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# Studies on Fungal Contamination of Current Egyptian Paper Banknotes

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**Abstract:** Survey of the currency bills in Egypt for fungal contaminations revealed the ability of the paper money to be infected. This study was on some banknotes denomination obtained from different occupational groups in Cairo, Egypt. The currencies were processed for fungal isolation using potato dextrose agar medium. The streaking technique was employed for the isolation and purification of the colonies. A total of 102 fungal isolates was recovered during the study, these were assigned to nine species. Identification and characterization revealed active participation of the following genera and species of organisms arranging in deascending order of percentage as *Aspergillus flavus* (27.45%), *A. niger* and *Penicillium* spp. (17.64%), *Alternaria tenuis* and *Trichoderma* spp. (10.78%), *Fusarium* spp. and *Trichoderma viride* (5.88%), *A. paraziticus* (2.94%) and *Sporotrichum* spp. (0.98%). Data also showed that only 7 out of 72 fungal isolate having a high ability to decompose CMC in Czapek's agar medium. Results showed that benlate fungicide was very effective against the three tested fungi followed in descending order by thymol, clove, cider and rosemary oils. Aflatoxin $B_1$ was produced by 7.14% of the *A. flavus* isolates with a mean of 7-30 ppm, while 33.33 % of the *A. paraziticus* isolates were able to produce aflatoxin $B_1$ with a mean of 8 ppm. On the other hand, data showed that only 2 out of 18 isolates of *Penicillium* spp. were able to produce ochratoxin A in amount ranging from 14-23 ppm.

**Key Words:** Fungi · Banknotes · Contamination · Cellulosic paper · Fungicide · Essential oil

## INTRODUCTION

Paper currency is widely exchanged for goods and services in most countries worldwide [1, 2]. Contamination from the anal region, wounds, droplets during coughing, sneezing, touching with hand placement on dirty surface are potential sources of transfer of microorganisms to currency notes during handling [3, 4]. Thus, it becomes obvious that anything that gets on hands get on money. Microorganisms on the skin can be transferred from cashiers, salespeople & the general public to currency notes that they handle [4]. There had been fewer studies on the microbial contamination of currency worldwide [5-7]. Kawo *et al.* [8] isolated genera of bacteria include, *Bacillus, Brucillus, Clostridium, Corynebacterium, Listeria and Micrococcus*, while fungi include *Aspergillus, Fusarium, Mucor, Penicillium and Rhizopus* from Nigerian abused naira denominations. A similar study in Egyptian reports that 65% of the bills had bacteria 5.0 $cm^2$, like *Staphylococcus* and *Klebsiella* [7]. Dirty money is not only confined to developing nations. Separate studies from the USA reported contamination of coins and paper bills [5]. Another survey isolated a total of 93 different types of microorganisms [6]. Oyero and Emikpe [1] reported that paper Nigerian currency is commonly contaminated with bacteria and fungi and this contamination may play a role in the transmission of antibiotic resultant or potentially harmful organisms. Bosch and Steyn [9] showed that 90% of South African banknotes were contaminated with either bacteria or fungi. The corresponding figure reported for Nigerian currency is 83% [10]. In Saudi Arabia, Alwakeel and Nasser [11] reported that of 390 currency notes, 282 (72.3%) were contaminated with fungi which include *Aspergillus niger, A. flavus, Candida* spp. *Penicillium* spp. and *Rhizopus* spp. and bacteria which included *Enterobacter, Klebsiella, Cedecea, Yersinia, Acinetobacte* and *Staphylococcus*. Feglo and Nkansah [12] revealed that 98.6% of the Ghanaian currency notes were bacterially contaminated, 12 (17.14%) had acid fast bacilli and 1.43% *Taenia* sp.

The objective of this study was to determining the types and population of the fungi on the Egyptian banknotes in circulation of Cairo province. So, as to determine, whether or not the paper banknotes constitute a potential source of mycotoxigenic producing fungi.

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Fig. 1: Deteriorated Egyptian pound showed different appearance of fungal infection

## MATERIALS AND METHODS

**Sample Collection:** A total of 60 pieces of Egyptian paper banknotes (20 pieces of each of the denominations of 25, 50 and 100 pt) were randomly collected from different locations in Cairo. Currency notes were obtained from supermarkets, bus conductors, taxi drivers traders and food sellers. Samples were obtained from different locations in Cairo using sterile gloves. Each currency was kept in separate sterile filter paper and transferred to the laboratory for analysis.

