

## Lignolytic and phosphate solubilizing efficiency of fungal species isolated from Arecanut husk waste

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**ABSTRACT:**

A wide range of microorganisms are capable of degrading lignin present in the Arecanut husk waste for their growth. The present study has been undertaken to isolate lignolytic and phosphate solubilizing fungi from Arecanut husk waste. Isolation of fungi by using serial dilution method and direct soil plate method was employed. Estimation of lignolytic and phosphate solubilizing efficiency of isolated fungi was carried out. Fifteen varieties of fungal species were isolated from the Arecanut husk waste. Of these fifteen fungal species isolated seven showed lignolytic property and nine showed phosphate solubilizing activity. The lignolytic ability shows that the zone of clearance was higher in *Gibberella fujikuroi* ( $0.8 \pm 0.02$  cms), medium in *Aspergillus niger* (strain-1) ( $0.6 \pm 0.06$  cms) and very low activity in *Aspergillus flavus* ( $0.2 \pm 0.02$  cms). The Phosphate solubilizing ability shows that the zone of clearance was higher in *Aspergillus terreus* ( $0.8 \pm 0.03$  cms), medium in *Botrytis cinerea* ( $0.5 \pm 0.08$  cms) and very low activity in *Aspergillus niger* (Strain-2) and Unidentified-3 ( $0.1 \pm 0.03$  cms). The Phosphate solubilizing activity was higher in Unidentified-2 ( $550 \pm 8.50$   $\mu$ g/ml), medium in Unidentified-1 ( $530 \pm 10.00$   $\mu$ g/ml) and very low activity in *Aspergillus niger* (strain-2) ( $40 \pm 2.52$   $\mu$ g/ml). Total biomass yield of the fungi showed the highest rate of growth for *Aspergillus niger* (strain-1) ( $0.31 \pm 0.02$  gm/days), medium growth rate in unidentified-2 ( $0.29 \pm 0.01$  gm/days) and very low growth rate in unidentified-1 ( $0.06 \pm 0.01$  gm/days) in twelve days incubation. An effective fungal Species is very essential for the degradation of lignocellulosic wastes and municipal solid wastes.

**Keywords:**

Lignolytic activity, phosphate solubilization, zone formation, Arecanut husk waste, Fungi.

**Article Citation:**

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## INTRODUCTION

Lignins are the major components in agriculture wastes, wood chips, municipal solid waste and Arecanut husk waste. Lignin is a highly complex biopolymer of phenylpropanoid structures with various carbon-carbon and ether linkages between monomeric units (Nazareth and Mavinkurve, 1987). Lignocellulose is a complex substrate and its biodegradation is not dependent on environmental conditions alone, but it also has the degradative capacity of the microbial population (Waldrop *et al.*, 2000; Malherbe and Cloet, 2002). Lignocellulose biodegradation is essentially a race between cellulose and lignin degradation (Reid, 1989; Malherbe and Cloet, 2002). This contest is even more extensive and complex in nature. Fungi with restricted metabolic capabilities develop mutualistic relationships and degrading cellulose, lignin etc. (Rayner and Boddy, 1988; Malherbe and Cloet, 2002). Most fungi are capable of cellulose degraders and fungi produce active polymer degrading enzymes, including cellulases and xylanases (Hodrova *et al.*, 1998; Malherbe and Cloet, 2002). Their cellulases are among the most active reported to date and able to solubilise both amorphous and crystalline cellulose (Wubah *et al.*, 1993; Malherbe and Cloet, 2002). The extra cellular enzymes of some white rot fungi are able to degrade lignin extensively (Rao, 1993; Selvam *et al.*, 2011). The ligninolytic enzymes are directly involved in the degradation of various xenobiotic compounds, including industrial dyes and polycyclic aromatic hydrocarbons (Metuku *et al.*, 2011; Bennet *et al.*, 2002). Lignocellulose biodegradation by prokaryotes is essentially a slow process characterized by the lack of powerful lignocellulose degrading enzymes, especially lignin peroxidases. Therefore, lignocellulose biodegradation by prokaryotes is of ecological significance, but lignin biodegradation by fungi, especially white-rot fungi is of commercial importance (Bennet *et al.*, 2002). The complexity of the lignin structure renders it fairly resistant to microbial attack and

