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ANTHRACNOSE CROWN ROT DISEASE OF STRAWBERRY PLANTS IN EGYPT AND ITS CONTROL

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ABSTRACT: ***Isolation and identification of fungal pathogens were purified and identified as: Colletotrichum acutatum, Alternaria tenius and others. C. acutatum and A. tenius were the most frequent fungi that isolated from samples of strawberry plant materials which collected from the different locations in both seasons 2013/ 2014 and 2014/ 2015. Susceptible strawberry cultivar Florida was highly susceptible one where infection reached to 100% infection by Colletotrichum acutatum isolate. 2. Also, Alternaria tenius was pathogenic to c..v Florida, but came in the second rank in pathogenicity tests. The most effective bioagent was T. viride. Also, treating strawberry plants with different concentrations of organic acids affected greatly C. acutatum and A.tenius Ascorbic acid at 200 ppm concentration is the best effective concentration in controlling both pathogens in vitro .. Gall extract affected greatly the growth of. both pathogens in all daily records from 1-6 days, followed by both plant extracts Mustard and Clove.. Two fungicides (recommended for up ground diseases of strawberry plants); i.e., Bellis 38% WG (with three doses; 0.4 g/L., 0.8 g/L. and 1.6 g/L.) and Amistar 25% SC (with three doses; 2.5 ml/l., 5 ml/l. and 10 ml/l.) were used in this experiments. . Complete inhibition of fungal growth was noticed in both high concentration of the tested fungicides and little growth was happened in the low concentration of Bellis and Amistar. A. tenius was revealed as less tolerant to fungicides than C. acutatum. All tested treatments against both pathogens in vitro under laboratory conditions were applied in vivo under field conditions on strawberry plants cv. Florida to investigate their effects on plants under natural infection in field. All growth characteristics of different parts of strawberry cv. Florida like leaves number, lateral branches, flower number, green fruits, colored fruits, TSS% and fruits weight were affected as a result of infection with pathogens and treated with different chemical, biological and agricultural treatments, there were great variations among all applied treatments in this respect, The best treatments were fungicides, followed by gall extract and un-mulched control treatment comparing with other treatment and control treatment (un-treated) , All disease symptomson strawberry cv. Florida were calculated on plant leaves, flower parts, vegetative fruit characteristics. Black spot, irregular spot, black margin, petiole lesions and dead leaf were calculated during the period ranged between 3-15 days at 15th - 30th March, 2015. Both chemical fungicides (Bellis 38% WG and Amistar 25% SC) controlled completely the leaf infection symptoms, followed by Galls 10%. Treating plants with Kocide 2000, Ascorbic acid 5% and T viride protected plants with various degrees of infection.***

Key words: ***Strawberry, Anthracnose, Crown rot, Colletotrichum acutatum, Alternaria tenius, Control.***

INTRODUCTION

The crown rot disease is one of the most important diseases limiting the cultivation and yield of strawberry (*Fragaria x ananassa* Duch.) in many countries. Major species that cause strawberry crown rot and anthracnose are *Colletotrichum acutatum* Simonds, *C.*

fragariac **Brooks and *C. gloeosporioides* (Penz) Penz. Sacc. (Howard *et al.,* 1992; Smith, 1986 and Smith and Black, 1990). Debode *et al.,* (2015) stated that *Colletotrichum acutatum* (spp. complex), has become a trouble some problem in strawberry production worldwide.) Debode *et al.,* (2015) recorded crown rot *(Phytophthora cactorum)* and leaf diseases (*Colletotrichum acutatum*) in strawberry, in Finland production fields. Poling (2008) reported that *colletotrichum acutatum* has become an even more serious threat to strawberry plant in major strawberry growing areas of North America. This highly virulent pathogen causes fruit rot, crown rot, root rot and Fesions on petioles and stolons. Embabv *et al*., (2010) published the first report of *colletutrichum acutatum* and *C. gloeosporioides* causing anthracnose diseases on strawberry (Fragaria x ananassa) fields in Kalubia and Ismailia governorate in Egypt. Arroyo *et al*, (2009) recorded that strawberry anthracnose, caused by *colletotrichum acutatum*, is one of the most important diseases of this crop in south western Spain. They added that lesions can occur on all parts of the plant but anthracnose crown rot is especially severe leading to wilt and death of plants. Freeman (2008) mentioned that *Colletotrichum* spp. are bored-rang pathogens, meaning that species can infect a single host and a single species can infect deserve hosts. Anthracnose is one of the major fungal diseases of strawberry occurring worldwide. In Israel, the disease is caused primarily by the species *C. acutatum*. The pathogen is most destructive when it causes root necrosis and crown rot, which usually kill the plants in nurseries and transplants in the field. Detpode *et al.,* (2015) reported that anthracnose, caused by *Colletotrichum acutatum* (species complex), has become a troublesome problem in strawberry production worldwide. Baroncelli *et al*, (2015) illustrated that *Colletotrichum acutatum* isolated from plant materials from different geographic areas in Europe.**

