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Occurrence of Stem and Leaf Spots on Lucky Bamboo (*Dracaena sanderiana* hort. ex. Mast.) Plants in Vase and its Control with Safe Means

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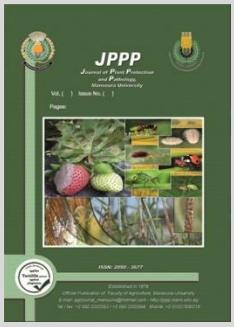

ABSTRACT

Lucky bamboo (*Dracaena sanderiana*) plants showing stem and leaf spots symptoms of each observed season were monitored during a survey that was conducted in four periods from 2018 to 2019. There were significant differences between the four periods, as well as the mean percentages of infection and disease severity were high (45.92, 44.41 % and 46.15, 55.67%, respectively) During the first (March-May) and third (August-October) study periods in 2018. The same disease symptoms occurred at the same time of observation during 2019 in the several surveyed nurseries, and the percentage of infection and disease severity recorded were in the same trend. There were twelve different fungal pathogens identified as *Absidia spinosa, A. cylindrospora, Alternaria alternata, Aspergillus flavus, A. niger, A. terreus, Aspergillus sp., Colletotrichum gloeosporioides, Fusarium avenaceum, F. oxysporum, F. solani,* and *Trichoderma harzianum* where *Colletotrichum gloeosporioides* was the most common (26.93 and 26.34 for leaves and stems, respectively). The pathogenicity tests were carried out, where *C. gloeosporioides* were the most aggressive. The systemic fungicide Kemazed® and biocontrol agents were demonstrated to be effective in controlling three of the most common aggressive fungi. i.e., *C. gloesporioides*, *F. oxysporum* and *A. alternata*, *in vitro* colony growth of fungal isolates was significantly reduced as compared to the untreated control. The bioagents tested had significant antagonistic activity against *C. gloesporioides* growth on bamboo in a vase, according to the results, with Kemazed® showing the most consistent highest inhibition effect, followed by Rhizo-N® and Plant Guard®, respectively, when compared to the untreated control.

Keywords: *Colletotrichum gloesporioides, Fusarium oxysporum,* pathogenicity, Kemazed®, biocontrol, *in vitro*, lucky bamboo

INTRODUCTION

Bamboos (*Dracaena sanderiana* hort. ex. Mast.) are the Asparagaceae family's most widely introduced member, it's bred as a vase plant of decorative foliage and for low-light interiorscapes (Damen *et al.*, 2018). Some of the common names for lucky bamboos include goddess of mercy plant, friendship bamboo, the golden bamboo, ribbon plant, belgian evergreen, belly bamboo, curly bamboo, Chinese water bamboo, pot bound, and bamboos (Hugh and Giam 2008). Bamboo plants have become the most common ornamental indoor plants in recent years, and they are being used to run over severe air and environmental pollution problems. Lucky bamboo is a valuable commodity in our lives because it plays an important role in the decoration of places both private and public, including homes, offices, schools, and shopping malls (Adnan, 2019).

However, several fungal diseases, including Antharacnoses caused by, *Colletotrichum gloeosporioides*, *C. dracaenophilum*, *C. petchii* and *C. boninense*, have been reported to affect bamboo plants in Egypt, as well as other countries around the world (Bobev *et al*., 2008, Liu *et al*., 2014, Macedo and Barreto, and Morsy and Elshahawy, 2016), *Fusarium oxysporum*, *F. proliferatum*, *F. phyllophitum*, *F. semitectum*, *F. solani*, and *F. subglutinans* as a result of stem rot, while leaf spots were caused by *Aspergillus niger* and *Alternaria alternata* (Abbasi and Aliabadi, 2008 and Hilal *et al.*, 2016).

According to Oraghi *et al*. (2011) and Sivasakthi *et al*. (2014) fungicides have remained an important part of disease control strategies for many dracaena ornamental plant manufacturers. Morsy and Elshahawy (2016) found that using a systemic fungicide (Kemazed®) in combination with other fungicide formulations can effectively control *Colletotrichum dracaenophilum* on bamboo diseases. Siddique *et al*. (2014) and Hilal *et al.* (2016), stated that although chemical control of plant diseases may be the simplest and quickest method, the environment, health risks, and the ongoing development of new strains of pathogens resistant to the chemicals used call for safer alternatives.

The use of microorganisms to prevent plant disease is a promising new approach to disease administration strategy. Biological agents against fungal diseases have been described by several microorganisms (Jayaraj *et al*., 2004, Kim *et al*., 2008, Jia-Yi *et al*., 2014, and Stamenković *et al*., 2018). Morsy and Elshahawy (2016) stated that *Bacillus subtilis* showed antifungal activity against a *Colletotrichum dracaenophilum* on lucky

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bamboo, and in the presence of its cells, *Colletotrichum dracaenophilum* mycelial growth was inhibited.

Furthermore, several studies (Leelavathi *et al*., 2014, Singh *et al*., 2018, and Halifu *et al*., 2019) found that *Trichoderma* spp. is a fast-growing fungus with powerful spore-forming abilities, a source of cell-wall-breaking enzymes, and an important antibiotic product implementation, biological control agents can also induce significant changes in plant metabolism, resulting in improved plant growth, increased nutrient availability, disease resistance, and enhanced yield production.