**Culturing, Isolation and Identification:** Currencies of the available denominations were processed for fungal isolation using potato dextrose agar (PDA). The conventional methods of swabbing and streaking were used [5]. The surface of the chosen bills was wiped under aseptic conditions by a sterile wet cotton swab (Fig. 1). The surfaces of PDA plates were streaked by the cotton swabs. The Petri dishes were incubated at 28±2°C for 7 days. During the incubation period any emerged fungus was isolated on PDA slants and purified by using the single spore technique of Manandhar *et al.* [13]. The obtained isolates were identified using the microscopic and cultural characteristic according to Gilman [14], Booth [15], Nelson *et al.* [16] and Barnett and Hunter [17]. The frequency occurrence of each fungal isolate was expressed as the percentage of sample a given organism.

**Selection of Cellulose Degrading Microorganisms:** For the screening of the cellulolytic activities, cultures of the isolated fungi grown on PDA plates (plus 5% avicel) were inoculated to 250 ml flasks each containing 50 ml sterilized Mandels and Weber liquid medium [18]. It contained g/l): 1.4 $(NH_4)_2SO_4$, 0.3 urea, 0.3 Ca $Cl_2$, 0.3 $MgSO_4$. 7 $H_2O$, 0.005 $Fe_2(SO_4)_3$, 0.0016 Mn $SO_4$, 0.002 $COCl_2$, avicel (1%), protease peptone (1%) and tween 80 (0.1ml) with final pH of 5.0. The flasks were incubated on a rotary shaker (180 rpm) at 28±2°C for 15 days.

**Effect of Cellulolosic Substrates on Fungal Radial Growth:** The effect of three different substrates including CMC, normal paper and paper bulp as carbon source on the radial growth of some isolates which gave high capability were studied using agar plates. A disc (5mm-dm) for pure cultures of tested fungi were inoculated individually in the centre of a Czapek's plate (without sugar) containing CMC, cellulosic paper and bulp paper at 1%. Plates were incubated at 26±2 °C and the diameters of the developing colonies were recorded in mm over a period of 10 days. Spore production also determined using the following scale, - = No spore produced, + = few, ++= moderate, +++ = high and ++++ = dense.

**Inhibitory Effect of Fungicides and Essential Oils on Fungal Growth:** Benlate fungicide (Methyl-1- (butyl carbamyl)- 2- benzimidazole carbamate, 50%) was used at concentration of 0, 3.12, 6.25 and 12.5 ppm based on the active ingredient. Thymol (Isopropyl-metacresol $(CH_3)$ $CHC_6H3$ $(CH_3)$ OH was also used at 0, 25, 50, 100 and 200ppm. The essential oils (cider, clove and rosemary) were applied in a set of 5 concentrations (0, 200, 400, 800 and 1600 ppm).The fungicides were added to PDA medium after sterilization, while still warm. Plates were inoculated with a disc from pure cultures of chosen fungi, incubated at 28±2°C and the colony diameter was recorded as mentioned before.

**Mycotoxins Assay:** Culture medium (Sterile rice, 50g, water 50 ml) in 500 ml Ehlenmeyer flasks with cotton plugs were inoculated with spore suspension of *Aspergillus flavus* and *Penicillium* spp. isolates. Cultures were incubated for 21 days at 28±2°C. After biosynthesis each culture was blended with chloroform and filtered through filter paper Whatman No.4. Chloroform layer was filtered through anhydrous $Na_2SO_4$, then concentrated under steam $N_2$ as described by El-Sayed *et al.* [19]. The mycotoxins (ochratoxin A and aflatoxin $B_1$) were determined quantitatively by TLC method as described by AOAC [20].

