Biochemical Changes in Stored Seeds due to *Aspergillus* Species

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ABSTRACT:

The aim of present study is to detect biochemical changes occur in stored plant seeds. *Aspergillus* species and other fungi are known biodeteriorats of stored plant seeds. The stored seeds of groundnut, bajra, black gram and neem were surface sterilised separately with 0.1 % $HgCl_2$ solution. In all six species of *A. niger, A. flavus, A. terreus, A. glaucus, A. sulphureus* and *A. ornatus* were isolated from stored plant seeds and biochemical changes tested . Estimation of ash, fat, protein and starch was done. Maximum loss in seed weight was occurred due to *A. niger* and increase in ash content was observed due *A. flavus* and *A. niger*, more reduction in starch content in groundnut and more or less reduction in protein contents due to *Aspergillus* tested species.

KEY WORDS: Biodeterioration, Ash, Dry weight, Fat, Protein, Starch, stored Plant Seeds.

INTRODUCTION:

Nutritional quality losses during storage are caused by poor post-harvest handling and the natural respiration of grain (Golob et al., 2002) and by damage caused by bio-deterioration (Rehman, 2006; Reed et al., 2007). *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera, Fusarium moniliforme* and *Rhizopus stolonifer* were found to be common and dominant on pulses. The fungi that appear mostly on the vended samples isolated are *A. flavus, A. niger, A. ochraceus, A. parasiticus, Fusarium* sp., *Rhizopus stolonifer*, yeast, and *Trichoderma koningii* (Kumar et al. 2008). Fungal contamination can occur in the field, or during harvest, transport and storage (Kader and Hussein, 2009). Postharvest loss accounts for direct physical losses and quality losses that reduce the economic value of crop, or may make it unsuitable for human consumption. In severe cases, these losses can be up to 80% of the total production (Fox 2013.) Maize (Zea mays L) commonly known as corn in the United States and Canada, is the third most important cereal grain worldwide after wheat and rice (Golob et al., 2004). Contamination of maize grain with mold and fungi is regarded as one of the most serious safety problems in the tropical countries and throughout the world (Kaaya and Kyamuhangire, 2006). Infection of maize grain by storage fungus results in discoloration, dry matter loss, chemical and nutritional changes and overall reduction of maize grain quality Chuck-Hernández et al., (2012).

Two different media are used for isolation of different groups of fungi that leads biodeterioration of plant seeds especially in storage. In all fourteen species of *Aspergillus* were isolated along with other fungi during study from different crops seeds were maintained on Potato Dextrose Agar. Out of which *A. flavus, A. niger, A. terreus, A. glaucus, A. sulphuerus* and *A. ornatus* were used in present study. These leads biochemical

changes so far. Hence, the present study was undertaken to determine the changes caused by *Aspergillus* species isolated from stored plant seeds

MATERIALS AND METHODS:

1. **Collection of seed samples:**

The methods described by Neergaard (1973) have been adopted for the collection of seed samples of groundnut, Black gram, bajra, and neem. Accordingly seed samples were collected from field, store houses and market places and from farmers at Aurangabad. A composite sample was prepared by mixing the individual sample together, preserved in cloth bags at room temperature during the studies.

2. Preparation of spore suspension:

Spore suspension of *Aspergillus* species were prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures of seed-borne fungi of crop plants maintained on PDA slants for seven days at room temperature. The slants were shaken and content was filtered through muslin cloth to separate mycelium and spore. The filtrate thus obtained was used as spore suspension.

