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ORIGINAL PAPER

Mango grafting failure disease: associated fungi and possible control

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ABSTRACT

Grafting failure (GF) disease is the main problem facing mango seedlings production in nurseries located at three governorates during 2019 and 2020 seasons. The highest percentage (GF) was recorded at El Manashy nursery, Giza governorate, followed by El Qanater nursery, Qalyoubiya governorate, while the lowest% disease incidence was observed at Ismailia nursery, Ismailia governorate. The highest values of (GF) disease incidence on permutations and combinations of rootstock/scion in the three nurseries during the two years were recorded on Zebda/Kiett, Zebda/Sedeek and Balady/Kiett, followed by Balady/Sedeek, Sokkary/Kiett and Zebda/Ewais. On the other hand, the lowest susceptible rootstock/scion combinations were Sokkary/Sedeek, Balady/Ewais and Sokkary/Ewais. *Lasiodiplodia theobromae* was the most frequently isolated fungus and the most pathogenic on all permutations and combinations of rootstock/scion, followed by *Fusarium solani*. During grafting under greenhouse conditions, dipping Kiett, Ewais and Sedeek scions in the suspension of each of the tested commercial products before dipping in spore suspension of each pathogenic fungus, *L. theobromae* and *F. solani* reduced the percentage of (GF) of all permutations and combinations of rootstock/scion compared to the untreated scions. Also during grafting under nursery conditions, dipping mango scions in each of the tested treatments increased the % grafting success of all permutations and combinations of rootstock/scion compared to the untreated scions. In this respect, Copper oxide nanoparticles and Kemazed were the most effective which recorded (96.1 and 97.2) and (92.7 and 96.1) % success of mango grafted seedlings during the two seasons, respectively, followed by Biocontrol T34, Serenade ASO and Lemongrass oil being (87.2 and 90.5), (85.5 and 88.3) and (83.3 and 87.2), respectively. While the lowest % success was recorded when scions were dipped in Hydrogen Peroxide and Jojoba oil being (81.1 and 86.6) and (80.0 and 84.4), respectively. It is concluded that improving mango seedlings production in nurseries can be achieved by producing compatible rootstock-scion through controlling (GF) disease by using alternative and eco-friendly products beside to chemical products to obtain much higher economic returns with the least possible damage to the environment.

Keywords: Mango, *Mangifera indica*, Graft failure, Fungal pathogens, Eco-friendly chemical control.

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INTRODUCTION

Mango (*Mangifera indica.* L.) is called king of all fruits due to its attractive appearance and delicious taste. Recently, the total area cultivated with mango in Egypt has increased covering 289,020 Feddan (Feddan= 0.42 Hectare) with an approximate production of 2,800,000 metric tons (Anon., 2020). Due to the expansion of mango cultivation on commercial orchard in new reclaiming desert, the demand for mango seedlings is increasing day by day. The propagation of mango can be performed by either planting seeds or through the grafting of mango rootstock seedlings with the desired cultivars. Balady, Sokkary and Zebda are the most common varieties that are used as rootstocks in mango nurseries as they produce uniform, vigorous seedlings that are compatible with other cultivars. Desirable growth, development, thick and size of mango rootstock seedlings have important effect on the graft success process as well as the productivity, quality and quantity of fruits (Mng'omba *et al*., 2010). Also, Hamed *et al*. (2021) reported that there is a relationship between mango rootstocks and the scions including vegetative, flowering characters and also yield in quality and quantity.

Grafting is the most recommended and economical method for vegetative propagation in fruit trees specially mango. Vegetative propagation by grafting is a suitable technique to maintain true to type of scion cultivar that enables to transfer quality parameters from mother to the offspring (Nakasone and Paull, 1998). Grafting should be performed during the warmest months of the year during April and September but grafting in April is more successful than in September and can be recommended. A graft involves the joining of scion and rootstock where the rootstock develops into the root system while the scion develops the upper fruiting part of the grafted tree. Selection of mango rootstock/scion and grafting time of the year based on the desirable growing conditions are the major factors affecting on the success rate of grafting (Mng'omba *et al*., 2010). Also, improving the skills and grafting techniques of people who perform grafting is important (Akinnifesi *et al*., 2008). Grafted mango seedlings are affected by grafting failure process (GF) during vegetative propagation that led to huge losses in mango seedlings. The site where the scion and rootstock meet is called the graft union. Sometimes the graft union of rootstock/scion is unsuccessful, resulting in the poor growth or death of the top part of plant. *Lasiodiplodia theobromae* has been associated with symptoms of (GF) and necrosis at the binding site of grafting on mango (Hassan, 2015). Abo Rehab *et al*. (2013) reported that the main reason for graft failure in grapevine is fungal infected graft unions. They also added that symptoms appear as complete separation occurs between scion and rootstock on a union area of grafting, this region turned into brown and black and presence of fungal growth on and within the grafting area and dieback may progress and appear on rootstock. Mycelium of the pathogenic fungi appears in graft union and on other buds on the scion cutting.

Hence, we should interest with the health of the grafted seedlings through controlling graft failure (GF) disease. Eco-friendly products have been designed for controlling plant diseases and to do the least possible damage to the environment. Hydrogen peroxide (H_2O_2) has an antifungal effect by inducing resistance against anthracnose in cucumber (*Cucumis sativus*) (Byun and Choi, 2004). Salem *et al*. (2016) observed that a complete inhibition of *Rhizopus stolonifer*, the causal agent of post-harvest rot of peaches *in vitro* was occurred at 1.5 ml/L and 20 g/L for lemongrass and calcium chloride, respectively. Also, reduction of both rot incidence and severity was achieved using lemongrass oil at 1.5 ml/L and calcium chloride at 1.5 g/L. Biological control is considered one of such eco-friendly, which has been applied successfully to protect plant diseases. This has been mainly due to the large input of pesticides, beside to it causes economic, environmental and safety concerns. Nowadays, there are many available products that contain *Bacillus* spp. and *Trichoderma* spp. Giovanni *et al*. (2019) reported that *Pseudomonas* spp. and *Trichoderma* spp., controlled Fusarium wilt of banana up to 79% and 70%, respectively. Suppression of Verticillium wilt of cotton by *Bacillus subtilis* is attributable to competition for space and nutrients, lysis of pathogen hyphae, antibiosis and induced systemic resistance (Li *et al*., 2013). Bramhanwade *et al.* (2016) reported that various crop diseases caused by *Fusarium* spp. can be controlled by using Cu nanoparticles and their antifungal activity makes them a good candidate for plant disease management.

