Fungal dissemination by housefly (*Musca domestica L.*) and contamination of food commodities in rural areas of South Africa

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Abstract

Several insects that act as vectors, including houseflies (*Musca domestica* L.), are often considered to be an important source of fungal contamination in human foods. Houseflies are also involved in the transmission of bacterial pathogens that may pose a serious hazard to human health. Thus, the rural population of South Africa, as typified by that in the Gauteng Province investigated in this study, is at high risk from fungal exposure disseminated by houseflies and it is therefore important to assess the role of flies in contaminating various food commodities. Eighty four samples of houseflies (captured from households and pit toilets) were studied for their potential to carry fungal spores into food commodities. The fungi occurring in samples of raw maize (15) and porridge (19) were also assessed. Fungal isolates were identified based on morphological characteristics by conventional identification methods. Fifteen genera of fungi were isolated and identified, of which *Aspergillus*, *Fusarium*, *Penicillium*, *Cladosporium*, *Moniliella* and *Mucor* were the most prevalent in all three sample types analysed. The incidence rates of fungal contamination per total fungal count isolated in houseflies, maize and porridge were recorded with mean fungal load of 2×10^8 CFU/ml, 1×10^7 CFU/g and 2×10^7 CFU/g respectively. Additionally, *A. flavus*, *A. parasiticus*, *F. verticillioides*, *F. proliferatum*, *P. verrucosum*, *P. aurantiogriseum* and *M. suaveolens* were the most frequent fungal isolates in houseflies with incidence rate of 34%, 11%, 27%, 21%, 22%, 17% and 32% respectively. *F. verticillioides*, *A. flavus*, *A. niger* and *P. oslonii* were the most prevalent species contaminating porridge and maize with incidence rate of 23%, 32%, 16% and 28% in maize samples, while incidence rates of 59%, 15% and 29% were

recorded in porridge samples with the exception of *F. verticillioides*. The prevalence of these genera of fungi may pose serious health risks.

Keywords: *Musca domestica* L., fungi, isolation, food contamination.

1. Introduction

The common housefly, *Musca domestica* L. (Diptera: Muscidae), lives in close proximity to humans all over the world and has plagued humans since recorded history (Forster et al., 2007; Omalu et al. 2009). The housefly is a disturbing agricultural insect and is regarded as a public health hazard, predominantly in parts of the world where sanitary and hygienic conditions are poor. Furthermore, hygiene and sanitation are often relatively poor in rural areas especially in the developing countries (Junaid et al., 2014; Khan et al., 2013). Houseflies can serve as vectors and reservoirs for foodborne diseases (Barro et al., 2006; Khobdel et al., 2008). They are also widely recognized as potential factors for transmitting bacterial diseases such as cholera, shigellosis and salmonellosis (Barin et al., 2010; De Jesus et al., 2004). In addition to being mechanical vectors, houseflies feed on various kinds of food- stuffs, garbage and human excreta where they can pick up and transport pathogens. These pathogens can be harboured in the alimentary canal for several days, contaminating external body parts while feeding on food or during defecation and regurgitation. Thus they can be transferred to exposed food, food preparation surfaces and storage containers used for food for human consumption (Tilak et al., 2010; Vasan et al., 2008).

Although insecticides can effectively reduce *M. domestica* populations, serious side effects from these chemicals can result from residuals in food and the environment, which may be harmful to humans (Siriwattanarungsee et al., 2008).

