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Evaluation of Fungal Flora and Aflotoxins from the Ground Nuts Collected from Various Regions of Warangal District

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Authors' contributions

This work was carried out in collaboration between all authors. Author MKT designed the experimental study protocol and wrote the first draft of the manuscript. Author RV helped during sample collection and as well during the detection of aflotoxins from collected samples. Authors EMKR and BSA reviewed the experimental design and all drafts of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the current study is to screen about twenty three ground nut samples for the identification and detection of mycotoxin-producing fungi and type of mycotoxin.
Study Design: The present investigation was designed to identify the type of mycotoxin in the ground nut samples collected from various regions of Warangal district of Telangana State, India.
Place and Duration of Study: Mahabubabad, Nekkonda, Thorrur, Palakurty, Jangon etc., of Warangal district, 2 years; from July 2014 to July 2016.
Methodology: The study includes: analysis of moisture content M.C. mycological analysis, isolation of sample-borne mycoflora, isolation of sample surface mycoflora, standard dilution plate, identification of the fungal genera, determination of potential toxigenic fungi using DRBC test, aflatoxin extraction and analysis by ELISA.

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Results: The M.C. of samples collected during wet season from Mahabubabad, Nekkonda, Thorrur, Palakurty, Jangon of different storage systems ranged between 13.1 – 17.0%, 17.7%, 12.8%, 15.4%, 14.1%, 9.0%, 12.5%, 10.6%, respectively. The freshly harvested ground samples showed highest M.C. percentage with 36.6%. Using Czapek Dox Agar (CDA) media we have isolated six fungal genera containing fourteen species from sterilized and unsterilized samples. Isolated genera were *Aspergillus* spp. *Cladospoium* sp., *Fusarium* sp., *Rhizopus stolonifer*, *Penicillium* spp. and yeasts and the fourteen species were isolated from the surface of the collected samples. The commonly observed species were *R. stolonifer, A. candidus*, *A. flavus*, *A. tamarii*, *A. wentii*, *A. ochraceus*, *A. niger*, *P. fellutanum*, *P. citrinum*, *A. ochraceus*, Yeast, *F. equiseti*, *P. aethiopicum*. The identification and the total Colony Forming Units (CFU) of isolated fungi were performed using standard plate technique. The total enumerated CFU of isolated fungi from the 23 collections was (114×103). High fungal contamination was observed in the sample kadiri-2 followed by Jyoti and JM-3. Detection of toxogenic fungi using DRBC media tested positive for several fungal species. Aflotoxin analysis using ELISA showed that Kadiri-2 (MK-374), Jyoti and JM-3 produced 8.6, 6.4, 7.0, ppb of aflatoxin content.

Conclusion: Using Czapek Dox Agar (CDA) six fungal genera comprising fourteen fungal species were isolated from sterilized and unsterilized samples. Identification and estimation of the total Colony Forming Units (CFU) of fungi was carried out using standard plate technique. The total enumeration of isolated fungi from the 23 collections was (114×103) CFU. Aflotoxin analysis using ELISA showed that the concentrations of aflatoxin in the samples are low and below permitted limits.

Keywords: Aflatoxin; mycotoxin; toxigenic fungi; Mahabubabad; Nekkonda; Thorrur; Palakurty; Jangon; Czapek Dox Agar; standard plate technique.

