

Mycotoxin Producing Fungi Associated With Some Medicinal Plant Produce And Induced Mycotoxin Level & Biochemical Changes

ABSTRACT

Mycotoxin contamination and induced biochemical changes studied in medicinal plants namely *Azadirachta indica*, *Embllica officinalis*, *Plantago ovata* and *Vitex negundo* collected from Uttarakhand region under field and storage conditions. Mycotoxin producing fungi like *A. flavus*, *A. ochraceus*, *F. moniliforme* and *Penicillium citrinum* were recorded on all samples under study. In comparison to other mycotoxigenic fungi, percentage toxigenicity was higher in *A. flavus*. In case of *E. officinalis*, 32.69% isolates of *A. flavus* were toxigenic and produced aflatoxins up to 21µg/ml in the liquid media followed by *A. indica* where 22.2% isolates produced aflatoxin in the range of 0.4-13.8 µg/ml. In case of *V. negundo* and *P. ovata* 14.2% and 8% isolates of *A. flavus* elaborated aflatoxin B₁B₂ and aflatoxin B₁ respectively in low concentration. Mycotoxin contamination in stored samples of *E. officinalis* and *V. negundo* showed aflatoxin B₁ as natural contaminant. Twenty percent (28%) samples of *E. officinalis* exhibited higher concentration of aflatoxins, up to 0.98µg/g whereas in case of *V. negundo* 6% samples were found naturally contaminated with aflatoxin B₁. Changes in the level of total alkaloid content in medicinal samples due to toxigenic strain of *A. flavus* was also carried out. There was an indication of inhibition in the total alkaloid content due to toxigenic strain of *A. flavus*. Statistical analysis of the results show a decline in the level of total alkaloid content due to fungal contamination significant at 5% level of significance.

KEY WORDS: Toxigenic fungi, Mycotoxins, Medicinal plant produce, Alkaloid content

INTRODUCTION:

Indian forests have been a rich source of medicinal plant produce and these produce are widely used in the prevention, treatment and cure of disorders and diseases since ancient times. Literature on traditional herbal medicine preparation and survey of products of leading ayurvedic medicine manufacturer indicate use of plant produce in pharmacopoeial preparations. The concern with the quality of these natural products is due to the risk of potential fungal contamination and presence of mycotoxins. Practices used in harvesting, handling, storage, production and distribution make medicinal plants subject to contamination by various fungi, which may be responsible for spoilage and production of mycotoxins.

In spite of the extensive research on the occurrence of mycotoxins in agriculture, horticulture, Tree Borne Oil Seeds (TBOS) and other edible commodities (Hesseltine, 1974; Bilgrami and Sinha, 1984; Zohari *et al.*,1987; Abdel Hafez and Sabah,1993; Singh *et al.*,2001; Shukla and Singh, 2006; Singh, 2012 & 2014). There are few reports available on the incidence of toxigenic mycoflora and mycotoxin contamination in medicinal plants and phytotherapeutic compounds world wide (Chourasia and Roy,1991; Aziz *et al.* ,1998; Abou-Arab *et al.*, 1999;Efuntoye, 1999; Singh, 2003; Mandeel, 2005).

In this background, present study was carried out on medicinal plant produce of *Azadirachta indica*, *Embllica officinalis*, *Plantago ovata*, *Vitex negundo* to assess mycotoxin producing fungi and induced mycotoxin level & biochemical changes. The incidence of mycoflora was studied and isolated fungi were screened for their mycotoxin producing potential. Natural contamination of mycotoxins in fresh and stored samples under study was also carried out. Aflatoxin induced biochemical changes in the

total alkaloid content of medicinal seeds/ fruits under study, due to infestation with toxigenic strain of *A. flavus*, was carried out. There was an indication of inhibition in the alkaloid content due to toxigenic strain of *A. flavus*.

2. MATERIAL AND METHODS

Fresh samples of, *Azadirachta indica*, *Emblica officinalis*, *Plantago ovata*, *Vitex negundo* were collected from the forests during their harvesting season whereas stored samples were collected from different storage centres and pharmaceutical industries.

