International Journal of
Life Sciences
International Peer Reviewed Open Access Refereed Journal

Special Issue A 12: March 2018:181-185
UGC Approved Journal No 48951

Original Article | Open Access

Seed Mycoflora of *Brassica carinata* and Biochemical Changes in Protein, Oil, Starch and Sugars under the Seed Storage

Ghugal SI

S.S.E.S. Amt's, Science College Pauni, Distt.- Bhandara.
Email ID: sanjughugal@rediffmail.com

Manuscript details:

Available online on
http://www.ijlsci.in

ISSN: 2320-964X (Online)
ISSN: 2320-7817 (Print)

Editor: Dr. Arvind Chavhan

Cite this article as:
Ghugal SI (2018) *Seed Mycoflora of Brassica carinata and Biochemical Changes in Protein, Oil, Starch and Sugars under the Seed Storage*, *Int. J. of. Life Sciences*, Special Issue, A12: 181-185.

ABSTRACT

The seeds of *Brassica carinata* were stored and isolated twenty two seed storage fungi in seed samples by agar plate method and blotter method throughout the year on a monthly basis. Major predominant fungi were *Alternaria alternata, Aspergillus flavus, A. candidus, A. fumigatus, A. niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxysprorum, Penicillium chrysogenum* and *P. Oxalicum.* Seed sample evaluated for the crude protein, oil, starch and sugars on monthly basis of one year storage and shows significant decreased subsequently with an increase in storage period. It was observed that the percentage change over control in protein, oil and starch was -31.79 %, -24.09 % and -52.74 % respectively at the end of year. Total sugar, reducing and non-reducing sugars were found to be decreased till 8th months of a year of the storage period but subsequently increased at the end of storage period.

Key words: Storage fungi, Protein, Oil, Starch.

INTRODUCTION

Brassica carinata (or Abyssinian mustard) almost certainly originated in North East Africa. Due to its limited distribution to Ethiopia and neighbouring countries, this *Brassica* oilseed has little exposure to modern plant improvement. It is high oil yielding with light brown seed coat colour. This oilseed crop grows in rabi season and the flowering and maturity period is similar to other oilseed Brassicas. Large number of fungi is known to bring about several biochemical changes in oilseeds and degrade seed constituents (Rai and Saxena, 1980). The seed mycoflora associated with the seeds may be pathogenic, weak parasites or saprophytes and may be external or internal. The present study deals with storage fungi associated with the seeds of *Brassica carinata* and also changes in protein, oil, starch and sugars due to the association of seed fungi.

METHODOLOGY

Brassica carinata seed samples were selected for experimental study and collected from different oilseed *Brassica* growers of North India and also from Department of Botany, RTM Nagpur University. Seed samples were bulked together and selected randomly for further investigations.

Isolation of fungi was done by both blotter as well as agar plate method as recommended by ISTA (1966). The seed sample was stored in small cotton bags under normal room temperature condition for one year. After every month 400 seeds were taken out randomly and isolated the storage seed mycoflora.

To study the effect of fungi on the seed constituents, the seeds were stored in separate cotton bags in a glass desiccator maintained at 96 percent relative humidity, prepared by saturated salt solution of sulphuric acid (Johnston and Booth, 1983). The control seeds (surface sterilized and dried) were kept in separate desiccators at a relative humidity of 96 percent. At an interval of every 30 days, the seed samples were taken out randomly. The fungal mycelium was removed by washing the seeds gently in running tap water and then dried at 60 ^{0}C for 48 hours. Both the infected as well as the control seeds were dried and powdered separately in a grinder which was followed by a quantitative estimation of nitrogen, protein, oil, starch and sugars (reducing, non-reducing and total sugar). Nitrogen percentage and crude protein estimation was calculated by micro-kjeldahl method suggested by Davys and Pirie (1969). To calculate the crude protein percentage, Nitrogen percent per gram was multiplied by the factor 6.25 (Sadasivam and Manikam, 1992). Oil percentage was determined with the help of Oxford 4000 NMR (Nuclear Magnetic Resonance Spectroscopy Analyzer) in a 2 ml assembly at Department of Botany, Nagpur. The mode was introduced, tuned and calibrated with pure oil.

