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MYCOFLORAL PATHOGENICITY ON CORN (*ZEA MAYS*) SEEDS AND ITS MANAGEMENT BY DIFFERENT STRATEGIES IN AZAD KASHMIR PAKISTAN

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Abstract

The study was conducted to evaluate mycfloral pathogenicity prevailing on corn (*Zea mays* L.) and indigenous management strategies in different districts of Azad Jammu & Kashmir (AJK) Pakistan. Fungi were grown on potato dextrose agar (PDA), isolated and identified by colony counter and microscopic analysis. Eighteen different fungal species of eight genera were found associated with maize seeds. For verification *In vitro* seeds inoculation tests and pathogenicity rate was measured. On infection rate basis *Fusarium moniliforme* was (80.75 %), *Aspergillus niger* (63.25%) and *Rhizopus stolonifera* (32.75%), respectively. Their phytogeographical prevalence was found in descending order in Bhimber (61.50%), Mirpur (60.25%) and Muzaffarabad (39.03%). Analysis of variance (ANOVA) demonstrated that effect of different species was quite dynamic and fluctuating not only for taxa based differences but also with climate and altitudinal variations. The impact of different fungal inoculations was tested by least standard deviation (LSD) which demonstrated that d. distilled water treatment had highest seed germination rate 75.87%, with *F. moniliforme* 53.64%, *Aspergillus niger* 62.55% and their synergetic infusion showed least value of 41.73%. To reduce or eliminate the detrimental impacts of these species, four different management strategies were evaluated in experimental plot and results were analyzed by LSD. The garlic extract treatment was the best with highest seed germination rate (85.75%), followed by Benomyl treatment (84.75%), hot water treatment (79%), and distilled water treatment (65%), respectively. It was observed that all the results were significantly different from each other but the interaction between treatments and localities showed various degrees of variations.

Introduction

Corn (Maize) is one of the world's top most cereal crop along with wheat and rice and it ranks 3rd in terms of total cultivation and yield (Anon, 2006). Maize (*Zea mays* L.) belongs to the family Poaceae having five phenotypes originating from common single ancestor. Maize is a main source of income in developing countries as it provides staple food, oil, fodder and confectionary products to many populations of various countries around the globe. In the world production of maize is 817 million tons while in Pakistan 3604.7 thousand tons are grown on area of 1051.7 thousand hectares during the year 2008 (Anon., 2006; 2007). The major part (97%) of total production comes from two provinces Khyber Pakhtunkhawah (57%) and Punjab (38%) whereas Baluchistan and Sindh contribute only (3%). Azad Jammu & Kashmir lies between longitude 73-75° and latitude 33-36° with an area of 5734 sq miles (12397 Km^2) with diverse an dynamic climate. Its geographical pictorial ranging from plains of Ifthkarabad (Chanmb) District Bhimber to glacial peaks of Neelam Valley is strategically very important (Ishtiaq *et al.*, 2012). Maize being one of the dominant cereals of AJK is grown in a wide range of agro-ecological zones and altitudes ranging from 1,828 meters to 3,656 meters elevation. The crop accounts for major part of the cultivated area in season constituting 41 percent of the annual cropped area (Anon., 2000). Different regions of AJK cultivate maize for domestic and/or commercial purposes and its growth and yield production per acre fluctuates due environmental and biological parameters. So this makes it an interesting to conduct comprehensive research on maize crop in A.K. There is little reliable empirical information available pertaining to farmers' maize production practices and inters agro-ecological zone (AEZ) variations in AJK observed the paucity of country (Ali *et al*., 2001).

Maize cultivation has been threatened or restricted yield due to many parameters such as drought, salinity, wild animals and microbial diseases all over the world ca. 11% (Tagne *et al.,* 2008; Ahmad *et al*., 2012). Past research previews that over sixty different types of diseases are caused by various pathogens affecting maize crop (Anon., 2007) and fungi is the dominant in this plethora. There are reported huge losses of maize grains in the field as well as in storage houses by seed-borne pathogenic fungi (Onifade, 2000). Fungi deteriorate food grains by producing mycotoxins and aflotoxins during storage consequently shedding menace on its nutritive quality (Park *et al*., 2004; Koirala *et al*., 2005; Domijan *et al*., 2005). The most predominant reported fungi infecting seed germ plasm were *Aspergillus* and *Fusarium* species (Askun, 2006; Fandohan *et al*., 2003). Anne *et al*., (2000), Curtui *et al*., (1998) and Susan *et al*., (2005) isolated several *Fusarium* species from maize seed in previous research from various regions of the world. *Fusarium* and *Aspergillus* species were found as common fungal contaminants of maize that produce high mycotoxins (Bakan *et al*., 2002; Verga *et al*., 2005).