2.1 ISOLATION AND IDENTIFICATION OF MYCOFLORA

In order to record the fungal flora associated with fresh and stored samples, blotter test as well as agar plate methods as recommended by International Seed Testing Association (ISTA, 1999) were followed. Isolation of mycoflora was done from the surface sterilized samples to isolate the internal fungi and unsterilized samples to isolate the surface fungi. For surface sterilization the samples were soaked in 2% sodium hypochlorite (NaOCl) solution for 10 minutes. Subsequently the seeds, fruits were thoroughly rinsed in sterile distilled water and aseptically placed in Petri plates containing three layered moistened blotter pads and Potato Dextrose Agar medium. The Petri plates were then incubated for seven days at $25 \pm 2^\circ\text{C}$. The Petri plates were regularly examined under stereoscopic binocular microscope from third day and developing fungal colonies were recorded, isolated, purified and maintained on PDA.

The isolated fungi were identified using research microscope with the help of standard literature and keys and matching the isolates with National Type Culture Collection at Forest Research Institute, Dehradun.

2.2 SCREENING OF FUNGAL ISOLATES FOR MYCOTOXIN PRODUCING POTENTIAL

The aflatoxin producing potentials of *Aspergillus flavus* isolates were tested in SMKY liquid medium (Diener and Davis, 1966). The constituents of the medium were, Yeast extract– 7g ; Sucrose– 200g ; Magnesium Sulphate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) – 0.5g ; Potassium Nitrate (KNO_3 - 3g ; Distilled water – 1 lt. Methods of Schwenk *et al.* (1958) and Davis *et al.* (1969) were followed for testing citrinin and ochratoxin producing abilities of *Penicillium citrinum* and *Aspergillus ochraceus* isolates, respectively. The composition of liquid medium used was, Sucrose - 40 g; Yeast extract - 20 g; Distilled water - 1 lt. Zearalenone producing ability of the different isolates of *Fusarium* was tested on moist - rice medium as suggested by Scott *et al* (1970). In all the cases mycotoxins were finally extracted with chloroform and the chloroform extract used for qualitative and quantitative detection of mycotoxins.

2.3 NATURAL OCCURRENCE OF MYCOTOXINS

The fresh and stored samples were extracted chemically for the presence of aflatoxins by the method of Thomas *et al.*(1975). Few samples, in which fungi producing other mycotoxins were associated, were extracted by the method of Roberts and Patterson (1975). The chloroform extract were used for qualitative and quantitative detection of mycotoxins

Qualitative and quantitative estimation of mycotoxin

Qualitative and quantitative estimation of the mycotoxins were carried out using Thin Layer Chromatography (TLC). Silica Gel - G (with 13 % CaSO_4 as binder) was used as stationary phase for the TLC. 50 μl of chloroform extract obtained for mycotoxin screening was spotted on TLC plates. The spotted chromatoplate was developed in the solvent system comprising Toluene: isoamyl alcohol: methanol (90: 32:2, v/v/v) . After developing, the plates were air dried and were observed under long (360nm) and short (260nm) wavelengths UV- light for the detection of mycotoxins. Chemical confirmation of aflatoxin was done by Trifluoroacetic acid (TFA) as suggested by Stack and Pohland

(1975). Presence of Ochratoxin on TLC plates was confirmed with ammonia fumes which changed blue green spot to deep blue colour (Davis *et al.*, 1969). Confirmation of citrinin was done by spraying TLC plates with freshly prepared mixture of 0.5 ml p- anisaldehyde in 85 ml of methanol containing 10 ml of glacial acetic acid and 5 ml of conc. H₂SO₄ and then by heating the plate at 130° c for 10 minutes. This changed yellow streak of citrinin to yellowish green under long wave UV- light (Scott *et al.*, 1970). Zearalenone was also confirmed by spraying TLC plates with acidic p- anisaldehyde solution (Scott *et al.*, 1970) by which greenish blue fluorescence turned faint brown (in visible light) and faint yellow in long wave UV- light.

Aflatoxin being most potent mycotoxin, the quantitative estimation for the same was carried out. Quantity of aflatoxin was estimated spectrophotometrically (Nabney and Nesbitt, 1965) with the help of UV- Spectrophotometer.

2.4 ESTIMATION OF TOTAL ALKALOID

Total alkaloid was estimated following the methodology of Waldi *et al.* (1961). 20 gm of powdered sample was soaked with 28% ammonium hydroxide solution and little dried up. Subsequently, the sample was soxhleted with a mixture of chloroform: ethanol (3:1, v/v) for 8 hrs. The extract was acidified with N/2 H₂SO₄. The acid extract was collected. The process was repeated thrice for complete extraction of alkaloids. The combined acid extract was made alkaline with dilute NH₄OH. The alkaloids were extracted from alkaline extract with 20 and 15 ml of chloroform. Chloroform was distilled on water bath until only few ml were left. The solvent was completely dried up and the residue was weighed on monopan balance to calculate the crude alkaloids. The crude alkaloids of each sample were dissolved in 0.5 ml of methanol. 50 µl of the solution was spotted on a TLC plate with the help of a micropipette. A mixture of cyclohexane, chloroform and diethyl amine (5:4:1, v/v/v) were used as solvent system. The air dried chromatoplates were then sprayed with Dragendroff reagent.

