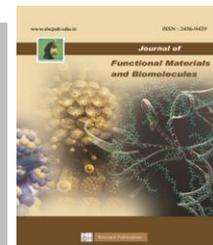




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Effect of novel vegetable peel waste culture medium on the growth of industrially beneficial fungi (*Aspergillus niger* and *Penicillium chrysogenum*)

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Abstract

The present study was aimed to study the effect of Vegetable peel waste Novel culture medium on the growth of Industrially Beneficial Fungi (*Aspergillus niger* and *Penicillium chrysogenum*). The Onion peel waste was the good low cost environmental free medium for the cultivation of an Industrially Beneficial Fungi *Penicillium chrysogenum* and *Aspergillus niger* when compared to the Garlic peel waste. It was also found that the Garlic peel has showed an average growth for *Penicillium chrysogenum* and the fungi *Aspergillus niger* did not showed any growth on Garlic peel medium. Garlic peel waste medium is the good source for *Penicillium chrysogenum* cultivation but not for the cultivation of *Aspergillus niger*. In comparison, the Onion peel waste act as a good source for the cultivation of Industrially Beneficial Fungi when compared to the Garlic peel waste.

Keywords: Onion peel waste, Garlic peel waste, *Aspergillus niger* and *Penicillium chrysogenum*.

1 Introduction

India is agriculture based country and the farming system have its special place in the universe from the ancient days. Among the various agricultural products produced in India, vegetable cultivation has occupied the major role due to its daily requirement for cooking purposes and the presence of various nutrients. India ranked second in the production of vegetables and marching towards the first rank because of the highest need due to the increase in population. Besides its beneficial role as a nutritive source in food, the peel wastes generated from the vegetable creates nuisance to the environment. The management of wastes has been considered as hot topic in various debates during recent times. The wastes generated from unspoiled vegetables are not harmful to the human health and environment but it is very essential to find a way for utilizing the vegetable peel wastes in a beneficial way. The disposal of unwanted agriculture wastes in the beneficial way is very challenging in the present scenario. It is sure that the designed research work will provide one best way for utilizing the waste vegetable peels in a beneficial way.

Microorganisms are the invisible creates which are ubiquitous in nature and present everywhere in this universe. Various researchers narrated the microorganisms as the “Double edged sword” because of its beneficial and harmful activities. The major categories of the microorganisms in this universe are bacteria, archaea, fungi, algae and protozoa. Among the all microbial types, the fungi take part an imperative role in the productions of various industrial products which are repeatedly used by human beings in their routine life. Fungi are the ubiquitous eukaryotes present in air and soil. Based on its, cellular nature, fungi are classified into two major groups viz., Molds and Yeast. The molds are multicellular and the yeast is unicellular in nature. The mold is widely spread in the air and soil in the form of spores and the presence of yeast is very less in an environment.

The percentage of beneficial role of fungi is equivalent to the harmful effects. The word “Double edged sword” is more suitable for the fungi when compared to other microorganisms. The fungal mold has finds its special role in the production of antibiotics, industrial enzymes, organic acids, organic solvents, biopesticides, biocontrol agents against phytopathogens and several polymers [1]. The first antibiotic Penicillin was discovered from the fungi *Penicillium notatum* by Sir Alexander Fleming, *Trichoderma viride* act as a biocontrol agent and *Beauveria bassiana* & *Metarhizium* sp. act as a Bioinsecticide. The Basidophytic fungi Mushrooms are well known for its role as a food for human diet and the presence of various bioactive compounds which was regularly used by the pharmacological industries. The role of fungal mold in the bioremediation of various wastewaters and textile dyes were investigated by various researchers. Unfortunately, the usage of fungal mold was limited in the wastewater treatment due to the production of mycelial mats and replaced by the bacteria due to its high efficient bioremediation in short duration.

