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Anthracnose Disease (*Colletotrichum* sp.) Affecting Olive Fruit Quality and Its Control in Egypt

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Abstract Olive anthracnose is the most important fungal disease of olive fruits worldwide. It occurs in humid olive-growing areas of many countries and causes heavy yield losses and lowering of oil quality. *Colletotrichum acutatum* was isolated and identified from rotten olive fruits. Pathogenicity test of *C. acutatum* was confirmed. It was found to be decreased all physical characteristics measured i.e. weight (gm), length (mm), diameter (mm) and volume (ml3). Also, *C. acutatum* was found to decrease the oil content of the fruits, while increasing their total titratable acidity and moisture content. Physiological studies resulted that, the highest growth rate and sporulation was recorded with PDA medium, PH 6.5 and Light/dark cycle treatments. Hot water treatments at 45, 50 and 55 ℃ were able to decreased spore viability of *C. acutatum* compared with untreated (control). The best treatment of hot water was recorded with 55 ℃. *in vivo*. Also, hot water treatment at 55 ℃ was successful in reducing the percentage of anthracnose disease incidence on olive fruits *in vitro*. All tested alternative substrates i. e. Ascorbic acid, Benzoic acid, potassium sorbate and citric acid used were able to reduce the linear growth rate of *C. acutatum in vitro*. Benzoic acid was found to be the best alternative substrate used which gave completely fruit protection (hundred of reduction percent) followed by Ascorbic acid, Potassium sorbate and Citric acid. This is thought to be the first report of anthracnose disease of olive fruits in Egypt.

Keywords: Olive Fruit rots, Colletotrichum, Nutrition, pH, Light/dark, Hot water, Ascorbic acid, Benzoic acid, Potassium sorbate and Citric acid

Introduction

Colletotrichum is a very common fungus that is wide spread in horticultural regions. The infected fruit will rot and some times shrivel before prematurely dropping from the tree. Anthracnose is a latent disease. This means that the fungus can infect the fruit when the environmental conditions are suitable but will remain dormant until the fruit begins to ripen. *Colletotrichum*

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acutatum and *C. gloeosporioides* (Penz.) Penz. & Sacc. are the two members of the genus that are most commonly associated with fruit rots in the literature. *Colletotrichum gloeosporioidesis* considered a cumulative species and is found on a wide variety of fruits, including almond, apple, avocado, citrus, mango, olive, and strawberry (Sutton, 1992). Anthracnose (*Colletotrichum acutatum* and *C. gloeosporioides*) disease causes soft circular rots on the fruit (Robert, 2005). Anthracnose is a fruit rot of olives caused by a strain of *Colletotrichum acutatum* resulting in an orange slimy mass of spores on the fruit surface (Kailis and Harris, 2007). Mummified olive fruits were observed when the fruits began to ripen (Sergeeva *et al*., 2008). Two anamorphic species of *Colletotrichum*, *C. gloeosporioides* (Penz.) Penz.&Sacc. and *C. acutatum* J. H. Simmonds, were reported to be associated to olive anthracnose (Sergeeva *et al*., 2010).

The disease is more severe with some varieties for example Manzanillo and Leccino (Moral *et al*., 2009 and Sónia *et al*., 2012). The genus *Colletotrichum* (Sordariomycetes, Ascomycota,) comprises ~600 species attacking over 3,200 species of monocot and dicot plants (ARS Fungal Databases, see URLs). Several species or genotypes of Colletotrichum have been indicated as responsible for olive anthracnose in different countries, including *C. gloeosporioides*, *C. acutatum* (both in a broad and narrow sense), *C. fioriniae*, and *C. simmondsii*. Recently, the molecular group A4 of *C. acutatum* has been described as a new species named *C. clavatum* (Cacciola *et al*., 2012).

Anthracnose disease caused by *Colletotrichum* sp. is one of the most economically important diseases reducing marketable yield from 10 to 80% of the crop production in some developing countries, particularly in Thailand. Anthracnose is a main problem on mature fruits, causing severe losses due to both pre- and post-harvest fruit decay. *C. acutatum* and *C. gloeosporioides* have been reported to be more prevalent on both young and mature green fruits (Ashwini and Srividya, 2012).

Hot water treatment (45 ℃) for 150 s, in addition to controlling rot, to a great level improves the fruit cortex (Larrigaudiere *et al*., 2002). Fruits were passed through hot dips for a few minutes at 49 ℃ to kill mold spores on citrus fruit. Practical systems have used either vapor heat or hot water. Fruits are dipped in water at 50-55 ℃ for 15 min before storage for control of fungus (Irtwange, 2006). Pre–storage hot water dips of fruit at temperatures above 40 ℃ have been shown to be effective in controlling storage decay, not only by reducing the pathogen but also by enhancing the resistance of fruit tissue, influencing host metabolism and ripening.

Different organic acids and salts showed various levels of either protective or therapeutic effect for coated lemon fruits against mould infection whatever the time of their artificial inoculation under in vivo conditions (El-Mougy *et al*., 2008). Potassium sorbate was used to control post-harvest decay caused by many fungi (Palou *et al*., 2001). Potassium sorbate was shown to reduce the incidence of sour rot under laboratory conditions (Smilanik *et al*., 2008 and Embaby *et al*. (2013, II&III). Ascorbic acid. Ascorbic acid (Vitamin C) can be added to the covering brines of processed green table olives to prevent discolouration by oxidation of brines and olives. Benzoic acid, an antimicrobial preservative against yeasts and moulds. Citric acid has antimicrobial activity because of its pH effect. It is used as a synergist to enhance the effectiveness of antioxidants such as ascorbic acid Kailis and Harris (2007).

