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Evaluation of four plant extracts in the control of post-harvest fungi tuber rot of Irish potato (S*olanum tuberosum*)

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Abstract

This study was conducted to isolate and identify pathogens associated with post-harvest tuber rot of Irish potato and to evaluate the efficacy of some plant extracts *in vitro* and *in vivo* in the control of rot-causing fungal pathogens. One hundred rotten Irish potato tubers were collected from Bodija, Sabo, Apata and Garki (Abuja) Markets. Fungi were isolated from rotten potato tuber samples identified using culture and morphological characteristics Four plant extracts namely *Zanthoxylum zanthoxyloides, Distemonathus benthamianus, Morinda lucida and Moringa oleifera* were evaluated against *Botryodiploidia sp.,* and *Fusarium verticilloides* using three (500, 1000 and 1500mg/ml) concentrations. Nine fungi species associated with Irish potato tuber rot were isolated and identified in this study. *Fusarium oxysporium* had the highest frequency of occurrence in Bodija market followed by *Fusarium verticilloides. F. verticilloides* had the highest frequency of occurrence in Sabo, Abuja and Apata market. There were significant differences ($P \leq 0.05$) in the rot induced by fungi which range between 7.07— 87.55%. *Botryodiplodia* sp, *Fusarium verticilloides*, *Macrophomina* sp., *Fusarium oxysporium, Penicillium digitatum*, *A. niger*, *Phoma* sp., were found to cause post-harvest rot on Irish potato. Growth inhibition of rot causing organisms in vitro varied with extract type, concentrations and pathogens. *D. benthamianus* extract was the most effective *in vitro* and *in vivo* of the four extracts evaluated. *Morinda lucida* and *Moringa oleifera* extracts inhibited *Botryodiplodia* sp. at 1500mg/ml concentration producing 16.67% and 16.27% inhibition respectively. Both extracts inhibited *Fusarium verticilloides* at all concentrations. *D. benthamianus* could serve as an alternative to synthetic chemical in controlling post-harvest tuber rot of Irish potato. However, this botanical should be further evaluated for its control of the potato rot-causing pathogens in storage as well as any side effect on potato quality especially taste.

Key words: Irish potato, tuber rots fungi, plant extracts, Mycelial inhibition.

Introduction

Irish or white potato (*Solanum tuberosum*) family Solanaceae, is one of the important crops in the world due to its high value for human nutrition (Desjardins *et al*., 1995; FAO, 2010). It is ranked the fourth most important food crop in the world after wheat, maize and rice (Otupa *et al*, 2003). The world total production is about 324.4 million tons planted on 18.6 million hectares of land (FAO, 2010). China is the world largest potato producer with about 74.8 million

tons, followed by India and Russia (FAO, 2012). Potato is an important tuber crop in Nigeria; Jos in Plateau State is the most important producing area with minimum temperatures range of about 9.4ºC (December - February) to 15°C (May to August) and maximum temperatures of about 34.4ºC (September –November). Potato is best known for its high carbohydrate content (8-28%) (Stanley *et al.,* 1986 and Ferretti, 2011). Potato is eaten boiled, fried, and in stews (Badiru and Sofela, 1985). It is used to brew alcoholic beverages and also used as food for domestic animals. The starch is used in the food industry as, thickeners and binders of soups and sauces, in the textile industry, as adhesives, and for the manufacturing of papers and boards (Gopal *et al.,* 2006).

Many fungi spp especially *Fusarium spp. Rhizopus stolonifer, Macrophomina phaseolina, Botryodiplodia theobromae, Penicillium* sp and *Aspergillus niger* have been reported to be associated with rottening of potato (Clark and Hoy 1994; Onuegbu, 2002 and Oyewale, 2006). These fungi infect the tubers during the pre-harvest stage while in soil and also through openings and physical damages incurred by the tubers during harvest operations and then manifest fully during storage (Amusa *et al.,* 2003). There have been attempts to control tuber rot of potato using improved cultural practices on the farm and synthetic pesticides in form of chemical dips such as the used of Dichloronitroanline against Rhizopus soft rot (Amienyo and Ataga 2006). However, the use of chemicals on crops is becoming less favourable due to its detrimental effect on both the farmer and environment. Pesticides, apart from producing undesirable effects, have become expensive, unavailable to peasant farmers who are regarded as the producers of food in Nigeria (Akinbode, 2010). This constraint has stimulated studies on the development of safer, cheaper and effective control measure on post harvest tuber rot of Irish potato. This has brought about the introduction of the use of natural plant extracts in the control of these diseases, which will have no side effect, cost effective, readily available and requires no skill for its application. Plant extracts have been used to control diseases in maize (Ekpo, 1991), cowpea (Amadioha and Obi, 1998) and banana (Okigbo and Emoghene, 2004), but limited work has been done on the use of botanicals in the control of the post-harvest rot of Irish potato tubers. Despite the various applications of the plant extracts in the control of human diseases, available information on the use of *Moringa oleifera*, *Morinda lucida, Zanthoxylum zanthoxyloides and Distemonanthus benthamianus* in the control of plant diseases are scanty. Therefore, the objectives of the study were to isolate and identify the fungi associated with Irish potato tuber rot and evaluate the efficacy of some plant extracts *in vitro* and *in vivo* in the control of Irish potato rot-causing pathogens.

