

RESEARCH REPORT

Comparative analysis of nutritional value in yellow mustard seeds infected by individual test fungi

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Manuscript details:	ABSTRACT
<p>Received: 22.10.2017 Revised: 22.11.2017 Accepted: 21.12.2017 Published: 31.12.2017</p> <p>Cite this article as: Ghugal SI (2017) Comparative analysis of nutritional value in yellow mustard seeds infected by individual test fungi, <i>Int. J. of Life Sciences</i>, Volume 5(4): 791-794.</p> <p>Copyright: © Author, This is an open access article under the terms of the Creative Commons Attribution-Non-Commercial - No Derives License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.</p> <p>Available online on http://www.ijlsci.in ISSN: 2320-964X (Online) ISSN: 2320-7817 (Print)</p>	<p>Yellow mustard (<i>Brassica juncea</i> cv Heera) developed at Department of Botany, Nagpur University Nagpur. The seed coat color is golden yellow. The oil content is 35 percent while the erucic acid is zero and glucosinolate is very low in defatted meal.</p> <p>In the present study, attempts have been made to evaluate and record the changes in protein, oil, total sugar, reducing sugar and non-reducing sugars due to infestation by the test fungi which are dominant storage fungi occurs under the seed storage. The test fungi are <i>Alternaria alternata</i>, <i>Aspergillus flavus</i>, <i>A. fumigatus</i>, <i>A. niger</i>, <i>Fusarium moniliforme</i> and <i>Penicillium oxalicum</i>.</p> <p>After 21 days of incubation with infested test fungi viz <i>A. fumigatus</i>, <i>A. niger</i> and <i>Penicillium oxalicum</i>, showed more decline in protein that is -32.04 percentage change over control in protein while test fungi <i>Aspergillus flavus</i> showed maximum decrease in oil content that is - 24.78 percentage change over control in oil. After the incubation of 21 days with infested test fungi, it was seen that the starch and sugars content of yellow seeds declined, the maximum decrease in starch occurred by <i>Aspergillus flavus</i> was - 36.356 percentage change over control while the maximum decrease in total sugars occurred by <i>Aspergillus niger</i> was -15.930 percentage change over control.</p> <p>Keywords: Mustard, Yellow seeds, Test fungi, Protein, Oil, Starch, Sugars.</p>
	<h3>INTRODUCTION</h3> <p>In India and certain countries in Africa and South America the losses of food grains due to storage fungi is about 30 percent of the annual harvest. In addition to field diseases, there are number of fungal infections, which occurs during storage period. Poor storage practices and mechanical injuries caused to the grains during harvesting facilitate easy entry of fungal pathogen during storage affecting various metabolites, viz. carbohydrates, protein, lipids, etc. The present study deals with the analysis of biochemical aspects that is protein, oil, starch and sugars content of yellow mustard seeds after the incubation of 7, 14, and 21 days in association of test fungi that is <i>Alternaria alternata</i>, <i>Aspergillus flavus</i>, <i>A. fumigatus</i>, <i>A. niger</i>, <i>Fusarium moniliforme</i> and <i>Penicillium oxalicum</i>.</p>

MATERIALS AND METHODS

The yellow mustard seeds (*Brassica Juncea* (L.) Czern, & Coss. cv. Heera) selected for experimental study. Isolation of fungi was done in every month of the storage period by both blotter as well as agar plate method as recommended by ISTA (1966). After collecting the data of percentage of fungal incidence, most frequent or dominant fungi were selected as, 'Test fungi'. The test fungi are *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium moniliforme* and *Penicillium oxalicum*.

The control seeds were artificially infected by spore suspension (10 spores / ml) of 'test fungi'. The spores suspension was prepared by adding 5 ml sterile distilled water and 2 drops of Tween 80 in 8 days old pure culture grown in the culture tube, shaking it well for one minute, and was transferred aseptically in 150 ml Erlenmeyer flask containing 10 g of seeds. After aseptic transfer of spore suspension, the flasks were shaken vigorously for two minutes for even distribution of spores. Extra water from flask was discarded and then incubated at $28 \pm 1^\circ\text{C}$ for 7, 15 and 21 days respectively. 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 protein, oil, starch and sugars (reducing, non-reducing and total sugar). Crude protein estimation was calculated by micro-kjeldahl method suggested by Davys and Pirie (1969) and 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. The mode was introduced, tuned and calibrated with pure yellow mustard 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

After completion of each incubation period the seeds were washed properly to remove mycelium, later on dried in oven and powdered. These seeds were analyzed for protein contents, oil content, starch content, total, reducing and non-reducing sugars and data is presented in Tabulation.

