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Research article

Mycobiota and Mycotoxins in sunflower seeds in pre and post-harvest condition from Bihar state, India.

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Abstract

The Study was conducted to determine the mycobiota and co-occurrence of mycotoxin in sunflower seeds during Pre harvest and Post harvest condition. A total of 196 isolates of 11 species belong to 6 different fungal genera from Pre harvested samples and 558 isolates of 21 species belong to 8 different fungal genera from Post harvested sample were isolated and identified. 51.6% isolates of *Aspergillus flavus*, 46.2% of *Penicillium citrinum* and 42.1% isolates of *Fusarium moniliforme* and 33.3% isolate of *Penicillium verrucosum* were toxigenic in Post harvested samples. Whereas in pre harvested samples 38.4% isolates of *A. flavus*, 40% isolate of *P. citrinum* and 28.5% of *F. moniliforme* were toxigenic. Natural occurrences of mycotoxins in sunflower seeds were analyzed by ELISA and TLC method. About 46% of post harvested samples were positive to aflatoxin B_1 and in pre harvested samples it was only about 14%. The mycotoxin detected from post harvested sunflower seeds were aflatoxin B_1 (AFB_1), aflatoxin G_1 (AFG_1), ochratoxin A (OTA), citrinin and zearalenone and the highest amount of AFB_1 was 1070 µg/kg and AFG_1 was 338 µg/kg detected. In pre harvest sample only AFB_1, AFG_1, OTA and citrinin were detected however zearalenone was not found. The result from present study revealed that sunflower seeds are prone to mycotoxigenic fungi in both pre harvest and post harvest condition and further mycotoxin production. This is the first report of occurrence of mycotoxigenic fungi and mycotoxins in standing crop of sunflower from India.

Keywords: Sunflower seeds, Mycotoxigenic fungi, Mycotoxins, Pre harvest, Post harvest

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Introduction

Fungi are ubiquitous plant pathogen and so are major spoilage agents of foods and feedstuffs. The infection of plants by various fungi not only results in reduction of crop yield, quality and economic loss but also contamination of grains with toxic fungal metabolites called mycotoxins which are hazardous for human health and animals [1-3]. Mycotoxins are toxic secondary metabolites produced by fungi that are usually belonging to *Aspergillus, Penicillium* and *Fusarium* genera. These naturally occurring chemical compound can be produced on a wide range of agricultural commodity and under diverge range of situation worldwide. The presence of these compounds in food chain had highly adverse effect on consumers when ingested in unrestricted manner along with food and feeds [4,5].

Sunflower (*Helianthus annuus* L.) is a major source of vegetable oil in the world. Its seed and oil contain healthy unsaturated fats, protein, fiber and important nutrients like vitamin E, selenium, copper, zinc, folate and iron. About 90% of the fat in sunflower seeds is mono and polyunsaturated fat which is good for heart [6]. Vitamin E is an antioxidant that may protect against heart diseases by getting rid of harmful molecules called free radicals that can lead to arterioscleroses.

India is one of the largest producers of oil seed crop in the world. Sunflower seeds are one of the major producers of vegetable oil in India mainly used as cooking medium. As with many other agriculture products, sunflower seeds may be exposed to a wide range of microbial contamination during pre and post harvest. Natural incidence of mycotoxigenic fungi and mycotoxin contamination in cereals, nuts and spices has been reported by different workers from different part of world including India [7-9]. However very few reports are available regarding association of mycotoxigenic fungal flora and natural occurrence of mycotoxins in sunflower seeds and these are mainly confined to aflatoxins contamination [10].The present study was conducted to ascertain the predominant mycoflora associated with sunflower seeds in pre and post harvest condition and the natural occurrence of mycotoxin contamination in these seeds in the state of Bihar. The present study revealed that the other potent mycotoxins such as ochratoxin A, citrinin, and zearalenone were also present in sunflower seeds and the amount were sufficiently high to induced toxicity in consumers [11]. The occurrence of mycotoxigenic fungi and mycotoxins from field crop of sunflower was reported for the first time from India.

