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IMPACT OF SAFE ALTERNATIVE TREATMENTS TO MANAGEMENT GRAFT FAILURE ON CITRUS SEEDLING CAUSED BY SOME PHYTOPATHOGENIC FUNGI

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ABSTRACT: Commercial citrus trees are composed of a scion grafted onto a rootstock. Because grafting is one of the most expensive methods of plant propagation, grafting efficiency is of large practical importance. Recently, Valencia orange trees on root-stocked Volkamer lemon, showed failure grafting. So, they were re-grafted again on a farm located in Um Ammar region of Ismailia governorate. Isolation trials resulted in *Alternaria alternate*, *Fusarium solani* and *F. equiseti* with frequencies *i.e.* 66.25% 31.37% and 2.11%, respectively from necrotic tissues. Also, the isolation trials were done from all the tested rootstock and scion in the nursery of Horticulture Research Institute (HRI) and the highest frequencies showed with *A. alternata* on Volkamer lemon (48%) while the lowest frequencies showed with Trifoliate orange (2%). As for *Lasiodplodia theobromae*, it was isolated only from Volkamer lemon. Also, the isolated fungi were identified using the traditional methods besides molecular bioassay. Pathogenicity experiment was conducted in greenhouse; for the two more frequencies fungi *i.e. A. alternate* and *F. solani*, the highest disease incidence percentage was 100% by *A. alternate* on Navel orange; Valencia scion with Sour orange and Volkamare lemon rootstock. Also, pathogenicity test of the plant samples of HRI nursery was done and the results showed that *A. alternata* recorded the highest disease incidence (100%) on Navel orange as scion with Sour orange as rootstock. On the opposite it recorded the least disease incidence on Valencia scion with Sour orange rootstock. In an *in vitro* experiment, the activity of five treatments **(**H_2O_2, indole acetic acid (IAA), xanthan gum, wood vinegar and control without treatment**)** was examined against the growth of the pathogenic fungi. IAA (1000 ppm) showed 100% inhibition of both tested fungi mycelial growth. Also *in vitro* fungicides' activity was estimated on growth inhibition against the two citrus pathogenic fungi mentioned before. Kemazed and Kinol (200 ppm) gave complete inhibition (100%), to *F. solani*. Also, Kemazed, Kinol, H_2O_2, IAA, and wood vinegar were accomplished in the nursery to assess their efficacy on failure grafting on either scion and rootstock. The wood vinegar and IAA as alternative fungicides gave superior activity against the fungi on the failure grafting percentage compared with the control treatments, whereas they recorded 77.77% reduction in case of *A. alternate*. Treatments of H_2O_2 gave the highest increase in enzyme activities, while wood vinegar increased the peroxidase, and polyphenol oxidase activities only.

Keywords: Citrus, failure grafting, scion, rootstock, alternative fungicides, wood vinegar

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INTRODUCTION

The total area of citrus cultivated in Egypt is about 486.650 Fadden which produced about 4,330,353 tons of fruits (A.E.R.I., 2018). The grafting process is considered the basis of planting citrus as it depends on rootstock and scion. Grafted trees are then used by citrus growers for planting production groves. Grafted trees are used because of the benefits of a composite rootstock/scion plant. Using citrus rootstocks provides at least three major benefits. One is a shorter juvenility phase compared to seedling-derived trees where juvenility can last for up to 10 years, and sometimes longer. Two, the rootstock confers enhanced resistance to environmental stress and diseases and thereby allows for the production of citrus in areas where the scion on its own roots could not be grown or would grow poorly. Three, enhanced effects on horticultural traits such as tree architecture and fruit yield and quality. Volkamer lemon, Sour orange, and Trifoliate orange are the most dominant citrus rootstocks in Egypt. The failure grafting process is the incompetence of workers and other causal factors. One of them will be cleared in this research. The rootstock is the lower portion of the trunk and root system. Sour orange (*C. aurantium* L.) is a universal rootstock for citrus and is widely used in the Mediterranean region (El-Kady *et al.*, 2007). Sour orange rootstock is mentioned to be suitable for heavy moist soil, producing a high yield of good citrus quality. The produced fruits are characterized by smaller size, thin and smooth skin, high TSS and low acidity (Hemeda, 2014). Volkamer lemon is used as a rootstock for citrus, due to its acceptable resistance to a large scale of citrus diseases. It has a significant enhancement effect on growth due to its suitability for unfavourable environmental (climatic and soil) conditions (El-Kady *et al.*, 2007).

Grafting is a type of plant propagation where part of one plant (the scion) is inverted into another the rootstock or stock, whereas they shape unite and grow as a single plant. Budding is a type of grafting, with the scion consisting of a single bud attached to a piece of bark and sometimes a thin sliver of wood underneath. Also, it is the method of propagating young citrus trees because it is easier to do than other types of grafting through bud wood, which is part of plant with buds used to propagate new seedlings. The grafting takes place in citrus seedlings in March and has a grafting success rate of 80 to 85% it depends on the skill of the grafting agent, the compatibility between the rootstocks and scions and the agricultural processes of removing the crabs and irrigation, there is another grafting date in September and the success rate is 70:75% (Renault *et al.*, 2007). The objective of this study was to use five safe alternative treatments compared with the recommended fungicides to solve the problem of the failure grafting of Navel/Valencia citrus scion and the orange, Volkamer lemon and Sour orange with two citrus pathogenic fungi.

MATERIALS AND METHODS

Experimental sites:

In the Agriculture Research Center at the greenhouse of the Fruit and Woody Trees Diseases Research Department, the pathogenicity test was accomplished. Then the *ex vivo* trial was conducted in a greenhouse at Citrus Research Department, Horticultural Research Institute, Agricultural Research Center, Giza, Egypt.

Samples of fungal pathogens:

Diseased plants were obtained from farms located in Um Ammar region of Ismailia governorate during 2019-2020 showing symptoms of failure grafting diseases.

During the duration of grafting seedlings, many trees failure occurred in the grafting union, this region changed to a blackish to dark brown and separation space occurred between scion and rootstock. Also, seedlings showed some fungal infections within the grafting union were collected. The different fungal isolates used were obtained from both scions grafted and rootstocks. The samples of

naturally infected grafting union were cut and thoroughly washed with tap water, cut into small pieces (one cm long), and sterilized by dipping in 2% sodium hypochlorite solution for 2 min, then washed twice in sterile distilled water. The surface sterilized pieces were dried on sterilized filter paper, then put individually in Petri plates, each containing 10 ml of PDA medium, then incubated at 25±2°C for one week and inspected for mycelial growth. The developed colonies were purified forward single spore techniques (Goh, 1999).

Identification of pathogens associated with grafting failure:

The purified fungi were identified according to their morphological characteristics as described by Leslie and Summerell (2006), Woudenberg *et al*. (2013), and Burgess *et al*. (2006). And confirmed by Mycology and Plant Diseases Survey Res. Dept., Plant Pathology Res. Inst., ARC, Egypt.

