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COMPARATIVE EFFICACY OF FUNGICIDES, COMMERCIAL BIOPRODUCTS, ESSENTIAL OILS AND PLANT DEFENSE INDUCERS IN CONTROLLING POTHOS ROOT ROT

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Abstract

Production of Pothos (*Epipremnum aureum* L.) plants in Egypt is faced by different soil-borne fungi and yet scarce information on these diseases and their pathogens are available. Thus, Surveying Pothos plant pathogen(s) in greenhouses located at Giza Governorate, Egypt was carried out during 2015 and 2016. The root rot symptoms were recorded on about 17-21% of the examined plants. Isolation trials yielded *Rhizoctonia solani*, *Pythium* sp., *Sclerotium rofsii*, *Botryodiplodia* sp., *Trichoderma* sp., *Penicillium* sp., *Fusarium semitectum*, *F. solani*, *F. oxysporum*, Macrophomina phaseolina and Aspergillus sp. Pathogenicity of isolates was investigated, *R. solani* and *S. rolfsii* were the most aggressive fungi. Three essential oils, *i.e.* Eucalyptus, rue and thyme with three concentrations (2.5, 5 and 7%) and two plant defence inducers, *i.e.* tannic acid and salicylic acid at two concentrations (300 and 700 ppm) were tested against *R. solani* and *S. rolfsii* in vitro. Rue oil at 7% was the most effective in reducing mycelium growth of both fungi. Also, salicylic acid at 700 ppm was more effective than tannic acid in this regard. In the greenhouse, three biofertilizers (rhizobacterin, phosphorin and potassiumag) at 5%, two biocides (Bio-Cure-F and Bio-Arc) at 3.5 g/l, Salicylic acid (700 ppm) and rue oil (7%) as well as two chemical fungicides (Carbendazim and Rhizolex-T) were tested. Salicylic acid, rue oil and Bio-Cure-F gave the highest protection against the tested fungi. Meanwhile, Carbendazim was more effective than Rhizolex-T and any other treatment else.

Key words: Biofertilizers, biocides, essential oils, plant defence inducers and *Trichoderma viride*.

Introduction

Epipremnum aureum (Linden and Andre) G.S. Bunting belongs to Araceae family extremely popular as cultivated plants worldwide. It is synonymous with Golden Pothos. It is usually produced in hanging baskets for the patio or in small pots for dish gardens (Meshram and Srivastava, 2014).

Production of Pothos plant is faced by different diseases especially those caused by soil borne fungal pathogens. Though, very little information are available about diseases affecting Pothos plant in Egypt. Phytophthora root rot, Rhizoctonia root rot, and Southern blight caused by *Phytophthora nicotianae*, *Rhizoctonia solani* and *Sclerotium rolfsii*, respectively are the most destructive diseases affecting Pothos plants worldwide (Norman and Ali 2018). Control methods relying on the cultural practices and fungicides. However, the regular use of fungicides could lead to adverse impacts to the public human and animal health. Therefore, in order to solve problems of extensive use of fungicides, several scientists have engaged safe alternatives means to control soil borne diseases such as essential oils, plant growth promoting rhizobacteria and plant defense inducers (Brown *et al.*, 2019). Plant essential oils are volatile natural complex compounds formed by different aromatic plants as secondary metabolites. These metabolites play an important role in competing plant pathogens both *in vitro* and *in vivo* (Brown *et al.*, 2019 and Han *et al.*, 2019). Plant products damage pathogens by different mechanisms such as cell membrane disruption and enzymes inactivation (Aye and Matsumoto, 2011). The major compounds in plants responsible for fungicidal or fungistatic activity are phenols, flavanoids, quinones, terpenes, tannins, alkaloids, lectins, polypeptides, saponins, sterols, etc. (Sajeena *et al.*, 2019).

Plant growth promoting microorganisms (PGPB)

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are another useful mean. These microorganisms belong to genera *Azotobacter, Bacillus, Klebsiella, Enterobacter, Arthrobacter, Burkholderia, Pseudomonas, Serratia*, etc and can be obtained from soil and different habitat. Because of their positive effects on the plant growth and health, different bioformulations contain one or more of them were developed in the world markets with various headings such biocides, biofertilizers and biostimulans. In general, PGPR play vital role in agriculture by preventing plant pathogens “Biocontrol”, improving the uptake of nutrients from the soil “Biofertilization” and synthesizing phytohormones “Biostimulants” (Kenneth *et al*., 2017).

