Pak. J. Sci. Ind. Res. 2004 ***47****(1) 46 - 49*

SEED - BORNE MYCOFLORA OF OATS IN THE PUNJAB

Altaf Hussain Tariq, Saeed Ahmad, Muhammad Arshad Hussain, Lal Hussain Akhtar*, Muhammad Arshad and Sabir Zameer Siddiqui

Regional Agricultural Research Institute, Bahawalpur, Pakistan

(Received May 10, 2003; accepted September 30, 2003)

Twenty - eight seed samples of eight oats (*Avena sativa* L.) cultivars were collected from Sargodha, Faisalabad and Bahawalpur districts of the Punjab, Pakistan during 1999 - 2000 and analyzed for seed - borne mycoflora during 2000 - 2001. Four genera and nine species of fungi were identified at different frequencies. *Drechslera avenae* and *D. sorokiniana*, known pathogens causing pre - and post - emergence seedling blight and leaf spot in mature plants were detected in 67.80 and 53.60% of the seed samples with maximum infection of 34.00 and 6.00%, respectively. *Phoma* sp. was found in 46.40% of the sample with a maximum infection of 16.00%. Phoma is a new pathogen recorded on oats in Pakistan. All these fungi were found equally pathogenic and caused 86.00, 67.80 and 86.70% pre - and post - emergence seedling mortality in pathogenicity test. These pathogens produced almost the same type of symptoms on roots and leaves. In two samples *Cephalosporium* sp. was recorded in high frequencies (up to 66.00%) but did not show any pathogenic effect on seeds and seedlings. The observed association of different fungi with oats seeds in the present study indicates the need of thorough survey for these and other pathogenic fungi.

Key words**:** *Avena sativa*, Seed - borne fungi, Seed germination.

Introduction

Fodder crops play a pivotal role in the dairy industry. These crops are equally important for draught - adapted animals such as camels, bulls, horses and mules etc. Generally, these crops are planted as secondary crops. Therefore, little attention is devoted to fertilization, planting density and plant protection measures. Efforts have been made to enhance the yield of fodder by different cultural means such as fertilizer, sowing methods, irrigation etc. However, little attention has been paid to fodder crops diseases that should be considered as one of the important causes for poor vigour and low yield. Oat (*Avena sativa* L.) is one of the major Rabi fodder crops. It is mainly sown around the big cities, at dairy farms and remount depots for feeding of cattle and draught - adapted animals.

Oats are attacked by many diseases, of which seedling blight and leaf spot [*Drechslera avenae* (Eidam) Scharif], seedling blight and root rot [*D. sorokiniana* (Sacc.) Subram and Jain], Victoria leaf blight [*D. victoriae* (Meehan and Murphy) Subram and Jain], snow mould and brown foot rot [*Fusarium nivalae* (Ces. Ex Berl. and Voglino)], leaf blotch [*Septoria avenae* (A. B. Franke)] and loose smut [*Ustilago avenae* (Pers.) Rostr] are of major importance in the world (Neergaard 1979). In Pakistan, efforts have been made to enhance the yield by developing high yielding cultivars and adopting advanced agronomical practices but little attention has been given to the health status of seeds of fodder crops that is one of the main reasons of low yield.

*Author for correspondence; E - mail: lhakhtar@yahoo.com

In the light of the importance of seed - borne pathogens in oats and the limited information on such aspects, present study was initiated to find out the health status of oats seeds in the Punjab and the influence of important fungi on germination of oats seeds.

Materials and Methods

Twenty - eight seed samples (about 1.5 kg) of oats cultivars; Algerian, Avon, Early miller, Fullgrain, Kent, Palestine, PD_2 LV65 and SSII were obtained from the Fodder Research Institute, Sargodha, the Fodder Research Sub Station, Faisalabad and the local markets of Bahawalpur from crops harvest of 1999 - 2000. Four hundred seeds taken randomly from each sample were analyzed for the presence of seed mycoflora by following standard blotter paper method (ISTA 1993) at the Regional Agricultural Research Institute, Bahawalpur. Twenty - five seeds were plated in each petri dish (9 cm dia) containing three layers of blotter paper well moistened with sterilized water. The petri dishes were incubated at 22 ± 2°C for seven days under 12 h alternate cycle of light and darkness. Fungi developing on seeds were examined and identified on the 8th day under the stereoscopic microscope and high magnification of the compound microscope (Ellis 1971; Paul *et al* 1983). The isolates were purified and maintained on potato dextrose agar (PDA) media for further confirmation of identification and pathogenicity studies. Heavily infected seeds of important isolates of *Drechslera avenae*, *D. sorokiniana*, *Phoma* sp. and *Cephalos-*

