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Research Article

Plants used as Biofungicides against Storage- Decay of Yam (*Dioscorea alata* L.) in Odisha, India

Akhtari Khatoon[1], Ashirbad Mohapatra[2], Kunja Bihari Satapathy[1]*

[1]P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar-751004, Odisha, India
[2]Sri Jayadev College of Education and Technology, Naharkanta, Bhubaneswar-752101, Odisha, India

***Corresponding Author:** Kunja Bihari Satapathy, P.G. Department of Botany, Utkal University, Vani Vihar, Bhubaneswar-751004, Odisha, India

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ABSTRACT

Petroleum ether and alcoholic leaf extracts of *Abutilon indicum* (L.) Sweet, *Ageratum conyzoides* L., *Alstonia scholaris* (L.) R.Br., *Artocarpus heterophyllus* Lam., *Averrhoa carambola* L., *Cassia fistula* L., *Centella asiatica* Urban. and *Dillenia indica* L. were tested for their antifungal activity against fungi responsible for storage decay of *Dioscorea alata* L. tubers namely *Aspergillus niger*, *Geotrichum candidum* and *Fusarium oxysporum* under aseptic laboratory condition. These fungi were isolated from the rotten yam tubers collected from different market places of Odisha, India. The fungitoxic potential of plant extracts was compared with four synthetic fungicides such as Blitox-50, Dhanustin, Indofil M-45 and Mancozeb. The results of the present study revealed that the plant extracts also had significant inhibitory effect against the three test fungi when compared to four synthetic fungicides. The results on the efficacy of eight plant extracts against three fungi indicated that against *Aspergillus niger* and *Geotrichum candidum*, the petroleum ether extract of *Ageratum conyzoides* was most effective in inhibiting its growth while against *Fusarium oxysporum*, petroleum ether extract of *Averrhoa carambola* was found most promising.

Keyword: Post-harvest decay; yam tubers; plant leaf extracts; antifungal activity; bio-fungicides

INTRODUCTION

Vegetables are vital source for human nutrition as well as a cheaper source of vitamins and minerals. They are responsible for maintenance of good health and also beneficial in protecting against some degenerative diseases. Different parts of vegetables are used for human consumption which may belong to different botanical groups with varying cultural and environmental requirements. The vegetables grouped under root and tuber category are most important as compared to other vegetables being non-perishable in nature. After harvest they are transported to store houses and consumed throughout the year. Yam (*Dioscorea alata*) is a cultivated tuber crop and it is unique for its food, medicinal and economic values [1]. About 100 g of yam tuber yields 118 calories of energy. It is also an important source of complex carbohydrates and soluble dietary fibre. It is reported to be a source of dietary fibre that help reduce constipation and decrease bad cholesterol (LDL) levels besides other medicinal uses such as reducing colon-cancer risk as well as regulating blood sugar level. The crop is drought tolerant and provides a wide harvesting window

which makes it available throughout the year. However, post-harvest storage decay of the tubers due to infestation of microorganisms is the important factor for the loss in its production and contributes largely to the unsuccessful storage practice of these root tubers [2-4]. In Odisha, the post-harvest rotting of these tubers during storage poses serious problem sustaining heavy losses particularly for the warm and humid climate in the state. To control phytopathogenic microorganisms of crop plants, chemical pesticides have been used to control the plant diseases in agriculture. A number of problems are also seen against the effective use of these chemicals in the agricultural areas where the fungal pathogens have developed resistance [5]. Besides overuse of the chemicals and fertilizers increased the risk of high-level toxic residues in the products which are hazardous for human consumption as well as the ecosystem. Thus, the problem has drawn the interest on the research of possible use of plant extracts for control of pest and diseases in agriculture which was less harmful to the human health and environment [6, 7].