Synthetic chemical inducers of plant immunity are structurally different from the natural plant defense elicitors. They may activate or prime plant immunity by simply mimicking the structures of natural immune inducers. Alternatively, they can also be structurally unrelated to natural elicitors and target a subset of defense signaling components (Zhou and Wang, 2019). As a major plant immune hormone, salicylic acid (SA) plays a pivotal role in the establishment of plant immunity. SA and its famous synthetic derivative, Aspirin, is among the first plant endogenous chemicals reported to induce systemic acquired resistant (SAR), which is accompanied by accumulation of pathogenesis related proteins (PR proteins) and resistance to tomato mosaic virus (TMV) in tomato plants (White, 1979).

This study aims to throw light on the causal fungi affecting Pothos plant production in the Egypt with respect to its possible management using fungicides, commercial bioproducts, essential oils and plant defense inducers.

Materials and methods

Disease survey

Epipremnum aureum (Linden and Andre) G.S. Bunting plants growing in commercial greenhouses located at Giza governorate were surveyed during 2015 and 2016 for the presence of root rot symptoms. Furthermore, affected cuttings and plants were collected and transferred into the laboratory to isolate the causal pathogens and associated microorganisms on PDA medium.

Isolation and identification of fungi and the microorganisms associated with root rot symptoms.

In order to isolate the causal fungi of Pothos root rot disease and its associated microorganisms, diseased roots of Pothos plants showing rotting discoloration symptoms were collected from different commercial greenhouses located at Giza governorate, Egypt. Infected stems and roots were washed several times under running tap water, cut into small pieces (0.5 cm long), surface sterilized by immersing in 2% sodium hypochlorite for 2 min, washed in several changes of sterile water and finally aseptically were transferred onto potato dextrose agar medium (PDA) in Petri plates (9 cm in diam,) and incubated at 25±1ºC for 7days. The emerged fungi were purified, then identified according to their morphological and microscopical characteristics as described by Booth (1977) and Barnett and Hunter (1986). The obtained culture isolates were maintained on PDA slants and kept in refrigerator at 5°C for further studies. The frequency of fungi emerged from 300 infected plant part was separately calculated.

Pathogenicity test

The most frequently isolated fungi were subjected to Pathogenicity tests in the greenhouse of Orman Botanical Garden, Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. Inocula of the tested fungi were allowed to grow in 500 ml milk bottles, each containing 75gm washed dried barley, 100gm washed dried coarse sand and 65ml potato decoction and incubated at 25±1ºC for two weeks. Formalin-disinfested plastic pots (15-cm-diam.) filled with Formalin-disinfested German peat moss and washed sand mixture (1:1 v/v) were infested with any of the tested fungi at the rate of 3% (w/w), 7 days before planting. Experimental blocks design, completely randomized with three replicated pots was used for each isolated fungus. Four apparently healthy Pothos cutting obtained from an nursery located at El-Manawat Village, Giza, Egypt were transplanted in each pot. All agricultural practices were carried out according to the recommendation of Ministry of Agric., Egypt. The percentage of the disease incidence was estimated 60 days after transplanting as follows:

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected plants}}{\text{Total no. of plants}} \times 100$$

Extraction of essential oils:

Aerial partsof Rue (*Ruta graveolens* L.), Eucaluptus (*Eucalyptus camaldulensis* L.) and Thyme (*Tymus vulgaris* L.) plants were collected from Medicinal, Aromatic and Poisonous Plants Experimental Station, Faculty of Pharmacy Cairo University, Giza, Egypt. The leaves were washed, then the volatile oils were separately extracted from the samples by hydrodistillation for 6 hr in a Clevenger type apparatus.

