

SEED-BORNE FUNGI IN WHEAT SEED AND THEIR CONTROL

BY

AMITAVA MAJUMDAR AND S. B. CHATTOPADHYA

*Division of Mycology and Plant Pathology, Faculty of Agriculture,
University of Kalyani, Kalyani, west Bengal.*

Study on pathology of wheat seeds of West Bengal was undertaken as per Elotter and agar method of International Seed Testing Association. Seeds of six varieties of wheat namely NP 710, NP 798, NP 824, NP 835, Sonora 63 and Sonora 64 were taken. Predominantly four pathogenic fungi namely *Helminthosporium sativum*, *Alternaria tenuis*, *Alternaria triticina* and *Fusarium culmorum* were found to be present in both apparently healthy as well as diseased seeds, though prevalence of these fungi were much less in apparently healthy seeds. These fungi were also detected in seeds which were put in blotter and petriplates containing PDA after surface sterilization with 0.1% mercuric chloride. Seeds of these six varieties after treatment with five fungicides namely Thiram, Ziram, Captan, Bisdithane and Agrosan GN were plated in petriplates containing PDA and sown in fields. It was observed that treatment of seeds resulted in control of seed borne fungi and reduction in mortality seedlings. Agrosan GN followed by Captan and Thiram gave best results.

INTRODUCTION

Relationship between seeds and diseases in wheat must have been observed before scientific studies were taken up. Transmission of seed-borne pathogens was convincingly demonstrated by Tillet (1755) and Prevost (1807) in case of bunt of wheat. Utility of treatments of seeds to get rid of infection particularly of smuts and bunts in cereals is known for a long time. Some major diseases of wheat are seed-borne. Extent to which the pathogens are carried in the seeds under local conditions has not been fully determined in each case, as yet. Hence, a programme of study was taken up on pathology of wheat seed.

Experiments were also taken up to determine the efficacy of different fungicides. Recently, use of organomercurials as seed dressing is tending to be restricted due to cost as well as toxicity. So, other fungicides (non-mercurial) were used in this study, along with organomercurial to find out their effectiveness in controlling seed-borne pathogens of wheat.

Beckwith (1910) found a species of *Helminthosporium* pathogenic on wheat and Johnson (1914) established pathogenicity of *H. sativum*. Stakman (1920) showed that root rot of wheat was due to *H. sativum*. Christensen (1922) studied in detail the parasitism of *H. sativum* on wheat. He observed the disease to overwinter mainly through mycelium in the seed. Later, Waldron (1936), Greaney (1944), Oswald (1950), Gorodilova (1960, 1967), Csuti (1963), de Tempe (1964) and Shipton and Chambers (1966) also reported seed-borne nature of *H. sativum*. In India, McRae (1923) first reported root rot of wheat caused by *H. sativum* from Madhya Pradesh. Mitra (1930) and Dastur (1932, 1942), reported the disease causing mainly leaf blight than foot-rot in India. Later on, Chattopadhyay and Sen Gupta (1955) and Chakraborti (1968) observed *H. sativum* to be the most important seed-borne pathogen causing foot-rot and leaf blight of wheat.

Alternaria tenuis was first recorded from wheat seed by Kulkarni (1924). Later on, Machacek and Greaney (1938), Brentzel (1944), Mehta (1950), Bose (1956), Gorodilova (1960), Parashar and Chouhan (1967) and Dharam Vir and Joshi (1968) reported seed borne nature of *A. tenuis*. *Alternaria triticina* was first reported by Prasada and Prabhu (1962) causing severe damage to wheat. Later on, they (1956, 1967) mentioned the fungus to be seed borne. Other important fungi occurring on wheat were *Fusarium culmorum* described by Bennett (1928) in England as seed-borne, and *F. avenaceum*. Later on, Russell (1931), Christensen (1936), Doyer (1938), Greaney and Machacek (1946), Noble *et al.* (1958), Hewett (1967), and Korshunova *et al.* (1967) reported seed borne nature of *F. culmorum*. Sadasivan (1939) and Bakshi (1951) also reported *F. culmorum* to be seed-borne.

