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Isolation and Identification of Fungal Pathogens Associated with Diseases of Onion Crop in District Swat, Pakistan

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Abstract

Onion is an important vegetable and a source of income for formers in District Swat, Pakistan. However, the crop is attacked by several pathogens i.e. fungi, bacteria, viruses and nematodes. Therefore, a study was carried out for estimation of fungal diseases of onion crop in district Swat. Within this study about 50 samples of diseased onion crop were collected from 17 different regions of District Swat. The most prevalent fungal borne onion diseases were black mold (38%), onion rust (32%), Blue Mold Rot (10%) and seedling. Fungal species were consistently isolated from all infected samples. In which *Aspergillus* was isolated from 82% samples, *Puccinia alli* was from 70%, *Alternaria porri* from 52%, *Fuserium spp* from 42% *Penicillium spp* from 40%, *Peronospora destructor* from 35%, *Pyhtaium* from 23% and *Rhizoctonia solani* from 18% of samples. These fungal species consistently isolated and cultured from diseased onion plant of different fields in Swat.

Keywords: Onion crop, Fungal diseases, *Aspergillus.*

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1. INTRODUCTION

Onion *(Allium cepa L.)* is an important vegetable crop cultivated worldwide which belongs to genus *Allium* of family *Allidaceae.* It may consume as a fresh salad and processed food dishes. Fresh onion is rich source of proteins, vitamins (B6 and C), Potassium, Zink and sulfur compounds i.e. thiosulfinates and thiosulfonates. These compounds have a range of health benefits such as antimicrobial and antioxidant activities which protect against cardiovascular diseases and cancer[1].

Commercial production of onion crop is varying among regions, mostly favors well-drained sandy loam and sandy soil with pH range from 6.2 to 6.5. In Pakistan it can grow in all provinces. Its average area of cultivation was 1,37,500, hectare in 2011 with the annual production of 18,58,500, tones in Pakistan. Whereas in Khyber Pakhtunkhwa (KPK) onion is cultivated on about 12000 hectares with annual production of 2,06,100 tones. Most part of onion crop in KPK is growing in district Swat[2]. This low production is due to several factors such as lack of improved raised beds, temperature, day length and several diseases.

Onion is attacked by large number of pathogens i.e. Fungi, Bacteria, Viruses and nematodes. It is estimated that over 30 – 40% of crop losses may be with these diseases. Basal rot is caused by *Fusarium oxysporum.* It is an important fungal disease[3]. Downy Mildew is airborne fungal disease; the pathogen belongs to oomycetes that need moisture in soil with low temperature. It causes by *Peronospora*

destructor. Purple blotch of onion is a well-documented air borne disease caused by *Alternaria porri*. Their spores are air-borne and transmit to susceptible host through wind. Rust is a sporadic disease of onion crop that generally causes significant economic damage. It is cause by *Puccinia allii* belongs to order Uridinae[4]. Black mold is an airborne and sometime soil borne disease mainly occurs during storage, processing and transport of onion crop[5]. This disease is world widely distributed and causes by *Aspergillus niger*[6]. Blue Mold Rot of onion is soil borne disease cause by *Penicillium species.* That is commonly found during storage and transportation of onion crop[7]. These all are the most common fungal diseases of onion crop which are worldwide distributed and mostly present in Pakistan. It causes a gross amount of decrease in onion weald every year in many onion growing countries of the world including Pakistan.

Common way to control fungal disease of onion is chemical fungicides. However frequently uses of these chemicals are associated with hazards to community health, damaged beneficial organisms and cause environmental pollution. It encourages the pathogen to develop resistance against these chemicals and reduce their effectiveness[8]. Recently a large no of chemical fungicides has been withdrawn from market in developed countries because of their high risk and toxicity to human and the environment[9]. Biological control is another way to lower down the population level of the target pathogen using natural enemies, which are commonly called biological control agent (BCA). That agents have some important features like they are, rapidly colonization, has persistent virulence and ability to bring the pathogen level below the economic threshold level. They are also easy to propagate and multiply, store easily, low cost, compatible with agrochemicals and must be safe. Use of fungi as biological control agent (BCA) against fungal plants diseases are well reported[8].

