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FIRST RECORD OF PEPPER ANTHRACNOSE DISEASE CAUSED BY *Colletotrichum coccodes* IN EGYPT.

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ABSTRACT

Six isolates of *Colletotrichum* were obtained from naturally infected sweet and hot pepper fruits obtained from greenhouses and open fields collected from Ismailia Governorate**.** Identification using morphological characterization indicate that the pathogen are *Colletotrichum coccodes.* Pathogenicity test of six *C.coccodes* isolates were tested on wounded and unwounded pepper fruits of Markony Spain hot pepper cultivar. Under lab and greenhouse conditions and *in vivo* indicate that isolate No.1 obtained from Abo-Swear was the virulent and aggressive one. Reactions of pepper cultivars to be infected by the virulent isolate of *C.coccodes* under laboratory conditions revealed that Markony cultivar was the most susceptible one followed by red hot pepper of the same cultivar, green 7182, and yellow Derby sweet pepper cultivars. On the other hand, Baramo orange sweet pepper cv. was moderately susceptible to *C.coccodes*. On the contrary, Ferrari red sweet pepper cv. was resistant to the highest virulent isolate of *C. coccodes.* In the same time, the rotted area on the fruits increased with increasing the period of incubation at 26±1 ^{o}C from 5 to 9 days. Host range reactions of some plant fruits to infect with *C.coccodes* indicate that the pathogen was able to attack tomato and apple fruits while fruits of eggplant, strawberry, peach, orange, lime, papaya, guava and mango showed no symptoms. Effect of plant extracts on the growth of *C.coccodes* revealed that garlic at the highest concentration (20%) was found to be the best in the inhibition of fungal growth followed by lime,onion , sweet basil and mint extracts .The fungicides Euparen, Octave and Swtich inhibited the growth of *C.coccodes* completely at all concentrations tested *in vitro* (100, 200, 300, 400 and 500ppm)*. Trichoderma harzianum* followed by *Chaetomium spirale* and *C. globosum* caused considerable reduction of *C.coccodes* mycelial growth. Hot water treatments of inoculated hot pepper fruits were more effective with increasing the periods of fruit dipping from 2 to 3 minutes and increasing temperature of water from 50^{o}C to55^{o}C but unfortunately the treatments decrease the fruit quality and fruit shelf life. Dipping inoculated pepper fruits in *T. harzianum* suspension reduced the lesions of anthracnose size compared with the control. Different plant extracts reduced the severity of pepper fruit rots. Lime extract was the effective one in decreasing area infected with *C.coccodes* followed by Sweet basil, Mint and pepper extracts

Keywords: *Colletotrichum coccodes*, morphological characterization, Markony Spain hot pepper, Derby sweet pepper, *Trichoderma harzianum, Chaetomium spirale* and *C. globosum*.

INTRODUCTION

Nowadays in Egypt colored pepper is one of the most important horticultural crops produce for export to foreign countries and also for local consumptions**.**

Anthracnose, incited by *Colletotrichum* spp, is a major disease of pepper, causing pre- and post – harvest decay of fruits. Park and Kim (1992) reported that decay of mature red fruits caused by anthracnose can result in substantial reduction in pepper yield. They also reported that five

anthracnose fungi, *Colletotrichum gloeosporioides, C.dematium, C.coccodes, C.acutatum* and *Glomerella cingulata* are pathogenic to different tissues of pepper plants. .Identification of *Colletotrichum* to species is usually based on more than one characteristic, such as colony morphology, conidial and appressoria shape, size and presence of setae, fungicides sensitivity, pathogenicity and other traits (Katan ,2000 and Cannon *et al* .2000) . Effect of some crude plant extracts on growth of *C.capsici* ,causal agent of pepper anthracnose was studied by Nduagu *et al*. (2008) .Their study demonstrate that crude extracts of some plants exhibited strong toxicity against anthracnose of sweet pepper . Andrivon *et al*. (1997) reported that the growth of five *C.coccodes* isolates *in vitro* were severely affected by five fungicides in reducing the growth of these isolates*. Trichoderma* species have been applied to control *Colletotrichum* species in chilli pepper with clear disease reduction (Boonratkwang *et al*. 2007). The objective of the present study was to isolate and identify the species of *Colletotrichum* causing pepper anthracnose in Egypt.Evaluations of different fungicides, bioagents and plant extracts against *C.coccodes,* the causal of pepper anthracnose, in Egypt were also investigated.

