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Efficacy of various compounds as pruning wound protectants against mango die back disease.

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Abstract

Mango (*Mangifera indica L.*) is one of the most important fruits cultivated in Egypt. Dieback of mango is among several diseases responsible to low crop production. Dieback disease has become one of the most severe problems on all mango cultivars at El Qanater El Khairia Horticultural Research Station Farm, Agricultural Research Centre. Mango cultivars were differed in their reactions against the disease. Hindi Sennara, Alphonso, Ewais and Keitt were the highly susceptible while, Langra Benares, Fagr Kelan and Naomi were the most resistant ones. Isolation trails from die-backed mango twigs/branches confirmed that *Lasiodiplodia theobromae* (*Syn: Botryodiplodia theobromae, Diplodia natalensis*) was more frequently isolated fungus than others. Pathogenicity tests proved that *L. theobromae* is a causative fungus of this disease. Pruning does not always make a clean cut. Wounds caused by pruning may provide an excellent entry point for infection and often the branches are shattered. Lateral branches grow from below the pruning wound, but then often die, so wound protection is the recommended control strategy. Field trials were conducted in 2015 and 2016 by spraying ten treatments with various compounds as pruning wound protectants after pruning diseased Hindi Sennara cv. twigs/branches. All treatments decreased number of infected twigs/branches and increased number of fruits compared to control. The best treatments were T4 (pruning + Kema Zed), T3 (pruning +Topsin M 70) and T6 (pruning + Aliette) which recorded less number of infected twigs/branches and more number of fruits, compared to T1 (control).

Key words: mango, pruning, dieback, *L. theobromae*, treatments and fruit yields.

Introduction

Mango (*Mangifera indica L.*) is a popular and an economically important fruit crop in Egypt introduced from Bombay, India in 1825. In Egypt, it is cultivated along the Nile valley and some desert areas (Abdalla *et al.* 2007). It is grown over an area of 265,350 feddans with an annual production of 927,352 tons (Anon., 2016). All the plant parts, namely, trunk, branch, twig, leaf, flower and fruit are subjected to attack with a number of diseases at all stages of its development from nursery till consumption of fruits. Some of the diseases are very severe on plants and have become a limiting factor in profitable mango orcharding (Rawal, 1998). Among the wide range of destructive fungal pathogens are members of the Botryosphaeriaceae. Botryosphaeriaceae is a genus-rich family in the Dothidiomycetes, containing numerous species with a cosmopolitan distribution (Crous *et al.* 2006). *Lasiodiplodia theobromae (Syn: Botryodiplodia theobromae*), a member of Botryosphaeriaceae, has been reported as a mango pathogen worldwide associated with several disease symptoms including dieback, stem-end rot, decline, gummosis and canker, in Australia (Slippers *et al.* 2005), Brazil (Costa *et al.* 2010), Egypt (Ismail *et al.* 2012). The disease on the tree may be noticed at any time of the year but it is most conspicuous during April – June. Various factors play significant roles in the predisposition of mango trees to attack by dieback such as mechanical injuries, mineral deficiencies and environmental factors (Ploetz *et al.* 1996).Winter pruning helps to devigorate the trees, control tree size and shape, promote shoot-flush during fruit set, prompt early fruit development, delay leaf hardening and minimizing branch dieback by removing inoculum from the canopy. Pruning does not always make a clean cut and wounds occur on the bark surface of twigs and branches may provide an excellent entry point for pathogenic fungus. Lateral branches grow from below the pruning wound, but then often die. Increasing the economic importance of dieback disease caused by *L. theobromae* led to study this disease. The present study has been conducted to find out the occurrence of the disease at El-Qanater El-Khairia Horticultural Research Station Farm, Agricultural Research Centre. All mango cultivars suffered from dieback disease especially twigs and branches. The problem was therefore taken-up on hand to ascertain the real cause of disease and their management. *L. theobromae* was the most frequently isolated fungi of all the isolates in the 2015 and 2016 seasons. Limited successes in controlling mango dieback emphasize the need and importance of developing effective alternative control strategies. This research evaluated some various compounds for their ability to protect pruning wounds against infection with mango dieback disease. Treating the pruning infected branches with wound protectants, timed to provide

optimum protection, may then ensure effective control of this damaging disease of mango.

Materials and Methods

1-Susceptibility of mango cultivars to dieback disease.

Disease incidence% and disease severity% were assessed during 2015 and 2016 seasons on twelve mango cultivars (15- year old) i.e. Hindi Sennara , Alphonso, Langra Benares, Naome, Zebda, Ewais, Keitt, Montakhab El-Qanater, Sedeek, Mestkawy,Timor and Fagri Kelan at El-Qanater El-Khairia Horticultural Research Station Farm, Agricultural Research Centre. The farm had a history of mango dieback where the trees are showing severe disease symptoms. Five symptomatic trees (15-year old) nearly uniform in size were chosen randomly as replicates and sampled for twigs/branches dieback in order to calculate the disease incidence and disease severity.