Materials and Methods

Sample collection

Total 240 samples of sunflower seeds were collected from 10 different area of the Bihar, 24 samples from each area (12 samples from the field before harvesting and 12 from the stored place after harvesting of same area).

Pre-harvested sample collection

After maturation of the sunflower plant in the field, the capitulum/heads were collected randomly and put it into the sterilized polythene bag and then into the sterilized brown envelope. Further the sunflower seeds were kept at 4°C to arrest any mycotoxin formation before analysis in laboratory.

Post- harvested sample collection

After harvesting of the same sunflower crop, the seeds were collected from different storage system from the farmers. The samples were randomly collected from different stored place and put into the sterilized polythene bag and then into sterilized brown envelope and kept at 4°C to arrest any mycotoxin formation before analysis.

Standard samples preparation

Standard samples were prepared by mixing of 12 samples of each area of pre harvest or post harvest. Then total 20 standard samples were prepared, 10 each from pre and post-harvested samples.

Moisture content of samples

100gm of each standard sunflower samples were dried at 70°C in triplicate for 48 to 72 hours until their weight remains constant. The difference in weight after drying is considered as moisture content.

Mycological studies

20 standard samples of both collected from pre harvest and post harvest had randomly placed on the freshly prepared Potato dextrose agar (PDA) and Czapek dox agar media in triplicate. Total 100 seeds were plated in 15 cm petriplate that each plate contains 25 seeds, and incubated at 28 ± 2°C for 7 days and examined daily. The counts were recorded after 3 to 5 days. After incubation all plates were examine visually and by binocular stereomicroscope. Fungal colonies of different morphological type were sub-cultured by hyphal tip method culture tube containing PDA media. Identification was carried out by morphological characteristics and followed the taxonomic schemes of Maren [12] for genus *Aspergillus*, Pitt [13] for *Penicillium* and Dugan [14] and Paul et al. [15] for other genera.

Screening of fungal isolates for mycotoxin producing potentiality

Aflatoxin producing potential of *A. flavus* isolates were examined in SMKY and YES broth media [16] and potentiality of *P. citrinum* and *A. ochraceus* for citrinin and ochratoxin were examined by the methods of Schwenk et al. [17] and Davis et al. [18]. Moist-rice medium [19] was used for assessing zearalenone producing potentiality of *Fusarium* spp. The suspensions of isolated fungi were prepared by Macfarland standard in normal saline that each ml of saline contains 10^6 spores [20]. In all cases 50µl of each suspension was inoculated in 25ml of freshly prepared broth media and incubated at 28 ± 2°C for 10 days then the culture was filtered with Watman No.1 paper. The cultured filtrate was extracted with 10 ml of chloroform. In case of CTN the culture filtered was acidified with 1N HCL to bring down the pH subsequently then it was extracted with chloroform. The chloroform extract was evaporated to dryness and residue was dissolved in 1 ml of chloroform and qualitative and quantitative estimation of mycotoxins producing potentiality of fungi were examined by Thin Layer Chromatography (TLC). TLC was done in 20 x 20 cm glass plates with silica gel (Sigma-Aldrich). The plate were dried at 110°C for one hour and stored at moisture proof container. For the analysis of aflatoxins, 50µl of each extract were placed together with the standard Aflatoxin mix (Sigma-Aldrich) with the help of micropipette and put into TLC chamber containing the mobile phase of Toluene : Isoamyl alcohol : Methanol (90:32:02, v/v/v). For Citrinin, ochratoxin A (OTA) and zearalenone, the extract were placed with citrinin, ochratoxin A and zearalenone standard mycotoxins (Sigma-Aldrich) and put into the chamber containing mobile phase of solvent Toluene : Ethylacetate : Formic acid (6:3:1, v/v/v) and Benzene : Methanol : Acetic acid (24:2:1, v/v/v) and the plates were observed under the long and short wave length UV-light (254 and 365 nm). The fluoresce and the Rf value of the sample spot on TLC plate were matched with the Fluorescent intensity and Rf value of standard.

