# Management of Black Rot Disease of Navel Orange

**Asmaa, Abbas[1], Heba, Abd El Nabi[2*] and Manal, Eid[2]**

[1]Agricultural Quarantine, Egypt. [2]Department of Agricultural Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt

***Received:*** *8/11/2023*

**Abstract:** Citrus is one of the most popular fruits in many tropical and sub-tropical countries. Numerous pathogens attack citrus fruits in the field or during harvesting and marketing causing considerable losses. A survey of citrus fruit diseases caused by *Alternaria citri* was carried out in three districts in Ismaillia Governorate during two years 2016 and 2017. The disease was found in all inspected citrus orchards i.e. Abo-swear, Al-Kassasin and Al-salhia districts during two seasons. The percentage of naturally infected citrus cultivations varied in the different locations as well as in the two seasons. During these two years, percentages of the natural infection ranged from 1% to 11% in citrus cultivations. On the other hand, the highest incidence of the disease was observed in Abo-swear citrus orchards which reached 11% during 2017. *Alternaria citri* may infect a plant through wounds on the plant surface or through the end of the stem of the fruit at the location called a stylet where natural openings exist. The internal decay causes the fruits to ripen and drop prematurely. Alternaria rot is a fungal disease that affects mainly navel oranges and lemons. Fruit infected with *A. citri* change color prematurely. Pathogenicity tests with *A. citri* were tested on mature citrus fruits. The pathogen recorded the higher infection in navel orange (Length and Width) at Abo-Swear district, followed by summer orange, grapefruits, blood orange, balady orange, succary orange, murrcot, and mandarin. However, lime fruits recorded the lower fruit rot. Studding the host range of *A. citri* indicate that the higher fruit rot was observed in pear fruits followed by yellow apple, apricot, gawafa , red apple and kiwi fruits .Testing the effect of three fungicides, Rizolex 50% WP, Topsin –M and Nasrzim 50 showed that in Rizolex 50% WP was the superior one inhibited the linear growth of *A. citri* at the lower tested concentrations (100 ppm). Antagonistic study using different bioagents against *A. citri* in Petri dishes indicate that interaction between *Alternaria citri* and *B. subtilis* 48 hrs after incubation showing that the first phase of antagonism occurred as contact followed by abnormal shape of *Alternaria* conidial spores in the second phase. Mode of action of this antagonism may be include lysis enzymes led to cell disruption.

***Keywords:*** *Alternaria* rot, Management of fungal, Rot disease, *B. subtilis*, *Trichoderma harzianum, T. virde*

## INTRODUCTION

Most of the fungi responsible for citrus infection are usually carried from the field associated with infected leaves, buds, flowers, or injured and rotting fruits through insect or air-borne fungi. The spores of many fungi carried on or in the fruits from the field as a blossom end rot or other wounds and initiate infection. Survey made in most orchards of citrus in different areas in Ismailia Governorate revealed that Alternaria alternate and *A. citri* were one of the most important pathogens responsible for the infection in the field. Consequently, the present work was initiated with the objective of studying the fungus which cause fruit rots in navel orange, and other citrus species survey of black rot disease under field conditions, and after harvest was studied. Occurrence of black rot disease in navel orange, mandarin and murcott fruits was carried out during two successive seasons 2016 and 2017. Isolation and identification of the causal organism and pathogenicity of fungal isolates obtained from infected navel orange fruits were tested. Susceptibility of different orange cultivars to infect with the pathogen was also studied. Host range of different hosts (non-citrus fruits) to infect with the black rot pathogens were evaluated. Chemical and biological control were also investigated.

## MATERIALS AND METHODS

**Isolation and identification of associated fungi:** Citrus fruits showed typical symptoms of black rot disease were collected from three districts belong to Ismailia Governorate i.e. El-Kassasin, Abo-swear and El-Manayef during 2016 and 2017 growing seasons. Isolation was undertaken from infected and apparently healthy fruits. Diseased fruits were washed in running water. Washed segments were cut to small pieces and dipped in 0.3 sodium hypochlorite solution for 15 sec., washes in sterile water and dried between two sterilized filter papers, then placed on PDA medium in Petri-dishes. Tree segments were placed in each dish and 2-3 dishes were used for each sample. Dishes were incubated at 25 degrees centigrade for a week. Hyphal tip or single spore of each colony was transferred to PDA for identification.

