International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 7, Issue 10 -2020

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.10.006

Isolation of seed borne mycoflora from different varieties of wheat crops under the irrigated system of Faisalabad, Pakistan

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Abstract

Wheat (*Triticum aestivum* L) is a staple food and cash crop in the world. The wheat seeds play a vital role in the transmission of pathogens caused diseases. The infections are present in nature in the form of seed, soil and air borne pathogens. The major seed borne diseases are loose smut (*Ustilago tritici*), Fusarium Rot (*Fusarium oxysporum*), Karnal bunt (*Tilletia indica*), Common bunt (*Tilletia laevis*), Ear cockle (*Clavibacter tritici* and *Anguina tritici*). The present research was conducted to isolate seed born mycoflora collected from different wheat varieties at the research area of Plant Pathology University of Agriculture, Faisalabad during Rabi season 2018-2019. The experiment was laid out with randomized complete block design and complete randomized design and the obtained results were statistically analyzed. *P.expansum* was recorded significant infection (30%) in blotter paper and *A. flavus* through PDA medium from Johar-2016. respectively with *Co-efficient of determination* (R^2 = 0.4). Similarly, *Aspergillus niger*, *Rhizopus spp.*, *Alternaria alternata* and *F. oxysporum* were isolated from Anaj-2017. Maximum Rhizopus incidence (40%) was significantly higher through PDA method showed week positive relationship (R^2 = 0.04, R^2 = 0.0182). The pathogen *F. oxysporum*, *A. acracious*, *P. expansum* were isolated from Galaxy-2013. Our results showed strong positive *co-efficient of determination* (R^2 = 0.92, R^2 = 0.96) in Galaxy-2013, however few seeds were germinated during incubation process. The result found that germinated seeds (50%) were recorded maximum in Gold-2016 compared to infected seed. The strong positive *co-efficient of determination* (R^2 = 0.80, R^2 = 0.77) showed polynomial curve recorded through PDA and blotter paper methods found the best fitness of the model. The pathogens isolated from Fsd-2008 found that *F. oxysporum*, *A. alternata*, *P. expansum*, *Rhizopus spp*., *A. niger* and *A. flavus* were isolated from Fsd-08. The maximum *A. niger* (25%) was found by blotter paper with moderate positive *co-efficient of determination* (R^2 = 0.56) and *F. oxysporum* (25%) with weak positive polynomial relationship (R^2 = 0.15) through PDA.

Keywords: blotter paper, Isolation, mycoflora, PDA, pathogens, seed borne, wheat, Pakistan

Introduction

Wheat belongs to the family Poaceae and it is a universal cultivated grass, grown mainly for food and cash earnings. Wheat is the staple food and it provides 40% to the world's population with 20% food calories (Rehman, *et. al.*, 2011). Wheat is the largest grain crop of Pakistan and it also the staple food on an area of 8,734 thousand hectares, however wheat crop is cultivated and it causes a decrease of 2.6% compared to eight thousand hectares during 2017-18. The production stood at 25.49 million ton's showing a deterioration of 4.4 percent over the last year manufacture of 26.67 million tons. Wheat contributed 9.1% in the value added in agriculture and also contributes 1.7% of GDP. Wheat is widely grown across the entire of United States, Europe, China and central Canada and in several other countries in the

Southern hemisphere. The 4th main wheat producing countries in the world are China, India, United States and Russian (Shuaib, *et al*., 2007 and FAO, 1996).

The production of wheat is low compared to the production of developed countries in the world (Ahmed, *et. al*., 2013). It is important to produce more wheat crops to the food security of the world and also to maintain sustainable supply to consumers. One way to save wheat crop throughout the world is to minimize the effects of biotic and abiotic agents (Chatrath, *et. al*., 2007). There are number of biotic and abiotic agents affected negatively to the wheat crops at different stages resulted to reduce growth, quality and quantity. Wheat crop is sensitive to a lot of infectious diseases like fungi, viruses, bacteria and nematodes (Bockus, *et. al*., 2010).

