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Developing Culture Media from Yam Species (*Dioscoreae rotundata*): Technique for Isolation of Tuber-Rot Fungi of Yam

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Abstract: In an experiment to develop yam (*Dioscorea rotundata*) based culture media for isolation of tuber-rot fungi of yam (*Dioscorea* sp.), culture media were prepared from three varieties of *Dioscorea rotundata*, namely Nwopoko, Obioturugo and Abi, for isolation of six causing rot fungi of yam with Potato Dextrose Agar (PDA) as standard culture medium. The six rot fungi were: *Botryodiplodia theobromae*, *Fusarium oxysporum*, *Penicillium sclerotigenum*, *Aspergillus niger*, *Rhizopus nodosus* and *Mucor circineloides*. The result of the study showed that the growth of the fungi on the yam-based culture media was equally as good as their growth on the Potato Dextrose Agar (PDA) used as standard medium, while some even gave better growth of the fungi with sporulation as high as 1.4×10^8 and 1.6×10^8 obtained, respectively on Nwopoko and Obioturugo-based agar medium.

Key words: Yam, culture, media, rot-causing, fungi, Nigeria

INTRODUCTION

Development of indigenous technology for the isolation of rot-causing fungi of yam (*Dioscoreae rotundata*) is a wise step to solving fungal disease complex of the crop especially under storage. This is to remove the perpetual ignorance which has been created due to the use of synthetic culture media in the market which could not support the growth/sporulation of disease (rot) pathogens. The use of ineffective culture media has been responsible for wrong control measures applied especially under storage when more damage is done to this important crop. It is clear that wrong laboratory result makes room for application of wrong control measures because when a problem is carefully defined and analyzed such problem is half-solved and with direction.

Yam is an essential food consumed in large quantity in Africa and some other parts of the world. The tuber contains vitamins and limited amount of protein (Wright and Peters, 2002). The rot of yam tubers has been traced to the activities of fungi which are described as primary invaders and even secondary invaders, respectively (Amusa, 2001; Nwawuisi *et al*., 2008). Among the fungi that cause damage to tubers are *Botryodiplodia theobramea*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Penicillium sclerotigenum*, *Penicillium oxalicum*, *Aspergillius niger*, *Rhizopus nodosus*, *Mucor circineloides*, etc. (Ikotun, 1983; Okigbo and Ikediugwu, 2000). Yam tubers succumb to rot at all stages of development, from tuber initiation through bulking to harvest and storage.

The global awareness of the importance of yam, calls for the need to develop culture media from yam species for the pathogenic fungi as alternative to commercial synthetic culture media. Therefore, this research was designed to develop yam media that could be used to sustain pathogen cultures for isolation and innoculum stock-keeping in yam as a guide for disease control at field and storage. Six rot-causing fungi were assessed to determine their growth and sporulation on culture made from three yam varieties of *Dioscorea rotundata*.

MATERIALS AND METHODS

The three yam varieties used in the experiment were Nwopoko, Obioturugo and Abi. These are among the popular yam varieties grown in South East Nigeria. The yam varieties were used to prepare yam-based media to culture six tuber rot-causing fungi: *Botryodiplodia theobromea*, *Fusarium oxysporum*, *Penicillium sclerotigenum*, *Aspergillius niger*, *Rhizopus nodosus* and *Mucor circineloides*. Stock cultures of these pathogens were grown at 25°C on agar slopes.

About 20 g of washed yam tuber of each variety (Nwopoko, Abi, Obioturugo) which had been weighed and cut into smaller fragments was put into 500 mL conical flask and about 100 mL of distilled water added.

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The mixture was boiled for 30 min. After cooling, it was filtered through a clean piece of cloth and the filtrate made up with distilled water. Addition of 12 g of agar powder to the medium solidified it. Liquid medium containing only agar powder was equally prepared. After cooling, the agar containing medium was poured into plates and left to solidify before inoculation. The medium without yam extract formed the control. This was distilled water with agar powder only.

Potato Dextrose Agar (PDA) was used as standard medium. The effect of the different media on the growth of the test fungi was determined by measuring the linear growth on the solid media. The average number of spores produced by each of the test fungi, on different solid media was also determined. Two lines intercepting at right angles in the center of the petridishes were drawn at the bottom of each plate. An innoculum of each of the test fungi was cut with a 5 mm diameter sterile cork borer and placed on each media at the point of interception of the lines. They were then incubated at room temperature and the colony diameter measured.