**Pathogenicity test:**

**Inoculum preparation spore suspension of *Alternaria citri*:** *Alternaria citri* inoculum maintained on potato dextrose agar (PDA) at 25 °C under fluorescent lights 12 hrs. each day. Cultures were transferred approximately 7-10 d before inoculation. To produce inoculum, conidia from approximately 2-wks-old cultures were washed from the dishes, vortexed for 1.5 min, filtered through cheesecloth, and suspended in 200 ml sterile water with two drops Twenty 20 (0.01%) as a surfactant. The spore concentration was adjusted to $5x10^6$ conidia/ml-L with the aid of a hemocytometer. Inoculations were performed in the early evening of April to maximize conditions for optimal infection. Eight fruits were used for each treatment and for the control. After the elapse of the required period, the percentage of decayed fruits was calculated. The amount of the decay was also estimated as a percentage of the length of the rotted part in proportion to the total length of the fruit. Re-isolation of the same fungus under study was tried from fruits developing typical rot symptoms.

*Corresponding author e-mail: hebaabdelnaby2002@gmail.com

**Susceptibility of certain citrus cultivars to infect with *Alternaria citri*:** Mature citrus fruits belonging to certain cultivars especially including those of the most commercial importance in Egypt, were used in the present tests. The present work included the following citrus cultivars: Navel orange, Mandarin, Summer orange, Grapefruits, Murcott, Blood orange, Lime, Balady orange, and Succary orange. Fruits were carefully prepared and inoculated as previously mentioned under pathogenicity tests, the percentage of infection in each case was determined. Severity of infection was estimated as a percentage of the length of the rotted part in proportion to the total length of the fruit.

**Biological and chemical control**

**Antagonistic study using different bioagents against *Alternaria citri* in vitro:**

**Pathogens and bio agents:** High virulent isolate of *Alternaria citri* was used and three antagonistic isolates namely *Trichoderma harzianum*, *Trichoderma viride* and *Bacillus subtilis* were tested. Three antagonistic organisms were kindly obtained from Central Lab. of Organic Agriculture, at Plant Pathology Research Institute, Agr. Res. Center, Giza, Egypt. These antagonists were tested for their efficacy against the selected pathogenic fungus *Alternaria citri*.

**Preparation of antagonistic microorganisms:** Inoculate of *T. harzianum* and *T. viride* were grown on potato dextrose broth medium and prepared in the form of a conidial suspension. Number of cells/1ml were adjusted using plate count technique. Conidia suspension was adjusted to be 106 spores / ml using hemocytometer slide method developed by Sivan et al. (1984).

**Antagonism studies:** In this study; slide culture technique was applied according to (Desai et al., 2002). Inoculated plates were incubated at 28°C until the fungal growth of the control plates reached the edge of the plate. The growth and reduction in mycelial growth of the pathogenic fungus was calculated according to Fokemma (1973). % Reduction in linear growth = 100 – {(G2/G1) X 100}. Where: G1: growth of pathogenic fungus in plates inoculated with the pathogen alone, G2: growth of pathogen against antagonist. In vitro growth inhibition of pathogen by *Trichoderma* was inspected under a light microscope (IX70; Olympus, Tokyo) with an attached camera (Olympus SC35). The hyphal interactions between the opposing colonies were photographed every 24 h. The first apparent physical contact between Trichoderma strains and its host was determined.

**Chemical control:** Sterile distilled water was used to prepare stock suspension of the formulations tested fungicides. The amount required for obtaining a known concentration of any fungicide was calculated according to (Shovan et al. 2008). and added aseptically to known amount of warm sterilized PDA agar medium and poured before solidification into plate (9 cm in diameter) then plates were inoculated at the center with equal discs 5 mm in diameter obtained from the periphery of 10 days old cultures of tested fungus *A. citri* grown on PDA medium. Plates contained media without any fungicide inoculated with the fungus was served as control treatment. Five plates were used for each concentration. All plates were incubated at 23±2oC. The experiment was ended when mycelia mats covered medium surface within control treatment. All plates were examined and linear growth formation of fungus in each treatment was measured and reduction in mycelia growth was calculated as percentage of fungal growth diameter in treatment relatively to the growth diameter in control.