Culture medium is an environment which favors the growth of microorganisms by providing various chemical

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nutrients and growth factors. Based on its properties, culture medium is classified into six types viz., Basal medium, Enriched medium, Enrichment medium, Differential medium, Selective medium and Transport medium. The nature of the culture medium was liquid, semi-solid or solid and technically the solid medium are referred as the agar medium and the liquid medium are called as broth. Sabouraud's dextrose agar which was familiarly abbreviated as SDA is the common culture medium used for the cultivation of fungi. Other than SDA, the commonly used culture medium are Martin Rose Bengal agar (RBA) and Potato dextrose agar (PDA) [2]. All the culture medium are rich in the chemical nutrients like carbon sources (usually carbohydrate sugars), nitrogen sources, phosphorous sources and sulphur sources for the utilization of microorganisms. The carbohydrate sugar Dextrose is considered as an important ingredient of fungal culture medium. The cost of commercial culture medium is rising day by day due to the highest need of researchers and less availability of raw material sources. In developing countries like India, tax system like Goods Service Tax (GST) increases the cost of culture medium and causing economic troubles for researchers and industrialists. For managing the economical issues, we are in need to find an alternative source for the cultivation of microorganisms. Utilization of enormous agricultural waste materials for the cultivation of microorganisms particularly industrially important fungi is the correct remedy for the replacement for synthetic culture medium. On that line, the present research was designed [3, 4]. The present research was designed to utilize the vegetable peel wastes for the cultivation of two industrially beneficial fungi (*Aspergillus niger* and *Penicillium chrysogenum*).

2 Experimental

2.1. Collection of Vegetable peels

Two types of vegetable peels viz., Onion peel waste and Garlic peel waste which was selected for the present study was collected from the Vegetable market in Tirupattur, Vellore district, Tamil Nadu, India. The collected vegetable peels were dried, powdered with the help of mixer, packed in plastic cover and stored at room temperature.

2.2. Platting of Industrially Beneficial Fungi in Vegetable peels waste medium

2.2.1 Inoculum preparation

The suspension of 3 days old cultures of Industrially Beneficial Fungi (*Aspergillus niger* and *Penicillium chrysogenum*) were used to study the qualitative and quantitative growth analysis. They were prepared in saline solution (0.85 % sodium chloride). The fungal cultures were inoculated into 50 ml of saline and incubated at room temperature for 5 hours.

2.2.2. Qualitative growth analysis of Industrially Beneficial Fungi in Vegetable peel wastes

The effect of two different vegetable peel wastes viz., Onion peel waste and Garlic peel waste on the growth of

two industrially beneficial fungal isolates viz., *Aspergillus niger* and *Penicillium chrysogenum* was studied in the present research. An amount of 4.0 grams of Vegetable peel waste and 1.5 grams of Agar - agar was added into the 100 ml of distilled water and sterilized by Autoclaving at 121 °C for 15 minutes. After sterilization, the Vegetable peel waste agar was cooled and then 0.1 ml of industrially important fungal inoculum (*Aspergillus niger* and *Penicillium chrysogenum*) was added. The prevalence of other fungal isolates in UG Biochemistry Laboratory and PG Biochemistry Laboratory of Sacred Heart College (Autonomous), Tirupattur, was also studied in the Vegetable peel waste agar. The inoculum added medium was incubated at room temperature for 3 to 6 days. The presence or absence of the fungal growth on Vegetable peel waste agar was visually observed and recorded from 3rd day to 6th day.

3 Results and Discussion

Agricultural wastes particularly the peels of vegetables and fruits are creating the nuisance to the environment in the universe. In most parts of the world, the regions around the vegetable market are looked like colorful garbage due to the presence of plenty of vegetable and fruit peels. The dumping of peels are also sometimes results in spreading of disease by indirect mechanism through insect vectors. In older days, before the invention of microbial culture medium, researchers utilized various food products like milk, fruits and vegetables for the cultivation of microorganisms and that time they are referred as Natural medium. In order to recycle the various vegetable peels, the present study was designed to formulate the low cost medium using the peel wastes of Onion and Garlic for cultivation of two industrially beneficial fungi, *Aspergillus niger* and *Penicillium chrysogenum*.

