IDENTIFICATION OF AIR BORNE FUNGAL CONTAMINANTS OF CURD IN INDOOR ENVIRONMENT

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ABSTRACT

Fungi are ubiquitous, chlorophyll-less, heterotrophic microorganisms. The spores of fungus are easily aerosolized and is considered as one among the chief contaminants of food products. Intrusive toxicity to normal human health may arise as a consequence from the intake of such contaminated food. Curd (yoghurt), a coagulated product from milk, has an ineluctable place in our regular diet. The present study attempted to identify the fungal contaminants of curd in indoor environment. Morphological features of the colony, hyphae and spores lead to the identification of species of four different genera. Three species of Penicillium namely P.chrysogenum, P.commune and P.citrinum isolates were identified. Other members such as Aspergillus niger, Cylindrocarpon sps and Rhizoctonia sps were also found from curd. The result evidenced that contamination of curd take place from fungal spores on exposure to atmosphere and the extent of contamination increases with duration of exposure. Further research is needed to establish the extend of fungal contamination as a function of temperature and pH.

Keywords: curd, fungus, contamination, *Penicillium*, *Aspergillus*, *Cylindrocarpon*, *Rhizoctonia*

INTRODUCTION

Fungi are ubiquitous, chlorophyll-less, heterotrophic, non-acid fast, gram positive eukaryotic organisms (Pal, 2014). Mostly, fungi derive nutrition from dead decaying organic matters (saprophytes), but some behave as parasites or symbionts (Babic *et al*., 2017)

Generally, fungal spores are produced in excess numbers which get easily aerosolized once released from the spore bearing structures. Thus, literally everything that is exposed and has moisture content is susceptible to fungal attack. Babic and the team isolated *Acremonium*, *Alternaria*, *Aspergillus*, *Cladosporium*, *Fusarium*, *Penicillium*, *Trichoderma* and Saccharomyces from drinking water (Babic *et al*., 2017). Fungus such as *Aspergillus*, *Penicillium* and *Trychophyton* sps pose serious contamination risk to pharmaceutical products

(Vijayakumar *et al*., 2012). *Penicillium*, *Chaetomium*, *Ulocladium*, *Aspergillus*, *Stachybotrys*, *Alternaria*, *Cladosporium*, *Acremonium*, *Paecilomyces*, *Trichoderma* and Phoma are deteriorating agents of building materials (Li and Yang, 2004). Eidi *et al*., (2016) isolated *Aspergillus*, *Penicillium*, *Yeast*, *Alternaria*,*Cladosporium*, *Stemphylium*, *Ulocladium*, *Stachyb otrys*, *Basidiobolus* and *Exophiala* from nasal cavity and indoor building environments of healthy subjects.

Several studies had encountered fungal contaminants from food samples and its consumption pose serious health risks. *Alternaria*, *Curvularia*, *Drechslera*, *Chaetomium*, *Epicoccum*, *Fusarium*, *Cladosporium*, *Nigrospora*, *Pithomyces*, *Trichoderma*, *Ulocladium*, *Aspergillus* and *Penicillium* are the common food and feed stuffs contaminants found in atmosphere (Udagawa, 1979). Sakai *et al*., (2004) detected *Aspergillus*, *Cladosporium* and *Penicillium* from bakery products and beverages and the victims complained of nausea, vomiting, diarrhea and stomach ache after consumption.

Milk and its products are vital source of nutrition for human health and hence billions of people consume it every day (Pal, 2014). Mostly, moulds (filamentous, muticellular and aerobic) and yeast (unicellular and facultative anaerobic) are associated with milk and milk product spoilage. Presence of fungal contaminants such as *Cladosporium*, *Penicillium*, *Saccharomyces* and *Aspergillus* from butter and *Penicillium* from cheese were previously reported (Pal, 2014). *Aspergillus*, *Penicillium*, *Geotrichum*, *Mucor*, *Cladosporium* and *Curvularia* were detected in milk and yoghurt (El-Diasty, *et al*., 2008). Contamination by *Aspergillus* sps, *Fusarium* sps and *Penicillium* sps are often associated with serious health hazards as they release toxic compound mycotoxins into the medium which interfere with human health (Pal, 2014).

