Agricultural Journal 7 (1): 53-57, 2012
ISSN: 1816-9155

Mycoflora of Cabbage (*Brassica oleracea* L. var *Capitata*) and Lettuce (*Lactuca sativa* L.) in Small Holding Peri-Urban Farms in the City of Accra, Ghana

Ebenezer Owusu and Seshi Dotse
Department of Botany, University of Ghana, Legon-Accra, Ghava

Abstract: Using the Plate Count Technique, fungal contaminants of leaves of cabbage (*Brassica oleracea* L. var *Capitata*) and lettuce (*Lactuca sativa* L.) obtained from farms at three localities, Dzorwulu, East Legon and Haatso in Accra, Ghana were investigated under laboratory conditions. The surfaces of the leaves were washed with 100 mL sterilized distilled water containing 0.1% peptone as diluent. Aliquots (1 mL) of samples were plated on DRBC, OGY, PDA and SGA media and incubated at 30°C. Seven fungal species belonging to five genera (Aspergillus, Cladosporium, Mucor, Penicillium and Rhizopus) were resident on the cabbage leaves obtained from Dzorwulu; six fungal species belonging to four genera (Aspergillus, Mucor, Penicillium and Rhizopus) were isolated from samples from the farm at East Legon while six fungal species belonging to the genera Aspergillus, Mucor, Penicillium and Rhizopus were encountered on cabbage samples obtained from the farm at Haatso. On the leaves of lettuce nine fungal species belonging to five genera (Aspergillus, Mucor, Penicillium, Rhizopus and Rhodoctila) were isolated from samples obtained from the farm at Dzorwulu while five species belonging to three genera (Aspergillus, Mucor and Rhodoctila) were associated with lettuce leaves obtained from the farm at East Legon. The mycoflora of the leaves of lettuce from the farm at Haatso showed 7 species belonging to the genera Aspergillus, Mucor and Penicillium. The study concluded that fungal species were resident on the leaves of cabbage and lettuce obtained from the three farms with toxigenic *Aspergillus flavus* and *Aspergillus niger* being the most predominant species. Since, the two vegetables are mostly eaten raw, there is the need to educate consumers to wash them severally and thoroughly before consumption.

Key words: Fungal contaminants, fungal species, mycoflora, cabbage, lettuce, Ghava

INTRODUCTION

Vegetables have important nutritional, health and economic value (Chrispeels and Sadava, 1977). Recently, the market demand for minimally processed vegetables has increased because of the busy lifestyles of urban dwellers, increasing purchasing power and the increasing health conscious of consumers (Leistner and Gorris, 1995). The cultivation of vegetables in urban areas has also increased tremendously because of their high demand and the fact that it serves as a source of livelihood for unemployed urban migrants due to the relatively high returns gained from vegetable farming. Unfortunately, the production of vegetables is associated with many challenges such as post harvest losses, contamination of farm produce with pathogens and health problems associated with the consumption of vegetables which are contaminated with toxic substances. Although, the original source of contamination of vegetables is not definitely known, manure from farm animals, fungal spores from the air, water and soil on farms and human handling have been regarded as primary sources of contamination (Beuchat, 1996; Doyle, 2000a, b). Cabbage and Lettuce are among the most popular vegetables and important components of various organic food products grown and consumed in the urban and peri-urban areas of Ghana. Notably, the cultivation of these vegetables has increased tremendously because of the high demand by the ever increasing urban population and the fact that it serves as source of livelihood for some urban and peri-urban migrants.

The marketing and pricing of these vegetables are determined by the seasonality of demand. The demand in Accra peaks around Christmas and extends throughout the dry season (November-December). At that time of the year, the farmers usually irrigate their farms with polluted water likely to contaminate the vegetables with chemicals and microorganisms, compounding the inevitable contamination by air-borne fungi (Horna *et al*., 2006). Some of these microorganisms develop intensively on these vegetables making them unfit fir consumption. These include alternaria (carrots, cabbages, cauliflowers, peppers and tomatoes) and Fusarium (carrots) (Bullcarelli and Brackett, 1991). The level of airospora in

Corresponding Author: Ebenezer Owusu, Department of Botany, University of Ghana, Legon-Accra, Ghava

these areas recorded by Teye (1994) was almost as high in the dry season as in the rainy season. Indeed, Teye (1994) recorded high frequencies of phylloplane fungi of okra (*Abelmoschus esculentus* L.) pepper (*Capsicum annum* L.) and tomato (*Lycopersicum esculentum* Mill.) and there was a direct relationship qualitatively between the airflora and fungi detected on the surfaces of fruits and leaves of these vegetables. However, the levels of percentage occurrence of particular species on the different plants were not the same.

