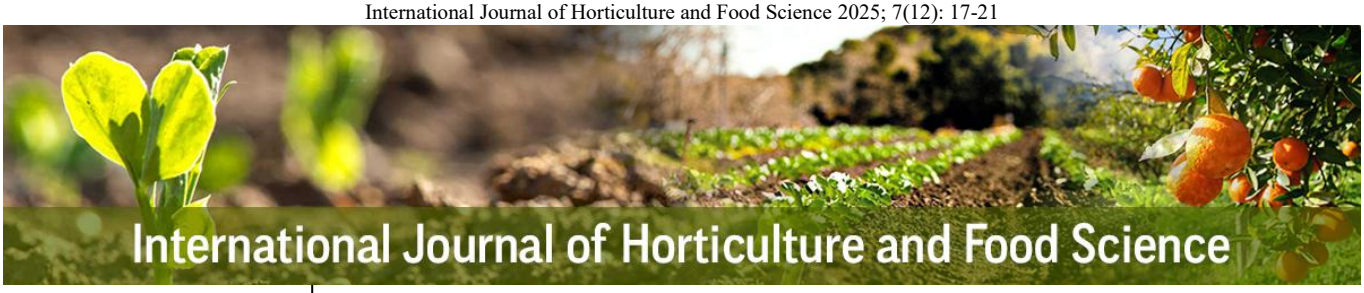

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HS Shirsath
Student (M.Sc. Agriculture), Department of Plant Pathology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India

TP Uchitkar
Student (M.Sc. Agriculture), Department of Plant Pathology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India

DH Sarnobat
Assistance Professor, Department of Plant Pathology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India

SR Zanjare
Senior Scientist (Seed Pathology), Department of Plant Pathology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India

Corresponding Author:
HS Shirsath
Student (M.Sc. Agriculture), Department of Plant Pathology, Post Graduate Institute, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahilyanagar, Maharashtra, India

Efficacy of fungicides and bioagents on incidence of seed borne pathogens associated with black point disease, their effect on seed germination and seedling vigour index of wheat

HS Shirsath, TP Uchitkar, DH Sarnobat and SR Zanjare

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Abstract
The present investigation was carried out on the **"**Studies on Seed borne Mycoflora of Wheat with Special Reference to Black Point Disease", with a view to assess seed borne pathogens associated with black point disease 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. Three seed borne pathogens associated with black point disease of wheat *viz., Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* were detected on wheat seeds. The internally two fungi identified were *Alternaria alternata* and *Drechslera sorokiniana.* All the isolated pathogens were found pathogenic to wheat resulting into reduction in seed germination and seedling vigour index. The seed borne pathogen *viz., Alternaria alternata,* was found more dominant and damaging. Among the seed treatments of fungicides and bioagents fungicidal seed treatments were found significantly superior in reducing pathogens associated with black point disease of wheat, increasing seed germination and seedling vigour index. Among fungicides Carboxin 37.5% + Thiram 37.5% DS @ 0.3% was found most effective against *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata.* Among bioagents *Trichoderma harzianum* @ 10g/kg seed was found most effective against all detected seed pathogens associated with black point disease of wheat.

Keywords: Bioagent, *Alternaria alternata*, *Drechslera sorokiniana*, vigour index, seed germination

