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Identification of Seed Borne Mycoflora of Economically Important Vegetables of District Abbottabad and their Effect on Seed Germination and Seedling Vigor

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Research Article

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ABSTRACT

Seed samples of three economically important vegetables viz Tomato, Pea and Chilli were collected from local seed shops of Nawasher, Abbottabad city, Nathiagali and Qalandarabad locations of District Abbottabad. Seed borne infection, percent germination and vigor index of seeds were tested and twelve fungi were isolated and identified as *Alternaria alternata*, *Alternaria solani*, *Aspergillus niger*, *A. flavus*, *A. fumigatus, A. terrus*, *Cladosporium spp*., *Curvularia spp*, *Colletotrichum capsici*, *Fusarium oxysporum*, *Penecillium spp* and *Rhizopus spp*. The total percentage fungal incidence in tomato was 25-42%, in pea (13-33%) and in chilli (5-32%). The highest % seed germination was recorded in Qalandarabad (70%) and lowest in Abbottabad city (43%) in tomato seeds, similarly vigor index ranged from 107.6-1115.1 in tomato seeds. In case of pea seeds maximum % germination was found in samples of Nawasher (72%) and minimum in Qalandarabad location (20%) and vigor index was highest in Abbototabad city (572.8) and lowest in Qalandarabad (147). In chilli seeds % germination range from 10-45% and vigor index was highest in Qalandarabad (289.1) and lowest in Nawasher (14.1) seed samples.

INTRODUCTION

Vegetables are the important food of considerable nutritive value particularly as sources of vitamins (C, A, B1, B6, B9, E), phytonutriceuticals, minerals, phytochemicals, dietary fiber and carbohydrates that are essential for health and disease prevention [1,2]. Four hundred and two vegetable plants are cultivated globally, representing sixty nine families and two hundred and thirty genera [3].

Above 36 types of vegetables are cultivated in numerous ecological zones in Pakistan [4]. Summer vegetables includes tomato, chilies, cucumber, brinjal, potato, gourds and okra while in winter season cauliflower, cabbage, lettuce, spinach, onion, potato, carrot, radish, turnip, coriander, fenugreek and peas are commonly cultivated [5].

Khyber Pakhtunkhwa (KPK) is one of the five provinces in Pakistan where vegetables and fruits play a major role in the economic system of KPK [5]. At higher elevation, off season vegetables are grown and are sold at finest price in the local as well as in other big cities throughout summer seasons. Abbottabad is considered as major district for growing off-season vegetables. Vegetables are vulnerable to many pathogenic organisms that reduced yield by damaging entire plants and make them unmarketable. 26% of yield losses are due to the plant diseases worldwide [6]. Seed is considered as primary origin for crop production and is the principal input factor available for farmers [7]. In Pakistan many small farmers utilize their produced seeds for further planting and these seeds are frequently of poor quality, infected with pathogens [8]. Numerous studies indicated that many of these pathogens are seed-borne fungi [9]. Seed-borne fungi are of significance due to their effect on germination, overall fitness and ending crop stand within the field [10]. Fungal pathogens may be related to the seeds as contaminants, as intra or extra embryo and internally or externally seed-borne [11].

Tomato (*Lycopersicon esculentum*) is infected globally by numerous fungal diseases such as early blight, late blight, *Fusarium* wilt, *Septoria* leaf spot, *Sclerotium* rot, Collar rot and damping-off [12]. A great variety of these fungal diseases are seed-borne [13].

Pea (*Pisum sativum*) is one of the economically essential legumes in temperate regions. Except, different reasons of low pea yields in Pakistan; seed associated fungi additionally play an essential role [14]. 50-75% yield losses are due to pathogenic fungi which causes infections in plant tissues [15]. *Fusarium* spp*, Ascochyta pisi*, *Phoma pinodella*, and *Alternaria* spp, are the most important fungal species involved in diseases of field pea [16].

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Chilli (*Capsicum annum*) is the fourth predominant vegetable crop grown globally. 400 unique varieties of chilies are grown around the world [17]. In Pakistan, chilli is considered as beneficial cash crop and is used for both vegetable and spice crop. In chilles the most considerable seed borne fungi are *Alternaria solani*, *Botrytis cineria Fusarium*, *Colletotrichum* spp., *Cercospora capsici*, and *Sclerotinia sclerotiorum* [18,19].

For crop production a crucial input is seed and huge yield losses are caused by microorganisms which cause numerous seed borne diseases [20,21]. Keeping in view the economic significance of these vegetables and the losses caused by seed-borne fungi the present research work has been designed in order to isolate and identify seed-borne mycoflora of locally available seed in District Abbottabad, their % incidence and the effect of seed borne fungi on seed germination and seedling vigor.