Curd (yoghurt), a coagulated product from milk, has an inevitable place in our daily diet. Nearly, one sixth of milk is consumed as curd (Pal, 2014). Many factors are responsible for contamination of milk and other milk products. Fungal contaminants can enter from air, equipments or from packaging materials making it unfit for consumption. Further, the composition of fungus in environment may vary from place to place. So, it is necessary to know the potential fungal contaminants in immediate surroundings to take proper preventive steps. Thus, the present study attempted to isolate and identify the air borne fungal contaminants of curd present in indoor conditions.

MATERIALS AND METHODS

Study material

Fresh curd (stock) obtained from local market (Kottayam, Kerala) was used as the study material. Three set of petriplates (set 1,2 and 3) were categorized with 5 plates in each set. To each added 20 ml of curd from stock. All set of plates were exposed to atmosphere so that fungal spores in air may fall on curd. Set 1, 2 and 3 plates were exposed for 1, 5 and 10 hrs respectively. After exposure all the set of plates were kept inside sterilized refrigerator at 8°C

for 20 days. Evaluation of colony development were made on every 5th day. Unexposed curd was used as standard.

Culture medium preparation

Culture vessels, plates were sterilized by hot air oven at 160°C for 2 hours.The composition of sabouraud dextrose agar (SDA) medium consisted of peptone (1g), dextrose (0.4g), agar (2.4g) in 100ml distilled water (Nagano *et al*., 2008). The pH of the medium was adjusted to 5.6 and boiled the medium to dissolve the agar properly. Autoclaved the mixture at 121°C for 15 minutes and cooled at 45 to 50°C. The medium was then poured into sterilized petridishes.

Transferring sample into cultural medium, sub culturing and establishing pure culture

Fungal samples from each colonies developed on curd were streaked on separate SDA containing petriplates using sterilized loops. Petriplates were incubated at 30°C for 4 days. Isolated colonies were sub cultured on SDA plates to establish pure culture.

Morphological studies of the fungal colony

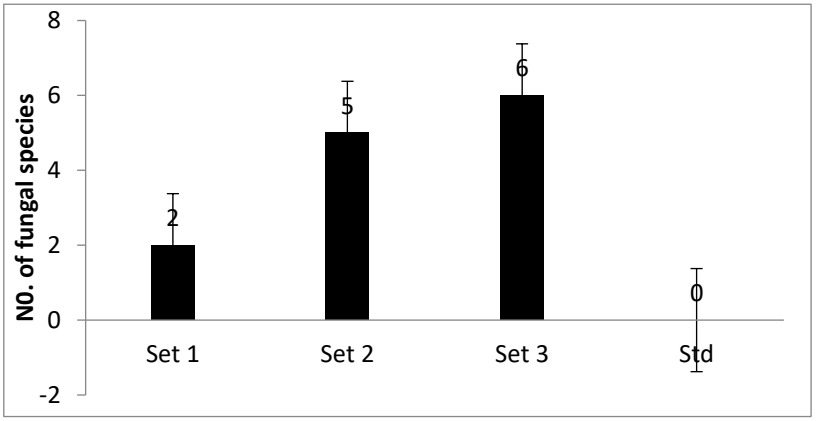
Colony morphology and microscopic characteristics on cotton blue staining were used for identification (Heritage and Killington, 1996).

Statistical analysis of result.

Experiments were carried out in triplicates. Results were expressed as mean value±standard error.

RESULTS AND DISCUSSION

All three set of curd samples exhibited development of fungal colonies from the 10th day onwards. The number of species recovered from each set showed variation. More colonies identified from set 3 followed by set 2 and set 1 i.e., the number increased with increase in time of exposure (Fig. 1).



Mean value±Standard Error

Fig.1: No. of fungal species recovered from different set of plates after 20 days.

Pure colonies of six distinct fungal samples were observed. Three of the plates (a,b and c) shared more or less similar features on primary observation as shown in Fig 2.

(a) (b) (c)

Fig.2a,b,c: Pure colonies of three fungal species

Colonies in all the three plates (a,b and c) exhibited rapid growth. Morphologically, colonies found to be flat and velvety in texture. Though the colour of colonies in the initial stage observed as white later it turned blue green, gray green, olive gray, yellow or pinkish with time.

