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Comparison of cockroach fungal contamination in the clinical and non-clinical environments from Iran

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Abstract

Cockroaches have been associated with human environments threatening human health. A cross-sectional study about cockroach fungal contamination in the hospital, restaurant and household environments from Khorramabad, Lorestan province of Iran between August 2015 and March 2016 was done. Sampling was uniformly carried out monthly from the randomly selected sites. Fungi were isolated from the external surfaces of cockroaches using standard method. *Periplaneta americana* (66.7%) was found the most infested cockroach, less percentage were observed in *Blattella germanica* (18.6%) and *Blatta orientalis* (14.7%). Hospitals (66.7%) were found the most infested places in comparison with households (18.6%) and restaurants (14.7%). Households (64.3%) were found the most cockroach fungal contaminated places than hospitals (49.0%) and restaurants (59.1%). The highest and the lowest infestation of cockroaches were observed in January (30.0%) and March (7.0%) respectively, while the highest and the lowest cockroach fungal contamination were observed in November (73.3%) and March (14.3%). *B. germanica* (60.7%) was the most fungal contaminated cockroach. *Candida*, *Geotrichum* and *Penicillium* were the most frequent cockroach fungal contaminating genera. Statistical analysis revealed significant differences between cockroach infestation places (P=0.022), locality (P=0.03), and monthly (P=0.0001) cockroach fungal contamination, respectively. As a conclusion, the highest cockroach fungal contamination was observed in *B. germanica*, followed by *P. americana* and *B. orientalis*. Some human fungal opportunistic pathogens were also found among the fungal contaminated cockroaches. Recent epidemiological survey showed that the mortality rates of the infective diseases were increased, indicating cockroaches involved in their transferring.

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Introduction

Cockroaches have been associated with human living environment due to very high compatibility with human habitats (Nasirian, 2016). As infestation of cockroaches are increasing, may be not seen a place that is safe from the presence of cockroaches (Nasirian, 2007; 2008; 2017b; Nasirian *et al*., 2006e; Limoee *et al*., 2012; Nazari *et al*., 2016). Because of presenting in the dirty places, vomiting the eaten foods, defecating feces in the environment and producing bad smell and allergens, cockroaches are harmful to human health (Doroodgar *et al*., 2005; Stypułkowska-Misiurewicz *et al*., 2005). The ability of cockroaches to run and move quickly from seams and narrow paths also increases their ability to escape from control methods and their role in the transmission of pathogens and reveals their medical importance more than ever.

Like houseflies the role of cockroaches is clear in the spread of nosocomial infections (Davari *et al*., 2010; Khoobdel & Davari, 2011; Davari *et al*., 2012; Nasirian, 2010; 2017a). Many studies have been conducted about natural cockroach contamination to

pathogenic fungi. As well as advances in the diagnosis and treatment of diseases and new discovery of diagnostic or therapeutic methods have increased the patients' age, ascending infections due to saprophytic fungi agents are observed.

Recent epidemiological survey showed that the mortality rates of the patients admitted to Shohada Hospital of Khorramabad are increased and the mortality rates of infective diseases were 4.2% (Mahmoudi *et al*., 2014).

The city of Khorramabad, the capital of Lorestan province, has a sweet nature with four weather seasons and is a benefit thanks the rich natural and tourist attractions. A cross-sectional study about contamination of cockroaches to fungi in the hospital, restaurant and household environments of Khorramabad, Lorestan province was carried out between August 2015 and March 2016.

Materials and methods

Site of study

The sites of study were randomly selected from the hospital, restaurant and household environments in Khorramabad. The selected hospitals were Shohaday-e-Ashayer Medical Education Center and Shahid Madani Specialized hospital. The Shohaday-e-Ashayer Medical Education Center is the largest and the best equipped Medical Education Center of Lorestan University of Medical Sciences with 350 beds. It has more than 20 Specialized and Super-specialized units. Shahid Madani Specialized hospital has 60 beds. We also selected 6 households and 6 restaurants from Khorramabad city including one from the each north, south, east, west and 2 from the center.