Grellet - Bournonville, *et al*., (2012) examined the participation of the salicylic acid (SA) signaling pathway involved in the response of Fragariae x ananasa cv. Pajaro plants to *Colletotrichum* spp. pathogens. Results obtained supports the hypothesis that strawberry plants activate a SA mediated defense mechanisms that is effective against a casual agent of anthracnose. Awad and Al-Shennawy (2015) reported that *in vivo* experiments, all plant extracts with different concentrations which applied as dipping treatment decreased gray mould rot disease severity of strawberry fruits especially Galls, Clove and cinnamon extracts. Abril *et al*., (2009) studied that the steroidal saponin from cayenne pepper, CAY-1 was tested as potential fungicide in detached leaf assays and field trials. Efficacy of CAY-1 against strawberry anthracnose was compared to the commercial fungicide azoxystrobin. CAY-1 reduced the growth of several fungal pathogens in lab assays and prevented anthracnose development in detached-leaf assays, but it did not control foliar or fruit-rot diseases of strawberry in field trials. Porras *et al*., (2003) studied that they evaluated the effect of non-chemical alternatives to control colletotrichum crown rot and anthracnose in strawberry plants. Azoxystorbin, pyraclostrobin, or thiophanate-metlyl applications should be applied when weather conditions are highly favourable for disease development and the activity of contact fungicides such as captan or thiram might be comparomised. Daughvish *et al*., (2009) inoculated daughter plants of strawberry (fragariae x ananassa) with *Colletotrichum acutatum*. Plants were subsequently dipped in fungicide solutions. Fungicide treatments reduced

plant dieback by up to 92% in fruit production fields.

This work aimed to throw the light on the iportance. of anthracnose crown rot disease in Beheira governorate in Egypt through isolation and identifecation the causal pathogens- Also, controlling the disease using some biotic and abiotic agents.

MATERIALS AND METHODS

This work was concerned with crowns causal organisms and different methods of control these important diseases and minimizing strawberry fruit losses. All laboratory trials were carried out at Plant Pathology Research laboratories, Agricultural Botany Department, Faculty of Agriculture, Menoufia University, Shebin El-Kom, Egypt, while the field trials were conducted at Badr District Behaira governorate, West Nile Delta, Egypt.

Isolation of causal organisms fungi: Samples of diseased strawberry plants showing crown rot symptoms were collected for determination the occurrence of fungi associated with diseased plants. They were obtained occasionally over a two-year period of 2014 and 2015 seasons, from open Fields. Samples were obtained from four fields of four farms where plants were suffered from crown rot disease symptoms. The infected crown rot, short stems, roots, runners were used for pathogens isolation.

Isolation and identification of biological agents: The Dilution Plate Method (DPM) was used for the isolation of Biological agent fungi. Soil and rhizosphere samples were taken from strawberry fields by uprooting the infected plants with great care to obtain most of the intact root system. Trichoderma Selective Medium (TSM) was used also for isolation of *Trichoderma* species isolates from soil (Elad *et al*., 1981).

Purification and Identification of the isolated fungi: Purification of the isolated fungi was carried out using hyphal tip and /or single spore techniques according to Dhingra and Sinclair (1985) methods. The developed fungal colonies were identified using the morphologic al and macroscopically characteristics according to Barnett and Hunter (1972), Domsch *et al*. (1980). Cultures were multiplied and maintained on PDA slants and kept at 5°C for further studies. On the other hand, isolates of *Trichoderma* spp. as well as *Bacillus* spp. obtained from the rhizosphere of strawberry were identified after growing them on 20% malt extract agar which they were incubated for two days at 25°C according to Rifai (1969) and Bissett (1991). Stock cultures were maintained on PDA slants in a refrigerator at 5°C and were subculture on fresh medium every 6-8 weeks.

Pathogenicity tests: Transplants representing strawberry commercial cultivar namely Florida kindly provided by Strawberry Improvement Center, Faculty of Agriculture, Ain-Shams University during the progress of the present investigation. Apparently healthy transplants were carefully selected. The inoculum was mixed with sterile water with the desired rate 50g/l L.water and checked on shaker and spore concentrations were adjusted at the rate of lx10^6/ml. The plants were inoculated by spraying the spore suspensions where the concentrations were adjusted at the rate of lx10^6spore/ml) individually on each plant. In check experiment, equal amounts sterile water were sprayed on each plant. Each of both selected isolated fungi (*Colletotrichum acutatum* and *Alternaria tenius*) was represented by one isolate according to its highest frequency, obtained from a certain

localities, during isolation trials. Disease incidence assessment in different experiments was carried out through percentage of infection (infection %). The percentage of infection was calculated according to the following formula:

Infection % = Number of infected plants / Total number of plans

Control of anthracnose crown rot pathogens in vitro

The selective two pathogenic fungi; *Colletotrichum acutatum* and *Alternaria tenius* were subjected to some laboratory tests to study their effects on both pathogens in Petri dishes under laboratory conditions to select the more effective treatments for applying under field conditions

Antagonistic activities of *Trichoderma* spp. in dual culture against *Colletotrichum acutatum* and *Alternaria tenius* were studied in Petri dishes according to the method described by Fokkema (1973) and Zahra (1990).

Organic acids control: Organic acids i.e., Ascorbic acid, Salicylic acid and Oxalic acid at different concentrations (12.5, 25, 50, 100 and 200 ppm) were used for controlling crown rot disease on strawberry plants using more virulent pathogens (*C. acutatuum* and *A. tenius*).

Plant extracts control: Plant water extracts of three plants i.e. Gall (*Quercus infectoria*), Mustard (*Brassica alba*) and clove (*Eugenia coryophyllata*) in three concentrations of each (i.e. 2.5, 5 and 10%) were tested against both virulent pathogens.

Effect of fungicides and chemical compound on strawberry crown rot pathogens *in vitro*: Two fungicides (recommended for up ground diseases on strawberry plants) i.e.: Bellis 38% WG with three doses (0.4 g/L, 0.8 g/L and 1.6 g/L). Amistar 25% SC with 3 doses (2.5 ml/L., 5 ml/L and 10 ml/L). Also, two chemical compounds, each of them was treated with three doses for con trolling both crown rot pathogens, i.e.: Kocide 2000 53.8% WP with 3 doses (0.9 g/L., 1.8 g/L. and 3.6 g/Litre). Potssium citrate 5% solution with three doses (2.5 ml/L., 5 ml/L. and 10 ml/Liter).

