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STUDIES ON SEED BORNE MYCOFLORA AND EFFECT OF BIOAGENTS AND FUNGICIDES ON WHEAT SEED HEALTH

P. R. BHOYAR[1], V. D. CHANDANKAR[1], V. L. BAGDE[2], DIPALI B. BORKAR[2] AND J. S. SONONE[1]
[1]Department of Plant Pathology, Post Graduate Institute,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola - 444 104, M.S., INDIA
[2]Biotechnology centre, Post Graduate Institute,
Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola - 444 104, M.S., INDIA
e-mail:

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***Corresponding author**

ABSTRACT
The study revealed that, untreated and pre-treated seed samples exhibited association of eight fungi *Viz Alternaria altermata*; *Alternaria triticina*; *Aspergillus flavus*; *Aspergillus niger*; *Bipolaris sorokiniana*; *Curvularia lunata*; *Drechslera tetramera* and *Fusarium semitectum* belonging to six genera. Less association of seed borne fungi was exhibited by pre-treated seed samples over untreated ones. For arresting the mycelia growth fungicidal seed treatments *Viz*. Thiram (0.3%), Carbendazim (0.1%), Thirum + Carbendazim (2:1) and Carboxin (0.2%) were found significantly superior over rest of the treatments. Increased seed germination, shoot and root length and seedling vigor index was observed in Thirum + Carbendazim (2:1) and Carboxin (0.2%) seed treatments followed by *Tricoderma harzianum* Rifai seed treatment.

INTRODUCTION

Wheat (Triticum aestivum L.) family Poaceae is one of the major widely cultivated cereal food crops in India and world. It is one of the most important staple foods of man and is grown in almost all the temperate and sub-tropical region of the world.

Primary source of the infection for some of the diseases is the grain itself (Ali and Fakir, 1982). Seed borne pathogen may cause seed abortion, seed rot, seed necrosis or reduction in germination as well as seedling damage by systemic or local infection resulting in the development of disease at later stages of plant growth (Khanzada *et al.*, 2002).

Seed health play an important role for successful cultivation and yield exploitation of a crop species and seed borne pathogens of wheat are responsible to cause variation in plant morphology and reducing yield up to 15-19 per cent if untreated seeds are grown in the field (Wiese, 1984).

Seed borne mycoflora of wheat reported recently included *Alternaria alternata, Drechslera sorokiniana, Fusarium monilliforme, Fusarium avenacearum, Fusarium gramineanum, Fusarium nivale, Fusarium culmorum, Fusarium equiseti, Fusarium sporotirchaids, Cladosporium herbarum, Stemphylium botryosum* (Glazek, 1997 and Mirza *and* Qureshi, 1978).

The presence of the mycelium in seed indicating that disease is internally seed borne (Kumar and Arya, 1973). *Alternaria triticina* Prasada and Prabhu causing leaf blight of wheat is externally as well as internally seed borne (Shabana and Kumar, 2001).

The severities of infection of individual seed fungus differ depending upon the varieties and the location (Singh *et al.*, 1977). The severity of infection by seed borne fungus differs with varieties and locations. Many scientists made attempts to minimize the several pathogens on wheat seed to increase the economic yield (Lodhi *et al.*, 2002; Basak *et al.*, 1987; Ravi *et al.*, 1999; Sudhirkumar and S. C. Jain, 2004).

Present investigation were undertaken for detection of seed borne mycoflora and efficacy of bioagents and fungicides with storage study.

MATERIALS AND METHODS

Seed samples of twenty four wheat cultivars were collected from four different locations *viz*. Akola, Washim, Niphad and Wellington. These seed samples were soaked in 0.1 % $HgCl_2$ solution for one minute followed by three times washing with sterile distilled water (Bharti, 2000).

The seeds were soaked in distilled water as a control treatment. These seed samples were used for detection of seed borne mycoflora by using standard blotter paper method with some modifications (ISTA, 1985 and Goulart, 1998).