3. Detection of Seed Mycoflora:

The procedure for blotter test and agar plate methods was followed as described by International Seed Testing Association, ISTA (1966) De Tempe (1970), Neergaard (1973) and Agarwal (1976).

i) Blotter Test Method:

A pair of white blotter paper of 8.5 cm diameter was jointly soaked in sterile distilled water, placed in pre-sterilized corning Petriplates of 10 cm diameter. Ten seeds per plate were placed at equal distance on the moist blotters. One hundred seeds were tested for each treatment. The plates were incubated at 25±20C under diurnal condition. On 7^{th} day the seeds were examined under stereoscopic microscope for the preliminary determination of *Aspergillus.* Identification and further fungi occurred on seeds was made by preparing slides of the fungal growth and observing under compound microscope.

ii) Agar Plate Method:

In this method, pre-sterilized corning glass Petri-plates of 10 cm diameter were poured with 25 ml of autoclaved Potato Dextrose Agar (PDA) medium. On cooling the medium, 10 seeds per plate were equispaced aseptically; incubation condition and other details were same as described for the blotter test method. In order to isolate only internal seed mycoflora, seeds were pre-sterilized with 0.1% solution of mercuric chloride for 1

minute. Subsequently, thoroughly washed twice with sterile distilled water and placed on agar plates. Seeds without any such pre-treatments were employed for the total seed mycoflora (control).

4. Artificial Infestation of Plant Seeds:

The seeds of groundnut, bajra, black gram and neem were surface sterilized separately with 0.1 % $HgCl_2$ solution and washed twice with sterile distilled water. Excess water was drained. The seeds were distributed into pre-sterilized conical flask (25 g/ flasks) and were inoculated separately with 2 ml spore suspension of different species of *Aspergillus*. The flasks were incubated at room temperature for 15 days and were harvested for assessment of chemical changes in the seeds due to the *Aspergillus*. For which the seeds were thoroughly washed under running tap water in order to remove mycelial growth from their surfaces. Subsequently, the seeds were dried at 60^0C for 48 hours and crushed into fine powder for the estimation of different chemicals. Seeds were incubated in a similar manner but without inoculating the spore suspension of *Aspergillus* as control.

i) Extraction and Estimation of Ash:

One g of seed powder was placed in a previously weighed crucible and it was subjected to heating on a hot plate for about 30 minutes till the sample was sufficiently turned black. Then it was placed in muffle furnace pre-heated to 60^0C for two hours with automatic control. Crucibles were transferred directly to dessicator, cooled and weighed immediately. Weight of ash was obtained and reported as percent ash.

ii) Extraction and Estimation of Fat:

Fat estimation was done by ether extraction method. One g of seed powder was placed in a thimble, placed in an extractor at 40-45^0C for one hour. Then the solvent ether along with extracted seed fat was poured in a pre-weighed disc. Ether was evaporated under fan and residue was dried overnight in an oven at 60^0C. The dish was immediately transferred in a dessicator. On cooling, it was adjusted and the amount of fat extracted was reported as percent crude fat.

iii) Extraction and Estimation of Protein:

Estimation of crude protein was made by MicroKjeldahl method (A. O. A. C 1960) 300 mg dry powder of seeds was placed in 50 ml MicroKjeldahl flask. 60 mg catalyst and 7.5 ml H_2SO4 were added in the flask. The flasks were heated for 6-8 hours (Digestion). After, this on cooling flasks 5 ml of the aliquot was introduced in Markham's distillation unit through the side tube funnel to which glass stopper was fitted. 10 ml of 40 % NaOH was allowed to run it into the digest. NH_3 liberated was collected in 50 ml conical flasks containing 10 ml of 2 % Boric acid with indicator and the distillation was titrated against 0.035 NHCL till end point was achieved. The crude protein was calculated as percent N X 6.25 + crude protein.

iv) Extraction and Estimation of Starch:

It was estimated by Anthrone reagent method (A. O. A. C. 1966). 100 g seed powder was mixed with 5 ml of distilled water, 6.5 ml 52 % perchloric acid was added to it in order to dissolve the total starch. The mixture

was placed at 0^{O}C for 20 minute, centrifuged and retained the supernatant extract. The residue was repeatedly extracted with the addition of fresh perchloric acid. The combined extract was diluted to 100 ml. The diluted extract (0. 5 ml) was taken in a test tube and to it 4.5 ml distilled water was added. 10 ml of cold Anthrone sulphuric acid reagent was mixed with 5 ml above extract in an ice-bath heated for 8 minutes at 100^{0}C and cooled rapidly to room temperature. Then the O.D. was measured at 630 nM, calibration curve was prepared by using glucose solution. Starch content was calculated by multiplying by 0.9 the equivalent.