In Pakistan, wheat is mostly affected by fungi (Smut, Rust, Bunt) and environmental conditions favorable for their growth (Bux, *et. al*., 2012). Although the frequency of all pathogens critical however rusts tend to be more ubiquitous globally as well as in Pakistan (Bux, *et. al*., 2012). Leaf rust of wheat (*Puccinia trilicina* f. sp. *tritici*) is more destructive pathogen of wheat crops that initially appears on foliar parts. The pathogen infection spreads very rapidly in favorable climatic factors that can significantly reduce the grain yield (Hassan, 1979). In Pakistan, significant pathogen infection was recorded in the wheat growing areas (Tahir, 1978). There are many physiological races of leaf rust pathogen (Johnston and Browder, 1996), however, wheat varieties are infected from these virulent races of the rust pathogens (Nayer, *et. al*., 1987).

Intensive cultivation and monoculture have led to the incidence pathogen infection like Smut; Bunt, black tip disease etc. Severe infection of pathogen infections were recorded upto 1990 resulted high qualitative and quantitative losses. The aim of the study was conducted to isolate seed borne mycoflora collected from wheat varieties through different methods under irrigated fields of Plant Pathology University of Agriculture, Faisalabad during Rabi 2018-2019.

Materials and Methods

This field experiment was conducted at the Plant Pathology Research area, Department of of Plant Pathology University of Agriculture Faisalabad during Rabi season 2018-2019 to isolate seed borne pathogen from different varieties of wheat by different methods. The experiment was executed by randomized complete block design (RCBD) with three replications in field conditions. The wheat varieties Galaxy- 2013, Faisalabad-2008, Gold-2016, Johar-2016 and Anaj-2017 were used in the experiment.

Fig 1. Non uniform germination due to *Fusarium* and harvesting of infected seedlings

Sample collection and storage

The associated mycoflora seeds of different varieties of wheat were harvested from field area of Plant Pathology UAF, Faisalabad. Samples of Johar-2016, Anaj-2017, Galaxy-2013, Gold-2016, and Fsd-2008 were collected and stored in seed health testing lab at 4^0C (Fig. 1).

Pathogen identification

The seed associated mycoflora was detected through incubation test 1) Standard Blotter Method and 2) Agar Plate Method (ISTA, 1976). In agar plate method, 200 gram of potatoes was boiled in 1200 ml of water for about an hour, cool down and stained the mixture. Then 20g of glucose and 17g of ager were added and boiled again for one minute. After that 100 ml PDA was poured in 300 ml conical flask and sealed. The material was autoclaved at 121°C at 15 PSI for 20 minutes. After cooling, PDA was poured into sterilized petri plates. After pouring plates were placed until the media was solidify. When media become solidify 10 seeds per plate were placed and plates were incubated at 25°c for 4-6 days (Sikandar *et. al*., 2019).

After examination under stereoscopic binocular microscope mycelial growth of seed associated fungi were isolated with the help of sterilized needle and transferred into petri plates containing PDA media by single hyphal tip method. All this procedure was done in laminar flow chamber in sterilize condition. Inoculated plates were kept at 28°C in the incubator with 12 hr of light and 12 hr darkness for the colony emergence of pathogenic fungi. After the colony formation the pathogenic fungi were again transferred to PDA media by single spore technique for purification. These plates were kept in the incubator again with earlier mention control condition for next seven days. With the assistance of stopper borer 1cm plates shape the immaculate culture of *F. oxysporum* and put on the PDA containing petri plates. Petri plates were hatched at 25^oC and settlement distance across was measured when development of the test organism was finished in charge. Information was recorded 3, 6 and 9 days and was subjected to factual examination keeping in mind the end goal to exhibit the outcomes in scientific expressions. When pure colonies of seed associated fungi were appeared their colony and morphological characters were studied (Yaacoby and Seplyarsky, 2011; Wahyuno, 2012).

Mixing of seed with purified isolated culture

Fresh healthy seeds of 5 varieties Johar-2016, Anaj-2017, Galaxy-2013, Gold-2016 and Fsd-2008 were taken and surface sterilized with 1% NaOCl and two washing of sterilize water. Pure 7 days old culture of *Fusarium oxysporum* was taken and thick paste was made by mixing distilled water with culture. All seeds of different varieties were mixed with this paste one after the other. Rectangular trays having 28cm length and 18cm width were taken and seeds were sown in these trays. In each tray seeds of two varieties were sown in 6 rows. *Co-efficient of determination* (R^2) was conceded out for the model comparison between these two methods (Iqbal and Feng, 2020; Iqbal, *et. al.* 2019a; Iqbal, *et. al.* 2019b).