its degradation is the rate determining step in biodeterioration and bioconversion process for the utilization of lignocellulosic fiber (Malherbe and Cloet, 2002). A number of microorganisms utilize lignin as carbon source for their growth. Microbiological work is carried out on Arecanut husk waste for biodegradation and to increase the manorial value of the compost using microbes (Saxena and Joshi, 2002; Sharma *et al.*, 1991). Soil microbes have the ability to convert fixed form of phosphorus to soluble forms that can be easily taken up by plants species of *Aspergillus* and *Penicillium*, whose fungal isolates identified to have phosphate solubilizing capabilities (Yadav *et al.*, 2011). Filamentous fungi are widely used as producers of organic acids and in particular *Aspergillus niger* and *Penicillium* species have been tested in fermentation system or inoculated directly into soil in order to solubilize rock phosphate (Yadav *et al.*, 2011). Hence, the lignolytic and phosphate solubilizing activity of the microorganisms has great economic importance. According to Rose (1957) the phosphate solubilizing activity of fungi may be attributed to the acids produced by them. The efficiency of biological transformation of phosphorus depends on the ratio of organic matter to phosphorus. As a result of the transformation, soluble phosphates are derived from insoluble TriCalcium Phosphate (TCP) in calcareous and alkaline soils. The transformation of insoluble phosphate into soluble form is carried out by a number of microbes present in the soil. A large fraction of soil microbes can dissolve insoluble inorganic phosphates present in the soil and make them available to the plants. Pure insoluble inorganic phosphates like TriCalcium Phosphate (TCP) have been used for screening organisms for phosphorus solubilizing activity (Pradhan and Sukla, 2005). The use of an economically cheap phosphate source and properly selected solubilizing fungi can help replacing the currently used costly phosphatic fertilizers. The aim of the study was to investigate the isolation, identification and



characterization of lignolytic and phosphate solubilizing fungi from Arecanut husk waste.

## MATERIALS AND METHODS

### Isolation of Fungi by serial dilution method

One gram of Arecanut husk waste powder was taken in a conical flask containing nine milliliters of sterile distilled water and shaken well in vortex mixer for 30 minutes. From this stock, various dilutions were prepared from  $10^{-1}$  to  $10^{-7}$ , using sterile distilled water. One milliliters of the diluted sample was poured into Petri plates containing the Martin Rose Bengal agar medium and Potato Dextrose agar (PDA) medium. Distinct fungal colonies grown in Martin Rose Bengal agar medium and Potato dextrose agar medium were isolated from repeated plating. All the isolates were pure cultured, identified and studied for lignolytic and phosphate solubilizing activity. The fungal colonies were identified based on their cultural characteristics and structure of their conidiophore (Paul and Daniel, 2007; Aneja, 2001; Naveenkumar *et al.*, 2011).

### Isolation of Fungi by Direct soil plate (War cup soil plate) method

Arecanut husk waste powder was collected in sterilized polythene bag. Then, 0.15 g of soil sample was added to sterile plates with the help of a sterilized cooled loop or transfer needle. Then, 15-20 ml of melted, cooled ( $45^{\circ}\text{C}$ ) sabouraud agar media was added, supplemented with streptopenicillin and rose Bengal, to each soil inoculated Petri plate. Dispense the soil particles throughout the medium by gentle rotation of the Petri dishes and allowed the plates to solidify. Plates were incubated at room temperature ( $28^{\circ}\text{C}$ ) in an inverted position for 15 days.

