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RESEARCH ARTICLE

EFFECT OF ASPERGILLUS INFESTATION ON NUTRITIONAL VALUE OF CHICKPEA SEEDS

***Shirurkar Deepavali D.**

Annasaheb Magar Mahavidyalaya, Hadapsar, Pune 411028, MS, India

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ABSTRACT

Chickpea seeds are rich source of proteins and consumed as food and fodder. Chickpea seeds are contaminated with various fungi during storage. Fungi are the widespread pathogen and the rate of seed deterioration is noteworthy for the food industry. Association of mycoflora during storage is a common problem and the most dominant genera were *Aspergillus*. *Aspergillus niger*, *A. flavus, A. quericinus, A. Oryzae, and A. nidulans* are major *Aspergillus* species recovered from selected chickpea varieties. Fungal infestation affects seedling growths as well as reduction in nutritive values was also observed during investigation. During investigation decrease in dry weight, protein, carbohydrate and starch content was recorded.

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INTRODUCTION

The chickpea (*Cicer arietinum*) is one of the most important pulses, commercially grown in tropical, sub-tropical, semi-arid and Mediterranean regions of the world (Kashiwagi*et al*., 2015; Kandhare, 2015). It is an annual legume of the family Fabaceae, also known as gram. India is the world's leading producer of chickpeas (Taylor et al. 2016). It is one of the earliest cultivated crops that are consumed all over the world due to its high nutritional quality (Tripathi*et al*., 2015). It is a source of protein, dietary fiber, resistant starch, polyunsaturated fatty acids, vitamins, and minerals, especially folate, calcium, magnesium, and potassium (Tripathi*et al*., 2015). Infestation of various fungi like *Curvularia lunata, Helminthosporium sativum, Rhizopus nigricans, Alternaria alternata, Fusarium oxysporum, Macrophomina phaseolina, Penicillium italicum, Sclerotium rolfsii*, *Aspergillus flavus*, *A. niger*, *A. oryzae*, *Penicillium italicum, Penicillium notatum* and *Mucor* sp. were reported by many workers from chickpea during storage (Kiran Sing 2005, Kaur *et al*., 2015; Muhammad *et al*., 2015; Leo *et al*., 2015; Kandhare, 2015; Zaidi and Pathak, 2015; Kushwaha, 2017; Arshad, 2019). Seed mycoflora affects seed texture, physiology and content. Seed mycoflora affect adversely to nutritive value of pulses.

***Corresponding author: Shirurkar Deepavali D.,**
Annasaheb Magar Mahavidyalaya, Hadapsar, Pune 411028, MS, India.

Ability of various fungal species to utilize seed carbohydrates from different crops and varieties have been reported by various workers. Adisa (2006) found decrease in carbohydrate content in maize grains due to infestation of *A. clavatus,A. clavatus,* A. *nidulans* and *A. nidulans*. Embaby and Mona (2006) reported biochemical analysis of artificially infected of some legume seeds with mycotoxin produced isolate of *Aspergillus flavus* decreased the percentage of carbohydrate content compared with the healthy of legume seeds like bean, cowpea & lupin. Reduction in the carbohydrate content in seeds of cowpea (*Vigna sinensis)* infested with *Aspergillus* sp. recorded by Ushamalini et.al, (1998) and Morkunas et.al. (2005). Maheshwari and Mathur(1987) reported reduction in the reducing and non-reducing sugars in seeds of cowpea (*Vigna sinensis*) when infested with *Aspergillus nidulans* and *A. terreus* under different temperature and Infection by *A. nidulans* was more deleterious than by *A. terreus. According to* Aziz and Mahrous (2004) *Aspergillus flavus* utilizes carbohydrate of seeds for its growth and aflatoxin production, then decrease lipids and carbohydrate contents of wheat and soybean seeds. Degradation of starch due to seed borne fungi in seeds is a serious fact. Breakdown and utilization of starch from the seed of green gram due to *Aspergillus* species have been reported by Vidyasekaran and Kandaswamy (1972). Similarly, association of *A. niger* and *A. flavus* in cowpea (Vijayakumari and Karan, 1981) caused reduction in starch content to the considerable level. Utilization of starch content of cereal seeds by the species of *Aspergillus* has been studied by Premlata and Sinha (1985) where they found that wheat seeds infested with *A. parasiticus* showed loss in starch content.