Fungal pathogens of onion usually cause alteration of root system of onion plant which leads to disturb plant physiology like damage in vascular system of plant resulting reduction in rate of photosynthesis that decrease onion crop qualitatively as well as quantitatively. The study of fungal borne diseases onion crop in swat distract is very important because the climate of Swat is favorable for well Growth of onion crop. Range of temperature during the onion season is 12 to 30 C˚ which is favorable for onion crop. The soil aeration and PH is also favorable for onion growth. Thus, the aim of the present study was to isolate and identify fungal diseases of onion crop in district Swat.

2. MATERIALS AND METHODS

2.1 Sampling areas

The samples were collected from 17 different areas of Swat district (Table 1) where onion crop is predominantly cultivated. Three samples were collected from each site. Plant that showed symptoms of fungal diseases were selected (Fig. 1). Each plant was uprooted; the roots were cut about 5cm above ground level. Then these roots and shoot along with the infected soil were labelled and preserved in the Central Laboratory of Center for Microbiology and Biotechnology until further processing.

Table 1. Areas selected for sampling of onion plants in distract Swat

S/No	Area	S/No	Area
1	Kota Swat.	2	Aboha Bazaar.
3	Barekot Swat.	4	Shamozo Swat.
5	Ghaligay Swat.	6	Manyar Swat.
7	Thana Malakand	8	Gogdarra Swat
9	Mingora Swat	10	Charbagh Swat
11	Khwaza khela Swat	12	Madyan Swat
13	Kalam Swat	14	Bahrain Swat
15	Maindam Swat	16	Matta Swat
17	Kabal Swat		

2.2 Media preparation for fungal isolation

Potato Dextrose Agar (PDA) was used for isolation of the fungi that can grow on artificial medium. The media was prepared as; 200gm of potato were sliced and boiled in 1 liter of distilled water. The extract was sieved through a clean muslin cloth. The broth was then added with 1.7% of agar and 2.0% of dextrose sugar. The media was autoclaved at 121°C for 15 minutes. It was cooled at 45°C and supplemented with streptomycin at 5ml per litre and poured in sterilized Petri plates under the aseptic condition in the Laminar Flow Hood (LFH) and level all the Petri plates for an overnight.

2.3 Isolation of fungal species from shoot portion

About 5mm infected part of leave and shoots were cut and surface sterilized by 5% Silver Chloride for 30 seconds then three time washed with sterilized distilled water for 3 minutes, so all the chemicals was washed down. These specimens were then put on Agar plates in LFH and incubate at 28°C for 3 to 4 days or more depending on the growth state of different fungi.

2.4 Isolation of fungi from root portion

Roots showing infected symptoms were cut in small pieces, washed under running tap water to remove all soil debris, then about 5mm size specimen were cut and sterilized with 5% Silver Chloride for 30 seconds then three time washed with sterilized distilled water. Ten root specimens were plated on PDA medium in LFH. Plats were then incubated at 28°C for 3 – 4 days or more depending on the growth stat of fungi.

2.5 Isolation of fungus from soil

For the isolation fungal species from soil sample, that was serially diluted from 10^{-1} to 10^{-9} concentration. For that 1gram of sieved soil sample was put in sterilized test tube containing 9ml of sterilized distilled water. The soil solution was then shacked thoroughly to form a homogeneous mixture. the sample was then subsequently serially diluted from 10^{-2} to 10^{-9}. From 10^{-6} soil sample 1ml solution was taken with the help of a sterilized pipette and gently spread on the PDA plates in LFH. The culture plates at 28°C for 3 – 5 days or more depending on the growth state of different fungi.