Materials and Methods

Sources of materials

Irish Potato tubers were collected from three local markets in Ibadan (Southern western Nigeria) namely Bodija, Sabo, and Apata markets and Garki market in Abuja (North central Nigeria). Thirty infected (rotten) and 5 healthy potato tubers were collected from three markets in Ibadan and 10 infected tubers from Abuja making 100 infected and 15 healthy tubers. All infected potato tubers were carefully inspected for rotten areas before sampling, kept in clean polyethylene bags and brought to the Laboratory for further analysis. *Zanthoxylum zanthoxyloides roots and Distemonathus benthamianus stems* were purchased from Apata market in Ibadan, Oyo state and authenticated at the Federal Research Institute of Nigeria (FRIN). *Morinda lucida leaves were* collected from National Center for Genetic Research and Biotechnology (NACGRAB) *with Moringa oleifera* leaves from NACGRAB and International School Ibadan (ISI) in Ibadan Oyo-State.

Isolation and Identification of isolated fungi from rotten Irish potato tuber samples

Each rotten tuber sample was washed in running tap water and cut to expose the fresh necrotic tissues from the areas in advance of the necrosis. Pieces of tissue were surface-sterilized with 0.5% Sodium Hypochlorite (NaOCl) for 2 minutes, and rinsed in five changes of sterile distilled water before plating on Potato Dextrose Agar (PDA). The inoculated plates were incubated at a temperature of 28±2°C for 3 to 4 days and examined for fungal growth.

Various fungal isolates from each of the samples were sub-cultured on PDA to obtain pure cultures for identification. The structural features of colony, colour, extent of growth, presence or absence of mycelia, spores and the nature of colony surface were observed. Microscopic examination involved slide mounts of the test isolates, stained with Lacto phenol cotton blue. Structural features of the organisms were compared with those described in a standard manual of fungi (Barnett and Hunter, 1987).

Pathogenicity test

The experiment was set up in a completely randomized design with three replicates. Inoculation of tubers and storage of inoculated tubers were carried

out in the Pathological Laboratory of Crop Protection and Environmental Biology (CPEB) University of Ibadan using the same fungi isolated from the rotten Irish potato tubers. All the fungal isolates obtained from infected tubers were inoculated into healthy tubers of Irish potato to determine whether they could induce similar symptoms on re-inoculation. They were re-isolated in order to establish Koch's postulates. Fresh healthy Irish potato tubers were washed in running tap water to remove soil and other debris on the tuber surface. The whole tubers were surface-sterilized in 0.5% solution of NaOCl after which they were rinsed with Sterile distilled water. Each tuber was bored to a depth of 1cm, using a flame-sterilized diameter cork borer at middle of the tuber, 3 mm mycelial discs of the test pathogen were placed at the bottom of the hole and covered with the tuber piece earlier removed and then sealed with Vaseline to prevent extraneous infection. The control set-up consist of tubers that were similarly bored into but inoculated with sterile PDA discs of 3mm, and covered with Vaseline. All inoculated tubers were enclosed in polyethylene bags moistened with sterile cotton wool soaked with sterile distilled water to maintain a high relative humidity and incubated at 28 $\pm 2^0$C for 7 days. At the end of the incubation period, the tubers were cut open at the point of inoculations and observed for rot development. Rot severity was calculated using the method of Ayodele and Iwhiwhu (2010):

$$\text{Rot severity} = \frac{\text{area of infected tissue}}{\text{total tissue area}} \times \frac{100}{1}$$

Where: area of rotted tissue= $^1/_3 \times \pi \times D \times H$
d= diameter of lesion,
h= depth of lesion and
Total area of tuber= $1/3 \times \pi \times D \times H$
(D= diameter of tuber, H=length of tuber).

