Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

DETECTION OF WHEAT SEED MYCOFLORA WITH SPECIAL REFERANCE TO *DRECHSLERA SOROKINIANA*

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A B S T R A C T

Wheat seed samples collected from different locations of three districts viz Sialkot, Narrowal and Gujranwala, were analyzed in the Seed Pathology laboratory to study the associated mycoflora by using the Standard blotter method (SBM). A total of 14 genera and 22 species of fungi were isolated, among which *Drechslera sorokiniana* was recovered with maximum mean frequency (18.1%),other pathogenic fungi include *D. tetramera* (15.66), *D. teres* (12.5), *Alternaria alternata* (9.75), *A. tritici* (4.33), *A. triticola* (6.41), *Fusarium semitectum* (10.58), *Cercospora spp*. (2.75) *F. solani* (1.08), *F. oxysporum* (1.66), *Stemphylium solani* (5.66), *S. botryosum* (2.55), *Cladosporium herbarium* (3.41), *Phoma spp* (6.5) and *Sclerotinia sclerotiorum* (3.25). Among five tested fungicides as seed treatment and poisoned food technique, Score® (Difenoconazole) and Topsin-M® (Thiophanate methyl) exhibited the best performance to inhibit the mycelial growth of *D. sorokiniana* followed by AmistarTop ® (Azoxystrobin + Difenoconazole) while Halonil ® (Chlorothalonil) and Curzate-M ® (Cymoxanil + Mancozeb) were least effective.

Keywords: Wheat Seed Mycoflora, *D. sorokiniana*, Standard Blotter Method, Fungicides.

INTRODUCTION

Wheat (*Triticum aestivum L.*) is one of the earliest cereals known to have been cultivated and major source of nutrition for the people of Pakistan. Wheat is the vital staple food and has the largest acreage of any crop in Pakistan. Area under wheat enlarged to 8693 thousand hectares in 2012-13 from 8650 thousand hectares viewing an increase of 0.5 percent over last year's area but the production stood at 24.2 million tons which is 5.1 percent less for this period. Its growth and production is threatened due to different diseases and pests. Seed health plays an important role for successful cultivation and yield exploitation of a crop species (Rajput *et al*., 2005). Seed borne diseases are generally transmitted through the seed. Among various factors that affect the seed health, the most important are the seed borne fungi that not only lower seed germination but also reduce seed vigor causing disease to the newly emerged seedlings or growing plants and also contaminate the soil by establishing its inoculums which results in low (Hasan *et al*., 2005; Kutama and Aliyu, 2008; Umar *et al*., 2009). Seed borne diseases cause 10-15 % yield losses if untreated diseased seeds are grown in the field (Wiese, 1998). Seed borne pathogens of wheat which affects its yield include *Alternaria alternata, Cladosporium herbarum, Drechslera sorokiniana, D. tetramera* and post-harvest fungi include species of *Aspergillus* and *Penicillium* (Ilyas *et al*., 1998). Genera of *Bipolaris, Fusarium, Alternaria, Drechslera, Stemphylium, Cladosporium, Rhizopus, Aspergillus* and *Penicillium* has been the most commonly isolated fungi from wheat seeds (Rehman *et al*., 2011).

Drechslera sorokiniana is a seed and soil borne pathogen and causes spot blotch in cereal grains and grasses. Seeds affected by *D. sorokiniana* may cause 15% yield loss (Wiese, 1998). For the management of any disease, the basic step is the use of disease free and certified seed. The present investigation dealt to record the frequency of wheat seed mycoflora from Sialkot, Narrowal and Gujranwala districts by using SBM technique and to find out the most effective fungicide against the most frequently isolated pathogen *i-e D. sorokiniana* from all the above said samples by food

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poisoned technique and seed treatment.

MATERIALS AND METHODS

30 seed samples were collected from three districts viz., Sialkot, Gujranwala and Narrowal of Punjab province. Seeds were assessed for the association of seed borne fungi by using standard blotter paper method (ISTA 1985). A working sample of 400 seeds was randomly taken from each composite sample for isolation and identification of fungal pathogens. 10 seeds were plated in 9 cm sterilized Petri plates on moistened three layered sterilized blotter paper and incubated at 25 ± 2 ^{0}C for one week under alternating cycle of 12 hours fluorescent light and darkness. The plates were moistened on daily basis with sterilized distilled water. After 7 days, the seeds were examined to record the frequency %age of different fungi from each seed on the basis of morphological characteristics (Barnett and Hunter, 1972).

Isolation and Purification*:* For isolation*, D. sorokiniana* from the 7 days old growing colonies on wheat seed was shifted on PDA plates and incubated at 25 ± 2˚C or 7 days. The isolated pathogen was multiplied by single spore technique. The fungus was identified with the help of keys and literature (Barnett and hunter, 1972; Chidambaram *et al*.,1973).