Field program experiments *in vivo*: Strawberry cv. Florida was selected for field experiments i.e. biological control, systemic and contact fungicides, in addition to mineral salts, antioxidants, agricultural practices (irrigation, fertilization and non-mulch plants). This cultiver growing in Egypt in commercial scale because its stability in fruit production and well known as genetic stability and resulted from tissue culture propagation techniques. All above mentioned field trials were applied at Om Saber Village – Badr district, Behaira Governorate-under field conditions. The field experiment was started at 1st march, 2014 to 30th march, 2014. This represents the best time of high productivity for this selected cultiver and appropriate temperature for growth and spread of both selected pathogens of crown rot in strawberry fields (*Colletotrichum acutatum* and *Alternaria tenius*). The nursery soil was prepared by adding the recommended treatments/ feddan. With regard to the field soil of strawberry plantation, it prepared as follows: Animal manure 30 m^3/ feddan, Poultary manure 10 m^3/ feddan, Agricultural calcium sulphate 500 kg/ feddan, El Mowffer fertile 125 kg/ feddan, Agricultural sulpher 125 kg/ feddan, Super phosphate calcium 15.5%, 500 kg/ feddan.

All fertilizers were well spreaded and soil plowed orthogonal four times, and then fermented with sprays with 8 hours/ day for three days. Lines widith divided into 1.25 m with intervals 50 cm, then sterilization was done and washing of soil was done for three days, and stomp

extra (pendamethalin) pre plantation was sprayed with 1 Liter/ 100 Liter for controlling the grasses. The irrigation system was done using 2 plastic tabes 16 mm (GR 30) for every trac (40 cm between) plus tube for mist irrigation 3 m x 3 m with 20 L/hours. Florida cultiver was planted at August, 15th, 2014 with spaces 25 x 25 cm. At flowering stage, the fertilization was done as follows: 5 kg ammonium nitrate + 6 kg potassium sulphate + 1 kg phosphoric acid + 1 kg Magnisium sulphate + 250 g element mixture for five days – 5 Liter green + ½ kg Amino acid + 3 kg Calcium nitrate for one dose/ weekly. Re-transplant another transplants instead of the dead one a new transplants grown in plastic pots with the same age. Cutting of purlins and pre-flowering flowers was done daily and the grasses were removed also.

Covering of plants with thin layer of non-colored plastic 90 micron with 110 kg/ feddan. The field trials were planned by select four wide lines for each strawberry variety separately and must be in the middle of the field to avoid any external effects on plant growth during the time of experiments. Strawberry plants were planted on the wide line at 1.25 meter width, and the distance between plants 25 x 25 cm, at the meantime.

The following treatments were applied on strawberry plants (cv.Florida) as follows: Systemic fungicides: Amistar (Azoxystrobin) 25% SC-50 ml/100 L. Water. Bellis (Bosclid 25.2% w/w + pyrachlorstrobin 12.8% w/w)- 80 g/ 100 L. Water. Contact fungicides: Kocide 2000 (Cupper hydroxide)- 180 g/ 100 L. Water. Mineral salts: Potassium citrate 5% -1 Liter/ 100 L Water. Organic acids : Ascorbic acid 5%. Biological control agents: *Trichoderma viride.* Plant extract: Gall extract 10%/ Liter.

Experimental plants were treated twice on the plants in each compound separately for the first time before 14 days of infection – the second before 7 days of zero time of disease estimation under natural infection under field conditions. The control treatments were treated by spraying with water at the mean time of the field treatment, and the plants were examined for disease infection at the same time.

EXPERIMENTAL RESULTS:

Isolation and identification of fungal pathogens causing strawberry crown rot and anthracnose disease: **Data presented in Table (1) illustrate that 898 plant materials out of 2400 strawberry plant materials were clear diseased by crown rot and anthracnose diseases with typical symptoms in both seasons 2014 and 2015. Number of diseased samples was higher in the second season 2015 (454 samples) in comparing with the first season 2014 (444 diseased samples). Also, data show that means of crown rot and anthracnose diseases incidence of strawberry at different localities ranged between 19 to 51% during 2015 season with average 37.0% while, during 2015 season means of crown rot disease incidence ranged between 28 to 50% with average 37.83%. Isolation was made on PDA medium yielded a group pathogenic fungi, either singly or in combinations groups. The isolated fungi were purified and identified as: *Colletotrichum acutatum, Colletotrichum* spp*, Fusarium oxysporum, Alternaria tenius, Alternaria alternata, Alternaria solani, Trichoderma* spp and others as shown in Table (1). Data presented in Table (1) indicate that *Colletotrichum acutatum,* was the most frequent fungus that isolated from samples of strawberry plant materials which collected from the different locations in both seasons 37.68% in 2013/ 2014 season, while it was 37.61% in 2014/ 2015 season, followed by *Alternaria tenius* (18.36% and 22.48%) in the same**

seasons, respectively. Other isolated pathogens were isolated in both seasons with low frequencies, in comparing with the most frequent two pathogens. The least frequent fungus was *Alternaria solani* (10.15%) in the first season, while *Colletotrichum fragareae* was the least frequent one (7.34%) in the second season. Also, the biological control agents were isolated from diseased strawberry materials that collected from the different locations at the four villages belonging to Badr district in Behaira governorate, i.e. *Trichoderma harzianum, T. hamatum* and *T. viride* .