MATERIALS AND METHODS

Isolation and identification of the pathogen from infected fruit pepper

The pathogen was isolated from pepper fruits of Markony Spain hot pepper cv. and 4408 sweet pepper cv. showing anthracnose disease symptoms grown under greenhouses and open fields, located at Abo-Swear and El-Ferdan, Ismailia Governorate during 2005 – 2006 season .The disease samples of fruits were isolated in moisten chamber and brought to laboratory .The diseased fruits were cut at the advanced margin of lesions in to small pieces (3mm x 3mm) and then surface sterilized with 5 % Clorox for 1 min, followed by washing in sterile distilled water and transferred on PDA medium in Petri dishes. Plates were incubated at 25 ^{o}C for 7-10 days. The colonies of the fungus were identified according to the *Colletotrichum* description reported by Sutton, 1992 and kindly confirmed by the Assouit Mycological Center, Faculty of Science; Assouit University .Pure cultures were stored at 4 ^{o}C on PDA slants for further studies.

Morphological studies

Colony characteristics of *C.coccodes* which including colony morphology on PDA, color of the culture, presence or absence of setae , microsclerotia pigmentation and size & shape of conidia, 10 days after growing at 25 ^{o}C were studied. In the same time , the mean of about 30 replicates of sizes (length and width) of acervuli,sclerotia,20 replicates of the sizes (length and width) of setae and 50 replicates of sizes (length and width) of conidia were recorded. Small specimens were taken from *C.coccodes* grown in PDA, seven days old for Scanning Electron Microscopy (SEM) .The specimens were immediately fixed in glutaraldehyde (2.5%) for 24 h at 4 ^{o}C, then post fixed in osmium tetroxide (1 %OSo4) for one hour at room temperature (Harley and Ferguson 1990). Then dehydrated through scending

concentrations of acetone and critical point dried. Finally, samples were sputter coated with gold. The examination and photographing were done through a Jeol Scanning Electron Microscope (T.330A) in the Central Laboratory, Faculty of Agriculture, Ain Shams University.

Pathogenicity test

Inoculum preparation.

The inoculum of the six isolates of *C.coccodes* were prepared from cultures grown on PDA medium for10 days under continuous fluorescent light (Salazar *et. al.,* 2007).Conidia were washed from cultures with distilled sterilized water. A final conidial suspension containing 2 x10^6 conidia / ml was prepared .The artificial inoculation was conducted with two methods:

In vitro:

Under lab. Conditions: Apparently healthy fruits of Markony Spain hot pepper cv. were selected and surface – sterilized by immersing in 70% ethanol for 1 min, followed by 1 % sodium hypochlorite solution for 1 min. Finally fruits were rinsed in sterile distilled water and air- dried under aseptic conditions. Fruits then were placed on paper towels in plastic box and 8 wounded and unwounded fruits per treatment were sprayed with spore suspensions containing 2 x10^6 conidia / ml of each isolate using a hand – held atomizer. Control treatment was sprayed with distilled sterilized water. Inoculated and non – inoculated fruits were incubated at 25±1 ^oC for 7days. The diameters of rotted tissues were recorded 7 days after inoculation.

In vivo:

Greenhouse experiment: Wounded and unwounded fruits on pepper plants of Markony cv. were sprayed with spore suspensions containing 2 x10^6 conidia /ml of each isolate using a hand – held atomizer. Control treatment was sprayed with distilled sterilized water. Inoculated fruits were covered with polyethylene bags for 72 hrs at 26±1 ^oC. Disease severity assessment was determined as lesions diameter on fruit for each isolate 7 days after inoculation.

Reactions of pepper cultivars to infect by the virulent isolate of *C.coccodes* under lab. conditions.

Susceptibility of 5 pepper cultivars green and red hot pepper of Markony cv., sweet green pepper of 7182 cv., sweet yellow pepper of Derby cv., sweet orange pepper Baramo cv. and red pepper of Ferrari cv. were evaluated. Apperantly healthy fruits were selected and surface – sterilized as mentioned above. Fruits then were placed on paper towels in plastic boxes and 6 fruits per treatment were sprayed with spore suspensions containing 2 x10^6 conidia / ml of the virulent isolate (No.1) using a hand – held atomizer. Control treatment was sprayed with distill sterilized water. Inoculated and non – inoculated fruits were incubated at 26±1 ^oC under lab. conditions and disease response was recorded 5and 9 days after inoculation. Disease severity assessment was determined as lesions diameter on fruits.