Plate “a” on microscopic observation exhibited filamentous, septate, hyaline hyphae with dry chains of conidiospores from brush-shaped conidiophores. The penicilli were terverticillate in branching. The colour of conidia ranged from blue to blue-green and were spherical to elliptical in shape. Conidiophore was visible arising from a foot cell. The tip of the stalk or conidiophore was swollen forming the vesicle. Sterigmata arise from the vesicle and its distal end bears long chains of smooth, unicellular and unbranched conidia (Fig. 3). Morphologically, the colonies appeared velvety and sulcate, with blue-green colour and white border. The reverse side of the colonies showed yellow coloration. The colony and microscopic morphological features shows the sample in plate “a” is *Penicillium chrysogenum.*

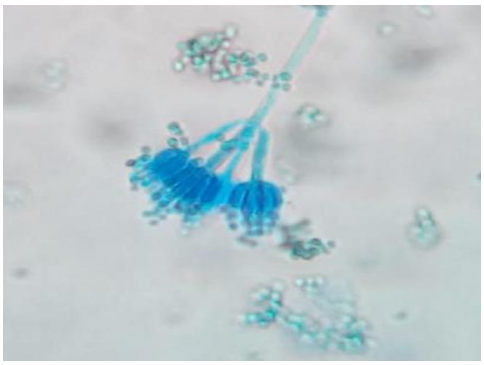


Fig.3: Cotton blue stained hyphae with condiospores of *P. chrysogenum*

Plate “b” also exhibited similar characters as that of plate “a”. The mycelium was filamentous, and septate. The colonies were initially white but later turned into gray-blue green in colour with white border. The colony surface appeared velvety and sulcate. The reverse side of the colonies was yellowish-cream in colour. Microscopic examination revealed that the penicilli were biverticillate meaning that the conidiophore can branch and the metulae and phialides extend from these branches (ie., each branch arising from conidiophore (stipe) branches once and then have a fruiting structure involving metulae and phialides). Metulae found longer than phialides. The phialides were flask shaped with globose (round) to sub-globose conidia (Fig. 4). The conidia were smooth walled and had a greenish-yellow colour. The morphological and cultural characters were found similar to that of *P. citrinum.*

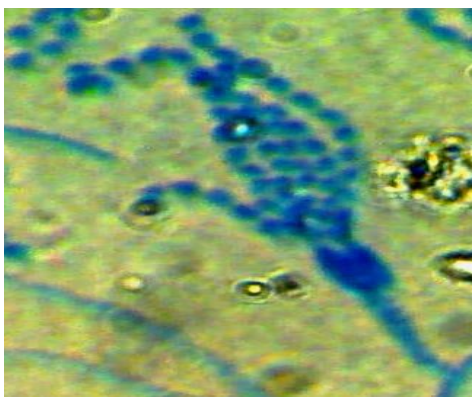


Fig.4: Cotton blue stained hyphae with condiospores of *P. citrinum*

Plate “c” showed fast mycelial growth in medium. Morphologically colonies were soft, velvety and grey blue in colour with white margin. The underside of colonies found to be yellow coloured. Smooth and spherical conidiospores were borne in disordered chains on conidiophores with rough-walled stipes. The conidium-bearing stalks were either produced singularly or in bundled groups known as fascicles (Fig. 5). Conidia were dull grey green or grey turquoise in colour. The morphological and cultural characters helped to distinguish the sample as *P. commune*. These observations are in tune with the descriptions of *P. chrysogenus*, *P. citrinum* and *P.commune* made by Ehgartner *et al*., (2017), Houbraken *et al*., (2010) and Bandh *et al*., (2011) respectively.

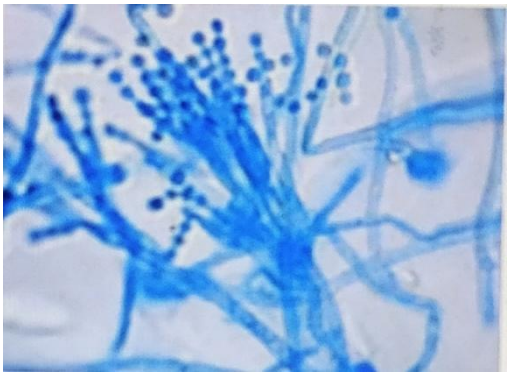


Fig.5: Cotton blue stained hyphae with condiospores of *P. commune*

Fourth culture were filamentous and colony exhibited a dark brown colour. On microscopic observation smooth walled conidiophore appeared slightly darkish with dark brown globose conidial head. Conidia liberated from biserite conidial head were dark brown in colour and had a rough surface (Fig. 6). These morphological characters are peculiar to *Aspergillus niger* (Zulkifli and Zakaria, 2017).

