Fungal diversity associated with the commercial grades of walnut kernels sold in the markets of Jammu and Kashmir State (India)

¹,Rohini Sharma, ²,Geeta Sumbali Department of Botany, University of Jammu, Jammu-180006, India.

ABSTRACT: Three grades of walnut kernels viz., in-shell kernels, shelled half kernels and shelled broken kernels, were collected from various wholesale and retail shops of Jammu and Kashmir. Mycodiversity associated with these different grades were investigated from 90 market samples. A total of 95 fungal species representing 21 genera were recovered using surface washing technique. Of these, Zygomycota were represented by 5 genera (9 species), Ascomycota by 2 genera (2 species) and mitosporic fungi by 14 genera (84 species). Maximum number of fungal species (58) were recovered from the in-shell kernels, followed in decreasing order from shelled broken kernels (55 species) and shelled half kernels (49 species). Five measures of diversity are considered viz., species richness (S), Shannon-Wiener's diversity index (H'), Simpson dominance index (Cd), Pielou's evenness index (J') and Berger Parker's dominance index (d'). High values of these diversity indices for fungal species was obtained which might be due to the surplus substrate, ambient temperature and humidity, which favour growth and multiplication of fungal species. Presence of ample nutrients and similar kind of substrate, that is, walnut kernel produced nearly similar kind of fungal diversity in all the three grades that were investigated.

KEY WORDS: Diversity indices, walnuts kernels, fungal species, Juglans regia.

I. INTRODUCTION

Walnut (Juglans regia L.) is an important tree nut, which belongs to the angiospermic family Juglandaceae. Every part of the plant has some utility and as such it has carved a special place in socio-religious and economic well being of the people. India is the 8th largest producer of walnut in the world and J&K stands first in the country, accounting for 92% of the production. The major walnut growing states of India are Jammu and Kashmir, Uttarakhand, Himachal Pradesh and Arunachal Pradesh. Among these, Jammu and Kashmir occupies the largest share in total area and production. In J&K, cultivation of walnut is common in Bhaderwah, Poonch, Rajouri, Kupwara, Baramulla, Bandipora, Ganderbal, Budgam, Srinagar, Anantnag and other temperate areas.The kernel is the edible part of the walnut, which is enclosed in a brown seed coat that contains antioxidants to protect the oil rich seed from atmospheric oxygen to prevent rancidity. It is rich in fats, proteins, minerals, vitamins and a substantial quantity of dietary fibres and is therefore, a concentrated source of energy. It contains about 60% oil, which may vary depending upon the location and cultivar [1]. The oil of walnut kernel contains major fatty acids, such as oleic acid, linoleic acid and linolenic acids, which are widely used in cosmetic industry as it contains moisturizing and antioxidants properties [2]. Considerable interest has been generated in walnut kernel as it is believed to possess plasma cholesterol-lowering properties [3]. Traditionally, walnut is used to treat cough, stomach ailments and cancer in Asia and European countries [4]. In addition, walnut is a popular ingredient of the baked foods and are frequently served in-shell during fall and winter seasons.

Walnuts are available in Indian markets in two forms. These are in-shell walnuts (kernel inside the shell) and shelled walnuts (kernels obtained by shelling whole walnuts). Being a dry fruit, walnut has an inherent potential for prolonged storage. However, its shelf- life is governed by the physical characteristics of in-shell nuts, moisture content of the kernels and microbial status of the kernels after shelling. Porous shell morphology, corky suture and loose seal provide an easy entry for microorganisms and acts as foci of infection in storage and thus reduces its shelf life potential. Among the various microbes, fungi are known to play a significant role in the spoilage and loss of stored plant products [5]. During the storage period, the fungal organisms thrive well on dehydrated commodities by utilizing some active ingredients like phenols, alkaloids and proteins [6]. Among the various fungi associated with dried commodities, xerophilic fungi are capable of growing at a water activity below 0.85 and are, therefore most important spoilage causing organisms [7]. Generally, species of *Aspergillus* and *Penicillium* exist in maximum number in low water activity habitats [8].Contamination of walnuts by fungi may occur at three different stages. In the first instance, it may occur prior to harvesting.

At this stage, particularly when the nuts have ripened and the hulls have opened, they are often attacked by air-borne and insect-borne spores of fungal species. The second stage of contamination may occur after harvesting, when the nuts are de-hulled, washed, dried and graded. The washing water can be a source of contamination, and if the nuts are allowed to remain wet for a long period, they may become highly susceptible to mould growth and their toxic contaminants. The third stage of contamination can be in storage, especially when nuts are packed and stored under adverse conditions of temperature and relative humidity. Infact, postharvest operations are expected to have major influence on the microbial contamination of nuts [9]. Realizing the fact that post-harvest fungal contamination of dried walnut kernels has not received the attention that the magnitude of the problem warrants and since no such work has been attempted from Jammu and Kashmir State, which is a large reservoir of medicinal plants, an investigation was undertaken to report the occurrence and diversity indices assessment of fungal species associated with different grades of walnuts marketed in Jammu and Kashmir State.

