



Full Length Article

Chemical and Biological Control of *Dracaena marginata* Leaf Spots in Northern Egypt

A. Hilal¹, Eman El-Argawy², A. El. Korany² and Taghreed Fekry¹

¹Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt

²Plant Pathology Dept., Faculty of Agriculture, Damanhour University, Egypt

*For correspondence: dreman_elargawy@yahoo.com

Abstract

Six common fungicides, Dithane M45, Kocide 2000, Ridomil Gold Plus, Score, Equation Pro and Kemazed were tested for controlling the dracaena leaf spot fungi. All the tested fungicides significantly decreased the *in vitro* growth of the tested dracaena leaf spot fungi. Percentage of inhibition on colony diameter ranged between 49.64% with Dithane M45 to 82.18% with Score which was followed by Kemazed (69.15%). However, inhibition of spore germination was the highest (72.8%) by Dithane M45, while inhibition of germ tube reached 89.91% with Kemazed. Meanwhile, in the *in vivo* tests, treatment with Kemazed decreased disease incidence of leaf spots on potted plants to 16.67% compared to 100% for the untreated inoculated control. On the other hand, the bacterial and fungal biocontrol agents tested proved to be effective against the dracaena leaf spot fungi. The *in vitro* assays conducted showed that the bacterial biocontrol agents namely, *Stenotrophomonas maltophilia*, *Pseudomonas ultimum*, *P. fluorescens*, *P. putida*, *P. aeruginosa*, *Bacillus subtilis*, *B. megaterium*, significantly decreased colony growth diameter (76.84–45.69%) of dracaena leaf spot fungi as well as spore germination (48.80–23.20%) and length of germ tube (88.77–65.65%) of the germinated spores compared to untreated control. Treatment with *S. maltophilia* showed the most consistent highest inhibition effect, followed by *P. ultimum*, *B. subtilis*, and *B. megaterium*, respectively. The *in vivo* tests of the most effective bacterial bioagents supported the *in vitro* results. Treatment with *S. maltophilia* showed the highest effect as it decreased disease incidence and disease index to 29.33% and 7.6%, respectively in comparison with 100% and 83.6% for the untreated inoculated control in the potted dracaena plants (cv. Bicolor). Meanwhile, the *in vitro* tests of the three *Trichoderma* spp., i.e. *T. harzianum*, *T. hamatum* and *T. album* showed significant inhibition effect on colony growth diameter of the tested dracaena leaf spot fungi in which *T. harzianum* showed the highest inhibition effect (75.02%), while *T. album* was most effective to inhibit spore germination (48%) and germ tube (90.21%) of the germinated spores. The *in vivo* tests of the fungal biocontrol agents supported the *in vitro* results. *T. album* (Bio-Zeid) showed the highest effect and decreased disease incidence and disease index on potted plants to 19.99% and 4.39%, respectively compared with 100% and 92.8% for the untreated inoculated control. © 2016 Friends Science Publishers

Keywords: Dracaena; Leaf spots; Chemical; Biological control

Introduction

Dracaena is one of the most popular and widespread ornamental plants all over the world including Egypt. It is becoming an economically important part of the horticultural industry. Unfortunately, dracaena plants are attacked by several pathogens causing leaf spot diseases. Fungal leaf spots have become increasingly serious and such diseases can lead to heavy defoliation that causes serious losses in the nursery production (Hilal *et al.*, 2000).

Chemical protection is a primary strategy in the control of plant diseases. Using fungicides is the most common way to protect plants from diseases and this is a widely accepted in the absence of resistant source. However, application of fungicides is not always efficient. In addition, the hazards and the environmental pollution

caused by agrochemicals used for plant diseases control is a worldwide problem. Bio and natural disease control agents might have less risk to the other living beings and also to the environment. However, little literature is available concerning the use of bacterial biocontrol agents against leaf spot diseases of dracaena.

Trichoderma is a fast growing fungus, strong spore producer, source of cell wall degrading enzymes and an important antibiotic producer. Application of *Trichoderma* spp. as biocontrol agents can also, bring substantial changes in plant metabolism to promote plant growth, increase nutrient availability, improve crop production and enhance disease resistance (Harman *et al.*, 2004).

In Egypt, little information exists on the chemical and biological control of dracaena fungal leaf spots in Egypt.

Therefore, the present study was conducted to determine the efficacy of certain chemical fungicides *in vitro* and *in vivo* for controlling dracaena leaf spot fungi, and the efficacy of certain bacterial and fungal biocontrol agents against dracaena leaf spot fungi.

Materials and Methods

Chemical Control

In vitro Effect of Fungicides on Colony Growth of Dracaena Leaf Spot Fungi

The tested fungi: Five of the most pathogenic leaf spot fungi of dracaena namely *Botryodiplodia theobromae*, *Fusarium graminearum*, *F. proliferatum*, *F. verticillioides* and *Gibellulopsis nigrescens* were obtained from fungal collection established by the authors in Faculty of Agriculture, Damenhour University.

The tested fungicides: Six fungicides commonly used to control leaf spots were tested *in vitro* in the present study. Fungicides were used as diluted solutions or suspension in water at their recommended doses. Fungicides name (commercial and/or common), formulation, rate of application and manufacture source are listed in Table (1).

The recommended rate of each fungicide was added to sterilized PDA medium before solidification to obtain the desired concentrations. Precisely 20 mL of un-amended (control) or amended PDA for each treatment was poured into each of five Petri dishes. After solidification of the medium, each dish was inoculated centrally with a mycelial disc (5 mm diameter) taken from the margins of actively growing mycelium from PDA cultures of each tested fungal isolate. Plates were incubated at $26 \pm 2^\circ\text{C}$. The average of colony diameter was measured when the untreated control mycelia had just covered the whole plate.

Percentage of inhibition (efficacy): The percentage of inhibition (efficacy) for each fungicide in Petri dishes was calculated according to the following formula (Amer, 1995):

$$\text{Inhibition \% (Efficacy)} = (A - B / A) \times 100$$

Where: A =Diameter of fungal colony in un-amended medium (control).

B =Diameter of fungal colony in amended medium.

Effect of Fungicides on Spore Germination and Germ Tube Length

Spore suspension of each tested fungus was prepared by pouring 20 mL of sterile distilled water on fungal culture in each Petri dish with gentle rubbing of culture surface to harvest spores. Then fungicides were added to spore suspension and fungicide concentrations were adjusted. Three drops of 0.1 mL of each tested fungicide containing spore suspension were put on clean glass of germination

slide, other slides with drops of sterilized distilled water were used as control and they were incubated at $26 \pm 2^\circ\text{C}$, for 6 h. Then slides were examined under light microscope to count the germinated and ungerminated spores with a haemocytometer. Length of germ tube of the germinated spores was measured, using ocular micrometer which was calibrated by a micrometer slide (Shama and Massoud, 2001). The values were converted to microns using the conversion multiplier established when the microscope was calibrated. The length of at least 10 germinated spores was measured and recorded.

In Vivo Effect of Fungicides on Leaf Spot Incidence

The *in vitro* most effective fungicides for controlling dracaena leaf spot fungi were tested *in vivo* under greenhouse conditions on *Dracaena marginata* (cv. Bicolor).

Fungi and Inocula

The five most pathogenic dracaena leaf spots fungi previously tested *in vitro* were used for the *in vivo* test. Pure cultures of the tested fungi were grown separately on 100 mL autoclaved potato dextrose liquid (PDL) in 250 mL conical flasks and incubated at $26 \pm 2^\circ\text{C}$ for 15–20 days to obtain sufficient mycelial growth. The fungal mycelium of each pathogen was collected and blended with 100 mL sterilized distilled water in a blender for one minute. Concentration of the fungal propagate suspensions (spores or mycelia fragments of the sterile fungus) was determined (El-Abd, 2002). The spore concentration was adjusted using a haemocytometer and concentration of inocula were 18×10^6 spores/mL for *Botryodiplodia theobromae*, 09×10^6 for *F. graminearum*, 05×10^6 for *F. proliferatum*, 07×10^6 for *F. verticillioides* and 04×10^6 for *Gibellulopsis nigrescens* according to pre-experiment conducted by the authors.