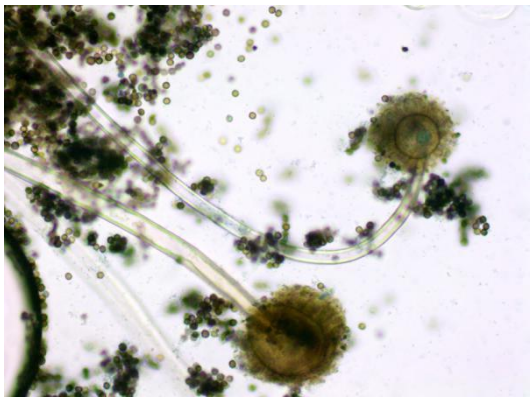


Fig.6: Cotton blue stained hyphae with condiospores of to *Aspergillus niger*

Fifth culture showed fast growth, hyaline and woolly colony. Phialides were simple and cylindrical. Macro-conidia smooth walled, cylindrical, straight with oval or flat base and unicelled (Fig. 7). Chlamydospores were thick walled. These features are the characteristics of *Cylindrocarpon* and are similar to the description made by Kid *et al*., (2016).

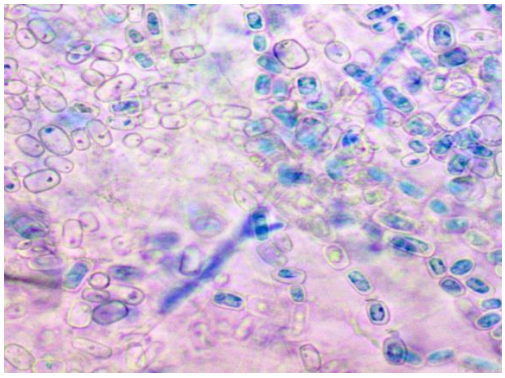
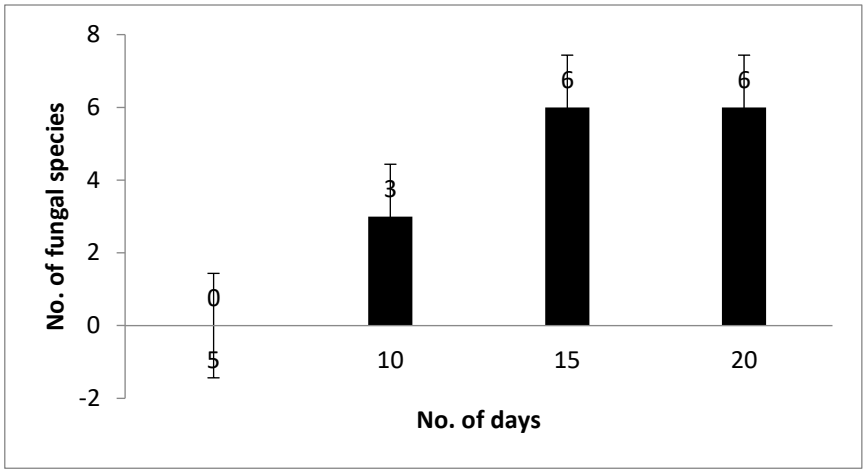


Fig.7: Cotton blue stained hyphae with condiospores of *Cylindrocarpon* sps

Sixth fungal culture were filamentous, branched and brownish in colour. Multinucleate, septate hyphae exhibited branching at right angle just above the distal septum. Branches had septum just above near the point of origin. Dolipore septum and absence of clamp connection reveals the identity of sample as *Rhizoctonia* (Fig. 8). These observations is in accordance with the findings of Al-Fadhal *et al*., (2019)

Fig.8: Cotton blue stained hyphae with condiospores of to *Rhizoctonia* sps

Penicillium chrysogenum, *P.commune*, *P.citrinum* were recovered from set 3 plates on 10^{th} day whereas colonies of *Aspergillus niger*, *Cylindrocarpon* and *Rhizoctonia* appeared on 15^{th} day. (Fig 9).



Mean value±Standard Error

Fig. 9: No. of fungal species recovered from set 3 of plates on 5th, 10th, 15th and 20th days.