The role of *M. domestica* in disseminating disease-causing organisms and food-borne human illnesses has led to increase in published research (Ahmad et al., 2007; Holt et al., 2007; Macovei et al., 2008). Wanaratana et al. (2011) revealed that *M. domestica* is also recognized as potential transmitter of bird flu virus, causing threats to humans worldwide. In addition, it is worth noting that the association of *M. domestica* and spoilage fungi has been verified by several authors (Srivoramas et al., 2012; Zarrin et al., 2007). Dissemination of spoilage fungi by *M. domestica* on exposed human foods and feeds may result in contamination by mycotoxin producing fungi including *Aspergillus, Fusarium* and *Penicillium*. Therefore, contamination by these fungi may not only reduce food quality but also lead to production of mycotoxins (Sultan and Magan, 2010). Mycotoxins have attracted worldwide attention due to their common occurrence and their hazardous impact on human health (Wagacha and Muthomi, 2008). Important mycotoxins are aflatoxins, fumonisins, ochratoxins, deoxynivalenol, trichothecenes, citrinin, patulin, zearalenone and T-2 toxin (Pitt and Hocking, 2009; Wild and Gong, 2010). In some severe cases, exposure to mycotoxin contaminated food and feed may lead to acute and chronic consequences such as carcinogenic, teratogenic, mutagenic and immunosuppressive effects (Binder et al., 2007). Some of the common symptoms of mycotoxicosis in humans include diarrhoea, vomiting, and gastrointestinal problems (Bhat et al., 2010).

Houseflies are capable of disseminating fungi, so this study was designed with an aim to isolate and identify filamentous fungi that are picked up by houseflies and carried over to human food. Consequently populations in rural farming communities in developing countries, particularly in South Africa, are at risk of fungal contamination of food, disseminated by houseflies. Finding ways to control the population of houseflies and to improve the general health of the rural population is of concern.

2. Material and methods

2.1. Materials

2.1.1. Fly traps

Fly traps with a propylene material (19 cm in diameter and 25 cm height) were purchased from Marco Plastics, South Africa.

2.1.2. Ringer's tablets and antibiotics

Ringer's tablets were purchased from Merck, Germany, and Streptomycin and Chloramphenicol from Sigma-Aldrich.

2.1.3. Fungal isolation culture media and identification

Potato Dextrose Agar (PDA) HGOOC100.500, Ohio Agricultural Experimental Station Agar (OAESA), Czapek Yeast Agar (CYA), Malt Extract Agar (MEA) 1038434, were used for fungal isolation and cultivation. Lactophenol blue solution, (Merck, Germany) and light microscope BX51 model, Ultra 20 soft imaging system (Olympus, Japan) were used to identify fungi.

2.2. Experimental methods

2.2.1. Sampling and sample preparation

Fly traps were placed in different households and pit toilets among the rural population in the Gauteng Province of South Africa. The fly traps were installed according to the method described in Phoku et al. (2012). Houseflies and food (raw maize and porridge) samples were also collected. The maize and porridge samples were placed in sealed zip-lock bags. Due to the distance between the sampling area and the laboratory all samples were put in sealed plastic bags placed in cooler boxes, and taken to the Food, Environment and Health Research Group (FEHRG), University of Johannesburg, South Africa, where they were stored at −4 °C until analysed. Under aseptic conditions the houseflies were separated in the laboratory according to their place of capture (households and pit toilets) and were transferred in sterile test tubes. Prior to analysis, maize samples were milled using a sterile laboratory mechanical blender (IKA M20, Merck, Darmstadt, Germany), whereas the porridge samples were freeze-dried and further crushed into powder using a sterile pestle and mortar.

2.2.2. Isolation of fungi and identification

In a laminar flow chamber, 9 ml of sterile Ringer's solution was transferred into each test tube containing fly samples and vortexed for 1–2 min to create a wash from each housefly for fungal isolation purposes. For maize and porridge samples, 1 g from each was weighed into sterile test

tube and diluted in 9 ml of sterile Ringer's solution. A serial dilution technique was then employed where by 1 ml of the solution was diluted in 9ml sterile Ringer's solution, vortexed and serially diluted down to 10−6. An aliquot of 1 ml of the dilutions from each tube was inoculated onto solidified PDA and OAESA media plates containing chloramphenicol and streptomycin to inhibit bacterial growth according to Srivoramas et al. (2012). These inoculated plates were incubated at a temperature of 25 °C for up to 7 days and then checked for fungal colonies. After incubation period, the colonies were counted, then single spore isolations from houseflies, maize and porridge were sub-cultured on PDA, CYA and MEA and then incubated for 7 days at 25 °C. The pure isolates were identified according to their macroscopic and microscopic characters following keys for fungal identification in Klich (2002), Pitt and Hocking (2009) and Samson and Varga (2007). The fungal isolates were stained with lactophenol blue for mounting between the slides and cover slides. An optical microscope was used to observe the micro-morphological characteristics for species identification.

