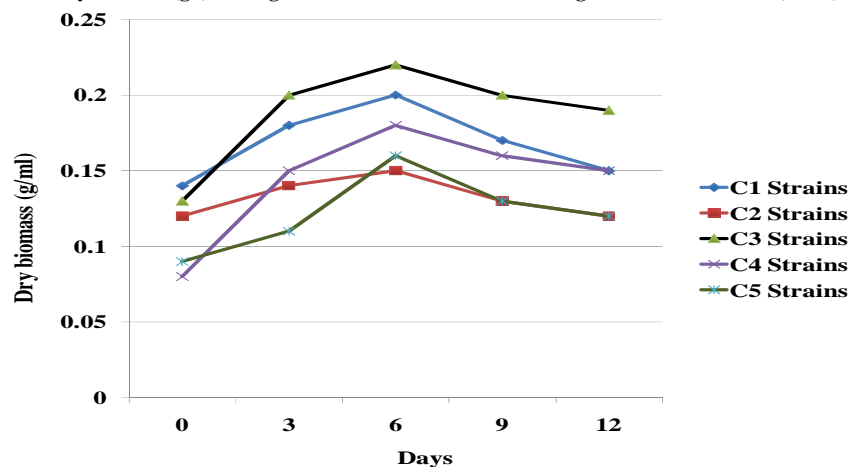


Figure 2: Dry biomass (g/l) of fungal strains inoculated broth having banana flower stalk (0.5%) I strains

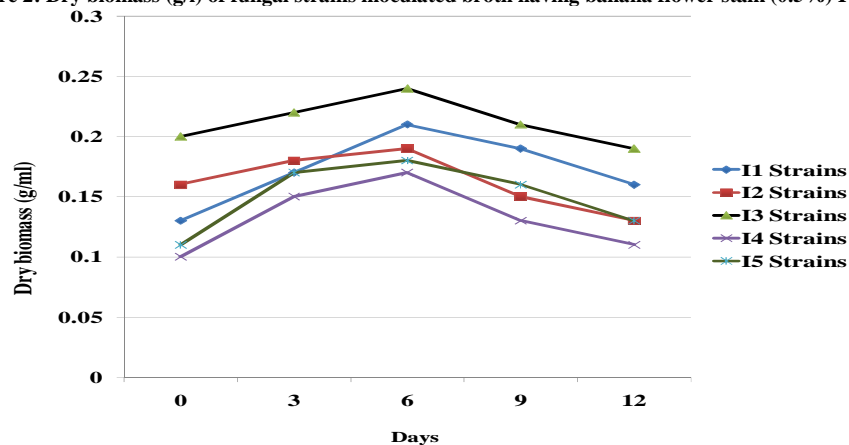


Figure 3: Dry biomass (g/l) of fungal strains inoculated broth having banana flower stalk (0.5%) T strains

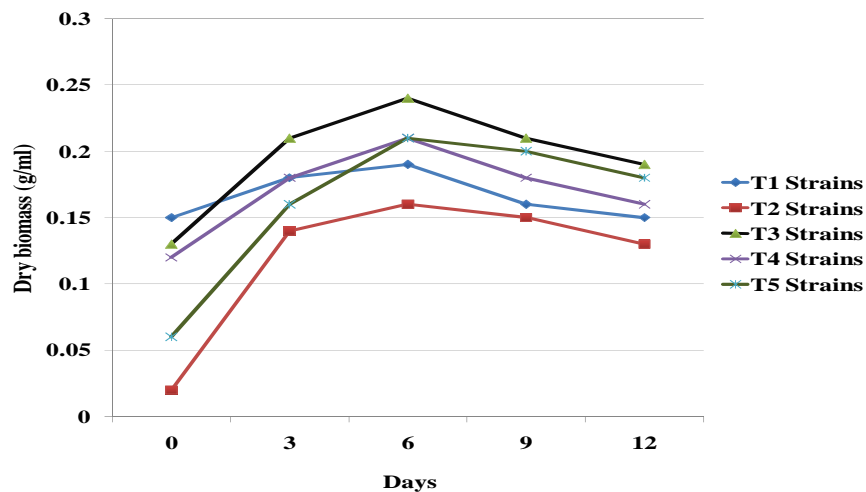


Figure 4: Dry biomass (g/l) of fungal strains inoculated broth having coir pith(0.5%) C strains