The control seeds exhibited 29.04% crude protein. The seeds infested by *Alternaria alternata*, *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Fusarium moniliforme* and *Penicillium oxalicum*, showed moderate depletion in Crude proteins. As the incubation period increased, there was decrease in protein content and almost all test fungi affected the existing proteins present in seeds. Out of these test fungi *Aspergillus fumigatus*, *A. niger*, *Penicillium oxalicum* shows higher percent change over control in 21 days i.e. -32.04%, -32.04%, -30.42% and -32.04% respectively (Table 1).

The control yellow mustard seed showed 34.99 % oil content. Infested seeds showed decrease in oil content with increase in incubation period. *Aspergillus flavus* showed higher depletion in oil content in infested seeds i.e. 26.32% oil with 24.78% decrease over control after completion of 21 days incubation period followed by 22.46% decrease over control in infested seeds by *Alternaria alternata*. Other test fungi also reduced the oil content in infested seeds (Table 1).

Table 2 shows the changes in seed constituents about brought by test fungi during different incubation periods. The surface sterilized seeds showed 60.92% starch, 5.656 mg/g total sugar, 2.893 mg/g reducing sugar and 2.763 mg/g non-reducing sugars and shows no change at different incubation periods. The results of individual test fungi are as follows.

Alternaria alternata –The infested seed shows decrease in starch up to 42.84% after 21 days indicating 29.682% decreased over control. Likewise, total, reducing and non-reducing sugars also decrease with increase in incubation period. For 7 to 14 days, there is low alteration, while for 14 to 21 day there was moderate depletion in contents.

Table 1: Changes in protein and oil contents in yellow mustard due to association of test fungi.

S. N.	Name of Organism	Protein percentage	% Change in protein over control	Oil percentage	% Change in oil content over control	
1	Control	a*	29.06	0.00	34.99	0.00
		b*	29.06	0.00	34.99	0.00
		c*	29.06	0.00	34.99	0.00
2	<i>Alternaria alternata</i>	28.70	-1.24	33.64	-3.86	
		27.24	-6.26	29.62	-15.35	
		26.75	-7.95	27.13	-22.46	
3	<i>Aspergillus flavus</i>	27.84	-4.20	32.26	-7.80	
		25.62	-11.84	28.72	-17.92	
		22.75	-21.71	26.32	-24.78	
4	<i>A. fumigatus</i>	25.94	-10.74	33.08	-5.46	
		23.04	-20.72	32.14	-8.15	
		19.75	-32.04	30.00	-14.26	
5	<i>A. niger</i>	28.50	-1.93	32.12	-8.20	
		27.00	-7.09	29.00	-17.12	
		19.75	-32.04	27.80	-20.55	
6	<i>Fusarium moniliforme</i>	27.14	-6.61	32.18	-8.03	
		26.44	-9.02	30.91	-11.66	
		20.75	-28.60	27.50	-21.41	
7	<i>Penicillium oxalicum</i>	25.42	-12.53	32.24	-7.86	
		24.89	-14.35	30.74	-12.15	
		19.75	-32.04	28.10	-19.69	

a* - 7 days of incubation b* - 14 days of incubation

c* - 21 days of incubation

Table 2: Changes in starch, total, reducing and non-reducing sugars in yellow mustard seeds due to association of test fungi.