Waldron (1936) from North Dakota USA recommended Ceresan against black point disease of spring wheat. Machacek and Greaney (1938) from USA achieved control of black point disease of wheat due to *Alternaria* spp. and *H. sativum* by use of organomercury dust. Hanson and Christensen (1944) recommended treatment of wheat seeds with 'Semesan' to control *H. sativum* and *Fusarium* spp. Later on, Greaney and Machacek (1946), Stapel *et al.* (1961), Csuti (1964), Prasada and Prabhu (1964), Mehata and Joshi (1965) and Gorodilova (1967) recommended different chemicals and also hot water treatment of wheat seeds for controlling seed-borne fungi like *H. sativum*, *Alternaria tenuis*, *Alternaria triticina* and *Fusarium* spp.

MATERIALS AND METHODS

*Materials**Seeds*

Wheat seeds of following varieties were used in present study :

- | | | |
|-----------|--------------|---------------|
| 1. NP 710 | 2. NP 798 | 3. NP 824 |
| 4. NP 835 | 5. Sonora 63 | 6. Sonora 64. |

Seeds were obtained from the Wheat Specialist, Directorate of Agriculture, Govt. of West Bengal, Kalyani, and from Farm Superintendent, College Farm, Haringhata, University of Kalyani, West Bengal.

b. Fungicides

Following fungicides were used :—

(Dosage 0.3% in all cases)

1. *Agrosan G N.* (1% mercurial dust in the form ethyl mercury chloride, 0.1% and Phenyl mercury acetate 0.9%) 2. *Thiram* (75% tetramethyl thiam disulphide) 3. *Ziram* (methyl dithiocarbamate, 80%) 4. *Ciptan* (SR-406) (n trichloromethyl mercapto 4. *Cyclohexene* 1, 2-dicarboximide) 5. *Bisidithane* (Manganese and zine salt of a dithiocarbamate 65% and inert ingredient 35%) 6. *Maneb* (Dithene M-45 (Zinc & Manganese ethylene bidithiocarbamate).

Methods

Seed health testing in the laboratory was done according to ISTA rule 1966, ie, Blotter method and Agar plating method.

Standard blotter method

Seeds were first surface sterilized with 0.1% mercuric chloride, washed thoroughly thrice by sterile distilled water and dried in between dry blotters. Dried seeds were placed on moist blotter (3 blotter/plate). 25 seeds were placed per plate.

Agar plating method

In most of the experiments *potato dextrose agar* was used as the medium. Seeds were surface sterilized washed in the usual manner and dried between sterilized blotter. Ten seeds were spaced out in each petriplates, containing 20 ml of the medium.

A. Detection of fungi in seeds

To detect the presence of different fungi associated with wheat seeds, the following experiments were conducted :

(a) To find out the relative distribution of different seed-borne fungi seeds of wheat varieties NP 798, NP 824, Sonora 63, Sonora 64, were taken. Apparently healthy seeds were sorted out. Two kinds of seeds (100 seeds for each variety) namely apparently healthy and diseased were placed without surface sterilization in petriplate (10 seeds/plate) containing potato dextrose agar and incubated at $27^{\circ} \pm 1^{\circ}\text{C}$ for 7 days (Table 1).

(b) Seeds (surface sterilized with 0.1% mercuric chloride) were put to moist blotter and potato dextrose agar tests for detection of fungi. 25 seeds per plate in case of blotter test and 10 seeds per plate in agar plating methods were used. They were incubated at $27^{\circ} \pm 1^{\circ}\text{C}$ for 7 days for both the tests. Microorganisms were identified in usual way (Table 1). 500 seeds were used in each case.

(c) Treatment of seeds with different fungicides

Seeds were taken in conical flask and the required amount of fungicides was taken, then mixed thoroughly. The excess of fungicide if any was sieved through fine muslin cloth. For evaluation of fungicides, the agar plate technique was used.

B. Experiments with seedlings

(1) Field experiments

Seedlings were raised from treated seeds in the field in the one metre square plots. In each plot 4 rows were made ; distance between row to row was 18 cm and extreme two rows were kept 22 cm apart from the border.

In each row 50 seedlings were grown i. e. 200 seedlings per plot. So the data on the seedlings were taken from these 200 seedlings at a interval of 7, 17, 24 and 34 days. Five replications were taken in each case and one 'control' set without any treatment was maintained throughout the experiment in all the cases.

