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E: ISSN NO.: 2349-980X **Shrinkhla Ek Shodhparak Vaicharik Patrika**

Micromycetes on Surface of Some Imported Ripened Fruits

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Abstract

Investigations on surface mycoflora of imported fruits was conducted. It was revealed that the marketed imported fruits were contaminated by propagules of various micromycetes. The obtained results allow the conclusion that riped fruit being juicy and rich sugar can become a good substrate for mycotoxin producing micromycetes. Some microorganisms especially of micromycetes belonging to the *Penicillium* Link, *Aspergillus* Mich. *Alternaria*. and other genera, are commonly found on fruits surface during investigation which are known to produce secondary metabolites (mycotoxins) of various compositions that are toxic to plants, animals and humans. Their detection frequency on the investigated fruit was determined.

Keywords: Mycoflora, Micromycetes, Contamination, Imported Fruit, Products.

Introduction

In terms of providing food with healthy nutrition, free from contaminants and long survived, the variety of fruits are imported from various countries. Regulations on contamination of micromycetes and mycotoxins have been set and strictly enforced by most importing countries. Imported fruit are juicy, rich vitamins, carbohydrates, organic acids, calcium, phosphorous and iron. All these constituents make these fruits vulnerable to post harvest attack by a variety of micromycetes to which they are resistant during the period of development on the plants.

Review of Literature

Most of the fungi are opportunists that may invade the harvested fruit directly or more usually through wounds attained during harvesting, transportation and poor handing practices(1). A few researchers have monitored the fungal spectra of fruits surface and their relative role in causing spoilage under storage (2). Many of these commodities facing a high risk of mycotoxin contamination. As imported fruits are often kept in unsuitable conditions like high humidity and temperature which is more favorable for microorganism spread, spoilage and mycotoxins contamination(8,9,10) which usually causes considerable economic losses . However, no work was done on regarding the surface micromyctes of imported fruits and their efficacy in spoilage in Jammu province(Jammu and Kashmir).

Aim of the Study

In view of this, investigation was conducted to monitor the surface micromyces of imported fruits and its efficacy to causes spoilage was also evaluated by artificial inoculation.

Materials and Methods

Isolation of surface mycoflora

For isolation of the surface mycoflora associated with imported fruits, sample were taken in a sterilized polythene bags and brought to the laboratory and micromycetes were isolated by surface washing technique. In this method, 25 g of fruit peel was taken in a Erlenmeyer flask(250 ml containing 50 ml sterilized distilled water.) and subjected to horizontal shaking for 30 min. on rotatary shaker. The liquid was then centrifuged at 3000rpm for 15 minutes and the residue thus obtained was mixed in10 ml sterilized distilled water and shaken vigorously to obtain a homogenous suspension. This suspension was poured in sterilized petriplate at the rate of 2 ml/plate. Czapeks's Dox Agar medium (CDA) modified by adding streptomycin sulphate (0.06g/l) and rose Bengal (0.20g/l) was used and five replicates were maintained for each sample. The plates were incubated at 28+ 2^{o}C for 7 days and the resulting fungal colonies subcultured on potato dextrose agar medium and identified.

Pathogenicity Test

It was conducted to check the ability of the recovered surface mycomycetes and assess their relative importance in the spoilage of fruits. For these tests, fruits of similar size and approximate of same maturity were surface sterilized with 70% alcohol and then incubated with the fungus by pin prick method (3). Inoculums used in each case was 5days old culture. After inoculation, the fruits were placed in sterilized glass chamber maintained at 28+2oc and relative humidity. Extent of spoilage was evaluated after 10 days of incubation.

Results and Discussion

Results depicted in table 1. that 33 micromycetes were found to be associated with the surface of fruits imported from abroad and sold in Jammu Market during 2017-18 i.e apple from California, Pohland; grapes from south Africa; Kiwi from Italy; Mango from Pakistan ; Orange from Egypt and Pear from China .