Qualitative and Quantitative detection of Mycotoxins by Enzyme linked immunosorbent assay (ELISA) and Thin-layer Chromatography

The Samples were analyzed by different Agraquant Mycotoxin ELISA kits, Agraquant Aflatoxin B_1 (COKAQ8000) for AFB_1, Agraqunt Ochratoxin (COKAQ2000) for OTA and Agraquant ZON (COKAQ5000) for zearalenone from ROMER LAB (ASTRIA) for qualitative and quantitative studies. For the presence of mycotoxins the 20 gm of sample were grinded and added 100 ml of 70% methanol blended for 3 minute. The solutions were filtered and the supernatant was collected. 4ml of extract was transferred through cleanup columns then the presence of specific mycotoxins were detected with specific ELISA kits and the optical density was recorded by the ELISA reader (MERK mios mini)
[21] using a 450 nm filter with a differential filter of 630 nm. Standard curve was prepared with standard solution provided with ELISA kits. The optical densities of the samples were compared to the optical density of standards and interpretative results were determined. Qualitative and quantitative estimation of Citrinin and other mycotoxins were carried out by the method of Robert and Patterson [22] and Wilson [23] using TLC technique.

Result & Discussion

In our study, total 196 isolates of 11 species of 6 different genera of fungi were isolated from pre harvested samples of sunflower seeds and 558 isolates of 21 species of 8 different genera were isolated from post harvested samples (Table 1) and identified as *Alternaria alternata, Alternaria helianthi, Aspergillus candidus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus ochraceus, Aspergillus terreus, Chaetomium globosum, Chaetomium indicum, Curvularia lunata, Drechslera howaiiensis, Fusarium moniliforme, Fusarium oxysporum¸ Fusarium semitectum, Penicillium citrinum, Penicillium fellutanum, Penicillium islandicum, Penicillium verrucosum, Rhizopus nigricans, Rhizopus oryzae.* In this context, Sharfun-nahar et al. [24] also reported the association of some of these fungi with sunflower seeds. Fungi classified into two broad ecological categories: Pre harvested fungi and Post harvested fungi. Pre harvested fungi attack living plants before harvest and post harvested fungi are those which can attack on plant after harvesting, during processing, transportation and at high moisture condition found in store. In this investigation it has been observed that *A. flavus, P. citrinum, P. verrucosum* and *F. moniliforme* were frequently present in standing crop. (Fig. 1 and Fig. 2).

In Pre harvested samples *A. flavus, P. citrinum, F. moniliforme and A. helianthi* and in post harvested samples *A. flavus, P. citrinum, F. moniliforme, A. helianthi, A.fumigatus, A. ochraceus, A.niger, P. verrucosum and R. nigricans* were the dominant among most of the examined sunflower seeds samples (Table 1), the present finding supports the report of Kakde et al. [25]. The mycofloral frequency is also higher in post harvested sample than pre harvested samples (Fig. 3). Incidentally, In pre harvested samples 38.4% of *A. flavus*