The colony diameter measurement was taken daily for 7 days along these lines and the growth rate per day calculated from the final colony diameter, after subtracting 5 mm which was the diameter of the initial innoculum. Final values were obtained by finding the average diameter of the 3 plates.

The mycelium or pure colony of each of the test fungi in a petri dish after 10 days of incubation was carefully seeped with a filter and a known volume of water added and stirred very well to release the available spores. The mixture was filtered with cotton wool and the spore suspension made. A drop of it was placed on haemocytometer and covered with cover slip. The cover slip was pressed harder to ensure no bubble of air. The haemocytometre was mounted and viewed under light microscope and the spores on each of the squares on the haemocytometer were counted. This was reported for the next 4 squares and average number of the spores for 5 different squares was recorded. Final volume of the number of spores of the mycelium on each plate was obtained by finding the average value (t) for a particular test medium containing a particular test fungus. Formula for calculating number of spores produced by a fungus on a particular solid medium was represented as: $d \times t \times 8 \times 10^4$, where:

d = Volume of water used in preparing the spores suspension
t = Average number of spores produced

RESULT AND DISCUSSION

Results of the experiment showed that the various yam media effectively supported the growth of the test fungi. Table 1 and 2 show the linear growth of *Botryodiplodia theobromae* and *Penicillium sclerotigenum* on the media. The growth was very vigorous, especially *B. theobromae* culture that filled the petri dishes 4 days after plating while *P. sclerotigenum* gradually progressed in its growth. Table 3 and 4 show the same trend in the growth of the test fungi on yam media which was effectively comparable with that of the standard (PDA). The growth of *Mucor circinelloides* and *Rhizopus nodosus* appeared spontaneous on the media

Table 1: Average rate (mm) of growth of *Botryodplodia theobromae* on the various solid media

	No. of days						
Names of media	1	2	3	4	5	6	7
Potato dextrose agar	42	69	Xx	Xx	Xx	Xx	Xx
Nwopoko agar	13	27	42	Xx	Xx	Xx	Xx
Obiaoturugo agar	11	24	33	X	X	Xx	Xx
Abi agar	12	23	38	X	X	X	X
SED	7.51	11.11	15.69	-	-	-	-

Measurement in mm; X = Slightly filled up; Xx = Totally filled up; Xxx = Heavily filled up

Table 2: Average rate of growth (mm) of *Penicillium sclerotigenum* on the various solid media

	No. of days						
Names of media	1	2	3	4	5	6	7
Potato dextrose agar	13	23	31	43	62	73	Xx
Nwopoko agar	18	32	41	54	62	74	Xx
Obiaoturugo agar	10	21	34	43	50	61	70
Abi agar	10	22	31	39	47	55	60
SED	1.89	2.53	2.36	3.22	3.94	4.64	10.31

Table 3: Average rate of growth (mm) of *Fusarium oxysporum* on the various solid media

	No. of days						
Names of media	1	2	3	4	5	6	7
Potato dextrose agar	13	23	31	43	62	73	Xx
Nwopoko agar	10	15	21	35	40	55	63
Obiaoturugo agar	9	14	18	26	38	45	55
Abi agar	9	12	19	28	58	46	54
SED	0.94	2.42	2.98	0.35	6.13	6.49	10.86

Table 4: Average rate of growth (mm) of *Aspergillius niger* on the solid media

	No. of days						
Names of media	1	2	3	4	5	6	7
Potato dextrose agar	8	17	26	36	44	51	62
Nwopoko agar	8	16	26	37	45	56	65
Obiaoturugo agar	7	13	19	27	34	40	50
Abi agar	10	16	26	36	45	55	66
SED	0.63	0.87	1.75	2.35	2.68	3.66	3.68

(Table 5 and 6). Barely 3 days after inoculation/ incubation the mycelia had already filled the plates. Although, the three yam media effectively supported the growth of the test fungi, Nwopoko medium showed impressively greater result on the fungal growth. However, the yam media compared favourably with the standard (Potato Dextrose Agar (PDA)) in linear growth of the fungi. The result equally showed that all the media effectively supported sporulation of the pathogenic fungi except *B. theobromae* which did not sporulate on PDA and Abi media while *R. nodosus* did not sporulate on all the media (Table 7).

