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ORIGINAL PAPER

Protecting Avocado Seedlings from Grafting Failure During Propagation in Nursery Using Biological and Chemical Control

Monir, G.A.*; Radwan, M.A. and Hassan, M.S.S.

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ABSTRCT

During propagation of avocado by grafting in 2019 and 2020, the maximum % grafting failure (GF) of avocado seedlings was recorded on cv. Hass and cv. Fuerte, followed by cv. Benkerton and cv. Reed. While cv. Etinger was the less susceptible one. *Lasiodiplodia theobroma*e was the most frequently isolated from graft failure seedlings in all avocado cultivars, followed by *Fusarium moniliforme*. While *Alternaria alternata* recorded the lowest frequency. The highest percentage of grafting failure and death of scions was obtained by the fungus *L. theobroma*e, followed by *F. moniliforme* and the lowest percentage of grafting failure was observed for *A. alternata* in all avocado cultivars. The efficacy of biological and chemical control was tested *in vitro* and *in vivo* as well as under nursery conditions for controlling GF on avocado seedlings. Amistar Top and Bellis gave the highest reduction in colony growth of the three pathogens, followed by Kocide (2000) and Star copper. While Serenade ASO and Bio Zeid were the lowest ones. Dipping scions in each of the tested suspensions before dipping in spore suspension of each fungus suppressed the spores of the pathogen during grafting and increased the percentage of success of grafted seedlings in the two tested cultivars Hass and Fuerte compared with the control. In this respect, Amistar Top and Bellis were the most effective treatments for controlling the pathogens in the two tested cultivars, followed by Kocide (2000) and Star copper. The lowest efficacy was recorded when scions were dipped on Serenade ASO and Bio Zeid. Also, dipping scions in the suspension of each tested commercial product during grafting increased the percentage of grafting success for all tested cultivars compared with the control. Considering the means of efficacy of the tested treatments on the tested cultivars in the two seasons, Amistar Top, Bellis and Kocide (2000) were the most effective which recorded (91.00 and 94.00%), (85.00 and 87.00%) and (84.00 and 87.00%), respectively (%) success of avocado grafted seedlings on all cultivars in the two years, followed by Serenade ASO (83.00% and 87.00%). While the least (%) success of avocado grafted seedlings on all cultivars in the two seasons was observed on Star copper and Bio Zeid (81.00 and 86.00%) and (81.00 and 84.00%), respectively. Also, the highest effect of all treatments was on cv. Etinger, cv. Reed and cv. Benkerton, while the lowest effect was on cv. Fuerte and cv. Hass in the two seasons. The highest values of production of peroxidase and polyphenoloxidase activities in the two tested avocado cultivars Hass and Fuerte were observed after dipping the scions in Amistar Top and Bellis, followed by Kocide (2000) and Serenade ASO. While the lowest values of peroxidase and polyphenoloxidase activities were recorded after dipping the scions in Star copper and Bio Zeid.

Keywords: Avocado, Graft Failure, Fungal Pathogen, Biological and Chemical Control.

***Correspondence:** Monir, G.A.
E-mail: gege_monir_07@yahoo.com
Gehan A. Monir
https://orcid.org/0000-0003-3701-103X
Mahmoud A. Radwan
Mabrouk S.S. Hassan
https://orcid.org/0000-0002-3477-339X
Plant Pathology Research Institute, Agricultural Research Center, 12619, Giza, Egypt.

INTRODUCTION

Avocado (*Persea americana* Miller) is an evergreen tree, cultivated in both tropical and Mediterranean regions for its delicious and nutritious fruits. In 2016, the world's total avocado production was 5 567 044 metric tons, and the production area was 563 916 hectares. Mexico, Dominican Republic, Peru, Brazil, Indonesia, and Colombia are known to be the largest producers in the world (FAO, 2018). In Egypt, El-Qanater El-Khairia Horticulture Research Station is famous for commercial production of grafting avocado seedlings of several commercial cultivars. Grafting is the most recommended and economical method for vegetative propagation in avocado. Sometimes the graft union is unsuccessful, resulting in the poor growth or death of the scions, this called grafting failure process that can cause huge losing in the number of avocado seedlings.

Grafting failure (GF) is the most serious disease during vegetative propagation by grafting of avocado in nursery which has a great economic importance on avocado grafted seedlings. Grafting failure can be caused by different factors

such as poor formation of the graft union (due to problems with anatomical mismatching, poor grafting technique, adverse weather conditions and poor hygiene), mechanical damage to the graft union and graft incompatibility (Spilmont *et al*., 2007). Fungal infected graft unions in French vineyards were found to be associated with the graft union of symptomatic plants *i.e*., *Phomopsis* spp., *Verticillium* spp., *Alternaria* spp., *Lasiodiplodia* spp. and *Eutypa* sp. (Spilmont and Bourisquot, 2002). Atia *et al.* (2003) reported that *Botryodiplodia theobromae, Phomopsis viticola* and *Fusarium solani* can cause necrosis in xylem parenchyma and xylem vessels of grapevine and can colonize the tissues with their hyphae. *Lasiodiplodia theobromae* has been associated with symptoms of dieback and necrosis at the binding site of grafting on guava (*Psidium guajava* L.) (Cardoso *et al*., 2002), on cashew (*Anacardium occidentale* L.) (Freire *et al*., 2002), on mango (Hassan, 2015), on citrus (Davis *et al*., 1987), on grapevine (Aroca *et al*., 2008 and Abo Rehab *et al*., 2013).

The main objective of the present study is to assay the incidence and possible causal pathogen(s) of avocado grafting failure (GF). Also, to investigate the effectiveness of biological and chemical control as single treatment for their ability to minimize avocado GF and to increase percentages of success avocado grafted seedlings under nursery conditions and their effects on the activity of defense-related enzymes (peroxidase and polyphenoloxidase).