The disease incidence (D.I %) was measured by the following formula:

D.I % = (Number of infected twigs/ branches ÷ Total number of inspected twigs/ branches) × 100. Disease severity (D.S. %) was measured according to the modified scale of Wicks and Davies (1999) as follows:

$$D.S.\ \% = \Sigma\ n \times V \div 5\ N$$

Where:

n = The number of diseased twigs/branches in each infected category.

V= Numerical value of the grade as in Table 1.

N = Total number of the inspected twigs/branches.

5 = Maximum disease severity grade.

Table 1.Dieback disease scoring chart.

Disease score/level	Disease aspects on infected twigs/branches
0	0% (no infections on twigs/branches)
1	1.0 - 10 % (slight infection)
2	10.1 - 25 % (twigs/branches¼ infected)
3	25.1 – 50 %(twigs/branches ½ infected)
4	50.1 – 75 %(twigs/branches¾ infected)
5	more than75% (twigs/branches dying up or dead)

2-Isolation and identification of the causal organism(s).

From April to June 2015 and 2016, samples of diseased twigs/branches of tested mango cultivars were collected then transferred to the laboratory for the isolation of associated pathogens. In this respect, samples were thoroughly washed under running tap water, cut into small pieces 3–5 mm Ø between the healthy and infected tissues, then surface sterilized with dipping in 0.1% sodium hypochlorite solution for 2 minutes, followed by three subsequent washings of sterile distilled water. The surface sterilized pieces were blotted dry on sterilized filter paper, and transferred individually to petri dishes, each containing 20 ml potato dextrose agar (PDA) medium, then incubated at 25°C for 5 days and inspected for fungal growth. The developed fungal colonies were purified using hyphal tip (Brown, 1924) and single spore (Zhang *et al.* 2013) techniques. The purified fungi were identified according to their morphological characteristics as described by Punithalingam (1976) and Barnett and Hunter (2006). Fungal isolates were grown onto potato dextrose agar (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.

3- Pathogenicity test.

Pathogenicity test was carried out under greenhouse conditions on transplants (one-year old) of cv. Hindi Sennara. Seeds obtained from an apparently healthy trees were surface-sterilized with 5% sodium hypochlorite solution and sown in plastic pots (26-cm Ø) containing sterilized sandy-loam soil. In each experiment, apparently healthy looking plants were selected while the infected plants were excluded in this study. Twelve transplants were used as four replicates for each isolate and arranged in a complete randomized design. The epidermis of the stem was disinfected with 70 % ethanol, washed with sterile distilled water and left to dry. Wooden toothpicks were autoclaved for sterilizing, then transferred occasionally as triangle around the inoculum disk of the isolated fungi on the prepared PDA plates prior to inoculation with an equal disc (5mm Ø) of each one of the tested fungi for each particular PDA plate then left to grow for one week at 25°C. Inoculated toothpicks were removed and inserted into mango shoots (for 3 mm. depth). Twelve seedlings were inoculated with sterilized woody toothpicks free of inoculants as control treatment. The inoculated shoots were covered with plastic bags to maintain the relative humidity around inoculation site of each one of the tested fungi to allow the fungal isolates to grow well for 48 hrs. . All transplants were kept under greenhouse conditions and watered as needed. Data were recorded as the average length of necrotic area (mm) at 15, 25, 35 and 45 days post inoculation with the tested fungi. Koch's postulates were confirmed by re-isolation of the original pathogen from artificially inoculated twigs/branches.

4. Field experiment:

4.1. Protection of pruning wounds.

This trial was designed in the mango farm at El-Qanater El-Khayria Horticultural Research Station, Agricultural Research Center where hand-pruning is common during 2015 and 2016 seasons to study the effect of ten treatments as pruning wound protectants on incidence of twigs/branches dieback and number

of fruits under field conditions. The orchard revealed an increase of dieback problem where trees showed severe disease symptoms. Mango trees cv. Hindi Sennara (15- year old) were chosen in this experiment. Five symptomatic trees nearly uniform in size were chosen randomly as replicates per each treatment. Before the first spray four main branches representing four geographical directions of each tree were chosen and labeled to perform pruning of diseased twigs/branches in December. The trees were pruned to eliminate dead twigs and branches, the cuts were made, 5 cm bellow the visible infected area of these branches. Ten treatments (Table 2) i.e. four fungicides (Topsin M 70, Kema Zed, Ridomil Gold MZ and Aliette), two locally biofungicides (Bio Zied and Bio Arc), two pure-grades of natural oils (Jojoba oil and Thyme oil), sterilized clippers with ethanol and untreated control were thoroughly sprayed four times with 15 days interval on branch pruning wounds in the field to minimize spreading of pathogen. Jojoba oil (*Simmondsia chinensis L.*) was obtained from Harraz Factory for Natural Oils, Cairo- Egypt and Thyme oil (*Thymus vulgaris L.*) was obtained from Elcaptain Company for Natural Oils, Cairo- Egypt were stored in dark glass bottles at 4°C and dissolved in 5% gum solution and 5% Tween 20. As for T1 and T2 treatments, ten symptomatic trees were pruned, five of them (clippers without sterilizing) served as control treatment and the others (clippers sterilized with 70 percent ethanol) and all sprayed with water. All treatments were dissolved in water to get a final concentration of recommended dose.