## RESULTS AND DISCUSSION

**Survey of Fungal Isolates:** Table 1 comprises the dominant fungal species associated with various banknote bills. Results showed that without exception, all the screened money bills were contaminated with fungi. Generally, the lower denomination (25pt and 50 pt) had the lower fungal counts, while the higher one (100pt) hade the higher microbial count (61.14%) and this expected in Egypt. The reasons for this might be that the higher denominations are probably more frequently exchanging hand than those of lower denomination and also this denomination of one pound was widely used and exchanged many times, especially amidst artisans and lower economic class. On the other hand, previous reports showed reverse results as lower denomination pass through more hands than higher one [3, 8, 21]. Several investigators reported that paper currencies recovered from meat sellers and food vendors had the highest percentage of contamination [1, 4]. This may be associated with the fact that paper money provided a large surface area as a breading ground for fungi. Currencies therefore, may play a role in the transmission of potentially harmful organisms [1].

Table 1 also shows that a total of 103 fungal isolates were recorded during the study, from the examined banknotes. The cultural and morphological properties of these isolates showed that they belonged to nine species and can arranged in descending order of percentage as: *Aspergillus flavus* (27.18%), *A. niger* (17.48%, *Penicillium* spp. (18.45%), *Alternaria tenuis* and *Trichoderma* spp. (10.68%), *Fusarium* spp. and *Trichoderma viride* (5.83%), *A. paraziticus* (2.91%) and *Sporotrichum* sp. (0.97%). The study revealed similar pattern of fungal contamination as those obtained by Oyero and Emikpe [1], Pope *et al.* [6] and El-Dars and Hassan [7]. Generally, *A. flavus* was the most isolated fungi {28 (27.18%)}, while *Sporotricum* sp. was the least {1 (0.97%)}. These fungi could have come in contact with the money through soil, clothing, food and/or hands of users. Some of these fungi are potential mycotoxin producer agents, i.e. *Aspergillus, Penicillium* and *Fusarium* spp. and could elaborate mycotoxins which are dangerous to human health [22].

**Selection of Cellulose Degrading Fungi:** Table 2 represents data on the cellulolytic activity of various representative fungal isolates. The degree of decomposition of CMC differed considerably between microorganisms. Data showed that only 7(9.72%) out of 72 fungal isolates having a high ability to decompose CMC in Czapek's agar medium. Other 9 (12.5%) and 27 (37.5%) isolates gave moderate and slight effect respectively, while 29(40.28%) isolates showed no growth. The most active of the seven fungal isolates comprised two from each of *Aspergillus flavus* and *Penicillium* spp. and one isolate from each of *Alternaria tenuis*, *Trichoderma viride* and *Fusarium* sp. These results are in agreement with that recorded by Sahaba [23], Caneva *et al.* [24] and Darwish and Sahab [25], they reported that the most active cellulolytic organisms belonged to fungal genera of *Aspergillus* and *Penicillium* spp.

**Influence of Cellulosic Substrates on Fungal Growth:** Table 3 shows data on the cellulolytic activity of various representative fungal isolates. The degree of decomposition of CMC, normal paper and paper bulp were differed considerably between isolates.

**Growth on CMC-Czapek's Agar Medium:** Data given in Table 3 shows that *Alternaria tenuis* (N0.1), *Penicillium* sp. (No.4) and *Trichoderma viride* (No.6) gave maximum linear growth and sporulation on solid CMC medium. The same result was also obtained by Darwish and Sahab [25], Domsch *et al.* [26] and Darwish [27], they reported that the above fungi are good cellulose decomposers.

**Growth on Cellulosic Paper and Bulp- Czapek's Agar Medium:** *Trichoderma viride* isolate (No. 6) showed a high ability to decompose normal paper in Czapek's agar medium, while isolates of *Penicillium* spp. (N0s 4 and 5) showed low ability to grow and sporulate (Table 3).

**Effect of Some Fungicides and Essential Oils on Growth of Some Fungal Isolates:** The experiment was undertaken to determine the effect of some fungicides and essential

Table 1: Relative occurrence and frequency of different isolates of fungi isolated from current banknotes in Cairo province, Egypt

| | Currency denomination and frequency | | | | |
|---|---|---|---|---|---|
| Isolated fungi | 25pt | 50pt | 100pt | Total isolates | Frequency % |
| *Alternaria tenuis* | 3 | 1 | 7 | 11 | 10.68 |
| *Aspergillus flavus* | 9 | 1 | 18 | 28 | 27.18 |
| *Aspergillus niger* | 3 | 1 | 14 | 18 | 17.48 |
| *Aspergillus paraziticus* | 1 | - | 2 | 3 | 2.91 |
| *Fusarium spp.* | 2 | 1 | 3 | 6 | 5.83 |
| *Penicillium spp.* | 3 | 2 | 14 | 19 | 18.45 |
| *Spororticum sp.* | - | - | 1 | 1 | 0.97 |
| *Trichoderma viride* | 4 | - | 2 | 6 | 5.83 |
| *Trichoderma spp.* | 5 | 3 | 3 | 11 | 10.68 |
| Total | 30(29.13%) | 9(8.73%) | 64(62.14%) | 103 | -- |