Sampling of cockroaches and identification

Sampling was uniformly carried out monthly for three hours from each sites of the study using Nasirian *et al*. methods (Nasirian *et al*., 2006c; 2009) between August 2015 and March 2016, 150 samples were collected to allow a good statistical estimate. Cockroaches were transferred to a sterilized wide mouth glass container after collecting by hand catch. Each container was allocated only to one cockroach to avoid mixing contamination of cockroaches to each other. Then the samples were transported to the laboratory after closing their mouth by a piece of cloth and elastic ring around it. The cockroaches were identified with cockroach taxonomic keys after their anesthetizing by placing them in the freezer for 5 minutes in the laboratory.

Fungal isolation and identification

In order to avoid possible microorganisms contamination from the external surfaces of cockroaches, each cockroach was placed into a sterile glass containing 5 ml of normal saline solution. Then they were shaken strongly and washed. To isolate fungi from the external surfaces of cockroaches, 2 mL of the obtained suspension was centrifuged for 10 min at 2000 rpm. Then the residues were cultured on medium plates of dextrose agar with the addition of Chloramphenicol. The plates were incubated at 25°C for one week. When fungal identification was impossible, the colony was cultured using culture slides (Ash & Greenberg, 1980; Zaeim *et al*., 2008). Finally, the fungi were recognized based on the fungal identification keys and the data were filed for statistical analysis among cockroach infestation places, locality contamination, monthly fungal contamination and species fungal contamination.

Statistical analysis

Chi-square test was used to compare the frequency of fungal pathogens using SPSS version 16 software. In the all statistical tests $P<0.05$ was considered as statistically significant level.

Results

Table 1 and Figure 1 show cockroach infestation between August 2015 and March 2016 in the sites of study. Three cockroach species including *Blattella germanica* (German cockroach), *Blatta orientalis* (Oriental cockroach) and *Periplaneta americana* (American cockroach) were identified. *Periplaneta americana* was found the most infested cockroach species (Figure 1A). As observed (Figure 1C) the cockroach infestation ranged from 4.7 % in March to 20.0 % in January. Hospitals (66.7%) were found the most cockroach infested places than households (18.6%) and restaurants (14.7%). Statistical analysis revealed significant differences between cockroach infestation places including households, hospitals and restaurants (P=0.022) (Table 2).

Table 3 shows the lists of isolated fungal pathogens from collected cockroaches and locality of selected sites of the study. Among cockroaches collected from the selected hospitals (n=100), 51 cockroaches were without any fungal contamination and 49 samples were contaminated with fungal pathogens. The most cockroach fungal contamination was related to *Penicillium* and yeast. Among cockroaches collected from the selected households (n=28), 10 cockroaches were without any fungal contamination

Table 1. Cockroach infestation between August 2015 and March 2016 in the sites of study.

Species	Sites	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Sites, total No.	Sites, total %	Cockroach, %
B. germanica	Hospitals	2	2	5	4	4	0	0	0	17	11.3	18.6
	Households	1	2	3	0	0	0	0	0	6	4.0	
	Restaurants	2	1	2	0	0	0	0	0	5	3.3	
B. orientalis	Hospitals	0	0	3	0	0	0	0	0	3	2.0	14.7
	Households	4	5	0	0	0	0	0	0	9	6.0	
	Restaurants	2	3	0	5	0	0	0	0	10	6.7	
P. americana	Hospitals	5	4	6	6	9	30	13	7	80	53.3	66.7
	Households	2	2	4	0	5	0	0	0	13	8.7	
	Restaurants	2	2	1	0	2	0	0	0	7	4.7	
Total	No	20	21	24	15	20	30	13	7	150	100	
	%	13.3	14.0	16.0	10.0	13.3	20.0	8.7	4.7	100	-	

and 18 samples were contaminated with fungal pathogens. The most cockroach fungal contamination was related to *Geotrichum* and *Candida*. In addition, among cockroaches collected from the selected restaurants (n=22), 9 cockroaches were without any fungal contamination and 13 samples were contaminated with fungal pathogens. The most cockroach fungal contamination was related to *Geotrichum* (Table 3 and Figure 2A,C).

