



CHEMICAL CONTROL OF GUAVA DIE-BACK AND RESPONSE OF GUAVA CULTIVARS TO THE DISEASE IN EGYPT

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ABSTRACT

Guava (*Psidium guajava* L.) die-back disease caused by *Botryodiplodia theobromae* Pat., is a destructive disease, considered one of the most important and economic disease of guava. Amistar Top 325-32.5% E.C. gave the highest decreasing of linear growth and amount of growth of isolate code No., Q.K.4., followed by Camzin 50% W.P., Monceren 25% W.P. and Montro 30% E.C. Meanwhile, Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. exhibited a lowest effect in reducing of linear growth and amount growth of *B. theobromae* isolate code No., Q.K.4. Amistar Top 325-32.5% E.C. was the most efficient fungicide in reduce disease incidence and disease severity infield of *B. theobromae* isolate code No., Q.K.4., followed by Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C. Meanwhile, Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. showed a lowest efficient in reducing the disease incidence and disease severity. Soil drench method of fungicides application was the higher efficient method in elimination of percentage of disease incidence and disease severity followed by foliar spray application, relatively. Banaty transplant was the most susceptible cultivar to the all tested *B. theobromae* isolates, whereas cultivar Gizy Ahmr was the lowest susceptible with the same isolates, information about cultivar reaction of guava transplants against *B. theobromae* is still scanty.

Key words: Guava, *Psidium guajava* L., *Botryodiplodia theobromae* Pat., Guava Die-back, Chemical Control, cultivar reaction

INTRODUCTION

Guava trees (*Psidium guajava* L.) suffer from several serious and destructive diseases at all stages of its life. Several fungi attack guava causing seedling root rots (Baiuomy et al 2003), antracnose (Dharam and Gaur, 1973), black spot (Mishra and Sitansu, Pan, 2007), stem canker (Cardoso et al 2002, Kamhawy, 2011), stem-end rot (Mishra and Sitansu, Pan, 2007), die-back (Bokhari et al 2008, Safdar, Asma et al 2015) and wilt diseases (Pandit and Samajpati, 2002; Pandit and Samajpati, 2005, Dwivedi, Neetu and Dwivedi, 2016).

Under the Egyptian environmental conditions, several most important serious diseases attack guava foliage, roots and fruits. Guava die-back disease an important and economic serious problem causing severe losses in both nurseries and orchards in Egypt (Abdel-Gawad, 2000, Baiuomy, et al 2003 and Kamhawy, 2011 and Rashed et al 2014).

Guava die-back is a complex disease to extensive root rot caused by *Botryodiplodia theobromae* Pat. on the shoot causing fruit rot (Rana, 1981, Adisa, 1985, Rawal and Ullasa, 1988, Majumdar and Pathak, 1997 and Baiuomy, et al 2003), fruit *Botryodiplodia* rot (Junqueira, 2001), fruit dry rot (Rana, 1981, Sitansu, Pan and Mishra, 2010), die back. The typical symptoms of guava die-back disease include chlorosis, scorching of leave margins and leaf drop, yield reduction and plant death within months caused by *Botryodiplodia theobromae* Pat. Several investigators have been recorded that *Botryodiplodia theobromae* Pat. is a soil borne phytopathogen fungus and attack guava trees and wide spread in the world (Rana, 1981, Mishra and Sitansu, Pan, 2007 and Kamhawy, 2011 and Safdar, Asma et al 2015).

Although the recent strategy of control plant diseases is depend upon minimizing of fungicidal utilization to avoid environmental pollution and keep human health, fungicides are one of Integrated Pest Management (IPM) weapons, fungicides are still one of the most important means to control the causal pathogens of different diseases either in *in vitro* or in *in vivo*, accordingly this trials were carried out to the present study. The effect 24 fungicides differed in their active ingredients and chemical groups including systemic and non-systemic ones were tested on *Botryodiplodia theobromae* growth and their efficiency on the die-back disease.

Information about cultivar reaction of guava transplants against *Botryodiplodia theobromae* is still scanty. Cultivar reaction of guava trees is one of the most important factors affecting percentage of disease incidence and disease severity of guava die-back disease and considered one of Integrated Pest Management (IPM) weapons to control the causal pathogens of different diseases.

The strategies of the present study were designed to chemical control against *Botryodiplodia theobromae* Pat., the causal agent of guava die-back disease, as well as response of guava cultivars to the disease were also investigated.

MATERIALS AND METHODS

In a previous study; *Botryodiplodia theobromae* Pat. isolate Q.K.4 was the highest pathogenic to produce typical symptoms of guava die-back under greenhouse conditions. Such isolate was used throughout of this study (the author, in Press).

1. Inocula preparation

Discs 5mm. of the mycelial growth of the pathogen was taken from 7 days old cultures of Q.K.4 isolate of *B. theobromae* Pat. grown on PDA medium. Each disc was set on the surface of sterilized corn meal medium in each 500ml. glass bottle. Corn meal medium was prepared by adding 200g. of corn meal to 100g. sand and 200ml. water in each glasses bottles (500ml.). All bottles were autoclaved at 120°C for one hour at 1.5 IB square inches. A set of 5 bottles were used as replicates of each treatment. A set of 5 bottles of sterilized corn meal sand medium were used without fungal inoculation as control treatment. All inoculated as well as uninoculated bottles were incubated at 25±2°C for two weeks to obtain sufficient mycelial growth of different isolates.

2. Soil infestation

Soil infestation was carried out using corn meal inoculated with a 5mm. disc from isolated *B. theobromae* (Q.K.4) isolate taken from 7 days old cultures. Plastic pots 25cm. in diameter were sterilized by immersing in 5% formalin solution for 15 minutes and left to dry for two weeks for complete evaporation of formalin, and then were filled with autoclaved at 120°C for one hour at 1.5 IB square inches; sand and clay soil mixture (1: 1 w/w) was prepared for soil infestation. Both sterilized pots and soil mixture were left for few days under greenhouse before infestation. The prepared inocula of the fungus on corn meal medium were mixed individually with the autoclaved soil sand mixture at the rate 5% (w/w). The inoculum was thoroughly mixed with the upper surface of soil and irrigated regularly 7 days before planting homogenous guava transplants to ensure the establishment of tested isolate. Thirty infested plastic pots (each pot contained 5kg. of soil sand mixture) were used for isolate (Q.K.4). The soil was infested with isolate at the rate 5% of soil weight. Control treatment was applied using fungus free corn meal medium. One guava transplant cultivar Banaty (one year old) was cultivated in each pot. Five pots were replicates used for each treatment; each treatment contained six guava transplants. Plants were examined for guava die-back disease after 15, 30, 45 and 60 days from inoculation. Pots were arranged in complete randomized design. Re-isolation from inoculated guava transplants was made as mentioned before, to confirm Koch's postulates.

Chemical Control of *Botryodiplodia theobromae* Pat., the causal pathogen of guava die-back disease

1. Laboratory studies (in *in vitro* experiments)

1.1. Effect of fungicides on mycelial linear growth (mm.)

Accordingly, these trials were carried out to study the effect of twenty four fungicides differed in their active ingredients and chemical groups including, systemic and non-systemic fungicides ones (Table 1).

The calculated quantity of each fungicide for each concentration was weighed and dissolved in 5ml. of distilled water and made up to 100 ml, 25ml. of each concentration was added to each plate (9cm.) in the freshly prepared PDA (Potato

Dextrose Agar) separately and allowed to cool to a pouring temperature of 40-45°C. Twenty five ml. of these PDA amended with different fungicide at different concentration poured into 9cm. in diameter sterilized petri dishes. Each plate including the control (without fungicide) on solidification PDA medium was inoculated in the middle with uniform mycelial disc (5mm. in diameter) of the most virulent isolate of *Botryodiplodia theobromae* code (No. Q.K.4) obtained from 7 days old culture by using sterilized inoculating needle. Each concentration of each fungicide including control (media without any fungicides) were placed in an incubator at 25°C±2 for 7 days and observed daily for mycelial growth. Radial mycelial growth was measured when growth of control treatment completely filled any plate.