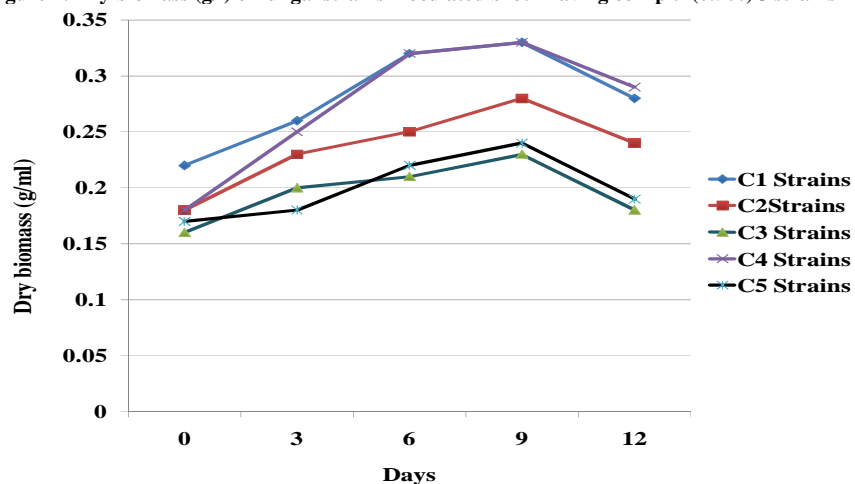


Figure 5: Dry biomass (g/l) of fungal strains inoculated broth having coir pith(0.5%) I strains

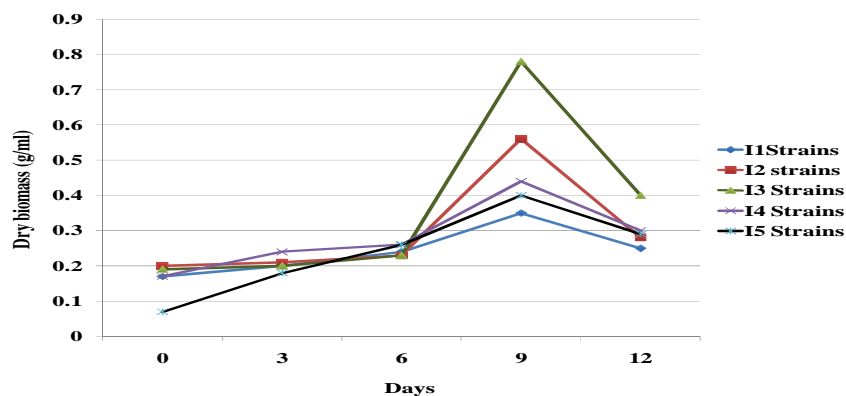


Figure 6: Dry biomass (g/l) of fungal strains inoculated broth having coir pith (0.5%) T strains