In Pakistan the most common seed-borne mycoflora recorded on maize seeds are *Fusarium* spp., *Alternaria* spp., *Aspergillus* spp., *Curvularia* spp., *Helminthosporium maydis, Monilia* spp., *Penicillium* spp., *Rhizopus* spp., and *Trichoderma* spp., (Ghafoor & Khan, 1976). *Fusarium moniliform* produces gibberela ear rot, kernel rot, stalk rot, seedling blight, seed rot, wilt and stunt (Thiel *et al*., 1991). *Aspergillus* species affects systemically and produces

aflatoxin in seedling of maize and damage stored corn (Blat, 1969). In Azad Kashmir incidence and severity of pathogenic fungi is more prevalent due to humid and dynamic climate conditions and hitherto no research has been reported on it.

To control loss of maize yield due to mycofloral toxicity, pathogen free seeds are prerequisite. For better yield of any crop prior to sowing, different seed treatment mechanism is an effective management strategy (Neergaard, 1974). Literature survey demonstrates that various methods are applied to purify seed germplasm such fungicidal chemicals: Benomyl, Acetic acid (5%), Urea (5%), Sodium acetate (5%), Thiabendazole (Hepperly *et al*., 1989). Ying *et al.,* (2005) studied the effects of different doses of various fungicides; however the results were not so significant. Nasim *et al.,* (2004) evaluated different pea varieties for the efficacy of different treatments. Seed samples were treated with 1% chlorox, heat treatment, and fungicide to assess their effectiveness. Chapman & Harris (1981) demonstrated that chemical method is not appropriate because they may cause health hazards and environmental pollution. Secondly, chemicals are beyond the approach of poor farmers due high expensiveness. Sometime different plants' extracts have also been employed to control the *Aspergillus* and *Penicillium* species in maize germplasm (Pinto *et al*., 2005).

The purpose of this research was multifarious: (i) to explore and prepare a checklist of mycoflora associated with maize crop in Azad Jammu and Kashmir, (ii) to determine incidence and prevalence of different fugal species on maize grown in different areas of Azad Kashmir and (iii) to evaluate efficacy of different management strategies in controlling seed associated pathogenic fungal inoculums and their impact on the germination of seeds.

Materials and Methods

Sample collection: Experimental seed samples of variety "Kashmiri gold" were collected by complete randomized pattern from different districts: Mirpur, Bhimber, Kotli, Sudhnoti, Poonch, Bagh, Muzaffarabad and Neelum of Azad Jammu & Kashmir. One kilogram composite sample was taken at random from a source of size one ton seeds from each sampling site. The samples were gathered in triplicate from each district, packed in polythene bags and properly labeled prior to laboratory analysis. These samples were transported in polythene bags for investigation in Plant Pathology Laboratory, The University of Azad Jammu & Kashmir Muzaffarabad. These maize seed samples were analyzed for mycoflora screening and determination of pathogenicity. All samples were grown in University model plot and/or green house in duplicate fashion.

Isolation of fungi: All seeds of different zones of AJK were isolated in different beakers and sterilized by immersing in 10% household chlorine bleach ($NaClO_2$) for 5 minutes, then rinsed in distilled water for 5 min and dried for 2 min (Elmer *et al*., 2001). For growing of maize seed associated mycoflora, potato dextrose agar (PDA) media was prepared according to recipe of Tanveer *et al*., (2011); Onkar & Sinclair (1985). PDA media was poured in sterilized petri dishes and Five seeds were placed in each Petri dish with ten replicates. The all experimental runs were incubated at 30 ± 2°C for seven days for fungal growth. The slide was prepared from each petri dish sample and observed under microscope (10 X, 30X, 50 X) and snaps were captured using Camera Lucida. The species type, vegetative size and number was as suggested by Dhingra & Sinclair (1985) and Tanveer *et al.,* (2010) recorded using digital fungal Colony counter technique (CCT). The isolated fungi were identified by using taxonomic features such as conidia and hypha (Benoit & Marthur, 1970; Tanveer *et al*., 2011) from manuals and herbaria specimen/slides preserved at the Department of Botany, University of Azad Jammu & Kashmir Muzaffarabad Azad Kashmir.

Frequency of prevalence: The frequency of prevalence (FP) was determined as per protocol of El-Awadi (1993). The total infection percentages of the component plating and seedlings tests were calculated by using following formulae:

$$\text{Prevalence (\%)} = \frac{\text{No. of pathogenic seeds}}{\text{Total seeds}} \times 100$$

Pathogenicity evaluation: To evaluate the pathogenicity of isolated and identified fungi from seeds an experiment based on RCD with 5-replicates was performed in Lab. Collected seed germplasm was sterilized in solution of Sodium Hypochlorite (13% v/v) for 5 min. The sterilized seeds were washed in d. dist. water and blotted on sterile filter paper. Then seeds were placed on 1% water agar (WA) plates and subsequently incubated at 25°C. Fifty seeds (10/plate) from the collected seed samples were used for the fungal positivity test. To check pathogenicity 4-5 days old seedlings grown under aseptic conditions were aseptically transferred to slants of WA in test tubes (1/tube). Five seedlings were inoculated per test tube and then incubated in the growth chamber at 22°C. Fungal infection free seeds and seedlings under the same experimental conditions were grown as control in a model plot of botanic research. The control samples were sprayed only with distilled water. The symptoms developed on the inoculated maize seeds and seedlings were compared with the control.

