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Plant materials as probable growth promoters for certain fungi

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ABSTRACT

Plants and plant products purchased from the local markets of Khartoum State in the Sudan were tested as culture media (solid and liquid) for cultivation of fungi. The pH values of the plants extracts were determined. It was found that the plants extracts varied in their pH values, semi- natural, alkaline and acidic. Curvularia lunata, Aspergillus niger, Aspergillus flavus, and Fusarium oxyaporum were screened initially for growth in 16 different plants extracts (broth media). These strains were able to grow on all of these extracts, but with some variations. The plant extracts exhibited very good growth for these strains were selected for formal assessment of growth. These plants extracts were Tomato, Aubergine, Wheat flour, Dukhun and Pigeon pea, amended with agar powder to solidify. The results showed that all the fungal, species namely C. lunata, A. niger, A. flavus and F. oxyaporum, were capable of growth on these extracts media (solid) and Potato Dextrose Agar (PDA) as control. The growth varied with the different isolates as reflected by colony diameters.

Key words: Plants, plant product, culture media**,** fungi strains, growth

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INTRODUCTION

This study is aimed to develop and evaluate of culture media (liquid and solid) derived from plant products, because the culture media sold as dehydrated preparations have become very expensive in the local market and in most instances are not available.

Generally microorganisms grow naturally in almost all types of habitats. They need essential sources of nutrients to maintain growth and reproduction. Microorganisms generally, need sources of energy, carbon, nitrogen, phosphorus, sulfur and various minerals [1]. Knowledge of microorganism's normal habitat is often useful in selecting an appropriate culture medium because its nutrient requirements reflect its natural surroundings. In addition, specialized media are essential in the isolation and identification of some microorganisms, these media include testing of antibiotic sensitivities, water and food analysis, industrial microbiology and other activities. The precise composition of a satisfactory medium will depend upon the species to be cultivated. Frequently, a medium is used to select and grow specific microorganisms or to help identify a particular species. In such cases, the function of the medium also will determine its composition [1] [2].

Culture media are solid, semi solid and liquid preparations employed for the cultivation and identification of microorganisms. Growth factors in culture media are absolutely necessary for the successful cultivation of most

microorganisms. Failure of an organism to grow on a certain medium is probably due to the absence of one or more of the essential growth accessory substances. Media are usually selected to support good growth [3].

The common ingredients of culture media include water, peptone, meat extracts, yeast extracts, minerals, carbohydrates and agar. Water is essential for the growth of all microorganisms. It must be free from any chemicals which inhibit their growth. Demonized or distilled water must be used in the preparation of culture media, if the local water supply has a high mineral content [3] In fact the water can be classified as nutorious material [4], it has been estimated that approximately 80% of the living weight of a bacterial cell is water . The water can be considered as a solvent, and all the metabolic activities occur in aqueous medium. Water acts as a catalyst for many enzymatic reactions [5].

Fungi
Microbiologists use the term fungus to include eukaryotic, spore-bearing organisms with absorptive nutrition and no chlorophyll that reproduce sexually and asexually. [(8)] Fungi are saprophytic or parasitic microorganisms, distinct from plants and animals [6].

Distribution:
Fungi are primarily terrestrial organisms although few are fresh water or marine. Many are pathogenic and infect plants and animals. Fungi also form beneficial relationships with other organisms [7].

Importance:
Fungi are important to human in both beneficial and harmful ways. With bacteria and few other groups of heterotrophic organisms, fungi act as decomposers, a role of enormous significance. In this way, carbon, nitrogen, phosphorous and other critical constituents of bodies are released and made available for other organisms [8]. Fungi are the major cause of plant diseases and over 5,000 species attack economically valuable crops and garden plants and also many wild plants. In like manner, many diseases of animal and humans are caused by fungi [9].

