

Influence Of Post-Harvest Fungi On Biochemical Changes Of Selected Edible Nut Seeds Stored In Commercial Markets In Marathwada

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ABSTRACT

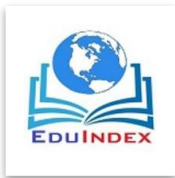
The role of fungi which were associated with edible nuts and their influence on change in biochemical studies have been carried on some widely consumed nuts namely, almond, cashew nut and pistachio nut which were collected from different market places of Marathwada region of Maharashtra. Fifteen samples of each nut were utilized for isolation of fungi by using. It was possible to isolate, the fifteen fungal species belonging to six genera. Percent frequency of fungal incidence was varied in almond, cashew nut and pistachio nut from which highest count of *Aspergillus niger* was observed in pistachio nut followed by cashew nut (100% and 73% respectively) and *Rhizopus* species was found about 80% in almond.

Ten species of isolated fungi were utilized in order to know their role in biochemical change in proteins, fats and carbohydrates in almond, cashew nut and pistachio nut. The results were remarkable to note that the highest degradation of protein, fat and carbohydrate were found in cashew nut as compared with almond and pistachio nut.

Keywords:- *Aspergillus niger*, nuts, post-harvest, *Rhizopus* species.

Introduction

Nuts are used in many industries as a raw material as well as for a direct consumption. They contain an important amount of protein and fat and their products have wide acceptance as food throughout the world. Globally, the most popular and commercially important edible nuts are peanuts (*Arachis hypogaea*), almond (*Prunus dulcis*), cashew (*Anacardium occidentale*), pistachio (*Pistachia vera*), Brazil nut (*Bertholletia excelssa*), hazelnut (*Corylus*



vellana), macadamia (*Macadamia integrifolia*), pecan (*Carya illinoensis*), pine nut (*Pinus pinea*), and walnut (*Juglans regia*) (Mahesh and Shridhar, 2006). They have attracted interest as a potential source of supplementary protein for human food because of the relatively high cost of animal protein (Watt and Merrill, 1950).

Fungal invasion of food and feed has resulted in a foremost socio-economic crisis throughout the world. Array of microorganisms subject nuts to infection and cause spoilage or produce metabolites that are toxic to humans (Molyneux *et al.*, 2007). Shejiny *et al.*, (1989) have described the variety of fungi, primarily species of *Aspergillus*, *Alternaria*, *Rhizopus*, *Cladosporium* and *Penicillium* which were found on almonds, cashew nuts, pistachio nuts, walnuts and hazelnuts. They had been infected during or after harvesting, storage and transition (Bilgrami and Choudhary, 1990; Bruce *et al.*, 2003). The storage temperature, moisture content, presence of oxygen and gaseous composition are the most important factors influencing the development of fungi during storage (Kubatova, 2000).

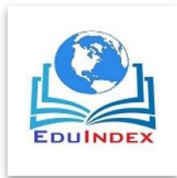
Although the natural contamination by mycoflora and mycotoxins of various kinds of nuts such as almond, Brazil nut, cashew nut, coconut, hazelnut, peanut, pistachio nut and walnut, and different nut products were investigated in many parts of the world (Abdel *et al.*, 1993; Burdaspal *et al.* 1990; El-Magraby *et al.* 1987; 1988; FAO 1979; Jimenez *et al.* 1991 and Stoloff L. 1976), but very few studies were reported on mycoflora and deterioration of nuts in Marathwada region. Considering these point this study was undertaken to determine the frequency of mycoflora and its influence on biochemical changes of several commercial samples of almond, cashew nut and pistachio nut collected from markets in Marathwada region of Maharashtra state.

Materials and Methods

Collection of samples

Seed samples were collected as per method described by Neergaard (1973) has been adopted. Accordingly random samples of seeds were collected from market places of Nilanga, Latur, Aurangabad.

Detection of seed mycoflora



The seed mycoflora was isolated by using standard agar plate method (APM) as recommended by ISTA (1966), Neergaard (1977), Agarwal (1976) and Pitt and Hocking (2009).

Potato Dextrose Agar (PDA)

Peeled potatoes 200 gm were boiled until soft and passed through muslin cloth. Then 20 gm dextrose was added in it and final volume of solution was made up to 1000 ml. by adding distilled water. 20 gm Agar was added in the solution and pH was adjusted to 5.6.