Table (1): Fungicides used in the study and its specifications

| Commercial name | Common name | Active ingredient | Application rate / Liter |
|---|---|---|---|
| **Topsin M WP 70%** | Thiophanate methyl | (Dimethyl[1,2-phenylene])-bis(iminocabonothioyl) bis [carbamate]). | 1.5 g/l |
| **Rizolex T WP 50%** | Telcolofos-methyl/ thiram | 20% Telcolofos-methyl (0,2,6 dichloro-4-methylphenyl 0,0 dimethyl phosphorothioate) and 30% thiram. | 3 g/l |
| **Nasrzim 50%** | Carbendazim | BCM, Methyl 2-benzimidazolecarbamate, Methyl benzimidazol-2-ylcarbamate | 0.3-0.5 g/l |

## RESULTS

**Survey of citrus navel orange disease in different orchards in Ismailia Governorate**: A survey of citrus fruit diseases caused by *A. citri* was carried out in three districts in Ismailia Governorate during two years 2016 and 2017. The disease was found in all inspected citrus orchards i.e. Abo-swear, Al-Kassasin and Al-salhia districts during the two seasons. The percentage of naturally infected citrus cultivations varied in the different locations as well as in the two seasons. During these two years, percentages of the natural infection ranged from 1% to 11% in citrus cultivations. On the other hand, the highest incidence of the disease was observed in Abo-swear citrus orchards which reached 11% during 2017. It has been observed that incidence of the diseases increased with increasing temperature degree after citrus fruit-set. Data presented in Table (3) and figs 1,2,3,4 indicate that the pathogen can be capable of causing severe or moderately severe rot to citrus fruits. The pathogen recorded the higher infection in navel orange (Length and Width) at Abo-Swear district, followed by summer orange, grapefruits, blood orange, balady orange, succary orange, murrcot, and

mandarin. However, lime fruits recorded the lower fruit rot. On the other hand, similar results were observed in inoculated fruits obtained from El-Kassen District. Data presented in Table (3) and Fig. (1) indicate that the pathogen can be capable of causing severe or moderately severe rot to citrus fruits. The pathogen recorded the higher infection in navel orange (Length and Width) at Abo-Swear district, followed by summer orange, grapefruits, blood orange, balady orange, succary orange, murrcot, and mandarin. However, lime fruits recorded the lower fruit rot. On the other hand, similar results were observed in inoculated fruits obtained from El-Kassen District. Data in Table (4) indicate that the pathogen under study can infect the 6 tested hosts through wounds. The higher fruit rot caused by *A. citri* was observed in pear fruits followed by yellow apple, apricot, gawafa, red apple and kiwi fruits.

Table (2): survey of navel orange disease at three orchards in three districts in Ismailia Governorate during two seasons. 2016 and 2017

| Districts | 2016 | 2017 |
|---|---|---|
| El-kasaseen | 6 % | 7% |
| Abu-Suweer | 8 % | 11% |
| El-Salheya | 2% | 1 % |

Table (3): Pathogenicity test on different mature fruits of citrus cultivars in vitro obtained from three locations in Ismailia

| Mature citrus fruits of different cultivars | Mean of 8 replicates (cm) | | | | | |
|---|---|---|---|---|---|---|
| | Average of internal length of rotted areas (cm) | | | | | |
| | Abu-Suwir | | El-Kasaseen | | El-Salheya | |
| | Length | Width | Length | Width | Length | Width |
| **Navel orange** | 8.62 | 4.26 | 6.08 | 2.34 | 4.38 | 2.86 |
| **Summer orange** | 8.04 | 2.43 | 7.11 | 1.92 | 7.08 | 2.07 |
| **Blood orange** | 6.12 | 1.86 | 6.34 | 2.09 | 5.88 | 1.96 |
| **Balady orange** | 5.24 | 1.97 | 6.21 | 1.07 | 5.25 | 1.12 |
| **Succary orange** | 5.32 | 2.01 | 5.43 | 1.02 | 6.13 | 1.14 |
| **Mandarin** | 3.02 | 1.04 | 5.04 | 1.06 | 4.51 | 1.08 |
| **Murrcot** | 4.56 | 3.22 | 4.08 | 1.52 | 4.35 | 1.43 |
| **Grapefruit** | 7.04 | 9.01 | 7.41 | 7.73 | 8.05 | 7.93 |
| **Lime fruits** | 3.02 | 1.01 | 2.54 | 1.03 | 2.25 | 1.07 |