Table 1
Frequency of fungi recorded on oats seeds by Blotter Method in 28 seed samples

Sr. No.	Fungi	Infected samples (%)	Infection range (%)
1	*Cephalosporium* sp.	53.60	0.25 - 66.00
2	*Drechslera avenae*	67.80	0.50 - 34.00
3	*D. biocolor*	10.70	0.50 - 2.50
4	*D. rostrata*	14.30	0.25 - 1.00
5	*D. sorokiniana*	53.60	0.50 - 6.00
6	*Fusarium equiseti*	25.00	0.25 - 2.00
7	*F. moniliforme*	35.70	0.50 - 6.00
8	*F. semitectum*	64.30	0.50 - 14.00
9	*Phoma* sp.	53.60	0.25 - 16.00

porium sp. were transferred from blotter to pots having sterilized soil to observe the effects of these pathogens on seeds and seedlings of oats. These observations were later compared with inoculated seeds in pathogenicity test.

One sample (cv. Early miller) with lowest visible infection of *D. avenae*, *D. sorokiniana* and *Phoma* sp. was selected. Two hundred seeds pre-treated with 1.00% available chlorine for 5 min were placed for 48 h on top of 10 days old viable pure cultures of each test pathogen. All the seeds were then transferred to test tubes (one seed per test tube) having 1.00% agar in order to examine the effects of these fungi on germination and seed to seedling transmission of the diseases. Surface sterilized seeds plated on water agar at the same time served as check. Data on germination, seedling mortality and shoot and root length were recorded 16 days after placing the seeds in agar slants. The symptoms appeared on seedling were also recorded.

Results and Discussion

From 28 seed samples of oats, *Cephalosporium* sp., *Drechslera avenae*, *D. bicolor*, *D. rostrata*, *D. sorokiniana*, *Fusarium equiseti*, *F. moniliforme*, *F. semitectum* and *Phoma* sp. were recorded in different frequencies (Table 1). *D. avenae* was recorded in 67.80% of the seed samples. The fungus was recovered from 0.50 to 34.00% from five cultivars Algerian, Avon, Fullgrain, Palestine and SSII with a maximum infection of 34.00% on Fullgrain followed by 23.00% in Algerian (Table 2). In most of the cases, the seeds were covered with profuse growth of mycelia and conidia causing lesions on emerging radicals. Small black Pycnidia, the perithecial state of *Pyrenophora avenae*, were also recorded on few seeds. These results are in accordance with those of Linns (1989) who found *D. avenae* associated with 90.00% of 232 samples from 7 oats cultivars from the 1987 harvest and that most of

Table 2
Incidence (%) of important fungi on oats seeds of different cultivars in the Punjab

Cultivar	No.of samples examined	Important isolates	Infection range (%)
Algerian	5	*Cephalosporium* sp.	0.00 - 66.00
		Drechslera avenae	0.50 - 23.00
		D. sorokiniana	0.00 - 4.50
		Phoma sp.	0.00 - 2.50
Avon	6	*Cephalosporium* sp.	0.50 - 6.75
		D. avenae	0.00 - 7.50
		D. sorokiniana	0.00 - 4.50
		Phoma sp.	0.00 - 3.00
Early miller	2	*D. sorokiniana*	0.00 - 0.50
Full grain	6	*D. avenae*	0.00 - 34.00
		D. sorokiniana	0.00 - 1.50
		Phoma sp.	0.00 - 4.50
Kent	1	*Cephalosporium* sp.	8.50
		Phoma sp.	1.75
Palestine	5	*D. avenae*	0.00 - 12.00
		D. sorokiniana	0.00 - 2.50
		Phoma sp.	3.00 - 16.00
PD_2 LV65	2	*Cephalosporium* sp.	1.00 - 6.50
		D. sorokiniana	0.50 - 6.00

the samples were only 1 - 9.00% infected with one sample showing 84.00% infection. Moreover, no differences were reported in infection rate among cultivars (Linns 1989). Langaro *et al* (2001) reported that the fungus *D. avenae* (*Pyrenophora chaetomioides*) the causal agent of *Helminthosporium* leaf spot of oats (*Avena sativa*) survives as mycelia in crop residues and in infected seeds.