Five glass petri-dishes 9cm. in diameter were used for each particular concentration as well as, control treatment (media without any fungicides).

3.1.2. Effect of fungicides on amount of mycelial growth (mg.)

The effect of twenty four tested fungicides (**Table 1**) on the amount of mycelial growth was studied of the most virulent isolate of *Botryodiplodia theobromae* code (No. Q.K.4) investigated by using eleven different used concentrations i.e., 0, 5, 10, 25, 50, 100, 200, 300, 400, 500 and 600 ppm. of each tested fungicide were prepared according to (**Sharvell, 1962**) to determine their ability to inhibit the amount of mycelial growth. One hundred ml. of sterilized Czapek's broth medium were placed in glass Erlenmeyer conical flasks (250ml. capacity) five replicates of each concentration for each fungicide (treatment) were inoculated with mycelial disc 5mm. in diameter obtained from 7 days old culture of the desired of the most virulent isolate of *Botryodiplodia theobromae* code (No. Q.K.4). All glass Erlenmeyer conical flasks were incubated at 25°C±2 for 7 days. Fungal mates were collected on previously weighed filter papers, washed with distilled water, dried at 70°C for constant weight and weighed. The amount of mycelial growth of each tested fungicide for each concentration was determined. Data was being the average of five single determinations.

2. *In vivo* evaluation the efficacy of different fungicides against guava die-back disease in pot experiments under greenhouse conditions

The same twenty four tested different fungicides in laboratory studies (in *in vitro* experiments)

were used to evaluate their efficiency against guava die-back disease on cultivar Banaty. Homogenous transplants one year old were cultivated in pots (25cm.). Pots contained infested soil with isolate Q.K.4 of the pathogen. One transplant was cultivated for each pot. By using rates of application (as recommended dose) of each tested fungicides. The different fungicides (**Table 1**) were applied by two methods; soil drench and foliar spray to determine their efficacy of reduction the disease incidence and disease severity of guava die-back disease.

Meanwhile a set of pots containing autoclaved soil mixture used as control treatment was amended a mixed with fungus-free corn meal medium (uninoculated corn meal medium). Pots were filled with either of the mixture as required. Guava transplant cultivar Banaty (one year old) were surface sterilized by using 2% sodium hypochlorite solution for one minute, washed with sterilized distilled water, then one transplant was planted in each plastic pots (25cm. in diameter) containing soil mixture infested with the tested *Botryodiplodia theobromae* isolate code (No. Q.K.4). Percentage of toxicity was calculated according to the formula suggested by (**Topps and Wain, 1957**) as the following:

Toxicity or reduction of disease incidence or disease severity % = $\frac{B-A}{B} \times 100$.

Where:

A= disease incidence or disease severity after fungicides application (treatment).

B= disease incidence or disease severity before fungicides application (control).

2.1. Soil drench application

The same twenty four fungicides tested in laboratory studies were used to evaluate their efficiency in pot experiments under greenhouse conditions against guava die-back disease of cultivar Banaty one year old by using rates of applications (as recommended dose) of each tested fungicides individually were used as suspensions by solving rates of application (as recommended dose) of each tested fungicides per liter of water for each plastic pot (25cm. in diameter). The percentage of disease incidence and disease severity were assessed before the application with fungicides treatment.

One transplant per/pot and pots soil were drenched with one liter of each fungicidal suspension or solution to saturate the pots soil and main-

tain a 1-cm. layer of fungicidal suspension or solution over the surface for one time.

Five replicates for each treatment; each replicate contained six homogenous guava transplants one year old, planted in six pots (one transplant per/pot) and also a set of pots for the control treatment (treated with water only without any fungicides), soil drench treatment was applied for one time. Pots were kept for 60 days under greenhouse conditions at $25^{\circ}\text{C}\pm 2$ and irrigated regularly with tap water as needed used for each treatment. After 60 days from fungicidal soil drench treatment, the disease incidence and disease severity were assessed.

2.2. Foliar spray application

The same twenty four fungicides tested in laboratory studies were used to evaluate their efficiency in pot experiments under greenhouse conditions against guava die-back disease of cultivar Banaty one year old, infected previously with the most virulent isolate of *Botryodiplodia theobromae* code (No. Q.K.4), the same method in pathogenicity test of inoculation was used as mentioned before. By using rates of applications (as recommended dose) of each tested fungicides. Each plastic pot (25cm. in diameter) were covered with plastic sheet upon soil surface to prevent solutions or suspensions of tested fungicides from fall into soil of each pot and fungicides were used a suspension by solving rates of application (as recommended dose) of each tested fungicides per liter of water and were sprayed from all direction until runoff by using a normal manual pressure sprayer 1.5 liter in size separately for one time. The percentage of disease incidence and disease severity were assessed before the application with fungicides treatment.

Five replicates for each treatment, six homogenous transplants for each replicate (one guava transplant per/pot), the same number of guava transplants were grown in infested soil, also, a set of pots treated with water only without any fungicides as a control (treatment). Pots were kept for 60 days under greenhouse conditions at $25^{\circ}\text{C}\pm 2$ and irrigated regularly with tap water as needed used for each treatment. After 60 days from fungicidal foliar spray treatment, the disease incidence and disease severity were assessed, and the reduction of the percentage of disease incidence % D.I. and percentage of disease severity % D.S. were calculated as mentioned previously in soil drench application.

4. Cultivar reaction

The reaction of four guava transplant cultivars i.e., Banaty, Malisy Ahmr, El-Mobaker and Giza Ahmr one-year-old were kindly obtained from Production Unit of Fruit Section, Horticulture Research Institute, Agriculture Research Center (ARC) were used in this investigation to infection with ten isolates of *Botryodiplodia theobromae* Pat. which were characterized in a previous study (the author, in Press) i.e., code numbers B.W.1, A.B. 2, D.S. 3, Q.K. 4, A.A. 5, B.N. 6, K.Q. 7, Q.S. 8, D.B. 9 and K.D. 10 were evaluated in plastic pots experiment under greenhouse conditions of each cultivar.

All *Botryodiplodia theobromae* isolates associated with guava die-back symptoms used for testing pathogenicity on guava transplants cultivars for pathogenicity screening against the isolates of *Botryodiplodia theobromae* and mentioned under greenhouse conditions (10-14h. light-and- dark cycles) at $25^{\circ}\text{C}\pm 2$. Five replicates for each treatment, each treatment contained six homogeneous guava transplants for each cultivar individually one transplant for each pot and the same number for each cultivar of guava transplants individually was used in control treatment and pots were arranged in a randomized design.

4.1. Inocula preparation

Discs 5mm. of the mycelial growth of different isolated *Botryodiplodia theobromae* Pat. isolates were taken from 7 days old cultures on PDA medium. Each disc was set in the surface of sterilized corn meal medium in each 500ml. glass bottle. Corn meal medium was prepared by adding 200g. of corn meal to 100g. sand and 200ml. water in each glasses bottles (500ml.). All bottles were autoclaved at 120°C for one hour. A set of 5 bottles were used as replicates of each isolate. A set of 5 bottles of sterilized corn meal sand medium were used without *Botryodiplodia theobromae* Pat. isolates inoculation as control treatment. All inoculated as well as uninoculated bottles were incubated at $25\pm 2^{\circ}\text{C}$ for two weeks to obtain sufficient growth of different isolates.

4.2. Soil infestation

Soil infestation was carried out using corn meal inoculated with a 5mm. disc from each of *Botryodiplodia theobromae* Pat. isolates taken from 7 days old cultures. Pots of 25cm. in diameter were sterilized with 5% formalin solution and left for a

week for formalin evaporation, then were filled with autoclaved sand and clay soil (1: 1 w/w). The soil was infested with each isolate alone at the rate of 5% of soil weight. The inoculum was thoroughly mixed with the upper surface of the soil and irrigated regularly seven days before planting to ensure the establishment of the isolates.