Healthy Dracaena Plants

The used dracaena plants (cv. Bicolor) were obtained from private farm of Bissar "Violette" nurseries at Ras El-Soda, Alexandria, where they were imported from Holland. They were used as seedlings of 11 months old.

Soil

Soil used for filling the pots in pathogenicity tests consisted of a mixture of sand, peat moss, loam and perlite at volumes rates of 4:3:2:1, respectively. Sand and loam were sterilized by autoclaving at 121°C for 30–60 mins during a period of sequenced three days. Pots of 25 cm in diameter were used through the greenhouse trials for planting (1 plant/pot). Pots were sterilized by immersing them in 5% formalin solution for 15 min and left to dry and to get rid of formalin odor.

The Detached Leaves Assay

Apparently healthy leaves (No. 6–8) of dracaena (cv. Bicolor) were detached, sterilized with 70% ethanol and placed in separate plastic bags with moistened tissue paper to create humid conditions. Then the leaves were inoculated with the appropriate concentration of each fungal spore suspension, five replicates for each treatment. The leaves were then incubated at room temperature $23 \pm 5^\circ\text{C}$ and daily examined over two weeks of incubation for full development of lesions. Then disease incidence and disease index were assessed (Jackson *et al.*, 2008; Boydom *et al.*, 2013).

Potted Plants Assay

This experiment was conducted under greenhouse conditions at $23\text{--}26^\circ\text{C}$ during the day and at $17\text{--}19^\circ\text{C}$ at night using dracaena plants (cv. Bicolor) of 11 month old seedlings. Fungicides were sprayed, 24 h after fungal inoculation (curative treatment). Five pots (replicates), each containing one plant were used for each treatment. Untreated plants (control) were sprayed with distilled water. All plants were covered with polyethylene bag for 48 h to maintain high relative humidity. Plants were kept in greenhouse under daily observation for 14 days. Measurements of disease incidence and index (severity) were carried out.

Disease Incidence

Disease incidence was determined as the percentage of spotted plants (or leaves) relative to the total number of the examined ones according to Morang *et al.* (2012) as follows:

$$\text{Percentage of infection} = \frac{\text{No. of infected plants(or leaves)}}{\text{Total No. of examined plants(or leaves)}} \times 100$$

Disease Index

Each plant (or plant leaf) showing symptoms of the leaf spots was examined with care and a rating was given according to Abd El-Zaher *et al.* (2005), applying the following numerical ratings:

- 0= No disease symptoms (apparently immune).
- 1= Few scattered lesions covering about 1–25% of the leaf (mild infection).
- 2= Spots covering about 25–50% of the leaf (moderate infection).
- 3= Spots coalescing and covering about 50–75% of the leaf (high infection).
- 4= Infection with coalescing lesion covering more than 75% of the leaf (very severe infection).
- 5=100% of leaf covered with spots (extremely severe infection).

Disease index was estimated according to the equation suggested by Abd El-Zaher *et al.*, (2005).

$$\text{Disease index (\%)} = \frac{\Sigma (\text{nr})}{5 \text{ N}} \times 100$$

Where: $\Sigma (\text{nr})$ = Total [number of plants (or leaves) under scale degree \times scale degree].

5N = Degree of freedom of scale degrees (5) \times total number of plants (or leaves) examined.

Biological Control

The tests were conducted using certain bacterial and fungal biocontrol agents (Table 2) *in vitro* and *in vivo* to evaluate their efficacy against the most pathogenic fungi of the dracaena leaf spots previously tested under chemical control. The fungal isolates tested were obtained from the authors fungal collection in Plant Pathology Dept. Faculty of Agriculture, Damanhour University. The used biocontrol agents were obtained from Plant Pathology Research Institute, Agric. Res. Center, Giza, Egypt.

Efficacy of Bacterial Biocontrol Agents for Controlling Dracaena Leaf Spot Fungi

***In vitro* effect on colony growth:** The bacterial biocontrol agents were grown in nutrient agar medium in 250 mL flasks containing 50 mL of the medium. Seventy two hours after incubation, 10 mL of physiologic solution was added to each flask with shaking to harvest bacteria and then bacterial concentrations were adjusted. Bacterial suspension was added to warm sterilized PDA medium at the rate of 10%. Approximately 20 mL of non-amended (control) or amended PDA for each treatment were poured into each of five Petri dishes. Each dish was inoculated centrally by a mycelial disk (5 mm diameter) of pathogenic fungi taken from the margins of actively growing mycelium of PDA cultures. Plates were incubated at $26 \pm 2^\circ\text{C}$.

Fungal growth diameters were measured after fungal growth covered completed any one of the untreated plates and percentage of inhibition (efficacy) of bio-agents, as reduction in colony diameter, was calculated as previously mentioned.

***In vitro* effect on spore germination and germ tube length:** The experiment was conducted as previously mentioned under effect of the fungicides on spore germination and germ tube length.

***In vivo* effect of bacterial biocontrol agents on dracaena leaf spot disease:** The most effective bacterial bio-agents, *in vitro*, were tested under greenhouse conditions for controlling fungal leaf spot on *D. marginata*. The experiment was conducted as previously mentioned under the *in vivo* effect of fungicide and the bacterial bio-agents were prepared as mentioned under the *in vitro* test of bacterial biocontrol agents.

Efficacy of Fungal Biocontrol Agents for Controlling *Dracaena* Leaf Spot Fungi

In vitro effect on colony growth: Bio-control agent cultures of *Trichoderma* spp. were prepared from Plant-guard® and Bio Zeid® by adding one ml of stock solution in water of each to pure PDA medium plates before solidification with shaking. All plates were incubated at 26±2°C for 5 days.

Two plugs (5-mm diam.) were taken, one from the advancing margins of seven day old cultures of the tested fungi and the other from the biocontrol agents *Trichoderma* spp. (Table 2). The two plugs were placed at 1.5 cm from the edge of the plate at opposite sides (Mueller et al., 1985).

Plates were then incubated at 26 ± 2°C for 7 days. Control plates were inoculated with plugs of PDA free of the bio-agents. Five replicates were used for each treatment. Diameter of the developed tested fungal colonies was measured and percentage of inhibition (efficacy) was calculated as previously mentioned under the effect of fungicides.

In vitro effect on spore germination and germ tube: The experiment was conducted as previously mentioned under the effect of fungicides on spore germination.

In vivo tests: The most effective *Trichoderma* biocontrol agents which were tested firstly *in vitro* on dracaena leaf spot fungi were tested also under greenhouse conditions on *D. marginata* "cv. Bicolor". The test was conducted as previously mentioned under the *in vivo* effect of fungicide.

Statistical Analysis

The obtained data were statistically analyzed according to Gomez and Gomez (1984) using SAS (Statistical Analysis System) version 9.2, 2001. Before carrying out analysis of variance (ANOVA), the percentages were transformed to angular transformation according to Steel and Torrie (1984). Means of treatments were compared using the least significant difference test at 5% level of probability.

Results

Chemical Control

In vitro effect of fungicides on colony growth of dracaena leaf spot fungi: The tested fungicides obviously suppressed the colony diameter of all the tested leaf spot fungi (Fig. 1). Score fungicide followed by Kemazed proved to be the most effective. Their means of inhibition of colony diameter were 82.18% and 69.15%, respectively (Table 3). Meantime, Kocide 2000 exhibited 59.70% of inhibition followed by Ridomil Gold, Equation Pro and Dithane M45 where 57.69%, 55.24% and 49.64% of inhibition were recorded, respectively (Table 3).

In vitro effect of fungicides on spore germination and germ tube of dracaena leaf spot fungi: All fungicides

tested significantly inhibited spore germination of the tested dracaena leaf spot fungi (Table 4). Overall means of percentage of inhibition ranged between 55.6% with Kemazed to 72.8–72.4% with each of Dithane M45 and Kocide 2000 (Table 4). Meanwhile, the fungicides significantly inhibited growth of germ tube of the germinated spores of the different fungi, where Kemazed and Score exhibited the highest inhibition effect being 89.91% and 88.25%, respectively. This was followed by Ridomil Gold, Equation Pro, Kocide 2000 and Dithane M45 where inhibitions were 84.26, 81.32, 77.45 and 74.89%, respectively (Table 4).

