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Full Length Research Paper

Fruit borne mycoflora of *Capsicum annum* L (pepper), *Abelmoschus esculentus* L. Moench (okra), and *Lycopersicon esculentum* Mill. (tomato) from Accra metropolis

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Fungal species composition on fresh *Capsicum annum* L, *Abelmoschus esculentus* L. Moench, and *Lycopersicon esculentum* Mill. obtained from Madina, Makola, Mallam Atta and Agbogbloshie markets in the Accra metropolis were studied. Using two media types Potato dextrose agar (PDA) and dichloran-glycerin (DG18) agar, a total of 18 fungal species belonging to 8 genera were isolated from surface sterilized and non sterilized fruits. From fruits of all four markets, species in the genera *Aspergillus* (5 species), *Cladosporium* (2 species), *Fusarium* (3 species), *Penicillium* (4 species), *Mucor* (1 species), *Neurospora* (1 species), *Trichoderma* (1 species) and Yeasts species were isolated. The highest (2.79 $\log_{10}$ CFU/g) microbial load of fungi was recorded in samples of pepper obtained from Agbogbloshie market on PDA medium whilst the least fungal load (1.69 $\log_{10}$CFU/g) was resident on tomato samples from Makola and Mallam Atta markets isolated on DG18. *A. flavus* was the most common species occurring on all the fruits from all the markets surveyed. The fresh fruits therefore ought to be handled properly to eliminate or minimize mycofloral contaminants which may prevent seed germination and its adverse effect on humans in the event of toxin production.

Key words: Mycoflora, fungal load, conidia, fruits.

INTRODUCTION

In Ghana, traditional vegetables are important source of vitamins and nutrients for rural populations as many nutritional studies have shown (Mnzava et al., 1999). Farmers have cultivated and collected these vegetables for generations as an additional food source. Again, convenience, freshness and the ready to use nature of vegetables have led to their increasing demand in recent years. According to Lapido (1997), trading in some of these traditional vegetables was mainly local but is slowly changing to a regional and even international trade, especially, *Gnetum africanum*, *Amaranthus*, okra and other fresh vegetables.

Vegetables production in certain areas in Ghana is inefficient and little attention is paid to produce quality and efficient marketing systems. A proportion of these traditional vegetables are rendered unsalable on farms and in markets as a result of physical, chemical and microbiological defects. It is estimated that about 20-25% of the harvested fruits are decayed by pathogens during post-harvest handling even in developed countries (Droby, 2006; Zhu, 2006).

Post harvest losses of fruit vegetables and other foodstuffs may threaten food security in view of the staggering population growth rate. Micro-organisms, especially fungi have been reported to cause extensive deterioration of fruits and vegetables (Fajola, 1979; Erinle, 1982; Amadioha and Uchendu, 2003). Some of these micro-organisms cause rotting, discoloration or fermentation of the fruits which affect their preservation.

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Infection may occur prior to harvest on the field and during or after harvest which frequently arise from mechanical damage or physiological injuries. According to Hernandez-Brenes (2002), fruit or vegetable micro-flora is due to contaminants from the soil, dust and surroundings; or may be introduced through poor production and handling practices such as the application of untreated manure, the use of irrigation water or unsanitary handling practices.

Fruits rot caused primarily by microorganisms (fungi and bacteria) does not only pose a major challenge to food security but also to human health in the event of toxin production by the microbes. Fungi are able to utilize the nutrients of the fruit vegetables and may cause deterioration and decay. According to Singh and Sharma (2007), fruits contain high levels of sugars and nutrients element and their low pH values make them particularly desirable to fungal decay. It has been estimated that there are over 100,000 fungi whose spores may become airborne (Kendrick, 1990) which may eventually settle on fruit vegetables upon exposure. Muhammad et al, 2004 found eight different fungi, *Aspergillus niger, Aspergillus ochraceous, Aspergillus flavus, Aspergillus* fumigates, *Penicilium citrinum, Curvolaria lunata* and *Sclerotium rolfsi* associated with rotten tomato fruits obtained from five different markets in Nigeria. *A. flavus* and *A. niger* had the highest rate of occurrence among the isolated fungi (Muhammad et al., 2004). Fruits of vegetables and vegetable products are susceptible to spores or conidia and mycelium fragments from the environment. According to Quaicoe (1991) *Cladosporium herbarum* is the most predominant species isolated from the air in the Greater Accra region followed by *Fusarium* and *Aspergillus* species. The susceptibility of these vegetables to fungal pathogens is a source of worry because of the losses they cause, including the loss of seed viability in some cases. In the present study, mycoflora associated with fresh fruits of *Capsicum annum* L, *Abelmoschus esculentus* L. Moench, and *Lycopersicon esculentum* Mill. from different markets in the Accra metropolis has been examined.

