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Composition of Agar Media for Fungi in the Laboratory Using Leaf Decoctions of Cassava, Cowpea, Sweet Corn and Banana

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Abstract
Imported culture media for culturing fungi are too expensive. It has therefore become highly necessary to compose standard fungal media from cheap locally available materials. This study was conducted to investigate the growth of three fungal pathogens (*Colletotrichum gloeosporioides*, *Fusarium oxysporum*, and *Curvularia lunata*) on various concentrations of maize (*Zea mays*), cowpea (*Vigna unguiculata*), cassava (*Manihot esculenta*) and wild banana (*Musa sapientum*) leaf decoctions. To the decoctions was added 15g of agar to solidify the medium. The ability of the decoctions to support radial growth and sporulation of the fungi with or without dextrose was observed. The consistency, smoothness and fluffiness of fungal cultures in comparison with their growth characteristics on a standard laboratory fungal culture medium on Potato dextrose Agar (PDA) were observed. Some of the various concentrations of the leaf decoctions tested promoted better radial growth of the fungi than PDA. Maize leaf decoction at 0.2kg, 0.50kg/L and 1kg/L and maize leaf decoction without dextrose supported the growth of *Fusarium ozysporum* more than PDA whereas banana leaf decoction at 0.25kg/L and 1kg/L without dextrose, 1kg/L with dextrose and cassava leaf decoction at 1kg/L with dextrose supported the growth of *Curvulania lunata* better than PDA. These are recommended for culturing the three fungal pathogens.

Keywords: Imported culture media; leaf decoctions; *Fusarium oxysporum; Curvularia lunata; Colletotrichum gloeosporioides;* Potato dextrose agar

Introduction
All fungi require several specific elements for growth and reproduction. The requirements for growth are generally less stringent than for sporulation, so it is often necessary to try several types of media when attempting to identify a fungus in culture (Stamets, 1997). Not all media are equally good for all fungi. So also there is no universal substrate or artificial medium on which all fungi can grow well (Thangamani *et al.,* 2011). Media generally contain a source of carbon, nitrogen and vitamins. Glucose is the most widely utilizable carbon source hence it is the most commonly used in growth media.

Most pathological studies require culturing of a pathogenic fungus either to increase propagules for inoculation or to study its taxonomy and genetics. Consequently techniques for producing vegetative, asexual or sexual propagules have been developed for many pathogens (Adewami, 1988).

Potato dextrose agar (PDA) is commonly used for the isolation and growth of a wide range of fungi in laboratories and its composition is well defined (Sharma and Pandey, 2010). Several workers informed that PDA is the best medium for mycelial growth (Xu *et al.,* 1984; Maheshwari *et al., 1999* and Saha *et al.,* 2008) most fungi thrive on PDA but this can be too rich in nutrient thus encouraging mycelial growth with ultimate loss of ability to sporulate (UKNCC, 1998).

However, with dwindling resources for research, it is becoming increasingly difficult for mycologists in developing countries to purchase imported culture media, as their cost is prohibitively high (Adesemoye and Adedire, 2005). In the last few years, some researchers started work on alternatives to the standard PDA using cheap locally available materials (Adesemoye and Adedire, 2005; Rajasab, 2007 and Marikar, 2009). Already one can use fresh potato tuber and add dextrose and agar to prepare a local form of PDA. Even though workers successfully cultivated different types of fungi on alternative media, in all cases, they still required the addition of imported agar as a solidifying agent. In 2010, Sharma and Pandey observed the growth rate, colony characteristics and sporulation patterns of ten fungal isolates using three different culture media. All ten isolates of fungi grew well and sporulated heavily on the three culture media.

Likewise, Omemu *et al,* (2008) demonstrated how six fungi grew and sporulated on viriously processed waste maize cobs in agar media.

Following on the results obtained by earlier workers, it is the aim of this study to investigate the rate of growth and of sporulation of three fungal pathogens, namely; *Colletotrichum gloeosporioides, Fusarium oxysporum* and *Curvularia lunata*, on various concentrations of leaf decoctions of maize, (sweet corn) cowpea. (ife brown), cassava (TMS 30572) and banana (Prata). These crops are locally available and their leaves are easy to obtain. The ability of the decoctions to support growth and sporulation with or without dextrose, the consistency, smoothness and fluffiness of cultures in relation to those on PDA are observation points of this study.