Table. (4): Host ranges of *Alternaria citri* on mature fruits of different horticulture commodity (non-citrus hosts)

| Medium of internal fruit rot fruits (mm,) *in vitro* | | |
|---|---|---|
| | Length | Width |
| **Red Apple** | 3.5 | 3.2 |
| **Yellow Apple** | 7.8 | 8.5 |
| **Kiwi** | 4.6 | 4.4 |
| **Apricot** | 6.5 | 7.9 |
| **Pear** | 17.9 | 16.7 |
| **Gawafa** | 5.1 | 4.8 |

**Chemical and Biological control:** Results in Table (5) and Fig. (2) indicate that all fungicides (Topsin M, Rhizolex T 50%, & Nasrzim) were significantly reduction the linear growth of *A. citri* at 6 concentrations.

**Biological control:** Antagonistic study using different bioagents (*B. subtilis*, *Trichoderma* spp.) agent *Alternaria citri* in Fig. (3, 4, 5, 6) showing that the first phase of antagonism occurred as contact followed by abnormal shape of Alternaria conidial spores, in the second phase, the lysis of *A. citri* mycelium response to the antagonist.


Fig. (1): Artificially inoculated orange fruits with spore suspension of *Alternaria citri*, after 25 Days from incubation at 22-25°C: A- Navel Orange, B- Summer Orange, C- Grapefruit D- Blood Orange, E- Orange Balady and F-Lime fruits

Table (5): Effect of different concentrations of three fungicides on the growth of *A. citri* grown on PDA *in vitro*

| **Fungicide** | **Redaction %** | | | | |
|---|---|---|---|---|---|
| | **Concentrations (ppm)** | | | | |
| | 100 | 200 | 300 | 400 | 500 |
| **Topsin –M** | 68.08 | 70.0 | 71.01 | 71.5 | 71.9 |
| **Rhizolex –T 50%** | 92.2 | 92.1 | 92.4 | 92.9 | 94.2 |
| **Nasrzim 50%** | 68.5 | 70.5 | 70.6 | 71.4 | 71.9 |
| **Control** | zero | zero | zero | zero | zero |