II. MATERIALS AND METHODS

Samples of different walnut grades were collected in pre-sterilized polythene bags from various wholesale and retail shops of Jammu and Kashmir. The sample bags were brought to the laboratory, sealed and kept in the refrigerator at 5-7°C to prevent undesirable changes till further studies were conducted. Fungal spores associated with the market samples were recovered by following the method of [10]. In this method, 5g sample was taken in an Erlenmeyer flask (250 ml capacity), containing 45ml sterilized distilled water and shaken vigorously on a rotary shaker for 30 minutes to obtain a homogenous suspension. Ten fold serial dilutions were prepared and 1ml portion of suitable dilution was poured in petriplates by using a sterilized pipette. For recovery of maximum number of fungal propagules from each sample, three different media-modified Czapek Dox agar (CDA), dichloran 18% glycerol agar (DG-18) and malt salt agar (MSA) were used. For each of these three media, 5 replicates were maintained. The medium was poured by making a gentle rotational movement of the petriplates to ensure uniform spreading of the sample. These petriplates were incubated for 7 days at $28\pm2^{\circ}$ C. After incubation, the colonies were counted and the results were expressed as average colony forming units in thousands per gram of the sample (10^{4} cfu/g) using the following formula [11]: $cfu/g = a \times d$

s _____s

where, a = average number of colonies on the petriplates, d = dilution factor (10,000) and s = dry weight of the sample.

Percentage frequency of occurrence (%) was calculated by using the formula: Frequency (%) = <u>Number of samples from which an organism was isolated</u> X 100 Total number of samples tested

The data was used in making comparison of different diversity indices as shown below. Shannon-Wiener's index (H') [12] and Simpson's Dominance index (Cd) [13] were calculated to determine the heterogeneity of fungal diversity. Pielou's evenness index (J') [14] and Berger- Parker Dominance index [15] were also calculated to augment the interpretation of Shannon values.

Diversity indices	Formula used
Shannon–Wiener's index (<i>H'</i>)	$-\sum_{i=1}^{s} p_i \ln p_i$
Simpson's dominance index (Cd)	$\sum_{i=1}^{s} (p_i)^2$
Pielou's evenness index (<i>J</i> ')	$\frac{H'}{H'_{\rm max}}$
Berger- Parker dominance index (d')	$\frac{n}{N}$

Where, pi is the relative importance value of species i; H max is the maximum value of H; n = number of individuals in a species and N = total number of individuals of all species.

III. RESULTS

During the period of investigation, a total of 90 market samples of walnut kernels were procured from different markets of Jammu and Kashmir State. These samples were screened for the mycobial load by using surface washing technique and three media (Czapek Dox agar, dichloran 18% glycerol agar and malt salt agar) of different chemical composition. While recovering surface mycoflora, Czapek Dox agar medium trapped maximum number of fungal species, dichloran 18% glycerol agar medium helped to recover a wide range of non-fastidious xerophilic fungi including most of the Penicillium and Aspergillus species, whereas, malt salt agar was more useful in recovering the members of Aspergillus glaucus group. This indicates that nutritional requirements of various fungi differ and there is no single medium, which can help in the recovery of all the fungi. During the present investigation, a total of 95 fungal species belonging to 21 genera were recovered. Of these, Zygomycota was represented by 5 genera (9 species), Ascomycota by 2 genera (2 species) and mitosporic fungi by 14 genera (84 species) (Fig. 1). Mycological survey of the in-shell kernels from J&K state showed an association of 58 fungal species belonging to 16 genera. Out of these, Zygomycota was represented by 5 genera (7 species), Ascomycota by 2 genera (2 species) and mitosporic fungi by 9 genera (49 species). Shelled half kernels of walnuts collected from the markets revealed an association of 19 fungal genera (49 species), of which, Ascomycota was represented by 2 genera (2 species), Zygomycota by 4 genera (6 species), whereas mitosporic fungi was represented by 13 genera (41 species). Shelled broken kernels showed contamination by 15 fungal genera (55 species). Zygomycota were represented by 2 genera (5 species), Ascomycota by 1 genus (1 species) and mitosporic fungi by 11 genera (49 species). The percent colonisation frequency of all the grades is shown in table 1. Recovery of a large number of fungal species indicates that dried walnuts provide ample nutrients to the surface invading fungi. Earlier, few other mycologists have also detected a variety of mycoflora from walnuts and other tree nuts of India [16] [17] [18]. Similarly, from other countries also some researchers have reported various fungal species from the kernels of walnuts and other nuts [19] [20] [21]. However, there is no previous work on the mycobial load of walnut kernels from Jammu and Kashmir State.