MATERIALS AND METHODS

1. Observation of avocado grafting failure in nursery:

During adaptation in commercial nursery of avocado at El Qanater El-Khairia Horticulture Research Station, Agricultural Research Center, percentage of grafting failure (GF) was evaluated on five avocado cultivars namely Hass, Reed, Fuerte, Eitinger and Benkerton throughout 2019 and 2020. Avocado seedlings were grafted in (April-May) and (August -September). Twenty seedlings as four replicates for each cultivar were chosen randomly. Success and failure of grafted seedlings were counted after 45 days from grafting and expressed as percentage using the following formula:

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

2. Isolation and identification of the associated fungi:

Naturally infected grafted seedlings were collected from avocado nursery during adaptation and brought to the laboratory to identify the associated fungal species. The collected samples were thoroughly washed under running tap water, cut into small pieces (1 cm-long) and surface sterilized with dipping in 1% sodium hypochlorite for 2 minutes. Small pieces were rinsed in sterile distilled water for several times, then dried between two pieces of sterilized filter paper. The sterilized pieces were transferred individually to Petri plates, each containing 20 ml potato dextrose agar (PDA) medium. All petri plates were incubated at 25 ± 1°C for 5 days and examined daily for fungal growth. Hyphal tip and single spore techniques were used to purify the associated organism(s) on PDA slants. Most frequent fungal colonies were purified and transferred to new Petri plates with PDA. The purified fungi were identified according to colony characteristics and conidial morphology as described by Booth (1971), Nelson *et al*. (1983) and Barnett and Hunter (1986). Pure culture stocks of the isolated fungi were kept in PDA slants at 5ºC for further studies. 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:

To perform the pathogenic potentialities of the isolated fungi, scions and rootstocks were immersed in the spore suspensions of the isolated fungi for one minute before joined. Inoculum was prepared by inoculation (∅9cm) sterilized Petri plates containing PDA medium with equal disks of the fungus *Lasiodiplodia theobroma*e, *Fusarium moniliforme* and *Alternaria alternata*, each alone then incubated at 25-28°C for 7 to 10 days. Five ml sterilized distilled water were added to each plate to free the spores from pycnidium and or conidia using sterile glass rod and added to 500 ml distilled water. Suspensions were filtered through three layers of cheesecloth to remove mycelial fragments. Spore concentration was adjusted using a haemocytometer to obtain (10^6 conidia/ml). Spore suspension was used directly for pathogenicity test according to Hassan, (2015). Before grafting, rootstocks were disbudded with a sharp knife, with the exception of the bottom buds un-removed. Scion wood was cut 12 cm

long. The rootstock and scion were joined together and tying with poly-ethylene. Avocado cultivars *i.e.*, Hass, Reed, Fuerte, Etinger and Benkerton were used to study their susceptibility to the pathogenic fungi during the grafting, four replicates of each treatment and five seedlings were used for each replicate. Data were calculated after 45 days from grafting as % seedlings with GF as mentioned before.

4. Control of GF of avocado seedlings:

4.1. *In vitro* assessment of different commercial products against GF causal agents:

Three concentrations of different commercial products (Table 1) each alone were evaluated *in vitro* for their efficacy against growth of *L. theobroma*e, *F. moniliforme* and *A. alternata*. Fungal mycelia plug (5 mm ∅), from the growing edge of one-week-old cultures, were placed in the center of petri plate with PDA amended with the concentrations of the tested treatments. Untreated PDA plates inoculated with each fungus were served as control. Each treatment included three plates as replicates and the whole experiment was repeated twice. Plates were incubated at 26±2°C and colony growth (mm) was measured in each treatment and the control after 5-7 days of incubation. Reduction in colony growth was calculated using the formula suggested by Abd-Alla *et al.* (2014) as following:

Reduction in colony growth (%) =

$$dc - dt/dc \times 100$$

Where:

dc = average colony diameter in the control.

dt = average colony diameter in the treatment.

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

Commercial name	Active ingredient	Dose used in basic medium *in vitro* and *in vivo*/ L water
Bio Zeid 2.5%WP	*Trichoderma album*	1, 2 and 3 g
Serenade ASO1.34%SC	*Bacillus subtilis* (QST713)	0.3, 0.5 and 1 ml
Bellis 38%WG	Boscalid 25.2% + Pyraclostrobin 12.8%	0.3, 0.5 and 1 g
Amistar Top 32.5% SC	Azoxystrobin 20% + Difenoconazole 12.5%	0.3, 0.5 and 1 ml
Star copper 50% WP	Copper oxychloride	1, 2 and 3 g
Kocide (2000) 53.8 % DF	Copper hydroxide	1, 2 and 3 g

4.2. Efficacy of different commercial products against causal agents under greenhouse conditions:

Apparently healthy scions were chosen, uniform from mother's avocado cultivars around 20-year-old trees. Under nursery conditions, avocado scions of each of cv. Hass and cv. Fuerte for each treatment were prepared for grafting on the same number of rootstocks. Scions were dipped in the concentration that gave the best inhibition of the tested pathogens in *in vitro* study (3g of Bio Zeid, Star copper and Kocide (2000) and (1 ml or 1g) of Serenade ASO, Amistar Top and Bellis as shown in Table (1) for five minutes and left to air-drying for 5 minutes. Then scions were artificially infested with dipping separately in the spore suspensions of each of *L. theobromae*, *F. moniliforme* and *A. alternata* for 20 seconds. Twenty avocado scions, as four replicates of each cultivar, were grafted on the same number of rootstocks. The same number of scions and rootstocks were artificially infested with the three tested fungi each alone to serve as control . Both scions and rootstocks were wrapped with polyethylene strips and kept under control for adaptation under nursery conditions. Efficacy of each treatment was calculated after 45 days from grafting using the formula suggested by Hassan (2015) as follows:

$$E = C\text{-}T / C \times 100$$

Where:

E = Efficacy (%).

C = Control: Number of infested and untreated failure scions.

T = Treatment: Number of infested and treated failure scions.