Statistical analysis of the results was carried out using SPSS package. Results of control and contaminated samples were tested for their differences by paired t- test using SPSS package.

3. RESULTS

Mycoflora associated with fresh and stored samples of *Azadirachta indica*, *Embllica officinalis*, *Plantago ovata* and *Vitex negundo* was recorded. In case of fresh samples nine fungi namely *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *A. ochraceus*, *Cladosporium cladosporioides*, *Curvularia lunata*, *Fusarium avenaceum*, *F. solani* and *Penicillium citrinum* were recorded. Among them, *A. niger* was the most common fungus appearing on all three samples except *E. officinalis*. *A. flavus* and *A. ochraceus* was recorded on samples of *A. indica* and *E. officinalis* whereas *P. citrinum* was detected only on *V. negundo* (Table-1).

All hues of known mycotoxin producing fungi i.e. *A. flavus*, *A. ochraceus*, *F. moniliforme* and *Penicillium citrinum* were recorded on the stored samples of the medicinal plant produce under study. Eight fungi namely *A. flavus*, *A. niger*, *A. ochraceus*, *A. spinulosus*, *F. semitectum*, *F. solani*, *P. citrinum* and *Penicillium* sp. were recorded on fruit of *E. officinalis*. In case of *P. ovata*, six fungi i.e. *A. flavus*, *A. niger*, *F. moniliforme*, *P. citrinum*, *Penicillium* sp. and *R. stolonifer* were found associated with the samples. On the other hand, seed mycoflora of *A. indica* comprised of *A. flavus*, *A. niger*, *Cladosporium cladosporioides*, *C. lunata*, *F. moniliforme*, *F. solani*, *Penicillium citrinum* and *Mucor hiemali*, and only four fungi *A. flavus*, *A. niger*, *A. ochraceus* and *R. stolonifer* were corded on *V. negundo*.

3.1 Mycotoxin Producing Potential Of Fungi

Aflatoxins were the most common mycotoxins elaborated by different isolates of *A. flavus* isolated from samples of *Azadirachta indica*, *Embllica officinalis*, *Plantago ovata*, *Vitex negundo*. In case of

Azadirachta indica 14 isolates of *A. flavus* out of 63 screened produced aflatoxin. Afl. B₁ was produced by 9 isolates whereas afl. B₁B₂ were produced by 5 isolates. In *E. officinalis* 17 out of 52 isolates produced aflatoxin. Out of which 9 produced afl. B₁ and 8 afl. B₁B₂. In case of *P. ovata* only 4 out of 25 isolates produced afl. B₁ in traces. In case of *V. negundo*, 3 isolates produced afl. B₁ out of 32 and 21 isolates screened respectively. The amount of aflatoxins produced by the toxigenic isolates of *A. flavus* was in the range of 0.4 - 13.8 µg/ml in *Azadirachta indica*, 0.4 - 21.0 µg/ml in *E. officinalis* and 0.23 - 4.7 µg/ml in *V. negundo*, however, in case of *P. ovata*, aflatoxins were present in traces only.

The percentage of toxigenic isolates was comparatively low in other mycotoxin producing fungi isolated from all the samples. In case of *A. ochraceus*, 7 out of 37 from *E. officinalis*, and 3 out of 15 from *V. negundo* elaborated och. A in liquid medium. Zearalenone was produced by 3 isolates of *F. moniliforme* isolated from *Azadirachta indica* (out of 26), 3 from *P. ovata* (out of 22) whereas Citrinin was elaborated by 21.8% isolates in *A. indica*, 14.81% in case of *E. officinalis*, 22.22% in *P. ovata* (Table-2).

3.2 Mycotoxin Contamination Of The Medicinal Plant Produce

Natural occurrence of mycotoxin contamination in fresh and stored samples was analyzed by following the standard procedures. Mycotoxin contamination in stored samples of *E. officinalis* and *V. negundo* showed aflatoxin B₁ as natural contaminant. Twenty percent (28%) samples of *E. officinalis* exhibited higher concentration of aflatoxins, up to 0.98 µg/g whereas in case of *V. negundo* 6% samples were found naturally contaminated with aflatoxin B₁ and mycotoxins were not detected in case of *A. indica* and *P. ovata* (Table-3).

