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Incidence of mycotoxigenic penicillia in feeds of Andhra Pradesh, India

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Incidence of different species of *Penicillium* in poultry feeds (starter, breeder, boiler and layer) and cattle feeds was analyzed. In all twenty three species of *Penicillium*, *Penicillium aethiopicum*, *Penicillium alli*, *Penicillium aurantiogriseum*, *Penicillium brevicompactum*, *Penicillium camemberti*, *Penicillium caseifulvum*, *Pchrysogenum*, *Penicillium citrinum*, *Penicillium commune*, *Penicillium crustosum*, *Penicillium digitatum*, *Penicillium dipodomyis*, *Penicillium discolor*, *Penicillium expansum*, *Penicillium flavigenum*, *Penicillium griseofulvum*, *Penicillium italicum*, *Penicillium nalgiovense*, *Penicillium nordicum*, *Penicillium olsonii*, *Penicillium roqueforti*, *Penicillium rubrum*, *Penicillium tricolor* and *Penicillium verrucosum* could be recorded in 400 samples of feed samples, poultry feed (280) and cattle feed (120) was analyzed for fungus isolation by dilution plate method, screening of 483 *Penicillium* species by thin layer chromatography (TLC) and spray reagents for mycotoxin production, 299 strains were positive to be mycotoxigenic which elaborates a variety of mycotoxins such as Citrinin, Cyclopiazonic acid, Mycophenolic acid, Ochratoxin A, Patulin, Penitrems, PR toxin, Roquefortine C, Rubratoxin and Terric acid etc.

Key words: *Penicillium* species, mycotoxins, cattle feed, poultry feed.

INTRODUCTION

In recent times poultry and dairy farming became an important agro-business, millions of small and marginal farmers use crop residues and natural herbage to feed their live stock. The availability and type of feed depends on local resources, climatic and the socioeconomic condition of the people, lack of scientific knowledge on improper processing during harvest, unseasonal rains and high moisture content provides an ideal condition for the proliferation of moulds and mycotoxins production in foods and feeds (Frisvad, 1995; Fazekas et al., 1996; Trucksess, 2001; Ana et al., 2009). These mycotoxins can be very stable to food processing (Molinie et al., 2005) can be present in fungal product. *Penicillia* from moldy feeds may cause infections, provoke allergic responses in sensitized objects or poison with toxic metabolites (Answorth and Austick, 1973; Lacey, 1975; Abramson, 1997). Hence, the present investigation was aimed to undertake an extensive and intensive survey of different feeds and feed ingredients for the incidence

of *Penicillium* species.

MATERIALS AND METHODS

An extensive survey of different feeds (cattle and poultry) of different geographical regions of Andhra Pradesh State (A.P.), India was undertaken. The samples were collected randomly, and analyzed for the presence of *Penicillium* species by dilution plate technique (Waksman, 1922). Specific medium such as Czapek Yeast Autolysate (CYA) agar (Pitt, 1979) medium was employed for isolation of *Penicillium* species. In addition macro morphology of structure and branching of the conidiophores, the shape and ornamentation of conidia, colony characters that including growth rate, conidium color and reverse color of the colony, diffusing pigment characteristics for few species were observed and documented. Most of the *Penicillium* isolates inoculated on four enriched media such CYA agar (Pitt, 1979) Blakeslee Malt extract Autolysate (MEA) agar (Raper and Thom, 1949) Yeast extract sucrose (YES) agar (Frisvad et al., 1992) and Creatine sucrose (CREA) agar (Frisvad, 1985) for their identification, and these media gave characteristics aerial and reverse colour on type of media. The sub genus *Penicillium* species were identified by with the help of standard manuals and protocols. (Hyde, 1990; Filtenberg et al., 1992; Svendsen and Frisvad, 1994; Pitt et al., 2000; Samson et al., 2002; Frisvad and Samson, 2004).