MATERIALS AND METHODS

Source of samples

Fresh undamaged fruits samples of *Capsicum annum* L (pepper), *A. esculentus* L. (okra), and *L. esculentum* Mill. (tomato) were purchased from Madina, Makola, Mallam Atta and Agbogbloshie markets of the Accra metropolis. Fifteen samples of each of the vegetables were randomly purchased from each market and sent to the laboratory in clean transparent zip lock bags and used for isolation of mycoflora.

Preparation of aqueous fruit extracts

Fruit extracts were prepared by blending 250 g of tomato, 50 g each of pepper and okra, in 250 and 500 ml of sterilized distilled water, respectively, using the National MX-J210PN blender. Each was then filtered with clean absorbent cotton wool and filter paper using Compton vacuum pump.

Isolation of fruit surface mycoflora

Fruit surface mycofloral load were isolated by washing 100 g of pepper, 200 g each of okra and tomato in 1000 and 2000 ml of sterilized 0.1% peptone solution, respectively. The resultant solution served as the stock spore suspension from which serial dilutions were made up to $1:10^3$. Using the dilution plate method, Johnson and Curl (1972) and two media Potato dextrose agar (PDA) and dichloran-glycerin (DG18) agar, 1 ml of the spore suspension was put into separate sterile Petri dishes and 20 ml of sterilized media was poured into each Petri dish. Three replicates were made and incubated at 30°C. The fungal colonies were enumerated on the third day and expressed as $\log_{10}$ Colony Forming Unit per gram ($\log_{10}$CFU/g). The species were identified on the seventh day of the incubation period using cultural, morphological and microscopic characteristics according to Sampson and Reenen-Hoekstra (1988), Barnett and Hunter (1972), and Von Arx (1970).

Isolation of fruit-borne mycoflora

Washed fruits samples were surface sterilized by immersing in 1% sodium hypochlorite solution for two minutes and rinsed in sterile distilled water three times. The fruits were cut transversely into discs of four with flame sterilized knife and plated onto DG18. The plates were incubated for seven days at 30°C. The fungal colonies were counted on the third day, isolated and identified at the end of the incubation period.

Germination of conidia in fresh fruit extracts of okra, pepper and tomato.

Spore germination test was performed to determine the effect of the fruit extracts on conidial germination. A spore suspension of the test fungi was prepared by removing a 6 mm disc of a five day old culture with sterile cork borer into McCartney tubes containing the fruit extracts. The spore suspension was shaken by hand to give a uniform dispersion. The number of conidia in suspension for every germination test was standardized to 30 to 40 spores under the high power (X40 objective) microscope field (Figure 3).

Two separate drops were delivered with a micropipette onto a cooled flamed sterilized slide (7.5 × 2.5 cm) supported on a V-shaped glass rod placed in a sterile Petri dish with relative humidity of 100%. Twelve replicates were made. The conidia in drops of suspension were then incubated. Three slides were withdrawn at 4 h intervals and examined for germinating spores. Percentage germination for each treatment was based on not less than 300 observed conidia from all the six suspension drops using the formula:

$$\%\,Germination = \frac{A}{A+B} * 100$$

Where: A = Number of germinated spores and B = Number of ungerminated spores.

The lengths of 20 representative germ tubes were also measured with an eye piece graticule calibrated with a stage micrometer and the mean length of the germ tubes calculated.

