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RESEARCH ARTICLE

MYCODIVERSITY ASSOCIATED WITH LOWER DENOMINATION CURRENCY NOTES IN CIRCULATION IN JAMMU CITY, INDIA.

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Abstract

A survey of soiled and mint currency notes of lower denomination i.e., rupees five, ten, twenty and fifty was undertaken for the assessment of mycobial diversity associated with them. By using dilution plate technique and modified Sabouraud Dextrose Agar medium (SDA), 37 fungal species belonging to 15 genera were recovered from the sampled currency notes. Mint notes were found to be less contaminated (17 fungal species) as compared to the soiled notes (37 fungal species). Diversity indices computed for the fungal species recovered from the notes of lower denomination showed differences in values of Shannon-Wiener's diversity index (H') and Simpson Dominance index (Cd). These values varied with the denomination of notes and whether the notes were soiled or mint. Higher values of indices were obtained for soiled note samples in comparison to mint currency notes.

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Introduction

Trade has been a part of mankind from time immemorial and money is an indispensable part of it. We use money as a measuring unit in pricing a transaction, offer it as a medium for exchange of goods and services, settlement of debts, for deferred payments in economic activities and make it a store of value for our savings. Money can be segmented universally with regard to coins and paper currency. Paper currency is used repeatedly in exchange for goods and services (Oyero and Emikpe, 2007).

In day to day transactions, money is handled by persons of varying health and hygienic standards and also stored under varying environmental and personal hygienic conditions. An individual living in unhygienic conditions and having unhygienic habits will contaminate the notes e.g., keeping currency notes in socks, shoes and pockets, under the carpet or rugs and squeezing them in the hand frequently introduces an array of microbes to the notes. Moreover, in India, poor currency handling culture is widespread, and there is indiscriminate abuse of currency notes. For instance, attitudes such as the wetting of hands or fingers with saliva or use of contaminated water to lubricate the hand in counting money and use of food contaminated fingers in handling currency notes may not only enhance the contamination of currency notes but may also increase the risk of infection from contaminated ones. In addition, microorganisms on the skin can be transferred from cashiers, salespeople and the general public to the currency notes that they handle. Paper currency can also be contaminated by droplets during coughing, sneezing, touching with previously contaminated hands or other materials and by their placement on dirty surfaces. Contamination from the anal region, wounds and nasal secretions are also potential sources of transfer of microorganisms to currency notes during handling (Igumbor et al., 2007). Source of contamination could also be due to poor or negative money handling practices like spraying during ceremonies where such notes may be trampled upon when they fall on the ground (Ogo et al., 2004). These notes pass through many hands, during which pathogens become imposed on them and thus act as vehicles delivering microbes to contaminate the hands of the next user. Further, storage of these notes in polythene, cotton or leather bags in humid and dark conditions also favours the growth of fungal and bacterial organisms on them. Currency notes of lower denomination receive the most rough handling as they are circulated among people from various occupations and walks of life, like beggars, street food vendors, shoe-shiners, school children, butchers, etc. Therefore, there are chances of higher levels of

microbial contamination on lower denomination notes. The older the paper note, the more accumulation of microbes may occur (Ghamdi et al., 2011).

Studies on the contamination of money with microbial agents is lacking in most of the developing countries. Shortage of information may contribute to the absence of public health policies regarding currency usage, handling and circulation (Ghamdi et al., 2011). In India, very few studies have been conducted on the the mycobial colonization of currency notes (Wanule et al., 2011 and Abirami et al., 2012). The situation is further compounded by the inability of the Indian government to consistently withdraw old, worn-out and mutilated currency notes from the circulation as these could elevate their contributory role in transmission of some pathogens, thereby constituting potential public health hazard. In view of this, present study was undertaken to screen the lower denomination currency notes circulating in Jammu city for fungal contamination.