For this test, 400 seeds of each sample (25 seeds / plate) were placed on three layers of moisten blotter papers in plastic Petri plates (90 mm diameter). These plates were incubated at 27 ± 2ºC for seven days. After seven days of incubation fungal

species growing on the surface of seeds were identified and their per cent frequency (PF) of occurrence was calculated by applying the following formula (Javaid *et al.*, 2006)

PF = (No. of seeds on which fungus appear/ Total No. of Seeds) x100

Efficacy of fungicides *viz.*, Thiram, Carbendazim, Thiram+Carbendazim (2:1), Carboxin, Champonion, Curzate M-8, Benomyl and Chlorothalonil (Sudheerkumar and S. C. Jain, 2004) and bioagents *Trichoderma harzianum*, *Pseudomonas fluorescens, Bacillus subtilis and P. fluorescens + B.subtilis* (1:1) were tested against naturally infected wheat seeds. These treated seeds were kept for one month storage at room temperature in cotton bags while untreated seeds were served as control (Machenahalli *et al.,* 2014; Ravi *et al.*, 1999).

After one month of storage period, 400 seeds of each treatment were tested by blotter method for reduction of fungus incidence. Also seeds of each treatment were sown equidistance on two layer of moist paper towel of 45 x30 cm^2 which folds and kept in growth chamber for seven days. At the end of incubation period, observations of seedling vigour index were calculated (Shakshi Singh, *et al.,* 2014. Gilbert and Tekauz, 1997).

RESULTS AND DISCUSSION

Eight fungal species namely, *Alternaria alternata, A. triticina, Bipolaris sorokiniana, Curvularia lunata, Drechslera tetramera, Aspergillus flavus, A. niger and Fusarium semitectum* were detected from twenty four wheat cultivars seeds by standard blotter paper methods and result were given in Table 1. It was observed that when seeds were pre treated with 0.1% of $HgCl_2$ showed drastic decline in the incidence of seed borne mycoflora as compared to untreated seeds.

Incidence *of Alternaria alternata* (41.5%) was dominant followed by *A. triticina* (38.5%) on seeds of cultivars collected from Wheat Research Unit, Akola. While highest incidence of *Aspergillus flavus* (37%) followed by *A. niger* (31.75%) were observed on seed of cultivars collected from ARS, Washim and *A. alternata* (31.5%) followed by *A. niger* (21.25%) on ARS, Niphad. Among the six cultivars received from IARI Regional Station, Wellington, highest incidence of *Aspergillus flavus* (24.5%) followed by *A. niger* (23%) were observed.

The highest frequency of seed mycoflora was observed on wheat cultivar AKAW-3722 (Vimal) followed by WSM-1472 and lowest fungal frequency was recorded NIAW-1621 followed by NIDW-295. Similar fluctuations in incidence of seed mycoflora were observed by Rajput *et al.* (2005) on wheat cultivars from Sindh region of Pakistan and Singh *et al.* (1977) from seven states of India.

The effect of bioagents and fungicides on the incidence of seed mycoflora after one month of storage was tested by blotter paper test and data obtained is given in Table 2. The treatments of Thiram+ Carbendazim (2:1) and Carboxin (0.2%) were most effective in reducing the incidence of seed born mycoflora (100 %) followed by *T. harzianum* (70.58 %). Whereas seeds was kept for storage study at one month storage, highest seed germination (78.33%) was observed in treatment of thiram+ carbendazim (2:1) followed by *Pseudomonas fluorescens*

Table 1: Incidence of seed-borne fungi with untreated and pretreated (0.1 per cent $HgCl_2$) wheat seeds tested by blotter paper method

