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Management of Seed Borne Mycoflora of Wheat (*Triticum Aestivum* L.) Using Fungicides and Bioagent

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Abstract

The present investigation was carried out on the "Studies of Seed borne Mycoflora of Wheat (*Triticum aestivum* L.)", with a view to assess seed borne mycoflora associated with the seeds of wheat, their effect on seed germination and seedling vigour index, effects of fungicides and bioagents on seed borne mycoflora, seed germination and seedling vigour index All the isolated pathogens were found pathogenic to wheat resulting into reduction in seed germination and seedling vigour index. The seed borne mycoflora *viz*., *Aspergillus falvus, Aspergillus niger, Alternaria alternata, Fusarium oxysporum* and *Curvularia lunata* were found more dominant and damaging. Among the treatments of fungicides and bioagents fungicidal seed treatments were found significantly superior in reducing seedborne mycoflora, increasing seed germination and seedling vigour index. Among fungicides Tebuconazole @ 50 % + Tryfloxystrobin @ 25% WG @ 0.1% were found most effective against *Aspergillus niger*, *Aspergilus flavus* and *Fusarium oxysporum*, whereas Carboxin 37.5% + Thiram 37.5% DS @ 0.3% were found most effective against *Curvualria lunata* and *Alternaria alternata*. Among bioagents MPKV Fungal Consortium @ 10 gm kg^{-1} seed were found most effective against all detected seed mycoflora of wheat.

Key words : Bioagent, Seedborne mycoflora, Vigour index, Seed germination.

Wheat (*Triticum aestivum* L.) is one of the main cereals. Wheat belongs to the family Poaceae. For centuries bread wheat (*Triticum aestivum*) and durum wheat (*Triticum durum*) have been cultivated in the West to provide humans with energy and essential nutrients. Wheat crop has wide adaptability. Which rank in the first place, in production among the grain yields of the world It can be grown not only in the tropical and sub-tropical zone but also in the temperate zone. Studies were carried out to study the composition of seed borne mycoflora occurring in wheat. It has been found that the seed-borne fungi generate a considerable effect on the reduction of crop productivity as the external and internal distribution of seed-borne pathogens causes seed abortion, seed necrosis, elimination of seed germination capacity, and damage to seedlings.

Seed borne mycoflora is one of the major components reducing the wheat yield. Mycoflora associated with seeds both internally and externally are responsible for seed abortion, mortality of grains, reduction in germination capacity, seed necrosis and at the end cause destructive to serious diseases during different stages of plant growth (Niaz and Dawar, 2009). Yield losses due to seed borne fungi have been reported between 15 to 90% of untreated seeds grown in field. Seed borne pathogens of wheat include *Alternaria alternata, Cladosporium oxysporum, Curvularia lunata, Drechslera sorokiniana, D. tetramera, Fusarium graminearum, Helminthosporium sativum*, and post-harvest fungi include species of *Aspergillus* and *Penicillium*. Genera of *Tilletia, Ustilago, Bipolaris, Fusarium, Alternaria,*

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Drechslera, Stemphylium, Curvularia, Cladosporium, Rhizopus, Aspergillus and *Penicillium* has been the most common isolated fungi from wheat seeds. Major impact of seed borne diseases in wheat is not only the yield reduction but also deteriorate marketable quality of grains. Early detection of pathogens is a crucial step in diagnosis and management programmes in wheat. The present investigation is carried out to detect various seed mycoflora on wheat.

Material and Methods

The seeds of wheat varieties/genotypes will be collected from ARS, Niphad. The ISTA's standard blotter test as described by Neergaard (1979) was used for detection of external seed borne mycoflora of Wheat. ISTA's standard agar plate method was used to detect internally seedborne mycoflora associated with seeds of the wheat variety. (Neergard 1979). Kotchs postulate was used for proving the pathogenicity.

The effect of seed borne mycoflora of Wheat on seed germination and seedling vigour index was studied by adopting the procedure of rolled towel paper described by ISTA (Anonymous, 1999). Total four hundred seeds of wheat variety were inoculated with the individual pathogen by dipping the seeds in concentrated spore suspension (106 cfu ml^{-1}) for 12 hours. Then these inoculated seeds were dried in shade for 12 hours (Agarwal and Sinclair, 1993). On one towel paper, fifty seeds were placed and rolled carefully in order to avoid disturbances of seeds from their places. For each pathogen, eight towel papers were used, each with 50 seeds (for a total of 400 seeds). In a seed germinator, the wrapped towel papers were kept in a slanting position andincubated at 20-25°C with a RH over 85%. (Anonymous, 1999). After seven days, a count of normal seedlings was recorded. Germination was estimated in percentages for normal seedlings with full radical and plumule development. The root and shoot length (cm) of randomly selected ten normal seedlings from each towel paper were measured with the use of scale and seedling vigour index was calculated using formula which was given by Abdul-Baki and Anderson (1973).

