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# Screening of fungal diseases infecting onion plants in Lower Egypt

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#### **ABSTRACT**

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Onion (*Allium cepa* L.) is worldwide and cultivated crop, it also has an economic, medicinal and nutrient importance. Currently, in Egypt, five onion species are cultivated; Tantawy red and Giza white are commonly cultivated in Lower Egypt. Different diseases attack onion during the growth stage such as fungal disease which causes huge crop losses due to climatic conditions which are suitable for plant infection. There are thirteen fungal diseases in Egypt reported until now. The aim of present study is to survey and identify the different fungal diseases that infect onion in four governorates in Lower Egypt during the harvest season 2016–2017. The results identified three different diseases caused with three different fungal species were *Alternaria sp.*, *Aspergillus sp.* and *Botrytis sp.* 

Keywords: Onion, fungal diseases, Lower Egypt, Alternaria sp., Aspergillus sp. and Botrytis sp.

#### Introduction

Onion is one of the main crops grown for bulb production (Campbell *et al.*, 1986). *A. cepa* including seed propagated onions and most shallot types are only known from cultivation (Baudoin *et al.*, 1994). Onion plants are infected with a wide spectrum of diseases; bacterial, viral, fungal and nematodes (Fritsch and Friesen, 2002). The plant is infected with a wide spectrum of fungal diseases as they are 23 diseases as Conn *et al.* (2012) surveyed. In Egypt, onion plants are most infected with fungal diseases than any other diseases due to climatic conditions which are suitable to infect the plant. It was reported that onion in Egypt is infected with thirteen fungal diseases; the basal rot, black mold, black stalk rot and damping off (El-Helaly *et al.*, 1962), neck rot and pink root (Ali *et al.*, 1979), purple blotch (Abdel-Hafez *et al.* 2013 a and b), downey mildew and rust (Elarosi, 1964), smut (El-Shehaby, 1995), southern blight (El-Helaly *et al.*, 1962), stemphylium leaf blight (Hassan *et al.* 2007) and white rot (Satour *et al.* 1989). In Egypt, there are five onion species cultivated; Modified Giza 6 species, Giza 20, Shandaweely, Tantawy red and Giza white. Only are Giza 20, Tantawy red and Giza white species are cultivated in Lower Egypt (ARI, 2015).

Black mold disease generally increases at the neck of the bulbs of the injured or necrotic leaf tissue. However, it can develop on injured or diseased roots, or on bruised or split outer scales along the side of bulbs (Wani and Taskeen-Un-Nisa, 2011). Infected bulbs may develop a black discoloration at the neck. Clusters of black spores generally form along duct and on or between the outer papery scales of the bulbs. The infected tissues first symptom is a water-soaked manifestation and over time it will dry and fade. No symptoms can be visible on the infected bulbs. Soft rot bacteria can follow the infection by this fungus (Dhir, 2017). Causal agent is *Aspergillus niger* (Edens *et al.*, 2006). Spores of this fungus are widespread in air and soil. The disease is distributed in Egypt (Metwally *et al.*, 2015). It was first reported in Egypt by El-Helaly *et al.* (1962) as it was observed on the onion bulbs. Black mold is widespread when the temperatures are more than 30°C in the field or 24°C in storage. Free moisture for six hours or longer on the onion surface is necessary for infection to occur (Elarosi, 1964).

While the neck rots disease; onions are attacked by at least seven *Botrytis* species, of these, Brewster, (1994) distinguished three species that causes neck rot: *B. aclada Fresen (syn. B. allii* 