Pathogenicity tests:

Four isolates from the most frequent two pathogens, i.e. *Colletotrichum acutatum* and *Alternaria tenius,* each isolate representing one village and isolated from diseased materials that collected from different strawberry growing areas in Bader district, Behaira governorate were tested for their virulence on three commercial strawberry cultivars under controlled conditions in greenhouses. The selected strawberry cultivar was Florida. Data in Table (2) illustrate that, susceptible strawberry cultivar FLORIDA was highly susceptible one and infection reached to 100% infection by *Colletotrichum acutatum* isolate No. 2 that isolated from Sokhna village with clear crown rot symptoms, followed by isolate No. 3 (96.15%). Also, *Alternaria tenius* was pathogenic to Florida cultivar, but came in the second rank in pathogenicity tests. The most percentage of crown rot disease symptoms was 72.32% on plants infected by isolate No. 3 (that isolated from Nabil El-Wakkad village), followed by 70.15% on Florida plants infected by isolate No. 2 (Sokhna village).

Table (1): Frequency of isolated fungi from diseased crowns and roots of strawberry plants –samples collected from four villages during 2013/2014 and 2014/2015 seasons in some localities in Behaira governorate of West Nile Delta of Egypt.

Isolated Fungi	Season – villages – frequency (%)											
	2013-2014						2014-2015					
	Om Saber	Sokhna	Nabil El-Wakkad	Ahmed Oraby	Total	%	Om Saber	Sokhna	Nabil El-Wakkad	Ahmed Oraby	Total	%
Fusarium oxysporum	3	5	2	3	13	6.28	2	7	6	3	18	8.26
Cholletotrichum acutatum	23	16	22	17	78	37.68	21	23	17	21	82	37.61
Cholletotrichum fragareae	15	6	6	2	29	14.01	7	2	2	5	16	7.34
Alternaria alternata	4	6	3	9	22	10.63	3	6	7	7	23	10.56
Alternaria tenius	10	9	11	8	38	18.36	13	11	9	16	49	22.48
Alternaria solani	7	3	7	4	21	10.15	5	8	6	2	21	9.63
Trichoderma spp	1	2	0	3	6	2.89	0	3	4	2	9	4.13
Total	63	47	51	46	207		51	60	51	26	218	

Table (2): Virulence of two strawberry pathogens on strawberry cv florida under greenhouse conditions .

Fungus	Isolate	Location	Symptoms	Infection %	Healthy %
Colletotrichum acutatum	1	Om Saber	Crown rot	91.40	8.60
	2	Sokhna	Crown rot	100.00	00.00
	3	N. Wakkad	Crown rot	96.15	3.85
	4	Ahmed Oraby	Crown rot	88.20	11.80
Alternaria tenius	1	Om Saber	Crown rot	36.73	63.27
	2	Sokhna	Crown rot	70.15	29.85
	3	N. Wakkad	Crown rot	72.32	27.68
	4	Ahmed Oraby	Crown rot	40.00	60.00
Control				00.00	100.00

As for Florida strawberry cultivar, data in Table (2) show that this cultivar was reacted with 100.00% crown rot symptoms when infected by isolate No. 3 of *C. acutatum* , followed by 93.33% on strawberry plants infected by isolate No. 2 of the sane pathogenic fungus. Other two isolates (isolate 1 & 4) were highly pathogenic also and reacted by 90.00 and 89.13% respectively. *Alternaria tenius* also infected Florida cultivar with low percentages of infection in comparing to *C. acutatum*. The maximum % of infection was 73.32%, followed by 70.30% when plant infected by isolates No. 3 & 4, respectively.

Physiological studies: The selective two pathogenic fungi; *Colletotrichum acutatum* and *Alternaria tenius* were subjected to some laboratory tests to study their effects on both pathogens in Petri dishes under laboratory conditions to select the more effective treatments for applying on plants under field conditions

Antagonistic activities of *Trichoderma* spp. in dual culture against *Colletotrichum acutatum* and *Alternaria tenius* were studied in Petri dishes. The effect of different isolates of bioagents i.e., *Trichoderma harzianu and T. viridi* on radial growth and the percentage of growth inhibition against *Colletotrichum acutatum* and *Alternaria tenius,* the causal pathogens of crown rot and anthracnose of strawberry plants was studied *in vitro.* The above mentioned isolates of bioagents were tested individually against both isolates of fungal pathogens. Clear differences in radial growth of the different tested pathogens under stress of bioagents. Also, there were clear differences between the pathogenic isolates in dual cultures and the control treatments which included the pathogen isolates without biocontrol agents. The least linear growth of *C. acutatum* isolate that affected by *T. viride* isolate (26.0 mm), followed by *T. harzianum* isolate (33.0 mm), three tested biological agents acted with *Alternaria tenius* with the same trend of *C. acutatum*. All tested isolates of bioagent affected linear growth of the pathogen isolates of (*A. tenius*) in dual

cultures. The most effective bioagent was *T. viride*, while the least growth inhibition against *A. tenius* was 31.1%. *Trichhoderma viride* isolate only grow over growth of *A. tenius* with 2.8 mm, while *T. harzianum* inhibition zone between growth of *A. tenius* and its growth (2.3 mm). Generally, *Trichoderma viride* isolate was effective against both pathogenic isolates.

Organic acid control: Three antioxidants i.e., Ascorbic acid, Salicylic acid and Oxalic acid were used in this study. Each organic acid with five concentrations i.e., 12.5, 25, 50, 100 and 200 ppm were used for controlling anthracnose and crown rot diseases on strawberry plants using more virulent fungal pathogens (*Colletotrichum acutatum* and *Alternaria tenius*). The fungus *C. acutatum* was affected by treated organic acids with various concentrations. Ascorbic acid was greatly affected the fungal growth in comparing to both other organic acids.. From noticed results of organic acids and their effects on fungal growth, it was clear that increasing of organic acids concentration from 12.5 ppm to 200 ppm, gradually decrease the fungal growth *in vitro*.These results indicate that ascorbic acid at 200 ppm concentration is the best effective concentration in controlling *C. acutatum in vitro* .As for *Alternaria tenius* pathogen, the same trend of results with *C. acutatum* was noticed also with *A. tenius*, but the means of linear growth were less in *A. tenius* in comparing to these means with *C. acutatum*. It is also clear that a ascorbic acid with 200 ppm concentration was noticed as the best treatment for controlling the fungal growth of *A. tenius in vitro*.