In Egypt, lucky bamboo is affected by several fungal diseases, especially stem and leaf spots, which harm plant life in vases. As a result, the current study aimed to identify the causal pathogens that infect bamboos, to determine the severity and timing of disease occurrence, pathogenicity, and the efficacy of one standard systemic fungicide and some bioagents in controlling bamboo stem and leaf spot fungal pathogens.

MATERIALS AND METHODS

The current research was carried out at "Plant Pathology Research Institute, Ornamental, Medicinal, and Aromatic Plant Diseases Research Department, Agricultural Plant Protection Research Station, El-Sabihia-Alexandria" during the two successive years of 2018 and 2019.

1. Survey for stem and leaf spots of bamboo:

The occurrence of stem and leaf spots was constantly monitored on lucky bamboo plants in vases in two Egyptian Governorates (Alexandria and Cairo), the observation was carried out in some nurseries and bamboo stores that sell and retail bamboo. The four periods of disease monitoring were: 1st (March-May), 2nd period (June-July), 3rd period (August-October), and 4th period (November-February) of both 2018 and 2019.

Infection percentages and disease severity were calculated in keeping with Thongkantha *et al.* (2008) and Morang *et al.* (2012) in the following manner:

$$\text{Infection (\%)} = \frac{\text{No. of infected plants}}{\text{Total No. of examined plants}} \times 100$$

In addition, each plant displaying fungal disease symptoms was carefully inspected and given a rating, after which the severity of the disease was calculated in keeping with Abd El-Zaher *et al*. (2005) as follows:

Zero indicates that there are no signs or symptoms of the disease.

1= There are a few scattered lesions that occupy less than 25% of the plant.

2= Spots that cover more than 25 to 50% of the plant.

3= Spots coalescing and covering more than 50% of the plant until it reaches 75%.

4= Infection resulting in a coalescing lesion that covers more than 75 percent of the plant until it dies.

$$\text{Percentage of disease severity} = \frac{\text{Total No. of plants under scale degree} \times \text{scale degree}}{\text{Scale degrees (4)} \times \text{total No. of plants testeds}} \times 100$$

2. Sampling, isolation and identification of the associated fungi:

During the survey, samples of lucky bamboo plants with stem and leaf spots were collected, washed thoroughly with running tap water, sterile distilled water, and sterilized for two minutes with five percent sodium hypochlorite, after that fragments of infected tissues were aseptically plated onto PDA (potato dextrose agar) was used, and it was incubated at 27±2°C for 4 to 5 days. using method of a single spore isolation technique was used to purify the recovered fungal colonies (Thongkantha *et al*., 2008 and Cai *et al*., 2009).

According to Nelson *et al*. (1983), Barnett and Hunter (1987) and Domsch *et al*. (2007), the recovered fungal species were first identified based on morphological characteristics and then verified by the Mycology Research and Plant diseases Survey Department, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Then, according to Bhattacharyya *et al*., (2017), the frequencies of these fungi were calculated as follows:

$$\text{Frequency of fungal isolates (\%)} = \frac{\text{No. of single fungal species colonies}}{\text{Total No. of all fungal colonies}} \times 100$$

3. Pathogenicity studies on the fungal species recovered:

The pathogenicity tests were performed as prescribed by Hassan *et al*. (2015), and Morsy and Elshahawy (2016).

Outwardly healthy lucky bamboo plants (of mean length stem 75 cm and internode segment 3 cm) were bought from a well-known Cairo commercial nursery, and to make certain they're in good health were sown for 45 days in glass bottles containing 500 ml distilled water. Before being sown, all glass bottles were sterilized by submerging them for 5 minutes in 5% sodium hypochlorite and then washed in sterile distilled water. To keep the bamboo scraps from fading during the trial era, alexandrany wax was added to the cut area after cutting it. Five replicates were used (one plant/ bottle). Pathogenicity tests were prepared and inoculated as follows:

- One isolate of good growth of each fungal species recovered in the survey were grown for 8-10 days at 27±2°C on PDA and 0.5cm culture discs of the active margins were used for inoculations of one hundred-ml autoclaved potato dextrose (PD) medium in 250 ml conical flasks and incubated at 27±2°C for 10-15 days to obtain enough mycelial growth and the fungal growth of each pathogen was collected, then blended with 100 ml sterilized distilled water in a blender for one minute. The suspension of the spores was set to 1×10^6 spores/ml, the spore concentration was determined using a Haemocytometer slide (Hassan *et al*., 2015).
- Aseptically 25 plant segments of 1-cm long and 2-cm in diameter were cut from internodes 2-6 (from plant basal) and placed horizontally on filter paper in Petri dishes, 5 segments for each. A 0.5-cm culture disc of the tested fungi was placed separately in the center of the horizontal surface of the segment and monitored for six days. Then, the percentage of the horizontal surface colonized by the tested fungus to the whole surface was calculated.
- Also, 25 internodal segments of 3-cm long were prepared from internodes 7-11 and were placed in 300 ml size sterile jars lined with filter-paper, 5 segments in each, inoculation was performed by entering the mycelial agar plug sporulating (diameter 0.5 cm) was inserted with a sterile blade into a cut in the stem segment, and a sterile

PDA agar plug of similar size was used as a negative control. After that, parafilm strips were used to cover the inoculated areas. Jars were sealed and monitored for 10 days, at 27±2°C according to Morsy and Elshahawy (2016) and the severity of the developed disease was calculated as previously described.