Table 2. Application program and test treatments

Treatment No	Trade name	Active ingredient* or Botanical name**	Usage dosage/100L water
T1	Pruning only (untreated control)	H_2O*	--
T2	Pruning + sterilized clippers with ethanol	C_2H_5OH*	--
T3	Pruning +Topsin M 70	Thiophanate methyl *	65g
T4	Pruning + Kema Zed	Carbendazim*	75g
T5	Pruning + Ridomil Gold MZ	Metalaxyl M+Mancozeb*	250g
T6	Pruning + Aliette	Fosetyle almonium*	250g
T7	Pruning + Bio Zied	*Trichoderma album**	250g
T8	Pruning + Bio Arc	*Bacillus megaterium**	250g
T9	Pruning + Jojoba oil	*Simmondsia chinensis L* **	500g
T10	Pruning + Thyme oil	*Thymus vulgaris* L**	500g

Efficiency of each treatment on mango trees was evaluated by calculating the number of infected twigs/branches after pruning per treated and untreated trees as follow:

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

Where:
A= number of infected twigs/branches per untreated trees.
B= number of infected twigs/branches per treated trees.

Regular and uniform cultural practices were followed and average number of fruits was calculated before harvest by counting the fruits of four main branches/ tree representing all geographical directions on treated and untreated (control) trees. Increment% for each treatment in relation to the control was calculated according to the following formula:
Increment% of average number of fruits/ tree = average number of fruits in the treatment - average number of fruits in the control/ average number of fruits in the control ×100.

Statistical analysis.

The experiment was arranged in a randomized complete design and the obtained data were subjected to analysis of variance and significant differences among means according to Snedecor and Cochran (1980). In addition, significant differences among means were distinguished according to the Duncan's multiple test range (Duncan, 1955).

Results

1-Susceptibility of mango cultivars to dieback disease.

In this trail, twelve different mango cultivars were evaluated for their susceptibility to natural dieback infection. Dieback disease was observed in all examined cultivars during the two successive growing seasons 2015 and 2016 as clear in Table 3. Clear significant variations in the assessed disease reactions (D.I% and D.S %) were recorded among the inspected cultivars. Among the twelve cultivars,

none of the cultivars showed resistant to dieback disease. Hindi Sennara , Alphonso, Ewais and Keitt were the highly susceptible cultivars while, Sedeek, Zebda, Mestkawy, Montakhab El-Qanater and Timor were the moderately susceptible ones. Whereas, the lowest average of dieback disease incidence and severity was recorded on Langra Benares , Fagr Kelan and Naomi. On the other hand, the highest percentages of disease severity and dieback incidence were scored during seasons 2016.

Table 3. Natural infection of mango dieback during the two successive seasons 2015 and 2016.

Cultivar	Season 2015		Season 2016	
	D.I.%	D.S.%	D. I. %	D. S. %
Hindi Sennara	31.25A	24.75A	33.00A	25.10A
Alphonso	31.00A	22.90B	32.25A	23.50AB
Langra Benares	19.50G	10.35GH	22.75E	12.30G
Naomi	15.00H	7.70I	16.50F	8.80H
Zebda	24.75DE	15.80E	26.75BC	18.95D
Ewais	30.00AB	21.95B	32.00A	23.10AB
Keitt	28.25BC	20.00C	29.00B	21.10C
Montakhab El-Qanater	22.50EF	13.00F	23.25DE	14.75EF
Sedeek	26.25CD	17.65D	27.25BC	19.60CD
Mestkawy	24.25DE	15.40E	25.50CD	16.35E
Timor	21.25FG	11.80FG	23.25DE	13.50FG
FagrKelan	16.00H	8.80HI	18.25F	9.60H

D.I= disease incidence D.S= disease severity

2- Isolation and identification of the causal organism(s).

Data in Table 4 reveal that the isolated fungi from diebacked mango branches during seasons 2015 and 2016 were identified as *L. theobromae, Fusarium solani, Alternaria alternata* and *Aspergillus niger* which belonging to four different genera and species. Of these, *L. theobromae* was the most abundant fungus which isolated from all samples. The fungus initially produced cottony white fluffy growth on potato dextrose agar, which covered the Petri dish (90 mm) within 4 to 5 days in moist condition. The growth was depressed in the center (around the point of inoculation) and fluffy, raised in the rest of the area, which touched to lid of the dish. After 2 days colour of the colony changed from white cottony to olivaceous dark. Under the lower surface of the dish, completely blackish background was observed. Seven days after growth, black pycnidial bodies were visually observed more in the center and less towards periphery. The single spore isolation technique was adopted for purification of fungus. Periodical transfers of the fungal culture were made on potato dextrose agar for further study. All other fungi were isolated occasionally with very low frequencies.

