ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 6 (1): 40-44, 2007
ISSN 1682-296X
© 2007 Asian Network for Scientific Information

In vitro Analysis of Growth Media and the Control of Yam Minisett-rot

[1]E. Asare-Bediako, [1]F.A. Showemimo, [1]Y. Opoku-Asiama and [2]D.H.A.K. Amewowor
[1]Department of Crop Science, School of Agriculture,
[2]Department of Botany, Faculty of Science, University of Cape Coast, Ghana

Abstract: Yam minisetts are susceptible to rot caused by microorganisms in both the sprouting media and mother seed yam. This study was conducted to determine the most effective treatment of yam minisetts against rot organisms. Five different protectants/disinfectants were used *in vitro* and replicated thrice. The result revealed that disinfectants (sodium hypochlorite and aqueous neem leaf extract), protectants (lime, wood ash and Benlate) either suppressed or inhibited the growth of fungi *in vitro*. Benlate inhibited the growth of all the test fungi, except *Rhizopus stolonifer* that was tolerant to the fungicide. Aqueous neem leaf extract was the least effective among the disinfectants in controlling fungal growth *in vitro*. Both quicklime and wood ash suppressed the growth of *Aspergillus flavus* and *Rhizopus stolonifer* and completely inhibited the growth of *Sclerotium rolfsii* and *Penicillium* sp. Sodium hypochlorite completely inhibited the growth of *Sclerotium rolfsii* and suppressed the growth of *Aspergillus flavus*, *Fusarium* sp., *Rhizopus stolonifer* and *Trichoderma* sp. Higher concentrations of the disinfectants and protectants were more effective in controlling the growth of these fungi than the lower concentrations.

Key words: Concentrations, disinfectants, fungi, growth inhibition, yam minisett

INTRODUCTION

Microbial rot of yam minisetts had been identified to be responsible for poor sprouting and growth of yam minisetts (Osai and Ikotun, 1994). Microbes such as; *Sclerotium rolfsii*, *Trichderma longibrachyatum*, *Botryodiplodia theobramae* and *Penicillium oxalic* are the most pathogenic fungi responsible for losses ranging from 38.2 to 70.2% among *Dioscorea rotundata* cultivars. Cornelius (1998) identified Pona as the most economically important white yam cultivars in Ghana. Increased production of Pona through yam minisett propagation technique has suffered a serious setback as a result of rapid rotting of the minisetts in the sprouting medium or nursery, (Emehute *et al*., 1998; Asare-Bediako, 2003).

Aspergillus flavus, *A niger*, *A. ochraceus*, *A. tamari*, *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., *Cladosporium* sp., *Sclerotium rolfsii*, *Rhizopus stolonifer* and a bacterium *Corynebacterium* sp. had been identified to cause yam minisetts rot (Osai and Ikotun, 1994; Cornelius, 1998; Asare-Bediako, 2003). According to Ikotun (1983) the sources of rot pathogens were the infected mother seed yam and the sprouting medium. Meanwhile, Emehute *et al.* (1998) reported that, the fresh cut surfaces of yam minisetts serve as entry points of microorganisms and the growth media encourages the microbial growth. To avoid or reduce the incidence of attack of yam minisetts by disease organisms, Otoo *et al.* (1987) recommended that the minisetts should be treated with a mixture of fungicide and wood ash.

Pona minisetts, however, had been found to be very susceptible to rot pathogens and require proper treatment before high percentage sprouting can be obtained. This study, was therefore, aimed at assessing *in vitro* the effect of different disinfectants and protectants in the control of yam rot diseases in Pona.

MATERIALS AND METHODS

The study was carried out in the laboratory using 4 day old cultures of *Aspergillus flavus, Fusarium* sp., *Cladosporium* sp. *Sclerotium rolfsii*, *Trichoderma lignorum* and *Rhizopus stolonifer* isolated ealier from rotten Pona minisetts and maintained on Potato Dextrose Agar (PDA). Each of the fungi was used to inoculate different plates in each treatment. Inoculums plugs were obtained with 1cm-diameter- cork borer from the growing margin.

The treatment consist fungal isolates on PDA amended with *Senna siamea* wood ash (APDA), quicklime (Calcium oxide) (LPDA), benomyl (BPDA), sodium hypochlorite (SPDA) and aqueous neem leaf extract

Corresponding Author: Dr. F.A. Showemimo, Department of Crop Science, School of Agriculture, University of Cape Coast, Ghana

(NPDA), respectively. The treatment levels were 0.5, 1.0 and 1.5 g of both wood ash and lime; 6 mL each of 0.8, 1.0 and 1.2 g L^{-1} of benomyl; 6 mL each of sodium hypochlorite dilutions of 1:5, 1:10 and 1:15 and 6 mL each of aqueous neem leaf extracts of 100, 200 and 300 g L^{-1} 15 mL of PDA in a 90 mm petri dish. The pH of *Senna siamea* wood ash solution (50%) and quicklime solution (50%) were used as determined by pH meter.

