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Nutritional changes in oilseeds due to *Aspergillus* spp.

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ABSTRACT: In present research work experiments were carried out to study the deteriorative changes in oilseeds under the influence of *Aspergillus* sp. It was found that fat content in groundnut and soybean was reduced due to *Aspergillus flavus*. In groundnut the maximum loss in protein content was due to *A. terreus.* On the other hand, *A. niger, A. terreus, A. parasiticus* and *A. fumigatus* were found to reduced the protein content in soybean. *A. versicolor* effectively reduced maximum amount of reducing sugar content in groundnut and soybean.

Key words: *Aspergillus* sp., Fat content, Protein content, Reducing sugar, Biodeterioration

Introduction

The oil seeds production scenario in India has witnessed a dramatic turn. The country achieved a status of 'self sufficient and net exporter' during early nineties, rising from the net importer state, with an average annual production of nearly eleven million tonnes from the annual oil seed crops, up till the year 1986-87. In a span of just a decade, an all time record oil seeds production of 25 million tonnes from annual oil seed crops was attained during 1996-97. This transformation has been tamed as 'The yellow revolution' and could be primarily attributed to the institutional support.

In agriculture, seeds play very important role for the production of healthy crop. About 90% of the crops all over the world are produced by using seeds. Seeds in the field as well as in ill storage conditions interact with several microbes which deteriorate the seeds, both qualitatively and quantitatively (Christensen and Kaufman, 1969). The microorganisms grow on the seeds by the consumption of easily digestible components. The successful invasion or colonization, however, depends largely upon the efficiency of microorganisms to degrade complex molecules into simpler forms (Bilgrami and Verma, 1978). Fungi growing on stored grains reduce the germination rate, carbohydrate, protein, total oil content, increase moisture content and also enhancing other biochemical changes of grains (Bhattacharya, 2002). Such seeds are unfit for human consumption and are also rejected at the industrial level.

Like other plant products, oil seeds also carry a variety of microorganisms which originate from soil, air and plant source. However, presence of these microorganisms in or on products of human interest is not harmful. But under certain conditions they may start consuming such products for growth and reproduction and their activity is likely to cause undesirable change of varied nature in the product concerned including quality of nutrients, loss of constituents nutrients, poisoning of the products by mycotoxins, loss of germinability. The seeds are also found to be responsible for disease transmission because they carry number of pathogens, which get associated either in the field or in the post harvest storage condition. The associated moulds spoil the seeds, the process is known as seed biodeterioration.

It is reported in the literature that during storage several microbes including bacteria, nematodes, fungi etc contaminate seeds. The species of *Aspergillus, Penicillium, Fusarium, Rhizopus* and *Alternaria* are found commonly occurring as post harvest molds in storage condition. Most of the species of *Aspergillus* are dominant and play vital role in the seed biodeterioration.

Considering this fact emphasis is given on to study the biochemical changes like change in crude fat, change in protein and change in reducing sugar in oilseeds under the influence of *Aspergillus* spp.

Materials and Methods

Ten dominant *Aspergillus* spp were isolated from abnormal oilseeds and selected to study their impact on nutritional changes in oilseeds. Healthy seeds of groundnut, soybean, sesame, safflower and sunflower were surface sterilized with 0.1% mercuric chloride solution and subsequently washed and soaked in sterile distilled water for four hours. Excess water was decanted from the seeds. The seeds were distributed into flasks (l00g per flask) and were inoculated separately with 10ml spore suspension of the test fungi. The flasks were incubated at room temperature for 14 days. At the time of harvest, seeds were thoroughly washed under running tap water in order to remove complete mycelia mat from their surface. Subsequently, the seeds were dried at 60°C for 48 hours and crushed into fine powder for the estimation of chemical changes in the seeds. Seeds incubated in a similar manner but without inoculating spore suspensions of fungi served the control.