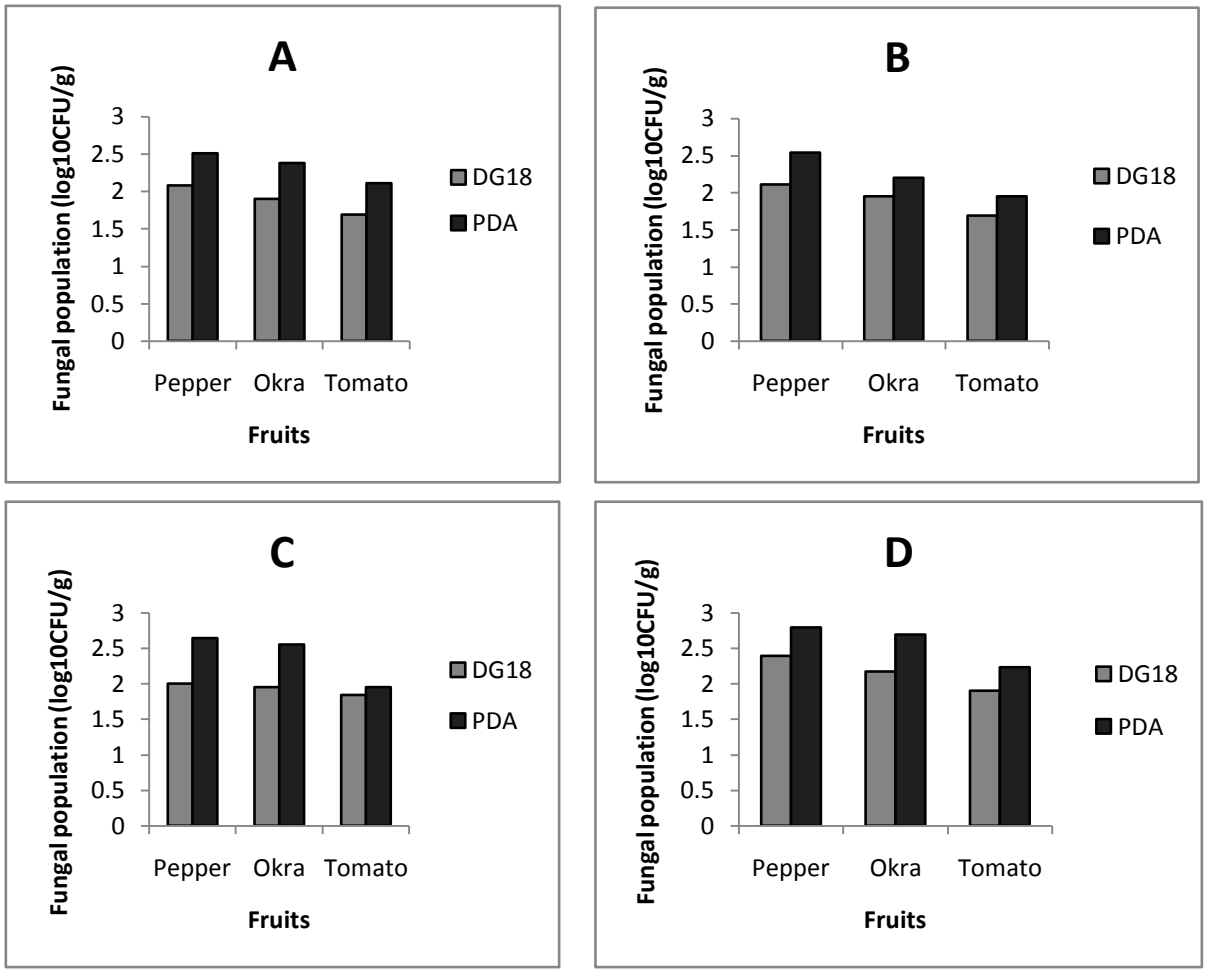

Figure 1. Fungal load of fresh fruits of pepper, okra and tomato from different markets isolated on DG18 and PDA, (A) Madina, (B) Makola, (C) Mallam Atta and (D) Agbogbloshie.

RESULTS

The microbial load expressed as $\log_{10}$ Colony Forming Unit (CFU/g) from each market and the fungal species identified are presented in Figure 1 and Table 1 respectively. The results obtained show clearly that the three fruits obtained from the four markets, Agbogbloshie, Madina, Makola and Mallam Atta, are contaminated by propagules of various fungi. Fruits obtained from Agbogbloshie market recorded the highest fungal population, followed by Mallam Atta market, Madina market and lastly Makola market (Figure 1). The highest (2.79 $\log_{10}$CFU/g) microbial load of fungi was recorded in samples of pepper obtained from Agbogbloshie market on PDA medium (Figure 1) whilst the least fungal load (1.69 $\log_{10}$CFU/g) was resident on tomato samples from Makola and Mallam Atta markets isolated on DG18 (Figure 1).