Prasad and Pathak (1987) observed the quantitative biochemical change in protein, starch and carbohydrates contents in cereal seeds under different storage condition. Biodegradation of protein content of pulses by common and dominant seed-borne fungi like *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Drechslera tetramera*, *Fusarium moniliforme*, *Rhizopus stolonifer* etc. has been reported by Kandhare, (2016). Sinha and Prasad, (1977) reported decrease in protein content of the arhar and mung seeds due to infestation of fungi like *A. niger, A. flavus* and *Fusarium moniliforme*.

MATERIALS AND METHODS

Collection of Samples: Seed samples of Chickpea varieties Kabuli and Desi were collected from field and market places of Pune; Maharashtra, India. A composite seed sample for each of the variety was made by mixing the individual seed sample together and preserved in cloth bags at room temperature during the studies (Neergaard, 1973).

Preparation of Spore Suspension: The isolated seed-borne fungi of chickpea varieties were identified on the basis of colony character, texture, colour and sporulation with naked eye and microscopically. Identifications were confirmed from Agharkar Research Institute, Pune. Pure cultures of the isolated identified fungi were maintained on potato dextrox agar (PDA) slants. Preparation of spore suspension was carried out from pure cultures slants at room temperature. Spore suspension was prepared from *Aspergillus* species of chickpea varieties from spores taken from the 7-day-old culture by adding 10 ml of saline water into the pure cultures slants. The slants were shaken and content was filtered through muslin cloth to separate mycelium and spore. The obtained filtrate was used as spore suspension.

Artificial infestation of chickpea seeds: Artificial infestation of chickpea seeds was carried out by adopting procedure of Shirurkar and Wahegaonkar (2012).Chickpea seeds of the Desi and Kabuli varieties were surface sterilized with 0.1% mercuric chloride solution for 2 min. and consequently washed with sterile distilled water. Surplus water was poured and 100 g. Chickpea seeds were inoculated separately with 10ml spore suspension of the test fungus in 250 ml flask. Inoculated flasks were incubated at room temperature. Harvesting was done on the 10^{th} day. During harvesting, seeds were thoroughly washed under running tap water in to remove complete mycelial mat from their surface and oven dried seeds were stored in air tight containers. Estimation of protein, carbohydrates, starch was carried out from above oven dried seeds powder.

Estimation of Dry weight: Above artificially infested seeds after harvesting was washed and dried with blotting paper and weighted (fresh weight/ initial weight).The seeds were then oven dried at 60^0C temperature till constant weight obtained (dry weight / final weight). Percent dry weight calculated as follows.

$$\% \text{ Dry weight} = \frac{\text{Dry weight}}{\text{Fresh weight}} \times 100$$

Estimation of Protein, carbohydrates and starch: Protein estimation was done by Lowry's method; estimation of total sugars and starch was carried out by Anthrone method which is given in Sadasivam and Manickam's book (2008) using spectrophotometer.

RESULTS AND DISCUSSION

Occurrence of *Aspergillus* species: Kabuli and Desi varieties were infected with many fungal species, among them *Aspergillus* species were dominant one. *Aspergillus niger*, *A. flavus, A. quericinus, A. oryzae, and A. nidulans* are major *Aspergillus* species recovered from selected chickpea varieties.

Change in dry weight: Dry weight of seeds in artificially infested seeds was reduced in both varieties of chickpea seeds (table 1, figure 1). In Desi variety % reduction in dry weight over control was maximum when seeds are artificially infested with *A. niger* followed *A. oryzae A. fiavusA. quericinus* and *A. nidulans.* In Kabuli variety % reduction in dry weight over control was maximum when seeds are artificially infested with *A. niger* (32.64) followed by *A. oryzae* (21.92), *A. fiavus* (17.13), *A. nidulans* (11.45) and. *A. quericinus* (0.41). Percent reduction in seed dry weight was more in Kabuli variety than Desi variety by artificial infestation of *A. niger* and *A. oryzae*.

