

CONTROL OF *LASIODIPLODIA THEOBROMAE* ON AVOCADO IN EGYPT

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ABSTRACT: This study was performed during 2015-2016 seasons to identify fungal species associated with dieback, grafting failure process during vegetative propagation in commercial nursery and stem-end rot of avocado (*Persea americana* Mill.). *Lasiodiplodia theobromae* (syn. *Botryodiplodia theobromae*, *Diplodia natalensis*) was the most pathogenic as well as the most frequently isolated fungi whereas, the other isolated fungi (*Alternaria solani*, *Fusarium moniliforme*, *F. solani*, *Aspergillus niger* and *Rhizopus stolonifer*) were negligible. Hass and Fuerte were the most susceptible cultivars to dieback and stem-end rot diseases, followed by Eitinger, Duke and Reed, while Bekon and Benkerton recorded the lowest percentages of infection. Hass and Fuerte were susceptible to grafting failure process. All tested fungicides suppressed completely the avocado dieback infection when shoots were sprayed at the same time of inoculation with *L. theobromae*, followed by 7 days pre inoculation. On the other hand, spraying the avocado shoots 7 days post inoculation with *L. theobromae* was the least effective treatment in controlling the disease under greenhouse conditions. Dipping the scions in the tested fungicides before dipping it in spore suspensions of *L. theobromae* during grafting increased the percentage of success of grafted seedlings. Dipping avocado fruit after harvest in the tested fungicides before placing mycelial agar discs (5 mm diameter) of *L. theobromae* into a hole on the pedicel (stem) that was made with a 5-mm-diameter cork borer reduced the infection of stem-end rot. Generally, KemaZed, Amistar Top, Topsin M70 and Occidor were the most effective in controlling the avocado three diseases. This study confirms that stem-end rot and failure grafting followed by dieback of avocado can be initiated by fungus causing branch dieback and stresses the importance of controlling branch dieback.

Key words: Avocado, cultivars, dieback, grafting failure process, stem-end rot, *Lasiodiplodia theobromae*, fungicides.

INTRODUCTION

There are 57 avocado (*Persea americana* Mill.) producing countries in the world. The main producing countries in the world are Mexico, U.S.A, Colombia and Indonesia, with 36.8 % of the world production in Mexico. The land that is currently destined for the growing of avocado totals 348,769 ha, producing 3,583,226 tons a year (FAO, 2013). The avocado area is still small in Egypt, although, it was stated that avocado was introduced into Egypt between 1870 and 1880 as a botanical specimen (Milad, 1936). Old trees are very scarce in Egypt and nobody appreciated their existence. In late years, El-Qanater El-Khairia Horticultural

Research Station made fresh production of avocado commercial cultivars including Hass, Fuerte, Duke and others. The nutritional and medicinal uses of avocado have been confirmed through numerous studies. (Elosaily, 2015) in Egypt, reported that avocado fruit and its pericarp fixed oil are usually considered as efficient components of healthy antioxidant diets. Essential oils of all cultivars showed good antimicrobial activities against *Streptococcus mutans* and *Salmonella typhimurium* (IC50 values, 0.4-20 μ L/mL). The mesocarp was found to contain higher amounts of vitamins A, C and E.

Dieback caused by *Lasiodiplodia theobromae* is the most important disease

attacking Avocado. (Sutton ,1980) has adopted the name *Lasiodiplodia theobromae*. *Lasiodiplodia theobromae* (syn. *Botryodiplodia theobromae*, *Diplodia natalensis*) is the pycnidial state of *Botryosphaeria rhodina*, a pathogen that causes cankers and dieback in many woody and herbaceous species. Dieback symptoms appear at different stages of growth kills twig, shoot, branch and trunk wood causing great reduction in yield of diseased fruitful trees. It often attacks plants weakened by environmental stress or other pathogens and has caused death of many infected plants (Burgess *et al.*, 2003).

Death of scions is a severe disease during propagation by grafting of avocado in El-Qanater El-Khairia Horticultural Research Station. Pathogenicity data showed that *Lasiodiplodia theobromae* is the causal agent of graft failure process of avocado. The two tested avocado cultivars Hass and Fuerte which inoculated onto the binding site with *Lasiodiplodia theobromae* were highly susceptible to infection with failure grafting seedlings and dieback. This may be the first study that provides isolation and pathogenicity data about a disease caused by *L. theobromae* during the avocado grafting process.

Stem-end rot of avocado fruit caused by *Lasiodiplodia theobromae* develops in the field, as well as after harvest during storage or shipment. The fungus enters through mechanically injured areas on the stem or skin. The fungus grows from the pedicel into a circular black lesion around the pedicel. Stem-end rot symptoms are dark spots with defined edges on the epidermis of the fruit that emanate from the stem end. The affected areas becomes soft and watery .

Lasiodiplodia theobromae was identified as branch cankers and dieback, dieback of avocado grafts during vegetative propagation in commercial nursery and stem-end rot of fruit on avocado trees in Egypt. In pathogenicity test, *Lasiodiplodia theobromae* has been associated with

symptoms of dieback, necrosis at the binding site of grafting and stem- end rot. Hass cv. is the most popular and potential cultivar found to be highly susceptible to the three diseases. *Lasiodiplodia theobromae* has been associated with symptoms of dieback and necrosis at the binding site of grafting on cashew (*Anacardium occidentale* L.) propagative material (Freire *et al.*, 2002) and grapevine (Aroca *et al.*, 2008) , as well as stem-end rot in guava (*Psidium guajava* L.) (Cardoso *et al.*, 2002), dieback in citrus (Davis *et al.*, 1987). The objective of this study was to evaluate the preventative ability of some commercial fungicides against fungus associated with avocado dieback, grafting failure process and stem-end rot.