MATERIALS AND METHODOLOGY

Collection of rotten samples, Isolation and Identification of associated Fungi

Yam tubers showing the symptoms of rotting were randomly collected from different market places and store houses of Odisha and brought to the Laboratory of Microbiology, Post Graduate Department of Botany, Utkal University, Bhubaneswar, Odisha (India) for further phytopathological analysis. The diseased samples were washed with tap water and sterilized the surface with 0.1% mercuric chloride solution for 2-3 minutes. Small piece of rotten tissue from the rotten sample was taken with a sterile knife and placed on potato dextrose agar (PDA) medium and incubated at room temperature for 24 hours. Representative colony types were purified by sub-culturing on fresh PDA plates. Pure cultures were transferred to slants of PDA and the isolates were grown singly on PDA for identification. The isolates were identified with the help of the available literature. Authentication of identification was made in the Department of Mycology and Plant Pathology, College of Agriculture, Odisha University of Agriculture and Technology (OUAT), Bhubaneswar, India.

Collection and identification of plant material

The leaves of eight medicinal plants such as *Abutilon indicum* (L.) Sweet, *Ageratum conyzoides* L., *Alstonia scholaris* (L.) R.Br., *Artocarpus heterophyllus* Lam., *Averrhoa carambola* L., *Cassia fistula* L., *Centella asiatica* Urban. and *Dillenia indica* L. were collected from the "Chandaka reserve forest" area near Bhubaneswar, Odisha in March, 2015 (Fig 1). The test plants were identified by following the "Flora of Orissa" [8]. The voucher plant specimens were deposited in the herbarium of Post Graduate Department of Botany, Utkal University, Vani Vihar, Bhubaneswar. The leaves were collected in bulk amount, washed in tap water, dried under shade and made to coarse powder form to be utilized for their antifungal study.

Processing of plant material and preparation of extract

The leaves collected were shade dried and ground to form coarse powder and successively extracted with the solvent petroleum ether and methanol by Soxhlet apparatus [9] and the extract was recovered under reduced pressure in a rotatory evaporator. The extracts were kept in desiccators for further use.

In vitro evaluation of antifungal efficacy of plant extracts

For the evaluation of the efficacy of the fungitoxicity, the petroleum ether and methanolic leaf extracts were diluted with 50 % dimethyl sulfoxide (DMSO) @ 20mg/ml. Then antifungal efficacy of the plant extracts was carried out following the method of Satish et al. (2007) with some modification [10]. For sample treatment, 1 ml of diluted plant extract was mixed with 19 ml of Potato Dextrose Agar (PDA) and the mixture was poured into each petri plate, mixed thoroughly and allowed to solidify, making the concentration of plant extract on PDA medium 1mg/ml. PDA medium without the plant extracts was used as control. Disc of 0.5 cm culture of the test fungi was placed at the centre of the petri plate following poison food technique (both sample and control) and incubated at 28 ± 1 ºC for respective days (e.g. *Aspergillus niger* for 8 days, *Fusarium oxysporum* for 10 days and *Geotrichum candidum* for 11 days) of their growth up to 8 cm diameter (complete growth).

The antifungal effect of each plant extract was estimated by measuring the radial growth (cm) of the fungal pathogen using ruler. The fungitoxicity of the plant extracts in terms of percentage inhibition was calculated by using formula as below:

% Inhibition = (X-Y)/X x 100

where X= average increase in mycelial growth in control, Y= average increase in mycelial growth in treatment [10].