Fungal morphology was studied macroscopically by observing colony features (colour and surfaces) and microscopically by staining with lactophenol cotton blue and observe under compound microscope for the conidia, conidiophores and arrangement of spores (Aneja, 2001;

Barnett, 1975; Booth, 1971; Domsch *et al.*, 1980; Fundar, 1961; Subramanian, 1983; Naveenkumar *et al.*, 2011).

### Lignolytic activity

Fifteen fungal species isolated from Arecanut husk waste were screened for lignolytic activity by the method described by (Paul and Daniel, 2007). Then, 1.5 percent of malt extract and 2 percent agar were dissolved in 850 ml of water in a flask. Then, 0.5 percent of tannic acid was dissolved in 150 ml distilled water in another flask. These flasks were sterilized at  $121^{\circ}\text{C}$  for 15 minutes. The solutions in two flasks were mixed just before pouring them in to plates. After solidification, the plates were separately inoculated with the fifteen fungal isolates and incubated at room temperature for six days. The diameter of the colony and the brown zone developed around the colony were examined and measured (Paul and Daniel, 2007).

### Phosphate solubilizing activity

#### Primary screening

Fungi isolated from Arecanut husk waste were plated in Pikovskaya's medium containing 250 mg of TriCalcium Phosphate (TCP) the  $\text{PO}_4$  source to screen the efficacy of phosphate solubilization. The plates were incubated for five days. The zones of clearing around the microbial colonies indicated the extent of phosphate solubilization. Which was measured and based on the formation of the zone of clearance, nine fungal species were selected among them for further phosphate solubilization tests (Rose, 1957; Jackson, 1973).

### Quantitative measurement of phosphate solubilization

The efficacies of the selected nine fungal cultures in the solubilization of insoluble phosphates (TCP) were determined. These cultures were grown in 100 ml of Pikovskaya's liquid medium containing 250 mg of TCP as the phosphate source for 12 days at  $28 \pm 2^{\circ}\text{C}$  in a shaker. Every three days the sample was taken and filtered using Whatman No.1 filter paper. The biomass of

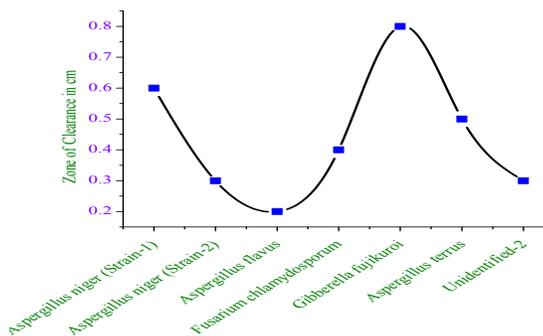
the fungus was measured on dry weight basis and the pH of the filtrate was measured with a digital pH meter. From filtrate, the phosphate was measured by the method of Jackson (Aneja, 2001; Sadasivam and Manickam, 1997; Selvi et al., 2011).

**RESULTS AND DISCUSSION**

In this study, the isolation and screening of Arecanut husk degrading fifteen fungal species were recorded. The results of the lignolytic activity of the fifteen fungal species isolated from the Arecanut husk waste is given in table-1. As indicated in the table, four fungal isolates, i.e., *Aspergillus clavatus*, *Aspergillus wentii*, *Penicillium* sp., Unidentified-3, did not show any zone of clearance and also mycellial growth. *Aspergillus fumigatus* showed mycellial growth of  $4.6 \pm 0.10$  cms, *Botrytis cinerea* showed mycellial growth of  $0.8 \pm 0.10$  cms, *Gibberella avenacea* showed mycellial growth of  $1.1 \pm 0.10$  cms and Unidentified-1 showed mycellial growth of  $0.9 \pm 0.06$  cms, but did not show a zone of clearance. The other seven fungal isolates, namely *Aspergillus terreus*, *Aspergillus flavus*, *Aspergillus niger* (Strain-1), *Aspergillus niger* (Strain-2), *Gibberella fujikuroi*, Unidentified-2 and *Fusarium chlamyosporum* showed a zone of clearance as well as mycellial growth. Lignin and tannic acid are closely related substances and

therefore evidence for tannic acid degradation can be taken for lignin degradation (Garcia et al., 2007). Tannic acid degradation was shown by the formation of a brown zone around the fungal colony (Table-1) (Fig-1).