During the present study, most of the fungal species recovered from dried walnut kernels were mesophilic, that is, capable of growing between the temperature range of 10-40°C, showing optimum growth at 15-30°C. However, some of the recovered fungal species were thermo-tolerant, that is, capable of growth within the mesophilic range. These fungi included Aspergillus terreus, A. fumigatus, A. flavus, A. ochraceus, Paecilomyces variotii and Scopulariopsis brevicaulis. Many of the fungal species recovered during the present investigation were moderately xerophilic. Among these, Aspergillus and Penicillium species were the most dominant represented by 25 and 34 species respectively. Some of the common xerophilic aspergilli and penicilli recovered from the walnut samples included Aspergillus flavus, A. niger, A. japonicus, A. fumigatus, A. ochraceus, A. versicolor, A. tamarii, A. sydowii, Penicillium brevicompactum, P. chrysogenum, P. citrinum, P. fellutanum, P.griseofulvum and P.waksmanii. Dominance of Aspergillus and Penicillium species from dried fruits and nuts has also been reported earlier also [16] [22]. Species of Aspergillus and Penicillium are considered versatile in their water activity (a_w) requirements and these are most numerous in low a_w habitats [23]. Aspergilli and penicilli grow on a large number of substrates and their ability to thrive at high temperatures (30-40°C) and relatively low available water (xerophilic nature) makes them well suited to colonize a number of nut crops. Previous study conducted by Nawar [24] has shown that relative humidity plays a vital role in the development and spread of fungal contamination. Infact, fungal contamination rate may also vary as a result of adverse pre-harvest conditions of temperature and humidity in the field and improper post-harvest handling and storage conditions [25]. The increase in the frequency of Aspergillus can also be explained by the fact that this genus is a storage fungus that can grow well at low moisture content [26].

From the present investigation, it was found that colonization frequency (CF%) was maximum for *Aspergillus* in all the grades. Earlier, few other researchers have also reported black aspergilli as one of the most important food contaminant especially involved in the post-harvest decay of nuts and dried fruits [27] [28]. This is probably due to their pH tolerance, fast growth and abundance in many environments. The presence of *Aspergillus* in walnuts is considered serious since this fungus initially colonizes the substrate and then results in aflatoxin B_1 and B_2 production [29]. *Aspergillus* was followed in decreasing order by *Penicillium* species. Among the three grades, highest colonization frequency was found in case of in-shell kernels followed in decreasing order by shelled broken kernels and shelled half kernels of walnuts. The reason for highest fungal contamination level in the in-shell kernels may be due to wash water, which acts as a source of *A.flavus* contamination and it appeared in some nuts that the infection took place even prior to harvesting and was deep seated [30]. Mean number of colony forming units (cfu/g) of fungal species for all the grades was also calculated (Table 1).

During the present investigation, mycological analysis revealed that the most frequently recovered fungal genus from all the grades of walnut kernels was Aspergillus with spore count ranging from 0.3x10²- 50.8×10^2 cfu/g. Earlier, Sejiny et al [31] also reported Aspergillus as the predominant fungus in 41-50% of the unshelled nuts with a total count of 3x10³cfu/g. Later, Adebajo and Diyaolu [32] found A. niger, A. flavus, and A. fumigatus as the predominant aspergilli with a total spore count of 80-16,300 cfu/g in cashew nuts from Nigeria. Similarly, Lubna [33] found A. niger and A. flavus associated with pistachio nuts with spore count of 2.5×10^2 and 1.25×10^2 cfu/g respectively. Recently, Nadjet et al [34] found incidence of Aspergillus flavus from peanuts with mean value count ranging from 1.5×10^2 to 5.7×10^2 cfu/g. Colony forming units of *Penicillium* species were comparatively lesser than that of Aspergillus species and were in spore count range of 0.3×10^2 - 39.4×10^2 cfu/g. Nadjet et al [34] also revealed a high contamination level of *Penicillium* species from peanuts, with total number of viable propagules varying between 4.74x10²-5.77x10² cfu/g.Besides aspergilli and penicilli, some other xerophiles recovered from dried walnut kernels included Emericella nidulans, E. nidulans var. echinulatus and Paecilomyces variotii. These species are usually the first colonizers of dried commodities and are considered to be the most destructive as they increase the a_w of the substrate and allow other fungal species to grow [24]. Dried fruits and tree nuts typically have one or more pre-harvest insect pests that feed directly on the product and are capable of causing considerable damage and quality loss. Many of these may be present at the time of harvest and are often brought into storage [35]. Feeding damage by these insects also provides entry to many moulds, some of which may be toxin producers [36] [37].