MATERIALS AND METHODS

1- Disease severity of avocado dieback in orchards, percentage of the grafted failure seedlings in nursery and percentage of stem-end rot were evaluated from April to August 2015 and 2016 seasons in orchards, commercial nursery in El-Qanater El-Khairia Horticultural Research Station, Agricultural Research Centre.

1- a- Disease severity of avocado dieback in orchards.

Five symptomatic trees nearly uniform in size on seven avocado cultivars *i.e.* Hass , Fuerte , Duke , Reed, Eitinger ,Bekon and Benkerton were chosen randomly as replicates and sampled for branch dieback in order to calculate the disease severity (DS) according to the modified scale of (Barakat *et al.*,1990) as follows:

$$DS = \frac{\sum n \times V}{5N} \times 100$$

Where:

n = the number of diseased branches in each infected category.

V = Numerical value of the grade as follows:

0 = No infection (healthy).

1 = 1.0 - 10 % infection.

2 = 10.1 - 25 % infection.

3 = 25.1 – 50 % infection.

4 = 50.1 – 75 % infection.

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5 = 75.1 – 100 % infection.

N = Total number of the branches inspected.

5 = Maximum disease severity grade.

1- b- Percentage of the grafted failure seedlings in avocado nursery.

In April 2015 and 2016, twenty five seedlings were chosen randomly as five replicates on two avocado cultivars, *i.e.* Hass and Fuerte to evaluate the grafted failure seedlings during adaptation in commercial nursery. Seedlings were grafted during February. Data were evaluated after 45 days from grafting.

1- c- Percentage of stem-end rot in avocado fruit.

In August 2015 and 2016 five symptomatic avocado trees nearly uniform in size were chosen randomly as replicates on seven avocado cultivars, *i.e.* Hass, Fuerte, Duke, Reed, Eitinger, Bekon and Benkerton to investigate the percentage of stem-end rot.

2-Isolation and identification of the associated fungi.

Isolations were made from symptomatic material of diseased avocado collected from tip dieback of branches, grafted plants with scion dieback symptoms and diseased fruits. Samples were washed thoroughly with tap water then small pieces (4–5 mm) of tissue were taken from the margin between necrotic and apparently healthy tissue sterilized by dipping in (0.1%) sodium hypochlorite for 3 min., followed by rinsed several times in sterilized distilled water and dried between two pieces of sterilized filter paper and placed in petri dishes with potato dextrose agar culture medium (PDA). The dishes were incubated at 25°C for seven days. Hyphal tip and single spore techniques were used to purify the associated organism on PDA slants. Most frequent fungal colonies were purified and transferred to new petri dishes with PDA. Fungal isolates were grown onto (PDA) plates or slant and maintained at 5°C in a

refrigerator for further studies. Stock cultures were routinely sub-cultured on fresh slant or plates every 6-8 weeks. Pure culture of the isolated fungi was identified (through culture characteristic and microscopic observation).

3- Pathogenicity test.

a- Pathogenicity test on branches.

The pathogenic capability of the isolated fungi *L. theobromae*, *A. solani*, *Fusarium moniliforme*, *F. solani*, *A. niger* were done in Plant Pathology Greenhouse by using wooden toothpicks. Wooden toothpicks were autoclaved for 20 minutes, at 121°C, and transferred occasionally as triangle around the inoculum disk of the isolated fungi on PDA plates. After incubation for one week, inoculated toothpick were removed and inserted into avocado green shoots (for 3 -5 mm depth). Twenty seedlings two-year old were used as four replicates. The same number of avocado seedlings was inoculated with only autoclaved toothpicks (un- inoculated) and served as control. Seedlings were incubated at normal temperature, dieback and/or the average length of necrotic (mm) of each inoculated site was measured and data was recorded. Koch's postulates were confirmed by re-isolation of the original pathogen from artificially inoculated shoots (Swart *et al.*, 2003).

b-Pathogenicity test during grafting.

The operation conducted in this section was according to (Gaser, 2007) with minor modification. The inoculum for the pathogenicity tests for scions infection were prepared by adding 10 mL of sterile distilled water into (Ø9cm) sterilized petri dishes containing PDA medium and pure cultures of *each fungus alone* and scooping with a heat sterilized spatula to dislodge the conidia. The conidia suspension was filtered through an 8 layer cheese cloth. The concentration of conidia suspension for each isolate was adjusted to 1×10^6 conidia /mL using a haemocytometer. A surfactant (Tween 20) was added to the conidia suspensions to

give a more uniform suspension of spores that will spread more evenly over the plant surface. Before grafting, rootstocks were disbudded with a sharp knife, with the exception of the bottom buds un-removed. Grafting was done in February. Scions of avocado Hass and Fuerte cvs. wood was cut 12 cm long. Scions and rootstocks were immersed on the spore suspensions of each fungus for 20 minutes before joined. Each treatment contains four replicates and each replicate consists of five grafted seedlings. The same number of scions from each cultivar were dipped in the sterile distilled water and served as control. The rootstocks and scions were joined together and tying with poly-ethylene. %Seedlings with failure grafting (scions breakdown and collapse followed by dieback) were calculated after 45 days from grafting.

c- Pathogenicity test on fruit.