Solidified 15 mL amended PDA in a Petri dish was inoculated at the centre with a 1 cm disc of agar medium bearing the mycelium of the test fungus. There were three replicate plates per isolate. The inoculated petri dishes were enclosed in clean polythene bags and incubated in the laboratory under ambient temperature ranging from 26 to 30°C in a completely randomized design layout. At the end of seventh day, the colony diameter of each isolate was measured with a rule. Square root of data transformation was used for Analysis of variance, mean separation was done using Least Square Difference (LSD).

RESULTS

The analysis of variance revealed highly significant differences among disinfectants, levels of disinfectant concentration, fungal responses and their interactions. Thus, mean data averaged over replications were presented in this study.

The quicklime either suppressed or totally inhibited the growth of the test fungi (Table 1). The lime, at all concentrations, completely inhibited the growth of *Penicillium* sp. and *Sclerotium rolfsii* and significantly suppressed the growth of other fungi. Highest concentration of lime (1.5 g 15 mL PDA) significantly inhibited the growth of *Cladosporium* sp. but low concentrations; 0.5 and 1.0 g 15 mL PDA suppressed its growth, recording colony diameters of 9.3 and 5.0 mm, respectively.

Table 1: Growth of fungi on media ammended with different levels of quick lime (calcium oxide) incubated at 27-31°C for 7 days

	Mean colony diameter (mm) on PDA amended with indicated level of lime				
Fungal species	0.0	0.5	1.0	1.5	Fungi means
Aspergillus flavus	54.6	22.0	10.0	3.3	22.5b
Cladosporium sp.	30.7	9.3	5.0	0.0	11.3bc
Fusarium sp.	71.3	48.3	38.3	23.0	45.3a
Penicillium sp.	27.7	0.0	0.0	0.0	6.9c
Rhizopus stolonifer	90.0	31.7	15.0	6.7	35.8a
Sclerotium rolfsii	90.0	0.0	0.0	0.0	22.5b
Trichoderma lignorum	90.0	33.3	23.3	6.7	38.3a
Means	64.9a	20.7b	13.1c	5.6d	
SE		0.29			0.39
CV (%)		32.49			

Means followed by the same letter(s) are not significantly different by LSD at 1% level. pH of 50% lime suspension was found to be 12.00, which is highly alkaline

Table 2: Growth of fungi on media amended with different levels of *Senna siamea* wood ash incubated at 27-31°C for 7 days

	Mean colony diameter (mm) on PDA amended with indicated level of wood ash (g 15 mL PDA)				
Fungal species	0.0	0.5	1.0	1.5	Fungi means
Aspergillus flavus	51.7	23.7	10.0	0.0	21.3c
Cladosporium sp.	30.7	19.7	7.3	0.0	14.4d
Fusarium sp.	69.7	38.3	27.7	10.0	36.4a
Penicillium sp.	27.0	0.0	0.0	0.0	6.8e
Rhizopus stolonifer	90.0	20.0	0.0	0.0	28.3b
Sclerotium rolfsii	90.0	0.0	0.0	0.0	22.5c
Trichoderma lignorum	90.0	0.0	0.0	0.0	22.5c
Means	64.16a	14.53b	6.43c	1.43d	
SE		0.20			0.10
CV (%)		9.78			

Means within the column bearing identical letter(s) are not significantly different by LSD at 1% level. pH of wood ash at 50% suspension was found to be 11.73, which was highly alkaline

The mean colony diameter of 64.9 mm recorded for fungi which grew on the unamended PDA (control) was significantly higher than those on lime-amended PDA at different concentrations which also differed significantly from each other.

Table 2 shows the mean colony diameters of fungi on PDA amended with different levels of *Senna seamea* wood ash and incubated at 27-31°C. All levels of ash concentrations significantly inhibited ($p<0.01$) the growth of *Trichoderma lignorum*, *Penicillium* sp. and *Sclerotium rolfsii* and suppressed the growth of others. The higher concentration of wood ash (1.5 g/15 mL PDA) completely prevented the growth of all the test fungi except *Fusarium* sp. Mean colony diameters of the fungal species on the unamended PDA was signifcantly higher ($p<0.01$) than those on the ash-amended PDA, which also differed significantly from each other. The mean colony diameters of the test fungi differed significantly.