- Also, 25 healthy-looking leafy stem cuttings of elven nodal long were prepared from the internodal top of the plant. Following that, all surfaces were disinfected for one minute with 5% sodium hypochlorite, rinses with sterile distilled water, and finally dried with sterilized filter paper. Inoculation was conducted as sterile syringes were used to inject one ml of conidial suspension into the cortex region beneath the epidermis in the basal stem of the healthy bamboo plant, which was then sown in 250 ml sterile distilled water in glass bottles, with dipping 5 cm of its basal stems in these bottles. Sterile distilled water was used as the control. All bottles are covered with a thick sponge stopper around the stalk of the bamboo to prevent water evaporation and reduce the entry of bacteria from the surrounding air, the plants were covered with polyethylene plastic bags for 72 hours to provide wet conditions. Then, kept under room conditions for 45 days and the percentages of infection and severity of the developed disease were calculated as previously described (Bobev *et al*., 2008 and Vanita *et al*., 2015).
- To ensure that the pathogen was associated with the developed symptoms, it was constantly reisolated from symptoms that had been artificially developed.

4. Efficacy of bioagents and Kemazed fungicide to control stem and leaf spots fungi of bamboo:

Four bioagents i.e., Bio Zeid, Bio Arc, Plant Guard, and Rhizo-N as well as the fungicide Kemazed were tested to evaluating their effectiveness against the most three common fungal species revealed in the conducted survey of the bamboos, i.e., *Colletotrichum gloesporioides*, *Fusarium oxysporum* and *Alternaria alternata.* Five replicates were conducted for each treatment as follows (Table 1):

Table 1. The tested fungicides and bioagents their, Common name, rate and source.

Treatment	Commercial name and formulation	Common name (Active ingredients)	Recommended rate	Source (manufacture)
T_1	Kemazed ® 50% WP (Powder)	Carbendazim	75g/100 l.	Kafr-El-Zayat
T_2	Bio Zeid®2.5% WP (Powder)	*Trichoderma album*	$25*10^6$ spores/g at 250 g/100 l.	Organic Biotechnology
T_3	Plant Guard® (Liquid)	*T. harzianum*	$30*10^6$ spores/ml at 250 cm^3/100 l.	Bayou-Tech for biopsies
T_4	Rhizo-N® (Powder)	*Bacillus subtilis*	$30*10^6$ cell/g at 4g/l.	and pesticides
T_5	Bio Arc®6% WP (Powder)	*B. megaterium*	$25*10^6$ cell/g at 250 g/100l.	Organic Biotechnology
Control	sterilized distilled water			

4.1. The *in vitro* inhibition effect of bioagents and Kemazed:

According to Hilal *et al.* (2016), before hardening the PDA medium, the Kemazed at the recommended rate (0.075g/100ml) was added. After the PDA had solidified, each dish was centrally inoculated with a mycelial disc (1 cm) of the tested fungi. The colony's average diameter was measured after the untreated control mycelia had just covered one plate.

The tested biocontrol agent was tested by adding (*Trichoderma album* 2.5 g/100ml and *T. harzianum* 0.006 cm3/100ml PDA medium) of the commercial product to PDA medium before solidification with shaking, followed by pouring into the dishes and all plates were incubated at (27±2°C) for five days. A one-centimeter mycelial disc was taken from the active mycelium margins of each isolate. Every plate was divided into of equal size of two halves. Two one cm diameter plugs were taken. One disk came from the tested fungi margins of seven-day-old advancing cultures, while the other came from *Trichoderma* spp., on the plate's opposite sides both plugs were positioned 1.5 cm from the edge of the plate. At the end of the test, the colony growth of the tested fungi was determined in every treatment and the percentage reduction in the tested fungi's colony growth was calculated using the formulas of Ferreira *et al.* (1991) and Uribe and Loria (1994) as follows:

$$\text{Reduction (\%)} = \frac{\text{Distance of mycelial growth of control - distance of mycelial growth of pathogen toward the plugs of } \textit{Trichoderma}}{\text{Distance of mycelial growth of control}} \times 100$$

Also, according to Hilal *et al.* (2016), bacterial powder of the commercial product of *Bacillus subtilis* 0.4 g/100ml and *B. megaterium* 0.25 g/ 100ml was grown on nutrient agar and incubated at 30±2°C for 24 hours. Then streaking solid PDA medium plates, then at a distance of 25 mm, on the opposite side, one disc (0.5 cm) from a 7-day-old culture of one of the tested fungi was placed. At 27±2°C, the plates were incubated. At the end of the experiment, the colony diameter of the pathogenic fungi was determined each and every treatment, and the percentage reduction in colony diameter of the tested fungi was calculated using the formula of Amer (1995) as follows:

$$\text{Reduction (\%)} = \frac{\text{Control colony diameter of pathogen - treatment colony diameter toward the bioagents}}{\text{Control colony diameter of pathogen}} \times 100$$

4.2. The *in vivo* effect of bioagents and Kemazed:

According to Vanita *et al.* (2015), and Morsy and Elshahawy (2016), bottles of 1000 ml capacity were treated with each of (Kemazed® at 0.375g/500 ml, *Bacillus subtilis* 2g/500 ml, and *T. harzianum* 0.03g/500 ml) sterile distilled water, after one day artificial inoculated with *Colletotrichum gloesporioides* (most common and most aggressive fungal species by two ml of concentration of inoculum spores' ($1x10^6$ spores/ ml). Inoculation and sown were conducted as above mentioned in the leafy stem segments in pathogenicity. For 45-60 days, five replicates (one plant/ bottle) were observed daily until anthracnose symptoms appeared on control plants. The infection percentages and severity of disease were then calculated as previously described.