Estimation of crude fat

The crude fat was estimated by the standard Soxhlet method given in A.O.A.C. (1970). The fat present in the seed material was extracted in the solvent mixture of chloroform and methanol in Soxhlet extraction assembly. 2g dry seed powder was placed in a thimble of Whatman filter paper No.l. The mouth of thimble was plugged with fat free absorbent cotton. Solvent was added in dry 250ml receiver flask from the soxhlet assembly just to reach the level of the neck. The thimble with sample was introduced into the soxhlet. The apparatus was assembled and placed on heating mental with temperature controlling device. The fat was extracted for 8 hours at 60°C. When the extraction was over, thimble was removed from soxhlet. Apparatus was again assembled and heated to recover most of the solvent from the receiver flask. About 25ml solvent along with the extracted fat was left in the receiver flask, the receiver flask was disconnected. The solvent was then transfered in a clean, previously weighed beaker. After drying in a hot air oven at 95°C, it was then cooled in a dessicator and weighed. The amount of fat was measured form extracted per 2g of the sample and amount of crude fat as percent of dry matter (DM) was calculated.

Estimation of reducing sugar

The sugar content in the plant material was estimated by the procedure recommended by Oser (1979). 500mg of seed powder was taken in 50ml distilled water and boiled it, then filtered it and the filtrate was diluted up to 100ml. Three Folin-wu tubes were taken and to it following content were added. (1) Blank tube - D. W. 2ml (2) 2ml glucose 'C' solution. (3) 2ml filtrate. In each tube 3ml alkaline solution of copper was added. Then tube was boiled in boiling water bath for 8 minutes. Cooled the tubes under tap water and 2ml of phosphomolybdic acid solution was added which gave blue colour. Then this solution was diluted up to 25ml distilled water and optical density determined at 420nm and the amount of reducing sugar present in seed powder was calculated.

Estimation of crude protein

Crude protein was by estimating Nitrogen content in the samples with the help of microkjeldahl technique (AOAC, 1970). The amount of N content was multiplied by 6.25 factors which gave crude protein content of the samples. 300mg seed powder were taken in Kjeldahl flask along with 250mg H_2SO_4 and 40mg $CuSo_4$ and kept overnight. This was digested till the mixture become white. After complete digestion the flasks were allow to cool. The digest was processed for distillation with the help of Markham's distillation set.

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Digest was diluted to 50ml volumetric flask, 5ml aliquots were taken and introduced in distillation unit through the side tube funnel. The glass stopper was immediately fitted. To this 10ml 40% NaOH was added into digest. NH_3 is liberated into 10 ml 2 percent boric acid (with mixed indicator) containing 50ml conical flask. After distillation green colored ammonium borate was titrated against 0.035 NHCl till the end point (faint pink) was obtained (This gave 1ml 0.035 NHC1 = 0.5mg N% crude protein = %N x 6.25). Crude protein of seeds was calculated as percent nitrogen liberated x 6.5.

Fig. 1: Deteriorated oilseeds A) Groundnut B) Soybean C) Sunflower and D) Sesame due to *Aspergillus* spp. 1. *Aspergillus flavus*, 2. *A. fumigatus*, 3. *A. glaucus*, 4. *A. nidulans*, 5. *A. niger*, 6. *A. oryzae*, 7. *A. parasiticus*, 8. *A. terreus*, 9. *A. ustus*, 10. *A. versicolor* and c-Control.