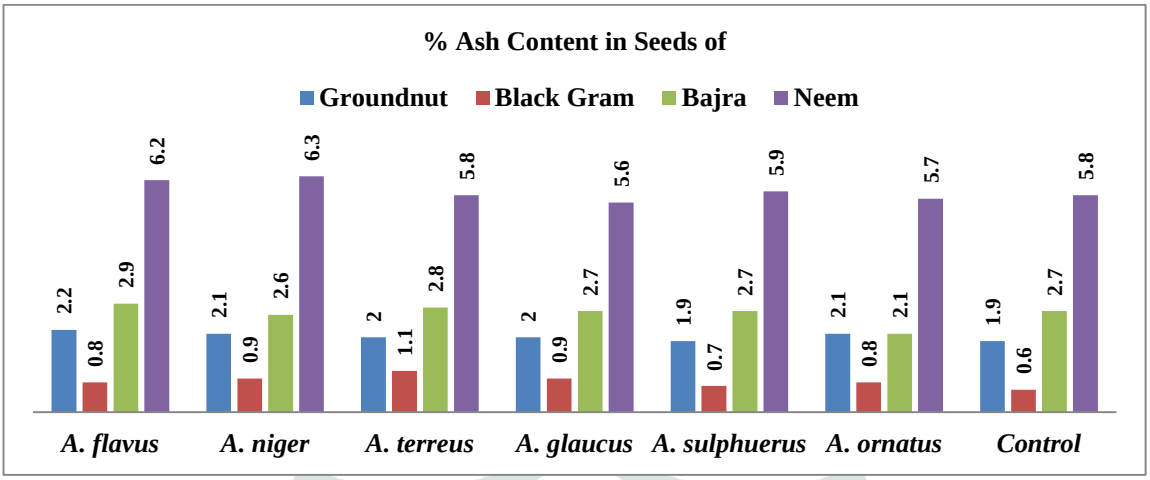
Anthrone sulphuric acid reagent-200 mg (Anthrone was dissolved in 100 ml of cold 95 % H_2SO_4 and stored at 0^{0}C).

RESULTS AND DISCUSSION:

Table 1: Changes in Ash Content of Seeds due to Species of *Aspergillus* Associated with Different Plant Seeds.

Sr. No.	Seeds Infested With	% Ash Content in Seeds of			
		Groundnut	Black Gram	Bajra	Neem
1	*A. flavus*	2.2	0.8	2.9	6.2
2	*A. niger*	2.1	0.9	2.6	6.3
3	*A. terreus*	2.0	1.1	2.8	5.8
4	*A. glaucus*	2.0	0.9	2.7	5.6
5	*A. sulphuerus*	1.9	0.7	2.7	5.9
6	*A. ornatus*	2.1	0.8	2.1	5.7
7	Control	1.9	0.6	2.7	5.8

Graph 1: Changes in Ash Content of Seeds due to Species of *Aspergillus* Associated with Different Plant Seeds



The seeds of groundnut, black gram, bajra and neem infested separately with six species of *Aspergillus* were incubated for 10 days as described earlier and after that they were estimated for percent ash content. The results were summarised in table no 2 and graph no 2**.**

It is evident from the results summarised in table 02 that irrespective of the crop and all the species of *Aspergillus* with more or less degree caused increase in ash content of the seeds. However, this was maximum due to *A. flavus* and *A. niger*.

Changes in fat Content of Seeds due to Species of *Aspergillus* Associated with Different Plant Seeds:

Utilization of seed fat was studied by growing the species of *Aspergillus* separately on seeds of groundnut, black gram, bajra and neem at room temperature for a period of 10 days. Then estimation of fat was carried out and was compared with that in control seeds.

