Effect of Some Plant Powders against *Fusarium* spp. and *Macrophomina phaseolina* Under Greenhouse Conditions

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Abstract: Chemical fungicides cause serious environmental problems. Plant based pesticides considered to be better alternatives as they are known to have minimal environmental impact in contrast to synthetic pesticides. The aim of this study was to assess the efficiency of some plant powders on controlling damping-off disease incited by the tested pathogens under greenhouse conditions. Seed treatment with the tested plant powders significantly reduced TIP values incited by the tested seed-born pathogens. The highest reduction rate (37.5%) was detected in R. raetam / F. semitictum / CREDO cv. treatment, whereas the least rate (4.55%) was realized in C. trifurcatum F. semitictum and F. moniliforme interactions in ANANAS cv.. The highest TIP reduction rates (33.33%, 23.53% and 22.22%) were observed in R. raetam / F. solani, R. raetam / F. moniliforme and R. raetam / M. phaseolina treatments, respectively. The highest reduction rate (25.0%) was detected in P. dactylifera / F. semitictum and F. solani interactions in CREDO cv. Significant reduction in PRD values was detected as a result of seed treatment with the tested plant extract, attaining maximum rates in R. raetam / F. solani (43.75%), F. semitictum (33.33%), M. phaseolina (28.57%) and F. moniliforme (23.07%), while in P. dactylifera treatment gave the highest reduction rates (37.5%, 26.32% and 25.0%) were observed in F. solani / CREDO cv., F. solani / ANANAS cv. and M. phaseolina / ANANAS cv. Significant reductions PTD in damping-off incidence in all cantaloupe cultivars were obtained due to treatment with most of the tested plant powders, but in some treatments were non effected, however, R. raetam was more efficient in reducing damping-off incited by F. semitictum, F. moniliforme and F. solani (42.86%, 28.57% and 27.27%, respectively compared with control). On the other hand, P. dactylifera and C. trifurcatum were more efficient in controlling damping-off caused by F. semitictum, where reduction%, compared damping-off incidence. Generally, R. raetam was more effected to controlling the tested pathogenic fungi followed by P. dactylifera.

Keywords: Damping-off pathogens, Plant powders, cucurbit seed-borne fungi, Antifungal activity

1. Introduction

Fungicides like many other chemicals that used for pathogens control have negative environmental consequensis. So, the modern approach in plant disease control is directed toward minimizing the fungicidal use to decrease environmental pollution and finding alternatives to chemical fungicides. Hence, in recent time application of plant powders as well as plant metabolites for plant disease management has become important viable component of integrated Pest Management, as these plant products are ecofriendly where botanicals place an important role (Sahayaraj et al., 2009). Several investigation studies have been conducted in order to screening different plants for their antifungal properties (Ja Choi et al., 2004; Stephan et al., 2005; Satish et al., 2010) and biochemical compounds that these plants have. Studies revealed a highly significant antifungal activity of some water extracts or essential oils of plants. Since some plants are already known to possess several biological activities (Amin et al., 2009; Barrera-Necha et al., 2009; Belabid et al., 2010). Therefore, the objective of this work was to controlling the isolated damping-off pathogens, using some plant powders under greenhouse conditions.

2. Materials and Methods

2.1 Disease Control

2.1.1 In vivo experiments

Soil was autoclaved at 1.5 Kg/cm2 for 90 minutes and then left to aerate for 7 days before adding the inoculum. Inocula were prepared by growing each of the tested pathogens (F. solani, F. moniliforme, F. semitectum and M. phaseolina) on PD medium in 250 ml conical flasks, each containing 50 ml of medium and incubated at 25°C for 15 days. After 15 days incubation, fungal mats were collected, blended with sterile water. The inocula were used at rate of 3g/Kg autoclaved soil.

Seeds of cantaloupe cultivars (CREDO F1, ISI 54139 F1 and ANANAS) were soaked in a 5% sugar solution for 30 seconds after surface disinfestations with 3% sodium hypochlorite. Pots (12 cm in diameter) were sterilized by immersing them in 5 % formalin solution for 15 minutes, then left for several days to get rid of the poisonous effect of formalin. Autoclaved soil mixture (50 % sand and 50% loam) was applied. The soil infested with the tested fungi was left for one week for pathogen establishment. The sticky seeds were fully mixed with the dried powder of the extracts, namely: *Retama raetam* (mixed of flowers, Stems and fruits) and *Phoenix dactylifera* (seeds) were collected from Misurata region in north Libya, while *Chrysanthemum trifurcatum* (mixed of leaves, stems and roots) in the target treatment. The proportion of the extract was about 10% of

Volume 6 Issue 7, July 2017 <u>www.ijsr.net</u> Licensed Under Creative Commons Attribution CC BY the weight of the seeds. Seed dressing was carried out by mixing the seeds thoroughly with the tested plant extracts before sowing. Pots filled with sterile sandy clay soil were inoculated with each of the tested fungi and sown with treated seeds as mentioned in the pathogenicity test (10 seeds/pot). Four replicates (4 pots) of each treatment were used. Similar treatments with untreated seeds were used as a control. Data were recorded 14 days after germination. Percentages of seedling infection were recorded as a reflection for pre- and post-emergence damping-off percentages (Bahraminejad, 2012).