Host range of *C.coccodes*:

Fruits of tomato(*Lycopersicon esculentum*), eggplant (*Solanum melongena*) Family Solanaceae ,orange *(Citrus sinensis*) ,lime (*Citrus aurantifolia*) Family Rutaceae, peach (*Prunus persica L*.) strawberry (*Frgaria* x *ananassa*) ,apple (*Malus domestica*) Family Rosaceae , papaya (*Carica*

papayaa) Family Caricaceae, mango (*Mangifera indica*) Family Anacardiaceae and guava (*Pisidium guajava*) Family Myrtaceae were inoculated as mentioned before with spore suspension of isolate No.1, the virulent isolate of *C.coccodes* in order to study the pathogenic capability of *C.coccodes* on these fruits .Other group of such fruits were inoculated with sterilized water as a control .Six fruits as replicates were used for each host . All tested fruits were incubated at 26±1 ^{o}C for 4 and 7days according to the shelf life of pepper fruits and lesions diameter .

Laboratory evaluation of plant extracts, antagonistic fungi and fungicides on the growth of *C. coccodes*

A- Plant extracts

Test was conducted *in vitro* to determine the inhibitor effect of plant extracts on the fungal growth of *C.coccodes* following poison food technique as described by Begum and Bhuiyan (2006). Water extracts of garlic (*Allium sativum*), onion (*Allium cepa*), sweet basil (*Ocimum basilicum)*, mint (*Mentha spicata*) and lime (*Citrus aurantifolia*) leaves were tested. Stock solutions of the materials were prepared by blending 100g of each plant material in 100 ml of sterilized water in a blender. PDA medium was amended with diluted individual extract at 0, 5, 10 and 20% (v/v). After mixing with plant extracts the medium was autoclaved and poured into 90 mm Petri dishes. The plates were inoculated by placing 5 mm discs of 10 days old PDA cultures of *C. coccodes* and incubated at 25 ^{o}C for 7 days. Thereafter, inhibition percentages of *C. coccodes* growth were calculated based on the growth of the pathogen on PDA plates following the formula suggested by Sundar *et al.* (1995).

$$\% \text{ Inhibition} = \frac{X-Y}{X} \times 100$$

Where, X= Growth on control plate. Y= Growth on the plant extract treated plate, fungicides or antagonistic fungi.

B- Antagonistic fungi:

An *in vitro* screening experiment was conducted to find out the antagonistic effect of three antagonistic fungi (*Trichoderma harzianum* , *Chaetomium spirale* and *Chaetomium globosum*) kindly supplied by The Assouit Mycological Center , Assouit University against *C. coccodes* on PDA using dual culture technique (Dhingra and Sinclair 1985). Discs of (5 mm diameter) of the virulent fungal isolate (No.1) were cut from the edge of an actively growing fungal colony with a cork borer. One disc of each antagonistic and one disk of the fungal pathogen was placed simultaneously on the edge of each PDA Petri plate at opposite direction. Three replicates were used for each antagonistic fungus. The plates were incubated at 25 ^{o}C until mycelium of the tested pathogen *C. coccodes* cover the whole control plate. Inhibition of the fungal growth was calculated as colony diameter on treated and untreated control plates using the same formula (Sundar *et al.* 1995) as described above.

C-Fungicides

Six different concentrations (0.0,100, 200, 300, 400 and 500ppm) of each tested fungicide (Rovral 50WP, Topsin M70 %, Euparen M50, Tecto 500 SC,

Swtich M and Octave) were tested to investigate their effect on the growth of *C. coccodes in vitro*. The poison food technique described by Dhingra and Sinclair 1985 were used in this experiment. Fifteen ml of the poisoned sterilized PDA medium was poured in each 9 cm sterilized Petri dish. After solidification, the plates were inoculated with 5 mm discs of *C. coccodes,* 7 days old cultures. Non fungicidal plates were used as control treatment .Three replicates were used for each particular fungicide. The inoculated plates were incubated at 25 ^{o}C and data on the fungal colony diameter was recorded 8 days after incubation when the growth of the control plates completely covered the plate. Diameter of the colonies on PDA with and without fungicide was measured from the bottom side of the Petri dishes.

Post harvest treatments

A lesion (0.2 cm long) was made on the surface of pepper fruits .The wounded fruits were sprayed with a spore suspension (2 x10 ^{6} spores /ml) of *C.coccodes* and incubated for 2h at26±1 ^{o}C. (Sivakumar *et al.* (2000).

1- Effect of hot water treatment on the control of pepper fruit rots

Wounded and non wounded fruits of pepper artificially inoculated with *C.coccodes* were dipped in hot water at 50 and 55 ^{o}C for 2 and 3 minutes. Fruits were allowed to dry in the air and stored at room temperature 26±1 ^{o}C for a week and severity of infection was determined.