Two different fungal molds viz., *Aspergillus niger* and *Penicillium chrysogenum* was selected for the present research because that two fungal isolates were highly utilized for the production of various industrial microbial products which are required by the human beings for various purposes. *Aspergillus niger* is a Black mould which was commonly referred as "Common Laboratory Contaminant". It is an easily isolated fungi which was commonly occurred as black colour colonies in Sabouraud's dextrose agar. It is both harmful (for humans and plants) and beneficial. Some of the industrial products like Bioethanol, Citric acid, Amylase, Cellulase and Pectinase are produced by *Aspergillus niger*. It is also playing a major role in the bioremediation of Textile dyes, Textile wastewater, Tannery effluent, Heavy metals, Sugar mill effluent and Paper mill effluent. The *Penicillium* sp. is a well know fungi for its Antibiotic production. Sir Alexander Fleming discovered the inhibitory activity of *Penicillium notatum* against the Gram positive bacteria *Staphylococcus aureus* and introduced the first antibiotic Penicillin (cell wall inhibitor) for this world. In older days, the antibiotic Penicillin was prepared from *Penicillium notatum* but nowadays Penicillin was prepared from *Penicillium chrysogenum* due to its high efficiency and yield. Naturally,

the fungi *Penicillium* sp. are not harmful to the humans and but in some cases it causes some diseases to plants.

3.1. Growth of Industrially beneficial fungi other fungal isolates on Onion peel waste medium

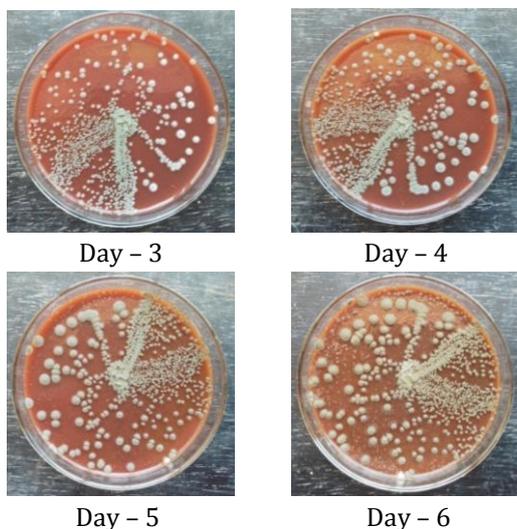


Figure - 1: Growth of *Penicillium chrysogenum* on Onion peel waste medium

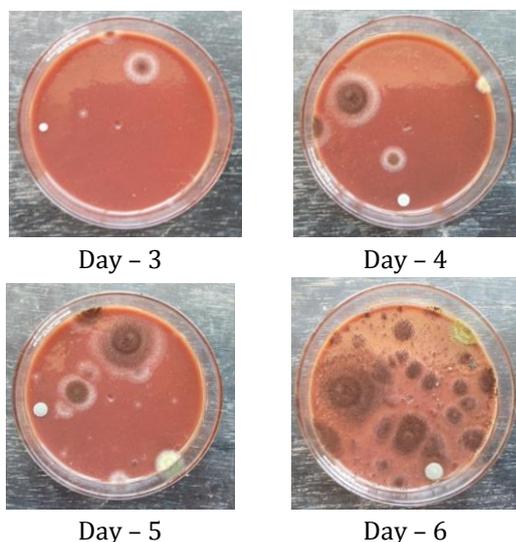


Figure - 2: Growth of *Aspergillus niger* on Onion peel waste medium

The growth of industrially beneficial fungi other fungal isolates on Onion peel waste medium was studied in the present research and the findings were furnished in Table - 1 and Figure - 1 to Figure - 4. The culture of *Penicillium chrysogenum* and *Aspergillus niger* are inoculated in the centre of the Onion peel waste agar medium. The presence of fungi in the UG Biochemistry Laboratory and PG Biochemistry Laboratory was also studied in the Onion peel waste agar medium by Open plate method.

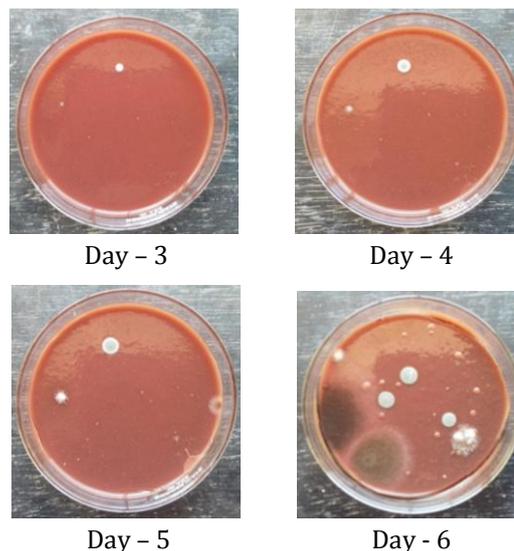


Figure - 3: Prevalence of fungal colonies in PG Biochemistry Laboratory on Onion peel waste medium