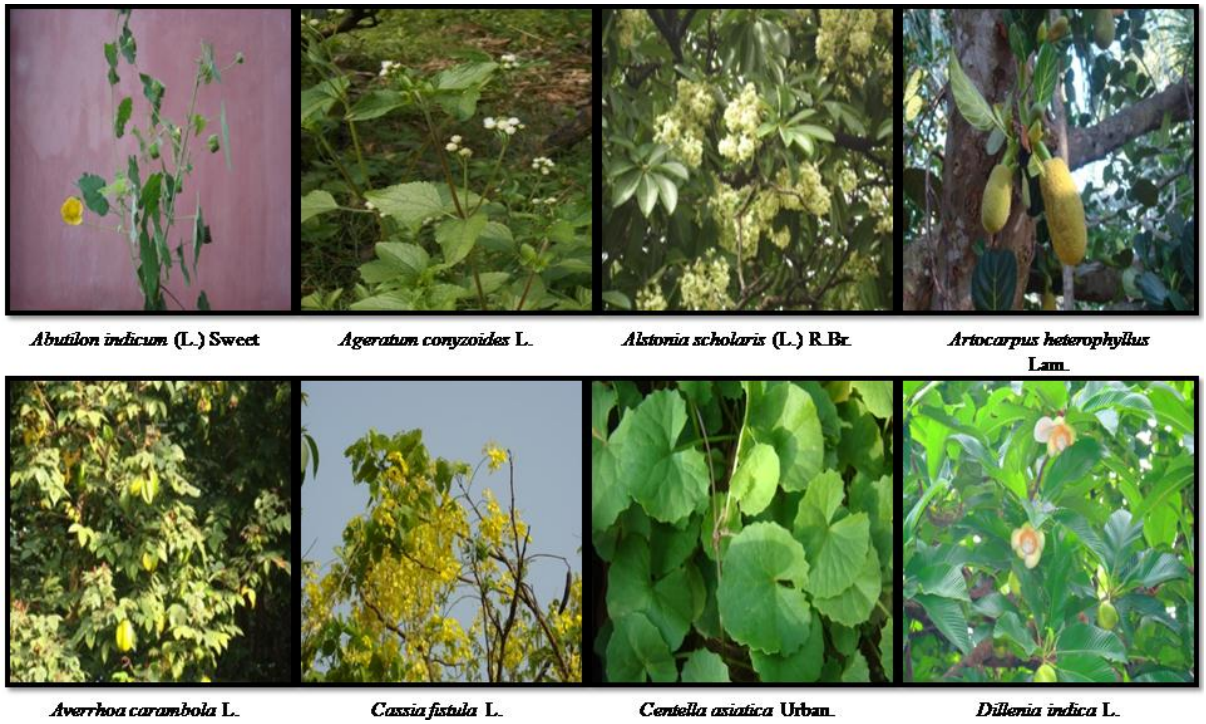

Fig 1: Photo of test plant materials

Comparison of the efficacy of selected fungicides and plant extracts:

A separate experiment was conducted for comparison of the relative efficacy of four synthetic fungicides namely Dhanustin, Mancozeb, Blitox-50, Indofil M-45 and 16 leaf extracts (petroleum ether and methanolic extracts) of eight selected plants against the test fungi under study. The concentration of fungicides was 0.05 mg/ml. Fungicides (0.5 mg/ml) and plant extracts (1 mg/ml) were incorporated after sterilization in PDA medium. Five test fungal isolates were inoculated into the medium in three replicates and were incubated for respective days of their growth as described earlier. At the end of incubation period, the colony diameter of each fungus treated with chemical/ plant extract combination was measured and transformed to percentage of mycelial growth inhibition as per the method described earlier.

RESULTS AND DISCUSSION

Isolation of fungi from the rotten sample

From the present investigation it was found that *Aspergillus niger*, *Geotrichum candidum* and *Fusarium oxysporum* were associated with storage-decay of yam tubers in market places and store-houses of Odisha.

***In vitro* antifungal efficacy plant extracts and synthetic fungicides**

In-vitro fungitoxic efficacy of eight medicinal plants against three plant pathogenic fungi revealed that all the test plants were more or less effective in inhibiting the mycelial growth of the fungi causing storage rot of yam tubers during the present investigation. The petroleum ether extract of *Ageratum conyzoides* significantly controlled the growth of *Aspergillus niger* and *Geotrichum candidum*, while petroleum ether extract of *Averrhoa carambola* had significant inhibitory effect on *Fusarium oxysporum*. The mycelial growth inhibition by the medicinal plants extracts was estimated

which ranged from 23.33 ± 1.65 % to 89.03 ± 0.98 % (Table 1). The efficacy of plant extracts of eight medicinal plants were compared with four synthetic fungicides namely Blitox-50, Dhanustin, Indofil and Mancozeb and the result revealed that against *Aspergillus niger*, Dhanustin was most effective and against *Geotrichum candidum*, Mancozeb showed maximum inhibition of mycelial growth; while the growth of *Fusarium oxysporum* was completely inhibited by Blitox-50 (Table 1).