C. Statistical analysis and transformation (Field Expt.)

Change of scale for analysis of variance

The basic assumptions underlying the analysis of variance of the experimental data are :

- (1) that the observations are normally distributed about their true or expected value,
- (2) that the observations are independent,
- (3) that the variance of the observations is constant,
- (4) the model is additive in nature,

Percentage data on the mortality of seedlings do not satisfy all the above assumptions. So, in order to fulfil the conditions for application of analysis of variance in the present case, the percentage data have been transformed into angular scale by $\sin^{-1}\sqrt{x}$ transformation (Fisher and Yates, 1963) with necessary interpolation was used for this purpose. Significant differences applicable to the mean of the transformed data were calculated from the analysis of variance and comparison of different treatments and varieties were done with the table of means of the transformed values and the critical difference calculated.

Transformed values of table of means were retransformed into actual percentage data to get an idea of the differences of the actual mean values.

RESULTS

1. Detection of fungi in seeds

The following observations were made from the data presented in Table 1.

The distribution of the four main pathogens of wheat namely, *Helminthosporium sativum*, *Alternaria tenuis*, *Alternaria tritici* and *Fusarium culmorum* in seeds (both apparently healthy and heavily spotted) of the four varieties of wheat were noted. It was observed that all the four fungi were also present in apparently healthy seeds though their prevalence was much less than what was observed in heavily spotted seeds. In moist blotter and potato dextrose agar seeds after surface sterilization, showed also four fungi namely *H. sativum*, *A. tenuis*, *A. tritici* and *F. culmorum*. *H. sativum* and *A. tenuis* were the most predominant fungi in both the tests.

2. Effect of seed treating chemicals on control of seed-borne infection

In the evaluation of different fungicides, following experiments were conducted namely :

- (i) Potato dextrose agar test (in which the treated seed were placed on PDA to find the presence of different fungi).
 - (ii) Field experiments to determine the extent of primary infection in the seedling stage.
- (i) Tests in petriplates containing potato dextrose agar

Seeds of six different varieties namely, NP 798, NP 824, Sonora 32, Sonora 64, NP 710 and NP 835 were treated with five different fungicides namely, Ziram, Thiram, Captan, Bisdithane and Agrosan GN and were put in petriplate (standard size, containing 20 ml of potato dextrose agar). 10 seeds were put in each petriplate and altogether 10 replicates (100 seeds) were used in each treatment. A similar set was kept as 'control' for each variety. Presence of different fungi in the treated seeds or in control were recorded after 7 days.

Results presented in Table 2 showed that the fungicides used for seed treatment gave good control of fungi in almost all the cases. In 'control set' the following seed borne fungi were recorded in fairly high number namely *H. sativum*, *A. tenuis*, *A. tritici* and *F. culmorum*. In treated seed lots though *H. sativum* only recorded rather in high number but still less than 'control set' in each case and in the variety. *F. culmorum* and *A. tritici* though observed in treated seed lots but practically very less in comparison to 'control set' in each case.

1. Distribution in percentage of four major wheat fungi in both apparently healthy and heavily spotted seeds on potato dextrose agar (Surface sterilized seeds), and growth of four major wheat fungi both on blotter and potato dextrose agar after surface sterilization.

		<i>H. sativum</i>				<i>Alternaria tenuis</i>				<i>Alternaria triticiua</i>				<i>Fusarium culmorum</i>			
	ASH on PDA	Sp. seed on PDA	S. S. Seeds on		AHS on PDA	Sp. seed on PDA	S. S. Seeds on		AHS on PDA	Sp. seed on PDA	S. S. Seeds on		AHS on PDA	Sp. seed on PDA	S. S. Seeds on		AHS on PDA
			BL	PDA			BL	PDA			BL	PDA			BL	PDA	
8	4.00	16.00	13.20	8.40	3.25	13.00	7.60	4.20	1.75	6.00	0.00	0.40	4.00	10.00	3.40	2.40	
4	2.50	24.25	10.20	8.20	5.75	15.50	9.20	4.20	1.50	3.50	0.00	1.40	5.75	13.25	4.00	1.40	
63	5.00	12.75	8.00	6.20	4.00	12.50	12.40	5.40	12.25	50	0.00	2.20	2.25	8.00	2.60	3.20	
64	5.50	13.50	7.40	8.00	4.25	13.00	11.60	2.60	1.25	4.75	0.00	2.20	2.75	4.25	4.40	1.00	
10	—	—	6.40				11.00	6.40			0.00	1.00			5.00	1.80	
35	—	—	8.20	5.40			9.60	2.20			0.00	0.00			3.60	4.30	

AHS=Apparently healthy seeds. Sp. seed=Spotted seeds BL=Blotter method, PDA=Potato dextrose agar method,
S. S.=Surface sterilized seeds.