Materials and Methods

Experimental location

This study was carried out in the Plant Pathology Laboratory of the Department of Crop Protection and Environmental Biology (CPEB), University of Ibadan.

Sources of inocula (plant pathogens) and plant materials

Cultures of *Colletotrichum gloeosporioides* and *Fusarium oxyporum* were obtained from the existing stock in the Plant Pathology Laboratory while *Curvularia lunata* culture was obtained from the Department of Botany, University of Ibadan.

Sources of plant leaves used for preparing decoctions. Leaves of maize (*Zea mays*), cowpea (*Vigna unguiculata*), banana (*Musa sapientum*) and cassava (*Manihot esculenta*) were collected from CPEB Crop Garden.

Leaf decoction preparation

The decoctions were prepared by separately blending 1kg, 500g and 250g of each of the crop leaves in 1L of water in a Binatone blender for `15 minutes. The resulting slurry was filtered through an eight-layer cheese-cloth. The filtrate was heated for 15 minutes to inactivate microorganisms present in the filtrates. To each of the decoctions containing extracts from 1kg, 0.50kg and 0.25kg/L of water, was added 10g dextrose and 15g of BACTO agar. The resulting mixture from each litre of the decoction, dextrose agar was heated to dissolve the agar completely and dispensed in 100ml amounts into 250ml Erlenmeyer flasks, corked with non-absorbent cotton wool and the neck and top

was wrapped with aluminium foil. They were then autoclaved for 15 minutes at 121^{0}C (pressure of 1.05kg/cm^2).

Comparison of rate of growth of fungi on different media

Each medium was tested for its capacity to support mycelial growth of *Curvularia lunata, Fusarium oxysporum* and *Colletotrichum gloeosporioides* as follows: From the advancing margin of 5 day old cultures of the pathogens on PDA, 5mm diameter discs were aseptically removed using a sterile corkborer and transferred face up to the centres of the plates containing media to be assayed. The assay was carried out in triplicates for each of the concentrations of the decoctions. Inoculated plates were incubated at laboratory temperature of 28±2^{0}C (under diffuse light). Radial growth of resulting colonies was measured at 24h intervals for seven days using the method of Adesemoye and Adedire (2005).

Ability to support sporulation

To evaluate sporulation, wet mounts of 14 day old mycelia on glass slides were examined under an Olympus compound microscope at low (10x10) and high (10x40) power magnifications., cultures with less than five spores per slide view were deemed to have light sporulation. Those that produced more than five but less than fifteen spores/view were deemed to have moderate sporulation while those that produced more than fifteen spores per view were deemed to sporulate heavily.

Influence of media on growth morphology of pathogens

The consistency, smoothness and fluffiness of the cultures were compared with those on potato dextrose agar which served as control. The amount of aerial mycelia and gross morphology were visually assessed after seven days of incubation.

Cost implication of using leaf decoctions compared with PDA

The cost of using local materials such as leaf decoctions in formulating a growth medium for fungi was studied and compared with that of PDA.

Statistical analyses

Means were separated using Duncan's new multiple range test for comparison of means at P (0.05) or f (5%).

Results

Comparison of rate growth of fungi on different decoction media with those on PDA

All leaf decoction agar media used in the study supported the growth of the pathogenic fungi although at varying degrees.

Colletotrichum gloeosporioides had its best growth of 4.06cm in diameter on 0.50kg/L of maize leaf decoction followed by 4.00cm diameter on 0.25kg/L., whereas growth of 3.10cm diameter was achieved on PDA.

The fungus had very poor growth on same leaf decoctions without dextrose (Fig. 1). It however had its lowest growth (1.50cm diameter on 0.50kg/L) on cowpea leaf decoction without dextrose. This was not significantly different from its growth on the various concentrations of maize leaf decoction without dextrose.

