RESEARCH ARTICLE

Incidence of microorganisms in Mustard seeds during the storage and its toxic effect on seed germination and seedling diseases.

Ghugal SI[1] and Thakre RP[2]

[1.] *Department of Botany, S.S.E.S. Amt's Science College, Pauni, Dist.- Bhandara.*

[2.] *Ex-Prof. Post Graduate Teaching Department of Botany, R.T.M. Nagpur University Campus, Nagpur.*

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ABSTRACT

This work aims to isolate and to identify the fungal microorganisms associated with mustard (*Brassica juncea* cv. Pusabold) seed during storage. The seed mycoflora was isolated from mustard seeds at an interval of a month for a period of one year at laboratory condition by blotter and agar plate method. Altogether 28 storage fungi were confined to mustard seed samples throughout the year. Major predominant fungi included *Alternaria alternata*, *Aspergillus flavus, A. fumigatus, A. niger, Fusarium moniliforme, Penicillium multicolor, P. oxalicum* and *Curvularia lunata.* Mustard seeds were inoculated with the fungal culture filtrate of above test fungi and incubated for 7,14, and 21days. The pathological symptoms such as weak root, yellowing of leaf, stunted growth, blackening of radical, seedling rot, no primary root etc. were distinct symptoms of disease due to fungal filtrate of storage fungi. Culture filtrate of longer duration inhibited the seed germination and retarded the seedling growth.

Key words: Mustard, storage fungi, test fungi, fungal filtrate.

INTRODUCTION

In India and certain countries in Africa and South America the losses of food grains due to association of microorganisms is about 30 percent of the annual harvest. The fact that particularly some tropical developing countries suffer from these losses makes the problem of storage fungi, a major one in world agriculture. *Brassica juncea* (L.) Czern. & Coss. Cv. Pusabold also known as mustard is one of the important and widely distributed oilseed crop. It is cultivated in the cooler agricultural regions and at higher elevation as well as winter crop in India and other countries of the temperate zones. Large numbers of fungi are known to bring about several biochemical changes in mustard seeds and degrade seed constituents (Rai and Saxena, 1980). The microorganism associated with seed may be pathogenic, weak parasites or saprophytes and may be external or internal. The present investigation deals with the incidence of microorganisms in mustard seeds during the storage and also effect of fungal filtrates of predominant fungi (test fungi) on seed germination and Seedling diseases.

MATERIALS AND METHODS

Seed samples of Mustard was selected for experimental study and collected from different oilseed Brassica growers of north India and also from department of Botany, Nagpur. Seed samples were mixed 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 i. e. from Jan 1999 to Dec 1999. After every month 400 seeds were taken out randomly and percentage incidence of fungi were recorded. Total percentage of fungal incidence was calculated by using the formula suggested by Sahai and Mehrotra (1982). To study the toxic effects, the metabolites from culture filtrate of test fungi were obtained for 7, 14 and 21 days incubation period. Surface sterilized one hundred seeds were soaked in culture filtrates for 24 hours. Seed soaked in sterilized czapek's broth served as control. The treated and control seeds were allowed to germinate on moist blotter paper in triplicate at laboratory condition. After six days of incubation at 28 ± 1^0 C, per cent seed germination as well as seedling pathogenic diseases symptoms were recorded.

RESULT AND DISCUSSION

The seed mycoflora obtained on Pusabold seeds from January 99 to December 99 is presented in Table 1. Altogether 28 fungi were isolated, out of which some were recorded throughout the year included *Alternaria alternata, Aspergillus flavus, A. fumigatus, A. niger, Fusarium oxysprorum, Penicillium chrysogenum* and *P. oxalicum.*