Table 4. Frequency % of isolated fungi from mango twigs/ branches naturally infected with dieback disease**.**

Isolated fungi	frequency %	
	Season 2015	Season 2016
L. theobromae	68.06	72.22
A. alternata	13.89	9.72
F. solani	11.11	12.50
A. niger	6.94	5.56
Total	100	100

3- Pathogenicity test

After 45 days of inoculation, *L. theobromae* was capable of infecting and developing disease symptoms on healthy twigs of cv. Hindi Sennara transplants. Transplants inoculated with *L. theobromae* showed typical symptoms of the disease as observed during the survey. After five days of inoculation, brownish dark dots were observed at the point of inoculation. Thereafter, brown, necrotic bark lesions developing around the inoculation sites gradually enlarged downwards and also towards the tip of twig, leading to wilting and drying of the apical as well as the terminal leaves. As it advanced entire twig turned black, started shriveling and drying. Cracking of the stem cortex was observed, and fungal structures (pycnidia and mycelium) developed on the necrotic lesion around the inoculation site. Re-isolation from the dead branches of *L. theobromae* inoculated transplants showed up to 97% recovery of the fungus**.** While *F. solani* was

able to induce mango necrotic lesion (necrosis) around the inoculation sites, but it was not able to induce dieback. However, *A. alternata* and *A. niger* were not able to induce necrosis or dieback symptoms in artificially inoculated mango seedlings after 45 days. It is clear also from data that increasing the incubation period from 15-45 days led to increasing necrotic length (mm) and the dieback symptoms on inoculated mango shoots at 45 days post inoculation. Control plants did not exhibit the typical symptoms and remained normal and healthy. It clearly indicated that the dieback is caused by *L. theobromae* (Table 5).

Table 5. Necrotic length around inoculation site (mm) on cv. Hindi Sennara transplants inoculated by the tested fungi under greenhouse conditions.

Isolated fungi	average length of necrotic area (mm) after inoculation days				Mean
	15	21	35	45	
L.theobromae	14.50d	46.50c	74.75b	97.75a	58.38A
A. alternata	0.00h	0.0h	0.00h	0.00h	0.00C
F. solani	2.75g	4.00g	7.00f	10.25e	6.00B
A. niger	0.00h	0.00h	0.00h	0.00h	0.00C
Control	0.00h	0.00h	0.00h	0.00h	0.00C
Mean	3.45D	10.10C	16.35B	21.60A	12.88

4. Field experiment.

4.1. Protection of pruning wounds.

All the diseased twigs/branches were pruned prior to foliar application with ten treatments. On the basis of their efficacy, treatments in the two seasons 2015 to 2016 were grouped into three categories. The 1st category (i.e., T4: pruning + Kema Zed, T3: pruning + Topsin M 70 and T6: pruning + Aliette) comprises of the most effective treatments which recorded the less number of infected branches, compared to T1: Pruning (control). Followed by the 2nd group (i.e., T5: pruning + Ridomil Gold MZ, T7: pruning + Bio Zied and T8: pruning + Bio Arc). Remaining three treatments (i.e., T9: pruning + Jojoba oil, T10: pruning + Thyme oil and T2: Pruning + sterilize clippers) represented the 3rd group compared to T1: Pruning (control). In untreated control trees, disease incidence was increased with increasing the time. None of the treatments elicited any phytotoxic response under the field conditions. Spraying ten treatments as pruning wound protectants with 15 days interval on branch pruning wounds in the field against the disease increased average number of fruits/tree compared to the untreated control. The highest increases were recorded with T4 (pruning + Kema Zed), T3 (pruning +Topsin M 70) and T6 (pruning + Aliette), followed by T5 (pruning + Ridomil Gold MZ), T7 (pruning + Bio Zied) and T8 (pruning + Bio Arc).While the lowest increases were recorded with T9 (pruning + Jojoba oil), T10 (pruning + Thyme oil) and T2 (pruning + sterilized clippers). Present results revealed that the mango yield increased when the treatments were applied after pruning compared to the untreated control. Percentages of increase in average number of fruits/ tree ranged from 38.88% to 145.37% in season 2015 and 34.06% to 174.72% in season 2016 (Table 6 and 7).

Table 6. Efficiency of ten treatments on mango dieback disease and number of fruits after pruning under field conditions in season 2015.