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Munn), causal agent of sclerotial neck rot; Botrytis byssoidea (teleomorph Botryotinia allii), causal agent of mycelial neck rot; and Botrytis squamosa, causal agent of small sclerotial neck rot of white onion cultivars. The growing crop seldom shows symptoms until harvest. However, this disease can be devastating on stored onions. The fungus can pervade the youthful leaves, but it usually infects the neck directly or through wounded tissue. This tissue becomes soft and spongy as the fungus continues to grow into the bulb (Zhang et al., 2013). Infected parts of the bulb are brown and water-soaked, and the infected tissue finally collapse and becomes spongy. A white to gray mycelial growth eventually develops between the bulb scales and masses of small black sclerotia may develop on the outer scales around the neck (Hafez et al., 2013 and 2015). In addition to neck rot, Botrytis allii has been involved in causing a soil-line rot. Other *Botrytis* species can also cause this disease. The fungus penetrates the outer scales of the bulb initiating a rot that is exacerbated by secondary invaders (Hussein et al., 2014). It was observed the first time in Egypt on 1976 (Ali et al., 1979). Under extended moist conditions the fungus can sporulate on dead tissue in the field as well as from sclerotia. Wind readily disseminates these conidia to other plants where they can infect the neck of the plant through wounds or cuts. Disease is spread rapidly during moderate temperatures with high humidity, rainfall or overhead irrigation (El-Neshawy et al., 1999).

Meanwhile the purple blotch disease; older leaves tend to be more susceptible than younger leaves. Symptoms begin as water-soaked lesions that usually have a white center. Edges of lesions become brown to purple and the leaf turns yellow above and below the lesions. With time, dark brown to black concentric rings form throughout the lesions. These are areas of sporulation of the fungus (Abdel-Hafez *et al.*, 2013 a and b). As the disease progresses, lesions may girdle the leaf causing it to collapse and die. Similar symptoms occur on seed stalks and infected stalks can collapse resulting in shriveled seed development. When bulb infection occurs, it is normally through the neck. If the fungus invades the bulb, the infected area is initially bright yellow, but eventually turns a characteristic red wine color (Abdel-Hafez *et al.*, 2014). Its causal agent is *Alternaria porri*. It was observed first time in Egypt on 1956 (Elarosi, 1964).

#### **Materials and Methods**

The present work was carried out at Plant Protection and Bio-molecular Diagnosis Department, Arid Lands Cultivating Research Institute, City of Scientific Research and Technological Applications (SRTA-CITY), New Borg El-Arab City, Alexandria, Egypt and Agricultural Botany Department, Faculty of Agriculture Saba Basha, Alexandria University, Alexandria, Egypt. Onion plants showing symptoms of fungal diseases were collected in April 2017, from Alexandria, El Behairah, Kafr El Sheikh and El Garbiyah Governorates, Lower Egypt.

### 1. Survey of natural infection with different fungal diseases on onion plant

Naturally diseased onion leaves of the two examined cultivars infected with varied degrees of fungal diseases were collected from various districts in Alexandria (Abees area), El Behairah (Etay Elbaroud, El Noubaria, Kafr Eldawar and Damanhour), Kafr El Sheikh (Foah and Elhamoul) and El Garbiyah (Tanta and Kafr Elzayat) Governorates.

## 2. Isolation and purification of the causal organism

The collected diseased onion leaves were washed in running tap water followed by sterile water. Using sterilized scalpel, leaves were cut into small pieces  $(1-2 \text{ cm}^2)$  containing the infection in the center. The pieces were then transferred into 1 % hypochlorite solution (disinfectant solution) for three minutes for surface sterilization. Surface sterilized piece were then washed several times with sterilized water to wash out the remaining disinfectant solution. The pieces were then dried on sterilized filter paper. Using sterilized forces, plot dried pieces were then transferred into petri dishes containing Potato Dextrose Agar medium (PDA) supplement with antibacterial agent. The dishes were then incubated at 25 °C. The petri dishes were then checked for microbial growth two days after planting. Purification of the resulting isolates was done using the single spore technique to obtain them in pure cultures (Shabana, 1987).

### 3. Identification of the causal organism

Pure cultures of the isolated fungi were identified according to the cultural properties, morphological and microscopic characteristics described by Mew and Gonzales (2002). Identification was confirmed morphological through the Agricultural Botany Department, Faculty of Agriculture Saba Basha, Alexandria University, Alexandria, Egypt.