Aim of this study: Interestingly, this work centered on the *Colletotrichum* sp. fungus (the causal agent of anthracnose disease) associated with olive fruits, affecting fruit production and fruit quality (causing olive fruit decay), pathological and physiological studied of this fungus and to assess the effect of hot water and some alternative fungicide treatments in controlling this disease.

Materials and methods

Colletotrichum was isolated from rotten olive fruits then, identified in Plant Pathology Department, National Research Centre (NRC) based on cultural and morphological characteristics using a light microscope and the available of literature described by Bernstein *et al*. (1995), Litz (1997) Embaby and Abd-Ellatif (2013).

Pathogenicity test: Colletotricum fungus was grown on potato dextrose agar (PDA) at 22±2℃ oC for 10 days. Inoculums' was prepared by flooding dishes with sterile distilled water, scraping the surface gently with a glass rod, and filtering the resulting suspension through sterile cheesecloth. Spore concentration was adjusted to ($1X10^5$) spores' ml-1 in sterile water for inoculation. Healthy of olive fruits were surface sterilized by70% ethanol and washed by rinsing sterilized distilled water for three times and dried with sterilized filter paper, then were slightly injured with sterile needles aseptically and were inoculated by spraying spore suspensions of conidia ($1X10^5$ conidia ml^{-1} water). A hand atomizer was used for spraying the inoculums suspension of each isolate and atomizer was pre sterilized with 90% ethanol before the spraying of the inoculums each time Lakshmi *et al*. (2011); Embaby and Abd-Ellatif (2013). Three replications each containing ten fruits were inoculated. Control treatment was carried out by sterilized water only. All fruits were incubated in moist chambers and covered with perforated polythene bags to

maintain a high humidity necessary for infection and maintained at room temperature. Symptoms appearance was examined. Infected fruits were recorded after 7 days from inoculation. Koch's postulates were proved using tender olive fruits. Colletotricum fungus was re-isolated from infected fruits and its identity was confirmed (Jayasinghe and Fernando, 2009; Lakshmi *et al*., 2011; Embaby and Abd-Ellatif, 2013). Disease developments (percentage of the incidence) were recorded after 7 days from inoculation. The disease incidence of infected olive fruits in relation to the total number of fruits in each replicate according to the following formula (Zeng *et al*., 2006 and Sónia *et al*., 2012).

$$\text{Infection}(\%) = \frac{\text{Number of rotted fruits}}{\text{Total number of tested fruits}} \times 100$$

Physical attributes (Physical parameters)

General physical features of olive fruit are as follows:

Fruit length cm, diameter of size cm, weight from grams, olive shapes include pear, egg and heart, other features include asymmetry, nipples and points. These attributes of quality are measured by applying principles of physics and measuring the response of fruit Stan Kailis and David Harris (2007). Varieties with large fruit size are used for table olive processing. The time of harvesting for table olive cultivars depends on the type of the final product and it is a function of climatic conditions. The determining factors include the following Jeff Atherton *et al.* (2009).

Fruit weight loss %

Fruit weight loss is a basic parameter of quality and has to be measured precisely on digital balance/weighing machine to calculate loss in weight of fruit (Fig, 5). It should be in replicates of 3 fruits. Weight loss was approximated as percent of original weight. For this purpose initial and final weight of the fruits was determined at zero time (Milind, 2008; Abdel and Rashid, 2012; Gautam *et al*., 2012 and Nevarez *et al*., 2012). The percentage of weight loss was calculated using the following formula.

Physiological studies:

Effect of nutrient media: Four types of different media i. e. olive leaf extract medium (OLE), olive fruit extract (OFE), potato dextrose agar (PDA) and mixed medium (OLE and OFE) were tested to determine the effect of nutrients on mycelial growth and Colletotrichum sporulation. Mycelia discs (5mm in size) were cut from the advancing margins of 7 days old culture of the pathogen using a flame-sterilized cork-borer. One 5 mm disk of a pure culture of this pathogen was placed at the center of a Petri dish 9cm containing 10 ml of each medium. Each treatment was replicated three times. The dishes were incubated at 22 ± 2 ℃ for 7 days. Radial of mycelial growth of the pathogen was measured daily along two diameters as the means of the percentage of growth of the dishes for this fungus (Mello *et al*., 2004; Wokocha *et al*., 2010 and Ashwini and Srividya, 2012).

To determine effect of different media on sporulation (Spore density), the plate surface was flooded with 5ml of distilled water and brush in order to release conidia into the water. The resulting spore suspensions were separately filtered through double-layered muslin cloth into sterile test-tubes. The number of spores (conidia) per suspension of the fungus was estimated using a haemocytometer and tally counter (Mello *et al*., 2004 and Wokocha *et al*., 2010). Spore production was quantified and calculated.

Effect of different pH concentrations: To evaluate pH effects on growth rate and sporulation, the pH of PDA medium was adjusted before autoclaving to 3.5, 5.0, 6.0, 6.5, by buffers with the help of 0.1M HCl and sodium hydroxide buffer was used to adjust the pH to 7, 7.5, before sterilizing in an autoclave at 121 ℃ for 20 minutes (Sarkar *et al*., 2011). One 5 mm disk by using a flame-sterilized cork-borer of a pure culture of the pathogen 7 days old culture was placed at the center of a Petri dish containing potato dextrose agar (PDA). Plates were incubated at 22 ± 2 ℃. Each treatment was replicated three times. After 7 days, the mycelium growth diameter of the pathogen was measured. Spore production was quantified using a hemacytometer and calculated (Ding *et al*., 2007; Wokocha *et al*., 2010; Ashwini, and Srividya, 2012).

