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RESEARCH ARTICLE :

Study of cellulolytic activity of fungi involved in decomposition of organic waste

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SUMMARY : Partially rotten leaves were collected from plantation of Dr. B. S. K. K. V., Dapoli. For isolation of fungi involved in decaying process. During repeated isolations seven fungal isolates *viz*., *Pestalotiopsis palmarum, Phoma* spp., *Aspergillus niger, Trichoderma harzianum, T. viride* and two sterile fungi were obtained. Maximum growth on filter paper was observed by *Pestalotiopsis palmarum* (87.3 mm) followed by *A. niger* (62.3 mm), followed by *T. harzianum* (58.0mm) after 7 days of incubation. Minimum growth was recorded by isolates *Phoma* spp. (4.6 mm), Sterile Fungus-1(4.3 mm) and Sterile Fungus-2 (6mm). The plate screening assay recommended by International Union of Pure and Applied Chemistry (IUPAC) were used in the investigation. Cellulolytic fungi were evaluated after 7 days for the production of cellulolytic enzymes by staining with 1% Iodine' The diameter of clear zone on fungal plates, gave an approximate indication of cellulase activities. In starch hydrolysis test, maximum clearing zone was produced by *Trichoderma harzianum* (68 mm), followed by SF1 (56 mm) and then by *T. viride* (52 mm).

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KEY WORDS :
Cellulolytic activity, *Pestalotiopsis palmarum, Phoma* spp., *Aspergillus niger, Trichoderma harzianum, T. viride*

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BACKGROUND AND OBJECTIVES

Agricultural wastes contain a high proportion of cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes. Lignin is an integral cell wall constituent, which provides strength and resistance to microbial degradation. The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Biological degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic as well as toxic waste. These wastes have been insufficiently disposed off leading to environmental pollution.

Fungi are the chief cellulase producing micro-organisms, so also many bacteria and actinomycetes have also been reported to possess cellulolytic activity. Fungal genera like *Trichoderma* and *Aspergillus* are known to be cellulase producers and crude enzymes produced by these micro-organisms are commercially available for agricultural use. Members of genus *Aspergillus* degrade

cellulose producing significant amount of cell free cellulase capable of hydrolyzing cellulose into fermentable soluble sugars such as glucose; an important raw material in chemical industries. *Aspergillus* and *Trichoderma* specie are well known efficient producers of cellulases. Several studies have been carried out to produce cellulolytic enzymes from bio waste degradation process by many micro-organisms including fungi such as *Trichoderma, Penicillium* and *Aspergillus* species etc.

In general, plant residues contain 15-60 % cellulose, 10-30% hemicellulose, 5-30% lignin and 2-15% protein and 10% sugars, amino acids and organic acids. The enzymes involved in the composting process includes cellulases, which depolymerise cellulose, β-glucosidases which hydrolyse glucosides, proteases and urease involved in N mineralization, phosphatases that remove phosphate groups. Therefore, enzymatic activity should indicate the ability of compost to degrade a wide range of common organic substrates.

Amylase is an exoenzyme that hydrolyses starch, a polysaccharide into disaccharides and some mono saccharides such as glucose. Starch is a complex carbohydrate composed of glucose molecules linked together by glycosidic bonds.

Majority of organic matter is cellulolytic in nature and it takes about 10-15 months for decomposition under natural condition. In order to decrease the period of decomposition of various planting material and also to make easily available cheap compost or manure, it is important to find out efficient strains of fungi which can play important role in decomposition and accelerate the entire recycling process. (Kamat *et al.*, 1978).

This main aim of the present study is to isolate fungal isolates which are found naturally and examine their cellulolytic ability to degrade agricultural wastes.

RESOURCES AND METHODS

Collection of sample :

Partially rotten leaves of mango, cashew, sapota, coconut and glyricidia were collected during rainy season (August, 2015) in plastic bags from plantation of Department of Horticulture, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli. Samples were brought to the laboratory and kept in plastic bags for further decomposition for a period of two weeks. For providing aeration, plastic bags were randomly punctured with pin in all directions.