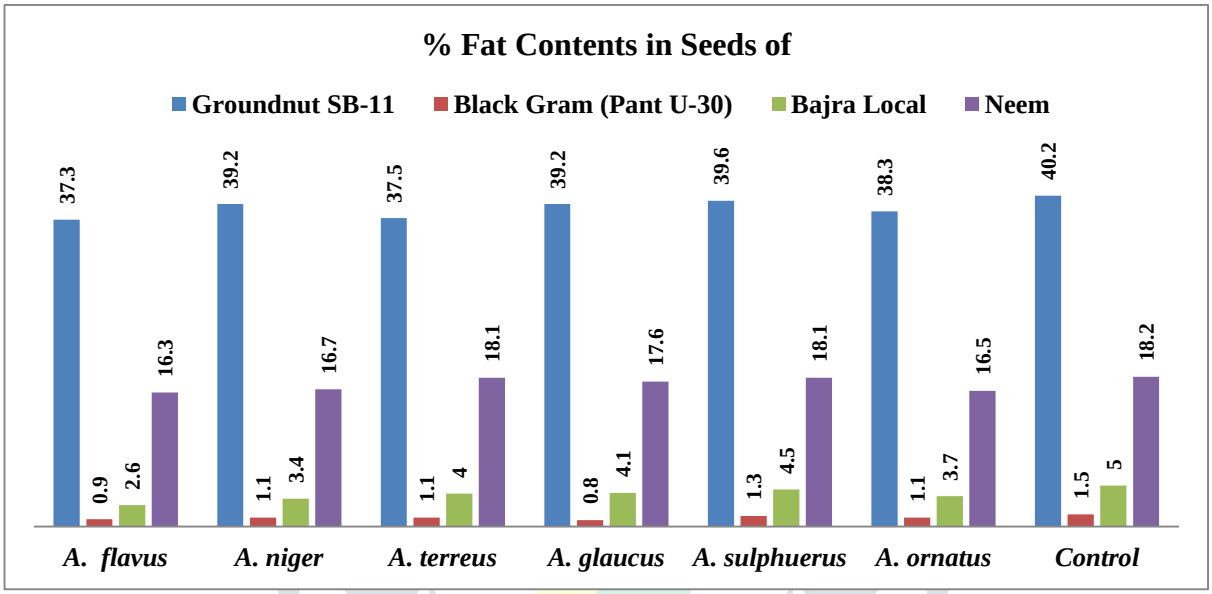
It is clear from the results given in table 02 that all the six species of *Aspergillus* have been found to reduce fat contents in the seeds of tested crops. The loss in fat content was found to be more due to *A. flavus* and *A, terreus* in groundnut due to *A. terreu*s and *A. glaucus* in black gram due to *A. flavus* and *A. niger* in bajra while due to *A. flavus, A. niger* and *A. ornatus* in neem seeds. A dominant seed-borne fungi *A. flavus* caused quantitative and qualitative damage in *Arachis hypogea* by reducing sugar and oil content of the seeds (Naikoo *et al.*, 2013)..

Table 2: Changes in Fat Content of Seeds due to Species of *Aspergillus* Associated with Different Seeds

Sr. No.	Seeds Infested With	% Fat Content in the Seeds of			
		Groundnut SB-11	Black Gram (Pant U-30)	Bajra Local	Neem
1	*A. flavus*	37.3	0.9	2.6	16.3
2	*A. niger*	39.2	1.1	3.4	16.7

3	*terreus*	37.5	1.1	4.0	18.1
4	*A. glaucus*	39.2	0.8	4.1	17.6
5	*A. sulphuerus*	39.6	1.3	4.5	18.1
6	*A. ornatus*	38.3	1.1	3.7	16.5
7	Control	40.2	1.5	5.0	18.2

Graph 2: Changes in Fat Content of Seeds due to Species of *Aspergillus* Associated with Different Seeds



