



## Purple Blotch as Seed-Borne Disease of Onion and its Control

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Aya H Abo-Zaid\*, Karima G Helmy, Hala Abdel Wahab,  
Magdy G El-Samman

Plant Pathology Dept, Fac of Agric, Ain Shams Univ, P.O. Box 68, Hadayek Shoubra 11241,  
Cairo, Egypt

\*Corresponding author: [ayaabo-zaid@agr.asu.edu.eg](mailto:ayaabo-zaid@agr.asu.edu.eg)

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### Abstract

*Alternaria porri* (Ellis). Ciffen causes serious problems in onion plants. It is transmitted with naturally infected seeds and bulbs from the previous crop to infect the new flower stalk of the next crop. Also, onion bulb infection decreases seed vitality. Infected sample were collected from four locations; Giza, Sharkia, Kalubia and Menofia governorates to measure disease severity. Experiments have been done on naturally infected seeds and bulbs from the previous year by cultivating infected bulbs. Infection was classified into five categories based on the percentage of disease severity. Pathogen transmission by the onion seed was investigated, either as internal or external infection type, Five different ratios of blub infection was determined as; 14.2%, 25.2%, 41.5%, 61.1%, 87.5%. Also, trials were carried out in the current study to evaluate the efficiency of some botanical, biological and chemical as seed dressing treatments for infected seeds before sowing it, *i.e.* (Achook, *Bougainvillea* sp., *Bacillus* sp., *Trichoderma* sp. and Score). Results showed that disease severity was increased in the blub produced from previous crop, the number of flower stalk was reduced, and its disease severity was increased. Seeds produced from infected blub showed reduction in germination percentage. The most effective seed dressing treatment on seed germination were obtained by using score & Achook treatment compared with other seed dressing treatments. All treatments including Score, Achook, *Bacillus* sp., *Bougainvillea* sp., and *Trichoderma* sp.; respectively showed variable seed stimulation of germination compared with plants in the control treatment.

**Keywords:** Purple blotch, Onion, Seed-borne, Control

### 1 Introduction

Onion plant (*Allium cepa* L.) is a very essential crop, occupies the high-ranking position among cultivated vegetable in Egypt (Bayoumi et al 2019). The total production of onion in Egypt was 2379035 tons (FAOSTAT, 2017).

*Alternaria porri* (Ellis). Ciffen causes Purple Blotch (PB) on onion is the extremely worldwide serious disease on onion according to Chaput (1995), Cramer (2000) and Schwartz et al (2005). Suheri and Price (2000) reported that purple blotch occurred especially during warm-wet conditions. Richardson, 1990 said that onion plant-seed harvest deteriorates of twenty-two different mycological infections. Gupta (1998), Lakra (1999) and Papu (2010) reported that *A. porri* caused PB disease in many onion growing countries including Egypt is able to affect and caused huge reduction in the bulb and seed of onion production ranged from 2.5 to 97%.

Under subtropical environments, Munoz et al (1984) and Kumar (2020) reported that purple blotch severely affected seed production through reduction in seed yield by breaking of floral stalks. Abo-Elyousr (2014) mentioned that the *A. porri* produce 80-100% deficiency in Egypt as causative organism of PB. *A. porri* can affect each part of the plant above ground as well the bulb and start indications occur on eldest leaves, usually during late summertime. Spores are carried on contaminated wreckage. La Forest (2011) reported that old plant is additionally sensitive to spread fungus infection

with fields under rainwater and wind. This led to overwinter as internal and external infestation of crop debris and may transmitted as seed borne. Wet weather period experiments at IAR, Samaru, Nigeria have related a decrease in production which was ascribed to incident with leaf pathogens especially *A. porri* (Ellis). (Cif Green 1972). According to Suheri and Price (2001) and Bock (1964) mentioned that the fungus attacks both leaves and flower stalks and reducing foliar production by 62–92%. Everts and Lacy (1990), Bayoumi et al (2019) reported the disease can occasion a produce loss of 30%, and according to Daljeet et al (1992), Schwartz (2004) and Agale et al (2014) stated that the weather conditions are favorable going to lose 100% of the seed yield.