Management strategies: Maize seed samples associated with mycoflora were analyzed for different management strategies. For the purpose of evaluating different management strategies for fungal flora, the seeds were plated by using blotter technique subject to the ISTA rules (ISTA, 2001). Fungi grown on the incubated seeds were identified under the stereo-microscope (Lectophenol blue stain used for permanent slides) following the key of Mathur & Kongsdal (2003). A sample of 400 seeds for each locality was taken for each treatment in 4 replications, 100 for each. Three treatments namely, Hot-water, Plant diffusate and chemical were used to assess their effects on the mycoflora during the germination of maize seeds under investigation. One set of experiments was kept for controls by giving distilled water only.

Distilled water (control): For treatment with distilled water, seeds were soaked in autoclaved water for 5 minutes and then dried on blotting paper. Seeds were then allowed to germinate by using Standard Towel Method. These seeds were placed on anchor brand paper (size 24x48) in three rolls. Each roll was of 100 seeds. Papers were then incubated at 22 ± 2 °C for 7 days under an alternating cycle of 12 hours day and night using fluorescent tube light. Moisture was provided to keep the papers wet. Infection percentage was determined by grading seeds and seedlings.

Hot-water treatment: Seeds were soaked in distilled water for 5 minutes and then used to place in water bath at 53°C for 15 minutes and then used for experiment. These seeds were then placed on anchor brand paper (size 24x48) in three rolls. Each roll was of 100 seeds. Papers were then incubated at 22 ± 2°C for 7 days under an alternating cycle of 12 hours day and night using fluorescent tube light. Moisture was provided to keep the papers wet. Seeds and seedling were examined for grading into categories.

Plant diffusate treatment: Garlic bulb extracts was used for the antifungal activity. Material was crushed and make up to 400ml solution by using distilled water. The diffusate then filtered with Whatman's No.1 filter paper. Seeds were soaked in extract for 15 minutes and then dried on blotter paper. These seeds were then placed on anchor brand paper (size 24x48) in three rolls. Each roll was of 100 seeds. Papers were then incubated at 22 ± 2°C for 7 days under an alternating cycle of 12 hours day and night using fluorescent tube light. Moisture was provided to keep the papers wet. Seeds and seedling were examined for grading into categories.

Chemical treatment (fungicide): Benomyl, a fungicidal chemical was used for seed dressing at the rate of 2.5g/kg seeds and sampled seeds were soaked in the solution for 5 minutes and dried on the blotter paper. These seeds were then placed on anchor brand paper (size 24x48) in three rolls. Each roll was of 100 seeds. Papers were then incubated at 22 ± 2°C for 7 days under an alternating cycle of 12 hours day and night using fluorescent tube light. Moisture was provided to keep the papers wet. Seeds and seedling were examined for grading into categories. Germination % was also recorded after 7 days of incubation at 22 ± 2°C.

Statistical analysis: For statistical analysis "Analysis of Variance (ANOVA)" technique was employed suing MVSP and Matlab softwares and means were compared by using least standard deviation (LSD) @ 0.05 probability levels (Steel *et al*., 1996).

Results

The research was designed to study the prevalence, infection of different fungi associated with seeds of maize and its subsequent impacts on seeds/and seedlings growth. The other purpose was to devise some better methodology to lessen or eradicate pathogenicity by optimization of management protocol. During the project survey in year 2009-2012, twenty four spatial and temporal locations from eight different districts (marked as L_1-L_8) of Azad Jammu & Kashmir were selected, marked and examined for sample collection (Table 1). The seed germplasm of corn variety called "Kashmiri gold" was collected and preserved in Mycology and Plant Pathology Laboratory of University of Azad Kashmir (Muzaffarabad) for further experiment.

Table 1. Maize seeds associated mycoflora and its infection values (%) in different areas of Azad Kashmir.

S. #	Identified fungal species	Sampling sites and their infection values								
		L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	Total infection
1.	*Aspergillus niger*	13.75	7.0	8.25	1.5	15.0	6.25	7.5	4.0	63.25
2.	*A. flavus*	1.5	-	-	1.0	-	1.5	3.75	4.25	12.0
3.	*A. clavatus*	-	5.25	-	-	0.75	-	-	-	6.0
4.	*A. Fumigatus*	-	-	-	-	1.25	-	-	-	1.25
5.	*Fusarium moniliforme*	14.5	0.75	1.25	4.0	0.25	27.25	27.75	5.0	80.75
6.	*F. semitectum*	1.25	-	0.75	-	-	-	-	0.25	2.25
7.	*F. graminearum*	-	-	0.25	-	-	0.75	0.75	-	1.75
8.	*F. equisetti*	-	0.25	1.25	-	0.25	-	-	0.75	2.50
9.	*Alternaria alternate*	0.75	2.5	1.5	3.5	3.25	2.5	3.0	3.75	20.75
10.	*Drechslera maydis*	0.5	3.25	1.5	0.25	4.5	6.75	3.0	3.5	23.50
11.	*Curvularia lunata*	0.25	2.25	1.25	4.25	1.25	5.75	3.75	4.25	23.0
12.	*Rhizopus stolonifera*	6.25	-	6.25	4.5	3.5	3.5	3.5	5.25	32.75
13.	*Penicillium galaucum*	-	2.75	2.0	4.25	1.0	3.0	2.75	-	15.75
14.	*Mucor* spp.	1.0	1.25	-	-	3.25	2.25	2.5	3.5	13.75
15.	*Macrophomina phaseolina*	0.25	-	-	-	-	0.75	1.5	2.75	5.25
16.	*Cephalosporium acremonium*	-	1.25	1.5	-	-	-	1.0	0.75	4.50
17.	*Nigrospora oryzae*	-	-	-	0.25	-	-	0.75	0.75	1.75
18.	*Diplodia maydis*	-	1.25	-	-	1.75	-	-	-	3.0
	Total infections	**39.03**	**27.75**	**25.75**	**23.5**	**36**	**60.25**	**61.5**	**38.75**	