It is also revealed from the present study that plant extracts have better potential in inhibiting the mycelial growth of the test fungi as compared to synthetic fungicides. The use of plant extracts to control plant diseases is an eco-friendly approach and can be a suitable alternative to toxic synthetic fungicides. The use of commercial fungicides is further leading to potential danger to both the farmer and environment and cost-intensive for most of the farmers [11]. But the plant derived fungicides are eco-friendly and less cost effective and many of the higher plants have already reported to have antibacterial, antifungal and insecticidal properties [10, 12-17]. Further the study make it clear that the isolated compounds from plants can effectively be used as natural fungicides for the control of plant diseases.

Table 1: *In-vitro* antifungal effect of plant extracts and commercial fungicides

Sl. No.	Test plants		Percentage of Inhibition of mycelial growth		
			Aspergillus niger	***Fusarium oxysporum***	***Geotrichum candidum***
1	*Abutilon indicum* (Malvaceae)	A	30.11 ± 0.4	26.45 ± 0.31	56.08 ± 0.82
		B	35.07 ± 0.28	35.07 ± 0.53	55.19 ± 1.38
2	*Ageratum conyzoides* (Asteraceae)	A	62.54 ± 2.04	59.80 ± 0.76	89.03 ± 0.98
		B	46.51 ± 1.09	57.41 ± 1.05	79.08 ±1.12
3	*Alstonia scholaris* (Apocynaceae)	A	34.32 ± 0.63	58.55 ± 0.22	73.41 ± 3.24
		B	54.72 ± 1.71	57.33 ± 1.51	80.14 ± 1.79
4	*Artocarpus heterophyllus* (Moraceae)	A	36.38 ± 1.23	55.45 ± 0.76	77.16 ± 1.64
		B	42.57 ± 1.94	50.19 ±0.59	82.5 ± 2.04
5	*Averrhoa carambola* (Averrhoaceae)	A	27.8 ± 0.21	73.5 ± 0.62	77.75 ± 1.13
		B	45.47 ± 1.31	58.57 ± 0.44	70.46 ± 1.45
6	*Cassia fistula* (Caealpiniaceae)	A	47.24 ± 4.82	50.25 ± 1.43	76.4 ± 2.72
		B	38.22 ± 2.32	52.5 ± 1.17	72.57 ± 2.8
7	*Centella asiatica* (Apiaceae)	A	39.75 ± 2.76	36.69 ± 0.99	60.66 ± 2.49
		B	33.4 ± 2.27	23.33 ± 1.65	63.5 ± 3.34
8	*Dillenia indica* (Dilleniaceae)	A	24.08 ± 2.25	46.53 ± 1.73	55.25 ± 3.43
		B	51.93 ± 3.61	46.08 ± 1.41	81.33 ± 4.98
Test commercial fungicides					
1	Blitox-50		17.66 ± 0.6	0	17.09 ± 1.37
2	Dhanustin		81.41 ± 0.71	86.7 ± 0.69	31.41 ± 0.71
3	Indofil M-45		22.75 ± 1.13	29.33 ± 0.54	31.41 ± 0.71
4	Mancozeb		18.11 ± 1.06	79.53 ± 1.1	61.05 ± 0.92

Results expressed as mean ± S.E.M. of three determinations, A = Petroleum ether extract, B = Methanol extract

CONCLUION

In this study, the effects of the extracts of botanicals and their use against fungi causing post-harvest decay of yam tubers were studied which revealed that they have tremendous inhibitory activity which is most likely due to presence of antifungal bioactive principles present in plant extracts. However, the exact phytoconstituents and their controlling mechanism to prevent storage rots need to be further studied. Since the leaves of all these plants are easily available without any cost and the leaf extracts do not have any phytotoxic effects for human consumption they can suitably be utilized as biofungicide.

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CONFLICT OF INTEREST

The authors confirmed that there is no conflict of interest for this research paper.

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