2.6 Identification of obligate fungal species

All the pure fungal isolated species were identified under the microscopic examination. For fungal identification, a small piece of the infected part was macerated with the help of back side of the needle and kept on the slide under covered glass containing stain of lacto Phenol solution and then observes under the microscope for fungal identification. Observed characteristics were recorded and compared with the established identification key; illustrated key of phytopathology[10].

2.7 Pure culture of fungal isolate

For the isolation of pure culture of fungal isolates, the isolates fungal cultures were transferred into fresh pre-sterilized Petri plates and incubated until fungal colonies appeared. The separated colonies were subculture on PDA Petri plates to obtain the pure fungal culture. The fungal colonies obtained were then identified.

2.8 Fungal microscopy and identification

A single mycelia plug was carefully isolated from each plate through sterilized pin-point forceps and placed on slide, cover slips were placed carefully to avoid air bubbles assimilation. All fungal species were identified on the base of disease symptoms, growth rate, culture characteristics and spore morphology like sporangiospores size, sporangiospores structure, sporangia size, conidiophores structure, conidial size and shape, hyphae hyaline and diameter [11, 12] and by its pathogenicity to the onion bulb[10].

3. RESULTS AND DISCUSSIONS

3.1 Farmer's perceptions about fungal diseases of onion

The results obtained from the interviews of onion cultivators from various areas of Swat district on the prevalence, spread, economic impact and control of fungal borne diseases are presented below.

3.2 Farmers' perceptions about prevalence and symptoms of fungal diseases that affects onion crop in the study area

Majority (70%) of the farmers considered black mold, (38%) and onion rust (32%), are the most prevalent fungal borne onion diseases and in the area followed by dumping off (20%), Blue Mold Rot (10%) and seedling (Table 2).

3.4 Diagnosis of different diseases

Detail physical examination of disease symptoms and their effects on plant show that the Onion crop of district Swat is more than 90% likely to be infected with fungi.

3.5 Fungal species associated with onion diseases in swat districts

The fungal species consistently isolated and cultured from diseased onion plant of different fields in Swat (Table 3). The technique of James and Natalie (2001) were used for identification of the isolated fungi[13].

Fig. 1. (A, B, C) Infected onion plants collected from various region of swat district. (D) Infected nursery of onion crop.