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

S. No.	Name of 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	*Alternaria alternata* a*	15.89	10.00	5.00	-	-	9.09	21.00	18.00	12.50	5.00	16.00	16.50
	b*	7.00	4.00	-	-	-	-	8.00	6.50	14.00	12.00	-	13.00
2	*Alternaria brassicae*	22.00	16.00	-	-	-	-	9.00	-	-	10.00	-	14.54
		8.00	-	-	-	-	-	-	5.50	-	14.54	2.00	5.05
3	*Aspergillus candidus*	7.62	-	-	5.72	-	3.00	-	-	-	-	-	10.00
		-	-	7.68	10.00	-	-	-	6.00	-	-	-	-
4	*Aspergillus flavus*	8.76	9.09	33.33	42.00	30.19	42.85	23.86	57.14	33.33	33.33	28.52	9.52
		5.05	-	-	40.00	-	-	-	10.00	14.28	61.90	38.09	-
5	*Aspergillus fumigatus*	-	37.50	47.00	20.20	47.61	9.52	19.04	19.04	12.08	-	42.85	13.27
		7.65	-	3.00	-	52.38	76.19	33.33	38.09	-	-	13.27	-
6	*Aspergillus glaucus*	-	-	-	-	-	-	8.76	10.00	19.52	14.28	-	16.70
		4.76	-	-	-	5.00	-	-	-	-	9.52	-	-
7	*Aspergillus nidulans*	16.00	8.33	5.00	10.00	9.00	-	-	-	-	-	-	15.50
		4.76	-	-	-	-	-	-	-	-	-	-	-
8	*Aspergillus niger*	4.54	-	-	47.61	52.38	33.33	57.14	66.66	61.90	19.52	-	13.60
		-	-	-	-	-	-	-	14.28	-	-	15.74	15.00
9	*Aspergillus versicolor*	-	-	9.09	5.54	-	8.00	-	-	-	-	-	-
		-	-	4.74	-	-	-	-	-	-	-	-	-
10	*Aureobasidium sp.*	-	8.96	-	10.62	12.00	-	-	-	-	-	-	-
		9.52	-	-	-	-	5.76	-	-	-	-	-	-
11	*Chaetomium bostrychodes*	-	-	10.00	-	-	-	-	15.16	15.60	9.05	-	-
		-	-	-	-	-	-	-	5.00	-	-	-	-
12	*Curvularia lunata*	22.72	30.17	15.42	-	-	-	4.76	15.16	-	-	-	9.52
		-	-	-	9.05	-	3.00	-	-	-	-	-	15.00
13	*Fusarium moniliforme*	4.54	25.00	31.00	-	4.76	-	-	-	15.00	27.00	19.05	-
		-	4.76	5.00	-	-	-	-	14.28	-	-	-	-
14.	*Fusarium oxysporum*	18.18	45.58	61.38	65.05	20.00	18.18	30.19	-	23.80	-	39.12	-
		5.00	-	9.05	12.00	-	-	-	-	25.00	11.00	21.05	22.23
15.	*Haplosporangium sp.*	-	-	-	-	-	4.76	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
16	*Memnoniella sp.*	-	-	-	-	-	-	-	-	-	5.00	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
17	*Penicillium chrysogenum*	-	28.57	12.00	-	-	-	38.50	-	-	28.57	19.05	9.52
		4.76	4.76	-	10.00	-	-	-	-	-	4.76	-	15.00
18	*Penicillium frequentans*	-	-	-	-	-	-	-	-	10.00	16.55	-	14.50
		-	-	-	-	-	-	-	-	-	-	5.00	-
19	*Penicillium multicolor*	-	30.00	-	-	30.54	-	19.05	11.76	-	13.49	28.09	-
		-	4.76	-	-	12.65	-	10.00	-	-	-	-	-

Table 1: Continued…

S. No.	Name of 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
20	*Penicillium notatum*	18.18	20.00	21.51	19.09	-	4.76	26.32	33.33	9.00	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
21	*Penicillium oxalicum*	21.47	-	9.54	30.00	10.00	-	47.61	18.18	23.00	30.00	23.80	33.33
		-	-	-	-	-	-	-	-	4.76	5.00	4.76	-
22	*Phoma lingum*	-	-	-	4.76	-	5.00	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	5.54	-
23	*Phytophthora undulata*	-	-	-	4.54	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
24	*pythium sp.*	-	-	5.00	-	-	-	-	-	-	-	-	-
		-	-	-	-	10.00	-	-	-	-	-	-	-
25	*Rhizopus nigricans*	-	-	20.00	-	-	-	-	-	-	6.90	43.25	10.00
		-	-	-	-	-	-	-	-	-	-	-	-
26	*Stachybotrys sp.*	-	-	-	4.76	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
27	*Syncephalastrum sp.*	-	-	-	9.52	12.00	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
28	*Trichoderma viridae*	-	-	-	-	7.71	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	25.50

Table 2: Effect of culture filtrate on seed germination and disease symptoms on B. juncea cv Pusabold.