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The percentage of incidence, frequency and abundance of each fungus with special emphasis on *Penicillium* was calculated by the following formulae:

% of incidence = (No. of colonies of a species in all plates / Total no. of colonies of the all the species in all plates) × 100

% of frequency = (No of observations in which a species appeared / Total no. of observations) × 100

% of abundance = (No. of colonies of species in all observations / Total no. of colonies in all observations) × 100

Penicillium mycotoxins (Extrolites) were analyzed by employing thin layer chromatography (TLC) (Frisvad and Filtenberg, 1989; Filtenberg et al., 1983, 1992; Lund, 1995). The TLC plates (Silica Gel GF 254) were impregnated in 10% solution of oxalic acid in methanol solution for 10 min, after heating at 110°C for two minutes the plates were kept for cooling and immediately the mycotoxin extract (20 µl) was spotted on activated and cooled TLC plates (Smedsgaard, 1997). The spotted plates were developed in suitable solvents system (Samson and Pitt, 2000) by ascending chromatography. The compounds thus separated were identified either by the color of the fluoresce under (U.V.333 nm) or by the R_f value, they were further confirmed by chemical tests using different spray reagents (Pitt and Hocking, 1996, and Frisvad et al., 2004), and U.V Spectrum (U.V-10 VIS). The R_f value was calculated by the following formulae:

$R_f = \frac{\text{Distance traveled by the compound}}{\text{Distance traveled by the solvent}}$

RESULTS AND DISCUSSION

The identification of subgenus *Penicillium* species is difficult (Thom, 1930; Raper and Thom, 1949; Smith 1960; Ciegler et al., 1969; Frisvad, 1981, Samson and Pitt, 1990; Larsen and Frisvad, 1995) because the micro morphology of the strains is very similar. In total twenty three *Penicillium* species were associated with both poultry and cattle feed (Table 1) collected from different geographical region of Andhra Pradesh with the employment of CYA agar media. *Penicillium rubrum*, *Penicillium citrinum* and *Penicillium olsonii* occurred with highest percentage of incidence followed by *Penicillium chrysogenum*, *Penicillium aethiopicum* *Penicillium alli* and *Penicillium aurantiogriseum* could be isolated only feed collected from Adilabad Districts. The incidence of *Penicillium* species was dominated and followed by *Aspergillus* species. *Penicillium brevicampactum* was associated with all the samples except in Nalgonda and Guntur. *Penicillium flavigerum* was associated with all the samples except the sample collected from Khammam, Warangal, and Nalgonda Districts. *Penicillium nalgiovense* could be detected in all the samples except those collected from Adilabad. *Penicillium roqueforti* and *P. rubrum* were associated with the samples of both poultry and cattle feed. *Penicillium verrucosum* was detected in all poultry feed samples of Warangal, similarly. *Penicillium caseifulvum* could not be detected

in poultry feed samples of Adilabad. On the other hand, *P.chrysogenum*, *P.citrinum* and *Penicillium commune* were common to all samples. The incidence of *Penicillium crustosum* could not be recorded in cattle feed samples of Warangal, Khammam, Nalgonda, Krishna, Guntur and Adilabad. The incidence of *Penicillium* species more in poultry feeds than in cattle feeds.

Screening for toxigenic potential of different species of *Penicillium* revealed (Table 3) that, large numbers of *Penicillium* isolates were mycotoxigenic. However, the percentage of mycotoxigenic strains varied with the species and place of collection. All the isolates of *Penicillium* sub genus were mycotoxigenic and many cases more than one mycotoxin was detected thus so called OSMAC (one strains many compounds), (Bode et al., 2002).