***In vitro* evaluation of different fungicides against *D. sorokiniana* by Poisoned Food Technique:** *In vitro* five fungicides i-e Score, Topsin-M, Amistar Top, Halonil and Curzate-M were tested against the predominant fungal pathogen by food poisoned technique, using different concentrations (50, 100, 150ppm) of each fungicide with three replications. Data on radial mycelia growth was recorded after 3, 6 and 9 days interval and compared with the control to calculate the percent decrease in growth of pathogen over control by using the following formula:

$$\%\ disease = \frac{Control - treatment}{Control} \times 100$$

Evaluation of fungicides as seed treatment for control of *D. sorokiniana*: Seed samples from Kotnaina (District Narrowal) were evaluated for the effectiveness of different fungicides to control the pathogen. Seeds were artificially infested with 10 days old culture of the *D. sorokiniana* and incubated for 24 hrs at 25^{o}C. The inoculated seeds were then treated with the fungicides under study using their recommended dose rate. These treated seeds were plated on blotter paper and incubated for 7 days at 25 ± 2^{o}C. The efficacy of each fungicide was evaluated on the basis of recovery of the pathogen from the seeds.

RESULTS

From 30 tested wheat samples, a total of 14 genera and 22 species of fungi were isolated and identified as given in table 1. Among the mycoflora isolated, *D. sorokiniana* (18.1%) was the most frequently isolated pathogen. Other fungi of pathogenic nature viz. *D. tetramera* (15.66%), *D. teres* (12.5%), *Alternaria alternata* (9.75%), *A. tritici* (4.33%), *A. triticola* (6.41%), *Fusarium semitectum* (10.58%), *Cercospora spp*. (2.75%) *F. solani* (1.08%), *F. oxysporum* (1.66%), *Stemphylium solani* (5.66%), *S. botryosum* (2.55%), *Cladosporium herbarium* (3.41%), *Phoma spp* (6.5%) and Sclerotinia *sclerotiorum* (3.25%) had a low %age of prevalence to the samples collected from all the three districts. Keeping in view the frequency of prevalence of *D. sorokiniana* with all the samples, further studies were carried out for the control of this pathogen as seed treatment and Poisoned Food Technique because it can be considered a threat to wheat cultivation in the future.

Table 1. Mean Frequency %age of different fungi associated with wheat seeds.

Sr. No	Fungus	Frequency %age of isolated fungi			
		Sialkot	Narrowal	Gujranwala	Mean
1	*Alternaria alternate*	13.5	8.25	7.5	9.75
2	*A. tritici*	4.0	5.5	3.5	4.33
3	*A. triticola*	7.5	6.0	5.75	6.41
4	*Aspergillus flavus*	52	43	49	48
5	*A. niger*	27	17	23	22.33
6	*Chaetomium globosum*	11.5	13.5	15.5	13.43
7	*Cercospora* spp.	3.25	2.75	2.25	2.75
8	*Drechslera tetramera*	14.75	16.75	15.5	15.66
9	*D. teres*	12.5	14.5	10.5	12.5

Continue…

10	*D. sorokiniana*	29.5	22	33	18.1
11	*Fusarium oxysporum*	2.25	1.5	1.25	1.66
12	*F. semitectum*	9.0	12.5	10.25	10.58
13	*F. solani*	1.0	0.5	1.75	1.08
14	*Rhizopus stolonifer*	32.25	38	29	33.08
15	*Penicillium digitatum*	67.5	52.0	58.5	59.33
16	*Stemphylium solani*	7.0	5.75	4.25	5.66
17	*S. botryosum*	2.25	1.5	3.75	2.5
18	*Cladosporium herbarium*	4.25	2.75	3.25	3.41
19	*Nigrospora* spp.	12.5	13.0	15.5	13.66
20	*Phoma* spp.	7.5	6.25	5.75	6.5
21	*Sclerotinia sclerotiorum*	3.0	2.5	4.25	3.25
22	*Botrytis* spp.	5.25	4.75	5.0	5.0

Poisoned food technique data (Table 2) recorded after an interval of 9 days revealed that Score (0.56cm) was proved to be the most effective with a value of 95.55% decrease over control to check the mycelial growth of the fungus followed by Topsin-M (0.80cm) with a value of 91.04% decrease over control and Amistar Top (1.80cm) with a value of 83.20% decrease over control even at the lowest concentration while Halonil (4.3cm) and Curzate-M (7.5cm) were least effective in reducing the mycelial growth of *D. sorokiniana*.

Table 2. In vitro evaluation of different fungicides against D. sorokiniana by poisoned food technique.