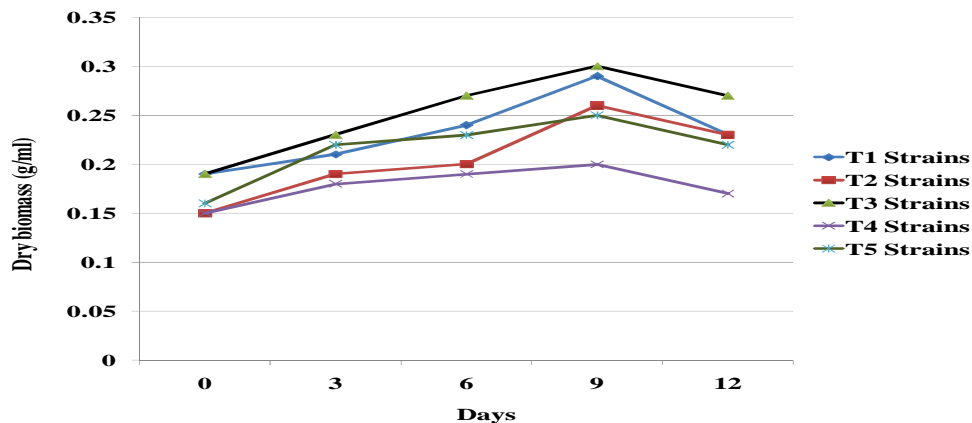


Plate 11: Secondary Screening of fungal strains grown in broth for 5 days

1

2



1. Flower stalks of banana inoculated separately with 5 (screened) fungal strains

2. Coir pith of coconut inoculated separately with 5 (screened) fungal strains

Figure 7: pH profile of fungal strains inoculated broth having banana flower stalk(0.5%)

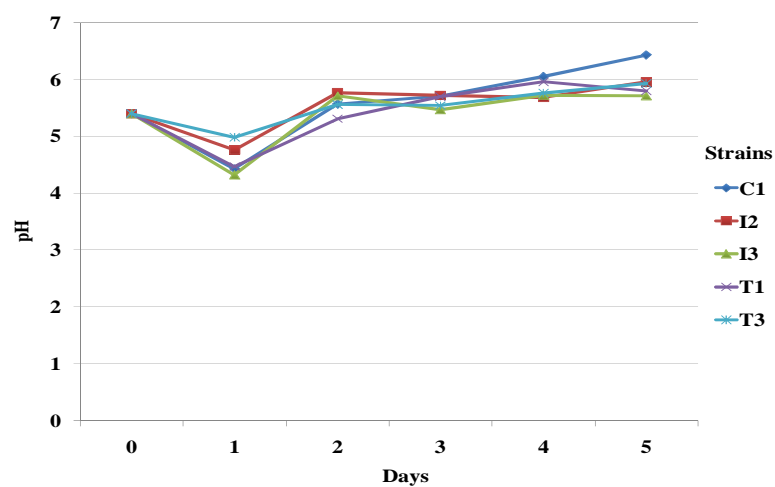


Figure 8: pH profile of fungal strains inoculated broth having coir pith(0.5%)

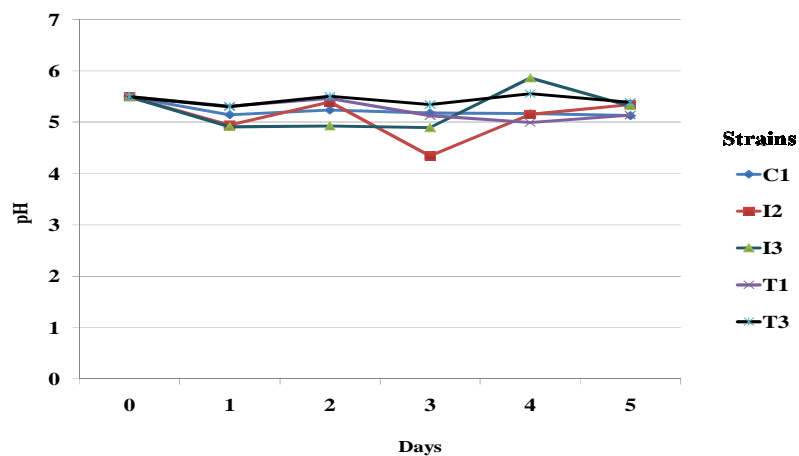


Figure 9: Dry biomass (g/l) of fungal strains inoculated broth having banana flower stalk (0.5%)