3.3 Changes In The Alkaloid Content Of Medicinal Plant Produce

Changes in the level of alkaloid content in medicinal seeds/ fruits due to infestation of toxigenic strain of *A. flavus* have been carried out. *A. flavus* caused considerable changes total alkaloid content of the medicinal seeds/ fruits under study during their infestation (Table-4). There was an indication of inhibition in the total alkaloid content due to infestation with toxigenic strain of *A. flavus*. The percent amount of alkaloid in healthy medicinal seeds/fruits was 0.065%, 0.020%, 0.004%, 0.042% in, *A. indica*, *P. ovata*, *E. officinalis*, and *V. negundo* respectively. On the other hand, in infested fruits/seeds of *A. indica*, *P. ovata*, *E. officinalis*, and *V. negundo*, the percent amount of total alkaloid recorded was 0.062%, 0.020%, 0.003%, and 0.039% respectively. Statistical analysis of the results shows that a decline in the level of total alkaloid content due to fungal infestation in the substrates is significant at 5% level of significance.

4. DISCUSSION

The results of the present investigation indicate that a large number of fungi were associated with medicinal plant produce of *Azadirachta indica*, *Emblica officinalis*, *Plantago ovata* and *Vitex negundo*, Association of fungi as well as their incidence are governed by the nature of the substrates, methods of storage and prevailing environmental conditions. Earlier reports also indicate varied pattern of fungal incidence with *E. officinalis* (Chourasia, 1995) and *Azadirachta indica* (Singh et al., 1999).

Four mycotoxigenic fungi viz. *Aspergillus flavus*, *A. ochraceus*, *Fusarium moniliforme* and *Penicillium citrinum* were commonly associated with the stored samples, however their incidence in fresh samples was comparatively low. The range of toxin production by these fungi in liquid medium varied with the type of the substrate. It was also noted that aflatoxin B₁ was produced by majority of the toxigenic isolates of *A. flavus*, however, the frequency of aflatoxins other than B₁ was comparatively low.

The results of the natural occurrence of mycotoxin contamination of the stored samples under study indicate that Aflatoxin B₁ was detected in both fresh and stored samples of *E. officinalis* and the concentration of aflatoxin B₁ in the stored samples was quite high i.e. up 0.98 µg/g in stored samples. *V. negundo* also exhibited presence of aflatoxins as natural contaminant. The variation in natural occurrence of mycotoxins may be due to differences in their moisture contents and constitutional make up. Earlier reports also indicate association of large number of mycoflora and mycotoxins in edible and medicinal fruits/seeds of forest origin (Zohari *et al.*, 1987; Abdel Hafez & Saber, 1993; Singh *et al.*, 2001; Singh, 2003, 2012, 2014). There is an indication of decline in the level of total alkaloid in infested substrates. Inhibition in the level of total alkaloid content might be due to their utilization or degradation into simpler forms. Dutta (1988) worked on deterioration in total alkaloid content of *Strychnos nux-vomica* by some fungi and reported that *A. flavus* and *P. citrinum* significantly inhibited the level of alkaloid content. Bilgrami *et al.* (1983) also reported significant biochemical changes in dry fruits during aflatoxin elaboration by *A. flavus*.

Presence of high concentration of aflatoxins in the medicinal plant produce is a matter of great concern as these raw materials are commonly used for the preparation of herbal drugs. The use of herbal drugs as alternative medicines world wide, it is necessary to set up appropriate standards for screening the crude herbal drugs and medicinal plant produce to be used as raw material in the pharmacopoeial industry.

Table-1: Mycoflora Associated With Fresh Samples Of Medicinal Plant Produce

S.N.	List of Mycoflora	% Incidence of mycoflora associated with different substrates*			
		<i>Azadirachta indica</i>	<i>Emblica officinalis</i>	<i>Plantago ovata</i>	<i>Vitex negundo</i>
1	<i>A. alternata</i>	-	-	-	18.5 ± 1.25
2	<i>A. flavus</i>	37.0 ± 1.67	9.4 ± 1.4	-	-
3	<i>A. niger</i>	45.2 ± 1.28		13.5 ± 0.95	20.0 ± 0.89
4	<i>A. ochraceus</i>	14.2 ± 1.39	47.4 ± 1.98	-	-
5	<i>C. cladosporioides</i>	11.0 ± 1.09	-	-	-
6	<i>C. lunata</i>	12.4 ± 1.07	-	-	-
7	<i>F. avenaceum</i>	15.4 ± 0.92	9.75 ± 1.03	-	-
8	<i>F. solani</i>	-	40.5 ± 2.21	-	14.8 ± 1.01
9	<i>P. citrinum</i>	-	-	-	14.0 ± 0.95