Penicillium chrysogenum, *P.commune*, *P.citrinum*, *Aspergillus niger*, *Cylindrocarpon*, *Rhizoctonia* are the fungal contaminants identified from curd in this study. *Penicillium* sps and *Aspergillus* comes under Trichocomaceae (division-Ascomycota), whereas *Cylindrocarpon* falls in Nectriaceae (Ascomycota) and *Rhizoctonia* in Ceratobasidiaceae (Basidiomycota). Previous reports infer association of all the three *Penicillium* sps and *Aspergillus niger* with food spoilage. *P.chrysogenum* is ubiquitous in indoor air, dust and damp building materials. It is prevalent in regions with temperate and subtropical climate and can even found growing on salted food products and a main responsible factor for the deterioration of food quality (Houbraken *et al*., 2011). *P.citrinum* has a worldwide distribution and is anamorphic and mesophilic in nature. *P.citrinum* is often seen on several fruits and occasionally on tropical spices and cereals (Olagunju *et al*., 2018). *P.commune* is commonly associated with spoilage of food materials such as cheese, meat, nuts, margarine, fermented sausages, yogurt, sour cream, lactose powder, and high fat-filling cakes (Pitt and Hocking, 2009). Interestingly, besides food products, *P. commune* has also been isolated from disposed used oil (Esmaeili and Sadeghi, 2014). Only few reports available regarding *Cylindrocarpon* and *Rhizoctonia* as food contaminants. In New Zealand, *Cylindrocarpon* is considered as one of the the main post harvest pathogens of Kiwi fruits (Burdon and Lallu, 2011). Generally, *Cylindrocarpon* is well known as a pathogen of several plants. Root-rot and rusty root diseases of ginseng crops are cause by *Cylindrocarpon destructans* var. *destructans* (Farh *et al*., 2018). Similarly, *Rhizoctonia* has a well reputation of causative organism of several serious plant diseases rather than a contaminant of food. *Rhizoctonia* is associated with seedling damping-off disease (Feher, 1993), root diseases in *Asparagus* and carrot (Blancard, 2012), dry and wet root rot disease of chickpea (Knights and Hobson, 2016), root rot disease of *Solanum sessiliflorum* (Duarte, 2011) and sheath blight of rice (Liang *et al*., 2015).

Several health complications were previously reported concomitant with consumption of fungal contaminated foods. Mok *et al*., (1997) reported *P.citrinum* infection causing fatal pulmonary and pericardial complications in an immuno-compromised host. The asexual spores produced by *P. chrysogenum* are considered as human allergens (Shen *et al*., 2003). *P.*

commune known to produce mycotoxins such as cyclopiazonic acid and regulovasine A and B and it is the only species capable of producing neurotoxins penitrem A and roquefortine (Wagener *et al*., 1980). *A. niger* also has harmful effects on animals. A few studies reported *A. niger* as potential causative agent of pneumonia (Person *et al*., 2010). *Cylindrocarpon* in human, is associated with post traumatic keratitis, athlete's foot, mycetoma following injury and peritonitis (Champa *et al*., 2013). *Rhizoctonia solani* is associated with extensive human mycosis (Kaore *et al*., 2012).

Aspergillus, *Fusarium*, *Alternaria*, *Penicillium*, *Cladosporium*, *Eurotium*, *Mucor*, *Rhizopus*, and *Emericella* are some of the common contaminants found in food (Kocic-Tanackov and Dimic, 2013). In the present work *Penicillium chrysogenum*, *P.commune*, *P.citrinum*, *Aspergillus niger*, *Cylindrocarpon* and *Rhizoctonia* were identified from curd (yoghurt). Fungi, besides being serious contaminants of agricultural products, deteriorate the quality of food stuffs as well. Secondary metabolites released from certain fungus are highly toxic and the syndromes caused are collectively called mycotoxicoses (Babic *et al*., 2017; (Kocic-Tanackov and Dimic, 2013). Mycotoxins from filamentous fungi such as Aspergillus, Fusarium and Penicillium are harmful to animals and humans. Mycotoxins namely Ochratoxin and aflatoxin are potent carcinogens. Fumonisins (mycotoxin) are associated with equine leuko-encephalomalacia and porcine pulmonary oedema syndrome. These toxins get into the body through the consumption of food contaminated by Fusarium sps (Adeyeye, 2016). Further, considering the fact that some mycotoxins are heat resistant augment the risk of food poisoning from the ingestion of fungal contaminated food particles (Kocic-Tanackov and Dimic, 2013).