*Alternaria species* spores carried on the seed surface as contaminants rather than infection (Neergaard 1977). The hyphae in infected blub penetrated the seed coat. fungal infected onion seeds and effect on their germination & vigor seedlings. McDonald (1999 and 2004) said that *A. porri* was observed to be internally seed-borne and external infection on the seeds. Seed borne *A. porri* reduced the seed germination (Yalamalle et al 2019). infection of some seed is not visible and such seed germinates without external signs of disease (Strandberg, 1983). According to Nainwal (2013) Seedling raised from naturally infected seeds exhibited characteristic symptoms of PB indicating the transmission of *A. porri* from seed to plant. However, none of the seedling showed the symptom of Stemphylium blight. Moreover, the seed infected with *A. porri* reduced seed germination.

Several antagonistic microorganism including fungi and bacteria have been reported to decreased the activity of *Alternaria porri*, On the other hand, using other trials of disease management, e.g. plant extract, chemical control and agricultural practical

as seed dressing observed by Mude (1977) and Yadav (2017). Soaking due to decreased infection by way of 90% but negatively affected germination. Transplanting high-quality seed and healthy control strategies for PB (*A. porri*) by planting a seedling Schwartz et al (2004). using crop rotation for three-years to non-hosts was also effective. Decrease pathogens' survival of overwintering through culls onion wreckage. The objective of work was to study the transmission PB disease as seed borne pathogen; as well as to study the efficacy of onion seed treatment with plant extract, biological and fungicide to control associated *A. porri* with seeds.

## 2 Materials and Methods

### 2.1 Sample collection and Diagnosis

The occurrence of a PB on onion in Egypt recorded in winter 2017-2018. Four locations Giza, Sharkia, Kalubia, and Menofia were selected to record the symptoms. PB symptoms on onion leaves and cluster stalk were spotted on onion fields and taken a sample of infected leaves. Leaves showing blotch symptoms were accumulated from the select localities and taken to the laboratory in polyethylene bags for investigation regarding the concerned fungus. Scrapping was raised by a needle and put upon a glass slide in a drop-water then checked under a light microscope for the existence of conidiospores. (Islam 2013).

### 2.2 Disease assessment

Disease severity (DS) as (percentage leaf area disease and flower stalk). The evaluation was estimated by using the scale **Figs 1 and 2** followed by Islam et al (1999) as indicated in the below:

Scale range	
0	No disease symptom.
1	A few spots towards tip covering 10 percent leaf area.
2	Several purplish brown patches covering up to 20 percent of leaf area.
3	Several patches with paler outer zone covering up to 40 percent leaf area.
4	Leaf streaks covering up to 75 percent leaf area or breaking of the leaves from center.
5	Complete drying of the leaves or breaking of leaves from center.



Fig 1. Leaf area infection (0-5) scale



Fig 2. Flower stalk infection (0-5) scale

The formula to calculate percentage of DS used by Wheeler (1969) followed:

$$\text{Percent disease severity} = \frac{\sum (\text{class rating} \times \text{class frequency})}{\text{total number of samples} \times \text{highest rating}} * 100$$

### 2.3 Isolation and identification of the causal pathogen

Infected onion leaves were cut into one-four mm size pieces. The pieces were surface sterilized by sodium hypochlorite solution 0.1% concentration for 2 minutes then washed 3 times by sterilized distilled water and dried between two layers of sterilized filter paper. The sterilized leave pieces were transferred into sterilized peri-dishes containing water agar (WA) medium then incubated in incubation on 25°C. according to Dhingra and Sinclair, 1985 fungal colonies purification was done using a single spore or hyphae tip method. Pure culture of each isolated fungus was transferred on fresh potato dextrose agar (PDA). The plate was incubated on 25°C±1 for 10days. Purified cultures of isolated fungi were identified according to cultural morphological and microscopically characters described by Ellis (1971), Barnett and Hunter (1986) and Simmons (2007).

### 2.4 Investigation on seedborne nature and influence of *A. porri* on seed germination

Seeds and bulbs of onion were produced from seedlings which were cultivated the previous year and infected a natural. Effect of *A. porri* on sterilized or unsterilized seed germination was studied which infected naturally last year. Actually, seeds of each plant infected were taken after flower stalks dried on the plant.

