Environment and Ecology 41 (1A) : 266—272, January—March 2023
ISSN 0970-0420

Isolation of Mycoflora Associated with Wheat Seed and their Management by Chemicals

Yamleshwar, R. K. Ranjan, Dinesh Rai

Received 28 September 2022, Accepted 8 December 2022, Published on 6 February 2023

ABSTRACT

Wheat (*Triticum aestivum* L.) belonging to the family Gramineae is a major cereal food crop in the world. Most of the wheat diseases are transmitted by seeds which are the important limiting factor getting good quality seeds and good yield. Total 70 seed samples of different variety were collected from the farmers of different villages of Samastipur and Muzaffarpur Districts of Bihar state for studies of mycoflora associated with wheat seeds and their management. Three methods for isolation of seed associated mycoflora i.e., standard blotter paper method, standard agar plate method and standard paper towel method, were used. Five seed associated mycoflora i.e. *Bipolaris sorokiniana*, *Fusarium moniliforme, Alternaria triticina*, *Aspergillus* sp. and *Penicillium* sp. were detected and isolated from seed samples. In study of different chemicals against isolated pathogens, Propiconazole (GI% 67.74) found most effective against *B. sorokininana*, followed by Mancozeb + Carbendazim (GI% 65.59) and Mancozeb (GI% 64.51) at lowest concentration (0.1%). In case of *Fusarium moniliforme, Alternaria triticina* and *Penicillium* sp., Propiconazole has shown highest percent inhibition of growth at lowest concentration. However in case of *Aspergillus* sp., Mancozeb + Carbendazim (GI% 62.62) found most effective followed by, Propiconazole (GI% 60.60) at lowest concentration (0.1%).

Keywords Wheat seed, Mycoflora, Chemical management.

INTRODUCTION

Wheat (*Triticum aestivum* L.) belonging to the family Gramineae is a major cereal food crop in the world. India the second largest wheat producing country in the world after China has the production of about 98.61 million tons with an average productivity of 33.18 q/ha and cultivated over an area of about 29.78 million hectare (ICAR-Indian Institute of wheat and barley research 2018). The main wheat growing states in India are Uttar Pradesh, Punjab, Madhya Pradesh, Haryana, Rajasthan and Bihar. Bihar rank 6th in wheat production and cultivated over an area of about 2.04 million ha, production 5.74 million tonnes and productivity 28.16 q/ha (ICAR-Indian Institute of Wheat and barley research 2018). In India, the farmers are growing wheat crop mainly for consumption purpose and they save a portion of produce to use them as a seed for next season. These farmers saved seeds are not tested for their quality. Obviously, the farmers

Yamleshwar[1], R. K. Ranjan[2]*, Dinesh Rai[3]
[1]Young Professional-II under Biotech Kisan Hub ICAR DBT at IGKV, Raipur, CG 492012, India
[2, 3]Assistant Professor, Department of Plant Pathology, Dr Rajendra Prasad Central Agricultural University, Pusa, Samastipur, Bihar 848125, India
Email : rkrrau@rediffmail.com
*Corresponding author

saved seeds are of poor health and quality and often infected by seed borne pathogens. These poor qualities of infected wheat seeds fail to germinate and if germinated the young seedlings from infected seeds die in few days, resulting in germination failure, post emergence damping off and cause seedling blight. The seed borne pathogens present in the seeds or associated with seeds will remain alive in dormant condition with seed lots as long as the seed remains viable.

Wheat crop is affected by approximately 120 different diseases, among them, 42 diseases are seed borne and 35 diseases are caused by fungi (Hasan *et al*. 2005). Coincidentally, seed borne diseases of wheat are more destructive in nature and it occurs worldwide. Seeds provide natural substrate for the growth of fungi gets associated with externally or internally or both to the seeds. Fungi associated with seeds as contaminant can cause seed abnormalities, poor germination as well as seedling damage resulting in development of disease at later stages of plant growth by systemic or local infection (Khanzada *et al.* 2002). For better crop improvement there is need to produce healthy and disease free seeds. A number of chemical agents are used to control these pathogens, fungicides are being used in the form of dusting, slurry and soaking treatment. Seed dressing fungicides have long been applied to cereal seeds to prevent seed decay, damping-off and seedling blight and seed-borne fungi (Kadege and Lyimo 2015). Seed treatment is the safest and cheapest practice to control seed-borne fungal pathogens (Chandler 2005). Thus, farmers saved wheat seeds can be infected by many seed borne pathogens. Seed associated mycoflora and seed borne diseases are mainly responsible for low yield and production of poor quality wheat seeds in Bihar. However, the successful wheat cultivation largely depends on the use of quality and healthy seeds. In view of the above facts and importance of seed associated mycoflora, the present investigation has been undertaken to isolate the associated mycoflora in wheat seeds and their management by chemicals.