The results of the primary screening of the fifteen fungal species isolated from the Arecanut husk waste for phosphate solubilizing ability is given in table-2. Only fourteen out of fifteen species showed mycellial growth and six species out of these fourteen, viz, *Fusarium chlamyosporum*, *Penicillium* sp, *Aspergillus clavatus*, *Aspergillus fumigatus*, *Gibberella avenacea* and *Aspergillus wentii* did not show a zone of clearance. Hence the nine species, i.e. *Aspergillus terreus*, *Gibberella fujikuroi*, *Botrytis cinerea*, *Aspergillus flavus*, *Aspergillus niger* (Strain-1), *Aspergillus niger* (Strain-2),



**Figure: 1. Lignolytic activity observed during the selected seven fungal species isolated from Arecanut husk waste.**

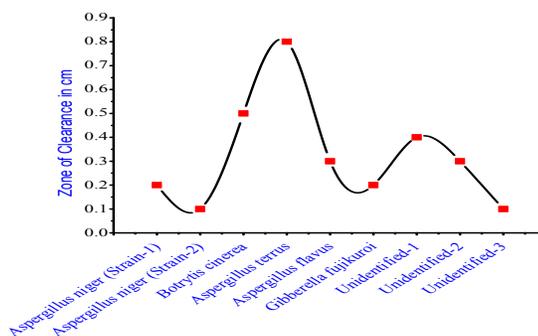
**Table: 1. Lignolytic activity of the selected fifteen fungal species isolated from Arecanut husk waste.**

Sl.No.	Name of the organisms	Mycelial growth (cm)	Zone of clearance (cm)	Total diameter (cm)
1	<i>Aspergillus niger</i> (Strain-1)	$8.1 \pm 0.10$	$0.6 \pm 0.06$	$16.3 \pm 0.18$
2	<i>Aspergillus niger</i> (Strain-2)	$4.3 \pm 0.06$	$0.3 \pm 0.04$	$8.6 \pm 0.02$
3	<i>Botrytis cinerea</i>	$0.8 \pm 0.10$	-	$1.6 \pm 0.07$
4	<i>Penicillium</i> sp	-	-	-
5	<i>Aspergillus flavus</i>	$2.4 \pm 0.25$	$0.2 \pm 0.02$	$4.8 \pm 0.09$
6	<i>Aspergillus wentii</i>	-	-	-
7	<i>Fusarium chlamyosporum</i>	$5.2 \pm 0.09$	$0.4 \pm 0.03$	$10.5 \pm 0.10$
8	<i>Aspergillus clavatus</i>	-	-	-
9	<i>Gibberella fujikuroi</i>	$6.1 \pm 0.10$	$0.8 \pm 0.02$	$12.3 \pm 0.01$
10	<i>Aspergillus terreus</i>	$4.6 \pm 0.10$	$0.5 \pm 0.01$	$9.3 \pm 0.03$
11	<i>Gibberella avenacea</i>	$1.1 \pm 0.10$	-	$2.3 \pm 0.02$
12	<i>Aspergillus fumigatus</i>	$2.3 \pm 0.20$	-	$4.6 \pm 0.10$
13	Unidentified-1	$0.9 \pm 0.06$	-	$1.8 \pm 0.01$
14	Unidentified-2	$3.6 \pm 0.20$	$0.3 \pm 0.02$	$7.3 \pm 0.10$
15	Unidentified-3	-	-	-



unidentified-1, unidentified-2 and unidentified-3. This showed a zone of clearance of  $0.1 \pm 0.06$  to  $0.8 \pm 0.03$  cms were selected and further tested for phosphate solubilizing activity. The zone of clearance was high in *Aspergillus terreus* and was formed due to the solubilization of insoluble phosphates by acidification associated with either proton extrusion or organic acid secretion (Bardiya and Gaur, 1974; Darmal *et al.*, 1989; Yadav *et al.*, 2011) (Table-2) (Fig-2).