Growth of the pathogen on 0.50kg/L of cowpea leaf decoction with dextrose

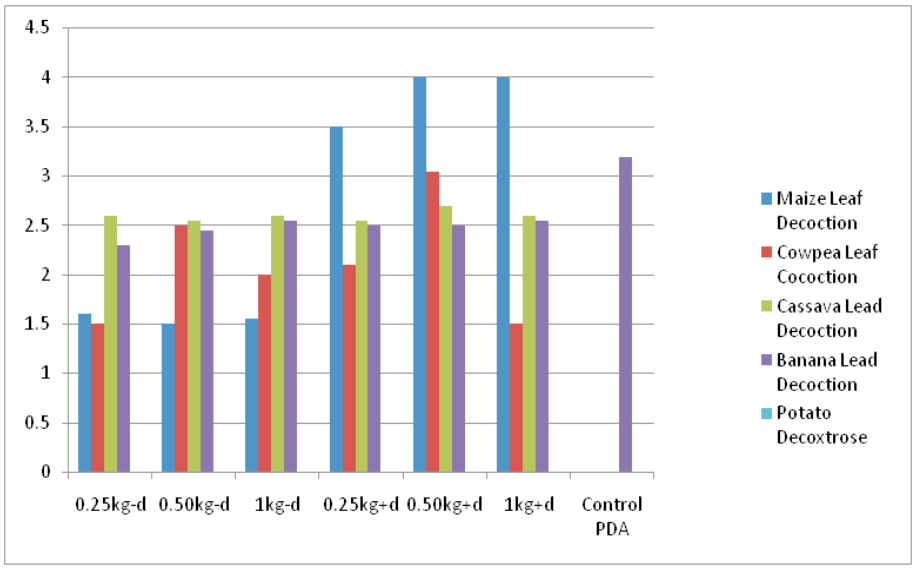

Figure 1: Radial growth of *C. gloeosporioides* on different media, five days after inoculation (-d = without dextrose; +d = with dextrose)

(3.10cm diameter) was comparable to PDA (3.23cm diameter).

The radial growth of *Fusarium oxysporum* on maize leaf decoction agar containing 0.25kg/L without dextrose did not grow well in the different decoction concentration of cowpea, cassava and banana. It grew better on PDA.

Better growth of *Curvularia lunata* (3.22cm diameter) was observed on maize leaf decoction agar (1kg/L) when compared with PDA (2.33cm diameter). (Fig. 3).

PDA supported significantly higher growth than any concentration of banana leaf decoction agar. *C. lunata* had better radial growth on PDA than on 0.25kg and 0.50kg/L of cassava leaf decoction. Only 1kg/L concentration of cassava leaf decoction performed better than PDA (Fig. 3).

Ability of leaf decoctions to support sporulation compared with PDA

Colletotrichum gloeosporioides did not sporulate on maize leaf decoction (1kg/L of distilled water) with dextrose. It however, had heavy sporulation on PDA, on maize leaf decoction agar with dextrose at concentrations of 0.25kg and 0.50kg/L of distilled water. Cowpea leaf decoction agar without dextrose at 0.25kg/L of distilled water), without dextrose and 0.25kg/L with dextrose also supported heavy sporulation. Banana leaf decoction agar at 0.50kg/L and 1kg/L of distilled with and without dextrose also gave heavy sporulation. The fungus sporulated moderately on all the remaining media.

Fusarium oxysporum was observed to produce more than fifteen spores/field of view on maize leaf decoction agar

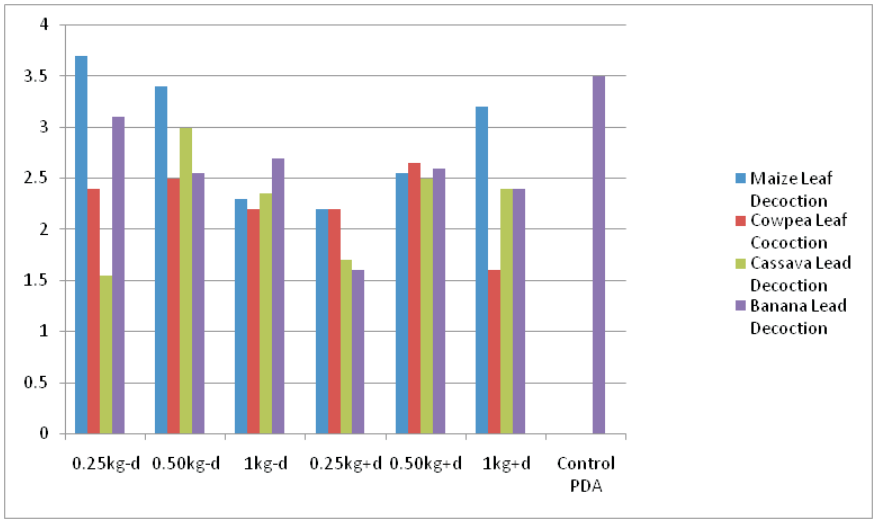

Figure 2: Radial growth of *F. oxysporum* on different agar media, five days after inoculation. (-d = without dextrose; +d = with dextrose)