Assessment of rot development was done by destructive sampling. Measurement of the rotten area was expressed as a percentage of the total surface area of the potato tuber. Rot severity was ranked as follows:

0 = No rotting
1 = Very mild rotting (<5% of tuber rotted)
2 = Mild rotting (≥5-10% of tuber rotted)
3 = Moderate rotting (>10-25% of tuber rotted)
4 = Severe rotting (> 25% of tuber rotted
5 = Very severe rotting (>50% of tuber rotted)

Re-isolation and identification of the fungi was done to establish Koch's postulates.

Preparation of plant extracts

Selected parts (leaves/stems/roots) of the plant samples were washed thoroughly with sterile distilled water to remove dust and air-dried at room temperature 28±2°C for two weeks, powdered and stored in air-tight container. Two hundred grammes each of the powdered plant samples were separately soaked in 1000ml of 90% methanol at room temperature for 7 days. The mixture was then filtered using sterile muslin cloth. The filtrates were concentrated to dryness in vacuum using a rotary evaporator and the extracts were kept in air-tight bottles at 4°C temperature of prior to use. The plant extracts were reconstituted with sterile distilled water at different concentrations 1500mg/ml, 1000mg/ml and 500mg/ml (Oloyede *et al*. 2012).

***In vitro* screening of plant extracts for fungal growth**

The experiment was set up in a 4 × 3 × 2 factorial experiment, laid out in CRD with 3 replicates. Method of Amadioha and Obi (1998) was used to determine the effect of the extracts on fungal growth. Two perpendicular lines were drawn at the bottom of the Petri dishes to create four equal sections. Ten milliliters PDA was dispensed into each of the plates and an aliquot of 1ml of each plant extracts was separately introduced into these Petri dishes. A 5mm diameter mycelial disc was obtained from the colony edge of 7-day-old culture of each fungus (*Fusarium verticilloides* and *Botryodiploidia* sp.) and separately placed at the center of the extract impregnated PDA. Control experiments contained PDA without the addition of plant extract. All plates were incubated at 28±2°C and radial growth was measured daily for 5 days as the mean growth along two directions on the perpendicular lines drawn on the reverse side of the plates. Toxicity to fungi were recorded in terms of percentage colony inhibited and calculated according to the formula of *Pandey et al.* (1982):

$$\text{Growth inhibition (\%)} = \frac{\text{DC - DT}}{\text{DC}} \times \frac{100}{1}$$

Where DC = average diameter of control
DT = average diameter of fungal colony with extract treatment

***In vivo* screening of plant extracts for tuber rot control**

The experiment was set up in a CRD with four replicates. 1000mg/ml of *D. benthamianus* methanol extract that yielded complete *in vitro* inhibition of the test pathogens was used. Clean healthy-looking tubers of Irish potato were surface-sterilized. One

centimeter deep hole was created with 3mm cork borer at the middle of the potato tuber and filled with 1 ml of the extract. Then, 3mm mycelial discs of each of *F. verticiloides and B. theobromae* were placed at the bottom of the hole, covered with the tuber piece earlier removed and then sealed with Vaseline to prevent extraneous infection. Control tubers consisted of holes filled with sterile distilled water. The tubers were incubated in polyethylene bags moistened inside with sterile cotton wool soaked in sterile distilled water to maintain a high relative humidity. Tubers were incubated at 28±2^{0}C for 7 days and assessed for rot development. Assessment of rot development was done using destructive sampling of the tubers by calculating the percentage area of Irish potato infected by the pathogens in relation to the total area of potato using the method of Sangoyomi (2004):

$$\text{Percentage area of rotten tissue} = \frac{\text{Area of rotted tissue}}{\text{Total surface area}} \times \frac{100}{1}$$

Statistical analysis

Data were analyzed by ANOVA with general linear model (PROC GLM) using a statistical analysis system (SAS, 2008) package, version 9.2. Means with significant differences were separated using Duncan Multiple Range Test (DMRT) and Least Significance Difference (LSD) at 5% level of significance

Results

Frequency of occurrence of fungal pathogens from rotten tuber of Irish potato across the four locations

Table 1 shows the frequency of occurrence of fungal pathogens from rotten tuber of Irish potato across the four markets. *Fusarium oxysporium* had the highest frequency of occurrence (31.48%) in Bodija market followed by *Fusarium verticilloides* (22.22%) while *Phoma sp*. had the lowest occurrence (3.70%). *Fusarium verticilloides* occurred most frequently (28.85, 23.81 and 28.57 %) in Apata, Sabo and Abuja markets with either *A. flavus* or *Phoma* sp. having the lowest frequency of occurrence in these markets.