Location	Name of variety/Genotype	Seed borne fungi																Total fungi (variety-wise)	
		Alternaria alternate		*Alternaria Triticina*		*Aspergillus flavus*		*Aspergillus niger*		*Bipolaris sorokiniana*		*Curvularia lunata*		*Drechslera Tetramera*		*Fusarium semitectum*			
		UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT
Akola	AKAW-3722 (Vimal)	9.5	3.5	12.5	5.0	14.0	5.0	9.0	3.0	2.75	-	2.0	-	-	-	4.25	1.0	54.0	17.5
	AKDW-4021	5.25	3.0	6.5	2.5	3.0	1.0	3.0	1.0	-	-	-	-	-	-	-	-	17.75	7.5
	AKAW-4073	7.0	2.5	4.25	3.0	7.25	2.0	6.25	2.5	-	-	4.25	1.0	4.0	2.5	-	-	33.0	13.5
	AKAW 1071(Puma)	7.5	2.0	5.0	3.0	-	-	-	-	2.5	1.0	-	-	2.0	-	4.0	1.5	21.0	7.5
	AKDW-4432-3	5.75	3.5	5.25	2.5	4.75	2.0	4.5	1.5	-	-	2.5	-	1.25	0.5	-	-	24.0	10.0
	AKDW-3931-2	6.5	2.5	5.25	2.0	5.5	2.0	3.75	2.0	2.25	1.0	1.25	-	-	-	4.0	1.0	28.0	10.5
	Total Fungi (Species-wise)	41.5	17.5	38.5	18.0	34.5	12.0	31.75	10.0	7.5	2.0	10.0	1.0	7.25	3.0	12.2	3.5	183.5	67.0
Washim	Bijga yellow	1.5	-	4.5	1.5	6.5	3.0	4.5	2.0	1.25	-	-	-	-	-	4.0	1.0	22.25	7.5
	PDKV WSM-1472	6.5	4.0	4.5	2.75	7.0	2.5	7.75	3.0	2.25	1.0	3.25	1.0	2.75	1.0	2.5	-	36.5	14.75
	MACS-1967	3.0	1.0	4.0	1.5	6.75	2.5	5.75	1.5	-	-	-	-	-	-	2.0	-	21.0	14.75
	N-59	3.5	-	2.5	1.0	3.0	-	2.5	-	1.5	0.5	-	-	1.75	-	-	-	14.75	1.5
	AKAW-3997	6.0	3.5	5.0	2.5	9.0	3.0	7.25	4.0	2.5	0.5	-	-	-	-	3.0	-	32.75	13.5
	NI-5439	4.0	1.0	2.5	1.0	4.75	-	4.0	-	-	-	-	-	-	-	-	-	14.15	2.0
	Total Fungi (Species-wise)	24.5	9.5	23.0	10.25	37.0	11.0	31.75	10.5	7.5	2.0	3.25	1.0	4.5	1.0	11.5	1.0	143	46.25