Table 2 revealed that out of 483 strains of *Penicillium* species isolated from cattle and poultry feed 299 strains were mycotoxigenic and 65 toxigenic out of 95 strains of *P. citrinum*, *P. expansum*, *P. nordicum* and *P. verrucosum* were screened for citrinin production. More strains of *P. citrinum* were toxigenic from the cattle feed samples collected from Khammam and poultry feed samples of Warangal, Nalgonda and Krishna Districts. Out of 25 strains of *P. camemberti* and *P. commune* were screened, 17 strains produced Cyclopiazonic acid. Out of 40 strains of *P. verrucosum* and *P. nordicum*, 28 strains produced Ochratoxin A. Comparatively more numbers of strains were toxigenic in cattle feeds of Khammam, Guntur, Adilabad and poultry feed of Krishna districts. Out of 23 strains of *P.expansum* and *P.dipodomyis* screened, 7 strains were positive for Patulin elaboration. The incidence of mycotoxigenic strains were more in cattle feed samples collected from all districts of Khammam and Nalgonda. Out of 16 strains of *P. flavigenum*, 10 strains produced penitrem (penitrem A). Contamination of penitrem was comparatively more in poultry feeds of Guntur and Adilabad District. When 19 strains of *P.aurantiogriseum* and *P.alli* from Adilabad districts were screened, 8 strains were positive for Penicillic acid, Similarly 93 strains of *P. alli*, *P. chrysogenum*, *P. crustosum*, *P. flavigenum*, *P. expansum* and *P. roqueforti* when screened for their mycotoxigenic potential, 57 strains were found to produce roquefortin C toxin, However, *P. alli* failed to produce the toxin. When 50 strains of *P. rubrum* were screened, 36 strains were positive for rubratoxin B. The incidence of this mycotoxin was comparatively more in poultry feeds of Khammam, and cattle feed of Adilabad. When 18 strains of *P. crustosum* and 53 strains of *P. chrysogenum* and *P. roqueforti* were screened, 5 strains were terric acid positive and 30 produced PR toxin. Similarly out of 41 strains of *P. brevicampactum* and *P. roqueforti*, 33 strains elaborated mycophenolic acid.

The order of percentage of contamination of different secondary metabolites by *Penicillium* species were

Table 1. Incidence of mycotoxigenic *Penicillia* in feed samples.

Name of fungus	Incidence												Frequency		Abundance	
	Khammam		Warangal		Nalgonda		Krishna		Guntur		Adilabad		A	B	A	B
	A	B	A	B	A	B	A	B	A	B	A	B				
<i>P. aethiopicum</i>	--	--	--	--	--	--	--	--	--	--	2.1	1.3	16.6	16.6	0.85	0.46
<i>P. alli</i>	--	--	--	--	--	--	--	--	--	--	0.6	1.4	16.6	16.6	0.24	0.48
<i>P. aurantiogriseum</i>	--	--	--	--	--	--	--	--	--	--	1.3	0.8	16.6	16.6	0.53	0.28
<i>P. brevicampactum</i>	5.2	1.4	2.2	1.6	--	--	--	1.5	2.9	--	1.4	3.7	50	66.6	4.77	2.93
<i>P. camemberti</i>	0.9	2.1	--	3.7	3.1	1.4	2.5	6.7	--	1.3	2.2	--	50	83.3	3.55	5.44
<i>P. caseifulvum</i>	1.5	1.3	2.6	4.4	1.2	6.2	1.6	4.4	2.4	2.8	--	1.1	83.3	100	3.7	7.24
<i>P. chrysogenum</i>	4.6	3.4	3.1	4.8	1.6	2.1	5.1	1.8	1.9	5.3	3.9	4.3	100	100	8.24	7.77
<i>P. citrinum</i>	2.7	5.9	6.2	1.8	5.8	1.8	4.6	5.3	2.3	4.6	4.2	5.1	100	100	10.2	8.78
<i>P. commune</i>	2.2	1.5	1.6	0.6	2.4	1.2	1.8	0.6	3.8	2.3	3.8	1.3	100	100	6.36	2.68
<i>P. crustosum</i>	1.6	--	--	2.8	--	--	--	2.1	7.2	--	1.9	2.7	50	50	4.36	2.72
<i>P. digitatum</i>	--	2.8	--	2.6	--	--	2.6	2.9	--	2.7	2.1	1.6	33.3	83.3	1.91	4.51
<i>P. dipodomyis</i>	--	--	--	--	--	--	0.6	--	--	--	1.9	2.2	33.3	16.3	1.02	0.78
<i>P. discolor</i>	2.1	1.8	--	--	--	1.9	--	1.3	3.1	2.8	1.6	2.7	50	83.3	2.77	3.76
<i>P. expansum</i>	2.3	4.5	2.7	0.8	2.7	5.9	3.8	0.8	--	1.1	3.3	2.3	83.3	100	6.04	5.51
<i>P. flavigenum</i>	0.9	--	1.9	--	1.9	2.5	0.7	2.9	0.5	2.1	4.5	1.8	66.6	100	4.24	3.33
<i>P. italicum</i>	--	1.2	--	1.5	--	4.2	--	1.4	2.6	1.5	0.7	3.1	33.3	100	1.34	4.62
<i>P. nalgiovense</i>	3.2	3.6	4.2	--	4.2	2.1	2.5	3.3	0.2	2.9	--	--	83.3	66.6	5.83	4.26
<i>P. nordicum</i>	1.5	2.6	3.1	3.7	3.1	0.9	5.4	4.7	1.2	2.2	--	0.9	83.3	100	5.83	5.37
<i>P. olsonii</i>	2.4	3.8	2.8	5.4	2.8	2.6	6.5	4.2	1.7	5.4	2.1	2.7	100	100	7.4	8.63
<i>P. roqueforti</i>	2.3	2.8	1.4	4.5	1.4	2.2	2.3	4.9	2.5	2.3	--	0.3	83.3	100	4.04	6.09
<i>P. rubrum</i>	6.4	4.9	2.9	3.2	2.9	3.1	2.9	1.4	1.2	2.4	2.3	4.1	100	100	7.59	6.84
<i>P. tricolor</i>	0.2	0.9	1.2	--	--	--	0.4	1.1	0.5	--	--	--	66.6	33.3	0.93	0.71
<i>P. verrucosum</i>	2.9	4.1	3.4	--	3.9	3.7	2.7	2.2	2.3	4.6	3.7	4.2	100	83.3	7.71	6.73
Otherfungi =	57.1	51.4	60.7	58.6	63.0	58.2	54.1	46.5	63.7	53.7	56.4	52.4	100	100		