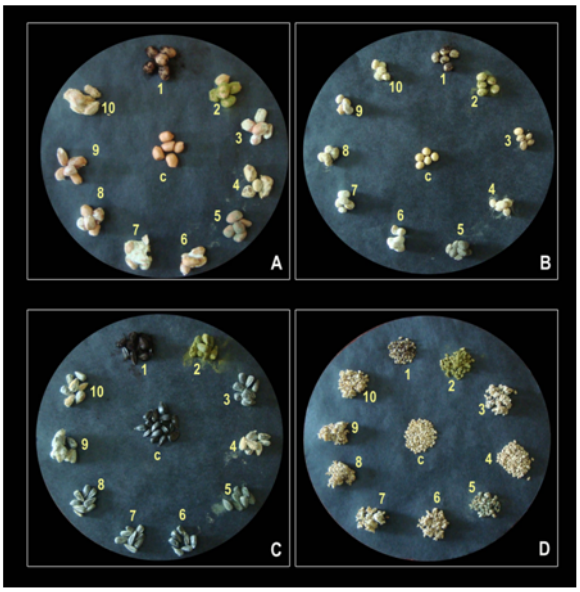

Fig. 2: Steriomicrophotographs of Aspergillus deterioated oilseeds. a-Soybean seed, b- Groundnut seed, c-Sesame seed, d-Soybean seed, e,f,g and h- sunflower seed.

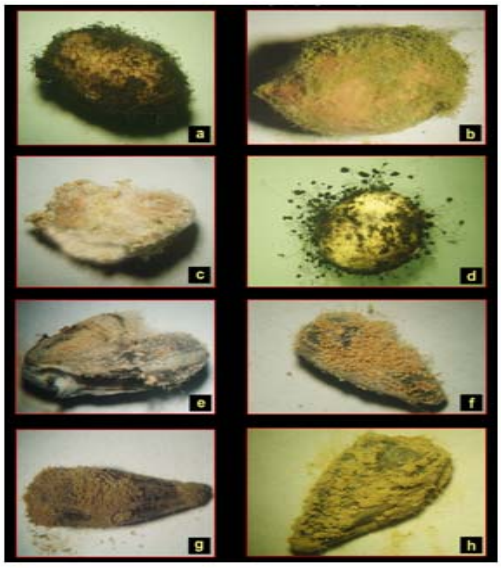

Table 1. Change in fat content of oil seeds due to different *Aspergillus* species

Aspergillus	**Oil Seeds**				
Species.	**Groundnut**	**Soybean**	**Sesame**	**Sunflower**	**Safflower**
A. flavus	35.5	08.0	42.3	29.8	25.3
A. fumigatus	45.5	12.3	39.9	34.0	28.4
A. glaucus	42.0	13.0	44.3	37.9	29.3
A. nidulans	39.0	15.0	41.9	43.5	29.0
A. niger	43.1	18.8	47.0	37.5	26.6
A. oryzae	44.3	16.0	43.3	43.0	30.9
A. parasiticus	47.0	16.1	43.7	44.0	28.5
A. terreus	40.3	12.3	44.0	33.9	30.4
A. ustus	46.8	15.2	46.2	35.3	31.0
A. versicolor	42.9	17.0	48.0	39.4	31.5
Control	49.0	20.0	50.0	52.9	32.4

Values are expressed in%

Table 2. Change in reducing sugar of oil seeds due to different *Aspergillus* species

***Aspergillus* species**	**Oil seeds**				
	Groundnut	**Soybean**	**Sesame**	**Sunflower**	**Safflower**
A. flavus	1.8	2.43	1.82	2.81	1.82
A. fumigatus	2.58	1.25	1.19	1.72	1.27
A. glaucus	2.13	2.34	2.11	2.24	2.12
A. nidulance	3.67	1.25	2.04	2.75	1.93
A. niger	2.22	2.88	2.14	2.62	2.27
A. oryzae	4.28	1.25	0.81	1.10	1.533
A. parasiticus	2.07	2.55	2.11	1.79	1.64
A. terreus	4.06	1.90	0.92	1.02	1.59
A. ustus	3.13	2.43	1.82	2.24	2.04
A. versicolor	2.05	1.06	0.80	2.33	1.96
Control	7.20	6.90	2.40	3.14	2.63

Values are expressed in %

Table 3. Change in protein content of oil seeds due to different *Aspergillus* spp.