Isolation:

Isolation was carried out by leaf tissue method using 0.1% mercuric chloride on PDA (Potato Dextrose Agar) medium. The bags were checked periodically for fungal growth. After 2 weeks, whitish fungal growth was observed on leaves, then isolation was carried out. During repeated isolations, seven fungal isolates were obtained. (Table A).

Table A : List of fungi isolated

Isolate No.	Substrate used	Fungal isolates
1	Coconut leaves	*Pestalotiopsis palmarum*
2	Glyricidia leaves	*Phoma* spp.
3	Sapota leaves	Sterile fungus -1
4	Mango leaves	*Trichoderma harzianum*
5	Coconut leaves	*Aspergillus niger*
6	Cashew leaves	Sterile fungus -2
7	Mango leaves	*Trichoderma viride*

Among these species, *Trichoderma* spp. were most dominant in decomposed organic waste

Morphological and cultural characteristics of isolated organism:

The morphological characters and cultural behaviour of these fungi were studied on PDA. The pure fungal cultures isolated from partially rotten leaf tissue were tentatively identified on the basis of colony and morphological characters in the laboratory itself. The identity of cultures was further confirmed from Agharkar research Institute, Pune. Morphological characters of isolated fungi were studied by preparing temporary mounts.

Cellulose decomposing ability of test cultures:

Circular shape of filter paper, having dimensions 60 mm were cut and oven dried at 60ºC for 48 hrs. Each strip was then placed in a Petri plate containing around 10 ml of water agar medium. Each Petri plate was inoculated aseptically with an agar bit at centre from 7 days old fungal culture grown in a Petri plate. Three replications for each fungus were maintained. An uninoculated Petri plate with medium and filter paper was kept as a control. The Petri plates were incubated at 27 ± 2ºC for 10 days. After completion of incubation period, the radial growth of fungal isolates was recorded.

Amylase production test (Starch Hydrolysis):

Starch agar medium (minimal medium) was used for this test. After autoclaving and cooling upto 450C the medium was poured onto the autoclaved Petri plate. After solidification, using sterile cork borer make small bits of organism and inoculate bits into the centre of the respective plate by inoculating needle. Incubate the plates for 72-96 hours at 27 ± 2ºC in an inverted position. The plates were than flooded with iodine solution with a dropper for 30 seconds. Pour off the excess iodine solution. Examine the plates for starch hydrolysis zone around line of growth of each organism. Diameter of clear zones were measured.

OBSERVATIONS AND ANALYSIS

Test isolates were inoculated on filter paper in Petri plate already poured with water agar medium. After 8 days observations were recorded. (Table 1).

Table 1 : Cellulose decomposition of selected fungal isolates on filter paper

Tr. No.	Isolates	Fungal growth (diam.in mm)*
1	*Pestalotiopsis palmarum*	87.3
2	*Phoma* spp.	4.60
3	Sterile fungus -1	4.30
4	*Trichoderma harzianum*	58.0
5	*Aspergillus niger*	62.3
6	Sterile fungus -2	6
7	*Trichoderma viride*	11.6

*****Each value represents mean of three values.

Cellulose decomposing ability of isolated fungi:

Maximum decomposition of cellulose on filter paper was recorded by *Pestalotiopsis palmarum* (87.3 mm) followed by *Aspergillus niger* (62.3 mm) and then by *Trichoderma harzianum* (58.0mm) after 7 days of incubation. Minimum decomposition was done by isolates *Phoma* spp. (4.6 mm), SF_1 (4.3 mm) and SF_2 (6mm) as these were slow growing. Data on cellulose decomposing ability of isolated organism was statistically significant.

