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Management of Turfgrass Root-Rot using Botanical Oils, Green Chemicals, and Biocides

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ABSTRACT

Turfgrasses play an important role in improving the environment atmosphere, and functional values in modern cities. Soil-borne diseases are one of the most significant constraints to ornamental nursery outputs. Among several fungi isolated from the roots of turfgrass plants collected from different land landscape gardens located at Alexandria, Cairo, and Giza, *Rhizoctonia solani*, *Fusarium semitectum*, and *Fusarium oxysporum* were the most aggressive soil-borne isolates against both Bermuda and seashore *Paspalum* cv. Other fungi were isolated from the same areas included, *Helminthosporium* sp., *Pythium* spp. (fungi like), *Alternaria* sp., and *Macrophomina* sp. Essential oils and elicitors, were investigated in vitro at three concentrations for their antifungal activities against the growth of these fungi. These agents as well as the fungicide Topsin-M 70%, for a comparison, were evaluated in a greenhouse for their efficacy in controlling root-rot diseases on both Bermuda and seashore *Paspalum* cv. They were also evaluated for their effect on the plant contents of phenols and chlorophyll. It was found that Bio-Arc[®] at a concentration of 6 g l⁻¹ was the most effective one among all treatments tested for suppressing the linear growth of *F. oxysporum* and *M. phaseolina*, while clove oil was the most effective growth inhibitor against *F. semitectum*, the fungus like *P. splendens*, and *R. solani*. For the greenhouse experiment, the most effective treatment in reducing root-rot disease incidence was Topsin-M followed by clove oil and Bio-Arc[®]. For the biochemical tests in turfgrasses, all tested treatments increased the phenols and chlorophyll compared with the untreated control. From the results, it is noted that the Bermuda grass cv. is more resistant to root-rot diseases than the seashore *Paspalum* cv.

Keywords: Turfgrass, disease management, root-rot, total phenols, chlorophyll a & b.

INTRODUCTION

Turfgrass has a number of beneficial environmental effects, including lowering local temperatures (Douset and Gourmelon, 2003), energy use reduction, potential phytoremediation uses and erosion control (McPherson, 1994; Olson *et al.* 2003 and Kowalczyk *et al.* 2011). Greenscapes, such as turf, have also been demonstrated to alleviate stress and improve cognitive capacities. These advantages help to explain why grass is so commonly grown and used around the world (Douset and Gourmelon, 2003). A turfgrass lawn can contribute to a better quality of life, better environmental conditions, and improved functional values in modern cities. In addition, the turfgrass sector is a billion-dollar industry with a significant economic impact. The Poaceae (Gramineae) family includes the majority of grasses. Because of its capacity to develop quickly and cover the ground with beautiful form and color, seashore *Paspalum* (*Paspalum vaginatum* Sw.), a warm-season turf used in many places, can be covered with wonderful form and color (Sharaf El-Din *et al.* 2017). Due to its high tolerance to heat and drought and quick recovery, Bermuda grass (*Cynodon dactylon* L.) turf is the most widely used sod in urban landscapes (Ihtisham *et al.* 2020). It provides a high-quality turf and is the most

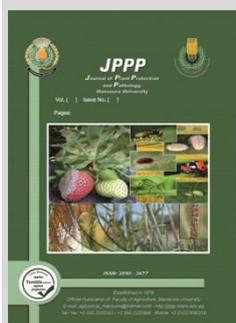
widely used warm-season turfgrass on athletic fields, lawns, parks, and urban landscapes.

Plant diseases caused by fungi, bacteria, viruses, and nematodes have a significant impact on the turfgrass sector, which is worth billions of dollars (Stackhouse *et al.* 2020). Among the significant constraints to ornamental nursery output are soil-borne diseases (Elumalai and Rengasamy, 2012). Soil-borne pathogens (the fungi like *Phytophthora* spp., *Rhizoctonia* spp., the fungi like *Pythium* spp., *Sclerotinia* spp., *Fusarium* spp. and *Verticillium* spp.) cause approximately 50 to 75% economic losses of the possible yield for many crops. Young seedlings are more sensitive to root rot disease than older plants most of the time (Dreistadt, 2001 and Lewis and Lumsden, 2001). To control soil-borne diseases, insects, and pests, ornamental nursery growers rely extensively on conventional fungicides and pesticides (Jones and Benson, 2001 and 2004). Because of the growing threat of fungicide resistance among plant pathogens, as well as serious environmental issues, researchers have been working on developing alternate methods to employing chemical pesticides to manage soil-borne plant pathogens in recent years (Larkin *et al.* 1998). Several techniques and approaches are being investigated in order to reduce the use of conventional fungicides, use fewer toxic ingredients, and introduce alternatives such as biological and bio-rational fungicides

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in turfgrass cultivation. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of the induced resistance. In both SAR and ISR, plant defense is preconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen. Only limited number of research studies was done on seashore Paspalum and Bermuda root-rot diseases in Egypt. Therefore, more research is necessary with green fungicides and biocontrol products to determine their efficacy in controlling turfgrass diseases. Thus, the present study aims to study in detail the most serious fungal diseases of seashore Paspalum and Bermuda grass and managing them using botanical oils and green chemicals or bioagents as safe alternatives to conventional chemical fungicides.