Statistical analysis:

The data were statistically analysed using the Statistix program by accordance with Snedecor and Cochran (1989), and the means were compared using the LSD test at a probability level of 5%.

RESULTS AND DISCUSSION

1. Occurrence of stem and leaf spots of bamboo:

The present investigation was conducted during the two successive years of 2018 and 2019, four consecutive periods were monitored, depending on the severity of the infection i.e., season: 1st period (March-May), 2nd period (June-July), 3rd period (August-October) and 4th period (November-February). Data presented in Table 2 showed that the highest mean percentages of infection and disease severity were found in the first and third study periods. Concerning percentages of infection, the obtained values in the first period were as high as 45.92 and 42.74 % for the first and the second year, respectively, compared with 44.41 and 40.74 % at the 3rd period, for the two years of the survey, respectively. However, the other two periods of the investigation showed much lower infection values in the two years of the survey.

As regards the developed disease severity, a similar trend was revealed, where the 1st and 3rd period of investigation showed the highest severity values with an even higher mean value for the 3rd period of the investigation being 54.33 % over the two years of the survey.

Other researchers in Egypt and the world have come to harmonious conclusions (Musavi and Balakrishnan 2013, Sharma *et al*., 2014, Morsy and Elshahawy, 2016 and Kamel *et al.*, 2018). The obtained high percentage of infection and severity at the 1st (March-May) and the 3rd (August-October) period may be explained in view that moisture and temperature at these two periods may lead to the emergence of such pathological symptoms of some fungi latent in bamboo and latent infection on plants may persist and develop when appropriate conditions are provided for the growth of some fungi such as *Colletotrichum* spp. and *Fusarium* spp. (Sharma *et al*., 2014).

Table 2. Percentages of infection and disease severity of stem and leaf spots in a survey of lucky bamboo plants over four periods during 2018 and 2019 consecutive years.

Period	Infection %			Severity%		
	Year		Means	Year		Means
	2017/2018	2018/2019		2017/2018	2018/2019	
1st March-May	45.92	42.74	44.33 a*	46.15	52.76	49.45 a
2nd June-July	17.71	16.08	16.99 b	28.09	28.33	28.21 b
3rd August-October	44.41	40.74	42.57 a	55.67	53.00	54.33 a
4th November-February	13.08	12.72	12.90 b	29.69	29.34	29.51 b
Mean*	30.28 A	28.07A		39.90 A	40.86 A	
LSD. $_{0.05}$	4.32		6.12	4.19		5.91

*** Values followed by different letter (s) are significantly different at 0.05 of probability.**

2. Fungi associated with stem and leaf spots of bamboo:

Data in Table 3 showed that twelve different fungal species were isolated from leaves and stems of bamboos plant showed stem and leaf spots symptoms during the conducted survey, where *Colletotrichum gloesporioides* showed the highest frequency being 26.93 and 26.34 % of the total isolates recovered from leaves (505 isolates) and stems (1253), respectively, followed by *Fusarium oxysporum* with 14.26 and 15.80 % and *Alternaria alternate* with *F. solani* and *F. avenaceum* for leaves and stems, respectively. *Aspergillus* sp. the only fungus recovered from stems, had the lowest occurrence (0.00 and 1.52 %). These findings were in harmony with reports from Egypt and other parts of the world on lucky bamboo (Mohanan, 1997, Komaki *et al*., 2012, Musavi and Balakrishnan, 2013, Sharma *et al*., 2014, Abedi-Tizaki *et al.,* 2016, and Morsy and Elshahawy, 2016).

Table 3. Frequency of the isolated fungi from stem and leaf spots of lucky bamboo samples collected from Alexandria and Cairo Governorates during the 2018-2019 period.

Isolated fungi	Number of total fungal isolates		Frequencies (%)	
	Leaves	Stems	Leaves	Stems
Absidia spinosa	00.00	60.00	00.00 e	04.79 ef
A. cylindrospora	00.00	49.00	00.00 e	03.91 f
Alternaria alternata	63.00	148.0	12.47 c	11.81 c
Aspergillus flavus	00.00	49.00	00.00 e	03.91 f
A. niger	41.00	94.00	08.12 d	07.50 de
A. terreus	00.00	60.00	00.00 e	04.79 ef
Aspergillus sp.	00.00	19.00	00.00 e	001.52 f
Colletotrichum gloeosporioides	136.0	330.0	26.93 a	26.34 a
Fusarium avenaceum	36.00	92.00	07.13 d	07.34 de
F. oxysporum	72.00	198.0	14.26 c	15.80 b
F. solani	45.00	110.0	08.91 d	08.78 cd
Trichoderma harzianum	112.0	44.00	22.18 b	03.51 f
Total	505	1253	100	100
LSD. $_{0.05}$	2.93	8.79	2.54	3.32

*** Values followed by different letter (s) are significantly different at 0.05 of probability**

3- Pathogenicity tests:

Pathogenicity tests for the recovered fungal species were tested on lucky bamboo (*Dracaena sanderiana*). As shown in Fig.1, the tested fungal species clearly induced infection to varying degrees in both tested techniques. Meanwhile, data presented in Table 4 showed that inoculation of the stem segments surfaces with of the tested isolates revealed that *Colletotrichum gloeosporioides* was the most pathogenic and covered 100% of segment surface, followed by *F. oxysporum*, *Alternaria alternata*, and *Aspergillus niger* with 97, 96 and 89 %, respectively, while the other fungal species covered 77-63 % of the horizontal

segment surface. Similar trends were also revealed for the severity of infection with side stem segment inoculation where *C. gloeosporioides* was the most pathogenic and incited 97 % disease severity followed by *F. oxysporum* (95 %), and *A. alternata* (91 %).