Light requarment: Light studies were conducted at 22 ± 2 ℃. Comparable plates were incubated under constant illumination (continuous light, CL), 40 Watt: FL 40T9D/38 Fluorescent Lamp, constant darkness (continuous darkness, CD) or a 12 h light/ dark photoperiod (12:12 L: D), CL, 40 Watt: FL 40T9D/38 Fluorescent Lamp. PDA medium were inoculated with 0.5mm disc, 7days old culture of the tested Colletotricum fungus then, divided for three groups with three replicates. First group was incubated with continuous light, CL, 40 Watt: FL 40T9D/38 Fluorescent Lamp, second group

under continuous dark while, third group with light/dark cycle 12/12h. The growth rates were calculated and sporulation density was quantified using a hemacytometer and calculated as maintained (Mello *et al*., 2004).

Control olive fruit decay caused by Colletotricum sp.

Effects of hot water treatments on spore viability in vitro: A tested Colletotrichum fungus was surface harvested after 10 days old culture from incubation. Spores were harvested by adding 9 ml of water sterile from the plate surface with a sterile glass rod, and passing through two layers of cheesecloth to removing the mycelial growth. The suspension was diluted with sterilized water to an optical density (OD). The fungal suspension was adjusted at 2 x 10^5 spores/ml **(**Plaza *et al*., 2004; Zamani *et al*., 2009 and Abd el-Azem, 2013**).**

Spore suspension (0.2 ml) of a concentrated fungus was added to sterilize distilled water in the sterile glass tubes, to achieve a final concentration of 2 x 10^5 spore's ml^{-1}. All sterile glass tubes were placed in water bath at 45, 50, and 55 ℃, and allowed to equilibrate for 5 min. After 5min., tubes were removed from the water bath and placed immediately on ice. Aliquots (5 ml) of the spore suspensions were transferred to Potato Dextrose Agar (PDA) kept in Petri dishes padded with treated spore suspension, and incubated for3 days at 22 ±2^{o}C in darkness. The numbers of colony-forming of viable spores were counted for each treatment after three days of incubation. These treatments included: hot water (45, 50 and 55^{o}C) for 5 min, comparing with un-treated control treatment were recorded (Plaza *et al*., 2004, Fatemi and Hassan, 2011; Abd el-Azem, 2013).

Effects of hot water treatment in vivo: Healthy olive fruits were wounded using sterilized needle. Twelve olive fruits constituted each replicate that included the inoculated wound from each fruit. The wounded sites were inoculated with 20 ml spore suspension of the tested fungus (10^5 spores ml^{-1}), and the fruits were kept for incubation in plastic trays, at 22 ±2^{o}C under humid conditions. After 24 h, the fruit were rinsed in hot water at 55 ℃ for 5min and kept for incubation under the same conditions. The percentage of infected wounds was determined 7 days after inoculation comparing with un-treated control treatment. Following drying (each treatment was separately packed in plastic bags and along with control. At the end of 7 days, appraised parameters including disease incidence, and decreased weight loss of fruits were measured as mentioned before (Fatemi and Hassan, 2011; Abd el-Azem, 2013).

Effect of some alternative fungicides in vitro: A tested Colletotrichum fungus was surface harvested after 10 days old culture from incubation. Spores were harvested by adding 9 ml of water sterile from the plate surface with a

sterile glass rod, and passing through two layers of cheesecloth to remove the mycelial growth. The suspension was diluted with sterilized water to an optical density (OD). The fungal suspension was adjusted to 1 x 10^5 spores/ml **(**Zamani *et al*., 2009); Embaby *et al*. (2013 II&III)**.**

The efficacy of the tested compounds i. e. Ascorbic acid, Benzoic acid, Citric acid and Potassium sorbate on the inhibition growth of Colletotricum was evaluated in vitro. The tested compounds were added to sterilized PDA medium, before solidifying to obtain the proposed concentrations Ascorbic acid, Benzoic acid, Citric acid (6, 8, 10 g/l) and Potassium sorbate (1, 2& 3 g/l) then, gentle rotation was done for five minutes to ensure the equal distribution of the added compound(s). After that, the concentrations were dispensed in sterilized Petri plates (9-cm-diameter). PDA medium free of these compounds was used for the check treatment (Control). All plates were inoculated at the center with discs (5-mm-diameter) of 10 day old culture of Colletotricum. Three replicates were used for each treatment. Inoculated plates were incubated at 22 ± 2^oC. The average linear growth was measured after 5-7 days when the fungi reached full growth in the check treatment. Then, reduction (%) in mycelial growth was calculated in all treatments relative to the fungal growth (9-cm-diameter) in the control one Embaby *et al*. (2013 II&III).

Ability of some alternative fungicides in vivo: Healthy olive fruits were obtained from the field and disinfested with 70% ethanol then, fruits were rinsed three times with sterilized distilled water and blotted dry on sterilized filter paper. The fruits were wounded by a sterilized needle (Abdel and Rashid, 2012). Each treatment consisted of 12 fruits and replicated three times with 4 fruits per replicate. The fruits were dipped individually into the proposed concentrations Ascorbic acid, Benzoic acid, Citric acid (6, 8 &10 g/l) and Potassium sorbate (1, 2 & 3 g/l) for 5 minutes. Fruits were kept at room temperature and allowed to air dry. The fruits were artificially infected by spraying them with spore suspension ($2x10^5$ spore/ml) by collecting the surface growth of 10 days old cultures of *Colletotrecuim* with sterilized brush in sterilized distilled water. Thereafter, all treated fruits were placed into carton boxes, covered with plastic sheets to maintain a relative humidity– RH (90-95%), and stored in a fruit store at room temperature, for 7 days. The percentage of disease incidence (of infection) was calculated periodically after 7 days as previously described. The quality and quantity measurements were determined at the end of the storage period (7 days) under different treatments as previously described. In check treatment, fruits were dipped individually into sterilized water without any chemicals and then inoculated with the pathogenic fungus. Three replicates were used for each treatment as well as cheeks.

