Evaluation of the Effect of Extract Of Monodora Myristica And Xylopia Aethiopica On The Growth Of Fungi In Sweet Potatoe Juice

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ABSTRACT

Aqeous exract of Monodora myristica and Xylopia aethiopica were assayed for the antifungal effect on Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer isolated from deteriorating sweet potato. Aqueus extracts of Monodora myristica and Xylopia aethiopica showed inhibitory effect against Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer in sweet potato juice. 3%(v/v) aqueous extract of Monodora myristica or Xylopia aethiopica reduced the growth of the fungi; however a combination of 2% each of both plant extracts retarded the growth better. Partial purification of the aqueous extract of Monodora myristica and Xylopia aethiopica showed that ethyl acetate fraction of the plant extracts exhibited the highest level of inhibition of growth of the test fungi compared with diethyl ether and n -hexane fractions. Extract of Monodora myristica and Xylopia aethiopica may be important sources of preservative of root juices.

Keywords: Antifungal, inhibition, extract, fungi, growth, preservative.

INTRODUCTION

Sweet potato is one of the root crops which provide the major part of the calorie need of people in the tropics. It is a good source of vitamin C and beta-carotene (Wolf, 1992). Sweet potato cannot be stored for very long at temperatures below 13°C. They develop chilling injury at temperatures from 0°C to 10°C (Ihenkoronye, 1995). Chilling leads to increase in sugar content and accelerate respiratory activity.

The chilling injury can lead to increased susceptibility to decay and failure to sprout. Chilling also produces such physiological effects as loss of ascorbic acid and increase in chlorogenic acid (Leistener, 1992).High level of chlorogenic acid is associated with discolouration upon exposure to air, inability to synthesize carotene and accumulation of carbon dioxide in the root during chilling. Infection of sweet potato by fungi progresses into various stages of decay during storage. Soft rot in sweet potato is caused by species of *Rhizopus* that produces soft decay that consumes the root quickly, even at 16°C (Ogundana, 1972). Surface rot and end rot are caused by species of *Fusarium* that grow slowly; it may take several weeks for the entire root to be destroyed (Wolf, 1992). Storage temperature above 16°C encourage the development of virus disease that cause the development of corky areas in susceptible varieties (Matern and Kneusel,1998).

Loss of weight is associated with high temperature and low humidity storage, resulting in development of pithiness in the root. At harvest 5 – 10% of sweet potato tissue is made up of intercellular spaces. As weight losses exceed volume losses during storage, these spaces increase and eventually become viable (the root becomes pithy). Sprouting of sweet potato occurs at temperature of above 16°C. A high relative humidity encourages growth if if the temperature is high enough. Sprout growth contribute to the development of pithiness. Monodora myristica seed are used as condiment in West Africa, a decoction of the seed is used to treat guinea worm infection. The seeds are used as a remedy for constipation, when mixed with palm oil. Roasted and powdered seeds of the plant are very effective in curing stomach ache. The seeds are rubbed on the forehead to cure head ache. (Gill, 1992).

Xylopia aethiopica (West African pepper tree) is a slim, tall, evergreen aromatic tree. It is about 60.7cm in diameter with straight stem , the leaves are simple, alternate, oblong, elliptic to ovate. The flower is bisexual, solitary or in
branched spikes, or cyomes, up to 5.5 by 0.4cm and creamy-green. The fringe forest in the savanna zones of Africa, but is found mainly in widely distributed in the humid forest zones especially along rivers in
the drier area of the region.

Crushed powdered fruits and seeds of Xylopia aethiopica are used as pepper substitute. The seeds have an aromatic pungent taste. The dried fruits are important as flavourings to prepare local soups in West Africa. The fruit is used against cough, stomachache, dizziness, bronchitis and dysentery.An investigation was conducted on the effect of Monodora myristica seeds and Xylopia aethiopica on the growth of three spoilage fungi : Aspergillus niger, Aspergillus flavus and Rhizopus stolonifer in potato juice.

MATERIALS AND METHODS

Plant materials and microorganisms

The microorganisms used were *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer*. The organisms were isolated from deteriorating sweet potatoes and identified by standard microbiological procedures. *Monodora myristica* and *Xylopia aethiopica* used in this study were obtained from Bida Niger State.

