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# Influence of *Aspergillus* Spp. on Biochemical Characters of Groundnut Seed

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**ABSTRACT**

Influence of major seed borne fungi *Aspergillus niger* and *A. flavus* on biochemical characters of 13 different genotypes of groundnut seed was investigated. Both *A. niger* and *A. flavus* caused decrease in seed protein, total soluble sugar and oil contents and increase in total phenol content irrespective of the genotype. *A. niger* caused 8.82, 21.79 and 1.65 per cent decrease in protein, total soluble sugar and oil contents whereas *A. flavus* caused 7.10, 12.08 and 1.33 per cent reduction in protein, total sugar and oil contents respectively. The per cent increase in phenol content was 13.22 and 11.69 in *A. niger* and *A. flavus* inoculated seed, respectively. Between the two fungi, *A. niger* resulted in maximum changes in seed quality and biochemical characters over uninoculated control.

**Keywords:** *Aspergillus, Groundnut, Oil content, Phenol content, Seed protein and Soluble sugars.*

Groundnut is the world's fourth most important source of edible oil and the third important source of vegetable protein. It is the 13th most important food crop of the world. Groundnut kernel is also called as "poor man's almond" because of its rich nutritive value, high oil (48%) and protein content (26%) along with vitamins. Kernels are used as confectionery item all over the country and after extraction of oil, groundnut kernel cake obtained forms a protein rich meal for livestock and poultry. Approximately every 100 g of groundnut contains 25.8 g proteins, 49.24 g oil and supplies 567 K cal energy (https://ndb.nal.usda.gov/ndb).

Seeds get deteriorated both qualitatively and quantitatively in the field as well as under poor storage conditions as they interact with several microbes (Christensen and Kaufman, 1969). Fungi grown on stored grains reduce carbohydrate, protein, total oil content and increase moisture content (Bhattacharya, 2002). Such grains are unfit for human consumption and are also rejected at the industrial level. In groundnut *A. flavus* and *A. niger* are the dominant storage fungi and play a major role in seed deterioration. *A. flavus* is reported to cause spoilage of stored groundnuts and reduction of the quality and quantity of oil produced (EL-Wakil *et al.*, 2001). In the present study, influence of these fungi on crude protein, total soluble sugar and total oil contents of different genotypes of groundnut was observed.

## MATERIAL AND METHODS

Apparently healthy seed of groundnut cultivars *viz*., Abhaya, Amaravati, Chitravati, Dharani, Haritandhra, ICGV 00 350, Kadiri 6, Kadiri 9, Narayani, TAG 24, TCGS 1073, TCGS 1616 and TCGS 1694 were surface sterilized and soaked in conidial suspensions of *A. niger* and *A. flavus* containing $1\times10^6$ CFU for 20 min and dried at room temperature overnight. The seeds of control treatments were similarly treated except that they were

soaked in sterile distilled water. These seeds were kept aside for two weeks and biochemical changes were recorded on 15th day after seed inoculation.

Crude protein was calculated by estimating nitrogen content in the samples with the help of Microkjeldahl technique (AOAC, 1970). The amount of N content was multiplied by the factor 6.25 to get the crude protein content of the samples.

Total soluble sugar content in groundnut samples was estimated as suggested by Dubois *et a1*. (1956). Glucose was dehydrated to hydroxymethyl furfural in hot acidic medium which formed a golden yellow coloured complex with phenol and had absorption maximum at 490 nm. The sugar content was calculated from a standard graph prepared by using glucose solution as a standard in the range of 20-100 µg. The total soluble sugar content was expressed as mg per gram of oven dried sample.

Phenols from groundnut seed were estimated using folin-ciocalteau reagent (FCR) method suggested by Swain and Hillis (1959). To estimate phenol content a standard graph was prepared using catechol in the range of 20-100 µg. The total phenol content was expressed as mg per gram of oven dried sample.

Total oil content was estimated non-destructively through NIRS technique (Near Infrared Reflectance Spectroscopy) by using Foss Grain Analyzer. Total oil content was expressed in percentage.