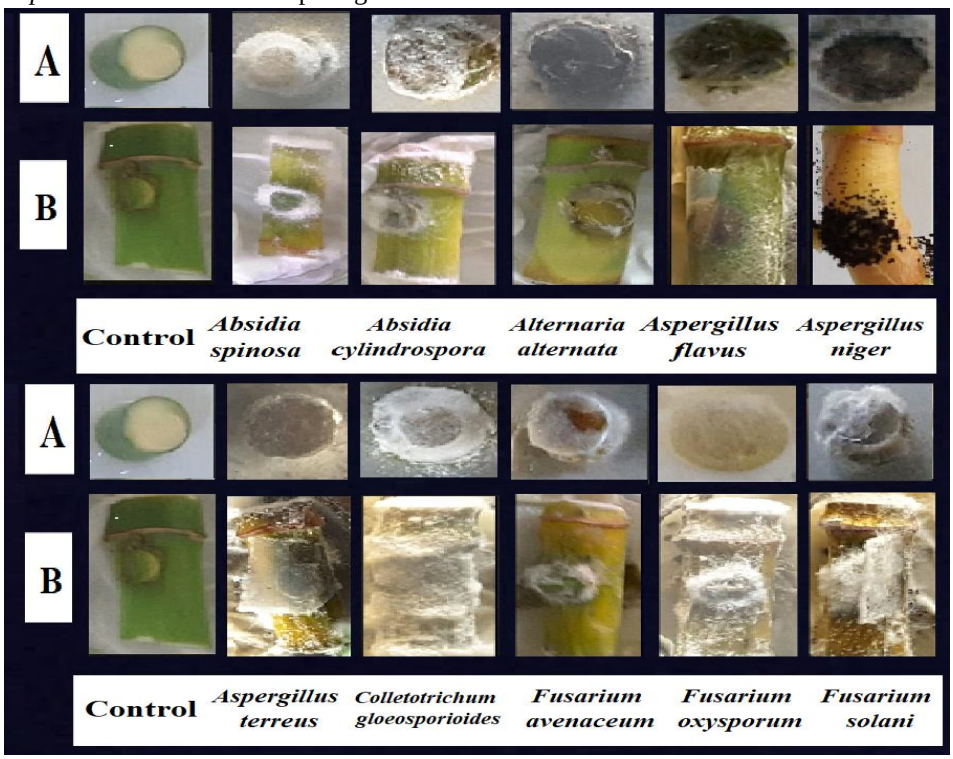

Fig. 1. Pathogenicity tests of the fungal species associated with stem and leaf spots of lucky bamboo. A = Cutting internode horizontal surface, six days after inoculation. B = internodal segments, 10 days after inoculation.

Table 4. Disease severity of the most aggressive fungi on lucky bamboo in pathogenicity test.

Isolated fungi	Infected area (%) **	Severity (%)
Absidia spinosa	76* c	69.00 e
A. cylindrospora	73 cd	83.83 bc
Alternaria alternata	96 ab	91.00 ab
Aspergillus flavus	63 e	74.00 de
A. niger	89 b	87.00 b
A. terreus	70 cde	78.00 cd
Colletotrichum gloeosporioides	100 a	97.00 a
Fusarium avenaceum	68 de	85.00 bc
F. oxysporum	97 a	95.00 a
F. solani	77 c	87.00 b
Control	00 f	00.00 f
LSD. 0.05	7.66	7.19

****Percentage of the covered area with fungus growth relative to the whole horizontal surface inoculated of the cutting internode.**

*** Values followed by different letter (s) are significantly different at 0.05 of probability.**

Data presented in Table 5 showed that inoculation of the leafy stem segments with the tested isolates revealed that mean percentages of infection and disease severity were the highest in *C. gloeosporioides* treatment (88.9 and 95.0 %), followed by *F. oxysporum* (86.7 and 95.0 %) and *A. alternata* and (84.4 and 90.0 %), respectively. The interaction between the ten fungal isolates and the tested stems and foliage systems was shown in the artificial inoculation (Fig. 2). Depending on the ability of each fungus to infect the plant, symptoms developed to varying degrees. After 30 days of inoculation, a bamboo plant became infected with a specific fungus that grew fast on the stems. On the other hand, infection on foliage was relatively slow (45 days after inoculation). Around the stems, developed symptoms were discovered, the middle of the stem has brown discoloration (rotting) from colonizing vascular tissues. Moreover, various stains spread across the leaves, resulting in light brown lesions that turned purple to dark brown. In comparison to healthy plants, the hard tissues in the stem and foliage became soft, and then plants died 60 days after inoculation. water and nutrient transportation are thought to be hampered by the impairment and maceration of stem and vascular tissues. Furthermore, fungal growth in tissues resulted in leaf wilt and, eventually, plant death. Meanwhile, Table 5 and Fig. 2 showed a similar trend on the inoculated leafy segments. The obtained data were in harmony with that acquired by Komaki *et al*. (2012), Abedi-Tizaki *et al*. (2016), Wang *et al*. (2016) and Adnan (2019).

Table 5. Percentage of infection and severity on lucky bamboo after 45 days of the leafy stem segments in pathogenicity test.