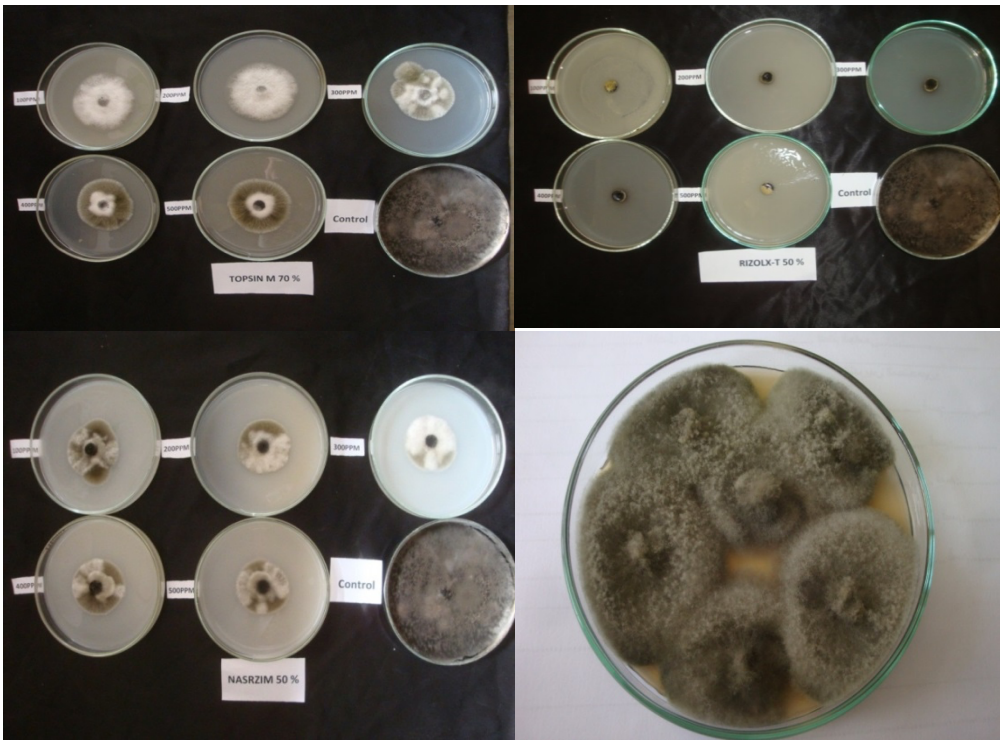


Fig. (2): Effect of different concentrations of three fungicides on the linear growth of *A. citri*

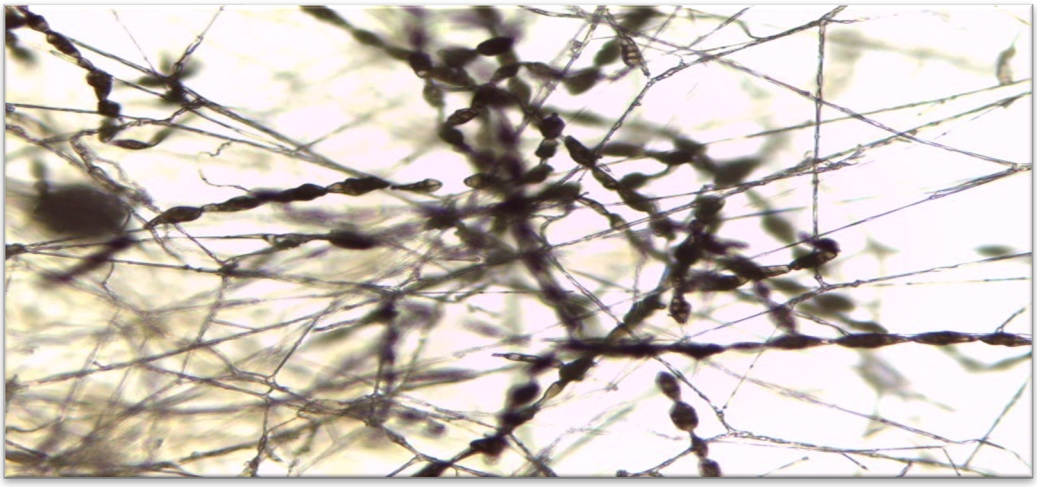

Fig. (3): Normal shape of *Alternaria citri* in Petri dish 6 days after incubation at 25 ºC.
Notes that the chain of conidiospors usually more than5 spores on each conidiophore.

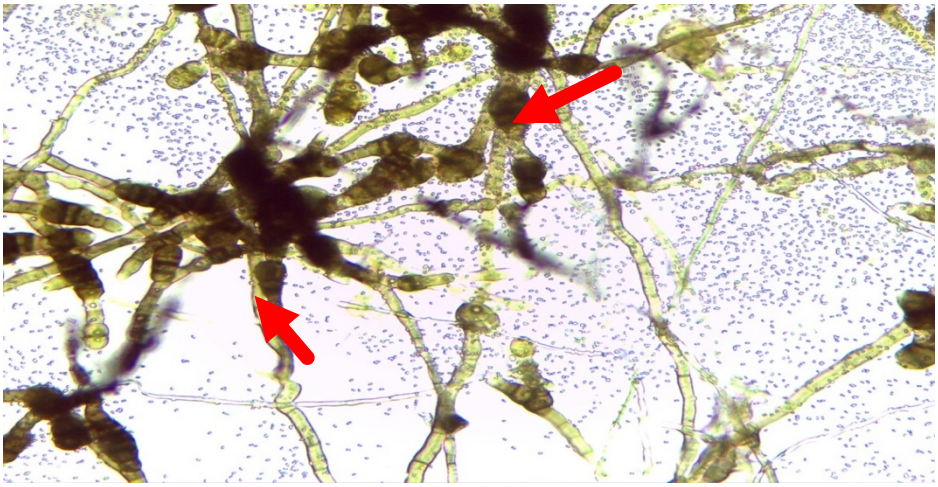

Fig. (4): *Alternate citri* and *B. subtilis* race1 showing the abnormal and malformed conidiospores