Otherfungi: *Aspergillus*, *Fusarium*, *Mucor*, *Rhizopus*, *Neurospora*, *Cladosporium* species.

Rubratoxin, Citrinin, Ochratoxin Roquefortine followed by Patulin, PR, Mycophenolic acid, Cyclopiazonic acid, Penicillic acid and Terric acid respectively.

The critical perusal of Table 1 reveals that the cattle feed was most ideal substratum for the

proliferation of *Penicillia* and mycotoxin elaboration. The mycotoxigenic potential of *Penicillium* species isolated from poultry feed was intermediate. Hyde (1990) has isolated *Cladosporium herbarum*, *Alternaria tenuissima* and *Aspergillus fumigatus* from feeds, which are

responsible for various allergic diseases in cattle and farm workers. Fungal growth in feeds may also deplete the nutritive value and can alter the availability of micronutrients (Zohri et al., 1993; Broster, 1998). In all Cyclopiazonic acid followed by Patulin, Citrinin, Ochratoxins

Table 2. Contamination of Mycotoxins produced by *Penicillium* spp.

Name of fungus	TS	PS	Ts (%)	Name of the toxin
<i>P. citrinum</i>	40	28	70	Citrinin
<i>P. verrucosum</i>	30	22	73	
<i>P. nordicum</i>	10	6	60	
<i>P. camemberi</i>	10	5	50	Cyclopiazonic acid
<i>P. expansum</i>	15	9	60	
<i>P. commune</i>	15	12	80	
<i>P. verrucosum</i>	30	22	70	Ochratoxin A
<i>P. nordicum</i>	10	6	60	
<i>P. dipodomyis</i>	8	2	25	Patulin
<i>P. expansum</i>	15	5	45	
<i>P. flavigenum</i>	16	10	62	Penitrem A
<i>P. aurantiogriseum</i>	14	6	42	Penicillic acid
<i>P. alli</i>	5	2	40	
<i>P. chrysogenum</i>	35	24	68	Roquefortine C
<i>P. crustosum</i>	9	4	44	
<i>P. expansum</i>	15	9	60	
<i>P. flavigenum</i>	16	8	50	
<i>P. roqueforti</i>	18	12	66	
<i>P. rubrum</i>	50	36	72	Rubra toxin B
<i>P. crustosum</i>	18	5	27	Terric acid
<i>P. chrysogenum</i>	35	24	68	PRtoxin
<i>P. roqueforti</i>	18	6	33	
<i>P. brevicompactum</i>	33	25	75	Mycophenolic acid
<i>P. roqueforti</i>	18	11	66	
	483	299		

TS= Total strains; PS= Positive strains; %Ts= Percentage of toxigenic strains.

Table 3. Detection *Penicillium* producing Mycotoxin by different spray reagents.