Observation from the present study showed that the growth of the test fungi on the media prepared using varieties of yam (Nwopoko, Obioturugo and Abi) were equally as good as on the Potato Dextrose Agar used as the standard medium while some even gave better growth of the test fungi.

Some of the test fungi such as *Penicillium sclerotigenum* and *Aspergillus niger* showed poor linear growth on all media but produced highest number of spores. It is interesting to note that *Botryodiplodia theobromae* which needs illumination to induce sporulation produced pycnidia on Nwopoko agar and Obioturugo agar media. These show that these varieties of yam (*Dioscorea* sp.) probably contain the essential elements that could be supplied into growth media to compensate for exposure to light needed for sporulation of the fungi (Okonkwo, 2002; Okonkwo *et al*., 1988). Furthermore, it is remarkable that *Botryodiplodia theobromae* sporulated on yam varieties of *Dioscorea* sp. (Nwopoko and Obioturugo) media because the fungus has variously been associated with the storage rot of yams (Adesiyam and Odihirin, 1975; Ohazurike and Obi, 2000). This result is also in agreement with result of Amusa *et al*. (2003) who showed that pycnidia were formed in mummified yam tissues infected by *Botryodiplodia theobromae*.

The better growth result obtained from some of these yam varieties over the standard medium (PDA) may probably be due to the presences of some natural essential growth factors contained in the Nwopoko, Obioturugo and Abi yam varieties.

Table 5: Average rate of growth (mm) of *Mucor circinelloides* on the solid media

	No. of days						
Names of media	1	2	3	4	5	6	7
Potato dextrose agar	90	Xx	Xx	Xxx	Xxx	Xxx	Xxx
Nwopoko agar	10	32	Xx	Xx	Xx	Xx	Xx
Obiaoturugo agar	10	62	Xx	Xx	Xx	Xxx	Xxx
Abi agar	9	35	Xx	Xx	Xx	Xx	Xx
SE±	20.08	15.77	-	-	-	-	-

Table 6: Average rate of growth (mm) of *Rhizopus nodosus* on the different solid media

	No. of days						
Names of media	1	2	3	4	5	6	7
Potato dextrose agar	80	Xx	Xx	Xxx	Xxx	Xxx	Xxx
Nwopoko agar	12	X	Xx	Xx	Xx	Xx	Xx
Obiaoturugo agar	10	40.5	X	Xx	Xx	Xx	Xx
Abi agar	10	22	60	X	X	X	x
SED	17.33	-	-	-	-	-	-

Measurement in mm; X = Slightly filled up; Xx = Totally filled up; Xxx = Heavily filled up

Table 7: Sporulation of the test fungi on the yam media

	Test fungi		
Name of media	*B. theobromae*	*P. sclerotigenum*	*F. oxysporum*
PDA	-	1.50×10^{8}	1.000×10^{7}
Nwopoko agar	2.40×10^{6}	1.40×10^{8}	1.400×10^{7}
Obioturugo agar	4.80×10^{6}	1.60×10^{8}	1.000×10^{7}
Abi agar	-	7.60×10^{7}	4.800×10^{6}
SED	0.12×10^{7}	1.89×10^{7}	0.19×107
Name of media	*A. niger*	*M. circinolloids*	*R. nodosu*
PDA	2.10×1.0^{8}	6.00×10^{7}	-
Nwopoko agar	7.20×10^{7}	5.90×10^{7}	-
Obioturugo agar	6.80×10^{7}	7.50×10^{7}	-
Abi agar	2.90×10^{7}	6.90×10^{6}	-
SED	3.96×10^{7}	1.49×10^{7}	-

CONCLUSION

Since these varieties of yam were able to support the growth of the test fungi, they can be utilized in laboratory work, for the culture of the tested fungi or in any mycological and pathological engagement having in mind that routine work does not involve their nutritional requirement. This would save the money that could have been spent on purchase of synthetic media.

RECOMMENDATION

The tested culture media been yam-based are hereby recommended for mycological work in yam research and laboratory analyses as the material for the preparation is readily available, cheap to procure and the product is effective.

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