Molecular characterization of isolates:

Alternaria and *Fusarium* species were identified based on a combination of the internal transcribed spacer (ITS) region of ribosomal DNA was amplified using ITS1 and ITS 5.4s with ten 5-mm-diameter plugs of each fungal isolate were transferred to 250 ml autoclaved medium containing 30 g/l potato dextrose broth (PDB; Difco) supplemented with 0.5 ml distilled water solution containing 0.72 g $Fe(NO_3)_3 \cdot 9H_2O$, 0.44 g $ZnSO_4 \cdot 7H_2O$ and 0.2 g $MnSO_4 \cdot 4H_2O$ g/l at pH 5.1, and shaken in a 500 ml Erlenmeyer flask at 24±1 °C under continuous light for 1 week. Mycelium was collected by vacuum filtration on a Buchner funnel through two layers of Mira cloth (40 µm). Fungal DNA was extracted from freshly collected mycelium with phenol-chloroform as described by Lee and Taylor (1990) and diluted to a final concentration of 10 $ng\mu l^{-1}$for PCR reactions. r DNA from the ITS region (ITS1, ITS 4·8S,) was amplified with primers ITS4 and ITS1(White *et al.,* 1990). Thermal cycling conditions involved an initial denaturation step at 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 sec, 50 °C for 1 min and 72 °C for 1.5 min, and a final extension step at 72 °C for 10 min. PCR product purifications were carried out with the QIAquick™ PCR purification kit (Qiagen Inc.) Successful PCR reactions resulted in a single band observed on a 1.5% agarose gel.

The internal transcribed spacer (ITS) regions of two isolates were sequenced in this study. Sequencing reactions were performed with primers ITS4 and ITS1 using the CEQ DTCS QuickStart Kit™ (Beckman Coulter, Inc.). Chromatograms were determined with a CEQ 200XL capillary automated sequencer Beckman Coulter, Inc.). Nucleotide sequences were compiled with Sequencer

Pathogenicity test:

Processing of spore suspension methods:

Fungal spores were obtained from two weeks of *A. alternate* culture and *Fusarium solani*. Five ml of sterile water, containing 0.05% (v/v) Tween 80 (Sigma, St. Louis, MO) was added in suspension to improve their properties of the solution, and the spores were isolated from the surface with a sterile glass rod, and suspensions were filtered through three layers of cheesecloth to remove fragments of mycelium. Spore concentration was adjusted using a haemocyto meter to obtain 5×10^6 conidia/ml for each of *Alternaria alternate*, and *Fusarium solani* (Vloutoglou and Kalogerakis, 2000).

Grafts preparation method:

At the greenhouse of the Plant Pathology Research Institute, active axillary buds of a one-year-old shoot from cv. Valencia root-stocked with Volkamer lemon were used as a source of grafting material. All grafting material used for this study is kept humid. Also, they transferred to the nursery of Horticulture Research Institute (HRI) for side-grafting technique with the previously mentioned cultivars. Attached buds from the scion, then vertical cutting with a sterilized grafting knife in bark equally for cambial alignment contact of each scion and rootstock, then binding with vinyl wrapping

tap (Kamanga *et al*., 2017). Periodically irrigated and fertilized as recommended.

Inoculation of Valencia scion grafted with Volkamer lemon rootstock under greenhouse conditions:

In a controlled greenhouse with three plots, each plot has three replicates in 5 grafted seedlings pots each plot was inoculated with *A. alternate* or *F. solani*, and another plot blank plot was kept without treatment. The grafted seedlings were sprayed with the spore suspensions, of each fungus alone and were left for five minutes before joining. The grafted seedlings were maintained, irrigated and fertilized following the recommendations and observed for one month to record the development of appearing symptoms. Results were recorded after 30 days using the following formula:

The successful grafted plants % =

(Total grafted plants - failure grafted plants)/total grafted plants × 100

Controlling the causal organisms in the laboratory:

Evaluate alternative fungicides on linear growth of the pathogenic fungi in the laboratory:

Tested compounds (Table, 1) were prepared at four concentrations to evaluate their effects on the growth of *A. alternate* and *F. solani*. Oxygen peroxide (H_2O_2) (with a concentration of 500, 1000, 2000. 4000 ppm), indol acetic acid (IAA) (with a concentration of 250, 500, 1000, 2000 ppm), xanthan gum (with a concentration of 1000, 2000, 4000, 6000 ppm), and wood vinegar (with a concentration of 50, 100, 200, 400 ppm), were added to flasks containing PDA centrally inoculated with disks (5 mm) of the 14-days-old culture of the tested fungi. Five plates were used as replicates for each treatment. The tested plates were incubated at 25±2 °C. The average linear growth of the tested fungi was recorded after the complete growth of the control plates (Bauiomy, 1997). The percentage of reduction in colony diameter was calculated as follows:

Reduction % colony of diameter =

$$d_e\text{-}d_t\,/d_t \times 100$$

Whereas:

d_e= average growth diameter in the control set

d_t= average diameter of growth in treatment set

Effect of different fungicides on growth of the pathogenic fungi *in vitro*:

Tested fungicides (Table, 1) were prepared at four concentrations to evaluate their effects on the growth of *A. alternate* and *F. solani* and added to flasks containing PDA medium to obtain the desired concentration of 500, 1000, 2000, and 4000 ppm, then dispensed in Petri plates and centrally inoculated with disks (5 mm) of two weeks culture old of the tested fungi. Five plates were used as replicates for each treatment. Inoculated plates were incubated at 25±2 °C. The average linear growth of the tested fungi was recorded after the complete growth of the control plates (Bauiomy, 1997). The percentage of reduction in colony diameter was recorded as mentioned above.

Grafting preparation:

By using six hundred and forty-eight of root stock seedlings mentioned before at Citrus Res. Dep., HRI, the contained many buds grafting flutes were soaked in the aforementioned alternative fungicides and/or fungicides each one alone for 20 min and at the mentioned before their concentration. Buds grafting were attached to the tested rootstock and sprayed with a spore suspension of *A. alternata* or *F. solani* at (5×10^6 conidia/ml concentration alone for 20 min, and the buds were bound by vinyl wrapping tap before 5 min of spraying with spore suspension (Kamanga *et al*., 2017), periodically irrigated and fertilized as a recommended.

Table 1. All the tested compounds, fungicides, and alternative fungicides used in the experiments.