Results and Discussion

The seed borne mycoflora were assessed by blotter paper and agar plate method. Two hundard seeds collected from each variety were taken and acessed for presence of mycoflora from which 100 were plated on blotter paper and 100 were plated on agar medium. The plated seeds were incubated for 8 days at 25°C in case of blotter paper and for 6 days in case of PDA media. After incubation seeds were observed under stereoscopic microscope and mycoflora was identified. On each variety number of seed infected with specific pathogen was recorded from each plate and their percentage on PDA medium and blotter paper was recorded and deppicted. *Aspergillus flavus, Penicillium expansum, Alternaria alternata* and *Fusarium oxysporum* were isolated from Johar-2016. *P.expansum* was recorded significant infection (30%) in blotter paper and A. flavus through PDA medium respectively (Figure 2A) with *Co-efficient of determination* (R^2 = 0.4). However, *Aspergillus niger, Rhizopus spp., Alternaria alternata* and *F. oxysporum* were isolated from Anaj-2017. Maximum Rhizopus incidence (40%) was significantly higher through PDA method showed week positive relationship (R^2 = 0.04, R^2 = 0.0182). Mostly seeds were free from pathogens after the incubation only some seeds were infected. The result found that some seeds were germinated during incubation. The pathogen *F. oxysporum, A. acracious P. expansum* were isolated from Galaxy-2013 (Fig. 2C). Our results showed strong positive *co-efficient of determination* (R^2 = 0.92, R^2 = 0.96) in Galaxy-2013, however few seeds were germinated during incubation process. The result found that germinated seeds (50%) were recorded maximum in Gold-2016 compared to infected seed (Fig. 2D). The strong positive *co-efficient of*

determination (R^2 = 0.80, R^2 = 0.77) showed polynomial curve recorded through PDA and blotter paper methods found the best fitness of the model (Fig. 2D). The pathogens isolated from Fsd-2008 found that *F. oxysporum*, *A. alternata*, *P. expansum*, *Rhizopus spp*., *A. niger* and *A. flavus* were isolated from Fsd-08. The maximum *A. niger* (25%) was found by blotter paper with moderate positive *co-efficient of determination* (R^2 = 0.56) and *F. oxysporum* (25%) with weak positive polynomial relationship (R^2 = 0.15) through PDA (Fig. 2E). Our results are in line with the researcher who reported that seed borne mycoflora in wheat crops (Marcinkowska, 1998; Iqbal *et. al.*, 2014).