IV. DISCUSSION

From these results, it was observed that differences in the number and type of fungal species associated with the three grades of walnut kernels may be due to the fact that the shelled half kernels and shelled broken kernels undergo processes like sun-drying and sorting before marketing, which leaves behind lesser number of contaminated kernels. However, differences detected in the mycoflora of shelled half kernels and shelled broken kernels might be due to the fact that broken grade of walnut kernels provide larger surface area for the colonization of fungal species than that of the shelled half kernels. Perusal of results also show that the maximum number of fungal species were associated with the in-shell grade of walnuts as it provides natural congenial atmosphere for the growth of fungal species on the kernels within the shell. In addition, temperature and humidity are also maintained within the shell, which favour the growth and multiplication of a large number of fungal species. Freire et al [38] considered that the penetration, spore germination and infection of nuts may happen even during inflorescence or during seed formation, suggesting invasion and colonisation of the premature tissues. Similarly, Bayman et al [9] also noted internal colonisation of Brazil nuts by fungal species like Aspergillus flavus, A.tamarii, A. niger, A. nidulans, A. fumigatus, Penicillium species and Rhizopus species and concluded that the internal colonisation rate in Brazil nuts was much higher than in other tree nuts. It is possible that a similar situation may occur in walnuts also, which may add to fungal invasion and kernel deterioration.Diversity indices computed for the fungal species recovered from the in-shell kernels, shelled half kernels and shelled broken kernels are given in table 2. These diversity indices show differences in values of species richness (S), Shannon-Weiner's diversity index (H'), Simpson dominance index (Cd), Pielou's evenness index (J') and Berger-Parker's dominance index (D).

Among all the grades, highest species richness (S) and Shannon-Weiner's diversity (H) was recorded from in-shell kernels (S=58 species and H = 1.683), closely followed by shelled broken kernels (S=55 species and H'=1.670), whereas least values were observed for shelled half kernels (S=49 species and H'=1.617). These values clearly indicate high diversity of fungal species in the in-shell walnuts. This may be probably due to the ambient temperature and humidity, which favour growth and multiplication of fungal species inside the shell. Insect infestation inside some walnut shells may also selectively favour colonisation and metabolism of some fungi. In addition, mycobial interaction creates microclimate inside the shell of the walnut, which may favour high species richness. Simpson's dominance values are very low (Cd=0.24 for both in-shell and shelled broken kernels and Cd=0.27 for shelled half kernels). These low values of dominance imply that no particular fungal species is dominant in the investigated samples and there is more homogeneity of fungal species recovered from all the grades of walnuts. This homogeneity in mycoflora is attributed to similar kind of substrate that is available to the fungal species for growth as well as similar storage conditions prevailing in the domestic market. Maximum evenness value was shown by the shelled broken kernels (J'=0.96), which implies that most of the mycoflora recovered from the shelled broken kernels also occurred in the other two grades of walnuts. Thus, there is little variation in the fungal species detected from broken kernels.Berger-Parker's values range from 0.059 (shelled broken kernels) to 0.067 (shelled half kernels). These values indicate that the proportionate representation of different species of a particular fungal genus is maximum in shelled half kernels, whereas in the other samples, there is relatively more representation of Aspergillus and Penicillium species than species of other fungal genera, which lead to low Berger-Parker's values of these samples.

V. CONCLUSION

In the present investigation, a large number of fungal contaminants harbouring all the three grades of kernels, clearly indicates that they are not completely safe for human consumption as these contaminant may produce mycotoxins during post-harvest storage. In view of the fungal contaminants detected from the market samples, an urgent need for proper storage and packing of these walnut kernels in polythene bags or preferably in airtight containers and vacuum packs after microwave treatment is recommended.

VI. ACKNOWLEDGEMENT

The authors are thankful to Head, Department of Botany, University of Jammu for providing laboratory facilities.