MATERIALS AND METHODS

Seed Samples Collection

The seed samples of 3 vegetable crops i.e., Tomato, Chilli, and Pea were collected from seed shops of local markets of different locations (Nawasher, Nathiagali, Abbottabad city and Qalanderabad) of District Abbottabad during 2017. Three samples from different shops for each vegetable were collected from each location and combined to make a representative composite sample for a location. The isolation and identification of seed borne fungi from the samples were conducted at Hazara Agriculture Research Station Abbottabad.

Isolation and Identification of Seed-Borne Mycoflora

Agar plate method was employed for isolation of fungal mycoflora of the collected samples of each vegetable. One hundred seeds of each of three vegetables from every location were surface disinfected with sodium hypochlorite (NaOCl, 3%) for three minutes [22], rinsed thrice with sterilize distilled water (SDW) and blotted dry on sterilize filter paper before platting on potato dextrose agar (PDA) medium. Petri dishes were incubated at 25°C for seven days.

Mycoflora growing from seeds were identified by using taxonomic features such as colony characteristics and conidial size after referencing to mycological keys [23,24]. Total fungal incidence percentage and percent incidence of individual fungus were calculated by the following formula.

% Fungal incidence=(Number of infected seeds/Total number of seeds) × 100

% Incidence of individual fungus=(Number of seeds infected with particular species/Total number of seeds) × 100

Germination Test

To determine the effect of seed borne fungi on seed germination, blotter method was used following the International Rules for Seed Testing Association (ISTA) 2001 [25]. One hundred seeds of each of three vegetables of each location were surface sterilized with 3% Sodium hypochlorite (NaOCl) for 2 minutes, washed thrice with Sterilized distilled water (SDW), and asceptically placed @10 seeds of tomato and chilli and 5 seeds of pea per petri plate on two layers of sterilize filter paper. An aliquot of 10 ml of sterilized distilled water (SDW) was added to each petri plate in order to keep the filter paper moist. The plates were incubated at 25°C for a period of one week. Data was recorded on % seed germination by using formula:

Percent seed germination=(Number of seeds germinated/Total number of seeds) × 100

Determination of Abnormal Seedlings

As against normal seedlings, abnormal seedlings do not possess all essential structures and are unable to produce normal plants. During germination test abnormal seedlings with no, short or brown colored roots; and decayed or coiled/twisted shoots were also observed and counted to know the effect of the pathogens on % abnormal seedlings. The following formula was used for calculation of % abnormal seedling.

% abnormal seedling= (Number of abnormal seedling/Total number of seeds) × 100

Vigor Test

Hundred seeds of each vegetable from each location were surface disinfected with 3% Sodium hypochlorite (NaOCl) for 2 minutes, dried on sterilized filter papers and incubated on water agar in petri plates. The plates were incubated at 25☐ for two weeks. Shoot and root length and germination percentage were recorded. A seed was considered germinated when the minimum length of its plumule was 1.25 cm [26]. Vigor index was calculated according to the equation: VI=(RL+SL) GP (where VI=Vigor Index; RL=Root Length; SL=Shoot Length and GP= Germination Percentage [27].

Analysis of Data

The experiments were conducted following completely randomized design (CRD) and the recorded data were subjected to analysis of variance (ANOVA). Means were compared using least significance difference (LSD) at 5% probability level.

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RESULTS

Percent Incidence of Seed borne Mycoflora of Tomato, Chilli and Pea Seeds Collected from Different Locations of District Abbottabad

Several seedborne fungi were isolated and identified from the seeds of the three vegetables i.e., tomato, chilli and pea. Overall percent incidence of seed borne mycoflora on tomato seeds varied among different locations **(Table 1)** with 45% fungal infection was recorded on seeds of Nathiagali whereas lowest 25% on Nawasher. Regarding the individual fungal species isolated from tomato seeds the *Alternaria solani* 6%, *Penicillium* spp (8%) were recorded only from Qalandarabad **(Table 2)**. *Aspergillus flavus* and *A. niger* were isolated from the samples of all four locations **(Table 2)**. *Aspergillus fumigatus* was detected in Nawasher and Nathiagali seed samples with only 1% infection **(Table 2)**. *Rhizopus* spp. 6%, and *Cladosporium* spp (3%) was isolated only from Qalandarabd and Nawasher, respectively **(Table 2)**.

Table 1. Percentage incidence of seed borne fungi of tomato, chili and pea seeds collected from different locations of District Abbottabad.

Locations	% Total fungal incidence		
	Tomato	Pea	Chilli
Nawasher	25^{a}	20^{ab}	14^{b}
Abbottabad city	35^{a}	18^{b}	13^{b}
Nathiagali	45^{a}	13^{b}	5^{b}
Qalandarabad	42^{a}	33^{a}	32^{a}

Table 2. Percentage incidence of individual fungal infection of Tomato seeds collected from different locations of District Abbottabad.

