

# Seed Borne Mycoflora of different Berseem Cultivars from Pakistan

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## Abstract

This study was aimed at isolating and identifying fungi associated with seeds of four cultivars of Berseem by using standard Blotter paper and Rag doll method. Eight fungi viz. *Aspergillus niger*, *Alternaria alternata*, *Cladosporium herbarum*, *Curvularia lunata*, *Drechslera tetramera*, *Fusarium solani*, *Penicillium* sp. and *Colletotrichum capsici* were isolated and identified. The most common fungal pathogen reported was *Fusarium solani* found on seeds of all cultivars. The germination test is done to have an idea about germination percentage as affected by seed born fungi. The germination percentages of four varieties were 92%, 85%, 80% & 70% for Berseem cultivars like Peshawari, Faisalabadi, S1 and Agaiti Berseem. The identified fungal pathogens affected germination by 10-30%. *Aspergillus niger*, *Cladosporium herbarum*, *Curvularia lunata*, *Drechslera tetramera* *Penicillium* sp. and *Colletotrichum capsici* are new records to Berseem crop.

**Index Terms**- Seed borne, Mycoflora, Fungi, Berseem cultivars, Pakistan.

## I. INTRODUCTION

Berseem (*Trifolium alexandrinum* L.) is an important Rabbi Legume crop cultivated for fodder purpose. This is native to Egypt and known as Egyptian clover and is the major forage legume crop of India and Pakistan. Owing to the highest fodder yield (100-125t ha-1) and capable to produce 5-7 cuts of forages, it is called the king of fodder. Besides, this crop is grown for reclaiming saline soils especially rice growing areas of the Punjab [1].

This crop was introduced first in Sindh during 1904 and then bought into cultivation in the Peshawar region in 1924 from where, it was then moved to the irrigated plains of Punjab [2].

This is an important Rabi fodder crop that is grown in the area of 797 thousand hectares in Punjab yielding 23671.7 tones [3]. It grows well in a moderate climate, however temperature below 20°C retard the growth of crop. It has adaptability on variety of soil ranging from medium to heavy loam. Berseem is resistant to alkaline soil and its growth is best in soil pH range of 7.0-8.5. The crop is very popular due to its palatability, fodder supply for a long period, protein contents and increase milk yield in cattle. Since this crop is leguminous nature, therefore it enriches the soil and minimizes the cost of N<sub>2</sub> fertilizer [4].

Seed-borne diseases involve in deterioration the growth and productivity of various crops [5,6,7]. The pathogens present externally or internally on seeds as contaminant, may cause seed abortion, seed rot & necrosis and reduce germination capacity as well as seedling damage. This will result in development of disease at later stages of plant growth by systemic or local infection [8,9].

The important seed born fungal genera recorded from the major crops of Pakistan are: *Alternaria*, *Aspergillus*, *Ascochyta*, *Botryodiplodia*, *Botrytis*, *Cephalosporium*, *Colletotrichum*, *Curvularia*, *Fusarium*, *Phoma*, *Phomopsis*, *Drechslera*, *Rhizoctonia*, *Sclerotium*, *Septoria*, *Spomgospora*, *Stemphylium*, *Tilletia*, *Tricochonis*, *Ustilago* and *Verticillium* mostly identified from wheat, rice, maize, cotton, vegetable legume and oil crops seeds [10].

Like many other crops, Berseem is also attacked by a number of fungal diseases. So far, root rot, wilt and leaf spot diseases are recorded in Pakistan. Root rot and leaf spot diseases alone cause substantial losses every year in chilies [11]. Some sporadic information is available on the existence of seven fungal pathogens viz. *Arthrobotrys* spp., *Alternaria alternata*, *Cephalosporium* spp., *Fusarium solani*, *F. semitectum*, *Stemphylium sarciniforme* and *Verticillium* spp. that attacked on Berseem crop [12]. However detailed information is missing to report severity, frequency and effect on germination of seeds of Berseem cultivars. Keeping in view, present study was designed to identify and isolate fungal pathogen associated with seeds of four Berseem cultivars and their effects on the germination percentage.

## II. MATERIAL AND METHODS

The study was carried out at plant pathology Lab., PMAS-Arid Agriculture University, Rawalpindi.

### Collection of Materials:

The seeds of four Berseem varieties such as Peshawari, Faisalabadi, S1 and Agaiti Berseem were collected from National Agriculture Research Council (NARC), Islamabad. The seed were stored at 4°C in refrigerator till used.

### Germination of seeds:

Two standard methods i.e. Blotter-paper and Rag doll methods were used for the germination of seeds (Anon., 1976). In Blotter-paper method, four varieties of Berseem, each having 200 seeds were surface sterilized with Clorox bleach (25%) and placed on

moist filter paper spreading in Petri plates inside laminar flow. After 3-4 days, the germination percentage was noted. After 6-7 days, fungal attacked seeds were observed under stereomicroscope. In Rag doll method, 100 seeds were placed on two well moistened blotters and then covered with another well moistened blotter of same sizes. The blotters were wrapped in plastic bag and incubated at 20°C for seven days. After seven days, the germination percentage was recorded.