Isolated fungi	Infection (%)	Severity (%)
Absidia spinosa	40.0* c	40.0 ef
A. cylindrospora	35.6 c	55.0 de
Alternaria alternata	84.4 a	90.0 ab
Aspergillus flavus	31.1 c	35.0 f
A. niger	42.2 bc	80.0 abc
A. terreus	80.0 a	65.0 cd
Colletotrichum gloeosporioides	88.9 a	95.0 a
Fusarium avenaceum	82. 2 a	85.0 ab
F. oxysporum	86.7 a	95.0 a
F. solani	57.8 b	75.0 bc
Control	00.0 d	0.0 g
LSD. 0.05	15.98	18.91

*** Values followed by different letter (s) are significantly different at 0.05 of probability.**

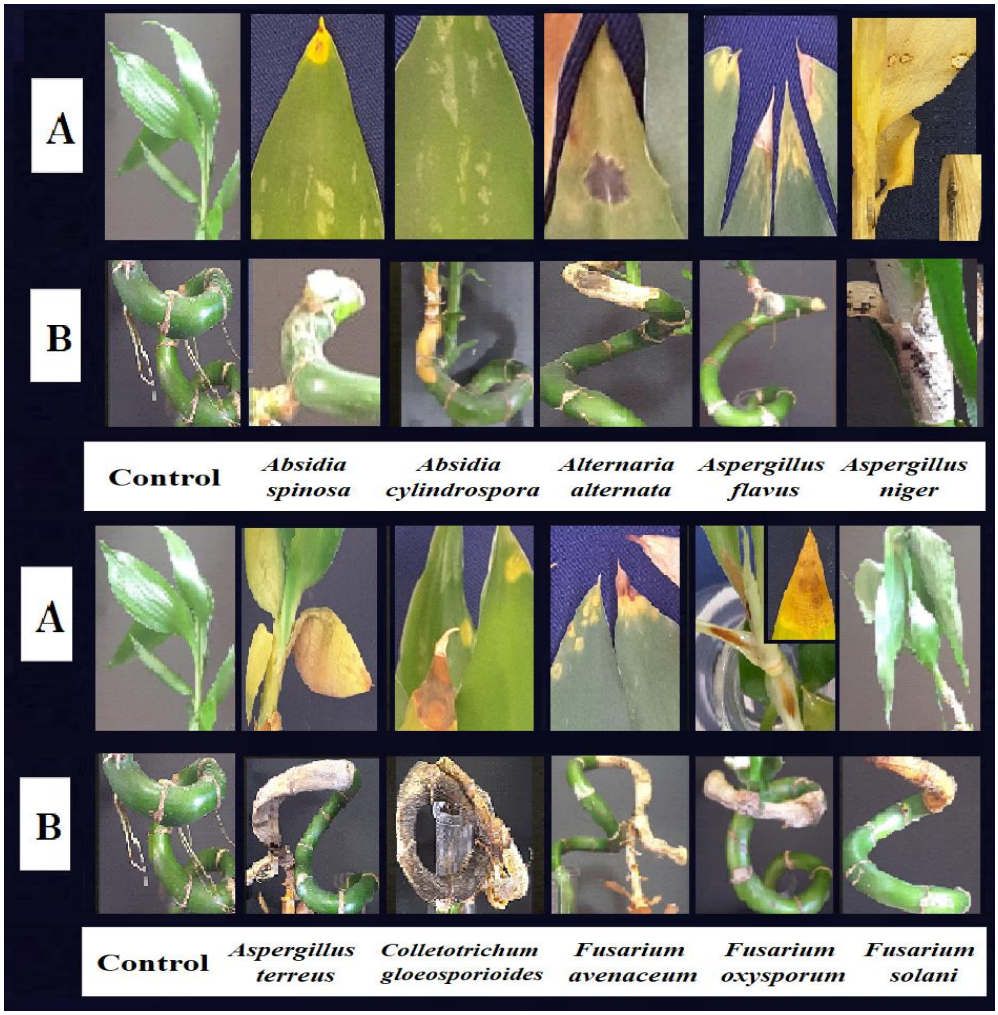

Fig. 2. Symptoms of artificial inoculation leafy segments of lucy bamboo with the stem and leaf spots fungal species recovered in the survey after 45 days of inoculation as compared with control.

(A) Showing different yellowing, wilting, and anthracnose symptoms on foliage. (B) Conidia, spores and acervuli on stem.

4. Efficacy of the tested bioagents in controlling bamboo stem and leaf spots:

Four bioagents, i.e., Bio Zeid, Bio Arc, Plant Guard and Rhizo-N, as well as the fungicide Kemazed were tested for their efficacy against the most three common fungal species in the conducted survey, i.e., *C. gloeosporioides*, *F. oxysporum* and *A. alternata in vitro* and under greenhouse conditions.

4.1. The *in vitro* effect:

Data in Table 6 showed that *Bacillus subtilis* significantly decreased colony growth diameters, with the most invariable highest inhibition effect, followed by *Trichoderma harzianum*, *T. album*, and finally *B. megaterium*. Kemazed, on the other hand, significantly reduced the *in vitro* growth of the three fungi, with an overall mean inhibition of colony diameters (80.81 %), followed by *Bacillus subtilis* (73.78 %), and finally *T. harzianum* (68.15 %). *F. oxysporum* had the strongest effect, followed by *A. alternata* and *C. gloeosporioides*, in that order. Meanwhile, it was evident from Fig. 3 that the four tested bioagents as well as the Kemazed fungicide decreased colony growth of the tested fungi to different degrees. These findings were consistent with those of several other researchers (Oraghi *et al.*, 2011, Siddique *et al.*, 2014, Sivasakthi *et al.*, 2014, and Morsy and Elshahawy 2016).