In vivo Effect of Fungicide on *Dracaena* Leaf Spot Disease

Effect on detached leaves: Data presented in Table (5) and illustrated in Fig. (2) showed that foliar application with the tested fungicide as curative treatment significantly inhibited disease incidence and disease index of dracaena leaf spots caused by the inoculation with five pathogenic leaf spot fungi of dracaena. Kemazed was the most effective and decreased mean percentage of infection to 31.11% compared to 100% for the infected untreated control. This was followed by Score, where percentage of infection decreased to 48.89%, while Equation Pro exhibited the lowest effect. Similar trend was revealed for the disease index (Table 5; Fig. 2). However, Kemazed exhibited more pronounced effect as it decreased the leaf spot disease index 7.56% compared to 92.8% for the inoculated untreated control. This was followed by Score with 12.44% disease index while Equation Pro exhibited the lowest effect being 27.15% disease index (Table 5; Fig. 2).

Effect on potted plants: Data in Table (6) showed that spraying Kemazed, which was most effective in the detached leaf assay, to dracaena potted plants (cv. Bicolor) previously inoculated with dracaena leaf spot fungi, significantly decreased mean percentage of infection to 16.67% compared to 100% for the untreated inoculated control. Meantime, the developed mean disease index decreased to 4.13% compared to 83.6% for the untreated inoculated control.

Biological Control

Efficacy of bacterial biocontrol agents – in vitro effect on colony diameter of dracaena leaf spot fungi: Incorporating the biocontrol bacterial bio agents into PDA obviously inhibited colony growth of the tested leaf spot fungi (Fig. 3). Meanwhile, data in Table (7) showed that *Stenotrophomonas maltophilia* exhibited the highest mean inhibition value (76.84%). This was followed by *Pseudomonas ultimum* (60.18%) and *Bacillus subtilis* (60.53%) with no significant variation between the last two bacterial bioagents (Table 7). This was followed by *P. aeruginosa*, *B. megaterium* and *P. fluorescens*, where

Table 1: The tested fungicides, common name, source and rate of application

Commercial name and formulation	Dithane M45® 80% WP	Kocide 2000® 53.8% WP	Ridomil Gold plus® 42.58% WP	Score® 25% E.C	Equation Pro® 52.5% WG	Kemazid® 50% WP
Recommended rate/100 L	20 g	180 g	200 g	50 ml	40 g	75 g
Common name	Mancozeb	Cu-hydroxide	Copper oxychloride	Difenocon + Famoxadone	+ Cymoxanil + Famoxadone	+ Carbendazim
Source (Manufacture)	Egyptian grow	Sentic	Syngenta	Novartis	Syngenta	Kafr EL-Zyat Co. for pesticides (Egypt)

Table 2: List of biocontrol agents and their rate of application used in the present study

Biocontrol Agents	Scientific name (preparation)	Commercial name (Common name)
Bacterial Agents	<i>Bacillus megaterium</i> ; Powder (25*10 ⁶ cell/g) and used at concentration of 250 g/100 L water	Bio Arc®
	<i>B. subtilis</i> ; Powder (30 * 10 ⁶ cell/g) and used at concentration of 4 g/L water	Rhizo-N®
	<i>B. cereus</i> (28*10 ⁶)	isolate
	<i>B. thuringiensis</i> (28*10 ⁶)	isolate
	<i>Stenotrophomonas maltophilia</i> (40*10 ⁶)	isolate
	<i>Pseudomonas fluorescens</i> (33*10 ⁶)	isolate
	<i>P. putida</i> (37*10 ⁶)	isolate
Fungal Agents	<i>P. ultimum</i> (36*10 ⁶)	isolate
	<i>P. aeruginosa</i> (36*10 ⁶)	isolate
	<i>Trichoderma album</i> ; Powder (25 * 10 ⁶ spores/g) and used at concentration of 250 g/100 L water	Bio Zeid®
	<i>T. harzianum</i> ; (8 * 10 ⁷ spores/ml) and used at concentration of 6 g /100 L /water	Plant Guard®
	<i>T. hamatum</i> (8 * 10 ⁶)	Isolate

Table 3: The *in vitro* colony diameter of five pathogenic dracaena leaf spot fungi on PDA amended with six different fungicides

Leaf spot fungi	Fungicides	<i>Botryodiplodia theobromae</i>	<i>F. graminearum</i>	<i>F. proliferatum</i>	<i>F. verticillioides</i>	<i>Gibellulopsis nigrescens</i>	Means	
							Colony diameter	Inhibition (efficacy) %
Dithane M45	6.60	2.70	3.76	2.98	6.62	4.53	49.64 F	
Kocide 2000	5.58	1.56	4.46	4.28	2.24	3.62	59.73 C	
Ridomil Gold	7.20	1.50	3.80	4.52	2.02	3.80	57.69 D	
Equation Pro	5.32	2.38	4.20	3.66	4.58	4.03	55.24 E	
Kemazed	2.22	1.84	3.50	2.66	3.66	2.78	69.15 B	
Score	1.50	1.64	1.72	1.66	1.50	1.60	82.18 A	
Control	9.00	9.00	9.00	9.00	9.00	9.00	0.00 G	

Values are means of five replicates, Means followed by different letter are significantly different at p=0.05; Percentage of colony growth inhibition (efficacy) = ((A-B)/A) × 100; Where, A= diameter of non-amended media, B= diameter for amended media; Control = no fungicide treatment

Table 4: Percentage of spore germination, length of germ tube and inhibition of five pathogenic dracaena leaf spot fungi caused by the tested six fungicides

Fungi	Spore Germination (%)					Mean inhibition (%)	Germ tube length (µm)					Mean inhibition (%)
	<i>B. theobromae</i>	<i>F. graminearum</i>	<i>F. proliferatum</i>	<i>F. verticillioides</i>	<i>G. nigrescens</i>		<i>B. theobromae</i>	<i>F. graminearum</i>	<i>F. proliferatum</i>	<i>F. verticillioides</i>	<i>G. nigrescens</i>	
Dithane M45	26.00	20.00	26.00	34.00	30.00	72.80A	0.27	0.19	0.25	0.17	0.18	74.89E
Kocide 2000	26.00	24.00	24.00	24.00	40.00	72.40A	0.21	0.18	0.25	0.15	0.16	77.45D
Ridomil Gold	22.00	24.00	32.00	36.00	44.00	68.40B	0.13	0.15	0.16	0.11	0.12	84.26B
Equation Pro	38.00	30.00	30.00	34.00	30.00	67.60B	0.11	0.14	0.18	0.14	0.26	81.32C
Kemazed	36.00	38.00	56.00	52.00	40.00	55.60C	0.10	0.10	0.05	0.07	0.14	89.91A
Score	52.00	28.00	50.00	32.00	46.00	58.40C	0.06	0.15	0.08	0.15	0.09	88.25A
Control	100.00	100.00	100.00	100.00	100.00	0.00D	0.75	1.064	0.774	0.938	0.906	0.00F

Values are average of ten readings for each replicate, Control =no fungicide treatment; Inhibition length of germ tube % = (Control of germ tube length -Treatment of germ tube length/Control of germ tube length) × 100; Means followed by different letter for each parameter, are significantly different at p = 0.05

inhibitions were 57.29%, 56.89% and 55.38%, respectively. Rest of the bacterial bioagents, *i.e.* *B. thuringiensis*, *P. putida* and *B. cereus* exhibited inhibition less than 50%.

***In vitro* effect on spore germination and germ tube:** All bacterial bioagents tested significantly decreased spore

germination of the tested fungal isolates (Table 8). Percentage of inhibition of spore germination ranged between 48.8% with *S. maltophilia* to 44.0% with *B. subtilis* and 36% with *P. ultimum*. The bioagents *i.e.*, *P. putida*, *B. megaterium*, *P. aeruginosa*, came next with inhibition effect

ranged between 29.6% and 28.0%, while *P. fluorescens* exhibited the lowest effect (23.20%). Bacterial bioagents also significantly decreased germ tube length (µm) of the germinated spores of the tested dracaena leaf spot fungi (Table 8). Percentage of inhibition ranged between 88.77% with *S. maltophilia* to 67.15% with each of *B. cereus* and *B. thuringiensis* (Table 8). However, *B. subtilis*, *P. ultimum*, *B. megaterium*, *P. aeruginosa*, and *P. putida* showed percentage of inhibition of 84.97%, 80.45%, 73.88%, 73.0% and 72.15%, respectively (Table 8).