Rot severity and ranking of healthy potato tubers artificially inoculated with fungi isolates

Rot severity and ranking of healthy Irish potato tubers artificially inoculated with isolated fungi and incubated at 28±2^{o}C for 7days are presented in Table 2. All the test pathogens differed significantly (P= 0.05) in their rot-causing ability on Irish potato. *Fusarium verticilloides* proved to be more virulent among the test pathogens with mean rot value of 87.55% which was not significantly different from that observed in *Botryodiplodia theobromae* (67.82%) and *Macrophomina sp.* (64.84%). Rot severity of 30.73%, 13.54% and 7.07% observed from *A. niger, A. flavus and Trichoderma* sp. were not significantly different from that of control.

***In vitro* screening of plant extracts for growth inhibition of tuber rot fungi**

Mycelial growth inhibition (%) of four botanicals on *Botryodiplodia theobromae*

Inhibitory effects of the four botanicals on the mycelial growth of *B. theobromae in vitro* are shown in figure 1. *Z. zanthoxyloides* mycelial growth inhibition increased with concentrations. The mycelial growth inhibition of the extract was not significantly different at 1500 mg/ml and 1000 mg/ml while inhibition at 1000 mg/ml was not higher than that observed at 500mg/ml. In *D. benthamianus* extracts, there was no significant difference in the mycelial growth inhibition at 1500 mg/ml and 1000 mg/ml but were different from inhibition at 500 mg/ml. *Morinda lucida* and *Moringa oleifera* were only effective at 1500 mg/ml extract concentration

Mycelial growth inhibition (%) of four botanicals on *Fusarium verticilloides*.

Figure 2 presents the inhibitory effects *in vitro* of the four botanicals on the mycelial growth of *Fusarium verticilloides*. *Z. zanthoxyloides* produced 100% mycelial growth inhibition of the fungus at all concentrations (500, 1000, and 1500 mg/ml). *D. benthamianus* also produced 100% mycelial growth inhibition at 1500 and 1000 mg/ml which were significantly higher than the inhibition (40.67%) observed at 500 mg/ml. *M. lucida*, 1500 mg/ml extract concentration gave the highest mycelial growth inhibition of 20.41% which was significantly higher (P= 0.01) than 13.31% and 12.53% inhibitions produced at 1000 mg/ml and 500 mg/ml respectively. Mycelial growth inhibition of *M. oleifera* were not different at all concentrations.

***In vivo* screening of plant extract for the control of tuber rot causing pathogens**

Inhibitory effects of *Distemonanthus benthamianus* methanol extract on rot development in tubers inoculated with *F. verticilloides* and *Botryodiploidia theobromae* at 7 days after inoculation is shown in Table 3. There were significant differences (p= 0.05) in the rot development between the inoculated tubers treated with and without the extracts. The extract

caused a very low rot development in tubers inoculated with *B. theobromae* (1.67%) and *Fusarium verticilloides* (9.02%) compared with that (67.82% and 87.55%) of the inoculated tubers without extract.

Discussion

Nine fungi species belonging to seven genera associated with Irish potato tuber rot were isolated and identified. *Fusarium oxysporium* had the highest frequency of occurrence in Bodija market followed by *Fusarium verticilloides. Fusarium verticilloides* had the highest frequency of occurrence in Sabo, Abuja and Apata market. This result agrees with the findings of previous workers who associated these same pathogens with post-harvest rot of sweetpotato (Amienyo and Ataga 2007; Onuegbu, 2002). *Botryodiplodia* sp, *Fusarium verticilloides*, *Macrophomina* sp., *Fusarium oxysporium*, *Penicillium digitatum., Aspergillus niger* and *Phoma* sp. caused rot on Irish potato tubers while *Aspergillus flavus,* and *Trichoderma sp.,* could be secondary invaders producing moderate and mild rottening respectively. These fungi have been reported by several workers on Irish potato (Crop Protection Compendium, 2007) and sweet potatoes in storage (Clark and Hoy, 1994; Oyewale, 2006).