MATERIALS AND METHODS

Isolation, purification and identification of the causal pathogens:

Both Bermuda grass and seashore Paspalum plants showing typical symptoms of root-rot diseases were collected from several gardens in Cairo, Giza, and Alexandria governorates during 2016 and 2017. The infected roots were excised and carefully washed with tap water to remove any adhesive soil. Small segments of the infected roots were superficially sterilized in a diluted solution of sodium hypochlorite (5%) for 2 min. Then the fragments were rinsed several times in sterilized distilled water, blotted to dry on sterilized filter papers, and then placed on potato dextrose agar (PDA) plates. All the plates were incubated for 7 days at room temperature (27±2°C). Percentages of each of the isolated fungi were calculated. The isolated fungi and fungi like were purified by single spore technique and/or hyphal tip method. Colony characteristics and spore morphology were described and identified according to Booth (1971) and Domsch (1980). The identification was confirmed by the Fungal Taxonomy Department, Plant Pathology Research Institute, Agricultural Research Center, Giza.

Pathogenicity test

The pathogenicity test was carried out using the most frequent eight fungi isolated, namely *Fusarium oxysporum*, *Fusarium semitectum*, the fungus like *Pythium splendens*, *Rhizoctonia solani*, *Macrophomina phaseolina*, *Alternaria* sp., *Curvularia* sp., and *Helminthosporium* sp. This experiment was conducted in the greenhouse of the Ornamental, Medicinal and Aromatic Plants, Plant Diseases Research Department, Plant Pathology Research Institute, Agricultural Research Center. Plastic pots (30-cm diam.) were sterilized by immersing them in 5% formalin solution for 15 min. and then left to dry for 10 days. Nile silt and sand (1:1, v/v) were sterilized by 5% formalin solution and covered tightly with polyethylene sheets for seven days (Abd-Kader *et al.*, 2010), then left uncovered for ten days to evaporate formaldehyde odor. Pots were filled with the sterilized soil. Inoculum of each fungus was prepared by growing each fungus in 500-ml glass bottles containing cornmeal-sand sterilized medium (100g cornmeal, 50g washed sand, and 100ml water).

The bottles were incubated at 25°C for 2 weeks and shaken for one min. every three days to ensure uniform distribution of the fungal growth. Soil infestation was achieved by mixing the inoculum of each fungus with the 15-cm upper layer of the soil at a rate of 3% of soil weight, and irrigated 7 days before transplanting to stimulate fungal growth and ensure its homogeneous distribution in the soil. Control treatment was prepared using the same amount of the sterile cornmeal-sand medium (fungus-free) (El-Sheshtawy *et al.*, 2018). Ten healthy transplants of any of Bermudagrass and seashore Paspalum were transplanted in each pot. Six replicates were used for each particular treatment. The percentage of disease incidence of root-rot was recorded 15, 45, and 60 days after transplanting. Also, re-isolation from the infected turfgrass plants grown on the infested soil was carried out to fulfill Koch's postulates (Grainger (1949).

In vitro evaluation of essential oils and elicitors for their antifungal activities against the growth of the isolated pathogens

Two commercial essential oils (clove and lemon grass) and two elicitors (potassium silicate (10% K₂O+25% SiO₂) and Bio-Arc® (*Bacillus megaterium*)) were obtained from Agricultural Research Center and used at three concentrations from each to evaluate their antifungal activities against the growth of the recovered fungi. Essential oils of clove and lemon grass were used at concentrations of 6, 8, and 10 mL⁻¹. The elicitors, potassium silicate and Bio-Arc® were used at 2, 4, and 6 g L⁻¹. Petri plates (9-cm diam.), each containing 20 mL of sterile PDA were inoculated with 0.5-cm-diam. disc taken from the edge of a 7-day-old culture of the pathogen and placed in the center of the plate. Three replicates (plates) were used for each treatment. The inhibition of the fungal growth was calculated after 7 days as inhibition percentage of radial growth comparing to the control (Menzies *et al.* 1991).

In vivo evaluation of essential oils and elicitors for their efficacy in controlling root-rot diseases under greenhouse conditions

Clove oil, lemon grass oil, potassium silicate, commercial Bio-Arc®, and the chemical fungicide Topsin M 70% were used to control the root-rot diseases of Bermuda grass and seashore Paspalum under greenhouse conditions (Adjouet *et al.*, 2012).

Clove and lemon grass oils, potassium silicate, and Bio-Arc® were used at a concentration of 5 g L⁻¹ water. However, Topsin-M 70% was used at the recommended rate of 3 g L⁻¹ water. Plants were separately treated with each material by soaking in a preparation of any of the tested materials for 30 min, just before transplanting. Ten treated plants (preferable cuts) were transplanted in the infested soil in sterilized pots (25 cm diameter) or 30 cm. Plants soaked into these materials as described before but transplanted in sterilized (fungus-free) soil were used as a control. Six replicates (pots) were used for each treatment. Disease incidence was recorded 60 days after transplanting (Adesemoya and Klopffer, 2009).

Estimation of the total phenols and chlorophyll (a & b) contents

Total phenols and photosynthetic pigments (chlorophyll a & b) contents in seashore Paspalum and Bermuda grass leaves in all treatments were determined

according to Singleton and Rossi (1965); Arnon (1949); and Maclachlan and Zalik, (1963).

