J. Plant Prot. and Path., Mansoura Univ., Vol.9 (9): 577- 585, 2018

Control of Avocado (*Persea americana* Miller) Damping-off and Root Rot Diseases in Egypt

Radwan, M. A. and M. S. S. Hassan
Plant Pathol. Res. Inst., Agric. Res. Center, Giza, Egypt
drmahmoudawwad67@yahoo.com& mabrouk2276@yahoo.com

ABSTRACT

Damping-off and root rot are the most troublesome and common fungi diseases attack avocado (*Persea americana* Miller) which affecting seedling production and causing serious losses in the number of seedlings in nurseries and transplants in new orchards. Nursery and greenhouse experiments on pre- and post-emergence damping-off of avocado were conducted at El-Qanater El-Khairia Horticulture Research Station, Agricultural Research Center throughout 2016 and 2017 growing seasons. Hass, Eitinger and Fuerte were the most susceptible cultivars to damping-off and root rot diseases, followed by Duke. Whereas, the lowest percentage of pre- and post-emergence damping-off and the highest percentage of survival plants were recorded on Reed, Benkerton and Bekon. Pathogenicity tests on avocado seeds and seedlings under greenhouse conditions showed that *F. solani, F. moniliforme, F. semitectum* and *L. theobromae* which isolated from roots of either seedlings in nursery or transplants in new orchards were pathogenic but varied at different degrees in their pathogenicity. *Fusarium solani* was the most pathogenic fungi causing the highest percentage of pre- and post-emergence root-rot followed by *F. moniliforme.* On the other hand, the least pathogenic ones were *F. semitectum* and *L. theobromae*. To overcome this problem, single treatment of nine compounds *i.e.* Topsin M70WP, DithaneM-45 80%WP, Kema-Z 50% WP, Rizolex–T 50% WP, Salicylic acid, Bio Zeid 2.5%, Bio Arc 6.0 %, Lemongrass and Thyme oil were used as seed dressing and soil drenching to investigate their effect in reducing damping-off and root rot disease incidence under greenhouse conditions. All compounds reduced the avocado damping-off and root rot disease incidence and increased the percentage of survival plants comparing with untreated seeds or untreated soil. Seed dressing and soil drenching with fungicides (Topsin M70, Kema-Z, Rizolex–T and Dithane M-45) showed a superior effect which produced the highest level of the disease control with the highest number of survived plants. Treatments with biofungicides (Bio Zied and Bio Arc) gave moderate effect. Essential oils (Thyme and Lemongrass) and Salicylic acid reduced the disease and increased mean survival plants of avocado transplants. Best results were obtained when the compounds were applied as seed dressing.

Keywords: Avocado, damping-off, root rot, fungicides, biofungicides, essential oils and salicylic acid.

INTRODUCTION

Avocado (*Persea americana* Miller) as an evergreen tree is cultivated in both tropical and Mediterranean regions for its delicious and nutritious fruits. World avocado production is around 3,583,226 metric tons harvested from 348,769 ha FAO (2013).The fruit of the avocado is green- or purple- to black-skinned and spherical, pear- or egg-shaped which contains one seed. Avocado fruit has very high value not only for its high nutritional value but also for its role in the cosmetic and health industries. In Egypt, Elosaily (2015) reported that essential oils of seven cultivars i.e. Duke, Ettinger, Bekon, Penkerton, Gwen, Hass and Fuerte showed good antimicrobial. The mesocarp contains higher amounts of vitamins A, C and E. Avocado fruit and its pericarp fixed oil are usually considered as efficient components of healthy antioxidant diets.

Damping-off caused by a variety of soil-borne pathogens that can attack seeds and seedlings prior to emergence (pre-emergence damping-off) and can attack seedlings after emergence (post-emergence damping-off) which result in seeds rot or death of seedlings. Avocado seeds and seedlings in nursery and transplants in new orchards were attacked by damping-off and root rot diseases that caused by a variety soil-borne fungi. Pre-emergence damping-off occurs when avocado seeds are infected and germinating seedlings are killed before they can appear above soil surface. Post-emergence damping-off occurs when avocado seedlings emerged above soil surface, causing collapse and eventual death Omokhua *et al*. (2009).These diseases lead to high reduction in the numbers of avocado seedlings in nurseries and transplants in new orchards. In this investigation, damping-off and root rot diseases are caused by a complex fungi *i.e. Fusarium solani*, *Fusarium moniliforme*, *Fusarium semitectum* and *Lasiodiplodia theobromae*. All fungi were pathogenic to avocado seeds and seedlings under greenhouse conditions, causing pre- or post-emergence damping-off. These effects of the tested fungi on avocado seeds and seedlings may be due to seeds rot prior to emergence (pre-emergence damping-off) and damage of roots system after emergence (post-emergence damping-off). Killing roots system lead to reduced absorption surface and uptake water and essential nutrients Porter *et al*. (1990). Saleh (1997) reported that the infected ground nut with seed rot, root rot and wilt seedlings diseases exhibited chlorosis of leaves that causes a reduction in photosynthetic capacity and net photosynthesis.

This investigation aimed to study the effectiveness of certain compounds (four fungicides, two biofungicides, two essential oil and Salicylic acid) as single treatment for their ability to minimize damping-off and root rot diseases and increase survival plants of avocado under greenhouse conditions.

MATERIALS AND METHODS

1- Disease incidence of avocado damping-off and root rot diseases.

In nursery.

The natural infection of pre-emergence damping-off, post-emergence damping-off and survival plants were evaluated on seven avocado cvs. namely Hass, Fuerte, Duke, Reed, Eitinger, Bekon and Benkerton in commercial nursery at El Qanater El-Khairia Horticulture Research Station, Agricultural Research Center, throughout 2016 and 2017 growing seasons. Twenty seeds as four replicates for each cultivar (one seed/pot) were sown in plastic pots (10 cm in diameter). Disease assessment was recorded as percentage of damping-off (pre- and post-emergence) after 30 days and 60 days from sowing. Healthy survived plants were counted and expressed as percentage 90 days after sowing using the following formula:

$$\text{Pre}-\text{emergence}\ \% = \frac{\text{No. of non-emerged seedlings}}{\text{Total no. of sown seeds}} \times 100$$

$$\text{Post}-\text{emergence}\ \% = \frac{\text{No. of dead emerged seedlings}}{\text{Total no. of sown seeds}} \times 100$$

Healthy (survival) plants% = 100 – (pre-emergence% + post-emergence%).