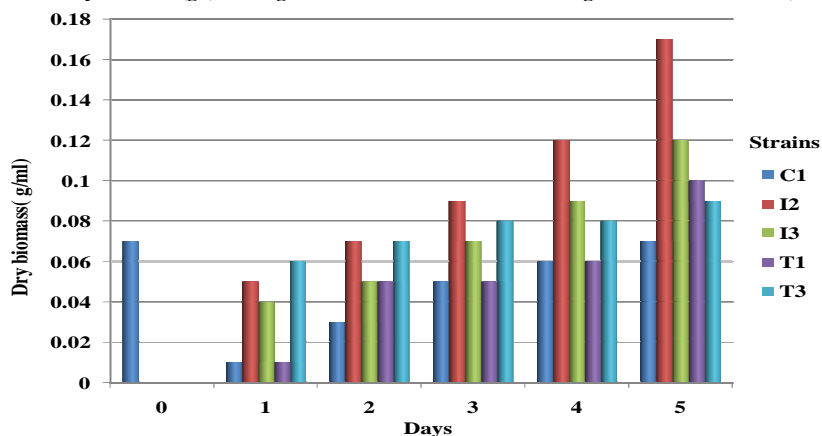


Figure 10: Dry biomass (g/l) of fungal strains inoculated broth having coir pith(0.5%)

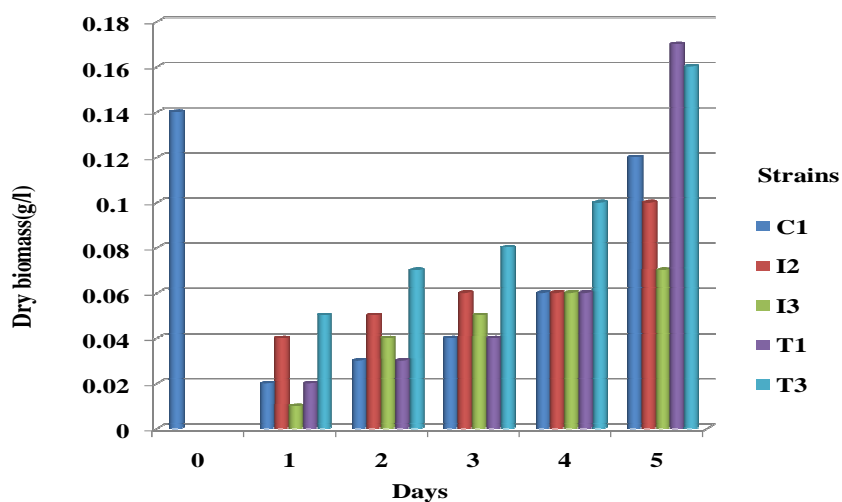
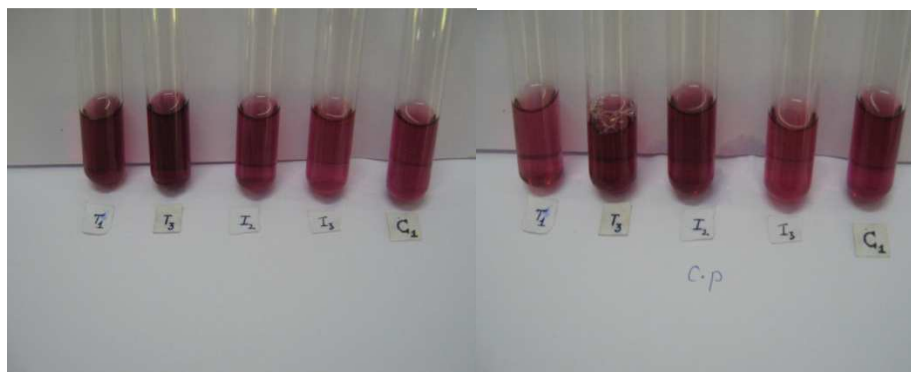
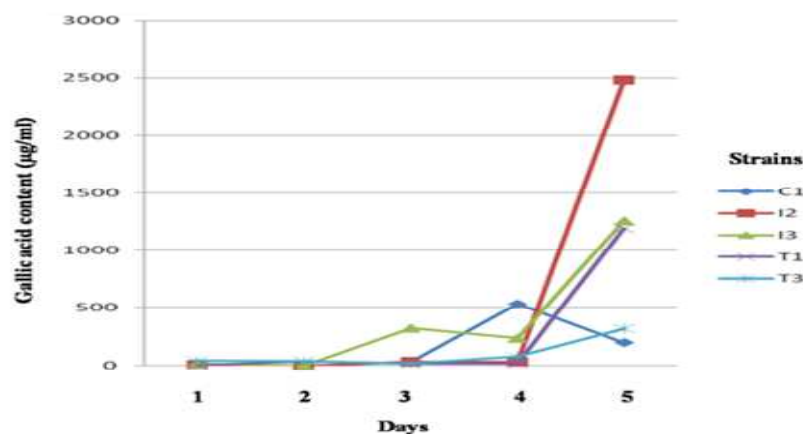
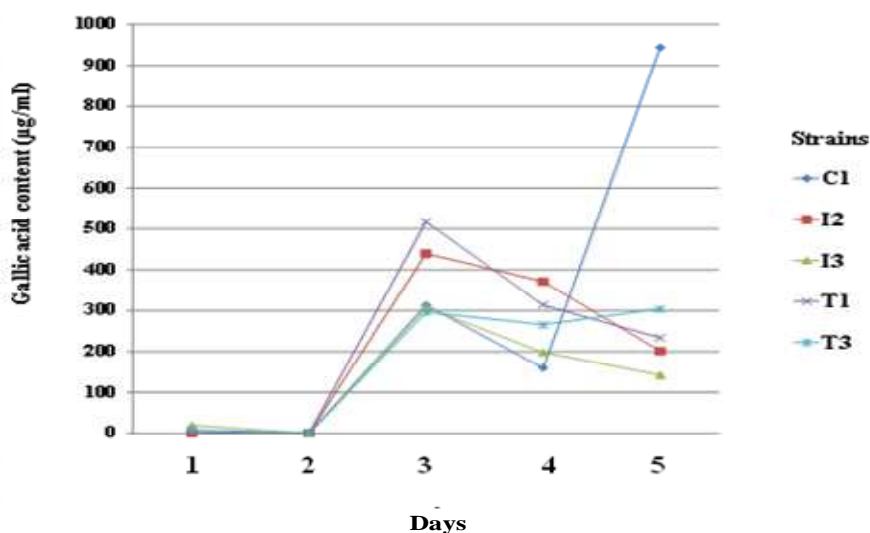
Plate 12: An Evidence for the presence of Gallic acid in Fungal strains inoculated plant wastes.
Flower stalks of bananaCoir pith from coconut