The growth intensity of *D. avenae* on individual seeds in the blotter test was directly correlated with the amount of damage to these seeds measured in loss of seed and seedling in the soil. The infected seeds by this fungus when transferred from blotter to sterilize soil in pots, gave 86.00% pre - and post - emergence loss of seedlings (Table 3). The infection of roots and coleoptiles either arrested the germination or caused death of seedlings for 10 - 15 days after emergence. The parts of dead seedlings and rotted seeds from these pots yielded 97.90% *D. avenae* when incubated for 7 days at 22 ± 2°C (Table 3). These results agree with the studies conducted by Ruland *et al* (1989) who reported that infection of *D. avenae* at seedling stage in the field was proportional to the infection of heads and flowers at the time of grain formation. *D. avenae* is of world wide importance and known to cause economic looses to oats in the forms of pre- and post- emergence seedling blight and

Table 3
Percentage of seedling mortality and recovery of pathogens from infected parts of dead seedlings

No.of infected sample	Pathogen	No.of infected seeds transferred from blotter to soil	Pre- and post-emergence seedling mortality (%)	Pathogens recovered from dead seedlings	Recovery (%)
2	*Cephalosporium* sp.	100	3.00	-	-
2	*Drechslera avenae*	100	86.00	*Drechslera avenae* *D. sorokiniana* *D. avenae* + *F. semitectum*	97.90 1.05 1.05
3	*D. sorokiniana*	56	67.80	*D. sorokiniana* *D. avenae* + *Phoma* sp.	83.30 16.70
2	*Phoma* sp. *Phoma* sp. + *D. avenae*	60	86.70	*Drechslera avenae* *D. avenae* + *Phoma* sp. *Phoma* sp.	64.60 12.50 23.00

Table 4
Effect of pathogens on seed germination and seedling of *Avenae sativa* in pathogenicity test (200 seeds)

Pathogen	Germinated seeds (%)	Decrease over control (%)	Average shoot length (cm)	Average root length (cm)	Symptoms on seedlings
Cephalosporium sp.	96.50	1.50	16.00	6.00	No symptoms
Drechslera avenae	84.00	14.30	7.80	3.40	Blighting of coleoptiles and roots with brown flecks with light brown to yellowish white centers on leaves spreading up and down, covering entire leaf blade, roots stunted with brown lesions darkened when specks coalesced
D. sorokiniana	85.00	13.30	6.50	3.00	Shoot appearing weak with brown to black flecks on leaves spread up and down, roots stunted, decayed with brown lesions
Phoma sp.	85.50	12.70	9.10	3.10	Reddish brown flecks with grey cottony mycelium on coleoptiles reddish brown lesions with yellow halos on leaf sheet, roots stunted with brown colour
Un-inoculated seed (check)	98.00	-	15.40	5.60	No symptoms

as leaf spotting of mature plants (De Tempe 1964; Malone and Muskett 1964).

D. sorokiniana was found in 15 seed samples from six cultivars and severity of infection ranged from 0.50 to 6.00%. Maximum severity (6.00%) was recorded in PD_2 LV65 (Table 2). *D. sorokiniana* has a wide host range and causes seedling blight, root rot and blotch of oats, brome grass, barley and wheat (Noble and Richardson 1968). Khan and Bhutta (1994) isolated *D. sorokiniana* in high frequencies as a main pathogen from 1267 wheat seed samples of 25 cultivars collected during 1985 - 1990 in Pakistan. They also recorded *Fusarium moniliforme* (*Gibberella fujikuroi*) and *Cephalosporium acremonium* (Corda) in different frequencies along with some saprophytic fungi. Fifty - six seeds infected with *D. sorokiniana* transferred from blotter to sterilized soil in pots resulted in 76.80% pre - and post - emergence seedling mortality (Table 3). In Brazil, Goulart (1996) studied the transmission of *Bipolaris sorokiniana* [(Sacc.) Shoemaker] from seed of wheat coleoptiles and

found positive and significant correlation between incidence on seed and its transmission to seedling coleoptiles both in field and green house studies. The seeds of wheat cv. Anahuac were found to be 16 - 90.50% naturally infected, and that the seeds with 90.50% infection resulted in 1.1: 1 transmission index. The loss of wheat due to root rot (*B. sorokiniana*) was assessed in field experiments by Zhang *et al* (1999) in Heilongjiang province (China) and reported that the percentage of ear bearing tillers decreased at the seedling stage while grain weight decreased significantly due to the disease causing necrotic leaf spots and head blight at the ripening stage.