In orchard.

Twelve transplants of seven avocado cultivars i.e. Hass, Fuerte, Duke, Reed, Eitinger, Bekon and Benkerton were chosen randomly as three replicates and sampled for root rot disease incidence (declining or died soon after were transplanted into orchards) during 2016 and 2017 growing seasons under field conditions. The disease incidence of root rot disease was determined as follow:

Disease incidence% = Number of infected transplants / Total number of transplants ×100

2. Isolation and identification of the causal pathogens.

Root rotted samples of avocado seedlings and transplants were collected from nursery and new orchards, washed under tap water, parts showing disease symptoms were cut into small pieces (1cm in length), dipped in 3% sodium hypochlorite solution for 4 min for surface sterilizing. Small pieces washed with sterile distilled water for several times, then dried between two filter papers. The sterilized pieces were transferred individually to Petri dishes, each containing 20 mL potato dextrose agar (PDA) medium, then incubated at 25°C for 5 days and examined daily for fungal growth. The developed fungal colonies were purified using hyphal tip or single spore isolation techniques. The purified fungi were identified according to fungal morphological and microscopical characters as described by Nelson *et al*. (1983), Booth (1985) and Barnett and Hunter (1986). The obtained isolates were maintained on PDA slants and kept in refrigerator at 5°C for further study. The frequency of the isolated fungi from the root rotted samples was separately calculated according to the following formula:

Fungal frequency % = No. of isolates of each fungus / Σ of all isolates × 100.

3. Pathogenicity tests.

On avocado seeds.

Pathogenicity tests of the 4 fungal isolates were carried out under greenhouse conditions of Fruit and Woody Trees Diseases Research Department at El-Qanater El-Khayria Horticulture Research Station, Agricultural Research Center, throughout 2016 and 2017 growing seasons. To prepare fungal inocula required for the pathogenicity tests, 100g barely grains and 100 mL of distilled water were put in each conical flask and were autoclaved, subsequently inoculated with the fungal isolates using about 5~10 of (1 cm) fungal discs, then incubated at 25°C for 14 days with shake every day. Soil was also sterilized with formalin solution (5%), then covered with a polyethylene sheet for 7 days to retain the gas and left to dry for 2 weeks until all traces of formaldehyde disappeared. The sterilized soil was individually infested with the inocula of the tested fungi at the rate of 3% (30g fungal inoculum / 1 kg soil). Plastic pots (10 cm in diameter) were sterilized by 5% formalin solution then filled with infested soil, except control (which it's sterilized soil was received the same amount of the sterilized non-inoculated barely grains)Abdel-Monaim *et al*. (2014). The pots were irrigated regularly for three times a week before planting to ensure the distribution and establishment of the inoculated fungus in the soil. Avocado Hass cv. seeds were surface disinfected by immersing in sodium hypochlorite (2%) for 2 min, and washed several times with sterilized water, then dried between two sterilized layers of filter paper. One avocado seed was sown in each pot and twenty seeds (pots) were used as four replicates for each treatment. Data of pre-and post-emergence damping-off as well as survival plants were recorded 30, 60 and 90 days from sowing, respectively as mentioned above.

On avocado transplants.

The same method which mentioned before was used to prepare inocula required for the pathogenicity tests on avocado transplants. Plastic pots 30 cm in diameter were dipped for 15 min. in formalin solution (5%) , then left for 2 weeks to dry. The sterilized soil was individually infested with the inocula of the tested fungi at the rate of 3% (30g fungal inoculum / 1 kg soil).The sterilized pots were filled with infested soil (2 Kg/pot). The pots were irrigated regularly for three times a week before planting to ensure even distribution of the inoculated fungus in the soil. One avocado transplant (one year old) was cultivated in each pot and twelve transplants (pots) were used as three replicates for each treatment. Another group of pots contained uninoculated medium was kept as control. Seedlings were incubated at normal temperature and disease incidence (%) was recorded after three months from transplanting in pots. To meet Koch’s postulates, samples of seedlings showing the same symptoms were taken from different treatments for re-isolation procedures of the original pathogens from artificially inoculated roots Swart *et al*.(2003).

4. Disease control:

Greenhouse experiment was carried out to evaluate the effect of single treatment of four fungicides, two biofungicides, two Pure-grade of the essential oils which were obtained from Cairo Company for oils and aromatic extractions (CID), Egypt and were stored in dark glass bottles at 4 ºC and Salicylic acid Table1 as seed dressing and soil drenching on the disease incidence of damping-off and root rot of avocado in pot experiment during 2016 and 2017 growing seasons.

Effect of nine compounds as seed treatments.

Avocado seeds were immersed in 2% sodium hypochlorite for 3 min. for surface disinfecting, washed several times with sterilized water, then dried under room temperature. Avocado seeds were separately mixed with nine tested compounds at rate of commercial dose plus glue suspension as adhesive material (10m/kg seeds) in polyethylene bags and vigorously shaked-up for 10 minutes to ensure even distribution of the added materials. To obtain an emulsion feature of essential oil, few drops of the emulsifier Tween 20 (Sigma Co.) were added to essential oil volumes. The treated seeds were then left on a plastic tray to air dried. After seed dryness, the treated seeds were sown in pots (10 cm diameter) containing autoclaved soil infested separately with one of the tested fungi, i.e. *F. solani*, *F. moniliforme*, *F. semitectum* and *L. theobromae* which were grown on autoclaved barley grain medium at the rate of 3% (w/w) as mentioned before. One seed /pot and eighty pots were used for each treatment (20 pots/fungus). In control treatment, untreated seeds were sown in potted soil infested separately with one of the tested fungi (control 1) and untreated seeds were sown in potted disinfested soil (control 2). Disease incidence was recorded as the percentage of pre-emergence damping-off and post-emergence damping-off as well as survival plants for each treatment after 30, 60 and 90 days from sowing, respectively as mentioned before.