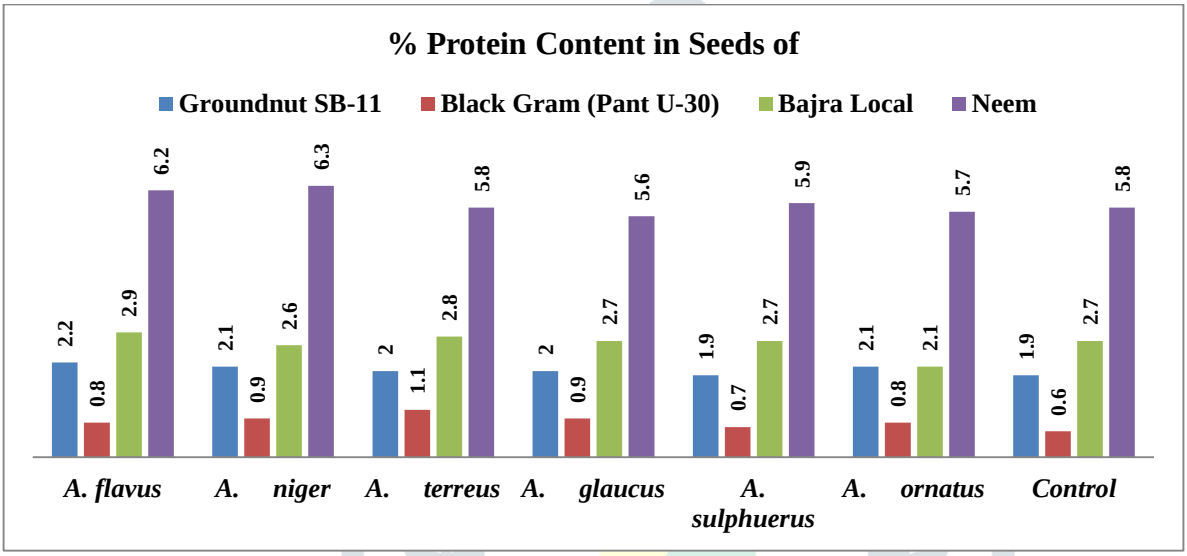
Reports regarding loss in fat content of seeds due to different species of *Aspergillus* have been made in many cases. Ward and Diener (1961) observed significant loss of oil content in groundnut seeds due to *A. tamari, A. ruber, A. chevalier and A. restrictus*. Neera and Mehrotra (1990) observed the same in case of sunflower due to *A. flavus and A. fumigatus*. While, Sinha and Singh (1993) in Mahua reported loss in oil contents due to *A. niger*, *A. ochracerus* and *A. tamari*

Table 3: Changes in Protein Content of Seeds due to Species of *Aspergillus* Associated with Different Plant Seeds

Sr. No.	Seeds Infested With	% Protein Content in the Seeds of			
		Groundnut SB-11	Black Gram (Pant U-30)	Bajra Local	Neem
1	*A. flavus*	2.2	0.8	2.9	6.2
2	*A. niger*	2.1	0.9	2.6	6.3

3	*A. terreus*	2.0	1.1	2.8	5.8
4	*A. glaucus*	2.0	0.9	2.7	5.6
5	*A. sulphuerus*	1.9	0.7	2.7	5.9
6	*A. ornatus*	2.1	0.8	2.1	5.7
7	Control	1.9	0.6	2.7	5.8

Graph 3: Changes in Protein Content of seeds due to Species of *Aspergillus* Associated with Different Plant Seeds



Degradation and utilization of protein by six different species of *Aspergillus* was studied by growing them on the seeds of groundnut, black gram, bajra and neem as described earlier. The results are summarised in table 03 and graph no 03.

The results regarding utilization of seed protein in four different crops by six species of *Aspergillus* summarized in table no 4 are found to be very much informative. All the species of *Aspergillus* are found to be capable of utilizing seed proteins but the rate of utilization was variable among the species. Srivastavaand (2013) reported maximum protein content of *Jatropha crucas* L. was reduced by the seed-borne fungi *Fusarium chlamydosporum*. In all test pulses, *Fusarium moniliforme* caused maximum reduction in protein content followed by *Aspergillus niger and A. flavus*.