4.3. Efficacy of different commercial products on the percentages of successful avocado grafted seedlings:

The present study was carried out at El-Qanater El-Khayria Horticulture Research Station, Qaliobiya governorate to study the effect of different commercial products on the percentages of successful avocado grafted seedlings.1.5-year-old avocado seedlings were used for grafting under nursery conditions. The scions of cvs. Hass, Reed, Fuerte, Etinger and Benkerton were collected from 20-year-old trees showing good growing and were prepared for grafting during April 2019 and 2020. Scions were dipped in the above-mentioned best concentrations of the commercial products as shown in Table (1) for five minutes and left to air-drying for five minutes. Each treatment

consisted of four replicates and each replicate consisted of five seedlings. The same number of seedlings of each cultivar were dipped in distilled water and served as control. Successful seedlings were counted after 45 days from grafting and expressed as percentage of successful grafted seedlings as follow:

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

5. Effect of the tested commercial products on peroxidase and polyphenoloxidase activity:

To determine the effect of the tested commercial products with the concentration 3 g/L or 1ml or g/L on the defense enzymes activities in two avocado cvs. Hass and Fuerte, samples of scions were taken 1hr. and 24 hrs. of dipping in the tested compounds to determine the peroxidase (POD) and polyphenoloxidase (PPO) activities.

Scions' extraction:

Fresh scions samples were ground with sodium phosphate buffer (0.1 M, pH 7.0) using a glass homogeniser at a concentration of 100 mg/1 ml buffer. Homogenates were centrifuged at 10,000 r.p.m. for 30 min at 4 °C. The supernatant was used to determine enzyme activities (Tuzun *et al.*, 1989)**.**

1. Peroxidase activity (POD):

Peroxidase assay (based on oxidation of pyrogallol to purpyrogallin in the presence of H_2O_2) was determined according to the method described by Allam and Hollis (1972). The reaction mixture contains 0.3 ml of the *in vitro* crude enzyme + 0.5 ml phosphate buffer solution (pH_7) + 0.3 ml pyrogallol + 1 ml H_2O_2. The mixture was completed with distilled water up to 3 ml. Peroxidase activity was expressed as the change in the absorbance of the mixture every 0.5 minute for 5 minutes' period at 425 nm by Spectrophotometer (Spectronic 601 Milton ROY).

2. Polyphenoloxidase activity (PPO):

The activity of polyphenoloxidase was measured as mentioned by Matta and Dimond (1963). The reaction mixture contains 1ml of the *in vitro* crude enzyme + 1ml phosphate buffer solution (7.1 pH) + 1ml catechol and completed with distilled water to 6.0 ml. Polyphenoloxidase activity was expressed as the change in the absorbance of the mixture every 0.5 minute for 5 minutes' period at 495 nm by Spectrophotometer (Spectronic 601 Milton ROY).

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. Observation of avocado grafting failure in nursery:

Symptoms of grafting failure (GF) and death of scions appeared on recently grafted seedlings in nursery in all avocados examined cultivars as wilting and death of the scions followed by dieback, gradual drying and cracking of the bark from the apex and moving down toward the base of the scion. After 20 days from grafting the union of the graft presented longitudinal extended necrosis. 35 days after grafting, abundant light gray mycelial growth was observed covering most of the scion and the graft union. Scions after GF showed necrosis and dieback appeared in the rootstock (Figs. 1 and 2).

Fig. (1): Symptoms of grafting failure (GF) appear as completely separation occurred between scion and rootstock on joined grafting area (A), this region turned into a brown and black (B), fungal growth on and within the grafting area (C), comparing with successful grafting (D).

Fig. (2): Death of scion after grafting (A), fungal growth on and within the grafting area (B and (C), after (GF) dieback progress appeared on rootstock (D), comparing with successful grafting E.

Data presented in Table (2) show that the % GF of avocado seedlings was higher during April-May than in August -September. The maximum mean of disease incidence in the two periods in the two years (47.5 and 42.5%) were recorded on cv. Hass and cv. Fuerte, respectively, followed by cv. Benkerton and cv. Reed (35.0 and 33.7%), respectively. In this respect, cv. Etinger was the less susceptible which recorded (30.0%) mean of disease incidence in the two periods.

Table (2): % Grafting failure of avocado seedlings in El Qanater El-Khairia nursery in 2019 and 2020.

Cultivars	% Grafting Failure (GF)				Mean (%)
	Season 2019, periods of grafting		Season 2020, periods of grafting		
	April-May	August – September	April-May	August - September	
Hass	55.0	50.0	45.0	40.0	47.5
Reed	40.0	30.0	35.0	30.0	33.7
Fuerte	45.0	40.0	45.0	40.0	42.5
Etinger	35.0	30.0	30.0	25.0	30.0
Benkerton	40.0	35.0	35.0	30.0	35.0
Mean (%)	43.0	37.0	38.0	33.0	37.7
L.S.D.at 5%	P.= 0.71; Se.=0.65; Cvs. =0.92; P.× Se.= 1.24; P.× Cvs. = 1.29; Se. ×Cvs. = 1.05; P. × Se. × Cvs. = 2.29				

P. = Periods of grafting; Cvs. = Cultivars; Se. = Seasons

2. Isolation and identification of the associated fungi:

Data in Table (3) show that fungi isolated from unsuccessful grafted seedlings and dead scions were *Lasiodiplodia theobromae, Fusarium moniliforme and Alternaria alternata*. *L. theobromae* was the most common isolated fungus (72.6 %) on all tested cultivars, followed by *F. moniliforme* (21.5). While *Alternaria alternata* recorded the lowest mean of frequency on all tested cultivars, being 5.9%.

Table (3): Frequency (%) of fungal species isolated from joined grafting area of avocado cultivars showing grafting failure.