Antifungal activity of eucalyptus, rue and thyme oils against *R. solani and S. rolfsii*

The antifungal activity of Eucalyptus, rue and thyme oils with three different concentrations (2.5, 5 and 7%) against *R. solani* and *S. rolfsii* was determined by using the poisoned food technique described by Adjou *et al*., (2012). Once the fungus mycelium fill the plates in any plate, the radial growth (mm) was measured and percent inhibition in radial growth (I) was calculated using the formula suggested by Philippe *et al.*, (2012) as follows.

I(%) = (C - T)/C × 100, Where, I = Inhibition of mycelia growth, C = Growth of fungus in control, T = Growth of fungus in treatment

Antifungal activity of salicylic acid and tannic acid against *R. solani and S. rolfsii*

The antifungal properties of salicylic acid and tannic acid against *R. solani* and *S. rolfsii* were determined by using poisonous plates where, the desired salicylic acid and tannic acid concentrations (300 and 700 ppm) were separately mixed in PDA medium before solidification, then poured in a sterile Petri dishes (9 cm). Three replicated plates for each concentration were used. Each plate inoculated with 5 mm fungal disc of each fungus. Treatments were kept at 25±1°C. The linear growth of each fungus was measured when the full growth of the tested fungi was observed in the control treatment. The mycelial growth (mm) of the fungi both in treated (T) and control (C) was measured and percent inhibition in radial growth was calculated as mentioned before.

Comparative efficacy of rue oil, salicylic acid, commercial bioproducts and fungicides in controlling Pothos root rot caused by *R. solani* and/or *S. rolfsii* in the greenhouse.

Rue oil (7%), salicylic acid (700 ppm), two biocides (Bio-Cure-F, Bio-Arc), three biofertilizers (Phosphorin, rhizobacterin, potassiummag) and two fungicides (Carbendazim and Rhizolex-T) were tested against Pothos root rot caused by *R. solani* and *S. rolfsii* under greenhouse conditions. The source, composition and the recommended doses of the tested biofertilizers, biocides and fungicides are shown in Table (1). Apparently healthy Pothos cuttings were separately soaked in rue oil, salicylic acid and Bio-Cure-F as well as in the tested fungicides for 30 min, then transplanted in plastic pots (15 cm-diam) containing peat moss pre- inoculated with 3% of any of the tested fungi. Extra 50 ml of rue oil, salicylic acid and Bio-Cure-F were separately added around the root system at the time of transplanting. The biofertilizers were tested as soil amendment at the time of transplanting. Experimental blocks design, completely randomized with three replicated pots was used for each isolated fungus. Four apparently healthy Pothos cuttings were transplanted in each pot. The percentage of the disease incidence was estimated 60 days after transplanting as mentioned before.

Statistical analysis

Data were subjected to statistical analysis using WASP 2.0 program. The least significant difference (LSD., $p \leq 0.05$) for comparison between the means of treatments was used as mentioned by Gomez and Gomez (1984).

Results and Discussion

Disease survey

Epipremnum aureum (Linden and Andre) G.S. Bunting plants growing in commercial greenhouses located at Giza governorate, were surveyed during 2015 and 2016 for the presence of root rot symptoms. Disease symptoms were recorded on about 17-21% of the examined plants.

Isolation, identification and frequency of fungi associated with Pothos root rot.

Different fungi belonging to 10 genera were recovered from 300 infected Pothos tissues. *Rhizoctonia solani* Khun. was the most dominant fungus. It was isolated from 22.33% of the infected plant parts. *Fusarium semitectum* Berk & Rav., Fusarium solani (Mart.) Sacc., *Pythium* sp. Pringsheim, Fusarium oxysporum Schlecht. and *Sclerotium rofsii* Sacc. ranked second by 16.67, 15.0, 9.0, 9.0 and 9.0%, respectively. Additionally, Alternaria sp. Pringsh, *Botryodiplodia* sp. Pat., *Trichoderma* sp. Pers., *Penicillium* sp. Link, Macrophomina phseolina (Tassi) Goid and Aspergillus sp. Micheli were isolated from the affected plant tissues with the lowest frequent percentages being, 4.0, 3.67, 3.0, 3.33, 2.33 and 2.0%, respectively. These results are in harmony with those obtained by El-Morsy (2000) who found that Fusarium solani, *R. solani* and *Pythium* splends were the most frequent fungi isolated from Pothos plants in Egypt.

