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Effect of Storage Fungi on the Seed Quality Parameters of Different Mustard Varieties

Tamoor Khan*, K. Khan and Muhammad Zahoor ul Haq
Food Technology Division, PCSIR Laboratories, Mastung Road, Quetta, Pakistan

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Abstract. Seven seed storage fungi (*Aspergillus flavus, Alternaria brassicae, Helminthosporium brassicae, Penicillium* sp, *Pythium* sp, *Rhizoctonia solani* and *Fusarium oxysporum*) were isolated from four mustard seed varieties (B-raya, Y-raya, B-M-1 and S-9). *A. flavus* was the most predominant fungus found on seeds of all the four mustard seed varieties. The germination percentage of the fungal infected seeds of B-raya decreased significantly, followed by Y-raya, B-M-1 and S-9 mustard seed varieties, both in the laboratory and the pot-scale studies. It was noted that weight and oil contents of seeds of all the four varieties decreased in those that were infected by fungi during storage in comparison with the seeds that were stored in sterilized bottles. The fungal infected seeds also had lower glucosinolate and erucic acid contents than those of the non-infected seeds.

Keywords: storage fungi, mustard seed, seed quality parameters, glucosinolate in mustard, erucic acid in mustard, stored seed germination

Introduction

Mustard (*Brassica juncea* L) is commonly known as "sarson" in the Indo-Pakistan sub-continent. It is an annual winter crop and a major oil seed source in Pakistan. It is cultivated in the temperate regions of the world, in Europe, China, India and Pakistan (Shafi *et al.,* 1994). In Pakistan, it is cultivated in all the provinces while the province of Punjab is its major area of cultivation. The total area under the cultivation of mustard in Pakistan is 33950 hectares, averaging 859 kg/ha (PARC, 1998). The average oil and protein content in the mustard seed is 38-45% and 28%, respectively (Bhatti and Soomro, 1994). Mustard plant, during all the stages of its growth, is attacked by a number of diseases, such as downy mildew (*Peronospora parasitica)*, white rust *(Albugo candida),* powdery mildew *(Erysiphe cichoracearum)*, blight *(Alternaria brassicae),* wilt *(Fusarium oxysporum)*, stem and root rot *(Sclerotinia sclerotiorum),* and seed storage rots caused by species of *Rhizoctonia, Aspergillus, Penicillium* and *Fusarium* (Rakesh *et al.,* 1995). The seed mycoflora associated with abnormal wrinkled and discoloured mustard seeds include *Chaetomium indicum*, *Cladosporium fulvum, Penicillium rubrum*, *Alternaria brassicae, Rhizopus arrhizus, Fusarium* sp, *Alternaria brassicae* (Kumar *et al.,*1986); *Rhizoctonia brassicae, Pyrenopeziza brassicae*, *Botrytis cinerea, Fusarium oxysporum, Macrophomina phaseolina, Pythium butteri, Urocystis brassicae* (Saha and Singh, 1988); and *Altenaria curvularie, Aspergillus* sp*, Rhizopus* sp and *Mucor* sp (Rani *et al.,* 1995). Healthy seeds play an important role in increasing plant population, improving seed quality and yield of any crop species (Hafiz, 1986).

Keeping in view the importance of mustard seed crop in the production of oil in the country, the present studies were carried out to evaluate the effect of storage fungi on the seed quality parameters of different mustard varieties.

Materials and Methods

Collection of mustard seeds. Seed samples of four mustard varieties, namely, B-M-1, S-9, brown raya (B-raya) and yellow raya (Y-raya) were obtained from the Oil Seed Section of Agricultural Research Institute, Quetta, Pakistan. 1 kg seeds of each variety were stored for 6 months at 25 °C and 8-20% relative humidity in open atmosphere. Furthermore, 1 kg seeds of each variety were stored for 6 months in sterilized glass bottles at 25 °C and 8% relative humidity.

Isolation and identification of storage fungi. 200 seeds of each variety, stored in the open, were thoroughly washed under running tap water for about 20 min. These seeds were sterilized by rinsing in 0.01% mercuric chloride for 1-2 min and washing twice with distilled water for about 2-3 min. 25 seeds of each variety were placed in petri dishes containing sterilized potato dextrose agar medium. All plates were kept at room temperature (25±1 °C) for 12 days in three replications. The fungi associated with each seed were identified on the basis of their specific mycelial and spore characteristics using the genera identification protocol (Agrios, 1978) . The percentage of infection in the storage fungi was determined using the

*Author for correspondence

following formula:

$$\% = \frac{\text{number of seeds infected by each fungus species}}{\text{total number of seeds incubated}} \times 100$$