MATERIALS AND METHODS:

(i) Collection of currency notes

Mint currency notes of lower denominations i.e., rupees five, ten, twenty and fifty were collected aseptically from the bank and soiled currency notes of same denomination were collected from various occupational groups like traders, beggars, butchers, food vendors and shoe shiners . The collected samples were brought to the laboratory in pre-sterilized polythene bags for assessing the mycobial diversity associated with them.

(ii) Isolation of mycoflora

For the isolation of mycoflora, dilution pour plate method was used. According to the technique, the sample (currency note) weighing approximately 0.5g was transferred aseptically to 49.5 ml of sterile water in a 100 ml conical flask and stirred on a rotary shaker at 180 rpm for 30 minutes at room temperature. Then 1 ml of the final diluted suspension was poured in each sterilized Petri plate and plated with 15-20 ml of modified Sabouraud Dextrose Agar medium (SDA) supplemented with Rose Bengal (0.2g/1000 ml) and streptomycin sulphate (50mg/1000ml).The Petri plate was then swirled to mix the inoculum properly. Five replicates were prepared and incubated for 7 days at $28^{o} \pm 2^{o}C$ till proper growth of the fungal colonies was obtained.

(iii) Identification of fungal species

The recovered fungal species were identified by studying their macro and micro-morphological characters. For the purpose of identification, the isolated fungal species were grown and made to sporulate on different culture media, such as potato dextrose agar medium (PDA), malt extract agar medium (MEA), Czapek yeast agar medium (CYA), potato carrot agar medium (PCA) and water agar medium (WA). Identification of fungal species was done by using various keys and relevant literature given by (Brown and Smith (1957), Ames (1961), Raper and Fennel (1965), Tandon (1968), Rifai (1969), Booth (1971), Ellis (1971), Pitt (1979) and Onions et al. (1981)

(iv) Calculations

Percentage colonization frequency (CF %) and cfu were calculated for each fungal species. Colony forming units (cfu /g) were calculated by following Parikh and Shah (2006). The data was also used in making comparison of different diversity indices i.e., Shannon-Wiener index (H´) was used for the calculation of species richness (Shannon and Wiener, 1963) and Simpson's Dominance index (Cd) was calculated to determine the concentration and homogeneity/heterogeneity of fungal species among the note sample (Simpson, 1949). These calculations were made by using formulae given in table 1.

RESULTS:

During the period of study, soiled and mint notes of lower denomination (rupees 5, 10, 20 and 50) were assessed for the associated mycobial diversity. A total of 37 fungal species belonging to 15 genera were recovered from the sampled currency notes (Table 2). Mint notes were found to be less contaminated as compared to the soiled notes. In total, 37 fungal species were recovered from the soiled note samples, whereas only 17 species were recovered from the mint notes. Mycodiversity recovered from the contaminated currency notes included members of Zygomycotina, Ascomycotina and mitosporic fungi. Zygomycotina was represented by one species each of *Absidia (A. corymbifera), Mucor (M. hiemalis), Rhizopus (R. oryzae)* and *Syncephalastrum (S. racemosum),* which accounted for 10.8% of the recovered fungal species. Ascomycotina was represented by *Chaetomium globosum, C. indicum, Emericella nidulans var. echinulatus* and *Eurotium chevalieri,* which contributed 10.8% of the total recovered mycodiversity, whereas representation of mitosporic fungi was maximum, amounting to 78.4% of the recovered fungal species. The mitosporic fungi included eleven species of *Aspergillus (A. flavus, A. fumigatus, A. japonicus, A. nidulans, A. niger, A. ochraceous, A. parasiticus, A. sydowii, A. terreus, A.terricola* var. *americana and A. versicolor),* seven species of *Penicillium (P. brevicompactum, P. chrysogenum, P. citrinum, P.expansum P.*

griseofulvum, P. oxalicum, P. waksmanii), four species of *Fusarium (F. oxysporum, F. pallidoroseum, F. solani* and *F. verticilloides),* two species each of *Curvularia (C. lunata and C. pallescens)* and *Paecilomyces (P. fusisporus* and *P. liliacinus),* and one species each of *Cladosporium (C. cladosporioides), Trichoderma (T. koningii)* and *Alternaria (A. alternata).*