Table 2. Effect of different seed treating chemicals on control of wheat seed-borne fungi in petriplates containing potato dextrose agar (figures in the tables represent per cent of occurrence).

Variety :	Fungicides :	<i>H. sativum</i>	<i>A. tenuis</i>	<i>A. triticina</i>	<i>F. culmorum</i>
NP 798	Ziram	7	4	—	—
	Thiram	5	3	—	—
	Captan	5	1	—	—
	Bisdithane	4	1	2	—
	Agrosan GN	5	—	—	—
	Control	25	31	4	9
NP 824	Ziram	3	1	3	—
	Thiram	5	1	—	2
	Captan	3	—	2	—
	Bisdithane	2	—	2	3
	Agrosan G N	4	1	1	—
	Control	15	5	17	13
Sonora 63	Ziram	5	—	—	—
	Thiram	4	1	1	—
	Captan	2	2	2	—
	Bisdithane	3	2	1	—
	Agrosan G N	5	3	—	—
	Control	18	7	3	3
Sonora 64	Ziram	5	1	1	—
	Thiram	4	—	—	—
	Captan	3	—	—	—
	Bisdithane	5	2	3	1
	Agrosan GN	3	1	1	1
	Control	17	7	3	7
NP 713	Ziram	6	3	1	—
	Thiram	4	2	1	1
	Captan	—	1	1	—
	Bisdithan	3	2	—	1
	Agrosan GN	4	2	—	1
	Control	20	8	3	6
NP 835	Ziram	6	3	—	1
	Thiram	3	4	—	—
	Captan	5	3	—	—
	Bisdithane	3	—	—	1
	Agrosan GN	5	—	—	2
	Control	22	18	1	9

Temp. $27^{\circ} \pm 1^{\circ}\text{C}$, 7 days incubation, fungicides (0.3% a the cases).

Table 3. Effect of different seed treating chemicals on different wheat varieties in field experiments under West Bengal conditions (Figures represent percentage of mortality of seedlings)

Fungicides	Year	NP 798	NP 824	Sonora 63	NP 835	NP 710
Thiram	1st year	12.80	13.00	13.10	13.00	15.30
	2nd year	12.70	9.60	6.30	9.60	11.70
Ziram	1st year	13.90	11.40	10.10	13.50	14.00
	2nd Year	9.40	10.20	10.20	11.00	9.40
Captan	1st year	11.40	11.90	13.80	10.60	8.10
	2nd year	6.10	5.90	7.50	7.20	9.10
Bisdithane	1st year	11.20	12.10	8.90	10.50	7.10
	2nd year	13.70	12.70	8.30	13.80	13.90
Agrosan GN	1st year	10.70	7.40	11.70	13.20	4.70
	2nd year	8.60	9.90	9.10	8.00	8.60
Control	1st year	35.80	30.30	34.30	38.50	35.80
	2nd year	33.70	32.50	30.30	27.20	35.00

Field=5 replications, 1000 seedlings

Statistical analysis after transformation

Analysis of variance

1st year (Field)				2nd year (Field)			
Source	S.E.m.	C.D.at 5%	C.D.at 1%	Source	S.E.m.	C.D.at 5%	C.D at 1%
Treatment	0.703	2.5556*	4.0079*	Treatment	0.65	2.3630*	3.7058*
Variety	0.64	N.S.	N.S.	Variety	0.59	N.S.	N. S.
Variety x Treatment interaction	0.49	1.4453	N.S.	Variety x Treatment interaction	0.463	N.S.	N.S.

N. S. = Not significant

* = significant

Practically no difference could be observed amongst the different seed treating chemicals used regarding their efficacy in control of seed-borne fungi. Also, very little differences were noted amongst the different varieties in respect of relative prevalence of different seed-borne fungi of wheat.