.

MATERIALS AND METHODS

Collection of wheat seed samples

Total 70 seed samples of different variety were collected from the farmers of different region of Bihar state. The collected seed samples were kept in cloth bag and stored in a well ventilated room of the laboratory in Department of Plant Pathology, RPCAU, Pusa, Bihar, India.

Isolation and identification of seed mycoflora associated with wheat seed samples

Seventy seed samples of wheat collected from different locations were used for the isolation of mycoflora by using (i) Standard blotter paper method (ii) Standard agar plate method and (iii) Standard paper towel method (ISTA 2001) were employed for this purpose. After 24 - 48 hrs, fragments of hyphal tip from the growing point were transferred to fresh PDA slants for pure culture preparation. Pure culture was maintained on PDA media slants for further studies.

Evaluation of different chemicals against isolated pathogens

Different chemicals were tested in *in-vitro* against pathogens at different concentrations by poison food technique. Following chemicals were used in this study :

Sl. No.	Chemicals
1	Copper oxy chloride 50% WP
2	Carbendazim 50% WP
3	Mancozeb 75% WP
4	Carbendazim 12% + mancozeb 63% WP
5	Propiconazole 25% SC

Chemicals were evaluated at 0.1, 0.2 and 0.3% concentrations against isolated pathogens. Desired concentrations were prepared by thoroughly mixing of each weighted chemicals in 300 ml of sterilized PDA media. These mixtures were poured in Petri plates aseptically and allowed to solidify. Then the plates were inoculated with 5.0 mm disc of culture by using cork borer. Control plate was also inoculated with 5.0 mm disc of culture without adding any chemical in media. Three replications were maintained for each treatment. Inoculated plates were incubated in the BOD at 28 ± 1°C. Observations were recorded after 48 hrs of incubation.

Table 1. Effect of different chemicals against *Bipolaris sorokiniana*. I = Percentage inhibition over control.

Different chemicals	Average growth of *Bipolaris sorokiniana* in mm at different concentration of chemicals					
	0.1%	I	0.2%	I	0.3%	I
Mancozeb	11.000	64.516	10.000	67.742	9.000	70.968
Mancozeb + Carbendazim	10.667	65.591	9.333	69.892	9.000	70.968
Propiconazole	10.000	67.742	9.000	70.968	9.000	70.968
Copper oxy chloride	21.667	30.108	21.333	31.183	9.667	68.817
Carbendazim	16.333	47.312	13.667	55.914	11.333	63.441
Control	31.000	0.000	31.000	0.000	31.000	0.000
Factors		C.D. at 5%		SEm±		
Chemicals (B)		1.461		0.507		
Concentrations (A)		1.033		0.359		
Interaction (A × B)		2.53		0.878		

Inhibition percentage was calculated by using formula :

$$\text{Inhibition percentage} = \frac{(C-T) \times 100}{C}$$

Where,

C = Radial growth of pathogen in mm in control plate.

T = Radial growth of pathogen in mm in treated plate.

RESULTS

The seed borne mycoflora associated with wheat seeds were isolated by standard incubation techniques and isolated by culturing on potato dextrose agar medium in petri plates. The plates were incubated at 28±1°C for the fungal growth. Pure cultures thus obtained were maintained for further investigations. The seed borne mycoflora was identified on the basis of cultural and morphological characters was confirmed as *Aspergillus* sp., *Alternaria triticina*, *Bipolaris sorokiniana, Fusarium moniliforme* and *Penicillium* sp. The efficacies of different chemicals were tested against isolated pathogen from wheat seeds.

Evaluation of different chemicals against isolated pathogens

Different chemicals (Copper oxy chloride 50% WP, Carbendazim 50% WP, Mancozeb 75% WP, Carben-

Table 2. Effect of different chemicals against *Fusarium moniliformae*. I = Percentage inhibition over control.