***Aspergillus* species**	**Oil seeds**				
	Groundnut	**Soybean**	**Sesame**	**Sunflower**	**Safflower**
A. flavus	13.05	26.15	14.57	09.37	20.47
A. fumigatus	09.37	27.06	10.41	12.49	28.49
A. glaucus	10.41	26.03	17.18	11.97	24.23
A. nidulance	08.33	25.51	17.74	12.49	22.97
A. niger	07.28	21.86	14.57	08.85	25.23
A. oryzae	13.53	26.59	17.71	10.93	27.48
A. parasiticus	12.49	22.90	15.61	10.41	26.90
A. terreus	03.12	21.86	17.70	09.89	24.89
A. ustus	07.28	23.94	16.74	08.33	26.79
A. versicolor	07.28	24.46	15.09	08.33	28.57
Control	14.57	29.15	19.78	13.01	30.02

Values are expressed in %

Results and Discussion

Changes in fat content

It is clear from the results given in table 1, that all *Aspergillus species* were capable of reducing the fat content. *Aspergillus flavus* was responsible for maximum deplition of fat content in all oil seeds. In case of safflower, soybean and sesame seed, *A. fumigatus* reduced the fat content. In groundnut and safflower *A. nidulance* showed maximum reduction in fat content while, *A. terreus* decreased fat content in soybean and sunflower.

Change in reducing sugar content

From table 2 it is clear that, all *Aspergillus* species reduced sugar in all oil seeds. It was interested to note that *A. versicolor* was found to the most effective fungi among all the ten *Aspergillus* species which reduced maximum amount of sugar content in groundnut, soybean and sesame. *A. fumigatus* was responsible for maximum deterioration of sugar content in soybean, sesame and safflower while, *A. oryzae* deplited maximum fat content in all the oilseeds except groundnut.

Change in protein content

From table 3, it was observed that, all *Aspergillus* species reduced protein content in sunflower, sesame, soybean, safflower and groundnut. In groundnut the maximum loss in protein content was due to *A. terreus* followed by *A. niger, A. ustus* and *A. versicolor.* The fungi like *A. niger, A. terreus, A. parasiticus* and *A. fumigatus* were found to reduced the maximum protein content very effectively in soybean. Where as, *A. fumigatus* reduced the protein content in sesame followed by *A. niger, A. flavus, A. versicolor* and *A. parasiticus.* In case of sunflower the maximum loss in protein content was observed due to *Aspergillus ustus, A. versicolor, A. flavus* and *A. terreus. Aspergillus* species like *A. flavus* followed by *A. nidulans, A. glaucus* and *A. terreus* reduced the protein content in safflower seeds.

It is clear from the literature that, seed-borne fungi cause nutritional changes in seeds. Nutritional value of groundnut (Ward and Diener, 1961 and Singh et al., 1974) and other oilseeds (Sharma, 1977) is affected due to seed-borne fungi which are found to damage or discolor the kernels consequently, affect fat and reducing sugar contents. Mathur and Sinha (1978) reported that in case of bajra seeds *Aspergillus candidus, A. nidulans, A. flavus, A. fumigatus, A. niger* and *A. terreus* caused initial increase in reducing sugar but latter on it was found to be decreased gradually. Bilgrami et al. (1976) studied that, infesting seeds with *A. flavus* results in significant decrease in the seed protein. Iyayi (2004) observed the changes in the protein and sugar of three agroindustrial by products after fermentation with *Aspergillus niger, Aspergillus flavus* and *Penicillium* sp. They found that protein and sugar content were increased due to these fungi. Kakde and Chavan (2011) found that, storage fungi are responsible for decrease in fat, protein and resucing sugar content in oilseeds. From results it can be concluded that, decreased in reducing sugar and protein may be due to fungi utilized sugar as a substrate for their growth. There is a decrease in crude fat, it is because of fungi might have degraded the lipids by lipase enzyme.

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