1. INTRODUCTION

Mycotoxins are secondary metabolites secreted by toxigenic fungi, that commonly grow in various agricultural products including food and feed stuffs. These are a potential threat to the health of human beings and animals. The corresponding health risks of mycotoxin consumption, their existence in many foodstuffs has drawn the recognition of scientists and the general public round the globe. Human consumption of plant-derived foods which are contaminated with different fungal moulds and may result in carry-over of mycotoxins in animal products viz., meat and eggs. According to the Council for Agricultural Science and Technology, 25% world wide of crops are affected by mycotoxin annually which would lead to loss of billion dollars [1-2]. Currently, more than 300 mycotoxins are identified from various sources. Aflatoxins are the major class of mycotoxins secreted by the Aspergillus sp, They were isolated and characterized from mold-contaminated peanut meal which caused turkey X disease [3-4]. Using fluorescence under blue or green UV light and relative chromatography till the date the major aflatoxins identified are B_1, B_2, G_1, and G_2, M_1 and M_2 (D'Mello and MacDonald [4]). Among these Aflatoxins, B1 is reported as the most potent natural carcinogen produced by toxigenic strains [5]. Rapid detection for the presence and level of aflatoxin contamination can be achieved by the mini-column method. Telangana is well known for large cultivation of groundnut crops in India. This crop is grown on a large scale approximately 70-80 million tons (35-60 million hectares) in Warangal, Karimnagar, Mahaboobnagar districts. The average rainfall 570- 1090 mm per year, making these district areas prone to drought and leading to favorable environment for the growth of aflatoxin producing fungi. According to the Regional agricultural Research Station, Warangal, the rain fall notices per year is 1059 mm, which is favorable for the growth of fungal species. The overall production of ground nut in both seasons is 1239 kg/ hector (565 Kg/hector in Kharif and 674 Kg/hector in Rabi) in overall land of 1100 hector. Due to limited monitoring and surveillance activities for detection of mycotoxin contamination in Warangal ground fields, the present study was framed out to conduct the survey on ground fields surrounding Warangal district and to determine the presence of mycotoxins and the fungi responsible for the production of aflotoxins.

2. MATERIALS AND METHODS

Samples were collected during August to November and October to March) different varieties of ground nut. samples of the varieties Kadiri-2 (MK-374), Kadiri-3 (Robout-33-1), Kadiri-4, Kadiri-5, Kadiri-6, Kadiri-7, Kadiri-8, Kadiri-9, Kadiri-71-1 (Virginia Group), Abhaya (TPT-25),

Table 1. Survey of ground nut fields used for the collection of ground nut seeds from various Mandals of Warangal District

S. no	Mandal	Area (hectares)		Variety
		Kharif	Rabi	
1	Bhupallpalle	33.87	25.5	Kadiri-2 (MK-374)
2	Cheriyal	41.96	52.6	Narayani (TCGS-29)
3	Dornakal	38.59	30.1	Kadiri-4
4	Duggondi	38.0	36.8	Rars-T-1
5	Eturnagaram	38.7	44.9	Jyoti
6	Hanamkonda	25.8	----	JM-2
7	Hasanparthy	37.2	----	Kadiri-8
8	Jangon	59.3	63.3	Kadiri-9
9	Kesamudram	40.5	41.4	JM-24
10	Lingalaghanpur	55.5	64.2	Abhaya (TPT-25)
11	Maddur	76.1	84.8	Kadiri-7
12	Mahabubabad	88.1	102.3	ICGV-91114
13	Mulugu	45.6	62.3	Jagtial-88 (JCG-88)
14	Narsampet	52.5	59.1	Kalahasti (TCGS-320)
15	Nekkonda	72.3	----	Kadiri-3 (Robout-33-1)
16	Palakurty	60.8	72.7	Prasana (TCS-341)
17	Parkal	33.6	39.8	Kadiri-5
18	Raghnathpalle	45.3	55.5	Rars-T-2
19	Raiparthy	24.6	----	Tirupati-4 (TCGS-30)
20	Regonda	47.5	23.6	Kadiri-6
21	Shyampet	39.1	20.6	Kadiri-71-1 (Virginia)
22	Thorrur	78.8	43.6	JM-3
23	Wardhannapet	45.7	22.3	Greeshma

Greeshma, Icgv-91114, Jagtial-88 (JCG-88), Kalahasti (TCGS-320), Narayani (TCGS-29), Prasana (TCS-341), Rars-T-1, Rars-T-2, Tirupati-4 (TCGS-30), Jyoti, JM-2, JM-3, JM-24 were taken from various District Mandals of Warangal (above Table 1).

2.1 Sample Preparation

A total of twenty three samples, representing various varieties of ground nuts were used in the study. For each sample, 3 replicates were taken to prepare one composite sample. All the samples were sealed and stored at 3-5℃ for mycoflora and mycotoxin determination. Samples were finely ground in a common household blender and rinsed in 85% alcohol. The powder stored at 4℃ for further analysis.

3. MOISTURE CONTENT DETERMINATION

Percent moisture content of kernels was determined by the oven method [6]. Three replicates were used for each sample. The kernels were ground and dried in the oven at 130℃ for 2 hours.

The moisture content was determined using the formula:

$$\text{Moisture content} = (Mo - Ml) \times \frac{100}{Mo}$$

Where;

Mo is the initial mass, in gram, of the test portion.