REFERENCES

- [1] RBN Prasad, Walnuts and pecans. In Encyclopaedia of Food Science, Food Technology and Nutrition, *London: Academic Press*. 1994,4828-4831.
- [2] JC Espin, C Soler-Rivas, HJ Wichers, and C Garcia-Viguera, Anthocyanin-based natural colorants: anew source of antiradical activity for foodstuff, J Agric Food Chem. 48, 2000, 1588-1592.
- [3] J Sabate, GE Fraser, K Burke, S Knutsen, H Bennett, and KD Lindsted, Effect of walnut on serum lipid levels and blood pressure in normal men, *New England J. Med*, *328*, 2003, 603-607
- [4] T Fakuda ,H Ito and T Yoshida , Antioxidative polyphenols from walnuts (*Juglans regia* L.), *Phytochem*, 63, 2003, 795-801.
- [5] CM Christensen, and HH Kaufmann, Storage of cereal grains and their products. (Am. Assoc. Cereal Chem. St. Paul. Minnesota, 1974 pp. 549).
- [6] AK Roy, Mycological problems of crude herbal drugs: overview and challenges, *Indian Phytopathol 56*, 2003,1-13.
- [7] JI Pitt, *Xerophilic fungi and the spoilage of foods of plant origin*. (In: Water relations of food (Ed. Duckworth RB). Academic Press, London, 1975, pp. 273-307).
- [8] N Magan, Mycotoxin contamination of food in Europe: Early detection and prevention strategies, *Mycopathology*, 162, 2006, 245-253.
- [9] P Bayman, JL Baker, and N Mahony, *Aspergillus* on tree nuts: incidence and associations. *Mycopathology*, 155, 2002,161-169.
- [10] W Harrigan, *Laboratory methods in food microbiology*. (Academic Press, San Diego, 1998 pp. 359-375).
 [11] SC Parikh, and HR Shah, Soil microbial diversity and sustainability of some agricultural area of the Sabarkantha district
- (North Gujarat region), *Adv. Biosci. and Biotec, 5*, 2006, 19-24.
- [12] CE Shannon, and W Wiener, *The Mathematical Theory of Communities*. University of Illinois Press, Urbana, 1963 pp. 117.
- [13] EM Simpson, Measurement of diversity. *Nature*, *163*, 1949, 688.
- [14] EC Pielou (1975), *Ecological Diversity*. Wiley, New York, 1975.
 [15] WH Berger, and FL Parker, Diversity of planktonic foraminifera in deep-sea sediments. *Science*,
- [15] WH Berger, and FL Parker, Diversity of planktonic foraminifera in deep-sea sediments. *Science*, *168*, 1970, 1345-1348.
- [16] P Sekar, N Yumnam, and K Ponmurugan, Screening and characterization of mycotoxin producing fungi from dried fruits and grains. Adv. Biotech, 2008, 12-15.
- [17] M Javanmard, Occurrence of mould counts and Aspergillus species in Iranian dried figs at different stages of production and processing, J. Agr. Sci. Tech. 12, 2010, 331-338.
- [18] PK Singh, Assessment of mycotoxins in edible tree borne oil seeds (TBOS), J. Food. Res, 1, 2012, 91-101.
- [19] YH Leong, N Ismail, AA Latif, and R Ahmad, Aflatoxin occurrence in nuts and commercial nutty products in Malaysia, Food Contr. 21, 2010, 334-338.