## RESULTS AND DISCUSSION

### Crude protein content

The protein content in different genotypes ranged between 22.18% and 27.13% in seed inoculated with *A. flavus* and 21.89% and 27.73% in *A. niger* inoculated seed while in control seed it was between 25.00% and 29.48% (Table 1). Seed protein content ranged from 27.46% (control) to 25.55% (*A. flavus*).

The reduction in protein content was more pronounced in seeds inoculated with *A. niger* than those inoculated with *A. flavus*. The maximum per cent reduction in protein content due to *A. niger* was recorded in Amaravati (14.29%) followed by ICGV 00 350 (12.24%) and due to *A. flavus* it was Amaravati (13.19%) followed by Dharani (10.59%). (Fig 1). *A. flavus* associated decrease in protein content in groundnut was reported by Deshpande and Pancholy (1979) and Advier and Kumar (2015) while Chavan (2011) observed *A. terreus* to cause maximum loss in protein content. Bindu and Kumar (2003) recorded reduction in the total protein and starch content in groundnut due to *A. flavus* infection. This indicates that seed protein serves as a primary source of readily available carbon and nitrogen for growth and metabolism of the invading fungi (Robinson *et al*., 1974).

### Total soluble sugar content

The soluble sugar content in seeds of different genotypes varied between 105.88 mg/g and 106.08 mg/g when inoculated with *A. flavus* and between 91.45 mg/g and 97.98 mg/g with *A. niger* inoculation as against 115.05 mg/g and 126.70 mg/g in control with suspection species. The average soluble sugar content in seeds decreased from 120.56 mg/g in control to 105.99 mg/g and 94.29 mg/g in seeds inoculated with *A. flavus* and *A. niger,* respectively (Table 1) and it was more pronounced in seeds inoculated with *A. niger* than those inoculated with *A. flavus* (Fig 2) with maximum reduction in Dharani (26.12%) followed by Amaravati (25.31%).

Chavan (2011) reported that *A. versicolor* resulted in maximum reduction of reducing sugar in groundnut and soybean. Advier and Kumar (2015) noticed reduction in total sugar, reducing sugar and

**Table 1. Effect of *Aspergillus* spp. on crude protein and total soluble sugar contents of groundnut genotypes**

| Genotype | Protein content (%) | | | | Total soluble sugar content (mg/g) | | | |
|---|---|---|---|---|---|---|---|---|
| | *Aspergillus niger* | *Aspergillus flavus* | Control | Mean | *Aspergillus niger* | *Aspergillus flavus* | Control | Mean |
| Abhaya | 24.23 | 24.23 | 26.56 | $25.00^{c}$ | 93.85 | 105.88 | 123.00 | $107.58^{a}$ |
| Amaravati | 22.77 | 23.06 | 26.56 | $24.13^{b}$ | 92.88 | 106.00 | 124.35 | $107.74^{a}$ |
| Chitravati | 23.06 | 23.64 | 25.69 | $24.13^{b}$ | 93.00 | 105.98 | 124.30 | $107.76^{a}$ |
| Dharani | 21.89 | 22.18 | 25.00 | $23.03^{a}$ | 93.60 | 105.90 | 126.70 | $108.74^{a}$ |
| Haritandhra | 25.98 | 26.56 | 28.31 | $26.95^{ef}$ | 91.45 | 105.95 | 121.25 | $106.22^{a}$ |
| ICGV 00 350 | 25.10 | 25.69 | 28.60 | $26.46^{d}$ | 92.05 | 105.98 | 121.83 | $106.62^{a}$ |
| Kadiri 6 | 27.73 | 27.73 | 29.48 | $28.31^{i}$ | 94.55 | 106.00 | 115.05 | $105.20^{a}$ |
| Kadiri 9 | 26.85 | 27.44 | 28.90 | $27.73^{h}$ | 96.70 | 106.08 | 115.68 | $106.15^{a}$ |
| Narayani | 26.27 | 27.14 | 28.41 | $27.27^{fg}$ | 94.93 | 106.05 | 118.53 | $106.50^{a}$ |
| TAG 24 | 25.98 | 26.56 | 27.73 | $26.76^{de}$ | 97.98 | 105.95 | 117.28 | $107.07^{a}$ |
| TCGS 1073 | 26.85 | 27.14 | 27.83 | $27.27^{fg}$ | 95.43 | 106.03 | 119.03 | $106.83^{a}$ |
| TCGS 1616 | 22.77 | 23.64 | 25.20 | $23.87^{b}$ | 94.18 | 106.08 | 118.35 | $106.20^{a}$ |
| TCGS 1694 | 26.56 | 27.14 | 28.70 | $27.47^{gh}$ | 95.15 | 106.00 | 121.93 | $107.69^{a}$ |
| Mean | $25.08^{A}$ | $25.55^{B}$ | $27.46^{C}$ | | $94.29^{A}$ | $105.99^{B}$ | $120.56^{C}$ | |
| | G | T | | GxT | G | T | | GxT |
| SEm | 0.143 | 0.0685 | | 0.247 | 1.454 | 0.6985 | | 2.519 |
| CD (p=0.05) | 0.359 | 0.2827 | | 0.597 | 3.669 | 2.8845 | | 6.094 |
| CV (%) | 1.642 | | | | 4.079 | | | |