A set of pots as control treatment were applied using free from any *Botryodiplodia theobromae* isolate corn meal medium. One guava transplant, cultivar Banaty, Malisy Ahmr, El-Mobaker and Gizy Ahmr (one year old) were cultivated for each cultivar individually, one transplant planted in each pot and five replicates were used for each treatment, each treatment contained six homogeneous guava transplants. All transplants were examined for guava die-back disease after 15, 30, 45 and 60 days after inoculation. Re-isolation from inoculated guava transplants was made as mentioned before, to confirm Koch's postulates.

5. Disease assessment

A method of visual estimation of the disease was assessed on samples consisted 160 guava trees from each inspected locality where used for assign disease. The disease incidence (D.I.) and the disease severity (D.S.) percentages were calculated for each season through the following formula (Cooke et al 2006).

$$\% \text{ Disease incidence (D.I.)} = \frac{\sum x}{N} \times 100$$

Where:

$\sum x$ = sum number of diseased transplants; N = total number of inspected transplants

$$\% \text{ Disease severity (D.S.)} = \frac{\sum (nxv)}{Nx} \times 100$$

Where:

n = number of examined transplants; v = numerical rating of the scale (0-4); N = total number of transplants; x = maximum value (5) of evaluation scale

Disease reading was determined for each transplant according to the disease severity rating by determining the area of infected part to include the diameter of the area of the transplant. The following numerical rates were suggested for disease severity:

0 = healthy transplants, no symptoms; 1 = 1-25% infected part of the transplants; 2 = 26-50% infected part of the transplants; 3 = 51-75% infected part of the transplants; 4 = 76-100% infected part of the transplants

6. Statistical Analysis

All experiments were laid out in a completely randomized design (C.R.D.) there were five replicates for each treatment; each treatment contained six homogenous guava transplants, one transplant planted per/plastic pot (25cm. in diameter) experiment under greenhouse conditions.

Statistical Analysis carried out in Agricultural Informatics And Arithmetic Unit, Faculty Of Agriculture, Ain Shams Univ. Data were subjected to ANOVA by using SAS statistical software (SAS Institute, 2009) and significant difference among the treatments was portioned by least significant difference test (LSD) at probability levels of P = 0.05 (Steel et al 1997).

RESULTS

1. Chemical Control of *Botryodiplodia theobromae* Pat., the causal pathogen of guava die-back disease

1.1. *In vitro* studies

1.1.1. Effect of the tested fungicides on mycelial linear growth (mm.) of *Botryodiplodia theobromae* Pat. (Isolate code No., Q.K.4)

Data in Table (1) indicate the effect of twenty four tested fungicides at different concentrations i.e., 5, 10, 25, 50, 100, 200, 300, 400, 500 and 600 ppm., to growth PDA medium on the mycelial linear growth (mm.) of *Botryodiplodia theobromae* Pat. isolate code No., Q.K.4 (the most virulent isolate). The fungicide Amistar Top 325-32.5% E.C. was highly effective in reducing the rate of mycelial growth (mm.) on PDA medium at 100 ppm., followed by Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C., Topsin-M 70% W.P., Alliette 80% W.P. and Daconil 72% S.C. at 200 ppm. conc., these set of fungicides exhibited the highest effective in reducing the rate of mycelial growth (mm.) of *Botryodiplodia theobromae* Pat. (isolate code No., Q.K.4.). Meanwhile, Bellis 38% W.G. and Dithane M45 80% W.P. were stopped the fungal linear growth (mm.) at 300 ppm. conc. followed by, Tilt 80% W.P., Penazole 10% E.C., Opus 12.50% S.C. at 400 ppm. conc., followed by, Master 25% E.C. and Punch 40% E.C., inhibited mycelial linear growth (mm.) at 500 ppm., these set of fungicides showed a moderate effective in reducing the rate of mycelial growth (mm.).

1.1. *In vitro***Table 1.** Effect of different concentrations (p.p.m) of twenty four fungicides on mycelial linear growth (mm.) of *Botryodiplodia theobromae* Pat. isolate code number (Q.K.4) on PDA medium at 25±1°C degree.

Fungicides		Fungicides concentrations (p.p.m)											Mean
Trade Name	Common Name	0 (Control)	5	10	25	50	100	200	300	400	500	600	
Alliette 80% W.P.	Fosetyl aluminium	90.00	80.00	76.00	63.00	36.00	20.00	0.00	0.00	0.00	0.00	0.00	33.18
Amistar Top 325-32.5% E.C.	Azoxystrobin-Difenoconazole	90.00	80.00	56.00	26.00	11.00	0.00	0.00	0.00	0.00	0.00	0.00	23.91
Bellis 38% W.G.	Pyroclorobin+Boscoild	90.00	80.00	76.00	38.00	26.00	18.00	6.00	0.00	0.00	0.00	0.00	30.36
Camzin 50% W.P.	Carbendazim	90.00	70.00	61.00	43.00	20.00	5.00	0.00	0.00	0.00	0.00	0.00	26.27
Copral 50% W.P.	Copper oxychloride	90.00	90.00	90.00	90.00	90.00	20.00	90.00	75.00	50.00	20.00	0.00	70.75
Daconil 72% S.C.	Chlorothalonil	90.00	84.00	81.00	63.00	36.00	28.00	0.00	0.00	0.00	0.00	0.00	34.73
Dithane M45 80% W.P.	Mancozeb	90.00	84.00	78.00	68.00	60.00	42.00	10.00	0.00	0.00	0.00	0.00	39.27
Eminent 12.50% E.W.	Tetraconazole	90.00	90.00	86.00	78.00	74.00	66.00	53.00	46.00	38.00	22.00	0.00	58.45
Fungshow 12.50% W.P.	Diniconazole	90.00	88.00	86.00	68.00	61.00	44.00	26.00	16.00	10.00	6.00	0.00	45.00
Master 25% E.C.	Prochloraz	90.00	86.00	83.00	61.00	43.00	36.00	14.00	7.00	4.00	0.00	0.00	38.54
Monceren 25% W.P.	Pencycuron	90.00	84.00	66.00	60.00	10.00	5.00	0.00	0.00	0.00	0.00	0.00	28.64
Monstro 30% E.C.	Difenoconazole-Propiconazole	90.00	76.00	66.00	28.00	10.00	8.00	0.00	0.00	0.00	0.00	0.00	25.27
Opus 12.50% S.C.	Epoxiconazole	90.00	90.00	90.00	90.00	90.00	75.00	40.00	18.00	0.00	0.00	0.00	53.00
Penazole 10% E.C.	Penconazole	90.00	90.00	90.00	90.00	90.00	65.00	33.00	14.00	0.00	0.00	0.00	51.09
Punch 40% E.C.	Flusidazole	90.00	90.00	90.00	90.00	90.00	90.00	60.00	38.00	26.00	0.00	0.00	60.36
Ridomil Gold/Plus 42.50% W.P.	Copper oxychloride+Metaloxyl M	90.00	90.00	90.00	90.00	90.00	90.00	83.00	68.00	45.00	18.00	0.00	68.54
Rizolex T 50% W.P.	Thiram-Tolclofosmethyl	90.00	90.00	90.00	90.00	90.00	90.00	78.00	46.00	34.00	16.00	0.00	64.91
Rovral 50% W.P.	Iprodione	90.00	90.00	90.00	90.00	90.00	90.00	85.00	65.00	40.00	15.00	0.00	67.73
Score 25% E.C.	Difenoconazole	90.00	90.00	78.00	65.00	51.00	48.00	42.00	26.00	21.00	6.00	0.00	47.00
Tecto 50% S.C.	Thiabendazole	90.00	90.00	90.00	90.00	90.00	70.00	50.00	30.00	20.00	10.00	0.00	57.27
Thiovat Jet 80% W.G.	Sulfur	90.00	90.00	90.00	90.00	90.00	90.00	90.00	80.00	55.00	30.00	0.00	72.27
Topsin-M 70% W.P.	Thiophanate methyl	90.00	90.00	83.00	20.00	14.00	11.00	0.00	0.00	0.00	0.00	0.00	28.00
Tilt 80% W.P.	Propiconazole	90.00	90.00	90.00	81.00	62.00	40.00	17.00	10.00	0.00	0.00	0.00	43.63
Vectra 10% S.C.	Bromuconazole	90.00	90.00	90.00	90.00	90.00	80.00	80.00	50.00	20.00	5.00	0.00	62.27
Mean		90.00	86.33	81.92	69.25	58.92	47.12	35.71	24.54	15.12	8.42	0.00	-----