In vivo Effect of Bacterial Biocontrol Agents on Dracaena Leaf Spot Disease

Effect on detached leaves: Data presented in Table (9) illustrated in Fig. 4 showed that treatment with the tested bacterial bioagents (curative) to detached

dracaena (cv. Bicolor) leaves inoculated with dracaena leaf spots fungi, significantly decreased disease incidence and disease index of leaf spots developed by the inoculated fungi. Meanwhile, *S. maltophilia* showed the highest effect as decreased percentage of infection to 53.33% compared to 100% for the inoculated untreated control. This was followed by *B. megaterium*, which decreased percentage of infection to 64.44%. The other bacterial bioagents i.e., *P. ultimum* and *B. subtilis*, decreased percentage of infection to 73.33% and 75.55%, respectively compared to 100% for the untreated infected control. Similar trend was revealed for the disease index as *S. maltophilia* significantly decreased the leaf spot disease index to 16.0% compared to 92.8% for the inoculated untreated control (Table 9; Fig. 4). This was followed by *B. megaterium* where disease index was 18.67%, while the other two bacterial

Table 5: Percentage of infection and disease index of leaf spots developed on dracaena (cv. Bicolor) inoculated with five dracaena leaf spot fungi and sprayed with certain fungicides, using the detached leaves technique

Dracaena leaf spot fungi	Percentage of infection (%)				Disease index (%)			
	Infected and treated			Infected un treated	Infected and treated			Infected un treated
	Equation Pro	Kemazed	Score		Equation Pro	Kemazed	Score	
<i>Botryodiplodia theobromae</i>	88.89	44.44	66.70	100.00	26.67	13.33	17.80	90.00
<i>Fusarium graminearum</i>	88.89	33.33	44.44	100.00	35.56	8.89	13.30	96.00
<i>Fusarium proliferatum</i>	77.78	11.11	44.44	100.00	20.00	2.22	11.10	92.00
<i>Fusarium verticillioides</i>	88.89	44.44	55.56	100.00	33.33	8.89	13.30	94.00
<i>Gibellulopsis nigrescens</i>	77.78	22.22	33.33	100.00	20.20	4.44	6.70	92.00
Mean	84.45 B	31.11 D	48.90 C	100 A	27.15 B	7.56 D	12.44 C	92.80 A

Means for each parameter followed by different letter, are significantly different at p = 0.05

Table 6: Disease incidence and disease index of leaf spots developed on potted dracaena plants (cv. Bicolor) inoculated with dracaena leaf spot fungi and sprayed with kemazed fungicide under greenhouse conditions

Dracaena leaf spot fungi	Percentage of infection (%)			Disease index (%)		
	Infected and treated	Infected un treated	Inhibition (%)	Infected and treated	Infected un treated	Inhibition (%)
<i>Botryodiplodia theobromae</i>	23.33	100.00	76.67 E	6.00	82.00	92.68 B
<i>Fusarium graminearum</i>	20.00	100.00	80.00 D	5.33	90.00	94.07 AB
<i>Fusarium proliferatum</i>	10.00	100.00	90.00 A	2.00	80.00	97.50 A
<i>Fusarium verticillioides</i>	16.67	100.00	83.33 C	4.67	84.00	94.44 AB
<i>Gibellulopsis nigrescens</i>	13.33	100.00	86.67 B	2.67	82.00	96.70 A
Mean	16.67 B	100 A	83.33	4.13 B	83.60 A	

Means, for each parameter, followed by different letter are significantly different at p = 0.05

Table 7: The *in vitro* colony diameter and percentage of inhibition of five dracaena leaf spot fungi on PDA amended with bacterial biocontrol agents

Leaf spot fungi	Bacterial bio agents	<i>Botryodiplodia theobromae</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium verticillioides</i>	<i>Gibellulopsis nigrescens</i>	Mean	
							Colony diameter (cm)	Inhibition (%)
	<i>Stenotrophomonas maltophilia</i>	2.60	2.78	1.92	1.60	1.52	2.08	76.84 A
	<i>Pseudomonas ultimum</i>	8.56	3.34	2.04	2.42	1.56	3.58	60.18 B
	<i>P. fluorescens</i>	8.86	4.02	2.40	3.30	1.50	4.20	55.38 D
	<i>P. putida</i>	8.18	7.40	3.54	3.36	1.56	4.81	46.58 F
	<i>P. aeruginosa</i>	8.68	3.30	3.46	2.26	1.52	3.84	57.29 C
	<i>Bacillus subtilis</i>	7.72	2.86	2.82	2.84	1.52	3.55	60.53 B
	<i>B. megaterium</i>	8.38	3.32	2.96	2.96	1.78	3.88	56.89 C
	<i>B. cereus</i>	8.74	7.08	1.94	1.92	4.76	4.89	45.69 F
	<i>B. thuringiensis</i>	8.86	7.48	1.82	1.78	2.82	4.55	49.42 E
	Control	9.00	9.00	9.00	9.00	9.00	9.00	0.00 G

Values are means of 5 replicates. Control = no bacterial treatment; Inhibition % = ((A-B)/A) × 100 where A = Colony diameter of the control (cm); B = colony diameter of treatment (cm); Means followed by different letter, are significantly different at p= 0.05

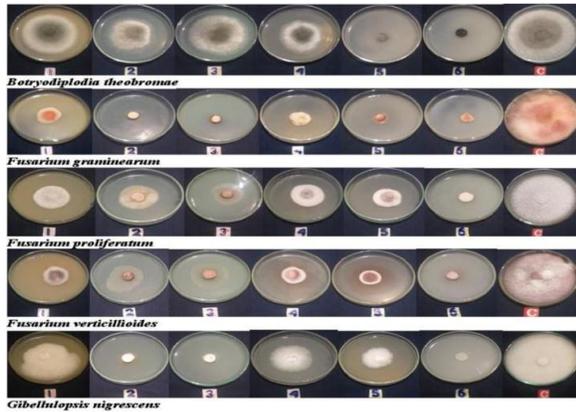


Fig. 1: The *in vitro* effect of six fungicides: (1) Dithane M45, (2) Kocide 2000, (3) Ridomil Gold, (4) Equation pro, (5) kemazed and (6) Score, on colony diameter of five pathogenic dracaena leaf spot fungi, (C) = Control

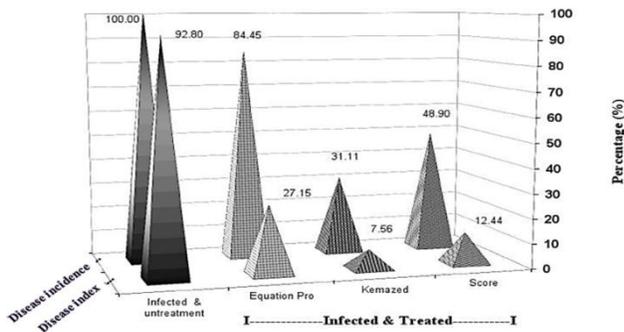


Fig. 2: The *in vivo* effect of foliar spray with Equation pro, kemazed and Score fungicides (curative) on disease incidence and disease index of leaf spots developed on detached leaves of dracaena (cv. Bicolor) previously inoculated with leaf spot fungi

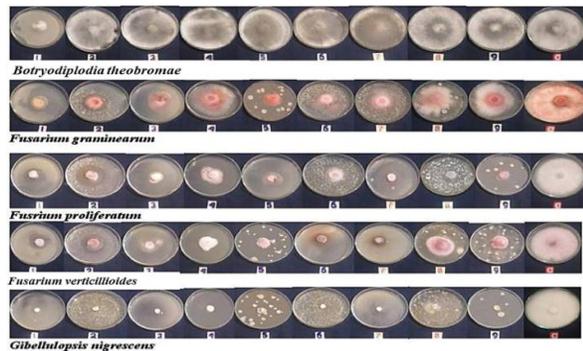


Fig. 3: The *in vitro* effect on PDA of nine bacterial biocontrol agents *i.e.*, (1) *Stenotrophomonas maltophilia*, (2) *Pseudomonas ultimum*, (3) *P. fluorescens*, (4) *P. putida*, (5) *P. aeruginosa*, (6) *Bacillus subtilis*, (7) *B. megaterium*, (8) *B. cereus* and (9) *B. thuringiensis*, on the colony growth diameter of five pathogenic dracaena leaf spot fungi

biocontrol agents *i.e.*, decreased disease index to

20.0–20.89%, respectively with no significant difference between them (Table 9; Fig. 4).