Treatment	Commercial name	Chemical composition, active ingredient (AI), and dose used fungicide	Manufacture or source
Kemazed	Kemazed 50% WP	Methyl benzimidazol-2-ylmethl-carbamate 75 g 100% l., AI= Benzimidazoles	Rotam Agrochemical Hong kong (Kafer Al-Zayt).
Kinol	Kinon l25% EC	(±)-1-(2-(2, 4-dichlorophenyl)-4-propyl-1, 3-dioxolan-2-ylmethl)-IH-1, 2, 4-triazole 25 cm^3/100 l.	Kanza group
Bellis®	Bellis® 38% WG 38%WDG	Boscalid = 2-chloro-N-(4-chlorobiphenyl-2-yl) nicotinamide Pyraclostrobin = methyl N-[2-[[1-(4-chlorophenyl) pyrazol-3-yl] oxymethyl] phenyl]-N-methoxycarbamate AI=25.2% Boscalid and 12.8% Pyraclostrobin 38% WG used at 30 g/100 l	Basf Corporation
Ridomil Gold Plus	Ridomil Gold Plus 71.5% WP	methyl 2-(*N*-(2-methoxyacetyl)-2,6-dimethylanilino) propanoate	Syngenta
Topsin®	Topsin® M70	Thiophanate-methyl = methyl N-[[2-(methoxycarbonyl carbamothioylamino)phenyl] carbamothioyl]carbamate	Sumitomo chemical Japan
		Fungicide alternatives	
Hydrogen peroxide	Hydrogen peroxide	H_2O_2	Al-Gomhorya Co.
Indol acetic acid	Indol acetic acid	1H-indole-3-acetic acid, monosodium salt	
		GRAS- Compounds	
Xanthan gum	Xanthan gum	6-[6-[6-(acetyloxymethyl)-2-[3-[3,4-dihydroxy-6-(hydroxymethyl)-5-phosphanyloxyoxan-2-yl] oxy-5-hydroxy-2-(hydroxymethyl)-6-(phosphanylmethyl)oxan-4-yl]oxy-4,5-dihydroxyoxan-3-yl]oxy-2-carboxy-4,5-dihydroxyoxan-3-yl]oxy-7,8-dihydroxy-2-methyl-4,4a,6,7,8,8a-hexahydropyrano[3,2-d][1,3]dioxine-2-carboxylic acid Molecular Formula: $C_2H_4O_2$	Al-Gomhorya Co.
Wood vinegar	Pyroligneous Acid (Natural)	It consists of three major compounds *ie*. 2.6dimethoxyphenol,2-methoxyphenol and3.5-Dimethoxy-4-hydroxytoluenel	Sigma Aldrich

Percentage of failure grafting of different treated citrus cultivars:

Ex vivo treatments regarding the inhibitory activity of different fungicides and alternative fungicide compounds:

The tested compounds were prepared to evaluate their effects on mycelial growth reduction of *A. alternate* and *F. solani*. Fungicides *i.e.* Kemazed 50% WP, Kinol 25% EC, at *i.e.* 50, 100, 200 and 400 ppm concentration, and the hormone and antioxidants of H_2O_2, IAA, and wood vinegar, at different concentrations, *i.e.* 500, 1000, 2000, 4000 ppm, 250, 500, 1000, 2000 ppm, and 1000, 2000, 4000, 6000 ppm respectively, were tested in to evaluate their effects against fungal pathogens in the nursery at Citrus Research Department, HRI. Activity buds of scions, Navel orange and Valencia were soaked in the abovementioned compounds solution for 20 min and left till dried before being installed in the rootstock (Abo Rehab, 2013), then the spore suspension was applied when installing the graft by spraying it with 5×10^6 conidia/ml (Vloutoglou and Kalogerakis, 2000) with *Alternaria alternate*, and *Fusarium solani*.

The concentration of inoculum was optimized for all the treatments *i.e.*: two

cultivars, three rootstocks, two fungal isolates, and five chemical compounds. Each treatment had three plants in three groups (nine plants per plot). The control treatments were sprayed with spore suspension with treated with compound mentioned before.

The IAA was dissolved in absolute ethyl (90%) alcohol (Yadav, *et al.*, 2022) before the application, Also, all chemical substances dissolved directly in water. The experiment was observed in the nursery of Hort. Res. Inst., ARC, Giza, for two months, and disease incidence was calculated as follows:

The successful grafted plants % =

(Total grafted plants - failure grafted plants)/total grafted plants × 100

Extraction of enzymes:

Samples of tissues (2 g/pot) were taken soon after plant removal and ground in a mortar in the presence of purified sand plus 4 ml of 0.1 M sodium phosphate buffer (pH 7.1) according to Goldschmidt *et al*. (1966). The homogenate was strained through four layers of cheesecloth then the filtrates were centrifuged at 3000 rpm for 20 min at 6 °C.

The obtained supernatant fluids (crude enzyme extracts) were used for assaying activities of peroxidase, polyphenol-oxidase (PPO) and chitinase enzymes at 425, 420 and 540 nm, respectively using a Spectrophotometer (acculab, model: UVS90). Enzyme extract was replaced by distilled water in the control blank cuvette.

Peroxidase assay:

Peroxidase activity was determined according to the method described by Allan and Hollis (1972). The cuvette contained 0.5 ml. 0.1 M potassium phosphate buffer at pH 7.0 + 0.3 ml of enzyme extract + 0.3 ml 0.05 M pyrogallol + 0.1 ml 1.0% H_2O_2 and distilled water to bring cuvette contents to 3.0 ml. The reaction mixture was incubated at 25°C for 15 min. Peroxidase activity was expressed as the increase in absorbance at 425 nm/g fresh weight the method described by Allan and Hollis (1972).

Polyphenol-oxidase assay:

The polyphenol-oxidase activity was determined according to the method described by Matta and Dimond (1963). The reaction mixture contained 0.2 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0 and 1.0 ml 10-3 M catechol and was complete with distilled water up to 6.0 ml. The reaction mixture was incubated for 30 min at 30 °C. The polyphenol-oxidase activity was expressed as the increase in absorbance at 420 nm/g fresh weight, by Matta and Dimond (1963).

Statistical analysis:

Obtained data were subjected to analysis of variances (ANOVA) through Co-Stat 3.4 software as the usual technique of analysis of variance with grouping information using the Fisher LSD Method and 95% confidence as Gomez and Gomez (1984).

RESULTS

Data in Table (2) show that *A. alternate* recorded high-frequency percentage (66.52%) while *F. equiseti* was the least ones (2.11%) in this respect.

Data in Table (3) and Figs. (1 and 2) show that there are significancant differences between the two tested pathogenic fungi in the pathogenicity test. Also, both fungi were pathogenic to citrus seedlings compared to the check treatment.

Data in Table (4) show that *A. alternate* was the most dominant fungus in isolation trials compared with the other isolated fungi, whereas it recorded 86-mean percentage from all the tested citrus varieties. On the other hand, *Lasiodiplodia theobromae* isolated only from Volkamer lemon and ranked in the least degree in this respect.

Molecular Characterization of isolates:

Successful PCR reactions resulted in a single band shown on a 1.5% agarose gel amplified fragments of isolates IMI289962, IMI178784. Purified PCR products yielded sequences of 545–595 bp in length.

Table 2. Frequency % of fungi which isolated from rootstock and scion of Valencia on Volkamer lemon in the Um Ammar farm.

Isolated fungi	Frequency %
Alternaria alternata	66.52
Fusarium solani	31.37
Fusarium equiseti	2.11
Total	100

Table 3. Pathogenicity test of fungi isolated from scion of Valencia citrus on rootstock Volkamer lemon done in Fruits Pathology greenhouse.

Fungi	Disease % incidence of pathogenic fungi
Alternaria alternata	80.00
Fusarium solani	66.66
Control (with DDW*)	00.00
LSD at 5%	14.34

*** DDW: doubled distilled water.**

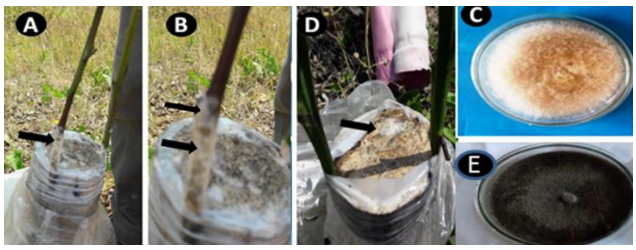

Fig. 1. Fungal mycelium observed on the surface of scion and rootstock (A, B and D), C, *Fusaruim solani* (C), *Alternaria alternata* (E).