For each isolate, twenty Hass fruit as four replicates freshly harvested in August by clipping were surface disinfected by dipping into a (0.1%) sodium hypochlorite solution for 1 min, rinsed with water, and allowed to air-dry. Mycelial agar discs (5 mm diameter) from the edge of actively growing cultures were placed into a hole on the pedicel (stem) that was made with a 5-mm-diameter cork borer. After 15 days, fruit were examined and % disease incidence and % disease severity were calculated. Small pieces of the flesh removed from the margin of the rot fruits were placed on PDA. Fungal colonies were compared morphologically to those of isolates used in the inoculations (Smith *et al.*, 2012).

Disease control.

I- Effect of some fungicides on dieback of avocado seedlings.

Under greenhouse in 2015 and 2016 seasons, seven fungicides were used in this experiment *namely* *Kemazed* (carbendazim), *kocide 2000* (copper hydroxide), *Amistar Top* (Difenoconazole + Azoxystrobin), *Topsin M70* (Thiophanate

methyl), *Antracol* (dithiocarbamate), *Occidor* (benzimidazol) and *Coprotech* (copper oxychloride) with recommended commercial doses were evaluated to control avocado seedlings dieback. Sterilized atomizer filled with 500 mL of each tested fungicides. Avocado seedlings, two years' old (Hass cv.) were inoculated by using toothpick with *L. theobromae* as described in pathogenicity test. Twenty seedlings were used as 4 replicates for each treatment. Sprayed with tested treatment were done at the same time and pre or post 7 days of inoculation. 20 seedlings were sprayed with distilled water and served as control. Average length of necrotic dieback infection (mm) of inoculated seedlings was calculated after 15, 21, 35 and 45 days from inoculation.

II- Efficiency of some fungicides on the percentage of succeeded avocado grafted seedlings.

In February 2015 and 2016, scions were chosen healthy in uniform (1cm thick and 12cm tall) from Hass trees (the most susceptible cultivar), (around 20 years). Under greenhouse, twenty avocado scions of Hass cultivar for each treatment as four replicates were prepared to grafting on the same number of rootstocks. Immediately before pathogen inoculation, scions were dipped in one of each seven tested fungicides for one minute and left to air-drying for one minute. Scions were artificially inoculated with dipping separately in the spore suspensions of *L. theobromae* for 5 seconds. Twenty avocado scions were inoculated with tested pathogenic fungus, then grafted on the same number of rootstocks without any treatments where served as control. Both scions and rootstock were wrapped with polyethylene strips and kept under control for adaptation. The results as the percentage of grafting successes were recorded after 45 days from grafting. The efficiency of each treatment was calculated as follows:

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$$\% \text{ Efficiency} = \frac{A - B}{A} \times 100$$

Where:

A= number of inoculated seedlings (control).

B= number of inoculated and treated seedlings (treatment).

III- Effect of some fungicides on % stem-end rot of avocado fruit.

In August 2015 and 2016 seasons, post harvest dipping trials were carried out on Hass fruit to determine the effect of seven fungicides on the incidence of stem-end rot, compared to fruit left untreated or dipped in water. Twenty Hass fruit as four replicates per treatment were surface disinfected by dipping into a (0.1%) sodium hypochlorite solution for 1 min, rinsed with water, and allowed to air-dry. Mycelial agar discs (5 mm diameter) from the edge of actively growing cultures of *L. theobromae* were placed into a hole on the pedicel (stem) that was made with a 5-mm-diameter cork borer. The following day, dipping treatments on the tested fungicides were done. The same number had been left untreated or treated with water only as control treatment. Once fruit had been dipped they were air dried and then re-packed into fruit boxes before being moved to a controlled environment ripening room set to 25°C and 80% relative humidity. After 12 days, fruit were examined and %disease incidence was calculated (Smith *et al.*, 2012).

Statistical analysis:

The experiment was arranged in a randomized complete blocks design and the obtained data were subjected to analysis of variance and significant differences among means according to Snedecor and Cochran (1984). In addition significant differences among means were distinguished according to the Duncan multiple test range.

RESULTS AND DISCUSSION

1- Symptoms on trees in groves and on seedlings in nursery.

The dieback disease on branches is characterized by drying of twigs and branches followed by complete defoliation, which gives the tree an appearance of scorching by fire. Tip die back disease occurs on the branches/ trunk of infected trees that start drying slowly at first and suddenly branches become completely dried /killed resulting gummy substance oozes out or remains hanging on the tree Fig. 1 (a & b). Avocado branch canker has long been known as *Dothiorella* canker because the pathogen most often isolated was known as *Dothiorella gregaria* Sacc. (teleomorph *Botryosphaeria ribis*) (Halma, and Zentmyer, 1953). Symptoms of avocado branch canker can occur on twigs, branches, or trunks. Canker infections are initiated by spores entering the host plants through fresh wounds such as pruning wounds, mechanical injuries, or injuries resulting from sunburn or frost damage (McDonald and Eskalen, 2011). Cankers on avocado may exude brownish red or reddish sap that dries to form a whitish-beige powder. The bark and xylem under the canker is killed and turns red-brown to brown. In older cankers, the dead bark can be easily removed, and if the xylem is colonized, leaves quickly turn brown but remain attached Fig. 1 (c). This may lead to the collapse of branches or entire trees. Canker infections become severe following stress caused by drought, flooding, insect attack, nutrient deficiencies, or any other factor that weakens the tree (Menge and Ploetz, 2003).