Isolated fungi	% incidence of individual fungus			
	Nawasher	Abbottabad city	Nathiagali	Qalandarabad
Alternaria solani	0^{b}	0^{b}	0^{b}	6^{a}
Aspergillus flavus	5^{a}	17^{a}	14^{a}	10^{a}
Aspergillus niger	14^{a}	18^{a}	30^{a}	12^{a}
Aspergillus fumigatus	1^{a}	0^{a}	1^{a}	0^{a}
Cladosporium spp.	3^{a}	0^{a}	0^{a}	0^{a}
Penecillium spp.	0^{b}	0^{b}	0^{b}	8^{a}
Rhizopus spp.	2^{a}	0^{a}	0^{a}	6^{a}
Mean values followed by different letters are significantly different from one another at 5% level of probability in column.				

Percent Incidence of Seed Borne Mycoflora of Pea Seeds Collected from Different Locations of District Abbottabad

A total of nine different fungi were isolated from pea seeds i.e., *Alternaria* alternate, *Aspergillus niger*, *Aspergillus terrus*, *Aspergillus flavus*, *Fusarium oxysporium*, *Curvularia* spp, *Penicillium* spp, *Rhizopus* spp and *Cladosporium* spp. Overall percent incidence of seed borne fungi was high in seed samples of Qalandarabad location (33%), followed by Nawasher (20%) and Abbottabad city (18%). The lowest percent incidence was recorded in Nathiagali location (13%) **(Table 3)**. In case of individual fungal species *Fusarium oxysporum* percent incidence was found highest (15%) among all the fungi isolated from pea seeds and was recorded only in samples of Qalanadarabad location **(Table 3)**. *Aspergillus niger*, *Penicillium* spp and *Cladaosporium* spp were isolated from all the four locations. *Aspergillus terrus* was only detected from Abbottabad city location with incidence of 2% **(Table 3)**.

Table 3. Percentage incidence of individual seed borne fungus on Pea seeds collected from different locations of District Abbottabad.

Isolated fungi	% Incidence of individual fungus			
	Nawasher	Abbottabad city	Nathiagali	Qalandarabad
Alternaria alternata	0^{b}	2^{ab}	6^{a}	0^{b}
Aspergillus flavus	2^{a}	1^{a}	0^{a}	0^{a}
Aspergillus niger	1^{a}	1^{a}	1^{a}	1^{a}
Aspergillus terrus	0^{b}	2^{a}	0^{b}	0^{b}
Cladosporium spp.	12^{a}	7^{a}	3^{a}	5^{a}
Curvularia spp.	1^{b}	0^{b}	0^{b}	9^{a}
Fusarium oxysporum	0^{b}	0^{b}	0^{b}	15^{a}
Penecillium spp.	4^{a}	4^{a}	3^{a}	1^{a}
Rhizopus spp.	0^{a}	1^{a}	0^{a}	2^{a}
Mean values followed by different letters are significantly different from one another at 5% level of probability in column.				

Percent Incidence of Seed Borne Mycoflora of Pea Seeds Collected from Different Locations of District Abbottabad

The isolated fungi from the chilli seeds were *Alternaria solani, Colletotricum capsici, Cladosporium* spp*, Aspergillus flavus*, *Fusarium oxysporum*, *Aspergillus niger*, *Penicillium* spp, *Rhizopus* spp and *Aspergillus terrus* **(Table 4)**.

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Table 4. Percentage incidence of individual seed borne fungus of chili seeds collected from different locations of District Abbottabad.

Isolated fungi	% Incidence of individual fungus			
	Nawasher	Abbottabad city	Nathiagali	Qalandarabad
Alternaria solani	3[a]	0[a]	2[a]	2[a]
Aspergillus flavus	0[b]	6[a]	0[b]	5[a]
Aspergillus niger	4[a]	3[ab]	0[b]	0[b]
Aspergillus terrus	0[b]	0[b]	0[b]	9[a]
Cladosporium spp	5[a]	2[a]	3[a]	4[a]
Colletotricum capsici	0[b]	0[b]	0[b]	6[a]
Fusarium oxysporum	0[a]	1[a]	0[a]	0[a]
Penecillium spp.	2[a]	0[a]	0[a]	6[a]
Rhizopus spp.	0[a]	1[a]	0[a]	0[a]

Mean values followed by different letters are significantly different from one another at 5% level of probability in column.

Total percent incidence of seed borne mycoflora varied among different locations **(Table 1)** with highest % fungal infection on Qalandarabad location (32%). Among the isolated fungi *Fusarium oxysporum* and *Rhizopus* spp showed the minimum percent incidence (1%) and were found only in samples of Abbottabad city **(Table 4)**. *Aspergillus terrus* was isolated only from Qalandarabad seed samples with percent incidence of 9% whereas *Cladosporium* spp was detected in the seed samples of all locations **(Table 4)**.

Cultural and Microscopic Characteristics of Fungi Isolated from Tomato, Pea and Chilli Seeds

Alternaria solani

Alternaria solani appeared as grayish black in color **(Figure 1C)** . Conidiophores were branched and olivous brown in color. Conidia were in chain, with typical oblong shape and possessed up to 6 transverse and several longitudinal septa. Conidial size varied from 25-87 × 10-13 µm in diameter **(Figure 2A)** . These characteristics of the fungus matched with those reported for *Alternaria solani* [23,24].