Fungal colonies at Day - 4



Fungal colonies at Day - 5



Fungal colonies at Day - 6

Figure - 4: Prevalence of fungal colonies in UG Biochemistry Laboratory on Onion peel waste medium

The *Penicillium chrysogenum* exhibited the good growth on the Onion peel medium than the *Aspergillus niger* and other fungal isolates. In Day - 3, the growth of *Penicillium chrysogenum* was observed in full plate without any other fungal contamination. The same strategy was continued on 4th day, 5th day and 6th day. It is very rare to see the single fungal culture in one plate without any other

fungal contamination and the rare case was observed in our research in Onion peel medium.

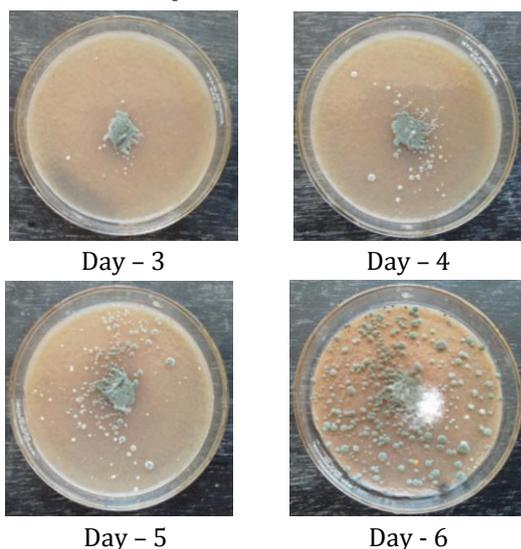


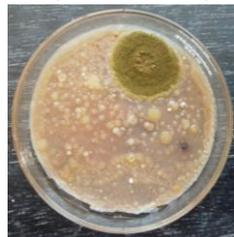
Figure - 5: Growth of *Penicillium chrysogenum* on Garlic peel waste medium



No fungal growth but bacterial growth was observed on Day - 4



No fungal growth but bacterial growth was observed more on Day - 5 than Day - 4



Aspergillus flavus growth and more bacterial growth was observed on Day - 6

Figure - 6: Growth of fungal isolates in PG Biochemistry Laboratory on Garlic peel waste medium

A single colony of *Aspergillus niger* was started to grow on the Onion peel medium at 3rd day. On the 4th day, two colonies of *Aspergillus niger* are started to grow on the Onion peel medium. One *Aspergillus niger* colony is small in size and another colony is large in size. Fungi belongs to other species was also started to grow on the edges of Onion peel medium. On 5th day, two colonies of *Aspergillus niger* are matured in its growth. Some development was also noticed in the other fungal colony which was grown on the edges of the Onion peel waste medium. On 6th day, the *Aspergillus niger* colonies are spreaded all over the plates of the Onion peel waste medium. The fungal colony which was grown on the edges of the Onion peel waste medium was matured as *Aspergillus flavus*.

In the Onion peel waste medium plate which was opened in the PG Biochemistry Laboratory, a single small fungal colony was started to grow on the 3rd day. In Day - 4, some maturation was observed on the single fungal colony. On 5th day, Single colony was developed again more. One fungi was also started to grow on the edges. On the 6th day, the fungal population was average. The fungi which was grown on the edges was emerged as *Aspergillus niger*.



No fungal growth was observed from Day - 3 to Day - 5



Little amount of fungal growth was observed on Day - 6

Figure - 7: Growth of fungal isolates in UG Biochemistry Laboratory on Garlic peel waste medium.

In the Onion peel waste medium plate which was opened in the UG Biochemistry Laboratory, no fungal colony was started to grow on the 3rd day. In Day - 4, very little fungal growth was observed. On 5th day, little fungal growth was observed. On 6th day, more *Penicillium chrysogenum* colonies, little *Aspergillus niger* colonies and very little *Aspergillus flavus* colonies were observed.