Fungi, especially the yeasts, are essential to many industrial processes involving fermentation. They also play a major role in the preparation of some cheeses and soy sauce; in the commercial production of many organic acids and certain drugs; and in the manufacture of many antibiotics and the immunosuppressive drug cyclosporine. Fungi have been also exploited by geneticists and molecular biologists as model systems for the investigation of a variety of eukaryotic processes [10].

Structure:
Most fungi consist of microscopic branching filament called hyphae. These are normally divided into cells by cross-walls referred to as septa. A visible mass of inter moven hyphae is called a mycelium. Unlike bacterial colonies, fungal colonies spread radially by the hyphae continually extending and the peripheral hyphae producing branching growth. The rate of growth, texture, colour and form of the colony is distinct for each species and these characteristics are there-fore important in identification.[(13)] Fungi which reproduce by budding are generally referred to as yeasts [11].

Nutrition and Metabolism:
Fungi grow best in dark, moist habitats, but they are found wherever organic material is available. Most fungi are saprophytes, securing their nutrients from dead organic material. Like many bacteria, fungi can secrete hydrolytic enzymes that digest external substrates. They then absorb the soluble products. They are chemoorgano-heterotrophy and use organic material as a source of carbon, electrons and energy [8].

Fungi are usually aerobic. Some yeast, however, are facultatively anaerobic and can obtain energy by fermentation, such as in the production of ethyl alcohol from glucose. Obligate anaerobic fungi are found in the rumen of cattle [8].

Microbial Media Derived From Plant Materials for Fungal Growth:
The selection of a satisfactory medium for stimulating growth and sporulation of a particular fungus can only be found by tests. Most fungi grow well on media having a pH 6-6.5. Any rich carbohydrate source will support fungal growth [9].

Some plant [12] products extracts have been used as culture media for fungal growth as the following: Lima bean agar, hay infusion agar, potato carrot agar, prune agar and oat meal agar. Moreover [13] reported some plant products used for fungal growth. These were: wood extract, canned tomato juice, bean juice from canned green bean, bean meal agar, carrot agar, corn (maize) steep agar, garlic agar, pea agar and potato extract agar.

MATERIALS AND METHODS

1. Collection of samples:

i) Plant materials:

Sixteen plant items, such as fresh vegetables, legumes and seeds, were purchased from local markets of Khartoum state, Sudan. These plant materials were tested for growth promoting effect on fungi. The plant items used in the present work were presented in Table (1).

ii) Preparation of microbial media derived from plant materials:

-Seeds:

Seeds of Wheat and Sorghum (Fatarita) were washed by rinsing several times in tap water and allowed to dry in air. Then the seeds were ground to fine powder (flour) using a mill in the Food Research Center (FRC) at Shambat (Khartoum North, Sudan).

-Fresh vegetables and fruits:

The fresh vegetables and fruits after being purchased from the market they were immediately cleaned from dirt and other foreign plant if there were any, and they were washed thoroughly with tap water and then peeled and chopped.

iii) Liquid plant materials extract (broth media)

PROCEDURE

A specific weight of each sample cited in Table (1) was weighed in a flask, and then 500 ml of distilled water were added. The slurry was mixed thoroughly, boiled in an autoclave at 121°C for 15 minutes. The slurry was then removed from the autoclave with caution and allowed to cool down at room temperature. Each slurry was filtered through a thick cotton fabric (Domoria), then the filtrate was collected and made up to one liter by adding distilled water. The diluted filtrate was mixed thoroughly, and dispensed to appropriate containers that could be plugged and sterilized at 121°C for 15 minutes in an autoclave and kept at 4°C for use. These diluted filtrates constituted the starting materials for microbial media used in this study. Depending on the test, they were used as a broth or supplemented with agar to make a solid medium.