Table 1. Trade name, active ingredient and recommended doses of the compounds that used.

Trade name	Active ingredient	Usage dose/1L water or/1kg seeds
Topsin M70WP	Thiophanate methyl	2.0g
DithaneM-45 80%WP	Mancozeb	2.5g
Kema-Z 50% WP	Carbendazim	2.0g
Rizolex–T 50% WP	20%Tolclofos – methyl +30% Thiram	3.0g
Salicylic acid	$C_7H_6O_3$	15.0 mM
Bio Zeid 2.5%	*Trichoderma album*	2.5g
Bio Arc 6.0%	*Bacillus megaterium*	2.5g
Lemongrass	Citrol or citral, about 70 % up to 85 %	4.0mL
Thyme oil	Thymol, carvacrol, geraniol, thymol methyl ether, α -terpinene	4.0mL

Effect of nine compounds as soil drenching.

One apparently healthy seed were sown in pot (10 cm in diameter) containing autoclaved soil infested separately with one of the tested fungi, namely *F. solani, F. moniliforme*, *F. semitectum* and *L. theobromae* and eighty pots were used for each treatment (20 pots/fungus) as four replicates. In control treatment, untreated seeds were sown in potted soil infested separately with one of the tested fungi (control 1) and untreated seeds were sown in potted disinfested soil (control 2). The recommended dose of each compound that tested was applied as soil drenching at rate of 50mL/pot at the time of sowing. Pre-emergence damping-off and post-emergence damping-off as well as survival plants for each treatment were recorded 30, 60 and 90 days from sowing, respectively as mentioned before. These essential oils were stored in dark bottles at 4ºC for further studies. Essential oils Lemongrass and thyme solutions (4mL oil was added to 1 litre sterilized water and 1mL Tween80). Salicylic acid at concentration 15mM added to 1 litre sterilized water.

Statistical analysis

The obtained data were subjected to statistical analysis of one way analysis of variance and means were compared using L.S.D test to differentiate the differences between various treatments Snedecor and Cochran (1990).

RESULTS

Symptoms on seedlings in nursery and transplants in new orchard.

Several soil-borne pathogens can attack avocado seeds, seedlings and transplants, causing severe deterioration in nurseries and new orchards. These fungi are able to surviving in the soil in the absence of their host plants. Under favorable conditions they invade the succulent tissue of germinating seedlings or transplants that leads to decay and early death. Damping-off attacks seeds and germinating seedlings prior to emergence above soil surface (pre-emergence damping-off) and can attack seedlings after emergence (post-emergence damping-off), usually occurs within 30 to 60 days after sowing. The only evidence of pre-emergence damping-off in nursery beds is that the germinating seedlings are sparse and patchy (Fig1a).When post-emergence damping-off occurs in the cotyledon stage, causes wither and collapse to seedlings (Fig1b).The most obvious indicator of post-emergence damping-off is the collapse of the transplants. The first symptoms of the disease are observed in the seedlings. Pale green, often wilted with brown tips appears on small leaves that drop readily. Shoots die from the tips, and eventually the seedlings are reduced to a bare framework of dying leaves (Fig 2 d and f).When the pathogen penetrates the succulent root-collar tissue or the roots, the disease is referred to as soil-infection damping-off. On diseased seedlings and transplants, the small feeder roots appear black, brittle and decayed (Fig 2 e and g). In the advanced stages of decline the small feeder roots on diseased seedlings may be absent, in contrast to healthy seedlings, creamy-white feeder roots are abundance (Fig 2 c).

Fig 1. Avocado nursery shows seeds failure of grow expressed by pre-emergence damping-off (a) and post-emergence damping-off (b)

Fig 2. Healthy avocado tree (a), healthy avocado seedlings (b) and roots of healthy avocado seedling (c) (control). Natural infected transplant in the new orchard (d), infected roots of infected transplant (e), natural typical root rot symptoms of infected seedling (f) and roots rot symptoms on roots of avocado seedling, note the dark brown/black necrotic root in the centre, and other roots with brown lesions in natural infected seedling (g).

1. Disease assessment of avocado damping-off and root rot.

In nursery.

Pre- and post-emergence damping-off % as well as survival plants % of seven avocado cultivars under natural infection in the nursery were recorded during two successive seasons 2016 and 2017 in Table2. Data indicate that Hass, Eitinger and Fuerte were the most susceptible cultivars to damping-off and root rot diseases where they had the highest percentage of pre- and post- emergence damping-off and the lowest percentage of survival plants, followed by Duke. Whereas, the lowest percentage of pre- and post- emergence damping-off and the highest percentage of survival plants were recorded on Reed, Benkerton and Bekon.

Table 2. Natural infection of damping-off % and survival plants% of avocado seedlings in nursery.

Cultivar	Disease assessment in nursery					
	Season 2016			Season 2017		
	Pre-emergence %	Post-emergence %	Survival plants %	Pre-emergence %	Post-emergence %	Survival plants %
Eitinger	30.00	20.00	50.00	35.00	25.00	40.00
Benkerton	20.00	10.00	70.00	20.00	10.00	70.00
Bekon	15.00	10.00	75.00	20.00	10.00	70.00
Reed	20.00	10.00	70.00	25.00	10.00	65.00
Hass	35.00	20.00	45.00	40.00	25.00	35.00
Duke	20.00	15.00	65.00	25.00	15.00	60.00
Fuerte	30.00	15.00	55.00	30.00	20.00	50.00
LSD	17.58	20.80	30.10	17.28	17.28	24.44

In orchard.

Also Table 3 shows the natural percentage of infected new transplants of seven avocado cultivars in orchard. Root rot symptoms found in all examined cultivars during the two successive growing seasons 2016 and 2017. Data also show that Hass, Eitinger and Fuerte were the most susceptible cultivar which recorded the highest percentage of wilted seedlings, followed by Reed and Duke. while Benkerton and Bekon recorded the lowest percentage of wilted seedlings.

Table 3. Natural infection of wilted transplants% of avocado in the new orchard.