### 4. Pathogenicity test and cultivar reaction

Pathogenicity test was carried out using thirty-three isolates form three different fungi of the causal organisms *Aspergillus sp.*, *Botrytis sp.* and *Alternaria sp.*, which were isolated from different districts as described, and the most aggressive isolate among them was used for further studies. Spore suspension (5 x 10<sup>5</sup> spores / ml) of the pathogenic isolates were prepared from five days old cultures of *Aspergillus sp.*, *Botrytis sp.* and *Alternaria sp.* isolates to be tested. The onion cultivars Tantawy red and Giza white were used in this experiment and were obtained from the Agricultural Research Institute, Ministry of Agriculture, Egypt. Pots (20 cm in diameter) were filled with disinfested soil and the rate of 3 - 5 seedling plants were put into each pot. Three pots were used for each isolate. In the seedling stage, after cultivating with 3 - 5 days the spore suspensions were sprayed. Three pots were sprayed with water only to serve as a control treatment. The pots were then covered with transparent plastic covers for 24 hours. All pots were kept outdoor and plants were checked for appearance of disease symptoms (Rashad, 1996).

#### **Results and Discussion**

The surveys result showed three different fungi with different isolates which obtained and purified by single spore technique. These isolates were isolated from the infected leaves on PDA media then identified under the microscope as; *Alternaria sp.*, *Aspergillus sp.* and *Botrytis sp.* according to the morphological and microscopic characteristics of the cultures.

## 1. Isolation, microscopic identification and grouping of the fungi

# 1.1. Disease syptoms and fungal characterization

The morphological examination and microscopic examination showed that the thirty-three isolates are grouped into three different species; *Alternaria sp.*, *Aspergillus sp.* and *Botrytis sp.*, as follow:

#### 1.1.1. Alternaria sp.

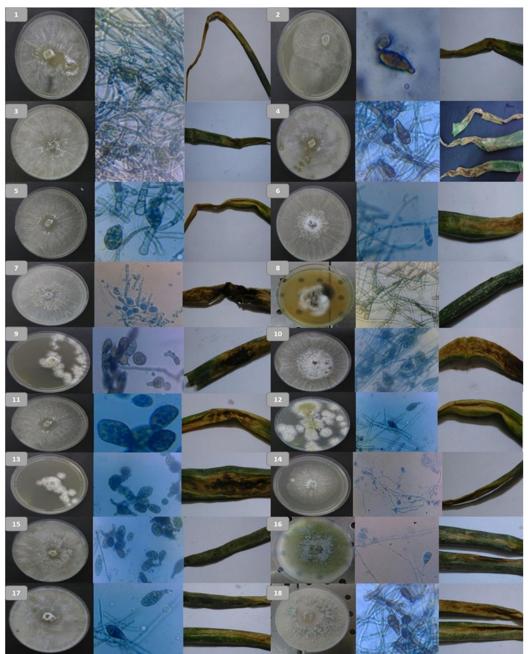
Eighteen isolates showed the purple blotch disease morphological symptoms as the plant edges color is brown to purple and also the leaf seems to be yellow higher and below the lesions as most of the collected samples, this in the early injury. In late injuries, dark brown to black concentrically rings type throughout the lesions, as these dark areas are the sporulation of the fungus. The obtained results agreed with Abdel-Hafez *et al.* (2013a and b). The purified isolates gave single colonies on the petri dishes that led us to identify the conidia of the fungus by the light microscopy examination as the *Alternaria sp.* which is the causal agent of the purple blotch disease. They appear pale brown color to light brown, short conical beak or beak less at the tip, its surface is smooth and its size is 20.63 x 9.18 μm as shown in Figure (1) these results agree with Abdel-Hafez *et al.* (2014) and Abo-Elyousr *et al.* (2014).

#### 1.1.2. Aspergillus sp.

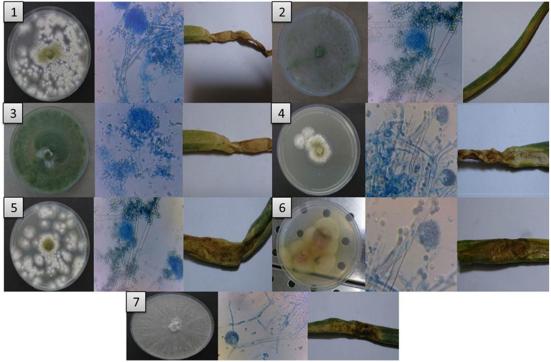
Seven isolates showed morphological symptoms on the leaves seems as necrotic tissue as the clusters of black spores is found along the duct on or between the outer papery scales of the bulbs as these symptoms agree with Bishop and Davis, (1990). The purified samples gave single colonies; the microscopic examination shows that the conidia consist of a compact dark-brown to black conidial heads when staining with the coomassie blue it turns into dark blue as shown in Figure (2). Conidial heads are large (up to 3 mm, 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. That led us to identify the fungus microscopically as the *Aspergillus sp.* which is the causal agent of the black mold disease, these results agree with Wani and Taskeen-Un-Nisa, (2011).