Table 4 shows the lists of isolated fungal pathogens during the study. The highest and the lowest cockroach fungal contamination were observed in the January (n=16) and March (n=1), respectively (Figure 2B). Furthermore, the most abundant fungi were *Penicillium*, yeast and *Geotrichum* in order (Figure 2). The most cockroach fungal contamination was related to *Penicillium* and yeast (Figure 2C). The Oriental cockroaches were mostly contaminated by *Geotrichum*. The American cockroaches were mostly contaminated by *Penicillium* and yeast while the German cockroaches were mostly contaminated by *Candida* (Figure 2C). Statistical analysis didn't reveal significant differences between fungal contamination of the cockroach species (P=0.076) (Table 2).

Figure 3 shows cockroach fungal contamination between August 2015 and March 2016 in the sites of study. *B. germanica* cockroaches (60.7%) was mostly contaminated to fungi than the other species (Figure 3A), even though statistical analysis didn't reveal significant differences between fungal contamination of the cockroach species (P=0.076) (Table 2). According to cockroach collection locality, households (64.3%) were found the most cockroach fungal contaminated places than hospitals (49.0%) and restaurants (59.1%) (Figure 3B). Statistical analysis revealed significant differences between the fungi isolated from cockroaches collected from hospital and household environments (P=0.03) (Table 2). The cockroach fungal contamination during the study ranged from 14.3 % for March to 73.3 % for November (Figure 3C,D). Statistical analysis revealed significant differences between monthly cockroach fungal contamination (P=0.0001) (Table 2).

Discussion

In the present study three cockroach species including *Periplaneta americana* (66.7%), *Blattella germanica* (18.6%) and *Blatta orientalis* (14.7%) were found. Similar results were found by Zarchi & Vatani (2009) in Tehran (Zarchi & Vatani, 2009). *P. americana* and *B. germanica* species were also found as dominant cockroach species in Zanjan, Sari and Kashan from Iran. The highest and the lowest cockroach infestation during the study were observed in January (20.0%) and March (4.7%), respectively (Figure 1C). Statistical analysis also revealed significant differences between cockroach infestation places including households, hospitals and restaurants (P=0.022) (Table 2). Cockroach populations were increased at the beginning of winter season in coincidence with turning on of heating appliances, while population of cockroaches were decreased at the end of the cold season when heating appliances were turned off.

Our study confirms that hospitals (66.7%) were found the most cockroach infested places respect to households (18.6%) and restaurants (14.7%). Different groups of fungi were isolated from the cockroaches in the hospital, restaurant and household environments. The diversity and dominancy of fungal contamination in cockroach species was higher in *P. americana* than in *B. germanica* and *B. orientalis*. Some human fungal opportunistic pathogens were also found among the cockroach contaminated fungi threatening human health (Tables 3 and 4). Statistical