For quantitative determination of sugars and starch, 0.5 – 1.0 g of dried, powdered seed sample was taken in round bottom flask. 125 ml of 50 % ethyl alcohol was added to it and the mixture was refluxed for 4 hours with an air condenser. The contents were kept overnight and centrifuged at 3000 rpm for 10 minutes. The supernatant was evaporated to few ml by heating gently. This extract was diluted to 100 ml with distilled water. From this aqueous extract, 50 ml was taken for reducing sugar and 50 ml was kept for non- reducing sugar estimation. The residue after centrifugation was preserved in deep freezer for starch analysis (Agrawal *et al*. 1992). The amount of reducing sugar was determined by Phenol-Sulfuric Acid suggested by Dubois *et al*, (1956).

RESULTS AND DISCUSSION:

Table 1 represents the data on *Brassica carinata* seeds exhibited the association of 22 fungal organisms of which *Alternaria alternata, Aspergillus flavus, A. candidus, A. fumigatus, A. niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxysprorum, Penicillium chrysogenum* and *P. oxalicum* were recorded throughout the year. *Fusarium chlamydosporum* and *Penicillium multicolor*, which were confined to winter season only. The fungal organisms chiefly confined to summer season were *Aspergillus nidulans, Aspergillus glaucus*, and *Rhizopus nigricans*. *Aspergillus versicolor, Chaetomium bostrychodes, Phytophthora undulata, Penicillium notatum, Penicillium purpurogenum, Pythium sp.* and *Syncephalastrum sp.* were associated sporadically.

Table 1: Incidence of Seed Mycoflora

Occurrence of storage fungi			
Throught out the year	**Summer months**	**Winter months**	**Sporadically**
Alternaria alternate, Aspergillus flavus A. candidus, A. fumigatus, A. niger, Curvularia lunata, Fusarium moniliforme, Fusarium oxysprorum, Penicillium chrysogenum,P. oxalicum	*Aspergillus nidulans Aspergillus glaucus Rhizopus nigricans*	*Fusarium chlamydosporum Penicillium multicolor*	*Aspergillus versicolor, Chaetomium bostrychodes, Phytophthora undulata Penicillium notatum, Penicillium purpurogenum, Pythium sp, Syncephalastrum sp*

Table 2: Changes in Protein and oil contents in *B. carinata* seeds due to fungal association during storage.

Incubation Period	Protein Percentage		% Change in Protein over control	
(Months)	a*	b*		
0	30.54	30.54	0.00	0.00
1	30.02	30.18	-1.70	-1.18
2	30.02	30.18	-1.70	-1.18
3	29.76	29.81	-2.55	-2.39
4	29.50	28.99	-3.41	-5.08
5	28.98	28.65	-5.11	-6.19
6	28.98	25.26	-5.11	-17.29
7	28.72	23.96	-5.96	-21.55
8	28.46	22.40	-6.81	-26.65
9	28.20	21.88	-7.66	-28.36
10	27.94	21.09	-8.51	-30.94
11	27.42	20.83	-10.22	-31.79

a* - control seeds. b* - infested seeds.

Table 3: Changes in starch, total, reducing and non-reducing sugars in *B. carinata* seeds due to fungal association during storage