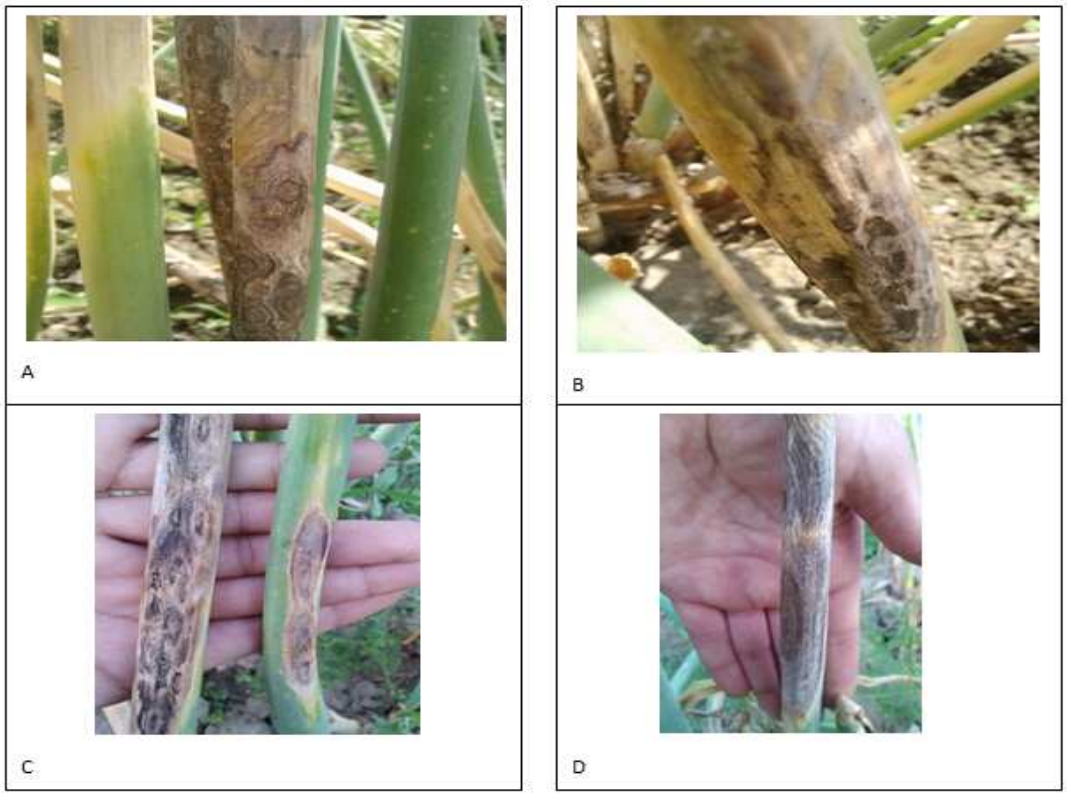

Fig. 2. (A, B, C, D) Symptoms base identification of fungal diseases.