Percentages of diseases incidence of decayed fruits were determined Embaby *et al.* (2013 II&III).

Statistical analysis: All data, were set up in a completely randomized factorial design with three replications. Data were analyzed with the analysis of variance (ANOVA) procedure of MSTAT-C program. When significant differences were detected, treatment means were compared using Duncan's Multiple Range test (DMRT) at 5% level of significance (Duncan, 1955 and Samir *et al*., 1999).

Results and discussions

This search was focused on *Colletotrichum* sp. the major causal agent of anthracnose disease and affecting olive fruit quality. Typical symptoms of natural postharvest infection by anthracnose disease on olive fruits are observed as soft circular rots, dark, sunken lesions. (Figs. I&2). Resulting in an orange slimy mass of spores on the fruit surface. Mummified olive fruits were observed when the fruits began to ripen. All or part of the infected fruit starts to rot and dries up, shrivels and becomes mummified. The spores live on in infected, mummified fruit.

Fig. 1. a-Typical symptoms of natural postharvest infection by anthracnose disease on olive fruits are observed as soft circular rots, dark, sunken lesions, b-Produces an orange slimy mass of spores on the fruit surface.

Fig. 2. a- Shrivels and mummified olive fruits were observed when the fruits began to ripen, b- Spores of *Colletotrichum* sp. under light microscope (200x)

Sutton (1992), Litz (1997), Robery (2005) and Kailis and Harris (2007) reported that, the infected fruit will rot and sometimes shrivel before prematurely dropping from the tree. Anthracnose (*Colletotrichum acutatum* and *C. gloeosporioides*) disease causes soft circular rots on the fruit, usually on the shoulder, and at high humidity produces an orange slimy mass of spores on the fruit surface. Orange slimy conidial masses can be formed as the acervulli matured.

Pathogenicity test: *Colletotrichum acutatum* was found to be pathogenic to olive fruit cultivars and causing fruit rot symptoms except for Egizzi-Balady cultivar which was found to be resistant. Data in Table (1) presented that, Zarayr cultivar was the most susceptible cultivar which record 14.o % infection, followed by Manzanillo cultivar 9. 3 % infection while Chimlali (2) cultivar gave 4.7 infection percent. No infected symptoms were appeared with un-inoculated fruits (control treatment). Similar results were reported by Said, (1978) and Hashish (1996).

Table 1. Pathogenicity test of *C. acutatum* (the causal agent of anthracnose disease) after 7 days from inoculation

Tested olive cultivars	Use	Infection%	
		Inoculated fruits	Un- Inoculated fruits
Egizi-Balady	Table	0.00	0.00
Chimlali(2)	Oil	4.7	0.00
Zarayr	Oil	14.o	0.00
Manzanillo	Dual	9.3	0.00

Effect of *Colletotrichum acutatum* on some physical characteristics: Table (2) show that, *C. acutatum* was found to be decreased all physical characteristics measured i.e. weight (g), length (mm), diameter (mm) and volume (ml^3). *C. acutatum* reduced 21.27% of Manzanillo fruit weight, 8.22%

of fruit length, 12.37% of diameter and 22.22% of volume. The reduction percent of Chimlali olive fruits was 8.94% of weight, 6.46% of length, 12.13% of diameter while it did not cause any change in the volume of fruits. *C. acutatum* reduced 23.80% of Zaraye fruit weight, 10.95% of fruit length, 17.11% of fruit diameter and 22.22% of its volume. Egizi-Balady cultivar was found to be resistant to the infection of *C. acutatum*.

Table 2. Changes of some physical characters of olive fruits caused by *C. acutatum* the causal agent of anthracnose disease

Tested olive cultivars	Physical characteristics											
	Weight (g)			Length (mm)			Diameter (mm)			Volume(ml^3)		
	H	I	%L	H	I	%L	H	I	%L	H	I	%L
Chimlali(2)	1.23	1.12	8.94	17.62	16.48	6.46	11.78	10.35	12.13	1.0	1.0	0.00
EgiziBalady	10.76	10.76	0.00	28.13	28.13	0.00	26.56	26.56	0.00	10.6	10.6	0.00
Manzanillo	4.7	3.7	21.27	24.18	22.19	8.22	19.32	16.93	12.37	4.5	3.5	22.22
Zarayr	1.89	1.44	23.80	17.98	16.01	10.95	13.79	11.43	17.11	1.8	1.4	22.22

H: Healthy, I: Infected, %L =Loss

Effect of C. acutatum on some chemical characteristics: Data in Table (3) show that, *C. acutatum* was found to decrease 18% of Manzanillo oil content, while it can be increased the total titratable acidity by 0.0224% and the moisture content of this fruits 12.01%. Oil content of Zarayr fruits was decreased by 18%, while the total titratable acidity of the fruits was increased by 0.002% and the moisture content was increased by 3.34%. *C. acutatum*, decreased oil content of Chimlali (2) fruits by 18%, increased 0.0128 of total titratable acidity and increased 16.7% of moisture content with the infected fruits. EgiziBalady cultivar was found to be resistant to the infection by *C. acutatum.*