2-Effect of plant extracts on the control of pepper fruit rot :

Healthy leaves without damage selected from the collected lime (*Citrus aurantifolia*), pepper (*Capsicum annuum*), sweet basil(*Ocimum basilicum*) and mint(*Mentha spicata*)were rinsed in sodium hypochlorite (5%) and distilled water , air – dried and stock solutions of the materials were prepared by blending 100g of each plant material in 100 ml of sterilized water in blender . Non – inoculated and inoculated pepper fruits with *C.coccodes* were dipped in different plant extracts for 10 minutes at concentration of 10 % diluted stock solution. Fruits were allowed to dry in the air and stored at room temperature (26±1 ^{o}C) for 7 days and severity of infection was determined.

3-Effect of biological control on the incidence of pepper fruit rots artificialyl inoculated with *C.coccodes*.

Fruits of pepper inoculated with *C.coccodes* and non - inoculated were dipped for 15 min in the culture filtrate (10^{9}conidia /ml) of *Trichoderma harzianum* and incubated at room temperature (26±1 ^{o}C) for 7 days and disease severity was determined.

Statistical analysis:

The obtained data were statistically analyzed by analysis of variance (ANOVA) using the Fisher LSD method. Means were separated by Fisher's protected least significant differences (LSD) at $P < 0.05$ level (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Isolation and identification of the causal fungus of pepper anthracnose :

Six isolates of *Colletotrichum* were isolated from naturally infected sweet and hot pepper fruits obtained from greenhouses and open fields collected from Ismailia Governorate.

Note: the honey spore masses on the fungal growth (A_1)

Fig.(1): Morphological characteristics of *C.coccodes* 10 days old showing the fungal growth on PDA (A_1) and on reveres (A_2), black microsclerotia (B),acervuli and setae (C),individual acervuli with setae (septate) and conidiophores (D) fusiform shape conidia with acute or pointed ends under light microscope (E).

Fig.(2): Scanning electron micrograph showing : acervuli on PDA media 7days old(A), setae and conidia (B),setae slightly swollen at the base and tapered at the apex (C),abundant conidiospores (D) and starting of conidial germinated spores(E)

Identification using morphological characterization of *C.coccodes* grown on PDA media are dominated by white, sparse aerial mycelium and by abounded, black sclerotia that abundant. Using light and scanning electron microscopes indicate that the sclerotia are spherical, and distributed evenly over the agar surface. Acervuli produced in culture and on pepper fruits normally in association with sclerotia, which are round to elongate ,

approximately 200-300 µm in diameter , and produced setae , approximately 90 – 160, µm long by 2.5- 4.8 mm wide µm , 1-7 septate, slightly swollen at base ,tapered to paler acute apex ,dark globose, smooth – walled (Fig.1)and branched conidiophores .Conidia are formed in honey , orange to salmon – coloured mass and are 21-24 µm long by 3.5 – 4.3 µm wide ,straight ,fusiform and tapered to each end (Fig.2). Such, criteria of *C.coccodes* from pepper as reported by Sutton, 1992, Davis and Johnson 2002 and Hugo *et al* .2007 were observed.

Symptomatology:

Symptoms of naturally rotted pepper fruit initially begin as water – soaked lesions that become soft, slightly sunken, and become tan .The fungus develop in this lesion and lesion can cover most of the fruit surface and multiple lesions occur (Fig.3A). The surface of the lesions becomes covered with wet, gelatinous spores with salmon – colored fungal fruiting bodies (acervuli) with numerous black spines setae .Concentric rings of the acervuli and sclerotia (Fig.3B) are common within the fruit spots (Isaac 1992and Hugo *et al*. 2007).

Fig. (3) Naturally infected sweet (A) and hot (B) pepper fruits with anthracnose, from which *C.coccodes* was isolated. Notice the numerous acervuli and microsclerotia scattered surrounding the lesion.

Pathogenicity test

Pathogenic capabilities of six *C.coccodes* isolates obtained from infected pepper fruits were tested on wounded and unwounded pepper fruits of Markony cv. *in vitro* and *in vivo* under greenhouse conditions for pathogenicity test .Data in Table (1) and (Fig.4) indicate that the isolate No.1 obtained from Abo-Swear was the virulent and aggressive one as the diameter of anthracnose lesions reached 45.5 mm in wounded and 24 mm in unwounded inoculated pepper fruits *in vitro* (25±1 ^{o}C).However, the corresponding figure *in vivo* under greenhouse at 26±1 ^{o}C showed 46.2 mm and 26.9 mm in wounded and unwounded pepper fruits,7 days after inoculation followed by isolate No.2 (31- 20mm) and No.3 (29 - 15 mm) for wounded and unwounded pepper fruits *in vitro*, respectively .

Fig. (4): Pathogenicity test with the virulent *C.coccodes* isolate No.1 on green pepper fruits of Markony cv. under lab. Conditions (A) and under greenhouse conditions (B), 7 days after inoculation, induced typical symptoms of anthracnose disease on wounded and unwounded Spain hot red pepper fruits. However, the control wounded or unwounded fruits showed no symptoms(C).