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05

Least Significant Difference (LSD) for tested fungicides= 0.8286

Least Significant Difference (LSD) for fungicides concentrations= 0.5609

Least Significant Difference (LSD) for interaction between fungicides and concentrations= 2.7481

However, the other fungicides i.e., Vectra 10% S.C., Fung show 12.50% W.P., Score 25% E.C., Tecto 50% S.C., Rovral 50% W.P., Rizolex T 50% W.P., Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. inhibited mycelial linear growth (mm.) at 600 ppm. and exhibited a lowest effect in reducing of

mycelial linear growth (mm.). Data also, indicate that the inhibiting effect of all the previously tested fungicides were gradually increased by increasing the concentration of each fungicide and inhibited the mycelial linear growth (mm.) from 5 to 600 ppm., respectively. Data also, recorded the maximum linear growth of *Botryodiplodia theobromae*

Pat. isolate code No., Q.K.4 was 90mm. in diameter on PDA medium at 0 ppm. concentration as the control treatment.

1.1.2. Effect of the tested fungicides on amount of growth (dry weight mg.) of *Botryodiplodia theobromae* Pat. (Isolate code No., Q.K.4)

As for the effect of the previous twenty four tested fungicides on the total amount of growth (dry weight mg.) on Czapek's medium of *Botryodiplodia theobromae* Pat. isolate code No., Q.K.4 (the most virulent isolate), the Data recorded in **Table (2)** indicate that Amistar Top 325-32.5% E.C. tended the same trend as with mycelial linear growth where it stopped completely the fungal growth of *Botryodiplodia theobromae* Pat. at 100 ppm., however, the other fungicides showed different trend followed by, Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C., Topsin-M 70% W.P., Alliette 80% W.P. and Daconil 72% S.C. at 200 ppm. conc., these set of fungicides exhibited the highest effective in reducing the total amount of growth (dry weight mg.) on Czapek's medium of *Botryodiplodia theobromae* Pat. isolate code No., Q.K.4. Meanwhile, Bellis 38% W.G. and Dithane M45 80% W.P. were stopped the amount of growth (dry weight mg.) at 300 ppm. conc. followed by, Tilt 80% W.P., Penazole 10% E.C., Opus 12.50% S.C. at 400 ppm. conc., followed by, Master 25% E.C. and Punch 40% E.C., inhibited amount of growth (dry weight mg.) at 500 ppm., these set of fungicides showed a moderate effective in reducing the amount of growth (dry weight mg.) of *Botryodiplodia theobromae* Pat. (isolate code No., Q.K.4.). However, the other fungicides i.e., Vectra 10% S.C., Fungshow 12.50% W.P., Score 25% E.C., Tecto 50% S.C., Rovral 50% W.P., Rizolex T 50% W.P., Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. inhibited amount of growth (dry weight mg.) at 600 ppm., and exhibited a lowest effect in reducing of amount of growth (dry weight mg.). Data also, indicate that the inhibiting effect of all the previously tested fungicides were gradually increased by increasing the concentration of each fungicide and inhibited the amount of growth (dry weight mg.) from 5 to 600 ppm., respectively.

1.2. *In vivo* disease control with the tested fungicides under greenhouse conditions experiment

Greenhouse experiments (*in vivo*)

Application of twenty four fungicides as foliar spray or soil drench to guava plants under greenhouse conditions. The efficiency of 24 fungicides (**Tables 3 and 4**) applied as foliage spray or soil drench the suppression for estimation their effect on guava die-back diseased trees. **Data in Table (3)** proved that soil drench was the higher efficient method in elimination of disease incidence followed by foliar spray. Such result was true for all fungicides. Amistar Top 325-32.5% E.C. was the most efficient fungicide in reduce disease incidence, followed by Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C., Topsin-M 70% W.P., Alliette 80% W.P. and Daconil 72% S.C., these set of fungicides exhibited the higher efficient in reducing the disease incidence. Meanwhile, Bellis 38% W.G., Dithane M45 80% W.P., followed by Tilt 80% W.P., Penazole 10% E.C., Opus 12.50% S.C., Master 25% E.C. and Punch 40% E.C., these set of fungicides showed a moderate efficient in reducing the disease incidence. However, other fungicides i.e., Vectra 10% S.C., Fungshow 12.50% W.P., Score 25% E.C., Tecto 50% S.C., Rovral 50% W.P., Rizolex T 50% W.P., Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. showed a least efficient in reducing the disease incidence.

Data in **Table (4)** proved that soil drench was the higher efficient method in elimination of disease incidence followed by foliar spray. Such result was true for all fungicides. Amistar Top 325-32.5% E.C. was the most efficient fungicide in reducing the disease incidence, followed by Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C., Topsin-M 70% W.P., Alliette 80% W.P. and Daconil 72% S.C., these set of fungicides exhibited the higher efficient in reducing disease incidence. Meanwhile, Bellis 38% W.G., Dithane M45 80% W.P., followed by Tilt 80% W.P., Penazole 10% E.C., Opus 12.50% S.C., Master 25% E.C. and Punch 40% E.C., these set of fungicides showed a moderate efficient in reducing the disease incidence.

Table 2. Effect of different concentrations (p.p.m) of twenty four fungicides on the amount of mycelial growth (mg.) of *Botryodiplodia theobromae* Pat. isolate code (No.Q.K.4) on Czapek's growth medium at 25±1°C degree.

Fungicides			Fungicides concentrations (p.p.m)										Mean
Trade Name	Common Name	0 (Control)	5	10	25	50	100	200	300	400	500	600	
Alliette 80% W.P.	Fosetyl aluminium	260.50	209.80	152.60	117.50	96.60	62.80	0.00	0.00	0.00	0.00	0.00	81.80
Amistar Top 325-32.5% E.C.	Azoxystrobin-Difenoconazole	260.50	140.60	88.60	38.50	17.40	0.00	0.00	0.00	0.00	0.00	0.00	49.60
Bellis 38% W.G.	Pyroclostrobin+Boscoild	260.50	200.40	110.80	88.50	46.80	23.60	16.50	0.00	0.00	0.00	0.00	67.92
Camzin 50% W.P.	Carbendazim	260.50	154.80	58.60	47.80	38.50	36.80	0.00	0.00	0.00	0.00	0.00	54.27
Copral 50% W.P.	Copper oxychloride	260.50	260.50	260.50	260.50	230.50	198.60	169.80	146.80	59.60	45.60	0.00	172.08
Daconil 72% S.C.	Chlorothalonil	260.50	231.60	196.80	146.50	118.80	88.50	0.00	0.00	0.00	0.00	0.00	94.79
Dithane M45 80% W.P.	Mancozeb	260.50	212.80	163.60	153.80	121.40	70.80	27.30	0.00	0.00	0.00	0.00	91.84
Eminent 12.50% E.W.	Tetraconazole	260.50	254.60	240.80	212.40	181.60	146.60	114.60	106.60	80.50	66.50	0.00	151.34
Fungshow 12.50% W.P.	Diniconazole	260.50	216.80	186.60	142.60	98.60	43.30	24.60	16.50	8.80	4.40	0.00	91.15
Master 25% E.C.	Prochloraz	260.50	215.80	150.60	115.60	88.60	53.60	20.00	16.60	10.40	0.00	0.00	84.70
Monceren 25% W.P.	Pencycuron	260.50	156.80	78.50	66.40	53.40	41.80	0.00	0.00	0.00	0.00	0.00	59.76
Montro 30% E.C.	Difenoconazole- Pro-piconazole	260.50	228.50	166.60	146.50	73.80	48.40	0.00	0.00	0.00	0.00	0.00	84.03
Opus 12.50% S.C.	Epoxiconazole	260.50	248.80	172.60	153.30	134.80	114.80	98.80	35.30	0.00	0.00	0.00	110.81
Penazole 10% E.C.	Penconazole	260.50	216.80	193.80	136.80	100.80	88.80	60.60	18.50	0.00	0.00	0.00	97.87
Punch 40% E.C.	Flusidozole	260.50	260.50	260.50	260.50	196.50	162.80	69.80	32.60	17.30	0.00	0.00	138.27
Ridomil Gold/Plus 42.50% W.P.	Copper oxychloride+Metaloxyl M	260.50	260.50	260.50	260.50	260.50	191.80	168.50	112.60	52.50	38.00	0.00	169.63
Rizolex T 50% W.P.	Thiram-Tolclofos-methyl	260.50	260.50	260.50	260.50	186.70	108.80	142.30	98.00	33.80	28.60	0.00	149.11
Rovral 50% W.P.	Iprodione	260.50	260.50	260.50	260.50	260.50	173.30	148.30	126.60	46.50	20.00	0.00	165.20
Score 25% E.C.	Difenoconazole	260.50	200.80	182.60	121.50	100.60	90.50	41.20	18.80	12.80	8.60	0.00	94.35
Tecto 50% S.C.	Thiabendazole	260.50	228.80	203.80	198.80	141.40	126.60	53.00	28.60	20.60	16.40	0.00	116.23
Thiovat Jet 80% W.G.	Sulfur	260.50	260.50	260.50	260.50	260.50	210.80	186.50	155.60	118.80	80.60	0.00	186.80
Topsin-M 70% W.P.	Thiophanate methyl	260.50	252.60	186.80	156.40	76.50	56.50	0.00	0.00	0.00	0.00	0.00	89.94
Tilt 80% W.P.	Propiconazole	260.50	214.50	160.50	120.60	95.60	86.60	30.80	16.00	0.00	0.00	0.00	89.55
Vectra 10% S.C.	Bromuconazole	260.50	260.50	260.50	260.50	193.80	153.60	124.60	48.80	34.60	3.50	0.00	145.54
Mean		260.50	225.35	188.24	166.13	132.26	99.15	62.38	40.75	20.68	13.01	0.00	-----