3.2. Growth of Industrially beneficial fungi and other fungal isolates on Garlic peel waste medium

The growth of two industrially beneficial fungal isolates viz., *Penicillium chrysogenum* and *Aspergillus niger*, and other fungal isolates on Garlic peel waste medium was

studied in the present research and the findings were tabulated in Table – 2 and Figure – 5 to Figure - 7. Among the two industrially beneficial fungal isolates tested,

Penicillium chrysogenum alone showed the growth in Garlic peel waste medium and the *Aspergillus niger* did not exhibited any growth on the test medium.

Table – 1: Growth of Industrially beneficial fungi other fungal isolates on Onion peel waste medium

Industrially Important Fungi	Onion Peel Waste Medium			
	Day - 3	Day - 4	Day - 5	Day - 6
<i>Penicillium chrysogenum</i>	The growth of <i>Penicillium chrysogenum</i> was observed in full plate without any other fungal contamination.	The growth of <i>Penicillium chrysogenum</i> was observed in full plate without any other fungal contamination.	The growth of <i>Penicillium chrysogenum</i> was observed in full plate without any other fungal contamination.	The growth of <i>Penicillium chrysogenum</i> was observed in full plate without any other fungal contamination.
<i>Aspergillus niger</i>	A single colony of <i>Aspergillus niger</i> was started to grow.	Two colonies of <i>Aspergillus niger</i> are started to grow. One colony is small in size and another colony is large in size. Fungi belongs to other species was also started to grow on the edges of Onion peel medium.	Two colonies of <i>Aspergillus niger</i> are matured in growth. Some development was also noticed in the other fungal colony which was grown on the edges of the Onion peel waste medium.	The <i>Aspergillus niger</i> colonies are spreaded all over the plates of the Onion peel waste medium. The fungal colony which was grown on the edges of the Onion peel waste medium was matured as <i>Aspergillus flavus</i> . The fungal population was average. The fungi which was grown on the edges was emerged as <i>Aspergillus niger</i> . More <i>Penicillium chrysogenum</i> colonies, little <i>Aspergillus niger</i> colonies and very little <i>Aspergillus flavus</i> colonies were observed
Open plate in PG Laboratory	A single small fungal colony was started to grow.	Some maturation was observed on the single fungal colony.	Single colony was developed again more. One fungi was also started to grow on the edges.	
Open plate in UG Laboratory	No fungal growth was observed.	Very little fungal growth was observed.	Little fungal growth was observed.	

A cluster of *Penicillium chrysogenum* growth was observed in the centre of Garlic peel waste medium plate on the 3rd day without other fungal contamination. In Day – 4, growth of *Penicillium chrysogenum* was more than Day - 3 without other fungal contamination. On 4th day, growth of *Penicillium chrysogenum* was more than Day - 4 without other fungal contamination. On 5th day, the *Penicillium chrysogenum* growth covered the full plate with the contamination of white fungal colony.

The growth of *Aspergillus niger* on Garlic peel waste medium was analyzed from Day – 3 to Day – 6. The

Aspergillus niger does not showed any growth on the Garlic peel waste medium till 6th day.

In the Garlic peel waste medium plate which was opened in the PG Biochemistry Laboratory, no fungal growth was observed on the 3rd day. On 4th day, no fungal growth was observed but the bacterial growth was observed in mucoid form. The bacterial culture was may be *Klebsiella pneumoniae* because that bacteria is the only one which exhibited mucoid colonies. On 5th day, no fungal growth was observed but the bacterial growth was observed in mucoid form more than the Day - 4. On 5th day, The mucoid bacterial growth covered the complete part of

the medium and the large single green colony of *Aspergillus flavus* was observed on Day - 6. In UG Biochemistry Laboratory, the opened Garlic peel waste medium plate does not showed any microbial growth on Day - 3 to Day - 5 but it showed Yeast like non - mycelial colonies, a small pinch of fungal mold colonies on 6th day.