Fungus	Frequency (%)					Mean (%)
	Hass	Reed	Fuerte	Etinger	Benkerton	
L. theobromae	74.5	77.8	69.7	68.9	72.2	72.6
F. moniliforme	20.4	18.7	23.9	21.6	22.9	21.5
A. alternata	5.1	3.5	6.5	9.6	4.9	5.9
Total	100	100	100	100	100	100
L.S.D.at 5%	F. = 0.56; Cvs. = n.s.; F.× Cvs. = 0.58					

n.s. = insignificant; F. = Fungi; Cvs. = Cultivars

3. Pathogenicity test:

Results concerning the pathogenicity test show that all the recovered fungal species had the capabilities to cause the grafting failure and death of scions on the five cultivars at different degrees. In this respect, data in Table (4) and Fig. (3) show that the highest mean of (GF) and death of scions on all cultivars were caused by the fungus *L. theobroma*e (100%), followed by *F. moniliforme* (57%). The lowest percentage of (GF) and death of scions was observed for *A. alternata* (12%) in all cultivars. Concerning to the five cultivars, Hass and Fuerte were the most susceptible cultivars where % GF and death of scions was (63.3% and 60.0), followed by Benkerton and Reed (56.6 and 53.3). While value of % Grafting failure (GF) was 48.3% on cv. Etinger.

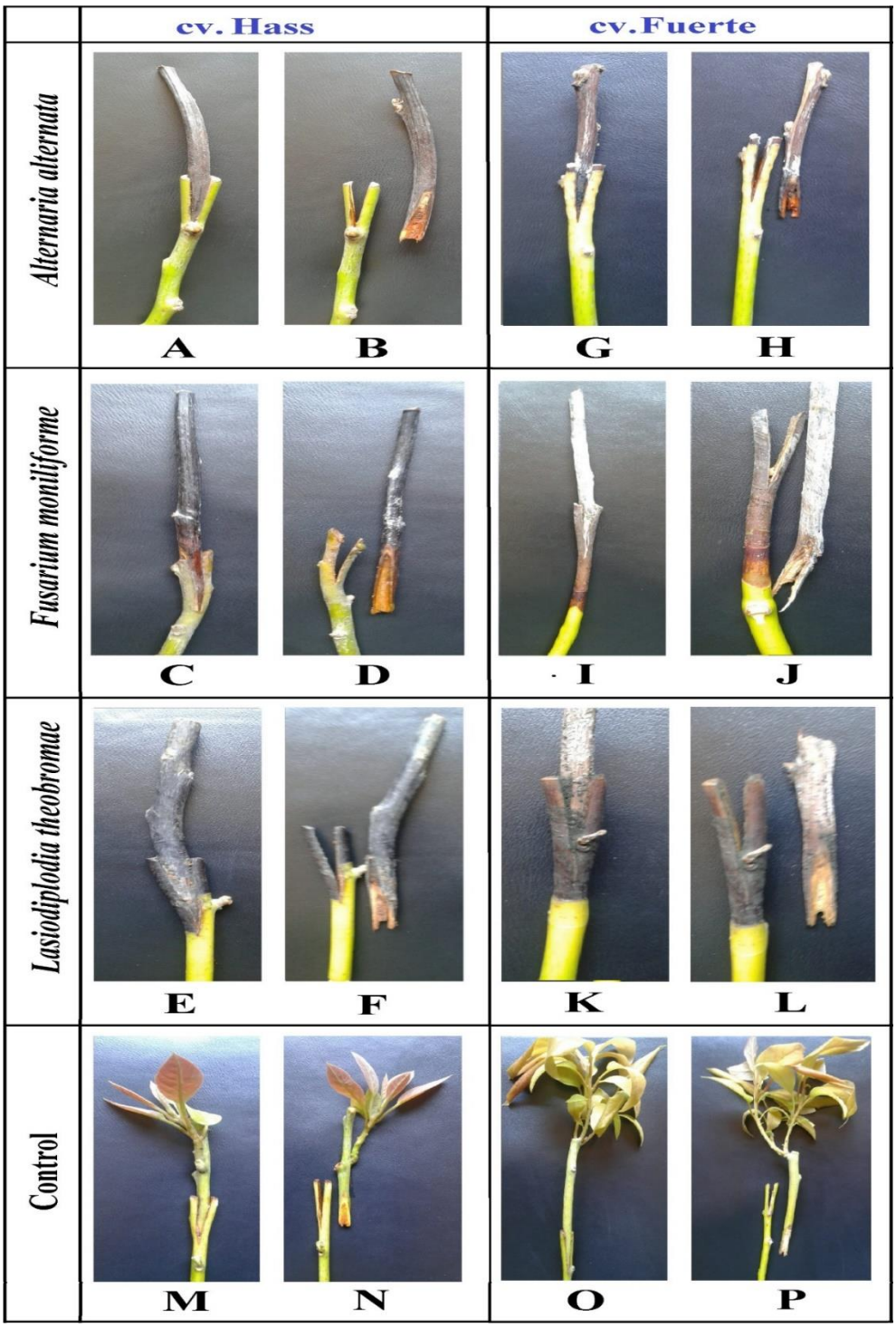

Fig. (3): Pathogenicity test for the avocado seedlings cv. Hass and cv. Fuerte using the isolated fungi, *Alternaria alternata* (A, B and G, H), *Fusarium moniliforme* (C, D and I, J), *Lasiodiplodia theobromae* (E, F and K, L) and control (M, N and O, P).

Table (4): Pathogenicity test of the isolated fungi expressed as the percentage of grafting failure on five avocado cultivars.

Fungus	% Grafting failure (GF) on cvs.					Mean (%)
	Hass	Reed	Fuerte	Etinger	Benkerton	
L. theobromae	100.0	100.0	100.0	100.0	100.0	100.0
F. moniliforme	70.0	50.0	65.0	40.0	60.0	57.0
A. alternata	20.0	10.0	15.0	5.0	10.0	12.0
Mean (%)	63.3	53.3	60.0	48.3	56.6	56.3
L.S.D. at 5%	F. = 1.65; Cvs. = 1.33; F. × Cvs. = 2.19					

F. = Fungi; Cvs. = Cultivars

4. Control of GF of avocado seedlings:

4.1. *In vitro* assessment of different commercial products against GF causal agents:

Data in Table (5) show the effectiveness of three different concentrations of six commercial products against the colony growth of the isolated pathogenic fungi. Generally, all products completely inhibited the colony growth of all the tested fungi at 3g/L and 1ml or g/L. At concentration 2g and 0.5 ml or g/L water, the treatments differed in their ability to inhibit the colony growth of the three pathogens. Considering the average of efficacy of the tested fungicides at concentrations 2g and 0.5 ml or g/L water against *L. theobromae, F. moniliforme and A. alternata*. Amistar Top and Bellis gave the highest reduction in colony growth of all pathogens (92.4 and 87.3%), followed by Kocide (2000) and Star copper (85.9 and 83.1%). While Serenade ASO and Bio Zeid recorded the lowest efficacy in reducing the colony growth of the three pathogens being 81.4 and 80.5%.