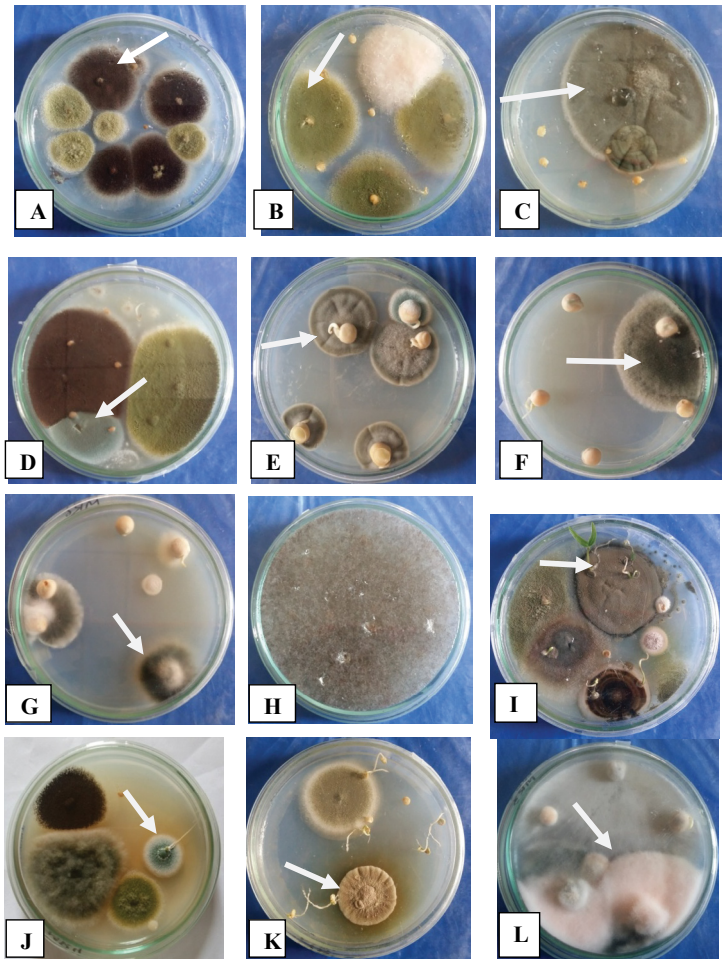

Figure 1. Colonies of *Aspergillus niger* (A); *Aspergillus flavus* (B); *Alternaria solani* (C); *Aspergillus fumigatus* (D); *Cladosporium* spp. (E) *Alternaria alternata* (F); *Curvularia* spp (G); *Rhizopus* spp. (H); *Colletotrichum capsici* (I); *Penecillium* spp. (J); *Aspergillus terrus* (K); and *Fusarium oxysporum* (L) isolated from seeds on PDA incubated at 25 °C for seven days.

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Aspergillus flavus

Colonies of *Aspergillus flavus* were granular bright to dark yellowish green in color with whitish periphery and distinct margin **(Figure 1B)**. Conidial heads were radiate (75-77.5 µm in diameter) and conidiophores were hyaline in color **(Figure 2B)**. Conidia were globose to subglobose with range up to 3.75-5 µm in diameter. These characteristics matched with those reported for *Aspergillus flavus* [23,24].

Aspergillus niger.

Colonies of *Aspergillus niger* were appeared as black and powdery **(Figure 1A)**. Conidial heads were large, globose and dark brown to black in color and 220-225 µm in diameter. Conidiophores were smooth walled and hyaline. Conidia were globose to subglobose, size varied from 3-5 µm in diameter **(Figure 2C)**. The characteristics matched with those reported for *Aspergillus niger* [23,24].

Aspergillus fumigatus

On PDA colonies of *Aspergillus fumigatus* were blue-green in color **(Figure 1D)**. Conidial heads were typically columnar (60-70µm in diameter) and uniserate **(Figure 2D)**. Conidiophores s olive green to brown in color. Conidia were produced in long chains and were globose to subglobose with range up to 2.5-5 µm in diameter. The characteristics were matched with those reported for *Aspergillus fumigatus* [24].

Penicillium spp.

Colonies of *Penicillium* spp. were dense, powdery and bluish green in color **(Figure 1J)**. Conidiophore was hyaline and smooth walled. Phialides were flask shaped and conidia were produced in long chains. Conidia were globose and light green in color with size ranged up to 2.5-5 µm in diameter **(Figure 2E)**. These characteristics matched with those reported [23,24].

