



STORAGE FUNGI ASSOCIATED WITH SEEDS OF *BRASSICA CARINATA* AND CHANGES IN PROTEIN, OIL, STARCH AND SUGARS UNDER THE SEED STORAGE

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Abstract:

Twenty two seed storage fungi were isolated in *Brassica carinata* 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 oxysporum*, *Penicillium chrysogenum* and *P. Oxalicum*.

The crude protein, oil and starch decreased 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. 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.

Material and Methods:

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 the percentage incidence of fungi was recorded. Total percentage of fungal incidence was calculated by using the formula suggested by Sahai and Mahrotra (1982).

$$\% \text{ Frequency} = \frac{\text{Total no. of seeds in which particular fungus appeared}}{\text{Total no. of seeds studied}} \times 100$$

To study the effect of fungi on the seed constituents, the seeds were stored in separate

cotton bags in a glass desiccators 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 °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 oxysporum*, *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.

From Jan.99 to Dec. 99 total of 10,10,11,10,9,13,16,17,16,10,10 and 9 fungi were detected respectively and their total frequency of incidence was 307.79, 374.11, 325.67, 194.02, 329.91, 370.54, 453.88, 416.73, 380.62, 429.37, 315.92 and 224.68 percent respectively. In the month of April the total frequency of incidence was least as compared to the full storage period. **[Fig. 1]**

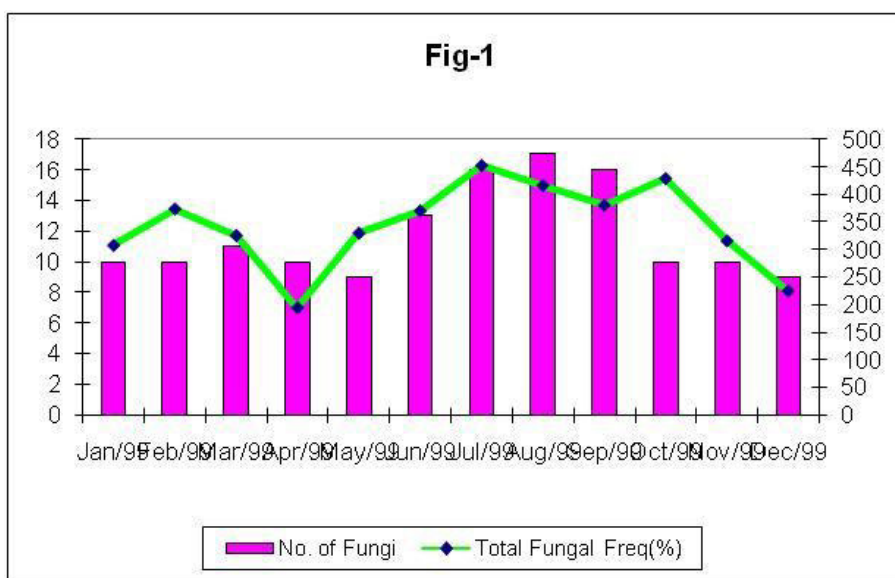


Table - 1 : Monthly incidence of fungi during storage on *Brassica carinata* seeds using blotter and agar plate method.