2.3. Data analysis

Total mean colonies of fungi were calculated by dividing the total number of CFU by the number of plates from each household and results were expressed as CFU ml−1 and CFU g−1.

3. Results

Data on fungal contamination of houseflies, maize and porridge based on the incidence rates and fungal load are presented in Table 1. In total, 497 fungal isolates were identified from 84 samples of captured houseflies of which 72 samples were captured from different households and 12 captured from pit toilets. In addition to mycological analysis of houseflies, a total of 188 isolates belonging to the same species as those from houseflies were also isolated from maize (146) and porridge (42) (Table 1). Overall, mycological analysis of the external surfaces of houseflies showed a wide range of fungi. *Fusarium* species, *Penicillium* species and *Aspergillus* species were the most common fungi in maize, whereas Aspergillus species and *Penicillium* species were common in porridge (Table 1). Of the *Aspergillus* isolates from flies, 172 were from flies captured from the households, and 14 were from flies captured from the toilets (Table 2). There were 85 *Fusarium* isolates from houseflies captured in the households and pit toilets (Table 2).

F. verticillioides was the sole species of *Fusarium* isolated from houseflies captured from the toilets. Results in Table 2 show that *Penicillium* was also identified among the most prevalent fungi, and these were isolated from houseflies captured from households and pit toilets.

Interestingly, similar isolates belonging to the same species of *Aspergillus* isolated from houseflies namely, *A. carbonarius*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. parasiticus* were also isolated from maize and porridge samples. The genus Fusarium was also frequently isolated from maize, accounting for 66 of the 146 fungal isolates recovered, with *F. proliferatum* being the most dominant followed by *F. oxysporum* and *F. culmorum* (Table 2). *Penicillium* species accounted for 42 of the total 146 fungi isolated, with *P. oslonii* the most dominant in maize. In addition, *P. verrucosum* and *P. oslonii* were the most frequently isolated in 7 of the total 42 fungi isolated in porridge. This study also revealed the presence of other species of fungi including *Cladosporium herbarum*, *Moniliella suaveolens*, *Mucor plumbeus*, *Rhizopus microsporus* and *Scopulariopsis brevicaulis* in houseflies and maize, whereas from porridge only *C. herbarum*, *Candida krusei*, *M. suaveolens* and *M. plumbeus* were isolated (Table 3).

4. Discussion

This study was designed to investigate the role that *M. domestica* play in the dissemination of fungal spores, as there is inadequate data on the incidence of fungal species disseminated by *M. domestica* and the dangers of contamination of food commodities and consequent effects on the health of the rural community. To meet the objective houseflies, maize and porridge samples were collected from the rural areas of Gauteng Province, South Africa. Mycotoxin producing fungi of importance in food commodities are *Aspergillus*, *Penicillium* and *Fusarium*, whereas *Moniliella* and *Cladosporium* are important spoilage species (Table 1). Interestingly, a correlation between the fungal species from houseflies, maize and porridge samples was observed with some samples yielding more than one fungal species. This confirmed the hypothesis that houseflies can be vectors for mycotoxigenic fungal spores which could be deposited on foods and feeds, which may then result in mycotoxin production with adverse public health impact. Human exposure to filamentous fungi in maize has been widely investigated, however there is little information about alternative sources of contamination. Thus, the study gives a new aspect on the hygienic importance of the *M. domestica* and their role in infections, and possibly how fungi isolated from the flies may contaminate foods and produce mycotoxins.