Pathogenicity test

Data illustrated in Table 3 indicate that all the tested fungi were able to infect Pothos plants but with different levels. The most aggressive fungi were *R. solani* and *S. rolfsii*. Each of them affected 83.33 % of the tested plants. On the other hand, *Pythium* sp., M. phaseolina and *Botryodiplodia* sp. were less aggressive. They affected about 58.33, 25.0 and 33.33% of the tested plants, respectively with 41.67, 75.0 and 66.67% survived plants, respectively. These results are in harmony with those obtained by El-Morsy (2000) who found that *R. solani*, P. splendes and *F. solani* were the most pathogenic fungi on Pothos plants in Egypt. Also, Harmon and Bledsoe (2015) mentioned that Rhizoctonia root rot can cause

Table 1: Commercial bioproducts and fungicides used against pathogenic fungi.

Compound trade name	Constituents or active ingredient	producer	Recommen--ded dose
Phosphorin	*Bacillus megaterium* var. *Phosphaticum* 1 x 10^9 cfu/ml	General Organization for Agric. Equalization, Fund, ARC, Giza, Egypt	5%
Rhizobacterin	*Azotobacter chroococcum* 3 × 10^7 cfu/g		5%
Potassiumag	*Bacillus verculanes* 1 × 10^8 cfu/g		5%
Bio-Arc	*Bacillus subtilis* 3 × 10^7 cfu/ml	El-Nasr Co.	3.5g/l
Bio-Cure-F	*Trichoderma viride* 1 x 10^9 spore/ml	T. STANES&COMPANY LIMITED	3.5 g/l
Carbendazim 50% WP	Carbendazim	Agriphar S.A., Bllgium	2 g/l
Rhizolex-T 50% WP	Tolclofosm methyl + Thiram	Sumitomo Chemical Co., Ltd., Japan	2 g/l

significant damage to Pothos cuttings during the rooting stage. *S. rolfsii* was isolated by Orlikowski and Ptaszek (2013) for the first time in Poland from 90% of the infected plant parts.

Antifungal activity of eucalyptus, rue and thyme oils against *R. solani and S. rolfsii*

Data presented in Table 4 show that the tested oils significantly reduced the growth of both fungi. The percent of mycelium reduction was varied from 60.03 to 73.70% on the average for *R. solani* and from 53.73 to 66.70% on the average for *S. rolfsii*. Increasing the concentration of the tested oils resulted in increasing of the mycelium growth reduction. Rue oil was the most effective against *R. solani* (73.70% inhibition on the average) and *S. rolfsii* (66.70% inhibition on the average).

Antifungal activity of tannic acid and salicylic acid against *R. solani* and *S. rolfsii*

The antifungal activity of tannic acid and salicylic acid was tested against *R. solani* and *S. rolfsii* with two different concentrations (300 and 700 ppm). The activity of both compounds in reducing the mycelium growth was increased by increasing of the tested concentration. The percentages of mycelium inhibition are shown in Table 5. Increasing the concentration of the tested treatments to 700 ppm resulted in increasing of the mycelium growth reduction. The mycelium reduction was varied from 16.5 to 32.6% in case of *R. solani* and from 0.2 to 68.6% on the average in case of *S. rolfsii*. Salicylic acid was more effective than tannic acid in this respect where the percentage of inhibition reached the maximum value in plates treated with Salicylic acid 32.6% on the average for *R. solani* and 68.6% on the average for *S. rolfsii*.

Comparative efficacy of rue oil, salicylic acid, commercial bioproducts and fungicides in controlling Pothos root rot caused by *R. solani* and *S. rolfsii* in the greenhouse

Data presented in Table 6 show that salicylic acid, rue oil and Bio-Cure-F were highly effective in reducing the disease incidence caused by the tested fungi. As for the effect of the three treatments against *R. solani*, they reduced the disease incidence by 66.67, 66.67 and 66.67%, respectively. The results of the three treatments against *S. rolfsii* haven't changed much in comparison with their effect on *R. solani* where they reduced the disease incidence by 66.67, 66.67 and 58.34%,

Table 2: Frequency (%) of fungi isolated from naturally infected Pothos plants collected from commercial greenhouses of Giza governorate.