Key: L_1: Muzaffarbad, L_2:Poonch, L_3: Sudhnoti, L_4: Bagh, L_5: Neelam, L_6: Mirpur, L_7: Bhimber, L_8: Kotli

There was diverse data generated from the study depending on altitudinal and latitudinal variations. In the research total 18 species of 12 different genera were found from maize seeds with dynamic prevalence and infection rates (Table 1) and some of those identified species are shown in Fig. 8. The highest frequency was associated with genera *Aspergillus* and *Fusarium* having four taxa each, followed by one species of other eight genera (Table 1). The species *Fusarium moniliforme* was leading with infection rate of 80.75% and *Aspergillus niger* showed 63.25% infection value while *Rhizopus stolonifera* was third in this context with 32.75% concentration (Fig. 1). The phytogeographical analysis depicted that highest prevalence was found in following districts with descending order: Bhimber (61.50%), Mirpur (60.25%) and Muzaffarabad (39.03%). The least impacts on fungal species were demonstrated in district Bagh with 23.5% value (Table 1).

To explore impacts of mycoflora on maize seed germination in different treatments, an analysis of variance (ANOVA) was formulated. Its statistical data demonstrated that effect of different species was quite dynamic and fluctuating not only for taxa differences but also with climate and altitudinal variations (Table 2). For further comprehensive analysis least standard deviation (LSD) was determined for all the sampling sites in comparison with all available fungal species using different treatments during seed germination. The results demonstrate that distilled water treatment has highest seed germination rate with 75.87% and other treatment having *F. moniliforme* and *Aspergillus niger* infusion showed least value ca. 41.73 C (Fig. 2). In eco-climatic perspectives efficacy of different treatments on seed germination depicted different paradigm where Muzaffarbad sample had highest value (66.57 A), followed by Bhimber (64.10A) and Sudhnoti (62.55 A) respectively, and least difference (44.50 C) was shown in Kotli sample treatment trial (Table 3).

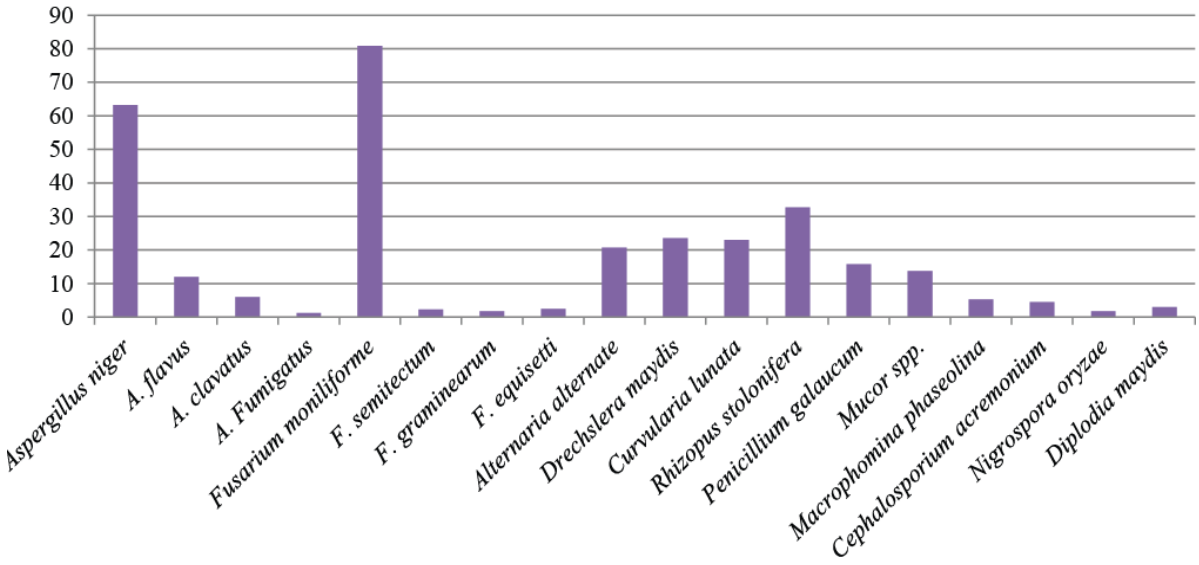

Fig 1. Fungal Infection rate (%age) in different localities of experiment of Azad Kashmir.