Introduction
Wheat (*Triticum aestivum* L.), a principal member of the family *Poaceae*, is among the most significant cereal crops cultivated globally. As a fundamental dietary staple, wheat plays a vital role in ensuring food security by providing a substantial portion of the daily caloric and nutritional intake for human populations across the world. Optimal growth conditions for wheat include cool and dry climates, particularly during the germination and early vegetative phases. The ideal temperature range for germination lies between 20°C and 25°C. India is currently ranked as the second-largest producer of wheat globally, a position that underscores its strategic importance in the nation's food economy. According to the Department of Agriculture and Farmers Welfare, wheat production in India reached a record-breaking 113.29 million tonnes during the 2023-24 crop year. Numerous infections harm the wheat crop, with the majority being seed-borne. Karnal bunt (*Tilletia indica*), loose smut (*Ustilago nuda tritici*), head blight or scab (*Fusarium* spp.) and tundu or ear cockle (*Clavibacter tritici* and *Anguina tritici*) are the major seed borne diseases of wheat. One of the main factors lowering wheat production is seed borne mycoflora. Internally and externally, mycoflora related to seeds are the cause of seed abortion, grain mortality, decreased germination ability, seed necrosis, and ultimately, harmful to severe illnesses at various phases of plant development (Niaz and Dawar, 2009) [12].
Black point, also referred to as kernel smudge, is a notable seed borne disease of wheat that has been reported in multiple wheat growing regions globally, including across diverse agro-ecological zones in India. The disease is primarily characterized by dark brown to black discoloration, typically localized at the embryonic end of the wheat kernel. In mild to moderate infections, the discolouration is confined to this region; however, under severe conditions, the entire grain may exhibit extensive blackening, shrivelling, and deformation.

El-Gremi *et al.,* (2016) [6] reported that *Alternaria alternate* (Fr.) Keissl, *Cochliobolus sativus*, *Drechsler* spp. and *Fusarium graminearum* had been repeatedly isolated from kernel black pointed wheat and found to be pathogenic either alone or in combination. Ghosh *et al*., (2018) [7] reported that black point is a disease of cereal seeds, exhibiting a brown to black tip at the embryo end of the grain. The disease is caused by *B. sorokiniana.* In addition, the association of *Alternaria alternata, Fusarium* spp. *and Penicillium* spp. with wheat seeds developing black point symptoms. Early pathogen detection is an important stage in wheat diagnosis and control strategies. 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 seedborne pathogens associated with black point disease of wheat.

Materials 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) [11] was used for detection of pathogens associated with black point disease of wheat. ISTA's standard agar plate method was used to detect internal pathogens associated with black point disease of the wheat seed. (Neergard 1979) [11]. Kotch's postulate was used for proving the pathogenicity.

The effect of seedborne pathogens associated with black point disease of wheat on seed germination and seedling vigour index was studied by adopting the procedure of rolled towel paper described by ISTA's (Anonymous, 1999) [5]. Total four hundred seeds of wheat variety were inoculated with the individual pathogen by dipping the seeds in concentrated spore suspension (10^6 cfu/ml) for 12 hours. Then these inoculated seeds were dried in shade for 12 hours (Agarwal and Sinclair, 1993) [3]. 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 and incubated at 20-25^{0}C with a RH over 85%. (Anonymous, 1999) [5]. 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) [1].

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) [13].

The seed germination and seedling vigour index was studied by adopting the procedure of rolled towel paper described by ISTA's (Anonymous, 1999) [5].

The effect of fungicides and bioagents on artificially inoculated seeds was evaluated using the standard blotter test previously described. Under aseptic conditions, the seeds of the wheat variety Samadhan 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^{0}C with an alternating cycle of 12 hours darkness and 12 hours light. On the eighth day, the number of seeds infected with mycoflora was observed using a sterio-binocular microscope, the per cent incidence of each mycoflora was recorded and the results were analysed statistically. The artificially inoculated seeds treated with fungicides and bioagents were kept for germination by using between paper method as described earlier. Untreated seeds served as control. Seed germination, root and shoot 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 and bioagents was studied. In the laboratory, the experiment was conducted in a Completely Randomized Design (CRD) with four replication. The data recorded in per cent to seed mycoflora and seed germination were converted into arc 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) [13].

Results and Discussion

Three pathogens *viz., Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* were found associated with black point disease of wheat. Internal pathogens associated with black point disease of wheat seeds showed that two fungi *viz., A. alternata* and *Drechslera sorokiniana* were found internally associated with black point disease of wheat seeds.