(ii) *Field experiments*

Experiments on the effect of fungicides tested in the laboratory in petriplates was conducted also in the field. Seeds of five different varieties of wheat namely, NP 798, NP 824, Sonora 63, NP 835 and NP 710 were treated with five different fungicides namely, Thiram, Ziram, Captan, Bisdithane and Agrosan GN. A control plot for each variety was maintained throughout the experiments. So there were five levels of variety and six levels of treatments (including control), and hence the design can be regarded as a 5×6 factorial randomized block design.

Experiment was conducted for two consecutive years i. e., 1966-67 and 1967-68.

Data were recorded on 200 seedlings (see Methods) and statistically analysed. Data are presented in Table 3. Observations recorded in above table showed mortality of seedlings in control plots was between 30.30 to 38.50 per cent and 27.20 to 35.00 per cent for first and second year respectively. Similarly, the mortality in plots with treated seeds (different fungicides) ranged between 4.70 to 15.30 and 5.90 to 13.90 per cent respectively during the same periods. All the fungicides used, effectively reduced the percentage of seedling mortality. None however was found to control the disease completely in the field.

Fungicides showed some significant differences amongst themselves. Agrosan GN was found to be the best than the others in both the years. Then the performance of Captan and Thiram was much better than rest two fungicides in both the years. Difference though little, could be noted amongst different varieties, and all the fungicides behaved alike in different varieties in first and second year. Percentage of mortality of seedlings were less during second year compared to that of first year in all the varieties though the treatments were same for both the year.

DISCUSSION

Seed pathologists all over the world, already showed that quite a number of serious pathogenic fungi of wheat are perpetuated through seed and the diseased seeds act as primary source of infection of wheat seedlings in the field. Method of controlling these seedborne fungi is normally seed treatment. Because of high benefit cost ratio treatment of seed is considered to be a popular and easily acceptable plant protection measure.

Damage due to seed-borne infection is two-fold. First of all, the stand is reduced, and secondly from the infected seedlings the disease spreads rapidly to healthy ones in the field and cause wide spread infection. The present study demonstrated the effectiveness of blotter test simple methods in the laboratory to judge the health of wheat seeds. This method can easily be adopted by seed testing organizations connected with certification of seeds. Though Agrosan GN gave better results in this present study, still other non-mercurial safe fungicides like Captan, Thiram etc. may be used to replace the toxic and costly mercurial fungicides (Agrosan GN).

ACKNOWLEDGMENT

The Senior author is thankful to the University of Kalyani, for award of research scholarship for undertaking this work.