Table (1): The plant materials used in the present work, their quantity and pH values

No.	Common Name	Genus and Species	Quantity (g)	pH
1	Aubergine	*Solanum melongena*	100.0	5.0 ± 0.2
2	Banana	*Musa* spp	100.0	5.6 ± 0.2
3	Beet	*Beta vulgaris, L.*	100.0	5.7 ± 0.2
4	Carrot	*Daucus carota, L.*	200.0	5.3 ± 0.2
5	Dukhun	*Pennisetum typhoides*	50.0	5.2 ± 0.2
6	Faba bean	*Vicia faba (A)*	50.0	5.9 ± 0.2
7	Guar	*Cyamopsis tetragonoloba*	50.0	5.6 ± 0.2
8	Guava	*Psidium guajava*, L.	100.0	4.7 ± 0.2
9	Mango	*Mangifera indica*, L.	100.0	4.6 ± 0.2
10	Pigeon pea	*Cajanus cajan (A)*	50.0	6.0 ± 0.2
11	Rocket	*Eruca sativa*, Mill	100.0	6.7 ± 0.2
12	Sorghum (Fatarita)	*Sorghum bicolor (* seeds *)*	50.0	6.8 ± 0.2
13	Squash	*Cucurbita pepo, L.*	100.0	5.7 ± 0.2
14	Sweet potato	*Ipomoea batatas*, Lam	200.0	5.4 ± 0.2
15	Tomato	*Lycopersicon esculentum*, Mill	200.0	4.1 ± 0.2
16	Wheat	*Triticum vulgare* (flour)	50.0	5.7 ± 0.2

-pH measurement:

Determinations of the pH of plant extracts were done using the potentiometer method which employs a glass electrode. This method was selected because it is more accurate than the colorimetric method and is not affected by colour that develops with some extracts. The pH meter(Kent-E11,England) was calibrated using standard phosphate buffer solutions.

iv) Screening for fungal ability to grow on test media:
Sixteen different plant materials extracts (broth media) were prepared with similar procedure previously described (in iii), and distributed in 200 ml amount in to sterile flasks then closed tightly with cotton wool and autoclaved at 120°C for 15 min. The flasks were allowed to cool at room temperature. The inocula of the pure and actively growing subcultures of *Curvularia lunata*, *A. niger*, *A. flavus* and *Fusarium oxyaporum* (Table 2) were aseptically transferred on the point of a sterile needle to the flasks .The inoculated flasks were incubated at 28°C for 7- 15 days. The growth rate of each fungus recorded ever day.

Table (2): Furgal isolates used in this study.

No.	Fungi	Division	Main Medical Importance
1	*Aspergillus niger*	Ascomycotiona	Some strains are pathogens of plants, for crown rot of groundnuts and boll rot of cotton, used in the production of citric acid, glucoinc acid and other products[10].
2	*Aspergillus flavus*	Asocomycotiona	Acommon soil fungus, may infest products such as ground nut meal and dried foods and produce carcinogenic material called aflatoxin known to induce liver cancer in man and poultry. Ear infections in human[4].
3	*Curvularia Lunata*	Deutromycoina	Cause different disease on rice and other crops, leaf spots, leaf blights kernel rot, root rot, grain discol orations and grain deformations [10].

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Assessment of fungal Growth on plant extracts (solid media):
Five plant extracts which supported the fungal growth in the screening investigation were selected for formal assessment of growth. These extracts were prepared from tomato, aubergine ,pigeon pea, wheat flour and dukun seeds, as previously described , adding agar powder autoclaved at 120c for 15 min and were distributed in to sterile Petri plates (9.0cm) .The inocula of some selected fungi (Table 2): *Curvularia lunata*, *A. niger*, *A. flavus Fusarium oxyaporum* from 3-5 old cultures were aseptically transferred using sterile needles to inoculate the middle of the plant extracts media plates, and Potato Dextrose Agar(PDA) as control. The inoculated plates, three replicates for each fungus, were incubated at 28°C for 7 days. The fungal growth was determined by measuring the colony diameter of each fungus daily and the average was recorded for each fungus [12].