A total of seventeen fungal species belonging to eight genera; *Aspergillus, Cladosporium, Fusarium, Mucor, Neurospora, Penicillium, Trichoderma* were isolated from the unsterilized fruits surface (Table 1). The highest number (13) of fungal species was isolated from the fruits from the Agbogbloshie market whereas the least (10) was encountered on the fruits from the Mallam Atta market. The Makola and Madina markets recorded eleven and twelve species respectively. Samples from Agbogbloshie yielded quantitatively as well as qualitatively more fungi than samples from Madina, Makola and Mallam Atta markets. *Aspergillus flavus* was the only fungus isolated on all the fruits from the four markets surveyed. *Neurospora crassa* and *Trichoderma viride* were recorded on fruits from Agbogbloshie market only whilst *Penicillium nalgiovense* was isolated on pepper from Mallam Atta market. Species such as *A. flavus, A. niger, Cladosporium herbarum, Fusarium sp.* and Yeasts were common in the four markets surveyed whilst *T. viride, N. crassa, P. nalgiovense, Fusarium oxysporum* and *Aspergillus nalgiovense* were encountered occasionally. The fungal species were encountered on pepper, okra and tomato fruits were 14, 13 and 9 respectively. It is interesting to note that

Table 1. Fungal species isolated from the fruits of pepper, okra and tomato obtained from different markets in Accra.

Species	Maadina			Makola			Mallam Atta			Agbogbloshie		
	Pepper	Okra	Tomato	Pepper	Okra	Tomato	Pepper	Okra	Tomato	Pepper	Okra	Tomato
**Aspergillus flavus*	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus fumigatus	+	-	-	+	+	+	+	-	-	-	-	-
**Aspergillus niger*	+	+	+	+	-	-	+	+	-	+	+	+
Aspergillus sp.	-	+	-	-	+	-	-	-	-	+	+	-
***Aspergillus sulphureus*	-	+	-	-	+	-	-	-	-	-	-	-
**Cladosporium herbarum*	-	+	+	-	+	+	+	+	+	+	+	+
***Fusarium oxysporum*	-	-	-	-	-	-	-	-	-	-	-	+
Fusarium sp.	+	+	-	+	+	-	-	+	-	+	+	-
Fusarium verticilloides	+	-	-	+		-	-	-	-	-	+	-
Mucor sp.	-	+	-	-	+	-	-	-	-	+	+	-
***Neurospora crassa*	-	-	-	-	-	-	-	-	-	+	+	+
**Penicillium expansum*	+	+	+	-	-	-	+	-	+	+	+	+
Penicillium glabrum	-	-	-	-	-	-	+	-	-	+	-	+
Penicillium sp.	+	+	-	+	-	-	+	-	-	-	-	-
***Penicillium nalgiovense*	-	-	-	-	-	-	-	+	-	-	-	-
***Trichoderma viride*	-	-	-	-	-	-	-	-	-	+	-	-
**Yeasts*	+	-	+	+	-	+	+	-	+	+	-	+

+ Present, -Absent, *-Common, **Occasional.

A. flavus, A. niger, C. herbarum, P. expansum and Yeasts were most frequent and recorded 100, 75, 83.3, 66.7 and 66.7% respectively (Figure 2).

The fruit borne fungi isolated from surface-sterilized fruits reduced in species richness from the four markets. In all, eight fungal species belonging to five genera; *Aspergillus*, *Cladosporium, Fusarium*, *Mucor* and *Penicillium* were isolated from the fruits (Table 2). Six fungal species ascribed to five genera, were isolated from fruits from the Makola market while fruits from the Agbogbloshie market harboured six fungal species belonging to three genera (Table 2). The fungi resident in fruits from Madina market were five species in three genera, whilst fruits from Mallam Atta market recorded four fungal species belonging to two genera (Table 2). Incidentally, nearly all the fungal species that were internally fruit-borne were also encountered on the surface of the fruits with the exception of *Cladosporiummacrocarpum* which was absent from the fruits surface.