Preparation of extracts of *Monodora myristica* and *Xylopia aethiopica*

The seed of *Monodora myristica* and *Xylopia aethiopica* were shade dried at ambient temperature and ground into powder. Ten grams of ground dry seed sample of *Monodora myristica* and *Xylopia aethiopica* were then agitated in 250ml of hot (70 °C) sterile water contained in two separate 500ml capacity flasks. The flasks were plugged with cotton wool wrapped in aluminum foil, shaken vigorously and allowed to stand in the refrigerator for 72hrs. The filtrate was obtained by suction and concentrated using a water bath (BT101) at 80 °C until a brown viscous residue remained.

Extraction of potato juice

Potato roots were washed and immersed in 10% hypochlorite solution for 10mins. The roots were then peeled and the juice extracted manually.

Determination of effect of different concentration of plant extracts on growth of fungi potato juices.

A 20ml sample of the juice was introduced into 100ml capacity flasks. Extract of *M.myristica* and *X.aethiopica* were then added to give concentrations (v/v) ranging from 1.0% to 5.0%. Thereafter, they were inoculated with 1.0ml of aqueous suspension containing 10^{10} spores of test fungi obtained by serial dilution and incubated for 7 days at room temperature (28 ± 2^{o}C). The developing mycelia of four replicates were subsequently recovered by filtration using pre-weighed whatman No 1 filter paper and dried to constant weight at 70 ^{o}C in a hot air oven. Control experiments were performed without the extracts. The weight differences were analysed by analysis of variance and Duncan Multiple Range (DMR) test.

Determination of effect of combination of plant extract on growth of fungi in potato juice

The procedure for effect of different concentration of plant extraction in potato juice was was repeated using the following combination of the two plant extracts (*M. myristica* and *X. aethiopica*); 1:1, 1:2, 2:1, and 2:2. Control flasks contain no plant extract.

Determination of antifungal effect of organic solvent soluble fractions of aqueous extract of *M. myristica and X.aethiopica*.

The method of Isao *et al* . (1982) for separation of organic compounds with a slight modification was used to determine the antifungal effect of organic solvent soluble fraction of aqueous extract of *M.myristica and X.aethiopica*. Aqueous extract of *M.myristica* and *X.aethiopica* was partitioned between water and sequentially n-hexane, diethyl ether and ethyl acetate. Each fraction was collected and allowed to evaporate to dryness using a hot were inoculated with 1.0mlaqueous suspension containing 10^{6} at room temperature (28 ± 2^{o}C). The developing mycelia of three filtration using pre-weighed Whatman No 1 filter paper and dried to constant weight at 70^{o}C. Control experiments were performed without the extracts. The weight differences were analyzed by analysis of variance and Duncan Multiple Range (DMR) test.

RESULTS AND DISCUSSION

The plant extracts showed inhibitory effect against *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer* in sweet potato juice (Table 1). The application of *M.myristica* showed a significant reduction of fungal biomass at 3.0% concentration while 4.0% concentration of *X. aethiopica* showed a significant reduction of fungal biomass. The effect of plant extract on microorganisms may depend on the type as well as the medium (Obeta and Uguanyi, 1995). Spices contain phenols and essential oils, which are inhibitory to microorganisms (Nakatani , 1994).The effect on microorganisms may depend on the type as well as the medium (Giese, 1994). It was reported that fat and proteins bind or solubulise phenolic compounds thereby reducing their availability for antimicrobial activity (McMance and Widdowson, 1993; McNeil and Schmidt, 1993).

This may partly explain why the concentrations of the extract used in this study were overcome by fungi. The combination of the extracts reduced the growth of *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus stolonifer*; however it did not impose enough stress to stop the growth of the fungi. The higher level of inhibition of growth by combined extracts when compaered to the single exract merely suggest that the effect was only additive.

Ethyl acetate fraction of aqueous extract of *M.myristica* and *X.aethiopica* exhibited the highest level of inhibition of growth when compared with n-hexane and diethyl ether fractions (Tables 3 and 4).This may suggest the suitability of ethyl acetate for the separation of the active constituents from aqueous extract of *M.myristica and X.aethiopica*. The results suggest that the extracts of the plant may be important sources of preservative of root juices.