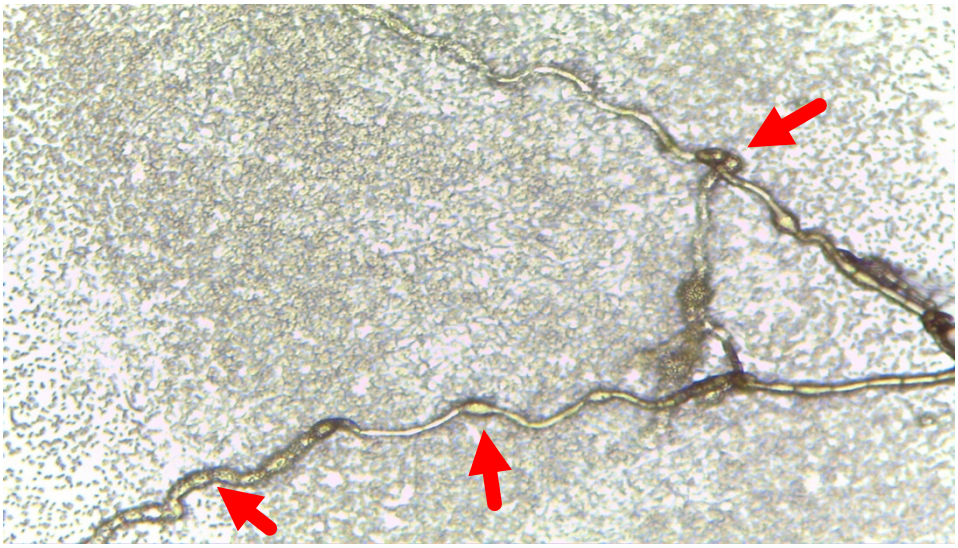

Fig. (5): *Alternate citri* and *B. subtilis* race1 showing malformed and abnormal mycelium.

Fig. (6): Interaction between *Thichderma verde* and *A. citri*, notice the lyses of the conidiospores and the malformed mycelium.