It is understood from the table No. 03 that all the six species of *Aspergillus* caused reduction in protein content of the seeds in all the four crops studied. However, the amount of protein reduction was found to be variable with the species of *Aspergillus*. It was interesting to note that loss in protein content of neem seeds was found to be less than the other three crops

Changes in Starch Content of Seeds due to Species of *Aspergillus* Associated with Different Plant Seeds

The seeds of groundnut, black gram, bajra and neem were subjected for the growth of the six species of *Aspergillus* as described for the studies on estimation of dry weight. The starch content of such seeds was estimated and results are given in (Table 04**,** Plate No-01 Artificial infestation of plant seed by *Aspergillus* species**)**

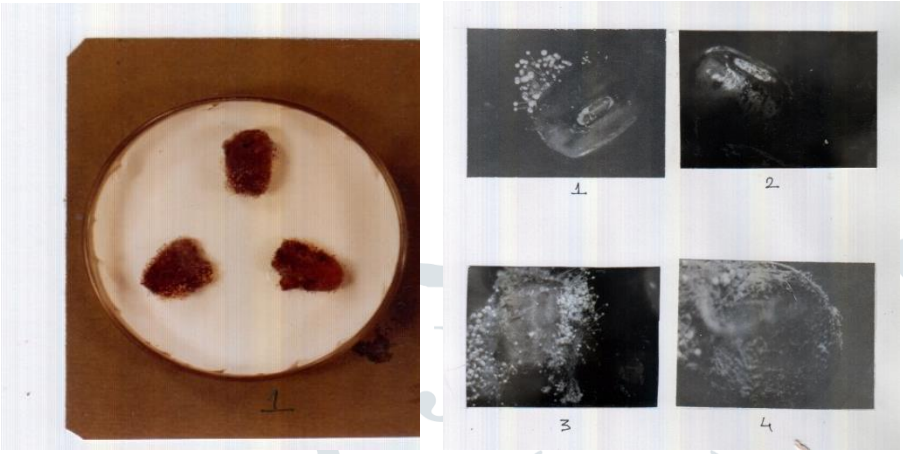


Plate No. 1. Artificial infestation by *Aspergillus* species

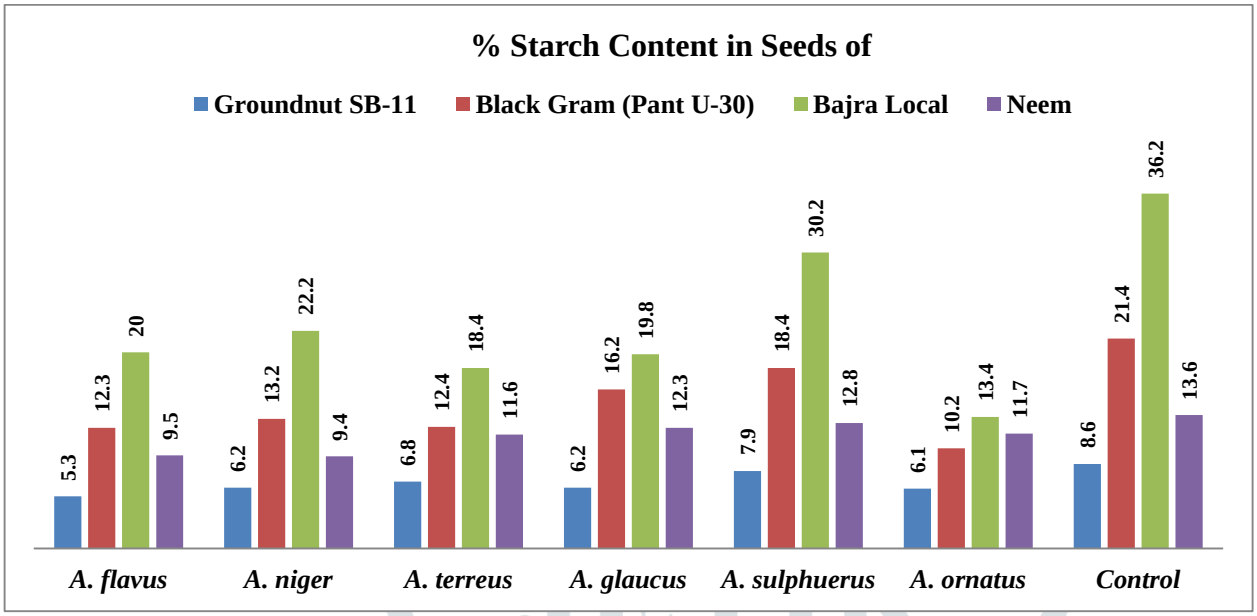
(Neem seeds) (Black Gram)