Effect of storage fungi on the germination of different mustard varieties

a. Laboratory-scale germination studies. Germination studies in petri plates were undertaken by taking randomly 200 seeds of each variety stored in the open, as well as those stored in sterilized bottles. Seeds were surface sterilized with 0.01% mercuric chloride, washed twice with distilled water and then placed in petri plates containing two layers of sterilized moist filter papers. Observations were recorded after 12 days, when the plumules of the germinated seeds were about 1.25 cm long. The percentage of germination was determined.

b. Pot-germination studies. Seed germination studies were carried out in earthen pots (22 cm dia) containing sterilized soil with 25 seeds of each variety prepared in the same manner as was done for labortory-scale germination studies. Seeds stored under both the storage conditions, namely, in the open and in the sterilized bottles were used for pot germination. The pots were irrigated whenever needed. The germination percentage was recorded after 32 days when seedlings were about 4 cm long.

Effect of storage fungi on the mustard seed quality

a.1000- seed weight. Three replicates of 1000 seeds of each variety stored under the two sets of conditions were randomly selected. Seed weights of all the replicates were recorded.

b. Determination of oil content. Oil content was determined by taking three replicates of 50 g seeds of each variety kept under the two storage conditions. The seeds were oven-dried for 1 h at 130 °C, followed by grinding of the samples. 5 g of each sample was extracted with petroleum ether in Dickey John analyzer for oil extraction by heating for 2 h. The recovered oil percentage was calculated as:

$$\text{oil (\%)} = \text{oil recovered} \times 100/5$$

c. Determination of erucic acid and glucosinolates. Erucic acid and glucosinolates were determined by gas liquid chromatography (GLC) in accordance with Smith *et al*. (1993).

Data analysis. Data were analyzed for the percentage of isolted fungi, seed germination and chemical composition by analysis of variance (Steel and Torrie, 1984).

Results and Discussion

Isolation and identification of storage fungi. Seven species of storage fungi were isolated and identified from each of the four mustard varieties, which were *Aspergillus flavus, Alternaria brassicae, Helminthonsporium brassicae, Penicillium* sp, *Pythium* sp, *Rhizoctonia solani* and *Fusarium oxysporum.* There were similarities and disparities among the mustard varieties with regard to the frequency of their presence in different varieties of mustard seed (Table 1). Overall, the most common fungus was *Aspergillus flavus*. The percentage of this fungus was the highest on the seeds of three varieties (S-9, Y-raya, B-raya), but the second highest in one variety (B-M-1). The second most common fungal species present on the three varieties, S-9, Y-raya and B-raya was *Alternaria brassicae*. However, *Helminthosporium brasicae* was the most common on the seeds of mustard variety, B-M-1, followed by *Aspergillus flavus* as the second most common, and

Table 1. Frequency of storage fungi isolated from four mustard varieties stored under conditions of open atmosphere*

Mustard variety	Storage fungi isolated	Fungi isolated (number)	Percentage of infection (%)
S-9	*Aspergillus flavus*	80	40.0
	Alternaria brassicae	35	17.5
	Helminthosporium brassicae	26	13.0
	Penicillum sp	24	12.0
	Pythium sp	4	2.0
	Rhizoctonia solani	13	6.5
	Fusarium oxysporum	18	9.0
B-M-1	*Aspergillus flavus*	60	30.0
	Alternaria brassicae	32	16.0
	Helminthosporium brassicae	80	40.0
	Penicillum sp	9	4.5
	Pythium sp	13	6.5
	Rhizoctonia solani	4	2.0
	Fusarium oxysporum	2	1.0
Y-raya	*Aspergillus flavus*	87	43.5
	Alternaria brassicae	79	39.5
	Helminthosporium brassicae	6	3.0
	Penicillum sp	14	7.0
	Pythium sp	3	1.5
	Rhizoctonia solani	5	2.5
	Fusarium oxysporum	6	3.0
B-raya	*Aspergillus flavus*	103	51.1
	Alternaria brassicae	48	24.0
	Helminthosporium brassicae	16	8.0
	Penicillum sp	10	5.0
	Pythium sp	4	2.0
	Rhizoctonia solani	6	3.0
	Fusarium oxysporum	13	6.0

*200 seeds of each variety were tested for recording fungal infection

Alternaria brassicae as the third most common. The experiment thus indicated some degree of resistance in the mustard gene pool to most of the fungus species, except *Aspergillus flavus, Alternaria brassicae and Helminthosporium brassicae.*

Effect of storage fungi on the germination of four mustard varieties. The results given in Table 2 show that the germination percentage of seeds of all the four varieties infected by fungi during storage was significantly decreased as compared to those stored in sterilized bottles. The overall lowest germination was noted for B-raya, which ws 15% and 16% under laboratory conditions and pot germination, respectively. Varieties S-9 and B-M-I were affectd to a lesser extent by storage fungi, followed by Y-raya and B-raya.

