International Journal of Pharmacy and Biological Sciences-IJPBS™ (2020) 10 (3): 01-05
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | OA Journal | MCI Approved | Index Copernicus

Isolation of Mycoflora From Dry Fish in North Telangana Region

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Received: 07 Mar 2020 / Accepted: 19 Apr 2020 / Published online: 1 Jul 2020
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Abstract
Dry fish samples were collected from various fish markets of different districts of northern Telangana region and analyzed for fungal infestation and mycotoxin contamination. In all 25 fungal species representing 11 genera were isolated such as Aspergillus, Alternaria, Cladosporium, Curvularia, Penicillium, Rhizopus, Fusarium, Trichoderma, Chaetomium, Acremonium and Drechslera. However, the incidence of different fungi varied with the type of dry fishes and condition of sample. Significant percentages of moulds were toxigenic and elaborated variety of mycotoxins.

Keywords
Dry fishes; Sterigmatocystin; Patulin; Ochratoxin; Aflatoxin.

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INTRODUCTION
Fish is a good alternative meat and is one of the most important sources of protein. It has essential nutrients such as high-quality proteins, unsaturated essential fatty acids, minerals, water soluble and fat-soluble vitamins to nourish body [1]. Dried fish plays an important role in the traditionally accepted diet in many developing countries as a major source of protein [2]. Dried fish is more easily digested than beef or any other types of proteinaceous food [3].

Edible fishes are preserved after removal of moisture. Fish drying is the removal of the water content from the fish, where the activity of the muscle enzyme is reduced to its minimum level [1]. This method helps in prolonging the shelf-life of the food product. Fish salting and drying are some of the ancient and traditional methods for long term storage of dry fish. Air-drying and sun-drying are the most common methods in preserving fish due to its low cost and easy process [1].

Generally, fish spoilage is accompanied by various physical and chemical changes which are clearly visible; hence it must be subjected to some forms of processing as soon as captured to avoid contamination [4].

The poor handling and poor storage facilities lead to rapid post-harvest deterioration and limit the availability of fish. The nutrients present in fish provide a good medium for microbial growth. Fungal growth on dried fish indicates the onset of spoilage and deterioration of the product [5]. Though fish are smoke dried there are chances of contamination with moulds so that it is not fit for human consumption [6], [7].

During storage of dried fish wholesalers and retailers, store them in less ventilated stores where pests can gain access and may directly inoculate them with fungi. The marketplaces where the dried fish displayed for sale are mostly neither clean nor hygienic. Dry fish are kept in open trays without any protective covering on them. This kind of exposure allows the dust and fungal spores to settle on the dry fish and lead to fungal invasion, production of toxins and spoilage [2].

Prolonged consumption of dried fish with these metabolites (mycotoxins) exhibit toxic, mutagenic effects in human beings [8], [2]. Hence in the present investigation fungal infestation and mycotoxin contamination of dry fish were analyzed.

MATERIALS AND METHODS

Sample Collection

Three varieties of dry fish (A-*Anguilla bengalensis*, B-*Puntius chola*, C-*Salmostoma bacaila*) were collected from various markets of different districts in Northern Telangana. The collected samples were kept in sterile polythene bags and brought to the laboratory for fungal analysis.

2.3.2 Isolation of Fungi

Isolation of fungi from the dry fish samples was made by dilution plate method [9]. For dilution plating 10grams of each fish samples namely (A- *Anguilla bengalensis*, B- *Puntius chola*, C- *Salmostoma bacaila*) were weighed aseptically and all the samples were surface sterilized using 70% ethanol and rinsed with sterile distilled water before cut into small pieces. The purpose of surface sterilization was to remove fungal spores from the surface of dry fish and ensure enumeration of those fungi actually invaded and contaminated dry fish [10]. Then the fish samples were taken into 250ml conical flask with 90ml of distilled water and stirred the total content for 30min on electrical stirrer. In serial 10-fold dilution was made up to 10^{-4} and spread on to Saboraud Dextrose Agar medium in three replicates and incubated at 25^0 C for seven days. Streptomycin was added to saboraud dextrose agar to suppress the growth of bacteria and to ensure the growth of fungi only. All observed colonies were subcultured to obtain pure cultures which were isolated and identified using morphological characters under microscope. The mycotoxigenic fungi were grown in 25ml of saboraud dextrose broth medium contained in 250ml Erlenmeyer conical flask at 29 ± 2º C for 15 days. At the end of incubation period, the culture filtrate was employed for different mycotoxins with the help of TLC and Long wave (360nm) UV light. Based on florescence, different mycotoxins were identified, and they further confirmed with colour tests. [11].