Cultivar	Wilted transplants % in orchard	
	Season 2016	Season 2017
Eitinger	33.33	41.66
Benkerton	16.66	25.00
Bekon	8.33	16.66
Reed	25.00	33.33
Hass	41.66	50.00
Duke	25.00	41.66
Fuerte	33.33	33.33
LSD	27.02	27.02

2. Isolation, Identification of the causal organism (S)

Isolation from rotted roots of diseased seedlings and wilted transplants of avocado which collected from nursery and new orchards resulted in *F. solani, F. moniliforme, F. semitectum* and *L. theobromae*. *F. solani* showed the highest isolation frequency 39.17, 41.49% in nursery and 35.73, 37.78% in new orchard, respectively during 2016 / 2017 seasons, followed by *F. moniliforme* which shows the 24.84, 27.98% isolation frequency in nursery and 27.04, 23.33 % in new orchard, respectively during the two successive seasons. Also, *F. semitectum* and *L. theobromae* were recorded at moderate frequencies (Table 4 and Fig 3).

3.Pathogenicitytests.

Before starting the control tests, the pathogenicity tests for pathogens that isolated from rotted roots were performed under greenhouse conditions by sowing healthy avocado Hass cv. seeds and healthy avocado Hass cv. transplants in soil artificially infested separately with *F. solani*, *F. moniliforme*, *F. semitectum* and *L. theobromae* and assessed by occurrence of the typical symptoms of pre- and post-emergence damping-off and disease incidence.

Table 4. Fungal frequency % isolated from naturally infected roots of avocado seedlings collected from nursery and transplants collected from new orchards.

Isolated fungus	Isolation frequency %			
	Season 2016		Season 2017	
	Nursery	New orchards	Nursery	New orchards
F. solani	39.17	35.73	41.49	37.78
F.moniliforme	24.84	27.04	27.98	23.33
F. semitectum	19.16	21.17	19.44	20.0
L. theobromae	16.83	16.06	11.09	18.89
Total	100	100	100	100

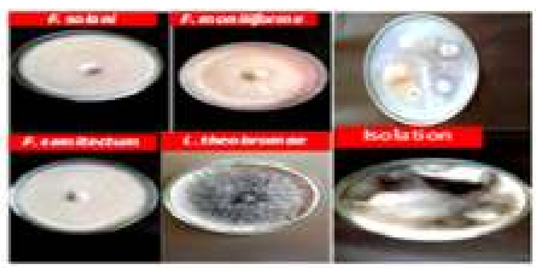

Fig 3. Colonies of *Fusarium* spp .and *L. theobromae* isolated from rotted roots of avocado 5 days after transferring to selective media.

On avocado seeds.

Results for each isolated fungus were expressed as an average of pre-emergence damping-off calculated 30 days after seed sowing, post-emergence damping-off recorded another 30 days later and survival plants calculated 90 days after seed sowing. Data in Table 5 show that all the 4 fungal isolates were pathogenic under greenhouse conditions and the disease symptoms that appeared on the infected seed and seedlings during the pathogenicity test were exactly similar to those appeared on seeds and seedlings under natural infection in nursery. The tested fungi attacked avocado seeds and germinating seedlings prior to emergence, causing seeds rot and loss of roots system that lead to death of germinating seedlings. The symptoms that appears on seedlings above the soil surface were similar to that appeared on nursery, were the infected seedlings exhibited symptoms as shown in fig 4 and 5. *Fusarium solani* was the most pathogenic fungi causing the highest percentage of pre- and post-emergence root-rot and the lowest percentage of survival plants, followed by *F. moniliforme and F. semitectum.* On the other hand, the least pathogenic one was *L. theobromae*. re-isolation procedures of the original pathogens from roots of artificially inoculated seedlings that appeared the same symptoms yielded the same fungi originally inoculated.

Table 5. Pre- and post-emergence damping-off caused by the isolated fungi on Hass cultivar under greenhouse conditions.

Isolated fungus	Season 2016			Season 2017		
	Pre-emergence damping-off (%)	Post-mergence damping-off (%)	Survival plants (%)	Pre-emergence damping-off (%)	Post- emergence damping-off (%)	Survival plants (%)
F. solani	60.00	20.00	20.00	60.00	25.00	15.00
F.moniliforme	55.00	10.00	35.00	55.00	20.00	25.00
F. semitectum	45.00	15.00	40.00	50.00	15.00	35.00
L. theobromae	15.00	25.00	60.00	20.00	25.00	55.00
Control	0.00	0.00	0.00	0.00	0.00	0.00
Mean	35.00	14.00	31.00	37.00	17.00	26.00
LSD	19.46	16.51	16.96	15.07	16.05	33.19

2. On avocado transplants.

Fusarium spp. and *L. theobromae* caused essentially similar symptoms on infected transplants as shown in Fig (4, 5 and 6).Also, data presented in Table 6 show that all the tested fungi were pathogenic to avocado transplants. The pathogenic fungi isolates exhibited different degrees of pathogenic capabilities. *F. solani* and *F. moniliforme* caused the highest wilted plants, followed by *F. semitectum*. While, *L. theobromae* caused the lowest wilted plants compared with non-infected soil in the two seasons.

Table 6. Wilted transplants of Hass cultivar caused by the isolated fungi under greenhouse conditions.

Isolated fungus	Disease incidence (%)	
	Season 2016	Season 2017
F. solani	75.00	83.33.
F.moniliforme	66.66	75.00
F. semitectum	50.00	58.33
L. theobromae	33.33	33.33
Control	0.0	00.0
LSD	33.22	28.77

Fig 4. Different stages of root rot symptoms produced on avocado transplants after artificial inoculation with *F. solan*i and *F. moniliforme* each alone. In *F. solani* , control seedling shows no disease symptoms. (a) Yellowing of leaves from up to down. (b) Wilting and death of apical bud followed by died appeared in rootstock and the leaves drop readily. (c) Shoots die from the tips, the leaves fall down and drop readily leaves from the apex progressed towards base. (b) Wilting and leaves death appeared without leaves fall down, followed by yellowing and necrosis eventually the seedlings are reduced to a bare framework of dying leaves. In *F. moniliforme,* control seedling shows no disease symptoms. (a) Yellowing of. (c) Once sudden collapse and complete wilt with brown color.