Change in total carbohydrate content: Results of carbohydrate content in seeds of artificially infested gram varieties are recorded in table 2, figure 2. From results it is cleared that in Desi variety % reduction of carbohydrate is more when seeds are artificially infested with *A. niger* (20.36), followed by *A. quericinus* (12.21), *A. fiavus* (11.63), *A. nidulans* (11.61) and *A. oryzae* (10.47). In Kabuli variety % reduction of carbohydrates was maximum when seeds are artificially infested with *A. fiavus* (26.20) followed by *A. niger* (17.92), *A. quericinus* (11.30), *A. oryzae* (10.71) and *A. nidulans* (10.12). % reduction of carbohydrate was much more, when seeds are artificially infested with *A. niger*and *A. fiavus.* In *Kabuli* variety % reduction of carbohydrates is more than Desi variety when it is infested *A. fiavus, A. oryzae* while % reduction of carbohydrate is less when seeds are artificially infested with *A. quericinus* and *A. niger.*

Change in starch content: Results of change in starch content presented in table 3, figure 3. Starch content was decreased in both varieties of gram when artificially infested with *Aspergillus* species. In Desi variety % reduction of starch content was maximum when seeds are artificially infested with *A. niger* (32.68) followed by *A. quericinus* (18.30), *A. fiavus* (14.70), *A. nidulans* (14.70) and *A. oryzae* (11.76). In Kabuli variety % reduction of starch content was maximum when seeds are artificially infested with *A. niger* (41.12) followed by *A. fiavus* (22.12), *A. oryzae* (15.87), *A. nidulans* (15.76) and *A. quericinus* (15.58). % reduction of starch content was much more, when seeds are artificially infested with *A. niger* in both varieties of gram. *A. fiavus, A. oryzae* and *A. quericinus* cause moderate reduction of starch content.

Change in protein content: Protein content was decreased (table 4, figure 4) in both varieties of gram when artificially infested with recovered major *Aspergillus* species. In Desi variety % reduction in protein content was maximum when seeds are artificially infested with *A. niger* (17.83) followed by *A. oryzae* (17.68), *A. fiavus* (16.81), *A. nidulans* (10.65) and *A. quericinus* (8.05).

Sr. no.	Seeds infestation	% Reduction Over Control	
		Desi	Kabuli
1	*A. niger*	27.54	32.64
2	*A. oryzae*	19.49	21.92
3	*A. flavus*	17.82	17.13
4	*A. quericinus*	15.52	14.41
5	*A. nidulans*	11.68	11.45

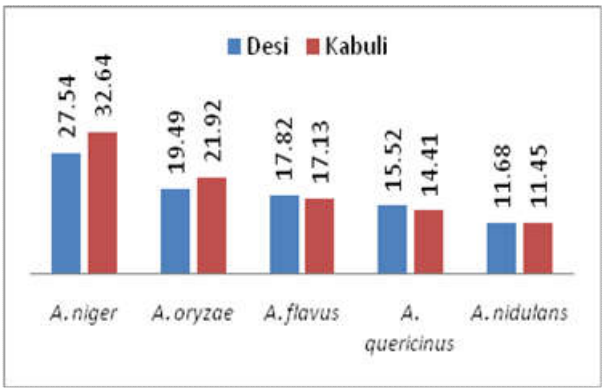

Table 1 and Figure 1. % Reduction in dry weight in artificially infested seeds over control

Sr. no.	Seeds infestation	% Reduction Over Control	
		Desi	Kabuli
1	*A. niger*	20.36	17.92
2	*A. oryzae*	10.47	10.71
3	*A. flavus*	11.63	26.20
4	*A. quericinus*	12.21	11.30
5	*A. nidulans*	11.61	10.12