Treatment	Season 2015			
	IB*	% Eff.*	N*	% I*
T1:Pruning (control)	35.0	--	21.60	--
T2:Pruning+ sterilized clippers)	23.20	33.71	30.00	38.88
T3:Pruning +Topsin M 70	4.80	86.28	51.60	138.88
T4:Pruning + Kema Zed	4.00	88.57	53.00	145.37
T5:Pruning + Ridomil Gold MZ	7.80	77.71	46.60	115.74
T6:Pruning + Aliette	6.20	82.28	49.80	130.55
T7:Pruning + Bio Zied	9.80	72.00	45.40	110.18
T8:Pruning + Bio Arc	10.40	70.28	44.00	103.70
T9:Pruning + Jojoba	13.20	62.28	41.60	92.59
T10:Pruning + Natural Thyme oil	14.00	60.00	41.00	89.81

IB* = mean number of infected twigs/ branches per five trees.
% Eff.* = % Efficiency.
N* = mean number of fruits per five trees.
% I* = % increment of average number per five trees.

Table 7. Efficiency of ten treatments on mango dieback disease and number of fruit after pruning under field conditions in season 2016.

Treatment	Season 2016			
	IB*	% Eff.*	N*	% I*
T1:Pruning (control)	38.20	--	18.20	--
T2:Pruning+ sterilized clippers	25.60	32.98	24.40	34.06
T3:Pruning +Topsin M 70	5.60	85.34	48.20	164.83
T4:Pruning + Kema Zed	5.00	86.91	50.00	174.72
T5:Pruning + Ridomil Gold MZ	8.60	77.48	41.60	128.57
T6:Pruning + Aliette	6.60	82.72	44.80	146.15
T7:Pruning + Bio Zied	11.80	69.10	39.60	117.58
T8:Pruning + Bio Arc	12.00	68.58	38.40	110.98
T9:Pruning + Jojoba	15.20	60.20	34.80	91.21
T10:Pruning + Natural Thyme oil	15.80	58.63	33.40	83.51

IB*= mean number of infected twigs/ branches per five trees. % Eff.* = % Efficiency.
N* = mean number of fruits per five trees. % I* = % increment of average number per five trees.

Discussion

Mango (*Mangifera indica L.*) is considered one of the most important fruit crop grown in Egypt, which is ranked third after citrus, grapes. Among the wide range of diseases affecting mango production, dieback has become increasingly important disease. Mango dieback disease has become one of the most severe problems in all cultivars at El- Qanater El-Khairia Horticultural Research Station Farm. The disease on the trees may be noticed at any time of the year but it is most conspicuous during April – June. In affected plants, dieback is characterized by a progressive drying out of the branches. The disease begins from the top to downward, progresses toward the trunk and in more severe cases, can result in the death of the tree. The young green twig start withering first at the base and then extending outwards along the veins of leaf edges also a black ring was observed at the joint portion of infected and healthy parts of the branch. The affected leaf turns brown and its margins roll upwards. Leaves scorch and fall, leaving a dead branch. In severe conditions, branches start drying one after another in a sequence resulting in death of the whole tree (Ploetz and Ploetz, 2003). Haggag, (2010) reported that dieback is one of the serious diseases of mango in Egypt. Khanzada *et al*. (2004) reported that since the late nineties, mango dieback disease has become one of the most severe problems in mango orchards of the Sindh province. In most cases, the disease has been characterized by the exudation of gum, wilting, dieback, vascular browning and death of the whole tree (Narasimhudu and Reddy, 1992; Khanzada *et al.*, 2004). In Oman, since 1999, up to 60% of trees were found affected in parts of the Al Batinah region (Al Adawi *et al.* 2003).

The twelve mango cultivars screened for resistance against dieback under field conditions. Dieback has been observed in all mango growing cultivars under the study. The significant variations were found to disease reaction among the inspected cultivars, maximum disease incidence and disease severity were recorded in Hindi Sennara , Alphonso, Ewais and Keitt, followed by Sedeek, Zebda, Mestkawy, Montakhab El- Qanater and Timor whereas, the minimum disease incidence and disease severity were observed in Langra Benares , Fagr Kelan and Naomi. Our results are in accordance with Sharma and Badiala (1994) who reported that "Alphanso" is the most susceptible but none of the cultivars were completely resistant to the dieback disease. Also Banik *et al*. (1998) noted that Totapuri and Zardalu were moderately resistant to *Diplodia natalensis* (*L. theobromae*) whereas Ashadhio and Dudhpendo were moderately susceptible. Ten varieties viz., Kesar, Amrapali, Jamadar, Alphanso, Payari, Langra, Fasali, Nilam, Jahargir and Rajapuri were found susceptible.