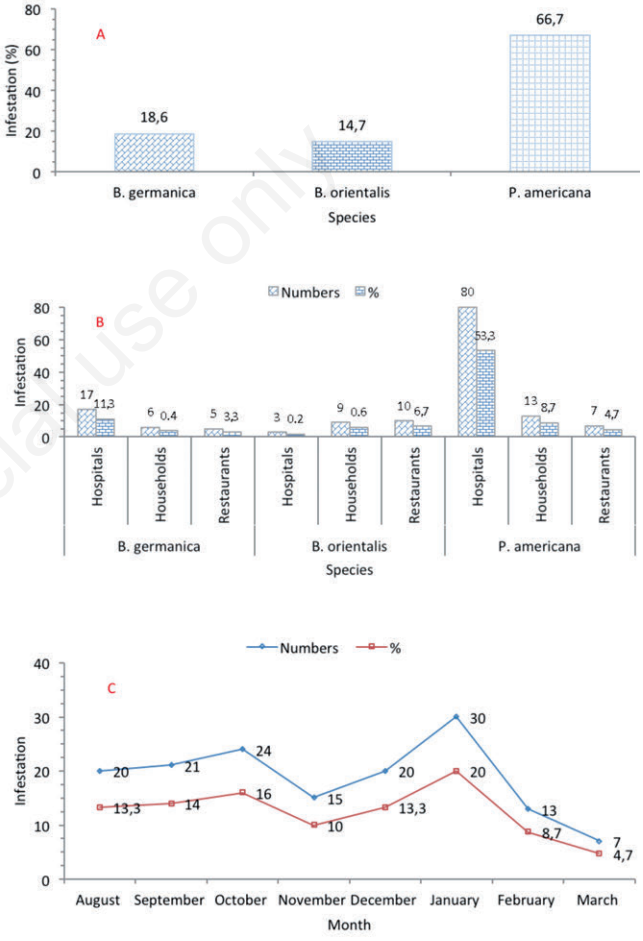

Figure 1. Cockroach infestation between August 2015 and March 2016 in the sites of study. A) Cockroach species infestation. B) Cockroach infestation in the sites of study. C) Trend of cockroach infestation during the study.

Table 2. Pearson Chi-Square statistical analyses, August 2015-March 2016.

Pearson Chi-Square tests	Value	df	Sig. (2-sided)
Between cockroach infestation places	67.281	46	0.0220
Between cockroach locality contamination	23.250	8	0.0300
Between cockroach monthly fungal contamination	228.847	161	0.0001
Between cockroach species fungal contamination	60.373	46	0.0760

analysis didn't reveal significant differences between fungal contamination of the cockroach species (P=0.076) (Table 2). It means that the importance of cockroach species to be contaminated with fungal agents is usually similar. The German cockroaches were mostly found in the clinical environments and the American cockroaches were mostly found in the non-clinical environments (Pai *et al*., 2004).

In this study different groups of fungi were isolated from the cockroaches. Some human opportunistic pathogens were found among them threatening human health. The increasingly resistance of cockroaches to various insecticide groups is an additional problem (Nasirian *et al*., 2006a; 2006b; 2006d; 2011; Limoee *et al*., 2010; Paksa *et al*., 2011, 2012; Ladonni *et al*., 2013). In accordance with the study conducted by Fotedar & Banerjee (1992), the present study revealed significant differences between the fungi isolated from cockroaches collected from hospital and household environments (P=0.03), and between monthly cockroach fungal contamination (P=0.0001) (Table 2). Naderinasab and Moravvej (2012) also found significant differences between the fungi isolated from cockroaches collected from different wards of hospitals and residential areas. Similar to the present study, Chitsazi *et al*. (2013) found *Penicillium* as the most cockroach fungal contaminant in the hospital environments (Fotedar & Banerjee, 1992; Naderinasab & Moravvej, 2012; Chitsazi *et al*., 2013).

The highest and the lowest cockroach fungal contamination during the study were observed in November (73.3%) and March (14.3%), respectively (Figure 3B,C). Statistical analysis also revealed significant differences between monthly cockroach fungal contamination (P=0.0001) (Table 1), understanding the reason of this observation needs further investigation.

Conclusions

Cockroaches are the major transmitter of fungal pathogens and allergen-causing agents, so their presence in the public places such as hospital, restaurant and household environments is dangerous and threaten human health. Therefore, in this study, we examine to consider and compare the dominant cockroach fungal contaminants in the hospital, restaurant and household environments of Khorramabad.

Table 3. List of fungi isolated from locality and cockroach species fungal contamination, August 2015-March 2016.