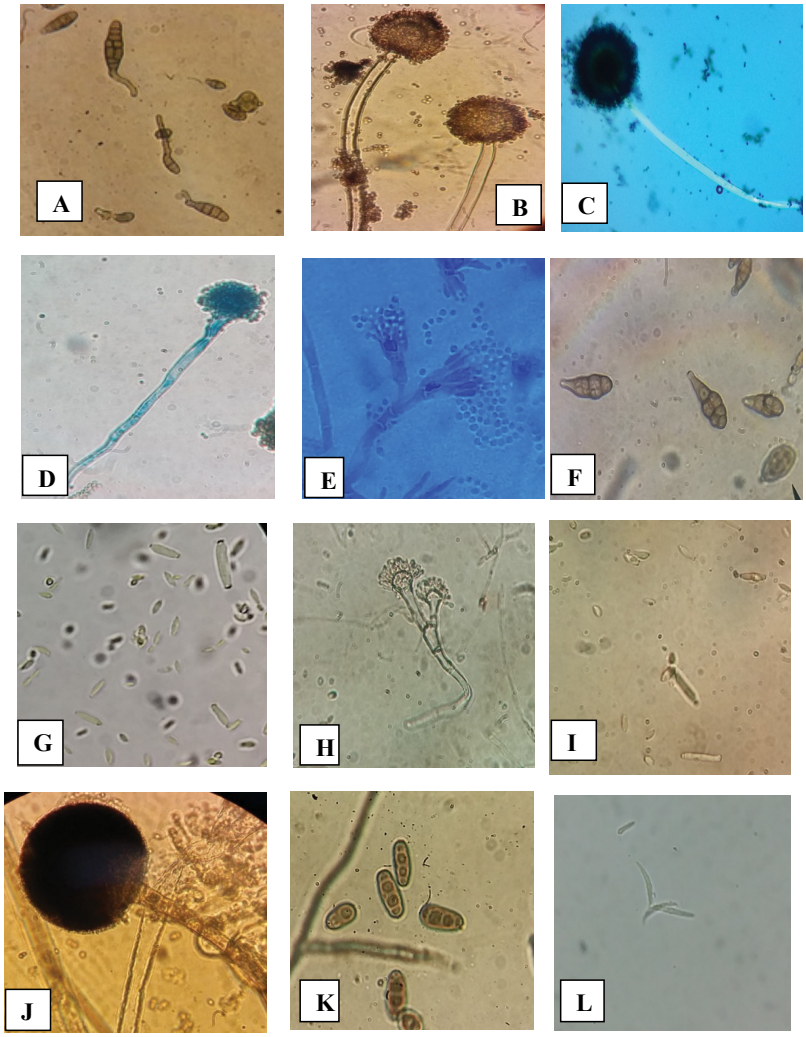

Figure 2. Conidia of *Alternaria solani* (A); condial head of *Aspergillus flavus* along with conidiophores (B); conidial head along with conidiophore of *Aspergillus niger* (C); conidial head with conidiophores of *Aspergillus fumigatus* (D); conidiophore and penicillus of *Penicillium* spp. (E); conidia of *Alternaria alternata* (F); conidia of *Cladosporium* spp. (G); conidial head with conidiophore of *Aspergillus terrus* (H); conidia of Colletotrichum capsici (I); Sporangia along with sporongiphore of *Rhizopus* spp. (J); conidia of *Curvularia* spp. (K) and Macroconidia of *Fusarium oxysporum* (L) produced in pure culture on PDA after incubation at 25°C for seven days (400x).

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Rhizopus spp.

Colonies of *Rhizopus* spp. were white cottony at first and becoming brownish grey on sporulation **(Figure 1H)**. Sporongiophores were smooth walled and non septate. Sporangia were globose and black. Size of sporangia ranged from 150-162.5 µm in diameter **(Figure 2)**. Spores were globose to sub globose, varied in size from 2-7.5 µm in diameter **(Figure 2J)**. The characteristics matched with those reported for *Rhizopus* spp [23,24].

Cladosporium spp.

Colonies of *Cladosporium* spp. were olivous brown sometimes grey in color with distinct white margins, velvety floccose like and often become powdery due to production of conidia **(Figure 1E)**. Conidia were olive green in color and produced in chains and had a prominent attachment scar called hilum **(Figure 2G)**. Conidial size varied from 7-17.5 × 2.5-5 µm in diameter. The characteristics confirmed with those reported for *Cladosporium* spp [24].

Alternaria alternate

Colonies of *Alternaria alternata* appeared grayish to black in color **(Figure 1F)**. Microscopically, conidiophores were short, branched and brown in color. Conidia were produced in chains, ovoid in shape with conical beak, light brown in color having up to five transverse and 2-3 longitudinal septa. Conidial size varied from 27.5-52.5 × 10-12.5 µm **(Figure 2F)**. The characteristics of *Alternaria* alternate matched with those reported [23,24].

Curvularia spp.

Colonies of *Curvularia* spp. appeared as grey to black **(Figure 1G)**. Conidiophore solitary and brown in color ranged from 110-147.5 × 2.5-5 µm in diameter. Conidia cylindrical, rounded at the end and pale brown in color usually possessed three septation. The conidial size ranged up to 25-32.5 × 7.5-12.5 µm in diameter **(Figure 2K)**. These characteristics of *Curvularia* spp matched with the characteristics [24].