From Figure 1 It is observed that maximum number of micromycetes were reported on apple (25 species) and Pear (25 species) followed by mango(21 species), orange(20 species), grapes(17 species), Kiwi (17 species). Members of genus *Aspergilus* and *Penicillium* were predominant followed by *Fusarium* species. Other genera isolated as components of the micromycetes included *Alternaria, Botrytis, Curvularia, Cladosporium, Chaetomium, Colletotrichum, Emericella, Geotrichum, Lasiodiplodia, Mucor, Phoma, Rhizopus, Trichothecium, Trichurus* and *Paecilomyces.* These genera may have reached the fruit surface directly from the orchard during picking and packing operation or they may have been carried along the packing material (6) .

Data presented in table 2 shows that most of the genera like *Alternaria alternate* (Fr) keissel*, Aspergillus flavus* link ex. Fries*, A. ochraceus* Wilhelm, *A.fumigatus, A.niger* Van Tiegham*, Botrytis cinere Pers* ex Fries*, Fusarium moniliforme* J.shed, *Lasiodiplodia theobromae* (Pat) Griff and Mauble *, Penicillium expansum* link ex Grey*, P. chrysogenum* Thom and *Rhizous stolonifer* (Ehrenb) Lind. were frequently present on the surface of all the fruits. Because of its frequently present on the fruit surface, known fasting growing and have enough potential to cause fruit loss(5) ,these may cause deterioration of these respective fruits in the open market and even under cold storage. According to few researchers, propagule of these fungi causes fruit loss after injuries occurs on fruit surface (5).These injuries provide the entry of such mycopropagules resulting in rot development. In addition, as the fruits continues to respire even after harvesting, the resulted heat accelerates respiration and aging, which in turn makes the fruit susceptible to attack by the surface micromycetes (7).

After inoculation by their respective frequent occurred fungal species, it was also observed that maximum causes fruit rot when incubated at 28±2^{o}C for 10 days with 100% relative humidity (table 3). In addition, among these fungal species, *Alternaria alternate, Aspergillus flavus, A. niger, Botrytis cinera,F. moniliform, Lasiodiplodia theobromae, Penicillium expansum, P. chrysogenum*, and *Rhizpus stolonifer* showed maximum extent of rot in respective fruits while *Aspergillus ochraceus* cause minimum rot. *Aspergillus fumigatus* have not shown any rot may be not posses such specific macerating enzymes necessary for pathogenesis. Similarly , there are some reports on rot causing pathogens from apple (5), grapes(13), Kiwi(12),Mango(13) , Orange(14,15) and Pear(5)

If fruits are damaged by such frequently occurred micomycetes and have ability to produce toxic metabolite, their product can be responsible to cause a severe and chronic diseases in human and animals. Similary, it is also reported in fruit by Santos et al.,2016. `Therefore from this study it is observed that there is more chance of availability of mycotoxins in our children's food and also lead to huge loss in finance from our state.

Conclusion

In view of this above study, it ardent need to check on mycoflora, uses of contaminated imported fruit in making their product. The aim of the present work was to monitor the occurrence of kinds of micromycetes on the surface of imported market fruits and these have potential to cause spoilage and to produce mycotoxins.

Endnotes

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Table 1: Micromycetes Detected on the Imported Fruits from Jammu