The mean colony diameters of fungi on PDA amended with different levels of Benlate (benomyl) incubated at 27-31°C for 7 days is shown in Table 3. All levels of Benlate concentration completely inhibited ($p<0.01$) the growth of *Aspergillus flavus*, *Cladosporium* sp., *Fusarium* sp., *Trichoderma lignorum*, *Penicillium* sp. and *Sclerotium rolfsii* but was able to reduce the growth of *Rhizopus stolonifer* as the level of Benlate concentration increases. Generally, the fungi, which grew on unamended PDA, recorded significantly higher ($p<0.01$) mean colony diameter of 63.9 mm than 14.4, 9.4 and 6.0 mm recorded on PDA amended with first, second and third levels of Benlate. The colony diameters of the various fungi differed significantly from each other. The highest value of 61.3 mm was recorded for *Rhizopus stolonifer* and the least value of 6.5 mm was recorded for *Cladosporim* sp.

Table 3: Mean colony diameter of fungi growing on media amended with different levels of benlate (benomyl) incubated at 27-31°C for 7 days

Fungal species	Colony diameter (mm) on PDA amended with indicated level of benlate				Fungi means
	L_0	L_1	L_2	L_3	
Aspergillus flavus	54.3	0.0	0.0	0.0	13.6cde
Cladosporium sp.	26.0	0.0	0.0	0.0	6.5e
Fusarium sp.	70.6	0.0	0.0	0.0	17.7cd
Penicillium sp.	26.7	0.0	0.0	0.0	7.7de
Rhizopus stolonifer	90.0	80.0	60.0	41.7	61.3a
Sclerotium rolfsii	90.0	0.0	0.0	0.0	29.2b
Trichoderma lignorum	90.0	0.0	0.0	0.0	22.5bc
Means	63.9a	14.4b	9.4c	6.0d	
SE	0.19				0.28
CV (%)	9.47				

Means within the column and bearing identical letter(s) are not significantly different by LSD at 1% level. L0-L3 for media amended with 0.0, 0.8, 1.0 and 1.2 g, respectively, of Benlate 250 mL^{-1} distilled water 15 mL^{-1} PDA in a 90 mm petri dish

Table 4: Growth of fungi on media amended with different levels of sodium hypochlorite incubated at 27- 31°C for 7 days

Fungal species	Colony diameter (mm) on PDA amended with Indicated level of sodium hypochlorite				Fungi means
	L_0	L_1	L_2	L_3	
Aspergillus flavus	51.3	16.7	9.3	5.0	20.6abc
Cladosporium sp.	28.0	8.0	2.2	0.0	9.5bc
Fusarium sp.	74.0	13.3	9.3	2.3	2.5ab
Penicillium sp.	28.0	2.0	1.0	0.0	7.8c
Rhizopus stolonifer	90.0	30.7	12.3	3.7	34.2a
Sclerotium rolfsii	90.0	0.0	0.0	0.0	22.5abc
Trichoderma lignorum	90.0	26.7	15.0	7.3	34.8a
Means	64.5ª	13.9b	7.0c	2.6d	
SE			0.39		0.51
CV (%)			15.53		

NS: Not significant at 5% level. Means with identical letter(s) are not signficantly different by LSD at 1% level. L_0-L_3 for media ammended with 0.0 (Control), 1:5, 1:10 and 1:15 dilution resprectively of sodium hypochlorite 15 mL^{-1} PDA in a 90 mm petri dish

Sodium hypochlorite completely inhibited the growth of *Sclerotium rolfsii* while it suppressed the growth of other fungi. An increase in concentration of sodium hypochlorite decrease the colony diameters of the fungi (Table 4). The mean colony diameter of the fungi which grew on the unamended media was significantly higher ($p<0.01$) than those which grew on the media amended with various levels of sodium hypochlorite which also differed sgnificantly from each other.

Colony diameters of fungi cultured on PDA amended with different levels of aqueous neem leaf extract are presented in Table 5. The neem extract was effective in completely controlling the growth of *Penicillium* sp, while the higher concentrations (200 and 300 g L^{-1} of distilled water) also completely inhibited the growth of *Cladosporium* sp. It also suppressed the growth of *Sclerotium rolfsii*, *Rhizopus stolonifer*, *Fusarium* sp.,

Table 5: Growth of fungi on media amended with different levels of aqueous neem leaf extract