- [20] AA Saddiq, and SA Kalifa, Impact of fungal contents of some Arabic nuts to induce kidney toxicity and agonistic action of natural resources, *Afr. J. Microbiol. Res. 5*, 2011, 1046-1056.
- [21] MI Nejad, and AD Farahani, Aflatoxins in raw walnut kernels marketed in Tehran, Iran, Food Add. Contam, 5, 2012, 8-10.
- [22] A Kumar, R Shukla, P Singh, B Prakash, and NK Dubey, Chemical composition of *Ocimum basilicum* L. essential oil and its efficacy as a preservative against fungal and aflatoxin contamination of dry fruits, *Int. J. Food Sci. and Tech.* 46, 2011, 1840-1846.
- [23] AD Hocking, *Xerophilic fungi in intermediate and low moisture foods*. (In: Handbook of applied mycology. Vol. 3. Foods and feeds. (eds. Arora D.K., Mukerji, K.G. and Marth, E.H.), Marcel Dekker Inc, 1991).
- [24] LS Nawar, Prevention and control of fungi contaminated stored pistachio nuts imported to Saudi Arabia, *Saudi J. Biol. Sci,15*, **2008**, 105-112.
- [25] VK Nakai, LD Rocha, E Goncalez, H Fonseca, EMM Ortega, and B Correa, Distribution of fungi and aflatoxins in a stored peanut variety, *Food Chem. 106*, 2008, 285-290.
- [26] MT Hedayati, S Kaboli, and S Mayahi, Mycoflora of pistachio and peanut kernels from Sari, Iran. Jundi J. Microbiol. 3, 2010, 114-120.
- [27] JI Pitt, and AD Hocking, Fungi and Food spoilage 2nd ed, (Blackie Academic and Professional publishers, London.1997).
- [28] G Perrone, A Susca, G Cozzi, K Ehrlich, and J Vargas, Biodiversity of Aspergillus species in some important agricultural products, Students Mycol. 59, 2009, 53-66.
- [29] UL Diener, RJ Cole, and RA Hill, Epidemiology of aflatoxin formation by Aspergillus flavus. Ann. Rev. Phytopathol. 25, 1987, 249-270.
- [30] H Mojtahedi, D Danesh, B Haghighi, and RC Barnett, Post harvest pathology and mycotoxin contamination of Iranian pistachio nuts, *Phytopathol.* 68, 1987,1800-1804.
- [31] MJ Sejiny, FM Thabet, and MK Elshaieb, Microbiol contamination of various nuts stored in commercial markets in Jeddah, *J.K.A.H. Sci.* 1, 1989, 61-71.
- [32] LD Adebajo, and SA Diyaolu, Mycology and spoilage of retail cashew nuts, Afr. J. Biotechnol ,2, 2003, 369-373.
- [33] SN Lubna, Prevention and control of fungi contaminated stored pistachio nuts imported to Saudi Arabia, *Saudi. J. Biol. Sci 15*, 2008, 105-112.
- [34] GT Nadjet, N Bouras , and S Mokrane , Aflatoxigenic strains of *Aspergillus* section Flavi isolated from marketed peanuts (*Arachis hypogaea*) in Algiers (Algeria), *Ann. Microbiol*, 63, 2013, 295-305.
- [35] JA Johnson, PV Vail, DG Brandl, JS Tebbets, and KA Valero, Integration of non-chemical treatments for control of post harvest pyralid moths (Lepidoptera: Pyralidae) in almonds and raisins, *J. Economic Entomol*, *95*, 2002, 190-199.