It is clear from the data that utilization of starch in case of groundnut seeds was found to be more due to *A. flavus, A. niger* and *A. ornatus* and very poor due to *A. sulphureus*. Similar type of trends of utilization of starch has been seen in the seeds of black gram and bajra. It was interesting to note that utilization of starch from neem seeds was maximum by *A. flavus* and *A. niger* while, remaining fungi utilized starch very poorly from neem seeds.

Table 4: Changes in Starch Content of Seeds due to *Aspergillus* Species Associated with Different Seeds

Sr. No.	Seeds Infested With	% Seeds of Starch Content			
		Groundnut SB-11	Black Gram (Pant U-30)	Bajra Local	Neem
1	*A. flavus*	5.3	12.3	20.0	9.5
2	*A. niger*	6.2	13.2	22.2	9.4
3	*A. terreus*	6.8	12.4	18.4	11.6
4	*A. glaucus*	6.2	16.2	19.8	12.3
5	*A. sulphuerus*	7.9	18.4	30.2	12.8
6	*A. ornatus*	6.1	10.2	13.4	11.7
7	Control	8.6	21.4	36.2	13.6

Graph 4: Changes in Starch Content of Seeds due to *Aspergillus* Species Associated with Different Plant Seeds



Starch degradation ability was found to be variable among the species of *Aspergillus*. It was more in case of *A. flavus, A. niger* and *A. ornatus* and extremely poor in *A. sulphureus.* Among six species of *Aspergillus* studied, *A. flavus* has been found an efficient to degrade starch while, *A. sulphureus* proved to be less efficient for the same purpose. A gradual loss of carbohydrate (both soluble and insoluble) and protein was recorded due to storage fungi of maize, groundnut and soybean (Bhattacharya and Raha, 2006).

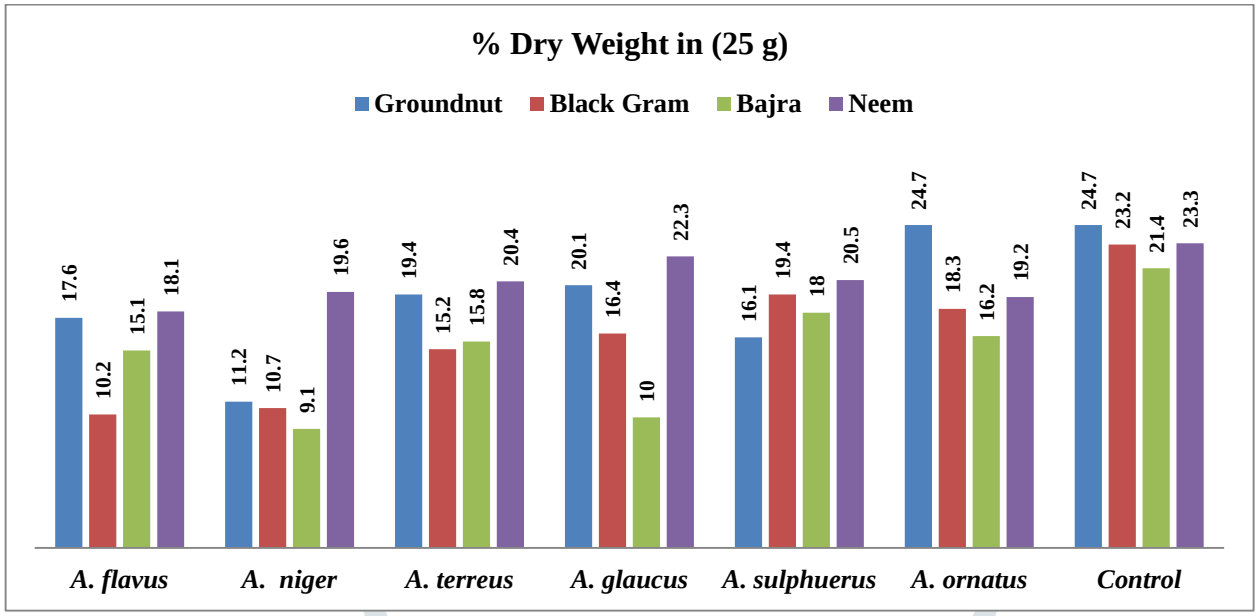
Changes in Dry Weight of Seeds due to *Aspergillus* Species Associated with Different Plant Seeds