Effect on potted plants: Treatment with *S. maltophilia*, (curative), which was the most effective in the detached leaves assay, to dracaena (cv. Bicolor) previously inoculated with dracaena leaf spot fungi, significantly decreased mean percentage of infection with leaf spots to 29.33% compared to 100% for the inoculated untreated control. More pronounced effect was observed for disease index as *S. maltophilia* decreased disease index to 7.6% compared to 83.6% for the inoculated untreated control (Table 10).

Efficacy of Fungal Biocontrol Agents

In vitro effect on colony growth of dracaena leaf spot fungi:

All the tested *Trichoderma* spp., *i.e.* *Trichoderma harzianum*, *T. hamatum* and *T. album* obviously inhibited growth diameter of the tested dracaena leaf spot fungi. Data in Table (11) showed that percentage of colony diameter inhibition ranged between 64.22% and 75.02%, where *T. harzianum* showed the highest inhibition effect. This was followed by *T. hamatum* and *T. harzianum* where percentages of inhibition were 67.73% and 64.22%, respectively (Table 11).

In vitro effect on spore germination and germ tube:

All *Trichoderma* fungal bioagents significantly inhibited spore germination of the tested dracaena leaf spot fungi and percentage of inhibition ranged between 48.00% with *T. album* to 30.00% with *T. harzianum* (Table. 12). Meantime, the different biocontrol agents of *Trichoderma* spp. significantly decreased length of germ tube of the germinated spores of the different fungi (Table 12). The *T. album* was the most effective as exhibited mean inhibition effect on the tested leaf spot fungi as high as 90.21%. This was followed by *T. hamatum* and *T. harzianum*, where inhibition effects were 84.56% and 80.33%, respectively (Table 12).

In Vivo Effect of *Trichoderma* on Dracaena Leaf Spot Disease

Effect on detached leaves: Data presented in Table (13), illustrated in Fig. 5 showed that treatment with *Trichoderma* spp. (curative) significantly decreased percentages of infection and disease index of leaf spots developed on detached leaves of dracaena (cv. Bicolor), previously inoculated with the tested five dracaena leaf spot fungi. The *T. album* showed the highest effect and decreased percentage of infection to 35.54%, compared to 92.80% in the inoculated untreated control and also, decreased the developed disease index to 9.34% compared to 100% for the inoculated, untreated control.

Effect on potted plants: Data presented in Table (14), illustrated in Fig. (6) showed that treatment with *T. album* (curative), which exhibited the highest effect in the detached leaves assay, to potted plants of dracaena (cv. Bicolor)

Table 8: The *in vitro* percentage of spore germination, length of germ tube and inhibition of five pathogenic dracaena leaf spot fungi treated with nine bacterial biocontrol agents

Leaf spot	Spore Germination (%)					Mean inhibition (%)	Germ tube length (µm)					Mean inhibition (%)
	<i>Botryodiplodia theobromae</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium verticillioides</i>	<i>Gibellulopsis nigrescens</i>		<i>Botryodiplodia theobromae</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium verticillioides</i>	<i>Gibellulopsis nigrescens</i>	
<i>Stenotrophomonas maltophilia</i>	40.00	50.00	60.00	58.00	48.00	48.80A	0.10	0.05	0.15	0.14	0.07	88.77A
<i>Pseudomonas ultimum</i>	66.00	62.00	66.00	62.00	64.00	36.00B	0.15	0.15	0.20	0.20	0.17	80.45C
<i>Pseudomonas fluorescens</i>	70.00	68.00	80.00	80.00	86.00	23.20D	0.23	0.25	0.28	0.39	0.37	65.65E
<i>Pseudomonas putida</i>	68.00	72.00	72.00	74.00	66.00	29.60C	0.23	0.27	0.22	0.22	0.30	72.15D
<i>Pseudomonas aeruginosa</i>	74.00	64.00	80.00	76.00	60.00	28.00C	0.23	0.22	0.21	0.22	0.31	73.00D
<i>Bacillus subtilis</i>	56.00	60.00	48.00	56.00	60.00	44.00A	0.10	0.13	0.10	0.14	0.21	84.97B
<i>Bacillus megaterium</i>	76.00	74.00	66.00	72.00	68.00	28.80C	0.21	0.22	0.17	0.26	0.30	73.88D
<i>Bacillus cereus</i>	76.00	68.00	72.00	76.00	72.00	27.20C	0.23	0.33	0.30	0.24	0.35	67.15E
<i>Bacillus thuringiensis</i>	72.00	72.00	80.00	70.00	86.00	24.00D	0.27	0.26	0.31	0.24	0.35	67.15E
Control	100.00	100.00	100.00	100.00	100.00	0.00E	0.77	1.07	0.80	0.95	0.85	00.00F

Values are average of five replicates and ten readings for each replicate; Means followed by different letter, are significantly different at p = 0.05; Inhibition of germ tube length (%)= (Control of germ tube length -Treatment of germ tube length/ Control of germ tube length) × 100 Control = no bacterial treatment

Table 9: The *in vivo* effect of four bacterial biocontrol agents (curative) on disease incidence and disease index on detached leaves of dracaena (cv. Bicolor) inoculated with five dracaena leaf spot fungi

Dracaena leaf spot fungi	Disease incidence					Disease index (%)				
	Infected and treated				Infected untreated	Infected and treated				Infected untreated
	<i>S. maltophilia</i>	<i>P. ultimum</i>	<i>B. subtilis</i>	<i>B. megaterium</i>		<i>S. maltophilia</i>	<i>P. ultimum</i>	<i>B. subtilis</i>	<i>B. megaterium</i>	
<i>Botryodiplodia theobromae</i>	66.67	88.89	88.89	66.67	100	26.67	28.89	31.11	17.78	90.00
<i>Fusarium graminearum</i>	66.67	77.78	88.89	88.89	100	15.56	31.11	24.44	24.44	96.00
<i>Fusarium proliferatum</i>	33.33	55.56	55.56	44.44	100	11.11	11.11	13.33	13.33	92.00
<i>Fusarium verticillioides</i>	44.44	77.78	66.67	66.67	100	13.33	22.22	15.56	22.22	94.00
<i>Gibellulopsis nigrescens</i>	55.56	66.67	77.78	55.56	100	13.33	11.11	15.56	15.56	92.00
Mean	53.33D	73.33B	75.55B	64.44C	100 A	16.00D	20.89 B	20.00 B	18.67C	92.80 A

Values followed by different letter, for each parameter are significantly different at p = 0.05

Table 10: Disease incidence and index of leaf spots developed on dracaena plants (cv. Bicolor) inoculated with five pathogenic leaf spot fungi and sprayed (curative) with *S. maltophilia* bacterial biocontrol agent

Dracaena leaf spots fungi	Percentage of infection (%)			Disease index (%)		
	Infected & treated	Infected untreated	Inhibition (%)	Infected & treated	Infected untreated	Inhibition (%)
<i>Botryodiplodia theobromae</i>	36.67	100	63.33 D	10.7	82.00	86.95 B
<i>Fusarium graminearum</i>	33.3	100	66.67 CD	10.0	90.00	88.88 AB
<i>Fusarium proliferatum</i>	20.0	100	80.00 A	4.7	80.00	94.12 A
<i>Fusarium verticillioides</i>	26.7	100	73.33 A	6.7	84.00	92.02 AB
<i>Gibellulopsis nigrescens</i>	30.0	100	70.00 BC	6.0	82.00	92.68 AB
Mean	29.33	100	70.67	7.60	83.60	90.93

Values for each parameter followed by different letter, are significantly different at p = 0.05

inoculated with five dracaena leaf spot fungi significantly decreased mean percentage of infection to 19.99% compared to 100% for the inoculated untreated control. Meanwhile, *T. album* decreased the developed disease index to 4.93% compared to 83.60% for the inoculated untreated control (Table 14; Fig. 6).