Plant extracts control: All tested aqueous plant extracts affected greatly the growth of *Colletotrichum acutatum* and *Alternaria tenius* isolates on media mixed with adjusted concentrations of each of the three tested plant extracts. Plant aqueous extracts were Gall (*Quercus infectoria*), Mustard (*Brassica alba*) and Clove (Eugenia coryophyllata) in three concentrations of each i.e., 2.5, 5 and 10% were added individually to PDA medium in Petri dished. Gall extract affected greatly the growth of *C. acutatum* in all daily records from 1-6 days, followed by both plant extracts Mustard and Clove. Plant extracts affected greatly the growth of *A. tenius* comparing to the fungus *C. acutatum*. Gall extract was the best treatment in inhibition the fungal growth *in vitro*.

Chemical control of anthracnose and crown rot pathogens *invitro:* Two fungicides (recommended for up ground diseases of strawberry plants); i.e. Bellis 38% WG (with three doses; 0.4 g/L., 0.8 g/L. and 1.6 g/L.) and Amistar 25% SC (with three doses; 2.5 ml/l., 5 ml/l. and 10 ml/l.) were used in this experiments by adding these fungicides individually to Czapeck's medium in Petri dishes with the above mentioned concentrations. Also, in these experiments, two chemical compounds; i.e. kocide 2000, 53.8% WP (with three doses; 0.9 g/L., 1.8 g/L. and 3.6 g/L.) and potassium citrate 5% solution (with three doses 2.5 ml/l., 5 ml/l. and 10 ml/l.).

All tested treatments decreased the pathogen growth on to medium in vitro in comparing to control treatment. In this respect, *Collectotrichum acutatum* growth on medium was greatly affected as a result of treated fungicides where it disappeared in most plates containing two concentrations of the fungicide Bellis 38% WG i.e. 0.8 g/l. and 1.6 g/l. at all the 6 days of growth measuring, while the growth of the same fungus was recorded few growth rates between 2 to 6 days of the experiment time (6 days). Mean time, Amestar 25% SC recorded the maximum effect on *C. acutatum* growth. Complete inhibition of fungal growth was recorded

by the applied concentrations, except the 5th and 6th day in the case of the least concentration of this fungicide (25 ml/l.), while the growth was inhibited completely by treatment with 5 and 10 ml/l. Regarding the effect of both recommended two fungicides, Amistar 25% SC recorded the best effect on fungal growth (mean diameter of fungal growth in Petri dishes). It recorded 0.56 mm after incubation for 6 days on 26± 2°C, followed by Bellis 35% WG. Data indicate also that the chemical compound copper hydroxide 53% WP (Kocide 2000) was affected the growth of *C. acutatum* in Petri dishes, and a complete inhibition of fungal growth was achieved by the concentrations of 1.8 g/l. and 3.6 g/l., Mean while the least concentration (0.9 g/l.) was affected the pathogen growth to values similar to those recorded with the first concentration of Bellis (0.4 g/L). The mean diameter of fungal growth was 3.94 mm that recorded by the three treated concentrations.

As for the effect of potassium citrate, the three treated concentrations 2.5, 5 and 10 ml/l., affected the growth of *C. acutatum*, but with low effect in comparing with both tested recommended fungicides and the copper hydroxide 53% WP. The pathogen *Alternaria tenius* also was affected by fungicides and chemical compounds treatment in Petri dished under laboratory condition. The same trend of results was similar on this pathogen as revealed with *C. acutatum* but with some differences between them. Complete inhibition of fungal growth was noticed in both high concentration of the tested fungicides, and little growth was happened in the low concentration of Bellis and Amistar. *A. tenius* revealed as less tolerant to fungicides than *C. acutatum.*

Field program experiments *in vivo*:

The infections and symptoms were examined on plants as leaf infection symptoms, flower parts symptoms, fruit infection symptoms and the vegetative and fruit characteristics of strawberry cultivars after 30 days from planting under field conditions during four weeks post plantation (15th , March to 30th , March, 2015). With regard to leaf infection under field condition. Data indicate that, both used fungicides; Bellis and Amistar stopped leaf infection on cv. Florida. Also, Gall 10% plant extract also, controlled leaf infection under field conditions. All rest treatments, were varied in their effects on leaf infections, where kocide 2000 controlled black spot and irregular spot at the 4 examination, periods but black margin, petioles lesion and dead leaf were noticed at the last examination period at 45 days from trial start ,on the other hand, Ascorbic acid 5% had no effect on leaf infection while black spot and irregular spot were noticed at 7, 11 and 15 days of examination. Also, black margin and petiole lesion were recorded at 11 and 15 days examination while dead leaf was noticed at last period of examination at 45 days from trial start. However, *Trichoderma viride* had moderate effect, leaf infection symptoms, black spot were noticed at 45 days post inoculation; petiole lesion and dead leaf, while irregular spot and black margin symptoms were not noticed in the case of *T. viride*. Regarding to agricultural practices; irrigation with 1/2 amount, 1/2 fertilization ratio, non mulch and potassium citrate, it was clear that they affected the infection symptoms with clear differences among them where the symptoms almost appeared in the fourth examination period on one plant or two plants of the five tested plants without stable trend. As for the control plants, the leaf infection symptoms were not noticed

Regarding the control plants without treatments, the infection symptoms leaves on leaves were noticed at 7, 11 and 15 days post plantation under field conditions as shown in Tables (3 – 7).