The propagules of these fungi float passively in the atmosphere and their dispersal may be achieved by the physical mechanisms of gravity, wind, water and animals (Kasprzyk, 2008). Dispersal of fungi as with plants is an important reproductive function in order to maintain the species, extend the existing habitat range and also, the spread genetic variability when it occurs throughout the population.

Also, many fungi have active mechanisms to effect the release of their spores which are then dispersed by these external agencies (Gover, 2001). Spores deposited on the plant act as propagules of disease if they attain the inoculum potential that is the energy growth available for infection of a host at the surface of the host organ to be infected (Garrett, 1960). Equally damaging to human health are the noxious metabolites of the spores that do not invade the internal tissues but the mycelial growth is on the surface of the plant. The ability of such fungal propagules to adapt to changing environmental conditions enables them to contaminate these vegetables and cause spoilage.

In order to achieve adequate nutrition, clean environment are basic factors predetermining the quality of human health. Healthy food and vegetables ought to be biologically valuable, free of chemical pollutants and microbiological contamination. A safe organic food supply requires the development of detection methods that can detect and identify microflora and mycotoxin risks in the production chain and determine critical points of harvesting storing or processing. This study was carried out to identify the mycoflora of cabbage (*Brassica oleracea* L. var *Capitata*) and lettuce (*Lactuca sativa* L.) leaves, determine the percentage occurrence of the individual fungal species and to compare the mycoflora in three small holding urban farms in Accra that might suggest the state of the health of cabbage and lettuce grown in Accra.

MATERIALS AND METHODS

Study site: The farms were located in three localities, Dzorwulu, East Legon and Haatso representing the varieties at an approximately 2 km sided equilateral triangle.

Cabbage and lettuce leaves: The four oldest leaves were collected from cabbage and lettuce plants of the same age from each of the three farms on the same day. The leaves were kept in previously sterilized desiccators and transported to the laboratory. In each case, 30 g samples were used.

Isolation of fungi: The initial mycoflora of cabbage and lettuce samples collected from the three farms was determined by aseptically transferring 30 g of the leaves of cabbage or lettuce in 250 mL Erlenmeyer flasks containing 100 mL of sterilised distilled water with 0.1% peptone water as diluents. Each flask was shaken at 140 rev^{-1} for 20 min in an orbital shaker (Gallenkamp, England). Decimal serial dilutions of 10^{-1} to 10^{-3} were prepared and 1 mL aliquots plated on 20 mL Oxytetracycline-glucose-yeast extract (OGY) medium, Dichloran Rose Bengal Chloramphenicol Agar (DRBC Oxoid 727), Sabouraud Dextrose Agar (SGA) and PDA for the growth and isolation of fungi. The various media were prepared as specified in the manufacturer's manuals where necessary, streptomycin sulphate (0.033 g L^{-1}) was added to prevent bacterial growth. The plates were then incubated at 30°C for 7 days. The pure isolates of fungal species encountered were sub-cultured on potato dextrose agar and incubated for 1 week at in an incubator at 30°C after which fungal isolates growing were identified and counted.

Morphological identification: Morphological identification and quantification of fungi encountered in these investigations were carried out with light microscope to directly observe fungal reproductive structures on the media. The fungi were removed with the aid of flame-sterilised needle and squash mounted on sterile distilled water or lactophenol cotton blue and examined with a LEICA DME microscope. The fungi were identified by their colour, culture and morphological characteristics using the conventional identification manuals (Barnett and Hunter, 1972; Founder, 1953; Ramirez, 1982; Smith, 1969; Samson, 2000).

RESULTS

The results obtained indicate that fungal species are resident on the leaves of both cabbage and lettuce obtained from the three farms (Fig. 1 and 2). Using the plate count method, a total of 10 fungal species belonging to 5 genera (Aspergillus, Cladosporium, Mucor, Penicillium and Rhizopus) were isolated from the leaves of cabbage (Table 1) whereas 13 fungal species belonging

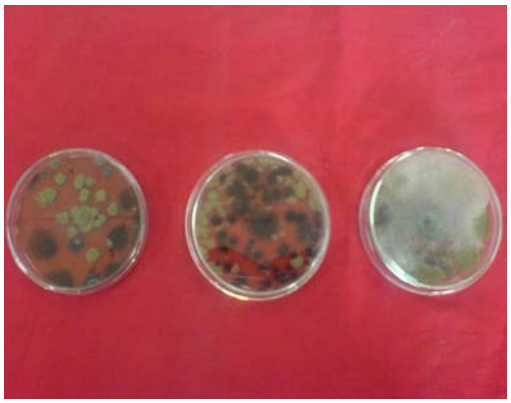

Fig. 1: Photograph showing some of the fungal colonies isolated from the leave of cabbage (×5/9)