L. theobromae was the most frequently isolated fungus among the other isolates during the two seasons. The fungus is commonly known as *Botryodiplodia theobromae (Pat.).* However, Sutton (1980) has adopted the name *Lasiodiplodia theobromae*. *L. theobromae* have been reported to cause mango dieback diseases and widespread in all major mango-growing countries. *L. theobromae*, a member of Botryosphaeriaceae, is a cosmopolitan fungus occurring predominantly throughout tropical and subtropical regions (Burgess *et al.* 2006). *Lasiodiplodia theobromae (Syn: Botryodiplodia theobromae)* is a causative fungus of mango dieback disease (Khanzada *et al.* 2004). Simone (1999) also reported *L. theobromae* and *Botryospheriaribis* as the cause of dieback in Florida. In other parts of the world, similar association between *L. theobromae* and mango decline has been observed by many research workers. Also Al Adawi *et al.* (2003) reported that *Diplodia theobromae* [*Lasiodiplodia theobromae*] as a cause of the mango decline in Oman. Mahmood *et al.* (2002) also isolated *L. theobromae* along with *Colletotrichum gloeosporioides, Fusarium oxysporum, F. semitectum* and *F. solani* from declining mango plants in Faisalabad, Punjab.

Plants inoculated with *L. theobromae* showed typical symptoms of the disease as observed during the survey, while *F. solani* was able to induce necrosis around the inoculation sites, but it was not

able to induce dieback. Similarly, Khanzada *et al*. (2004) reported that *L. theobromae* was the most isolated fungi, while *F. solani* was very rarely isolated from the infected mango branches. After one month of inoculation, plants inoculated with *L. theobromae* alone or in combination with *F. solani* showed typical symptoms of the disease, whereas, no such symptoms were observed on plants inoculated with *F. solani* alone. Results of the present study would suggest that *L. theobromae* is the causative agent of mango dieback in Egypt.

Pruning wounds are the only important infection site for *L. theobromae*, the causal of mango dieback and they also contribute to tree stress. Pruning does not always make a clean cut and often the branches are shattered. Lateral branches grow from below the pruning wound, but then often die. It appears that the shattered branches may provide an excellent entry point for *L. theobromae* which infect through wounds and sporulate on woody stems and green shoots throughout the year (Johnson, 2008). Infection of mango by this wood pathogen is usually through pruning and trimming wounds, so control has primarily relied on protecting pruning wounds with fungicides. In field experiment, the treatments were applied after pruning in December. Kema Zed, Topsin M 70 and Aliette proved to be the highly effective treatments for controlling dieback disease, followed by Ridomil Gold MZ, Bio Zied and Bio Arc. While, Jojoba oil, Natural Thyme oil and sterilizing clippers with Ethanol were the least effective treatments. Present results revealed that mango yield increased when the treatments were applied after pruning compared to the untreated control.

Several research studies have attempted to find fungicides that may be effective against the pruning wounds fungi. In California, Rolshausen *et al*. (2010) investigated the efficacy of four wound-dressing products (pyraclostrobin, thiophanate methyl, cyproconazole iodocarb and boric acid) in preventing infection of field vines by nine inoculated fungi (four petri disease pathogens, *E. lata* and four botryosphaeriaceous species (*B. dothidea, D. seriata, Dothiorella viticola* and *L. theobromae*). Their results demonstrated that thiophanate methyl was the most effective overall, but the efficacy of all products varied between the fungi. In South Africa, Bester *et al*. (2007) investigated the efficacy of ten fungicides as chemical pruning wound protectants against botryosphaeriaceous species *D. seriata, N. australe, N. parvum*and *L. theobromae*, in greenhouse studies using rooted grapevine cuttings. Their studies indicated that reductions in incidence due to benomyl, tebuconazole, flusilazole and prochloraz wound dressings, but their efficacy varied between species. In India mango dieback disease was effectively controlled by pruning the affected portions and spraying the wounded areas with 5:5:50 Boradeaux mixture (Parkash and Raoof, 1989). Mahmood *et al*.(2002) reported that 1stfoliar spray of Topsin-M (Thiophanate methyl) @1 g-1L water reduced the infestation of *L. theobromae* to 10% and 2nd spray of the same fungicide completely inhibited the fungus as no tissue yielded this fungus. Similarly, Rawal (1998) observed that dieback of mango caused by *L. theobromae* was controlled by spray of Carbendazim @ 0.1%, Thiophanate methyl @ 0.1% or Chlorothalonil @ 0.2% at fortnightly interval. Lonsdale and Kotze (1993) reported that broad-spectrum systemic fungicides are beneficial for the control of mango dieback disease. Khanzada *et al*. (2005) reported that in field experiment, Carbendazim proved to be the highly effective fungicide for the control of decline disease, followed by Thiophanate methyl and Aliette. The fungal infection in treated mango trees gradually reduced with the number of fungicidal sprays. It was accompanied with a gradual reduction in the disease severity and disease incidence in treated trees as compared to untreated control trees.