The results of the selected nine fungal isolates tested for phosphate solubilizing activity is given in table -3. As indicated in this table, the phosphate solubilizing activity increased with the increase in the number of culture days but, after reaching a maximum, the phosphate solubilizing activity slowly decreased for all the nine fungal isolates tested. The number of days taken for the maximum activity varied for various species (Gupta *et al.*, 2010). In 3<sup>rd</sup> day *Aspergillus terreus* and *Botrytis cinerea* showed high phosphate solubilizing activity. In 6<sup>th</sup> day *Aspergillus niger* (strain-1), *Botrytis cinerea*, *Aspergillus flavus*, *Gibberella fujikuroi*, Unidentified-1 and Unidentified-2 showed high phosphate solubilizing activity. In 9<sup>th</sup> day Unidentified-3 and *Botrytis cinerea* showed high phosphate solubilizing activity. The solubilization of phosphates in a liquid



**Figure: 2. Phosphate solubilizing ability observed during the selected nine fungal species isolated from Arecanut husk waste.**

medium depends on the nature of the phosphate source, the organic incubation period and the nature and quantity of the organic acids secreted in to the medium (Sujatha *et al.*, 2004; Sharma *et al.*, 2011; Rose, 1957). The number of days taken for the maximum activity varied for various species (Kapri and Tewari, 2010; Vassilev *et al.*, 1995) (Table-3) (Fig-3).

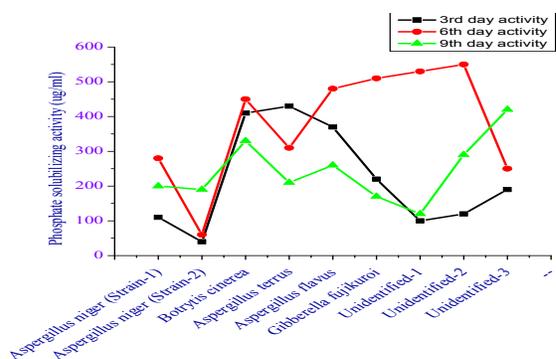
Fourteen days of incubation period was favorable for maximum phosphate solubilization by microorganisms in a liquid medium. Narsian *et al.*, (1995) observed that the phosphate solubilizing activity of *Aspergillus aculeatus* was the highest after 48h of fungal growth and that the fungal biomass production was more in the case of TriCalcium Phosphate than other

**Table: 2. Phosphate solubilizing ability of the selected fifteen fungal Species isolated from Arecanut husk waste in Pikovskaya’s solid medium.**

Sl. No.	Name of the organisms	Mycelial growth (cm)	Zone of clearance (cm)	Total diameter (cm)
1	<i>Aspergillus niger</i> (Strain-1)	1.2 ± 0.10	0.2 ± 0.10	2.4 ± 0.05
2	<i>Aspergillus niger</i> (Strain-2)	1.2 ± 0.06	0.1 ± 0.06	2.4 ± 0.02
3	<i>Botrytis cinerea</i>	1.3 ± 0.13	0.5 ± 0.08	2.75 ± 0.03
4	<i>Aspergillus terreus</i>	1.6 ± 0.10	0.8 ± 0.03	3.33 ± 0.02
5	<i>Aspergillus flavus</i>	1.4 ± 0.06	0.3 ± 0.03	2.95 ± 0.24
6	<i>Gibberella fujikuroi</i>	1.3 ± 0.01	0.2 ± 0.01	2.6 ± 0.09
7	<i>Fusarium chlamydosporum</i>	2.3 ± 0.07	-	4.65 ± 0.03
8	<i>Aspergillus clavatus</i>	6.2 ± 0.10	-	12.5 ± 0.26
9	<i>Aspergillus wentii</i>	2.7 ± 0.05	-	5.45 ± 0.15
10	<i>Penicillium sp.</i>	4.7 ± 0.12	-	9.5 ± 0.28
11	<i>Gibberella avenacea</i>	1.3 ± 0.10	-	2.75 ± 0.05
12	<i>Aspergillus fumigatus</i>	4.8 ± 0.06	-	9.7 ± 0.13
13	Unidentified-1	1.6 ± 0.19	0.4 ± 0.01	3.2 ± 0.10
14	Unidentified-2	2.0 ± 0.12	0.3 ± 0.02	4.15 ± 0.07
15	Unidentified-3	1.5 ± 0.15	0.1 ± 0.03	3.15 ± 0.10