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05

Least Significant Difference (LSD) for tested fungicides= 1.0495

Least Significant Difference (LSD) for fungicides concentrations= 0.7105

Least Significant Difference (LSD) for interaction between fungicides and concentrations= 3.4807

1.2. *In vivo*:

Table 3. Therapeutic effect of twenty four fungicides at recommended dose for each fungicide by two methods of application i.e. foliar spray and soil drench on disease incidence (%) of guava die-back disease on transplants cultivar Banaty one year old, interval period of spray and soil drench 15 days for three times for each fungicide, two months after fungicides application in pot experiments under greenhouse conditions during 2015 and 2016 year.

Tested Fungicides	Methods of Application at recommended dose for each fungicide													
	Foliar Spray						Mean	Soil Drench						Mean
	Disease incidence % before application		Disease incidence % 60 days after application		Efficacy of fungicides %		Efficacy of fungicides %	Disease incidence % before application		Disease incidence % 60 days after application		Efficacy of fungicides %		Efficacy of fungicides %
	2015	2016	2015	2016	2015	2016	2015 and 2016	2015	2016	2015	2016	2015	2016	2015 and 2016
Alliette 80% W.P.	33.33	36.67	16.67	20.00	49.98	45.46	47.72	33.33	36.67	13.33	16.67	60.00	54.54	57.27
Amistar Top 325-32.5% E.C.	33.33	36.67	6.67	10.00	80.00	72.73	76.37	33.33	36.67	3.33	6.67	90.00	81.83	85.92
Bellis 38% W.G.	33.33	36.67	20.00	23.33	40.00	36.38	38.19	33.33	36.67	16.67	20.00	49.98	45.46	47.72
Camzin 50% W.P.	33.33	36.67	10.00	13.33	70.00	63.65	66.83	33.33	36.67	6.67	10.00	80.00	72.73	76.37
Copral 50% W.P.	33.33	36.67	30.00	33.33	10.00	9.11	9.56	33.33	36.67	30.00	33.33	10.00	9.11	9.56
Daconil 72% S.C.	33.33	36.67	16.67	20.00	49.98	45.46	47.72	33.33	36.67	16.67	20.00	49.98	45.46	47.72
Dithane M45 80% W.P.	33.33	36.67	20.00	23.33	40.00	36.38	38.19	33.33	36.67	16.67	20.00	49.98	45.46	47.72
Eminent 12.50% E.W.	33.33	36.67	30.00	33.33	10.00	9.11	9.56	33.33	36.67	30.00	33.33	10.00	9.11	9.56
Fungshow 12.50% W.P.	33.33	36.67	26.67	30.00	19.98	18.19	19.09	33.33	36.67	23.33	26.67	30.00	27.27	28.64
Master 25% E.C.	33.33	36.67	23.33	26.67	30.00	27.27	28.64	33.33	36.67	20.00	23.33	40.00	36.38	38.19
Monceren 25% W.P.	33.33	36.67	13.33	16.67	60.00	54.54	57.27	33.33	36.67	10.00	13.33	70.00	63.65	66.83
Montro 30% E.C.	33.33	36.67	16.67	20.00	49.98	45.46	47.72	33.33	36.67	13.33	16.67	60.00	54.54	57.27
Opus 12.50% S.C.	33.33	36.67	23.33	26.67	30.00	27.27	28.64	33.33	36.67	20.00	23.33	40.00	36.38	38.19
Penazole 10% E.C.	33.33	36.67	20.00	23.33	40.00	36.38	38.19	33.33	36.67	20.00	23.33	40.00	36.38	38.19
Punch 40% E.C.	33.33	36.67	23.33	26.67	30.00	27.27	28.64	33.33	36.67	23.33	26.67	30.00	27.27	28.64
Ridomil Gold/Plus 42.50% W.P.	33.33	36.67	30.00	33.33	10.00	9.11	9.56	33.33	36.67	26.67	30.00	19.98	18.19	19.09
Rizolex T 50% W.P.	33.33	36.67	30.00	33.33	10.00	9.11	9.56	33.33	36.67	26.67	30.00	19.98	18.19	19.09
Rovral 50% W.P.	33.33	36.67	26.67	30.00	19.98	18.19	19.09	33.33	36.67	26.67	30.00	19.98	18.19	19.09
Score 25% E.C.	33.33	36.67	26.67	30.00	19.98	18.19	19.09	33.33	36.67	23.33	26.67	30.00	27.27	28.64
Tecto 50% S.C.	33.33	36.67	26.67	30.00	19.98	18.19	19.09	33.33	36.67	23.33	26.67	30.00	27.27	28.64
Thiovat Jet 80% W.G.	33.33	36.67	30.00	33.33	10.00	9.11	9.56	33.33	36.67	30.00	33.33	10.00	9.11	9.56
Topsin-M 70% W.P.	33.33	36.67	16.67	20.00	49.98	45.46	47.72	33.33	36.67	13.33	16.67	60.00	54.54	57.27
Tilt 80% W.P.	33.33	36.67	20.00	23.33	40.00	36.38	38.19	33.33	36.67	20.00	23.33	40.00	36.38	38.19
Vectra 10% S.C.	33.33	36.67	23.33	26.67	30.00	27.27	28.64	33.33	36.67	26.67	30.00	19.98	18.19	19.09
Mean	33.33	36.67	21.94	25.28	34.16	31.07	-----	33.33	36.67	20.00	23.33	39.99	36.37	-----

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05
 Least Significant Difference (LSD) for methods of application= 0.3041
 Least Significant Difference (LSD) for tested fungicides= 1.0535

Table 4. Therapeutic effect of twenty four fungicides at recommended dose for each fungicide by two methods of application i.e. foliar spray and soil drench on disease severity (%) of guava die-back disease on transplants cultivar Banaty one year old, interval period of spray and soil drench 15 days for three times for each fungicide, two months after fungicides application in pot experiments under greenhouse conditions during 2015 and 2016 year.