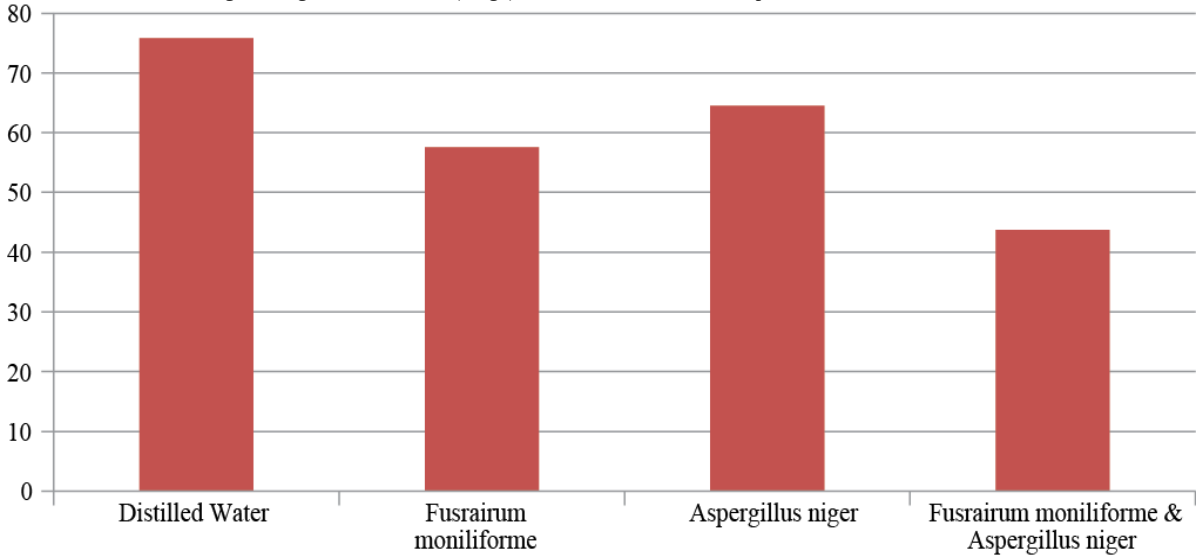

Fig. 2. Effect of fungal infusion trials on seed germination (%age) in different localities of Azad Kashmir.

Table 2. Analysis of variance of management treatments and different localities of Azad Kashmir.

	Effects of different mycoflora on seed germination (%)		
S. No.	**Factors**	**Df**	**Mean square**
1.	Treatments (A)	3	2869.794*
2.	Localities (B)	7	1921.217*
3.	AB	21	42.861

Table 3. Effects of different management treatments on seed germination (%) in different localities of Azad Kashmir by using L.S.D.

S. #	Localities	Different management strategies				
		Distilled water	**Hot water**	**Garlic extract**	**Benomyl**	**Means**
1.	Mirpur	68.00	83.25	81.00	83.00	78.81 A
2.	Bhimber	62.25	88.00	82.00	90.00	80.56 AB
3.	Kotli	72.75	88.75	82.75	93.25	84.37 A
4.	Sudhnoti	61.25	82.25	88.00	79.75	77.81 A
5.	Poonch	69.50	75.25	73.50	92.25	77.62 A
6.	Bagh	65.00	79.00	85.75	84.75	78.62 B
7.	Muzaffarabad	59.75	79.00	82.75	79.00	75.12 AB
8.	Neelum	64.75	83.25	73.75	92.75	78.62 AB
	Means	**65.37 B**	**82.34 A**	**81.19 A**	**80.59 A**	

Values in the same rows and column followed by the same letter are not significantly different (P=0.05)

Table 4. Effect of fungal treatments on seed germination (%) in different localities of Azad Kashmir by using L.S.D.

S. #	Treatments	% age of Seed Germination								Means
		L_1	L_2	L_3	L_4	L_5	L_6	L_7	L_8	
1.	Distilled water	85.50	81.25	79.90	72.00	86.50	66.20	78.40	57.20	75.87 A
2.	*F. moniliforme*	65.55	58.50	53.55	51.40	49.50	49.40	59.00	42.20	53.64 B
3.	*Aspergillus niger*	70.22	67.00	70.25	56.55	47.75	62.80	76.20	49.60	62.55 A
4.	*F. moniliforme* and *Aspergillus niger*	45.00	42.50	46.50	41.25	49.00	37.80	42.80	29.00	41.73 C
	Means	**66.57 A**	**62.31 A**	**62.55 A**	**55.30 B**	**58.18 B**	**54.05B**	**64.10A**	**44.50 C**	

*Key: L_1: Muzaffarbad, L_2:Poonch, L_3: Sudhnoti, L_4: Bagh, L_5: Neelam, L_6: Mirpur, L_7: Bhimber, L_8: Kotli