Table 1 Formulae used

$CF\ (\%) = \frac{\text{Number of note samples colonized by a specific fungi}}{\text{Total number of samples studied}} \times 100$
$cfu/g = \frac{a \times d}{s}$ Where,   a = average number of colonies on the petriplate   d = dilution factor (10,000)   s = dry weight of the sample (currency note)
Diversity indices : $\text{Shannon} - \text{Wiener index } (H') = -\sum_{i=1}^{s} p_i 1n\, p_i$ $\text{Simpson}'\text{s dominance index } (Cd) = -\sum_{i=1}^{s} (p_i)^2$ Where p_i is the relative importance value of species i.

Table 2 Colonization frequency (CF%) and colony forming units (cfu/g) of the recovered mycoflora

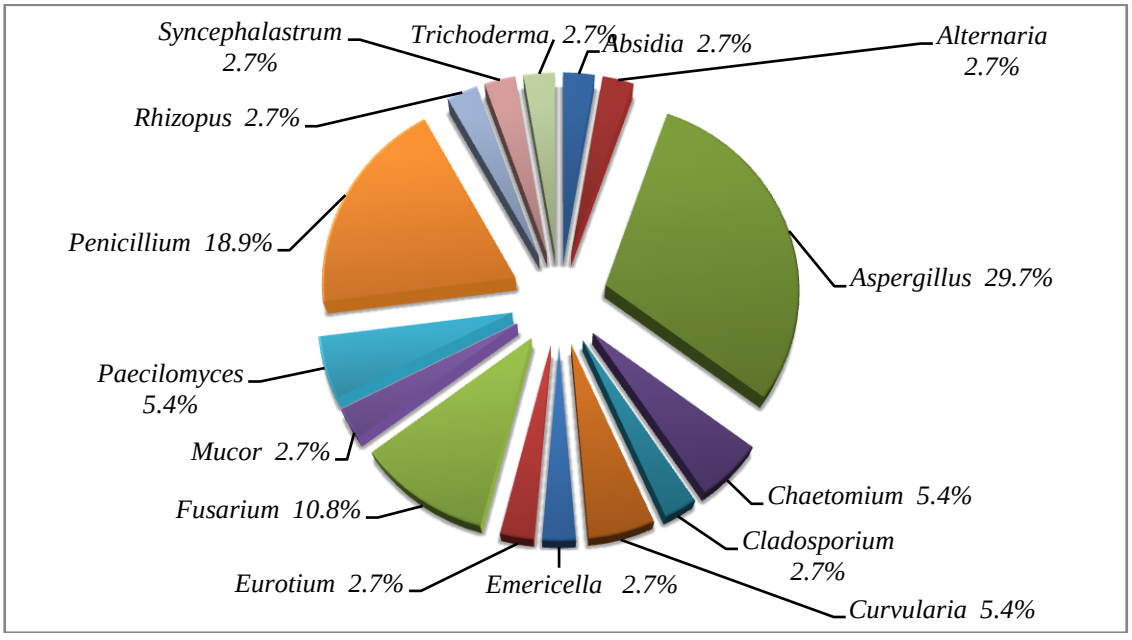
Fungal species recovered	Number of note samples analysed for each denomination=25															
	SOILED NOTE SAMPLES (Rupees)								MINT NOTE SAMPLES (Rupees)							
	5		10		20		50		5		10		20		50	
	CF(%)	Cfu/g	CF(%)	Cfu/g	CF(%)	Cfu/g	CF(%)	Cfu/g	CF(%)	Cfu/g	CF(%)	Cfu/g	CF(%)	Cfu/g	CF(%)	Cfu/g
Absidia corymbifera	–	–	12	1.6 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Alternaria alternata	20	2.8x10^3	20	3.2 x10^3	–	–	12	1.2 x10^3	–	–	–	–	–	–	–	–
Aspergillus flavus	20	2.8 x10^3	48	6.4 x10^3	12	1.6 x10^3	20	2.4 x10^3	8	0.8 x10^3	–	–	–	–	–	–
Aspergillus fumigatus	24	2.4 x10^3	16	2.0 x10^3	32	4.8 x10^3	–	–	–	–	–	–	–	–	–	–
Aspergillus japonicus	16	2.4 x10^3	28	4.0 x10^3	20	2 x10^3	28	4.4 x10^3	12	1.2 x10^3	–	–	4	0.4 x10^3	–	–