## Discussion

In recent years, the quality of Egyptian citrus has declined markedly. A major factor contributing to this decline has been the development of fruit rots which are the most widely occurring diseases either in the field or after harvest. Most of the fungi responsible for citrus infection are usually carried from the field associated with infected leaves, buds, flowers, or injured and rotting fruits through insect or air-borne fungi. The spores of many fungi carried on or in the fruits from the field as a blossom end rot or other wounds and initiate infection. Survey made in most orchards of citrus in different areas in Ismailia Governorate revealed that Alternaria alternate and *A. citri* were one of the most important pathogens responsible for the infection in the field. These results are in agreement with (Coates & Johnson, 1997). The percentage of naturally infected citrus cultivations varied in the different locations as well as in the two seasons. During these two years, percentages of the natural infection ranged from 1% to 11% in citrus cultivations. On the other hand, the highest incidence of the disease was observed in Abo-swear citrus orchards which reached 11% during 2017. It has been observed that incidence of the diseases increased with increasing temperature degree after citrus fruit-set. Similar results were reported by (Timmer et al., 2003). Alternaria rot is a fungal disease that affects mainly navel oranges and lemons. Fruit infected with Alternaria change color prematurely. The decay is softer on lemons than on oranges. Infections typically occur in the grove; disease often doesn't develop until after harvest, and most damage occurs during storage. On navel oranges, the disease is also called black rot, and results in dark brown to black, firm spots or areas at the stylar end or in the navel. If you cut the fruit in half, you can see the dark brown to black discoloration at the style end of navel fruits. These results are in agreements with those obtained by (Dewdney, 2015 and Timmer, 2013). Pathogenicity tests and varietal reactions of *Alternaria citri* obtained during the present work were tested on mature citrus fruits. It is clear that the pathogen can capable of causing severe or moderately severe rot to citrus fruits. The pathogen recorded the higher infection in navel orange (Length and Width) at Abo-Swear district, followed by summer orange, grapefruits, blood orange, balady orange, succary orange, murrcot, and mandarin. However, lime fruits recorded the lower fruit rot. On the other hand, similar results were observed in inoculated fruits obtained from El-Kassen District. Studding the host range of *A. citri* indicate that the pathogen under study can infect 6 tested hosts through wounds (non-citrus hosts). The higher fruit rot caused by *A. citri* was observed in pear fruits followed by yellow apple, apricot, gawafa, red apple and kiwi fruits. Testing the effect of three fungicides at different concentrations on the linear growth of *A. citri* in vitro indicate that all doses of Rizolex 50% WP completely inhibited the linear growth of *A. citri* at the lower tested concentrations (100 ppm). Meantime, the inhibition of linear growth by Topsin –M and Nasrzim 50% increased sharply, reaching the complete inhibition at the highest dose (500ppm). As for *A. citri* Rhizolex showed complete inhibited liner growth at the lower tested concentrations (100ppm). Nasrzim 50% had the 2nd rank after Rhizolex, that it reached its complete inhibition at dose 300 ppm; it was followed by Topsin - M which showed complete inhibition at 400 ppm. These results are in agreement with (Coates & Johnson, 1997). The interaction between *Alternaria citri* and *B. subtilis* 48 hrs after incubation showing that the first phase of antagonism occurred as contact followed by abnormal shape of Alternaria conidial spores in the second phase. The lysis of *A. citri* mycelium, response to the antagonist of *B. subtilis* observed up 10 days. (Fig.3). Mode of action of this antagonism may be include lysis enzymes led to cell disruption at the same time, *A. citri* and B. subtilis showing abnormal malformed conidiospores and abnormal mycelium. The present results are in conformity with the work of Elad, et al., (1983) who reported that Trichoderma meets its host then recognition occur, and it triggers the following steps. After the recognition occurs the coiling or sticking of Trichoderma around the host hyphae for structures or shaped like appressoria. After this, Trichoderma secret hydrolytic enzymes which are primarily i.e., chitinases, glucanases and proteases degrade the cell wall of the host (Haran et al., 1996) with the consequent assimilation .Mycoparasitism is being considered as a mechanism of biocontrol by various fungal antagonists like Trichoderma spp. and Gliocladium virens and many other antagonistic fungi. As far mycoparasitism as one of the important mechanisms of action of a fungal biocontrol. Other researchers reported that antagonistic effect of *Trichoderma* sp. was also studied by using slide culture method in which specially *T. viride* revealed high chemical antagonism by producing cell wall degrading enzymes which cause disintegration of cell wall of targeted pathogens with mycoparasitism activity by creating coiling structure around the mycelium of the targeted pathogens. These results agree with (Bardin et al., 2015). It could be recommended that biological control of plant diseases is an effective and sustainable alternative to the use of fungicides.

## REFERENCES

Bardin, M., Ajouz, S., Comby, M., Lopez-Ferber, M., Graillot, B., Siegwart, M., & Nicot, P. C. (2015). Is the efficacy of biological control against plant diseases likely to be more durable than that of chemical pesticides?. Frontiers in Plant Science, 6, 566.

Coates, L., & Johnson, G. (1997). Postharvest diseases of fruit and vegetables. Plant pathogens and plant diseases, 533-548.

Dewdney, M. M. "Alternaria Brown Spot". EDIS. University of Florida. Retrieved 22 October 2015.

Elad, Y., Chet, I., Boyle, P., & Henis, Y. (1983). Parasitism of Trichoderma spp. on Rhizoctonia solani and Sclerotium rolfsii-scanning electron microscopy and fluorescence microscopy. Phytopathology, 73(1), 85-88.