* Value are mean ± deviation of three replicates

256 **Table-2: Screening Of Mycoflora For Mycotoxin Producing Potential**
257

Substrate	Mycoflora	No. of Isolates Screened	No. of toxigenic isolates	% toxigenicity	Mycotoxin produced	Range of toxin production (µg/ml)
<i>A. indica</i>	<i>A. flavus</i>	63	14	22.22	Aflatoxin	0.4-13.8
			9	14.28	Aflatoxin B ₁	
			5	7.93	Afl. B ₁ B ₂	
	<i>F. moniliforme</i>	26	3	11.53	Zearalenone	-
	<i>P. citrinum</i>	32	7	21.87	Citrinin	-
<i>E. officinalis</i>	<i>A. flavus</i>	52	17	32.69	Aflatoxins	0.4-21
			9	17.30	Aflatoxin B ₁	
			8	15.38	Afl. B ₁ B ₂	
	<i>A. ochraceus</i>	37	7	18.91	Ochratoxin	-
	<i>P. citrinum</i>	27	4	14.81	Citrinin	-
<i>P. ovata</i>	<i>A. flavus</i>	25	2	8.00	Aflatoxin B ₁	Traces
	<i>F. moniliforme</i>	22	3	13.63	Zearalenone	-
	<i>P. citrinum</i>	18	4	22.22	Citrinin	-
<i>V. negundo</i>	<i>A. flavus</i>	21	3	14.28	Afl. B ₁ B ₂	Traces
	<i>A. ochraceus</i>	15	3	20.00	Ochratoxin	-

258
259

260
261 **Table-3: Natural Occurrence Of Aflatoxins In Medicinal Plant Produce**
262

S.N.	Substrate	% of Contaminated sample	Mycotoxin produced			Range of mycotoxin (µg/g)
			AFB ₁	AFB ₁ B ₂	AFB ₁ B ₂ G ₁	
1	<i>Azadirachta indica</i>	Nil	-	-	-	-
2	<i>Emblia officinalis</i>	28	15	6	2	0.14-0.98
3	<i>Plantago ovata</i>	Nil	-	-	-	-
4	<i>Vitex negundo</i>	6	1	-	-	traces

263
264

S.N.	Substrate		Total alkaloid (g/100g)	T- value	Significance CD (0.05)
			Mean ± S.E.		
1	<i>Azadirachta indica</i>	C	0.065 ± 0.001	4.630	0.044
		I	0.062 ± 0.000		
2	<i>Plantago ovata</i>	C	0.021 ± 0.000	2.60	0.122
		I	0.020 ± 0.000		
3	<i>Emblia officinalis</i>	C	0.004 ± 0.000	15.24	0.000

		I	0.003 ± 0.000		
4	<i>Vitex negundo</i>	C	0.042 ± 0.000	29.00	0.001
		i	0.039 ± 0.000		