Statistical analysis:

The results were expressed as (mean ± SE), data were subjected to analysis of variance (ANOVA) using the software; CoStat 6.4 (CoHort Software). Significant differences among treatment means were determined using least significant difference (LSD) at 0.05 (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Results

Pathogenicity test:

Pathogenic capabilities of the isolated fungi were carried out in (30 cm-diam.) plastic pots in a greenhouse. Data presented in Table (1) indicate that all the tested

fungi were able to infect seashore Paspalum and Bermuda grass plants as indicated by the disease incidence (DI) percent of wilt and root - rot.

R.solani, *F.semitectum*, and *F.oxysporum* were the most aggressive isolates with the percentages of DI, being 48.33, 43.33, and 38.33%, respectively on Bermuda grass and 28.33, 26.67, and 26.67%, respectively on seashore Paspalum plants 60 days after transplanting (Table 1). In this regard, the fungus like *P.splendens* and *M.phaseolina* came in these second place followed by *Curvularia* sp., *Alternaria* sp., and *Helminthosporium* sp. Thus, in this study we focused on the most aggressive fungi, i.e., *R.solani*, *F.semitectum*, *F.oxysporum*, the fungus like *P.splendens*, and *M.phaseolina* for further investigations.

Table 1. Disease incidence caused by the fungi isolated from diseased seashore Paspalum or Bermuda grass plants 15, 45 and 60 days after transplanting in artificially infested soil with the test fungi.

The test fungi	%Disease incidence (after days)					
	Seashore Paspalum			Bermuda grass		
	15	45	60	15	45	60
<i>Alternaria</i> sp.	6.67±0.248	10.00±0.202	16.67±0.248	3.33±0.046	8.33±0.128	16.67±0.075
<i>Curvularia</i> sp.	3.33±0.069	5.00±0.283	13.33±0.167	3.33±0.052	6.67±0.087	25.00±0.185
<i>Helminthosporium</i> sp.	5.00±0.254	11.67±0.179	11.67±0.248	3.33±0.167	6.67±0.110	16.67±0.248
<i>Rhizoctonia solani</i>	13.33±0.052	21.67±0.046	28.33±0.167	10.00±0.144	30.00±0.087	48.33±0.104
<i>Fusarium semitectum</i>	10.00±0.300	20.00±0.150	26.67±0.127	8.33±0.121	26.67±0.219	43.33±0.185
<i>Macrophominaphaseolina</i>	6.67±0.127	13.00±0.266	23.33±0.185	3.33±0.167	16.67±0.121	33.33±0.237
<i>Pythium splendens</i>	6.67±0.242	15.00±0.202	23.33±0.121	8.33±0.139	23.33±0.202	36.67±0.300
<i>Fusarium oxysporum</i>	10.00±0.202	20.00±0.150	26.67±0.104	10.00±0.00	26.67±0.00	38.33±0.046
Untreated Control	0.0±0.00	0.0±0.00	0.0±0.00	0.0±	0.0±	0.0±0.00
LSD at 5%	0.53	0.32	0.11	0.17	0.08	0.08

In vitro evaluation of essential oils and elicitors for their antifungal activities against the growth of the isolated pathogens

Data in Table (2) indicate that clove oil had the highest growth inhibition effect against three of the five fungi tested, namely *F. semitectum*, the fungus like *P.*

splendens, and *R. solani*. While, Bio-Arc® was the most suppressive agent against the growth of the other two fungi tested, i.e., *M. phaseolina* and *F.oxysporum*. It was found, also, that the inhibition effect increased with the increase of the agent's concentration tested (Table 2),

Table 2. Effect of clove oil, lemon grass, potassium silicate and Bio-Arc® at three concentrations on the linear growth (cm) of the most aggressive five fungi isolated from diseased turfgrass plants.