Fokkema, N. J. (1973). The role of saprophytic fungi in antagonism against Drechslera sorokiniana (Helminthosporium sativum) on agar plates and on

rye leaves with pollen. Physiological Plant Pathology, 3(2), 195-205.
Haran, S., Schickler, H., & Chet, I. (1996). Molecular mechanisms of lytic enzymes involved in the biocontrol activity of Trichoderma harzianum. Microbiology, 142(9), 2321-2331.
Khalili, E., Sadravi, M., Naeimi, S., & Khosravi, V. (2012). Biological control of rice brown spot with native isolates of three Trichoderma species. Brazilian Journal of Microbiology, 43, 297-305.
Shovan, L. R. (2012). Characterization of Botrytis cinerea, Trichoderma species and other fungi, including studies on inhibitory effects of chitosan and fungicides (Master's thesis, Norwegian Journal of Agricultural Sciences, Ås).
Sivan, A., Elad, Y., & Chet, I. (1984). Biological control effects of a new isolate of Trichoderma harzianum on Pythium aphanidermatum. Phytopathology, 74(4), 498-501.
Timmer, Lavern. M.; Tobin, L. Peever; Solel, Zvi; Akimitsu, Kazuya (2003). "Alternaria Diseases of Citrus - Novel Pathosystems". Phytopathology Mediterranea:99–112

# مكافحة مرض العفن الأسود في البرتقال أبو سره

**اسماء السيد محمد عباس[1] , هبة محمد محمد عبد النبي[2] , منال حسن عيد[2]**

[1] الحجر الزراعي المصري- [2] قسم النبات الزراعي – كلية الزراعة – جامعة قناة السويس

تعتبر الموالح واحدة من أكثر الفواكه شعبية في العديد من البلدان الاستوائية وشبه الاستوائية . تهاجم العديد من مسببات الأمراض ثمار الموالح في الحقل وأثناء الحصاد والتسويق مسببة خسائر كبيرة . وفي هذا البحث تم إجراء مسح لأمراض ثمار الموالح بثلاث مراكز تابعة لمحافظة الإسماعيلية خلال عامي 2016 و 2017 ، وقد تم العثور على المرض في جميع بساتين الموالح التي تم فحصها وهي : مركز أبو صوير ،مركز القصاصين ، ومدينة الصالحية الجديدة خلال فترة الدراسة . اختلفت نسبة الإصابة الطبيعية باختلاف المواقع وذلك خلال الموسمين 2016 و 2017 . من ناحية أخرى ، سجلت أعلى نسبة إصابة بالمرض في بساتين موالح أبو صوير حيث بلغت 11٪ خلال عام 2017 . وقد تحدث الإصابة بفطر *Alternaria citri* من خلال الجروح الموجودة على سطح النبات أومن خلال نهاية الثمرة عند السره ( الطرف الزهري ) حيث توجد الفتحات الطبيعية . ويتسبب العفن الداخلي في نضج الثمار وتساقطها قبل الأوان . والعفن الالترناري هو مرض فطري يصيب البرتقال والليمون بشكل رئيسي . والثمار المصابة بال *A. citri* يتغير لونها قبل الأوان وقد اختبرت القدرة المرضية بالفطر *A. citri* على ثمار الحمضيات الناضجة . و سجل الفطر الممرض أعلى نسبة إصابة في برتقال أبو سره في منطقة أبو صوير ، يليه البرتقال الصيفي ، ثم ثمار الجريب فروت ، والبرتقال ابو دمه ، والبرتقال البلدي ، والبرتقال السكري ، والموركيت ، واليوسفي . ومع ذلك ، سجلت ثمار الليمون انخفاض تعفن الثمار . تشير دراسة مجموعة العوائل من *A. citri* إلى وجود تعفن على ثمار الكمثرى يليه التفاح الأصفر والمشمش والجوافة والتفاح الأحمر وفاكهة الكيوي . اختبار تأثير ثلاثة مبيدات فطرية Rizolex 50% WP و Topsin –M Nasrzim 50 وأوضحت النتائج بان ال Rizolex 50% WP كان الأفضل في تثبيط النمو الخضري للفطر *A. citri* عند التركيزات المختبرة المنخفضة (100جزء في المليون). دراسة تأثير مسببات المكافحة البيولوجية بالكائنات الحية الدقيقة المضاده وغير الممرضة للنبات . تشير الى التفاعل بين *A. citri* و *B. subtilis* بعد 48 ساعة من الحضانة يوضح ان المرحلة الاولي من التضاد حدثت عند تلامس الكائن الحي بجراثيم *A. citri* وفي المرحلة الثانية يمكن ان تشمل طريقة عمل هذا العداء إنزيمات تحلل تؤدي الي اضطراب الخلية .