RESULTS

Plant Materials Media (broth):
Extracts of 16 plants materials were tested as media components for cultivation of fungi. Fungal isolates were able to grow on all media made of the different plant materials.

pH of the Media
Table (1) summarizes the results of the pH determination of the 16 types of media tested in this study. The media varied in their pH values, semi neutral, alkaline and acidic. Referring to Table (1) the most acidic media were that obtained from Tomato (pH 4.1), Mango (pH 4.6) and Guava (pH 4.7). The least acidic media were those obtained from the extracts of Rocket (pH 6.7) and Sorghum (Fatarita) (pH 6.8).

Growth characteristics of fungal isolates on microbial media derived from plant materials:
Table (3) summarized the results of the growth of fungal isolates of 5 plants extracts media amended with agar powder to solidify, and on Potato Dextrose Agar (PDA) medium as a control. It was found that all the fungal species namely *Curvularia lunata*, *Aspergillus niger*; *A. flavus* and *Fusarium*, *oxyaporum* were capable of growth on these media, but the growth varied with the different isolates as reflected by colony diameters. On PDA and plants extracts media from tomato, wheat flour, dukhun, pigeon pea, *Curvularia lunata*, grew rapidly covering 9.0 cm plates within 7 days at 28°C, where as on aubergine extract medium it covered the plates in 8 days. The cultures started a white to slightly grey in colour, and became darker by time. *Fusarium oxyaporum* exhibited a rapid growth, covering the plates within 7 days at 28°C on all the plants media, while it covered only 5.0 cm on PDA control plates. The cultures started as white colony growth, light orange in the middle media, and became intense orange by time. *A. niger* growth on the plants media was slow, colonies attained (3.3- 6.5 cm) in diameters after 7 days at 28°C. The fungus produced complete culture cover of the plates of plants extracts media in 12 days. On PDA. *A.niger* colony attained 5.5cm in diameters after 7 days at 28°C. The cultures started as white to dull yellow mycelium, and became black white forming spores. *A.flavus* also grew slowly on the plants extracts media, colonies attained (3.0- 5.3cm) in diameters within 7 days at 28°C. *A.flavus* produced a full plate culture in 12 days.

On PDA. *A.flavus* colony reached 4.1cm in diameters after 7 days at 28°C.The cultures started as white to slightly yellowish- green, becoming green with time.

Table 3: Average* colony diameters (cm) for some fungal species on different plant extracts media and PDA (control)

Incubation period in hours	Extract Medium	Colony diameter in cm			
		C. lunata	*A. niger*	*A. flavus*	*F. oxysporum*
24	Tomato	1.0	1.2	0.9	0.8
	Aubergine	1.1	0.9	0.6	0.8
	Wheat flour	1.3	1.0	1.1	0.6
	Dukhun seeds	1.0	1.3	1.3	0.8
	Pigeon pea	1.4	1.2	1.2	0.9
	PDA	0,5	0.6	0.7	0.6
48	Tomato	1.8	2.3	1.5	2.4
	Aubergine	2.7	1.4	0.9	2.9
	Wheat flour	2.8	1.5	1.4	2.8
	Dukhun seeds	3.0	1.6	1.9	3.2
	Pigeon pea	3.0	2.5	1.6	2.2
	PDA	3.0	2.3	1.5	1.4
72	Tomato	3.4	2.7	2.5	3.8
	Aubergine	3.0	1.8	1.3	4.7
	Wheat flour	4.5	2.3	1.6	4.2
	Dukhun seeds	4.5	2.1	2.2	4.5
	Pigeon pea	4.5	3.3	1.7	4.2
	PDA	4.5	2.8	2.8	2.2
96	Tomato	4.4	3.1	2.9	5.0
	Aubergine	5.7	2.0	1.7	5.3
	Wheat flour	5.5	3.0	2.3	5.1
	Dukhun seeds	6.5	2.8	2.4	5.1
	Pigeon pea	6.3	4.2	3.0	4.9
	PDA	5.6	3.2	3.0	2.8
120	Tomato	6.0	4.0	3.8	6.4
	Aubergine	6.4	2.3	2.0	6.0
	Wheat flour	7.0	3.7	2.5	7.0
	Dukhun seeds	7.7	3.8	2.8	6.3
	Pigeon pea	7.0	4.6	3.2	5.8
	PDA	7.0	4.0	3.5	3.4
144	Tomato	7.9	5.1	4.1	7.3
	Aubergine	8.0	2.5	2.4	8.0
	Wheat flour	8.5	5.0	3.1	8.5
	Dukhun seeds	8.5	4.1	3.0	7.4
	Pigeon pea	8.0	5.0	3.4	7.3
	PDA	8.5	5.0	4.0	4.2
168	Tomato	9.0	6.1	4.5	9.0
	Aubergine	8.5	3.3	3.0	9.0
	Wheat flour	9.0	6.5	5.3	9.0
	Dukhun seeds	9.0	5.1	3.2	9.0
	Pigeon pea	9.0	5.8	3.9	9.0
	PDA	9.0	5.5	4.1	5.0

