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The Effect of *Allium Sativum* and *Xylopia Aethiopica* Extracts on the Growth of Fungi in Sweet Potato (*Ipomoea Batatas*) Juice

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Abstract
A laboratory study was conducted to determine the effect of *Allium sativum* and *Xylopia aethiopica* on the growth of *Mucor* species, *Rhizopus stolonifer* and *Aspergillus niger* isolated from deteriorating sweet potato. While 3% (v/v) aqueous extract of *Allium sativum* and *Xylopia aethiopica* reduced the growth of the fungi; a combination of 2% each of both plant extracts retarded the growth better. Partial purification of aqueous extract of *Allium sativum* and *Xylopia aethiopica* showed that ethyl acetate fraction of the extracts exhibited the highest level of inhibition of growth of the test fungi compared with n-hexane and diethyl ether fractions. Extracts of *Allium sativum and Xylopia aethiopica* may be substitutes for conventional chemical preservatives in the processing of juices.

Key words: Preservative, conducted, isolated, deteriorating, extract, substitute, conventional

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Introduction

Sweet potato is an important neglected crop in the agricultural policies of many tropical countries including Nigera (Okonkwo *et al*., 2008).The crop has potential as raw material for the manufacture of a wide range of products. However its processing and utilization is not a common practice in Nigeria due to lack of processing technologies as well as high perishability occasioned by high moisture content and high metabolic activity after harvesting (FAO, 2002).

Both the leaves and the tubers are consumed in some communities in Nigeria. it has high fibre content and its complex carbohydrate appeals to diabetics. It contains a substantial amount of β-carotene. The tuber deteriorate rapidly post harvest by rotting, drying and enzymatic oxidative discolouration of the pulp under ambient conditions. Sweet potato develop chilling injury at temperatures from 0^0C to 10^0C (Ihenkoronye, 1995). Chilling leads to increase in sugar content and accelerates respiratory activity. The chilling injury can result in increased susceptibility to decay and failure to sprout. Chilling also produces such physiological effects as loss of ascorbic acid and increase in chlorogenic acid (Leistner, 1982).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.

Fungi infect sweet potato during storage; soft rot in sweet potato is caused by species of *Rhizopus* that produces soft decay that consumes the root (Ogundana , 1972). Soft 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 16^0C encourage the development of virus disease that cause the development of corky areas in susceptible varieties (Matern and Kneusel, 1988).In order to minimize tuber losses and enhance sweet potato utilization it must be processed into diverse forms (Iheagwara *et al*., 2008).

Allium sativum (garlic) has diuretic and emmenagic properties, it is used in the treatment of fever. A poultice of the bulb is used in the treatment of ringworm infection. The juice of Allium sativum is used as ear drop to treat earache (Gill, 1992). Decoction of the fruits of *Xylopia aethiopica* (Ethiopian pepper) is used as a remedy for stomach ache (Gill, 1992).The present study attempts to investigate the effect of *Allium sativum* and *Xylopia aethiopica* on the growth of fungi in sweet potato juice.

Materials and Methods

Plant materials and microorganisms: The microorganisms used in this study were *Mucor*

species, *Rhizopus stolonifer* and *Aspergillus niger*. The fungi were isolated from deteriorating sweet potato and identified by standard microbiological procedures. *Allium sativum*, and *Xylopia aethiopica* used in this study were obtained from Bida, Niger State, Nigeria. They were identified at forestry Research Institute of Nigeria (FRIN) Ibadan .Fresh sweet potato roots of red skin variety were obtained from Bida Niger state.

Preparation of extracts of Allium sativum and Xylopia aethiopica: The bulb of *Allium sativum* and fruits of *Xylopia aethiopica* were shade dried at ambient temperature and ground into powder. Ten grams of ground dry bulb sample of *Allium sativum* and fruits of *Xylopia aethiopica* were then soaked in 250ml of hot (70^0C) sterile water contained in two separate 500mlcapacity flasks .The flasks were plugged with cotton wool, wrapped in aluminum foil, shaken and allowed to stand in the refrigerator for 72h. The filtrate was obtained by suction and concentrated using a water bath (BT101) at 80^oCuntil a brown viscous residue remained (Banso and Sani, 2003).

Extraction of potato juice: Potato roots were washed and immersed in 10% hypochlorite solution for 10 min. The roots were then peeled and the juice extracted manually (Banso and Ayodele, 2005).

Determination of effect of different concentrations of plant extracts on growth of fungi in potato juice: Sample of the juice (20ml) was introduced into 100ml capacity flasks. Extracts of *Allium sativum* and *Xylopia aethiopica* were then added to give concentrations (v/v) ranging from 1% to 5%. Thereafter they were inoculated with 1ml of aqueous suspension containing 10^{10} spores of test fungi obtained by serial dilution and incubated for 7 days at room temperature (28 ± 2^oC). The developing mycelia of four replicates were subsequently recovered by filtration using pre- weighed whatman No 1 filter paper and dried to a constant weight at 70^oC. in a hot air oven (Banso and Ayodele, 2005) . 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 the effect of different concentrations of plant extract on the growth of fungi in potato juice was repeated using the following combination s of the two plant extracts (*A. sativum* and *Xylopia 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 s of A. sativum and X. Aethiopica: The method of Isao *et al*. (1992) for the separation of organic compounds with slight modifications was used to determine the antifungal effect of organic solvent soluble fraction of aqueous extract of *A. sativum* and *X. aethiopica*. Aqueous extract of *A. sativum* or *X. aethiopica* was partitioned between water and sequentially between n- hexane, diethyl ether and ethyl acetate. Each fraction was collected and allowed to evaporate to dryness using a hot plate. 20ml of 50% concentration of the residues was inoculated with 1ml aqueous suspension containing 10^6spores of the test fungi and incubated for 7 days at room temperature (28 ± 2^oC). The developing mycelia of three replicates were subsequently recovered by filtration using pre- weighed Whatman no 1 filter paper and dried to constant weight at 70^oC. 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