Abdullah and Atroshi (2016) [2] reported that several fungi were found to be associated with the black-point disease of wheat. *Alternaria, Cochliobolus, Fusarium*, *Cladosporium, Curvulavia, Penicillium, Aspergillus* and *Stemphylium* were predominant. Li *et al*., (2019) [9] reported that seed infection by *B. sorokiniana* can result in black point disease, which may result in root rot and seedling blight.

Efficacy of fungicides and bioagents on incidence of *Alternaria alternata, Drechslera sorokiniana and Curvularia lunata* (artificially inoculated to seed) of wheat

Among fungicides Carboxin 37.5% + Thiram 37.5% DS @ 0.3% was found most effective with the highest reduction in incidence of *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* among all fungicides followed by Carbendizim 25% + Mancozeb 50% @ 0.2%. Among bioagents *Trichoderma harzianum* @ 10g/kg seed was found most effective with the highest reduction in incidence of *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata.* The next best bioagents were *Trichoderma viride* @ 10 g/kg seed. Malaker and Main (2009) [10] reported that treating seeds with fungicides helps protect wheat seeds from infection. Some of the common fungicides used in seed treatment include fludioxonil and difenoconazole and Vitavax-200 (Carboxin 37.5% + Thiram 37.5%) and Homai-80WP (Thiophanate methyl 50% +

Thiram 30%). Hossain *et al*., (2015) [8] reported that the application of *T. harzianum*, has been identified as potential biocontrol agents for the management of various crop diseases. *Trichoderma* spp. have been found to be effective in reducing the foliar disease severity on wheat plants compared with untreated plants.

Efficacy of fungicides and bioagents on seed germination and seedling vigour index of wheat seeds artificially inoculated with *Alternaria alternata, Drechslera sorokiniana and Curvularia lunata*

Among fungicides Carboxin 37.5% + Thiram 37.5% DS @ 0.3% was found the most effective as it has shown maximum increase in seed germination against *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* as well as increase in seedling vigour index respectively over the inoculated control, was found to be the most effective among all fungicides followed by Carbendizim 25% + Mancozeb 50% @ 0.2%. Among bioagents *Trichoderma harzianum* @ 10g/kg seed was found the most effective as it has shown maximum increase in seed germination of *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata* as well as increase in seedling vigour index respectively. The next best bioagents were *Trichoderma viride* @ 10 g/kg seed. Anitha *et al.* (2013) [4] revealed that influence of seed treatment with Carbendazim 25% +Mancozeb 50% 0.2% which has systemic mode of residual action and additional benefit of Zn and Mn which helps in increasing the metabolic activities of the enzymes and those enzymes will make the enormous division and enlargement of root length and shoot length by plumule meristem along with shoot cells.

Conclusion

1. There are three pathogens associated with black point disease of wheat *viz., Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata*.
2. All pathogen were pathogenic and resulting into reduction in seed germination and SVI. Among them *Alternaria alternata* was found most damaging.
3. Fungicides treatment found most effective in reducing seed mycoflora and increasing seed germination and SVI followed by bioagents.
4. Among fungicides carboxin 37.5% + thiram 37.5% @ 0.3% most effective against *Alternaria alternata, Drechslera sorokiniana* and *Curvularia lunata*.
5. Among bioagents *Trichoderma harzianum* @ 10g/kg seed was found most effective against all detected pathogen associated with black point disease of wheat.