Table 3. Changes of some chemical characters of olive fruits caused by *C. acutatum* the causal agent of anthracnose disease

Tested Olive cultivars	Chemical characteristics								
	%Total titratable acidity (TTA)			%Moisture content			%Oil content		
	H	I	% Increase	H	I	%Increae	H	I	%L
Chimlali (2)	0.0128	0.0256	0.0128	37.7	54.4	16.7	46	28	18
EgiziBalady	0.0156	0.0156	0.00	60.87	60.87	0.00	N.F	N.F	T.
Manzanillo	0.015	0.038	0.023	44.2	56.21	12.01	38	20	18
Zarayr	0.030	0.032	0.002	45.27	48.61	3.34	44	26	18

H: Healthy, I: Infected, L%: Loss percent, T=Table

Physiological studies: Type of media: Table (4) presented that, *Colletotrichum acutatum* gave the highest growth rate and sporulation with potato dextrose agar (PDA) medium which record 74.07mm and 3500cfu1 x

102spore's ml-1. Olive leave extract (O.L.E) gave 55.73 and olive fruits extract (O.F.E) 54.19 mm of linear growth respectively with any sporulation formed. Complex medium was less supporting for the linear growth rate of *C. acutatum* that gave 41.69 mm, while it supported the sporulation of the fungus recording (1200 cfu 1 x 102spore's ml-1).

Ashoka (2005) reported that, among fourteen solid media evaluated, maximum radial growth of *C. gloeosporioides* was observed on potato dextrose agar (90.00 mm). The results are in confirmation with that of *C. capsici* Sudhakar (2000); Rani and Murthy,(2004) in case of *C. gloeosporioides.*

Table 4. Effect of different nutrient media on the linear growth rate and sporulation of *C. acutatum*

Type of media	Linear growth (mm)	Spore population (1 x 10^2spore's ml^{-1})
PDA	69.07 A	3500 A
O.L.E	55.73 B	0 C
O.F.E	54.19 B	0 C
Complex medium	35.02 C	1200 B

PDA =Potato dextrose agar, O.L.E=Olive leaves extract, O.F.E= Olive fruit extract, Complex= O.L.E + O.F.E

Effect of pH concentrations: Data in Table (5) show that, pH 6.5 gave the best linear growth rate and sporulation of *C. acutatum* which record 70.15mm linear growth rate and 5400cfu 1 x 102spore's ml-1, followed by pH 7 which gave a linear growth rate of 69.94 mm and a sporulation of (3433 cfu 1 x 102spore's ml-1), PH 6 and PH 7.5 which resulted in moderate growth rateof(67.79 and 62.16mm) and sporulation of (3950 cfu 1 x 102spore's ml-1 and 1917 cfu 1 x 102spore's ml-1) respectively. PH 5 and PH 3.5 recorded the least growth rates (55.72 and 40.9 mm) and sporulation (1900 and 433cfu 1 x 102spore's ml-1) respectively. The same results were obtained by ANOMA *et al*. (2006); Kailis and Harris (2007).

Kanappa (1998) and Biradar (2002) reported that maximum growth of *C. gloeosporioides* was found at an optimum pH of 6.0. Rajak (1983) claimed that, pH of 7.0 was optimum for *C. gloeosporioides.* Whereas, Naik, (1986) observed that maximum growth of *C. gloeosporioides* at a pH of 6.0 and 6.5 respectively.

Table 5. Effect of different pH concentrations on the linear growth rate and sporulation of *C. acutatum*

pH concentration	Linear growth (mm)	Spore population(1 x 10^2spore's ml^{-1})
7.5	62.16 AB	1917 BC
7	69.94 A	3433 AB
6.5	70.15 A	5400 A
6	67.79 A	3950 AB
5	55.72 B	1900 BC
3.5	40.9 C	433 C

Effect of light: Results in Table (6) presented that the highest growth rate and sporulation of *C. acutatum* was recorded with Light/dark cycle treatment (45.06 mm) of linear growth and 2500 cfu 1 x 102spores' ml-1. No differences significant in the linear growth rate and sporulation of *C. acutatum* in the continuous light and continuous dark treatments which gave 33.76 and 39.35 mm of a linear growth rate and 1100 and 1150 cfu1 x 102spore's ml-1 respectively.

Mishra and Siradhana (1980) stated that, disease was more when the pathogen was exposed to diurnal light compared to continuous light or darkness. Kanappa (1998) and Sudhakar (2000) reported that, the exposure of *C. gloeosporioides* to alternate cycles of light and darkness showed maximum growth and sporulation compared to continuous light or darkness.

Table 6. Effect of light/dark treatment on the linear growth rate and sporulation of *C. acutatum*

Treatments	Linear growth (mm)	Spore population(1 x 10^2spore's ml^{-1})
Continuous light	33.76 B	1100 B
Continuous dark	39.35 B	1150 B
Dark/light cycle	45.06A	2500 A

Effect of hot water on spore viability in vitro: Table (7) indicated that all hot water treatments i. e. 45, 50 and 55 ℃ were able to decrease spore viability of *C. acutatum* fungus. Hot water treatment at 55 ℃ gave the best results and reduced spore viability of *C. acutatum* with 84.51 % reduction, followed by 50 ℃ with 59.16% reduction of spore viability. Hot water treatment at 45 ℃ was less effective resulting 22.54 %. The same results were stated by Lemessa *et al*. (2004); Irtwange (2006); Inkha (2009); Abd el Azym (2013).