Ml is the mass, in gram, of the dry test portion.

We have selected only few areas for the collection of the samples for analysis of moisture content from normal and damaged kernels. The criterion for the selection was availability and non availability of water.

3.1 Mycological Analysis

3.1.1 Isolation of sample-borne mycoflora

The isolation of fungi was carried out using the method previously described by elsewhere [7]. 10 gm of each sample was decontaminated using 5-6% NaOCl (Sodium hypochlorite) for 1-2 min and rinsed with distilled water. The

disinfected samples were inoculated on the media that contain Czapek Dox Agar (CDA) supplemented with 0.5 mg chloramphenicol/mL to inhibit the bacterial growth. Three replicates were made and the plates were incubated at 25℃ for one week. The fungi colonies were identified according to morphological and microscopic characteristics.

3.1.2 Standard dilution plate for determination of colony-forming units

For fungal isolation, dilution method was used to determine total fungal counts in nut products samples. One grams of each composite sample (fine powder) were transferred into screw-capped medicinal bottle containing 9 mL of sterile distilled water and were mechanically homogenized at constant speed for 15 min. The sample-water suspension was allowed to stand for 10 min with intermittent shaking before being plated. Appropriate tenfold serial dilutions (1:10) were prepared and one mL portions of suitable dilutions of the resulting samples suspension (10-3) were used to inoculate Petri dishes each containing 15 mL Potato Dextrose Agar (PDA). Plates were then incubated for 7 days at 28℃. Three replicates plates per medium were used for each sample and the developing fungi were counted and identified according to several key processes. After incubation, the results were expressed in Colony-Forming Units (CFU) of samples; all plates were examined visually, directly and with a microscope [8].

4. DETERMINATION OF POTENTIAL TOXIGENIC FUNGI USING DRBC TEST

DRBC (Dichloran Rose Bengal chloramphenicol) is a selective medium that supports good growth of fungi. Dichloran reduces colony diameters of spreading fungi, rose bengal suppresses the growth of bacteria and restricts the size height of colonies of the rapidly growing moulds, chloramphenicol inhibits the growth of bacteria present in environmental and samples. The reduced pH of the medium from (7.2 to 5.6) helps inhibition of the spreading fungi [9-10]. The isolated fungi were inoculated in the solidified DRBC medium, after incubation for 7 days at 25℃, we looked for pigmentation and color change observed due to the secretion of toxigenic compound in comparison with CDA medium control. DRBC contains compounds that inhibit or reduce the spread of growth of moulds such as *Mucor* sp., *Rhizopus* sp., [11]. Dichloran and rose Bengal effectively slow down the growth of fast-growing fungi, thus readily allowing detection of other yeast and mold propagules, which have lower growth rates [12].

4.1 Aflatoxin Extraction and Analysis by Elisa

The homogenized samples (10 g each) were taken in 50 mL of 70% methanol separately and blended individually for 3 min. Samples were filtered and used for analysis. Commercially available immunoassay kit Veratox for quantitative analysis of aflatoxin test-NEOGEN Crop, Lansing, MI was used. The assay kit was based on Competitive Direct Enzyme Linked Immunosorbent Assay (CD-ELISA). The antibodies captured the analyte and conjugated to the enzyme (horse reddish peroxidase). Tetra methylbenzidine/hydrogen peroxide was used as a substrate for color development. Finally a stop solution was added to stop the reaction. The color intensity was inversely proportional to the mycotoxin concentration and measured with the ELISA reader. All necessary reagents were present in the kit. Concentration of mycotoxins was calculated by Log/logit Software Awareness Technology Inc. [13].