Freshly harvested surface sterilized dry seeds of groundnut, black gram, bajra and neem were infested separately with spore suspensions of 6 species of *Aspergillus* and were incubated at room temperature for 10th days, than dry weight of the seeds was recorded. Results are given in table 17 (Plate-XIII).

Table 05: Changes in Dry Weight of Seeds due to *Aspergillus* Species Associated with Different Plant Seeds

Sr. No.	Seeds Infested With	% Dry Weight in (25 gms)			
		Groundnut	Black Gram	Bajra	Neem
1	*flavus*	17.6	10.2	15.1	18.1
2	*niger*	11.2	10.7	9.1	19.6
3	*terreus*	19.4	15.2	15.8	20.4
4	*glaucus*	20.1	16.4	10.0	22.3
5	*sulphuerus*	16.1	19.4	18.0	20.5
6	*ornatus*	24.7	18.3	16.2	19.2
7	Control	24.7	23.2	21.4	23.3

Graph No 05: Changes in Dry Weight of Seeds due to *Aspergillus* Species Associated with Different Plant Seeds



It is evident from the results that all the species of *Aspergillus* showed their ability to degrade seed chemicals resulting it into the loss of seed weight. Similarly, the species vary in their ability to cause loss in seed weight in different crops. As *A. niger* showed maximum loss in seed weight of black gram, bajra and groundnut. This trend was followed by *A. ornatus* and *A. flavus* in case of groundnut. *A. flavus, A. terreus* and A. glaucus for black gram, *A. glaucus, A. terreus* and *A. flavus* for bajra. It was interesting to note that in case of neem loss in seed weight was found to be poor due to all the species of *Aspergillus*.

CONCLUSION:

It was interesting to note that all the species of *Aspergillus* caused increase in ash content of infested seeds as compared to the control.

Results regarding degradation of oil and fat contents of seeds from four crops due to *Aspergilli* are given in table no-1. It is understood from the results that all the species of *Aspergillus* degraded fat contents of seeds significantly. This clearly suggests their lipolytic nature. However, the fat content of neem seeds was found to be reduced poorly by all the species indicating presence of some inhibitory component in neem seeds.

Reports about ability of *Aspergillus* species to utilize seed proteins from different crops have been made by various workers. Bilgrami et al., (1976) found loss in protein content of black gram and green gram due to *A. flavus*. Sinha et al., (1987) observed the same in Arhar seeds due to *A. flavus* and *A. niger*. Similarly, Bilgrami et. al., (1981) in maize seeds due to *A. prasiticus*. Neeti and Karan (1981) in sunflower and sesame due to A. *flavus and A. niger*.

Reports regarding degradation of starch due to different species of *Aspergillus* have been recorded by different workers. Sinha et al., (1981) observed in pigeon pea due to *A. niger and A. flavus*, Prasad and Pathak

(1987) in case of wheat seed due to *A. niger, A. flavus, A. terreus, A. candidus and A. sydowi*. A dominant seed-borne fungi *Aspergillus flavus* caused quantitative and qualitative damage in *Arachis hypogea* by reducing sugar and oil content of the seeds (Naikoo Abbas *et al.,* 2013). Vegetable seeds were affected adversely due to association of seed-borne fungi during storage (Sethumadhava Rao *et al.,* 2014).

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