Dieback appeared in recently grafted plants, wilting and death of apical bud followed by dieback, gradual drying and cracking of the bark from the apex and moving down toward the base of the scion, developed 25 day after grafting. The union of the graft presented longitudinal extended necrosis. The vascular tissue of diseased scions showed hard texture (mummification of xylem and cortex) with a dark gray to black necrosis. When this happens, the flow of nutrients from the rootstock to the top growth (scion) becomes limited or stops completely and the top growth will gradually or suddenly deteriorate. Thirty days after

grafting, abundant light gray mycelial growth was observed covering most of the scion and the graft union Fig. 1 (f & g).

Decay from stem-end rot begins as slight shrivelling around the stem button. Fungal mycelium is often visible on fruit if the button is removed. Conspicuous dark decay with a well-defined margin develops at the stem end. As fruit ripens, decay spreads and rots the entire fruit, which becomes dark and shrivelled. Depending on the causal organisms, flesh may be watery and soft, or initially dry and corky, becoming watery later as secondary organisms colonize tissue Fig. 1 (c, d & e). Stem-end rot of avocado has been reported from all major production areas worldwide (Menge and Ploetz, 2003). (Johnson *et al.*, 1991) suggested that stem-end rot pathogens are endophytes (fungi growing inside the vascular system), living in the stems, branches and pedicels of the avocado canopy, which grow into the fruits

to initiate the stem-end rots. In commercial avocado production, stem-end rot mostly occurs during transit, in the packinghouse, and during or after marketing (Twizeyimana *et al.*, 2013). Stem-end rot are generally a relatively minor problem of avocados in California due to low relative humidity and rainfall during much of the growing season and harvest time (Faber *et al.*, 2008.). (Aaron *et al.*, 2006) reported that Stem-end rot is a post harvest disease that occasionally causes losses under suboptimal storage conditions. Fruit rot starts on the stem end and generally initiates as the fruit ripens or after harvest. Small, purplish-brown spots appear and can enlarge to involve the entire fruit surface. The pathogen invades the flesh causing tissue discoloration, degradation, and offensive odors, thus compromising the quality of the fruit.



Figure1. (a & b) avocado branches showed naturally typical dieback symptoms. (c) dieback appeared in shoot and fruit.(d & e) stem-end rot appeared on fruit in the field, small purplish-brown spots appear and can enlarge to involve the entire fruit surface. The spots gradually enlarge until much of the surface may be involved. (f) Natural dead scion after grafting. (g) Dieback appeared in the rootstock after grafting failure.

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2- %Disease severity of avocado dieback in orchards, %grafted failure seedlings in nursery and %stem-end rot.

Dieback disease, failure in grafted seedlings in nursery and stem-end rot were found in all examined cultivars during the two successive growing seasons 2015 and 2016 (Table1). The results show significantly differences in disease severity percentage between avocado cultivars. Hass and Fuerte cvs. were the most susceptible cultivars which recorded the highest percentage of %disease severity and %stem-end rot, followed by Eitinger, Duke and Reed, while Bekon and Benkerton cultivars recorded the lowest percentage of infection. The disease symptoms of failure in grafting and occurring dieback in seedlings were observed in the two examined cultivars. In this respect, Hass cv. was the most susceptible one which recorded (32.00 and 28.00) %failure in grafted seedlings in the two successive seasons respectively, while Fuerte cv. was the least susceptible which recorded (28.00 and 24.00) %failure in grafted seedlings in the two successive seasons respectively. Generally, Hass cv. was the most

susceptible cultivar for dieback disease, failure in grafted seedlings in nursery and stem-end rot while Benkerton was the least susceptible one. These data suggest that *L. theobromae* is a natural pathogen causing branches dieback during prune and causing grafting failure process when penetrates the vascular tissue through wounds or cuts made by the propagator or grafter. The fungus enters through mechanically injured areas on the stem or skin and grows from the pedicel into a circular black lesion around the pedicel. This coincides with (Fourie and Halleen, 2004 and Abo Rehab *et al.*, 2013) who affirm that the infected scions and rootstocks are the source of inoculum of pathogens that cause diseases during the vegetative grapevine propagation. (Tovar *et al.*, 2013) reported that dieback of mamey sapote (*Pouteria sapota*) grafts is the most important disease during vegetative propagation in commercial nurseries. *L. theobromae* was associated with this disease in 97% of necrotic rootstocks and scions samples from a nursery. (Darvas and Kotze, 1981) reported that Fuerte fruit proved the most susceptible and Ryan the least.

Table 1: Natural infection of dieback, failure in grafted seedlings and stem-end rot during two successive seasons 2015 and 2016.