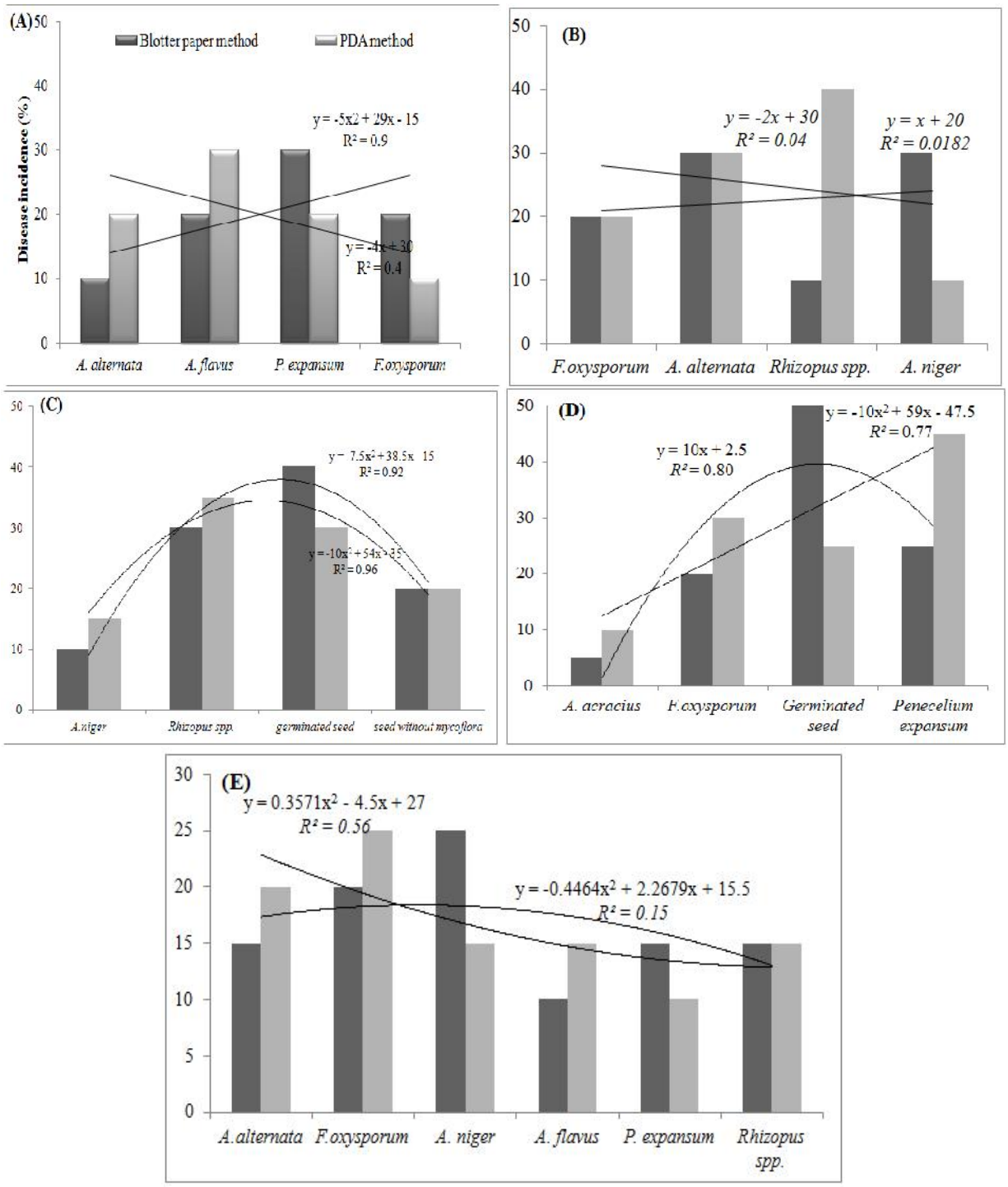

Fig 2. Seed mycoflora (%) isolated from Johar-2016 (A), Anaj-2017 (B), Galaxy-2013 (C), Gold-2016 (D), Fsd-2008 (E)

Whereas *A. flavus* (*Aspergillus flavus*), *P. expansum* (*Penicillium expansum*), *A. alternata* (*Alternaria alternata*) and *F. oxysporum* (*Fusarium oxysporum*), *Aspergillus niger* (*Aspergillus niger*)

The healthy seeds of wheat varieties like Johar-2016, Anaj-2017, Galaxy-2013, Gold-2016 and Fsd-2008 were taken and surface sterilized with 1% NaOCl with two washing of sterilize water (Fig. 3). At the point when wheat is assaulted by various seed borne pathogen it shows a few morphological changes in it. This exploration manages location of a few seed borne pathogen, their distinguishing proof and administration of *Fussarium* curse pathogen uncommonly. Seeds were plated and incubated and after incubation fungi were isolated. A specimen of 400 seeds of every variety, at 8 areas were collected and examined. Surface disinfected seeds were set on agar medium into Petri plates. Each plate contained 15 seeds.