Fungal species	Mean colony diameter (mm) on PDA amended with indicated level of aqueous neem leaf extract				Fungal means
	L_0	L_1	L_2	L_3	
*Aspergillus flavus**	52.0	16.7	11.7	11.7	23.1c
Cladosporium sp.	30.7	5.0	0.0	0.0	8.3d
Fusarium sp.	67.3	40.0	34.0	28.3	42.4b
Penicillium sp.	26.3	0.0	0.0	0.0	6.7d
Rhizopus stolonifer	90.0	71.7	40.0	21.7	55.8a
Sclerotium rolfsii	90.0	66.7	50.0	36.7	60.8a
Trichoderma lignorum	90.0	43.3	23.3	13.3	42.5b
Means	63.41a	34.77b	22.71c	15.96d	
SE			0.22		0.29
CV (%)			19.27		

Means bearing identical letter(s) are not significantly different by LSD at 1% level. L_0-L_3 for media amended with 10 mL each of 0.0 g (control), 100, 200 and 300 g, respectively of neem leaf 1000 mL^{-1} distilled water 15 mL^{-1} PDA. * *Aspergillus flavus* appeared on all the media amended with aqueous neem leaf extract

Trichoderma lignorum and *Aspergillus flavus* and increased concentration of the neem extracts reduced the growth of the fungi. Generally the mean colony diameters of the fungi on the unamended PDA was significantly higher than those on PDA amended with various levels of the neem extract, which also differed significantly from each other.

DISCUSSION

The effectiveness of lime to suppress or inhibit fungal growth could probably be due to the high pH produced in the culture medium. This might have raised the pH of the media beyond 6.5, thus inhibiting fungal growth and reproduction. The effectiveness of lime to either suppress or inhibit fungal growth has also been reported by Cornelius (1998). The observed reduction in growth of some fungi such as *Aspergillus flavus*, *Rhizopus stolonifer* and *Trichoderma* sp. with increased in lime concentration means that high concentrations of lime was required to control the growth of these fungi. *Fusarium* sp. was able to grow on lime amended PDA irrespective of the concentration thus indicating that *Fusarium* sp. was more tolerant or less sensitive to high pH than the other test fungi. The lower level of lime which significantly inhibited the growth of *Penicillium* sp. and *Sclerotium rolfsii* was indicative that these fungi were very sensitive to high pH levels.

The significant reduction and inhibition of mycelia growth by wood ash has also been reported by Oduro *et al.* (1997) and Cornelius (1998). This could be due to possible toxic compounds in the wood ash (Oduro *et al.*, 1997). Elements such as sulphur and copper present in the wood ash (Duta, 1995) are well known

fungicides. The presence of any of such elements could, therefore, suppress or inhibits fungal growth. The ability of wood ash to suppress fungal growth could also be attributed to the high pH of the amended growth medium (PDA) which is above 6.5 and can suppress fungal growth. This is expected because fungi grow best in media with initial pH of 5.0 to 6.5 (Cochrane, 1958). Wood-ash totally inhibited growth of fungi such as *Trichoderma* sp., *Penicillium* sp. and *Sclerotium rolfsii*. However, highest concentration of the ash could not inhibit growth of *Fusarium* sp. This indicated that different fungi grow well at different pH values.

The significant inhibition and slow rate of mycelia growth by benomyl (Benlate) *in vitro* might be due to its fungicidal property. Benlate is a well-known systemic fungicide used to disinfect seed and plant propagation stock against pathogens (Heitefuss, 1989). The inability of Benlate to completely inhibit the growth of *Rhizopus stolonifer* implies that the fungus is less sensitive to the fungicide. This is consistent with the result of Fry (1982) who reported that benomyl, a systemic fungicide, is especially effective against Ascomycetes and not effective against *Rhizopus stolonifer*, a phycomycete (Gilman, 1957).

The colony diameters of the fungi on unamended PDA were significantly higher than those on PDA amended with various concentrations of sodium hypochlorite (household bleach). Thus, indicating the ability of bleach to suppress or slow down the fungal growth. The observed reduction in colony diameters with increased concentration of bleach revealed that higher concentrations were required to effectively control the pathogens. *Sclerotium rolfsii* was unable to grow on PDA amended with even the lowest concentration of bleach. This could be attributed to high sensitivity of the fungus to the chemical composition of bleach. *Aspergillus flavus*, *Fusarium* sp., *Rhizopus stolonifer* and *Trichoderma lignorum* were less sensitive to the bleach because its highest concentration could not completely inhibit the growth of these fungi.