Cultivars	%Dieback disease severity		% failure in grafted seedlings		% stem- end rot		Mean	
	2015	2016	2015	2016	2015	2016	2015	2016
Hass	25.30A	23.96A	32.00A	28.00A	15.13A	12.23A	24.14	21.39
Fuerte	22.74B	21.04B	28.00B	24.00B	13.14B	11.51B	21.29	18.85
Duke	20.21C	18.62C	-	-	12.11BC	10.40BC	16.16	14.51
Reed	17.52D	16.26D	-	-	11.94BC	9.91C	14.73	13.08
Eitinger	21.12BC	20.22B	-	-	12.86B	10.75BC	16.99	15.48
Bekon	15.52E	13.03E	-	-	10.85C	8.32D	13.18	10.67
Benkerton	12.27F	11.27F	-	-	8.44D	6.38E	10.35	8.82

- = not detected

3- Isolation.

Isolation from infected branches, seedlings and fruits yielded six species representing five fungal genera. *L. theobromae* recorded the highest frequency percentages in two seasons, followed by *F. moniliforme* while the other pathogens *A. solani*, *F. solani*, *A. niger* and *Rhizopus stolonifer*, respectively recorded low percentages of frequency (Table 2). Diseases caused by species of *Botryosphaeriaceae* have been reported in California from avocado (Horne, 1932 and McDonald and Eskalen, 2011), almond (English *et al.*, 1996), grapevine (Urbez-Torres, 2006), pistachio (Michailides, 1991) and in México from mamey sapote (Tovar *et al.*, 2013). In Egypt from grapevine (Abo Rehab *et al.*, 2013) reported that *Phomopsis viticola* was the most frequent pathogen isolated from grafted failure seedlings, followed by *Lasiodiplodia theobromae*. Whereas, the other isolated fungi (*Phoma sp.*, *Fusarium solani* and *Alternaria solani*) were negligible. *Lasiodiplodia theobromae* generally recovered in greater numbers from stem-end rot of avocado fruit than other fungi and are often associated with severe rots (Rowell, 1983). Stem-end rot pathogens are present in living and dead branches, twigs, leaves, and in living pedicels (Hartill and Everett 2002).

4- Pathogenicity test.

a- Pathogenicity test on branches.

Data in (Table 3 and Fig. 2) show that, *L. theobromae* was able to induce avocado

dieback after 45 days from inoculation. Necrotic length (mm) was increased by increasing incubation period from 15-35 days before appearance typical dieback symptoms after 45 days from inoculation, while *F. moniliforme* and *F. Solani* were able to exhibit avocado twigs necrotic (necrosis) only, but a typical dieback disease symptoms did not appear. However, *A. solani* and *Aspergillus niger* were not able to exhibit necrosis or dieback symptoms in artificially inoculated avocado twigs. Observed symptoms in inoculated seedlings included brown, necrotic bark lesions around the inoculation sites extending upwards and downwards, leading to wilting and drying of the apical as well as the terminal leaves. Cracking of the stem cortex was observed and fungal structures (stromatic pycnidia and mycelium) developed on the necrotic lesions around the inoculation sites. Under the outer cortex, necrotic xylem vessels and brown discoloration extended along the length of the stems (Fig. 2c). These results are in agreement with (Abd El Aziz *et al.*, 2010) who reported that mango seedlings inoculated with *Lasiodiplodia theobromae* alone showed typical symptoms of the disease. No symptoms were observed on plants inoculated with other fungi. *Lasiodiplodia theobromae* caused dieback while the remaining pathogenic fungi caused necrotic area beginning from the site of artificial inoculation and vary in the necrotic parts.

Table 2: Fungi and their frequency (%) isolated from avocado branches, grafted seedlings and fruits naturally infected with dieback disease.

Isolated fungi	Frequency (%)							
	2015			2016			Mean	
	Branches	Grafted seedlings	Fruits	Branches	Grafted seedlings	Fruits	2015	2016
<i>L. theobromae</i>	58.34	51.39	56.94	55.56	54.17	55.56	55.56	55.09
<i>A. solani</i>	8.33	12.50	16.66	11.11	13.89	15.28	12.50	13.43
<i>F. moniliforme</i>	22.22	25.00	15.28	18.05	19.44	13.88	20.83	17.12
<i>F. solani</i>	8.33	9.72	2.78	11.11	12.50	4.17	6.94	9.26
<i>A. niger</i>	2.78	1.39	4.17	4.17	-	4.17	2.78	2.32
<i>Rhizopus stolonifer</i>	-	-	4.17	-	-	6.94	1.39	2.78

-= not detected

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Table 3: Necrotic length around inoculated stem and dieback (mm) on Hass cv. seedlings inoculated by the tested fungi.