Recently, the use of essential oils against fungal infection has gained highly importance because of acquired resistance against a large number of fungicides. The various environmentally friendly natural compounds as the biological control agents Bio Zied (*Trichoderma album*) and Bio Arc (*Bacillus megaterium*) and essential oils i.e. Jojoba oil *(Simmondsia chinensis L*.) and Thyme oil (*Thymus vulgaris L*.) were applied independently as alternatives to chemical fungicides were evaluated for their efficacy in controlling mango dieback.

This research has demonstrated that proper sanitation with at least four fortnightly sprays after winter pruning with Kema Zed, Topsin M 70 and Aliette will be helpful to devise management strategies for the management of mango dieback in Egypt and may then ensure effective control of this damaging disease and increased number of fruits under field condition.

Reference.

Abdalla, A.E.M; Darwish, S.M; Ayad, E.H.E. and El- Hamahmy, R.M. (2007). Egyptian mango by-product 1. Compositional quality of mango seed kernel. Food Chem., 103:1134–1140.

Al Adawi, A.O.; Deadman, M.L.; Al Rawahi, A.K.; Khan, A.J. and Al Maqbali, Y.M. (2003). *Diplodia theobromae* associated with sudden decline of mango in the Sultanate of Oman. Plant Pathology, 52: 419-419.

Anonymous (2016). Annual report of Agric. Statistical Dept. Egyptian Min. of Agric. A.R.E. (In Arabic).

Banik, A. K.; Kaiser, S.A. and Dhua, R.S. (1998). Evaluation of some systemic and non systemic fungicides against *Collectotrichum gloeosporioides* and *Diplodia natalensis* causing

post-harvest fruit rot of mango. J. of Soils and Crops, 8(2): 119-122.

Barnett, H.L. and Hunter, B.B. (2006). Illustrated Genera of Imperfect Fungi. 4th Edn., American Phytopathological Society (APS Press), St. Paul, Minnesota, Pages: 217.

Bester, W.; Crous, P.W. and Fourie, P.H. (2007). Evaluation of fungicides as potential grapevine pruning wound protectants against *Botryosphaeria* species. Australian Plant Pathol., 36:73–77.

Brown, W. (1924). Two mycelial methods. A method of isolating single strains of fungi by cutting out a hyphal tip. Ann-Bot., 28:402-404.

Burgess, T.I; Barber, A.; Mohali, S.; Pegg, G.; De Beer, W. and Wingfield, M. J. (2006). Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. Mycologia, 98(2):423–435.

Costa, V.S.O; Michereff, S.J.; Martins, R.B.; Gava, C.A.T.; Mizubuti, E.S.G. and Camara, M.P.S. (2010) Species of Botryosphaeriaceae associated on mango in Brazil. Eur J Plant Pathol., 127:509–519.

Crous, P.W.; Slippers, B.; Wingfield, M.J.; Rheeder, J.; Marasas, F.O.; Phillips, A.J.L.; Alves, A.; Burgess, T.; Barber, P. and Groenewal, J.Z. d. (2006). Phylogenetic lineages in the Botryosphaeriaceae. Stud Mycol., 55:235–253.

Duncan, D.B. (1955). Multiple range and multiple F test. Biometrices, 11:1- 24.

Haggag, W.M. (2010). Mango diseases in Egypt. Agriculture and Biology Journal of North America, 1: 285-289.

Ismail, A.M; Cirvilleri G.; Polizzi G.; Crous, P.W.; Groenewald, J.Z. and Lombard. L. (2012). *Lasiodiplodia* species associated with dieback disease of mango (*Mangifera indica*) in Egypt. Australian Plant Pathol., 41:649–660.

Johnson, G. I. (2008). Status of mango postharvest disease management R & D: Options and solutions for the Australian mango industry. Horticulture Australia Final report for project MG08017, 1–130.

Khanzada, M.A.; Lodhi, A. M. and Shahzad, S. (2004). Pathogenicity of *Lasiodiplodia theobromae* and *Fusarium solani* on mango. Pak. J. Bot., 36(1): 181-189.

Khanzada, M.A.; Lodhi, A. M. and Shahzad, S. (2005). Chemical control of *Lasiodiplodia theobromae* , the causal agent of mango decline in Sindh . Pak. J. Bot., 37(4): 1023-1030.

Lonsdale, J.H. and Kotze, J.M. (1993). Chemical control of mango blossom disease and the effect on fruit set and yield. Pl. Dis., 77: 558-562.

Mahmood, A.; Saleem, A. and Akhtar, K.M. (2002). Mango decline in Pakistan and its management. Pak. J. Phytopathol., 14: 40-43.

Narasimhudu, Y. and Reddy, P.S.N. (1992). A note on gummosis of mango. Indian Phytopathol., 45(2): 261-262.

Parkash, O. and Raoof, M.A. (1989). Dieback disease of mango (*Mangifera indica L.*), its distribution, incidence, cause and management. Fitopathologia Brasiliensis, 14: 207-215.