SVI = [Mean root length (cm) + Mean shoot length (cm)] x Seed Gemination (%)

The per cent reduction in seed germination and seedling vigour index of seeds artificially inoculated with the pathogens over control was calculated. The data was statistically analysed (Panse and Sukhatme, 1985).

The seed germination and seedling vigour index was studied by adopting the procedure of rolled towel paper described by ISTA (Anonymous,1999)

The effect of fungicides and bioagents on artificially inoculated seeds was evaluated using the standard blotter test previously described. Under aseptic conditions, the seedsof the wheat variety Mann were plated in petriplates according to the treatments. Seeds that had not been treated served as a control. In each treatment, total 400 seeds were used. With four replications, the experiment was performed using a Completely Randomized Design (CRD). For 7 seven days, the plates were incubated in an incubation room at 20 ± 2°C with an alternating cycle of 12 hours darkness and 12hours light. On the eighth day, the number of seeds infected with mycoflora was observed using asterio-binocular microscope, the per cent incidence of each mycoflora was recorded and the resultswere analysed statistically. The artificially inoculated seeds treated with fungicides, bioagents and botanicals were kept for germination by using between paper method as described earlier. Untreated seeds servedas control. Seed germination, root and shoot

Table 1. Efficacy of seed treatment of fungicides on incidence of seedborne (artificially inoculated to seed) and their effect on seed germination and seedling vigour index of wheat

Treatments	Incidence of Seedborne mycoflora (%)					Reduction in incidence over control (%)					Seed germination (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Carbendazim 50% WP @ 0.2%	16.67 (24.09)	16.00 (23.57)*	13.00 (21.13)*	17.00 (24.35)*	17.33 (24.60)*	63.77	64.18	68.22	65.54	65.57	76.33 (63.91)	81.00 (64.15)*	82.00 (64.89)*	81.67 (64.64)*	78.67 (62.49)*
Thiophanate methyl 70% WP @ 0.2%	21.67 (27.74)	21.33 (27.50)	19.33 (26.08)	23.33 (28.88)	23.33 (28.88)	52.42	52.24	52.73	52.70	53.65	76.30 (60.89)	78.33 (62.25)	79.67 (63.19)	79.00 (62.72)	75.00 (60.00)
Carboxin 37.5% + Thiram 37.5% @ 0.3%	4.00 (11.53)	4.60 (12.38)	4.00 (11.53)	8.33 (16.57)	6.17 (14.37)	91.30	89.70	90.22	83.11	87.75	90.33 (71.88)	89.00 (70.63)	94.33 (76.22)	89.00 (70.63)	87.67 (69.44)
Azoxystrobin 18.2% + Difenconazole 13.9% EC @ 0.1%	12.67 (20.84)	14.00 (21.97)	7.67 (16.07)	13.67 (21.69)	12.67 (20.88)	72.46	68.66	81.26	72.30	74.84	83.67 (66.16)	84.00 (66.42)	86.67 (68.58)	85.00 (67.21)	81.33 (64.40)
Picoxystrobin 6.78% + Tricycazole 20.33% EC @ 0.1%	13.00 (22.24)	15.00 (22.78)	12.97 (21.10)	15.33 (23.05)	13.67 (21.69)	71.74	66.42	68.30	68.92	72.85	82.33 (65.14)	81.00 (64.15)	84.67 (66.94)	84.33 (66.68)	80.67 (63.91)
Tebuconazole 50% + Tryfloxystrobin 25% WG @ 0.1%	8.00 (16.42)	3.00 (9.97)	4.17 (11.77)	6.33 (14.57)	4.83 (12.70)	82.61	93.28	89.81	87.16	90.40	89.33 (70.93)	92.00 (73.57)	88.00 (69.73)	91.33 (72.87)	90.67 (72.21)
Tebucanazole 45% WP @ 0.1%	18.67 (25.59)	17.33 (24.60)	18.00 (25.10)	19.23 (26.01)	20.33 (26.80)	59.90	61.20	55.99	61.01	59.61	76.33 (60.89)	79.00 (62.72)	80.67 (63.91)	81.00 (64.15)	77.00 (61.34)
Control	46.00 (42.70)	44.67 (41.93)	40.97 (39.79)	49.33 (44.61)	50.33 (45.19)	0.00	-	-	65.54	-	61.83 (51.84)	62.17 (52.04)	63.17 (52.63)	60.63 (51.13)	61.0 (51.35)
S. Em. ±	0.72	0.46	0.46	0.48	0.50						0.70	0.59	0.70	0.65	0.78
C.D. at 1%	2.19	1.41	1.41	1.47	1.53						2.13	1.79	2.11	1.99	2.36
C.V. (%)	7.08	4.78	5.40	4.44	4.72						1.52	1.26	1.47	1.39	1.71