CONCLUSION

Present study aimed at isolation and characterization of potent air borne fungal contaminants of curd from indoor environment. Fungal colonies developed on curd were streaked on sabouraud dextrose agar plates for developing pure cultures. Pure colonies of six fungus were obtained. Morphology based studies and cotton blue staining lead to the identification of the colonies as *Penicillium chrysogenum*, *P. citrinum*, *P. commune*, *Aspergillus niger*, *Cylindrocarpon* sps *and Rhizoctonia* sps. Previous reports shows most of these members have deleterious effect on normal human health conditions. The result evidenced that contamination of curd take place from fungal spores on exposure to atmosphere and the degree of contamination increases with duration of exposure. The toxicity resulting from the intake of contaminated food also vary depending upon the species involved. Thus it is recommended to restrain from the usage of curd which were exposed to external environment. Further research is needed to establish the extend of fungal contamination as a function of temperature and pH.

REFERENCE

1. Adeyeye, S.A.O. Fungal mycotoxins in foods: a review. Cogent Food Agric., 2016, 2(1): 1-11.

2. Al-Fadhal, F.A., AL-Abedy, A.N., Alkhafije, D.A. Isolation and molecular identification of Rhizoctonia solani and Fusarium solani isolated from cucumber (Cucumis sativus L.) and their control feasibility by Pseudomonas fluorescens and Bacillus subtilis. Egypt J Biol Pest Co., 2019, 29(47): 1-11.

3. Babic, M.N., Gunde-Cimerman, N., Vargha, M., Tischner, Z., Magyar, D., Veríssimo, C., Sabino, R., Viegas, C., Meyer, W., and Brandao, J. Fungal contaminants in drinking water regulation? a tale of ecology, exposure, purification and clinical relevance. Int J Environ Res Public Health., 2017, 14(6): 1-44.

4. Bandh, S.A., Kamili, A.N., and Ganai, B.A. Identification of some Penicillium species by traditional approach of morphological observation and culture. Afr J Microbiol Res., 2011, 5(21): 3493-3496.

5. Barcus, A.L., Burdette, S.D., Herchline, T. Intestinal invasion and disseminated disease associated with Penicillium chrysogenum. Ann Clin Microbiol Antimicrob., 2005, 4(1): 1-4.

6. Blancard D. Diagnosis of parasitic and nonparasitic disease, Tomato diseases. 2nd edition. Academic press, Netherland, 2012, Pp. 35-411.

7. Burdon, J., Lallu, N. Kiwifruit (Actinidia spp.). In: Yahia, E. (Ed.), postharvest biology and technology of tropical and subtropical fruits cocona to mango, vol. 3. Woodhead Publishing Series in Food Science, Technology and Nutrition, 2011, Pp. 326-360.

8. Champa, H., Sreeshma, P., Prakash, P.Y., Divya, M. Cutaneous infection with Cylindrocarpon lichenicola. Med Mycol Case Rep., 2013, 2: 55-58.

9. Duarte, O. 1 - Cocona (Solanum sessiliflorum Dunal). In: Yahia, E. (Ed.), postharvest biology and technology of tropical and subtropical fruits cocona to mango, vol. 3. Woodhead Publishing Series in Food Science, Technology and Nutrition, 2011, Pp. 1-7.

10. Ehgartner, D., Herwig, C., and Fricke, J. Morphological analysis of the filamentous fungus Penicillium chrysogenum using flow cytometry-the fast alternative to microscopic image analysis. Appl Microbiol Biotechnol., 2017, 101(20): 7675-7688.

11. Eidi, S., Kamali, S.A., Hajari, Z., Fata, A., Hosseini, R.F., Naseri, A., and Bakhshaee, M. Nasal and indoors fungal contamination in healthy subjects. Health Scope., 2016, 5(1): 1-5.

12. El-Diasty, E., and El-Kaseh, P. Microbiological monitoring of raw milk and yoghurt samples collected from El-Beida city. Arab J Biotechnol., 2008, 12: 57-84.

13. Esmaeili, A., Sadeghi, E. The efficiency of Penicillium commune for bioremoval of industrial oil. Int J Environ Sci., 2014, 11: 1271-76

14. Farh, M.E., Kim, Y., Kim, Y., Yang, D. Cylindrocarpon destructans/Ilyonectria radicicola-species complex: causative agent of ginseng root-rot disease and rusty symptoms. J Ginseng Res., 2018, 42(1): 9-15.