Table 1: Effect of pathogen associated with black point disease of wheat on seed germination and seedling vigour index wheat

Sr. No.	Pathogen associated with black point of wheat seeds	Seed Germination (%)	Reduction in seed germination over Control (%)	Seedling Vigour Index	Reduction in SVI over Control (%)
1	*Alternaria alternata*	61.75 (51.79)	17.66	1015.00	20.62
2	*Drechslera sorokiniana*	62.50 (52.23)	16.66	1046.00	18.18
3	*Curvularia lunata*	63.75 (52.98)	15.00	1050.50	17.84
4	Control	75.00 (60.00)	-	1278.75	-
	S. E.m±	0.57		36.10	
	CD at 1%	2.49		109.20	
	C. V. (%)	1.75		6.57	

Table 2a: Efficacy of seed treatment of fungicides on incidence of pathogens associated with black point disease of wheat (artificially inoculated to seed) and their effect on seed germination and seedling vigour index

Sr. No.	Treatments	Incidence of Seedborne mycoflora (%)			Reduction in incidence over control (%)			Seed germination (%)		
		A	B	C	A	B	C	A	B	C
1	Carboxin 37.5% + Thiram 37.5% @ 0.3%.	4.33 (12.01)	4.83 (12.70)	4.50 (12.24)	91.22	90.30	90.72	92.17 (73.74)	91.33 (72.87)	90.33 (71.88)
2	Penflufen 13.28% + Trifloxystrobin 13.28% @ 0.2%	8.00 (16.43)	7.50 (15.89)	7.83 (16.25)	83.78	84.95	83.85	89.67 (71.24)	89.50 (71.09)	89.00 (70.63)
3	Thiophanate methyl 45% + Pyraclostrobin 5% @ 0.2%	9.33 (17.78)	8.50 (16.95)	8.83 (17.29)	81.08	82.94	81.79	88.83 (70.47)	88.83 (70.47)	88.50 (70.17)
4	Carbendazim 50% WP @ 0.2%	7.17 (15.52)	7.00 (15.34)	6.67 (14.96)	85.47	85.95	86.25	90.83 (72.37)	90.67 (72.21)	89.67 (71.24)
5	Carbendizim 25% + Mancozeb 50% @ 0.2%	5.33 (13.35)	6.17 (14.37)	5.33 (13.35)	89.19	87.62	89.00	91.83 (73.39)	90.83 (72.37)	89.83 (71.40)
6	Propiconazole @ 0.1%	10.33 (18.75)	9.17 (17.62)	9.33 (17.78)	79.05	81.60	80.76	88.33 (70.02)	88.00 (69.73)	87.00 (68.86)
7	Hexaconazole @ 0.1%	11.17 (19.52)	10.17 (18.59)	10.67 (19.06)	77.36	79.60	78.01	87.83 (69.58)	87.17 (69.00)	86.17 (68.16)
8	Control	50.50 (45.28)	49.83 (44.90)	48.50 (44.14)	-	-	-	76.33 (60.89)	76.67 (61.11)	76.17 (60.77)
	S. E.m±	0.33	0.24	0.30				0.60	0.52	0.52
	CD at 1%	1.39	1.00	1.26				2.51	2.14	2.17

Sr. No.	Treatments	Increase in seed germination over control (%)			Seedling Vigour Index (SVI)			Increase in SVI over control (%)		
		A	B	C	A	B	C	A	B	C
1	Carboxin 37.5% + Thiram 37.5% @ 0.3%.	21.02	19.13	18.59	1216.67	1210.00	1203.33	24.15	22.84	22.31
2	Penflufen 13.28% + Trifloxystrobin 13.28% @ 0.2%	17.70	16.73	16.84	1156.67	1153.33	1143.33	18.03	17.09	16.21
3	Thiophanate methyl 45% + Pyraclostrobin 5% @ 0.2%	16.60	15.86	16.19	1146.67	1136.67	1130.00	17.01	15.40	14.86
4	Carbendazim 50% WP @ 0.2%	19.25	18.26	17.72	1180.00	1173.33	1170.00	20.41	19.12	18.92
5	Carbendizim 25% + Mancozeb 50% @ 0.2%	20.58	18.47	17.94	1200.00	1193.33	1183.33	22.45	21.15	20.28
6	Propiconazole @ 0.1%	15.93	14.78	14.22	1140.00	1133.33	1126.67	16.33	15.06	14.52
7	Hexaconazole @ 0.1%	15.27	13.69	13.12	1133.33	1130.00	1113.33	15.65	14.72	13.16
8	Control	-	-	-	980.00	985.00	983.83	-	-	-
	S. E.m±				19.61	21.95	21.85			
	CD at 1%				81.01	90.68	90.29			