**Table: 3. Phosphate Solubilizing activity of the selected nine fungal species isolated from Arecanut husk waste in Pikovskaya's broth.**

Sl. No.	Name of the organisms	Phosphate solubilizing activity (ug/ml)			Biomass yield (gm/d)	
		3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	12 <sup>th</sup> day	15 <sup>th</sup> day
1	<i>Aspergillus niger</i> (Strain-1)	110 ± 5.00	280 ± 7.64	200 ± 2.52	0.31 ± 0.02	0.03 ± 0.00
2	<i>Aspergillus niger</i> (Strain-2)	40 ± 2.52	60 ± 3.61	190 ± 7.09	0.28 ± 0.01	0.05 ± 0.00
3	<i>Botrytis cinerea</i>	410 ± 6.81	450 ± 1.53	330 ± 2.52	0.13 ± 0.02	0.03 ± 0.00
4	<i>Aspergillus terreus</i>	430 ± 13.23	310 ± 5.51	210 ± 3.00	0.21 ± 0.03	0.01 ± 0.00
5	<i>Aspergillus flavus</i>	370 ± 20.21	480 ± 5.51	260 ± 4.04	0.11 ± 0.02	0.02 ± 0.00
6	<i>Gibberella fujikuroi</i>	220 ± 9.85	510 ± 6.03	170 ± 2.52	0.08 ± 0.02	0.02 ± 0.00
7	Unidentified-1	100 ± 7.64	530 ± 10.00	120 ± 2.08	0.06 ± 0.01	0.03 ± 0.00
8	Unidentified-2	120 ± 13.65	550 ± 8.50	290 ± 2.52	0.29 ± 0.01	0.04 ± 0.00
9	Unidentified-3	190 ± 5.00	250 ± 4.04	420 ± 1.53	0.08 ± 0.05	0.04 ± 0.00



**Figure: 3. Phosphate solubilizing activity observed during the selected nine fungal species isolated from Arecanut husk waste.**

phosphate sources. The pH variations observed in the all nine selected fungal species during the phosphate solubilizing activity for a period of 12 days. All the nine isolates showed a decrease in the pH during the initial days (0 to 3 days) and then it increased or remained at the same level during 3 to 6 days thereafter. There was not much change in the pH values up to the production of organic acids like citric acid, acetic acid, fumaric acid

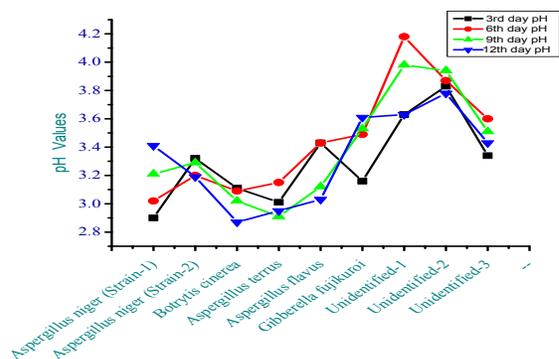
**Table: 4. p<sup>H</sup> variation observed during the phosphate solubilizing activity of the selected nine fungal species isolated from Arecanut husk waste.**