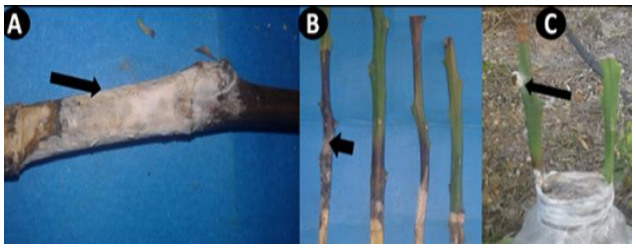

Fig. 2. Fungal mycelium of *Fusaruim solani* observed on the surface of scion (A, B and C).

Table 4. Percentage of frequency isolated fungi from rootstock and scion of citrus obtained from the nursery of Horticulture Research Institute.

Fungus	Frequency %					
	Sour orange	Volkamer lemon	Trifoliate orange	Navel orange	Valencia orange	Total
Alternaria alternate	4	4	2	48	28	86
Fusarium solani	4	0	2	4	3	13
Lasiodiplodia theobromae	0	1	0	0	0	1
Total	8	5	4	52	31	100

Pathogenicity test of the isolated fungus:

Data in Table (5) indicate that the pathogenicity test of *Alternaria alternate* resulted in a significant height infection percentage compared with the control treatments. All the data obtained with Navel orange were significant, whereas they recorded a 20% infection percentage except for *A. alternate* with rootstock Sour orange and Volkamer lemon whereas it destroyed all the test grafting eyes (100%). Also, the result obtained figured out the tested pathogenic fungus resulted in significant infection percentages with the Valencia, and the rootstock Volkamer lemon. On the other hand, the most sensitive rootstock to *Alternaria alternate* was Volkamer lemon compared with the other tested rootstock.

Table (6) demonstrated that all pathogenic tested fungi resulted in a height significant infection percentage compared with check treatments. All the data of the result of Navel orange were significant because they recorded a 20% infection percentage except for *F. solani* with rootstock Sour orange and Volkamer lemon whereas; it destroyed all the tested grafting buds (100%). Also, the result obtained to figure out all the tested pathogenic fungi resulted in significant infection percentages with Valencia variety, and the variety Volkamer lemon. On the other hand, the most sensitive rootstock to *Fusarium solani* was Volkamer lemon compared with the other tested rootstock.

The controlling experiments:

Data in Table (7) and Figs. (3 and 4) indicated all four treatments tested resulted in an increase in mycelial inhibition percentage compared with the control treatments. Also, the percentage of inhibition increased with increasing the concentration of the tested treatments. The tested treatments of H_2O_2 and IAA gave the same effects at the high-test concentration (4000 ppm) whereas they recorded 100% inhibition to both tested fungi *A. alternata* and *F. solani*. Also, xanthan gum and wood vinegar gave similar results in increasing the percentage of mycelial inhibition of both fungi. Whereas they recorded (29.88%) and (41.66%). IAA was the superior treatment in increasing the percentage of mycelial growth inhibition (100%). On the other hand, *F. solani* was the most sensitive fungus to all the tested treatment compared with *A. alternata*.

Data in Table (8) indicated that all the tested treatments decreased the growth of the isolated fungi significantly in comparison to the control treatment. The percentage of decreasing was correlated to the used concentration whereas, the inhibition increased with increasing the concentration, Kinol® fungicide was the superior treatment in decreasing the growth of pathogenic fungi, whereas it completely inhibited the mycelial growth by 100% at the minimum concentration used at 200 ppm the both tested fungi. On the other hand, Ridomil Gold Plus® as fungicide was the least active treatment by decreasing growth of the both tested fungi. *Fusarium equiseti* was the most sensitive fungus to Kemazed and Topsin-M70 fungicides compared with *A. alternata* under laboratory conditions.

Data in Table (9) and Fig. (5) show the activity of five treatments on the percentage of successful grafting of Navel orange and Valencia Scions on three rootstocks *i.e.* Trifoliate orange, Volkamer lemon and Sour orange with *A. alternata*. The superior treatments were IAA at 1000 ppm and wood vinegar at 400 ppm, whereas they recorded 77.77% successful grafting percentage. On the other hand, Valencia scion was affected with wood vinegar (400 ppm) and IAA (1000 ppm) whereas, it recorded 77.77% of successful grafting percentage for both treatments. On the opposite, H_2O_2 was the least effective treatment in this respect. Concerning fungicides, Kinol was the most active treatment as a fungicide whereas it recorded 44.44% of successful grafting.

Data in Table (10) and Fig (6) shows the effects of five different treatments on the percentage of successful grafting of Navel orange, Trifoliate orange, Volkamer lemon,

Table 5. Pathogenicity test of *Alternaria alternate* (GeneBank: Isolate OP26988) caused failure grafting in two orange varieties under Fruit Pathology Greenhouse condition.

Variety	Rootstock	Inoculation	Disease infection %	Grafting successful %
Navel orange	Sour orange	*Alternaria alternate*	100	0.0
	Control	without pathogen	20	80
	Trifoliate orange	*Alternaria alternate*	80	20
	Control	Without pathogen	8	92
	Volkamer lemon	*Alternaria alternate*	100	0.0
	Control	without pathogen	20	80
Valencia	Sour orange	*Alternaria alternate*	40	0.0
	Control	without pathogen	20	80
	Trifoliate orange	*Alternaria alternate*	80	20
	Control	without pathogen	20	80
	Volkamer lemon	*Alternaria alternate*	100	0.0
	Control	without pathogen	20	80
LSD at 5%	A (variety) = 12.96	B (rootstock) = 11.23	A×B = 22.46	

LSD was recorded for disease infection %.

Table 6. Pathogenicity test of *Fusarium solani* (Gene Bank: Isolate OR660591) caused failure grafting in two orange varieties under Fruit Pathology Greenhouse conditions.

Variety	Rootstock	Inoculation	Disease infection %	Grafting successful %
Navel orange	Sour orange	*Fusarium solani*	80	20
	Control	Without pathogen	20	80
	Trifoliate orange	*F. solani*	40	60
	Control	Without pathogen	20	80
	Volkamer lemon	*F. solani*	100	0.0
	Control	Without pathogen	20	80
Valencia	Sour orange	*Fusarium solani*	40	60
	Control	Without pathogen	20	80
	Trifoliate oramge	*F. solani*	80	20
	Control	Without pathogen	20	80
	Volkamer lemon	*F. solani*	100	0.0
	Control	Without pathogen	20	80
LSD at 5%	A (variety) =19.97	B (rootstock) =18.16	A×B =36.33	

LSD was recorded for disease infection %.

Table 7. Effect of the plant growth regulators (PGR) and alternative fungicide on mycelial growth (MG) of isolated fungi in lab.