S.no	Name of fruit	Imported from country	Micromycetes Species
1	Apple	California , Poland	*Alternaria alternate (Fr.) keissl.* *Aspergillus flavus link es.Fries* *A. ochraceus Wilhelm* *A. sydowii Thom and Church* *A.fumigatus Fresenius* *A.niger Van Tiegham* *A.niveus Blochwitz* *A.solani Sorauer* *A.terrius Thom* *Botrytis cinera Pers.ex Fries Curvularia lunata* *Cladosporium oxysporum Kunze ex Fries* *Colletotrichum gloeosporioides (Penz) sacc.* *Emericella nidulans (Eidam) Vuill* *F. moniliforme J.Sheld* *F. verticilliodes (Sacc.) niren-berg* *Fusarium sporotricoides Sherb.* *Lasiodiplodia theobromae (Pat) Griff and Manble* *P. chrysogenum Thom* *P. pupurogenum Stoll* *P.citrinum Thom* *Paecilomycetes variotii Bainier* *Penicillium expansum link ex. Grey* *Rhizopus stolonifer (Ehrenb) Lind* *Trichothecium roseum (Pers)link esx Fries*
2	Grapes(black)	South Africa	*Alternaria alternate (Fr.) keissl.* *Aspergillus flavus link es.Fries* *A. niger Van Tiegham* *A.terrius Thom* *A. ochraceus Wilhelm* *A.fumigatus Fresenius* *Botrytis cinera Pers.ex Fries* *Curvularia lunata* *Cladosporium oxysporum Kunze ex Fries* *Emericella nidulans (Eidam) Vuill* *F. moniliforme J.Sheld* *Geotrichum candidum* *Lasiodiplodia theobromae (Pat) Griff and Manble* *Penicillium expansum link ex. Grey* *P. chrysogenum Thom* *P. pupurogenum Stoll* *Rhizopus stolonifer (Ehrenb) Lind*
3	Kiwi	Italy	*Alternaria alternate (Fr.) keissl.* *Aspergillus flavus link es.Fries* *A. ochraceus Wilhelm* *A.fumigatus Fresenius* *A.niger Van Tiegham* *A.terrius Thom*

			Botrytis cinera Pers.ex Fries *F. moniliforme J.Sheld* *Geotrichum candidum* *Lasiodiplodia theobromae (Pat) Griff and Manble* *P. brevicompactum Dierckx* *P. chrysogenum Thom* *P.italicum Wehmer* *Penicillium expansum link ex. Grey* *Rhizopus stolonifer (Ehrenb) Lind* *Trichothecium roseum (Pers)link esx Fries* *Trichurus spiralis Hasselbring*
4	Mango	Pakistan	*Alternaria alternate (Fr.) keissl* *Aspergillus flavus link es.Fries* *A. ochraceus Wilhelm* *A.fumigatus Fresenius* *A.niger Van Tiegham* *A.terrius Thom* *Botrytis cinera Pers.ex Fries* *Cladosporium oxysporum Kunze ex Fries* *Colletotrichum gloeosporioides (Penz) sacc* *Curvularia lunata* *F. moniliforme J.Sheld* *F. verticilliodes (Sacc.) niren-berg* *Fusarium sporotricoides Sherb* *Geotrichum candidum* *Lasiodiplodia theobromae (Pat) Griff and Manble* *Mucor heimalis Wehmer* *P. chrysogenum Thom* *P. italicum Wehmer* *P.citrinum Thom* *Penicillium expansum link ex. Grey* *Rhizopus stolonifer (Ehrenb) Lind*
5	Orange	Egypt	*Alternaria alternate (Fr.) keissl* *Aspergillus flavus link es.Fries* *A. ochraceus Wilhelm* *A.fumigatus Fresenius* *A.niger Van Tiegham* *Botrytis cinera Pers.ex Fries* *Emericella nidulans (Eidam) Vuill* *Fusarium sporotricoides Sherb* *F. moniliforme J.Sheld* *F. verticilliodes (Sacc.) niren-berg* *Geotrichum candidum* *Lasiodiplodia theobromae (Pat) Griff and Manble* *Mucor heimalis Wehmer* *P. chrysogenum Thom* *Penicillium expansum link ex. Grey* *P.citrinum Thom* *P. italicum Wehmer* *P.digitatum* *Rhizopus stolonifer (Ehrenb) Lind*
6	Pear	China	*Alternaria alternate (Fr.) keissl.* *Aspergillus flavus link es.Fries* *A. ochraceus Wilhelm* *A. sydowii Thom and Church* *A.fumigatus Fresenius* *A.niger Van Tiegham* *A. niveus Blochwitz* *A.terrius Thom* *Botrytis cinera Pers.ex Fries* *Curvularia lunata* *Cladosporium oxysporum Kunze ex Fries*