Table 1: Effect of M.myristica and *X .aethiopica* on the growth of fungi in sweet

Extract(%v/v)		Biomas (mg dry weight/20ml) ± SD		
M. myristica	*X. aethiopica*	*A. niger* n = 4	*A. flavus* n = 4	*R. stolonifer* n = 4
Control		39.5±0.2	36.0±0.1	41.6±0.2
1.0	None	33.0±0.6[a]	31.0±0.2 [a]	37.6±0.1 [a]
2.0	None	27.4±0.02 [a]	25.5±0.01 [a]	30.4±0.1 [a]
3.0	None	21.6±0.3[b]	19.6±0.2 [b]	23.5±0.1 [b]
4.0	None	17.5±0.03 [b]	15.6±0.1 [b]	17.5±0.3 [b]
5.0	None	13.5±0.03 [b]	11.6±0.03 [b]	14.6±0.5 [b]
None	1.0	35.3±0.4	33.7±0.1 [a]	35.6±0.5 [a]
None	2.0	30.5±0.01 [a]	28.5±0.01 [a]	29.0±0.3 [a]
None	3.0	25.5±0.1 [a]	24.2±0.03 [a]	25.0±0.1 [a]
None	4.0	23.5±0.1 [b]	19.5±0.01 [b]	20.0±0.5 [b]
None	5.0	14.3±0.1 [b]	12.8±0.1 [b]	14.5±0.1 [b]

N = Number of samples, SD = Standard deviation, Control contain no *M.myristica* *and X.aethiopica*

Significant level of difference from control: [a] $P < 0.05$, [b] $P < 0.05$

Table 2: Effect of combination of aqueous extracts of *M. myristica* and *X. aethiopica* on the growth of fungi in potato juice

Extract (%v/v)		Biomass (mg dry weight/20ml juice) ± SD		
M.myristica	*X. aethiopica*	*A. niger* n = 4	*A. flavus* n = 4	*R. stolonifer* n = 4
Control		39.5±0.2	36.0±0.1	41.6±0.2
1	1	37.5±0.3	36.0±0.1	41.6±0.1
1	2	26.0±0.2	22.5±0.01	29.5±0.1
2	1	24.0±0.02	17.0±0.1	26.0±0.2
2	2	13.5±0.1	10.5±0.2	19.0±0.1

n = Number of samples, SD = Standard deviation, All treated cases are significantly different from control: ($P<0.05$), Control contain no *M.myristica* and no *X.aethiopica*

Table 3: Effect of organic solvent soluble fraction of *M. myristica* on the growth of challenge fungi in potato juice

Test organism	Biomass (mg dry weight/20ml juice) ± SD				
	Control	Ethyl acetate Fraction n = 4	N – hexane Fraction n = 4	Diethyl ether fraction n = 4	20% Dimethyl sulphoxide n = 4
A. niger	39.5±0.2	11.5±0.2	15.5±0.1	13.0±0.02	39.5±0.2
A. flavus	36.0±0.1	10.5±0.02	14.0±0.1	12.5±0.1	36.0±0.1
R. stolonifer	41.6±0.2	14.5±0.2	17.0±0.2	15.0±0.1	41.6±0.2

n = number of samples; All treated cases are significantly different from control (P<0.05); control contain no *M.myristica* and no *X.aethiopica*.

Table 4: Effect of organic solvent soluble fraction of aqueous extract of X. aethiopica on the growth of challenge fungi in potato juice

Test organism	Biomass (mg dry weight/20ml juice)SD				
	Control	Ethyl acetate fraction n = 4	n – hexane fraction n =4	Diethyl ether fraction n = 4	20% Dimethyl sulphoxide n = 4
A. niger	39.5±0.2	14.5±0.3	17.5±0.02	14.5±0.1	39.5±0.2
A. flavus	36.0±0.1	11.5±0.1	15.5±0.2	13.0±0.01	36.0±0.1
R. stolonifer	41.6±0.2	16.0±0.01	19.5±0.1	17.0±0.1	41.5±0.2

n = number of samples; All treated cases are significantly different from control (P<0.05); control contain no *M.myristica and* no *X.aethiopica*

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