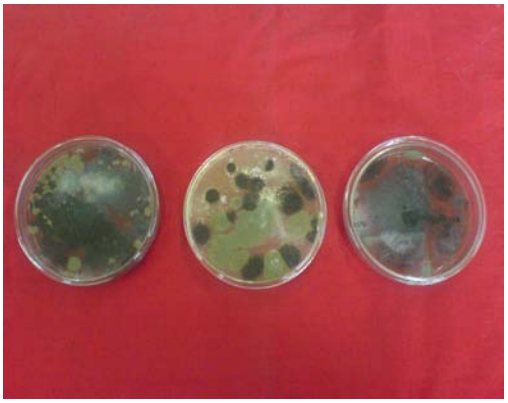

Fig. 2: Photograph showing some of the fungal colonies isolated from the leaves of lettuce (×5/9)

to 6 genera were obtained from the leaves of lettuce (Table 2). Out of the 10 fungal species obtained from the leaves of cabbage, Aspergillus was the most predominant genus and was represented by 5 species followed by Penicillium (2 species) while only one species of Cladosporium, Mucor and Rhizopus were isolated (Table 1).

The fungi isolated from the leaves of lettuce, Aspergillus was the most common genus and was represented by 7 species followed by Penicillium (2 species) while Mucor, Rhizopus, Rhodoctila and yeast were represented by one species each (Table 2). The fungi isolated form the leaves of lettuce, only 4 species viz., *Aspergillus oryzae*, *Aspergillus parasiticus*, *Rhodoctila* and yeast were not encountered on the leaves of cabbage (Table 2). It is worth noting that the genera composition of fungi isolated from vegetables from the three sampling sites did not differ much significantly. Whereas seven fungal species belonging to 5 genera (Aspergillus, Mucor, Cladosporium, Penicillium and Rhizopus) were

Table 1: Fungal species isolated from cabbage from three farms around legon

	Occurrence (%)		
Fungal species	Dzorwulu	East legon	Haatso
Aspergillus flavus Link	16.5	17.8	17.4
Aspergillus fumigatus Fres	5.4	-	-
Aspergillus niger Van Tieghem	30.6	42.9	41.3
Aspergillus ochraceus Wilhelm	-	-	9.8
Aspergillus sulphureus (Fres.) Thom and Church	-	5.8	-
Cladosporium cladosporioides (Fr.) de Vries	15.7	-	-
Mucor haemalis Wehmer	10.2	13.5	10.1
Penicillium citrinum Thom	-	-	12.2
Penicillium digitatum Sacc	12.4	12.5	9.2
Rhizopus oryzae Went and Prinsen geerlings	9.2	7.4	-

Table 2: List of fungal species isolated from lettuce obtained from three farms around legon

	Occurrence (%)		
Fungal species	Dzorwulu	East legon	Haatso
Aspergillus flavus Link and Pers.	26.4	12.5	25.4
Aspergillus fumigatus Fres	10.3	-	-
Aspergillus niger Van Tieghem	22.6	32.2	20.2
Aspergillus ochraceus Wilhelm	-	-	1.7
Aspergillus oryzae	-	22.1	15.4
Aspergillus parasiticus	-	-	17.8
Aspergillus sulphureus (Fres.) Thom and Church	12.9	-	-
Mucor haemalis Wehmer	-	28.5	-
Penicillium citrinum Thom	-	-	-
Penicillium digitatum Sacc	16.4	4.7	15.6
Rhodoctila sp.	9.1	-	
Rhizopus oryzae Went and Prinsen geerlings	2.3	-	-
Yeast	-	-	3.9

isolated from cabbage leaves obtained from the farm located at Dzorwulu, six fungal species belonging to 4 genera (Aspergillus, Mucor, Rhizopus and Penicillium) were isolated from cabbage leaves obtained from the farm at East Legon (Table 1). Fungal isolates identified at the farm in Haatso (around the E and S video complex) showed 6 species of 4 genera (Aspergillus, Mucor, Rhizopus and Penicillium). At all three farms, the most predominant fungal species were *Aspergillus flavus* and *Aspergillus niger*.