			Colletotrichum gloeosporioides (Penz) sacc. *Chaetomium globosumBerkand Cult.* *Emericella nidulans (Eidam) Vuill* *F. moniliforme J.Sheld* *F. verticilliodes (Sacc.) niren-berg* *Fusarium sporotricoides Sherb.* *Geotrichum candidum* *Lasiodiplodia theobromae (Pat) Griff and Manble* *Penicillium expansum link ex. Grey* *P. chrysogenum Thom* *P.citrinum Thom* *P. pupurogenum Stol* *Phoma sp* *Rhizopus stolonifer (Ehrenb) Lind* *Trichothecium roseum (Pers)link esx Fries*

Table 2: Micromycetes frequently occurred in imported fruits

S.No.	Micromycetes	Apple	Grapes	Kiwi	Mango	Orange	Pear
1	*Alternaria alternate (Fr.) keissl.*	+	+	+	+	+	+
2	*A.solani Sorauer*	+	--	-	-	-	-
3	*Aspergillus flavus link es.Fries*	+	+	+	+	+	+
4	*A. ochraceus Wilhelm*	+	+	+	+	+	+
5	*A. sydowii Thom and Church*	+	-	-	-	-	+
6	*A.fumigatus Fresenius*	+	+	+	+	+	+
7	*A.niger Van Tiegham*	+	+	+	+	+	+
8	*A. niveus Blochwitz*	+	-	-	--	--	-
9	*A.terrius Thom*	+	+	+	+	-	+
10	*Botrytis cinera Pers.ex Fries*	+	+	+	+	+	+
11	*Curvularia lunata*	+	+	-	+	-	+
12	*Cladosporium oxysporum Kunze ex Fries*	+	+	-	+	+	+
13	*Colletotrichum gloeosporioides (Penz) sacc.*	+	-	-	+	-	+
14	*Chaetomium globosum Berkand Cult.*	-	-	-	-	-	+
15	*Emericella nidulans (Eidam) Vuill*	+	+	-	-	+	+
16	*Fusarium. moniliforme J.Sheld*	+	+	+	+	+	+
17	*F. verticilliodes (Sacc.) niren-berg*	+	-	-	+	+	+
18	*Fusarium sporotricoides Sherb.*	-	-	-	+	+	+
19	*Geotrichum candidum*	-	+	+	+	+	+
20	*Lasiodiplodia theobromae (Pat) Griff and Manble*	+	+	+	+	+	+
21	*Mucor hiemalis Wehmer*	-	-	-	+	+	-
22	*Penicillium expansum link ex. Grey*	+	+	+	+	+	+
23	*P. chrysogenum Thom*	+	+	+	+	+	+
24	*P. pupurogenum Stoll*	+	+	-	-	-	+
25	*P. brevicompactum Dierckx*	+	-	+	-	--	-
26	*P. citrinum Thom*	+	_	-	+	+	+
27	*P. digitatum*	-	-	-	-	+	-
28	*P . italicum Wehmer*	-	--	+	+	+	-
29	*Phoma sp.*	-	-	-	-	-	+
30	*Rhizopus stolonifer (Ehrenb) Lind*	+	+	+	+	+	+
31	*Trichothecium roseum (Pers)link esx Fries*	+	-	+	-	-	+
32	*Trichurus spiralis Hasselbring*	--	-	+	-	-	-
33	*Paecilomyces variotii Bainier*	+	-	--	-	-	-