Treatment	Conc.** (ppm)	Mycelial growth inhibition %			
		A. alternata		*F. solani*	
		M.G.	Inhibition %*	M.G.	Inhibition %*
H_2O_2 (hydrogen peroxide)	500	2.56	71.55	3.35	62.77
	1000	1.75	80.55	1.550	82.77
	2000	0.0	100	0.0	100
	4000	0.0	100	0.0	100
Mean		1.07	84.03	1.22	81.84
IAA (Indol acetic acid)	250	0.72	92	0.77	91.44
	500	0.52	94.22	0.55	93.88
	1000	0.0	100	0.0	100
	2000	0.0	100	0.0	100
Mean		0.31	95.40	0.33	95.10
Xanthan gum	1000	5.25	41.66	6.31	29.88
	2000	3.84	57.33	4.75	47.22
	4000	2.75	69.44	4.18	53.55
	6000	2.42	73.11	3.18	63.88
Mean		3.56	60.38	4.60	48.63
Wood vinegar	50	5.25	41.66	6.31	29.88
	100	5.12	43.11	4.75	47.22
	200	2.75	69.44	4.18	53.55
	400	2.30	74.44	3.25	63.88
Mean		3.85	57.16	4.62	48.63
Control (without		9.0	0.0	9.0	0.0
L.S.D at 5%	A= 0.16, B = 0.14, C= 0.09, A×B= 0.31, A×C= 0.22, B×C = 0.19, A×B×C = 0.424				

LSD was recorded for mycelial growth.
Treatment (A), concentration (B), fungi (C).
*** Inhibition percentage according to the control treatment.**
**** The least tested concentration used as recommended by the production company.**

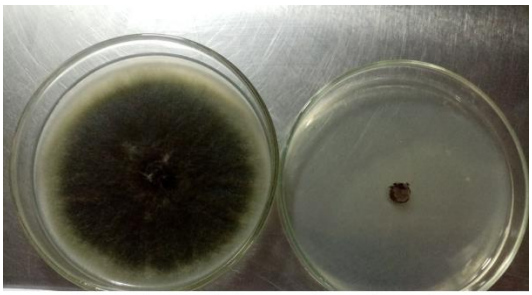

Fig. 3. Effect of concentration of 2% H_2O_2 on linear growth of *Alternaria alternata* shows complete inhibition.

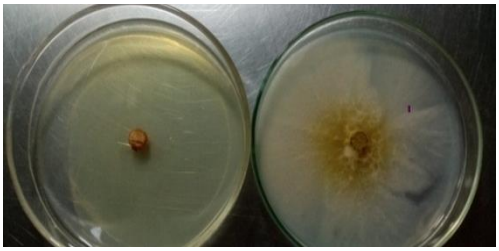

Fig. 4. Effect of concentration of 2% H_2O_2 on linear growth of *Fusarium solani* shows complete inhibition.

Table 8. Effect of five fungicides on mycelial growth (cm) isolated from the sites of the citrus grafting *in vitro*.

Fungicide (A)	Conc. ppm (B)	Linear growth of the isolated pathogenic fungi (cm)			
		A. alternata (C)	Inhibition %*	*F. solani* (C)	Inhibition %*
Kemazed® 50% WP	50	4.88	45.77	1.50	83.33
	100	4.36	51.55	1.25	86.11
	200	3.68	59.11	0.0	100
	400	3.37	62.55	0.0	100
Mean	-	4.07	-	0.68	-
Kinol® 25% EC	50	1.0	88.88	1.20	86.66
	100	0.0	100	1.0	88.88
	200	0.0	100	0.0	100
	400	0.0	100	0.0	100
Mean	-	0.25	-	0.55	-
Bellis® 38% WG 38% WDG	50	1.50	83.33	3.18	64.66
	100	1.32	85.33	2.92	67.55
	200	1.17	87.22	2.43	73
	400	1.0	88.88	1.15	87.22
Mean	-	1.24	-	2.42	-
Ridomil Gold Plus ®71.5%WP	50	7.43	17.44	7.43	17.44
	100	6.12	32.00	6.12	32
	200	4.37	51.44	4.63	48.66
	400	3.25	63.88	3.25	63.88
Mean	-	5.29	-	5.35	-
Topsin® M70 70%WP	50	4.37	51.44	3.31	63.11
	100	4.40	51.11	2.57	71.55
	200	4.20	51.11	2.0	77.77
	400	3.30	63.33	0.0	100
Mean	-	4.11	-	1.97	-
Control		9	0.0	9	0.0
LSD at 5%	A=0.062, B=0.041, C=0.037, A×B=0.125, A×C=0.082, B×C=0.065, A×B×C=0.174				

Treatments (A), concentration (B), fungi (C).
***According to the control treatment.**

Table 9. The percentage of the successful grafted plants of two citrus scions on three rootstocks and treated six treatments and inoculated with *A. alternata* under HRI nursery condition.

Scion (A)	Rootstock (B)	H_2O_2 2000 ppm	IAA 1000 ppm	Wood vinegar 400 ppm	Kemazed 400 ppm	Kinol 400 ppm	Control without treatment	Mean (AB)	Mean (A)
Navel Orange	Trifoliate orange	22.22	77.77	77.77	33.33	44.44	22.22	28.22	30.24
	Volkamer lemon	22.22	22.22	22.22	22.22	22.22	22.22	22.22	
	Sour orange	22.22	22.22	22.22	22.22	22.22	22.22	22.22	
Mean (AC)		22.22	40.73	40.73	25.92	29.62	22.22	-	
Valencia	Trifoliate orange	33.33	77.77	77.77	22.22	44.44	22.22	46.29	36.41
	Volkamer lemon	22.22	33.33	22.22	22.22	44.44	22.22	27.77	
	Sour orange	44.44	22.22	55.55	44.44	22.22	22.22	35.18	
Mean (AC)		33.33	44.44	51.84	29.62	29.62	22.22	Mean (B)	
Over All Means	Trifoliate orange	27.77	77.77	77.77	27.77	44.44	22.22	50.78	
	Volkamer lemon	22.22	27.77	22.22	22.22	33.33	22.22	30.94	
	Sour orange	33.33	22.22	38.88	33.33	22.22	22.22	34.91	
	Mean (C)	24.07	42.58	46.29	27.77	29.62	27.77		
LSD at 5%	A=7.246, B=8.873, C=12.546, A×B=12.546, A×C=17.747, B×C=21.728, A×B×C=30.728								