- BE Campbell, RJ Molyneux, and TF Schatzki, Current research on reducing pre- and post-harvest aflatoxin contamination of US almond, pistachio and walnut, *Tox. Rev.* 22, 2003, 225-266. RJ Molyneux, N Mahoney, JH Kim, and BC Campbell, Mycotoxins in edible tree nuts, *Int. Food Microbiol*, 119, 2007, 72-[36]
- [37] 78.
- FDCO Freire, Z Kozakiewicz Z, and RRM Paterson, Mycoflora and mycotoxins in Brazilian black pepper, white pepper and Brazil nuts, *Mycopathol, 149,* 2000, 13-19. [38]

Table 1: C	olonization fr	requency (CF%) a	nd colony for	ming units (cfu/	/g) of the	recovered	fungal species
------------	----------------	------------------	---------------	------------------	------------	-----------	----------------

Europi genero	In-shell kernels		Shelled half kernels		Shelled broken kernels	
recovered	Frequency (%)	cfu/g	Frequency (%)	cfu/g	Frequency (%)	cfu/g
ZYGOMYCOTA						
Absidia corvmbifera	10.0	2.0×10^2	-	-	-	-
Circinella simplex	17.0	0.8×10^2	13.0	$0.7 \text{ x} 10^2$	-	-
Mucor hiemalis	7.0	1.4×10^2	23.0	$2.6 \text{ x} 10^2$	7.0	$1.2 \text{ x} 10^2$
Mucor microsporus	-	-	17.0	3.9x10 ²	23.0	3.8×10^2
M. racemosus	20.0	4.3×10^2	-	-	-	-
Rhizopus microsporus	-	-	-	-	7.0	$1.4 \text{ x} 10^2$
R. oryzae	40.0	9.0×10^2	17.0	$1.8 \text{ x} 10^2$	27.0	$1.8 \text{ x} 10^2$
R. stolonifer	33.0	6.7×10^2	17.0	11.2×10^2	20.0	$4.9 \text{ x} 10^2$
Syncephalastrum racemosum	23.0	2.5×10^2	17.0	$23.0 \text{ x} 10^2$	-	-
АЗСОМУСОТА						
Chaetomium globosum	37.0	6.3×10^2	17.0	$1.6 \text{ x} 10^2$	-	-
<i>Emericella nidulans</i> var.	-	-	27.0	$4.8 ext{ x10}^2$	-	-
echinulatus						
Emericella nidulans	20.0	3.0×10^2	-	-	13.0	$10.3 \text{ x} 10^2$
MITOSPORIC FUNGI						
Acremonium implicatum	-	-	13.0	0.8×10^2	-	-
Alternaria alternata	37.0	8.2x10 ²	33.0	$9.7 \text{ x} 10^2$	27.0	$2.5 \text{ x} 10^2$
Aspergillus candidus	17.0	3.1×10^2	-	-	27.0	$1.0 \text{ x} 10^2$
A. carneus	14.0	0.6×10^2	3.0	0.3×10^2	36.3	$27.5 \text{ x} 10^2$
A.chevalieri	-	-	-	-	17.0	$1.9 \text{ x} 10^2$
A. clavatus	14.0	0.6×10^2	10.0	$0.7 \text{ x} 10^2$	-	-
A. ficuum	-	-	13.0	$0.7 \text{ x} 10^2$	-	-
A. flavipes	-	-	7.0	$5.6 \text{ x} 10^2$	10.0	$1.6 \text{ x} 10^2$
A. flavus	53.0	37.7×10^{2}	40.0	$15.5 \text{ x} 10^2$	33.0	$5.6 \text{ x} 10^2$
A. flavus var. columnaris	23.0	$11.7 \text{x} 10^2$	-	-	-	-
A. fumigatus	17.0	1.3×10^2	17.0	3.7×10^2	30.0	$4.0 \text{ x} 10^2$
A. glaucus	13.0	$15.4 \text{x} 10^2$	-	-	-	-
A. japonicus	33.0	4.6×10^2	13.0	$1.4 \text{ x} 10^2$	17.0	$4.8 ext{ x10}^2$
A. niger	63.0	50.8×10^2	50.0	21.7×10^2	43.0	$23.8 \text{ x} 10^2$
A. ochraceus	20.0	2.1×10^2	23.0	$7.1 \text{ x} 10^2$	17.0	$1.0 \text{ x} 10^2$
A. parasiticus	-	-	17.0	$5.5 \text{ x} 10^2$	-	-
A. oryzae	17.0	2.5×10^2	-	-	-	-
A. penicilloides	7.0	23.0×10^2	-	-	-	-
A. subolivaceus	-	-	-	-	17.0	$4.3 \text{ x} 10^2$
A. sulphureus	7.0	$0.8 \text{ x} 10^2$	-	-	-	-
A. sydowii	17.0	$5.6 \text{ x} 10^2$	7.0	$5.1 \text{ x} 10^2$	-	-
A. tamari	-	-	10.0	$0.8 \text{ x} 10^2$	17.0	$3.3 \text{ x} 10^2$
A. terreus	17.0	19.0×10^2	-	-	-	-
A. terricola var. americana	-	-	10.0	$0.6 \text{ x} 10^2$	-	-
A. terricola var.indicus	-	-	-	-	7.0	$0.6 \text{ x} 10^2$
A.tubingensis	-	-	-	-	10.0	$1.3 \text{ x} 10^2$
A. ustus	13.0	$0.9 \text{ x} 10^2$	0.8	$0.8 \text{ x} 10^2$	10.0	$3.5 \text{ x} 10^2$
A. versicolor	17.0	$1.6 \text{ x} 10^2$	-	-	13.0	$8.6 \text{ x} 10^2$
A. wentii	-	-	17.0	$2.1 \text{ x} 10^2$	-	-
Cladosporium oxysporum	10.0	3.3×10^2	-	-	7.0	$0.4 \text{ x} 10^2$
C. cladosporioides	10.0	14.0×10^2	33.0	$7.6 \text{ x} 10^2$	10.0	$1.8 \text{ x} 10^2$
C. sphaerospermum	10.0	$4.4 \text{ x} 10^2$	-	-	7.0	$1.6 \text{ x} 10^2$