Ploetz, R.C; Benscher, D.; Vázquez, A.; Colls, A.; Nage, J.l. and Schaffer, B. (1996). Mango decline: research in Florida on an apparently widespread disease complex. Proceeding of International Mango Symposium. Acta Hortic., 455:547–553.

Ploetz, R. and Ploetz, R. (2003). Diseases of mango. Diseases of tropical fruit crops, 327-363.

Punithalingam, E. (1976). *Botryodiplodia theobromae*. CMI descriptions of pathogenic fungi and bacteria, 519:1-2.

Rawal, R.D. (1998). Management of fungal diseases in tropical fruits. In: Tropical. Fruits in Asia: Diversity, Maintenance, Conservation and Use. (Eds.): R.K. Arora and V. Ramanatlia Rao. Proceedings of the IPGRI-ICAR-UTFANET Regional training course on the conservation and use of germplasm of tropical fruits in Asia held at Indian Institute of Horticultural Research, 18-3J May 1997, Bangalore, India.

Rolshausen, P.E; Urbez-Torres, J.R.; Rooney-Latham, S.; Eskalen, A.; Smith, R.J. and Gubler, W.D. (2010). Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. Am. J. Enol. Vitic., 61:113–119.

Sharma, I. M. and Badiyala, S. D. (1994). Effect of pre harvest fungicidal spray against stem end rot of mango in storage caused by *Botryodiplodia theobromae Pat.* in Himachal Pradesh. Indian J. Mycol. Pl. Pathol., 24(2): 141-142.

Simone, G.W. (1999). Disease Control in Mango *(Mangifera indica)*. Plant Pathology Department

Document PDMG-V3-22. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Slippers B.; Johnson, G.I.; Crous, P.W.; Coutinho, T.A.; Wingfield, B.; Wingfield, M.J. (2005) Phylogenetic and morphological re-evaluation of the Botryosphaeria species causing diseases of *Mangifera indica*. Mycologia, 97:99–1103.

Snedecor, G.W. and Cochran, W.G. (1980). Statistical methods. 7th Edition. Iowa State Univ. Press, Ames. Iowa, U.S.A.

Sutton, B. C. (1980). The Coelomycetes. Commonw. Mycolog.l Inst., Kew, Surrey, England.

Wicks, T. and Davies, K. (1999). The effect of Eutypa on grapevine yield. The Australian Grape grower & Winemaker. Annual Technical Issue: p. 15-16.

Zhang, K.; Su, Y.Y. and Cai, L. (2013). An optimized protocol of single spore isolation for fungi. Cryptogamie, Mycologie, 34 (4):349-356.

فعالية بعض المركبات كمواد واقية للجروح الناشئة عن التقليم فى مكافحة مرض موت الأطارف على المانجو

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تعتبر المانجو من أهم أنواع الفاكهة المنزرعة فى مصر. ومرض موت الأطارف أحد الأمراض المسببة لنقص إنتاج المانجو. ولقد أصبح مرض موت الأطارف من أخطر الأمراض التى تصيب المانجو المنزرعة فى مزرعة محطة بحوث البساتين بالقناطر الخيرية– مركز البحوث الزراعية. ولقد تم تقييم إثنا عشر صنفا من أصناف المانجو للإصابة بمرض موت الأطارف فوجد أنها قد إختلفت فى قابليتها للإصابة بالمرض حيث كانت الأصناف هندى بسنارة ، ألفونس ،عويس ، كيت هى أعلى الأصناف قابلية للإصابة بهذا المرض يليها الأصناف صديق ، زبدة ، مستكاوى ، منتخب القناطر ، تيمور والتى كانت متوسطة الإصابة بالمرض ، بينما أصناف لانجرا بنارس ، فجر كلان ، ناعومى هى أكثر الأصناف مقاومة للمرض. ولقد أثبتت تجارب العزل أن فطر لاثيوديبلوديا ثيوبرومى هو أكثر الفطريات تكرارا فى العزل كما كان أكثر الفطريات إحداثا لمرض موت الأطارف. وتعتبر الجروح الناشئة عن التقليم هى المكان المخصص لدخول الفطر وإحداث الإصابة بالمرض لذلك كان أستخدام المركبات الواقية لهذه الجروح أهم إستراتيجية فى مكافحة المرض. ولقد أجريت تجربة عامى 2015– 2016 برش عشر معاملات بمركبات مختلفة بعد التقليم مباشرة كواقيات للجروح الناشئة عن التقليم على الصنف هندى بسنارة. وقد أدت كل المعاملات إلى إنخفاض عدد الأفرع المصابة بالمرض وزيادة عدد الثمار مقارنة بالأشجار التى لم تعامل. وأحسن المعاملات كانت بمبيدات كيمازد ، توبسين إم 70 ، ألبيت حيث سجلت أقل عدد للأفرع المصابة بالمرض وأعلى عدد للثمارمقارنة بالأشجار التى لم تعامل.