### 2.5 Agar plate technique

Water-agar (WA) used as planting medium for seed. Seeds of onion were taken from 5 different infected degrees. Each seed samples used either sterilized seeds or unsterilized seeds. The seeds were plated aseptically on water-agar contained in Petri plates as described by Nainwal (2013). Ten seeds from each sample were placed in Petri plate an equal distance. Petri plates were incubated in an incubator on 25°C for 4-5 days before seed examination for fungal colonies on the seed surface or agar, and the seed germination percentage were recorded.

### 2.6 The efficiency of dressing of seed treatments in examination seed germination

Five treatments prepared from *Bougainvillea* sp. extract, *Bacillus* sp., *Trichoderma* sp. Achook (Azadirachtin) and score (Difenoconazole) used to study

the ability in examining seed germination in pots. Seeds of onion taken from five different infection degrees taken from the crop of the previous year showed natural infection was cultivated. The seeds were mixed thoroughly with Bougainvillea extract, *Bacillus* sp., *Trichoderma* sp., Achook and score (10seeds in every case). Seeds are sown in pots with soil (10seeds in every pot). The remaining seeds were sown directly (without treatments) in pots (10 seeds in each pot). Seed germination in every case was recorded after 10-15 days. The data obtained were analyzed statistically for difference among treatments.

### 2.7 Investigation on bulbs-borne nature and effect of *A. porri* on products flower stalk

Onion bulbs produced from seedlings cultivated in the previous year and showed natural infection was used. Infected bulbs were divided into five different infection degrees, and cultivation of these bulbs in the following year to study the influence of these infected bulbs on the production of flower-stalk and the production of seeds and disease severity on all of the leaves and flower stalks as described by Nainwal (2013).

### 2.8 Statistical analysis

Data of each experiment were statistically computed using SAS ANOVA (SAS Institute, 1992) with least significant difference test (LSD) with 95% probability level and the coefficient of correlation.

## 3 Result

### 3.1 Survey

Disease severity was carried during 2017-2018 winter season. Four locations Giza, Sharkia, Kalubia and Menofia were selected to the record the PB disease observations. PB disease was spotted on onion leaves, flower stalk fields and samples were taken from infected leaves. The data obtained presented in **Table 1**.

Mean of disease severity survey at the different locations indicate that the highest percentage of the disease was obtained in Kalubia (61.07%) followed by Menofia (57.6%) followed by Giza (57.1%), while the lowest percentage was obtained Sharkia (45.7%) Governorate respectively **Table 1**.

### 3.2 Efficacy of seed dressing treatments in checking seed germination

The observed disease severity percentages were (14.2%), (25.2%), (41.5%), (61.1%) and (87.5%) resembling five-different degrees of infected blubs. The data obtained are presented in **Tables 2 and 3**. The percentage of seeds germination and the percentage *A. porri* internal in seeds or external on seeds compared with disease severity of blubs were determined. External seed-borne and

seed germination percentages of disease severity of the five DS categories were (98.8%), (94%), (83%), (58.6%) and (53%; respectively) while the percentage of *A. porri* was (3.7%), (9%), (13.3%), (16.7%) and (20%). On the other hand internal seed-borne caused variable seed germination percentages; (96.3%), (88%), (81.4%), (76.4%) and (73.3%; respectively) and the percentage *A. porri* were (5.6%), (7.1%), (11.5%), (27.8%) and (66.6%; respectively).

**Table 1.** Survey of purple blotch disease on onion plants and number of isolates in different governorates during seasons 2017-2018

No.	Governorate	Location	No. of isolate	Disease severity (DS)	Mean
1	Giza	Geraza	10	57.1	50.4
2	Sharkia	El Gosak	44	45.7	
		Shalaqan	37	61.07	
3	Kalubia	kalyub	18	28.5	
		Shoubra Elkhama	10	52.5	
4	Menofia	El Sadat	35	57.6	

**Table 2.** Occurrence of external seed borne fungi isolated from blubs in different disease severity of purple blotch on onion

D.S. of blubs	No. cluster stalk	Occurrence of <i>A. porri</i> (%)	Occurrence of <i>Stemphylium</i> sp. (%)	Occurrence of other isolate <sup>2</sup> (%)	Germination (%)
14.2	8	3.7	0.0	96.3	98.8
25.2	5	9	4.3	86.7	94
41.5	7	13.3	0.0	86.7	83
61.1	11	16.7	3.7	79.6	58.6
87.5	15	20	16.1	63.9	53

<sup>1</sup> Disease severity of blubs infected naturally in pervious year

<sup>2</sup> others isolate (*Aspergillus* sp., *Curvularia* sp. and *Phoma* sp.)