### 1.1.3. Botrytis sp.

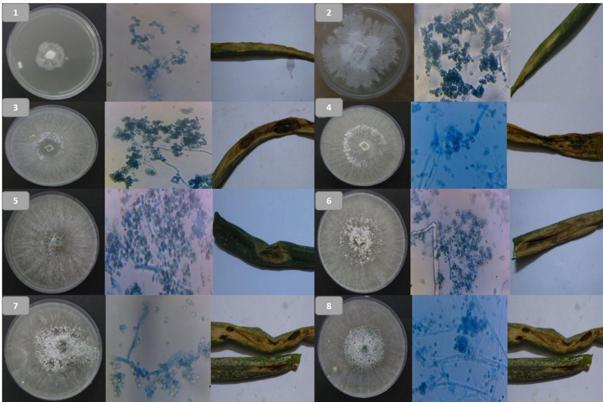
Eight isolates showed morphological symptoms as the tissue is brown, water-soaked, soft and spongy as shown in most of the collected samples. The late injured samples had a white to gray mycelia growth which was found on the outer scales around the neck as samples 26 and 27 as these symptoms agree with Zhang *et al.* (2013). The purified samples gave single colonies on the petri dishes then it was exanimated microscopically, the fungus looks like bunches of grapes. A large number of rounded conidia are budded off at the branched ends of the long (to 2 mm), stiffly upright conidiophores as shown in Figure (3). They are round shaped, brown and their size is  $10.2 \times 5.7 \,\mu m$  that led us to identify the spores of the fungus microscopically as the *Botrytis sp.* which is the causal agent of the neck rot disease that agrees with Hafez *et al.* (2013) and (2015).



**Fig. 1:** The eighteen fungal isolates (1-18) shows at the left in all samples the colony of the fungi on PDA media then in the middle the microscopic examination as the conidia of the *Alternaria* sp. isolates and the last one is the morphological symptoms of the disease on the infected onion leaves.



**Fig. 2:** The seven isolates (1-7) shows at the left in all samples the colony of the extracted fungi on PDA media then in the middle microscopic examination as the spores of the *Aspergillus sp.* isolates and the last one is the morphological symptoms of the disease on the infected onion leaves.



**Fig. 3:** The eight isolates (1-8) shows at the left in all samples the colony of the purified fungus on PDA media then in the middle the microscopic examination as the spores of the *Botrytis sp.* isolates and the last one is the morphological symptoms of the disease on the infected onion leaves.

## 2. Severity of the pathogenicity test according to the morphological symptoms.

Thirty-three isolates of the three fungi were subjected to the pathogenicity test to determine the most aggressive one. All isolates appear to have the potency to cause the disease. In order to determine the most susceptible onion cultivar for these three different fungal species (*Alternaria sp.*, *Aspergillus sp.* and *Botrytis sp.*), two onion cultivars that are commonly cultivated in Lower Egypt (Tantawy red and Giza white) were artificially infected with the different isolates of each fungus. The two tested onion cultivars manifested the disease symptom.

### 2.1. Tantawy Red onion

Their bulbs are solid; the veneer color is dark red. The color of the meat is dark red for all the leaves in the bulb and its normal storage period is 7 - 8 months. It is cultivated in Lower Egypt, Giza, Beni Suef and El Fayoum (Agriculture Research Institute, Giza, Egypt, 2016). All the isolated fungi were found to be pathogenic to the onion bulbs; however, some isolates were more virulent than others leading to rapid disintegration of the infected bulbs within 21 days of inoculation. This agrees with the reports of other researchers (Muhammad *et al.*, 2004; Dimka and Onuegbu, 2010) that fungi constitute a menace in the storage of many agricultural commodities including fruits, vegetables and nuts.