Locality cockroach fungal contamination		
Hospitals	Households	Restaurants
Acremonium; *Alternaria*; *Alternaria* and *Cladosporium*; *Candida*; *Candida* and *Geotrichum*; *Candida* and *Rhodotorula*; *Candida, Mucor* and *Aspergillus*; *Cladosporium*; *Geotrichum*; *Mucor*; *Penicillium*; *Penicillium* and yeast; *Penicillium, Cladosporium* and *Aspergillus*; *Rhizopus* and *Geotrichum*; Yeast.	*Acremonium*; *Aspergillus niger* and yeast; *Candida*; *Candida* and *Geotrichum*; *Candida* and *Rhodotorula*; *Cladosporium* and *Candida*; *Cryptosporidium* and yeast; *Geotrichum*; *Penicillium*; *Penicillium* and *Acremonium*; *Penicillium, Cladosporium* and *Aspergillus*; *Rhodotorula*; Yeast.	*Acremonium*; *Alternaria* and *Cladosporium*; *Aspergillus terreus*; *Candida*; *Candida* and *Geotrichum*; *Cladosporium*; *Geotrichum*; *Penicillium* and *Candida.*
Cockroach species fungal contamination		
P. americana	*B. germanica*	B. orientalis
Acremonium; *Alternaria Alternaria* and *Cladosporium*; *Candida*; *Candida* and *Geotrichum*; *Candida* and *Rhodotorula*; *Candida, Mucor* and *Aspergillus*; *Cladosporium*; *Cryptosporidium* and yeast; *Geotrichum*; *Mucor*; *Penicillium, Penicillium* and yeast; *Penicillium*; *Cladosporium* and *Aspergillus*; *Rhizopus* and *Geotrichum*; Yeast.	*Alternaria* and *Cladosporium*; *Aspergillus niger* and yeast; *Aspergillus terreus*; *Candida*; *Candida* and *Geotrichum*; *Candida, Mucor* and *Aspergillus*; *Cladosporium*; *Cladosporium* and *Candida*; *Geotrichum*; *Penicillium*; *Penicillium* and *Acremonium*; *Penicillium* and *Candida*; *Penicillium, Cladosporium* and *Aspergillus*	*Acremonium*; *Alternaria* and *Cladosporium*; *Candida*; *Candida* and *Rhodotorula Geotrichum*; *Penicillium* and *Candida*; *Rhodotorula*;Yeast

Table 4. List of monthly fungi isolated from cockroaches, August 2015 and March 2016.

Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar
Acremonium; *Aspergillus niger* and yeast; *Cryptosporidium* and yeast; *Yeasts*	*Acremonium*; *Alternaria*; *Alternaria* and *Cladosporium*; *Candida*; *Candida* and *Rhodotorula*; *Candida, Mucor* and *Aspergillus*; *Cladosporium*; *Cladosporium* and *Candida*; *Rhodotorula*	*Aspergillus terreus*; *Candida*; *Geotrichum*; *Mucor*; *Penicillium* and *Acremonium*; *Penicillium* and *Candida*; *Penicillium, Cladosporium* and *Aspergillus*; *Rhizopus* and *Geotrichum*	*Alternaria* and *Cladosporium*; *Candida*; *Candida* and *Geotrichum*; *Geotrichum*; *Penicillium*	*Candida*; *Candida* and *Geotrichum*; *Candida*; *Mucor* and *Aspergillus*; *Geotrichum*; *Penicillium*; *Penicillium*; *Cladosporium* and *Aspergillus*; Yeast	*Candida*; *Candida* and *Geotrichum*; *Penicillium*; *Penicillium* and yeast	*Candida* and *Geotrichum*; *Penicillium*; Yeast	Yeast