While in *vivo* isolate No.2 recorded (33.3,20.8mm) and isolate No.3 showed (30.5,19.8 mm) , respectively .On the other hand , isolates No. 4 and 5 were moderately pathogenic to pepper fruits *in vitro* and *in vivo* .In the same time ,Isolate No.6 was less pathogenic to pepper fruits *in vitro* and *in vivo*(14.3-9.5mm) and (16- 10.5mm), respectively .The chilli pepper anthracnose develops readily on pepper fruit of susceptible cultivars regardless of wounding and the disease severity enhanced by the wound inoculation compared to un-wounded one (Kim *et al* . 2004 and 2008).

Table .1. Artificial inoculation with different isolates of *C.coccodes* on pepper fruits of Markony Spain hot pepper cultivar, seven days after inoculation under lab. and greenhouse conditions.

Isolate No. and location	Mean diameter of anthracnose lesions on inoculated pepper fruits (mm)			
	Under lab. conditions. (25±1 °C) (*In vitro)*		Under greenhouse conditions. (26±1°C)(*In vivo)*	
	Wounded fruits	Unwounded fruits	Wounded fruits	Unwounded fruits
1-Abo-Swear	45.5	24	46.2	26.9
2-Abo-Swear	31	20	33.3	20.8
3-Abo-Swear	29	15	30.5	19.8
4-El-Ferdan	27.1	12	28.2	16
5- El-Ferdan	23.2	10.5	24.5	12.6
6- El-Ferdan	14.3	9.5	16	10.5
control	0.0	0.0	0.0	0.0

LSD 5 %: Isolates = 1.84 and artificial inoculation (*In vitro, In vivo*) = 1.062
Interaction between isolates X *in vitro – in vivo* (Wounded fruits) = non significant
" " X " " (unwounded fruits) =1.997.

Reactions of pepper cultivars to infect by the virulent isolate of *C.coccodes* under labrotary conditions

Sweet and hot pepper fruits varied in their susceptibility to *C.coccodes* based on the diameter of rotted lesions. Green hot pepper of Markony cultivar was the most susceptible one to *C.coccodes* followed by red hot pepper of the same cultivar , Green 7182cv. and sweet yellow pepper cultivar Derby. On the other hand, sweet orange pepper cultivar Baramo was moderately susceptible to *C.coccodes*. On the contrary, sweet red pepper Ferrari cv. showed no symptoms to the highest virulent isolate of *C. coccodes.* In the same time, the rotted area on the fruits increased with increasing the period of incubation from 5 to 9 days (Table 2). Reactions of pepper cultivars to infect by the virulent isolate of *C. coccodes* under study ,could be due to its chemical composition .Differences of these colored pepper cultivars to *C.coccodes* also my be explained by differences in biochemical properties of pepper fruits .In biochemical analyses of peppers Ko (1986) has demonstrated that green fruits had higher activities in peroxidase and polyphenoloxidase enzymes than red ones ,but lower levels of total phenolics, amino acids and carbohydrates ,some of which have been related to host resistance (Kim *et al*.1999).

Table 2. Reactions of pepper cultivars to infect by the virulent isolate of *C.coccodes* under lab. conditions.

Cultivars	Lesions size on the inoculated pepper fruits (mm)	
	Incubation period :	
	5 days	9 days
Hot green pepper (Markony cv.)	58.6	67.8
Hot Red pepper (Markony cv.)	53.2	64.2
Sweet green pepper (7182 cv.)	50.1	59.9
Sweet yellow pepper (Derby cv.)	40.3	50.2
Sweet orange pepper (Baramo cv.)	30	35
Sweet red pepper (Ferrari cv.)	0.0	15

***Green fruits are immature and turn red colour during the mature stage**
LSD 0.05: = Cultivars = 2.557, incubation period=1.476 and
Interaction between cultivars X incubation period = 4.294

Host range of *C.coccodes:*

Ten plant species belonging to six families were tested for their reactions to *C.coccodes* isolated from pepper fruit rot. Results of these reactions are recorded in Table (3). The pathogen was able to attack tomato and apple fruits while eggplant, strawberry, peach, orange, lime, papaya, guava and mango showed no symptoms . The diameter of anthracnose lesions increased by increasing the time of incubation. However Raid and Pennypacker (1987) reported that *C.coccodes* has a wide host range that includes at least 58 species in 17 families, primarily in Leguminosae, Solanaceae and Cucurbitaceae.