Aspergillus nidulans	20	2.0 x10^3	24	2.8 x10^3	28	3.6 x10^3	16	2.0 x10^3	4	0.4 x10^3	–	–	–	–	–	–
Aspergillus niger	56	6.8 x10^3	44	6.0 x10^3	44	6 x10^3	20	2.8 x10^3	12	1.6 x10^3	8	0.8 x10^3	4	0.8 x10^3	8	0.8 x10^3
Aspergillus ochraceus	40	4.8 x10^3	24	3.2 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Aspergillus parasiticus	16	2.0 x10^3	32	4.4 x10^3	16	3.6 x10^3	20	2.8 x10^3	–	–	8	0.8 x10^3	8	1.2 x10^3	4	0.4 x10^3
Aspergillus sydowii	–	–	–	–	20	3.6 x10^3	–	–	–	–	–	–	–	–	–	–
Aspergillus terreus	24	3.2 x10^3	–	–	–	–	–	–	–	–	4	0.4 x10^3	–	–	–	–
Aspergillus terricola var. americana	–	–	–	–	16	1.6 x10^3	–	–	–	–	–	–	–	–	–	–
Aspergillus versicolor	32	4.0 x10^3	20	2.4 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Chaetomium globosum	28	3.2 x10^3	–	–	–	–	8	1.6 x10^3	–	–	–	–	–	–	–	–
Chaetomium indicum	24	2.4 x10^3	8	1.2 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Cladosporium cladosporioides	–	–	–	–	20	3.2 x10^3	–	–	–	–	–	–	–	–	–	–
Curvularia lunata	–	–	8	1.2 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Curvularia pallescens	28	3.6 x10^3	32	4 x10^3	28	4.4 x10^3	16	2.4 x10^3	–	–	–	–	–	–	–	–
Emericella nidulans var. echinulatus	–	–	–	–	4	0.4 x10^3	8	1.2 x10^3	–	–	–	–	–	–	–	–
Eurotium chevalieri	–	–	20	2.8 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Fusarium oxysporum	–	–	–	–	24	3.2 x10^3	–	–	–	–	–	–	–	–	–	–
Fusarium pallidoroseum	20	2.4 x10^3	44	6 x10^3	–	–	16	1.6 x10^3	–	–	8	0.8 x10^3	–	–	8	0.8 x10^3