Fig 5. Different stages of root rot symptoms produced on avocado transplants after artificial inoculation with *F. semitectum* and *L. theobromae.* In *F. semitectum*, control seedling shows no disease symptoms. (a) Yellowing of leaves followed by distortion, wilt and necrosis. (b) The infected seedlings exhibited drooping of apices , wilting ,browning and shrinkage. (c) Chlorosis of leaves followed by complete wilting and death of seedlings. In *L. theobromae*, control seedling shows no disease symptoms. (a) The leaves show the slight pale colour. (b) After some time there was more pronounced discoloration yellowing and wilting of leaves. (c)After wilting, seedling decline may be fast with shrinkage of leaves without falling of leaves.

Fig 6. Note the dark brown/black necrotic roots, the small feeder roots on all infested seedlings become black, brittle and decayed. In severely affected seedlings by *L. theobromae* , *F . semitectum* , *F. moniliforme* and *F. solani* the small feeder roots system was lost in contrast to healthy transplants which have an abundance of creamy-white feeder roots.

4. Disease control.

Effect of seed treatment with nine compounds on the incidence of avocado damping-off under greenhouse conditions.

The effect of each of the tested compounds (applied as seed dressing) on pre- and post-emergence damping-off of avocado are presented in Table7. The treated seeds were sown in soil infested separately with one of the tested fungi *F. solani*, *F. moniliforme*, *F. semitectum* and *L. theobromae*. The results reveal that all nine compounds reduced disease incidence of pre- and pot-emergence damping-off and increased emergency and survival plants when compared with the untreated seeds sown in infested soil (control). Seed dressing with fungicides showed a superior effect against the tested fungi which produced the highest level of the disease control with the highest percentage of survival plants. In this regard, TopsinM 70 and Kema-Z were the most effective fungicides which gave 85% and 83.75%, respectively as mean survival plants followed by Rizolex–T which gave 82.50%mean survival plants. DithaneM-45 was the least effective one which gave the lowest percentage of survival plants. Seed treatment with biofungicides gave moderate effect as mean survival plants were (73.75 and 71.25%) for Bio Zeid and Bio Arc, respectively. Seed treatment with essential oils (Thyme and Lemongrass) and Salicylic acid caused the least reduction in the percentage of damping-off and root-rot incidence recorded as 68.75, 67.50 and 65% mean survival plants, respectively.

Table 7. Effect of treating avocado seed with nine compounds on the incidence of root rot (pre- and post-emergence damping-off) caused by the four fungi of Hass cultivar under greenhouse conditions.

Treatment	Seed treatment												Mean survival plants (%)
	F. solani			*F. moniliforme*			*F. semitectum*			*L. theobromae*			
	Pre-emergence %	Post-mergence %	Survival plants (%)	Pre-emergence %	Post-mergence %	Survival plants (%)	Pre-emergence %	Post-mergence	Survival plants (%)	Pre-emergence %	Post-mergence %	Survival Plants (%)	
TopsinM 70	10.00	5.00	85.00	10	5	85.00	5.00	5.00	90.00	10.00	10.00	80.00	85.00
DithaneM-45	20.00	5.00	75.00	20.00	10.00	70.00	20.00	5.00	75.00	10.00	5.00	85.00	76.25
Kema-Z	10.00	5.00	85.00	10.00	10.00	80.00	10.00	5.00	85.00	10.00	5.00	85.00	83.75
Rizolex–T	10.00	5.00	85.00	15.00	5.00	80.00	5.00	5.00	90.00	15.00	10.00	75.00	82.50
Bio Zeid	20.00	10.00	70.00	15.00	10.00	75.00	15.00	10.00	75.00	15.00	10.00	75.00	73.75
Bio Arc	15.00	10.00	75.00	20.00	10.00	70.00	15.00	15.00	70.00	20.00	10.00	70.00	71.25
Lemongrass	20.00	10.00	70.00	25.00	10.00	65.00	20.00	15.00	65.00	20.00	10.00	70.00	67.50
Thyme	25.00	10.00	65.00	20.00	10.00	70.00	20.00	15.00	65.00	15.00	10.00	75.00	68.75
Salicylic acid	30.00	10.00	60.00	20.00	15.00	65.00	25.00	10.00	65.00	15.00	15.00	70.00	65.00
Control 1	60.00	25.00	15.00	55.00	20.00	25.00	50.00	15.00	35.00	20.00	25.00	55.00	32.50
Control 2	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00	100.00
LSD	18.40	14.82	15.23	16.61	16.03	21.69	15.64	14.17	17.53	12.27	15.03	19.72	--

Control 1. untreated seeds sown in infested soil. Control 2 untreated seeds sown in non-infested soil.

Effect of soil drenching with nine compounds on the incidence of avocado root rot under greenhouse conditions.

The effect of nine compounds as soil drenching on pre- and post-emergence damping-off and root rot diseases of avocado under greenhouse conditions were shown in Table 8. All compounds reduced the disease incidence and increased the plant survivals when compared with untreated soil infested separately with one of the tested fungi *F. solani*, *F. moniliforme*, *F. semitectum* and *L . theobromae*. The four fungicides were the most effective in reducing pre- and post-emergence damping-off and in increasing the plant survivals, followed by biofungicides. Soil drenching with essential oils (Thyme and Lemongrass) and Salicylic acid caused the least reduction in the percentage of damping-off and root-rot incidence compared with untreated soil.

Table 8. Effect of treating soil with nine compounds on the incidence of root rot (pre- and post- emergence damping- off) caused by the four fungi of Hass cultivar under greenhouse conditions.