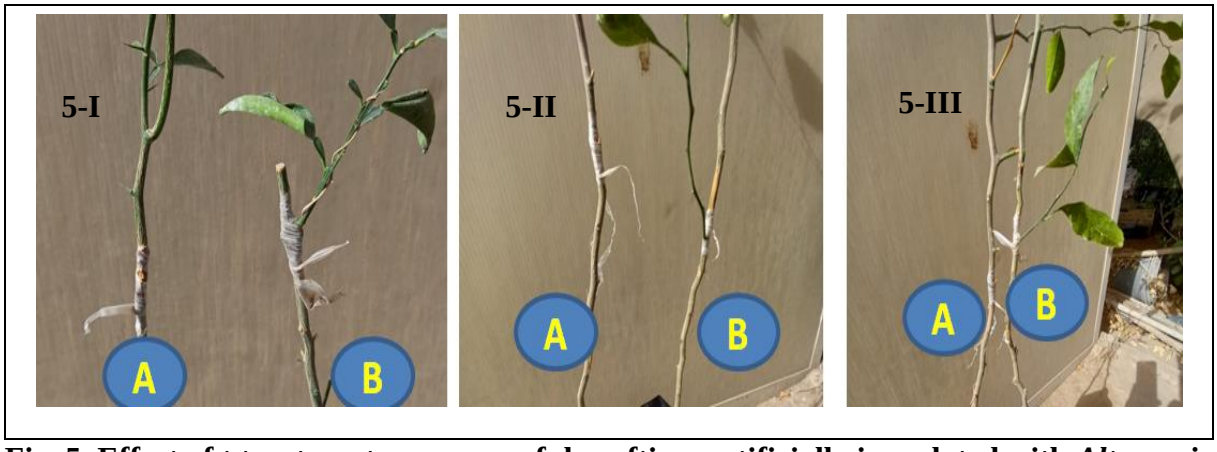

Fig. 5. Effect of t treatments on successful grafting, artificially inoculated with *Alternaria alternata* 5-I Treated with IAA in Navel orange on Trifoliate orange rootstock, 5-II Treated with Wood-vinegar in Navel orange on Trifoliate orange rootstock, 5-III Treated with IAA in Valencia orange on Trifoliate orange rootstock, (B) treatment, (A) control shows failure grafting and died the buds.

Table 10. The percentage of successfully grafted plants of two citrus scions grafting on three rootstocks inoculated with *F. solani* with five treatments in HRI nursery.

Scion (A)	Rootstock (B)	H_2O_2 2000 ppm	IAA 1000 ppm	Wood vinegar 400 ppm	Kemazed 400 ppm	Kinol 400 ppm	Control infected	Mean (AB)	Mean (A)
Navel Orange	Trifoliate orange	44.44	77.77	33.33	22.22	33.33	33.33	40.73	36.41
	Volkamer lemon	22.22	33.33	33.33	22.22	33.33	33.33	29.62	
	Sour orange	44.44	55.55	44.44	33.33	33.33	22.22	38.88	
Mean (AC)		37.03	55.55	37.03	25.92	33.33	29.62	-	
Valencia	Trifoliate orange	33.33	77.77	33.33	33.33	55.55	22.22	42.58	34.56
	Volkamer lemon	44.44	22.22	22.22	22.22	22.22	22.22	25.92	
	Sour orange	33.33	33.33	33.33	44.44	44.44	22.22	35.18	
Mean (AC)		37.03	44.44	29.62	33.33	40.73	22.22	Mean (B)	
Over All Means	Trifoliate orange	38.88	77.77	33.33	27.77	44.44	27.77	49.2	
	Volkamer lemon	33.33	27.77	27.77	22.22	27.77	27.77	34.12	
	Sour orange	38.88	44.44	38.88	38.88	38.88	22.22	44.43	
Mean (C)		37.03	49.99	33.32	29.62	37.03	37.03	-	
LSD at 5%	A=6.915, B=8.461, C=11.979, A×B=11.979, A×C=16.933, B×C=20.326, A×B×C=29.326								

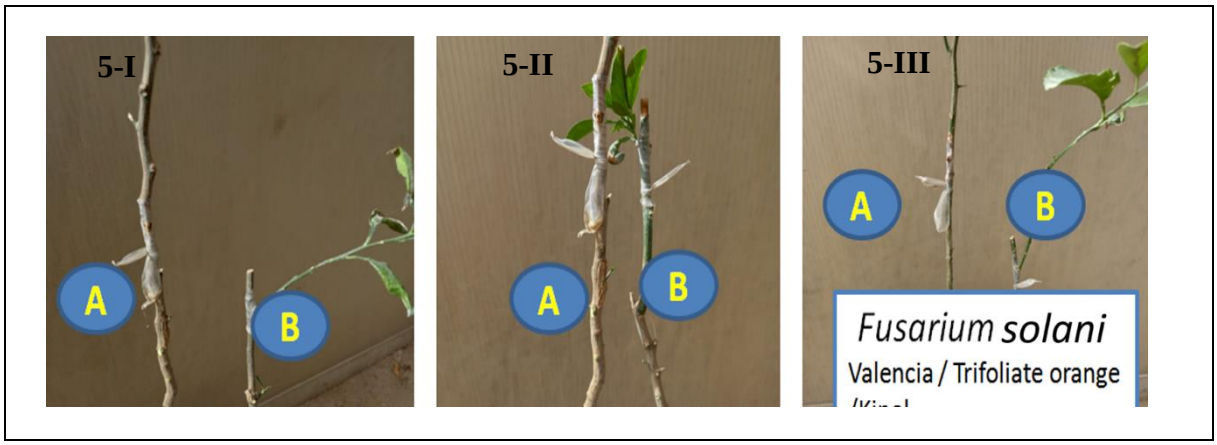

Fig. 6. Effect of t treatments on successful grafting, artificially inoculated with *Fusarium solani* 5-I Treated with IAA in Navel orange on Trifoliate orange rootstock, 5-II Treated with IAA in Valencia orange on Trifoliate orange rootstock, 5-III Treated with Kinol in Valencia orange on Trifoliate orange rootstock, (B) treatment, (A) control shows failure grafting and died the buds.

and Sour orange rootstock and inoculated with *F. solani*, citrus pathogenic fungus. The superior treatment was IAA at (1000 ppm) for the two tested scions when they grafted on Trifoliata, whereas they recorded 77.77% successful percentage for both scions. The least effective treatments showed with Valencia on Volkamer lemon with all the tested treatments except for H_2O_2 (at 2000 ppm as recorded 44.44%) whereas, they recorded 22.22% successful percentage and there was no significance when they compared with the control treatment. Also, Navel orange on Trifoliata as rootstock recorded 77.77% successful percentage when treated with IAA (1000 ppm).

Enzyme activities of the two orange scions with three different root stacks in response to different treatments:

Data in Table (11) demonstrate the effect of five different treatments on peroxidase activity in Navel/Valencia citrus scions and the three rootstocks of Trifoliate orange, Volkamer lemon, and Sour orange with two citrus pathogenic fungi. The superior treatment is H_2O_2 at 2000 ppm, the least effective treatment is Kemazed at 400 ppm.

Data in Table (12) show the effect of five different treatments on polyphenol oxidase activity in Navel/Valencia orange scion and the three rootstocks of Trifoliate orange, Volkamer lemon, and Sour orange with two citrus pathogenic fungi.

Table 11. Effect of treatments against pathogenic fungi in *ex vivo* on two scions and three rootstocks on peroxidase activity.