Different chemicals	Average growth of *Fusarium moniliforme* in mm at different concentration of chemicals					
	0.1%	I	0.2%	I	0.3%	I
Mancozeb	14.667	60.360	11.667	68.468	10.333	72.072
Mancozeb + Carbendazim	13.333	63.964	12.333	66.667	10.333	72.072
Propiconazole	11.667	68.468	11.000	70.270	9.000	75.676
Copper oxy chloride	23.333	36.937	21.333	42.342	13.667	63.063
Carbendazim	17.667	52.252	16.000	56.757	12.667	65.766
Control	37.000	0.000	37.000	0.000	37.000	0.000
Factors		C.D. at 5%		SEm±		
Chemicals (B)		1.154		0.401		
Concentrations (A)		0.816		0.283		
Interaction (A × B)		1.998		0.694		

Table 3. Effect of different chemicals against *Alternaria triticina*. I = Percentage inhibition over control.

Different chemicals	Average growth of *Alternaria triticina* in mm at different concentration of chemicals					
	0.1%	I	0.2%	I	0.3%	I
Mancozeb	14.000	56.250	11.333	64.583	10.000	68.750
Mancozeb + Carbendazim	13.333	58.333	11.000	65.625	9.667	69.792
Propiconazole	12.000	62.500	9.667	69.792	9.000	71.875
Copper oxy chloride	22.000	31.250	19.000	40.625	9.667	69.792
Carbendazim	16.333	48.958	14.667	54.167	13.000	59.375
Control	32.000	0.000	32.000	0.000	32.000	0.000
Factors		C.D. at 5%		SEm±		
Chemicals (B)		1.225		0.426		
Concentrations (A)		0.867		0.301		
Interaction (A × B)		2.123		0.737		

dazim 12% + mancozeb 63% WP and Propiconazole 25% SC) were tested in *in-vitro* against isolated pathogens at different concentrations at 0.1, 0.2 and 0.3% by "poisoned food technique". After 48 hrs of inoculation, observations on radial growth of fungus colony were recorded and percent inhibition of growth (GI%) was calculated.

Effect of different chemicals against *Bipolaris sorokiniana*

Different chemicals were tested against *B. sorokiniana* isolated from wheat seed samples and results were presented in Table 1, showed that at 0.1%, 0.2% and 0.3% concentrations, Propiconazole showed highest GI (%) i.e. 67.742%, 70.968% and 70.968 % respectively. At 0.3% concentration, Propiconazole, Mancozeb and Mancozeb + Carbendazim showed 70.968% of GI (%) (Table 1). Among tested chemicals Propiconazole found most effective to inhibit the growth of *B. sorokiniana* at lowest concentration.

Effect of different chemicals against *Fusarium moniliformae*

Different chemicals were tested against *F. moniliformae* isolated from wheat seed samples and results were presented in Table 2 showed that at all three concentrations 0.1, 0.2 and 0.3%, Propiconazole recorded highest GI (%) i.e. 68.468%, 70.270% and 75.676% respectively (Table 2).

Effect of different chemicals against *Alternaria triticina*

Different chemicals were tested against *A. triticina*

Table 4. Effect of different chemicals against *Penicillium* sp. I = Percentage inhibition over control.

Different chemicals	Average growth of *Penicillium* sp. in mm at different concentration of chemicals					
	0.1%	I	0.2%	I	0.3%	I
Mancozeb	14.667	26.667	13.000	35.000	11.667	41.667
Mancozeb + Carbendazim	13.000	35.000	12.000	40.000	11.000	45.000
Propiconazole	11.333	43.333	10.667	46.667	9.333	53.333
Copper oxy chloride	18.333	8.333	15.333	23.333	12.000	40.000
Carbendazim	18.000	10.000	16.333	18.333	13.667	31.667
Control	20.000	0.000	20.000	0.000	20.000	0.000
Factors		CD at 5%		SEm±		
Chemicals (B)		0.933		0.324		
Concentrations (A)		0.66		0.229		
Interaction (A × B)		1.616		0.561		

Table 5. Effect of different chemicals against *Aspergillus* sp. I = Percentage inhibition over control.