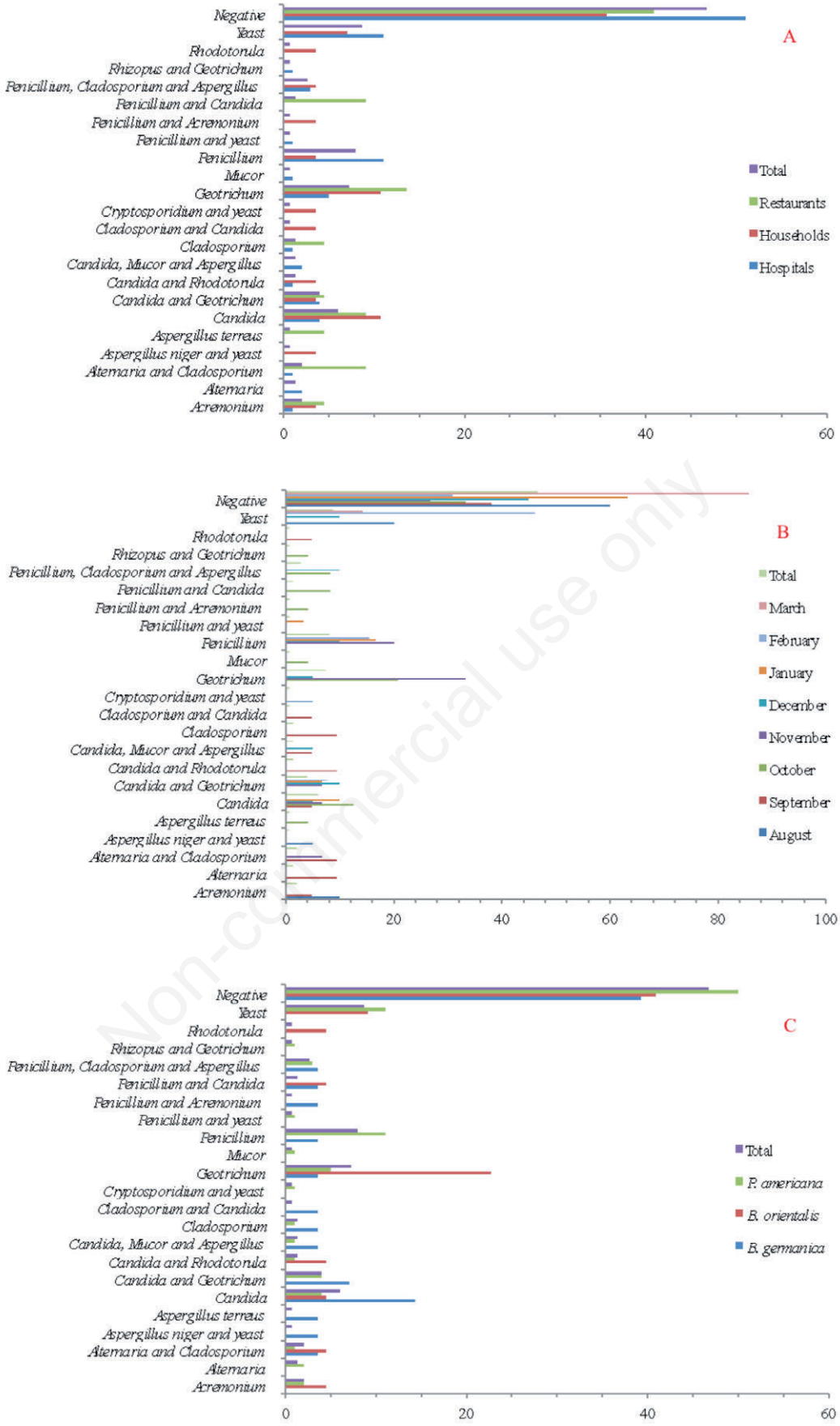

Figure 2. Cockroach fungal contamination (%), August 2015-March 2016. A) Locality cockroach fungal contamination. B) Monthly cockroach fungal contamination. C) Cockroach species fungal contamination.

In the present study three cockroach species including *P. americana*, *B. germanica* and *B. orientalis* were found. Different groups of fungi isolated from cockroaches in the hospital, restaurant and household environments. The diversity and dominancy of cockroach species to fungal contamination were *P. americana*, *B. germanica* and *B. orientalis* in order. Some human opportunistic pathogens were found among the cockroach fungal contaminants threatening human health. Recent epidemiological survey confirms that the mortality rates of the patients admitted to Shohada Hospital of Khorramabad are increased and the mortality rates of infective diseases were increased. It may be cockroaches involved in their transferring.

