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Detection of *Aspergillus flavus* Producing Aflatoxin in Groundnut

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ABSTRACT

To detect Aspergillus flavus producing aflatoxin in groundnut fifty groundnut samples were collected from local market shop of Muktainagar. All these fifty groundnut samples collected are of summer season produced only during the month of October 2023.All these collected samples were incubated on Czapek-Dox Rose Bengal Agar medium and examined for the fungal growth of Aspergillus flavus producing aflatoxin in groundnut in terms of percentage infection and colonization. The result of total fifty groundnut kernels samples screened for detection of Aspergillus flavus observed that out of total fifty groundnut kernels samples, the minimum and maximum infection range was found from 10 to 50 % and for colonization it stand between10 to 40%. For infection 21 sample (42%) was found completely nil, followed by13 (26%) with less than 25 % infection. Sixteen samples (32%) were observed between greater than 25 to 50 percent range of infection. The result for colonization is interesting where 25 samples (50%) was found completely nil followed by 17 (34 %) and 8 (16 %) found in the percentage range of less than 25 and greater than 25 to 50 for colonization respectively.

Keywords: Mycotoxin, aflatoxin, groundnut, infection, colonization.

INTRODUCTION

Mycotoxins (mykos = of fungal origin; toxikoses =toxins) are toxic substances produced mostly as secondary metabolites produced by fungi that grow on seeds and feed in the field, or in storage. The occurrence of mycotoxins in agricultural commodities is a major health concern for livestock and humans. Aflatoxins are the secondary metabolites of the fungi namely, *Aspergillus flavus* and *A. parasiticus*. Aflatoxins are further differentiated into sub types such as B1, B2, G1, G2 because of their blue (B) and green (G) fluorescence under ultra violet light respectively, based on structure, chromatographic and fluorescent characteristic. The aflatoxin which are found in milk are designated as M1 and M2. Both raw and processed fruits and vegetables are highly susceptible to mycotoxin contamination (Giryn and Szteke, 1995). *A. flavus* is often countered as a tomato fruit rot pathogen during post-harvest survey (Samyal and Sumbali, 2002). Spores of *A. flavus* are saprophytic in nature and once they become pathogenic, they are known to produce an array of toxic secondary metabolites including aflatoxins (Nallathambi and Umamaheswari, 2009). Aflatoxins are known to be highly carcinogenic and have been classified as group I carcinogens (IARC, 1993).

In our earlier surveyed carried out for awareness and assessment for aflatoxin of Muktainagar Taluka in Jalgaon district of Maharashtra it has been observed that only 36% individual were found aware about the aflatoxin and 64% were unaware about it and for other toxin 48% were aware and 52% were unaware (Yeole and Deshmukh, 2013a). The survey carried out for Malkapur Taluka in Buldhana district of Maharashtra the data collected on awareness about aflatoxin which indicates that 60% individuals were aware about the aflatoxin and 40% were found unaware about it.62% consumers were aware about the toxin, other than aflatoxin and 38% were unaware about this (Yeole and Deshmukh, 2013b). The survey carried out for awareness and assessment of Pune district of Maharashtra indicates that 14% were aware and 86% individuals were found unware about the aflatoxin and for toxin other than aflatoxin 32% were aware and 68% individuals were found unware (Yeole et. al., 2014).

The study conducted during the year 2016 for in vitro evaluation for *Aspergillus flavus* producing aflatoxin in groundnut the unsafe limit was found 14% minimum to 50% maximum but, highest was found 65% (Yeole *et. al.*, 2016). In last study carried out on *Aspergillus flavus* producing aflatoxin in groundnut concluded that only 40 to 44% samples are found safe to eat and 4 to 11% are found 100% infected and colonized which are found completely unsafe to eat. The remaining 55 to 45 % samples stood in between 25 to 99 percentage range of infection and colonization which might be a prone for producing aflatoxin in groundnut (Yeole,2021).Thus, aflatoxins have become of concern in agriculture as well as in animal and human health on a global scale.

More than 90% of the 1000 farmers surveyed in India do not know about the ill-effects of aflatoxin contamination and management options available. This was the results of a survey ICRISAT conducted in the important groundnut growing states of India such as Andhra Pradesh, Gujarat, Karnataka and Tamil Nadu. One of the reasons for poor adoption of aflatoxin management interventions is lack of awareness among the farming community about this menace. Other important interventions included identification of "Good Agricultural Practices" (GAPs) which significantly reduce pre- and post-harvest aflatoxin contamination. Tremendous progress was made in this area. Several technologies were identified, packaged and tested on-farm, such as soil amendments (e.g. farmyard manure, lime, and gypsum), moisture conservation techniques, pod drying methods and storage methods (ICRISAT, 15).