5. RESULTS AND DISCUSSION

Moisture content (M.C.) is one of the key factors for invading and development of fungi ground nuts [14]. In accordance with World Health Organization the minimum M.C. required for the development of *Aspergillus flavus* in groundnuts ranges from 9.0 - 10.0%. Tables 2 and 3 represent the percentage of M.C.'s and kernel damage of samples collected from different storage systems of various Mandals and villages of Warangal district during the wet and dry seasons. The percentage of moisture content and damaged kernels analysis was selectively conducted only in few samples from overall 23 sample collection (Based on the high availability of water for cultivation). The M.C. of samples collected during wet season from Mahabubabad, Nekkonda, Thorrur, Palakurty, Jangon of different storage systems (See Table 2) ranged between 13.1 – 17.0%, 17.7 %, 12.8%, 15.4%, 14.1%, 9.0%, 12.5%, 10.6%, respectively. The freshly harvested ground samples showed highest M.C. percentage with 36.6% (Table 2). Whereas, samples collected during dry season from Mahabubabad, Nekkonda, Thorrur, Palakurty, Jangon of different storage systems (See Table 3) exhibited less m.c and they were 8.1-13.0%, 12.1-15.9%, 10.1%, 12.5%, 7.1%, 11.9%, 8.6% and 11.1%.

Table 2. Moisture content and percentages of damaged kernels of ground nut seeds collected during wet season

Date & Place of Kernal collection	Source of sample	Nature of sample	Sample code	(%) of moisture content	(%) of damage
20th June 2015, Mahabubabad	Farmer storage sample (FSS)	Shelled	MK-M-S-15	13.1-17.0	28.3-37.1
14th Jan, 2015 Nekkonda	Wholesaler sample (WS)	Unshelled	MK-M-US-6	17.7	33.5-41.8
	Retailer sample (RS)	Unshelled	MK-M-US-21	12.8	20.9-28.6
8th Feb, 2015 Thorrur	House kitchen (HK)	Unshelled	MK-M-US-8	15.4	18.1-31.4
25th Mar, 2015 Palakurty	Wholesaler sample (WS)	Unshelled	MK-M-US-27	14.1	31.1-40.2
	Retailer sample (RS)	Unshelled	MK-M-US-19	9.0	15.5-21.2
15th May, 2015 Jangon	Wholesaler sample (WS)	Unshelled	MK-M-US-15	12.5	21.5-28.0
	Retailer sample (RS)	Unshelled	MK-M-US-2	10.6	17.1-25.3
12th July, 2016 Mulugu	Freshly harvested ground nut (FHG)	Shelled	MK-M-S-5	36.6	----------

Table 3. Moisture content and percentages of damaged kernels of ground nut seeds collected during dry season

Date & Place of Kernal collection	Source of sample	Nature of sample	Sample code	(%) of moisture content	(%) of damage
17th Aug, 2016 Mahabubabad	Farmer storage sample (FSS)	Shelled	MK-M-S-31	8.1-13.0	10.5-14.9
24th Aug, 2015	Wholesaler sample (WS)	Unshelled	MK-M-US-22	12.1-15.9	25.2-36.7
Nekkonda	Retailer sample (RS)	Unshelled	MK-M-US-7	10.1	13.0-18.1
11th Sep, 2015 Thorrur	House kitchen (HK)	Unshelled	MK-M-US-38	21.5	36.5-44.4
20th Sep, 2015	Wholesaler sample (WS)	Unshelled	MK-M-US-17	27.1	48.0-56.2
Palakurty	Retailer sample (RS)	Unshelled	MK-M-US-25	15.7	28.0-31.5
7th Oct, 2015	Wholesaler sample (WS)	Unshelled	MK-M-US-41	18.6	34.1-39.0
Jangon	Retailer sample (RS)	Unshelled	MK-M-US-12	11.1	16.4-20.3

The percentages of kernel damage of the samples collected during dry and wet seasons are represented in Tables 2 and 3. The percentages of damaged kernels from Mahabubabad, Nekkonda, Thorrur, Palakurty, Jangon were 12.0 - 51.8%, 10.2 - 38.0%, 8.7 - 27,9%, and 1.5 - 26.0%, respectively.

The percentages of damaged kernels of samples collected from different localities during the wet season are presented in Table 2. The damaged kernels from Cidolog were between 2.5 - 13.8% (FHG) and 9.9 - 31.7% (samples from FSS and MW), while those from Cianjur, Sukabumi and Bogor were between 13.0 - 61.0%, 18.0 - 55.0%, and 6.5 -56.0%, respectively.

A total of 23 samples were screened for enumeration of isolated fungal species. The results were presented in Table 4 and shows that fungi were isolated using Agar Plate Method (APM) plated on CDA medium. Agar plate method was commonly employed in this study. Two sets of sample are tested (unsterilized and surface sterilized) nut samples. Six types of fungal genera and fourteen species were identified and isolated on the

basis of their cultural and morphological characteristics.

