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Full Length Article

Effect of common and dominant seed-borne fungi on protein content of Pulses

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ABSTRACT

Seed-borne fungi affect adversely to nutritive value of pulses. Biodegradation of protein content of pulses by their common and dominant seed-borne fungi like *Aspergillusflavus*, *A.fumigatus*, *A. niger, Drechslera tetramera*, *Fusarium moniliforme*, *Rhizopus stolonifer* etc. has been reported through artificial infestation of the pulses like Green gram, Black gram, Chickpea and Pigeon pea. Results reveal considerable degradation in protein content of the test pulses. Green gram (120mg/g seed), Black gram (100mg/ g seed), Chick pea (104 mg/ g seed) and Pigeon pea (125 mg/ g seed).

Key Words: pulses, seed-borne fungi, seed content, Green gram, Black gram, Chick pea, Pigeon pea.

INTRODUCTION

Pulses are rich in protein, essential amino acids, and micronutrients. About 88% proteins consumed in India are of vegetable origin, dependence on animal proteins being very less. Green gram, Black gram, Chickpea, andpigeon pea are rich in protein content i.e. 24g/100g, 24g/100g, 20.5g/100g, and 20.4g/100g of seeds respectively (Shakuntala Manay and Shadaksharaswamy, 1987).

During study six fungi *Aspergillus flavus, A. fumigatus, A. niger, Drechslera tetramera, Fusarium moniliforme* and *Rhizopus stolonifer* were found to be common and dominant on four test pulses. Biligrami et.al (1976) reported that, seed of Green gram, Black gram were degraded due to *Aspergillus flavus* and *Fusarium semitectum*. Nager and Chauhan (1977) reported loss in protein content due to seed-borne fungi of groundnut (Nager and Chauhan, 1977). *Aspergillus niger*, *A. flavus*, *Fusarium moniliforme* caused decrease in protein content of the Arhar seeds (Sinha and Prasad, 1977). Shingare and Ade (2014) reported significant protein loss from groundnut kernels due to fungal infection. In *vigna radiata* L. Seed germination and seedling growth was retarded due to seed-borne fungi (Sinha and Prasad, 1981). Similarly, changes in sugar content of mango pulp due to different isolates of *Aspergillus niger* werereported (Gadgil and Chavan, 2009).

MATERIALS AND METHODS:

Collection of seed samples:

Seed samples of Green gram, Black gram, Chickpea, and Pigeon pea were collected from field, market places from Nanded by following standard methods of sampling (Neergaard Paul, 1977). A composite seed sample for each of the pulse crop was made by mixing the individual seed sample together and preserved in gunny bags at room temperature during the studies.

Preparation of spore suspension:

Spore suspension of common and dominant seed-borne fungi of pulses were prepared separately by adding 10 ml of sterile distilled water into the sporulating pure cultures of seed-borne fungi of pulses; 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.

Estimation of protein:

Protein estimation was done by Lowry's method (Wadje and Baig, 2006), using spectrophotometer. Standard calibration graph was prepared and based onit; protein content of each test pulse was estimated.

Reagents used:

i) 2% sodium carbonate (Na_2CO_3) in 0.1N NaOH (100 ml distilled water+ 0.4g NaOH + 2g sodium carbonate) (Reagent-a).

ii) 0.5% copper sulphate ($CuSO_4.5H_2O$) in 1% sodium potassium tartarate (100 ml distilled water + 1g sodium potassium tartarate + 0.5g $CuSO_4$) (Reagent-b).

iii) Alkaline copper solution: 50 ml of reagent-a + 1ml of reagent-b (freshly prepared)

iv) Folin- Ciocalteau reagent (Phenol reagent): The commercially available reagent was diluted with equal
volume of water at the time of use.

v) Standard protein solution (stock solution): 20 mg gelatin dissolved in 20 ml distilled water. The content transferred to 100 ml volumetric flask and volume made to 100 ml

Measurement of optical density (OD) and preparation of standard calibration graph:

Five sterilized borosil glass test tubes were serially arranged on test tube stand with sequential number labels on it. The protein stock solution was pipetted out with graduated pipette as 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1.0 ml added to the test tubes sequentially from 1st test tube to 5th test tube respectively. The volume of each of the test tube was made to 1 ml with distilled water. A tube with 1 ml of distilled water and with zero ml of protein solution served as blank. 5 ml of alkaline copper solution was added to all the test tubes; including blank. The test tubes were shaken thoroughly and allowed to stand for ten minutes at room temperature. 0.5 ml of diluted Folin-Ciocalteau reagent was added rapidly with immediate mixing; it resulted into production of blue colour in 30 minutes.