Therefore, due to the losses of mango seedlings in nurseries caused by (GF) disease, the aims of this study are to (i) determine the occurring and prevalence of (GF) disease on mango seedlings in three nurseries, (ii) identify the causal agent (s) associated with the disease and (iii) to find out the most effective alternative and eco-friendly commercial compounds to minimize the losses of grafted seedlings at mango nurseries with the least possible damage to the environment.

MATERIALS AND METHODS

1-Survey of grafting failure (GF) in different mango nurseries:

Survey was conducted during 2019 and 2020 to assess the occurrence and prevalence of grafting failure (GF) in three mango commercial nurseries located at El-Qanater El-Khairia, Qalyoubiya governorate, El Manashy, Giza governorate and Ismailia, Ismailia governorate. Grafting of mango seedlings was done during April by well-experienced personnel with above 10 years of experiences. Kiett, Ewais and Sedeek are considered the most popular cultivars for demand by growers and customers. Healthy and uniform-sized scions (15 cm long× 1.5cm thick) were collected from actively growing and bearing mango (10 to 12 year- old) respective mother's cultivars trees of each. Then scions were grafted on more than one- year old (at least 10-mm thick stem diameter) of the most desirable rootstock seedlings (Balady, Sokkary and Zebda) in different permutations and combinations of rootstock/scion (Balady/Kiett, Balady/Ewais, Balady/Sedeek, Sokkary/Kiett, Sokkary/Ewais, Sokkary/Sedeek and Zebda/Kiett, Zebda/Ewais, Zebda/Sedeek). After grafting, the graft unions were tied using polyethylene strips and smeared with paraffin to create airtight condition and to facilitate joining of the attached parts. Additionally, all necessary measures were done to make the plastic pots free from weeds and create a favorable environment to ensure proper growth and development of the grafted plants. The experiment was left to stand up to June (two months' duration). Twenty grafted seedlings were chosen randomly as four replicates from each permutation and combinations of rootstock/scion under the conditions of the three commercial nurseries. The (GF) seedlings during adaptation in the three commercial nurseries were estimated as follow:

$$\textbf{\% (GF) seedlings} = \frac{\textbf{Number of unsuccessful grafted seedlings}}{\textbf{Total number of grafted seedlings}} \times 100$$

2- Isolation and identification of fungi associated with graft failure process:

During 2019 and 2020, samples of grafted seedlings infected with (GF) disease were collected from the three mango nurseries during adaptation to identify the causal pathogen (s) of (GF). Each sample was washed thoroughly with tap water. Parts of each of infected scions and rootstocks were cut into small pieces (1cm in length), then dipped in 1% sodium hypochlorite for 3 min., small pieces were rinsed in sterile distilled water for several times, then dried between folds of sterilized filter paper. The sterilized pieces of scions and rootstocks were transferred individually to Petri plates, each containing 20 ml potato dextrose agar (PDA) medium. All the plates were incubated at 25±1°C for 5 days and examined daily for fungal growth. Hyphal tip and/or single spore techniques were used to purify the associated organism (s) on PDA slants. Most frequent fungal colonies were purified and transferred to other Petri plates with PDA. The purified fungi were identified according to colony characteristics and conidial morphology as described by Nelson *et al*. (1983), Booth (1985) and Barnett and Hunter (1986). Fungal isolates were kept at 5°C in a refrigerator for further studies. Stock cultures were routinely sub-cultured on fresh PDA slants or plates every 6-8 weeks. The frequency (%) of each fungal species was calculated using the following equation:

$$\textbf{Frequency (\%)} = \frac{\textbf{Number of colonies of each fungal species}}{\textbf{Total number of all fungi}} \times 100$$

3- Pathogenicity test during grafting:

Pathogenicity tests for fungi isolated from scions and rootstocks of grafted seedlings showing symptoms of grafting failure were performed under greenhouse conditions before starting control tests. To prepare the inocula for the pathogenicity tests, an amount of 10 ml of sterile water containing Tween 80 (0.05% v/v) were added to each Petri plate culture of any of the tested fungi, *Lasiodiplodia theobromae*, *Alternaria solani* and *Fusarium solani* each alone to improve the wetting properties of the solution. The fungal growth was gently dislodged from the surface with a sterile glass rod to separate the spores. Suspensions were added to 500 ml distilled water, then were filtered through three layers of cheesecloth to remove mycelial fragments. Spore concentration was adjusted using a haemocytometer to obtain 1×10^6 conidia/ml. Before grafting, rootstock seedlings were disbudded with a sharp knife, with the exception of the bottom buds un-removed. Healthy and uniform-sized scions (15 cm long× 1.5cm thick) were collected from actively growing and bearing mango (10 to 12 year-old) respective mother's cultivars trees of each of mango cultivars Kiett, Ewais and Sedeek and were grafted in April on more than one- year old rootstock seedlings (Balady, Sokkary and Zebda) in different permutations and combinations of rootstock/scion as mentioned before. For pathogenicity test, scions and rootstocks were immersed in the spore suspension of each fungus for 2 minutes before joined. The rootstocks and scions were joined together and tying with polyethylene strips. Each treatment comprised of four replicates, and each replicate consisted of five grafted seedlings. Data were calculated after 60 days from grafting as % seedlings with GF as mentioned before.