Table 1: Continue……

Location	Name of variety/ Genotype	Seed borne fungi *Alternaria alternata*		*Alternaria Triticina*		*Aspergillus flavus*		*Aspergillus niger*		*Bipolaris sorokiniana*		*Curvularia lunata*		*Drechslera tetramera*		*Fusarium semitectum*		Total fungi (variety-wise)	
		UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT	UN	PT
Niphad	NIDW-612	6.25	1.75	2.0	-	1.25	-	2.5	-	-	-	-	-	-	-	-	-	12.0	1.75
	NIDW-295	6.0	2.0	-	-	2.25	-	1.5	-	-	-	-	-	-	-	-	-	9.75	2.0
	NIAW-1621	2.5	0.5	-	-	4.25	0.5	2.25	-	-	-	-	-	-	-	-	-	9.0	1.0
	NIAW-1415	5.75	1.5	-	-	4.25	1.5	3.5	-	-	-	-	-	-	-	-	-	13.5	3.0
	NIAW-1609	3.5	1.5	-	-	2.0	-	4.75	1.5	1.0	-	-	-	-	-	-	-	10.25	3.0
	NIDW-577	7.5	2.0	-	-	2.5	-	2.75	-	-	-	-	-	-	-	-	-	12.75	2.0
	Total Fungi (Species-wise)	31.5	7.25	2.0	-	16.5	1.5	21.25	1.5	1.0	-	-	-	-	-	-	-	71.25	10.25
Wellington	HW-2045	3.0	1.0	4.0	1.0	4.5	-	5.5	1.75	1.0	-	-	-	1.5	-	-	-	19.5	3.75
	HW-5207	5.5	2.0	3.5	1.0	5.0	-	2.5	-	1.5	-	-	-	-	-	-	-	17.5	3.0
	HW-5001	2.5	0.5	-	-	3.75	-	4.5	1.0	-	-	-	-	-	-	-	-	12.25	1.5
	HD-2833	4.5		-	-	4.0	-	3.0	-	-	-	-	-	-	-	-	-	11.5	1.0
	HW-2044	2.5	0.5	3.5	-	5.0	1.0	3.5	-	-	-	-	-	-	-	-	-	14.5	1.5
	COW-(W)-1	4.5	1.0	2.5	1.0	3.0	-	4.0	0.5	3.5	-	-	-	-	-	-	-	14.0	2.5
	Total Fungi (Species-wise)	22.5	6.0	13.5	3.0	24.5	1.0	23.0	3.25	6.0	-	-	-	1.5	-	-	-	88.5	13.25

UN –Untreated, PT-Pretreated

Table 2: Effect of Bioagents and Fungicides on longevity of seed borne fungi and seed health of wheat (one month after storage)

Treatments	Dose(g/ kg seed)	Per cent fungi associated with seed								Total Fungi(%)	Reductiono ffungi over control (%)	Germination(%)	Shoot length (cm)	Root length(cm)	Seedling vigour index
		Aa	At	Af	An	Bs	Cl	Dt	Fs						
T. harzianum	4.0	1.5	2.0	2.0	1.5	-	-	-	0.5	7.5	70.58	73.66 (59.13)*	9.18	8.53	1304
P. fluorescens	10.0	1.25	1.0	2.0	2.0	1.0	0.75	-	-	8.0	68.62	75.66 (60.47)*	9.70	9.15	1426
B. subtilis	10.0	1.5	0.75	1.0	2.0	-	1.0	1.0	1.0	8.25	67.64	75.00 (60.06)*	9.05	8.74	1334
P. fluorescens + B.subtilis (1:1)	10.0	1.75	1.25	1.5	1.75	1.0	1.0	-	-	7.75	69.60	74.00 (59.36)*	9.30	8.64	1324
Thiram	3.0	1.25	1.0	1.25	1.25	-	-	-	-	4.75	81.37	77.00 (61.35)*	9.26	8.22	1345
Carbendazim	1.0	1.0	1.0	1.25	2.0	-	-	-	-	5.25	79.41	77.33 (61.59)*	8.82	8.24	1319
Thiram + Carbendazim (2:1)	3.0	-	-	-	-	-	-	-	-	0.00	100	78.33 (61.82)*	8.62	8.18	1315
Carboxin	2.0	-	-	-	-	-	-	-	-	0.00	100	77.00 (61.82)*	8.69	8.15	1296
Champonion	3.0	1.0	1.75	1.25	1.75	-	1.0	-	1.0	7.75	69.60	74.00 (61.35)*	8.62	8.29	1251
Curzate M-8	2.0	2.0	1.25	1.75	2.0	-	-	-	1.25	8.75	65.68	72.00 (58.05)*	8.79	8.86	1270
Benomyl	1.0	1.0	1.25	1.25	1.0	-	-	-	-	5.5	78.43	74.00 (59.36)*	8.31	8.42	1238
Chlorothalonil	1.0	2.0	1.5	2.0	1.5	1.0	2.0	1.25	1.0	9.75	61.76	70.00 (56.79)*	8.54	8.63	1201
Control	-	4.5	2.75	4.75	4.0	2.0	-	1.5	4.0	25.5	-	68.00 (55.34)*	7.33	7.48	1007
'F' test												Sig	Sig	Sig	Sig
SE(m)												1.43	0.22	0.18	45.29
CD (P = 0.01)												5.62	0.77	0.55	158.51