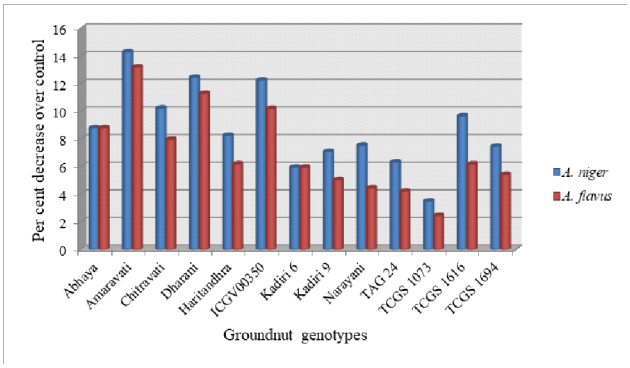


**Fig 1. Effect of *Aspergillus* spp on crude protein content in different genotypes of groundnut**

non-reducing sugar in *Aspergillus* inoculated groundnut seed. The reason for reduction in sugar might be due to utilization of sugar by fungi (Jamaluddin *et al.*, 1987).

**Total phenol content**

Significant increase in total phenol content was noticed in seeds of different genotypes inoculated with both *A. niger* and *A. flavus*. The total phenol content in different genotypes varied between 69.27 and 206.48 mg/g in seeds inoculated with *A. niger* and between 67.65 and 204.00 mg/g in *A. flavus* inoculated seeds as against 61.17 and 203.99 mg/g in control (Table 2).

The average total phenol content increased from 126.13 mg/g in control to 140.87 mg/g and 142.81 mg/g in seeds inoculated with *A. flavus* and *A. niger*, respectively (Fig. 2). The increase in total phenol content was more pronounced in seeds inoculated with *A. niger* than those inoculated with *A. flavus*. The maximum per cent increase in total