Figure 11: Gallic acid content ($\mu\text{g/ml}$) of fungal strains inoculated broth having banana flower stalkFigure 12: Gallic acid content ($\mu\text{g/ml}$) of fungal strains inoculated broth having Coir pithTable:6 R_f value of fungal strains inoculated culture filtrates

| S.NO | Plant Sources | Strains | R_f Value | | | | | |
|------|--------------------------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| | | | 0 th | 1 st | 2 nd | 3 rd | 4 th | 5 th |
| 1. | Flower Stalks of <i>banana</i> | Gallic acid | 0.90 | - | - | 0.89 | 0.87 | 0.93 |
| | | C ₁ | - | - | - | - | - | 0.92 |
| | | I ₂ | - | - | - | - | - | 0.91 |
| | | I ₃ | - | - | - | 0.82 | - | 0.93 |
| | | T ₁ | - | - | - | - | - | 0.91 |
| | | T ₃ | - | - | - | - | - | 0.92 |
| | | | | | | | | |
| 2. | Coir Pith from <i>coconut</i> | C ₁ | - | - | - | 0.90 | 0.70 | 0.89 |
| | | I ₂ | - | - | - | 0.90 | 0.85 | 0.92 |
| | | I ₃ | - | - | - | 0.89 | 0.79 | 0.89 |
| | | T ₁ | - | - | - | 0.88 | 0.82 | 0.94 |
| | | T ₃ | - | - | - | 0.86 | 0.81 | 0.91 |
| | | | | | | | | |
| | | | | | | | | |

CONCLUSION

Therefore, it is suggested that agricultural wastes containing tannin could be considered as alternative substrates for Gallic acid production. Utilization of the locally available cost free substrates, involving efficient fungal degraders, would be beneficial to produce Gallic acid to meet indigenous demand, export this product to other countries. Furthermore, the efficient use of cheap and enormously available plant waste are considered to be an alternative source for the production of value added products including organic acids.

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