Fungicides	Mycelia growth of pathogen (cm) at different concentrations								
	3 days			6 days			9 days		
	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm	50 ppm	100 ppm	150 ppm
Score	0.6 YZa	0.5 Za	0.4 a	0.7 XYZa	0.6 YZa	0.4 a	0.7 XYZa	0.6 YZa	0.4 a
Topsin-M	0.7 XYZa	0.6 YZa	0.4 a	0.9 VWXY	0.8 WXYZ	0.6 YZa	1.0 UVWX	0.8 WXYZ	0.6 YZa
Amistar Top	1.3 TU	1.1 UVW	0.8 WXYZ	1.5 ST	1.2 TUV	1.0 UVWX	1.8 RS	1.5 ST	1.2 TUV
Halonil	2.6 OP	2.1 QR	1.7 S	3.6 KL	3.2 MN	2.6 OP	6.0 F	5.2 G	4.3 HI
Curzate-M	3.5 LM	2.9 NO	2.4 PQ	5.2 G	4.6 H	4.3 HI	8.2 B	7.8 C	7.5 C
Control	3.9 JK	3.8 KL	4.2 IJ	6.4 DE	6.2 EF	6.6 D	9.0 A	8.8 A	9.0 A

LSD= 0.3237

Table 3. Mean mycelial growth of D. sorokiniana after 3,6, and 9 days of incubation after applying various fungicides by Poisoned Food Technique.

Fungicides	3 days (Mean)	6 days (Mean)	9 days (Mean)
Score	0.5 M	0.5 M	0.56 M
Topsin-M	0.56 M	0.76 L	0.80 L
Amistar Top	1.06 K	1.23 K	1.50 J
Halonil	2.13 I	3.13 G	5.16 D
Cruzate-M	2.93 H	4.70 E	7.83 B
Control	3.96 F	6.40 C	8.93 A

LSD= 0.1869

Table 4. Evaluation of various fungicides as seed treatment for control of seed borne fungi *D. sorokiniana.*

Fungicides	Active ingredient	Dose	Av. Recovery %age of pathogen	% Decrease over control
Score 250 EC	Difenoconazole	0.5mL/kg of seed	3.81	95.61
Topsin-M 70 WP	Thiophanate methyl	2g/kg of seed	5.93	93.16
Amistar Top 325 SC	Azoxystrobin Difenoconazole	2mL/kg of seed	10.12	88.34
Halonil 75 WP	Chlorothalonil	2g/kg of seed	46.5	46.43
Curzate-M 72 WP	Cymoxanil + Mancozeb	2g/kg of seed	58.12	33.04
Control			86.81	-

It is evident from the results given in table 4 that Score exhibited the best performance as seed treatment, it reduced 95.61% growth of the pathogen on treated seeds followed by Topsin-M (93.16%) and Amistar Top (88.34%). However, Halonil showed intermediate affect in reducing the mycelial growth on seeds inoculated with D. *sorokiniana* with a value of (46.43%) decrease over control. Whereas, Curzate-M was least effective seed treatment with a value of (33.04%) decrease over control.