**Table 2. Effect of *Aspergillus* spp. on phenol and oil contents of groundnut genotypes**

| Genotype | Phenol content | | | | Oil content | | | |
|---|---|---|---|---|---|---|---|---|
| | *Aspergillus niger* | *Aspergillus flavus* | Control | Mean | *Aspergillus niger* | *Aspergillus flavus* | Control | Mean |
| Abhaya | 138.09 | 138.09 | 124.16 | $133.45^{f}$ | 47.3 | 47.5 | 48.3 | $47.70^{de}$ |
| Amaravati | 69.27 | 67.65 | 61.17 | $66.03^{a}$ | 46 | 46.2 | 47 | $46.40^{a}$ |
| Chitravati | 119.19 | 116.27 | 108.49 | $114.65^{d}$ | 46.4 | 46.5 | 47.2 | $46.70^{b}$ |
| Dharani | 95.74 | 93.15 | 86.99 | $91.96^{b}$ | 47.9 | 48.2 | 49.1 | $48.40^{h}$ |
| Haritandhra | 127.4 | 124.81 | 114.11 | $122.10^{e}$ | 47 | 47 | 47.7 | $47.23^{c}$ |
| ICGV 00 350 | 104.06 | 102.23 | 98.55 | $101.61^{c}$ | 46.1 | 46.6 | 47.4 | $46.70^{b}$ |
| Kadiri 6 | 167.8 | 165.54 | 146.52 | $159.95^{h}$ | 47.4 | 47.6 | 47.9 | $47.63^{d}$ |
| Kadiri 9 | 183.9 | 183.04 | 161.75 | $176.23^{i}$ | 46.6 | 46.6 | 47.1 | $46.77^{b}$ |
| Narayani | 158.51 | 157.43 | 141.77 | $152.57^{g}$ | 47.5 | 47.5 | 48.1 | $47.70^{de}$ |
| TAG 24 | 206.48 | 204 | 173.1 | $194.53^{j}$ | 47.9 | 48 | 48.4 | $48.10^{g}$ |
| TCGS 1073 | 185.74 | 184.33 | 160.03 | $176.70^{i}$ | 47.5 | 47.7 | 48 | $47.73^{def}$ |
| TCGS 1616 | 138.85 | 136.8 | 122.86 | $132.84^{f}$ | 47.5 | 47.6 | 48.4 | $47.83^{ef}$ |
| TCGS 1694 | 161.43 | 157.97 | 140.15 | $153.18^{g}$ | 47.6 | 47.7 | 48.4 | $47.90^{f}$ |
| Mean | $142.81^{C}$ | $140.87^{B}$ | $126.13^{A}$ | | $47.13^{A}$ | $47.28^{B}$ | $47.92^{C}$ | |
| | G | T | | GxT | G | T | | GxT |
| SEm | 0.9304 | 0.4469 | | 1.6115 | 0.0604 | 0.02901 | | 0.10459 |
| CD (p=0.05) | 2.3451 | 1.8456 | | 3.8991 | 0.1522 | 0.11979 | | 0.25308 |
| CV (%) | 2.043 | | | | 0.382 | | | |