* Arc sine values; *Aa- Alternaria alternata, At- Alternaria triticina, Af- Aspergillus flavus, An-Aspergillus niger, Bs- Bipolaris sorokiniana, Cl- Curvularia lunata, Dt- Drechslera tetramera, Fs- Fusarium semitectum.*

Table 3: Effect of Bioagents and Fungicides on longevity of seed borne fungi and seed health of wheat (two month after storage)

Treatments	Dose(g/kgseed)	Per cent fungi associated with seed								Total Fungi(%)	Reduct-ionofb fungi over control (%)	Germination(%)		Shoot length (cm)	Root length (cm)	Seedling vigour index
		Aa	At	Af	An	Bs	Cl	Dt	Fs							
Trichoderma harzianum	4.0	1.0	1.0	1.5	1.25	-	-	-	-	5.25	73.41	73.00(58.69)*		9.02	8.51	1279
PseudomonasFluorescens	10.0	1.25	0.75	2.25	1.25	-	-	-	-	5.5	72.15	73.66(59.13)*		8.93	9.05	1324
Bacillus subtilis	10.0	1.0	-	1.25	1.5	-	0.5	0.5	1.25	6.0	69.62	73.33(58.93)*		9.11	8.07	1259
P. fluorescens + B.subtilis (1:1)	10.0	1.5	1.25	1.0	1.5	0.75	0.75	-	-	5.75	70.88	73.33(58.93)*		8.63	8.17	1231
Thiram	3.0	1.0	0.5	1.0	1.0	-	-	-	-	3.5	82.27	77.00(61.35)*		8.64	8.13	1291
Carbendazim	1.0	0.5	0.5	0.75	1.5	-	-	-	0.75	3.25	83.54	76.33(60.90)*		8.67	8.17	1285
Thiram + Carbendazim (2:1)	3.0	-	-	-	-	-	-	-	-	0.00	100	77.66(61.82)*		8. 45	7.62	1247
Carboxin	2.0	-	-	-	-	-	-	-	-	0.00	100	76.33(60.90)*		8.90	7.86	1279
Champonion	3.0	1.0	1.0	1.0	1.0	-	0.5	-	1.25	5.75	70.88	73.00(58.69)*		8.62	8.23	1230
Curzate M-8	2.0	1.5	1.0	1.25	1.75	-	-	-	1.0	6.5	67.08	71.33(57.64)*		8.49	8.44	1207
Benomyl	1.0	0.75	1.0	0.75	0.75	-	-	-	-	3.25	83.54	74.00(59.36)*		8.18	8.15	1208
Chlorothalonil	1.0	1.0	1.0	1.5	1.0	1.0	-	1.25	0.5	7.5	62.02	69.66(56.58)*		8.34	8.22	1153
Control	-	3.0	2.75	4.0	3.25	1.5	1.5	1.25	3.25	19.75	-	67.33(55.14)*		7.14	7.38	977
'F' test												Sig		Sig	Sig	Sig
SE(m)												1.21		0.25	0.18	34.45
CD (P=0.01)												4.79		0.76	0.54	137.8

* Arc sine values; *Aa- Alternaria alternata, At- Alternaria triticina, Af- Aspergillus flavus, An-Aspergillus niger, Bs- Bipolaris sorokiniana, Cl- Curvularia lunata, Dt- Drechslera tetramera, Fs- Fusarium semitectum.*

Table 4: Effect of Bioagents and Fungicides on longevity of seed borne fungi and seed health of wheat (three month after storage)