Note: C-Control, I- Infested

Table-4: Changes In Total Alkaloid Due To Fungal Contamination

REFERENCES

- Abdel- Hafez, A.I.I. and Sabah, M. Saber (1993). Mycoflora and mycotoxin in hazelnut (*Corylus avellana*) and walnut (*Juglans regia*) seeds in Egypt. *Zentralbl. Mikrobiol.* 148 (2) : 137 – 147.
- Abou-Arab, A.A.K.; Kawther, M.S.; El Tantawy, M.E.; Badeaa, R.I.; Khayria, N. Quantity estimation of some contaminants in commonly used medicinal plants in the Egyptian market. *Food Chem.*, 67, 357-363, 1999.
- Aziz, N.H.; Youssef, Y.A.; El-Fouly, M.Z.; Moussa, L.A. (1998). Contamination of some common medicinal plant samples and spices by fungi and their mycotoxins. *Bot. Bull. Acad. Sinica.*, 39(4), 279-285.
- Bilgrami, K.S., K.K. Sinha and Anjana Singh (1983). Chemical changes in dry fruits during aflatoxin elaboration by *Aspergillus flavus* Link ex Fries. *Current Science.*, 52 (10): 960-964.
- Bilgrami K S and K K Sinha 1984 Mycotoxin contamination of food and its control. *Indian Rev Life Sci* 4 19 - 36.
- Chourasia, H.K.; Roy, A.K. (1991). Effect of temperature, relative humidity and light on aflatoxin B₁ production in Neem and Datura seeds. *Int. J. Pharmacognosy*, 29(3), 197-202.
- Chourasia, H.K. (1995). Mycobiota and mycotoxins in herbal drugs of Indian pharmaceutical industries. *Mycol. Res.* 99 (6): 697- 703.
- Davis, N.D, J.W. Searcy and U.L. Diener (1969). Production of ochratoxin A by *Aspergillus ochraceus* in a semisynthetic medium. *Applied Microbiology*, 17: 742 – 744.
- Diener, U.L. and N.D. Davis (1966). Aflatoxin production by isolates of *Aspergillus flavus*. *Phytopath.*, 56 (12) : 1390- 1393.
- Dutta G.R. and A.K. Roy (1987) Mycoflora associated with Strychnos seeds and deterioration of their active principles under storage. *Indian Phytopathol* 40:520–524.
- Efuntoye, M.O. (1999). Mycotoxins of fungal strains from stored herbal plants and contents of Nigerian crude herbal drugs. *Mycopathologia*, 147, 43-48.
- Hesseltine CW 1974 Natural occurrence of mycotoxins in cereals. *Mycol Appl* 53 141-153.
- International Seed Testing Association 1999 *Seed Sci Technol* 27 (Suppl).
- Mandeel, Q.A. (2005). Fungal contamination of some imported spices. *Mycopathologia*, 159, 291-298.

- 303 Nabney J. and B.F. Nesbitt(1965). A spectrophotometric method for determining the aflatoxins.
304 Analyst. 90: 155- 160.
305
306
- 307 Roberts, B.A.,& Patterson, D. S. P. (1975). Detection of twelve mycotoxins in mixed animal
308 feedstuffs, using a novel membrane clean-up procedure. *J. Assoc. Off. Anal. Chem.*,
309 58, 1178-1181
310
- 311 Schwenk, E., G.J. Alexander, A.M. Gold and D.F. Stevens (1958). Biogenesis of citrinin. *J. Biol.*
312 *Chem.*, 233 (5): 1211 – 1213.
313
- 314 Scott, P.M. ,J.W. Lawrence and W. Van Walbeek (1970). Detection of mycotoxins by Thin layer
315 chromatography: application to screening of fungal extracts. *Appl. Microbiol.*, 19: 839 – 842.
316
- 317 Shukla, A.N. and Punam K. Singh (2006). Fungal infestation and aflatoxin contamination in in edible
318 oilseeds of forest origin. *J. Mycol & Plant Pathology* 36(1):96-100.
319
- 320 Singh, Punam Kumari (2003). Mycotoxin elaboration in Triphala and its constituents. *Ind.*
321 *Phytopathology*, 56(4), 380-383.
322
- 323 Singh, Punam Kumari, S.N. Khan and Kavita Tandon (1999). Seed mycoflora of *Azadirachta indica*
324 and its control. *Van Vigyan*, 37: 18-20.
325
- 326 Singh PK, Khan SN, Harsh NSK and Pandey,R (2001).Incidence of mycoflora and
327 mycotoxins in some edible fruits and seeds of forest origin. *Mycotoxin Research*
328 17(2) 46-58.
329
- 330 Singh, Punam Kumari (2012). Assessment of mycotoxins in Tree Borne Oil Seeds, *J. Food*
331 *Research*, Vol.1 (3), 92-101.
332
- 333 Singh, Punam Kumari (2014). Mycotoxin contamination and induced biochemical changes
334 in edible tree nuts. *J. Indian Bot. Soc.* Vol. 93 (3 & 4) 2014 : 222-228.
335
- 336 Stack, M. E. & Pohland, A.E. (1975). Collaborative study of a method for chemical
337 confirmation of the identify of aflatoxin. *Journal of the Association of Official*
338 *Analytical Chemists* 33, 110-113.
339
- 340 Thomas, F., Eppley, R.M. and Truckess, M.W. (1975). Rapid screening method for aflatoxins and
341 zearalenone in corn. *J. assoc. Off. Analyt Chem.* 58,114- 116.
342
- 343 Waldi, D. V., Sachnackerj and F. Munter (1961). *J. Chromatography*, 6: 61.
344
- 345 Zohari A.A., Kharia M Abdel- Gaward (1987)Survey of mycoflora and mycotoxins of some
346 dried fruits in Egypt. *J Basic Microbiol* 33 279–285.