Distemonanthus benthamianus and *Zanthoxylum zanthoxyloides* have antimicrobial potentials on test organisms *in vitro*. This implies that our locally occurring plants commonly used in folklore human medicine also possess anti-microbial effects on plant pathogenic fungi. This conformed to the results obtained from the studies of Olufolaji and Adeyeye (2002) and Olufolaji and Ojo (2005) on the bioassay of some plant extracts on some fungal pathogens. *Distemonanthus benthamianus* inhibited fungi investigated at varying concentrations *in vitro and in vivo.* This agrees with the study of Adekunle and Odukoya (2006) where ethanol extract of *D. benthamianus* had the highest antifungal activity among the four Nigeria chewing sticks tested against Candida albican and other investigated fungi. The antimicrobial activity of *D. benthamianus* stem may be due to the presence of secondary metabolites such as tannins, alkaloids, saponins or flavonoids (Adekunle and Odukoya, 2006).

The efficacy of these extracts is enhanced by the presence of active ingredients. Previous studies (Sofowora and Isaacs, 1971) indicated that chelerythrine, berberine and canthin-6-one are antibacterial components of *Zanthoxylum spp.* There has also been a report of antimicrobial activities of two groups of compounds (phenolic acids and alkaloids), which occur in the root of *Zanthoxylum zanthoxyloides (Islam et al., 2001). Morinda lucida and Moringa oleifera* could not completely control the test pathogens *in vitro*. The reduced efficiency of the botanicals may be due to tolerance of some of the fungi to the antimicrobial phenolics in plant extracts. Moreover, it has been established that some botanicals exert a stimulatory effect on plant pathogens. These plant species produce stimulatory lipopolysaccharides, which enhance the growth of the tested pathogens (Chan, 2006). Therefore, *Morinda lucida and Moringa oleifera* extracts that proved ineffective *in vitro* in this study may also have provided some stimulatory growth to the rot-causing fungi.

The active ingredients present in plants are influenced by many factors, which include the age of the plant, extracting solvent, method of extraction and time of harvesting plant materials (Amadioha and Obi, 1998; Okigbo, 2003). Presence of antifungal substances in the different extracts, which caused the inhibition of radial growth and spore germination *in vitro*, agrees with the reports of earlier studies (Dhaliwal, 1993; Enikuomehin *et al*., 1998). The differences recorded in the fungitoxic activity of the extracts may also be attributed to the solubility of the active ingredients in water or the presence of inhibitors to the fungitoxic principle. *D. benthamianus* extract was the most effective of all the botanicals tested causing inhibition of the growth of all tested pathogens *in vitro* and *in vitro* and this was followed by *Zanthoxylum zanthoxyloides.* Thus, *D. benthamianus extract* could serve as alternative to the synthetic chemicals in controlling post-harvest rot of Irish potato tubers. However, the effectiveness of *D. benthamianus* in the control of rot causing pathogens of Irish potato tubers in bulk storage and any side effect on potato quality especially taste should be further investigated.

Table 1: Frequency of occurrence (%) of fungi on infected Irish potato tubers from different Locations

Fungi	Bodija	Apata	Sabo	Abuja*
B.theobromae	7.41	7.69	6.35	-
Macrophomina sp.	5.56	7.69	6.35	9.52
F. oxysporium	31.48	19.23	12.70	19.05
Phoma sp.	3.70	7.69	4.76	4.76
Aspergillus niger	9.26	5.77	15.87	23.81
F. verticilloides	22.22	28.85	23.81	28.57
Aspergillus flavus	7.41	1.92	4.76	-
Trichoderma sp.	7.41	5.77	4.76	-
P. digitatum	5.56	15.38	20.63	14.29

*-, not found

Table 2: Rot severity and ranking of healthy Irish potato tubers artificially inoculated with fungal isolates and incubated at 28°C for 7days

Pathogen	**Rot severity (%)**	**Rank***
Botryodiplodia sp.	67.82ab	5
Macrophomina sp.	64.84abc	5
Fusarium oxysporium	46.98bcd	4
Penicillium digitatum	34.73de	4
Phoma sp.	33.41de	4
Aspergillus niger	30.73def	4
Fusariu verticilloides	87.55a	5
Aspergillus flavus	13.54ef	3
Trichoderma sp.	7.07ef	2
Control	0.00f	0

Values are means of three replicates. 0 = No rotting, 1=Very mild rotting (<5% of tuber rotten), 2 =Mild rotting (≥5-10% of tuber rotten), 3 = Moderate rotting (>10-25% of tuber rotted), 4 = Severe rotting (>25-50% of tuber rotten), 5 =Very severe rotting (> 50% of tuber rotten); mean with same letter along the column are not significantly different (P=0.05) according to DMRT.