, 40% of *P. citrinum*, 21.4% of *P. verrucosum*, 28.5% of *F. moniliforme* and 12.5% of *F. oxysporum* were the aflatoxin, citrinin, ochratoxin A and zearalenone producing fungi but none of the isolates of *Aspergillus ochraceus* and *Aspergillus terreus* were toxigenic (Table 2). Where as in the post harvested samples 51.6% of *A. flavus*, 22.5% of *A. ochraceus*, 28.4% of *P. verrucosum*, *42*.1% of *F. moniliforme*, 16.6 % of *F. oxysporum* and 46.3% of *P. citrinum* were aflatoxin, citrinin, ochratoxin A and zearalenone producing fungi reported in this study is alarming and is a matter of concern (Fig. 4). However none of the isolates of *A. terreus* was toxigenic. In this context Abdel-Mallek et al. [26] have reported the occurrence of mycotoxigenic fungi from sunflower seeds and recently Levic et al. [27] observed that *Alternaria* species were dominant on sunflower seeds (76.9%) in the Serbia and frequency of *Fusarium* species on sunflower seeds varied from 7% to 15%. Total 240 samples were analyzed to know the natural occurrence of mycotoxin contamination in sunflower seeds. Out of 120 Pre harvested samples, 16 were positive to AFB_1 and 11positive to AFG_1, whereas OTA was detected from 14 samples and only 11 samples were positive to citrinin. None of the pre harvested sample was zearalenone positive. The detected amount of AFB_1 and AFG_1 was 43-355 µg/kg and 24-85 µg/kg respectively, OTA was 49-212 µg/kg and citrinin was 23-65 µg/kg. whereas in 120 post harvested samples, total 56 samples were positive to AFB_1 and 36 samples were positive to AFG_1 whereas only 27 samples and 26 samples were positive to OTA and citrinin respectively. Zeralenone was detected from 7 sunflower samples. The detected amount of AFB_1 and AFG_1 was ranges from 463 – 1070 µg/kg and 129-338 µg/kg respectively and OTA, citrinin and zearalenone was 131-415 µg/kg, 65-433 µg/kg and 111-125µg/kg respectively (Table 3).

The natural occurrence of amount of mycotoxins in pre harvested sunflower seeds were less than post harvested samples but the amount is much higher than the permissible limit and able to induce toxicosis in consumers. In this context Jimenez et al. [28] found that 100% of the sunflower seeds samples show variable incidences of fungal contamination and these fungi have ability to produce different mycotoxins. Recently Konaneko and Burkin [29] had reported the contamination and co-occurrence of citrinin and ochratoxin A in sunflower oil-seed meal of poultry feed. Magnolia et al. [30] reported the occurrence of ochratoxin A in stored peanut seeds in Argentina. *Aspergillus spp*. (100%) *Penicillium spp.* (87%) and *Eurotium spp.* (6.4%) and 32% of peanut seeds sample were positive to ochratoxin A and it ranges from 0.5-170 ng/g. Konaneko and Burkin [31] had done the survey on the occurrence of citrinin in the feeds and their ingredients in Russia and found 28.9% of the sunflower oilseeds meal and cakes were contaminated with citrinin at the level at 14-394 µg/kg where as 3 soybean sample out of 148 were positive and contain citrinin in a range of 14-30 µg/kg. Earlier Torres et al. [32] reported occurrence of *Alternaria alternate* and several mycotoxins produced by this species such as alternariol, alternariol monomethyl ether and tenuazonic acid from sunflower seeds. The level of alternariol reported between 35-792 µg/kg.

The association of sunflower seeds with several toxigenic mycobiota and mycotoxins can cause carcinogenesis [33] and many other liver, kidney and digestive tract diseases in human beings and the animals. This study has been attempted to examine the sunflower seeds in pre harvested condition and post harvest condition to know that storage fungi which are usually not present or less present before harvesting but during storage they grow. Contamination occurs through the spores present in the standing crop subsequently enter into storage from handling, processing, transportation and storage structures or from spores already present in storage structures (Fig. 5 and Fig. 6). Under high temperature and moisture content of the storage system supports fungal growth and further mycotoxin production [34].

Abiotic factor which are responsible for growth of fungi on sunflower seeds is the moisture content of seeds and moisture content and temperature of the storage structure. The moisture content reported in this study is high (average 9.9%) in pre harvested sample but the temperature is not favorable for the growth of fungi in field. However the moisture content of post harvested sample (average 8.3%) is low in comparison to pre harvested sample as before storing the seeds were dried (Table 1). Toxins producing species usually grow at lower limit of moisture content than field fungi [35, 36].