Fungi	Clove oil (mL)					Lemon grass oil (mL)				
	0.0	6	8	10	Mean(A)	0.0	6	8	10	Mean(A)
<i>F.oxysporum</i>	9.00±0.029	3.17±0.052	2.47±0.023	2.27 ±0.046	4.23	9.00±0.064	4.23±0.046	2.97±0.069	2.73±0.023	4.73
<i>F. semitectum</i>	9.00±0.087	3.93±0.046	2.53±0.058	2.23±0.046	4.42	9.00±0.052	4.83±0.040	3.50±0.046	3.07±0.052	5.10
<i>M. phaseolina</i>	9.00±0.040	4.13±0.064	2.87±0.075	2.23±0.064	4.56	9.00±0.064	5.93±0.087	4.27±0.087	2.53±0.069	5.43
<i>P.splendens</i>	9.00±0.046	4.37±0.029	3.17±0.046	2.53±0.069	4.77	9.00±0.029	5.83±0.064	3.20±0.081	2.93±0.098	5.24
<i>R. solani</i>	9.00±0.069	4.53±0.052	3.07±0.064	2.70±0.081	4.83	9.00±0.064	5.47±0.081	3.90±0.052	3.77±0.087	5.54
Mean(B)	9.00	4.03	2.82	2.39	---	9.00	5.26	3.57	3.01	---
LSD at 5%		A=0.27	B=0.17	AxB=0.34			A=0.30	B=0.27	AxB=0.55	
Fungi	Potassium silicate (g L ⁻¹)					Bio-Arc® (g L ⁻¹)				
	0.0	2	4	6	Mean(A)	0.0	2	4	6	Mean(A)
<i>F.oxysporum</i>	9.00±0.052	5.27±0.064	3.80±0.035	3.03±0.052	5.28	9.00±0.035	4.20±0.035	2.87±0.052	2.20±0.040	4.57
<i>F. semitectum</i>	9.00±0.029	5.20±0.064	3.30±0.052	2.80±0.092	5.08	9.00±0.064	4.57±0.069	3.10±0.046	2.43±0.104	4.78
<i>M. phaseolina</i>	9.00±0.064	4.83±0.040	3.33±0.052	2.90±0.046	5.02	9.00±0.064	3.93±0.075	2.70±0.052	1.93±0.098	4.39
<i>P.splendens</i>	9.00±0.064	3.90±0.035	3.13±0.052	2.93±0.064	4.74	9.00±0.075	4.60±0.052	3.37±0.075	2.83±0.046	4.95
<i>R. solani</i>	9.00±0.035	4.93±0.087	3.10±0.046	2.73±0.052	4.94	9.00±0.029	5.20±0.052	3.70±0.035	3.17±0.069	5.27
Mean(B)	9.00	4.83	3.33	2.88	---	9.00	4.50	3.15	2.51	---
LSD at 5%		A=0.47	B=0.23	AxB=0.45			A=0.50	B=0.20	AxB=0.39	

In vivo evaluation of essential oils and elicitors for their efficacy in controlling root-rot diseases under greenhouse conditions

Data in Table (3) and Figures (1 and 2) showed that the use of essential oils (lemon grass and clove), potassium silicate, Bio-Arc® have a significant reduction to the root-rot incidence in comparison with the untreated control. However, in general, the chemical fungicide Topsin-M was the most effective treatment in controlling the root-rot disease caused by any of the five fungal pathogens tested. Nevertheless, there was no significant difference between the efficacy of Topsin-M

and Bio-Arc® in controlling *F. semitectum* on seashore Paspalum and between Topsin-M and clove oil in controlling *R. solani* on Bermuda grass. Topsin-M was followed by Bio-Arc® with all fungal pathogens on both grasses except for *M. phaseolina* and the fungus like *P. splendens* on seashore Paspalum for which, potassium silicate and clove oil were the second best, respectively (Table 3). It was noted that seashore Paspalum plants were more resistant to root-rot disease than Bermuda grass plants (Table 3).

Table 3. Effect of two essential oils, potassium silicate, Bio-Arc® and Topsin-M fungicide on root-rot incidence (%) on Bermuda grass and seashore Paspalum plants grown in soil artificially infested with some root-rot pathogenic fungi 60 days after transplanting.

Treatment	% Disease incidence																	
	<i>F. oxysporum</i>			<i>F. semitectum</i>			<i>M. phaseolina</i>			<i>P. splendens</i>			<i>R. solani</i>					
	Bermuda	Paspalum	Mean (A)	Bermuda	Paspalum	Mean (A)	Bermuda	Paspalum	Mean (A)	Bermuda	Paspalum	Mean (A)	Bermuda	Paspalum	Mean (A)			
Essential oil																		
Lemongrass oil	20.00 ±0.144	20.00 ±0.081	20.00	25.00 ±0.237	20.00 ±0.150	22.50	20.00 ±0.133	25.00 ±0.133	22.50	25.00 ±0.196	25.00 ±0.237	25.00	30.00 ±0.375	30.00 ±0.191	30.00			
Clove oil	15.00 ±0.358	15.00 ±0.202	15.00	20.00 ±0.202	20.00 ±0.081	20.00	15.00 ±0.283	25.00 ±0.121	20.0	20.00 ±0.069	20.00 ±0.185	20.00	15.00 ±0.139	25.00 ±0.237	20.00			
Elicitors																		
Potassium silicate	15.00 ±0.139	20.00 ±0.248	17.50	20.00 ±0.237	20.00 ±0.139	20.00	15.00 ±0.358	15.00 ±0.162	15.00	20.00 ±0.087	25.00 ±0.196	22.50	25.00 ±0.075	25.00 ±0.121	25.00			
Bio-Arc®	10.00 ±0.127	15.00 ±0.035	12.50	15.00 ±0.110	15.00 ±0.196	15.00	15.00 ±0.242	20.00 ±0.242	17.50	20.00 ±0.502	25.00 ±0.104	22.50	20.00 ±0.127	20.00 ±0.069	20.00			
Fungicide																		
Topsin-M	5.00 ±0.202	5.00 ±0.375	5.00	10.00 ±0.150	15.00 ±0.242	12.50	5.00 ±0.248	5.00 ±0.127	5.00	00.00 ±0.00	5.00 ±0.242	2.50	15.00 ±0.191	15.00 ±0.191	15.00			
Control	38.33 ±0.294	26.67 ±0.156	32.50	48.30 ±0.139	26.67 ±0.248	37.50	33.33 ±0.069	26.67 ±0.196	30.00	36.67 ±0.075	30.00 ±0.191	33.34	48.33 ±0.121	38.33 ±0.121	43.33			
Mean(B)	17.1	16.67	---	23.10	19.50	---	17.20	19.95	---	19.18	21.25	---	27.08	24.58	---			
LSD _{at5%}	A=2.41B=2.04AxB=2.88			A=1.98B=1.78xB=2.52			A=0.57			=0.41AxB=0.58			A=0.19B=31AxB=0.43			A=0.56B=0.43AxB=0.61		