RESULTS AND DISCUSSION

In this study twenty-five fungal species belong to eleven genera were isolated from nine different markets of various districts in Telangana. *Aspergillus flavus*, *A.niger*, *Cladosporium sp*, *Penicillium citrinum* were isolated from dry fish samples collected from all fish markets.

Eleven species of *Aspergillus* were isolated from different dry fish samples collected from different fish markets. *A. flavus* was isolated from three species of fish (A- *Anguilla bengalensis*, B- *Puntius chola*, C- *Salmostoma bacaila)* of all markets. *A.ochraceus* was present in dry fish of Jangoan, Jagityal, Korutla. *A.sydowii* was restricted for Jagityal, Mulugu, Warangal fish markets. *A. ustus* was detected in dry fish samples of Jagityal, Jangoan and Khammam. However, *A. nidulans* was not detected in Jangoan and Korutla, similarly *A.terreus* was not identified in Mahabubabad and Nirmal. *A.versicolar* was not recorded in Mahabubabad and Jagityal. *A. fumigatus* was not present in dry fish markets of Jangoan, Korutla and Mulugu.

Three species of *Penicillium* were detected in all three species of fish. *P.citrinum* was detected in all the nine districts of dry fish markets but *P. chrysogenum* was not found in Mahabubabad, Nirmal, Mulugu and Khammam. *P.expansum* was absent in Mahabubabad, Jagityal, Karimnagar, Korutla and Warangal. Earlier studies reported that *Aspergillus, Penicillium* identified with highest occurrence in salted fish [12].

Three species of *Rhizopus* were also identified i.e., *R. mucida* was identified in Mahabubabad, Warangal whereas *R. oryzae* was not recorded in Jagityal, Khammam and Warangal. *R. stolinifer* was absent in Karimnagar, Mulugu. Other species like *Drechslera* were restricted for Khammam, Warngal and *Chaetomium* for Mahabubabad and Korutla. The studies in the past reported that species of *Aspergillus, Rhizopus* and *Penicillium* were isolated from dry fish samples [13]. *Cladosporium sp. was isolated* from dry fish [14].

A critical perusal of Table 1 reveals that *Aspergillus flavus* was found with highest percentage of frequency (100.0%) followed by *A.nidulans, A. terreus, A. versicolor* and *R. stolinifer* (77.7%). *A. fumigatus*, *A. parasiticus, Curvularia* sps (66.6%), *A. clavatus, P. crysogenum* and *R. oryzae* (55.5%) were with intermediate percentage of frequency. On the other hand, *Chaetomium* sp, *Drechslera* sp. and *R. mucida* (22.2%) were recorded with low percentage of frequency.

A. flavus was with highest percentage of abundance followed by *A.niger, P. citrinum* and *Cladosporium* sp. On the other hand, *A. clavatus, A. ustus, Chaetomium* sp., *Rhizopus stolonifer*, *Fusarium* oxysporium, *R. oryzae, Drechslera* sp. and *Rhizopus mucida* were with least percentage of abundance. Rest of the fungi was associated with intermediate percentage of abundance.

ISSN: 2230-7605 (Online); ISSN: 2321-3272 (Print)
Int J Pharm Biol Sci.