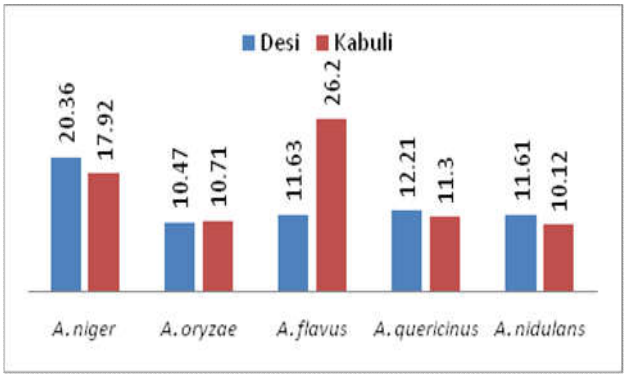

Table 2 and Figure 2. % Reduction in total carbohydrate content in artificially infested seeds over control

Sr. no.	Seeds infestation	% Reduction Over Control	
		Desi	Kabuli
1	*A. niger*	32.68	41.12
2	*A. oryzae*	11.76	15.87
3	*A. flavus*	14.70	22.12
4	*A. quericinus*	18.30	15.58
5	*A. nidulans*	14.70	15.76

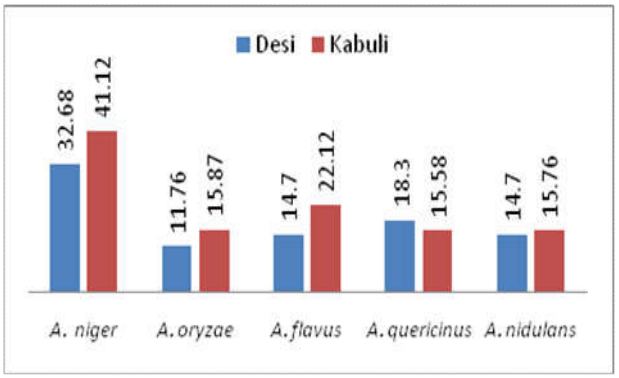

Table 3 and Figure 3.% Reduction in starch content in artificially infested seeds over control

Sr. no.	Seeds infestation	% Reduction Over Control	
		Desi	Kabuli
1	*A. niger*	17.83	28.11
2	*A. oryzae*	17.68	17.68
3	*A. flavus*	16.81	28.46
4	*A. quericinus*	8.05	7.65
5	*A. nidulans*	10.65	7.86

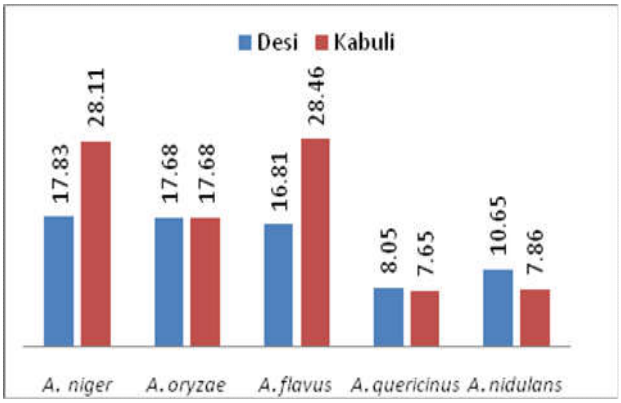

Table 4 and Figure 4. % Reduction in protein content in artificially infested seeds over control

(28.46), followed by *A. niger* (28.11), *A. oryzae* (17.68), *A. nidulans* (7.86) and *A. quericinus* (7.65). % reduction of protein was maximum in Kabuli variety of gram when seed are artificially infested with *A. fiavus* (28.46) and *A. niger* (28.11) than Desi variety. All the species of fungi which are artificially infested on gram are found to be capable of utilizing seed proteins but the rate of utilization was variable among the species. This indicates the ability of fungal species for their proteolytic nature which may also be variable for different varieties of seeds. This also gives a clue regarding different nature of proteins in different varieties of gram seeds.

Conclusion

Gram seeds were affected undesirably due to infestation of various *Aspergillus* species. A dominant seed-borne fungi *Aspergillus* species caused quantitative and qualitative damage in gram seeds by reducing dry weight, total sugars, starch and protein content of the seeds. It is very essential to protect seeds with natural biochemical sand avoid nutritive losses during storage.

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