4. Control of (GF) disease of mango seedlings:

4-1- Effect of different treatments on controlling *L. theobromae* and *F. solani*, the causal agents of grafting failure of seedlings under greenhouse conditions:

The present study was carried out at El-Qanater El-Khayria Horticultural Research Station, Qalyoubiya governorate to study the effect of different treatments (Table1) on the percentages of (GF) disease. During April, healthy and uniform sized mango scions (1.5 cm thick and 15 cm tall) representing the three mango cultivars (Kiett, Ewais and Sedeek) were collected from actively growing and bearing mango (around 12 year-old) respective mother's cultivars trees and were grafted on more than one- year old (at least 10-mm thick stem diameter) of each rootstock seedlings (Balady, Sokkary and Zebda) in different permutations and combinations of rootstock/scion as mentioned before. Before grafting, scions were prepared for grafting on the same number of rootstocks. Scions were dipped in the solutions of each of the tested commercial products alone for five minutes and left to air drying for 5 minutes. Then scions were artificially infested with dipping separately in the tested spore suspension of the most pathogenic fungi, *L. theobromae* and *F. solani* for 2 minutes. Each treatment comprised of four replicates, each replicate consisted of five scions. The same numbers of scions and rootstocks were artificially infested with the two tested fungi each alone to serve as control. Both scions and rootstocks were wrapped with polyethylene strips and kept under greenhouse conditions for adaptation. Percentages of grafting failure (GF) and disease reduction were recorded at 60 days after grafting as mentioned before.

Table (1): Trade names, active ingredients, and application rates of the commercial compounds.

Commercial name	Active ingredient	Dose/L water
Lemongrass oil	Citrol or citral, about 70 % up to 85 %	3 ml
Jojoba oil	Simmondisa chinesis	3ml
Hydrogen Peroxide 50% standard	H_2O_2	3 ml
Serenade ASO 1.34% SC	*Bacillus subtilis* (QST713)	1 ml
Biocontrol T34	*Trichoderma asperellum strain T34*	2g
Copper oxide nanoparticles	CuO NPs	1 ml
Kemazed 50% WP	Carbendazim	1g

4-2- Efficacy of different treatments on the percentages of successful mango grafted seedlings under nursery conditions:

Under nursery conditions during April 2019 and 2020, twenty healthy mango scions were distributed as four replicates, five scions per each were chosen from the three mother's mango cultivars (Kiett, Ewais and Sedeek) around 12-year-old trees. Scions were dipped in the solutions of each of the tested treatments (Table, 1) for five minutes and left to air-drying for five minutes. Scions were grafted on more than one- year old (at least 10-mm thick stem diameter) of each rootstock seedlings (Balady, Sokkary and Zebda) in different permutations and combinations of rootstock/scion as mentioned before. The same number of seedlings of each rootstock/scion were dipped in distilled water and served as control. Successful grafted seedlings were counted after 60 days from grafting and expressed as percentage of successful grafted seedlings as follow:

$$\textbf{Percentage of grafting success} = \frac{\textbf{Number of successful grafted seedlings}}{\textbf{Total number of grafted seedlings}} \times 100$$

5- Characterization of Copper oxide nanoparticles:

Copper oxide nanoparticles (NPs) were obtained from Biota EG Company for Nano Technology. Copper oxide nanoparticles (NPs) dispersibility and purity were determined using transmission electron microscope (TEM) (FEI Tecnai G2, FEI Company, Nano Tech Egypt) to obtain transmitted electron pictures (Hashim *et al.*, 2019). The TEM micrograph of copper oxide nanoparticles showed that their shape is spherical morphology with average sizes between 44.23 and 96.26 nm Fig. (1).

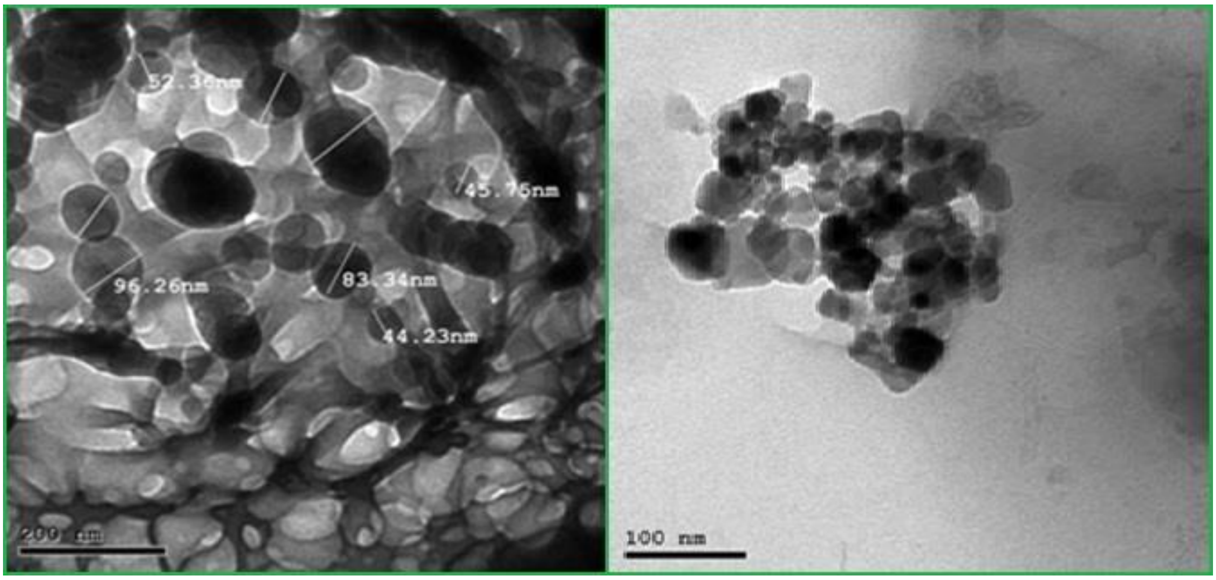

Fig. (1): Transmission electron microscopic image of copper oxide NPs, ranging from 44.23-96.26 nm.

6- Statistical analysis:

Data were statistically analyzed using the (F) test and the value of LSD (at 5%) according to Gomez and Gomez (1984).