Table (5): *In vitro* evaluation of various treatments against colony growth, of the tested fungi.

Treatments	Conc./1 L water	*L. theobromae*		*F. moniliforme*		*A. alternata*		Mean	
		Cg.	R%	Cg.	R%	Cg.	R%	Cg.	R%
Bio Zeid	1 g	33.3	63.0	30.3	66.3	28.3	68.6	30.6	65.9
	2 g	19.4	78.4	17.4	80.7	15.8	82.4	17.5	80.5
	3 g	00.0	100.0	00.0	100.0	00.0	100.0	00.0	100.0
Serenade ASO	0.3ml	30.4	66.2	27.6	69.3	27.1	69.88	28.4	68.5
	0.5 ml	16.8	81.3	16.0	82.2	17.4	80.7	16.7	81.4
	1 ml	00.0	100.0	00.0	100.0	00.0	100.0	00.0	100.0
Bellis	0.3g	16.1	82.1	13.8	84.7	17.1	81.0	15.7	82.6
	0.5 g	10.2	88.6	10.9	87.9	13.2	85.3	11.4	87.3
	1 g	00.0	100.0	00.0	100.0	00.0	100.0	00.0	100.0
Amistar Top	0.3ml	10.9	87.9	13.1	85.4	11.2	87.6	11.7	86.9
	0.5 ml	5.7	93.7	8.2	90.9	6.7	92.6	6.9	92.4
	1 ml	00.0	100.0	00.0	100.0	00.0	100.0	00.0	100.0
Star copper	1 g	24.6	72.7	26.3	70.8	27.2	69.8	26.0	71.1
	2 g	13.5	85.0	15.8	82.4	16.3	81.88	15.2	83.1
	3 g	00.0	100.0	00.0	100.0	00.0	100.0	00.0	100.0
Kocide (2000)	1 g	20.2	77.6	23.9	73.4	24.8	72.4	22.9	74.5
	2 g	10.5	88.3	11.8	86.9	15.7	82.6	12.6	85.9
	3 g	00.0	100.0	00.0	100.0	00.0	100.0	00.0	100.0
Control	--	90.0	--	90.0	--	90.0	--	90.0	--
L.S.D. at 5 % =		F. = 0. 98; Conc. = 0. 57; T. = 0.98; F.× Conc.= 1.00; F. × T. = 1.02; Conc.×T. = 1.04; F. × Conc. × T.= 1.97							

Cg. = Colony growth (mm); R% = Reduction; F. = Fungi; T. = Treatments; Conc. = Concentrations.

4.2. Efficacy of different commercial products against the causal agents under greenhouse conditions:

Data in Table (6) demonstrate that dipping scions in the suspension of each of the tested treatments suspensions before dipping in spore suspensions of each fungus led to reducing the (GF) during grafting and increased the percentage of grafting success in the two tested cultivars compared with control (infested and untreated failure scions). In this respect, Amistar Top and Bellis were the most effective treatments for controlling the pathogens in the two tested cultivars, followed by Kocide (2000) and Star copper. The lowest efficacy was recorded when scions were dipped in Serenade ASO and Bio Zeid.

Table (6): Efficacy of some different treatments against the causal agents of failure during grafting under greenhouse conditions.

Cultivars	Fungi	% Grafting failure											
		C	Bio Zeid		Serenade ASO		Bellis		Amistar Top		Star copper		Kocide (2000)	
			T	E (%)	T	E (%)	T	E (%)	T	E (%)	T	E (%)	T	E (%)
Hass	*L. theobromae*	20.0	4.0	80.0	3 .0	85.0	2.0	90.0	1 .0	95.0	3.0	85.0	2.0	90.0
	F. moniliforme	14.0	2.0	85.7	2 .0	85.7	1 .0	92.8	0 .0	100.0	2.0	85.7	2.0	85.7
	A. alternata	4.0	1.0	75.0	1.0	75.0	0 .0	100.0	0.0	100.0	1.0	75.0	1.0	75.0
	Mean%	12.6	2.3	80.2	2.0	81.9	1.0	94.2	0.3	98.3	2.0	81.9	1.6	83.5
Fuerte	*L. theobromae*	20.0	3 .0	85.0	2.0	90.0	2 .0	90.0	2.0	90.0	3.0	85.0	3.0	85.0
	F. moniliforme	13.0	1.0	92.3	1.0	92.3	1 .0	92.3	0.0	100.0	2.0	84.6	1.0	92.3
	A. alternata	3.0	1.0	66.6	1.0	66.6	0.0	100.0	0.0	100.0	0.0	100.0	0.0	100.0
	Mean%	12.0	1.6	81.3	1.3	82.9	1.0	94.1	0.6	96.6	1.6	89.8	1.3	92.4
L.S.D. at 5 % =		Cvs. = 0.57; F.= 0.87; T.= 0.86; Cvs. × F.= 0.68; Cvs. × T.= 0.98; F.×T.= 1.04; Cvs. ×F.×T.= 1.77												

C = Control: Number of infested and untreated failure scions; T. = Treatment: Number of infested and treated failure scions; E (%) = % Efficacy; Cvs. = Cultivars; F. = Fungi.