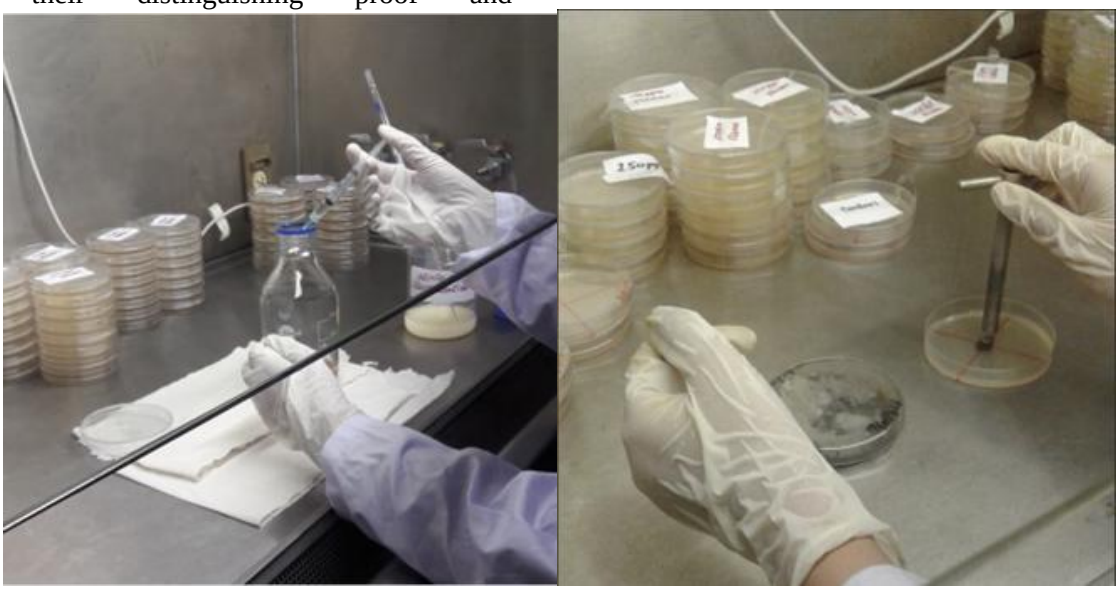

Fig 3. Laboratory studies showing inoculated wheat varieties by Food Poison technique

These plates were hatched, and information was recorded on eight day of plating. Distinguishing proof of parasites was finished after various keys (Marcinkowska 1998; 2001) and number of perceived species counted together for both illustrations. Larger piece of happening living beings that suggests nine customary species and 4 genera of more than one creature classifications (*Aspergillus, Cladosporium, Fusarium, Penicillium*) were isolated and perceived. Factual investigation was likewise accomplished for examination of recurrence of three species in *Ascochyta scourge* and *Fussarium oxysporum*. The most segregated growths from seeds, of various cultivars of various territories were *Fussarium oxysporum*, *Penicillium* spp. *S. botryosum* and *Penicillium* spp. The fungi were isolated and purified and after purification microscopic studies of some fungi was carried out and their morphological characters were studied. Khan, *et. al*., (1988) described pathogenicity test for important seed-borne fungi; the test inoculum was prepared by seed lots showed high incidence of infection (%) with *Fussarium oxysporum***.** The seeds were plated by blotter paper method and incubated. After incubation observed under stereoscopic microscope and spores of *Fussarium* were detached by single hyphal tip technique exchanged to PDA media in a Petri dish and brooded for a few days oblivious at 24±2°C. At the end it was concluded that strong maximum positive *coefficient of determination* recorded by blotter paper methods by Galaxy-2013 followed by Johar-2016. *Aspergillus flavus, Penicillium expansum, Alternaria alternata* and *Fusarium oxysporum* were isolated from Johar-2016 recorded significant infection (30%) of *P.expansum* through blotter paper and A. flavus through PDA medium respectively with moderate *Co-efficient of determination.* However, *Aspergillus niger, Rhizopus spp., Alternaria alternata* and *F. oxysporum* were isolated from Anaj-2017 recorded maximum *Rhizopus* incidence (40%) was significantly higher through PDA method showed week positive relationship.

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Quick Response Code

DOI:10.22192/ijarbs.2020.07.10.006

How to cite this article:
Muhammad Anas Bahoo. (2020). Isolation of seed borne mycoflora from different varieties of wheat crops under the irrigated system of Faisalabad, Pakistan. Int. J. Adv. Res. Biol. Sci. 7(10): 55-61.
DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.10.006