The identified genera were *Aspergillus* spp. *Cladospoium* sp., *Fusarium* sp., *Rhizopus stolonifer*, *Penicillium* spp. and yeasts. Samples which were sterilized resulted in minimal number of fungal species compared to untreated samples. Among the contaminated fungi *Aspergillus* spp was isolated from almost all the samples used in the study. This result correlates with the results of many investigations on seed pathology [15]. *Aspergillus* and *Penicillium* spp predominantly occur in the stored nuts [16-17]. Denizel et al. [18], mentioned in their report that *Aspergillus niger*, *Cephalosporium* sp. and *Trichoderma viridae* were the toxigenic fungi species commonly identified from the infected nuts in Turkey [19].

Previous research which was carried out globally to detect and isolate the different fungal species revealed that *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma* and *Cladosporium* were the common fungal genera that infects the nuts [20-21].

The results shown in Tables 5A1, 5A2 and 6 represent the identification and the total Colony Forming Units (CFU) of fungi isolated by standard plate technique. According to the results, all samples collected were contaminated with different fungal strains. The total enumeration of isolated fungi from the 23 collections was (114×10^3) CFU. High fungal contamination was observed with the sample kadiri-2 followed by Jyoti and JM-3 (See Table 6). Less contamination was noticed in sample Narayani (TCGS-29) followed by JM-2, Kadiri-8, JM-24. ICGV-91114 (See Table 6). The remaining samples showed minimum, average and moderate fungal contamination. Among the fungal strains identified, *A. niger* which in turn was followed by *R. stolonifer* with (21×103 CFU), *P. aethiopicum* (19×10^3) and Yeast (17×10^3), have all showed high infection in collected seeds compared to remaining samples (See Table 6). Our results correlated with previously reported articles and revealed that, most of the dominant fungal genera that infects peanut samples were *Aspergillus* and *Penicillium,* and the common species observed were *Aspergillus flavus*, *Aspergillus niger*, *Penicillium citrinum* [22-24].

Table 4. Fungal genera and species isolated from the surface of ground seeds using Agar Plate Method with and without treatment of sodium hypochlorite

S. no	Sample	Czapex dox agar	
		Untreated	Treated
1	Kadiri-2 (MK-374)	*A. candidus*, *A. flavus,*	*A. flavus*
2	Narayani (TCGS-29)	*A. flavus, A. tamari*	*A. flavus, A. tamari*
3	Kadiri-4	*A. niger, A. flavus,*	*A. niger*
4	Rars-T-1	*A. tamarii, A. candidus*	*A. candidus*
5	Jyoti	*A. wentii, A. fumigates,*	*R. stolonifer*
6	JM-2	*A. fumigatus*, *A. niger*	Yeast
7	Kadiri-8	*A. ochraceus, A. niger*	*F. equiseti*
8	Kadiri-9	*P. aethiopicum, A. candidus*	*P. fellutanum*
9	JM-24	*P. citrinum*, *A. fumigatus*	*A. tamarii*
10	Abhaya (TPT-25)	*A .fumigates, A. niger*	*P. citrinum*
11	Kadiri-7	*A. niger, A. candidus*	*A. candidus*
12	ICGV-91114	*A. candidus*, *A. flavus,*	*P. aethiopicum*
13	Jagtial-88 (JCG-88)	*A. flavus, A. niger*	*A. fumigatus*
14	Kalahasti (TCGS-320)	*A. ochraceus, A. candidus*	Yeast
15	Kadiri-3 (Robout-33-1)	*A. candidus*, *A. flavus,*	*A. niger*
16	Prasana (TCS-341)	*A. ochraceus, A. candidus*	*A. flavus*
17	Kadiri-5	*A. flavus, A. tamarii*	*A. tamarii*
18	Rars-T-2	*A. tamarii, A. niger*	*A. ochraceus*
19	Tirupati-4 (TCGS-30)	*P. citrinum, P. aethiopicum*	*A. tamarii*
20	Kadiri-6	*A. niger, A. flavus*	*A. candidus*
21	Kadiri-71-1(Virginia)	*A. ochraceus, A. candidus*	*R. stolonifer*
22	JM-3	*A. fumigates, A. flavus*	*P. fellutanum*
23	Greeshma	*A. niger, A. flavus*	*A. flavus*