Table 7. Reducing spore viability of *Colletotrichum acutatum* (the causal agent of anthracnose disease) by hot water treatment *in vitro*

Parameter	Treatments ℃		
	45 ℃	50 ℃	55 ℃
Total colony	55 B	29 C	11 D
Loss of colony	16	42	60
% spore viability	77.64	40.84	15.49
% Reduction	22.54	59.16	84.51
Un-treated	71 A		

Effect of hot water treatment in vivo: Table (8) presented that, hot water treatment at 55 ℃ for 5 minutes was successful in reducing the percentage of disease incidence of anthracnose with the inoculated olive fruits. Infection percent of Chimlali olive fruits was reduced from 33.43 to zero percent and gave hundred percent of fruit protection. The disease incidence of Zarayr olive fruits was decreased from 100% to 33.43 %, followed by Manzanillo olive fruits which decreased from 83.33 % to 50%. No infected symptom appeared with EgiziBalady. The same results were reported by Lemessa *et al*. (2004); Irtwange (2006); Inkha (2009); Abd el Azym (2013).

Table 8. Reducing the linear growth rate of *Colletotrichum acutatum* (the causal agent of anthracnose disease) by using some alternative fungicides *in vivo*

Tested cultivar	Use	Untreated		Treated		Mean
		T.I.F	%	T.I.F	%	
EgiziBalady	Table	N.F e	0.00	N.F e	0.00	0 D
Chimlali (1)	Oil	2 d	33.34	0 e	0.00	1 C
Zarayr	Oil	6 a	100.00	2 d	33.33	4 A
Manzanillo	Dual	5 b	83.33	3 c	50.0	4 A
Mean		3 A		1B		

T.I.F= Total of infected fruits, N.F= Not Found

Effect of some alternative fungicides on the linear growth rate in vitro: Data in Table (9) indicated that all alternative substrates were able to reduce the linear growth rate of *C. acutatum*. Both Ascorbic and Benzoic acid were the best alternative substrate used, which gave complete inhibition of the linear growth rate (100%) at the three concentrations used, followed by Potassium sorbate which recorded 72.03% at 1 g/L, 87.8% at 2 g/L and 100% at 3 g/L reduction of the linear growth rate respectively. Citric acid was less effective which record 37.3% at 6 g/L, 90.44% at 8 g/L and 100% at 10 g/L reduction of Colletotrichum linear growth rate respectively. Embaby *et al*. (2013, II&III)

reported that, all tested alternative fungicides were significantly able to reduce the growth rate of all tested fungi compared with control. Benzoic acid was the most effective substrate in *vitro*.

Table 9. Reducing the linear growth rate of *Colletotrichum acutatum* (the causal agent of anthracnose disease) by using some alternative fungicides *in vitro*

Substrae	Concentrations									Mean
	C1			C2			C3			
	Growth (mm)	Loss (mm)	% R	Growh (mm)	Loss (mm)	%R	Growh (mm)	Loss (mm)	%R	
A	0.00 f	73.59	100	0.00 f	73.59	100	0.00 f	73.59	100	18.39 C
B	0.00 f	73.59	100	0.00 f	73.59	100	0.00 f	73.59	100	18.39 C
C	56.40 b	17.19	23.23	44.8 c	28.79	38.99	0.00 f	73.59	100	43.69 A
P	15.92 d	57.67	78.36	5.44 e	68.15	92.60	0.00 f	73.592	100	23.73 AB
Mean	18.08 AB			12.56 BC			0.00 C			
Control	73.59mm A									

A =Ascorbic acid, B= Benzoic acid, C =Citric acid, P=Potassium sorbate, R =Redution C1,2 &3 Concentrate

Effect of some alternative fungicides in vivo: Results in Table (10) show that, Benzoic acid gave complete inhibition of the disease infection with all olive cultivars tested compared with untreated control treatment. Also, complete inhibition of disease incidence was recorded with Ascorbic acid with Egizi Balady, Manzanillo and Zarayrolive fruit cultivars while gave 16.66% infection percent with Chimlali (2) cultivar. Potassium sorbate was less efficient and record 50.0% infection percent with Chimlali (2) cultivar while completely prevented the infection of EgiziBalady, Manzanillo and Zarayr cultivars. Citric acid was the less effective which gave 50.0% and 16.66% infection percent with Chimlali (2) and Zarayr cultivars respectively while inhibited the infection of the other cultivars totally.

Embaby *et al*. (2013, II&III) reported that, all alternative fungicides were found to significantly decrease fungal decay (disease incidence compared with untreated control. Data also showed that benzoic acid substance was higher effective than others. Alternative fungicides were also found to significantly decrease disease severity, compared with untreated control. Benzoic acid substance was higher significantly effective in controlling disease severity compared with other substances followed by potassium sorbate and ascorbic acid while citric acid was less effective respectively.

Table 10. Reducing the percentage of anthracnose disease incidence (caused by *Colletotrichum acutatum*) by using some alternative fungicides *in vivo*

substrate	Conc (g/L)	Table Egizi-Balady		Oil Chimlali (2)		Oil Zarayr		Dual Manzanillo		Mean
		T.I.F	%	T.I.F	%	T.I.F	%	T.I.F	%	
A	10	0 e	0.00	1 d	16.66	0 e	0.00	0 e	0.00	0 D
B	6	0 e	0.00	0 e	0.00	0 e	0.00	0 e	0.00	0 E
C	10	0 e	0.00	3 c	50.0	1 d	16.66	0 e	0.00	1 B
P	3	0 e	0.00	3 c	50.0	0 e	0.00	0 e	0.00	0 C
Control		0 e	0.00	4 b	66.66	6 a	100.00	4 b	66.66	3 A
Mean		0 D		2 A		1 B		1 C		

T.I.F= Total of infected fruits, A =Ascorbic acid, B= Benzoic acid, C =Citric acid, P=Potassium sorbate

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References

Abdel Wahab, S. M. and Rashid, A. S. (2012). Safe post-harvest treatments for controlling Penicillium moulds and its impact maintaining navel orange fruits quality. American-Eurasian Journal of Agricultural & Environmental Sciences 12:973-982.