Period of incubation (Month)	Percent Starch	% Change in starch over control	Total Sugars (TS) (mg/g)	% Change in TS over control	Reducing Sugars (RS) (mg/g)	% Change in RS over control	Non-reducing Sugars(NRS) (mg/g)	% Change in NRS over control
0 a*	58.87	0.00	4.08	0.00	2.40	0.00	1.69	0.00
b*	58.87	0.00	4.08	0.00	2.40	0.00	1.69	0.00
1	58.87	0.00	4.08	0.00	2.40	0.00	1.69	0.00
	57.08	-3.03	3.76	-8.01	2.37	-1.25	1.39	-17.61
2	58.87	0.00	3.80	-6.93	2.39	-0.42	1.41	-16.18
	55.81	-5.19	3.46	-15.26	2.36	-1.50	1.10	-34.80
3	58.81	-0.10	3.73	-8.74	2.38	-0.67	1.35	-20.21
	55.35	-5.97	2.87	-29.64	2.35	-1.92	0.52	-69.00
4	58.79	-0.14	3.70	-9.38	2.33	-2.63	1.37	-18.97
	55.30	-6.07	2.83	-30.13	2.28	-4.84	0.57	-66.03
5	58.71	-0.27	3.81	-6.78	2.33	-2.92	1.48	-12.27
	39.40	-33.07	2.91	-28.66	2.04	-14.86	0.87	-48.25
6	58.69	-0.30	3.83	-6.29	2.27	-5.13	1.55	-7.94
	34.50	-41.39	2.99	-26.87	1.66	-30.59	1.32	-21.58
7	58.67	-0.34	4.16	-1.89	2.25	-6.26	1.91	13.46
	33.64	-42.86	3.05	-25.23	1.53	-36.02	1.52	-9.90
8	58.62	-0.42	4.17	2.03	2.33	-2.63	1.83	8.65
	33.35	-43.35	3.34	-18.20	1.80	-24.88	1.54	-8.71
9	58.59	-0.47	4.20	2.87	2.35	-1.80	1.85	9.48
	33.41	-43.25	3.83	-6.29	1.97	-17.65	1.85	9.84
10	58.55	-0.54	4.56	11.68	2.41	0.42	2.15	27.68
	33.47	-43.15	4.14	1.40	2.21	-7.93	1.93	14.64
11	58.48	-0.67	4.63	13.30	2.45	2.38	2.17	28.81
	27.82	-52.74	4.76	16.65	2.52	5.30	2.24	32.78

a* - control seeds. b* - infested seeds.

Table 2 shows the protein content in control and infested seeds. In the first month of storage period, it was recorded as 30.54%. Then, it gradually declined till the end of storage period. In the storage period of 1 to 11 month the protein contents were 30.18, 30.18, 29.81, 28.99, 28.65, 25.26, 23.96, 22.40, 21.88, 21.09 and 20.83% respectively as compared to 30.54% in control. At the end of storage period, in surface sterilized seeds, percent change in protein over control was -10.22% and in case of infested seeds it was -31.79%.

The oil content was 33.04% oil at the beginning of storage and declined its oil content regularly for one-year storage. At the end it showed 25.08% oil content with -24.09% change over control and that was -2.12% in surface sterilized seeds.

Table 3 indicates the quantitative changes in starch, total sugars, reducing and non-reducing sugars occurring during one year of storage period. Seeds (control and infested) were assayed on 0 day showed 58.87% starch found at start of storage. After interval of every month there was depletion in starch rapidly in infested seeds and reduced to 27.82% with 52.74% decrease over control. The same case happened with surface sterilized seed but the depletion was very small found to be 0.67% decreases over control at the end of storage.

Total sugar observed at 0 month of storage was 4.08 mg/g reduced rapidly up to 4 month of storage i.e. 2.83 mg/g with 30.13% decrease over control in infested seed. However, the reduction in total sugar observed till 9th month with 6.29% decrease over control and then it increased during 10th and 11th month. At the end of storage period, it was 4.76 mg/g with 16.65 % increase over control in infested seed. In surface sterilized seed there was increase in total sugar at the end of storage, found to be 4.63 mg/g.

In the beginning of the storage, the reducing sugar was observed 2.40 mg/g. Rapid depletion was observed till 7th month, however, the reduction in reducing sugar in infested seed was observed up to 11th month of storage i.e. 2.21 mg/g with 7.93% decrease over control. At the end of storage there was increase in reducing sugar in infested seed was found to be 2.52 mg/g with 5.30% increase over control. In surface sterilized seed the increase was observed at the end of storage i.e. 2.45 mg/g (+2.38% change over control).

1.69 mg/g non-reducing sugar was found at the start of storage, it shows depletion up to 8th month of storage in infested seed, after it shows rapid increase till the end of one year storage, found to be 2.24 mg/g with 32.78% increase over control in infested seed. In surface sterilized seeds the increase was +28.81% over control.