length were recorded also seedling vigour index was calculated Using the between paper method (Towel paper method), the seed germination of wheat seeds treated with fungicides, bioagents and botanicals was studied. In the laboratory, the experiment was conducted in a Completely Randomized Design (CRD) with four replications. The data recorded in per cent to seed mycoflora and seed germination were converted intoarc sin values. The final data of seed mycoflora, seed germination and seedling vigour index was statistically analysed by standard statistical method i.e., Analysis of variance.

The standard error (SE) for above mentioned parameters were calculated and compared the treatments, critical difference (CD) at 5 per cent level of significance was worked (Panse and Sukhatme, 1985).

Results and Discussion

The results showed that five fungi externally on seed of wheat namely, *Alternaria alternata, Fusarium oxysporum, Curvularia lunata, Aspergillus niger* and *Aspergillus flavus* on Wheat seeds. These results are in compatible with Hajihasani *et al.* (2012), Pathak and Zaidi (2013), Khan *et al.* (2023). The results of internally seedborne mycoflora associated with wheat seeds showed that four seedborne mycoflora *viz*., *Fusarium oxysporum*, and *Alternaria alternata* were found internally associated with wheat seeds. All of the pathogens detected were found to be pathogenic to wheat seeds. These results are in simillar with, Bhoyar *et al.* (2014), Zafar *et al*. (2014), Ulziijargal *et al*. (2019). All of the pathogens detected were found to be pathogenic to wheat seeds. Seed rotting, post-emergence damping-off of seedlings and black, brown-grey or greenish fungal growth on seed and seedlings were observed symptoms of pathogenic organisms. These results are in conformity with, Hussain *et al.* (2013), Bhoyar *et al.* (2014) and Shabana *et al.* (2021).

Among fungicides tebuconazole 50 % + tryfloxystrobin 25 % WG @ 0.1 % were found most effective against *Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum*, highest reduction in the incidence with 87.16, 90.40, and 93.28 per cent as it found increase in seed germination 50.64, 48.63, and 47.98 per cent as well as seedling vigour index 57.63, 58.90 and 55.25 per cent respectively over the untreated control whereas carboxin 37.5% + thiram 37.5% DS @ 0.3% were found most effective against Curvularia lunata and Alternaria alternata highest reduction in the incidence 90.22 per cent and 91.30 per cent also it show increase in seed germination 49.33 per cent and 46.10 per cent as well as increase in seedling vigour index 54.13 per cent and 56.42 per cent over the control respectively.

Among bioagents fungal consortium @ 10 gm kg^{-1} seed were found most effective against all detected seed mycoflora Aspergillus flavus, Aspergillus niger, Alternaria alternata, Fusarium oxysporum and Curvularia lunata of wheat highest reduction in the incidence with 62.25, 61.81, 56.74, 70.24, 56.69 per cent as it has shown maximum increase in seed germination with 36.52, 36.00, 46.10, 31.35, 33.69 per cent as well as increase in seedling vigour index 41.07, 41.56, 41.07, 40.27, 37.31 per cent over the control respectively.The results obtained are in conformity with Mathew *et al.* (2011), Islam and Borthakur (2012), , Bhoyar *et al*. (2014), Islam *et al.* (2015), Mahmoud *et al.* (2015), Dolas *et al*. (2018)

Conclusions

All the isolated pathogens were found pathogenic to seeds of wheat resulting into reduction in seed germination and seedling vigour index. The seedborne mycoflora *viz*.,

Table 1. Contd.