Isolated fungi	Isolation frequency (%)
Alternaria sp.Pringsh	04.0
*Aspergillus*sp.Micheli	02.0
Botryodiplodia sp. Pat.	3.67
*Fusarium oxysporum*Schlecht.	09.0
Fusarium solani(Mart.) Sacc.	15.0
*Fusarium semitectum*Berk&Rav.	16.67
Macrophomina phseolina(Tassi) Goid	2.33
*Penicillium*sp.Link	3.33
Pythium sp. Pringsheim	9.33
Rhizoctonia solani Khun.	22.33
*Sclerotiumrofesii*Sacc.	9.33
*Trichoderma*sp.Pers.	03.0
Total	100

Table 3: Pathogenicity of the isolated fungi to Pothos plants under greenhouse conditions 60 days after planting.

Fungi	Disease incidence (%)	Survived plants (%)
Botryodiplodia sp.	33.33	66.67
M. phaseolina	25.0	75.0
F. oxysporum	75.0	25.0
F. solani	66.67	33.33
F. semitectum	33.33	66.67
Pythium sp.	58.33	41.67
R. solani	83.33	16.67
S. rolfsii	83.33	16.67
Control	00.0	00.0
LSD 0.05%	5.49	-

Table 4: Antifungal activity of eucalyptus, rue and thyme oils against *R. solani and S. rolfsii.*

Oils	Inhibition (%) of mycelium growth							
	Concentrations (%)							
	R. solani				*S. rolfsii*			
	2.5	5.0	7.0	Mean	2.5	5.0	7.0	Mean
Eucalyptus	32.30	67.80	80.0	60.03	20.0	56.70	84.50	53.73
Rue	44.50	78.90	97.70	73.70	38.90	67.80	93.40	66.70
Thyme	34.50	72.30	94.50	67.10	30.00	65.50	90.0	61.83
Mean	37.10	73.0	90.73	-	29.63	63.33	89.30	-
LSD 0.05%	Oils(O)=0.13Con.(C)= 0.16O×C=0.27				Oil(O) =0.17 Con.(C) =0.19O×C=0.34			

respectively. Phosphorin and potassiumag were the lowest effective treatments. They recorded the lowest disease reduction percentages caused by *R. solani* and *S. rolfsii*, being 33.34-33.34% and 16.67- 8.34%, respectively. In general, chemical fungicides were more effective treatments. Carbendazim was the most suitable fungicide. It reduces the disease incidence by 91.67% for *R. solani* and 83.34% for *S. rolfsii*.

Different reports have been confirmed the potentiality of chemical resistance inducers such as salicylic acid in controlling root rot and wilt diseases under greenhouse and field conditions (El-Mohamedy *et al.*, 2015). In this respect, plants use a phytohormone, salicylic acid (SA), to induce defense responses against biotrophic pathogens. The SA-dependent signaling pathway leads to the production of antimicrobial molecules, such as pathogenesis-related proteins and phytoalexins, as well as the induction of hypersensitive responses (HR), often associated with programmed cell death in the infected area, which restricts pathogen colonization (Kouzai *et al.*, 2018). Also, SA has direct effect on the causal pathogen at the highest concentrations as reported in this study where it causes reduction in mycelium growth of *R. solani* and *S. rolfsii* at 700 ppm by 62.2 and 90%, respectively. Moreover, the high efficiency of rue oil against Pothos root rot during this study attributed to its high content of 2-undecanone as a major component (El-Sherbeny *et al.*, 2007).