Conclusion

On the basis of present study, it may be concluded that the occurrence of mycotoxigenic fungi and mycotoxin contamination is not confined only in the post harvested samples but it is also present in pre harvested samples which is new report from India. The amount of pre harvest samples contamination is lower than the post harvested samples. It has also been observed that potent mycotoxigenic fungi were present in this crop, hence care of crop in the field, processing, transportation, storage and handling is important to reduce these hazardous mycotoxins. Further research is needed to minimize mycotoxin level in this crop in field and storage condition.

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Table 1: Distribution of fungal species in Pre and Post-harvested samples of sunflower seeds and their moisture content.

Samples of sunflower seeds		Mycoflora[a]																				Total isolate	Moisture (%) ± S.E	Total Species
		A.al	*A.he*	*A.ca*	*A.fl*	*A.fu*	*A.ni*	*A.oc*	*A.te*	*C.gl*	*C.in*	*C.lu*	*D.ho*	*F.mo*	*F.ox*	*F.se*	*P.ci*	*P.fe*	*P.is*	*P.ve*	*R.ni*	*R.or*			
Pre- harvested samples	1F	-	6	-	5	3	-	-	-	-	-	-	-	1	1	-	1	-	-	4	-	-	22	9.7 ± 0.32	8
	2F	1	2	-	3	2	-	-	-	1	-	-	-	2	-	-	4	-	-	2	-	1	18	10.5 ±0.91	9
	3F	-	-	-	2	3	-	-	-	-	-	-	-	5	-	-	3	-	-	2	-	-	15	9.9 ± 0.66	5
	4F	2	-	-	2	-	-	-	-	-	-	-	-	3	1	-	4	-	-	3	-	-	15	11.3 ± 1.18	6
	5F	4	7	-	4	5	-	-	-	-	-	-	-	5	-	-	5	-	-	5	-	5	41	10.3± 0.26	9
	6F	-	-	-	7	-	-	-	-	-	-	-	-	3	-	-	5	-	-	-	-	2	17	9.9 ± 0.41	4
	7F	-	1	-	4	1	-	-	-	-	-	-	-	3	-	-	-	-	-	1	-	-	10	10.7 ± 1.11	5
	8F	-	-	-	2	-	-	-	-	-	-	-	-	2	2	-	3	-	-	2	-	-	11	9.0 ± 0.16	5
	9F	3	5	-	4	4	-	-	-	1	-	-	-	3	3	-	2	-	-	-	-	1	26	9.2 ± 1.12	9
	10F	-	4	-	6	1	-	-	-	1	-	-	-	1	1	-	6	-	-	2	-	1	21	8.8 ± 0.42	9
	Total	10	25	-	39	19	-	-	-	3	-	-	-	28	8	-	33	-	-	21	-	10	196		
	% density	5.1	12.7	-	19.9	9.7	-	-	-	1.5	-	-	-	14.3	4.1	-	16.8	-	-	10.7	-	5.1			
Post- harvested samples	1S	1	9	1	11	7	3	6	1	-	-	-	1	3	2	-	4	1	1	8	4	-	63	8.6 ± 0.56	16
	2S	1	3	-	7	6	1	4	-	-	-	1	-	5	2	-	7	-	1	5	3	-	47	9.2 ± 0.48	14
	3S	2	1	2	7	4	2	3	-	-	1	1	1	9	1	-	8	2	-	5	6	-	55	7.7 ± 0.31	16
	4S	2	-	1	5	2	2	2	-	-	-	-	1	5	4	2	6	1	-	4	-	-	37	8.0 ± 1.23	13
	5S	1	11	1	10	8	3	5	-	-	2	2	2	6	3	-	11	2	2	6	2	-	78	8.4 ± 0.69	18
	6S	1	1	3	15	1	6	3	1	-	-	3	4	4	1	1	8	-	-	3	4	-	59	7.5 ± 0.45	16
	7S	1	1	-	13	5	2	4	1	-	1	-	-	10	1	-	6	-	5	3	5	-	58	8.9 ± 1.21	14
	8S	2	-	1	6	-	3	4	1	-	-	-	1	4	2	1	5	1	2	5	3	-	39	8.1 ± 0.54	15
	9S	4	7	1	9	7	3	2	1	-	-	1	1	6	4	1	5	1	-	6	-	-	58	9.3 ± 0.25	16
	10S	1	4	-	9	4	5	7	1	-	1	1	2	5	4	-	7	-	1	8	3	-	64	8.1 ± 0.68	17
	Total	16	37	10	92	44	30	40	6	-	5	9	13	57	24	5	67	8	12	53	30	-	558		
	% density	2.8	6.6	1.8	16.5	7.9	5.4	7.2	1.1	-	0.9	1.6	2.3	10.2	4.3	0.9	12.0	1.4	2.1	9.5	5.34	-			