Chunata					13.0	2.0×10^2
Cumularia nallosoons	-	-	-	- 4.5 x 10 ²	15.0	2.0 X10
Drechslorg gustralionsis	- 12.0	- 0.5 v 10 ²	20.0	4.5 X10	- 7.0	- 0.6 x 10 ²
Directistera austratiensis	13.0	0.5 x10	-	$\frac{1}{1.0 \times 10^2}$	10.0	1.0×10^2
Dindwattensis	- 17.0	-	10.0	1.0×10^{2}	10.0	1.0×10^2
Fusarium pattaoroseum	17.0	0.4×10^2	7.0	1.0×10^2	7.0	1.5×10^{2}
F. solani	13.0	3.4×10^2	20.0	1.9 x10	10.0	0.9 x10
F. moniliforme var.	10.0	2.6 X10	-	-	-	-
Subglutinans E wartigillgidge					12.0	1.8×10^2
F.verilcilloides	-	-	- 7.0	-	15.0	1.6 X10
D famin agus	-	-	7.0	0.2 X10	-	- 1.0 v 10 ²
Provilomnoog lilgoinng	17.0	1.8×10^2	-	-	10.0	1.0 X10
Paecilomyces illacinus	10.0	1.8 X10	-	-	-	-
P. variolii	- 17.0	-	10.0	1.0×10^2	-	- 0.5 v 10 ²
	17.0	0.9×10^{2}	7.0	1.0 X10	15.0	0.5 x10
Penicillium arenicola	10.0	$7.2 \times 10^{-10^2}$	-	-	-	-
P. aurantiogriseum	7.0	0.6×10^{-10}	-	-	7.0	$2.2 \times 10^{-10^2}$
P. brevicompactum	37.0	36.3x10 ⁻	23.0	39.4 x10 ⁻	27.0	28.4 x10 ⁻
P. canescens	3.0	0.7×10^{2}	-	-	-	-
P. chrysogenum	33.0	14.5x10 ²	23.0	19.5 x10 ²	20.0	17.0 x10 ²
P. citrinum	27.0	19.9x10 ²	17.0	16.2×10^2	20.0	10.9 x10 ²
P.corylophilum	-	-	13.0	6.6 x10 ²	-	-
P. expansum	3.0	0.8×10^2	-	-	-	-
P. fellutanum	23.0	$5.5 \text{ x} 10^2$	7.0	16.4 x10 ²	10.0	2.2×10^2
P.fennelliae	-	-	-	-	7.0	$5.8 \text{ x} 10^2$
P. griseofulvum	20.0	$4.2 \text{ x} 10^2$	13.0	$3.3 \text{ x}10^2$	-	-
P.griseoroseum	-	-	-	-	7.0	$1.5 \text{ x} 10^2$
P.granulatum	7.0	$1.9 \text{ x} 10^2$	-	-	-	-
P.hirsutum	-	-	-	-	7.0	$1.1 \text{ x} 10^2$
P.islandicum	-	-	-	-	10.0	$3.4 \text{ x} 10^2$
P. italicum	7.0	$7.5 \text{ x} 10^2$	-	-	-	-
P.janczewskii	-	-	3.0	$1.5 \text{ x} 10^2$	-	-
P. daleae	7.0	$0.3 \text{ x} 10^2$	-	-	-	-
P. melinii	3.0	$0.6 \text{ x} 10^2$	-	-	-	-
P.miczynskii	-	-	7.0	$1.6 \text{ x} 10^2$	7.0	$0.9 \text{ x} 10^2$
P. olivicolor	7.0	$9.7 ext{ x10}^2$	-	-	-	-
P. olsoni	17.0	$1.3 \text{ x} 10^2$	-	-	13.0	$0.8 \text{ x} 10^2$
P. oxalicum	10.0	17.3×10^2	-	-	7.0	$5.5 \text{ x} 10^2$
P.paxilli	-	-	-	-	7.0	$5.6 \text{ x} 10^2$
P.piceum	-	-	3.0	$0.36 \text{ x} 10^2$	3.0	$0.26 \text{ x} 10^2$
P.pinophilum	-	-	-	-	20.0	$2.3 \text{ x} 10^2$
P. puberulum	7.0	$1.9 \text{ x} 10^2$	-	-	13.0	$3.2 \text{ x} 10^2$
P. purpurogenum	10.0	$5.7 \text{ x} 10^2$	-	-	-	-
P.restrictum	-	-	-	-	10.0	$1.8 \text{ x} 10^2$
P. variabile	-	-	13.0	$4.6 \text{ x} 10^2$	-	-
P.velutinum	-	-	10.0	$0.5 \text{ x} 10^2$	3.0	$1.9 \text{ x} 10^2$
P.verrucosum	-	-	10.0	$0.7 \text{ x} 10^2$	7.0	$2.9 \text{ x} 10^2$
P.viridicatum	-	-	-	-	7.0	$0.8 \text{ x} 10^2$
P. waksmanii	33.0	$4.5 ext{ x10}^2$	23.0	$25.8 \text{ x} 10^2$	-	-
Phoma glomerata	20.0	0.5×10^2	-	-	13.0	$1.1 \text{ x} 10^2$
Scopulariopsis brevicaulis	13.0	1.9×10^2	-	-	13.0	2.0×10^2
S.brumptii	-	-	10.0	0.8×10^2	-	-
S. candida	7.0	0.5×10^2	-	-	7.0	0.3×10^2
Trichoderma harzianum	-	-	-	-	7.0	1.3×10^2
T viride	-	-	7.0	0.3×10^2	-	-
Trichothecium roseum	-	-	10.0	1.0×10^2	-	-
2. ienomeenum roseum	1	1	10.0	1.0 ATO	1	1



Figure Legends

Figure 1: Fungal groups recovered from market samples of dried walnut kernels of different grades.