Isolated fungi	Necrotic length and dieback (mm) after inoculation days							
	15		21		35		45	
	Necrotic length	Dieback (mm)	Necrotic length	Dieback (mm)	Necrotic length	Dieback (mm)	Necrotic length	Dieback (mm)
<i>L.theobromae</i>	10.00	0.00	24.75	0.00	60.50	0.00	90.25	111.00
<i>A. solani</i>	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00
<i>F. moniliforme</i>	3.50	0.00	5.25	0.00	12.50	0.00	20.25	0.00
<i>F. solani</i>	2.50	0.00	2.75	0.00	5.00	0.00	8.00	0.00
<i>A. niger</i>	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

LSD at 0.5% for:

Necrotic (N) = 0.490
N x F = 0.422
N x F x D = 1.549

Fungi (F) = 0.548
N x D = 0.693

(Days) D = 1.096
F x D = 0.775



Figure 2. a - Morphology of isolated fungus *L. theobromae* on PDA culture colonies were initially white, soon becoming black and fast-spreading with immersed and superficial, branched, septate mycelium. Shiny black pycnidia were produced on the surface.

b-inoculated avocado seedlings using tooth pick method.

c- Typical dieback symptoms of avocado seedling after 45 days from stem inoculation showed wilting and death of leaves in apical region of an inoculated plant.

e- Failure grafted seedlings at the beginning of adaptation period compared with control (d).

f- Inoculated fruit with *L. theobromae* mycelial agar discs (5 mm diameter) from the edge of actively growing cultures.

b- Pathogenicity test during grafting:

Pathogenicity test of two avocado cultivars during grafting showed that Hass and Fuerte cultivars seedlings which inoculated with *L. theobromae* were collapse and breakdown and recorded (100% and 90%) failure grafting seedlings, respectively and recorded (110.00 and 100.75mm) dieback respectively (Tabel 4). All failure seedlings exhibited distinguished symptoms as grafted area is turned into a brown to black and oblique separation occurred between scion and rootstock. Fungal growth was seen at the grafting area. Necrosis appeared in recently grafted seedlings. Wilting and death of apical bud followed by dieback, gradual drying and cracking of the bark from the apex and moving down toward the base of the scion, developed 15 d after grafting. Longitudinal extended necrosis was presented in local union of the grafting. The vascular tissue of diseased scions showed hard texture (mummification of xylem and cortex) with a dark gray to black necrosis. 30 days after grafting, abundant light gray mycelial growth was observed covering most of the scion and the graft union after that dieback appeared in seedlings (Fig 2 e) compared with control (Fig 2 d). While *F. moniliforme* and *F. Solani* were able to exhibit % Seedlings with failure grafting, but they were not able to exhibit Seedlings with

dieback (mm). However, *A. solani* and *Aspergillus niger* were not able to exhibit %Seedlings with failure grafting or dieback symptoms. Wilting symptoms in apical buds, dieback of scions, and abundant grayish mycelial growth at the graft union were observed at 45 d of the inoculated plants. Rapid dieback after inoculation of grafted plants was probably due to the fungus infection during the formation of new vascular tissue in the rootstock and the scion, and prior to lignifications, which facilitated the development of this pathogen. The deep and extensive cuts made in the scions for the purpose of propagation probably facilitated pathogen penetration to vascular tissue (Ploetz *et al.*, 1996). Submersed and erumpent pycnidia were observed on the graft union area. These findings correlate with the symptoms reported in grafted plants infected with *L. theobromae* (Cardoso *et al.*, 2002). Dieback of mamey sapote [*Pouteria sapota*] grafts significantly reduces the success of grafting in Mexican nurseries .Pathogenicity and histopathological data showed that *Lasiodiplodia theobromae* is the causal agent of dieback of mamey sapote grafts (Tovar *et al.*, 2013). This may be the first study that provides a better understanding of a disease caused by *L. theobromae* during the grafting process of avocado.

Table 4: pathogenicity test on two avocado cultivars during grafting.

Cultivars	Hass		Fuerte	
	%Seedlings with failure grafting	Seedlings with dieback (mm)	%Seedlings with failure grafting	Seedlings with dieback(mm)
<i>L. theobromae</i>	100.00A	110.00A	90.00A	100.75A
<i>A. solani</i>	0.00D	0.00B	0.00C	0.00B
<i>F. moniliforme</i>	25.00B	0.00B	20.00B	0.00B
<i>F. solani</i>	10.00C	0.00B	5.00C	0.00B
<i>A. niger</i>	0.00D	0.00B	0.00C	0.00B

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c- Pathogenicity trial on fruit

(Table 5) show that after 15 days , *L. theobromae* was able to induce (100 and 75%) disease incidence and (56% and 42.25%) disease severity on Hass and Benkerton cultivars, receptively .While *A. solani*, *Rhizopus stolonifer* and *A. niger* exhated (15,10 %,10,10% and 10, 5 %) disease incidence and (6.25, 4.50%,4.75,3.25% and 3 , 2.25%) disease severity on Hass and Benkerton cultivars, receptively. However *F. moniliforme* and *F. solani* were not able to exhibit disease incidence and disease severity on Hass and Benkerton cultivars, receptively. Inoculations with *L. theobromae* only resulted in decay from stem begins as slight shriveling around the stem button. Fungal mycelium are often visible on fruit if the button is removed. Conspicuous dark decay with a well-defined margin develops at the stem end. As fruit ripens, decay spreads and rots the entire fruit, which becomes dark and shriveled. irregularly shaped, roughly circular black brown lesions appeared on the surface of the fruit (Fig 2 f).([Darvas and Kotze,1981](#)) reported that in tests on fruits of 4 cultivars *Rhizopus stolonifer*, *Botryodiplodia theobromae* and *Colletotrichum gloeosporioides* [*Glomerella cingulata*] were the most pathogenic of 9 stem-end rot isolates. In the United States, *B. Dothidea* was reported as the causal pathogen of stem-end rot (Menge and Ploetz, 2003). (McDonald and Eskalen, 2011) found that

the majority of fungi that cause avocado tree branch cankers are the same as those that lead to stem-end rot.The present study proved that *L. theobromae* associated with avocado branch dieback, grafting failure seedlings and stem-end rot .