Table 2 Colonization frequency (CF%) and colony forming units (cfu/g) of the recovered mycoflora

Fusarium solani	44	4.8 x10^3	32	4.8 x10^3	–	–	–	–	16	2 x10^3	–	–	–	–	–	–
Fusarium verticilloides	–	–	–	–	20	2.4 x10^3	12	1.6 x10^3	8	1.2 x10^3	–	–	–	–	–	–
Mucor hiemalis	8	1.2 x10^3	20	2 x10^3	–	–	–	–	–	–	4	0.4 x10^3	8	0.8 x10^3	4	0.4 x10^3
Paecilomyces fusisporus	–	–	–	–	12	2 x10^3	–	–	–	–	–	–	–	–	–	–
Paecilomyces lilacinus	–	–	12	1.6 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Penicillium brevicompactum	12	2.0 x10^3	8	0.8 x10^3	–	–	–	–	8	0.8 x10^3	–	–	–	–	–	–
Penicillium chrysogenum	–	–	28	5.6 x10^3	–	–	28	4 x10^3	1	0.4 x10^3	–	–	–	–	–	–
Penicillium citrinum	28	3.6 x10^3	40	6.8 x10^3	36	4.8 x10^3	20	3.2 x10^3	–	–	–	–	–	–	8	1.2 x10^3
Penicillium expansum	8	1.6 x10^3	36	6 x10^3	32	4.4 x10^3	–	–	–	–	–	–	4	0.8 x10^3	–	–
Penicillium griseofulvum	32	5.6 x10^3	56	9.2 x10^3	24	4.4 x10^3	24	3.6 x10^3	–	–	8	0.8 x10^3	–	–	–	–
Penicillium oxalicum	28	3.6 x10^3	36	5.6 x10^3	20	2 x10^3	8	1.6 x10^3	–	–	4	0.8 x10^3	–	–	4	0.8 x10^3
Penicillium waksmanii	–	–	28	4.8 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Rhizopus oryzae	8	1.2 x10^3	16	1.6 x10^3	20	2.4 x10^3	–	–	–	–	–	–	8	0.8 x10^3	8	0.8 x10^3
Syncephalastrum racemosum	–	–	20	2.4 x10^3	–	–	–	–	–	–	–	–	–	–	–	–
Trichoderma koningii	12	1.6 x10^3	–	–	–	–	16	2.4 x10^3	–	–	–	–	–	–	–	–

Out of 37 fungal species recovered from soiled and mint note samples, Aspergillus was found to be predominant, accounting for 29.7% of the total recovered mycoflora, followed in decreasing order by Penicillium (18.9%) and Fusarium (10.8%), whereas the remaining 12 fungal genera contributed 40.6 % of the total recovered mycodiversity (Figure 1). The fungal species of common occurrence included Aspergillus flavus, A. japonicus, A. nidulans, A.niger, Fusarium pallidoroseum and Penicillium griseofulvum.

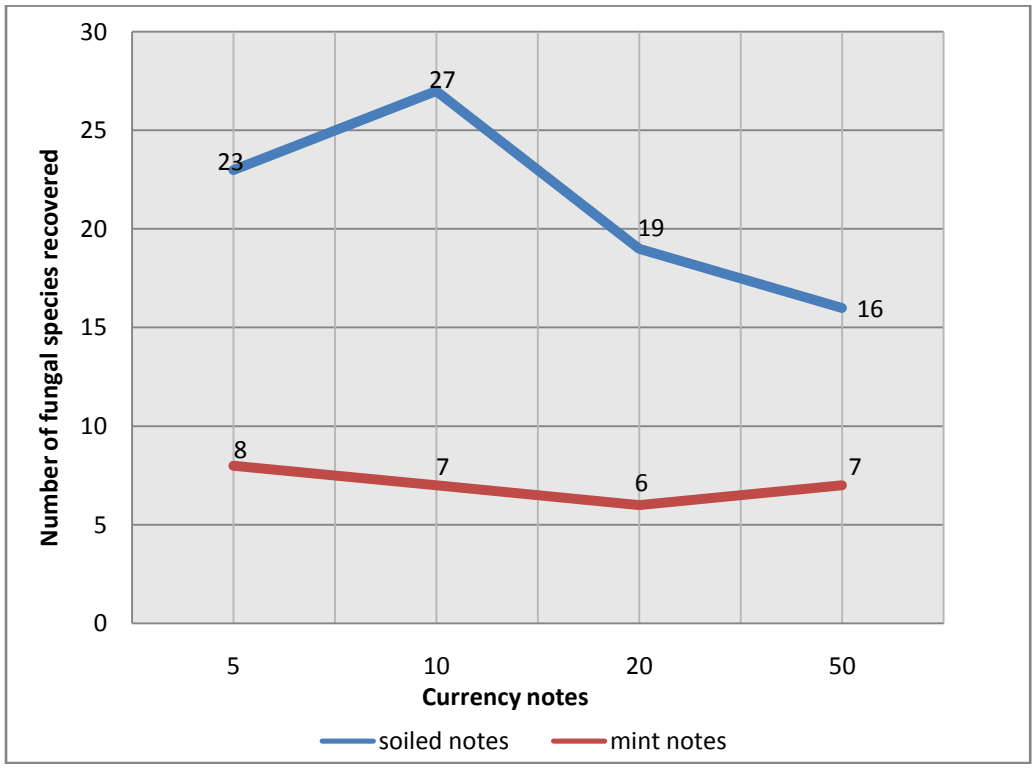
Figure 1 Percentage of fungal species recovered from lower denomination currency notes.