In order to isolate only internal seed-borne fungal species, nut seeds were pre-treated with 0.1% solution of mercuric chloride for 2 minutes subsequently; the seeds were thoroughly washed thrice with sterile distilled water and placed on Agar media. The 15 ml. of autoclaved Potato dextrose agar (PDA) medium was poured in the pre-sterilized corning glass Petri-plates. On cooling the medium five seeds per petri plate of the test sample were placed at equal distance aseptically and incubated at $25 \pm 20C$ for 7 days.

Identification of mycoflora

Detection and identification of different fungal species was made with the help of literature, Wollen Weber and Reinking (1935), Bessey (1950), Joseph Gilman (1960), Ramnath *et. al.*, (1971), Dubey (1990), Mukadam (1997), and Mukadam *et. al.*, (2006).

Bio-deterioration of nuts

Nut seeds were surface sterilized with 0.1% mercuric chloride solution and were washed twice by sterile distilled water and soaked in sterile water for four hours. Water soaked seeds were distributed in pre-sterilized conical flasks and were separately inoculated with 2ml spore suspension of the test fungi. Flasks were incubated at room temperature for 15- 20 days.

The inoculated seeds were harvested by thoroughly washing under running water and mycelial growths removed. Subsequently the seeds were dried at 60⁰C for 48 hours and were powdered for biochemical studies. Seeds were incubated without inoculating the spore suspension as control.

Estimation of fat

Fat was estimated by method recommended by AOAC (1995). A known weight of the sample was defatted in a Soxhlet apparatus using petroleum ether (boiling point range) 38.2-54.3 °C) as the solvent for 8 hr. Defatted samples were dried overnight (10-12 hr) in fume hood to remove residual traces of petroleum ether and calculated fat content.

$$\text{Fat (\%)} = \frac{[\text{initial wt. of full fat flour (g)} - \text{final wt. of defatted flour (g)}] \times 100}{\text{initial wt. of full fat flour (g)}}$$

Estimation of protein

The micro-Kjeldahl method AOAC (1960) was used to determine total proteins and calculated as per formula-

$$\frac{(\text{mL of H}_2\text{SO}_4 \text{ for sample} - \text{mL of H}_2\text{SO}_4 \text{ for blank}) \times \text{normality of H}_2\text{SO}_4 \times 1.4007\% \text{N}}{\text{wt. of sample (g)}}$$

Estimation of carbohydrates

Carbohydrates were calculated by sum of moisture, ash, protein and fat -100.

Result and discussion

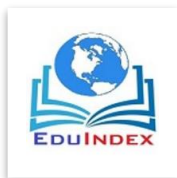
Table:- I - Incidence of fungi from different nuts

Sl. no.	Name of the fungi	Almond	Pistachio nut	Cashew nut
		% incidence		
1	<i>Aspergillus flavus</i>	40.00%	26.66%	93.33%
2	<i>Aspergillus terreus</i>	26.66%	13.33%	13.33%
3	<i>Aspergillus niger</i>	66.66%	73.33%	100.00%
4	<i>Aspergillus ruber</i>	-	-	60.00%
5	<i>Aspergillus parasiticus</i>	-	13.33%	-

<i>Sl. No.</i>	<i>Name of the fungi</i>	<i>Almond</i>	<i>Pistachio nut</i>	<i>Cashew nut</i>
1	<i>Aspergillus flavus</i>	24.87	23.43	15.92
2	<i>Aspergillus niger</i>	24.86	23.55	16.12
3	<i>Alternaria alternata</i>	24.62	23.56	14.85
4	<i>Alternaria tinuissima</i>	24.63	23.34	14.42
5	<i>Curvularia lunata</i>	24.87	23.12	15.50
6	<i>Curvularia pallescens</i>	24.85	22.81	15.69
7	<i>Fusarium oxysporum</i>	24.43	23.75	13.25
8	<i>Fusarium avenaceum</i>	24.41	23.53	13.52
9	<i>Penicillium chrysogenum</i>	24.68	23.81	14.38
6	<i>Alternaria alternata</i>	33.33%	26.66%	20.00%
7	<i>Alternaria tinnuissima</i>	13.33%	13.33%	-
8	<i>Curvularia pallescens</i>	-	26.66%	-
9	<i>Curvularia lunata</i>	26.66%	13.33%	20.00%
10	<i>Fusarium moniliforme</i>	13.33%	40.00%	40.00%
11	<i>Fusarium oxysporum</i>	33.33%	33.33%	53.33%
12	<i>Fusarium avenaceum</i>	13.33%	33.33%	26.66%
13	<i>Penicillium digitatum</i>	20.00%	26.66%	40.00%
14	<i>Penicillium chrysogenum</i>	26.00%	6.66%	-
15	<i>Rhizopus stolonifer</i>	80.00%	33.33%	46.66%