[a] Mycoflora are *A.al- Alternaria alternata, A.he- Alternaria helianthi, A.ca- Aspergillus candidus, A.fl- Aspergillus flavus, A.fu- Aspergillus fumigatus, A.ni- Aspergillus niger, A.oc- Aspergillus ochraceus, A.te- Aspergillus terreus, C.gl- Chaetomium globosum, C.in- Chaetomium indicum, C.lu- Curvularia lunata, D.ho- Drechslera howaiiensis, F.mo-Fusarium moniliforme, F.ox- Fusarium oxysporum, F.se- Fusarium semitectum, P.ci- Penicillium citrinum, P.fe- Penicillium fellutanum, P.is- Penicillium islandicum, P.ve- Penicillium verrucosum, R.ni- Rhizopus nigricans, R.or- Rhizopus oryzae.*

Table 2: Mycotoxin producing potential of different mycotoxigenic fungal flora isolated from sunflower seeds.

Mycoflora	Mycotoxins produced	Pre harvest samples				Post harvest samples			
		N.I.A[a]	N. I.P[b]	% of toxigenicity	Potentiality[c] (mg/ltr)	N.I.A[a]	N. I. P[b]	% of toxigenicity	Potentiality[c] (mg/ltr)
Aspergillus flavus	AFB_1, AFG_1	39	15	38.4	5.8-15.6	91	47	51.6	10.2 – 24.7
Aspergillus ochraceus	Ochratoxin A	--	-	-	--	40	09	22.5	3 - 8.
Aspergillus terreus	-----	--	-	-	--	06	00	00	-
Fusarium moniliforme	Zearalenone	28	8	28.5	0.6-3.1	57	24	42.1	0.6 – 3.65
Fusarium oxysporum	Zearalenone	8	1	12.5	0.1	24	04	16.6	0.1 – 0.28
Penicillium citrinum	Citrinin	30	12	40	0.1-1.5	67	31	46.2	0.5 – 3.4
Penicillium verrucosum	Ochratoxin A	21	4	19	0.5-5.8	51	12	23.5	3 – 8.5
	Citrinin	21	5	23.8	0.5-6.0	51	17	33.3	5 – 9.2

[a] Number of Isolates Analyzed.

[b] Number of Isolate Positive to different mycotoxins.

[c] Potential Range of Mycotoxin Produced by fungi.

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Table 3: Natural occurrence of mycotoxins in different samples of sunflower.