Interaction between *M. domestica* and fungi in stored food commodities could indirectly lead to increase in the production of fungal toxins, as *M. domestica* may spread the spores of toxigenic

moulds. Of great importance also is the possible distribution of harmful bacteria such as Salmonella, Streptococcus and Escherichia coli by grain infesting insects. Normally, freshly harvested maize, even before being milled into flour, is already contaminated with a range of insects and fungi from field and storage facilities. According to Sinha et al. (1998), a large amount of stored maize is contaminated by *M. domestica*, and they may be a key factor in the transmission of different types of fungi in stored maize. The presence of *M. domestica* in stored maize may lead to increased incidence of storage fungi particularly *Aspergillus*, *Fusarium*, *Penicillium* and *Cladosporium* species. Furthermore, the storage facilities may also provide favourable conditions for development of larvae (Allotey, 2011) which may explain why more *M. domestica* were captured in households than in pit toilets. Contaminated maize may be a route by which fungi are introduced into food during food production processes (Hageskal et al., 2006; Paterson et al., 2009). This may also explain the high incidence rate of fungal contamination in porridge samples.

5. Conclusions

This study revealed the similarity in the taxonomic composition of fungi in *M. domestica* and those of food commodities. The results revealed that none of the samples analysed were free of fungal contamination. The study has revealed the role in which *M. domestica* may play in spreading fungal spores in food commodities intended for human consumption. Guaranteeing human health and food safety in the rural communities thus requires control of transmission by houseflies of fungi, particularly mycotoxigenic species of *Aspergillus*, *Penicillium* and *Fusarium*.

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Table 1. Incidence rates of total fungi and fungal contamination per total fungal count isolated in houseflies, maize and porridge.

	Houseflies (n=84)			Maize (n=15)			Porridge (n=19)		
Isolated species	**No. of isolates**	**%**	**[a]CFU ml^{-1}**	**No. of isolates**	**%**	**[b]CFU g^{-1}**	**No. of isolates**	**%**	**CFU g^{-1}**
Aspergillus species	186	37	4 x 10^6	19	13	4 x 10^5	18	43	2 x 10^6
Fusarium species	85	17	8 x 10^6	66	45	2 x 10^6	-	-	
Penicillium species	106	21	4 x 10^6	42	29	2 x 10^6	7	17	5 x 10^6
Alternaria species	7	1.4	3 x 10^5	-	-	-	-	-	-
Chrysosporium species	8	2	10 x 10^6	-	-	-	-	-	-
Cladosporium species	1	0.2	2 x 10^4	7	5	2 x 10^6	4	10	8 x 10^5
Curvularia species	2	0.4	6 x 10^4	-	-	-	-	-	-
Epicoccum species	6	1	3 x 10^6	-	-	-	-	-	-
Eupenicillium species	6	1	5 x 10^6	-	-	-	-	-	-
Moniliella species	43	9	5 x 10^6	4	3	1 x 10^6	7	17	2 x 10^6
Mucor species	11	2	5 x 10^7	2	1	2 x 10^6	5	12	4 x 10^6
Nigrospora species	7	1	5 x 10^6	-	-	-	-	-	-
Rhizopus species	8	2	2 x 10^6	3	2	2 x 10^6	-	-	-
Scopulariopsis species	8	2	5 x 10^7	3	2	9 x 10^5	-	-	-
Yeasts species	13	3	6 x 10^6	-	-	-	1	2	2 x 10^6
Total	**497**	**100**	**2 x 10^8**	**146**	**100**	**1 x 10^7**	**42**	**100**	**2 x 10^7**

[a]CFU ml^{-1}: Colony forming unit per ml of sample; [b]CFU g^{-1}= Colony forming unit per gram of sample; n = number.

Table 2. Incidence rates of fungal contamination with *Aspergillus*, *Fusarium*, *Penicillium* and other species.