Discussion

Dracaena species are well known in Egypt and other parts of the world as an important indoor ornamental plants as well as important medicinal plants for curing a number of diseases (Wagih et al., 1989; Jia-Yi et al., 2014).

Unfortunately, dracaena plants are attacked by several plant diseases particularly the fungal leaf spots, which constitute a threat to dracaena cultivation and industry in Egypt and several parts of the world (Abd El-Zaher *et al.*, 2005; Thongkantha *et al.*, 2008).

For controlling dracaena leaf spot fungi, six common fungicides, namely Dithane M45® 80% WP, Kocide 2000® 53.8% WP, Ridomil Gold Plus® 42.5% WP, Score® 25 % E.C, Equation Pro® 52.5% WG and Kemazed® 50% WP were investigated *in vitro* and *in vivo*. All the tested fungicides significantly decreased the *in vitro* growth of the tested dracaena leaf spot fungi. Percentage of inhibition on colony diameter by the tested fungicides ranged between 49.64% with Dithane M45® to 82.18% with Score® which was followed by Kemazed® (69.15%). Also, inhibition of spore germination was the highest (72.8%) by Dithane M45® while inhibition of germ tube reached 89.91% with Kemazed®.

The *in vivo* tests using the detached leaves assay as well as the inoculation of potted plants of dracaena (cv. Bicolor) supported the *in vitro* results. Kemazed® decreased disease incidence of leaf spots in potted plants to 16.67% with 4.1% disease index compared to 100% and 83.6%, respectively for the untreated inoculated control. These results were in harmony with those obtained by Chase (2010), Siddique *et al.* (2014). The high positive efficacy of fungicides against fungal growth and their means of reproduction as well as the disease incidence and severity may ascribed to their toxic nature to fungal structure and its physiological activities, and to their long period of persistence on plant surfaces (Gill and Garg, 2014).

The bacterial and fungal biocontrol agents proved to be an effective means for controlling the dracaena leaf spot fungi (Heydari and Pessarakli, 2010). The *in vitro* assays conducted showed that the bacterial biocontrol agents *i.e.*, *S. maltophilia*, *P. ultimum*, *P. fluorescens*, *P. putida*, *P. aeruginosa*, *B. subtilis*, *B. megaterium*, *B. cereus* and *B. thuringiensis*, significantly decreased colony growth diameter (76.84–45.69%) of dracaena leaf spot fungi as well as spore germination (48.80–23.20%) and length of the germ tube (88.77–65.65%) of the germinated spores compared to the untreated inoculated control. Treatment with *S. maltophilia* showed the most consistent highest inhibition effect in this respect, followed by treatments with *P. ultimum*, *B. subtilis*, and *B. megaterium*, respectively. Meantime, the results of the *in vivo* tests on dracaena (cv. Bicolor) using the detached leaves method and the potted plants technique supported the *in vitro* results. *S. maltophilia* showed the highest effect as decreased disease incidence and disease index to 29.33% and 7.6%, respectively compared to 100% and 83.6% for the untreated inoculated control in the potted dracaena plants (cv. Bicolor) for the above mentioned disease parameters, respectively. These results were in agreement with those of Asaka and Shoda (1996); Liu and Wenshi (1997); Oraghi *et al.* (2011); Palaniyandi *et al.* (2011) and Abdalla *et al.* (2014). This

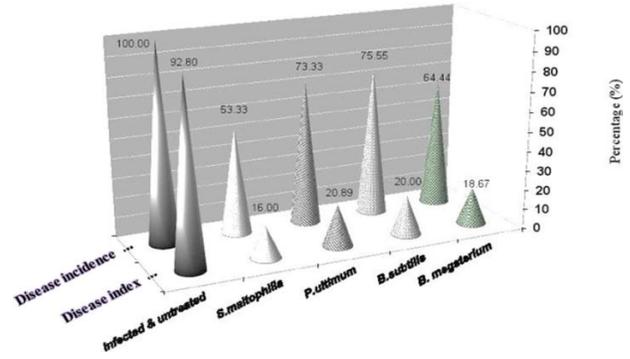


Fig. 4: The *in vivo* effect of foliar spray with four bacterial biocontrol agents (curative) on disease incidence and disease index of leaf spots developed on dracaena (cv. Bicolor) inoculated with dracaena leaf spot fungi using the detached leaves assay

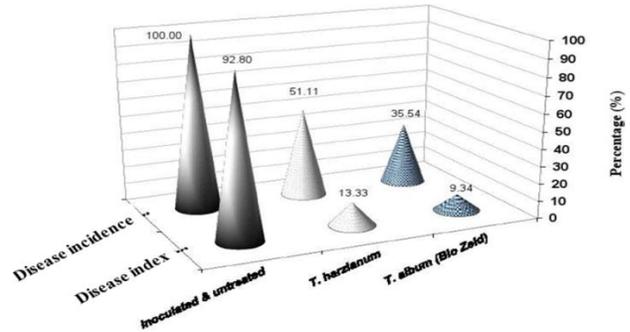


Fig. 5: Effect of two *Trichoderma* spp. (curative) on disease incidence and index of leaf spots developed on detached leaves of dracaena (cv. Bicolor) previously inoculated with five dracaena leaf spot fungi

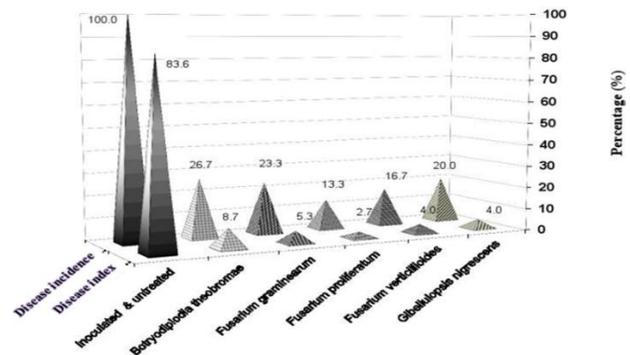


Fig. 6: Effect of *Trichoderma album* (curative) on leaf spots developed on potted plants of dracaena (cv. Bicolor) previously inoculated with five dracaena leaf spot fungi under greenhouse conditions

could be explained in view of Kloepper and Schroth (1981) and Sivasakthi *et al.* (2014) that bacterial bioagents promoted plant growth, increased availability of mineral nutrients and produced plant-growth regulators that may be

Table 11: The *in vitro* colony diameter and inhibition of five dracaena leaf spot fungi on PDA amended with three fungal biocontrol agents of *Trichoderma* spp

Leaf spot fungi bio agents	Fungal bioagents					Overall mean	
	<i>Botryodiplodia theobromae</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium verticillioides</i>	<i>Gibellulopsis nigrescens</i>	Colony diameter (Cm)	Inhibition (%)
<i>Trichoderma harzianum</i>	3.18	1.70	1.52	2.84	2.00	2.25	75.02 A
<i>T. hamatum</i>	4.28	2.26	1.60	3.44	2.94	2.90	67.73 B
<i>T. album</i>	5.26	1.44	1.84	3.76	3.80	3.22	64.22 C
Control	9.00	9.00	9.00	9.00	9.00	9.00	0.00 D

Values are means of 5 replicates, Percentage of inhibition = [(A-B)/A]×100 where: A = Colony diameter of untreated pathogen, B = Colony diameter of treated pathogen, Control = no biocontrol treatment; Means followed by different letter are significantly different at p= 0.05

Table 12: Percentage of spore germination, length of germ tube and inhibition of five dracaena leaf spot fungi treated with three fungal bio agents