Treatment	Soil infestation												Mean survival plants (%)
	F. solani			*F. moniliforme*			*F. semitectum*			*L. theobromae*			
	Pre-emergence %	Post-mergence %	Survival plants (%)	Pre-emergence %	Post-mergence %	Survival plants (%)	Pre-emergence %	Post-Mergence %	Survival plants (%)	Pre-emergence %	Post-emergence %	Survival plants (%)	
Topsin M70	15.00	5.00	80.00	10.00	5.00	85.00	15.00	5.00	80.00	15.00	10.00	75.00	80.00
Dithane M-45	20.00.00	10.00	70.00	20.00	15.00	65.00	20.00	15.00	65.00	10.00	10.00	80.00	70.00
Kema-Z	15.00	5.00	80.00	10.00	10.00	80.00	15.00	10.00	75.00	10.00	10.00	80.00	78.75
Rizolex–T	15.00	5.00	80.00	10.00	5.00	85.00	10.00	5.00	85.00	20.00	10.00	70.00	80.00
Bio Zeid	20.00	10.00	70.00	15.00	15.00	70.00	20.00	15.00	65.00	20.00	10.00	70.00	68.75
Bio Arc	15.00	15.00	70.00	20.00	15.00	65.00	20.00	15.00	65.00	20.00	10.00	70.00	67.50
Lemongrass	20.00	15.00	65.00	20.00	20.00	60.00	20.00	15.00	65.00	20.00	20.00	60.00	62.50
Thyme	20.00	20.00	60.00	20.00	15.00	65.00	20.00	15.00	65.00	20.00	15.00	65.00	63.75
Salicylic acid	25.00	15.00	60.00	25.00	20.00	55.00	20.00	15.00	65.00	20.00	15.00	65.00	61.25
Control 1	60.00	25.00	15.00	55.00	20.00	25.00	50.00	15.00	35.00	20.00	25.00	55.00	32.50
Control 2	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00	0.00	0.00	100.00	100
LSD	15.63	15.23	16.23	19.56	13.72	18.90	16.99	18.57	19.24	16.42	14.39	18.74	--

Control 1. untreated seeds sown in infested soil. Control 2. untreated seeds sown in non-infested soil.

DISCUSSION

post-emergence damping-off and root rot fungal pathogens, attempts to propagate avocado in the nursery has been met with little success. Soil borne diseases including damping-off and root rot can cause considerable losses in number of seedlings in nurseries and transplants in new orchards. Damping-off can cause rapid decay and mortality of avocado seeds and seedlings in avocado nursery during those first critical few weeks from germination to just after seedling emergence as the fungal invasion of the succulent tissue of seedlings leads to decay and early death. Soil borne fungi can attack seedlings both before emergence (pre-emergence damping-off) and after emergence (post-emergence damping-off) within 30 to 60 days after sowing. Some of the symptoms associated with root rot are browning and softening of root tips, root lesions that vary in size and color from reddish to brown and black, trimming of the roots and decay, yellowing, wilting of leaves and stunted plant growth Jung *et al.* (1999). In the present investigation, study was conducted during 2016 and 2017 seasons to determine the occurrence and frequency of various fungal pathogens associated with diseased avocado plants. The study showed differences in the frequency of the isolated fungi. *F. solani* showed the highest isolation frequency, followed by *F. moniliforme.* Also, *F. semitectum* and *L. theobromae* were recorded at moderate frequencies. All tested fungi were pathogenic but varied in their pathogenicity at different degrees. *Fusarium solani* was the most pathogenic fungi causing the highest percentage of pre- and post-emergence root rot, followed by *F. moniliforme.* On the other hand, the least pathogenic ones were *F. semitectum* and *L. theobromae*. Kotze (1985) reported that *Verticillium theobromae, Rhizoctonia solani, Fusarium oxysporum, F. moniliforme, Pythium* spp., *Cylindrocladium scoparium* and *Phytophthora cinnamomi* are associated with avocado root rot in South African avocado. Darvas (1978) reported that *Pythium debaryanum* was more often isolated from both soil and roots of avocado, *Fusarium oxysporum* were frequently found in the root zone as well as the roots. *F. moniliforme* was less frequently isolated. *Rhizoctonia solani* was often found in most of the orchards. *L. theobromae*(syn. *Botryodiplodia theobromae, Diplodia natalensis*) which isolated from infected avocado roots and was pathogenic to avocado transplantswas the most pathogenic as well as the most frequently isolated fungiof dieback, grafting failure process during vegetative propagation in commercial nursery and stem-end rot of avocado Radwan and Hassan (2016). Root rot disease was observed for the first time on sugar beet plants at Dakahliya and Gharbiya governorates, the causal agent was identified as *Botryodiplodia theobromae* Pat Abd-El Ghani and Fatouh (2005). Latha *et al.*(2009) reported that root rot and collar rot disease of physic nut was observed for the first time in India. *Lasiodiplodia theobromae* which was isolated consistently from the diseased roots of affected nut plants was pathogenic when artificially inoculated on 1-year-old plants.

To overcome this problem, nine compounds (four fungicides, two biofungicides, two essential oil and Salicylic acid) were treated as seed treatment and soil drenching to investigate their effect on reducing pre-emergence damping-off, post-emergence damping-off and root rot diseases under greenhouse trails. The results in the present investigation indicate that avocado seeds pre-treated with different compounds and treated soil at the time of sowing singly reduced roo trot disease incidence as well as increased survival plants when compared with the untreated seeds and untreated soil. Seed dressing and soil drenching with fungicides showed a superior effect against the tested fungi which produced the highest level of the disease control with the highest percentage of survival plants. Best results were obtained when the compounds were applied as seed dressing. Decreases of pre-emergence damping-off with the treated soil may be attributed to the effect of treatments on the pathogens attacking the seeds causing seed decay.

Biological control is considered apromising strategy for the replacement of extensive application of chemical fungicides which are harmful to human, living organisms and environment and cause development of resistance in the fungus. Biological control is now considered as an alternative to synthetic fungicides and chemical methods. *Trichoderma* sp. is used for over 80 years as bioagents to control plant diseases, because it has ability to parasite on plant pathogenic fungi and produces antibiotics Kucuk and Kivanc (2003). Ikotun and Adekunle, (1990) investigated the antagonistic effect of *Trichoderma harzianum*, *Bacillus cereus* and *B. subtilis* against cassava pathogenic fungus *Botryodiplodia theobromae*. Soil inoculation of the antagonists reduced root and tuber rot of cassava plants in the field. Abo-Rehab *et al.* (2013) reported that the three commercial biological control agents, *i.e.* Bio Zied (*Trichoderma album*), Bio Arc (*Bacillus megaterium*) and Rhizo-in (*Bacillus subtilis*) were efficient in suppressing fungal pathogens cause graft failure on grapevine under greenhouse conditions. The results in the present work indicate that seed treatment and soil drenching with biofungicides (Bio Zied and Bio Arc) gave moderate effect as shown for mean survival plants of avocado transplants.