Abd, E. A. and Faten, S. (2013). Studies on citrus rotted fruit in Egypt. (Master's thesis). Botany Department, Faculty of Science, Benha University Egypt.

Ashoka, S. (2005). Studies on fungal pathogens of vanilla with special references to *Colletotrichum gloeosporioides* (penz.) Penz. and Sacc. (Ph.D. thesis). UAS, Dharwad.

Ashwini, N. and Srividya S. (2012). Study of mycolytic enzymes of bacillus sp. Against *Colletotrichum gloeosporioides* causing anthracnose in Chili research article. Acta Biologica Indica 1:81-89.

Bernstein, B., Zehe, E. I., Dean R. A. and Shabi, E. (1995). Characteristics of Colletotrichum from peach, apple, an agar block technique pecan and other hosts. Plant Disease 79:478-482.

Biradar, S. V. (2002). Studies on *C. gloeosporioides* (Penz.) Penz. and Sacc., botryodiplodia theobromae pat. And fusarium semitectum berk. And rav. Causing fruit rot of custard apple (annona squamosa l.). (Master's thesis), University of Agricultural Sciences, Dharwad.

Cacciola, S. O., Faedda, R., Sinatra, F., Agosteo, G. E., Schena, L., Frisullo, S. and Magnano di San Lio, G. (2012). Olive anthracnose. Journal of Plant Pathology 94:1.

Ding, Y., Jian J., Chu, F., Zhen, G. U., and Zhuang, Q. J. (2007). Effect of different factors on growth and spore production of a *colletotrichum* sp., a Potential Biocontrol Agent for Water hyacinth. Journal of Aquatic Plant Management 45:129-131.

Duncan, D. B. (1955). Multiple range and multiple "f" tests. Biometrics 11:1-42.

El-Mougy, N. S., El-Gamal, N. G. and Abd-El-Kareem, F. M (2008). Use of organic acids and salts to control postharvest diseases of lemon fruits in Egypt. Archives of Phytopathology and Plant Protection 41:467-476.

Embaby, E. M. and Abd-Ellatif Amany, A. (2013). Species identification of Colletotrichum the causal agent of strawberry anthracnose and their effects on fruit quality and yield losses. Journal of Applied Sciences Research 9:3845-3858.

Embaby, E. M., Hazaa, M., Hagag, L. F., El-Sayed, T. I. and Abd El-Azem F. S. (2013). Decay of some citrus fruit quality caused by Fungi and their control: II- Control Alternaria rot or core rot decay by using some alternative fungicides. Journal of Applied Sciences Research 9:5671-5678.

El-Sayed E. M., Hazaa, M., Hagag, L. F., El-Sayed, T. I. and Abd El-Azem F. S. (2013). Decay of some citrus fruit quality caused by fungi and their control: III- Control blue and green mould decay by using some alternative fungicides. Journal of Applied Sciences Research 9:5086-5096.

Fatemi, S. and Hassan, B. (2011). The effect of physical treatments on control of *penicillium digitatum* decay orange cv. Valencia during storage period. African Journal of Agricultural Research 6:5757-5760.

Hashish, A. M. (1996). Studies on fungal rot of olive fruit and its control. (Ph. D. thesis). Faculty of Agricultural Zagazig University.

Hegde, Y. R. and Hegde, R. K. (1986). Studies an anthracnose of arecanut (*Areca catechu* L.) caused by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. Plant Pathology Newsletter 4:24-27.

Inkha, S., Boonyakita, D and Srichuwong, S. (2009). Effect of hot water treatment on green infection in tangerine fruit cv. Sai Num Pung. Cmu Journal of Natural Sciences pp. 1-8.

Irtwange, S. V. (2006). Hot water treatment: a non-chemical alternative in keeping quality during postharvest handling of citrus fruits. Agricultural engineering international: the CIGR E journal. Invited Overview. 5 pp.

Jayasinghe, C. K. and Fernando, T. H. P. S. (2009). First report of *colletotrichum acutatum* on *mangifera indica* in Sri Lanka. Ceylon Journal of Science (Biological Sciences) 38:31-34.

Kailis, S. and Harris, D. (2007). Producing table olives. Published by National Library of Australia Cataloguing-in-Publication. 328 pp.

Kanappa, B. C. (1998). Epidemiology and control of anthracnose of diseases of coffee incited by *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (Master's Thesis). University of Agricultural Sciences, Bangalore.

Lakshmi, B. K. M., Reddy, P. N. and Prasad, R. D. (2011). Cross-infection potential of *Colletotrichum gloeosporioides* Penz. Isolates Causing Anthracnose in Subtropical Fruit Crops. Tropical Agricultural Research 22:183-193.

Larrigaudiere, C., Pons, J., Torres, R. and Usall, J. (2002). Storage performance of clementines treated with hot water, sodium carbonate and sodium bicarbonate dips. Journal of Horticulture, Biology.

Lemessa, F., Shunli, W., Agravante, J., Amuoh, C. and Kiyimba, F. (2004). Effect of hot water treatment on the quality of citrus fruit. Unpublished group project report. International research and development course in postharvest biology and technology, the volcani center, Israel. Postharvest Biology and Technology 18:235-244.