Table 2: Fungal species associated with deteriorated Egyptian banknotes and their capability in decomposing carboxy methyl cellulose (CMC).

| | | Growth on CMC- Czapek's medium | | | |
|---|---|---|---|---|---|
| Isolated fungi | No. of tested isolates | Not effective | Feeble effective | Moderate effective | Highly effective |
| *Alternaria tenuis* | 11 | 9 | 1 | - | 1 |
| *Aspergillus flavus* | 28 | 18 | 6 | 2 | 2 |
| *Aspergillus niger* | 18 | 10 | 6 | 2 | - |
| *Aspergillus paraziticus* | 3 | 1 | 2 | - | - |
| *Fusarium spp.* | 6 | 2 | 2 | 1 | 1 |
| *Penicillium spp.* | 18 | 10 | 6 | - | 2 |
| *Spororticum sp.* | 1 | 1 | - | - | - |
| *Trichoderma viride* | 6 | 3 | 1 | 1 | 1 |
| *Trichoderma spp.* | 11 | 5 | 3 | 3 | - |
| Total | 72 | 29(40.28%) | 27(37.5%) | 9(12.5%) | 7(9.72%) |

- = not determined

Table 3: Influence of different substrates on the linear growth and spore production of different fungal isolates associated with deteriorated Egyptian banknotes under static condition and incubated at 28±2°C and 15 days incubation period.

| | Growth on CMC medium | | Growth on normal paper | | Growth on bulp paper | |
|---|---|---|---|---|---|---|
| Fungal isolates and NO. | Diam. (mm).* | Sporulation** | Diam. (mm). | Sporulation | Diam. (mm). | Sporulation |
| *Alternaria. tenuis* No1 | 90.0 | +++ | 75.2 | ++ | 82.5 | +++ |
| *Aspergillus. flavus* No.2 | 81.66 | +++ | 60.0 | + | 45.6 | ++ |
| *Fus. solani* No.3 | 70.0 | ++ | 62.0 | + | 66.34 | ++ |
| *Penicillium* sp. No.4 | 90.0 | +++ | 22.3 | ++ | 23.0 | + |
| *Penicillium* sp. No.5 | 58.0 | ++ | 21.20 | + | 28.62 | + |
| *Trichoderma viride* No.6 | 90.0 | ++++ | 90.0 | ++++ | 90.0 | ++++ |

*Each figure represents average of 3 replicates incubated at 28±2°C for 10 days.

** Sporulation scale: + = week, ++ = moderate, +++ = heavy and ++++ = vigorous

oils as agar amendments on the linear growth of *Alternaria tenuis, Penicillium* sp. and *Trichoderma viride*. Data in Table 4 indicated that the antifungal activity of different preventative materials was increased as the concentration increased. The linear growth of the three tested fungi was completely inhibited at 12.5 ppm benlate, 200 ppm thymol and clove oil at 800 ppm when those chemicals were incorporated into Czapek's medium plates. In another trails, benlate fungicide completely inhibited the linear growth of *Penicillium* sp. and *Trichoderma viride* at 6.25 ppm, while the growth of *Alternaria tenuis* was completely inhibited at 1600 ppm cedar oil. Benlate and thymol have been reported to inhibit the growth of several cellulolytic fungi isolated from old documents [23, 28, 29]. Data also showed that benlate fungicide was very effective against the three tested fungi followed in descending order by thymol, clove, cider and rosemary oils. Many experiments have been carried out aiming to detect the inhibitive effect of some essential oils on the growth of fungi [30- 32]. The use of such essential oils for controlling fungal growth during banknote circulations will help to combat

Table 4: Effect of some fungicides and essential oils in Czapek's medium on the linear growth (mm) of some fungal isolates associated Egyptian banknotes after 7 days incubation at 28±2°C.