Influence of the aqueous extracts of pepper, okra and tomato on conidial germination and mean germ tube length of *A. flavus* and *F. verticilloides* were assessed. The aqueous extracts variably promoted conidia germination of *A. flavus* and *F. verticilloides* (Table 3 and Figure 2). Over 80% of conidia of *A. flavus* germinated in the tomato aqueous extract whereas less than 15% germination occurred in both the pepper and the okra extract (Table 3). This may be as a result of differences in nutrient and inhibitory factors in the fresh fruit aqueous extracts. The mean germ tube length was also highest in the tomato extract reaching 140.6 µm at the end of the incubation period (Table 3). However, over 60% conidial germination of *F. verticilloides* occurred in all the aqueous fresh fruit extracts. The maximum conidia germination (88.2%) and highest mean germ tube (156.4 µm) were also recorded in the tomato extract (Table 3).

DISCUSSION

Post-harvest losses of perishable fruits such as pepper, okra and tomato pose a major challenge

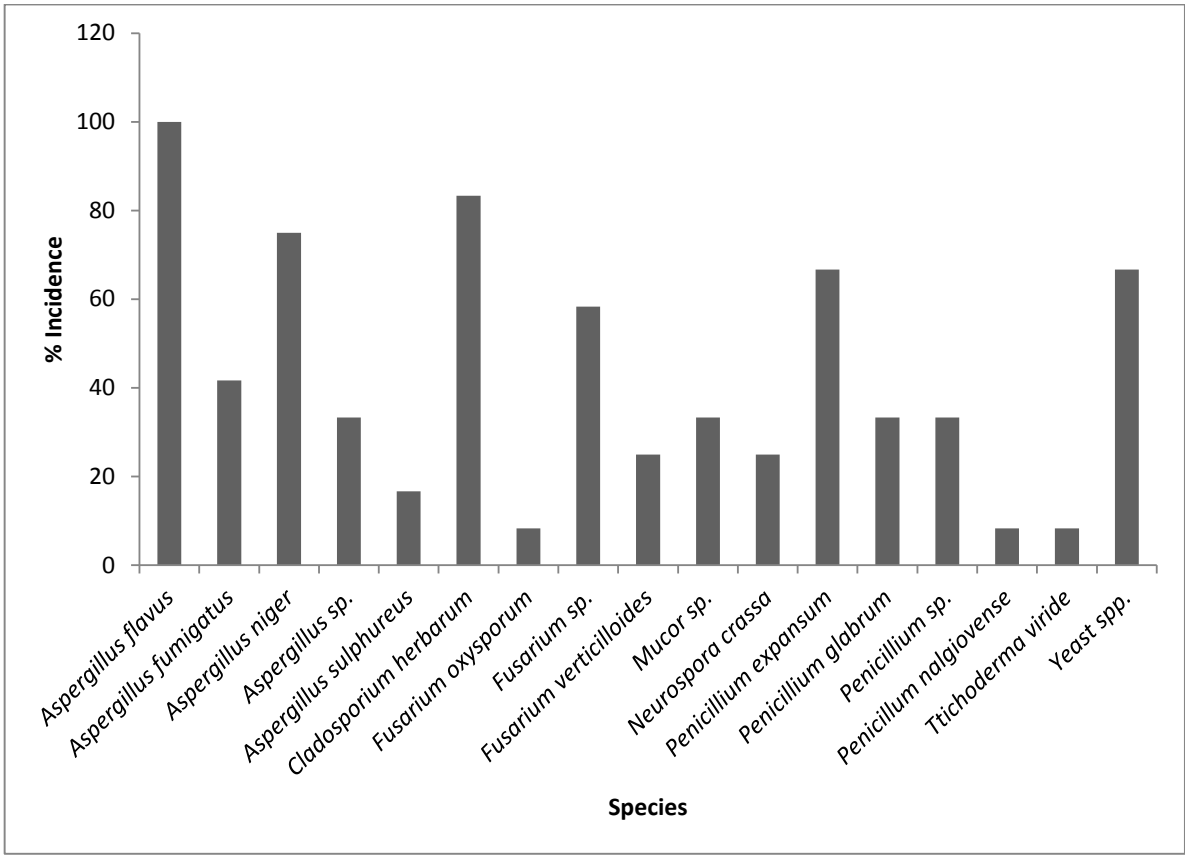

Figure 2. Incidence of fungi based on presence or absence on fruits of vegetables.