### 2.1.1. Alternaria sp. as causal effect of purple blotch

The eighteen isolates that were obtained from the survey in the four different governorates were used to test its pathogenicity. The samples gave different response to these isolates as we grouped it according to their severity on the plant after infecting the plants within 21 days of inoculation with the fungus. The most potent isolates were 2, 5, 8, 9, 13, 14 and 15 as shown in Figure (4). The lesions girdle the leaf crumples and die; as this is the last stage of the disease when infecting the onion. This result agrees with Abdel-Hafez *et al.* (2014). The moderate isolates showed resistance as shown in Figure (4) they were 11, 12 and 17. The infected area is bright yellow and crumbles in some samples. The less potent isolates were 1, 3, 4, 6, 7, 10, 16 and 18 as shown in Figure (4). Older leaves tend to be more liable than younger leaves, the leaf turns yellow higher than and below the lesions. These results agree with Abdel-Hafez *et al.* (2013 a and b).



**Fig. 4:** The most potent isolates of the *Alternaria sp.*: 2, 5, 8, 9, 13, 14 and 15, the moderate isolates 11, 12 and 17 and the less potent isolates: 1, 3, 4, 6, 7, 10, 16 and 18 on the cultivated Tantawy red onion (at the left) in comparison with the healthy one (at the right).

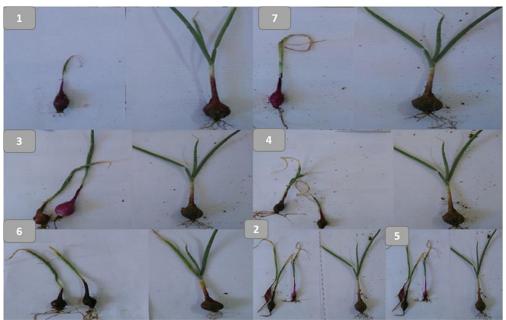
## 2.1.2. Aspergillus sp. as causal effect of black mold

The seven isolates were obtained from the survey in the four different governorates were used to test its pathogenicity. The samples gave different response to these isolates as we grouped it according to their severity on the plant after infecting the plants within 21 days of inoculation with the fungus. The most potent isolates were 1 and 7 as shown in Figure (5) as the symptoms are a water-soaked manifestation and over time it dries and fad, these results agree with Bishop and Davis, (1990). The moderate isolates were 3, 4 and 6 as shown in Figure (5) and the less potent isolates are 2 and 5 as

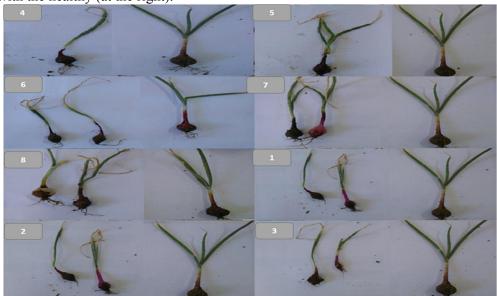
shown in Figure (5) as the fungus causes necrotic leaf tissue and dwarf in the outer papery scales of the bulbs, as these results agree with Edens *et al.* (2006).

# 2.1.3. Botrytis sp. as the causal agent of the neck rot disease

Based on the pathogenicity test eight isolates were obtained from the survey in the four different governorates. The samples gave different response to these isolates from the neck rot disease as we grouped it according to their severity on the plant after infecting the plants within 21 days of inoculation with the fungus. The most potent isolates were 4 and 5 (Figure 6) meanwhile the moderate isolates are 6, 7 and 8 (Figure 6) as the tissue is soft and spongy. The less potent isolates are 1, 2 and 3 (Figure 6) as the infected parts of the bulb are brown and water-soaked, these results agree with Brewster, (1994) and Zhang *et al.* (2013).



**Fig. 5:** The most potent isolates 1 and 7, the moderate isolates 3, 4 and 6 and the less potent isolates 2 and 5 of the *Aspergillus sp.* on the cultivated Tantawy red onion (at the left) with comparison with the healthy (at the right).



**Fig. 6:** The most potent isolates 4 and 5, the moderate isolates 6, 7 and 8 and the less potent isolates 1, 2 and 3 of the *Botrytis sp.* on the cultivated Tantawy red onion (at the left) and the healthy onion (at the right).