Sr. No.	Name of Organism	Percent Germination	% Change over control	Symptoms
1	a*	95.00	0.00	-
	Control b*	95.00	0.00	-
	c*	95.00	0.00	-
2	*Alternaria alternata*	90.00	-5.26	-
		87.00	-8.42	-
		82.50	-13.16	Weak root
3	*Aspergillus flavus*	91.00	-4.21	-
		86.50	-8.95	Weak root
		76.00	-20.00	Yellowing of leaves
4	*A. fumigatus*	93.00	-2.11	-
		88.00	-7.37	-
		85.00	-10.53	Stunted growth
5	*A. niger*	90.00	-5.26	-
		87.00	-8.42	-
		82.50	-13.16	Dark brown spot near emergence of root
6	*Curvularia lunata*	93.50	-1.58	-
		90.00	-5.26	-
		86.00	-9.47	Growth checked
7	*Fusarium moniliforme*	93.00	-2.11	-
		89.50	-5.79	Growth checked
		87.00	-8.42	Seedling rot
8	*Penicillium oxalicum*	90.50	-4.74	-
		87.50	-7.89	-
		80.50	-15.26	No primary root

a* - 7 days of incubation , b* - 14 days of incubation
c* - 21 days of incubation

Fungal organisms recorded only in winter were *Alternaria brassicae, Aspergillus glaucus, Chaetomium bostrychodes, Curvularia lunata, Fusarium moniliforme, Penicillium frequentans,* and *Rhizopus nigricans*. The fungi, confined to summer season, were *Aspergillus candidus, A. nidulans, A. versicolor, Aureobasidium sp., Penicillium multicolor, Penicillium notatum, Phoma lingum, Pythium sp.,* and *Syncephalastrum racemosum*. The fungi occurring rarely during storage period were *Haplosporangium sp., Memnoniella sp., Phytophthora undulata, stachybotrys sp.* and *Trichoderma viridae.*

The fungi occurring throughout the year showed varying percentage of incidence. These fungi are obviously more versatile in their food requirement and capacity of tolerance for varying environmental conditions. Bilgrami *et al.* (1979) studied seasonal variation in mixed seed samples of mung, urad, masoor and gram. Sahay (1988) studied the incidence of fungi on mustard seeds with respect to winter, summer and monsoon season of the year.

Effect of fungal filtrates on germination of seeds and effect on seedling growth given in Table 2. As per methodology stated earlier, the seed samples were inoculated with fungal filtrates of test fungi, incubation for 7, 14 and 21 days. The results obtained from present study indicates that the test fungi under study exerted marked effect on percentage of seed germination and symptom appearance at seedling stage of Brassica crops. Control seeds showed 95.00% seed germination. The effects exhibited by test fungi are as follows.

Alternaria alternata - Seed infestation with these test fungi shows reduction in germination i.e. –5.26%, -

8.42%, -13.16% with 7, 14 and 21 days old culture filtrates. There was normal growth with 7 and 14 days old fungal filtrate treatment while with 21 days filtrate weak root of seedlings were noted.

Aspergillus flavus – It shows maximum reduction i.e. –4.21%, 8.95% and –20.00% over control with 7, 14 and 21 days old culture filtrate treatment. 14 and 21 days old filtrate exhibited with weak root and yellowing of cotyledonary leaves of seedlings.

Aspergillus fumigatus – It shows reduction in seed germination with increase in incubation period. The seedlings grows normally for 7 and 14 days, but 21 days old filtrate shows stunted growth of seedlings.

Aspergillus niger – It decreased the seed germination with increase in incubation of fungi. 21 days old fungal filtrate showed dark brown spot near the emergence of root.

Curvularia lunata – treated seeds shows reduction i.e. –1.58%, -5.26% and –9.47% with respect to 7, 14 and 21 days incubation of fungi followed by checking the growth of seedlings with 21 days old fungal filtrate treatment.

Fusarium moniliforme – It shows less effect on germination of seeds. However 21 days old fungal filtrate treatment shows –8.42% reduction exhibited with seedling root.

Penicillium oxalicum – As the incubation period increased, there was gradual reduction in seed germination. 21 days fungal filtrate treated seeding exhibited with absence of primary roots.

Loss of viability is sensitive indicator of deterioration. Under some circumstances, at least, fungi are primary causes of loss of viability in seeds (Christensen, 1973). He also reported that the embryo of cereal grains is often preferentially invaded by fungi, mostly species of *Aspergillus* and *Penicillium.* Loss in seed viability culture filtrates reported by Arya *et al*. (1989), Bhajbhuje and Thakre (1989) and Barve (1995).

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