Tested Fungicides	Methods of Application at recommended dose for each fungicide													
	Foliar Spray						Mean	Soil Drench						Mean
	Disease severity % before application		Disease severity % 60 days after application		Efficacy of fungicides %		Efficacy of fungicides %	Disease severity % before application		Disease severity % 60 days after application		Efficacy of fungicides %		Efficacy of fungicides %
	2015	2016	2015	2016	2015	2016	2015 and 2016	2015	2016	2015	2016	2015	2016	2015 and 2016
Alliette 80% W.P.	53.33	56.67	13.33	9.33	75.00	83.54	79.27	53.33	56.67	13.33	14.67	75.00	74.11	74.56
Amistar Top 325-32.5% E.C.	53.33	56.67	3.33	4.67	93.75	91.76	92.76	53.33	56.67	5.33	6.67	90.00	88.23	89.12
Bellis 38% W.G.	53.33	56.67	18.67	16.67	65.00	70.58	67.79	53.33	56.67	16.67	18.67	68.74	67.05	67.90
Camzin 50% W.P.	53.33	56.67	4.67	5.33	91.24	90.59	90.92	53.33	56.67	6.67	8.00	87.49	85.88	86.69
Copral 50% W.P.	53.33	56.67	50.00	50.67	6.24	10.59	8.42	53.33	56.67	43.33	42.67	18.75	24.70	21.73
Daconil 72% S.C.	53.33	56.67	15.33	14.67	71.25	74.11	72.68	53.33	56.67	15.33	16.67	71.25	70.58	70.92
Dithane M45 80% W.P.	53.33	56.67	16.67	18.67	68.74	67.05	67.90	53.33	56.67	18.67	20.00	65.00	64.71	64.86
Eminent 12.50% E.W.	53.33	56.67	51.33	52.67	3.75	7.06	5.41	53.33	56.67	45.33	44.00	15.00	22.36	18.68
Fungshow 12.50% W.P.	53.33	56.67	37.33	35.33	30.00	37.65	33.83	53.33	56.67	29.33	31.33	45.00	44.71	44.86
Master 25% E.C.	53.33	56.67	25.33	28.67	52.20	49.41	50.81	53.33	56.67	23.33	26.67	56.25	52.94	54.60
Monceren 25% W.P.	53.33	56.67	6.67	6.67	87.49	88.23	87.86	53.33	56.67	7.33	9.33	86.25	83.54	84.90
Montro 30% E.C.	53.33	56.67	9.33	8.67	82.50	84.70	83.60	53.33	56.67	8.67	10.67	83.74	81.17	82.46
Opus 12.50% S.C.	53.33	56.67	23.33	26.67	56.25	52.94	54.60	53.33	56.67	22.67	24.67	57.49	56.47	56.98
Penazole 10% E.C.	53.33	56.67	21.33	23.33	60.00	58.83	59.42	53.33	56.67	21.33	23.33	60.00	58.33	59.17
Punch 40% E.C.	53.33	56.67	29.33	31.33	45.00	44.71	44.86	53.33	56.67	25.33	28.67	52.20	49.41	50.81
Ridomil Gold/Plus 42.50% W.P.	53.33	56.67	49.33	48.67	7.50	14.12	10.81	53.33	56.67	41.33	40.67	22.50	28.23	25.37
Rizolex T 50% W.P.	53.33	56.67	47.33	45.33	11.25	20.00	15.63	53.33	56.67	39.33	40.67	26.25	28.23	27.24
Rovral 50% W.P.	53.33	56.67	45.33	43.33	15.00	23.54	19.27	53.33	56.67	37.33	36.67	30.00	35.29	32.65
Score 25% E.C.	53.33	56.67	39.33	37.33	26.25	34.12	30.19	53.33	56.67	31.33	32.67	41.25	42.35	41.80
Tecto 50% S.C.	53.33	56.67	41.33	40.67	22.50	28.23	25.37	53.33	56.67	33.33	34.67	37.50	38.82	38.16
Thiovat Jet 80% W.G.	53.33	56.67	52.00	54.00	2.49	4.71	3.60	53.33	56.67	47.33	45.33	11.25	20.00	15.63
Topsin-M 70% W.P.	53.33	56.67	11.33	10.67	78.75	81.17	79.96	53.33	56.67	11.33	12.67	78.75	77.64	78.20
Tilt 80% W.P.	53.33	56.67	19.33	21.33	63.75	62.36	63.06	53.33	56.67	19.33	21.33	63.75	62.36	63.06
Vectra 10% S.C.	53.33	56.67	33.33	32.67	37.50	42.35	39.93	53.33	56.67	27.33	30.67	48.75	45.88	47.32
Mean	53.33	56.67	27.69	27.81	48.06	50.93	-----	53.33	56.67	24.61	25.89	53.84	54.29	-----

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05

Least Significant Difference (LSD) for methods of application= 0.0712

Least Significant Difference (LSD) for tested fungicides= 0.2467

However, the other fungicides i.e., Vectra 10% S.C., Fungshow 12.50% W.P., Score 25% E.C., Tecto 50% S.C., Rovral 50% W.P., Rizolex T 50% W.P., Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. showed a least efficient in reducing disease incidence of *Botryodiplodia theobromae* Pat. (isolate code No., Q.K.4.).

2. Cultivar reaction

Information about cultivar reaction of guava transplants against *Botryodiplodia theobromae* is still scanty, ten coded isolates of *Botryodiplodia theobromae* Pat., i.e., B.W.1, A.B.2, D.S.3, Q.K. 4, A.A.5, B.N.6, K.Q.7, Q.S.8, D.B.9 and K.D.10., based on molecular diversity characters obtained from ten different geographic locations of five governorates in Egypt were tested for their pathogenic capabilities on four different guava cultivars i.e., Banaty, Malisy Ahmr, El-Mobaker and Gizy Ahmr

during periods after inoculation (15, 30, 45 and 60 days) in pots experiments under greenhouse conditions. **Data in Tables (5, 6, 7 and 8)** showed that, the reactions of Banaty, Malisy Ahmr, El-Mobaker and Gizy Ahmr cultivars to such isolates. It is cleared that all the ten isolates of *Botryodiplodia theobromae* Pat. according to their capability to infection of all different four guava cultivars homogenous transplant one year old were pathogenic with 15, 30, 45 and 60 days after inoculation with different degrees.

Data also, indicated that all four guava cultivar reactions were differed from cultivar to another, where Banaty was the most susceptible cultivar to the all tested *Botryodiplodia theobromae* isolates, while Malisy Ahmr and El-Mobaker were moderate susceptible cultivars. Meanwhile, Gizy Ahmr was the lowest susceptible cultivar. Also, the disease incidence and disease severity of the tested isolates were increased by increasing periods after inoculation (15, 30, 45 and 60 days).

Table 5. Cultivar reaction of guava transplants (Banaty c.v. 30 transplants for each treatment) to infection with ten isolates of *Botryodiplodia theobromae* Pat. at 15, 30, 45 and 60 days after inoculation under greenhouse conditions during 2016 year.

Tested <i>B. theobromae</i> Isolates	Days after Inoculation								Mean	
	15		30		45		60			
	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**
B.W.1	0.00	0.00	13.33	9.33	16.67	18.67	20.00	28.67	12.50	14.17
A.B.2	0.00	0.00	0.00	0.00	10.00	8.67	13.33	19.33	5.83	7.00
D.S.3	16.67	14.67	30.00	25.33	33.33	38.67	36.67	43.33	29.17	30.50
Q.K.4	20.00	17.33	33.33	27.33	36.67	43.33	40.00	46.67	32.50	33.66
A.A.5	0.00	0.00	20.00	14.67	23.33	30.00	26.67	37.33	17.50	20.50
B.N.6	0.00	0.00	0.00	0.00	6.67	5.33	10.00	15.33	4.17	5.16
K.Q.7	0.00	0.00	16.67	11.33	20.00	27.33	23.33	33.33	15.00	17.99
Q.S.8	13.33	12.67	26.67	23.33	30.00	36.67	33.33	41.33	25.83	28.50
D.B.9	0.00	0.00	0.00	0.00	13.33	16.67	16.67	25.33	7.50	10.50
K.D.10	0.00	0.00	23.33	18.00	26.67	33.33	30.00	39.33	20.00	22.66
Control (un-inoculated)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Mean	4.54	4.06	14.85	11.76	19.69	23.51	22.72	29.99	15.45	17.33

Whereas: * = Diseases incidence, **= Diseases severity
 Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05
 Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease incidence=1.3444
 Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease incidence=0.4924
 Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 15 days of inoculation= 1.3084
 Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 30 days of inoculation= 2.1447
 Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 45 days of inoculation= 2.0812
 Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 60 days of inoculation= 2.1973
 Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease severity= 0.2712
 Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease severity= 0.0988
 Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 15 days of inoculation= 0.1663
 Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 30 days of inoculation= 0.212
 Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 45 days of inoculation= 0.3035
 Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 60 days of inoculation= 0.6791

Table 6. Cultivar reaction of guava transplants (Malisy Ahmr c.v. 30 transplants for each treatment) to infection with ten isolates of *Botryodiplodia theobromae* Pat. at 15, 30, 45 and 60 days after inoculation under greenhouse conditions during 2016 year.