On the leaves of lettuce, seven fungal species belonging to 4 genera (Aspergillus, Mucor Penicillium, Rhizopus) were isolated from the samples obtained from the farm located at Dzorwulu (Table 2). Five species belonging to three genera (Rhodoctila, Aspergillus and Mucor) were associated with lettuce leaves obtained from the farm at East Legon around (Table 2). The mycoflora of the leaves of lettuce from the farm at Haatso showed 6 species of 3 genera (Aspergillus, Mucor and Penicillium) (Table 2).

DISCUSSION

The incidence of food borne diseases has increased despite the introduction of preventive quality systems and the promulgation of regulations in food safety. Fresh whole and minimally processed vegetables are no exception due to the increased number of reported outbreaks of food borne illnesses related to produce (Beuchat, 1996).

These problems come about because more emphasis are placed on pest and disease management by fresh food farmers rather than the control of fungal contaminants some of which may be mycotoxigenic. Crop monitoring is an important farming practice which is usually ignored by farmers (Dimsey *et al*., 2005). Although, the role of fungi in the deterioration of food is documented in many books, apart from knowledge of fungi which cause spoilage and disease, there is no proper documentation of the native mycoflora of vegetables such as lettuce and cabbage in Ghana. Even though, some studies (Beuchat, 1996; Farkas *et al*., 1997) on microbial load of vegetables such as cabbage and lettuce have been documented, the emphasis was placed on bacterial flora such as *Escherichia coli* and other coliforms.

Results presented showed that fungal species are resident on the leaves of cabbage and lettuce obtained from the farms at Dzorwulu East Legon and Haatso. Ten fungal species belonging to 5 genera were isolated from the leaves of cabbage while 13 fungal species belonging to 6 genera were obtained from the leaves of lettuce. Many of these fungal species were Aspergillus species with toxigenic potential.

Contamination of vegetables by fungal propagules depends on various factors including their growing technologies and climatic conditions during the vegetative period (Lugauskas *et al*., 2004). It is known that the most common mode of dispersal of fungal spores is by wind. The percentage occurrence of propagules of *A. niger* on cabbage leaves was highest (41.3%) on samples obtained from the farm at Haatso while that for leaves of lettuce (32.2%) was recorded for samples obtained from the farm at East Legon. *Aspergillus niger* had been recorded earlier as the most prevalent genus on cotton seed cake in Saudi Arabia (Al-Falih and Al-Juliafi, 2002) and cabbage in preservation and selling premises (Lugauskas and Stakeniene, 2002). Lugauskas *et al.* (2005) also isolated 18 species of fungi on newly harvested cabbages. Some of the fungal contaminants detected methodologically in this study (Aspergillus, Penicillium and Mucor) were also encountered by these investigators. Other fungal species such as *Cladosporium*, *Fusarium*, *Mucor*, *Penicillium*, Rhizopus and *Rhodoctila* isolated from the leaves of cabbage and lettuce could have contaminated these vegetables from other sources such as wind which is by far the most common method of dispersal of terrestrial fungi. Wind-borne spores finally come to rest by sedimentation, impaction or rain-wash. Also, the active discharge of spores in the Ascomycetes and Basidiomycetes provides a means for the spores to avoid local settlement in this boundary layer and to reach the potentially turbulent air layer above (Alexopoulos *et al*., 1996).

It must be emphasized that the presence of heavily sporulating fungi of the genus Aspergillus on the vegetables obtained from the farms should be a matter of concern since they are considered as the most potent mycotoxin (aflatoxin, ochratoxins, cyclopiazonic acid, glotoxin) producers (Krikstaponis *et al*., 2001). A substantial fraction of these metabolites may be accumulated in air-borne spores and when inhaled can provoke acute or chronic mycotoxicoses. The Fusarium fungi are also known to be producers of DON, T-2, fumonisin and zearalenone toxins. The genera Mucor and Rhizopus are usually regarded as allergenic and their capabilities to produce mycotoxins have not been profoundly investigated in contrast to those of the fungi of Penicillium, Aspergillus or Fusarium (Krikstaponis *et al*., 2001). It should also be emphasized that the chemical composition of many fungal metabolites has not been studied and the fact that mycotoxins have not been reported for a species does not mean that the species does not produce mycotoxins. Therefore, the ability of such fungi to synthesize secondary metabolites some of them possible mycotoxins should be investigated in future studies.

CONCLUSION

The findings of this study have demonstrated that the two vegetables, cabbage and lettuce obtained from the three farms in Accra Ghana are contaminated with various fungal propagules. If the direct and considered benefits inherent in the consumption of these two vegetables are to be realized better production and more efficient post harvest handling and storage practices that allow decreasing contamination of the vegetables will be needed.

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