For OD measurement, spectrophotometer (Systronic Type-106) was set to zero with the help of blank solution at 660 nm. OD of each test solution was recorded separately one by one and standard graph was prepared by plotting OD versus protein quantity (mg).

Extraction and estimation of protein content of seeds of pulses:

One gram of seed of each four test pulse Green gram, Black gram, Chickpea and Pigeon pea were ground into fine flour with mortar and pestle separately. The powder of each pulse was extracted separately with an aliquot of ethanol and petroleum ether (2:1 v/v) mixture with constant stirring for few minutes. The sediments thus obtained were washed again with ether. The solvents of each seeds of pulses were filtered in conical flasks separately. The residue was collected and filtrates; containing lipids, carbohydrates and other non-proteinaceous compound was discarded. The collected residues of each seeds of pulses was dried at room temperature to remove all traces of ether and dissolved in 10 ml of 20% Trichloro acetic acid (TCA). The resultant mixture was kept at 4^{o}C for four hours. After cold treatment, the mixtures were subjected to centrifugation at 8000 rpm for ten minutes (Remi-R- 24). The precipitates obtained after centrifugation was dissolved separately in 5 ml of 0.1 N NaOH and made up to 50 ml with distilled water. These were treated as protein extracts of each of the test pulses. 0.1 ml and 0.2 ml protein extracts thus obtained were taken in different test tubes and similar procedure was followed as used earlier for standard proteins. Protein content of each test pulse was deduced from the standard graph of protein (Wadje and Baig, 2006).

RESULTS AND DISCUSSION:

The results in the Table show that, all common and dominant seed-borne fungi of pulses caused reduction in protein content of all test pulses in more or less quantity. In all test pulses, *Fusarium moniliforme* caused maximum reduction in protein content followed by *Aspergillus niger, A. flavus*. Minimum loss in protein was reported in Green gram, Chickpea, and Pigeon pea by *Rhizopus stolonifer*, in Black gram by *Aspergillus fumigatus* and *Drechslera tetramera*. This reduction in protein content of test pulses by the seed-borne fungi proves their proteolysis efficacy.

Srivastavaand Sweta (2013) reported maximum protein content of *Jatropha crucas* L. was reduced by the seed-borne fungi *Fusarium chlamydosporum*. Seed-borne fungi *Aspergillus flavus*, *A. niger*, *Macrophomina phaseolina*, *Fusarium oxysporum* caused reduction in carbohydrates and proteins (Ushamalini, 1998).

Table: Effect of common and dominant seed-borne fungi on total protein content of pulses. (After ten days of incubation).

Sr. No.	Infestation by common and dominant seed-borne fungi of pulses	Total protein content of seeds (mg/ g of seeds)			
		Green gram	Black gram	Chick Pea	Pigeon pea
1	*Aspergillus flavus*	121	120	107	136
2	*Aspergillus fumigatus*	130	136	158	159
3	*Aspergillus niger*	124	123	146	153
4	*Drechslera tetramera*	122	136	153	160
5	*Fusarium moniliforme*	120	100	104	125
6	*Rhizopus stolonifer*	133	131	162	170
7	Control	136	140	168	173

Vegetable seeds were affected adversely due to association of seed-borne fungi during storage (Sethumadhava Rao *et al.,* 2014). 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). 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).

Seed mycoflora of pulses is responsible for deterioration of the seed protein content of test pulses. Protein content degradation naturally makes seed low in protein rendering seed feeble for germination and seedling emergence. Overwhelming use of synthetic chemicals proved harmful to humans and animals ensuing biomagnification. In order to protect seeds from adverse effects of seed mycoflora, natural biochemicals are desirable. Some botanicals could be harnessed as pre treatment of seeds before sowing to protect seeds from protein content loss.

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