Application of essential oils and their components are gaining increasing interest as attractive method for controlling plant diseases, because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional uses Ormancey *et al*. (2001). Seed treatment and soil drenching with essential oils (Thyme and Lemongrass) reduced pre- and post-emergence damping-off and increased mean survival plants of avocado transplants compared with control treatment. Many researchers reported that essential oils inhibited large number of soil borne pathogens. El-Mougy *et al.* (2007) reported that geranium, rose, lemon and mint essential oils were used as seed dressing and foliar spray for controlling wilt and root rot incidence in *Phaseolus vulgaris* L. El-Mohamedy (2013) reported that all concentrations of lemongrass, thyme, citral and nerol oils inhibited mycelial growth of soil-borne pathogenic fungi (*Fusarium oxysporum radicis-lycopersici*, *F. oxysporum lycopersici, F. solani, Rhizoctonia solani, Sclerotium rolfsii, Macrophomina phaseolinae, Pythium* sp. *and Phytophthora* sp.) that cause tomato root rot. At 1.5% of all tested essential oils a complete inhibition of fungal growth was observed. Essential oils are promising alternative compounds which could be used as the main or as adjuvant antimicrobial compounds for controlling plant disease Kaur and Arora (1999). These plant compounds have different structures and different mode of action when compared with antimicrobials that used for controlling pathogens Nascimento *et al.* (2000). Essential oils have been used successfully in combination with antibacterial agents, mild heat and salt compounds on *Listeria monocytogenes* Karatzas *et al.* (2000). Naqui *et al.* (1994) reported that some Indian medicinal plants contain compounds able to inhibit the microbial growth.

Salicylic acid was used as disease resistance inducers against several soil-borne plant pathogens in various trials because it had been found to be active as antimicrobial. Chen-Chunquan *et al.* (1999) reported that Salicylic acid has role in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots. Mandavia *et al.* (2000)) recorded that Salicylic acid had inhibitory effects on spore germination and mycelial growth of *Fusarium oxysporum f.* sp. *cumini.* In this investigation seed treatment and soil drenching with Salicylic acid reduced the number of pre- and post-emergence damping-off and increased mean survival plants of avocado transplants compared with control treatment.

CONCLUSION

Because soil borne fungi that cause damping-off and root rot can survive in the plant debris, the infection can be increase every year. The following recommendations, application of fungicides, biofungicides, essential oils and Salicylic acid as seed dressing and soil drenching could be used for controlling damping-off and root rot diseases in addition to, improve on the successful propagation of avocado in the nursery and controlling root rot in new orchards.

REFERENCES

Abd-El Ghani, H.S.; H.M. Fatouh (2005) First record of sugar beet root rot disease caused by *Botryodiplodia theobromae* in Egypt. Egypt J. Phytopathol, 33(1):107–108.

Abdel-Monaim M.F.; M.E.A. EL-Morsi; M.E. Hassan (2014).Control of root rot and wilt disease complex of some evergreen fruit transplants by using plant growth promoting rhizobacteria in the New Vally Governorate, Egypt. Journal of Phytopathology and Pest Management, 1(3):23-33.

Abo Rehab, M.E.A.; A.K.M. Korra ; M.A.M. Kamhawy and K.Y.A Youssef (2013). Fungal species associated with graft union on grapevine, its impact on graft failure process and attempted solutions in Egypt. International Journal of Agriculture and Forestry, 3(2): 52-59.

Barnett, H.L. and B.B. Hunter (1986). Illustrated genera of imperfect fungi. 4th Ed., Macmillan Publishing Co., New York 218 pp.

Booth, C. (1985). The Genus *Fusarium* .Kew, Surrey.Commonwealth Mycol. Inst., 2nd Ed., 237 pp.

Chen-Chunquan, S.; R.R. Belanger; N. Benhamou; T.C. Paulitz and C.Q. Chen (1999). Role of Salicylic acid in systemic resistance induced by *Pseudomonas* spp. against *Pythium aphanidermatum* in cucumber roots . European J. Pl. Pathol., 105:477-486.

Darvas, J.M. (1978). Common root pathogens from avocados. South African Avocado Growers' Association Research Report for, 2:3-4.

El-Mohamedy, R.S.R; M.M. Abdel-Kader; F. Abd-El-Kareem and N.S. El-Mougy (2013). Essential oils, inorganic acids and potassium salts as control measures against the growth of tomato root rot pathogens in vitro. Journal of Agricultural Technology, 9(6):1507-1520.

El-Mougy, N.S.; N.G. El-Gamal; M.M. Abdel-Kader (2007). Control of wilt and root rot incidence in *Phaseolus vulgaris* L. by some volatile compounds. J. Plant Protection Res., 47 (3): 255–265.

Elosaily A.H. (2015). A comparative pharmacognostical study of certain avocado (*Persea Americana* Mill.) cultivars grown in Egypt. M. Sc. Thesis; Fac. Pharmacy CairoUniv., Egypt.

FAO (2013). Food and Agricultural Organization. California Avocado Commission. 2010-2011. unprecedented performance: Progress towards sustainable value. Calif. Avocado Comm. Annu. Rep. 12, Mauchly, Irvine, CA. http://faostat.fao.org.

Ikotun, T. and F. Adekunle (1990): Inhibition of growth of some plant pathogenic fungi by some antagonistic microorganisms isolated from soil. Journal of Basic Microbiology, 30(2): 95-98.

Jung, Y.S; Y.T. Kim; S.J. Yoo; H.G. Kim (1999). Mycological characteristics of *Fusarium solani* f. sp. Pisi isolated from pea, ginseng and soybean in Korea. Plant Pathology Journal, 15(1): 44-47.

Karatzas, A.K.; M.H. Bennik; E.J. Smid; E.P. Kets (2000). Combined action of S-carvone and mild heat treatment on *Listeria monocytogenes* Scott A. J. Appl. Microbiol., 89: 296–301.