Table. 3: Host range reactions of some plant fruits to infect with *C.coccodes.*

Hosts			Mean diameter of anthracnose lesions on the inculated fruits(mm) incubated for	
English name	Scientific name	Family	4 days	7 days
1-Tomato	*Lycopersicon esculentum*	Solanaceae	20.31	45.52
2- Eggplant	*Solanum melongena*	Solanaceae	0.0	0.0
3- Strawberry	*Frgaria x ananassa*	Rosaceae	0.0	0.0
4- Peach	*Prunus persica L.*	Rosaceae	0.0	0.0
5- Apple	*Malus domestica*	Rosaceae	3.22	4.11
6- Orange	*Citrus. sinensis*	Rutaceae	0.0	0.0
7- Lime	*Citrus aurantifolia*	Rutaceae	0.0	0.0
8- Papaya	*Carica papaya*	Caricaceae	0.0	0.0
9- Gauva	*Pisidium guajava*	Myrtaceae	0.0	0.0
0 – Mango	*Mangiferea indica*	Anacardiaceae	0.0	0.0

Effect of plant extracts on the growth of *C.coccodes* (*in vitro*):

Five plant extracts of garlic, onion, lime, sweet basil and mint were tested in the present study showed appreciable inhibition in colony growth of *C.coccodes* (Table 4). Among the tested plant extracts, garlic at the highest

concentration was found to be the best in the inhibition of fungal growth (81.1%) followed by lime extract (76.6 %). The rate of reduction was corroborated with its concentrations in case of all the tested plant extracts. Sweet basil gave the highest reduction 46 .3% in colony diameter at the highest concentration 20% (Table 4). Result of the experiment showed that the most effective material was garlic extract followed by lime, onion, sweet basil and mint. It is also clear that the higher concentrations (15-20 %) of garlic, onion and lime extracts prevent sclerotia formation on the inoculated media. These results supported the observation of other investigators (Singh *et al*. 1997, Harbant *et al.* 1999 and Ogbebor *et al*., 2007) they found that garlic extract was very effective in controlling the anthracnose pathogen in different crops. Kurucheve *et al.* (1997) observed that the variation in the inhibitory effect of plant extract may be due to qualitative and quantitative differences in antifungal principles.

Table. 4. Effect of plant extracts on the growth of *C. coccodes in vitro:*

Plant extract	Concentrations (%volume /volume)*	% Inhibition of fungal growth (mm)	Sclerotia formation
Garlic	5	36.5	+
	10	46.7	+
Allium sativum	15	58.4	-
	20	81.1	-
Onion	5	28.97	+
	10	37.51	+
Allium cepa	15	43.11	_
	20	53.41	_
Lime	5	35.3	+
Citrus aurantifolia	10	45.5	+
	15	57.6	_
	20	76.6	_
Sweet basil	5	22.83	+
Ocimum basilicum	10	27.13	+
	15	36.93	+
	20	46.3	+
Mint	5	18.5	+
Mentha spicata	10	22.9	+
	15	26.3	+
	20	31.8	+
Control	0.0	88.33	+
L.S.D 5%	Plant extracts =0.934 , Concentrations = 1.044 and interaction between plant extracts X concentrations= 2.084		

***100g of plant material blended in 100ml sterilized water to make a stock solution which diluted to 5, 10, 15 and 20 %.**

Effect of various fungicides and antagonistic fungi on the growth of *C.coccodes* (*in vitro*):

A- Fungicides

Six fungicides namely: Swtich M, Octave WP, Topsin- M , Euparen M50% , Tecto and Rovral 50 WP were added to PDA medium to study their effect on the growth of *C.coccodes.* Six concentrations of each fungicide, *i.e*., 0.0, 100,200,300,400 and 500, were tested and average diameter of colonies (mm) was recorded. It is clear from results presented in Table (5) and (Fig.5) that the increase in fungicide concentrations led to an obvious

decrease in the linear growth of *C.coccodes*. Euparen, Octave and Swtich were the most effective fungicides. Each one of them inhibited the growth of *C.coccodes* completely at all concentrations tested. In the same time , Tecto and Topsin at 500ppm inhibited the fungal growth (82.96% , 80.11%), respectively .However, Rovral, was the least effective fungicide on the mycelial growth of the tested fungus at all concentrations tested .Similar results were reported by Shovan *et al.m* (2008) .