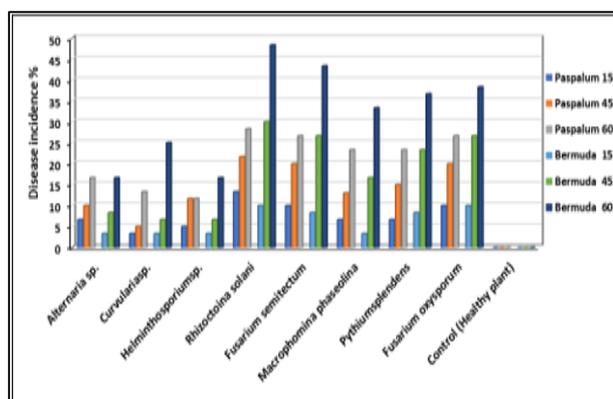


Figure 1. Disease incidence (%) caused by fungi isolated from either seashore Paspalum or Bermuda grass plants 15, 45 and 60 days after transplanting in artificially infested soil with the tested fungi.

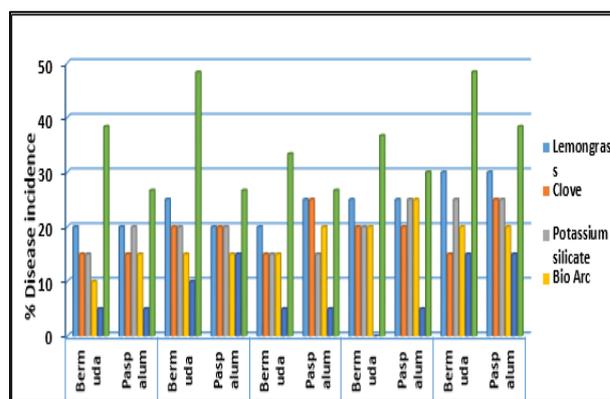


Figure 2. Effect of two essential oils, potassium silicate, Bio-Arc® and Topsin-M fungicide on root-rot incidence (%) on seashore Paspalum and Bermuda grass plants grown in soil artificially infested with the tested pathogenic fungi 60 days after transplanting.

Estimation of the total phenols and chlorophylla & b contents

In general, data presented in Table (4) and Figures (3, 4 and 5) show that all tested treatments significantly increased the total phenols and chlorophylla & b contents in grass plants in comparison with the untreated control. Overall, Bio-Arc® followed by lemon grass and potassium silicate treatments produced the highest level of total phenols in both grasses against all fungal pathogens tested (Table 4).

For Bermuda grass, the highest level of total phenols was obtained with the clove oil followed by potassium silicate on plants grown in soil artificially infested with *R. solani*. These were followed by lemon grass and Bio-Arc® against *F. oxysporum*, which were

significantly higher than the total phenols obtained with the chemical fungicide (Table 4).

For seashore Paspalum, the highest level of total phenols was obtained with the Bio-Arc® against the fungus like *P. splendens* followed by the clove oil on plants grown in soil artificially infested with *R. solani* and then lemon grass and Bio-Arc® against *F. oxysporum*, which were significantly higher than the total phenols obtained by the chemical fungicide (Table 4). However, the least phenolic contents were recorded in the untreated control followed by the chemical fungicide treatments (Table 4).

For both Bermuda grass and seashore Paspalum, the highest total chlorophyll (a & b) content was obtained by the clove oil, lemon grass, and the chemical fungicide (Table 4).

Table 4. Effect of two essential oils, potassium silicate, Bio-Arc® and a chemical fungicide on chlorophyll content and total phenol in 45-days old grass plants grown in soil artificially infested with some root rot pathogenic fungi.