Treatments	Increase in seed germination over control (%)					Seedling Vigour Index (SVI)					Increase in SVI over control (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Carbendazim 50% WP @ 0.2%	30.47	30.29	29.81	34.70	28.96	1322.67	1342.67	1362.67	1336.00	1282.67	39.47	38.66	37.88	43.91	41.21
Thiophanate methyl 70% WP @ 0.2%	23.40	26.00	26.11	30.30	22.95	1187.33	1207.33	1258.67	1167.33	1147.33	25.20	24.68	27.35	25.75	26.31
Carboxin 37.5% + Thiram 37.5% @ 0.3%	46.10	43.16	49.33	46.79	43.72	1483.33	1453.33	1523.33	1413.33	1393.33	56.42	50.09	54.13	52.24	53.40
Azoxystrobin 18.2% + Difenconazole 13.9% EC @ 0.1%	35.32	35.11	37.20	40.19	33.33	1381.33	1401.33	1448.00	1361.00	1341.67	45.66	44.72	46.51	46.61	47.71
Picoxystrobin 6.78% + Tricycazole 20.33% EC @ 0.1%	33.16	30.29	34.03	39.10	32.24	1351.33	1371.33	1391.33	1331.33	1311.33	42.50	41.62	40.78	43.41	44.37
Tebuconazole 50% + Tryfloxystrobin 25% WG @ 0.1%	44.48	47.98	39.31	50.64	48.63	1433.33	1503.33	1473.33	1463.33	1443.33	51.14	55.25	49.07	57.63	58.90
Tebucanazole 45% WP @ 0.1%	23.46	27.07	27.70	33.60	26.23	1257.00	1277.00	1296.00	1237.00	1227.00	32.55	31.88	31.13	33.25	35.08
Control		-	-		-	948.33	968.33	988.33	928.33	908.33	0.00	-	0.00		-
S. Em. ±						11.64	11.64	16.83	16.58	11.61					
C.D. at 1%						35.20	35.30	50.89	50.15	35.13					
C.V. (%)						1.55	1.53	2.17	2.24	1.60					

*Figures mentioned in parentheses indicates arc sin transformed values
Whereas; A: Alternaria alternata , B: Fusarium oxysporum, C: Curvularia lunata, D: Aspergilus niger, E: Aspergillus flavus

Table 2. Efficacy of seed treatment of bioagent on incidence of seedborne (artificially inoculated to seed) and their effect on seed germination and seedling vigour index of wheat

Treatments	Incidence of Seedborne mycoflora (%)					Reduction in incidence over control (%)					Seed germination (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Fungal consortium @ 5 gm kg^{-1} seed	21.33 (28.20)*	17.67 (24.85)*	20.00 (26.56)*	18.67 (25.59)*	19.33 (26.08)*	54.61	68.45	68.22	61.11	61.59	80.00 (62.25)*	81.00 (64.15)*	82.67 (65.39)*	82.00 (64.89)*	80.00 (63.43)*
Fungal consortium @ 10 gm kg^{-1} seed	20.33 (31.30)	16.67 (24.09)	18.33 (25.35)	18.33 (25.35)	19.00 (25.84)	56.74	70.24	52.73	61.81	62.25	81.00 (60.00)	82.00 (64.89)	84.00 (66.42)	83.00 (65.64)	81.00 (64.15)
Trichoderma viride @ 5 gm kg^{-1} seed	30.00 (33.62)	32.00 (34.44)	28.33 (32.16)	22.67 (28.43)	23.33 (28.88)	36.17	42.86	90.22	52.78	53.64	70.33 (58.69)	73.00 (58.69)	73.00 (58.69)	76.00 (60.66)	70.00 (56.78)
Trichoderma harzianum @ 5 gm kg^{-1} seed	29.67 (27.74)	30.33 (33.41)	26.67 (31.09)	21.67 (27.74)	23.34 (28.89)	36.88	45.83	81.26	54.86	53.63	72.00 (63.19)	76.00 (60.66)	75.00 (60.00)	76.33 (60.89)	72.00 (58.05)
Psudomonas flurosense + *Trichoderma viride* @ 5 gm kg^{-1} seed	24.00 (34.44)	20.33 (26.80)	24.67 (29.77)	20.67 (27.03)	21.67 (27.74)	48.94	63.69	68.30	56.94	56.95	75.00 (58.05)	78.00 (62.02)	75.00 (60.00)	76.67 (61.11)	75.00 (60.00)
Psudomonas flurosense + *Trichoderma harzianum* @ 5 gm kg^{-1} seed	23.67 (36.07)	18.67 (25.59)	22.00 (27.97)	19.67 (26.32)	20.33 (26.80)	49.65	66.67	89.81	59.03	59.60	77.00 (56.78)	80.00 (63.43)	79.67 (63.19)	78.33 (62.25)	77.00 (61.34)
Control	47.00 (43.47)	47.00 (43.28)	42.33 (40.58)	48.00 (43.85)	50.33 (45.19)	-	-	-	-	-	61.47 (51.53)	62.43 (52.19)	62.83 (52.43)	61.03 (51.37)	59.33 (50.37)
S. Em. ±	0.67	0.71	0.73	0.37	0.43						0.56	0.54	0.62	0.78	0.54
C.D. at 1%	2.04	2.18	2.28	1.15	1.33						1.71	1.67	1.92	2.39	1.68
C.V. (%)	4.12	4.73	4.95	2.70	2.98						1.31	1.24	1.42	1.77	1.29