Table - 2: Growth of Industrially beneficial fungi other fungal isolates on Garlic peel waste medium

Industrially Important Fungi	Garlic Peel Waste Medium			
	Day - 3	Day - 4	Day - 5	Day - 6
<i>Penicillium chrysogenum</i>	A cluster of <i>Penicillium chrysogenum</i> growth was observed in the centre of plate without other fungal contamination.	Growth of <i>Penicillium chrysogenum</i> was more than Day - 3 without other fungal contamination.	Growth of <i>Penicillium chrysogenum</i> was more than Day - 4 without other fungal contamination.	The <i>Penicillium chrysogenum</i> growth covered the full plate with the contamination of white fungal colony.
<i>Aspergillus niger</i>	No fungal growth was observed.	No fungal growth was observed.	No fungal growth was observed.	No fungal growth was observed.
Open plate in PG Laboratory	No fungal growth was observed.	No fungal growth was observed but the bacterial growth was observed in mucoid form. The bacterial culture was may be <i>Klebsiella pneumoniae</i> .	No fungal growth was observed but the bacterial growth was observed in mucoid form more than the Day - 4.	The mucoid bacterial growth covered the complete part of the medium and the large single green colony of <i>Aspergillus flavus</i> was observed.
Open plate in UG Laboratory	No fungal growth was observed.	No fungal growth was observed.	No fungal growth was observed.	Yeast like non - mycelial colonies, a small pinch of fungal mold colonies was observed.

Literarily, our study is the first study which tested the use of Onion peel waste and Garlic peel waste for the cultivation of *Penicillium chrysogenum*, *Aspergillus niger* and other fungal isolates. There were no previous studies on the present research. But, interestingly some research findings are available on the fruit peel wastes. The role of fruit peel wastes for the cultivation of industrially important fungi was reviewed by Saranraj *et al.* [5]. Anbu *et al.* [6] observed that the fruit peel wastes of Mango, Jack fruit, Pine apple, Green banana, Yellow banana and Pomegranate has showed an excellent source for the cultivation of industrially important fungal isolates like *Aspergillus niger*, *Penicillium chrysogenum* and *Rhizopus stolonifer*. On that line, the present study was designed to utilize the two vegetable peel wastes *viz.*, Onion peel waste and Garlic peel waste for the cultivation of two industrially important fungal isolates *viz.*, *Penicillium chrysogenum* and *Aspergillus niger*.

In the present study, the culture of *Penicillium chrysogenum* and *Aspergillus niger* are inoculated in the centre of the Onion peel waste agar medium. The presence of fungi in the UG Biochemistry Laboratory and PG Biochemistry Laboratory was also study in the Onion peel waste agar medium by Open plate method. It is very rare to see the single fungal culture in one plate without any other fungal contamination and the rare case was observed in our research in Onion peel medium with *Penicillium chrysogenum*. The Garlic peel waste medium also exhibited the growth of *Penicillium chrysogenum* but it was failed to show the growth of *Aspergillus niger*. Singh and Singh [7] and Smita Behera and Nibha Gupta [8] investigated the role of two vegetable (Drumstick and Carrot) wastes and one fruit waste (Papaya) for the cultivation of economically important Basidiomycetes fungi Mushroom. They exhibited to role of Mushroom vegetables in the production of low cost medium.

4 Conclusions

Onion peel act as a good source for the growth of *Penicillium chrysogenum* than *Aspergillus niger*. It is very rare to see the single fungal culture in one plate without any other fungal contamination and the rare case was observed in our research in Onion peel medium with *Penicillium chrysogenum*. Formulation of Selective medium for fungi is very challenging in the present scenario. This present research will provide a way for the production of low cost Selective medium for *Penicillium chrysogenum* and other fungal isolates. Garlic peel showed an average growth for *Penicillium chrysogenum* and the fungi *Aspergillus niger* did not showed any growth on Garlic peel medium. Finally, in our research we concluded that the Onion peel waste was the good low cost environmental free medium for the cultivation of an industrially important fungi *Penicillium chrysogenum* and *Aspergillus niger*. Garlic peel waste medium is the good source for *Penicillium chrysogenum* cultivation but not for the cultivation of *Aspergillus niger*. In comparison, the Onion peel waste act as a good source for the cultivation of Industrially Beneficial Fungi when compared to the Garlic peel waste. The present study will provide the concept of "Zero waste economy" to this society.

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