Treatment	Fungus	Total phenols		Chlorophyll a		Chlorophyll b		Total chlorophyll	
		Bermuda	Paspalum	Bermuda	Paspalum	Bermuda	Paspalum	Bermuda	Paspalum
Clove oil	<i>F. oxysporum</i>	0.22	0.24	2.00	1.98	4.90	4.50	6.90	6.48
	<i>F. semitectum</i>	2.32	2.40	2.00	1.98	4.90	4.50	6.90	6.48
	<i>M. phaseolina</i>	1.93	2.02	2.01	1.99	4.90	4.40	6.91	6.39
	<i>P. splendens</i>	1.35	1.36	2.04	2.00	4.80	4.40	6.84	6.40
	<i>R. solani</i>	7.83	5.75	1.96	1.94	4.90	4.50	6.86	6.44
	Mean	2.73	2.35	2.00	1.98	4.88	4.46	6.88	6.44
Lemon grass oil	<i>F. oxysporum</i>	5.33	5.34	2.01	1.99	4.90	4.50	6.91	6.49
	<i>F. semitectum</i>	5.11	4.74	2.00	1.96	4.70	4.30	6.70	6.26
	<i>M. phaseolina</i>	1.33	1.33	2.04	2.01	4.90	4.40	6.94	6.41
	<i>P. splendens</i>	0.37	0.37	1.91	1.91	5.00	4.50	6.91	6.41
	<i>R. solani</i>	4.12	3.76	2.76	2.59	4.20	3.90	6.96	6.49
	Mean	3.25	3.11	2.14	2.09	4.74	4.32	6.88	6.41
Potassium silicate	<i>F. oxysporum</i>	3.30	2.22	2.03	2.00	4.80	4.40	6.83	6.40
	<i>F. semitectum</i>	3.15	2.11	1.95	1.93	4.90	4.50	6.85	6.43
	<i>M. phaseolina</i>	3.68	2.47	2.02	1.99	4.70	4.30	6.72	6.29
	<i>P. splendens</i>	0.26	0.19	1.85	1.85	4.90	4.40	6.75	6.25
	<i>R. solani</i>	6.47	4.32	1.85	1.85	4.90	4.40	6.75	6.25
	Mean	3.37	2.26	1.94	1.92	4.84	4.40	6.78	6.32
Bio-Arc	<i>F. oxysporum</i>	5.30	5.31	2.18	2.08	4.10	3.80	6.28	5.88
	<i>F. semitectum</i>	2.99	3.00	1.42	1.59	6.90	6.00	8.32	7.59
	<i>M. phaseolina</i>	1.53	1.52	1.91	1.90	5.00	4.60	6.91	6.50
	<i>P. splendens</i>	7.25	7.32	1.92	1.91	4.90	4.50	6.82	6.41
	<i>R. solani</i>	3.58	2.40	1.67	1.71	5.10	4.60	6.77	6.31
	Mean	4.13	3.51	1.82	1.84	5.20	4.70	7.02	6.54
Topsin-M	<i>F. oxysporum</i>	2.21	1.49	1.51	1.58	5.30	4.70	6.81	6.28
	<i>F. semitectum</i>	2.67	2.46	1.68	1.72	5.10	4.60	6.78	6.32
	<i>M. phaseolina</i>	1.03	0.70	2.19	2.13	4.70	4.30	6.89	6.43
	<i>P. splendens</i>	2.28	2.86	2.48	2.37	4.60	4.30	7.08	6.67
	<i>R. solani</i>	1.06	0.72	2.19	2.13	4.70	4.30	6.89	6.43
	Mean	1.85	1.65	2.01	1.99	4.88	4.44	6.89	6.43
Untreated control	<i>F. oxysporum</i>	1.52	0.24	1.51	1.59	3.60	2.99	5.11	4.58
	<i>F. semitectum</i>	2.02	2.11	1.42	1.42	3.30	3.68	4.72	5.10
	<i>M. phaseolina</i>	0.70	0.80	1.91	1.71	3.90	2.99	5.81	4.70
	<i>P. splendens</i>	0.20	0.30	1.91	1.94	3.15	2.67	5.06	4.61
	<i>R. solani</i>	1.06	2.40	1.68	1.72	3.80	3.58	5.48	5.30
	Mean	1.10	1.17	1.69	1.68	3.55	3.18	5.24	4.86
LSD at 5%		0.18	0.19	0.16	0.17	0.19	0.19	0.22	0.29

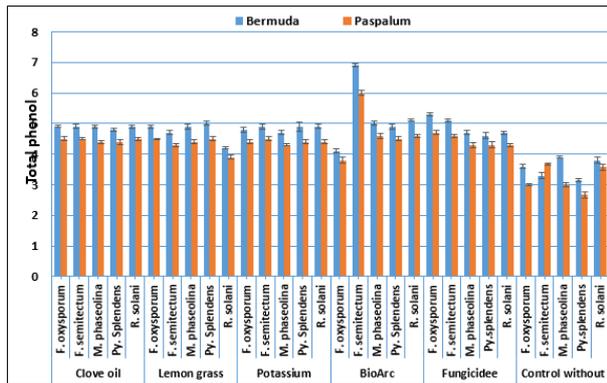


Figure 3. Effect of on total phenols content in 45-days old plants under infection by the fungi tested.

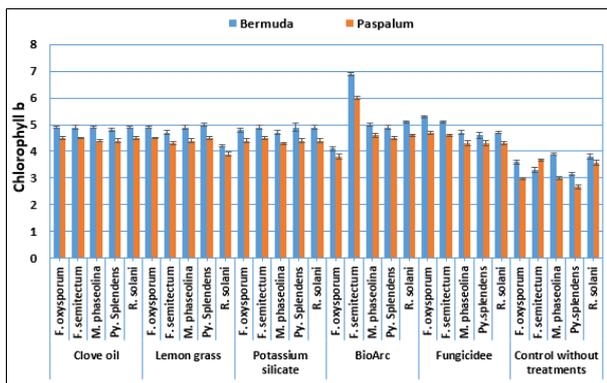


Figure 4. Effect of the treatments tested on chlorophyll a in 45-days old plants under infection by the fungi tested.