RESULTS

1. Survey of graft failure (GF) disease in different mango nurseries:

Grafting failure (GF) and death of scion's disease symptoms appeared on recently grafted seedlings in the three nurseries located in the three surveyed governorates of all mango's examined permutations and combinations of rootstock/scion during 2019 and 2020 as wilt followed by dieback and gradual drying and cracking of the bark from the apex and moving down toward the base of the scion that leads to death of scions. The union between the rootstock and the scion presented longitudinal extended necrosis after 30 days from grafting. Forty-five days after grafting, most of the scion and the graft union was covered with abundant light gray mycelial growth. After grafting failure, necrosis appeared on the scions and dieback appeared on the rootstock (Fig. 2). Data in Table (2) indicate that the highest means of incidence (%) of (GF) on all examined mango permutations and combinations of rootstock/scion were recorded in El Manashy nursery, Giza governorate (33.88 and 30.55%) during 2019 and 2020 respectively, followed by El Qanater nursery, Qalyoubiya governorate (27.78 and 23.33%). While the lowest means of incidence (%) of (GF) was recorded in Ismailia nursery, Ismailia governorate, being 22.22 and19.44% during the two grafting seasons, respectively. Regarding to the susceptibility of permutations and combinations of rootstock/scion to grafting failure, the maximum values of disease incidence in the three nurseries during the two years were recorded on Zebda/Kiett, Zebda/Sedeek and Baladyy/Kiett, followed by Balady/Sedeek, Sokkary/Kiett and Zebda/Ewais. On the other hand, the lowest susceptible rootstocks/scions were Sokkary/Sedeek, Balady/Ewais and Sokkary/Ewais.

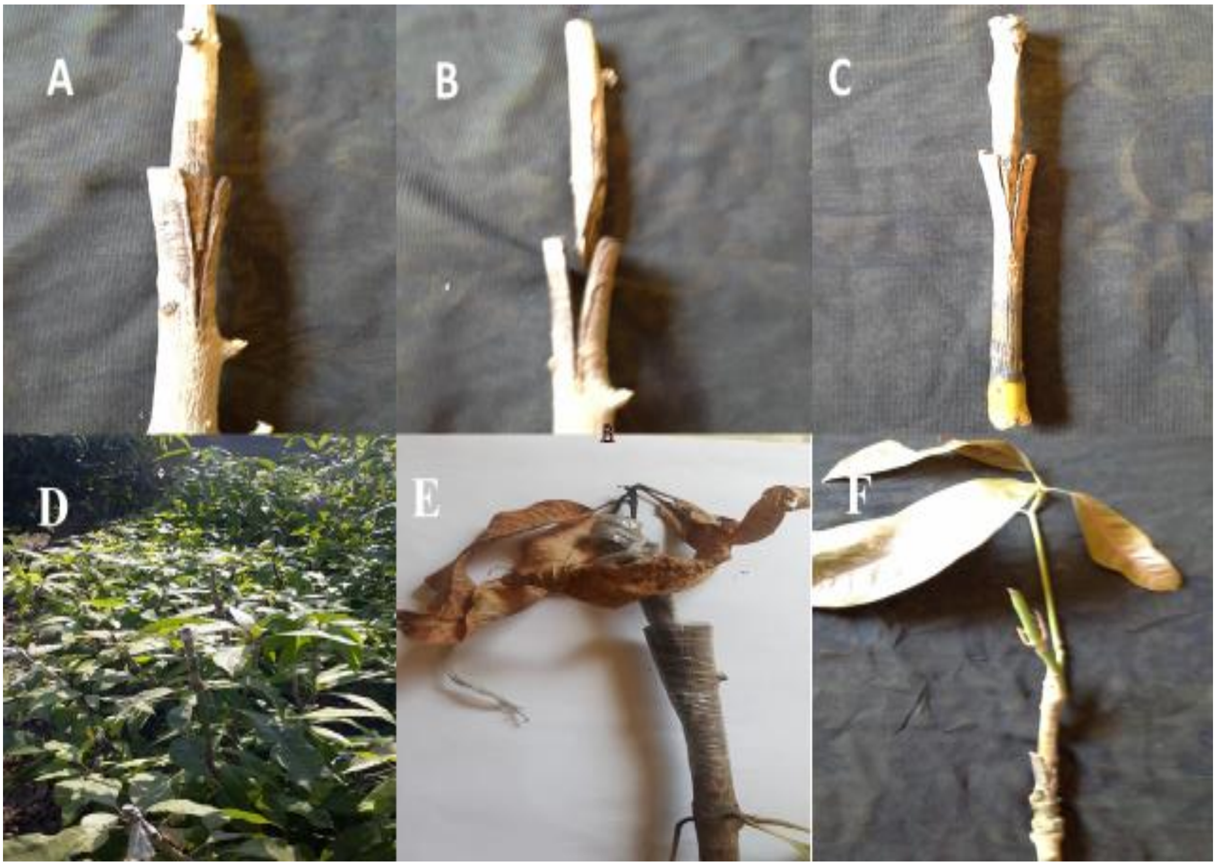

Fig. (2): Grafting failure (GF) symptoms appear on permutations and combinations of rootstock/scion as complete separation occurred between the scion and the rootstock on joined grafting area (A and B), this region turned into a brown and black, death of scion and dieback progress appeared on rootstock (C, D and E), compared with successful grafting (F).

Table (2): Disease incidence% of graft failure of some mango rootstock/scion in three governorates during 2019 and 2020 grafting seasons.