Table 6. Effect of the four bioagents and Kemazed fungicide on colony growth (diameter) of three causal fungal species of bamboo stem and leaf spots.

Treatments	*Colletotrichum gloeosporioides*	*Fusarium oxysporum*	*Alternaria alternata*	Overall mean
Kemazed®	76.44* a	84.00 a	82.00 a	80.81 A
Bio Zeid® (*Trichoderma album*)	43.56 d	64.44 c	62.00 c	56.6 7 D
Plant Guard® (*T. harzianum)*	56.22 c	77.78 ab	70.44 b	68.15 C
Rhizo-N® (*Bacillus subtilis)*	73.99 ab	79.55 ab	67.78 bc	73.78 B
Bio Arc® (*B. megaterium)*	71.33 b	73.56 c	31.56 d	58.81 D
Control	00.00 e	0.00 d	0.00 d	00.00 E
Overall mean	53.59 B	63.22 A	52.29 B	
LSD. 0.05	3.67	6.32 2.0778	5.84	2.94

*** At the 5% level, means denoted by the same letter (s) are not significantly different.**

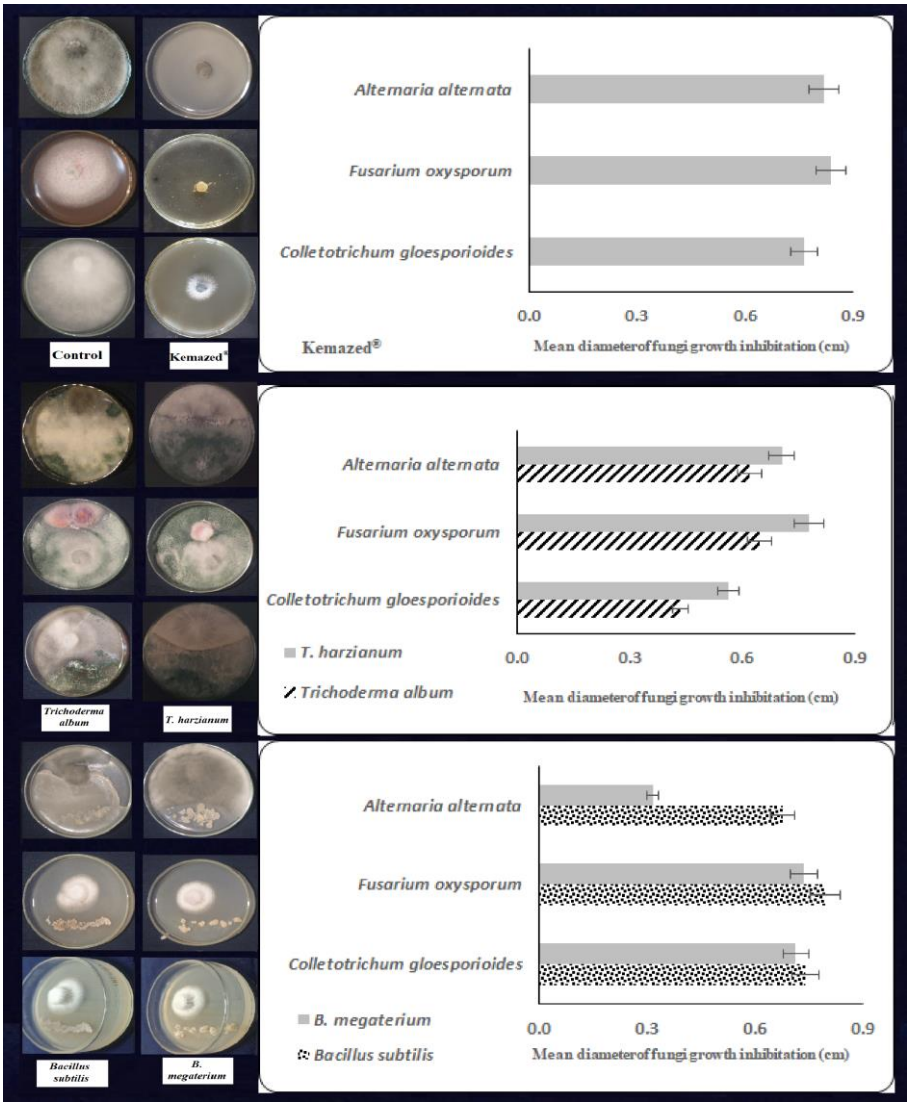

Fig. 3. The *in vitro* inhibitory effect of *Trichoderma album, T. harzianum, Bacillus subtilis* and *B. megaterium* bioagents and Kemazed fungicide on colony growth (diameter) of three stem and leaf spot fungal species recovered from lucky bamboo plants in a survey during 2018-2019 period.

4.2. Effect of the applied fungicide and bioagents on fungal inhibition in potted plants:

Data illustrated in Fig. 4 show that Kemazed, *B. subtilis*, and *T. harzianum* had significant antagonistic activity against the growth of *C. gloeosporioides*. Reduction of infection (%) and disease severity (%) were as high as 90.76 and 75.00 %, respectively, with Kemazed, followed by *B. subtilis* (86.79 and 73.33 %) which was followed by *T. harzianum* (82.94 and 71.94%). These findings agreed with those of Hilal *et al.* (2016), and Morsy and Elshahawy (2016).