The infection symptoms were disappeared on Florida plants sprayed by Bellis 38% WG and Amistar 25% SC fungicides as well as Galls 10% plant extract. Kocide 2000, Ascorbic acid 5% and *T. viride* were reacted with various levels of flower parts infection , whereas flower center infection symptoms were higher in the treatments of Ascorbic acid and *Trichoderma viride* with 3.3 and 5 and 2, 2 and 3 in 7, 11 and 15 days post plantation, respectively. The cultural treatments were varied in their effects on flower parts infection. The maximum flower death was happened in 1/2 fertilization, without mulch and potassium citrate (5 plants) treatments in comparison with control treatment. As for infected fruits on cv. Florida, both used fungicides controlled completely fruit infections. Also Gall 10% was the best effective treatment in controlling fruit petioles and green fruits, while, only one plant was infected on coloured fruits in this case. All rest treatments gave variable effect in controlling the fruit parts infection. The infected fruits in control treatment and/or cultural practices were noticed as high levels of infection on all fruit parts. Fruits petioles, green fruits and colored fruits were infected within 15 days plantation under field conditions.

All growth characteristics of different parts of strawberry cv. Florida like leaves number, lateral branches, flower number, green fruits, colored fruits, TSS% and fruits weight were affected as a result of infection with pathogens and treated with different chemical, biological and agricultural treatments, there were great variations among all applied treatments in this respect, the best treatments were fungicides, followed by gall extract and un-mulched control treatment comparing with other treatment and control treatment (un-treated). With regard to disease symptoms on Florida strawberry, all disease symptoms were calculated on plant leaves, flower parts, fruits and vegetative in addition to fruit characteristics. Black spot, irregular spot, black margin, petiole lesions and dead leaf were calculated during the period ranged between 3-15 days at 15th - 30th March, 2015. Both chemical fungicides (Bellis 38% WG and Amistar 25% SC) were controlled completely the leaf infection symptoms, followed by Galls 10%. Treating plants with Kocide 2000, Ascorbic acid 5% and *Trichoderma viride* protected plants with various degrees of infection.

Table (3): Growth characteristics of healthy FLORIDA cultivated strawberry variety at March, 2015.

Date of investigation	1st March								14th March							
Growth characteristices	leaf number	Flowers numbers	Lateral branches	Green fruits	Colored fruits	Fruit numbers	T.S.S. %	Friut weight gm	leaf number	Flowers numbers	Lateral branches	Green fruits	Colored fruits	Fruit numbers	T.S.S. %	Friut weight gm
Florida	17	9	3	9	5	5	8.1	40	20	11	3	11	8	8	8.3	35

Table 4

Table 5

Table (6): Characteristics of strawberry Fruits cv. Florida under field conditions at 3-15 days from starting during March 2015.

No. of infected Day of examination	Fruits Petiol				Green Fruits				Coloured Fruits			
Treatment	3	7	11	15	3	7	11	15	3	7	11	15
Bellis 38% WG	0	0	0	0	0	0	0	0	0	0	0	1
Amistar 25% sc	0	0	0	0	0	0	0	0	0	0	0	1
Kocide 2000	0	0	0	1	0	0	0	1	0	0	0	3
Ascorbic acid 5%	0	0	0	1	0	1	1	2	0	0	0	3
Trichoderma viride	0	0	0	1	0	0	0	2	0	0	0	2
Galls 10%	0	0	0	0	0	0	0	0	0	0	0	1
1/2 Irrigation Water	0	0	0	1	0	0	1	2	0	0	0	3
1/2 Fertilization-ratio	0	0	0	0	0	0	0	1	0	0	0	1
With out mulching	0	0	0	1	0	0	1	2	0	0	1	3
Potassium citrate	0	0	0	2	0	0	1	3	0	2	4	5
Control	0	0	0	0	0	0	0	0	0	0	0	0
Total number	0	0	0	7	0	1	4	13	0	4	11	23
Control (un-treated)	0	2	4	5	0	1	3	4	0	3	4	5

Table (7): Growth characteristics of strawberry cv. Florida under field condutuin at 3-15 days post trial starting (from 15th to 30th , March 2015).

Average	Leaves number		Lateral branches		Flower number		Green fruits		Colored fruits		TSS%		Fruits weight g	
Week	3	15	3	15	3	15	3	15	3	15	3	15	3	15
Bellis 38% WG	19	23	4	4	12	15	7	7	6	7	8.1	8.2	30	30
Amistar 25% sc	20	22	4	4	13	16	6	7	5	7	8.0	8.1	29	31
Kocide 2000	18	20	4	4	8	8	6	5	4	6	7.7	7.9	26	27
Ascorbic acid 5%	15	17	3	3	6	5	3	3	2	3	8.1	8.0	20	21
Trichoderma viride	19	19	4	4	4	4	3	4	4	4	8.3	8.1	23	26
Galls 10%	20	20	4	4	11	13	6	6	7	7	7.9	7.6	28	29
1/2 Irrigation Water	14	16	3	3	6	7	3	3	4	6	8.0	7.9	22	26
1/2 Fertilization-ratio	11	10	2	3	6	8	3	3	2	2	8.1	7.8	20	20
With out mulching	11	9	3	3	4	8	4	4	2	3	7.2	7.4	21	20
Potassium citrate	18	19	4	4	11	12	6	6	6	6	0.8	7.9	25	29
Control	20	24	4	4	15	19	7	9	7	7	8.3	8.6	31	29
Total number	8	6	4	4	3	3	2	2	2	1	6.8	6.1	20	18
Control(un-treated)														