**Values in the same rows and column followed by the same letter are not significantly different (P=0.05)

Another important part of this research was to develop, optimize and recommend some management strategy to control hectic and injurious impacts of seed associated mycoflora on corn crop. In this context it is worth to say that human being is trying to control different agricultural losses by using classical, conventional and scientific based methods since time immemorial. Our research study tried four different management strategies *viz*., distilled water treatment (DWT), hot water treatment (HWT), garlic extract treatment (GET) and Benomyl treatment (BT) on different sampling locations on crop variety "Kashmiri gold" (Tables 3&4). HWT run depicted the best results during seed germination (82.34 A) and DWT was seen as the least useful management trial (65.37 B). With reference to climate HWT treatment trial was found the most appropriate in district Kotli (88.75%), followed by district Bhimber sample run with 88% effectiveness (Fig. 4). To compare effects of different management strategies on control of mycoflora on corn seed samples ANOVA was designed and it depicted a major difference among the samples and within different treatment trials (Table 5). The other trials varied in results in different areas and GET was found good in district Sudhnoti and BT seemed appropriate in district Kotli trial run (Figs. 5&6).

Discussion

Fungi are integral part of biosphere with its pivotal role which may be useful or detrimental to human beings. Man since his emergence on earth has been using plants (crops) for livelihood and subsistence. Different crops are used in various areas of globe as food, fodder and medicines. *Zea maize* (corn) is called cereal in Pakistan with its huge role in economy of the country after wheat and rice.

Pakistan has diverse climate and among those Azad Kashmir is very dynamic region with all types of climate availability (Ishtiaq *et al*., 2007). In Azad Kashmir, people mainly rely on natural resources and agriculture for life and in different areas of the region various crops are grown but maize is very frequently grown in all regions. It provides a main stream source of staple food for the habitants.

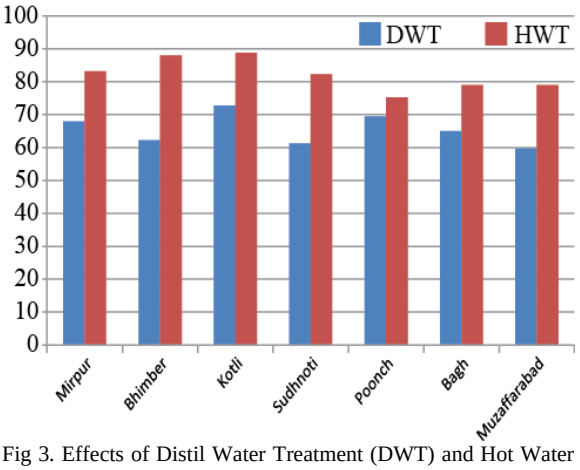

Fig 3. Effects of Distil Water Treatment (DWT) and Hot Water Treatment (HWT) on the Seed Germination (%) in different Localities of Azad Kashmir.

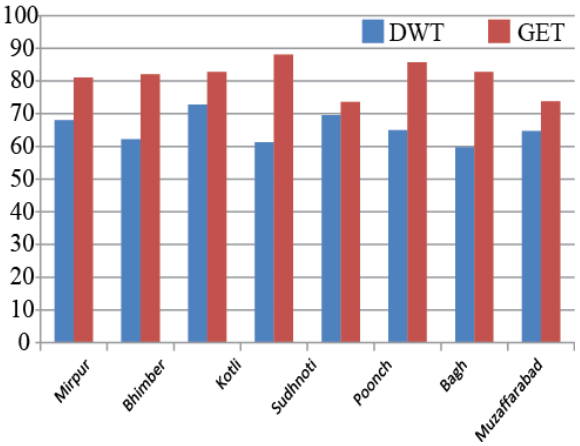

Fig. 4. Effects of Distil Water Treatment (DWT) and Garlic Extract Treatments (GET) on the Seed Germination (%) in different Localities of Azad Kashmir.

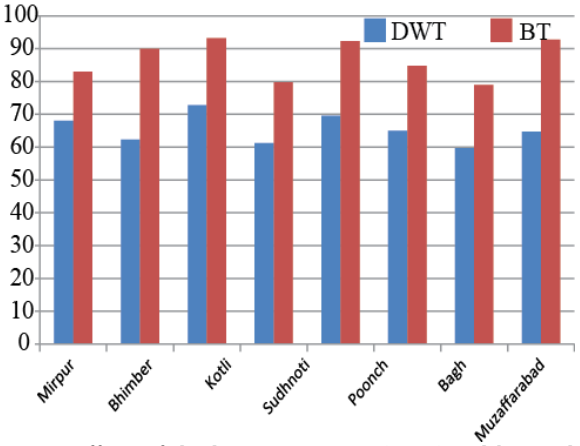

Fig. 5. Effects of distil water treatment (DWT) and benomyl treatments (BT) on the seed germination (%) in different localities of Azad Kashmir.