DISCUSSIONS:

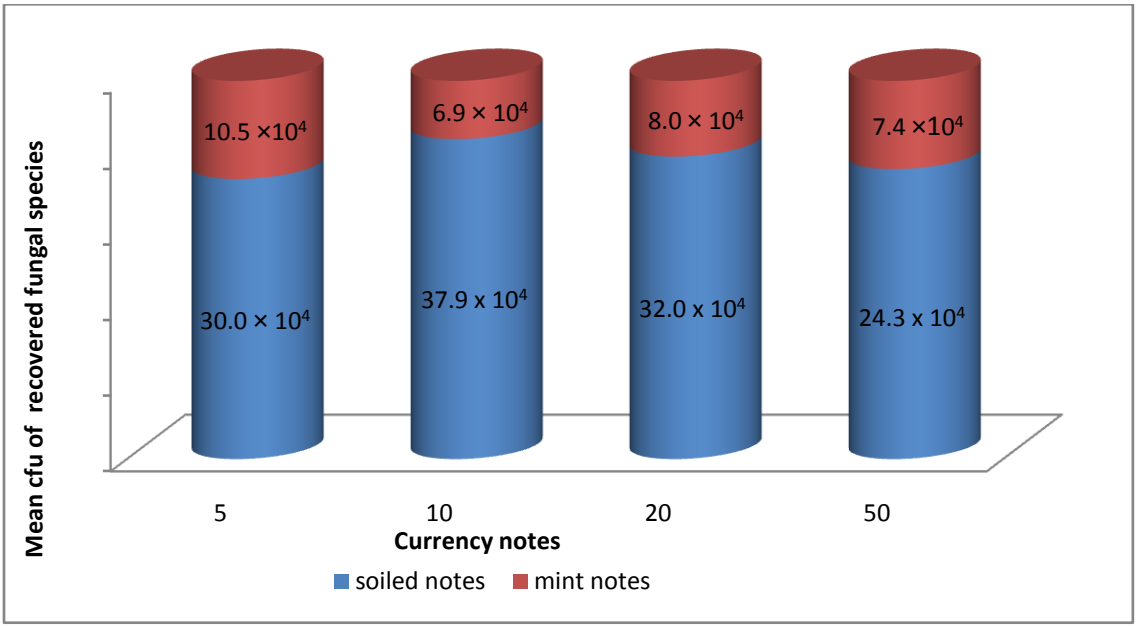
Out of 37 fungal species recovered from soiled and mint notes, Aspergillus was found to be predominant. Similar results were obtained by Wanule et al. (2011) who carried a survey of notes and coins from Nanded city, Maharashtra and reported heavy contamination of currency notes with Aspergillus, followed in decreasing order by Penicillium, Cladosporium, Rhizopus, Alternaria, Curvularia, Fusarium, and Trichoderma. Recently, Abirami et al. (2012) also detected Aspergillus species to be predominant among the fungal species recovered from Indian currency notes. The maximum number of fungal species (27) were recovered from the soiled notes of rupees ten denomination, followed in decreasing order by soiled notes of rupees five (23 fungal species), rupees twenty (19 fungal species) and rupees fifty (16 fungal species) as shown in figure 2. This shows that lower denomination notes harbor the greatest bulk of infectious agents, probably because they are exchanged more frequently than the higher denomination notes. An earlier study conducted among currencies from 10 different countries also showed that lower the index value of money, higher is the typical microbial content of the currency (Vrisekoop et al. 2010). Further, age of the currency notes and the material that is used to produce them also influences the contamination level (Vrisekoop et al.2010).