	Name of Standard Sample	No. of Sample Analyzed	No. of Sample Positive to AFB_1	Amount of AFB_1 (µg/kg) MEAN±S.E	No. of Sample Positive to AFG_1	Amount of AFG_1 (µg/kg) MEAN±S.E	No. of sample Positive to OTA	Amount of OTA (µg/kg) MEAN±S.E	No. of sample Positive to Citrinin	Amount of Citrinin (µg/kg) MEAN±S.E	No. of Sample Positive to Zearalenone	Amount of Zearalenone (µg/kg) MEAN±S.E
Pre Harvest samples	1F	12	2	152±15.28	2	24±15.21	3	132±18.26	2	23±12.24	-	-
	2F	12	1	246±0.0	1	17±0.0	2	212±12.11	1	45±0.0	-	-
	3F	12	3	169±10.53	2	38±18.35	-	-	-	-	-	-
	4F	12	-	-	-	-	3	126±20.23	-	-	-	-
	5F	12	-	-	-	-	-	-	2	65±18.26	-	-
	6F	12	4	58±12.86	3	85±16.20	2	49±21.33	-	-	-	-
	7F	12	-	-	-	-	-	-	3	44±15.31	-	-
	8F	12	1	43±0.0	1	69±0.0	-	-	2	25±12.28	-	-
	9F	12	2	355±16.31	1	53±0.0	1	84±0.0	-	-	-	-
	10F	12	3	60±18.80	1	28±0.0	3	121±14.31	1	24±0.0	-	-
Total	10	120	16		11		14		11		-	-
Post Harvest samples	1S	12	5	685±16.33	3	129±12.12	5	242±21.24	3	231±16.24	-	-
	2S	12	4	568±12.21	3	236±16.23	4	135±13.36	2	65±6.16	-	-
	3S	12	7	463±12.29	6	247±15.33	1	216±0.0	2	424±14.22	2	112±11.18
	4S	12	3	812±18.20	1	153±0.0	3	148±14.24	3	311±19.18	-	-
	5S	12	7	794±14.37	4	245±12.25	1	131±0.0	4	328±24.24	1	125±0.0
	6S	12	9	1070±20.31	6	338±19.28	4	246±13.24	2	218±16.15	-	-
	7S	12	3	610±10.41	1	148±0.0	2	415±18.28	2	433±29.25	-	-
	8S	12	6	490±13.24	3	131±9.18	1	212±0.0	4	248±12.18	2	111±16.24
	9S	12	5	744±18.22	3	135±11.24	2	121±16.22	1	339±0.0	-	-
	10S	12	7	916±12.38	6	242±16.30	4	248±19.28	3	325±17.15	2	125±13.26
Total	10	120	56		36		27		26		07	

Fig. 1. Mature heads of sunflower in field - the most susceptible stage for fungal infection

Fig. 2. A fully mature head showing fungal infection.

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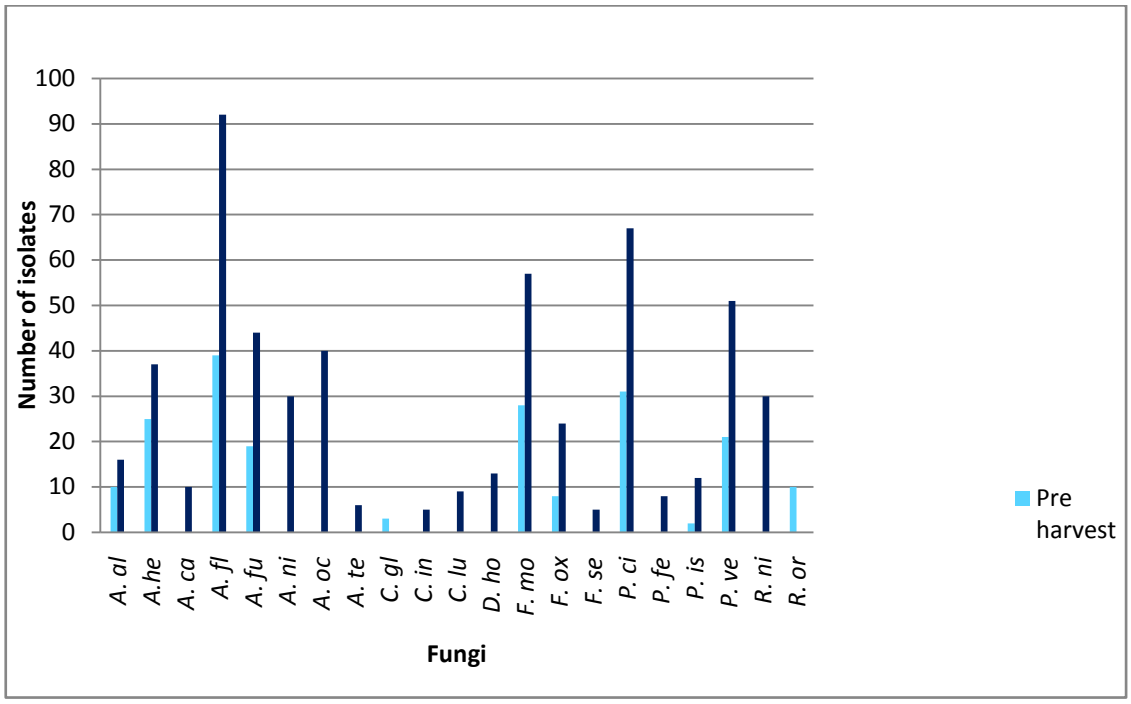