4.3. Efficacy of different commercial products on the percentages of successful avocado grafted seedlings under nursery conditions:

Data in Table (7) indicate that dipping scions in each suspension of the tested commercial products during grafting increased the percentage of grafting success for all tested cultivars compared with control. Considering the means of efficacy of the tested treatments on the tested cultivars, data also show that Amistar Top, Bellis and Kocide (2000) were the most effective which recorded (91.0 and 94.0%), (85.0 and 87.0%) and (84.0 and 87.0%), respectively (%) success of avocado grafted seedlings on all cultivars in the two seasons, followed by Serenade ASO (83.0 and 87.0%). While the least percentage of success of avocado grafted seedlings on all cultivars in the two years were observed on Star copper and Bio Zeid (81.0 and 86.0%) and (81.0 and 84.0%), respectively. Also, the highest effect of all treatments was on cvs. Etinger, cvs. Reed and Benkerton, while the lowest effect was on cvs. Fuerte and Hass in the two seasons.

Table (7): Efficacy of some commercial products on the percentages of successful avocado grafted seedlings.

Treatments	Conc. / 1L water	% Success of avocado grafted seedlings.										Mean%	
		Hass		Reed		Fuerte		Etinger		Benkerton			
		2019	2020	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Bio Zeid	3 g	75.0	80.0	85.0	85.0	80.0	80.0	85.0	90.0	80.0	85.0	81.0	84.0
Serenade ASO	1 ml	80.0	85.0	85.0	90.0	80.0	85.0	85.0	90.0	85.0	85.0	83.0	87.0
Bellis	1 g	80.0	85.0	85.0	90.0	80.0	85.0	90.0	85.0	90.0	90.0	85.0	87.0
Amistar Top	1 ml	85.0	90.0	95.0	95.0	90.0	95.0	95.0	95.0	90.0	95.0	91.0	94.0
Star copper	3 g	75.0	80.0	85.0	90.0	80.0	85.0	80.0	85.0	85.0	90.0	81.0	86.0
Kocide (2000)	3 g	80.0	80.0	85.0	90.0	85.0	85.0	85.0	90.0	85.0	90.0	84.0	87.0
Control	Water	45.0	55.0	60.0	65.0	55.0	55.0	65.0	70.0	60.0	65.0	57.0	62.0
Mean%	--	74.2	79.2	82.8	86.4	78.5	81.4	83.5	86.4	82.1	85.7	80.2	83.8
L.S.D.at 5 %		Cvs. = 0.67; Se. =0.80; T. = 0.88; Cvs. × Se.= 1.07; Cvs. × T.= 1.08; Se.× T. =1.05; Cvs. × Se. ×T.= 1.88											

Cvs. = Cultivars; Se.= Seasons; T. = Treatments

5. Impact of the tested commercial products on peroxidase and polyphenoloxidase activity:

Data in Figs. (4 and 5) show that all tested treatments increased peroxidase and polyphenoloxidase activities in comparison with control treatments in the two tested cultivars. Also, peroxidase and polyphenoloxidase activities were more after 1hr. of dipping the scions in the tested compounds than after 24 hrs. of dipping the scions in the tested compounds. In addition, peroxidase and polyphenoloxidase activities were more in cv. Fuerte than in cv. Hass. The highest values of peroxidase and polyphenoloxidase activities in the two tested avocado cultivars were observed after dipping the scions in Amistar Top and Bellis, followed by Kocide (2000) and Serenade ASO. While the lowest levels of peroxidase and polyphenoloxidase activities were recorded after dipping the scions in Star copper and Bio Zeid.

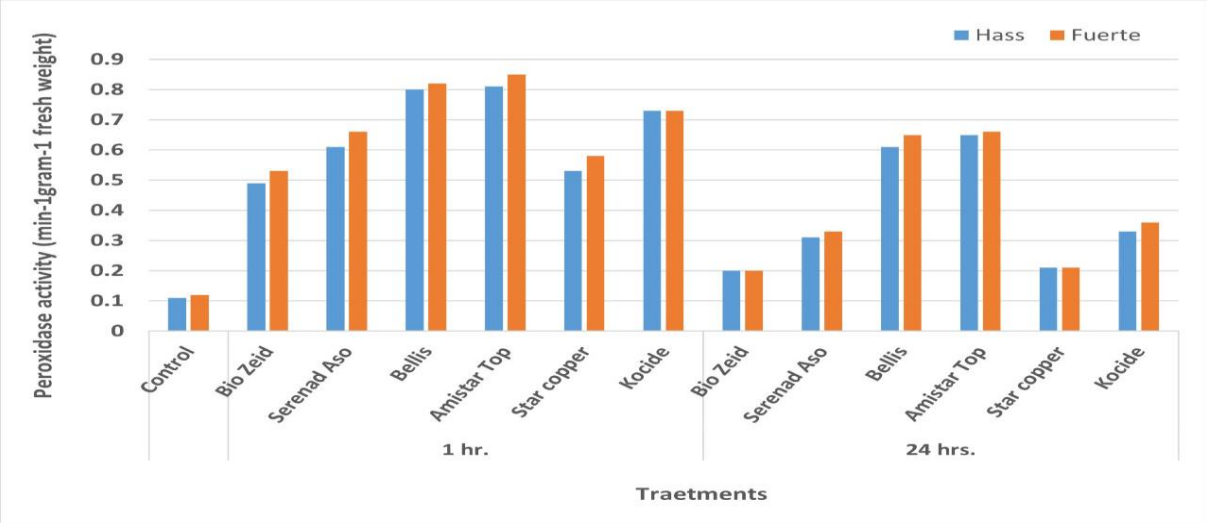

Fig. (4): Effect of the tested commercial products on the activity of peroxidase activity 1h. and 24h. after treatment. Enzyme activity is expressed as absorbance $min^{-1}gram^{-1}$ fresh weight.