#### 2.2. Giza White onion

Their bulbs are solid, the color of the crust is white and the flesh color is bright white. The storage period of this species is 8 - 9 months and the product is attached to the production conditions, it is cultivated in Upper and Lower Egypt (Agriculture Research Institute, Giza, Egypt, 2016). All the isolated fungi that were tested on the Tantawy red onion gave the same symptoms on the Giza white onion as it was found to be pathogenic to the onion bulbs. These results agree with the reports of other researchers (Muhammad *et al.*, 2004; Dimka and Onuegbu, 2010) that fungi constitute a menace in the storage of many agricultural commodities including fruits, vegetables and nuts.

## 2.2.1. Alternaria sp. as causal effect of purple blotch

The eighteen isolates that were obtained from the survey from the different governorates were used to test its pathogenicity. The samples gave different response to these isolates as we grouped according to their severity after infecting the plants within 21 days of inoculation with the fungus. The most potent isolates were 2, 5, 8, 9, 13, 14 and 15 as shown in Figure (7) as the lesions girdle the leaf crumples and die as this results agrees with Abdel-Hafez *et al.* (2014); the moderate isolates were 11, 12 and 17 as shown in Figure (7) as the infected area is bright yellow and the less potent isolates are 1, 3, 4, 6, 7, 10, 16 and 18 as shown in Figure (7) as the elder leaves tend to be more liable than younger leaves, the leaf turns yellow higher than and below the lesions. These results agree with Abdel-Hafez *et al.* (2013 a and b).



**Fig. 7:** The most potent isolates 2, 5, 8, 9, 13, 14 and 15, the moderate isolates 11, 12 and 17 and of the less potent isolates 1, 3, 4, 6, 7, 10, 16 and 18 *Alternaria sp.* on the cultivated Giza White onion in comparison between the healthy.

### 2.2.2. Aspergillus sp. as causal effect of black mold

When testing the pathogenicity test for the seven isolates the plant samples gave different response to these isolates as grouped according to their severity on the plant after infecting the plants within 21 days of inoculation with the fungus. The *Aspergillus sp.* isolates were grouped according to their severity infection on plant: the most potent isolates are 1 and 7 as shown in Figure (8) as their symptoms were a water-soaked manifestation these results agree with Bishop and Davis, (1990). Meanwhile the moderate pathogen isolates were; 3, 4 and 6 as shown in Figure (8) and the less potent isolates were isolates 2 and 5 as shown in Figure (8) as the fungus causes necrotic leaf tissue and dwarf in the outer papery scales of the bulbs, as these results agree with Edens *et al.* (2006).

### 2.2.3. Botrytis sp. as the causal agent of the neck rot disease

The eight isolates were obtained and then used to test its pathogenicity. The plant samples gave different response to these isolates from the neck rot disease as grouped according to their severity on the plant after infecting the plants within 21 days of inoculation with the fungus. The *Botrytis sp.* was grouped according to their virulence to; the most potent isolates were 4 and 5 as shown in Figure 9, the moderate isolates were 6, 7 and 8 as shown in Figure 9 and the less potent isolates were 1, 2 and 3 as shown in Figure 9 as the infected parts of the bulb are brown and water-soaked, These results agree with the results obtained by Brewster, (1994) and Zhang *et al.* (2013).



**Fig. 8:** The most potent isolates 1 and 7, the moderate isolates 3, 4 and 6 and the of the less potent isolates 2 and 5 *Aspergillus sp.* on the cultivated Giza White onion with comparison between the healthy one.



**Fig. 9:** The most potent isolates 4 and 5, the moderate isolates 6, 7 and 8 and the less potent isolates 1, 2 and 3 of the *Botrytis sp.* on the cultivated Giza White onion with comparison between the healthy one.

### Conclusion

It can conclude that three different fungal species; *Alternaria sp., Aspergillus sp.* and *Botrytis sp.* were commonly existed and have different potency for the disease causing. The surveyed regions are considered as suitable for the infection by the different diseases and they are concluded to be epidemic regions for one of the three diseases. So, we recommend searching another onion cultivar which could be resistant for the three fungal species.

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