Fig. 3. Frequency of mycoflora in pre and post-harvest condition

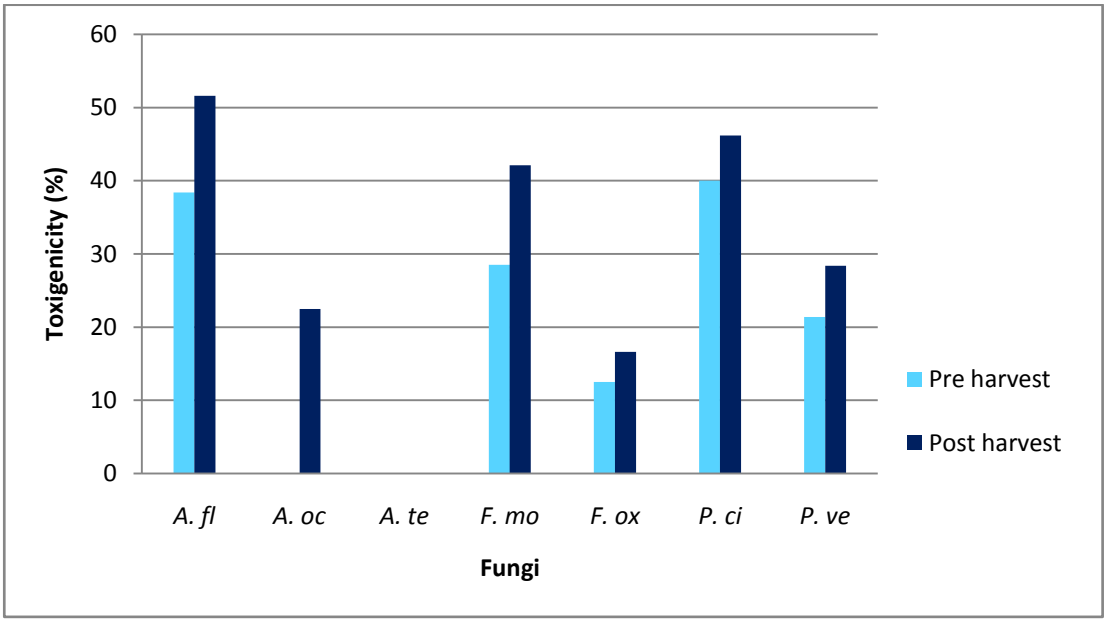

Fig. 4. Percent toxigenicity of toxigenic mycoflora in pre and post-harvest condition

Fig. 5. Harvested crop of sunflower showing ripened head

Fig. 6. Dried capitulum of sunflower and process of isolation of seeds