Table 3. Conidia germination of *A. flavus* and *F. verticilloides* in aqueous fruit extracts.

Fungus	Extract	pH	% Germination after hours				MGTL (µm) after hours			
			4	8	12	24	4	8	12	24
Aspergillus flavus	Pepper	5.50	0	0	0	14.3	0	0	0	70.1
	Okra	5.74	0	0	7	12.2	0	0	20.1	32.3
	Tomato	4.71	0	41.7	77.3	81.3	0	24	46.1	140.6
Fusarium verticilloides	Pepper	5.50	43.5	61.5	83.3	87.5	26.2	60.3	82.7	128.2
	Okra	5.74	26.3	48.4	58.3	62.5	12.3	58.1	68.3	114.0
	Tomato	4.71	41.7	73.3	83.2	88.2	28.3	46	156.4	-

Note: MGTL- Mean germ tube length, - Hyphae.

to developing countries. Losses of these perishable commodities may be due to physical, physiological and phytopathological factors. However, microbial spoilage plays a major role in losses of which fungi play a critical role. Losses of these vegetables can occur at all levels of the post-harvest system such as preprocessing on the field, during transportation, in storage and in wholesale and retail markets. Toma et al. (1990) reported that, “losses of tomatoes in developing countries may be as high as 50%”. Earlier, Onesironsan and Fatunla (1976) had reported that, up to 21% of the potential harvest of tomato fruits were lost to rots in the field while an additional 5 to 20% rotted in transit and in market.

Differences in fungal species isolated from the surface of unsterilized fruits of pepper, okra and tomato obtained from the four markets was apparent. The predominant fungal genera encountered on the surface of the unsterilized fruits obtained from the markets were

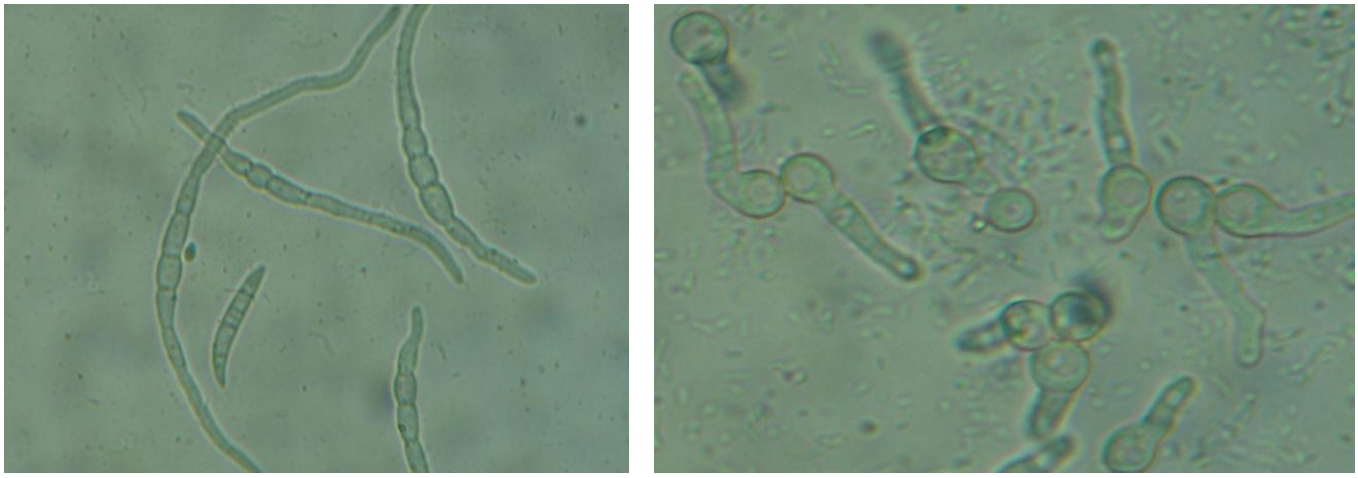

Figure 3. Photograph showing germinating conidia of *F. verticilloides* (left) and *A. flavus* (right) in pepper and tomato extracts respectively (X 1500).