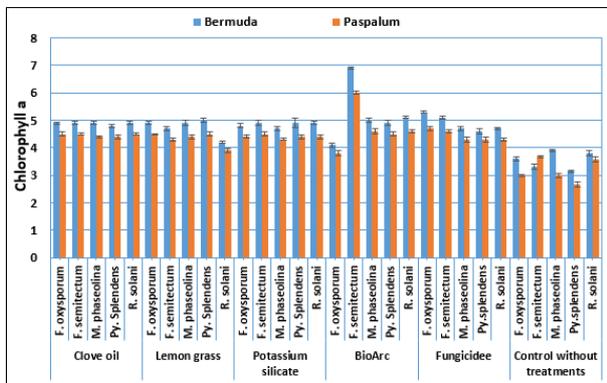


Figure 5. Effect of the treatments tested on chlorophyll b in 45-days old plants under infection by the fungi tested

Discussion

The present study showed that root-rot disease of seashore Paspalum and Bermuda grass are widely spread in Cairo, Giza, and Alexandria governorates. The disease caused a great reduction in the herb and healthy of plants, which cover wide area of lands in the gardens and important places in all governorates at Egypt.

Many fungi were isolated from naturally infected seashore Paspalum and Bermuda grass plants with typical wilt and root-rot symptoms collected from different gardens located in these governorates. The recovered fungi were purified, and then identified with the help of colleagues in the Department of Mycology and Plant Diseases Survey, Plant Pathology Research Institute, ARC, Giza, Egypt. Disease symptoms were previously described by Plates and

Vandler(1981); Skip and Christensen(1989) and Zhang *et al.* (2018). Most of fungi were isolated with different frequencies from the infected roots and rhizomes crown of the collected plants from the inspected governorates. The isolated fungi with highest frequency were *Alternaria* sp., *Curvularia* sp., *Helminthosporium* sp., *F. oxysporum*, *F. semitectum*, *R. solani*, *M. phaseolina* and the fungus like *P. splendens*. The obtained data were in agreement with those recorded by Hyakumachi *et al.* (1998); Kim and Park(1999); El-Hareedy(2004) and Adikaram(2017). These soil-borne pathogens have a broad host range in both non-cultivated and cultivated crops including turfgrasses. They damage roots and cause root-rot of plants, which lead to main economic losses in the production of turfgrass for landscape. Root-rot diseases are often difficult to control and cannot be managed solely using crop rotations, improved disease-resistant varieties. The application of fungicides and biocontrol products are also important management tools for soil-borne disease management (Baysal-Gurelet *et al.* 2012).

The use of plant oils such as clove and lemongrass oils inhibited the growth of the root rot fungi under present study. Clove oil was more effective than lemongrass oil in suppressing the tested pathogens growth. This finding is in agreement with those of Handique and Singh (1990), Brumet *et al.*(2014), and Kauret *et al.* (2019).

In this regard, essential oils from the aerial parts of *Mentha X piperita* showed a significant antifungal activity against *Alternaria alternata*, *Fusarium tabacinum*, *Penicillium* spp., *Fusarium oxysporum*, and *Aspergillus fumigatus* (Reddy *et al.* 2019). Clove oil provides antimicrobial activity against soil-borne fungi, plant pathogens, and mycotoxigenic fungi (Sreenivasa *et al.* 2011 and Morcia *et al.* 2012). Eugenol, acetyl eugenol, isoeugenol, and γ -caryophyllene are the primary chemical components of clove oil (Rahimi *et al.* 2012). The antibacterial and antifungal effects of essential oils are due to these phenolic molecules (Akthar *et al.* 2014). Our results are consistent with those of Sharma *et al.* (2017) and Furdos (2017). Similar studies were done by Abdulaziz and Younes (2010) who found that *Fusarium* sp. was sensitive to all of the essential oils examined, but clove oil was the most effective one.

The *in-vitro* studies in the present study showed the effect of potassium silicate in inhibiting the growth of the tested fungi. In parallel to this finding, Marschner (2012) explained that K is a major plant element which plays a major role in photosynthesis, a variety of physiological processes, maintenance of water status, and protein synthesis in plant tissues. In addition, potassium silicate modulates antioxidant system, while the antioxidant antimicrobial action could be, also, due to inhibition of the functions of many enzymes by oxidizing the membrane lipids as they interfere with the membrane functions including proteins, RNA and DNA synthesis (Nesci *et al.* 2003). Bekker *et al.* (2006) investigated the effects of soluble potassium silicate (20.7 percent SiO₂) on *Phytophthora cinnamomi*, *Sclerotinia sclerotiorum*, *Pythium* sp., *Mucor pusillus*, *Drechslera* sp., *Fusarium oxysporum*, *Fusarium solani*, *Alternaria solani*, *Colletotrichum coccodes*, and *Verticillium* sp. All fungi studied showed 100% inhibition of mycelial growth at 80