15. Feher, T. Watermelon: Citrullus lanatus (Thunb.), In: Kallo, G., Bergh, B.O. (Ed.), Genetic Improvement of vegetable crops. Pergamon Press, Oxford, U.K, 1993, Pp. 295-311.

16. Heritage, J., Evans, E.G.V., Killington, K.A. Introductory microbiology. Cambridge University Press, New York, Melbourne, 1996, Pp. 100.

17. Houbraken, J., Frisvad, J.C., and Samson, R.A. Taxonomy of Penicillium citrinum and related species. Fungal Diversity., 2010, 44: 117-133

18. Houbraken, J., Frisvad, J.C., Samson, R.A. Flemings's penicillin producing strain is not Penicillium chrysogenum but P. rubens. IMA Fungus., 2011, 2(1): 87-95.
19. Kaore, N.M., Atul, A.R., Khan, M.Z., Ramnani, V.K. A rare case of human mycosis by Rhizoctonia solani. Indian J Med Microbiol., 2012, 30(3): 361-363.
20. Kidd, S., Halliday, C., Alexiou, H., Ellis, D. Description of medical fungi. 3rd edition. Newstyle Printing, 41 Manchester Street, Mile End, South Australia, 2016, Pp. 23.
21. Knights EJ, Hobson KB. Chickpea Overview, Reference Module in Food Science, Elsevier publication, Australia, 2016.
22. Kocic-Tanackov, S.D., Dimic, G.R. Fungi and mycotoxins: Food contaminants. Hemijska Industrija., 2013, 67(4), 639-653.
23. Li, D., and Yang, C.S. Fungal contamination as a major contributor to sick building syndrome. Adv Appl Microbiol., 2004, 55: 31-112.
24. Liang, X., Xiao-han, W., Rui-ya, L., Shi-qiong, L., Ze-jian, G., Ming-an, W., Yang, L., Li-gang, Z. Secondary metabolites of rice sheath blight pathogen Rhizoctonia solani Kuhn and their biological activities. J Integr Agr., 2015, 14(1): 80-87
25. Mok, T., Koehler, A.P., Yu, M.Y., Ellis, D.H., Johnson, P.J., Wickham, N.W. Fatal Penicillium citrinum pneumonia with pericarditis in a patient with acute leukemia. J Clin Microbiol., 1997, 35(10): 2654-2656
26. Nagano, Y., Millar, B.C., Goilsmith, C.E., Walker, J.M., Elborn, S., Rendall, J., Moore, J.E. Development of selective media for the isolation of yeasts and filamentous fungi from the sputum of adult patients with cystic fibrosis (CF). J Cyst Fibros., 2008, 7(6): 566-572.

27. Olagunju, O., Mchunu, N., Venter, S., Guibert, B., Durand, N., Metayer, I., Montet, D. Fungal contamination of food commodities in Durban, South Africa. J Food Saf., 2018, 38(6): 1-10.
28. Pal, M. Spoilage of dairy products due to fungi. Bev Food World., 2014, 41(7): 37-40.

29. Person, K., Chudgar, S.M., Norton, B.L., Tong, B.C., Stout, J.E. Aspergillus niger: an unusual cause of invasive pulmonary aspergillosis. J Med Microbiol., 2010, 59: 834-838.
30. Pitt, J.I., Hocking, A.D. Fungi and food spoilage. Springer, Boston, MA, 2009, Pp. 401-421.
31. Sakai, A., Kawakami, K., Takatori, K., and Saito, Y. Foods with complaints of fungal contamination and physical problems caused by their ingestion. Shokuhin Eiseigaku Zasshi., 2004, 45(4): 201-206.

32. Udagawa, S. Fungal contamination of foods. Jpn J Med Mycol., 1979, 20(3): 171-176

33. Vijayakumar, R., Sandle, T., and Manoharan, C. A review on fungal contamination in pharmaceutical products and phenotypic identification of contaminants by conventional methods. Eur J Parenter Pharm Sci., 2012, 17(1): 4-18.

34. Wagener, R.E., Davis, N.D., Diener, U.L. Penitrem A and roquefortine production by Penicillium commune. Appl Environ Microbiol., 1980, 39(4): 882-887.
35. Zulkifli, N.A., and Zakaria, L. Morphological and molecular diversity of Aspergillus from corn grain used as livestock feed. Hayati J Biosci., 2017, 24: 26-34.