Aspergillus, Cladosporium, Fusarium and Penicillium whereas *Mucor, Neurospora* and *Trichoderma* occurred occasionally. The fungal species isolated from the fruit surface settled on the surface of the specimens from the field (field fungi) or in the market (storage fungi). Domsch et al. (1981) postulated that the contamination of feedstuffs with fungal species was as a result of natural extraneous contamination by dust during storage in humid conditions. Bankole (1996) reported of *Aspergillus, Cladosporium* and *Fusarium* as the most frequent fungal genera on non-surface sterilized tomato seeds. Earlier, Arinze (1986) and Oladiran and Iwu (1993) had reported the occurrence of the three fungal genera on rotten tomato fruits.

Eight fungal species belonging to five genera, *Aspergillus, Cladosporium, Fusarium*, *Mucor* and *Penicillium,* were isolated from surface-sterilized fruits obtained from the various markets. Surface sterilization of fruits reduced fungal species on all the fruits from the four markets. This confirms earlier findings by Omafuvbe and Kolawole (2004) that pretreatments such as steeping in boiling water alone or steeping combined with surface disinfection of fresh pepper fruits before drying, drastically reduced microbial load. Interestingly, the fresh pepper fruits which recorded the highest microbial load of 2.79 $\log_{10}$CFU/g (Figure 1) also recorded the least number (5) of fungal species (Table 2) after surface-sterilization. Andress et al. (2001) also reported a significant reduction in the microflora of spices when preliminary treatments such as washing with water and dipping in chlorine were employed.

Fungi which appear on fruits and seeds after surface sterilization give an indication of the fungal species that were resident within the produce from the field. Studies by Quaicoe (1991) showed that *Cladosporium herbarum* was the most predominant species isolated from the air in the Greater Accra region followed by *Fusarium* and *Aspergillus* species. This study however, found *A. flavus* as the most common followed by *C. habarum* and *A. niger* on the fruit surfaces. Peter et al. (1990) studied the fungal contamination of fruits and vegetables and indicated that *Aspergillus* was isolated from 79.5% of the samples.

Penicillium cyclopium had earlier been isolated from the surface of disinfected decaying fruits of pepper and tomato in storage whereas *P. expansum* was isolated from only tomato (Barkai-Golan, 1974). *Alternaria longissima*, *Aspergillus flavus*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium moniliforme (verticilloides)*, *Fusarium oxysporum* and *Phoma destructive* had been isolated as seed-borne fungi from two tomato varieties (Bankole, 1996).

The results of this study have confirmed that fungal species are resident on both the surface and within the tissues of the fruits of the three vegetables. The presence of fungal contaminants on fruits surface predisposes the produce to severe losses. Moreover the growth of conidia of *A. flavus* and *F. verticilloides* in the fruit extracts may have adverse effects on both humans and animals due to their potential to produce potent mycotoxins in the fruit extracts.

Conclusion

Investigation on mycoflora associated with fruits of pepper, okra and tomato from Agbogbloshie, Madina, Makola and Mallam Atta markets in the Accra metropolis shows an apparent difference in fungal composition. *Aspergillus flavus* occurred on all fruits from the different markets. Fresh fruits need to be handled with care during harvesting, transportation or marketing to reduce mycoflora load. *Aspergillus* and *Fusarium* are known to have strains that produce toxic metabolites. It is

recommended that the production of toxins by fruit borne *A. flavus* and *F. verticilloides* in extracts of pepper, okra and tomato be investigated.

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REFERENCE

Amadioha AC, Uchendu PN (2003). Post Harvest Control of Tomato Fruit Rot, caused by *Fusarium solani* with Extracts of *Azadirachta indica*. Discov. Innov., 15(1/2): 83-86.

Andress EL, Blackman IC, D'Sa EM, Harrison MA (2001). Microbiota of fresh herbs and whole spices used in home food preservation and effectiveness of microbial intervention methods. Proceedings of the Institute of Food Technologists Annual Meeting, New Orleans, LA. June 26, 88D-27.

Arinze AE (1986). Post-harvest diseases of tomato fruits in Nigeria. Fitopatholgia Brasileira, 11: 637-645.

Bankole SA (1996). The distribution and pathogenicity of the seed mycoflora of two tomato varieties cultivated in western Nigeria. Afr. Crop Sci. J., 4: 491-496.

Barkai-Golan R (1974). Species of *Penicillium* causing decay of stored fruits and vegetables in Israel. Mycopathologia et Mycologia applicata. 54: 141-145.