Name of toxin	TEF	U.V	Spray reagents				
	Rf		CesO ₄	2.4.DNP	FeCl ₃	<i>P.anisaldehyde</i>	AlCl ₃
Citrinin	0.52	y	Y	Bry	Br	--	LY
Cyclopiazonic acid	0.52	Y	Br	Y	Br	--	Lb
Ochratoxin A	0.32	B	B	Lo	Pbr	--	Lbr
Patulin	0.22	P	G	Y	--	--	Gr
Penitrem A	0.4	LP	Lo	P	G	--	LY
Penicillic acid	0.16	B	O	--	Lo	--	--
Roquefortine C	0.3	P	--	Gr	--	--	--
Rubra toxin B	0.35	Y	--	--	--	--	--
Terric acid	0.23	B	--	--	--	--	--
PRtoxin	0.19	PB	--	--	Br	--	--
Mycophenolic acid	0.36	Y	--	--	--	--	--

Detection color: Y=Yellow, LP=Light purple, B= blue, Pb= Purple blue, Br= brown, Ybr=Yellow brown, G= green, Lo= Light orange, O= orange, Bry= brown yellow, RBr= Red brown, Gr= grey, vo= violet, LY= Light yellow, Lb= Light brown, Ly= Light yellow, Lbr= Light brown, Pbr=Purple brown. Spray reagents: 1= CeSO₄ 1% IN 6N H₂SO₄, 2 = 2,4 DNP,3= FeCl₃ 3% in Ethanol, 4=p-anisaldehyde,5=50% H₂SO₄, 6 = 1%FeCl₃ in Ethanol,Iodine ,AlCl₃.Solvent system: TEF= Toluene, Ethyl acetate, Formic acid (6;3;1).

Rubratoxins, Roquefortine, Mycophenolic acid and Penicillin etc. could be spotted in feed samples

analyzed from different geographical places of Andhra Pradesh.