** = Three replicates.*

DISCUSSION

This work was undertaken to investigate and assess the potentiality of some plant materials as sources of nutrient for cultivation of fungi. Sixteen plants and/or plants products were investigated. Although the pH of the plants media were not adjusted to the pH range (5.5- 6.5), and there were no additives other than inert solidifying agar powder the fungal isolates; namely *Curvularia lunata*, *A. niger*, *A. flavus*, and *Fusarium oxyaporum were* able to grow on all the plants extracts used as culture media, with some difference in the colonies diameter of the various isolates. *Curvularia lunata* was able to grow on all plant extracts tested (100%). It produced complete condense cultures covering 9.0 cm plates of PDA and plant extracts (except aubergine) within 7 days at 28°C.

Fusarium oxyaporum also produced complete cultures covering 9.0 cm plates of plants extracts media These results, may be due to the fact that all these extracts have low pH values (acidic), and are rich in protein, fats,

carbohydrates and the essential elements for the growth of fungi. *A.niger* and *A.flavus* exhibited slow growth on these extracts media, covering the plates after 12 days. It is possible that this slow growth may be due to the absence of some essential growth factors that were particularly required by these two isolates.

In the present study *C. lunata* on PDA control, colony attained 9.0 cm growth within 7 days at 28°C.[16] reported that C. *lunata* on Malt Extract Agar (MEA) reached 4.0 cm in diameter after 5 days at 28°C. *Fusarium oxyaporum* colony on the control medium attained 5.0 cm in diameter after 7 days at 28°C. More over, [17] mentioned that *F. oxyaporum* colony on Potato Sucrose Agar (PSA), incubated at 28°C for 7 days was 3.5 to 5.2cm in diameter, where *F. solani* colony on PSA was 2.5 to 4.5 cm in diameter. Such difference within *F. spp*. Growth rates may be due to the differences in the media nutrients, extracts pH, incubation temperatures, and to the different species metabolism.

In this study *A. niger* and *A. flavus*, colonies on PDA control attained 5.5 cm and4.1cm diameter respectively in 7 days.[16] reported that *A. niger* and *A. flavus* colonies diameters on Czapek Agar (CZ) attained 5.5 cm and 5.6 cm in diameter respectively in 7 days. [17] reported that *A. niger* and *A. flavu* colonies diameters on Czapek Agar (CZ) attained media measured after 7 days incubation at 28°C in complete darkness 4.5 to 6.5 cm and4.0 to 4.5 cm respectively. The above findings were slightly different from the results obtained in this study, and this may be due to the fact that different media and different incubation temperature and light conditions were used.

Fungi grow [8] in dark, moist habitat, and wherever organic material is available.

CONCLUSION

1. The results of this study pointed to the potentiality of using plant materials as a nutrient media supporting growth of fungi
2. With the rising costs of manufactured dehydrated media in the local markets, these plant materials (sources of nutrients) would provide a cheaper alternate for use and demonstrative exercises.

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