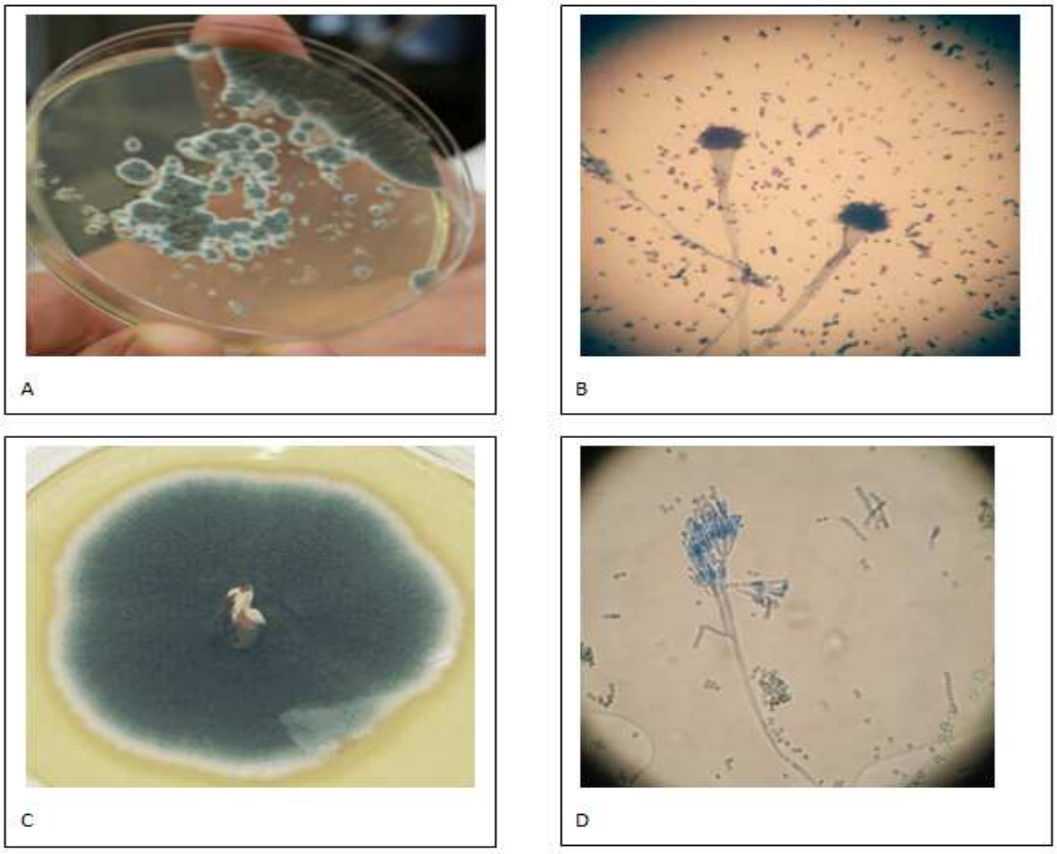

Fig. 3. (**A, B**) Aspergillus colony and microscopy. (**C, D**) Penicillium growth and microscopy.

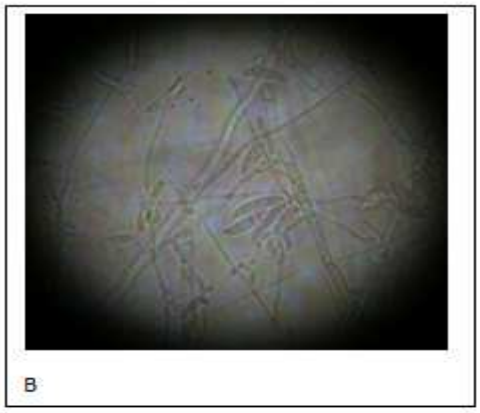

Fig. 4. (**A, B**) Fuserium growth on PDA and microscopy.

Table 2. Area wise distribution of fungal pathogens of onion plants in distract Swat

S/No	Area	Isolated fungal pathogen
1	Aboha	*Aspergillus*, *Puccinia allii*, *Penicillium*,
2	Bahrain	*Fuserium spp*, *Peronospora destructor*
3	Barekot	*Aspergillus, Puccinia allii*, *Penicillium*,
4	Charbagh	*Aspergillus*, *Pythium* spp, *Peronospora destructor*
5	Ghaligay	*Aspergillus, Puccinia allii*, *Penicillium*,
6	Gogdarra	*Puccinia allii*, *Penicillium*, *Peronospora destructor*
7	Kabal	*Aspergillus*, *Pythium* spp, *Peronospora destructor*
8	Kalam	*Fuserium spp*, *Peronospora destructor*
9	Khwaza khela	*Aspergillus, Puccinia allii Pythium* spp
10	Kota	*Aspergillus, Puccinia allii*, *Penicillium*,
11	Madyan	*Aspergillus, Puccinia allii Fuserium spp*, *Rhizoctonia solani*
12	Maindam	*Aspergillus, Fuserium* spp,
13	Manyar	*Aspergillus, Puccinia allii Penicillium*,
14	Matta	*Aspergillus, Puccinia allii*, *Rhizoctonia solani*
15	Mingora	*Aspergillus*, *Puccinia allii*, *Penicillium, Pythium* spp
16	Shamozo	*Aspergillus*, *Puccinia allii Penicillium, Peronospora destructor*
17	Thana Malakand	*Aspergillus, Puccinia allii, Rhizoctonia solani*

Table 3. Fungal species isolated from infected plants and soil samples.

S.NO	Isolated Fungal Species	Associated diseases
01	*Pyhtaium, Rhizoctonia* and *Fuserium* spp	Damping off
02	*Alternaria porri* (unable to culture)	Purple Blotch
03	*Aspergillus*	Black Mold
04	*Puccinia allii* (unable to culture)	Onion Rust
05	*Peronospora destructor* (unable to culture)	Downy Maldives
06	*Fusarium* spp	Black Stalk Rot
07	*Penicillium* spp.	Blue Mold Rot

Table 4. Quantification result of fungal pathogens in collected plants from Swat District

S.NO	Fungal pathogen	Percentage
01	*Aspergillus*	82%
02	*Puccinia allii* (unable to culture)	70%
03	*Alternaria porri* (unable to culture)	52%
04	*Fuserium spp*	42%
05	*Penicillium spp*	40%
06	*Peronospora destructor* (unable to culture)	35%
07	*Pyhtaium,*	23%
08	*Rhizoctonia solani*	18%