Sl. No.	Name of the organisms	pH Values (Number of days)			
		3 <sup>rd</sup> day	6 <sup>th</sup> day	9 <sup>th</sup> day	12 <sup>th</sup> day
1	<i>Aspergillus niger</i> (Strain-1)	2.90 ± 0.06	3.02 ± 0.01	3.21 ± 0.03	3.41 ± 0.02
2	<i>Aspergillus niger</i> (Strain-2)	3.32 ± 0.01	3.20 ± 1.62	3.29 ± 0.03	3.19 ± 0.02
3	<i>Botrytis cinerea</i>	3.11 ± 0.02	3.09 ± 0.02	3.02 ± 0.02	2.97 ± 0.01
4	<i>Aspergillus terreus</i>	3.01 ± 0.01	3.15 ± 0.01	2.97 ± 0.01	2.85 ± 0.03
5	<i>Aspergillus flavus</i>	3.43 ± 0.02	3.43 ± 0.02	3.12 ± 0.02	3.03 ± 0.03
6	<i>Gibberella fujikuroi</i>	3.16 ± 0.01	3.47 ± 0.01	3.53 ± 0.02	3.61 ± 0.03
7	Unidentified-1	3.63 ± 0.02	4.18 ± 0.01	3.98 ± 0.02	3.63 ± 0.03
8	Unidentified-2	3.83 ± 0.02	3.87 ± 0.02	3.94 ± 0.03	3.78 ± 0.01
9	Unidentified-3	3.34 ± 0.01	3.60 ± 0.10	3.51 ± .03	3.43 ± 0.01

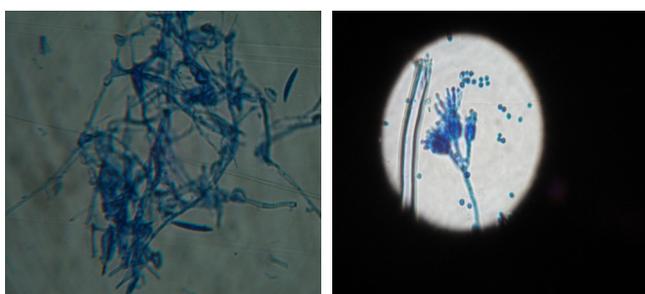
and malic acid during growth. The mechanisms used by microorganisms for inorganic phosphate solubilization have been attributed mainly to acidification, chelation and exchange reactions in growth environment (Molla and Chowdhury, 1984; Nopparat *et al.*, 2007; Cunningham and Kuiack, 1992) (Table-4) (Fig-4).

**CONCLUSION**

This study concluded that lignolytic activity and phosphate solubilizing efficiency of fungal species isolated from Arecanut husk waste. Few fungal species showed lignolytic activity, its ability for lignin degradation and utilization of lignin as a carbon source for their growth. Few fungal species showed phosphate solubilizing ability and utilization of Phosphate as a carbon source for their growth. In future fungal species are highly effective and very essential for the degradation of lignocellulosic wastes and municipal solid wastes.

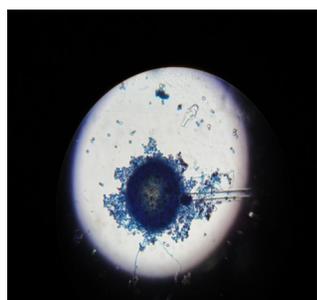


**Figure: 4.** pH variation observed during the phosphate solubilizing activity of the selected nine fungal species isolated from Arecanut husk waste.



*Fusarium chlamyosporum*

*Penicillium sp*



*Aspergillus niger*

**Figure: 5.** Photographs of fungal strains such as *Fusarium chlamyosporum*, *Penicillium sp* and *Aspergillus niger*.

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