Three basic approaches viz, prevention, removal and detoxification seem to be promising for aflatoxins control. Use of crop rotation and intercropping found useful in preventing aflatoxins contamination (Desai and Ghewande, 1999). Addition of calcium and gypsum also reduce pre-harvest aflatoxin contamination (Davidson et. al., 1983).Use of resistance genotype like Chitra (Desai et. al., 1990), PI-337409 (Pettit et. al., 1986) and other bold seeded genotype like ICG-239, B-95, B-99-1 supported to lowest aflatoxin production (Ghewande et. al., 1993) are useful in resistance breeding programme. Simple methods like exposure of oil to bright sunlight, use of common salt (10%) are useful even at household level for detoxification of aflatoxin (Shantha, 1987). The different factors responsible for aflatoxin contamination at different level are categories as preharvest level, at plant level, harvesting level and on at storage level i.e.postharvest level.

MATERIALS AND METHODS

To detect the growth of *Aspergillus flavus* in groundnut, summer produced fifty samples were collected from Muktainagar market shops and were processed after surface sterilization by 1.5 % Sodium hypochlorite (NaOCl or NaClO) or 1% Mercuric Chloride (HgCl₂) for five minutes and after cleaning two times with distilled water, were incubated on Czapek-Dox Rose Bengal Agar medium containing 1L distilled water,30 g sucrose, 1g K₂HPO₄, 0.5g MgSO₄, 0.5g KCl, 0.019g FeSO₄, 2g NaNO₃, 15g Agar, pH-7.3 (Aneja K,2003) with ten groundnut seeds in each petri dish, incubated in a incubator at 20-22 °C temperature and examined after 48 -72 hrs.for their fungal growth of percentage infection and colonization caused by *Aspergillus flavus* producing aflatoxin in groundnut.



Fig. 1 A. Czapek-Dox Rose Bengal Agar Media Preparation. B. Groundnut Seed inoculation C. Infected seed with *Aspergillus flavus*.

Sample No.	Infection %	Colonization %	Sample No.	Infection %	Colonization %
1	20	10	26	20	20
2	00	00	27	20	20
3	20	20	28	40	00
4	20	10	29	00	00
5	00	00	30	40	20
6	30	20	31	20	10
7	00	00	32	30	20
8	00	00	33	00	00
9	00	00	34	30	20
10	00	00	35	30	30
11	00	00	36	20	10
12	40	20	37	00	00
13	20	30	38	00	00
14	30	30	39	00	00
15	00	00	40	30	00
16	40	40	41	00	00
17	20	20	42	20	00
18	40	20	43	00	00
19	10	20	44	00	00
20	00	00	45	20	30
21	40	40	46	00	00
22	00	00	47	20	00
23	40	20	48	00	00
24	50	40	49	40	30
25	00	00	50	40	20

Sr. No.	Percentage range	Infection (No. of sample)	% of infected samples	Colonization (No. of samples)	Percentage of colonized samples
1	00 (Nil)	21	42	25	50
2	<25	13	26	17	34
3	>25 to 50	16	32	08	16
Total 50		50	100	50	100

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Table 2. Sample showing percent range	for infection and colonization	hy Asperaillius flavus in groundhuf
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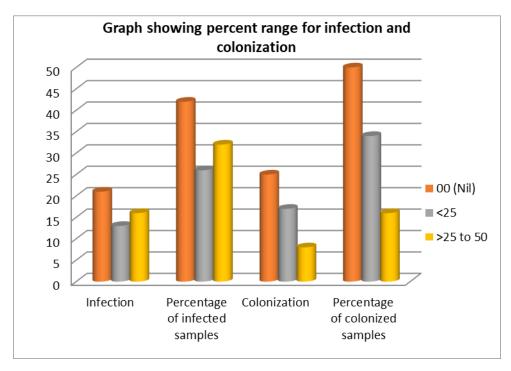


Figure 1: Percent range for infection and colonization by Aspergillus flavus in groundnut.

Result and Discussion:

The data in the table 1 indicates that out of total fifty groundnut kernels samples screened for detection of *Aspergillus flavus*. At the beginning the fungus growth looks white and then it becomes olive green in colour. The minimum and maximum infection range was found from 10 to 50 % and for colonization it stand between10 to 40%. For infection 21 sample (42%) was found completely nil, followed by13 (26%) with less than 25 % infection. Sixteen samples (32%) were observed between greater than 25 to 50 percent range of infection. The result for colonization is interesting where 25 samples (50%) was found completely nil followed by 17 (34%) and 8(16%) found in the percentage range of less than 25 and greater than 25 to 50 for colonization respectively.

CONCLUSION

The present study of research carried out for *Aspergillus flavus* Producing Aflatoxin in Groundnut, it is concluded that out of the fifty tested groundnut samples only 42 to 50% was found completely nil for infection and colonization respectively and safe to eat. From the table no.2 it is also concluded that the rate of infection of the fungus *Aspergillus flavus* more compare to that of colonization the result may vary with sample to sample and pre and postharvest management of groundnut crop including storage methods, etc.

Conflict of interest: The authors declare that they have no conflict of interest.

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