S. No.	Name of organism	% 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	
1	Control	a*	60.92	0.000	5.656	0.000	2.893	0.000	2.763	0.000
		b*	60.92	0.000	5.656	0.000	2.893	0.000	2.763	0.000
		c*	60.92	0.000	5.656	0.000	2.893	0.000	2.763	0.000
2	<i>Alternaria alternata</i>	55.34	-9.164	5.421	-4.155	2.754	-4.805	2.667	-3.474	
		49.06	-19.476	5.324	-5.870	2.698	-6.740	2.626	-4.958	
		42.84	-29.682	5.018	-11.280	2.587	-10.577	2.431	-12.016	
3	<i>Aspergillus flavus</i>	54.86	-9.946	5.327	-5.817	2.653	-8.296	2.674	-3.221	
		46.45	-23.754	5.054	-10.644	2.512	-13.170	2.542	-7.999	
		38.77	-36.356	4.800	-15.134	2.325	-19.634	2.475	-10.423	
4	<i>A. fumigatus</i>	57.24	-6.037	5.390	-4.703	2.680	-7.363	2.710	-1.918	
		51.24	-15.891	5.108	-9.689	2.544	-12.064	2.564	-7.202	
		46.02	-24.458	4.898	-13.402	2.462	-14.898	2.436	-11.835	
5	<i>A. niger</i>	54.95	-9.800	5.290	-6.471	2.660	-8.054	2.630	-4.814	
		47.01	-22.833	5.010	-11.421	2.526	-12.686	2.484	-10.098	
		39.27	-35.532	4.755	-15.930	2.388	-17.456	2.367	-14.332	
6	<i>Fusarium moniliforme</i>	55.49	-8.913	5.503	-2.705	2.748	-5.012	2.755	-0.290	
		52.11	-14.462	5.322	-5.905	2.690	-7.017	2.632	-4.741	
		46.35	-23.925	5.178	-8.451	2.572	-11.096	2.606	-5.682	
7	<i>Penicillium oxalicum</i>	54.32	-10.834	5.421	-4.155	2.659	-8.088	2.762	-0.036	
		50.42	-17.239	5.286	-6.542	2.534	-12.409	2.752	-0.398	
		44.81	-26.441	5.020	-11.245	2.470	-14.622	2.550	-7.709	

a* - 7 days of incubation

b* - 14 days of incubation

c* - 21 days of incubation

Aspergillus flavus – It affect the starch content in Heera seeds i.e. 54.86%, 46.45% and 38.77% after 7, 14 and 21 days of incubation period. There was constant decrease with increase in the incubation period in total, reducing and non-reducing sugars. The percentage decreased over control after 21 days in above sugars were -15.134%, -19.634% and -10.423% change over control respectively.

Aspergillus fumigatus – The percentage decrease in starch over control after 7, 14 and 21 days was -6.037%, -15.891% and -24.458%. Depletion in total, reducing and non-reducing sugars for 7 to 14 days incubation was faster than 14 to 21 days. Infested seeds after 21 days showed 4.898 mg/g total sugar, 2.462 mg/g reducing sugar and 2.436 mg/g non-reducing sugars.

Aspergillus niger – The starch content reduced to 39.27% with 35.532% decrease over control. Carbohydrate shows regular reduction with increase in the incubation period. It showed 15.930%, 17.456% and 14.332% decrease over control in total, reducing and non-reducing sugars respectively after 21 days.

Fusarium moniliforme – show low alteration in nutritive components as compared to other fungi.

Penicillium oxalicum – The starch content reduced to 54.32%, 50.42% and 44.81% after 7, 14 and 21 days respectively. It shows depletion in total, reducing and non-reducing sugar i.e. 5.020 mg/g, 2.470 mg/g and 2.550 mg/g after 21days.

In the present study, it was observed that there was reduction in protein content with variable incubation periods. Reduction in the protein content of the seeds is obviously due to their breakdown to amino acids and subsequent utilization by fungi for their growth. Breakdown of host proteins by fungal enzymes has also been reported by Bilgrami and Verma (1978) and Corhane (1958). 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. Maheshwari *et. al*, (1984) reported depletion in both values of reducing and non-reducing sugars in pulses.

CONCLUSION

The present study reveals that there was higher decrease in protein and oil content during 14 and 21 days of incubation period of infested seeds than 7 days incubation period. After completion of incubation period of respective days, infested seeds show reduction in starch content, total, reducing and non-reducing sugars.

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