The colony diameters of the fungi which grew on neem-extract amended PDA were significantly lower than those on the unamended PDA. This was due to the fungicidal or fungistatic action of the neem leaf extract as has also been observed by Singh *et al*. (1980) and Otoo *et al*. (1987) However, the neem extract could not completely inhibit the growth of *Sclerotium rolfsii*, *Trichoderma lignorum*, *Rhizopus stolonifer*, *Fusarium* sp. and *Aspergillus flavus*, suggesting that these fungi are tolerant or less sensitive to the aqueous neem leaf extract. A similar observation had was made by Bhatnager *et al*. (1990) who noted that aqueous neem leaf extract did not inhibit vegetative growth of *A. flavus* *in vitro*. The growth of *A. flavus* on the neem extract-amended PDA further supported the above assertion and therefore indicating the possibility of the neem leaf extract in stimulating vegetative growth and sporulation of *Aspergillus flavus*. This stimulatory effect could be due to nutrients supplied by the neem leaf extract as reported by Singh and Singh (1970). Higher concentrations of neem extract also completely inhibited the growth of *Cladosporium* sp. These observations clearly suggested that soils with *Penicillium* and *Cladosporium* as the dominant mycoflora could be used to pre-sprout yam.

This study concluded that Benlate inhibited or controlled the growth of all the test fungi as from 0.8 g 250 mL of water except *Rhizopus stolonifer*. Wood ash, quicklime and neem extract reduced or suppressed the growth of all the fungi. Common bleach suppressed the growth of *A. flavus* and *Rhizopus stolonifer* and inhibited the growth of *Sclerotium rolfsii* and *Penicillium* sp. It is suggested that Wood ash, quicklime and neem extract could serve as a better substitute to the expensive benlate and bleach for the control of yam minnisett rot.

REFERENCES

Asare-Bediako, E., 2003. The role of microorganisms and their control in the sprouting of minisetts of white yam (*Dioscorea rotundata*, *Poir*) cv. Pona in Ghana. M.Phil. Thesis (unpublished). School of Agriculture. U.C.C. Cape Coast, pp: 125.

Bhatnager, D., H.J. Zeringue and S. McCormick, 1990. Neem Leaf Extracts Inhibit Aflatoxin Biosynthesis in *Aspergillus flavus* and *Aspergillus* parasiticus. In: Neem's Potential in Pest Management Programs. Proc. SDA eem Workshop. Locke J.C. and R.H. Lawson (Eds.). USDA, ARS, 86: 118-127.

Cochrane, W.V., 1958. Physiology of Fungi. John Wiley and Sons, Inc. New York, USA., pp: 524.

Cornelius, E.W., 1998. Causes and control of tuber rot of white yam (*Dioscorea rotundata*, Poir varieties Araba, Asana and Puna). M.Phil. Thesis. U.G. Legon, pp: 123.

Data, A.C., 1995. Botany for Degree Student. Graphitech India Ltd., Bindhan, Nagar, Calcutta. Grown in Plastic House in Southeastern Spain. Plant Soil In: Rev. Plant Pathol., 1993, 42: 858-864.

Emehute, J.K.U., T. Ikotun, E.C. Nwauzor and H.N. Nwokocha, 1998. Crop Protection. In: Food Yams: Advances in Research, Orkwor, G.C., R. Asiedu and I.J. Ekanayake (Eds.). NRCRRI/IITA, Nigeria, pp: 143-186.

Fry, W.E., 1982. Principles of Plant Disease Management. Academic Press, New York, USA., pp: 151-174.

Gilmann, J.C., 1957. A Manual of Soil Fungi. Iowa State University Press, USA., pp: 450.

Heitefuss, R., 1989. Crop and Plant Protection: The Practical Foundations. Ellis Horwood Ltd., Chister, pp: 261.

Ikotun, T., 1983. Reduced germinability of yam setts caused by microbial deterioration. Fitopathol. Brasileira, 8: 445-459.

Oduro, K.A., H.B. Damptey and L.O. Adimora, 1997. Evaluation of some traditional control measures for diseases of stored white yam, *Dioscorea rotundata*, Poir var Gboko. Ghana J. Sci., 31-36: 3-10.

Osai, E.O. and T. Ikotun, 1994. Microbial rot of yam minisetts. Fitopatologia Brasileira, 19: 408-412.

Otoo, J.A., D.S.O. Osiru, S.Y. Ng and S.K. Hahn, 1987. Improved technology for seed yam production. 2nd Edn., IITA, Ibadan, Nigeria. pp: 165.

Singh, R.S. and N. Singh, 1970. Effect of oil-cake amendment of soil on population of some wilt causing species of Fusarium. Phytopath. Z., 69: 160-167.

Singh, U.P., H.B. Singh and R.B. Singh, 1980. The fungicidal effect of neem (*Azadirachta indica*) extracts on some soil-borne pathogens of gram (*Ciser arietinum*). Mycologia, 72: 1077-1093.