DISCUSSION

Crown rot diseases are one of the most important disease that affecting and limiting the fruit production of strawberry plantations in many parts of the world including Egypt. (Abada, 1986; Mostafa *et al.,* 1992; Fahim *et al.,* 1994 and Ragab Seham, 2007). Strawberry crown samples showing typical rot symptoms were collected from four villages belonging to Badr district in Behaira governorate from strawberry plants of different cultivars grown in commercial fields, during two successive seasons (2013/ 2014 and 2014/ 2015). The causal pathogens were isolated from diseased samples. 898 plant materials out of 2400 strawberry collected plant materials were clear diseased by crown rot disease with typical symptoms in both studied seasons. Strawberry crown samples showing typical rot symptoms were collected from four villages belonging to Badr district in Behaira governorate from strawberry plants of different cultivars grown in commercial fields, during two successive seasons (2013/ 2014 and 2014/ 2015). The causal pathogens were isolated from diseased samples. 898 plant materials out of 2400 strawberry collected plant materials were clear diseased by crown rot disease with typical symptoms in both studied seasons.

Number of diseased samples was higher in 2015 comparing with the first season; 2014;. The percentage of crown rot disease incidence of strawberry plants at different localities were lesser during 2014 season than the second season 2015. *Colletotrichum acutatum, Colletotrichum musa, Fusarium oxysporum, Alternaria tenius, A. alternate, A. solani, Trichoderma* spp. and others were isolated and identified from strawberry diseased samples. These results are confirmed by the findings of Smith (1986); Howard *et al.,* (1992) and Smith and Black (1990). Debode *et al.,* (2015) mentioned that *Colletotrichum acutatum* caused some problem of strawberry production worldwide. *Colletotrichum acutatum* were isolated from diseased crowns in many countries from cold-stored strawberry plants used as planting material in several European countries (Debode *et al.,* (2015); Calleja *et al.,* (2013) in UK since 1982 on plants originating from USA; Van Hemelrijck *et al.,* (2010) isolated *C. acutatum* from strawberry in Belgian fields; Parikka and Tuvinen (2014) recorded *Phytophthora cactorum* and *Colletotrichum acutatum* in strawberry plants in Finland production fields. Many investigators were recorded this disease on strawberry plants in many states in America, i.e., Smith (2008), Smith (2013), Polling (2008), Lewers *et al.,* (2007), Howard *et al.,* (1992). Also, this causal organism (*C. acutatum*) was recorded in south western Spain (Arroyo *et al.,* 2009); in western Australia & in Israel (Freeman, 2008) in Japan (Tanaka *et al.,2002* and Passos, 2002), in different countries of Europe (Debode *et al.,* 2015 and Baroncelli *et al.,* 2015). In Latin America, the pathogen was recorded in Argentina (Salazar *et al.,* 2007), in Brazil (Dias *et al.,* 2005). The pathogen was recorded in many counties worldwide, where strawberry production and cultivation, i.e., in China (Wu *et al.,* 2013), in many arab countries (El-Gali, 2008) in Libia. Moreover, in Egypt by many investigators. *Colletotrichum acutatum* was the most frequent fungus that isolated from samples of diseased strawberry materials which collected from different locations in both studied seasons (2013/2014 and 2014/2015) followed by *Alternaria tenius* in the same seasons, respectively. Other isolated

pathogens were isolated in low frequencies, in comparing to the most frequent two mentioned pathogens. These results were in accordance with those obtained by Embaby *et al.,* (2010), in Egypt, Smith (2008), Howard *et al.,* (1990), Freeman (2008), Debode *et al.,* (2015), Salazar *et al.,* (2007) and Baroncelli *et al.,* (2015). All tested isolates of both chosen pathogens were pathogenic to theall plants of the tested cultivar but with various degrees of infection. These results are in accordance with those resulted by Lilja *et al.,* (1998), Freeman (2008), Smith (2008), Arroyo *et al.,* (2009) and Hemelrijck *et al.,* (2010).

In control treatments of fungal growth in Petri dishes under laboratory conditions, all tested aqueous plant extracts affected greatly the growth of *Colletotrichum acutatum* and *Alternaria tenius* isolates on media mixed with adjusted concentrations of each of the three tested plant extracts. Gall (*Quercus infectoria*), Mustard (*Brassica alba*) and Clove (*Eugenia coryophyllata*) plant materials were involved in these studies. Gall extract inhibited fungal growth of the pathogen *C. acutatum* with highly effect, in comparing to both other tested plant extracts (Mustard and Clove). The same effect were agreed with the second pathogen *A. tenius* also, but the effect of the three plant extracts was effective on *Alternaria tenius* than on *C. acutatum* . Gall, Mustard and clove were affected greatly the linear growth of *A. tenius*. These results are in accordance with those obtained by Abril *et al.,* (2009), Xing *et al.,* (2010) and others. Ascorbic acid affected greatly the fungus growth, gradually by increasing the concentration from 12.5 to 200 ppm. Comparing to the other two organic acids with *C. acutatum* and *A. tenius*. These results are in agreement with Grellet-Bournville *et al.,* (2012)). *Trichoderma viride* was the most effective bioagent against both *C. acutatum* and *A. tenius* where the least linear growth of the pathogens as well as the maximum growth reduction. Both *Trichoderma* spp., grew over the pathogens grown in Petri dishes. The least effective bioagent was *B. Subtilis*. These results are agreement with those obtained by Porras *et al.,* (2003), Chalfoun *et al.,* (2011), Nam *et al.,* (2014) and Harender (2014). The superior effect was by both fungicides, i.e. Amistar and Bellis, respectively, followed by Kocide 2000, and Potassium citrate, respectively. These results were confirmed by the results of Smith and Black (1993), Santos *et al.,* (2002), Daughvish *et al.,* (2009), Mackenzie *et al.,* (2009) and Smith *et al.,* (2013). Concerning infections of both pathogens on strawberry plants under field conditions. The most effective treatment was Amistar 25%, followed by Bellis 38% WG. Meanwhile, Galls 10% plant extract was the best non chemical substance in controlling the infection of *C. acutatum* and *A. tenius* on strawberry plants under field conditions.