Phoma sp. was observed in 46.40% of the samples (Table 1). The recovery of the fungus ranged from 0.25 to 16.00% with one sample showing upto 16.00% infection in the variety Palestine. Most of the seeds were covered with pycnidia. This is a new record in Pakistan on oats. This fungus is responsible for foot rot of flax in most of the European countries where the crop is grown for the fiber. The disease may become epidemic if the crop is grown from infected seed (Malone and Muckett 1964). Bevilaqua and Pierobom (1995) studied the presence of seed - borne fungi in the 1992 - 1993 harvested seeds of *Avena strigosa* in Brazil. Of the 11 fungal genera identified, only *Helminthosporium* sp. and *Phoma* sp. occurred in 29.00 and 22.00% incidence, respectively. High fungal contamination was associated with reduced germination and rate of emergence in the field.

Seeds heavily infected with *D. avenae*, *D. sorokiniana* and *Phoma* sp. resulted in poor germination when transferred from blotter to sterilized soil in pots. Reddish brown spots with a light brown to yellowish white centre were observed covering the leaf in case of *D. avenae* and *Phoma* sp., while *D. sorokiniana* produced brown to black flecks on leaves (Table 4). Most of the seedlings failed to appear above ground while others reached the surface and produced aggregated loss of 76.80 to 86.70%. Isolations made from rotted seeds and diseased parts of the seedling yielded *D. avenae*, *D. sorokiniana* and *Phoma* sp. individually and in combination of these fungi in different frequencies (Table 3).

Two seed samples of the cv. Algerian were heavily infected (up to 66.00%) with *Cephalosporium* sp. (Table 2). In most of the cases, the fungus completely covered the seeds. Seeds infected with *Cephalosporium* sp. produced healthy seedlings and did not show any symptoms even after two months of growth (Table 4).

Pathogenicity test revealed that *D. avenae*, *D. sorokiniana* and *Phoma* sp. lowered the germination by 14.30, 13.30 and 12.70%, respectively, in comparison to the control and reduced the root and shoot length of seedlings. Similar symptoms developed on seedlings regardless of fungi (Table 4).

The present study indicated the need of thorough survey for presently identified pathogens as well as other pathogenic fungi. Such measures will permit the introduction of regulations of seed certification in order to provide healthy seed of improved cultivars to the farmers.

References

Bevilaqua G A P, Pierobom C R 1995 Sanitary and physiological quality of seeds of *Avena strigosa* Schreb from Southern Rio Grande do Sul. *Revista Brasileira de Sementes* **17**(1) 19 - 22.

De Tempe J 1964 *Helminthosporium* spp. in seeds of wheat, barley, oats and rye. *Proc Int Seed Test Assoc* **29** 117 - 140.

Ellis M B 1971 *Dematiaceous Hyphomycetes*, CMI. Kew, Surrey, England, pp 608.

Goulart A C P 1996 Transmission of *Bipolaris sorokiniana* from seeds of wheat coleoptiles. *Summa Phytopathologica* **22**(1) 5 - 9.

ISTA 1993 International Rules for Seed Testing. *Proc Int Seed Testing Assoc*, Zurich, Switzerland, pp 177.

Khan M O, Bhutta A R 1994 Seed - borne fungi of wheat cultivars in Pakistan. *Pak J Sci Ind Res* **37**(9) 397 - 398.

Langaro N C, Reis E M, Floss E L 2001 Detection of *Drechslera avenae* in oats seeds. *Fitopatologia Brasileira* **26**(4) 745 - 748.

Linns H 1989 Detection of oats leaf spot pathogen, *Drechslera avenae* in certified seed in Lippe, Westphalia. *Gesunde Pflanzen* **41**(2) 55 - 59.

Malone J P, Muskett A E 1964 Seed-borne fungi: Description of 77 fungus species. *Proc Int Seed Test Assoc* **29**(2) 179 - 384.

Neergaard P 1979 Economic significance of seed-borne diseases. In:*Seed Pathology*, Vol.I. The Macmillan Press Ltd., London, UK, pp 3 - 38.

Noble M, Richardson M J 1968 An annotated list of seed-borne diseases. *Proc Int Seed Test Assoc* **33** 1 - 191.

Paul E N, Tourson T A, Marasos W F O 1983 *Fusarium Species. An Illustrated Manual for Identification of Fusarium Species.* Pennsylvania State Univ Press, University Park, London, UK, pp 193.

Ruland W, Frahm J, Fehramann H 1989 Disease development of *Drechslera avenae* on oats. *Zeitschrift fiir Pflanzenkrankheiten and Rflanzenschutz* **96**(1) 39 - 46.

Zhang Y H, Chi P, TiRuo L, YanKun L, Zhibin Z, Ying L X, Yong L 1999 Loss of spring wheat caused by wheat root rot in Heilongjiang Province. *Acta Phytopathologica Sinica* **29**(4) 329 - 332.