ml (pH 11.7) and 40 ml (pH 11.5) soluble potassium silicate per liter of agar, with the exception of *Drechslera* sp. and *F. oxysporum* at 40 ml in one trial. At all soluble potassium silicate concentrations between 5 and 80 mL⁻¹ agar, only *Sclerotinia sclerotiorum* and *Phytophthora cinnamomi* were totally inhibited, while all other fungi were only moderately inhibited at potassium silicate concentrations of 5, 10, and 20 mL⁻¹ agar. Similarly, dose was positively linked with the percent inhibition. *In vitro*, inhibition of mycelial growth of phytopathogenic fungi cultured on potassium silicate-added media was demonstrated by Bekker et al. (2009). According to Nada et al. (2014) potassium silicate was more effective than calcium and sodium silicates in minimizing damping-off and enhancing coriander plant development characteristics. Also, according to Kanto et al. (2006) potassium and calcium silicates reduced Fusarium wilt of cucumber for three years longer than sodium silicate. Furthermore, Jayawardana et al. (2014) found that applying soluble potassium silicate to the roots and foliar reduced disease incidence and improved plant growth and fruit quality indices. Silicon, according to Liang et al. (2005) can limit pathogen entry into host tissues. Eventually, it's likely that the reduction in disease occurrence in plants treated with silicon sources under field settings isn't due to the fungistatic effects of silicon, but rather to silicon acting as a physical barrier against pathogen penetration or as an inducer of plant defensive response (Shenet et al. 2010).

Bio-Arc® (*Bacillus megaterium*), Bio-Zeid® (*Trichoderma album*) as biocides play a very useful role as effective and safe means in controlling root-rots. In this respect, similar results were obtained by Bardin et al. (2003) and Chavan et al. (2004) on the positive efficacy of treating with bio-agents. In this regard, microbial inoculants have paramount significance in integrated nutrient management systems to sustain agricultural productivity and healthy environment (Adesemoye and Kloepper, 2009). *Bacillus* spp. are capable of generating resistance in plants against a variety of diseases (Kloepper et al. 2004). *B. subtilis* activated induced systemic resistance (ISR) in rice against *R. solani* via jasmonic acid (JA), ethylene (ET), abscisic acid (ABA), and auxin signaling, according to Chandler et al. (2015). The same scientists found that *B. subtilis* LPs, such as fengycin and surfactin, are critical in the induced defense state. Fungicides, soil fumigants, and bioagents can all be used to control soil-borne plant diseases, including root-rot. Because of concerns about fungicide toxicity, there is a general trend to limit the amount of fungicide sprayed to soil.

In the present investigation, the effect of all tested treatments increased the total phenols and chlorophylla & b in grass plants. Overall, Bio-Arc® followed by lemon grass and potassium silicate treatments produced the highest level of total phenols in both grasses against all fungal pathogens tested. Amin and Diab (2020) found that essential oils had a positive effect on total sugar concentrations, level of total indoles and total phenols and total chlorophyll contents of cut flowers. Essential oils as an alternative substitute to chemical compounds had an excellent effect on cut flowers because of their antimicrobial activities and environmental friendly nature, where phenol content is known to play a vital function in plant resistance and serves as a defense mechanism against plant pathogen invasion.

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مقاومة اعفان جذور المسطحات الخضراء باستخدام الزيوت النباتية والمبيدات الحيوية والكميائية

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المخلص

تلعب المسطحات الخضراء دوراً مهماً في تحسين البيئة المحيطة ونوعية الحياة في المدن الجديدة، وتعتبر الأمراض التي تنتقل عن طريق التربة من أكثر المعوقات التي تعوق إنتاج نباتات الزينة. تم عزل وتنقية الفطريات الممرضة من جذور صنف نجيل الباسلم والبرمودا من الحدائق المختلفة في الإسكندرية والقاهرة والجيزة وتم تعريفها *Helminthosporium* sp., *Rhizoctonia* sp., the fungi like *Pythium* sp., *Alternaria* sp., *Fusarium* sp. and *Macrophomina* sp. تصل من ٥٠ إلى ٧٥% من المسطح الأخضر. تم إجراء تجريبه معملياً لدراسة تأثير كل من زيت القرنفل وزيت حشيشة الليمون وسليكات البوتاسيوم والمبيد الحيوي بيوارك على نمو الفطريات محل الدراسة على بيئة أجار البطاطس والدكستروز في أطباق بتري في المعمل. وقد وجد أن استخدام البيوارك بمعدل ٦ جم/لتر كان الأكثر فعالية في تثبيط نمو الفطريات محل الدراسة. أيضاً أجريت تجربة أصص تحت ظروف الصوبة الزجاجية لدراسة تأثير زيت القرنفل وزيت عشب الليمون، وسليكات البوتاسيوم والمبيد الحيوي بيوارك التجاري بالإضافة إلى المبيد الفطري (الكيموي) توبسين إم ٧٠% (للمقارنة) لدراسة كفاءتها في مكافحة أمراض تعفن الجذور. وقد أظهرت نتائج تجارب الصوبة أن أفضل العلاجات كان استخدام المبيد الكيموي توبسين إم ٧٠% كمبيد فطري فعال تلاه زيت القرنفل ثم البيوارك. أما بالنسبة لتأثير العلاجات المذكورة على التغيرات البيوكيميائية لنباتات المسطحات الخضراء محل الدراسة، وجد أن أفضل العلاجات كانت استخدام المبيد الحيوي بيوارك، حيث أدى إلى زيادة في محتوى الفينول الكلي والكلوروفيل. وبصفة عامة، وجد أن الصنف برمودا كان أكثر مقاومة لأمراض اعفان الجذور من صنف باسيلم.