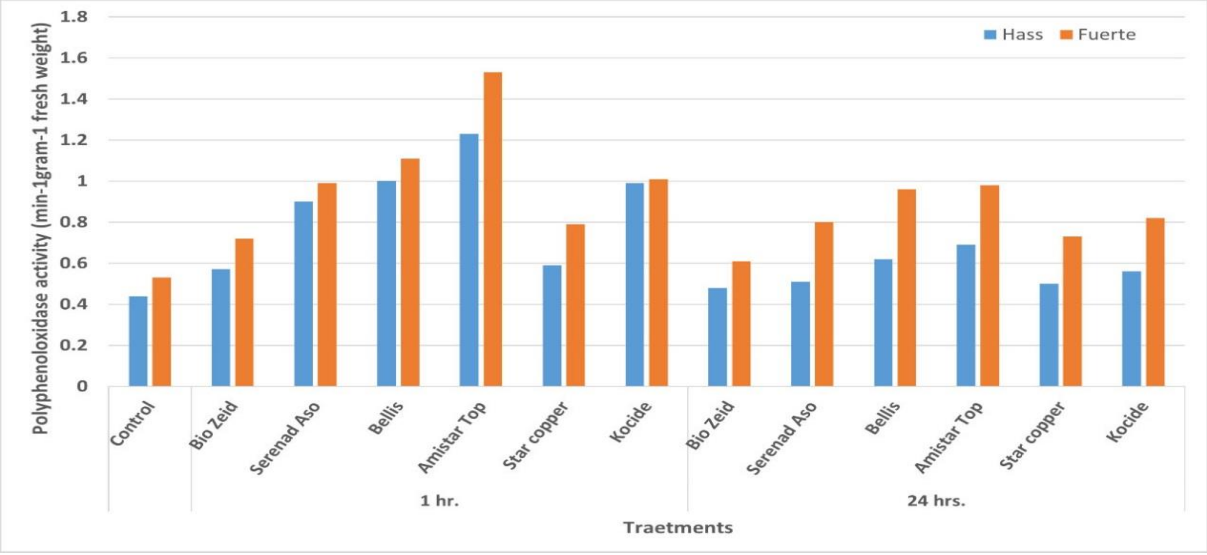

Fig. (5): Effect of the tested commercial products on the activity of Polyphenoloxidase activity 1h. and 24h. after treatment. Enzyme activity is expressed as absorbance $min^{-1}gram^{-1}$ fresh weight.

DISCUSSION

Grafting failure (GF) and death of scions is considered a severe disease which attacks avocado seedlings during propagation by grafting that can cause considerable losses in number of seedlings in nurseries and grafted plants in new orchards. Symptoms appear as completely separation occurs between scion and rootstock on

a union area of grafting, this region turned into a brown and black and presence of fungal growth on and within the grafting area and dieback may progress and appeared on rootstock. The union of grafting presented longitudinal extended necrosis after grafting failure. In this study, % grafting failure of seedlings was higher during April-May than in August -September. The maximum disease incidence was recorded on cv. Hass and cv. Fuerte, followed by cv. Benkerton and cv. Reed. In this respect, cv. Etinger, was the less susceptible. *L. theobroma*e, *F. moniliforme* and *A. alternata* were isolated from (GF) seedlings and dead scions. *L. theobroma*e was the most common isolated fungus on all tested cultivars, followed by *F. moniliforme*. While *A. alternata* recorded the lowest frequency. All the isolated fungi had the capabilits to cause the disease at different percentage of GF and dead scions on the five cultivars of avocado seedlings. In this respect, the highest percentage of (GF) and death of scions was obtained by the fungus *L. theobroma*e, followed by *F. moniliforme*. The lowest percentage of (GF) and death of scions was observed for *A. alternata* in all cultivars. Ploetz *et al.* (1996) and Pavlic *et al.* (2004) described grafting failure symptoms after 30 days of inoculation of mango and *Syzygium cordatum* grafted plants with *Lasiodiplodia gonubiensis* as wilting in apical buds, rapid dieback of scions and abundant grayish mycelial growth at the graft union. The deep and extensive cuts made in the scions and rootstocks facilitated pathogen penetration to vascular tissue. Tovar-Pedraza *et al.* (2012) indicated that 97% and 3% of *Lasiodiplodia* sp. and *Pestalotiopsis* sp., respectively were isolated from 20 of necrotic rootstocks and scions' samples of Mamey Sapote grafts from a nursery in Guerrero, Mexico that showed typical necrosis symptoms at the graft union and 82% and 18 % of *Pestalotipsis* sp. and *Lasiodiplodia* sp. were isolated from10 asymptomatic scions collected in nursery. Gramaje *et al.* (2009) reported that frequency of *Botryosphaeria* spp. which isolated from the scions, the graft union and rootstocks of grapevine was 23.1, 61.5 and 61.5%, respectively. Atia *et al.* (2003) noticed that hyphae of *Botryodiplodia theobromae*, *Phomopsis viticola* and *Fusarium solani* are able to colonize the tissues of grapevine and cause necrosis in xylem parenchyma and xylem vessels. Abo Rehab *et al.* (2013) reported that *Phomopsis viticola* was the most frequently isolated fungus from grafted failure grapes seedlings, followed by *Botryodiplodia theobromae*. While the least frequently isolated fungi were *Phoma* sp., *Fusarium solani* and *Alternaria solani*.