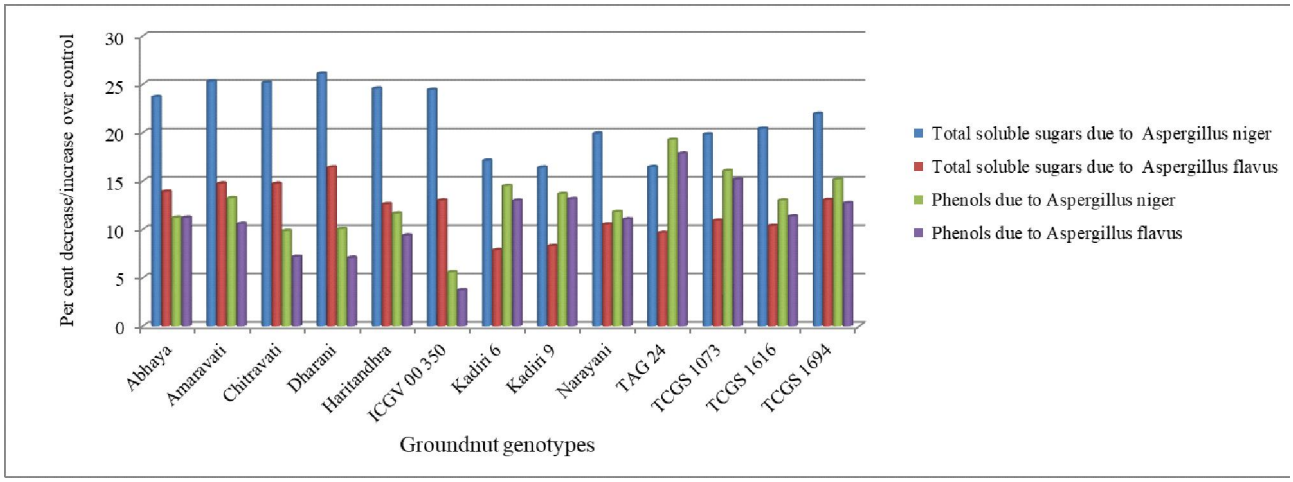


**Fig 2. Effect of *Aspergillus* spp on total soluble sugars and phenol content in different genotypes of groundnut**

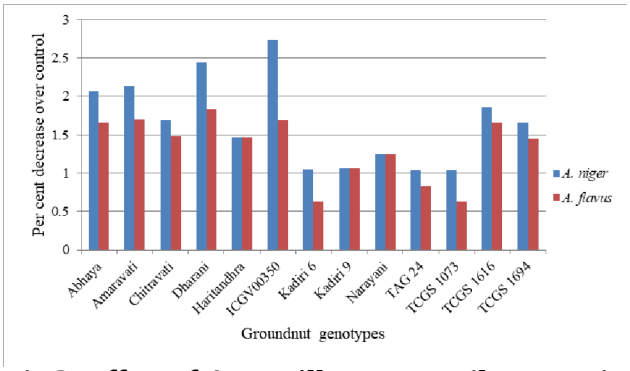


**Fig 3. Effect of *Aspergillus* spp on oil content in different genotypes of groundnut**

phenol content due to *A. niger* was recorded in TAG 24 (16.17) followed by TCGS 1073 (13.84). Similar results were observed in seeds inoculated with *A. flavus*. Minimum per cent increase was observed in ICGV 00 350 in seeds inoculated with *A. niger* and *A. flavus*.

Fajardo (1994) showed that peanut seeds infected by *A. flavus* could accumulate more phenolic compounds. Tanuja *et al.* (2012) also observed increase in phenol content after inoculation with *Aspergillus paraciticus*. The differences in induction of phenols may be specific to a particular variety (Shinde *et al*., 2016).

**Total oil content**

The oil content in different genotypes ranged between 46.2% and 48.2% in seeds inoculated with *A. flavus* and between 46% and 47.9% in seeds inoculated with *A. niger* as against 47% and 49.1% in control (Table 2). The average oil content decreased from 47.92% to 47.28% and 47.13% in seeds inoculated with *A. flavus* and *A. niger*, respectively. The reduction in oil content was more pronounced in seeds inoculated with *A. niger* than those inoculated with *A. flavus*.

The maximum per cent reduction in oil content due to *A. niger* was recorded in ICGV 00 350 (2.74) followed by Dharani (2.44) while maximum per cent reduction in oil content due to *A. flavus* was recorded in Dharani (1.83) followed by 1.70 in Amaravati (Fig 3). Negedu *et al.* (2014) observed decrease in oil content in castor due to *A. tamarii* while Chavan (2011), and Advier and Kumar (2015) made similar observations in groundnut due to *Aspergillus* spp. Nakrani and Patel (2018) observed minimum reduction of oil (4.94%) and also protein (2.92%) in the variety J-11. Invading fungus causes the oxidation of fatty acids and inactivation of enzymes and thus causes reduction in oil content.

## CONCLUSION

Maximum reduction in protein content was recorded in Amaravati in both *A. niger* and *A. flavus* inoculated seed. Maximum per cent reduction in soluble sugar content due to *A. niger* was recorded in Dharani followed by Amaravati in both *A. niger* and *A. flavus* inoculated seed. Maximum per cent reduction in oil content due to *A. niger* was recorded in ICGV 00 350 and in Dharani, due to *A. flavus*. These changes lower the nutritive as well as economic value of the seed warranting the prevention of storage fungi for preservation of seed quality by appropriate means.

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