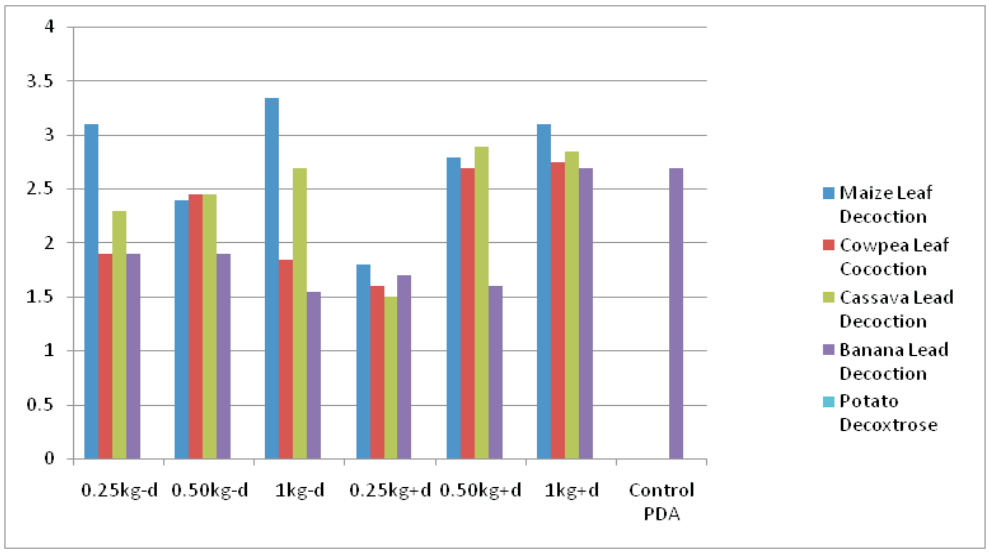

Fig. 3: Radial growth of *C. lunata* on different agar media five days after inoculation (-d = without dextrose; +d = with dextrose)

(0.25kg/L). This was deemed to be heavy sporulation. Heavy sporulation was also observed on cowpea leaf decoction agar at 0.25 and 0.50kg/L of distilled water without dextrose and at 0.25kg/L distilled water and dextrose.

Of all concentrations of banana leaf decoction (with and without dextrose) investigated and cassava leaf decoction agar (0.25kg/L and 0.50kg/L of cassava leaf decoction), only 1kg/L concentration of cassava leaf decoction performed better than PDA (Fig. 3).

All concentrations of banana leaf decoction (with and without dextrose) investigated and cassava leaf decoction agar (0.25kg/L and 0.50kg/L of distilled water without dextrose and 0.25kg/L distilled water with dextrose) gave heavy sporulation. They however, sporulated moderately on PDA (Table 1).

Curvularia lunata produced more than fifteen spores/field of view on maize leaf decoction agar at 0.50kg and 1kg/L of distilled water. The organism produced about 10 spores/field of view and was deemed to sporulate moderately on all other media except cassava leaf decoction agar with dextrose at 1kg/L of distilled water on which it produced six spores/field of view.

Characteristic colour

Characteristic colour of *C. gloeosporioides* was not variable on all the media tested. It

Table 1: Spore Production on different medial by study fungi

Fungus	Sporulation	Medium	No of Spores/Field
C. gloeosporioides	Heavy	PDA	15-25
		Maize leaf + dextrose at 8.25 and 0.50kg/L	15-25
		Cowpea leaf – dextrose at 0.25kg/L	17-28
		Cassava leaf – dextrose at 0.25, 0.50 and 1kg/L	15-20
		Cassava leaf + dextrose at 0.25kg/L	15-20
		Banana leaf + dextrose	18-30
		Banana leaf – dextrose at 0.05 and 1kg/L	16-25
F. oxysporoum	Heavy	PDA	20-30
		Maize leaf + dextrose at 8.25 and 0.50kg/L	15-20
		Cowpea leaf – dextrose at 0.25kg/L	17-25
		Cassava leaf – dextrose at 0.25, 0.50 and 1kg/L	15-25
		Bababa leaf + or - dextrose	
		Cassava leaf + dextrose at 0.25kg/L	20-22
		Banana leaf + dextrose	15-20
		Banana leaf – dextrose at 0.05 and 1kg/L	13-18
			28-35
C. lunata	Heavy	PDA	20-25
		Maize leaf + dextrose at 0.50 and 1.0kg/L	18-25
	Moderate Light	All other media	8-12
		Cassava leaf + dextrose	6-8

was white in the first five days and later turned to grey-blackcolour as the fungus grew older. *C. lunata* had no variation in colour on cassava leaf decoction agar, maintaining its dark brown colour which later turned brown-suede black.