Table 2. Contd.

Treatments	Increase in seed germination over control (%)					Seedling Vigour Index (SVI)					Increase in SVI over control (%)				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Fungal consortium @ 5 gm kg^{-1} seed	30.17	29.75	31.57	34.36	34.84	1413.00	1433.00	1453.00	1396.33	1413.00	40.27	39.49	36.56	41.43	40.27
Fungal consortium @ 10 gm kg^{-1} seed	31.79	31.35	33.69	36.00	36.52	1421.00	1441.00	1461.00	1397.67	1421.00	41.07	40.27	37.31	41.56	41.07
Trichoderma viride @ 5 gm kg^{-1} seed	14.44	16.93	16.19	24.53	17.98	1387.00	1407.00	1427.00	1366.67	1387.00	37.69	36.96	34.12	38.42	37.69
Trichoderma harzianum @ 5 gm kg^{-1} seed	17.15	21.74	19.37	25.08	21.36	1390.00	1410.00	1430.00	1370.00	1390.00	37.99	37.25	34.40	38.76	37.99
Psudomonas flurosense + *Trichoderma viride* @ 5 gm kg^{-1} seed	22.03	24.94	19.37	25.62	26.41	1395.00	1415.00	1435.00	1374.67	1395.00	38.48	37.74	34.87	39.23	38.48
Psudomonas flurosense + *Trichoderma harzianum* @ 5 gm kg^{-1} seed	25.28	28.14	26.80	28.35	29.78	1400.00	1420.33	1440.00	1380.00	1400.00	38.98	38.22	35.34	39.77	38.98
control	-	-	-	-	-	1007.33	1027.33	1064.00	987.33	1007.33	-	-	-	-	-
S. Em. ±		-			-	20.20	20.20	16.09	20.25	20.20					-
C.D. at 1 %						61.86	85.04	49.27	62.04	61.86					
C.V. (%)						2.86	2.56	2.00	2.64	2.60					

*Figures mentioned in parentheses indicates arc sin transformed values

Whereas; A: Alternaria alternata, B: Fusarium oxysporum, C: Curvularia lunata, D: Aspergilus niger, E: Aspergillus flavus

Aspergillus flavus, A. niger and *Alternaria alternata* were found more damaging. Among fungicides tebuconazole 50% + tryfloxystrobin 25% WG @ 0.1% which showed the higest reduction in the incidence, maximun increase in seed germination and seedling vigour index which found most effective against *Aspergillus niger, Aspergillus flavus* and *Fusarium oxysporum* among all fungicides. whereas carboxin 37.5% + thiram 37.5% DS @ 0.3% which showed the higest reduction in the incidence, maximun increase in seed germination and seedling vigour index of curuvalria lunata and Alternaria alternata over the inoculated control among all fungicides. Whereas, Tebuconazole 50% + Tryfloxystrobin 25% WG @ 0.1% and carboxin 37.5% + thiram 37.5% DS @ 0.3% they are statically at par to each other. Hence, both fungicides treatment effective among other. Among bioagents Fungal consortium @ 10 gm kg^{-1} seed was found most effective with the highest reduction in incidence, maximun increase in seed germination and seedling vigour index which found most effective against *Aspergillus flavus, Aspergillus niger, Alternaria alternata, Fusarium oxysporum* and *curuvulria lunata* respectively. The next best bioagents were Fungal consortium @ 5 gm kg^{-1} seed and *Psudomonas flurosense* + *Trichoderma harzianum* @ 5 gm kg^{-1} seed.

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