(- = No % incidence)

Table II - Change in protein content (gm / 100gm) due to different fungal species



10	<i>Penicillium digitatum</i>	24.63	23.77	14.17
11	<i>Control</i>	26.80	23.90	20.06

Table	Sl. no.	Name of the fungi	Almond	Pistachio nut	Cashew nut	III:-
	1	<i>Aspergillus flavus</i>	13.36	17.16	58.35	
	2	<i>Aspergillus niger</i>	13.42	16.96	58.27	
	3	<i>Alternaria alternata</i>	11.19	15.47	58.60	
	4	<i>Alternaria tenuissima</i>	10.69	16.20	58.27	
	5	<i>Curvularia lunata</i>	12.20	16.40	55.92	
	6	<i>Curvularia pallescens</i>	11.97	16.39	55.26	
	7	<i>Fusarium oxysporum</i>	13.89	16.46	56.20	
	8	<i>Fusarium avenaceam</i>	13.61	16.13	56.25	
	9	<i>Penicillium chrysogenum</i>	13.08	16.01	57.90	

Change in carbohydrate content (gm / 100gm) due to different fungal species

Table	10	<i>Penicillium digitatum</i>	13.10	16.00	57.44
	11	<i>Control</i>	14.03	17.32	62.72

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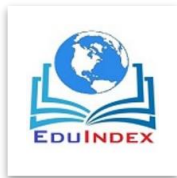
Change in fat content (gm / 100 gm) due to different fungal species

Sl. No.	Name of the Fungi	Almond	Pistachio nut	Cashew nut
1	<i>Aspergillus flavus</i>	55.09	52.70	42.50
2	<i>Aspergillus niger</i>	55.15	52.37	42.21
3	<i>Alternaria alternata</i>	55.61	53.00	34.00
4	<i>Alternaria tinuissima</i>	55.33	53.71	33.86
5	<i>Curvularia lunata</i>	56.11	52.62	32.00
6	<i>Curvularia pallescens</i>	55.70	52.14	31.68
7	<i>Fusarium oxysporum</i>	55.14	52.30	36.50
8	<i>Fusarium avenaceum</i>	54.92	52.19	36.71
9	<i>Penicillium chrysogenum</i>	56.08	52.81	44.00
10	<i>Penicillium digitatum</i>	55.74	52.60	43.82
11	<i>Control</i>	57.61	53.80	50.12

Conclusion

In order to know the percent incidence of fungi from almond, cashew nut and pistachio nut were used to determined the bearing capacity of fungi. Total 15 fungal species were obtained with more or less their percent incidence. It is remarkable to note that, the species of *Aspergillus niger*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Aspergillus flavus* proved to be their highest percent incidence on the tested nuts. The percent incidence of *Aspergillus ruber* which was only on the cashew nut neither on almond nor on pistachio nut. The maximum percent incidence was recorded by *Aspergillus niger* on cashew nut followed by pistachio nut and almond.

The role of the fungi on biodeterioration were estimated and to carry out the result. In case of the fat content 10 fungal species were screened their impact on change in fat content. The results were very much remarkable. The *Curvularialunata*, *Curvularia pallescens*, *Alternariatinnuissima* and *Alternariaalternata* were much efficient for maximum utilization of the fat from cashew nut. The maximum deterioration of fat from cashew nut and pistachio nut by *Curvulariapallescens*. Influence of *Fusariumavenaceum* also recorded and feeding behavior was

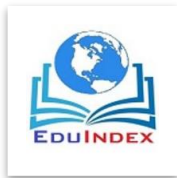


highest in case of almond. Similarly, the change in protein content were calculated. *Fusariumoxysporum* and *Fusariumavenaceum* play deteriorative role in case of almond and cashew nut followed by *Alternariaalternata* and *Alternariatinnuissima*. The majority of test fungi and their vital role in maximum deterioration of the protein in cashew nut except *Aspergillusflavus* and *Aspergillusniger*.

On similar line deterioration of carbohydrate content from almond, pistachio nut and cashew nut by the test fungi were variable in their nature. *Alternariaalternata* and *Alternariatinnuissima* were efficient in maximum change in carbohydrate content of almond and pistachio nut whereas, not prominent on cashew nut. Similarly, *Curvularialunata* and *Curvulariapallescens* were utilized maximum amount of carbohydrate in case of almond and cashew nut. It was interesting to note that the species of *Aspergillus* and their impact was not recorded in deterioration of carbohydrate.

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