Fungal agents	Spore Germination (%)					Germ tube length (µm)						
	<i>Botryodiplodia theobromae</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium verticillioides</i>	<i>Gibellulopsis nigrescens</i>	Mean inhibition (%)	<i>Botryodiplodia theobromae</i>	<i>Fusarium graminearum</i>	<i>Fusarium proliferatum</i>	<i>Fusarium verticillioides</i>	<i>Gibellulopsis nigrescens</i>	Mean inhibition (%)
<i>Trichoderma harzianum</i>	60.00	70.00	80.00	70.00	70.00	30.00C	0.12	0.12	0.16	0.18	0.22	80.33C
<i>T. hamatum</i>	60.00	60.00	70.00	60.00	70.00	36.00B	0.13	0.11	0.15	0.11	0.18	84.56B
<i>T. album</i>	60.00	50.00	40.00	50.00	60.00	48.00A	0.10	0.11	0.07	0.06	0.10	90.21A
Control	100.00	100.00	100.00	100.00	100.00	0.00D	0.79	1.05	0.77	0.95	0.89	0.00D

Values are average of five replicates and ten readings for each replicate; Means followed by different letter are significantly different at p = 0.05; Spore germination % = (Number of spores germinated in treated pathogen/Number of spores germinated in untreated control) × 100; Inhibition in germ tube length % = (Germ tube length of control - Germ tube length of treatment)/Control of germ tube length × 100; Control = no biocontrol treatment

Table 13: Disease incidence and disease index of leaf spots developed on detached leaves of dracaena (cv. Bicolor) inoculated with five pathogenic dracaena leaf spot fungi and treated (curative) with two *Trichoderma* spp

Dracaena leaf spot fungi	Percentage of infection (%)			Disease index (%)		
	Inoculated and treated			Inoculated and treated		
	<i>T. harzianum</i>	<i>T. album</i> (Bio Zeid)	Inoculated untreated	<i>T. harzianum</i>	<i>T. album</i> (Bio Zeid)	Inoculated untreated
<i>Botryodiplodia theobromae</i>	66.67	55.56	100	22.22	15.56	90.00
<i>Fusarium graminearum</i>	55.56	44.44	100	17.78	11.11	96.00
<i>Fusarium proliferatum</i>	44.44	22.22	100	8.89	6.67	92.00
<i>Fusarium verticillioides</i>	55.56	33.33	100	11.11	8.89	94.00
<i>Gibellulopsis nigrescens</i>	33.33	22.22	100	6.67	4.44	92.00
Mean	51.12 B	35.54 C	100.00 A	13.34 B	9.34 C	92.80 A

Values, for each parameter, followed by different letter are significantly different at p = 0.05

Table 14: Percentage of infection and disease index of leaf spots developed on potted plants of dracaena (cv. Bicolor) inoculated with five pathogenic dracaena leaf spot fungi and treated (curative) with *Trichoderma album*(Bio Zeid)

Dracaena leaf spot fungi	Disease incidence		Disease index (%)	
	Inoculated and treated		Inoculated and treated	
	<i>Trichoderma album</i> . (Bio Zeid)	Inoculated untreated	<i>Trichoderma album</i> . (Bio Zeid)	Inoculated untreated
<i>Botryodiplodia theobromae</i>	26.70	100	8.70	82.00
<i>Fusarium graminearum</i>	23.30	100	5.30	90.00
<i>Fusarium proliferatum</i>	13.30	100	2.70	80.00
<i>Fusarium verticillioides</i>	16.70	100	4.00	84.00
<i>Gibellulopsis nigrescens</i>	20.00	100	4.00	82.00
Mean	19.99 B	100.00 A	4.93 B	83.60 A

Values, for each parameter, followed by different letter are significantly different at p = 0.05

responsible for better healthy plants and consequently less disease parameters. Also, *Bacillus* and *Pseudomonas* genera are among the plant growth promoting rhizobacteria PGPR. The PGPR could act as biocontrol agents by production of antibiotics, triggering induced local or systemic resistance, or preventing the deleterious effects by acting as rhizoremediators (Glick et al., 2007). Meanwhile, Members of *Bacillus* spp. were able to produce various lytic enzymes

e.g., chitinase and β-1, 3-glucanase (Pankaj and Dubey, 2012), along with induction of systemic resistance of plants, such increasing the activities of plant defense related enzymes of peroxidase, polyphenol oxidase and phenylalanine ammonialyase (Jayaraj et al., 2004). Also, *P. fluorescens* was able to produce and secrete secondary metabolites that might play a role against activity and spore germination of plant pathogens (Sivasakthi et al., 2014).

On the other hand, The *in vitro* tests of three *Trichoderma* spp. i.e., *T. harzianum*, *T. hamatum* and *T. album* significantly inhibited colony growth diameter and spore germination of the tested dracaena leaf spot fungi. *T. harzianum*, however, showed the highest inhibition on colony diameter (75.02%) while, *T. album* was the most effective to inhibit spore germination (48%) and germ tube (90.21%) of the germinated spores. The *in vivo* assays of the fungal biocontrol agents supported the *in vitro* obtained results. Using the inoculation of the potted plants technique, *T. album* (Bio-Zeid) showed the highest effect as decreased percentage of infection and disease index to 19.99% and 4.93% compared to 100% and 83.6%, respectively for the untreated inoculated control. This could be explained in view that, the *Trichoderma* spp. could protect the plant by establishing themselves in the rhizosphere (root zone) and establish a barrier against pathogen attack (Hermosa *et al.*, 2012). Also, early applications of *Trichoderma* spp. protected plant roots by removing secreted nutrients that pathogens might use (Azarmi *et al.*, 2011). Meantime, many plant pathogens contain chitin as a component of their cell wall. *Trichoderma* release chitinases that have been shown to disrupt the cell wall of these pathogens. These enzymes dissolve the cell wall and create holes in the pathogen. Once damaged, the pathogen itself becomes the prey of other soil microflora (Hermosa *et al.*, 2012). Also, it has been shown that hyphae of *T. aureoviride* isolate grew and coiled around the hyphae of *F. oxysporum* as well as an intense vacuolation could be observed within the cytoplasm of the pathogens hyphae involved (Brzezinska, *et al.*, 2014). Also, it has been suggested that *Trichoderma* produced extracellular β -(1-4) glucanase and chitinase, which are key enzymes in the lysis of fungal cell walls (Sharma and Trivedi, 2010).

The active biocontrol agents such as species of *Trichoderma* have evolved numerous mechanisms that are involved in biological control. The mechanisms include competition for space and nutrients, antibiosis, antagonism, inhibition of pathogen enzymes and plant growth stimulation (Harman *et al.*, 2004; Abdel-Khair *et al.*, 2010). Also, different foliar diseases of plant were effectively controlled with each or more species of these bioagents when they were sprayed on susceptible plants (Bartmanska and Gladysz, 2006; Hilal *et al.*, 2009; Ragab *et al.*, 2011).

Conclusion

The obtained results surely can help to establish a sustainable, eco-friendly strategy for controlling the fungal leaf spots of dracaena.