Fusarium oxysporum

Colonies of *Fusarium oxysporum* appeared as white cottony colonies which becoming pinkish after sporulation **(Figure 1L)**. Conidiophores were arranged in densely branched clusters and hyaline. Macroconidia were fusiform, pointed at the tip and slightly curved **(Figure 2L)**. Size of macroconidia ranged up to 22.5-37.5 × 2.5-5 µ. The characteristics of *Fusarium oxysporum* matched with charecterisitcs [23,24].

Aspergillus terrus

Aspergillus terrus appeared as suede like and pale brown in color **(Figure 1K)**. Conidiophores were septate and hyaline. Conidia were produced in conidial head and its size varied from 15-22.5 × 10-12.5 µm in diameter. Conidial size ranged up to 2.5-5 µm in diameter **(Figure 2H)**. The characteristics of *Aspergillus terrus* were matched with those reported [24].

Colletotrichum capsici

Colonies of *Colletotrichum capsici* appeared as greyish to brown in color **(Figure 1I)**. Conidiophore was smooth and brown. Conidia were ovoid, single celled and hyaline. Conidial size varied from 7.5-15 × 2.5-5 µm in diameter **(Figure 2I)**. The characteristics of *Aspergillus terrus* were matched with the characteristics reported [24].

Germination Percentage and % Abnormal Seedlings of Chilli, Tomato and Pea Seeds Collected from Different Locations of District Abbottabad.

Data on percent germination **(Figure 3)** was recorded after seven days of incubation. The highest % germination in Chilli seeds was recorded in Abbottabad city (45%), while minimum (10%) in Nawasher samples. In tomato a significantly highest percent germination was recorded in Qalandarabad seed samples i.e., 70%, and lowest percent germination found in the samples of Abbottabad city (43%). In pea seed the highest percent germination was observed in Nawasher (72%), while seed of Qalandarabad location showed lowest percent germination (20%) **(Table 5)**. Also abnormal seedlings % was counted during germination test **(Table 5 and Figure 4)**. The three locations Abbottabad city, Nathiagali and Qalandarabad location showed 10% abnormal seedling in Chilli seeds, while in case of Pea and Tomato highest percentage of abnormal sealing i.e., 13% and 10% were recorded in the samples of Abbottabad city and Qalandarabad location respectively **(Table 5)**.

Vigor Test of Chilli, Tomato, Pea Seeds Collected from Different Locations of District Abbottabad

Seedling vigor test was conducted on water agar plates and the data on root/shoot length and germination percentage was recorded at 14 days of culturing. The vigor index was calculated by using the formula VI (RL + SL) GP Vigor index of Chilli seed samples **(Table 6)** varied significantly among different locations. The highest vigor index was recorded in Qalandarabad (289.1) and lowest in Nawasher (14.1) seed samples **(Table 6)**. Vigor index of tomato seeds was observed significantly higher in Qalandarabad (1115.1), followed by Abbottabad city (416.2) and Nathiagali location (368.1) whereas lowest vigor index was observed in seed samples of Nawasher location (107.6). In case of pea seeds the maximum vigor index was recorded in seed samples of Abbottabad city (572.8), followed by Nawasher (301.7) and Nathiagali (262.7) while the least in Qalandarabad (147),

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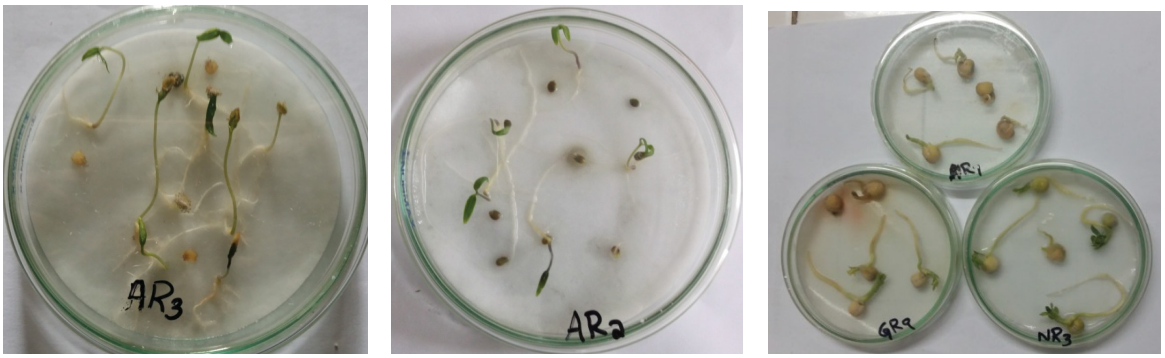

Figure 3. Chilli, tomato and pea seeds showing germination on blotter paper incubated at 25°C for seven days (left to right).

Table 5. Percentage germination and % abnormal seedling of chili, tomato and pea seeds collected from different locations of District Abbottabad.