5- Control.

I- Effect of some fungicides on dieback of avocado seedlings:

Data in (Table 6) show that time of application markedly affected the efficiency of the fungicides. All tested fungicides were effective in controlling avocado dieback infection when shoots were sprayed at the same time of artificial inoculation with *L. theobromae* where the average of dieback infected areas were zero at all incubation periods which ranged between 15-45 days ,followed by sprayed 7 days pre artificial inoculation with *L. theobromae*. On the other hand, spraying the avocado shoots 7 days post inoculation with *L. theobromae* was the least effective treatment in controlling the disease under greenhouse conditions in the two succesive seasons. Data also show that, spraying pre 7 days from inoculation exhibited more suppression than spraying post 7 days from inoculation. *Kemazed*, *Amistar Top* and *Topsin M70* proved to be the highly effective in reducing the Length of necrotic area (infected part mm), followed by *Occidor* and *Antracol* while *Kocide 2000* and *Coprotech* were the least effective ones.

Table 5: pathogenicity test of two avocado cultivars fruits.

Cultivars	Hass		Benkerton	
	%Disease incidence	Disease %severity	%Disease incidence	%Disease severity
<i>L. theobromae</i>	100.00A	56.00A	75.00A	42.25A
<i>A. solani</i>	15.00B	6.25B	10.00B	4.50B
<i>F. moniliforme</i>	0.00D	0.00D	0.00D	0.00D
<i>F. solani</i>	0.00D	0.00D	0.00D	0.00D
<i>A. niger</i>	10.00C	3.00C	5.00C	2.25C
<i>Rhizopus stolonifer</i>	10.00C	4.75BC	10.00B	3.25C

Table 6: Effect of different fungicides on the length of infected parts (mm) in Hass cv. inoculated with *L. theobromae* under greenhouse conditions.

Time of treatment	Fungicides	2015						2016					
		Length of necrotic area (infected part mm) after inoculation days											
		15	21	35	45	Mean	%reduction	15	21	35	45	Mean	%reduction
Control		10.00	24.75	60.50	90.25	46.37	-	10.50	25.50	64.25	93.0	48.31	-
7days pre -inoculation	<i>Kemazed</i>	2.00	4.50	7.00	8.25	5.43	88.28	2.00	5.25	7.25	9.50	6.00	87.58
	kocide 2000	6.00	9.50	11.50	14.00	10.25	77.90	5.50	9.00	11.75	14.00	10.00	79.30
	Amistar Top	2.25	4.25	7.00	9.50	5.75	87.59	2.00	4.50	6.50	9.00	5.50	88.62
	Topsin M70	3.00	5.50	8.00	10.00	6.62	85.72	4.00	7.00	8.75	12.00	7.93	83.58
	Antracol	4.00	6.25	10.50	12.75	8.37	81.94	5.75	9.25	11.00	13.00	9.75	79.82
	Occidor	3.00	7.50	10.00	12.00	8.12	82.48	4.25	6.50	9.00	12.25	8.00	83.44
	Copprotech	5.50	9.00	11.50	13.50	9.87	78.71	6.25	9.50	11.75	14.00	10.38	78.51
At the time	<i>Kemazed</i>	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
	kocide 2000	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
	Amistar Top	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
	Topsin M70	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
	Antracol	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
	Occidor	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
	Copprotech	0.00	0.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	0.00	0.00	100.00
7dys post -inoculation	<i>Kemazed</i>	5.75	9.25	11.50	14.00	10.12	78.17	6.25	10.25	11.75	14.00	10.56	78.14
	kocide 2000	6.75	12.75	15.75	19.25	13.62	70.62	11.00	13.25	16.00	20.50	15.18	68.57
	Amistar top	6.25	9.75	11.75	14.00	10.43	77.50	7.25	11.25	12.50	15.00	11.50	76.19
	Topsin M70	7.00	9.50	13.50	16.50	11.62	74.94	7.75	12.25	14.25	16.50	12.56	74.01
	Antracol	9.25	11.00	13.75	17.00	12.75	72.50	9.75	12.00	16.25	18.75	14.18	70.64
	Occidor	7.75	9.50	14.00	16.75	12.00	74.12	9.00	12.25	15.0	16.50	13.18	72.72
	Copprotech	7.00	13.00	16.25	20.00	14.06	69.67	11.50	13.75	16.75	20.75	15.68	67.54

LSD at 0.5% for:

In 2015 :

Time (T) = 0.2642

Fungicides (F) = 0.3051

Days (D) = 0.4315

T x F = 0.5285

T x D =0.7474

F x D =0.8630

T x F x D = 1.9440

in 2016 :

Time (T) = 0.2617

Fungicides (F)= 0.3022

Days (D) =0.4274

T x F = 0.5234

T x D = 0.7403

F x D = 0.8548

T x F x D = 1.9330

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II- Efficiency of some fungicides on the percentages of succeeded avocado grafted seedlings and stem-end rot.