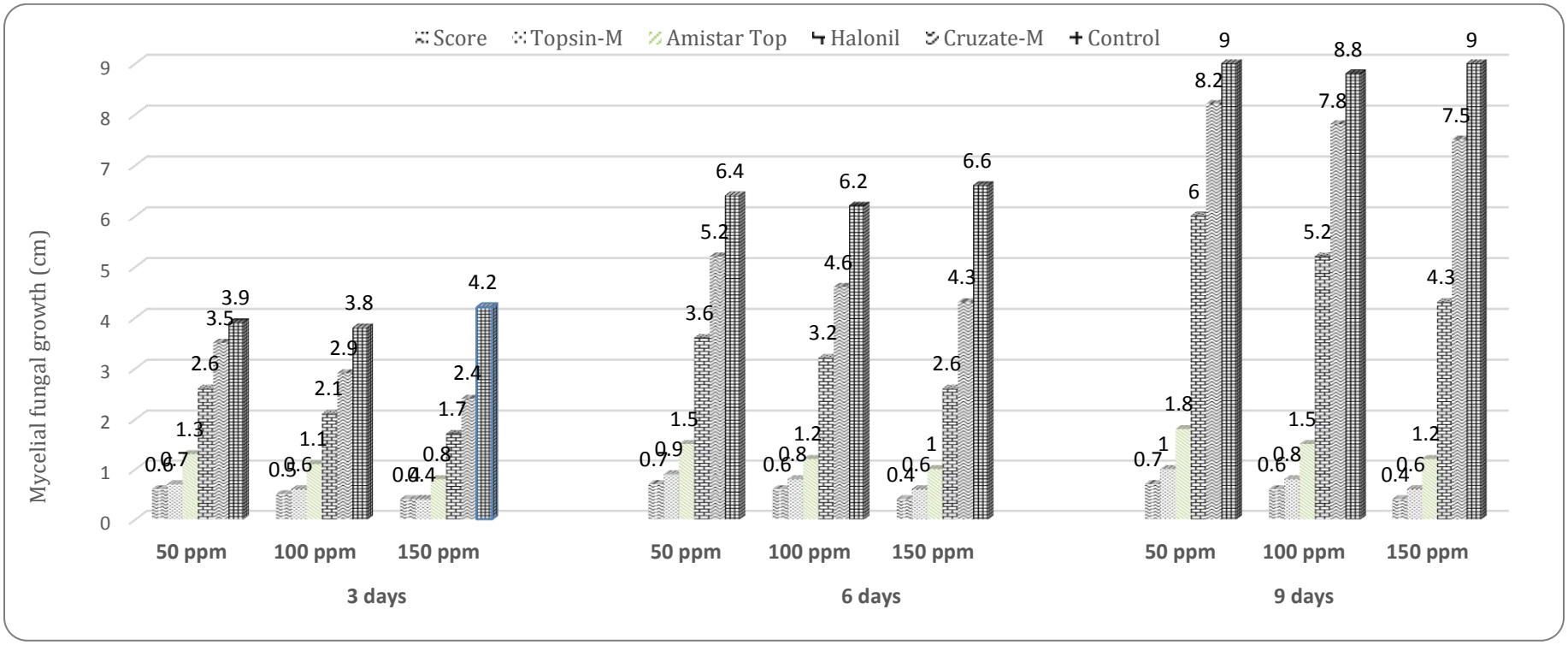

Figure 1. In vitro evaluation of different fungicides against *D. sorokiniana* by poisoned food technique.

DISCUSSION

Drechslera sorokiniana (Sacc.) Subram. B &.L Jain, the fungus *Cochliobolus sativus* is the teleomorph (sexual stage) of *Bipolaris sorokiniana* (anamorph) which is the causal agent of a wide variety of cereal diseases. The pathogen can infect and cause disease on the root (where it is known as common root rot). The two most common diseases caused by *D. sorokiniana* are spot blotch and common root rot, mainly on wheat and barley crops. Findings of present studies are closely in resemblance with that of Hussain *et al*., 2013 as most frequently isolated pathogen from wheat seeds of 10 commercial varieties was *D*. *sorokiniana* (11.125%). Other fungi included *A. flavus* (9.825%), *Alternaria alternata* (7.150%) and *A. niger* (6.225%). Hajihasani *et al.,* 2012 found 15 fungal species associated with the wheat seeds collected from Central Iran. Among which *D. sorokiniana* (17.4) had the maximum and *Ustilago tritici* (1.3%) minimum infection level. Singh *et al.,* 2011 reported 16 fungal species from wheat seeds of two cultivars and found that the blotter paper

method is better than the agar plate method for isolation of associated mycoflora. For the first time in Pakistan Fakhrunnisa *et al*, 2006 reported the presence of *Chaetomium globosum, Drechslera hawaiiensis, Fusarium subglutinens* and *Rhizoctonia solani* on wheat seeds collected from different localities by using blotter paper method. Rajput *et al*, 2005 investigated 12 wheat varieties for the association of fungal mycoflora. Among the five isolated seed borne fungi *A. tenuis* was predominant with an infection range from 22.5 to 47.5%. Among the four fungicides used at 0.75 R, 0.50 R and 0.25 R level of recommended (R) doses against *Drechslera australiensis,* Metalaxyl plus Mancozeb and Dithane M-45 except at 0.25 R completely checked the mycelia growth of the pathogen (Javaid *et al.*, 2006). The results of present studies are in close agreement with those of Habib *et al*., 2012 who found Dithane-M (86.67%) the most effective fungicide as a seed treatment followed by Derosal (85%) and Topsin-M (85%) as compared to control (35.83%), while testing against seed mycoflora of rice varieties.

It is established factor that fungal contamination reduces the viability and ultimately affects the germination of the wheat seeds (Enikuomehin 2005, Rajput *et al*., 2005, Hussain *et al*., 2013). So present studies were conducted to detect the fungal infection on wheat seeds collected from three districts having comparatively more rains especially during harvesting season in the year 2013. After identifying the pathogen chemical control studies showed the pathway to combat the situation. As healthy crop can only be harvested if healthy seed is sown so current studies can be considered as a milestone towards the destination of elevated quality and maximum yield of wheat crop.

Conclusion: *Drechslera sorokiniana* is a seed borne fungus responsible of low yield in wheat causing considerable losses, this evaluation of fungicides will be helpful in reducing the losses due to *D. sorokiniana* and will increase the farmer's income and contribute in food security.

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