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مرض عفن التاج الإنثراكنوزى على الفراولة فى مصر ومكافحته فى مصر

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الملخص العربى

اجريت عمليات عزل وتنقية المسببات المرضيه الفطريه تم تعريفها وكان الفطر كوليتوتريكوم اكيوتاتم يليه الفطر الترناريا تينيوس هما اكثر الفطريات المعزوله تكرارا وتلاهما مجموعه اخرى من الفطريات الممرضه الاقل تكرارا ويعض كائنات التضاد الحيوى ، وذلك من عينات مصابه ويظهر عليها اعراض الأمراض منزرعه فى موسمى ٢٠١٣/٢٠١٤ ، ٢٠١٤/٢٠١٥ . فى اختبارات العدوى تحت ظروف الصوبه كان الصنف التجارى فلوريدا عالى الحساسية للإصابة بالفطر اكيوتاتم حيث اصيب بنسبة ١٠٠% بالعزله رقم ٢ يليه فى القدره المرضية الفطر تينيوس . وكان الفطر ترايكودرما فيريدى اكثر فطريات التضاد الحيوى تأثيرا فى تثبيط نمو الفطرين الممرضين اكيوتاتم وتينيوس فى اطباق بترى . وكان حمض الأسكوربيك ٢٠٠ جزء فى المليون الأكثر تأثيرا ضمن الأحماض العضوية المختبره لتثبيط نمو كلا الفطرين الممرضين. وكان المستخلص النباتى بتركيز ١٠% لنبات العفص اكثر المستخلصات النباتية تأثيرا على نمو كلا الفطرين المختبرين متفوقا على مستخلصات نباتات الخردل والقرنفل.وقد تم إختبار مبيدين فطريين موصى بهما وهما بيليز ٣٨% ، اميستار ٢٥% بثلاثة تركيزات من كل منهما وكان اكثرها تأثيرا التركيز الأعلى لكل منهما . وقد صممت تجربه حقلية واسعه تم تطبيق كل معاملات المقاومة التى تم اختبارها فى المعمل على النباتات المنزرعه تحت ظروف الحقل وتم دراسة كل الصفات الخضرية والثمرية والمحتوى السكرى للثمار الناضجه وكمية المحصول على النبات فى الفتره من ١٥ مارس وحتى ١٥ ابريل ٢٠١٥ مقارنة بالكنترول غير المعامل بمعاملات المقاومة والنباتات المحمية من الإصابة وصولا لتوصيات يجب اتباعها فى مزارع الفراوله خاصة فى الزراعات المستهدف التصدير منها للأسواق الخارجية .

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Table (4): Leaf infection symptoms on strawberry cv. Florida at 3-15 days post trial starting during March 2015.

No. of infected Day of examination	Black spot				Irregular spot				Black Margin				Petiol lession				Dead leaves			
Treatment	3	7	11	15	3	7	11	15	3	7	11	15	3	7	11	15	3	7	11	15
Bellis 38% WG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amistar 25% sc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kocide 2000	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	2
Ascorbic acid 5%	0	1	2	2	0	1	2	2	0	0	2	2	0	0	2	2	0	0	0	2
Trichoderma viride	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Galls 10%	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1/2 Irrigation Water	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1
1/2 Fertilization-ratio	0	0	1	2	0	0	1	1	0	0	1	1	0	0	0	2	0	0	0	2
With out mulching	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0
Potassium citrate	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	2	0	0	0	2
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number	0	1	3	8	0	1	3	6	0	0	3	5	0	0	2	12	0	0	2	10
Control (un-treated)	0	1	2	2	0	3	4	5	0	1	1	3	0	2	2	4	0	0	2	3

Table (5): Characteristics of strawberry flowers cv. Florida variety at 3-15 days from starting trial (from 15th to 30th , March 2015).

No. of infected Day of examination	Flower petiol				Flower calyx				Flower center				Flower budes				Flower dead			
Treatment	3	7	11	15	3	7	11	15	3	7	11	15	3	7	11	15	3	7	11	15
Bellis 38% WG	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Amistar 25% sc	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kocide 2000	0	0	1	2	0	0	0	1	0	0	1	2	0	1	2	2	0	0	1	2
Ascorbic acid 5%	0	0	0	5	0	0	0	2	0	3	3	5	0	2	4	5	0	2	3	5
Trichoderma viride	0	0	1	1	0	0	1	1	0	2	2	3	0	0	4	4	0	0	3	3
Galls 10%	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1
1/2 Irrigation Water	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	2	2
1/2 Fertilization-ratio	0	2	4	4	0	0	0	1	0	2	3	3	0	1	2	4	0	1	4	5
With out mulching	0	0	0	3	0	0	2	2	0	1	4	4	0	4	5	5	0	3	5	5
Potassium citrate	0	0	0	3	0	0	1	2	0	1	1	5	0	2	3	3	0	1	5	5
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total number	0	2	3	20	0	0	4	9	0	9	14	24	0	10	20	25	0	8	23	28
Control (un-treated)	0	1	3	4	0	1	2	3	0	3	4	5	0	3	3	5	0	4	4	5