Table 5: Antifungal activity of tannic acid and salicylic acid against *R. solani* and *S. rolfsii.*

Treat-ments	Inhibition (%) of mycelium growth					
	Concentrations (%)					
	R. solani			*S. rolfsii*		
	300	700	Mean	300	700	Mean
Tannic acid	00.0	33.0	16.5	00.0	00.4	0.2
Salicylic acid	03.0	62.2	32.6	47.1	90.0	68.6
Mean	1.5	47.6	-	23.6	45.2	-
LSD 0.05%	Treatments(T)=0.1 Con.(C)=0.2TxC=0.3			Treatments(T)=0.4 Con.(C)=0.5TxC=0.7		

On the other hand, despite the efficiency of biocontrol agents such as *Trichoderma viride* and *Bacillus subtilis* in reducing different fungal diseases under controlled conditions, only few of them have been commercially available. The positive effect of the application of Bio-Cure-F in this study may be attributed to its contains of *T. viride*. The success of this beneficial fungus in competing the pathogenic fungi is attributed to different mechanisms including mycoparasitism, antibiosis, competition for space and nutrients, promotion of plant growth, stimulation of lateral root development, degradation of toxic compounds, enhanced nutrient solubility, organic acids and volatile compounds secretion and induction of systemic resistance (Salwan *et al.*, 2019). Furthermore, biofertilizers entrapping living microorganisms such as those belonging to genera *Azotobacter, Bacillus, Klebsiella, Enterobacter, Arthrobacter, Burkholderia, Pseudomonas, Serratia*, etc. that called plant growth promoting microorganisms (PGPB) play a vital role in controlling plant diseases as well as promoting of plant growth. These organisms promote the plant growth by different mechanisms including, phosphate solubilization, nitrogen fixation, production of siderophore, ammonia, vitamins, phytohormones, antibiotics, cell wall degrading enzymes and induced systemic resistance (ISR), (Singh *et al.*, 2019). Thus, such biofertilizers recommended by Abo-El-Ela (2003) to be an effective mean in competing soil-borne fungal diseases on the ornamental plants. In this study the biofertilizer rhizobacterin reduced the disease incidence caused by *R. solani* and *S. rolfsii* by

Table 6: Comparative efficacy of rue oil, salicylic acid, commercial bioproducts and fungicides in controlling Pothos root rot caused by *R. solani* and/or *S. rolfsii* in the greenhouse.

Treat-ments	*R. solani*		*S. rolfsii*	
	Disease incidence (%)	Reduction (%)	Disease incidence (%)	Reduction (%)
Bio-Cure-F	33.33	66.67	41.66	58.34
Bio-Arc	58.33	41.67	50.0	50.0
Rue oil	33.33	66.67	33.33	66.67
Salicylic acid	33.33	66.67	33.33	66.67
Phosphorin	66.66	33.34	83.33	16.67
Rhizobacterin	50.0	50.0	66.66	33.34
Potassiumag	66.66	33.34	91.66	08.34
Rhizolex-T	25.0	75.0	41.66	58.34
Carbendazim	08.33	91.67	16.66	83.34
Control	100	00.0	100	00.0
LSD 0.05%	6.64		5.37	

50 and 33.33%, respectively as shown in Table 6. This positive effect of rhizobacterin attributed to its contains of *Azotobacter chroococum* which fixes the atmospheric nitrogen by converting it to more usable organic forms in the soil, thereby making it available to plants (Itelima *et al.,* 2018). As the nutrients, water uptake and plant growth improved, the plants became more tolerant to abiotic and biotic factors.

This work also showed that Carbendazim was more effective than Rhizolex-T fungicide and any other treatment else in reducing the disease incidence caused by *R. solani* and *S. rolfsii*. These results are in harmony with those obtained by Sriraj *et al.,* (2014) who reported that Nativo and Bavistin were effective in reducing the mycelia growth and sclerotial production of *R. solani* at lower concentration. Also, Kumar *et al.,* (2017) found that Propiconazole and Carbendazim were highly effective against mycelia growth and sclerotial production of *R. solani* at 200 ppm.

Conclusion

The result of this work indicate that *R. solani* and *S. rolfsii* were the most aggressive fungi affecting Pothos plant in Egypt. Treatment of Pothos cuttings pre-planting with rue oil at the rate of 7%, salicylic acid at 700 ppm or *T. viride* that available in the commercial biocide namely, Bio-cure-F at the rate of 3.5 g/l significantly reduced the Pothos root rot caused by *R. solani* and *S. rolfsii*. Also, *Azotobacter chroococum* that commercially marketing as rhizobacterin fertilizer positively protect the plant from infection by both fungi.

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