Plants have to face different types of diseases and epidemics and if it is crop then it badly hampers the economy of peasants. In Azad Kashmir *Zea mays* is also infected and affected due to different microorganisms and other insects. Among these fungi have huge detrimental loss as it effects not only vegetative part of plant but also seed germplasm is also damaged. Fungi associated with seeds are addressed in this research project.

The results showed that there were eighteen different species from eight genera which cause seed infection (Fig. 7 &8). These fungi particularly *Fusarium moniliforme* and *Aspergillus niger* species were more prevalent in the sampled area and these findings are corroborative with previous results of different research conducted in Pakistan and other tropical zones of the world (Orisi *et al.,* 2000; Ghiasian *et al.*, 2004). It was found that analyzed taxa of mycoflora has more incidence and prevalence in Bhimber and Mirpur areas of Azad Kashmir which also lies in sub-topic zone and this might be due to hot and humid climate of the region which provide appropriate environment of fungal proliferation.

The seed pathogenicity of different mycoflora associated was tested by using different treatments and it proved that there is dynamic fluctuation in prevalence and pathogenicity from species to species and from area to area in Azad Kashmir. This phenomenon may be true due to edaphological and geographical differences ranging from plains of Iftekharabad (Chanmb) to sky-scrapper mountains of Neelam valley (Ishtiaq *et al*., 2012). The identified species showed infection rate with variations but hitherto do impede the germination of seeds and seedlings growth and similar type of results have been previously reported in various studies (Richardson, 1979; Ahmad *et al*., 1993; Fakhrunnisa & Hashmi, 1992; Ahmad *et al*., 1993). The findings showed that *Fusarium moniliforme* has highest infestation and infection rate in the area of Azad Kashmir and this property badly hampers the seed germination and seedling growth due to production of mycotoxins (Domijan *et al.,* 2005). The result as demonstrated in Table 1 are also supported by previous work of Ibrahim & Farag, (1965) who claimed that it was due to production of mycotoxins in different *Aspergillus* and *Fusarium* species. For further comprehensive analysis least standard deviation (LSD) was determined for all the sampling sites in comparison with all available fungal species using different treatments. It demonstrated that control had significant difference with other single or synergistic treatments by various fungal diffusiate as shown in Fig. 3. Interactive synergistic impact was more prevalent and significantly different in Muzaffarabad area trials as compared with all other seven experimental runs (Table 1, Fig. 1). There is crystal clear conformity between these findings with past work of Arif & Ahmed (1969). There was found that *Fusarium moniliforme* was more harmful (53.64 % seed germination) than *Aspergillus niger* (62.55 % seed germination) for the seed of corn however their synergistic impact was more severe and retarding (41.73% seed germination) than single infection dose (Table 3). All these taxa cause damage to seeds by rotting it and this corroborate the past work of Ibrahim & Farag (1965). In other reports on maize plant it was said that *Fusarium moniliforme* was the worst pathogenic fungus (CaldWell *et al.,* 1981). Pathogenicity of these indexed taxa as in Tables 1&3 have been reported in previous research on Sorghum seed and seedlings too (Randhawa

et al., 1998). As our findings demonstrate that *Fusarium moniliforme* and other species of the genus do impede detrimental impacts on seed germination of corn and same was also said by Mathur & Seghal (1964) in their work on other crop. The results also shows that Bhimber has the most infection rate (Table 3) that may be due to its humid and tropical features and plain area aiding spore dispersal by wind mechanism (Ishtiaq *et al.*, 2008; 2010).

The other important part of this research was management strategy and to develop, optimize and recommend authentic method for cure of these infections. In this context it is worth to say that we tried four different agricultural methods to reduce or eradicate this plethora. In our trial experiment we tried four different management strategies as mentioned in Table 4. Hot water treatment (HWT) technique was employed in conjunction with distilled water treatment (DWT) which was used as control (Table 4) and these findings are demonstrated in Figs. 3-8. The results proved that GET was the best treatment with seed germination (85.75%), subsequently followed by Benomyl treatment (BT) having 84.75% seed germination rate, HWT with 79% and DWT was seen as the least effective management trial (65.37%). The chemical method produced less effective results (80.59% seed germination rate) in our findings however Nasim *et al.,* (2004) evaluated different pea varieties for the efficacy of different treatments. Seed samples were treated with 1% chlorox, heat treatment, and fungicide to assess their effectiveness and it does not coincide each other. Ying *et al.*, (2005) studied the effects of different rates of seed coating with pharmaceuticals. The maximum un-germination and low rate of rotting counts in different locations may be due to the high dose of the chemical (2.5gm/Kg), so 2gm/Kg chemical dose would have been the best for better results. However the results were not significant. Fungicides used for seed purification prior to sowing are more costly and harmful for health too and furthermore chemical use also disturbs the environment and causes unfriendly ecosystem fluctuations (Chapman & Harris, 1981).

Fig. 6. Fungus (*Aspergillus*) growing on PDA in laboratory.