Colony colour of *F. oxysporum* was white in the first five days and later turned pink colour. This was also similar on all media tested apart from banana leaf decoction dextrose agar (1kg/L of distilled water) which maintained a whitish colour throughout the period of study.

Colony morphology

Colour morphology ranged from fluffy to smooth and scanty mycelia. The characteristic were variable on each medium in the same environment.

Curvularia gloeosporioides had fluffy and consistent mycelia on PDA, maize leaf and cowpea leaf decoction with dextrose agar. *C. lunata* had consistent and compact mycelia on PDA, maize leaf, cassava leaf and cowpea leaf decoction agar. *F. oxysporum* also had compact and consistent growth on PDA, maize leaf and cowpea leaf decoction agar. As the cultures advanced in age, more consistent mycelial mats were formed. Consistent growth was observed for *F. oxysporum* on cassava leaf decoction agar while its culture was sparse on banana leaf decoction agar.

Discussion and Conclusion

A medium suited to a particular species of plant pathogenic fungi may not prove satisfactory in the case of other species (Adewami, 1990). The range of conditions permitting vegetative growth and sporulation are divided into minimum, below which are maximum, above which no growth will occur and optimum condition under which it will grow best (Adewami, 1990). The optimum condition may be narrow or wide. However, there is no universal set of conditions for culturing pathogenic fungi.

Curvularia gloeosporioides had its best growth on maize leaf decoction while it had very poor growth on the same leaf decoction without dextrose. This is in support of the fact that carbon-rich media are used for good mycelia growth (Cooke, 2010). Sporulation was not supported by the highest concentration of maize leaf decoction used in this study. This may be due to over-enrichment of the medium with carbon which produced mycelial mass at the expense of sporulation (Frankland *et al.,* 1995; Cooke 2010). Its poor growth on cowpea leaf decoction agar which was not significantly different from its growth on the various concentrations of maize leaf decoction without dextrose suggests that cowpea leaves are not capable of supporting the growth of this species of the pathogen. *F. oxysporum,* had good growth at the lowest maize leaf decoction concentration and was comparable with its growth on PDA. Increase in concentration of maize leaf decoction did not result in the increase of growth of the pathogen. These suggest that both PDA and maize leaf decoction have similar capacity to support the growth of *F. oxysporum*. Better growth of *Curvularia lunata* on maize (which is one of its host plant) is in support of the works of earlier workers (Andrew and Pitt, 1986; Adewami, 1990).

The study is in consonance with the works of Adesemoye and Adedire (2005) and Omemu *et al.* (2008), who reported that using our local, readily available and

cheaper carbohydrates and leaf decoctions as alternatives to PDA is achievable.

Cost Implication of using leaf decoctions compared with PDA

The cost of imported PDA is about seventeen thousand Naira (N17,000.00) per 500g bottle. Agar-Agar costs eleven thousand naira (N11,000.00) per 500g bottle.

Since leaves can be obtained free of charge during the growing season in the locality, composing a culture medium with leaf decoctions can reduce cost by six thousand naira (N6,000.00) (41% of the cost of imported PDA) or by nine thousand five hundred naira (N9,500.00) (about 55.9% of the cost of imported PDA) for those that did not require dextrose. The use of leaf decoctions will not only save costs but will as well solve the problem of non-availability of culture media for fungal studies, since these leaves are readily available.

Recommendation

Based on the rate of growth of the fungal pathogens on the different concentrations of leaf decoctions the following are recommended.

For *Colletotrichum gloeosporioides* maize leaf decoction at 0.25kg/L of distilled water with dextrose is recommended. This will support its growth and sporulation as much as PDA. Also cowpea leaf at 0.50kg/L of distilled water, banana leaf at 1.0kg/L of distilled water without dextrose and 0.25kg of banana leaf of distilled water with dextrose could be used as they performed closely to PDA. These can be used if a slightly less fluffy culture is required.

For *F. oxysporum,* maize leaf decoction at 0.25kg/L of distilled water is recommended, as it supported its growth as much as PDA.

For *Curvularia lunata,* maize leaf decoction at 0.25kg without dextrose and cassava leaf decoction with or without dextrose are recommended.

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