References

Abdalla, A., A.A. Algam, A.I. Elshiekh and A.M. El-Naim, 2014. *In vitro* screening of *Bacillus* isolates for biological control of early blight disease of tomato in shambat soil. *World J. Agric. Res.*, 2: 47–50

- Abd El-Zaher, A. Effat, A.A. Hilal, I.A.M. Ibrahim and T.N. Mohamed, 2005. Leaf spots of ornamental foliage plants in Egypt with special reference to *Corynespora cassiicola* [(Berk. & Curt.) Wei] as a new causal agent. *Egypt. J. Phytopathol.*, 33: 87–103
- Abdel-Khair, H., M.R. Khalid and K.H.K. Haggag, 2010. Effect of *Trichoderma* species on damping-off incidence, some plant enzymes activity and nutritional status of bean plants. *J. Amer. Sci.*, 6: 122–132
- Amer, M.A., 1995. Evaluation of adjuvants to enhance fungicide efficacy against plant pathogens. *Ph. D. Thesis*, Faculty of Agricultural and Applied Biological Sciences, University of Gent, Belgium
- Asaka, O. and M. Shoda, 1996. Biocontrol of *Rhizoctonia solani* damping off of tomato with *Bacillus subtilis* RB 14. *Appl. Environ. Microbiol.*, 62: 4081–4085
- Azarmi, R., B. Hajieghrari and A. Gigloul, 2011. Effect of *Trichoderma* isolates on tomato seedling growth response and nutrient uptake. *Afr. J. Biotechnol.*, 10: 5850–5855
- Bartmanska, A. and J.D. Gladysz, 2006. Transformation of steroid by *Trichoderma hamatum*. *Enzyme Microbial Technol.*, 40: 1615–1621
- Boyd, A., W. Dawit and W. Ab. Getaneh, 2013. Evaluation of detached leaf assay for assessing leaf rust (*Puccinia triticina* Eriks.) resistance in wheat. *J. Plant Pathol. Microbiol.*, 4: 5
- Brzezinska, M.S., U. Jankiewicz, A. Burkowska and M. Walczak, 2014. Chitinolytic microorganisms and their possible application in environmental protection. *Curr. Microbiol.*, 68: 71–81
- Chase, A.R., 2010. Fungicides for leaf spot diseases of ornamentals. *Greenhouse Product News*, 12: 52
- El-Abd, Sawsan M., 2002. Studies on some fungal diseases infecting onion crop in Egypt. *Ph.D. Thesis*, p: 191. Alexandria University, Egypt
- Gill, H. and H. Garg, 2014. *Pesticides: Environmental Impacts and Management Strategies*, pp: 187–230. In *Pesticides-Toxic Aspects in Tech*
- Glick, B.R., B.J. Czamy, Z. Cheng, J. Duan and B. McConkey, 2007. Promotion of plant growth by bacterial ACC deaminase. *Crit. Rev. Plant Sci.*, 26: 227–242
- Gomez, K.A. and A.A. Gomez, 1984. *Statistical Procedures for Agricultural Research*. John Wiley Sons, Inc., New York, USA
- Harman, G.E., R. Petzoldt, A. Comis and J. Chen, 2004. Interaction between *Trichoderma harzianum* T22 and maize inbred line molt and effects of these interactions on diseases caused by *Pythium ultimum* and *Colletotrichum graminicola*. *Phytopathology*, 94: 147–153
- Hermosa, R., A. Viterbo, I. Chet and E. Monte, 2012. Plant-beneficial effects of *Trichoderma* and of its genes. *Microbiology*, 158: 17–25
- Heydari, A. and M. Pessarakli, 2010. A review on biological control of fungal plant pathogens using microbial antagonists. *J. Biol. Sci.*, 10: 273–290
- Hilal, A.A., A.M. Abo-El-Ela, A.A. Helmy and A.S. Ibrahim, 2000. Studies on fungal diseases of *Dracaena* spp., the ornamental foliage plant in Egypt. *Egypt. J. Appl. Sci.*, 15: 12–25
- Hilal, A.A., S.G.I. Soliman, O.Y. Shalaby and A.H. El-Shaer, 2009. Rusts of lemon grass (*Cymbopogon citratus*) and citronella grass (*Cymbopogon nardus*) in Egypt: New disease problems on aromatic plants and their management. *Egypt. J. Appl. Sci.*, 24: 68–89
- Jackson, E.W., D.E. Obert, J.B. Chong Avant and J.M. Bonman, 2008. Detached-leaf method for propagating *Puccinia coronata* and assessing crown rust resistance in oat. *Plant Dis.*, 92: 1400–1406
- Jayaraj, J., H. Yi, G.H. Liang, S. Muthukrishnan and R. Velazhahan, 2004. Foliar application of *Bacillus subtilis* AUBS1 reduces sheath blight and triggers defense mechanisms in rice. *J. Plant Dis. Prot.*, 111: 115–125
- Jia-Yi, F., Y. Tao, S. Chui-Mei, Z. Lin, P. Wan-Ling, Z. Ya-Zhou, Z. Zhong-Zhen and C. Hu-Biao, 2014. A systematic review of the botanical, phytochemical and pharmacological profile of *Dracaena cochinchinensis*, a plant source of the ethnomedicine “Dragon’s Blood”. *Molecules*, 19: 10650–10669
- Kloepper, J.W. and M.N. Schroth, 1981. Relationship of *in vitro* antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology*, 71: 1021–1024
- Liu, C. and W. Wenshi, 1997. Chemical and biological control of tomato early blight. *Plant. Pathol. Bull.*, 6: 132–140

- Morang, P., B.K. Dutta, B.S. Kumar and M.P. Kashyap, 2012. Growth promotion and biocontrol approaches of brown root rot disease of tea by *Pseudomonas aeruginosa* (PM 105). *J. Plant Pathol. Microbiol.*, 3: 129
- Mueller, J.D., M.N. Cline, J.B. Sincalair and B.J. Jacobsen, 1985. An *in vitro* test for evaluating efficacy of mycoparasites on sclerotia of *Sclerotinia sclerotiorum*. *Plant Dis.*, 69: 584–587
- Oraghi, A., N.A. Oraghi, S. Mohammad and M. Hamdi, 2011. Physiological effects of *Pseudomonas fluorescens* CHA0 on tomato (*Lycopersicon esculentum* Mill.) plants and its possible impact on *Fusarium oxysporum* f. sp. *lycopersici*. *Aust. J. Crop Sci.*, 5: 1631–1638
- Palaniyandi, S.A., S.H. Yang, J.H. Chengm and L. Meng, 2011. Biological control of anthracnose (*Colletotrichum gloeosporioides*) in yam by *Streptomyces* sp. MJM5763. *J. Appl. Microbiol.*, 3: 443–455
- Pankaj, K. and R.C. Dubey, 2012. Plant growth promoting rhizobacteria for biocontrol of phytopathogens and yield enhancement of *Phaseolus vulgaris* L. *J. Curr. Persp. Appl. Microbiol.*, 1: 6–37
- Ragab, Mona A.A., M.M. Saber, M.M. El-Gebaly and F.M. Farag, 2011. Application of antagonistic microorganisms to control powdery mildew of pea. *Egypt. J. Phytopathol.*, 39: 145–157
- Shama, S.M. and M.A. Massoud, 2001. New natural products as immunity inducers on cucumber (*Cucumis sativus*) against downy mildew caused by *Pseudoperonospora cubensis*. *Adv. Agric. Res.*, 6: 2001
- Sharma, P. and P.C. Trivedi, 2010. Evaluation of different fungal antagonists against *Fusarium oxysporum* infecting *Withania somnifera* (L) Dunal. *Assam Univ. J. Sci. Technol. Biol. Environ. Sci.*, 6: 37–41
- Steel, R.G. and J.M. Torrie, 1984. *Principles and Procedures of Statistics*. McGraw-Hill, Inc., New York, USA
- Siddique, S.S., M.K.A. Bhuiyan, R. Momotaz, G.M.M. Bari1 and M.H. Rahman, 2014. Cultural characteristics, virulence and *in-vitro* chemical control of *Fusarium oxysporum* f. sp. *phaseoli* of Bush bean (*Phaseolus vulgaris* L.). *The Agriculturists*, 12: 103–110
- Sivasakthi, S., G. Usharni and P. Saranraj, 2014. Biocontrol potentiality of plant growth promoting bacteria (PGPR) - *Pseudomonas fluorescens* and *Bacillus subtilis*. *Afr. J. Agric. Res.*, 9: 1265–1277
- Thongkantha, S., S. Lumyong, E.H.C. McKenzie and K.D. Hyde, 2008. Fungal saprobes and pathogens occurring on tissues of *Dracaena lourieri* and *Pandanus* spp. in Thailand. *Fungal Diversity* 30: 149–169
- Wagih, E.E., M.R.A. Shehata, A. Samia Farag and M.K. Dawood, 1989. *Dracaena* leaf proliferation, a newly recorded disease affecting *Dracaena sanderiana* in Egypt. *J. Phytopathol.*, 126: 7–16

(Received 28 May 2016; Accepted 05 September 2016)