B- Antagonistic fungi

It is evident from the results presented in Table (5) that *T.harzianum* followed by *Chaetomium spirale* and *C. globosum* caused considerable reduction of *C.coccodes* mycelial growth. This reduction might be due to secretions of harmful intracellular compounds and mycoparasitism. *Trichoderma* spp.are known to produce many cell-wall degrading enzymes such as glucanases and chitinases. Lin *et al.* (1994) revealed that activity of *Trichoderma* against other pathogenic fungi was due to the production of certain antibiotics suh as Tricholin , which inhibit the mycelial growth when spread in the medium .Concerning fungicides and antagonistic fungi, the present results are in partial agreement with some investigators such as Shovan *et al*. (2008) on their study to control anthracnose of soybean caused by *C.dematium* with fungicides ,plant extracts and *T.harzianum* .Other experimental results emphasized the supperession because of *Trichoderma* ability of producing lytic enzymes , which caused degradation of fungal cell wall (El – Abbasi *et al* ., 2003). Members of the genus *Chaetomium* occur widely in nature, and certain *Chaetomium* spp. can produce biologically active metabolites, such as Chaetochalasin A and Chaetoglobosins, which suppresses the growth of plant pathogens (Singh *et al*. 1997).

Fig. (5) Showing the effect of different fungicides on the growth of *C.coccodes in vitro*. Octave, Euparen and Swtich inhibited the growth of the fungus completely at all concentrations tested (A) compared with the Tecto, Topsin and Rovral fungicides (B). *c = control without fungicidal treatment.

Table 5. Percentage of *C.coccodes* growth reduction due to different concentrations of 6 tested fungicides and 3 bioagents *in vitro.*

Fungicides and bioagents	Concentration (ppm)	Average diameter of *C.coccodes* colony(mm)	% Inhibition
Octave WP	100	0	100
	200	0	100
	300	0	100
	400	0	100
	500	0	100
Euparen M50%	100	0	100
	200	0	100
	300	0	100
	400	0	100
	500	0	100
Swtich M	100	0	100
	200	0	100
	300	0	100
	400	0	100
	500	0	100
Tecto 500 SC	100	25	71.25
	200	20.3	76.67
	300	19.3	77.61
	400	16.7	80.80
	500	15	82.76
Topsin M70 %,	100	33.0	62.98
	200	27	68.13
	300	22.3	74.33
	400	21	75.60
	500	17.3	80.11
Rovral 50WP	100	66.7	23.3
	200	56.7	34.85
	300	56.3	35.53
	400	55	36.73
	500	53.3	43.13
Trichodermae harzianum	-	16.5	81.6
Chaetomium spirale	-	25.4	76.33
Chaetomium globosum	-	21.3	71.78
Control(without treatment)	-	90	0.0

L.S.D 5%: Fungicides = 0.913, Concentrations =0.215 and interaction between Fungicides X concentrations= 0.527

Effect of treatments with hot water , plant extracts and *T. harzianum* on anthracnose lesions diameter of inoculated pepper fruits cv. Markony Spain hot pepper 7 days after incubation at 20 – 25 ºC (*In vitro*).

1- Dipping in hot water :

Mature pepper fruits Markony Spain hot pepper cultivar treated with hot water were more effective with increasing the periods of fruit dipping from 2 to 3 minutes and increasing temperature of water from 50 ºC to 55 ºC.The period of 3 minutes dip in hot water at 55 ºC was sufficient decreasing infected area more than the dipping at 50 ºC .Generally, treatment with hot water as dipping for 2 to3 minutes at 50 ºC to 55 ºC were effective for controlling *C.coccodes*(Table 6) .Although these results are in harmony with those reported by Fallik *et al* .(2003) as they found that hot water dipping of sweet red pepper (*Capsicum annuum*) showed effective

control of grey and black moulds ,but unfortunately ,the treatment in the present paper decrease the shelf life of pepper fruits. .

Dipping in suspension of *T.harzianum*:

Data in the Table (6) indicate that dipping pepper fruits in *T. harzianum* suspension (10^9 conidia /ml) reduced the lesions of anthracnose size of pepper fruit rot (19mm) caused by *C.coccodes* compared with the control(44.6 mm) . These results are in agreement with those reported by Sivakumar *et al.* (2000).

Dipping in plant extracts :

Four plant extracts of lime, sweet basil, mint and pepper were tested and average diameter of lesions (mm) was recorded. It is clear from results presented in Table (6) that different plant extracts reduced the severity of pepper fruits rot. Lime was the effective one in decreasing area infected with *C.coccodes* followed by sweet basil and mint. On the contrary, pepper extract was less effective for controlling the decay caused by *C.coccodes* but the amount of decay still lower than that the control.Somada *et al* .(2007) reported that aqueous extract of *Cymbopogon citratus* exhibited the best control effect on seed infected by *Colletotrichum graminicola* and *Phoma sorghina .*

Table. 6. Effect of treatments with hot water, plant extracts and *T. harzianum* on anthracnose lesions diameter of inoculated pepper fruits cv. Markony Spain hot pepper,7 days after incubation under lab. conditions (26±1 ºC).