Table 5A1. Range of fungal infection of ground nut samples collected from different localities and different storage systems

Fungi	Mahabubabad				Nekkonda				Thorrur			
	D		W		D		W		D		W	
	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS
A. flavus	35-40	29-34	44-58	59-63	55-60	71-91	54-68	43-59	33-41	48-69	24-46	39-48
A. niger	30-44	49-66	70-89	39-48	66-78	25-29	31-52	25-41	22-38	25-45	34-49	26-38
A. fumigates	15-20	12-16	28-35	32-41	25-28	19-26	38-44	47-55	10-12	0-10	18-22	30-38
A. candidus	63-45	45-30	80-41	71-53	69-42	55-39	64-58	85-71	35-85	47-63	55-70	65-73
F. equiseti	----	----	----	----	----	----	----	----	----	----	----	----
R. stolonifer	----	----	----	----	----	----	----	----	----	----	----	----
P. fellutanum	05-08	07-09	14-16	12-17	05-10	15-20	24-30	18-23	18-28	30-41	58-63	45-59
P. aethiopicum	----	----	----	----	----	----	----	----	----	----	----	----
P. citrinum	----	----	----	----	----	----	----	----	----	----	----	----
Yeast	----	----	----	----	----	----	----	----	----	----	----	----

D- Dry sample, W- Wet sample, WS- Wholesale Sample, RS-Retail Sample

Table 5A2. Range of fungal infection of ground nut samples collected from different localities and different storage systems

Fungi	Palakurthy				Janagon				Mulugu			
	D		W		D		W		D		W	
	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS	WS	RS
A. flavus	55-75	49-70	36-64	73-82	18-29	20-24	10-35	23-42	20-29	29-53	18-24	07-15
A. niger	62-78	48-66	51-72	40-59	15-25	30-48	18-30	47-70	14- 22	52-70	17-25	20-28
A. fumigates	05-08	17-25	44-53	22-31	25-28	20-29	15-28	36-55	30-35	10-19	24-30	55-60
A. candidus	33-20	45-61	74-85	45-35	28-40	39-19	25-11	38-23	20-35	15-23	48-51	60-84
F. equiseti	----	----	----	----	----	----	----	----	----	----	----	----
R. stolonifer	----	----	----	----	----	----	----	----	----	----	----	----
P. fellutanum	15-20	27-36	58-69	36-51	12-18	04-06	18-26	12-16	04-05	01-05	11-14	08-14
P. aethiopicum	----	----	----	----	----	----	----	----	----	----	----	----
P. citrinum	21-26	18-24	11-13	08-10	----	----	----	----	----	----	----	----
Yeast	----	----	----	----	----	----	----	----	----	----	----	----

D- Dry sample, W- Wet sample, WS- Wholesale Sample, RS-Retail Sample

Table 6. Fungal isolates using standard dilution plate method and the Colony Forming Units samples

Fungi	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	TCFU
A. flavus	02	--	--	01	--	--	--	02	--	--	--	--	01	--	--	--	--	03	01	--	--	--	--	09
A. niger	--	--	05	01	02	01	--	02	01	01	01	02	02	--	01	01	01	--	02	01	02	01	--	27
A. fumigates	02	--	--	01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	03
A. candidus	02	--	--	01	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	04
F. equiseti	04	01	--	01	03	01	--	--	01	01	01	--	--	02	--	01	01	02	01	--	--	01	--	21
R. stolonifer	--	--	--	--	02	--	01	--	--	--	--	--	--	--	02	01	01	--	--	01	--	--	01	09
P. fellutanum	03	--	--	--	--	--	--	02	--	04	--	01	01	01	01	--	02	--	--	01	01	01	01	19
P. aethiopicum	--	--	--	--	--	--	--	--	--	--	--	--	01	--	--	--	--	--	--	--	--	--	--	01
P. citrinum	01	--	--	--	--	--	01	01	--	--	--	--	--	--	01	--	--	--	--	--	--	--	--	04
Yeast	03	--	02	01	02	--	--	--	--	--	--	--	--	01	--	01	01	01	--	--	--	05	--	17
Total	17	01	07	06	09	02	02	07	02	06	02	03	06	04	05	04	06	06	04	03	03	08	02	104×10^3