Different chemicals	Average growth of Aspergillus sp. in mm at different concentration of chemicals					
	0.1%	I	0.2%	I	0.3%	I
Mancozeb	26.333	20.202	22.667	31.313	19.333	41.414
Mancozeb + Carbendazim	12.333	62.626	11.000	66.667	14.000	57.576
Propiconazole	13.000	60.606	12.333	62.626	9.000	72.727
Copper oxy chloride	14.667	55.556	10.667	67.677	9.333	71.717
Carbendazim	16.333	50.505	14.000	57.576	10.667	67.677
Control	33.000	0.000	33.000	0.000	33.000	0.000
Factors		CD at 5%		SEm±		
Chemicals (B)		1.364		0.474		
Concentrations (A)		0.964		0.335		
Interaction (A × B)		2.362		0.82		

isolated from wheat seed samples and results were presented in Table 3 showed that at all three concentrations 0.1, 0.2 and 0.3%, Propiconazole showed highest GI (%) i.e. 62.500%, 69.792% and 71.875% respectively (Table 3).

Effect of different chemicals against *Penicillium* sp.

Different chemicals were tested against *Penicillium* sp. isolated from wheat seed samples and results were presented in Table 4, showed that at all three concentrations 0.1, 0.2 and 0.3%, Propiconazole has shown highest GI (%) i.e. 43.333%, 46.667% and 53.33% respectively (Table 4).

Effect of different chemicals against *Aspergillus* sp.

Different chemicals were tested against *Aspergillus* sp. isolated from wheat seed samples and results were presented in Table 5 showed that at 0.1% concentration, Mancozeb + Carbendazim showed highest GI (%) i.e. 62.626%, at 0.2% concentration, Copper oxy chloride showed 67.677% of GI (%) and at 0.3% concentration Propiconazole showed 72.727% of GI (%) (Table 5).

DISCUSSION

Standard blotter and agar plate method were recommended by ISTA for the isolation and detection of seed-borne mycoflora. No single method is adequate in the field of seed pathology for detecting seed-borne fungi associated with seeds. Isolation and identification of seed-borne fungi from wheat seeds were done through both standard blotter and agar plate methods (Ahmad *et al.* 2016).

In the present investigations, total 70 wheat seed samples of different variety were collected from the farmers of different villages of Samastipur and Muzaffarpur Districts of Bihar state to isolate and identify the seed mycoflora associated with seeds and their management by using chemicals.

In the present study, five fungus were recorded, which is found to associated with seeds i.e., *Aspergillus* sp., *Alternaria triticina*, *Bipolaris sorokiniana*, *Fusarium moniliforme* and *Penicillium* sp.

Tonu *et al.* (2017) in studied the health and quality of farmer saved wheat seeds. They recorded thirteen fungi in farmers' saved seed sample. The 5 major pathogenic fungi identified were *B.sorokiniana*, *A. tenuis,C. lunata, F. oxysporum* and *A. flavus*. Which is also confirm to our findings.

These isolated mycoflora were tested against different chemicals (Copperoxy chloride 50% WP, Carbendazim 50%WP, Mancozeb 75% WP, Carbendazim 12% + Mancozeb 63%WP and Propiconazole 25% SC) in *in-vitro* against isolated pathogens at different concentrations at 0.1, 0.2 and 0.3% through "poisoned food technique". Against the *Bipolaris sorokiniana* at 0.1%, 02% and 0.3% concentrations, Propiconazole has shown highest percent inhibition of growth. Among the tested chemicals Propiconazole, has shown highest percent inhibition of growth against *Fusarium* moniliforme, *Alternaria triticina* and *Penicillium* sp. at three different concentration 0.1, 0.2 and 0.3%. Propiconazole also showed highest percent inhibition of growth against *Aspergillus* sp. at 0.3% concentration followed by copper oxychloride, carbendazim. Mancozeb + Carbendazim, at 0.3% concentration.

Similar findings were also recorded by Samia *et al.* (2015), in their *in-vitro* study evaluated the effects of fungicides (mancozeb, carbendazim, propiconazoole and copper oxychloride) against *Bipolaris sorokiniana* isolates. Propiconazole found most effective, at the lowest concentration of propiconazole against all the isolates of *Bipolaris sorokiniana*, which is confirmation to our findings.

Khanzada *et al.* (2002), tested the effect of various fungicides against isolated seed borne fungi viz., *Alternaria tenuis, Aspergillus niger, Stemphylium herbarum, Fusarium moniliforme* and *Curvularia lunata* from wheat seeds. Among the tested fungicides Bayton, Benlate, Vitavax and Captan were found most effective. All tested fungicides were effective and they significantly increased the seedling emergence, number of grains per spike.