Locations	% Germination			% Abnormal seedling		
	Chilli	Tomato	Pea	Chilli	Tomato	Pea
Nawasher	10^{b}	47^{b}	72^{a}	4^{a}	9^{a}	6^{ab}
Abbottabad city	45^{a}	43^{b}	36^{b}	10^{a}	5^{ab}	13^{a}
Nathiagali	18^{b}	44^{b}	40^{b}	10^{a}	2^{b}	9^{ab}
Qalandarabad	22^{b}	70^{a}	20^{c}	10^{a}	10^{a}	3^{b}
Mean values followed by different letters are significantly different from one another at 5% level of probability in column (147).						

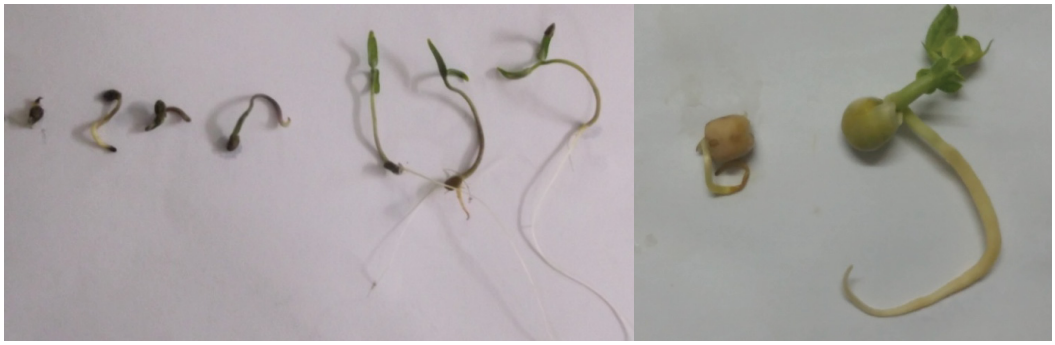

Figure 4. Tomato (left) and pea seeds (right) showing normal and abnormal seedlings.

Table 6. Vigor index of chili, tomato and pea seedlings obtained from samples collected from different locations of District Abbottabad and tested *in vitro* at 25°C for 14 days.

Location	Mean of shoot length (cm)			Mean of root length (cm)			Germination %			Vigor index (VI)		
	Chilli	Tomato	Pea	Chilli	Tomato	Pea	Chilli	Tomato	Pea	Chilli	Tomato	Pea
Nawasher	0.4^{b}	1.3^{b}	1.77^{ab}	10.7^{b}	1.7^{d}	3.93^{bc}	3^{b}	15^{c}	61^{a}	14.1^{b}	107.6^{c}	301.7^{b}
Abbottabad city	1.66^{a}	2.9^{a}	2.38^{a}	2.57^{a}	7.2^{b}	7.65^{a}	60^{a}	49^{b}	54^{a}	271.3^{a}	416.2^{b}	572.8^{a}
Nathiagali	1.1^{ab}	3.4^{a}	1.73^{ab}	0.81^{b}	5.3^{c}	5.17^{b}	6^{b}	38^{b}	28^{b}	35.5^{b}	368.1^{b}	262.7^{b}
Qalandarabad	31.6^{a}	3.5^{a}	1.31^{b}	2.23^{a}	10.2^{a}	1.94^{c}	60^{a}	82^{a}	34^{b}	289.1^{a}	1115.1^{a}	147^{b}
Mean values followed by different letters are significantly different from one another at 5% level of probability in column. VI (RL +SL) GP.												

DISCUSSION

Every seed is a latent harbor of a huge diversity of microflora, which includes seed borne pathogens inciting numerous diseases, leads to massive losses in the yield [28]. A number of seed borne mycoflora are also responsible for lowering seed germination and seedling death [21]. In the present research seed samples of Tomato, Pea and chilli collected from the main seed selling shops of different locations of District Abbottabad were tested against seed borne fungi. Nathiagali and Qalandarabad locations were found to be highly infected with seed borne fungi. This may be attributed to temperature and other climatic factors during seed development stage which support growth of fungal species. Climatic variations especially temperature and humidity are significant factors affect the incidence of seed borne fungi [29].

Eight genera of seed borne fungal pathogens i.e., *Alternaria*, *Aspergillus*, *Curvularia*, *Fusarium*, *Penicillium*, *Rhizopus*, *Colletotrichum* and *Cladosporium* were isolated from the seeds of tomato, pea and chilli [30]. Reported that the most of the seed mycoflora species exist inside the genera of *Fusarium* spp, *Aspergillus* spp and *Penicillium* spp that frequently arise in major food vegetation in the field and hold to infect them in storage [31] isolated *Aspergillus niger*, *Aspergillus flavus*, *Curvularia* spp, *Rhizopus*

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nigricans and *Alternaria* spp from Tomato seeds. Our results are also in line with the findings of [32] who reported in their study that *Alternaria* spp and *Fusarium* spp were the most common isolated seed borne fungi from pea seeds.