**Table 3.** Occurrence of internal seed borne fungi isolated from blubs in different disease severity of purple blotch on onion

D.S. of blubs <sup>1</sup>	No. cluster stalk	Occurrence of <i>A. porri</i> (%)	Occurrence of <i>Stemphylium</i> sp. (%)	Occurrence of other isolate <sup>2</sup> (%)	Germination (%)
14.2	8	5.6	0.0	94.4	96.3
25.2	5	7.1	0.0	92.9	88.0
41.5	7	11.5	0.0	88.5	81.4
61.1	11	27.8	0.0	72.2	76.4
87.5	15	66.6	0.0	33.4	73.3

<sup>1</sup> Disease severity of blubs infected naturally in last year

<sup>2</sup> others isolate (*Aspergillus* sp., *Curvularia* sp., *Fusarium* spp. and *Epicocum* sp.)

**Fig 3** refers to the Correlation coefficient between disease severity of blubs and infected seed germination which taken from these flower stalks, and relation with the presence of *A. porri* whether carried on external or internal infected seeds and effecting on germination.

### 3.3 Efficacy of seed dressing treatments in inspection of seed germination

Reliable results in different treatments that showed disease control effectively. Amongst different seed dressing treatment (*Bougainvillea* extract, bacillus sp., *Trichoderma* sp. A chook and score) on five disease severity different of blubs (14.2, 25.2, 41.5, 61.1 and 87.5) taken from last year, in 14.2% the per cent germination was higher (96%) in *Bougainvillea* sp. in percentage infected 25.2% was the germination maximum with score (84%) and the minimum with *Bougainvillea* sp. (56%). in 41.5% the per cent germination was higher (92%) in score. in percentage infected 61.1% was the germination

maximum with Achook (92%) in 87.5% the per cent germination was higher (100%) in score. consequently, the percentage of treated seeds germination for infected blubs degrees for (14.2%, 41.5%, 61.1%, 87.5%) was high as compared to the control. The data obtained are presented in **Table 4**.

### 3.4 Investigation on bulbs-borne nature and effect of *A. porri* on products flower stalk

During winter 2020, bulbs were cultivated which infection a natural the previous year the data explained submitted in **Table 5**, compared between disease severity of bulbs and No. Bulbs was (14), (18), (41), (35) and (17), and number flower stalk was (72), (49), (115), (68) and (30). the percentage of disease severity of leaves were read after 60day was (0.0%), (10.2%), (11.1%), (22.0%) and (22.9%), 90 days (2.0%), (16.4%), (19.2%), (25.1%) and (31.3%) from cultivation and the percentage of disease severity of leaves flower stalks was (39.9%), (41.3%), (54.6%), (56.6%) and (59.3%).