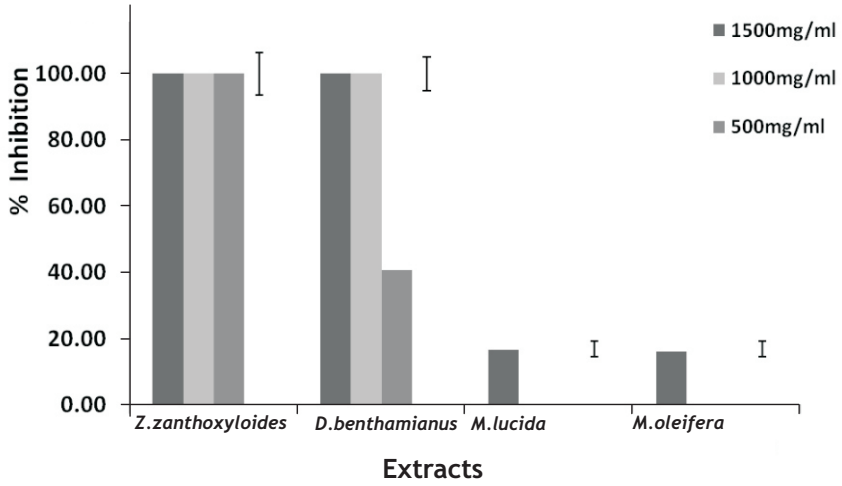

Figure 1: Percentage inhibition of four plant extracts on mycelial growth of Botryodiploidia theobromae

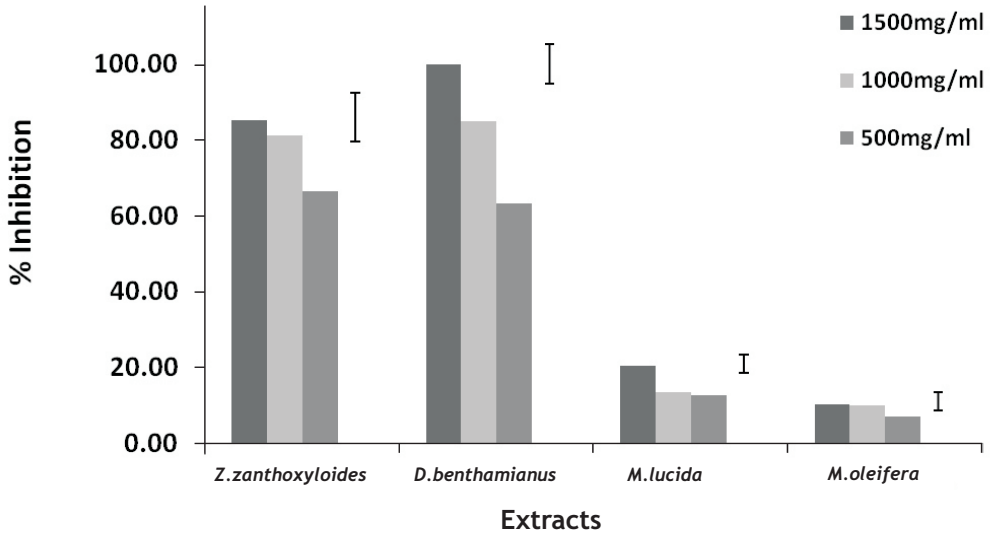

Figure 2: Percentage inhibition of four plant extracts on mycelial growth of *Furarium versitilloides*

Table 3: Effects of *Distemonanthus benthamianus* methanol extract on rot development in potato tubers inoculated with two rot causing fungi 7 days after inoculation.

Treatment	Area of rot tuber (%) induced by fungi	
	F. verticilloides	*Botryodiploidia theobromae*
With extract (1000mg/ml)	9.02b± 6.25	1.67b± 1.71
Without extract	87.55a±14.03	67.82a±12.81

Values are means of four replicates. Means with the same letter along the column are not significantly different (P= 0.05)

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