Rootstock/Scion	Incidence (%) of graft failure in							
	2019 season				2020 season			
	Qalyoubiya	Giza	Ismailia	M	Qalyoubiya	Giza	Ismailia	M
Balady/ Kiett	35	40	25	33.33	30	40	25	31.66
Balady/Ewais	20	25	10	18.33	15	20	10	15.00
Balady/Sedeek	30	40	20	30.00	25	35	15	25.00
Sokkary/Kiett	25	35	25	28.33	20	30	20	23.33
Sokkary/Ewais	15	20	10	15.00	10	15	10	11.66
Sokkary/Sedeek	20	25	15	20.00	15	25	10	16.66
Zebda/Kiett	45	45	40	43.33	40	45	35	40.00
Zebda /Ewais	25	30	25	26.66	20	25	20	21.66
Zebda/Sedeek	35	45	30	36.66	35	40	30	35.00
Mean	27.78	33.88	22.22	27.96	23.33	30.55	19.44	24.44
LSD at 5%	L = 1.17; R/S = 2.02; L × R/S =3.51				L = 1.16; R/S = 2.01; L × R/S =3.48			

L= location; R/S = rootstock/scion

2- Isolation and identification of fungi associated with graft failure process:

Graft failure was observed during adaptation of grafted mango seedlings in the three nurseries. The symptoms appeared in union area between the scion and the rootstock. This region turned into a brown and oblique separation was occurred between the scion and the rootstock and some fungal colonies within the grafting area were observed. Finally, death of scion and dieback progress appeared on the rootstock. The isolated fungi from both scions and rootstocks of unsuccessful grafted seedlings during vegetative propagation were identified according to their morphological and cultural characteristics as *Lasiodiplodia theobromae, Alternaria solani* and *Fusarium solani*. *L. theobromae* was the most frequent pathogen isolated from scions and rootstocks of unsuccessful grafted seedlings during the two seasons in the three nurseries whereas, *Fusarium solani* and *Alternaria solani* were the least frequent isolated fungi (Table, 3).

Table (3): Frequency (%) of the isolated fungi from infected scions and rootstocks of unsuccessful grafted seedlings in the three nurseries during two grafting seasons, 2019 and 2020.

Fungus	Frequency (%) of the isolated fungi in											
	2019 season						2020 season					
	Qaliobiya		Giza		Ismailia		Qaliobiya		Giza		Ismailia	
	Scion	Roots tock	Scion	Root stock	Scion	Roots tock	Scion	Root stock	Scion	Root stock	Scion	Roots tock
L. theobromae	82.36	86.66	84.52	87.88	87.12	85.34	85.24	83.98	83.76	88.34	84.53	82.68
A. solani	2.38	1.33	2.78	1.11	2.38	3.25	5.00	3.56	2.78	2.56	5.15	2.87
F. solani	15.26	12.01	12.70	11.01	10.50	11.41	9.76	12.46	13.46	9.10	10.32	14.45
Total	100	100	100	100	100	100	100	100	100	100	100	100
LSD at 5%	*L = n.s.; **F = 0.12; L× F = 0.20						L = n.s.; F = 0.10; L× F = 0.18					

*L= location; **F= fungi

3- Pathogenicity test during grafting:

Pathogenicity tests of the isolated fungi (Fig., 3) proved that *L. theobromae* is the principal causal agent of graft failure process of all permutations and combinations of rootstock/scion of mango and similar symptoms of graft failure (GF) appeared on the infected seedlings. The moderate percentage of disease incidence was recorded on all permutations and combinations of rootstock/scion of mango inoculated with *F. solani*, while *A. solani* recorded the lowest percentage of disease incidence. The highest disease incidence (%) of (GF) on permutations and combinations of rootstock/scion of mango was recorded on Zebda/Kiett, Zebda/Sedeek and Balady/Kiett, followed by Zebda/Ewais, Balady/Sedeek and Sokkary/Kiett. Meanwhile, the lowest percentage of disease incidence on permutations and combinations of rootstock/scion of mango was recorded on Balady/Ewais, Sokkary/Sedeek and Sokkary/Ewais.

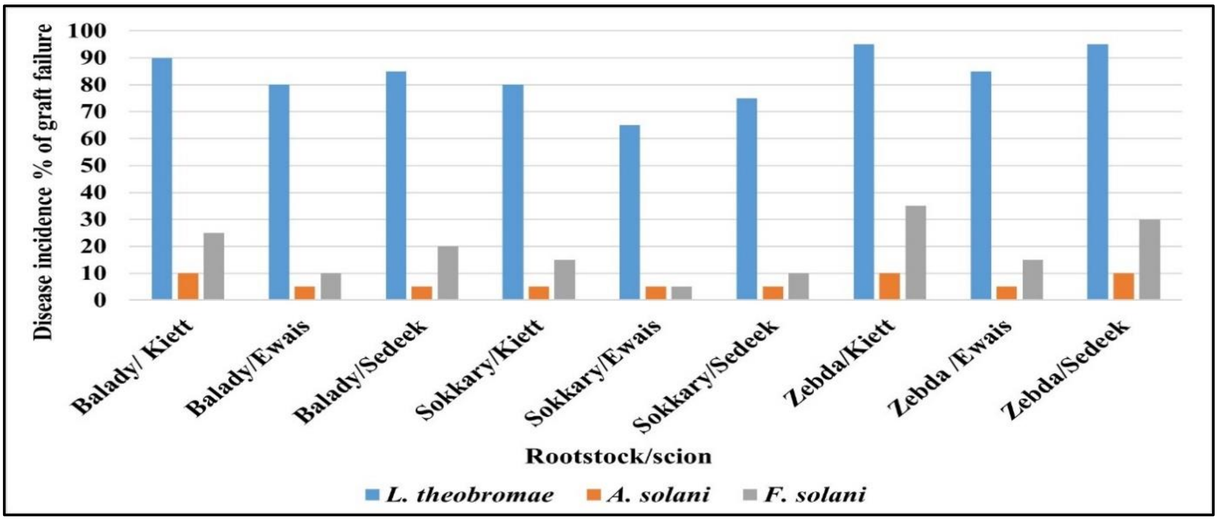

Fig. (3): Pathogenicity test of the isolated fungi of all permutations and combinations of rootstock/scion of mango expressed as the percentage of grafting failure under greenhouse conditions.