Barnett HL, Hunter BB (1972). Illustrated Genera of Imperfect Fungi 3rd edn. Burgess Publ. Co. Minneapolis, Minnesota. p. 331.

Domsch KH, Gams W, Anderson TH (1981). Compendium of Soil Fungi. Vol. 1 and 2, Academic Press, London.

Droby S (2006). Improving quality and safety of fresh fruits and vegetables after harvest by the use of biocontrol agents and natural materials. Acta Horticul., 709: 45-51.

Erinle ID (1982). The effect of some factors on keeping quality of tomato. Proc. 5th Annual Conf. Soc. Nigeria, Zaria. pp. 72-81.

Fajola AO (1979). The post harvest rots of tomato (*Lycopersicon esculentum*) in Nigeria. Die Naurung. 23: 105-109.

Hernandez–Brenes C (2002). Good Agricultural Practices, In: Improving the safety and quality of fresh fruit and vegetables: A training manual for trainers. University of Maryland.

Johnson LF, Curl EA (1972). Methods for research on ecology of soil borne pathogens. Burgess Publishing Co., Minneapolis, USA. pp. 247.

Kendrick (1990). Fungal allergens. In Sampling and identifying allergenic pollens and moulds (ed. E.G. Smith) Blewstone Press, San Antonio, USA. pp. 41-49.

Lapido DO (1997). Marketing and post-harvest constraints of traditional vegetables in Sub-Saharan Africa. In workshop on African indigenous vegetables. Jamay 13-18, 1998. Limbe, Cameroun. pp. 58-62.

Mnzava NM, Dearing JA, Guarino L, Chweya JA, de Koeijer H (1999). Bibliography of the genetic resources of traditional African vegetables. Neglected leafy green vegetable crops in Africa Vol. 2. International Plant Genetic Resources Institute, Rome, Italy. ISBN 92-9043-531-3.

Muhammad S, Shehu K, Amusa NA (2004). Survey of the market diseases and aflatoxin contamination of tomato (Mill.) fruits in Sokoto, northwestern Nigeria. 34: 72-76.

Oladiran AO, Iwu LN (1993). Studies on the fungi associated with tomato fruit rots and effects of environment on storage. Mycopathologia 121: 157-161.

Omafuvbe BO, Kolawole DO (2004). Quality Assurance of Stored Pepper (*Piper guineese*) Using Controlled Processing Methods. Pakistan J. Nutr., 3(4): 244-249.

Onesironsan PT, Fatunla T (1976). Fungal fruit rot of tomatoes in Southern Nigeria. J. Hort. Sci., 51: 473-479.

Peter M, Kiss E, Sabau M, Bedo C (1990). A study on the parasitic and fungal contamination of fruits and vegetables cultivated on soils irrigated with water from various sources. Rev. IG Med. Muncii. Med. Soc. Bacteriol. Virusal Parazitol Epidemiol Pneumoftiziol Ser. 39: 31-37.

Quaicoe RN (1991). Aerobiology of the Accra metropolitan Area (Legon) with special reference to fungi and its possible relation to plant disease. MPhil Thesis, University of Ghana, Accra, Ghana.

Sampson RA, Van Reenen-Hoekstra ES (1988). Introduction to Food Borne Fungi. 3rd Ed. Centraalbureau voor Schimmelcultures. Baarn Institute of the Royal Netherlands Academy of Arts and Sciences. pp 299.

Singh D, Sharma RR (2007). Postharvest diseases of fruit and vegetables and their management. In: Prasad, D. (Ed.), Sustainable Pest Management. Daya Publishing House, New Delhi, India.

Toma BR, Fansler LT, Knipe MT (1990). World Food Shortage: The Third Dimesion. Sci. Food Agric. J. Http://www.arec.umd.edu/arec365/castpharv.htm

Von Arx JA (1970). The genera of Fungi Sporulating in Pure Culture. Verlag Von J. Cramer, 3301 Lehre Germany. p. 288.

Zhu SJ (2006). Non-chemical approaches to decay control in postharvest fruit. In: Noureddine, B., Norio, S. (Eds.), Advances in Postharvest Technologies for Horticultural Crops. Research Signpost, Trivandrum, India, pp. 297-313.