The tested bioagents activity can be explained by the fact that Plant bioprotection is achieved through the production of plant growth regulators i.e., abscisic acid, indole-3-acetic acid and gibberellic acid by bacterial bioagents. the availability of mineral nutrients (bio fertilization) plant growth is boosted as a result, and disease parameters are reduced, have been directly linked to Phytohormone biosynthesis by these bacterial bioagents by the creation of od plant resistance through increasing peroxidase, polyphenol oxidase, and phenylalanine

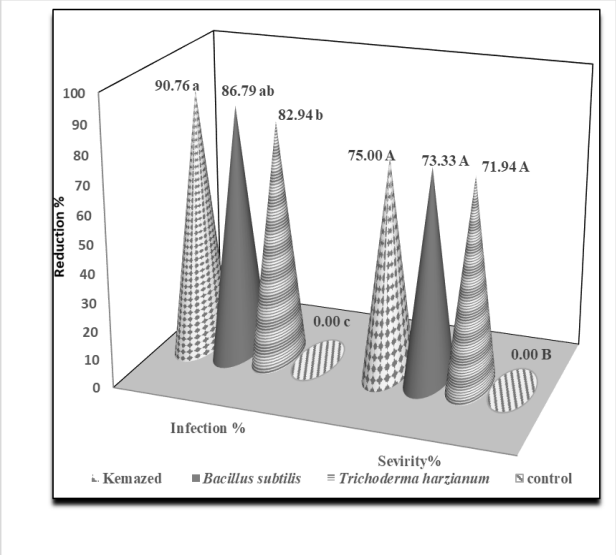

Fig. 4. Inoculated with *Colletotrichum gloeosporioides* and treated with *Bacillus subtilis* and *Trichoderma harzianum* bioagents as well as the fungicide Kemazed, lucky bamboo had a mean reduction in infection percentage and disease severity.

ammonia-lyase enzymes that are involved in plant defenses (Jayaraj *et al*., 2004, Kim *et al.*, 2008, Jia-Yi *et al.*, 2014 and Stamenković *et al*., 2018). *Trichoderma* spp. is a type of fungus that provides protection for plant roots by forming a barrier against pathogen attack by removing the used by pathogen nutrients. Meanwhile, secretion of chitinases dissolves the cell wall and creates holes in the pathogen, causing cell wall damage and lysis through the production of chitinase and extracellular-(1-4) glucanase, causing pathogen cell wall damage and lysis. (Oraghi *et al*., 2011, Leelavathi *et al*., 2014, Guzmán-Guzmán *et al*., 2018, Singh *et al*., 2018 and Halifu *et al*., 2019).

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حدوث تبقعات السوق و الأوراق على نبات البامبو (*Dracaena sanderiana* hort. ex. Mast.) في الزهريه ومكافحته بوسائل آمنة

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قسم بحوث أمراض نباتات الزينة والطبية والعطرية – معهد بحوث امراض النباتات - مركز البحوث الزراعية

تمت مراقبه نباتات البامبو lucky bamboo (*Dracaena sanderiana*) التى تظهر أعراض تبقعات بالسيقان والأوراق بكل موسم مرصود 2018- 2019 خلال أربع فترات الحصر الذي تم إجراؤه. وتم رصد فروق معنويه بين الفترات الأربع ، وسجل أرتفاع ملحوظ بمتوسط نسب الاصابه وشده المرض (45.92 و44.41 % و46.15 و55.67 %على التوالى) خلال الفترتين الأولى (مارس - مايو) والثالثة (أغسطس - أكتوبر) خلال عام 2018. وتم تسجيل نفس أعراض المرض فى نفس وقت الرصد خلال عام 2019 في العديد من المشاتل التي شملها الحصر. وسجلت نسبة الإصابة وشدة المرض فى نفس الاتجاه، وتم تعريف إثني عشر من مسببات الأمراض الفطريه المختلفة وهم (*Absidia spinosa, A. cylindrospora, Alternaria alternata, Aspergillus flavus, A. niger, A. terreus, Aspergillus sp., Colletotrichum gloeosporioides, Fusarium avenaceum, F. oxysporum, F. solani and Trichoderma harzianum*) ، وكان فطر (*Colletotrichum gloeosporioides*) الأكثر تكرارا (26.93 و 26.34 بالاوراق والسيقان على التوالى) اجريت اختبارات العدوى الصناعيه، وكان فطر (*C. gloeosporioides*) الأكثر عدوانيه ، أثبت كل من المبيد الفطرى الجهازى (كيمازيد) والمبيدات الحيوية فاعليتهم في السيطره على ثلاثه من أكثر الفطريات تكرارا وأكثرهم عدوانية ، وهم *C. gloesporioides* ، *F. oxysporum* و *A. alternata* ، مما أدى إلى انخفاض كبير في أقطار نمو المستعمرات الفطرية مقارنة بالفطريات غير المعالجة "الكونترول". وأظهرت الاختبارات التي تم إجراؤها أن جميع المبيدات التي تم استخدامها لها فاعلية مثبطه ضد نمو *C. gloesporioides* على نبات البامبو في المزهرية حيث أظهر الكيمازيد أعلى تأثير تثبيط ، يليه (Rhizo-N®) و(Plant Guard®) مقارنةً بالسيطرة غير المعالجة "الكونترول"، على التوالي.