In the present investigation on efficacy of different chemicals, against *Bipolaris sorokiniana*, Propiconazole showed best effect on inhibition of growth of fungus followed by Mancozeb and Mancozeb + Carbendazim. In case of *Fusarium moniliform*e *A*l*ternaria triticina* and *Penicillium* sp., Propiconazole has shown highest percent inhibition of growth at lowest concentration. However in case of *Aspergillus* sp., Mancozeb + Carbendazim found most effective followed by, Copper oxy chloride. However Propiconazol found most effective at lowest concentration to inhibit against all the tested pathogens.

Kakraliya *et al.* (2017), reported the effect of bioagents, botanicals and fungicides against *Alternaria trticinain in-vitro* and in field condition. Maximum growth inhibition percentage was recorded for Propiconazole (89.72%) followed by Hexaconazole (88.84%), *Vitavax* (87.70%), *Trichoderma harzianum* (85.50%), *T. viride* (83.30%), Pseudomonas fluo*rescens* (80.73%) and neem leaf extracts (73.57%).

In the year 2018, Lukman and Razia, evaluated three fungicides viz., Mancozeb, Carbendazim and Mancozeb + Carbendazim against seedbone pathogens by poisoned food technique and dual culture technique respectively. The results revealed that different concentrations of fungicides were found significantly effective against *Alternaria alternata, R. stolonifera, Mucor* spp., *Fusarium moniliforme* and *Aspergillus flavus*. Among tested fungicides, Mancozeb + Carbendazim was found most effective and showed highest growth inhibition at 300-ppm concentration followed by Mancozeb and Carbendazim as compared to control.

It was found that seed-borne fungi from the seeds may be destructive during germination of seeds or may be bringing about mortality soon after the emergence of seedlings, so it is desirable that seeds should be tested for seed health before planting. Management of seed-borne fungi was done to test the efficacy of fungicides *in vitro*. It was found that colony growth of this fungus inhibited significantly. The application of chemicals to the seed is a common and effective means of controlling the majority of seed-borne pathogens.

REFERENCES

Ahmad L, Neha Pathak, Razia K Zaidi (2016) Antifungal Potential of plant extracts against seed-borne Fungi Isolated from barley seeds (*Hordeum vulgare* L). *J Pl Pathol Microbiol* 7 : 350.

Chandler J (2005) Cost reduction in SIT programs using auto-dissemination as part of area wide integrated pest management. *Int J Pest Control* 42 (2) : 257—260.

Hasan MM, Chowdhury SP, Alam S, Hossain B, Alam MS (2005) Antifungal effects of plant extracts on seed-bo rne fungi of wheat seed regarding seed germination, seedling healthy and vigour index. *Paku J Biol Sci* 8 (9) : 1284—1289.

ICAR - Indian Institute of Wheat and Barley Research, Karnal 132001, Haryana, India (2018) Annual Report. ISTA (International rules for seed testing association), International rules for seed testing (2001) "Rules Amendments". *Seed Sci Technol* 29 : 1—127.

Kadege E, Lyimo HJF (2015) Prevalence and control of wheat (*Triticum aestivum* L.) seed borne fungi in farmer-saved seeds. *Archi Phytopathol Pl Prot* 48 (7) : 601— 610.

Kakraliya SS, Choskit D, Pandit D, Abrol S (2017) Effect of Bio-Agents, Neem leaf extract and fungicides against alternaria leaf blight of wheat (*Triticum aestivum* L.). *Natural Prod Chem Res J* 5 (8) : 1000295.

Khanzada KA, Rajput M, Aslam Shah, G. Sarwar, Lodhi A Mubeen, Mehboob Farrakh (2002) Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat. *Asian J Pl Sci* 1 (4) : 441—444.

Lukman Ahmad, Razia K Zaidi (2018) Effect of chemical and biological treatment for the control seed-borne mycoflora of Barley (*Hordeum vulgare* L.). *Acta Scientific Agric* 2 (6) : 06—11.

Samia T, Sultana S, Adhikary SK, Quddus KG (2015) Effect of fungicides against *Bipolaris sorokiniana* isolates collected from different wheat growing regions of Bangladesh. *Mycopath* 13 (2) : 81—88.

Tonu NN, Islam MR, Karim MM, Chowdhury MSM, Mia MAT (2017) Quality and health status of farmer saved wheat seed in Bangladesh. *Int J Res Sci Manag* 4 (9) : 10—24.