4. Control of (GF) of mango seedlings:

4-1- Effect of different treatments on controlling *L. theobromae* and *F. solani*, the causal agents of graft failure of seedlings under greenhouse conditions:

Data in Tables (4 and 5) indicate that all the tested treatments reduced (GF) when scions Kiett, Ewais and Sedeek cultivars were dipped in any of the tested treatments, and artificially infested by dipping separately in the spore suspensions of each of the most pathogenic fungi *L. theobromae* and *F. solani*. Then, scions were grafted on the seedlings of each rootstock (Balady, Sokkary and Zebda) in different permutations and combinations of rootstock /scion as mentioned before. Generally, considering the averages of efficiency of the tested treatments against *L. theobromae* and *F. solani*, Copper oxide nanoparticles and Kemazed were the most effective treatments for controlling fungal pathogens which cause graft failure, followed by Biocontrol T34 and Serenade ASO. While the lowest reduction in graft failure incidence of all permutations and combinations of rootstock/scion was recorded when scions were immersed in Hydrogen Peroxide, Lemograss oil and Jojoba oil. Data also show that means of the effect of all treatments on nine rootstocks/scions were varied from one to another. Also, the highest means of efficacy of all treatments were recorded for Sokkary/Ewais, Balady/Ewais and Zebda/Ewais, followed by Sokkary/Kiett, Zebda/Kiett, Sokkary/Sedeek and Zebda/Sedeek. Meanwhile, the lowest means of efficacy of all treatments were recorded for Balady/Kiett and Balady /Sedeek.

4-2-Efficacy of different treatments on the percentages of successful mango grafted seedlings under nursery conditions:

Data in Table (6) show that dipping mango scions in the solution of each of the tested treatments before grafting increased the grafting success percentage of all permutations and combinations of rootstock/scion compared with the untreated scions. Considering the averages of efficiency of the tested treatments of all permutations and combinations of rootstock /scion during the two seasons, Copper oxide nanoparticles and Kemazed were the most effective treatments which recorded the largest percentages of grafting success (96.1 and 97.2%) and (92.7 and 96.1%), respectively, followed by Biocontrol T34, Serenade ASO and Lemongrass oil (87.2 and 90.5%), (85.5 and 88.3%) and (83.3 and 87.2%), respectively. While the lowest effect was found when scions were dipped in each of Hydrogen Peroxide and Jojoba oil where the means of (%) success of mango grafted seedlings of all permutations and combinations of rootstock/scion in the two seasons were (81.1 and 86.6%) and (80.0 and 84.4%), respectively. Meanwhile, the highest means of success of mango grafted seedlings of all treatments during the two seasons were recorded on Sokkary/Ewais (92.5 and 95.6%), Sokkary/Sedeek (91.2 and 94.3%) and Balady/Ewais (90.0 and 93.1%), followed by Zebda /Ewais (86.8 and 90.6%), Sokkary/Kiett (86.8 and 89.3%) and Balady/Sedeek (81.8 and 85.6%). On the other hand, the lowest means of % success of mango grafted seedlings were recorded on Balady/Kiett (81.8 and 84.4%), Zebda/Sedeek (79.3 and 85.0%) and Zebda/Kiett (72.5 and 77.5%).

Table (4): Evaluation of different treatments used in controlling the pathogenic fungus of graft failure disease, *L. theobromae* under greenhouse conditions.

Treatment	% Graft failure due to *L. theobromae*																			
	Balady/ Kiett		Balady/ Ewais		Balady /Sedeek		Sokkary/ Kiett		Sokkary/ Ewais		Sokkary/ Sedeek		Zebda/ Kiett		Zebda /Ewais		Zebda/ Sedeek		Mean	
	*DI %	**Eff%	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff%
Lemograss oil	25	72.2	20	75.0	25	70.5	20	75.0	15	76.9	20	73.3	25	73.6	20	76.4	20	78.9	21.1	74.6
Jojoba oil	25	72.2	20	75.0	30	64.7	25	68.7	20	69.2	25	66.6	30	68.4	25	70.5	30	68.4	25.5	69.3
Hydrogen Peroxide	25	72.2	15	81.2	25	70.5	20	75.0	15	76.9	20	73.3	25	73.6	20	76.4	25	73.6	21.1	74.7
Serenade ASO	20	77.7	15	81.2	20	76.4	15	81.2	10	84.6	20	73.3	20	78.9	20	76.4	20	78.9	17.7	78.7
Biocontrol T34	20	77.7	15	81.2	20	76.4	15	81.2	10	84.6	15	80.0	20	78.9	15	82.3	25	73.6	17.2	79.5
Copper oxide nanoparticles	15	83.3	5	93.7	10	88.2	10	87.5	5	92.3	10	86.6	10	89.4	10	88.2	15	84.2	10	88.1
Kemazed	15	83.3	10	87.5	15	82.2	10	87.5	10	84.6	10	86.6	15	84.2	15	82.3	15	84.2	12.7	84.7
Control	90	--	80	--	85	--	80	--	65	--	75	--	95	--	85	--	95	--	83.3	--
Mean	29.4	76.9	22.5	82.1	28.7	75.5	24.3	79.4	18.7	81.3	24.3	77.1	30.0	78.1	26.2	78.9	30.6	77.4	26.0	78.5
LSD at 5%	♯T= 1.05; ♦R/S = 1.12; T × R/S =3.16																			

*DI= disease incidence; **Eff.= efficiency; ♯T= treatment; ♦R/S = rootstock/scion

Table (5): Evaluation of different treatments used in controlling the pathogenic fungus of graft failure disease, *F. solani* under greenhouse conditions.