Treatment	Navel			Valencia		
	Trifoliata	Volkamer	Sour	Trifoliata	Volkamer	Sour
			***Fusarium* spp.**			
Blank (T0)	0.062	0.063	0.063	0.065	0.062	0.064
AI (T1)	0.163	0.202	0.207	0.138	0.177	0.212
Kinol (T2)	0.176	0.214	0.211	0.091	0.106	0.173
Kemazed (T3)	0.153	0.107	0.210	0.106	0.103	0.145
H_2O_2 (T4)	0.197	0.250	0.247	0.186	0.295	0.250
W.V. (T5)	0.112	0.236	0.220	0.110	0.275	0.218
IAA (T6)	0.142	0.124	0.187	0.106	0.107	0.221
LSD at 5%	0.0032	0.0081	0.0043	0.0045	0.0060	0.0055
			***Alternaria* spp.**			
Blank (T0)	0.043	0.044	0.043	0.047	0.043	0.043
AI (T1)	0.185	0.406	0.191	0.158	0.168	0.195
Kinol (T2)	0.193	0.417	0.187	0.066	0.104	0.106
Kemazed (T3)	0.197	0.416	0.186	0.183	0.159	0.163
H_2O_2 (T4)	0.260	0.630	0.325	0.312	0.226	0.262
W.V. (T5)	0.242	0.416	0.309	0.117	0.221	0.260
IAA (T6)	0.126	0.452	0.219	0.113	0.121	0.126
LSD at 5%	0.0050	0.0062	0.0054	0.0157	0.0064	0.0056

Means that do not share a letter are significantly different.
AI: Artificial inoculation; W.V.: wood vinegar.

Table 12. Effect of treatments against pathogenic fungi in *Ex vivo* on two scions and three rootstocks on polyphenol oxidase activity.

Treatment	Navel			Valencia		
	Trifoliata	Volkamer	Sour	Trifoliata	Volkamer	Sour
			***Fusarium* spp.**			
Blank (T0)	0.0405	0.0418	0.0413	0.0274	0.022	0.0360
AI (T1)	0.0627	0.0401	0.0665	0.0422	0.0335	0..0410
Kinol (T2)	0.0532	0.0470	0.0438	0.0294	0.034	0.0335
Kemazed (T3)	0.0668	0.0347	0.0382	0.0149	0.040	0.0384
H_2O_2 (T4)	0.0794	0.0645	0.0871	0.0520	0.0444	0.0668
W.V. (T5)	0.0691	0.0474	0.0817	0.0516	0.0238	0.0463
IAA (T6)	0.0458	0.0488	0.0606	0.0264	0.022	0.0354
LSD at 5%	0.0051	0.0058	0.0039	0.0050	0.0059	0.0057
			***Alternaria* spp.**			
Blank (T0)	0.0491	0.0352	0.0426	0.0400	0.0397	0.0427
AI (T1)	0.0587	0.0417	0.0502	0.0445	0.0437	0.0671
Kinol (T2)	0.0776	0.0347	0.0426	0.0332	0.0370	0.0551
Kemazed (T3)	0.0730	0.0327	0.0420	0.0345	0.0389	0.0554
H_2O_2 (T4)	0.0872	0.0487	0.0911	0.0703	0.0635	0.0849
W.V. (T5)	0.0660	0.0378	0.0549	0.0438	0.0484	0.0678
IAA (T6)	0.0658	0.0349	0.0408	0.0436	0.0401	0.0657
LSD at 5%	0.0035	0.0040	0.0051	0.0046	0.0045	0.0057

Means that do not share a letter are significantly different.
AI: Artificial Inoculation; W.V.: wood vinegar.

The superior treatment is H_2O_2 at 2000 ppm; the least effective treatment is Kemazed at 400 ppm.

DISCUSSION

Grafting efficiency is economically important to the citrus nursery industry because it is expensive but how commercial citrus scion/rootstock trees are produced we selected treatments that might improve grafting efficiency sufficiently to be of value to commercial citrus tree producers. The frequency of the isolated fungi from citrus species obtained from Um Ammar under study recorded 66.52 and 31.37% of *A. alternata* and *F. solani* respectively while it recorded 48 and 28% from Navel orange and Valencia, in Horticulture nursery respectively. Gramaje *et al.* (2009) reported that the frequency of *Botryosphaeria* spp. which was isolated from the scions, the graft union, and rootstocks of grapevine was 23.1, 61.5 and 61.5%, respectively. Atia *et al.* (2003) reported that hyphae of *B. theobromae*, *P. viticola* and *F. solani* are able to colonize the tissues of grapevine and cause necrosis in xylem parenchyma and xylem vessels. Also, Abo Rehab *et al.* (2013) reported that *P. viticola* was the most frequently isolated fungus from grafted failure grapes seedlings, followed by *B. theobromae*. The least frequently isolated fungi were *Phoma* sp., *F. solani* and *A. alternata.*

PCR reactions resulted in a single band observed on a 1.5% agarose gel amplified fragments of isolates IMI289962, IMI178784. Purified PCR products yielded

sequences of 545–595 bp in length. Many investigators used PCR method to identify the causal organisms of different plant species (Joey *et al.*, 2016 and Adesemoye, 2014).

Pathogenicity test of the isolated fungi was done in Fruit Pathology Greenhouse (FPG) and nursery of HRI. *A. alternata* recorded disease incidence percentage (80%) on Valencia citrus grafted on Volkamer lemon rootstock in FPG, while it recorded 100% on both of Navel orange variety grafted on Sour orange rootstock and Volkamer lemon with Navel orange in the nursery of HRI, respectively, Also, it recorded 100% on Valencia citrus grafted on Volkamer lemon rootstock while, it recorded 40% on Valencia citrus grafted on Sour orange.

Pathogenicity test of *Fusarium solani* (GeneBank: Isolate OR660591) recorded (100%) on Navel orange grafted on Volkamer lemon and 40% Navel orange with Trifoliate of disease infection , while , it recorded 40% on Valencia with Sour orange.and 100% on Valencia citrus with Volkamer lemon rootstock. Abo Rehab *et al*., 2013 on effect of pathogenic fungi on grapevine failure grafting and Mounir *et al*.2021 stated that 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.

Under *in vitro*, four treatments were evaluated as antifungal agents H_2O_2 and IAA at 2000 ppm and 1000 ppm, respectively completely inhibited the mycelial growth of *A. altenata* and *F. solani* also, Kemazed and Kinol at 200 ppm completely inhibited the mycelial growth of *A. alternata* and *F. solani*. El-Banna *et al.* (2015) found that *in vitro* bioassay that some bacterial bio-agents reduced the colony growth of *L. theobromae* which causes die-back disease on grapevine. As well as, El-Banna *et al.* (2015) 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. Under laboratory condition, wood vinegar (400 ppm) was most active than xanthan gam (6000 ppm) whereas they recorded 63.88% inhibition of mycelial growth. These results are in harmony with those obtained by Wei *et al.* (2010) and Mungkunkamchao *et al.* (2013).

Under nursery conditions using IAA at 1000 ppm as soaking treatment to Navel orange flutes as a variety and trifoliata orange as a rootstock and Valencia as variety and Volkamer lemon as rootstock were the superior treatment whereas; successful grafting process by 77.77%. Also, Kinol at 400 ppm increased percentage of grafted plants by 44.44% in case of Navel orange as a variety and Trifoliata orange as a rootstock and Valencia as a variety and Volkamer lemonas as rootstock. Some results obtained by other researchers are in harmony with these results of Abo Rehab *et al.* (2013) who 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.