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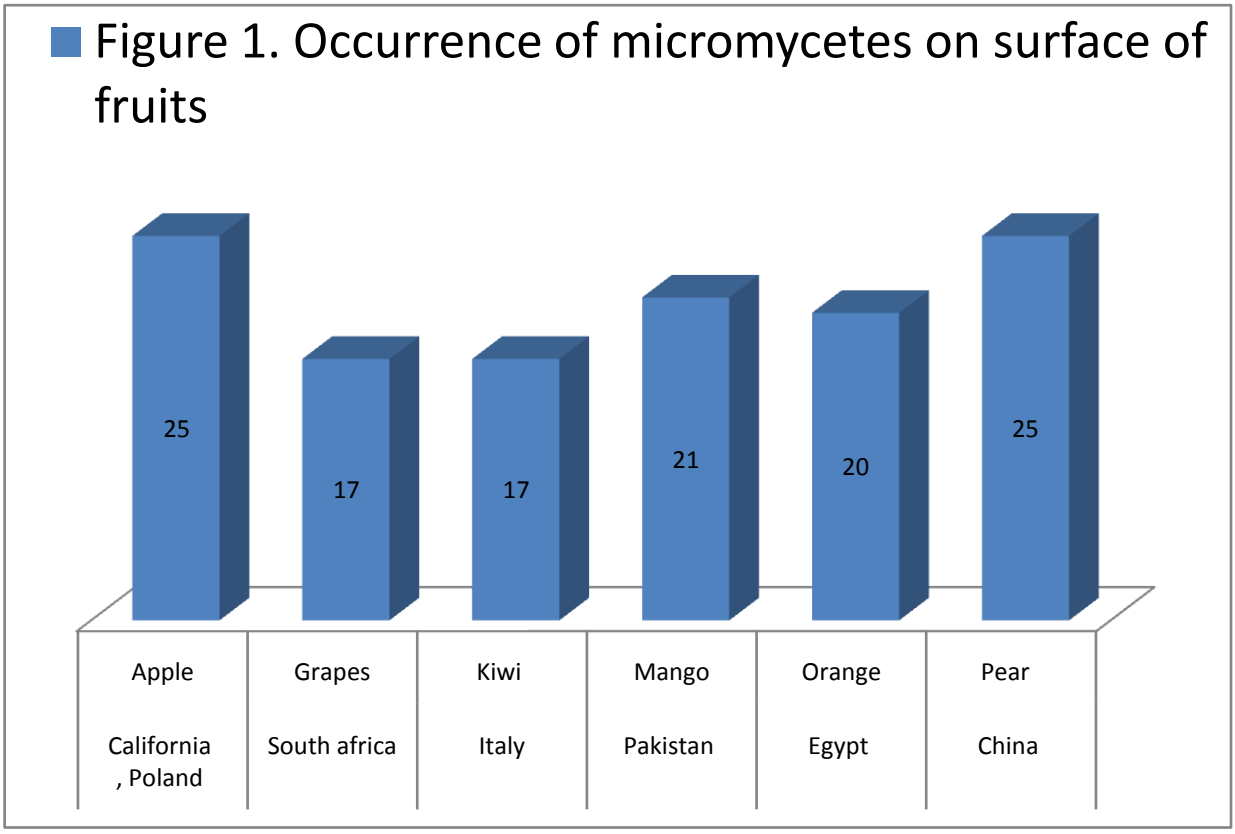

Table 3: Extent of Fruit Rots after Inoculation by Micromycetes

S. No	Name of species	Extent of fruit loss after incubation of 10 days at 28±2°C					
		Apple	Grapes	Kiwi	Mango	Orange	Pear
1	*Alternaria alternate* (Fr.) keissl.	+++	+++	++	+++	+++	+++
2	*Aspergillus flavus* link es.Fries	+++	+++	+++	+++	+++	+++
3	*A. ochraceus* Wilhelm	+	+	+	+	+	+
4	*A.fumigatus* Fresenius	-	-	-	-	-	-
5	*A.niger Van* Tiegham	+++	+++	+++	+++	+++	+++
6	*Botrytis cinera* Pers.ex Fries	+++	+++	+++	+++	+++	+++
7	*Fusarium. moniliforme* J.Sheld	+++	+++	+++	+++	+++	+++
8	*Lasiodiplodia theobromae* (Pat) Griff *a9nd Manble*	+++	+++	+++	+++	+++	+++
9	*Penicillium expansum* link ex. Grey	+++	+++	+++	+++	+++	+++
10	*P. chrysogenum* Thom	+++	+++	+++	+++	+++	+++
11	*Rhizopus stolonifer* (Ehrenb) Lind	+++	+++	+++	+++	+++	+++

+++= maximum rot, += minimum rot,- no rot