Figure 2 Number of fungal species recovered from soiled and mint notes of various denominations.



Comparison of mean colony forming units of fungal species recovered from soiled and mint notes is presented in figure 3. Soiled notes of rupees ten had highest value of cfu (mean = 37.9×10^4), followed in decreasing order by soiled notes of rupees twenty (mean = 32.0×10^4), rupees five (mean = 30.0×10^4) and rupees fifty (mean = 24.3×10^4). Among the sampled mint notes, rupees five showed highest cfu (mean = 10.5×10^4), followed in decreasing order by rupees twenty (mean = 8.0×10^4), rupees fifty (mean = 7.4×10^4) and notes of rupees ten (mean = 6.9×10^4). These results show that both the soiled and mint notes are contaminated with fungal spores. However, comparison of cfu data shows that the soiled notes are much more contaminated than the mint notes. Similar results were obtained earlier by Dehgani et al. (2011) while working on the Iranian currency notes.

Figure 3 Mean colony forming units of fungal species recovered from soiled and mint notes of different denominations.



Diversity indices calculated for the fungal species recovered from soiled and mint currency notes of lower denomination are given in table 3. These diversity indices show variation in values of Shannon–Wiener's index (H') and Simpson dominance index (Cd). These values vary with the denomination of notes and whether the notes are soiled or mint. Species richness was calculated by using Shannon–Wiener's index (H') and the highest value of species richness or that of Shannon–Wiener's index (H') was for soiled notes of rupees ten (3.14), closely followed in decreasing order by soiled notes of rupees five (3.04), rupees twenty (2.84), and rupees fifty (2.70). For mint notes, Shannon–Wiener's index (H') was less in comparison to that of soiled notes. It was 1.95 for five rupees note, 1.90 for ten rupees note, 1.88 for fifty rupees note and 1.75 for twenty rupees note. The concentration of dominance (Simpson's dominance index (Cd)) ranged between 0.05–0.07 for soiled notes and 0.15–0.18 for mint notes.