Tested <i>B. theobromae</i> Isolates	Days after Inoculation								Mean	
	15		30		45		60			
	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**
B.W.1	0.00	0.00	10.00	7.33	13.33	15.33	16.67	25.33	10.17	11.99
A.B.2	0.00	0.00	0.00	0.00	6.67	7.33	10.00	17.33	4.17	6.16
D.S.3	13.33	11.33	26.67	21.33	30.00	33.33	33.33	36.67	25.83	25.66
Q.K.4	16.67	15.33	30.00	24.67	33.33	38.67	36.67	41.33	29.17	30.00
A.A.5	0.00	0.00	16.67	12.67	20.00	25.33	23.33	31.33	15.00	17.33
B.N.6	0.00	0.00	0.00	0.00	3.33	4.67	6.67	13.33	2.50	4.50
K.Q.7	0.00	0.00	13.33	10.67	16.67	22.67	20.00	30.67	12.67	16.00
Q.S.8	10.00	10.67	23.33	20.67	26.67	33.33	30.00	36.67	22.67	25.34
D.B.9	0.00	0.00	0.00	0.00	10.00	13.33	13.33	19.33	5.83	8.16
K.D.10	0.00	0.00	20.00	17.33	23.33	30.67	26.67	34.67	17.50	20.67
Control (un-inoculated)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Mean	3.64	3.39	12.79	10.42	16.67	20.42	19.82	26.06	13.23	15.07

Whereas: * = Diseases incidence, ** = Diseases severity

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05

Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease incidence= 1.3945

Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease incidence= 0.4301

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 15 days of inoculation= 1.0605

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 30 days of inoculation= 2.0034

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 45 days of inoculation= 2.2173

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 60 days of inoculation= 2.0033

Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease severity=0.279

Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease severity= 0.0915

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 15 days of inoculation= 0.1414

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 30 days of inoculation= 0.2057

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 45 days of inoculation= 0.2282

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 60 days of inoculation= 0.6938

Table 7. Cultivar reaction of guava transplants (El-Mobaker c.v. 30 transplants for each treatment) to infect with ten isolates of *Botryodiplodia theobromae* Pat. at 15, 30, 45 and 60 days after inoculation under greenhouse conditions during 2016 year.

Tested <i>B. theobromae</i> Isolates	Days after Inoculation								Mean	
	15		30		45		60			
	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.	D.S.
B.W.1	0.00	0.00	6.67	5.33	10.00	12.67	13.33	22.67	7.50	10.17
A.B.2	0.00	0.00	0.00	0.00	3.33	5.33	6.67	11.33	2.50	4.16
D.S.3	10.00	9.33	23.33	18.67	26.67	30.67	30.00	33.33	22.50	23.00
Q.K.4	13.33	14.67	26.67	21.33	30.00	35.33	33.33	37.33	25.83	27.16
A.A.5	0.00	0.00	13.33	11.33	16.67	21.33	20.00	29.33	12.50	15.49
B.N.6	0.00	0.00	0.00	0.00	0.00	0.00	3.33	8.67	0.83	2.17
K.Q.7	0.00	0.00	10.00	8.67	13.33	14.67	16.67	27.33	10.00	12.67
Q.S.8	6.67	9.33	20.00	17.33	23.33	28.67	26.67	31.33	19.17	21.66
D.B.9	0.00	0.00	0.00	0.00	6.67	11.33	10.00	16.67	4.17	7.00
K.D.10	0.00	0.00	16.67	14.67	20.00	26.67	23.33	29.33	15.00	17.67
Control (un-inoculated)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Mean	2.73	3.03	10.61	8.85	13.64	16.97	16.67	22.48	10.90	12.83

Whereas: * = Diseases incidence, ** = Diseases severity

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05

Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease incidence= 1.3544

Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease incidence= 0.4156

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 15 days of inoculation= 1.0605

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 30 days of inoculation= 1.8987

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 45 days of inoculation= 2.0148

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 60 days of inoculation= 2.1022

Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease severity=0.1819

Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease severity= 0.0559

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 15 days of inoculation= 0.1007

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 30 days of inoculation= 0.199

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 45 days of inoculation= 0.2396

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 60 days of inoculation= 0.3625

Table 8. Cultivar reaction of guava transplants (Gizy Ahmr c.v. 30 transplants for each treatment) to infect with ten isolates of *Botryodiplodia theobromae* Pat. at 15, 30, 45 and 60 days after inoculation under greenhouse conditions during 2016 year.

Tested <i>B. theobromae</i> Isolates	Days after Inoculation								Mean	
	15		30		45		60			
	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**	D.I.*	D.S.**
B.W.1	0.00	0.00	3.33	4.67	6.67	10.67	10.00	18.67	5.00	8.50
A.B.2	0.00	0.00	0.00	0.00	3.33	4.67	3.33	8.67	1.66	3.33
D.S.3	6.67	7.33	20.00	15.33	23.33	27.33	26.67	31.33	19.17	20.33
Q.K.4	10.00	12.67	23.33	17.33	26.67	31.33	30.00	33.33	22.50	23.66
A.A.5	0.00	0.00	10.00	9.33	13.33	18.67	16.67	27.33	10.00	13.83
B.N.6	0.00	0.00	0.00	0.00	0.00	0.00	3.33	6.67	0.83	1.67
K.Q.7	0.00	0.00	6.67	6.67	10.00	11.33	13.33	23.33	7.50	10.33
Q.S.8	3.33	6.67	16.67	15.33	20.00	23.33	23.33	26.67	15.83	18.00
D.B.9	0.00	0.00	0.00	0.00	3.33	8.67	6.67	13.33	2.50	5.50
K.D.10	0.00	0.00	13.33	11.33	16.67	23.33	20.00	25.33	12.50	15.00
Control (un-inoculated)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Total	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----
Mean	1.82	2.42	8.48	7.27	11.21	14.48	13.94	19.51	8.86	10.92

Whereas: * = Diseases incidence, ** = Diseases severity

Statistical analysis system (SAS), Duncan's studentized range (HSD) test at alpha= 0.05

Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease incidence=1.3501

Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease incidence= 0.4009

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 15 days of inoculation= 0.9719

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 30 days of inoculation= 1.8507

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 45 days of inoculation= 2.003

Least Significant Difference (LSD) for days after inoculation as percentages for disease incidence after 60 days of inoculation= 2.0914

Least Significant Difference (LSD) for tested *B. theobromae* isolates as percentages for disease severity=0.1114

Least Significant Difference (LSD) for days after inoculation (15, 30, 45 and 60) as percentages for disease severity= 0.0518

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 15 days of inoculation= 0.0736

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 30 days of inoculation= 0.1328

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 45 days of inoculation= 0.157

Least Significant Difference (LSD) for days after inoculation as percentages for disease severity after 60 days of inoculation= 0.3055

DISCUSSION

Guava (*Pisidium guajava* L..) is considered one of the most important popular fruit crops to the Egyptian people. Guava die-back disease is an important and economic serious problem causing severe guava losses in both nurseries and orchards in Egypt (Baiuomy et al 2003 and Kamhawy, 2011) caused by *Botryodiplodia theobromae* Pat. (*Lasiodiplodia theobromae* Pat.). Several investigators have been recorded that *Botryodiplodia theobromae* Pat. is a soil borne phytopathogen fungus and attack guava trees (*Pisidium guajava* L.) and wide spread in the world (Rana, 1981, Adisa, 1985, Majumdar and Pathak, 1997, Junqueira et al 2001, Cardoso et al 2002, Pandit and Samajpati, 2002, Baiuomy, et al 2003, Pandit and Samajpati, 2005, Mishra and Sitansu, Pan, 2007, Bokhari et al 2008, Nunes et al 2008, Mishra et al 2009, Sitansu, Pan and Mishra, 2010, Kamhawy, 2011 and Asma Safdar, et al 2015).