The effectiveness of biological and chemical control was investigated *in vitro* as well as single treatment before grafting for their ability to minimize avocado GF and to increase survived seedlings after grafting under nursery conditions. All products completely inhibited the growth of all fungi at 3g/L and 1ml or g/L. At concentration 2g and 1ml or g/L water, the treatments were differed in their ability to inhibit the colony growth of the three pathogens. Amistar Top and Bellis gave the highest reduction in colony growth of all pathogens, followed by Kocide (2000) and Star copper. While Serenade ASO and Bio Zeid recorded the lowest efficacy in reducing the colony growth of the three pathogens. Also, our results, under greenhouse conditions, showed that dipping scions in each of the tested treatment suspensions before dipping in spore suspensions of each fungus led to suppress the spores of the pathogen during grafting and increased the percentage of success of grafted seedlings in the two tested cultivars compared with control (infested and untreated failure scions). In this respect, Amistar Top and Bellis were the most effective treatments for controlling the pathogens in the two tested cultivars, followed by Kocide (2000) and Star copper. The lowest efficacy was recorded when scions were dipped in Serenade ASO and Bio Zeid. Also, under nursery conditions, dipping scions in each suspension of the tested commercial products before grafting increased the percentage of grafting success for all tested cultivars compared with control. Data also showed that Amistar Top, Bellis and Kocide (2000) were the most effective which recorded the highest (%) success of avocado grafted seedlings on all cultivars in the two seasons, followed by Serenade ASO. While the least (%) success of avocado grafted seedlings on all cultivars in the two seasons were observed on Star copper and Bio Zeid. Twizeyimana *et al.* (2013) found *in vitro* test that azoxystrobin, fludioxonil, metconazole, and pyraclostrobin inhibited the mycelial growth of *Neofusicoccum america, N. luteum, N. parvum, and Phomopsis* sp. which are associated with branch cankers of avocado trees (*Persea americana*) in California. Also, In the field experiment azoxystrobin + propiconazole and metconazole can control avocado branch canker. Abo Rehab *et al.* (2013) found that Topsin M and Kema Zed gave the best results for controlling fungal pathogens causing

grafting failure of grapes, followed by Bellis, Saprol, Syllit and Conazol. Also added, Bio-Zied (*Trichoderma album*), Rhizo-in (*Bacillus subtilis*) and Bio-Arc (*Bacillus megaterium*) reduced the percentage of graft failure of grapes. There are different mechanisms that corroboration the effect of biological control on plant diseases, which are antibiosis, direct parasitism, competition, suppression, induced resistance hypovirulence and predation. The antagonistic activity has often been associated with production of secondary metabolites. Enzymes responsible for cell-wall degradation such as chitinases and glucanases have been associated with the ability of *Trichoderma* spp. to control plant pathogens (Matroudi *et al.,* 2009).

Regarding the changes in activities of defense related enzymes as a result of treating avocado scions with some fungicides or biofungicides, all tested treatments increased peroxidase and polyphenoloxidase activities in comparison with control treatment in the two tested cultivars. Also, peroxidase and polyphenoloxidase activities were more after 1hr. of dipping the scions in the tested compounds than after 24 hrs. of dipping the scions in the tested compounds moreover peroxidase and polyphenoloxidase activities were more on cv. Fuerte than cv. Hass. The highest values of peroxidase and polyphenoloxidase activities in the two tested avocado cultivars were induced after dipping the scions in Amistar Top and Bellis, followed by Kocide (2000) and Serenade ASO. While the lowest levels of peroxidase and polyphenoloxidase activities were recorded after dipping the scions in Star copper and Bio Zeid. Many investigators supported this idea, Retig, (1974) reported that in the roots and stems of the resistant and susceptible tomato plants peroxidase and polyphenoloxidase activities increased and induced resistance of tomato to Fusarium wilt after 1hr. of treating with Ethephon 2-chloroethylphosphonic acid. Yedidia *et al.* (2000) reported that inoculating roots of cucumber seedlings with *Trichoderma* sp. in an aseptic, hydroponic system showed higher activities of chitinase, β-1, 3-glucanases and peroxidase. Trichoderma-treated plants were more developed than nontreated plants. These results were observed for both the roots and the leaves of treated seedlings, providing evidence that *T. harzianum* may induce systemic resistance mechanisms in cucumber plants. Treating rice plants with carbendazim, benomyl, calixin and baycor controlled bacterial leaf blight by inducing defense-related enzymes (peroxidase, polyphenoloxidase, tyrosine ammonia lyase and phenylalanine ammonia lyase (Gomathinayagam *et al.*, 2008). Treating tomato plants with the endophytic bacterium *Pseudomonas fluorescens* strain 6328 increased resistance to *Fusarium oxysporum* by activating gene encoding defense enzymes *i.e*., peroxidase, β-1,3-glucanase, phenylalanine ammonia-lyase, chitinase (M'Piga *et al.*, 1997). Saravanakumar *et al.* (2008) noticed that *Metschnikowia pulcherrima* Strain Mach1 controlled the postharvest disease on apple caused by *Botrytis cinerea* compared to untreated controls and enhanced activities of defense-related enzymes *i.e*., peroxidase, polyphenoloxidase and phenylalanine ammonia lyase. Chakraborty *et al.* (2010) reported that brown root rot of tea caused by *Fomes lamaoensis* was reduced by treating tea seedlings with *Serratia marcescens* TRS-1 and increased the height, emergence of new leaves and branches and leaf biomass. Also, increasing in phenolics, peroxidase, chitinase, β-1,3-glucanase and phenylalanine ammonia-lyase were observed. Prakongkha *et al.* (2013) reported that anthracnose on grape caused by *Sphaceloma ampelinum* was controlled by foliar application with chitosan and benzo-(1, 2, 3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), also increased chitinase, ß-1, 3-glucanase and peroxidase activities levels in leaves over non-treated plants. El-Banna *et al.* (2015) found in *in vitro* bioassay that some bacterial bio-agents reduced the colony growth of *L. theobromae* which causes die-back disease on grapevine. Also, they cleared that secretion of enzymes endo, exo-β-1, 3-glucanase, chitinase and protease which be involved in the degradation of fungal cell walls were encouraged by treating with bacterial bio-agents.

CONCLUSIONS

Our results reported that *L. theobromae, F. moniliforme and A. alternata* may be the main pathogens causing grafting failure (GF) symptoms on avocado grafted seedlings. Dipping scions in the suspension of the tested commercial products just before grafting increased the percentage of grafting success. Amistar Top, Bellis and Kocide (2000) were the most effective which gave the highest (%) success of avocado grafted seedlings. There are positive relationships between peroxidase and polyphenoloxidase enzymes and resistance developed in cv. Fuerte and cv. Hass after dipping the scion in the treatments in comparison to control.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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