Treatments		**Lesions size (mm)**
Hot water	1- 50 ºC for 2 min	29.3
	50 ºC for 3 min	25.2
	2- 55 ºC for 2 min	20.6
	55 ºC for 3 min	15.8
Trichoderma harzianum	10^9 conidia /ml	19
Plant extracts (blended 100g.fresh weight in 100ml water)	1- Lime **(** *Citrus aurantifolia*)	15.3
	2-Sweet basil *(Ocimum basilicum)*	16.5
	3-Mint (Mentha spicata)	17.5
	4- Pepper (*Capsicum annuum*.)	30.4
Control (inoculated ,untreated fruits)		44.6

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اول تسجيل لمرض انثراكنوز الفلفل المتسبب عن الفطر *Colletotrichum coccodes* في مصر .

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يعتبر الفلفل احد المحاصيل البستانية الهامة في مصر سواء للتصدير او للسوق المحلي وقد تم عزل ٦ عزلات من الفطر *Colletotrichum* من ثمار الفلفل الباردوالحار مصاب طبيعيا تم الحصول عليها من الصوبات الزراعية ومن الحقل المكشوف بمحافظة الاسماعلية .

* تم تعريف هذه العزلات باستخدام الصفات المورفولوجية والتي اشارت الي ان المسبب هو الفطر *Colletototrichum coccodes* . اوضح اختبار العدوي الصناعية للعزلات الستة بالفطر *C. coccodes*علي ثمار فلفل الصنف ماركوني الاسباني الحار المجروحة والغير مجروحة بالمعمل وتحت ظروف الصوبة حيث وجد ان العزلة رقم(١) المعزولة من ابو صوير كانت اقوي العزلات المختبرة من حيث قدرتها المرضية .

*اختبار مدي قابلية اصناف الفلفل للاصابة بمسبب مرض الانثراكنوز اوضحت ان الصنف ماركوني كان اكثر الاصناف قابلية للاصابة بالعزلة القوية رقم (١) يليه الثمار الحمراء الناضجة لنفس الصنف يليه الصنف الحلو الاخضر ٧١٨٢ ثم الصنف الاصفر Derby علي الجانب الاخر ، كان الصنف البرتقالي Baramo متوسط الاصابة بالفطر المسبب وعلي العكس كان الصنف الاحمر الحلو اكثر الاصناف مقاومة لهذا المرض .وجد ان معدل الاصابة يزداد بزيادة مدة تحضين الثمار من ٥ الي ٩ ايام بعد العدوي .

*اوضح اختبار المدي العوائلي للفطر المسبب قدرته علي اصابة ثمار الطماطم والتفاح بينما لم تظهر اي اعراض مرضية علي ثمار الباذنجان – الفراولة – الخوخ- البرتقال – الليمون- البابا ظ- الجوافة والمانجو المعدية صناعيا بمعلق جراثيم الفطر المسبب *C. coccodes*.اوضحت دراسة تاثير المستخلصات النباتية علي تثبيط نمو الفطر *C. coccodes* في المعمل ان مستخلص الثوم المركز كان الاكثر تاثيرا في تثبيط نمو الفطر يليه مستخلص الليمون – البصل – الريحان والنعناع. المبيدات الفطرية ايوبارين ، اكتاف ، سوتش ثبطت بالكامل نمو الفطر *C. coccodes* بكل التركيزات المختبرة بالمعمل .

* كما وجد ان الفطريات المضادة *T.harizianum* يليه الفطر *C.spirale* والفطر *C.globosum* خفض بدرجة كبيرة نمو ميسليوم الفطر المسبب .

في مجال مكافحة المرض. وجد ان معاملة ثمار الفلفل المعدية صناعيا بالماء الساخن تزداد فاعليتها بزيادة غمر الثمار من ٢-٣ دقائق وبزيادة درجة الحرارة من 0٥—٥٥ م ولكن لسوء الحظ فان هذه المعاملة تقلل من فترة تسويق الثمار.

* وجد كذلك ان غمر ثمار الفلفل المعدية صناعيا في معلق *T.harizianum* يقلل من حجم بقع عفن الانثراكنوز علي ثمار الفلفل مقارنة بالثمار الغير معاملة .

* تم اختبار تاثير مستخلصات نباتية مختلفة علي شدة اصابة ثمار الفلفل المعدية صناعيا بالفطر المسبب ، فكان مستخلص الليمون هو الاكثر كفاءة في تقليل الاصابة بالفطر *C. coccodes* يليه مستخلص الريحان ومستخلص النعناع وعلي العكس كان مستخلص الفلفل الاقل تاثيرا في مقاومة التلف ولكن كمية التلف كانت مع ذلك اقل من مثيلتها في المقارنة.