Samples 1-23: Kadiri-2 (MK-374), Narayani (TCGS-29), Kadiri-4, Rars-T-1, Jyoti, JM-2, Kadiri-8, Kadiri-9, JM-24, Abhaya (TPT-25), Greeshma, Kadiri-7, Jagtial-88 (JCG-88), Kalahasti (TCGS-320), Kadiri-3(Robout-33-1), Prasana (TCS-341), Kadiri-5, Rars-T-2, Tirupati-4 (TCGS-30), Kadiri-6, Kadiri-71-1(Virginia Group), JM-3, ICGV-91114

Table 7. Determination of toxigenic fungi isolated from ground nut samples using culturing on DRBC agar media

S. no	Fungi	Dichloran rose Bengal chloramphenicol agar mediea
1	*A. flavus*	Positive
2	*A. niger*	Positive
3	*A. fumigates*	Positive
4	*A. candidus*	Positive
5	*P. fellutanum*	Negative
6	*P. aethiopicum*	Negative
7	*P. citrinum*	Negative
8	*F. equiseti*	Negative
9	*R. stolonifer*	Positive
10	Yeast	Negative

Table 8. Total aflatoxin content in collected samples by EISA method

S. no	Sample	OD	Results
	Aflotoxin content (ppb)		
1	Kadiri-2 (MK-374)	1.206	6.4
2	Narayani (TCGS-29)	0.856	1.3
3	Kadiri-4	0.243	0.9
4	Rars-T-1	0.729	0.2
5	Jyoti	0.831	0.2
6	JM-2	0.571	1.5
7	Kadiri-8	0.173	3.8
8	Kadiri-9	1.270	0.9
9	JM-24	0.962	0.5
10	Abhaya (TPT-25)	0.881	1.1
11	Greeshma	2.814	0.9
12	Kadiri-7	1.419	1.3
13	Jagtial-88 (JCG-88)	0.640	0.5
14	Kalahasti (TCGS-320)	0.917	0.8
15	Kadiri-3 (Robout-33-1)	0.444	0.3
16	Prasana (TCS-341)	0.810	1.2
17	Kadiri-5	0.383	0.5
18	Rars-T-2	1.892	0.1
19	Tirupati-4 (TCGS-30)	0.451	0.1
20	Kadiri-6	0.176	0.4
21	Kadiri-71-1 (Virginia group)	0.556	1.0
22	JM-3	0.827	2.8
23	ICGV-91114.	0.259	10

Detection of toxogenic fungi using DRBC media is shown in above Table 7. According to the test results, samples collected from different regions of Warangal District (during wet season) contained potential toxogenic fungi. The identified species were *A. flavus*, *A. niger*, *A. fumigatus*, *A. candidus*, *P. fellutanum*, *P. aethiopicum*, *P. citrinum*, *F. equiseti*, *R. stolonifer* and Yeast. Aflatoxin extraction and analysis by ELISA revealed that, among the samples tested Kadiri-2 (MK-374), Jyoti and JM-3 resulted in high 8.6, 6.4 and 7.0 ppb of aflatoxin content, respectively. The remaining samples, Kalahasthi (TCGS-320), Kadiri-3 (robout-33-1), Rars-T-2, Rars-T-1, Kadiri-6, Narayani (TCGS-29), Kadiri-9 showed minimal concentrations of aflatoxin content (see above Table 8). Most of the samples which tested positive for the production of aflatoxin were infected with *A. flavus*, *A. fumigates* and *A. niger*.

According to our results, samples collected during wet season contained high levels of aflatoxin compared to samples collected during dry season (Data of aflatoxin detection from dry samples were not shown). Our results are in contrary with investigations of Rajab [24] on the detection of aflatoxins in ground nut samples. By the current study, we came to know that the aflatoxin concentration in the collected samples was found in lower and safe limits for human consumption. The higher amount of mycotoxins in the human body can lead to severe damage to every part of the body [25].

6. CONCLUSION

Using Czapek Dox Agar (CDA) media we have isolated six types of fungal genera and fourteen species were isolated from sterilized and unsterilized samples. The identification and the total Colony Forming Units (CFU) of fungi was isolated using standard plate technique. The total enumerated of isolated fungi from the 23 collections was (114×10^3) CFU. The aflotoxin extraction using ELISA showed that the concentrations of aflatoxin in the samples are lower and in safer limits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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