	Houseflies (n=84)							
	Households (n=72)		Pit toilets (n=12)		Maize (n=15)		Porridge (n=19)	
Isolated species	**No. of samples**	**(%)**	**No. of samples**	**(%)**	**No. of samples**	**(%)**	**No. of samples**	**(%)**
***Aspergillus* species**								
A. candidus	1	1	-	-	-	-	-	-
A. carbonarius	16	8	1	1	4	21	1	4
A. clavatus	5	3	-	-	-	-	-	-
A. flavus	63	34	5	3	6	32	7	59
A. fumigatus	11	6	1	1	-	-	-	-
A. niger	25	13	4	2	3	16	4	15
A. ochraceus	15	8	2	1	1	5	1	4
A. oryzae	2	1	1	1	-	-	-	-
A. parasiticus	21	11	-	-	5	26	5	19
A. ustus	10	5	-	-	-	-	-	-
A. wentii	3	2	-	-	-	-	-	-
Total	**172**	**92**	**14**	**8**	**19**	**100**	**18**	**100**
***Fusarium* species**								
F. avenaceum	4	5	-	-	4	6	-	-
F. culmorum	9	11	-	-	10	15	-	-
F. equiseti	2	2	-	-	-	-	-	-
F. graminearum	3	3	-	-	7	10.5	-	-
F. nivale	2	2	-	-	6	9	-	-
F. oxysporum	12	14	-	-	11	17	-	-
F. poae	5	6	-	-	-	-	-	-
F. proliferatum	18	21	-	-	7	10.5	-	-
F. semitectum	3	4	-	-	3	4.5	-	-
F. sporotrichioides	3	4	-	-	3	4.5	-	-
F. verticillioides	23	27	1	1	15	23	-	-
Total	**84**	**99**	**1**	**1**	**66**	**100**	**-**	**-**
***Penicillium* species**								
P. aurantiogriseum	18	17	4	4	5	12	1	14
P. brevicompactum	16	15	1	1	3	7	-	
P. citrinum	5	5	-	-	7	17	1	14
P. crustosum	11	10	1	1	1	2	-	-
P. expansum	1	1	-	-	5	12	-	-
P. janthinellum	14	13	1	1	4	10	1	14
P. oslonii	10	10	-	-	12	28	2	29
P. sclerotiorum	1	1	-	-	-	-	-	-
P. verrucosum	23	22	-	-	5	12	2	29
Total	**99**	**94**	**7**	**7**	**42**	**100**	**7**	**100**

Note: Total number of isolates in houseflies: *Aspergillus* species = 186; *Fusarium* species = 85 and *Penicillium* species = 106. Total number of isolates in maize: *Aspergillus* species = 19; *Fusarium* species = 66 and *Penicillium* species = 42. Total number of isolates in porridge: *Aspergillus* species = 18; *Penicillium* species = 7.

Table 3. Incidence rates of fungal contamination with other species.

	Houseflies (n=84)							
	Households (n=72)		Pit toilets (n=12)		Maize (n=15)		Porridge (n=19)	
Isolated species	**No. of samples**	**(%)**	**No. of samples**	**(%)**	**No. of samples**	**(%)**	**No. of samples**	**(%)**
Other species								
Alternaria infectoria	7	6	-	-	-	-	-	-
Chrysosporium fornicola	6	6	-	-	-	-	-	-
Chrysosporium inops	-	-	1	0.8	-	-	-	-
Cladosporium herbarum	1	0.8	-	-	7	37	4	24
Curvularia lunata	2	2	-	-	-	-	-	-
Epicoccum nigrum	5	4	1	0.8	-	-	-	-
Eupenicillium javinicium	4	3	2	2	-	-	-	-
Moniliella suaveolens	39	32.4	4	3	4	21	7	41
Mucor circinellioides	2	1.6	-	-	-	-	-	-
Mucor piriformis	1	0.8	-	-	-	-	-	-
Mucor plumbeus	6	5	-	-	2	11	5	29
Mucor racemosus	2	2	-	-	-	-	-	-
Nigrospora oryzae	7	6	-	-	-	-	-	-
Rhizopus microsporus	6	4.8	-	-	3	15.5	-	-
Rhizopus oligosporus	1	0.8	-	-	-	-	-	-
Rhizopus stolonifer	1	0.8	-	-	-	-	-	-
Scopulariopsis brevicaulis	7	6	1	0.8	3	15.5	-	-
Candida krusei	11	9	1	0.8	-	-	1	6
Candida paralopsis	2	1.6	-	-	-	-	-	-
Total	**110**	**92.6**	**10**	**8.2**	**19**	**100**	**17**	**100**

Note: Total number of other species in houseflies = 120; maize = 19 and porridge = 17; n = number.

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