The overall rate of germination percentage of seeds stored in sterilized bottles of all the four varieties was (28-93%) as compared to the seeds infected during storage (15-53%).

Effect of storage fungi on seed quality of the four mustard varieties

a. 1000-seed weight. Seed weight was significantly reduced in variety B-raya followed by Y-raya, B-M-1 and S-9 in the seeds infected by fungi in comparison with the seeds stored in sterilized bottles (Table 3).

b. Oil content. The seeds of B-raya colonized with storage fungi had lower oil-content percentage as compared to Y-raya, B-M-1 and S-9 mustard seed varieties (Table 3).

Table 2. Effect of storage fungi on the seed germination of four mustard varieties in laboratory-scale and pot-scale studies*

Variety	Scale	Seed storage condition	Number of seeds germinated	Germination (%)
S-9	**laboratory**	sterilized bottles	185	92.5
		open atmosphere	107	53.3
	pots	sterilized bottles	166	83.0
		open atmosphere	119	59.5
Y-raya	**laboratory**	sterilized bottles	91	45.5
		open atmosphere	43	21.5
	pots	sterilized bottles	92	46.0
		open atmosphere	52	26.0
B-raya	**laboratory**	sterilized bottles	56	28.0
		open atmosphere	30	15.0
	pots	sterilized bottles	73	36.5
		open atmosphere	32	16.0
B-M-1	**laboratory**	sterilized bottles	186	93.0
		open atmosphere	101	50.5
	pots	sterilized bottles	174	87.0
		open atmosphere	128	64.0

*200 seeds of each variety were studied for germination

Effect of storage fungi on fatty acid and glucosinolate contents

a. Fatty acids. The fatty acid (erucic acid) percentage was significantly reduced in the fungal infected seeds of all the four mustard varieties, as compared to seeds stored under sterile conditions (Table 4). The overall erucic acid percentage was the lowest in the fungal infected B-raya variety.

b. Glucosinolate. The results given in Table 4 indicate that glucosinolate content was significantly reduced in the infected

Table 3. Effect of storage fungi on the seed quality of four mustard varieties in respect of weight and total oil content when stored in open atmosphere and sterilized bottles

Variety	Seed storage condition	1000-seed weight (g)	Oil concenration (%)
S-9	sterilized bottles	3.38 [a*]	38.0[a]
	open atmosphere	3.21[a]	34.51[b]
Y-raya	sterilized bottles	3.38[a]	38.0[a]
	open atmosphere	2.40[bc]	32.75[c]
B-raya	sterilized bottles	3.38[a]	38.0[a]
	open atmosphere	2.23[c]	30.35[bc]
B-M-1	sterilized bottles	3.38[a]	38.0[a]
	open atmosphere	2.46[b]	33.40[c]
lsd		0.19	1.13
cv		6.46%	2.90%

*values with different alphabets in each column are significantly different from each other; lsd: least significant difference at p = 0.05; cv: coefficient of variance

Table 4. Effect of storage fungi on the fatty acid and glucosinolate contents of four mustard varieties stored in the open and in sterilized bottles

Variety	Seed storage condition	Glucosinolate content (%)	Erucic acid (%)
S -9	sterilized bottles	0.25[a*]	41.50[a]
	open atmosphere	0.23[a]	38.75[b]
Y-raya	sterilized bottles	0.25[a]	41.50[a]
	open atmosphere	0.20[ab]	31.25[d]
B-raya	sterilized bottles	0.25[a]	41.50[a]
	open atmosphere	0.17[b]	29.67[e]
B-M-1	sterilized bottles	0.25[a]	41.50[a]
	open atmosphere	0.22[a]	37.00[c]
lsd		0.03	0.72
cv (%)		11.53	2.12

*values with different alphabets in each column are significantly different from each other; lsd: least significant difference at p = 0.05: cv: coefficient of variance

seeds of B-raya as compared to those of Y-raya, B-M-1and S-9 fungal infected varieties.

Conclusion

The undertaken studies revealed that all the seed quality parameters were severely affected by the seed mycoflora in all the four mustard varieties stored in open conditions, as compared with seeds stored in sterilized bottles. Fungal infection during storage also reduced the percentage germination of the seeds. It was further observed that poor quality seed lose oil yield and have poor market value. It is, therefore, suggested that healthy seeds should be used for sowing while all agricultural management practices should be carried out with the primary aim of reducing the incidence of storage fungi, thus preventing the erosion of seed quality parameters.

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