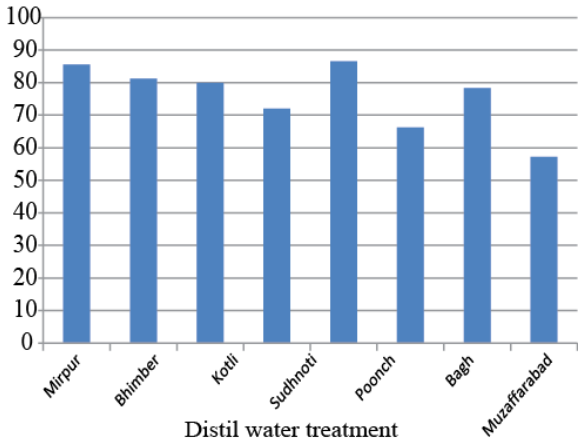

Fig. 7. Effects of distil water treatment (DWT) on the seed germination (%) as control in different Localities of Azad Kashmir.

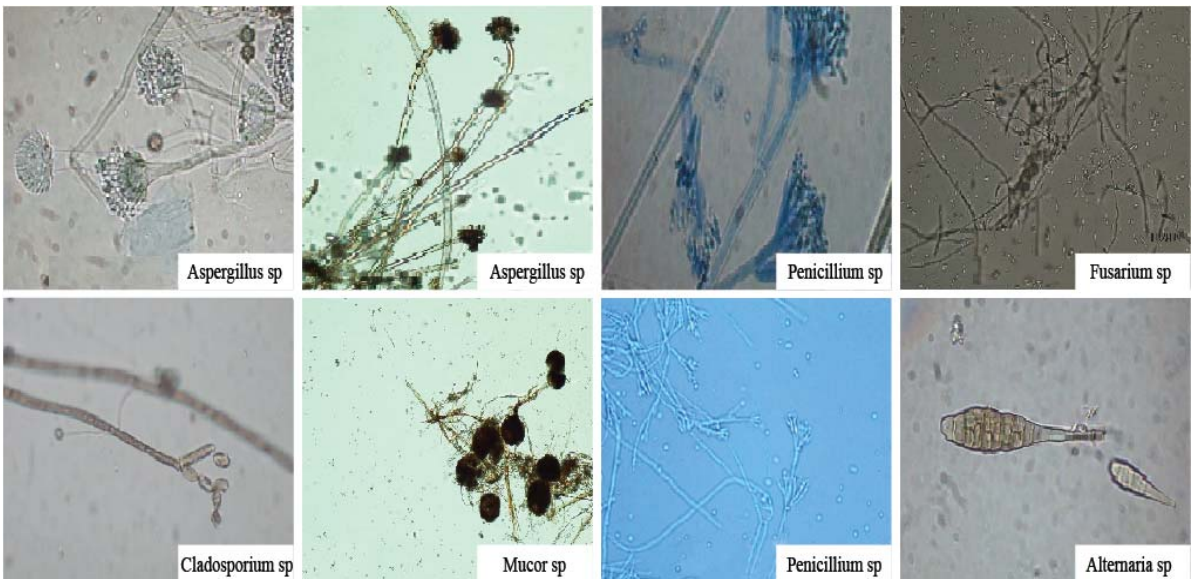

Fig. 8. Different species isolated corn seeds from different localities of Azad Kashmir.

Table 5. Analysis of variance of management treatments and different localities of Azad Kashmir.

	Effects of different management strategies on control of mycoflora		
S. No.	**Factors**	**Df**	**Mean square**
1.	Treatments (A)	3	5434.404*
2.	Localities (B)	8	243.169 NS
3.	AB	24	849.148*

It is worth to say that seed treatment becomes more economical and effective when it is carried out with respect to nature of pathogen and level of infection percentage (Neergaard, 1974) and our findings favour this report that GET trial is better than chemical treatment method. The use of plant diffusate and hot-water in comparison with chemical for controlling seed-borne mycoflora proved too better and our recommendations are in favour that farmers may use hot water treatment or garlic extract method to eradicate seed borne fungi. It was seemed that environment factor does have some influence in it and findings show that GET and HWT were better in Kotli (88.75%), Bhimber (88%) and Mirpur (78.81%) as shown in Fig. 4. In our findings comparison of different management strategies on control of mycoflora on corn seed samples was designed using ANOVA and it depicted a major difference among the samples and within different treatment trials (Table 4). The other trials varied in results in different areas and GET was found good in district Sudhnoti and BT was seemed appropriate in district Kotli trial run (Figs. 4&7).

Garlic extract treatment found to be very effective in controlling seed associated mycoflora as compared other treatments. It is more empirical, economical and safe compared to other management strategies (Table 4 & Fig. 1). The results of hot water treatment are also in conformity with other findings (Meah, 2004; Nega *et al.*, 2003 and Muniz, 2001). Although benomyl also gave some good results but studies showed that these synthetic fungicides produce harmful effects on the environment (Anastasiah *et al.*, 2001) and their continuous field application could lead to the development of resistant strains (Mehrota & Aggrawal, 2003).

So we recommend plant extract treatment using garlic or any other plant bearing fungicidal properties. In this context further detailed and comprehensive research is suggested that may try different plant extracts to control pathogenicity on seeds of corn and other cereals of the area.

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