S. No.	Organism	Period of Incubation											
		Jan-99	Feb-99	Mar-99	Apr-99	May-99	Jun-99	Jul-99	Aug-99	Sep-99	Oct-99	Nov-99	Dec-99
1	<i>Alternaria alternata</i> a*	30.00	21.84	4.76	4.76	-	9.52	15.31	-	-	43.52	28.76	14.28
	b*	-	4.76	-	-	4.76	-	-	9.60	-	21.80	-	-
2	<i>Aspergillus candidus</i>	-	4.76	-	-	-	-	30.00	28.80	40.25	-	-	-
3	<i>Aspergillus flavus</i>	15.50	-	4.76	5.00	-	-	4.76	-	9.52	-	4.76	4.76
		42.85	70.83	90.47	28.57	28.57	57.14	-	66.60	70.00	71.42	38.09	4.76
		76.19	66.66	57.14	9.52	-	-	66.66	33.33	33.33	71.42	15.14	10.00
4	<i>Aspergillus fumigatus</i>	-	-	-	-	71.42	4.76	-	23.80	19.04	14.28	28.57	42.85
		9.52	-	-	57.14	90.47	18.54	28.57	4.76	-	33.33	-	10.00
5	<i>Aspergillus glaucus</i>	-	-	-	-	9.52	4.76	-	19.04	9.52	-	-	-
		-	-	-	-	-	-	14.28	-	-	9.52	-	-
6	<i>Aspergillus nidulans</i>	-	-	4.76	10.00	-	4.76	-	9.52	21.80	-	-	-
		-	-	-	-	-	5.00	-	4.76	-	-	-	-
7	<i>Aspergillus niger</i>	47.61	52.38	41.66	19.04	33.33	42.85	23.80	25.57	33.33	33.33	42.85	47.61
		-	4.76	-	-	-	-	-	14.28	33.33	-	4.76	-
8	<i>Aspergillus versicolor</i>	-	-	-	-	-	-	4.76	-	-	-	-	-
		-	-	-	-	-	-	-	39.30	-	-	-	-
9	<i>Chaetomium bostrychodes</i>	-	33.33	-	-	-	-	-	-	-	-	-	-
10	<i>Curvularia lunata</i>	21.52	-	51.72	26.18	-	30.40	9.52	19.05	20.00	-	31.52	10.00
		-	4.76	-	-	4.76	-	-	28.56	-	15.00	-	4.76
11	<i>Fusarium chlamydosporum</i>	-	10.00	-	-	-	-	-	-	-	-	-	4.76
		-	-	19.54	-	-	-	20.21	4.76	-	-	-	-
12	<i>Fusarium moniliforme</i>	26.04	-	14.28	19.05	31.52	10.00	41.76	-	9.52	21.80	19.05	-
		-	-	-	-	15.00	-	10.00	4.76	-	-	4.76	-
13	<i>Fusarium oxysporum</i>	4.76	19.05	4.76	10.00	-	96.04	80.46	-	-	9.52	24.80	21.80
		-	4.76	-	-	9.52	31.52	14.28	-	19.05	9.52	4.76	-
14	<i>Penicillium chrysogenum</i>	-	19.09	10.00	-	21.52	31.68	-	10.00	4.76	-	33.33	23.80
		23.80	-	-	-	-	-	14.28	-	-	-	-	-
15	<i>Penicillium multicolor</i>	-	-	-	-	-	-	-	4.76	9.52	-	25.25	15.54
		-	-	-	-	-	-	-	-	-	10.00	-	-
16	<i>Penicillium notatum</i>	-	-	-	-	-	-	-	-	19.05	-	-	-
		-	-	-	-	-	-	-	9.52	-	-	-	-

17	<i>Penicillium oxalicum</i>	10.00	19.05	21.82	-	-	19.05	14.28	9.52	-	31.58	-	14.28
		-	4.76	-	4.76	9.52	-	21.82	-	5.00	-	-	5.00
18	<i>Penicillium perpurogenum</i>	-	-	-	-	-	4.76	-	-	-	-	-	-
19	<i>Phytophthora undulata</i>	-	-	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	9.52	-	-	28.57	-	-
20	<i>pythium sp.</i>	-	-	-	-	-	-	-	10.00	-	-	-	-
		-	-	-	-	-	-	-	-	4.76	-	-	-
21	<i>Rhizopus nigricans</i>	-	33.33	-	-	-	4.76	19.05	31.68	-	-	9.52	-
		-	-	-	-	-	-	4.76	-	14.08	-	-	-
22	<i>Syncephalastrum sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	9.52	-	-	-
	a* - Blotter test.		b* - Agar plate est.										

Results presented in 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.

Results presented in 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.

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

Incubation Period (Months)	Protein Percentage		% Change in Protein over control		Oil Percentage		% Change in Oil content over control	
	a*	b*			a*	b*		
0	30.54	30.54	0.00	0.00	33.04	33.04	0.00	0.00
1	30.02	30.18	-1.70	-1.18	33.04	33.00	0.00	-0.12
2	30.02	30.18	-1.70	-1.18	33.04	32.42	0.00	-1.88
3	29.76	29.81	-2.55	-2.39	33.00	30.73	-0.12	-6.99
4	29.50	28.99	-3.41	-5.08	32.94	29.82	-0.30	-9.75
5	28.98	28.65	-5.11	-6.19	32.90	29.12	-0.42	-11.86
6	28.98	25.26	-5.11	-17.29	32.84	28.67	-0.61	-13.23
7	28.72	23.96	-5.96	-21.55	32.72	27.65	-0.97	-16.31
8	28.46	22.40	-6.81	-26.65	32.65	27.10	-1.18	-17.98
9	28.20	21.88	-7.66	-28.36	32.60	26.23	-1.33	-20.61
10	27.94	21.09	-8.51	-30.94	32.52	25.48	-1.57	-22.88
11	27.42	20.83	-10.22	-31.79	32.34	25.08	-2.12	-24.09

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

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.

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

a* - control seeds.

b* - infested seeds.

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 7.93% 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) 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 and Pathak (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.

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