Traetment	% Graft failure due to *F. solani*																		Mean	
	Balady/ Kiett		Balady/ Ewais		Balady/ Sedeek		Sokkary/ Kiett		Sokkary/ Ewais		Sokkary/ Sedeek		Zebda/ Kiett		Zebda/ Ewais		Zebda/ Sedeek			
	*DI%	**Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff %	DI%	Eff%
Lemograss oil	10	60	5	50	10	50	5	66.6	0	100	5	50.0	15	57.1	5	66.6	15	50.0	7.7	61.1
Jojoba oil	10	60	5	50	10	50	5	66.6	0	100	5	50.0	15	57.1	5	66.6	15	50.0	7.7	61.1
Hydrogen Peroxide	5	80	0	100	10	50	5	66.6	0	100	5	50.0	15	57.1	5	66.6	15	50.0	6.6	68.9
Serenade ASO	5	80	0	100	10	50	5	66.6	0	100	0	100	10	71.4	5	66.6	10	66.6	5.0	77.9
Biocontrol T34	5	80	0	100	10	50	5	66.6	0	100	0	100	10	71.4	0	100	10	66.6	4.4	81.6
Copper oxide nanoparticles	5	80	0	100	5	75.0	0	100	0	100	0	100	5	85.7	0	100	5	83.3	2.2	91.5
Kemazed	5	80	0	100	5	75.0	5	66.6	0	100	0	100	5	85.7	0	100	5	83.3	2.7	87.8
Control	25	--	10	--	20	--	15	--	5	--	10	--	35	--	15	--	30	--	18.3	--
Mean	8.7	74.2	2.5	85.7	10.0	57.1	5.6	71.3	0.62	100	3.1	78.5	13.7	69.3	4.3	80.9	13.1	64.2	6.83	75.7
LSD at 5%	♯T = 0.92; ♦R/S = 0.97; T × R/S = 2.76																			

*DI= disease incidence; **Eff.= efficiency; ♯T= treatment; ♦R/S = rootstock/scion

Table (6): Efficacy of some commercial products on the percentages of successful permutations and combinations of rootstock/scion of mango grafted seedlings during 2019 and 2020 grafting seasons.

Treatment	% Success of mango rootstock/scion grafted seedlings																		Mean	
	2019 season									2020 season									2019	2020
	Balady / Kiett	Balady / Ewais	Balady / Sedeek	Sokkary/ Kiett	Sokkary/ Ewais	Sokkary/ Sedeek	Zebda/ Kiett	Zebda / Ewais	Zebda/ Sedeek	Balady / Kiett	Balady / Ewais	Balady / Sedeek	Sokkary/ Kiett	Sokkary/ Ewais	Sokkary/ Sedeek	Zebda/ Kiett	Zebda / Ewais	Zebda/ Sedeek		
Lemograss oil	80	85	80	85	90	95	70	85	80	85	90	85	85	95	95	75	90	85	83.3	87.2
Jojoba oil	75	85	80	80	90	90	65	85	70	80	90	80	85	95	95	70	85	80	80.0	84.4
Hydrogen Peroxide	80	90	80	85	90	85	65	85	70	80	90	85	90	95	90	75	90	85	81.1	86.6
Serenade ASO	85	90	80	90	90	90	75	90	80	85	95	85	90	95	95	75	90	85	85.5	88.3
Biocontrol T34	85	90	80	90	95	95	75	90	85	85	95	85	95	95	95	80	95	90	87.2	90.5
Copper oxide nanoparticles	95	100	95	95	100	100	90	95	95	95	100	95	95	100	100	95	100	95	96.1	97.2
Kemazed	90	100	90	95	100	95	85	90	90	95	100	95	95	100	100	90	95	95	92.7	96.1
Control	65	80	70	75	85	80	55	75	65	70	85	75	80	90	85	60	80	65	72.2	76.6
Mean	81.8	90.0	81.8	86.8	92.5	91.2	72.5	86.8	79.3	84.4	93.1	85.6	89.3	95.6	94.3	77.5	90.6	85.0	84.7	88.3
LSD at 5%	[♯]T = 0.96; [♦]R/S = 1.02 T × R/S = 2.90									T = 0.94; R/S = 1.00; T × R/S = 2.83										

[♯]T= treatment; [♦]R/S = rootstock/scion

DISCUSSION

Due to the expansion of mango cultivation on commercial orchards in new reclaiming desert, the demand of mango seedlings is increasing day by day. The propagation of mango can be performed by either planting seeds or through the grafting of mango seedlings with the desired cultivars. Balady, Sokkary and Zebda are the most common rootstocks that are using in mango nurseries because they produce uniform, vigorous seedlings that are compatible with other cultivars. Rootstocks play an important role for tree survival and establishment in the field, tree productivity and dwarfing of grafted fruit trees (Mng'omba *et al*., 2008). The graft success can be improved when rootstock selection is considered and based on desirable growth attributes of rootstocks (Simons, 1987).

During production of grafted mango seedlings in nurseries at El-Qanater El-Khairia, Qalyoubiya governorate, El Manashy, Giza governorate and Ismailia, Ismailia governorate in the two successive grafting seasons 2019 and 2020, grafted mango seedlings were found to suffer from grafting failure (GF) during vegetative propagation which limiting mango seedlings production and causing considerable losses in number of mango seedlings in nurseries.

Regarding to the susceptibility of permutations and combinations of rootstock/scion to grafting failure, data indicated that the maximum means of grafting failure disease incidence in the three nurseries during the two years were recorded on Zebda/Kiett, Zebda/Sedeek and Baladyy/Kiett, followed by Sokkary/Kiett, Zebda/Ewais and Balady/Sedeek. On the other hand, the less susceptible rootstocks/scions were Sokkary/Sedeek, Balady/Ewais and Sokkary/Ewais. Also, it was found that *L. theobromae* is the causal agent of graft failure process of all permutations and combinations of rootstock/scion of mango and similar symptoms of graft failure appeared on the infected seedlings. The moderate percentage of disease incidence was recorded on all permutations and combinations of rootstock /scion of mango inoculated with *F. solani*, while *A. solani* showed the least percentage of disease incidence.

Although several procedures were used to reduce (GF) disease in mango nurseries, investigations of eco-friendly disease control measures are urgently required. Due to the economic importance of mango, its export value and the losses caused by (GF) disease in mango nurseries, management practices were carried out under greenhouse and nursery conditions to find out eco-friendly cost effective and easily available control measures to manage the disease as the chemical fungicides are much expensive and have some health hazardous effects on human health and environment. Data showed that Copper oxide nanoparticles and Kemazed recorded the highest (%) success of grafted seedlings of all permutations and combinations of rootstock/scion in the two seasons, followed by Biocontrol T34, Serenade ASO and Lemongrass oil. While the lowest (%) success of grafted seedlings was recorded when scions were dipped in each of Hydrogen Peroxide and Jojoba oil.