The plant hormone auxin is critical for plant growth and development processes. Thayamini and Umadevi (2011) investigate its role in the incompatible Hm/Pt combination. Also, they stated that auxin signaling is initiated through binding of the hormone to the transport inhibitor response 1/auxin signaling F-Box protein (TIR1/AFB) and auxin/indole acetic acid (Aux/IAA) protein co-receptors, which results in degradation of the targeted Aux/IAA proteins. The degradation of Aux/IAA proteins allows the release of auxin response factors (ARFs), these facts showed in this study, further regulating grafting caused by *F. solani* by 77.77% in case of Navel orange as scion while Trifoliata orange as a rootstock, also it gave the same result in stat of Valencia as a scion and Trifoliata orange as a rootstock. All the tested treatments as anti-failure agents increased peroxidase activity of the tested orange variety, also Tallon and Olaya (2012) mentioned that the transfer of *in vitro* shoots to rooting media, containing different

concentrations of indole butyric acid (IBA) and indole acetic acid (IAA), resulted in regeneration of complete plantlets of trees of a lemon, Sour orange, and 'Cleopatra' mandarin citrus rootstocks. These results are similar to those reported by Sharma(2002) and Monir *et al*. (2021) they stated that the changes in activities of defense related enzymes as a result of treating avocado scions with some fungicides or biofungicides, all tested treatments such as fungicides or biocides increased peroxidase and polyphenoloxidase activities in comparison with control treatment in the two tested cultivars.

This study investigated the effect of five different treatments on peroxidase activity in Navel/Valencia citrus scions and the three rootstocks of Trifoliate orange, Volkamer lemon and Sour orange with two citrus pathogenic fungi the obtained results showed that the superior treatment was H_2O_2 at 2000 ppm; on the other hand, the least effective treatment is Kemazed at 400 ppm. Also, the aforementioned treatments on polyphenol oxidase activity in Navel/Valencia orange scion and the three rootstocks of Trifoliate orange, Volkamer lemon and Sour orange with two citrus pathogenic fungi. The superior treatment is H_2O_2 at 2000 ppm; the least effective treatment is Kemazed at 400 ppm (Retig, 1974).

CONCLUSION

In the nursery, the results showed that *A. alternata* recorded the least disease incidence on Valencia scion with Sour orange rootstock. *In vitro* experiment the activity of IAA (1000 ppm) showed 100% inhibition of the mycelial growth of *A. alternat*, and *F. solani*. Kemazed and Kinol (200 ppm) gave complete inhibition (100%) to *F. solani*. The wood vinegar and IAA gave superior activity against the pathogenic fungi as alternative fungicides on the failure grafting percentage as the reduction in case of *A. alternata*. Treatments of H_2O_2 gave the highest increase in enzyme activities, while wood vinegar increased the peroxidase, and polyphenol oxidase activities only.

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تأثير المعاملات البديلة الامنة لمكافحة فشل التطعيم على شتلات الموالح المتسبب عن بعض الفطريات الممرضة للنبات

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حديثاً ظهرت أعراض فشل التطعيم على أشجار صنف الصيفي عمر ثمانية سنوات والمطعوم على فولكا مارين وقد تم إعادة التطعيم مرة أخرى في مزرعة أم قمر في محافظة الإسماعيلية وكانت الأعراض عبارة عن تقرحات في منطقة التطعيم ذات لون أسود الى بني غامق وقد ظهرت على الأصناف علامات واضحة تدل على وجود الهيفات الفطرية للفطريات المسببة للمرض. ولقد ثبت من عمليات العزل من المناطق المصابة وجود جنسان من الفطريات الممرضة تتبع ثلاثة أنواع وهي *Fusarium equiseti* ، *Alternaria alternata* و *Fusarium solani* وكانت بتكرارية قدرها ٦٦,٢٥٪: ٣١,٣٧٪: ٢,١١٪ على التوالي وذلك عند عزلها من الأنسجة المتقرحة. ولقد تم إجراء العزل من الأصول والأصناف الموجودة في مشتل معهد بحوث البساتين ووجد أن الفطر *A. alternata* كان أكثر تكرارية عند عزله من الأصل فولكا مارين ليمون وكانت تقدر بـ ٤٨٪ في حين أن أقل تكرارية وجدت من عزلة البرتقال ثلاثي الأوراق وتقدر بـ ٢٪. أما فيما يخص الفطر*Lasiodiplodia theobromae* فقد عُزل من الأصل فولكا مارين ليمون فقط، وقد تم تعريف الفطريات المعزولة بإستخدام الطرق التقليدية بجانب التحليل بإستخدام البيولوجيا الجزيئية ITS1, ITS5. لقد تم اجراء العدوى الصناعية تحت ظروف الصوبة المتحكم فيها وذلك بإستخدام اكثر الفطريات تكرارية وهي *A. alternata* ، *F. solani*. ولقد ثبت أن أكثر نسبة إصابة (١٠٠٪) حدثت بالفطر *A. alternata* على أصناف أبو سرة والصيفى المطعومة على النارنج والفولكا مارين كأصول. تم أيضاً إجراء عدوى صناعية للأصناف الموجودة في مشتل معهد البساتين ولقد ثبت من التجربة أن الفطر *A. alternata* سبب إصابة بمقدار ١٠٠٪ على برتقال أبو سرة على أصل نارنج وعلى العكس من ذلك فقد تم تسجيل أقل نسبة إصابة على الصنف الصيفي على النارنج كأصل. تحت ظروف المعمل تم تقدير كفاءة أربعة من المعاملات وهي فوق أكسيد الهيدروجين وإندول حمض الخليك وصمغ الزانثان وخل الخشب بالإضافة الى الكنترول وذلك كمواد مثبطة للنمو الميسليومى للفطريات المختبرة. أثبت إندول حمض الخليك عند تركيز ١٠٠٠ جزء في المليون تثبيط ١٠٠٪ للنمو الميسليومى لكل من *A. alternata* ، *F. solani* . تم ايضا تحت ظروف المعمل تقدير كفاءة المبيدات الكيماوية وهى الكيما زد والكينول عند تركيز ٢٠٠ جزء فى المليون ولقد اعطا كلا من المبيدين تثبيط كاملا بنسبة ١٠٠٪ للفطر *F. solani* وكذلك تم تقييم كفاءة المبيد "توبسين إم" عند تركيز ٤٠٠ جزء فى المليون وقد اعطى تثبيط ١٠٠٪ للفطر *F. solani* وعلى الجانب الآخر

تم تقييم كفاءة الكيما زد والكينول و H_2O_2 وإندول حمض الخليك وخل الخشب كمواد مكافحة للإصابة لفشل التطعيم تحت ظروف المشتل في معهد البساتين على الصنف أبو سرة والصيفي على أصول النارنج والفولكا والبرتقال ثلاثي الأوراق كاصول، ولقد إتضح أن خل الخشب وإندول حمض الخليك أعطيا أفضل النتائج كبدائل للمبيدات الكيماوية حيث أنهما قللا نسبة الإصابة بنسبة ٧٧,٧٧٪ في حالة الفطر *A. alternata*. بالإضافة إلى ذلك ثبت أن إستخدام H_2O_2 أدى إلى زيادة في نشاط إنزيم البيروكسديز بينما خل الخشب أدى إلى زيادة في نشاط إنزيم البيروكسيديز والبولفينول أكسيديز.