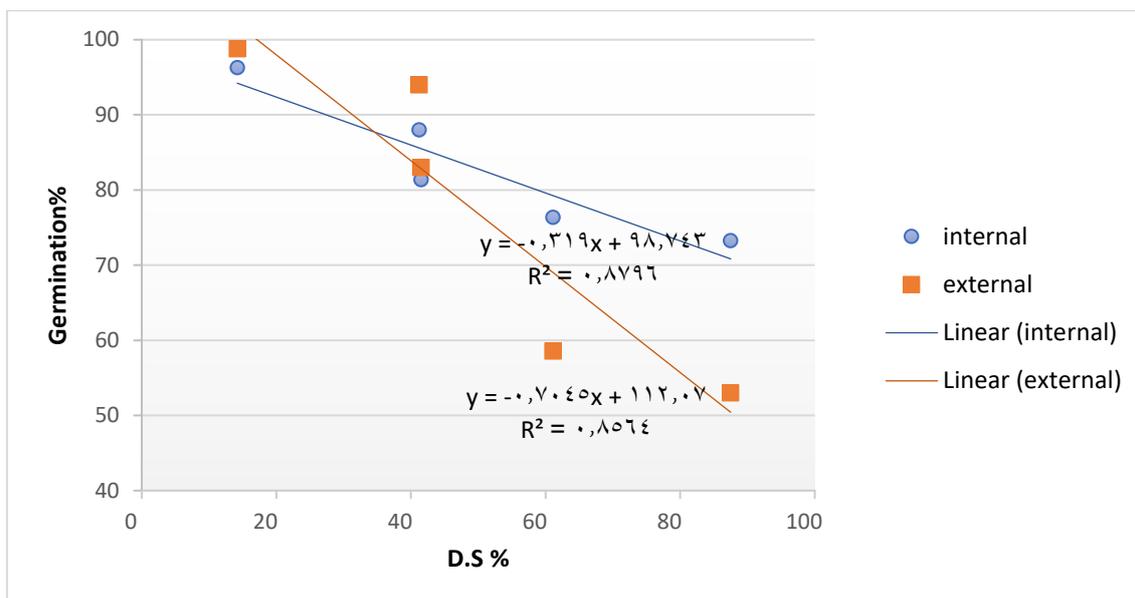


Fig 3. Correlation coefficient between disease severity of blubs and seed germination

Table 4. Efficacy of seed dressing treatment in onion seed germination in pot culture

Treatment	Percentage of seed germination after days (%)	D.S. of Blubs <sup>1</sup>					
		14.2	25.2	41.5	61.1	87.5	mean
Control	14ds <sup>2</sup>	72	60	64	64	68	16.4 <sup>A</sup>
	28ds <sup>3</sup>	80	76	64	68	68	17.8 <sup>B</sup>
Achook	14ds	60	72	76	84	72	18.2 <sup>A</sup>
	28ds	80	80	76	92	72	20.0 <sup>AB</sup>
<i>Bougainvillea</i> sp.	14ds	96	48	80	72	64	18 <sup>A</sup>
	28ds	96	56	80	76	80	19.4 <sup>B</sup>
<i>Bacillus</i> sp.	14ds	76	44	72	80	80	17.6 <sup>A</sup>
	28ds	88	60	76	80	92	19.8 <sup>B</sup>
<i>Trichoderma</i> sp.	14ds	76	60	64	88	64	17.6 <sup>A</sup>
	28ds	80	60	68	88	76	18.6 <sup>B</sup>
Score	14ds	92	52	84	76	68	18.6 <sup>A</sup>
	28ds	92	84	92	88	100	22.8 <sup>A</sup>

The same letters do not differ significantly followed Value (p ≤0.05)

<sup>1</sup> Disease severity of Blubs infected naturally in pervious year

<sup>2</sup>(14ds) 14days

<sup>3</sup>(28ds) 28days

**Table 5.** Effect of naturally infected bulbs on number flower stalk, number of seeds, seed weight (g) and diseases severity on all of leaves and flower stalk in field.

D.S. of blubs <sup>1</sup>	No. Bulbs	No. Flower stalk	D.S. of leaves <sup>2</sup> (%)		D.S. of flower stalk <sup>3</sup> (%)	No. seed	Seed weight (g)
			60 day	90 day	120 day		
14.2	14	72	0.0	2.0	39.9	3824	13
25.2	18	49	10.2	16.4	41.3	3503	11.9
41.5	41	115	11.1	19.2	54.6	2791	9.5
61.1	35	68	22.0	25.1	56.6	2156	7.3
87.5	17	30	22.9	31.3	59.3	1465	5.0

<sup>1</sup>Disease severity of Bulbs infected naturally in pervious year

<sup>2</sup>Disease severity of leaves infected naturally in recently year

<sup>3</sup>Disease severity of flower stalks infected naturally in recently year

#### 4 Discussion

PB has been considered to be a common disease on onion in all onion growing areas in Egypt that cultivated through 2017-2018. PB symptoms were shoed as Fusiform necrosis region in outer leaves which turned brown with age and became ornately laden with dark spore mass (zonate) and Fusiform shaped was observed. The severe foliar infection caused the loss of green leaves.

According to Neergaard (1945), Sohi and Puttoo (1972), Wu (1979), Boff et al (1995) and Nainwal (2013) reported Seed-borne nature of *A. porri* observed crop collected the previous crop, found it with external & internal seed borne and the infection has been in seed parts tested at seed coat area & in endosperm.

Gupta and Pathak, (1998) reported that some researchers have not been able to establish its seed-borne nature which does not agree with those who observed the seed-borne nature of *A. porri* in onion seeds. So, the researcher who did not observe *A. porri* in or on seeds might use seeds from evidently non-infected seed previously treated seeds. *A. porri* causes necrosis in the emerging seedlings and created pre & post-damping-off symptoms. Neergaard (1945) reported that the studies deeply point towards *A. porri* role in seedling count reduction in a nursery. Our observations are in agreement of the pervious findings.