Kaur, J. and D. Arora (1999). Antimicrobial activities of species. Int. J. Antimicrob. Agents, 12:257–262.

Kotze, J .M. (1985). Strategy for combating avocado root rot. South African Avocado Growers' Association Yearbook , 8:13-14.

Kucuk, C. and M. Kivanc (2003). Isolation of *Trichoderma spp.* and determination of their antifungal, biochemical and physiological features. Turk J. Biol., 27: 247-253.

Latha, P.; V. Prakasam; A. Kamalakannan; C. Gopalakrishnan; T. Raguchander; M. Paramathma and R. Samiyappan (2009). First report of *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl causing root rot and collar rot disease of physic nut (*Jatropha curcas L.*) in India. Australasian Plant Disease Notes, 4: 19–20.

Mandavia, M.K.; N.A. Khan; H.P. Gajera; J.H. Andaria and M. Parameswaram (2000). Inhibitory effects of phenolic compounds on fungal metabolism in host-pathogen interactions in *Fusarium* wilt of Cumin . Allelopathy Journal, 7:85-92.

Naqui, S.H.A.; M.S.Y. Khan and S.B. Vohora (1994). Antibac-terial, antifungal and anthelmintic investigation on Indian medicinal plants. Fitoterapia, 62:221-228.

Nascimento, G.G.; J. Locatelli and P.C. Freitas (2000). Anti-bacterial activity of plants extracts and phytochemical on antibiotic resistant bacteria. Braz. J. Microbiol., 31:247-256.

Nelson,P.E.; T. A. Toussoum and W.F.O. Marasa (1983). *Fusarium* species. An illustrated manual of identification .Pennsylvania Univ. Press, Univ.Park.193p.

Omokhua, G.E.; M.I. Godwin and V.C. Okereke (2009). Damping-off Disease of two Pulp and Paper Forest Species (*Pinus caribaea* Morelet and *Pinus oocarpa* Schiede) in the Nursery (pp.275-282).

Ormancey, X.; S. Sisalli and P. Coutiere (2001). Formulation of essential oils in functional perfumery. Parfums, Cosmetiques, Actualites, 157: 30–40.

Perez-Jimenez, R.M. (2008). Significant avocado diseases caused by fungi and oomycetes .The European Journal of Plant Science and Biotechnology, 2 (1): 1-24.

Porter, D.M; D.H. Smith and R. Rodiguez- Kabana (1990). Copendium of peanut disease. American Phytopathological Society (APS), St. MN., USA.

Radwan, M.A. and M.S.S. Hassan (2016). Control of *Lasiodiplodia theobromae* on avocado in Egypt. Menoufia J. Plant Prot., 1:173-188.

Saleh, O. L. (1997).Wilt, root rot and seed diseases of groundnut in El-Minia Governorate, Egypt. Egypt J. Phtopathol., 25:1-18.

Snedecor, G.W. and Cochran (1990). Statistical methods 7^{th}Ed. The Iowa. State Univ. Press. Ames. Iowa. USA P.593.

Swart, W.J.; R.M. Oelofse and M.T. Labuschagne (2003). Susceptibility of South African cactus pear varieties to four fungi commonly associated with disease symptoms. J. PACD, pp. 86-97.

مكافحة مرض موت البادرات وعفن جذورالأفوكادو (الزبدية) فى مصر

محمود عواد رضوان و مبروك سيد سيد حسن
معهد بحوث أمراض النباتات بالجيزة – مصر

موت البادرات وأعفان الجذور من أهم الأمراض الفطرية المزعجة التي تصيب الأفوكادو (الزبدية) فتؤثر علي إنتاج الشتلات مسببة نقص كبير في عدد الشتلات بالمشتل وعدد الشجيرات الحديثة الزراعة بالحقل. ولقد أجريت تجارب بمحطة بحوث البساتين بالقناطر الخيرية تحت ظروف المشتل والصوبة خلال عامي 2016- 2017. ولقد أثبتت النتائج أن الأصناف هاس، إيتينجر، فيورت هي أكثر الأصناف قابلية للإصابة بالمرض يليها الصنف ديوك، بينما أقل نسبة إصابة بالمرض وأعلى نسبة نباتات سجلت علي أصناف ريد، بينكيرتون، بيكون. ولقد أثبتت إختبارات القدرة الإمراضية تحت ظروف الصوبة علي البذوروالشتلات المزروعة في تربة معدية بفطريات فيوزاريوم سولانى، فيوزاريوم مونيليفورم، فيوزاريوم سيميتيكتم، لاثيوديبلوديا ثيوبرومى التى تم عزلها من جذور الشتلات المصابة بالمشتل والأشجار الحديثة بالحقل أن هذة الفطريات كانت قادرة علي إحداث المرض ولكنها إختلفت في قدرتها الإمراضية. وكان فيوزاريوم سولانى أكثر الفطريات إحداثا للمرض مسببا أعلى نسبة لموت البادرات يلية فيوزاريوم مونيليفورم بينما فيوزاريوم سيميتيكتم، لاثيوديبلوديا ثيوبرومى كانا أقل الفطريات إحداثا للمرض. ولكي نتغلب علي هذة المشكلة فلقد تم دراسة تأثير تسع مركبات مختلفة كمعاملة مفردة للبذرة والتربة كل علي حدة علي خفض المرض تحت ظروف الصوبة. ولقد أثبتت النتائج أن كل المركبات كانت قادرة علي خفض المرض وزيادة نسبة النباتات السليمة مقارنة بالبذرة والتربة التي لم تعامل. كما أن معاملة البذرة والتربة بالمبيدات الكيميائية كانت الأعلى تأثيرا حيث أعطت أعلى نسبة من النباتات السليمة. وأن المبيدين الحيويين بيوزيد وبيوأرك أعطا تأثيرا متوسطا فى مكافحة المرض. كما أدت المعاملة بالزيوت النباتية (زيت الزعتر ، حشيشة الليمون) وحمض السيلسيليك إلي خفض المرض وزيادة نسبة النباتات السليمة. وأفضل النتائج تم الحصول عليها في حالة معاملة البذرة.