The results are also in agreement with those [33] who confirmed the presence of *Alternaria* alternate, *Penicillium* spp, *Fusarium* spp, and *Ascochyta* pisi in pea seeds. In case of Chilli seeds, nine different fungi viz. *Alternaria solani*, *Colletotriticum capsici*, *Cladosporium* spp, *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terrus*, *Fusarium* spp, *Penicillium* spp and *Rhizopus* spp were recorded in our study.

The results also corroborate [34] who reported that an among the 35 fungal species isolated from the chilli seed samples, *Alternaria* alternata, *A. solani*, *Aspergillus flavus*, *A. niger*, *Botrytis cineria*, *Colletotrichum* spp, *Fusarium oxysporium*, *F. solani*, and *Rhizopus* spp were most common. Similar findings were obtained [35] who isolated *Aspergillus*, *Cladosporium* spp and many other fungi from seeds as well as pericarp of chilli.

The result of the research showed that seed borne fungi has great effect on seed germination, abnormal seedling and seedling vigor. In tomato seeds germination was lowest in Abbottabad city where *Aspergillus flavus*, *A. niger*, were dominant fungi. Similarly in pea seeds lowest germination was found in Qalandarabad location in which *Fusarium oxysporum* was predominant mycoflora and in chilli seeds Nawasher location has shown low percent germination and *Aspergillus niger* and *Cladosporium* as dominant fungi. The difference in seed germination % between locations may be due to the type of fungal specie predominantly isolated from the seed samples of a respective location. The possible reason can also be that at these locations the seeds are dried in open environment under sunlight and can be contaminated with airborne fungal spores as reported [36] that studied the mycological status of sundried okra fruit and found that it was highly contaminated with fungal spores and contained higher levels of toxins when tested. The result are in the line with findings of Ijaz (1988) [37,38] who reported that *Aspergillus flavus*, *A. niger* and *Fusarium* are harmful fungi which damage the radical, plumule and hypocotyl of seeds. *Alternaria* and *Fusarium* seed infection encouraged the Aflotoxin production, which had great impact on seed germination. *Fusarium* causes reduction in germination because it is recognized to invade the seed coat, embryo and endosperm [39]. *Curvularia* sp. related with seed, leads to abnormal seedlings appearance. Phytotoxins produced due to infection causes death of embryo which reduces the germination [40].

Seed borne mycoflora reduced root and shoot elongation by causing higher percentage of pre- and post-emergence death and then it also reduced vigour index. In tomato seeds minimum shoot and root length was observed in Nawasher location in which *Aspergilus niger* is a predominant fungus. Similarly in pea seeds fungal incidence was higher in Qalandarabad location with minimum shoot and root length and in Chilli seeds Nathiagali location shows minimum root and shoot length. Toxic metabolites produced by A. flavus results in shoot and root length reduction [41]. The above results correlate with the findings [42] found that *Aspergillus flavus, Aspergillus niger*, *Fusarium oxysporum*, *Alternaria* alternate, *Rhizopus*, *Curvularia* and *Penecillium* sp causes detoriation of seed value and lower down the germination in maize seeds [43] reported that *Fusarium*, Phoma and *Rhizopus* were responsible for lower seed germination in *Cucurbita* pepo seeds [44] found that *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* and *Rhizopus stolonifer* were dominant fungi in green grams and these fungi adversely seedling emergence and seed germination. It was reported that lowest incidence of Rhizopus stolonifer, Aspergillus spp, *Alternaria*, *Fusarium* spp, *Bipolaris oryzae*, *Phoma* spp, *Curvularia lunata*, *Penicillium* spp etc showed highest germination and vigor index of seeds of hybrid Rice varieties [45,46] found that *Alternaria* alternate, *Aspergillus* spp, *Curvularia lunata* and *Fusarium* spp significantly affect seed health status by reducing germination, root-shoot length and vigor index of Finger millet seeds.

CONCLUSION

It is concluded from results that twelve seed borne fungi namely, *Alternaria alternata*, *Alternaria solani*, *Aspergillus niger*, *A. flavus*, *A. fumigatus*, *A. terrus*, *Cladosporium*, *Curvularia* spp, *Colletotrichum capsici*, *Fusarium oxysporum*, *Penecillium* spp and *Rhizopus* spp were the major seed contaminants of different locations of District Abbottabad. Seed samples of Qalandarabad location were highly infected. In Tomato seeds *Aspergillus niger* and *Aspergillus flavus* prevailed in all locations while in pea seeds *Aspergillus niger*, *Cladosporium* and *Penecillium* were dominant fungi. Similarly, *Cladosporium* was predominant fungal species isolated from chilli seeds. These seed borne fungi also had adverse effect on seed germination and seedling vigor. Further research is needed on molecular characterization of these isolated fungi up to species level for their proper control in order to avoid major losses in production. Farmers need to be well trained on handling techniques of seed and needs proper care of seeds during storage.

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