Seed dressing is to protect the seed external and internal; therefore, the 5 treatments used against *A. porri* in seed culture in the pot. Analysis of data revealed that score was most effective in improvement of the seed germination followed by

Achook, *Bacillus* sp., *Bougainvillea* sp. and *Trichoderma* sp. The result presented in **Table 4** suggested that Neem (Achook) extract can be possible to be exploited to control infected seed-borne on onion as an environmentally friendly method. Similar results were reported by Hassanein (2008), Abo-El-yousr (2013) and Bhandekar (2019). Wherever smaller the bulb size, the fewer number flower stalk and the increasing severity of the disease on the leaves and flower stalk that refer to infected bulbs the previous year has carried the disease inside it when cultivated the following year to infect leaves and flower stalk.

Treating with Achook, *Bacillus* sp., *Bougainvillea* sp., and *Trichoderma* sp.; to be one of substitute at least to decrease the use fungicide (Score) as seed dressing.

It can be concluded from data that Score is most effective in controlling PB disease. Further experiment is needed to evaluate such, botanical, biological and chemical as Integrated Pest Management (IPM) system against Purple Blotch disease of onion under field condition.

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## اللطعة الأرجوانية كمرض محمول بالبذرة في البصل وطرق مكافحتها

[88]

آية حسام الدين أبو زيد\* - كريمة جابر حلمي - هاله عبد الوهاب - مجدي جاد الرب السمان

قسم أمراض النبات - كلية الزراعة - جامعة عين شمس - ص.ب 68 - حدائق شبرا 11241 - القاهرة - مصر

\*Corresponding author: [ayaabo-zaid@agr.asu.edu.eg](mailto:ayaabo-zaid@agr.asu.edu.eg)

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### الموجز

المصابة طبيعياً من العام السابق إلى خمس درجات مختلفة وهي: 14.2%، 25.2%، 41.5%، 61.1%، 87.5%. حيث لوحظ أن فطر *Alternaria porri* يحمل داخل البذور أكثر من خارجها لذلك تم معاملة البذور بأفضل المعاملات التي تم تجربتها في المعمل على فطر *Alternaria porri* حيث تم نقع البذور قبل الزراعة (جهنمية و Achook و *Bacillus sp.* score و *Trichoderma sp.*) ..... و أظهرت النتائج أن نسبة الإنبات الأعلى في درجه 14.2% عند معاملة البذور بمستخلص نبات الجهنمية حيث كانت نسبة الإنبات 96% بالمقارنة بالكنترول. أما عند النسبة المئوية للإصابة 25.2% كان أكبر نسبة إنبات (84%) عند المعاملة بمبيد score وعند درجه أصابه 41.5% كان أفضل نسبة للإنبات 92% عند المعاملة بمبيد score. والنسبة المئوية للعدوى 61.1% كان أفضل نسبة للإنبات 92% مع Achook. أما عند نسبة إصابة 87.5% كانت نسبة الإنبات 100% عند معاملة البذور بمبيد score.

أظهرت الدراسات أن فطر *Alternaria porri* يحمل بالبذور والابصال المصابة من المحصول السابق مما يؤدي إلى فقد في محصول البذور وحيويتها مما يتسبب في اضرار وخسائر كبيره للمحصول. لذلك.... تم إجراء التجارب على البذور والابصال المصابة سابقا. زرعت الأبصال المصابة طبيعياً من العام السابق حيث قسمت 5 درجات مختلفة من حيث شدة الإصابة (14.2%، 25.2%، 41.5%، 61.1%، 87.5%) وتم قياس شدة إصابة على الأوراق والشماريخ الزهرية الناتجة من الأبصال المصابة وتأثيرها على عدد الشماريخ الزهرية حيث كلما زاد شدة الإصابة للأبصال كلما قل عدد الشماريخ الزهرية وزيادة شدة الإصابة في الشماريخ الزهرية مما يؤدي إلى فقد كامل لمحصول البذور. وبدراسة كيفية انتقال المسبب المرضي بواسطة البذور، سواء داخلياً أو خارجياً، حيث قسمت الشماريخ الزهرية