| | | Tested fungi | | |
|---|---|---|---|---|
| Fungicides and Essential oils | Concentration (ppm) | *Alternaria tenuis* | *Penicillium sp.* | *Trichoderma viride* |
| Control | 0.0 | 90.0 | 90.0 | 90.0 |
| Benlate | 3.12 | 37.5 | 15.7 | 20.8 |
| | 6.25 | 20.4 | 0.0 | 0.0 |
| | 12.5 | 0.0 | 0.0 | 0.0 |
| Thymol | 25.0 | 80.3 | 72.0 | 87.0 |
| | 50.0 | 68.3 | 71.3 | 80.0 |
| | 100.0 | 34.6 | 38.5 | 40.3 |
| | 200.0 | 0.0 | 0.0 | 0.0 |
| Cider oil | 200.0 | 82.6 | 63.4 | 72.8 |
| | 400.0 | 61.3 | 36.2 | 38.4 |
| | 800.0 | 43.2 | 18.6 | 17.8 |
| | 1600.0 | 0.0 | 14.6 | 15.8 |
| Clove oil | 200.0 | 27.8 | 28.6 | 32.4 |
| | 400.0 | 14.5 | 18.4 | 20.2 |
| | 800.0 | 0.0 | 0.0 | 0.0 |
| | 1600.0 | 0.0 | 0.0 | 0.0 |
| Rosemary oil | 200.0 | 73.2 | 83.4 | 89.3 |
| | 400.0 | 65.3 | 43.2 | 46.3 |
| | 800.0 | 53.2 | 39.3 | 28.7 |
| | 1600.0 | 32.4 | 20.2 | 18.7 |

*Each figure represents average of 3 replicates incubated at 28±2°C for 10 days

Table 5: Production of mycotoxins by *Aspergillus* and *Penicillium* spp. isolated from Egyptian banknotes on rice sterile medium after 21 days incubation at 28±2°C.

| | No. of isolates | | Amount of toxins (ppm) | |
|---|---|---|---|---|
| Tested fungi | Tested | Toxigenic | Ochratoxin A | Aflatoxin $B_1$ |
| *Aspergillus flavus* | 28 | 2(7.14%) | - | 7-30 |
| *Aspergillus niger* | 18 | - ( 0%) | - | - |
| *Aspergillus paraziticus* | 3 | 1 (33.3%) | - | 8 |
| *Penicillium spp.* | 18 | 2 (11.0%) | 14-23 | - |

*Average of duplicates for positive sample

- (-) = Not detected

fungicide resistant strains of deteriorated fungi and to avoid pesticides residues from the environment and commodities.

**Screening Trail for the Detection of Mycotoxigenic Fungi:** Accordingly, the toxicity of 67 fungal isolates of *A. flavus, A. niger, A. paraziticus* and *Penicillium* spp. isolated from banknote bills was studied (Table 5). Data clearly showed that the spectrum of identified toxins from the extracts of the tested fungi include aflatoxin $B_1$ and ochratoxin A.

Regarding aflatoxin $B_1$ production, 7.14% of the *A. flavus* isolates were found to be able to produce aflatoxin $B_1$ with a mean of 7-30 ppm, while 33.33 % of the *A. paraziticus* isolates were able to produce aflatoxin $B_1$ with a mean of 8 ppm. Various reports indicate that aflatoxins were produced by *A. flavus* and *A. paraziticus* [19, 33, 34]. *Aspergillus* and *Penicillium* spp. a group of fungi that live soil and are found in the environment could also be transferred on money paper due to its placement on dirty surface or handling with dirty hands. On the other hand, data showed that only 2 (11.0%) out of 18 isolates of *Penicillium* were able to produce ochratoxin A in amount ranging from 14- 23 ppm. These results are in agreement with that recorded by El-Sayed *et al.* [19], Northolt *et al.* [35], Scott *et al.* [36] and Haggblo [37]; they reported that *Aspergillus* and *Penicillium* groups were able to produce ochratoxin A. A much larger study would be required in future concerning the pollution of banknote paper in Egypt with fungi and bacteria. Also, there needs to further investigate on the antibiogram and pathogenicity of the obtained fungal isolates.

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