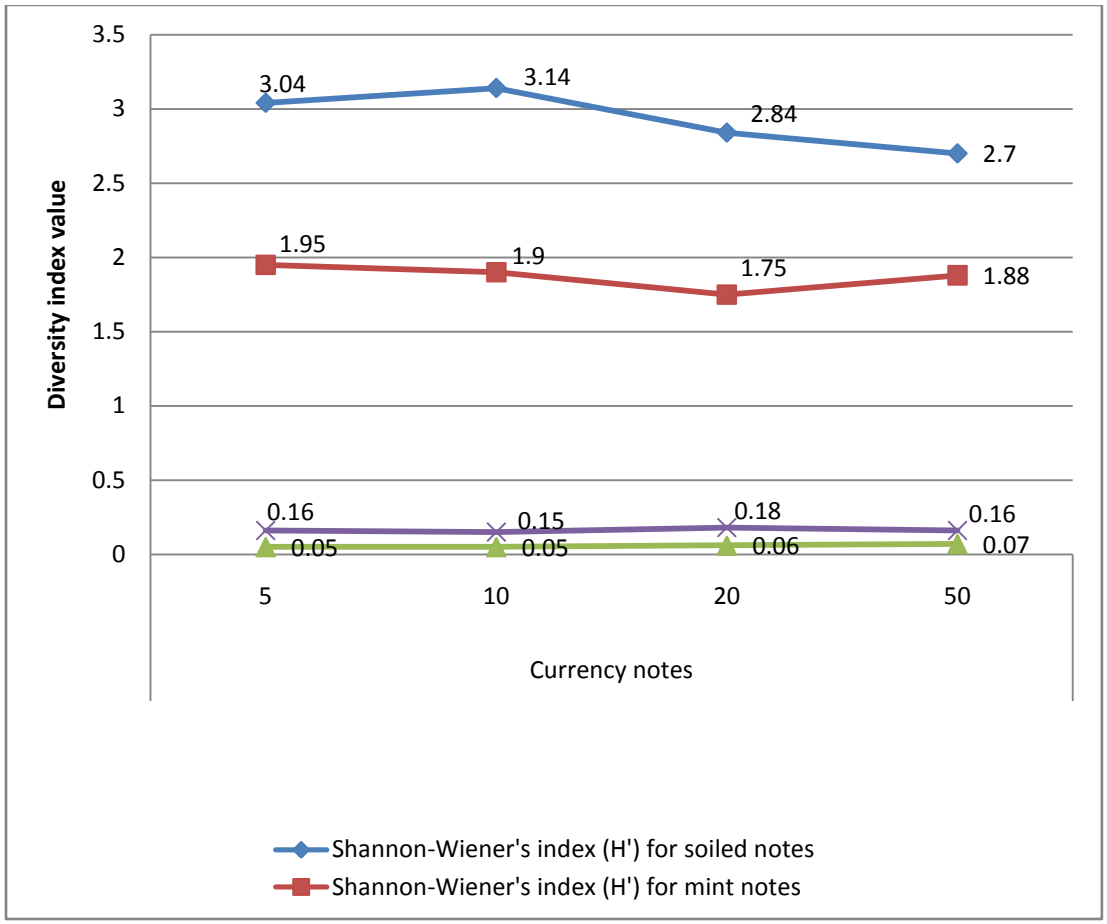
Table 3 Diversity indices calculated for the fungal species recovered from soiled and mint currency notes of lower denomination.

S. No.	Diversity indices	Sample notes	Currency notes (rupees)			
			5	10	20	50
1.	Shannon–Wiener's index (H')	Soiled notes	3.04	3.14	2.84	2.70
		Mint notes	1.95	1.90	1.75	1.88
2.	Simpson dominance index (Cd)	Soiled notes	0.05	0.05	0.06	0.07
		Mint notes	0.16	0.15	0.18	0.16

The values of Simpson's dominance index near 0 corresponds to low concentration and more homogeneity of fungal species among the note samples, which indicates that no particular fungal species is dominant among the studied note samples (Figure 4).The fungal organisms recovered from soiled currency notes may have come in contact with them through soil, clothing, food or hands of the users. Some of these organisms are potential disease causing agents. For example, Rhizopus species and Absidia corymbifera can be agents for zygomycosis and eye infections (Antoniadou, 2009), Penicillium species can cause pneumonia (Ekenna et al.,2007), Fusarium solani may cause a range of invasive mycosis and

opportunistic infections in immunocompromised patients (Zhang et al.,2006), Aspergillus fumigatus and A.niger spores when inhaled may cause aspergillosis (Schuster et al.,2002), Aspergillus flavus and A.parasiticus may produce aflatoxins Grundy and Grundy 1974; FAO 1979) , A.flavus may also cause pulmonary infection and cancer when it becomes invasive (Ozhak–Baysan et al.,2010).

Figure 4 Comparison of diversity indices for currency notes of different denominations



CONCLUSION:

Some of the associated fungal species are potential pathogens of human beings (e.g. Absidia corymbifera, Aspergillus fumigatus, Chaetomium globosum) and plants (e.g. Alternaria alternata, Aspergillus niger, A. flavus, A. parasiticus, Rhizopus oryzae, Fusarium solani, Cladosporium cladosporoides). Therefore, hygienic measures such as thorough hand washing with soap after using currency notes should be observed and the practice of keeping money in shoes and socks and under the carpets should be discouraged. Further, we should avoid the use of saliva during counting of currency notes as well as desist from placing money in the mouth and biting off corners of currency notes. Moreover, ready-to- eat food sellers should be educated to avoid possible cross contamination between currency notes and the food they sell.

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