REFERENCES

- Anonyms, 1966. International Rules for Seed Testing. *Proc. Int. Seed Testing Assoc.* **31** : 1-152.
- Bakshi, B. K. 1951. Seedling blight and foot rot of cereals caused by *F. avenaceum* (Fr.) Sacc. and *F. culmorum* (W. G. Sm.) Secc. *Indian Phytopathology*, **4** : 162-169.
- Beckwith, T.D. 1910. L' *Helminthosporium tetramera* Mck. Surble a Angola. *Compt. Rend. Soc. de Biol.* **103** : 1265-1266.
- Bennett, F. R. 1928. Fusarium diseases of cereals. *Ann. Appl. Biology*, **15** : 213-244.
- Brentzel, W.E. 1944. The black point disease of wheat. *Bull North Dakota Agri. Expt. Station.* **330** : 14 (Abs. Rev. Appl. Mycology **24** : 360).
- Bose, S. R. 1956. *Alternaria* within the pericarp of the wheat seed. *Nature*, **178** : 640-641.
- Chakraborti, K.B. 1968. Studies on the disease complex of wheat incited by different species of *Helminthosporium* Ph.D. Thesis, Plant Pathology Laboratory, Kalyani University, West Bengal.
- Christensen, J.J. 1922. Studies of the parasitism of *Helminthosporium sativum*. *Minst. Agr. Exp. Tech. Bull.* **11** : 42.
- „ „ 1936. Association of micro-organisms in relation to seedling injury arising from infected seeds. *Phytopathology* **16** : 1091-1105.
- Csuti, E. 1964. Experiments on the control of *Cochliobolus sativus* the causal agent of foot rot disease in wheat. *Rech. Inst. Plant Proc. Budapest : Novemytermeles*, **13** : 361-368.
- Csuti, I. 1963. *Cochliobolus sativus* a pathogen of the foot disease of wheat in Hungary. *Novemytermeles* **12** : 179-186 (Abst. Rev. Appl. Myc. **43** : 131).
- Dastur, J. F. 1932. Foot rot and black point diseases of wheat in Central Provinces. *Agric and Livestock in India.* 275-282.
- Dastur, J. F. 1942. Notes on some fungi isolated from black point affected wheat kernels in Central Provinces. *Indian J. Agric. Sci.* **12** : 731-742.
- De Tempe, J. 1964. *Helminthosporium* spp. in seeds of wheat, coats and rye. *Proc. Int. Seed Testing Assoc.* **29** : 117-140.
- Dharam Vir and Joshi, L.M. 1968. Preliminary note on the occurrence of black point disease of wheat in India. *Indian Phytopath.* **24** : 234.
- Doyer, L. C. 1938. *Manual for determination of seed borne disease.* ISTA, Wageningen, The Netherlands.
- Fisher, R. A. and Yates, F. 1963. *Statistical Tables for Biological Agriculture and Medical Research* (Table X). Oliver and Boyd. 6th Ed. Edinburgh Tweeddale Court. p. 146.
- Gorodilova, Mme, L. M. 1960 Black embryo of the grain of summer wheat and its seriousness in North Kazakhstan. *Trud. Nauch. Issled. Inst. Zerm. Khoz.* **1** : 206-216 (Rev. Appl. Mycology, **42** : 458).
- „ „ 1967. Root rot of wheat in North Kazakhstan. *Vest. Sel-Khoz. Nanki. Alma Ata.* **10** : 45-50 (Abs. Rev. Appl. Mycology, **47** : 28).
- Greaney, F. J. 1944. Symposium of seed borne diseases. *Proc. Canada Phytopath. Soc.* **12** : 18-21.
- „ „ and Machacek, J. E. 1946. The prevalence and control of seed-borne diseases of cereals. *Sci. Agric.* **16** : 59-78.
- Hanson, E. W. and Christensen, J. J. 1944. Report of the Milling and Baking meeting and Seventh Hard Spring Wheat Conference. 55 pages. (Rev. Appl. Mycology, **25** : 299).
- Hewett, P. D. 1967. A survey of seed borne fungi of wheat II. The incidence of common species of *Fusarium*. *Trans. Br. Mycol. Soc.* **50** : 175-182.
- Johnson, E. C. 1914. A study of some imperfect fungi isolated from wheat, oat and barley plants. *Jour. Agric. Res.* **1** : 475-489.
- Kulkarni, G. S. 1924. Report of work done in Plant Pathology Section during the year 1922-23. *Ann. Rept. Dept. Agric. Bombay Presidency Year 1922-23* : 167-171.
- Machak, J. E., and Greaney, F. J. 1938. The black point or kernel smudge disease of cereals. *Cand. Jr. Res. Sec. (C)* **16** : 64-113.

- Mehta, P. R. 1950. Some new diseases of plants of economic importance in U. P. *Plant Proc. Bull.* New Delhi, **2** : 50-51.
- Mehta, R. C. and Joshi, N. C. 1965. Seed borne diseases and trends in seed treatment in India. *Science and Culture*, **31** : 581.
- Mitra, M. 1930 A comparative study of species and strains of *Helminthosporium* in certain Indian cultivated crops. *Trans Br. Mycol. Soc.*, **15** : 254-293.
- Noble, M., Tempe J. de. and Neergaard, P. 1958. An annotated list of seed borne diseases. Comm. Mycol. Inst. Kew, Surrey, England.
- Oswald, J. W. 1950. Etiology of cereal root rots in California *Hilgardia*, **19** : 447-462.
- Parashar, R. D. and Chouhan, J. S. 1967. Effect of black point disease of wheat (*Helminthosporium sativum* P. K. and B and *Alternaria tenuis* Nees.) on seed germination under laboratory and field conditions and on yield. *Jr. Res. Ludhiana* **4** : 73-75.

(Accepted for publication 30th March, 1973)