Although the recent strategy of control plant diseases is depend upon minimizing of fungicidal utilization to avoid environmental pollution and keep human health, fungicides will be one of Integrated Pest Management (IPM) weapons, fungicides are still one of the most important means to control the causal pathogens of different diseases. The chemical control of guava die-back disease caused by *Botryodiplodia theobromae* Pat. by fungicides was concerned for a long time accounting for the great importance of this disease and high loss in yield in contaminated soils in different lands. The evaluation of twenty four systemic and non-systemic fungicides was tested in *in vitro* and in *in vivo* to evaluate their effect on the fungal growth, disease incidence and disease severity, respectively.

Data obtained showed that increasing fungicidal concentrations decreased the both of linear growth and amount growth of the tested *Botryodiplodia theobromae* isolate code No. Q.K.4. Although, effect of fungicides was differed, inhibition

of the linear growth and amount growth was at different concentrations of the tested fungicides. Amistar Top 325-32.5% E.C. gave the highest decrease of linear growth and amount of growth, followed by Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C., Topsin-M 70% W.P., Alliette 80% W.P. and Daconil 72% S.C., these set of fungicides exhibited the highest effective in reducing the rate of mycelial growth and amount of growth. Meanwhile, Bellis 38% W.G., followed by Dithane M45 80% W.P., Tilt 80% W.P., Penazole 10% E.C., Opus 12.50% S.C., Master 25% E.C. and Punch 40% E.C., these set of fungicides showed a moderate effective in reducing the rate of linear growth and amount of growth. However, the other fungicides i.e., Vectra 10% S.C., Fungshow 12.50% W.P., Score 25% E.C., Tecto 50% S.C., Rovral 50% W.P., Rizolex T 50% W.P., Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. exhibited a lowest effect in reducing of linear growth and amount growth of *Botryodiplodia theobromae* Pat. isolate code No., Q.K.4. The former results were in accordance relatively with **Li, Hong-Ye, 1995, Baiuomy et al 2003, Abd El-Aziz et al 2010, Korra et al 2014 and Safdar, Asma et al 2015.**

As such fungicides were applied under greenhouse conditions in two different trials, i.e. foliar spray and soil drench. The former method (soil drench) resulted in the highest efficiency on disease control. This result was related to the nature as soil borne fungus *Botryodiplodia theobromae* in its colonization to soil and high persistence as fruiting bodies (pycnidia) in it. The differences of fungicides efficiency may be due to the differences of their active ingredient and chemical groups.

Amistar Top 325-32.5% E.C. was the most efficient fungicide in reduce disease incidence and disease severity of *Botryodiplodia theobromae* Pat. (isolate code No., Q.K.4.), followed by Camzin 50% W.P., Monceren 25% W.P., Montro 30% E.C., Topsin-M 70% W.P., Alliette 80% W.P. and Daconil 72% S.C., Bellis 38% W.G., Dithane M45 80% W.P., followed by Tilt 80% W.P., Penazole 10% E.C., Opus 12.50% S.C., Master 25% E.C., Punch 40% E.C., these set of fungicides showed a moderate efficient in reducing the disease incidence and disease severity. However, other fungicides i.e., Vectra 10% S.C., Fungshow 12.50% W.P., Score 25% E.C., Tecto 50% S.C., Rovral 50% W.P., Rizolex T 50% W.P., Ridomil Gold/Plus 42.50% W.P., Copral 50% W.P., Eminent 12.50% E.W. and Thiovat Jet 80% W.G. showed a least

efficient in reducing the disease incidence and disease severity of *Botryodiplodia theobromae* Pat. (isolate code No., Q.K.4.). The former results were also similar like those obtained by **Ansar, 1994, Dwivedi and Dwivedi, 1994, Majumdar and Pathak, 1997, Banik et al 1998, Baiuomy et al 2003, Khanzada et al 2005, Bokhari et al 2008, Abd El-Aziz et al 2010, Kamhawy, 2011, Korra, et al 2014 and Asma Safdar et al 2015.**

Information about cultivar reaction of guava transplants against *Botryodiplodia theobromae* is still scanty. Cultivar reaction of guava trees is one of the most important factors affecting percentage of disease incidence and disease severity of guava die-back disease, were evaluated. Obtained data showed that all tested cultivars were susceptible but they revealed different reaction to each *Botryodiplodia theobromae* Pat. isolates. There was a clear rating for the 4 cultivars of guava according to their response to infection with *Botryodiplodia theobromae* Pat. isolates. This rating figured that Banaty cultivar was most susceptible, meanwhile Malisy Ahmar and El-mobaker were moderate susceptible, Gizy Ahmr was lowest susceptible as they recorded the initial disease incidence and disease severity after 60 days after soil infestation. The variation between reactions of guava cultivars may be due to the differences in their morphological, anatomical structures and chemical components, contrary reaction that occurred against *Botryodiplodia theobromae* Pat. isolates infection.

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المكافحة الكيميائية للموت الرجعي على الجوافه و رد فعل أصناف الجوافه للمرض في مصر

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في حين أظهرت المبيدات Ridomil Gold/Plus Eminent ،Copral 50% W.P. ،42.50% W.P. Thiovat Jet 80% W.G. ،12.50% E.W. في إختزال نسبة الإصابة وشدها. وجد أن طريقة التطبيق بإضافة المبيدات مع ماء الري soil drench أعلى فاعلية في تقليل نسبة المرض ونسبة شدة المرض تليها طريقة الرش foliar spray نسبياً. وجد أن شتلات الجوافه صنف بناتي هي أكثر الأصناف حساسية للإصابة بجميع عزلات فطر *B. theobromae* المختبرة، بينما كان صنف جيزي أحمر هو أقل الأصناف حساسية للإصابة بنفس العزلات المختبرة. لاتزال الدراسات السابقة عن رد فعل أصناف الجوافه ضد فطر *B. theobromae* ل نادرة.

الكلمات الدالة: جوافه، المكافحة الكيميائية، رد فعل أصناف الجوافه، الموت الرجعي على الجوافه، *Botryodiplodia theobromae* Pat. ،*Psidium guajava* L.

الموجز

يعتبر مرض الموت الرجعي على الجوافه المتسبب عن الفطر *Botryodiplodia theobromae* Pat. واحداً من الأمراض الهامة والإقتصادية على الجوافه. وقد حقق مبيد Amistar Top 325-32.5% E.C. أعلى إختزال في النمو الطولي وكمية النمو للفطر *B. theobromae* عزلة Q.K.4، يتبعه المبيد Camzin Montro ،Monceren 25% W.P. ،50% W.P. Ridomil 30% E.C. في حين أظهرت المبيدات Copral 50% W.P. ،Gold/Plus 42.50% W.P. Thiovat Jet 80% ،Eminent 12.50% E.W. W.G. أقل كفاءة في إختزال النمو الطولي وكمية النمو للفطر. وكان مبيد Amistar Top 325-32.5% E.C. هو أكثر المبيدات كفاءة في إختزال نسبة الإصابة وشدها للفطر *B. theobromae* عزلة Q.K.4، يتبعه المبيد Monceren 25% ،Camzin 50% W.P. ،W.P. Montro 30% E.C. ،W.P.