Litiz, R. E. (1997). The mango botany, production and uses. Common wealth agricultural buareaux, international, library of congress cataloging-in-publication data. pp. 257-280.

López-Escudero, F. J. and Mercado-Blanco J. (2010). Verticillium wilt of olive: a case study to implement an integrated strategy to control a soil-borne pathogen. Plant Soil.

Mello, A. F. S., Andressa, C. Z. M. and Ivan, P. B. (2004). Development of *Colletotrichum gloeosporioides* isolated from green pepper in different culture media, temperatures, and light regimes. Scientia Agricola 61:542-544.

Mishra, A. P. and Siradana, B. S. (1980). Utilization of carbon, nitrogen and vitamins by *colletotrichum graminicola* isolates. Journal of Turkish Phytopathology 9:107-114.

Moral, J., Bouhmidi, K. and Trapero A. (2009). Influence of fruit maturity, cultivar susceptibility, and inoculation method on infection of olive fruit by *Colletotrichum acutatum*. Plant Disease 92:1421-1426.

Munoz, A. M., Garcia, B. L., Candelas, A. V. and Marcos, J. F. (2011). Comparative analysis of the sensitivity to distinct antimicrobials among *penicillium* spp. causing fruit postharvest decay. Phytopathol Meiterr 50:392-407.

Naik, K. S. (1986). Studies on blight of coriander (*coriandrum sativum* linn.) Caused by *colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. (Master's thesis), University of Agricultural Sciences, Dharwad.

Nelson, T. and Marases (1983). Fusarium species: an illustrated manual for identification. London: The Pennsylvania State University Press University Park. pp. 142-150.

Palou, L., Smilanick, J., Usuall, J. and Vinas, I. (2001). Control of postharvest blue and green moulds of oranges by hot water, sodium carbonate, and sodium bicarbonate. Plant Dis., 85:371-367.

Plaza, P., Usall, J., Torres, R., Abadias, M., Smilanik, J. and Vinas (2004). The use of sodium carbonate to improve curing treatments against green and blue moulds on citrus fruits. Pest Manag Sci. 60:815-821.

Rajak, R. C. (1983). Nutritional requirements of two species of colletotrichum. India: Acta Botanica 11:155-160.

Rani, S. G. and Murthy, K. M. K. (2004). Cultural and nutritional characteristics of *colletotrichum gloeosporioides*, the causal organism in cashew anthracnose. Journal of Mycology and Plant Pathology 34:317-318.

Robert, S. H. (2005). Sustainable pest and disease management in australian olive production. A report for the rural industries research and development corporation. RIRDC Publication.

Samir, D., Ron P., Lea C., Batia W., Boris S., Sonia, P. H. and Shimon, M. (1999). Suppressing green mold decay in grape fruit with post-harvest jasmonate application Journal of the American Society for Horticultural Science 124:184–188.

Saied, K. (1978). Studies on spots of leaves and fruits disease of olive in the A. R. E. and its control. (Master's thesis). Zagazig University.

Sarkar, S., Girisham S. and Reddy, S.M. (2011). Cellulase activity in fungal infected Banana fruits: Effect of different synthetic media on cellulase production. Journal of Recent Advances in Applied Sciences (JRAAS) 26:12-18.

Sergeeva, V., R. Spooner-Hart, N. Nair (2008). Evidence of early flower infection in olives (Olea europaea L.) by Colletotrichum acutatum and C. gloeosporioides causing anthracnose disease. Australasian Plant Disease Notes, 3:81-82.

Sergeeva, V. (2010). Grapevine and olive diseases in australia. Petria -13th congress of the mediterranean phytopathological union 20:67-633.

Sistani, F., Ramezanpour S. S. and Nasrollanejadd S. (2009). Field evaluation of different fungicides application to control olive leaf spot. Australian Journal of Basic and Applied Sciences 3:3341-3345.

Smilanick, J. L., Mansour, M. F., Mlikota, Gabler, F. F. and Sorenson, D. B. (2008). Control of citrus post-harvest green mould and sour rot by potassium sorbate combined with heat and fungicides. Post-harvest Biology and Tecknology 47:226-238.

Sónia, G., Eunice B., Paula M. L., Teresa C. and Henrique, G. P. (2012). Infection process of olive fruits by *Colletotrichum acutatum* and the protective role of the cuticle and epidermis. Journal of Agricultural Science 4:2.

Sudhakar, S. (2000). Biology and management of stylosanthes anthracnose caused by colletotrichum gloeosporioides (penz.). Penz. and Sacc. (Master's thesis). University of Agricultural Sciences, Dharwad.

Sutton, B. C. (1992). The genus glomerella and its anamorph colletotrichum. In: colletotrichum: biology, pathology and control. In Bailey, J. A. and Jeger, M. J. (Eds.). Wallingford: CAB Int. pp. 1–26.

Wokocha, R. C., Nneke, N. E. and Umechuruba, C. I. (2010). Screening *Colletotrichum gloeosporioides* f. Sp. Manihotis isolates for virulence on cassava in akwaibom state of nigeria. Agro-science journal of tropical agriculture, Food, Environment and Extension 9:56–63.

Zamani, M., Sharifi T. A., Ahmadzadeh1, M., Housseininaveh, V. and Mostofy, Y. (2009). Control of *penicillium digitatum* on orange fruit combining pantoea agglomerans with hot sodium bicarbonate dipping. Australisian Plant Pathology 37:605-608.

Zeng, K. F., Cao, J. K. and Jiang, W. B. (2006). Enhancing disease resistance in harvested mango (*mangifera indica* l. Cv. "Matisu") fruit by salicylic acid. Journal of the Science of Food and Agricultur 86:694-698.

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