*Figures in parentheses indicates arc sin transformed values
A. *Alternaria alternata*
B. *Drechslera sorokiniana*
C. *Curvularia lunata*

Table 2b: Efficacy of seed treatment of bioagent on incidence of pathogens associated with black point disease of wheat (artificially inoculated to seed) and their effect on seed germination and seedling vigour index of wheat

Sr. No.	Treatments	Incidence of Seedborne mycoflora (%)			Reduction in incidence over control (%)			Seed germination (%)		
		A	B	C	A	B	C	A	B	C
1	*Trichoderma viride* @ 10 g/kg seed	11.87 (20.15)	12.13 (20.37)	12.50 (20.70)	75.38	74.40	73.33	85.00 (67.21)	85.00 (67.21)	84.75 (67.01)
2	*Trichoderma harzianum* @ 10g/kg seed	11.12 (19.48)	11.25 (19.59)	12.00 (20.26)	76.94	76.25	74.40	85.75 (67.82)	85.63 (67.71)	85.38 (67.51)
3	*Pseudomonas fluorescens* @ 10g/kg seed	13.50 (21.55)	13.63 (21.66)	14.88 (22.68)	72.02	71.24	68.27	83.00 (65.64)	83.25 (65.84)	83.00 (65.64)
4	*Trichoderma viride* + *Pseudomonas fluorescens* @ 5g each / kg seed	12.87 (21.02)	13.25 (21.34)	13.88 (21.86)	73.31	72.03	70.40	83.88 (66.32)	83.75 (66.22)	83.50 (66.03)
5	*Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5g each /kg seed	12.37 (20.59)	12.75 (20.92)	13.13 (21.24)	74.35	73.08	72.00	84.25 (66.61)	84.13 (66.51)	83.88 (66.32)
6	Control	48.25 (43.99)	47.38 (43.49)	46.88 (43.20)	-	-	-	75.00 (60.00)	75.75 (60.49)	75.25 (60.16)
	S. E.m±	0.27	0.21	0.17				0.41	0.42	0.50
	CD at 1%	1.12	0.87	0.71				1.70	1.73	2.05

Table 2: Continue

Sr. No.	Treatments	Increase in seed germination over control (%)			Seedling Vigour Index (SVI)			Increase in SVI over control (%)		
		A	B	C	A	B	C	A	B	C
1	*Trichoderma viride* @ 10 g/kg seed	13.33	12.21	12.62	1065.00	1057.50	1052.50	11.22	10.64	10.26
2	*Trichoderma harzianum* @ 10g/kg seed	14.33	13.03	13.45	1087.50	1082.50	1077.50	13.57	13.26	12.88
3	*Pseudomonas fluorescens* @ 10g/kg seed	10.66	9.90	10.29	1037.50	1035.00	1030.00	8.35	8.29	7.90
4	*Trichoderma viride* + *Pseudomonas fluorescens* @ 5g each / kg seed	11.83	10.56	10.96	1045.00	1040.00	1035.00	9.13	8.81	8.43
5	*Trichoderma harzianum* + *Pseudomonas fluorescens* @ 5g each /kg seed	12.33	11.05	11.46	1057.50	1052.50	1050.00	10.44	10.12	10.00
6	Control	-	-	-	957.50	955.75	954.50	-	-	-
	S. E.m±				9.46	9.37	8.03			
	CD at 1%				38.52	38.17	32.72			

*Figures in parentheses indicates arc sin transformed values
A. *Alternaria alternata*
B. *Drechslera sorokiniana*
C. *Curvularia lunata*

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