**Isolation and Identification of Fungal pathogen:**

Potato dextrose agar media (PDA) was prepared and autoclaved at 15 lbspsi for 15-20 minutes. Twenty ml of media poured in Petri plates and solidified. The identified fungus was transferred on media inside the laminar flow with the help of sterile needle. After 4-5 days, colonies were observed using stereomicroscope and identified fungus with the help of keys and manuals. For further confirmation, slides were prepared from culture and observed under microscope. The permanent slides were prepared using lectophenol and sealed with ordinary nail polish.

III. RESULTS & DISCUSSION

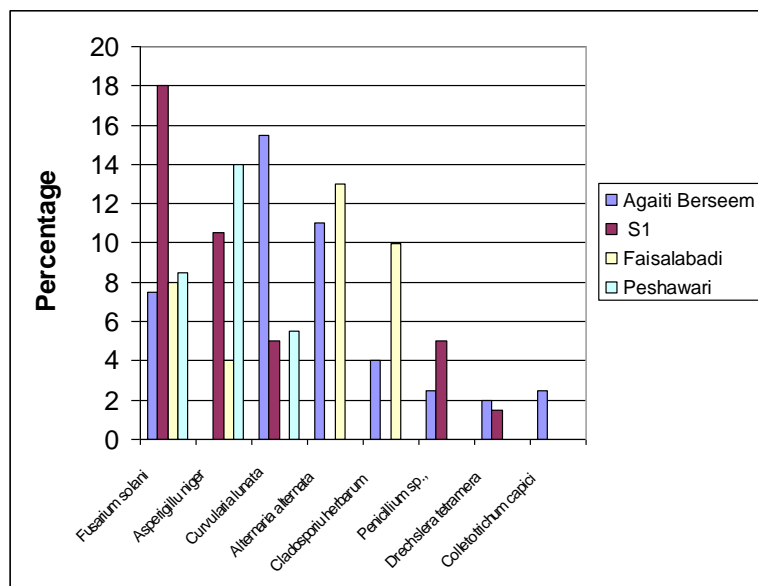
During the study, a total of eight fungi viz. *Aspergillus niger*, *Alternaria alternata*, *Cladosporium herbarum*, *Curvularia lunata*, *Drechslera tetramera*, *Fusarium solani* *Penicillium* sp. and *Colletotrichum capsici* were identify on the basis of their culture and morphological characteristic (Table 1). *Aspergillus niger*, *Cladosporium herbarum*, *Curvularia lunata*, *Drechslera tetramera*, *Penicillium* sp. and *Colletotrichum capsici* are new records on Berseem crop and not previously reported from Pakistan. Most of them are reported by various workers from crops other than Berseem [12,13,14].

Fig. 1 shows that Agaiti was highly susceptible variety which infected by seven fungal pathogens, followed by S1 (5 spp.), Faisalabadi (4 spp.) and Peshawari (3 spp.). On an average, *Fusarium solani* infected the highest number of seeds (10.5), followed by *Aspergillus niger* (7.12), *Curvularia lunata* (6.5), *Alternaria alternata* (6.0) and *Cladosporium herbarum* (3.5), while rest of three fungi infected little bit. Variety wise, Agaiti was heavily infected by *Curvularia lunata* (15.5%), followed by *Alternaria alternata* (11%) and *Fusarium solani* (7.5%), while rest of four fungi scarcely affected seeds of the said cultivar. The data also revealed that Peshawari variety is tolerant and less infection was observed on it (Table 1).

The germination percentage of seeds is provided in Table 2. It reveals that highest percentage of seed germination was found in Peshawari (92%), followed by Faisalabadi (85%), S1 (80%) and Agaiti (70%). On the other hand, highest percentage (45) of infected seeds were observed in Agaiti (Table 3), followed by Faisalabadi (35%), Peshawari (28.5%) and S1 (40%). Our result are in the agreement of [15] Ahmad *et al.*, (1993), who reported that seed infection is due to presence of seed born fungi which reduce the germination percentage.