Table-1: Mycoflora isolated from dry fish

Name of the Organism	% of Incidence																											Frequency	Abundance
	Area-I			Area-II			Area-III			Area-IV			Area-V			Area-VI			Area-VII			Area-VIII			Area-IX				
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C		
Acremonium sps.	-	-	-	-	-	-	-	-	-	-	-	-	5.26	-	-	-	-	-	8.69	-	-	-	6.25	-	-	4.16	-	44.44	1.14
Alternaria alternata	-	-	-	-	-	-	-	-	10.77	-	-	-	-	-	-	-	-	-	-	-	5.58	7.14	-	7.69	-	7.25	-	44.44	1.62
Aspergillus clavatus	-	-	-	-	8.17	-	-	-	-	-	7.14	-	-	-	-	-	-	21.42	-	7.69	-	-	-	-	-	-	3.84	55.55	1.78
Aspergillus flavus	30.76	28.57	31.05	22.38	26.66	35.71	23.07	20.19	21.73	23.52	28.57	26.66	26.31	23.07	21.42	8.33	29.41	28.57	21.73	23.07	17.96	21.42	12.5	23.07	17.97	16.66	17.69	100	24.08
Aspergillus fumigatus	-	-	5.26	-	-	-	13.87	-	-	-	-	6.66	-	-	-	-	5.88	-	-	-	-	7.14	-	-	-	8.33	-	66.66	1.83
Aspergillus nidulans	-	-	15.78	-	-	-	-	11.11	-	11.76	14.28	-	-	-	-	10.46	-	7.14	-	23.07	-	20.11	14.56	-	-	11.14	4.96	77.77	5.04
Aspergillus niger	23.07	18.35	10.52	-	13.33	14.28	18.59	16.66	17.39	13.9	-	17.84	21.05	15.38	14.28	23.55	29.41	14.28	17.39	15.38	10.29	14.28	16.69	15.38	21.78	12.5	7.69	100	15.59
Aspergillus ochraceus	-	-	-	5.88	-	-	-	8.09	13.04	-	-	-	-	7.69	-	-	-	-	-	-	-	-	-	-	-	-	-	33.33	1.62
Aspergillus parasiticus	-	-	-	8.02	-	-	-	-	4.34	-	-	-	15.78	-	7.14	-	17.64	-	13.04	-	-	-	-	-	9.52	-	-	66.66	3.21
Aspergillus sydowii	-	-	-	-	-	-	15.38	-	-	-	-	-	-	-	-	-	-	-	-	-	13.33	-	-	-	-	5.24	-	33.33	1.14
Aspergillus terreus	-	-	-	-	17.46	-	-	5.55	-	15.51	-	-	-	-	13.07	-	-	-	4.34	-	16.39	8.45	-	-	11.24	-	-	77.77	2.75
Aspergillus ustus	-	-	-	-	11.82	-	-	11.11	8.69	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5.66	-	-	-	22.22	0.68
Aspergillus versicolor	-	-	-	11.76	-	-	-	-	-	-	14.28	-	-	7.69	16.43	18.11	-	16.29	-	-	13.33	-	13.56	-	13.19	-	11.53	77.77	3.89
Chaetomium sps.	-	11.19	-	-	-	-	-	-	-	-	-	-	-	15.38	-	-	-	-	-	-	-	-	-	-	-	-	-	22.22	0.68
Cladosporium sps.	7.69	-	-	21.38	-	-	7.69	-	-	-	-	13.33	15.78	-	-	-	11.76	-	13.04	13.44	8.7	-	12.5	9.72	9.52	-	-	100	5.27
Curvularia sps.	-	7.14	-	5.88	-	-	-	-	6.61	-	-	8.82	-	-	-	-	-	-	-	-	7.68	-	-	7.69	-	-	-	66.66	1.78
Drechslera sps.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.46	17.49	-	-	9.23	22.22	1.3
Fusarium oxysporium	-	-	-	-	-	-	12.17	-	-	9.94	-	-	5.26	-	7.14	-	-	-	-	9.63	-	-	8.29	-	-	-	6.57	66.66	2.06
Penicillium chrysogenum	-	-	-	-	22.53	-	9.2	-	-	-	21.42	-	-	18.51	-	-	-	-	-	-	-	-	-	-	12.03	13.85	15.38	55.55	4.58
Penicillium citrinu	12.84	20.45	26.31	14.07	-	28.57	-	-	17.39	11.76	-	16.05	10.52	-	12.13	22.87	-	-	10.4	-	-	11.21	-	-	-	16.66	13.35	100	10.32
Penicillium expansum	-	-	-	-	-	14.28	-	-	-	-	-	-	-	-	-	16.66	5.88	-	6.98	-	-	-	-	13.27	-	-	-	44.44	2.26
Rhizopus stolinifer	15.38	-	-	10.59	-	-	-	18.69	-	-	-	-	-	12.25	-	-	-	12.27	-	-	-	10.21	-	-	4.76	-	3.84	77.77	2.98
Rhizopus mucida	10.23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	22.22	0.47
Rhizopus oryzae	-	-	10.52	-	-	7.14	-	-	-	7.68	14.28	-	-	-	8.35	-	-	-	-	7.69	-	-	-	-	-	-	-	55.55	1.83
Trichoderma viridae	-	14.28	-	-	-	-	-	5.55	-	5.88	-	10.61	-	-	-	-	-	-	4.34	-	6.66	-	5.19	-	-	-	5.87	66.66	2.06