In 2015 and 2016 trials, dipping the scions of Hass cv. in each of the used fungicides suspensions before dipping it in spore suspensions of *L. theobromae* led to suppress spore formation of the pathogen during grafting and increased the percentage of success of grafted plants in all seedlings compared to control (untreated). Amistar Top, Topsin M70, Kemazed and Occidor were the most effective treatments for controlling grafting failure. Antracol and Coprotech exhibited moderate effect while kocide 2000 was the least effective fungicide (Table 7). The obtained results of *in vivo* trails could be discussed in light the findings of (Abo Rehab *et al.* 2013) who found that Topsin M 70% and Kemazed gave the best results for controlling fungal pathogens cause grape graft failure.

Dipping avocado fruit after harvest in solutions containing the tested fungicides, compared to fruit left untreated or dipped in water reduced the infection of stem-end rot. Topsin M70, Amistar Top, Kemazed and Occidor resulted in the lowest levels of stem-end rot, followed by kocide 2000 and Antracol, while Coprotech was the least effective fungicides. These results could be interpreting in light the findings of (Muirhead *et al.*, 1982) who found that mature unripe Fuerte avocado fruit from 2 sources were dipped in carbendazim (1 g/litre) controlled stem-end rot, the percentages of disease-free fruit from the 2 sources were increased from 1 to 90%.

This study is thought to be the first in this attitude in Egypt. This confirms that stem-end rot and failure grafting followed by dieback of avocado can be initiated by fungus causing branch dieback and stresses the importance of controlling branch dieback.

Table 7: Efficiency of some fungicides against of *L. theobromae* on Hass cultivar during grafting and stem-end rot.

Treatments	2015						2016					
	%failure grafted seedlings			% stem-end rot			%failure grafted seedlings			% stem-end rot		
	C *	T*	E*	C**	T**	E**	C *	T*	E*	C**	T**	E**
Kemazed	20	1	95	20	2	90	20	1	95	20	1	90
Kocide20 00	20	3	85	20	3	85	20	2	90	20	3	85
Amistar Top	20	0	100	20	1	95	20	0	100	20	1	95
Topsin M70	20	1	95	20	0	100	20	0	100	20	1	95
Antracol	20	2	90	20	3	85	20	2	90	20	3	85
Occidor	20	1	95	20	2	90	20	2	90	20	3	85
Coprotech	20	2	90	20	4	80	20	2	90	20	3	85

C*=(control): number of failure untreated scions. T* = number of failure treated scions with fungicides. E*= % efficiency.

C**=(control):number of inoculated and untreated fruit. T**= number of inoculated and treated fruit with fungicides. E**=% efficiency.

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مكافحة لاثيوذيبلوديا ثيوبرومي على الأفوكادو في مصر

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أجريت هذه الدراسة في موسمي ٢٠١٥-٢٠١٦ لتعريف الفطريات المسببة لموت الأطراف، فشل عملية التطعيم في المشتل أثناء الأقامة، عفن قاعدة الثمار في الأفوكادو (بيرسيا أمريكانا) . ولقد وجد أن فطر لاثيوذيبلوديا ثيوبرومي (بتريوديبلوديا ثيوبرومي) أكثر الفطريات قدرة على أحداث الإصابة بالثلاثة أمراض بجانب أنه أكثر الفطريات تكرارا في العزل بينما كانت الفطريات (ألترناريا سولاني ، فيوزاريوم مونيليفورم ، فيوزاريوم سولاني ، إسبرجيلس نيجر ، ريزويس ستولونييفر) أقل الفطريات تكرارا في العزل وإحداث القدرة المرضية . وإختلفت أصناف الأفوكادو في قابليتها للإصابة بالثلاثة أمراض فلقد وجد أن صنفى هاس وفيورت أعلى الأصناف قابلية للإصابة يليهما إيتينجر وديوك وريد بينما بيكون وبينكرتون أقل الأصناف قابلية للإصابة بمرضى موت الأطراف وعفن قاعدة الثمار. وكان هاس وفيورت أكثر قابلية للإصابة بفشل عملية التطعيم أثناء الأقامة بالمشتل . كل المبيدات أزلت المرض تماما عندما تم رشها في نفس وقت العدوى بفطر لاثيوذيبلوديا ثيوبرومي المسبب لمرض موت الأطراف يليها الرش قبل العدوى بالفطر بسبعة أيام. وعلى الجانب الآخر رش أفرع الأفوكادو بعد العدوى بسبعة أيام كان أقل المعاملات كفاءة في مكافحة المرض تحت ظروف الصوبة. وعند غمس الأقسام المجهزة للتطعيم في المبيدات قبل غمسها في معلق جراثيم فطر لاثيوذيبلوديا ثيوبرومي أدى إلى زيادة نسبة النجاح في الشتلات المطعومة . وعند غمس الثمار بعد الحصاد في المبيدات قبل وضع قطعة الحقن لفطر لاثيوذيبلوديا ثيوبرومي في حفرة ٥ مم أنقصت الإصابة بعفن قاعدة الثمار . وعموما كيمازد ، أميستار توب ، تويسين إم ٧٠، أنتراكل كانت أكثر المبيدات كفاءة في مكافحة أمراض الأفوكادو الثلاثة. ويعتقد أن هذه الدراسة هي أول دراسة في مصر والتي أثبت بها أن مرضى عفن قاعدة الثمار وفشل عملية التطعيم على الأفوكادو يسببة الفطر المسبب لموت الأطراف ويؤكد أهمية مكافحة مرض موت الأطراف .