The nitrogen requirement for the growth of fungi comes from nitrates, ammonium and organic sources especially the amino acids. Proteins are the combination products of enzymes and amino acids. The nitrogen sources are good for growth and reproduction also. Nitrate nitrogen that is generally good for sporulation in several fungi (Bilgrami and Verma, 1978). Lalithakumari *et al* (1971) and Yadav et al (2014) reported that *Aspergillus flavus* reduced oil content of the groundnut remarkably. Similar were the findings of Rai and Saxena (1980) who reported that *Aspergillus flavus* was more effective in reducing oil content of Indian mustard. Agarwal (1965) and Prasad and Singh (1983) observed decrease in oil content due to fungi at higher relative humidity. Utilization and reduction of starch content by *Aspergillus parasiticus* shown by Singh and Sinha (1985) in arhar seeds while Prasad et al (1987) studied the quantitative biochemical change (protein, starch and carbohydrates) in cereal seeds under different storage condition. Singh *et al* (1973), showed reduction in starch content in sunflower seeds and infected groundnut seeds. In the present study, reduction in the total sugar content was observed in initial phase of storage and increase at end the of storage period. This may be due to its preferential utilization by some of the fungal species infesting the seeds. Preferential utilization of sugars by fungi also been reported by Cochrane (1958), Gupta and Gupta (1984), Bilgrami *et al* (1979), Bilgrami and Verma (1978). Similar results were obtained in case of reducing and non-reducing sugar.

REFERENCES

Agrawal PK, Dadlani M and Shrawa I (1992) Techniques in Seed Science and Technology.' Ed. S. Agrawal P. K. and M. Dadlani, South Asian Publisher, New Delhi. 207.

Bilgrami KS and Verma RN (1978) Physiology of fungi. Vikas Publishing House Pvt. Ltd. Delhi. 505.

Bilgrami KS, Prasad T and Sinha RK (1979) 'International Biosciences Monograph – 9. Changes in nutritional components of stored seeds due to fungal association.' Today and tomorrow's Printers and Pub. New Delhi. pp. 82.

Cochrane VW (1958) Physiology of fungi. John Wiley and Sons. Inc., New York.

Davys MNG and Pirie NW (1969) International Biological Programme (IBP). *Biotech. Bioengr*. 11: 528.

Dubois M, Gills KA, Hamilton JK, Robers PA and Smith E (1956) 'Methods in Microbiology' (eds). J. K. Norris and D.W. Ribbons. Acad. Press London, N. Y. 272.

Gupta PK and Gupta JS (1984). Storage deterioration of mung bean seeds by fungi. *Ind. J. Trop. Pl. Dis*. 2(2): 169-173.

Johnston A and Booth C (Ed.S) (1983). 'Plant Pathologists Pocket Books.' The Cambrian New Ltd. Aberystwyth, Wales. P.

Lalithakumari D, Govidaswamy CV and Vidhyasekaran P (1971) Effect of seed borne fungi on the physiochemical properties of groundnut oil. *Indian Phytopath*. 24: 283-289.

Prasad T and Singh BK (1983) Effect of relative humidity on oil properties of fungal infected sunflower seed. *Boil. Bull. India*, 5: 85-88.

Prasad BK, Prasad A, Shankar U and Kumar S (1987) Level of changes in carbohydrate in lablab bean FD 5 cv. Seed due to seed borne *Aspergillus niger. Indian J. Mycol. Pl. Pathol*. 17(2): 321-324.

Rai JN and Saxena A (1980) Effect of seed-borne fungi on the physio-chemical properties of the oil of Indian mustard. *Indian J. Agric. Sci*. 50: 769-772.

Sadasivam S and Manikam A (1992) 'Biochemical Method for Agricultural Science.' Wiley Eastern Ltd. New Delhi. 246.

Singh BP, Shukla BN and Sharma YK (1973) Changes in oil, protein and carbohydrate content of diseased safflower seeds. *P. K. V. Res. J.* 2(1): 72.

Singh Premlata and Sinha KK (1985) *Indian Phytopathol*. 38(3): 560-561.

Yadav VB, Bharud RW and DR Nagwade (2014) Biochemical changes associated with storage of summer groundnut (*Arachis hypogaea* L.) seeds. *Journal of Crop Science* ISSN: 0976-8920 & E-ISSN: 0976-8939, 5(1):112-115.