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REFERENCES

- Abramson D (1997). Toxicants of the genus *Penicillium*. In Flex: Flex D'Mello, J.P, (eds). Handbook of Plant and fungal toxicants. Florida: CRC, pp. 303-317.
- Answorth GC, Austwick PKC (1973). Fungal diseases of animals. Second edition. Fairham Royal: common Wealth Agricultural Bureau.
- Ana MP, Emilia CB, Hector HL, Gonzalez EM, White C, Elena JM, SilivaLR (2009). Fungal and fumonisins contamination and Argentinemaize (*Zea mays L.*) silico bags. J. Agric. Food Chem., 57: 2778-2781.
- Bode HB, Bethe B, Hof R, Zeeck A (2002). Effects from small changes: Possible ways to explore nature's chemical diversity. Chem. Bio. Chem., 3: 619-627.
- Broster WH, Broster VJ (1998). Body score of dairy cows. J. Dairy Res., 65: 155-173.
- Ciegler A. (1969). A tremorgenic mycotoxin from *Penicillium*. Appl. Microbiol., 18: 128-129.
- Fazekas B, Kis M, Haidu ET (1996). Data on the contamination of maize with fumonisins B1 and other fusarial toxins in Hungary. Acta Vet. Hung., 44: 25-37.
- Filtenberg O, Frisvad JC, Lund F, Thrane U (1992). Simple identification procedure for association of spoilage and toxigenic mycoco flora in foods .In modern method in food mycology ed.Samson R.A., Hoking, AD., Pitt, J.I. and King, A.D. Amsterdam: Elsevier, pp. 248-258
- Frisvad JC (1981). Physiological criteria and mycotoxin production as aids in identification of common asymmetric *Penicillia*. Appl. Environ. Microbiol., 41: 568-579.
- Frisvad JC (1985). Creatine sucrose agar, a differential medium for mycotoxin producing terverticillate *Penicillium* species. Lett. Appl. Microbiol., 1: 109-113.
- Frisvad JC (1995). Mycotoxins and Mycotoxigenic fungi in storage In: Stored-grain Ecosystems (Jayas, D.S., White, N.D.G. and Muir, W.E., Eds.), Marcel Dekker, New York, pp. 251-288.
- Frisvad JC, Filtenborg O (1989). Terverticillate *Penicillia* chemotaxonomy and mycotoxin production. Mycol., 81: 837-861.
- Frisvad JC, Filtenborg O, Lund F, Thrane U (1992). New selective media for the detection of toxigenic fungi in cereal products, meat and cheese. In modern methods in food mycology (eds) Samson, R.A., Hoking, A.D., Pitt, J.I and King, A.D. Amsterdam: Elsevier, pp. 259-268.
- Frisvad JC, Samson RA (2004). Polyphasic taxonomy of *Penicillium* subgenus *Penicillium*. A guide to identification of food and air-borne terverticillate *Penicillia* and their mycotoxins. Stud. Mycol., 49: 1-173.
- Frisvad JC, Smedsgaard J, Larsen TO, Samson RA (2004). Mycotoxins, drugs and other extrolites produced by species in *Penicillium* subgenus *Penicillium*. Stud. Mycol. (Utrecht), 49: 201-242.
- Hyde KD (1990). Intertidal mycota of five mangrove tree species. Asian Mar. Biol., 7: 93-107.
- Lacey (1975) Potential hazards to animals and man from microorganisms in fodders and grain. Transactions of the British Mycological Society.
- Larsen TO, Frisvad JC (1995). Chemosystematics of *Penicillium* based on profiles of volatile metabolites. Mycol. Res., 99: 1167-1174.
- Lund (1995). Differentiating *Penicillium* species by detection of indole metabolites using a filter paper method. Lett. Appl. Microbiol., 20: 228-231.
- Molini'e A, Faucet V, Castegnaro M, Pfohl-Leszkonicz A (2005). Analysis of some breakfast cereals on the fresh market for their contents of ochratoxin a, citrinin and fumonisins B1: development of method for simultaneous extraction of ochratoxin and citrinin. Food Chem., 92: 391-400.
- Pitt JI, Hocking AD (1996). Current knowledge of fungi and mycotoxins associated with food Commodities in Southeast Asia in Highley E, Johnson GI. (Eds) Mycotoxin Contamination in Grains. Canberra. Australian Centre for International Agricultural Research. ACIAR Tech. Reports, 37: 5-10.
- Pitt JI, Samson RA, Frisvad JC (2000). List of accepted species and their synonyms in the family Trichocomaceae In: Integration of Modern Taxonomic Methods for *Penicillium* and *Aspergillus* Classification (Samson, R.A. and Pitt, J.I., Eds.), Harwood Academic Publishers, Amsterdam, pp. 9-47.
- Pitt JI (1979). The genus *Penicillium* and its teleomorphic states *Eupenicillium* and *Talaromyces*. Academic Press Ed., London.
- Raper KB, Thom C (1949). Manual of the *Penicillia*. Williams and Wilkins, Baltimore, p. 875.
- Samson RA, Pitt JI (1990). Modern Concepts in *Penicillium* and *Aspergillus* Classification (eds). Plenum Press, New York, USA.
- Samson RA, Pitt JI (2000). Integration of Modern Methods for *Penicillium* and *Aspergillus*. Harwood Academic Publishers, Amsterdam, the Netherlands, p. 510.
- Samson RA, Hoekstra ES, Frisvad JC, Filtenborg O (2002). 6th Edn Introduction to Food- and Airborne Fungi, 202. Centraalbureau voor Schimmelcultures, Utrecht, pp. 379-381.
- Smedsgaard J (1997). Micro-scale extraction procedure for standardized screening of fungal metabolite production in cultures. J. Chromatogr., 760: 264-270.
- Smith G (1960). An Introduction to Industrial Mycology. 5th ed. Edward Arnold Ltd., London, p. 399.
- Svensden A, Frisvad JC (1994). A chemotaxonomic study of the terverticillate *Penicillia* based on high performance liquid chromatography of secondary metabolites. Mycol. Res., 98: 1317-1328.
- Thom C (1930). The *Penicillia*. Williams and Wilkins, Baltimore, p. 643.
- Truckess MW, Tang Y (2001). Solid phase extraction for Patulin in apple juice and unfiltered apple juice. In M.W.Truckees and A.F.pohland(ed.),Mycotoxin protocols. Human press, Totowa, N. J., pp: 205-213.
- Waksman SA (1922). A method for counting the number of fungi in the soil. J. Bot., 7: 339-341.
- Zohri AA, Abdei-Gawad KM (1993). Survey of mycoflora and mycotoxins of some dried fruits in Egypt, pp. 279-288.