Extracts of *A. sativum* and *X. aethiopica* showed inhibitory effect against *Mucor* species, *R. stolonifer* and *A. nger* in sweet potato juice (Table 1). The application of *A. sativum* sowed significant reduction ($P < 0.05$) reduction of fungal biomass At 3.0% while 4.0% concentration of *X. aethiopica* showed significant reduction of fungal biomass. The effect of plant extracts on microorganisms may depend on the type as well as the medium (Obeta and Ugwuanyi, 1995). Spices contain phenols and essential oils, which are inhibitory to microorganisms (Nakatani, 1994). It was reported that fat and proteins bind to solubilise phenolic compounds thereby reducing the availability for antimicrobial activity (McMance and Widdowson, 1993; McNeil and Schmidt, 1993). This may partly explain why the

concentrations of the extracts used in this study were overcome by the fungi.

The combination of the extracts reduced the growth of *Mucor* species, *R. stolonifer*, and *A. niger*; however it did not impose enough stress to stop the growth of the fungi.

Ethyl acetate fraction of aqueous extract of *A. sativum* 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 extracts of *A. sativum* and *X. aethiopica*. The results suggest that the extract of the plants may be important sources of preservative of root juices.

Table 1: Inhibitory effect of extract of *A. sativum* and *X. aethiopica* on the growth of fungi in sweet potato juice

Extract (% v/V)		Biomass(mg dry weight/20ml)±SD		
A. sativum	*X. aethiopica*	*Mucor species* n=4	*R. stolonifer* n=4	*A. niger* n=4
Control		40.5±0.1	36.5±0.01	39.5±0.2
1.0	None	35.6±0.01[a]	7.0±0.1[a]	28.5±0.1[a]
2.0	None	28.5±0.1[a]	25.0±0.2[a]	26.5±0.2[a]
3.0	None	23.5±0.2[a]	17.5±0.2[b]	18.2±0.1[b]
4.0	None	17.5±0.2 [b]	15.0±0.1[b]	16.5±0.2[b]
5.0	None	14.5±0.3[b]	11.5±0.3[b]	12.5±0.1[b]
None	1.0	33.5±0.5[a]	31.5±0.3[a]	32.5±0.1[a]
None	2.0	25.5±0.3[a]	23.6±0.1[a]	24.5±0.2[a]
None	3.0	22.5±0.3[a]	18.7±0.1[b]	20.3±0.4[a]
None	4.0	18.5±0.3[b]	16.6±0.5[b]	17.0±0.1[b]
None	5.0	14.5±0.1[b]	12.5±0.3[b]	13.5±0.3[b]

n= Number of samples, SD = Standard deviation, Control contain no *A. sativum* and *X. aethiopica*, Significant level of difference from control: $P^a > 0.05$, $P^b < 0.05$.

Table 2: Effect of combination of aqueous extracts of *A. sativum* and *X. aethiopica* on the growth of Fungi in potato juice

Extract (% v/V)		Biomass(mg dry weight/20ml)±SD		
A. sativum	*X. aethiopica*	*Mucor species* n=4	*R. stolonifer* n=4	*A. niger* n=4
Control		40.5±0.1	36.5±0.01	39.5±0.2
1	1	38.5±0.2	34.0±0.2	35.5±0.1
1	2	26.5±0.3	18.5±0.1	20.5±0.1
2	1	23.5±0.2	15.4±0.2	18.5±0.2
2	2	17.6±0.3	10.0±0.3	15.3±0.5

n = number of samples, SD = Standard deviation. All treated cases are significantly different from control (P<0.05). Control contain no *A. sativum* and no *X. aethiopica*

Table 3: Effect of organic solvent soluble fraction of *A. sativum* 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 sulfoxide fraction n=4
Mucor species	40.5±0.1	12.5±0.2	15.0±0.3	13.5±0.1	40.5±0.1
R. stolonifer	36.3±0.1	6.5±0.2	8.5±0.2	7.5±0.2	36.5±0.2
A. niger	39.5±0.2	8.6±0.2	10.5±0.2	9.5±0.1	39.5±0.2

n = number of samples; All treated cases are significantly different from control (P<0.05), control contain no *A. sativum*

Table 4: Effect of organic solvent soluble fractions of aqueous extract of *X. aethiopica* on the growth of challenge fungi in sweet 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 sulfoxide fraction n=4
Mucor species	40.5±0.1	10.5±0.1	13.5±0.1	11.5±0.1	40.5±0.1
R. stolonifer	36.5±0.1	4.5±0.2	6.0±0.3	5.0±0.1	36.5±0.1
A. niger	39.5±0.2	5.5±0.3	7.5±0.2	6.5±0.3	39.5±0.2

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

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