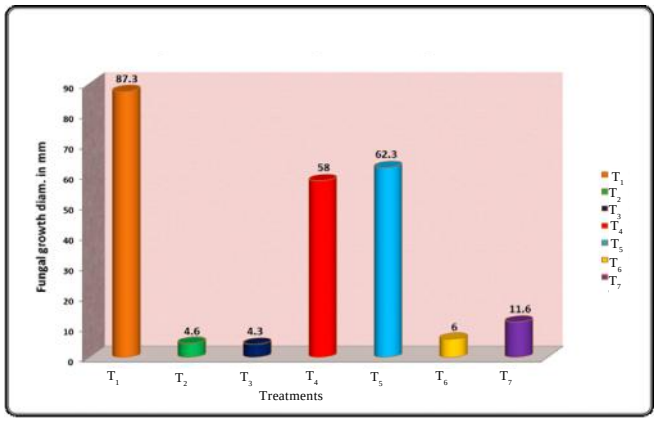

Fig. 1 : Cellulose decomposition of fungal isolates

P. palmarum, A. niger and *T. harzianum* were good cellulose decomposers as compared to the rest of the fungi under study. This was due to their high sporulation and fast-growing ability.

Good growth of fungi on filter paper indicated that they utilized the filter paper as carbon source and they can be considered as cellulolytic in nature. The findings of Kamat *et al*. (1978) indicated that *T. viride, T. aureoviride* grew well on filter paper strips than the media. He also reported that filter paper strip method was good to study the cellulolytic nature of fungi.

The findings are also in agreement with those reported by Reddy *et al*. (2014) and Irawan *et al*. (2014).

Starch hydrolysis test:

Data presented in Table 2 indicated that, maximum clearing zone was produced by *Trichoderma harzianum* (68 mm), followed by SF_1 (56 mm) and then by *Trichoderma viride* (52 mm). Minimum clearing zone was produced by SF_2 (18 mm) due to its dense mycelial growth and *Aspergillus niger* (25 mm) due to sparse fungal growth.

Table 2 : Starch hydrolysis activity of fungal isolates

Tr. No.	Fungal isolates	Clearing zone (mm)*
1	*Pestalotiopsis palmarum*	30
2	*Phoma* spp.	37
3	Sterile fungus-1	56
4	*Trichoderma harzianum*	68
5	*Aspergillus niger*	25
6	Sterile fungus-2	18
7	*Trichoderma viride*	52

*****Each value represents mean of three replications

Maximum clearing zone was produced by *T. harzianum* (68 mm), followed by SF_1 (56 mm) and then by *T. viride* (52 mm). Minimum clearing zone was produced by SF_2 (18 mm) due to its dense mycelial growth and *A. niger* (25 mm) due to sparse fungal growth. This indicated that *T. harzianum* was efficient starch hydrolyser or having highest amylase activity.

Saili *et al*. (2014) also reported that four *Trichoderma* isolates showed a significantly higher halo zone when flooded with iodine solution. However, Antarctic *Trichoderma* isolates exhibited apparently lower amylase activities with the halo zone sizes ranging from 1.53 to 3.90 cm.

Maximum starch hydrolysis was recorded in 6-7 days old culture of *Aspergillus fumigatus* and *Chaetomium globosum* by Sharma and Shukla (2008).

From the data given in Table 1, it is recorded that maximum growth on filter paper was recorded by *Pestalotiopsis palmarum* (87.3 mm) followed by *Aspergillus niger* (62.3 mm), followed by *Trichoderma harzianum* (58.0mm) after 7 days of incubation. Minimum growth was recorded by isolates *Phoma* spp. (4.6 mm), SF_1 (4.3 mm) and SF_2 (6mm) More or less similar results were obtained by Kaufmann *et al*., 1976; Lakshmikant and Mathur, 1990; Mandels and Reese, 1985; Sathe, 1998; Satyanarayan and Johri, 1980; Thorat, 1994 and Vijaya and Naidu, 1995.

Conclusion :

From the above discussion it can be concluded that, *P. palmarum*, *A. niger* and *T. harzianum* were good cellulose decomposers as compared to the rest of the fungi under study. This was due to their high sporulation and fast growing ability. *T. harzianum* was efficient starch hydrolyser or having highest amylase activity. More emphasis must be given on enzymatic study of cellulolytic fungi and their cost effective procedure.

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