Area- I – Mahabubabad; Area-I I – Jangoan; Area-III – Jagityal; Area- IV – Karimnagar; Area- V – Korutla; Area-VI – Nirmal; Area-VII – Mulugu; Area-VIII – Khammam; Area-IX – Warangal
A-*Anguilla bengalensis*, B-*Puntius chola*, C-*Salmostoma bacaila*

Table-2: Toxigenic potential of Fungi associated with dry fish

Name of the fungi	No. of strains screened	No. of positive strains for toxin production	% of Incidence	Name of the Toxin
Aspergillus clavatus	22	05	22.72	Patulin
A.flavus	62	43	69.35	Aflatoxin
	46	13	28.26	Sterigmatocystin
A.ochraceus	34	11	32.35	Ochratoxin
A.parasiticus	18	06	33.33	Aflatoxin
	18	04	22.22	Sterigmatocystin
A.terreus	26	08	30.76	Patulin
Penicillium chrysogenum	44	18	40.90	Ochratoxin
P. citrinum	42	24	57.14	Citrinin

From Table 2 it was evident that a number of fungi isolated from dry fish samples elaborated different mycotoxins. However, the incidence and toxigenic potential of different fungi varied. Out of 62 isolates of *Aspergillus flavus* screened, 43 isolates elaborated aflatoxin. When 46 isolates of *A. flavus* screened, 13 isolates elaborated sterigmatocystin. Out of 34 isolates of *A. ochraceus* screened, only 11 were positive for ochratoxin production. Six isolates of *A. parasiticus* elaborated aflatoxin out of 18 isolates were screened. Only 4 isolates of *A. parasiticus* were positive for production of sterigmatocystin out of 18 isolatess were screened. Out of 26 isolates of *A. terreus* screened, 8 isolates elaborated patulin. Eighteen isolates were positive for production of ochratoxin, when 44 isolates of *P. chrysogenum* were screened. Out of 42 isolates of *P. citrinum* screened, 24 isolates were positive for production of citrinin.

Different fungal isolates like *Aspergillus, Penicillium, Acremonium, Curvularia, Clodosporium, Fusarium, Rhizopus, Trichoderma, Chaetomium* and *Drechslera* from dry fish samples were collected in nine districts of northern Telangana.

In our study it is proved that the production of aflatoxin, ochratoxin, strigmatocystin, and patulin was carried out by *Aspergillus* species, whereas *Penicillium* species produce ochratoxin and citrinin.

Earlier studies also reported that *Aspergillus* species can produce aflatoxin and ochratoxin; *Penicillium* species can produce aflatoxin and citrinin from stock dry fish [15]. The studies in the past also proved that *Aspergillus* species produced aflatoxin, sterigmatocystin and *Penicillium* sp can produce citrinin in salted fish [12].

Among the all isolated fungal strains, *Aspergillus flavus*, *Penicillium chrysogenum P. citrinum* were recorded with highest toxigenic potentiality in dried fish. This same expression was also given in earlier, that was *A. flavus* and *A. parasiticus* were high potential toxigenic strains [7].

In our study among all *Aspergillus* species, *A. clavatus* was considered as low toxigenic potential strain which was isolated from dry fish.

CONCLUSION

In this study 25 fungal species belong to 11 genera were isolated from dry fish retail outlets of northern Telangana. Species of *Aspergillus, Penicillium* showed highest incidence followed by *Rhizopus* and *Fusarium* sps. Dry fish was recorded with highest susceptibility to species of *Aspergillus*, *Penicillium* which were produced different mycotoxins like Aflatoxin, Sterigmatocystin, Patulin, Ochratoxin and Citrinin. Therefore, it is suggested that the proper drying methods should be used and preserved in proper hygienic conditions to avoid contamination.

ACKNOWLEDGEMENTS

The authors are thankful to Dr. B. Lalaitha kumari, Head, Department of Botany, Kakatiya University, Warangal, for facilities.

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