Treatments	Dose(g/kgseed)	Per cent fungi associated with seed								Total Fungi(%)	Reduct-ionoffungi over control (%)	Germination(%)	Shoot length (cm)	Root length (cm)	Seedling vigour index
		Aa	At	Af	An	Bs	Cl	Dt	Fs						
Trichoderma harzianum	4.0	1.0	1.0	1.5	1.0	-	-	-	-	4.5	75.00	73.00(58.69)*	9.00	8.45	1273
Pseudomonasfluorescens	10.0	0.75	0.75	1.75	1.0	-	-	-	-	4.25	76.92	73.66(59.13)*	8.44	8.86	1274
Bacillus subtilis	10.0	0.5	-	0.75	1.5	-	0.5		0.75	4.0	77.77	73.33(58.93)*	9.02	7.88	1239
P. fluorescens + B.subtilis (1:1)	10.0	1.25	0.5	1.0	1.25	-	0.5	-	-	4.0	75.00	73.33(58.93)*	8.37	8.0	1200
Thiram	3.0	0.5	0.5	1.0	1.0	-	-	-	-	3.0	83.33	75.33(60.22)*	8.56	8.04	1250
Carbendazim	1.0	-	-	0.5	1.0	-	-	-	-	1.5	91.66	74.33(59.58)*	8.59	8.13	1242
Thiram + Carbendazim (2:1)	3.0	-	-	-	-	-	-	-	-	0.00	100	75.33(60.22)*	8.18	7.63	1190
Carboxin	2.0	-	-	-	-	-	-	-	-	0.00	100	76.00(60.61)*	8.67	7.64	1239
Champonion	3.0	1.0	0.75	-	1.25	-	-	-	0.75	3.75	79.16	72.33(59.84)*	8.56	8.03	1199
Curzate M-8	2.0	1.0	1.0	1.0	1.5	-	-	-	1.0	5.5	69.44	71.33(57.64)*	8.14	8.53	1189
Benomyl	1.0	0.5	0.75	0.5	0.5	-	-	-	-	2.25	87.50	72.00(59.15)*	7.95	8.02	1149
Chlorothalonil	1.0	1.25	1.0	1.75	1.75	-	-	0.5	0.5	6.5	63.88	69.33(56.15)*	8.14	8.09	1125
Control	-	2.25	2.5	3.0	3.0	1.5	1.25	1.25	3.25	18.00	-	67.00(54.93)*	7.10	6.99	944
'F' test												Sig	Sig	Sig	Sig
SE(m)												1.22	0.15	0.16	29.34
CD (P=0.01)												4.80	0.47	0.49	117.36

* Arc sine values; *Aa- Alternaria alternata, At- Alternaria triticina, Af- Aspergillus flavus, An-Aspergillus niger, Bs- Bipolaris sorokiniana, Cl- Curvularia lunata, Dt- Drechslera tetramera, Fs- Fusarium semitectum.*

(75.66%). Kamble *et al*. (1999) also reported similar results of fungicides seed treatment while working of vegetable seeds.

Data presented in the Table 3 revealed that when treated and untreated seed were tested by blotter method with two month after storage the fungicides reduced the incidence of seed borne fungi. No association of fungi were found in the treatment of thiram+ carbendazim (2:1) and carboxin (100%) followed by *T. harzianum* (73.41 %). Whereas seeds was kept for storage study at two month storage, highest seed germination (77.66%) was observed in treatment of thiram+ carbendazim (2:1) followed by *Pseudomonas fluorescens* (73.66%).

Data presented in the Table 4 revealed that when treated and untreated seed were tested by blotter method with three months after storage the fungicides reduced the incidence of seed borne fungi. No association of fungi were found in the treatment of thiram+ carbendazim (2:1) and carboxin (100%) followed by *B. subtillis* (77.77 %). Whereas seeds was kept for storage study at three months storage, highest seed germination was observed in treatment of carboxin (76%) followed by *Pseudomonas fluorescens* (73.66%). Srinivas *et al*. (2005) also reported increase in seed germination and seedling vigour index following seed treatment with bioagents and fungicides in brinjal.

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