**Table 1: Percentages of seeds of four varieties of Berseem infected by various fungi.**

Sr#	Fungal Pathogens	VARIETIES				Average
		Agaiti Berseem	S <sub>1</sub>	Faisalabadi	Peshawari	
1	<i>Fusarium solani</i>	7.50%	18%	8%	8.50%	10.54
2	<i>Aspergillus niger</i>	0%	10.50%	4%	14%	7.12
3	<i>Curvularia lunata</i>	15.50%	5%	0%	5.50%	6.5
4	<i>Alternaria alternata</i>	11%	0%	13%	0%	6
5	<i>Cladosporium herbarum</i>	4%	0%	10%	0%	3.5
6	<i>Penicillium sp.</i>	2.50%	5%	0%	0%	1.87
7	<i>Drechslera tetramera</i>	2%	1.50%	0%	0%	0.87
8	<i>Colletotrichum capsici</i>	2.50%	0%	0%	0%	0.62



**Fig. 1: Percentage of fungal pathogens in studied Berseem cultivars**

**Table 2: Germination percentage of four varieties of Berseem seeds by Blotter Paper Method.**

Variety	Germinated Seeds	Non-Germinated Seeds	Germination %age
Peshawari	184	16	92%
Faisalabadi	170	30	85%
S <sub>1</sub>	160	40	80%
Agaiti Berseem	140	60	70%

**Table 3: Percentage of infected Berseem seeds of four varieties by Blotter Paper Method.**

Variety	Infected Seeds	Non-Infected Seeds	Percentage of Infected Seeds
Berseem CV Peshawari	57	143	28.5%
Berseem CV Faisalabadi	70	130	35%
Berseem CV S <sub>1</sub>	80	128	40%
Agaiti Berseem	90	110	45%

#### IV. CONCLUSION

The results showed that fungi identified from the seed of Berseem cultivars affected the germination of seeds. It can be concluded that decrease in germination percentage of seeds will minimize plant per unit area required for better performance of agricultural crops. Therefore, management efforts are required to minimize the growth of identified pathogens for good vigor and germination. The alternate way for controlling of such diseases may be seed treatment before sowing crops. The yield of Berseem per hectare is less in world. The low yield is considered due to malignant practices or pest attack but this study shows that many seed born fungi may be responsible. The study provides baseline for future work about this much needed but neglected aspect of crop behavior

#### REFERENCE

- [1] Khalil, I.A., Jan, A. (2000). Cropping Technology. Millennium Ed. National Book Foundation, Islamabad, Pakistan. pp.169-203.
- [2] Khan, I., Jan, A.U., Khan, I., Ali, K., Jan, D., Ali, S. Khan, M.N. (2012). Wheat and Berseem cultivation: a comparison of profitability in district Peshawar. *Sarhad J. Agric.* 28(1): 83-88.
- [3] Anonymous. (1990). Punjab Agricultural Statistic 1980 to 1990. Director of Agriculture Crop Reporting Service, Punjab, Lahore.
- [4] Sanaullah, K., Khan, N. (1986). Effect of various NPK levels on green fodder yield of Berseem. *Sarhad J. Agric.* 2(4): 605-608.
- [5] Kubiak K.A., Korbas. (1999). Occurrences of fungal diseases on selected Winter Wheat cultivars. *Postepy w Ochrone Roslin.* 39(2): 801-804.
- [6] Weber R., Kita, W., Hrynczuk, B., Runowska-Hrynczuk, B. (2001). Effects of tillage method on occurrence of culm base disease in several winter wheat cultivars. *Electronic J. Polish Agric. Univ. Agronomy,* 4(2): 06.
- [7] Dawson, W.A.J.M., Bateman, G.L. (2001). Fungal communities on root of wheat and barley and effect of seed treatments containing fluquinconazole applied to control take-all. *Plant Pathology,* 50: 75-82.
- [8] Khanzada, K.A., M.A Rajput, G.S. Shah, A. M Lodhi and F. Mehboob. 2002 Effect of seed dressing

fungicides for the control of seed born mycoflora of wheat. *Asian Journal of Plant Sciences,* 1(4):441-444.

- [9] Bateman, G.L., Kwasana, H. (1999). Effect of number of Winter Wheat crop grown successively on fungal communities on wheat roots. *Applied Soil Ecology,* 13:271-282.
- [10] Bhutta, A.R., Ahmad, S.I. (2001). Seed pathological techniques and their application. National Book Foundation, Islamabad.
- [11] Saleem, A., Ansar, Hamid, K., Jamil, F.F. (1998). Effect of the physical parameters on the incidence of root and collar rot disease in chillies. *Pak. J. Bot.* 30:39-43.
- [12] Ali, S., Wahid, A., Saleem, A. (1991). Seed borne mycoflora of berseem (*Trifolium alexandrinum* L.) in Punjab, Pakistan. *J. Agri. Res.,* 29(4): 567-577.
- [13] Sinha, A., Singh, S.K., Qasair, J. (1999). Seed mycoflora of French bean and its control by mean of fungicide. *Der Tropenlandwirt,* 100(1): 59-67.
- [14] Zeeshan, Z.A., Haq, I., Raja, U. Sobia, C. (2003). Seed born mycoflora of sesame (*Sesamum indicum* L.). Annual proceedings of 4th National conference of plant pathology.
- [15] Ahmad, S.I., Bhutta, A.R. (1993). Wheat seed health testing for *Ustilago tritici* and *Tilletia indica*. *Pak J. Agric. Res.,* 11(2): 222-226.

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