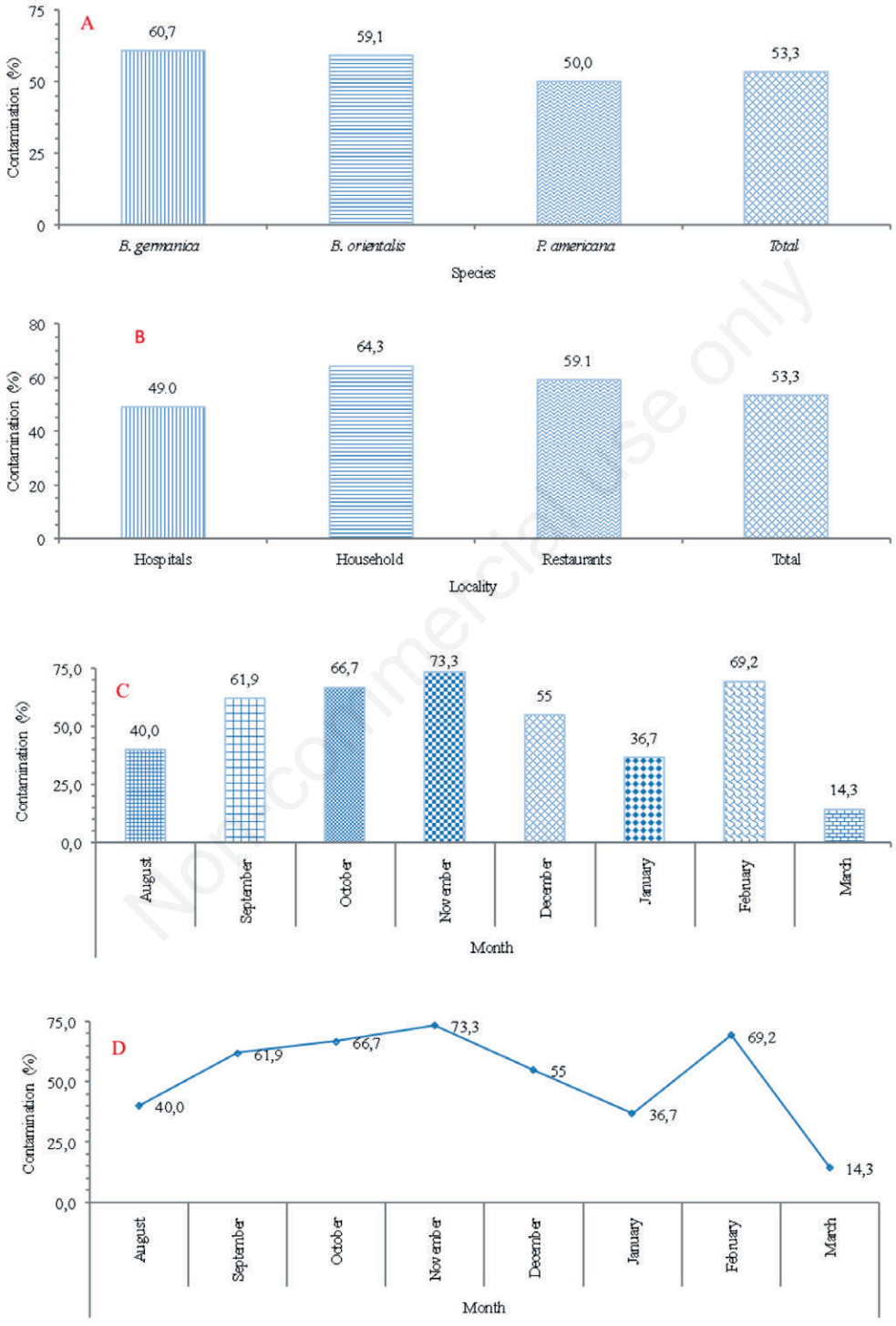

Figure 3. Cockroach fungal contamination, August 2015-March 2016. A) Cockroach species fungal contamination. B) Cockroach locality fungal contamination. C) Cockroach fungal contamination during the study. D) Trend of cockroach fungal contamination during the study.

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