The obtained results are in harmony with several studies, Mengal *et al*. (2015) found that all the tested botanical extracts at their highest doses significantly retarded the mycelial growth of *F. oxysporum* the causal agent of Fusarium wilt of mango in the nursery compared to control. *L. theobromae* and *C. gloeosporioides*, the causal agents of stem end rot disease and anthracnose disease in mango fruit, respectively are controlled with essential oil of *Eupatorium cannabinum* (Dubey *et al.*, 2007). Ozcan and Chalchat (2008) recorded that the growth of *Fusarium oxysporum*, *Alternaria alternata* and *Botrytis cinerea in vitro* was inhibited by Rosemary (*Rosmarinus officinalis*). Increasing the concentration of essential oil increased the effect against the growth of the three fungi. Muthukumar and Sanjeev (2012) reported that complete growth inhibition of *L. theobromae* was achieved by Lemongrass oil even at 0.005% concentration compared to other tested oils. In *in vitro* test, antifungal activity against *Colletotrichum coccodes*, *B. cinerea*, *Cladosporium herbarium*, *Rhizopus stolonifer* and *A. niger* was obtained by Lemongrass oil. Essential oils are volatile, natural, complex compounds characterized by a strong odor which can be used to delay or inhibit the growth of microorganisms. Essential oils can be obtained by hydro-distillation or steam plant materials such as leaves, flowers, fruits, seeds, twigs, wood, roots, herbs, bark and buds (Solgi and Ghorbanpour, 2014). Essential oils are widely used for bactericidal, insecticidal, virucidal, fungicidal, antiparasitical, medicinal and cosmetic applications due to prooxidant effects on the cellular level (Bakkali *et al.*, 2008).

Hydrogen peroxide (H_2O_2) plays an import role in activation of plant resistance against pathogen attacks as anthracnose in cucumber (*Cucumis sativus*) (Byun and Choi, 2004). Kotchoni *et al*. (2007) reported that H_2O_2 has an antibacterial activity against *Xanthomonas campestris pv. vignicola* causing cowpea bacterial blight when cowpea seeds and seedlings were pretreated with H_2O_2. Peng and Ku (1992) found *in vitro* test, that increasing H_2O_2 concentration gradually inhibited germination of sporangiospores of *Peronospora tabacina* causing tobacco blue mildew. Moussa *et al*. (2012) reported that the highest percentage of potato tuber dry matter was noticed when H_2O_2 was sprayed at 40mM. Taha and Ramadan (2017) found that the mycelial growth of *Alternaria alternata*, the causal agent of leaf spot disease of broad bean was inhibited with four concentrations (100, 200, 300, 400) mMol of H_2O_2 and this inhibition was increased with increasing the concentration. Also, higher increases in shoot fresh and dry weights, plant height, chlorophyll content of broad bean were achieved with using H_2O_2. Ali, (2018) reported that hydrogen peroxide (H_2O_2) at different concentrations (0.25 %, 0.50 %, 1 % and 2 %) reduced the linear growth of *Botryodiplodia* sp., *F. oxysporum*, *F. solani*, *M. phaseolina*, *Pythium* sp. and *R. solani in vitro*. However, complete inhibition of linear growth of all tested fungi was recorded at 2%. The same author Ali, (2018) added that under greenhouse conditions Hydrogen peroxide-controlled root rot and wilt diseases of thyme and increased plant survival compared to untreated plants. The role of Hydrogen peroxide in activation of host defense mechanisms, including stimulate activity of enzymes as chitinase and peroxidase followed by a significant increase in the suberin and lignin content. Also, hydrogen peroxide plays an essential role in lignification and a strengthening of cell walls at the site of pathogen attack (Copes, 2009).

Biological control is one of such eco-friendly, which has been applied successfully to protect fruit and vegetable crops from diseases (Wilson and Wisniewski, 1989). Nowadays, there are many products that contain *Bacillus* spp. and *Trichoderma* spp. are available. The effect of *Bacillus* spp. and *Trichoderma* spp. on plant diseases can occur through different mechanisms such as competition for space and nutrients, direct parasitism, antibiosis, lysis of pathogen hyphae, suppression, competition and induced systemic resistance. The antagonistic activity has often been associated with production of secondary metabolites. Enzymes responsible for cell-wall degradation such as chitinases and glucanase have been associated with the ability of *Trichoderma* spp. to control plant pathogens (Matroudi *et al.,* 2009 and Li *et al.,* 2013). In addition, *B. subtilis* increases N uptake, phosphate solubilization and siderophore and phytohormone production, promoting plant growth. *B. subtilis* has a demonstrated positive effect on disease suppression by altering the composition and function of soil microbial communities (You *et al*., 2016). Abo Rehab *et al*. (2013) noticed that Topsin M and KemaZed gave the best results for controlling fungal pathogens which cause grafting failure of grapes, followed by Bellis, Saprol, Syllit and Conazol. Also, they added that Bio-Zied (*Trichoderma album*), Rhizo-N (*Bacillus subtilis*) and Bio-Arc (*Bacillus megaterium*) reduced the percentage of graft failure of grapes.

Eco-friendly nanomaterials were used for controlling many crop diseases, Hashim *et al.* (2019) suggested that nanomaterials compounds *i.e.*, silica, chitosan and copper nanoparticles (NPs) have antifungal activity against *Botrytis cinerea*, the causal agent of gray mold on table grapes *in vitro* and *in vivo* tests.

CONCLUSIONS

Results of the present study suggest that *L. theobromae* is the main pathogen causing (GF) disease of mango seedlings in nursery, where artificial inoculation using this fungus induced typical symptoms on grafted seedlings. Also, nurserymen should beware of the effect of (GF) disease on scion- rootstock compatibility, this will be very helpful in minimizing seedling death at the nursery. Also, this research highlighted the potential for using eco-friendly treatments as essential oils, biofungicides and H_2O_2 beside CuO nanoparticles (NPs) and Kemazed as an attempt for management and reducing the losses of the number of mango seedlings in nurseries to obtain much higher economic returns and the least possible damage to the environment.

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