Discussions

Onion *(Allium cepa L.)* is an important vegetable crops that is a rich source of sugar, proteins, fats, vitamins and other nutrients[1]. It may use raw as salad and in vegetables processing. There are various factors like genetic makeup, physiological factors and various diseases which reduce onion production. Certain onion crop diseases are caused by bacteria, viruses, fungi and nematodes however diseases caused by fungi and nematodes are commercially more important. In the present study seventeen locations of swat district were selected for sampling for fungal disease. The results revealed that fungal diseases in swat district were with agreement of previous researchers[14,15].

Various types of plant pathogenic fungi have ability to infect of onion crop. In our study *Puccinia allii, Alternaria, porri, Fussiarum oxysporum, Peronospora destructor, Pyhtium* spp*., Rhizoctonia solani, Penicillium* spp and *Aspergillus* spp. were isolated from onion crop which is the confirmative of previous researcher[16]. *Peronospora destructor* causes downy mildew of onion, at the temperature of 15°C and relative humidity more than 95%. It was also observed in the study that it was isolated when there was high humidity and low relative temperature (14 – 20 °C). It was well reported that *Fusiarum oxysporium* is present in soil with high temperature (20 - 30°C) and less humidity. Our study in line with these finding as these pathogens were reported in areas with high temperature and less humid region.

Pythium spp. and *Rhizoctinia solani* cause damping off disease in vegetable crop including onion. In current study both *Pythium* spp. and *Rhizictonia* solani were present at high frequency *i.e*., 23 and 18%, respectively which was with agreement[17].

Result of our studies showed that *Alterneria porii* and *Puccenia allii* were the major pathogens of arial part of onion crop and were isolated with frequency of 52% and 70%, respectively. Similar reports have been reported in by[18,19.] Rust disease of onion caused by *Puccenia allii* was found in condition with moderate temperature and high relative humidity than 90% for 14 hours. Our study agreed with it and high frequency 70% of rust disease was reported in moderate temperature with relative humidity. *Alterneria*

porii favor little high temperature and low humidity condition[20]. Our study was conformity of these researchers. Our result is also in agreement with the finding of [20] who reported *Alterneria porii* caused severe loss at high temperature and Humidity.

Post harvested disease cause major crop loss at store house conditions. Various post-harvest pathogens are involved in onion crop. Important among them are *Aspergillus* spp. and *Penicillium* spp [21] *and* cause black mold and blue mold, respectively. In the present study *Aspergillus* spp. was isolated from 82% whereas *Penecillium* spp. was isolated from (42%) samples. These findings are like these of [22]. The reason for this high post-harvest disease is that both *Aspergillus* spp. and *Penicillum* spp. are air-borne fungi and Omni present in wide environmental conditions. This was also suggested by [22] and [23] that both pathogens present in high frequencies and survive in variable environmental conditions.

4. CONCLUSIONS

Fungal diseases in swat district also cause major loss in onion crop. There were eight fungus isolates namely; *Puccinia allii, Alternaria, porri, Fussiarum oxysporim, Peronospora destructor, Pyhtium* spp*., Rhizoctonia solani, Penicillium* spp and *Aspergillus* spp. were isolated from onion crop of Swat district because these fungal species can optimally grow in climate of Swat district. *Fusarium oxysporium, Pythium* spp. and *Rhizoctinia solani* were the major soil-borne diseases at seedling stage at high temperature and moist soil conditions. *Peronospora destructor*, *Alterneria porii* and *Puccenia allii* were the major pathogens of Arial part of onion crop. Post harvested diseases were caused by *Aspergillus* spp. and *Penecillium* spp.

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CONFLICT OF INTEREST

All the authors claim that there is no conflict of interest regarding the publication of this paper.

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