3. Experimental Results

This study was carried out to evaluate efficacy of some plant powders in controlling diseases caused by the tested seedborne pathogens *in vivo*. Seed treatment by different plant powders (10% w/w), i.e. *R. raetam* (flowers, fruits and Stems), *C. trifurcatum* (all of plant) and *P. dactylifera* (seeds), on the incidence of PRD, PTD and TIP, incited by four of the isolated cucurbitaceous seed-borne pathogens, i.e. *F. solani*, *F. moniliforme*, *F. semitectum*, *M. phaseolina*. PRD, PTD and consequently TIP values were then measured and presented in Tables (1- 4)

- 1. <u>F. solani (Table 1):</u>Results of Table (1) indicate that:
- 1) Significant reductions in PRD values were observed in cantaloupe cvs. as a result of seed treatment with any of the tested plant powders. On the other hand, all tested cantaloupe cvs. showed significant reductions in PRD incidence incited by *F. solani*, as a result of seed treatment with any of the tested plant powders, compared with control. *R. raetam* proved to the most effective in reducing infection by *F. solani* in all cantaloupe cvs., compared with the other plant powders. Reductions in PRD % with *R. raetam* were, 43.75%, 29.41% and

31.58%, followed by *P. dactylifera* 37.5%, 23.53% and 26.32% in CREDO F1, ISI 54139 F1 and ANANAS, respectively. Lower reduction rates were obtained with *C. trifurcatum* (31.25%, 17.65% and 21.05%, respectively.

- 2) Results of PTD occurrence after seed treatment with different plant powders revealed that, *C. trifurcatum* and *P. dactylifera* were ineffective and resulted in insignificant reductions in PTD, incited by *F. solani*, in both CREDO F1cv. and ANANAS cv. The other plant powders treatments significantly reduced PTD incidence, however, *R. raetam* treatment realized the highest reductions all in cantaloupe cvs. (12.50%, 27.27% and 9.10%, respectively). *C. trifurcatum* and *P. dactylifera*, since it reduced PTD values, in the previously ISI 54139 F1 9.10% and 18.18%, respectively, compared with control.
- 3) In general, cantaloupe seed treatment with the tested different plant powders significantly reduced TIP values incited by F. solani, however, suppression rates differed according to the tested different plant powders and cultivar. Generally, R. raetam was the most efficient plant powders in reducing TIP in all cantaloupe cvs., since reduction rates ranged from 23.33% to 33.33%, compared with control. In CREDO F1 cv., R. raetam was the most suppressive against damping-off disease than the other tested plant powders, attaining reduction rate 33.33%. P. dactylifera came next to R. raetam, since it realized less reduction rates (25%, 21.43 and 16.67% in CREDO F1, ISI 54139 F1 and ANANAS cvs., respectively, compared with control). Treatment of cantaloupe seeds with C. trifurcatum resulted in the least efficiency rates, since reductions in TIP % were (20.83%, 14.28% and 13.33% in CREDO F1, ISI 54139 F1 and ANANAS cvs., respectively, compared with control).

by r. soluni.											
Treatment	Percentage of seedling infection (PSI) Varieties										
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	1	
	F. solani + R. reatam	22.5	17.5	40	30	20	50	32.5	25	57.5	49.17
F. solani + C. trifurcatum	27.5	20	47.5	35	25	60	37.5	27.5	65	57.50	
F. solani + P. dactylifera	25	20	45	32.5	22.5	55	35	27.5	62.5	54.17	
Control (1)(F. solani alone)	40	20	60	42.5	27.5	70	47.5	27.5	75	68.33	
Control (2) (Untreated)	0.0	0.0	0.0	2.5	0.0	2.5	2.5	0.0	2.5	1.67	
L.S.D	1.88	1.57	2.24	1.78	1.49	2.66	1.92	1.39	2.98		

Table 1: Efficiency of Seed coating with some Plant powders agents on controlling damping-off of cantaloupe cvs., incitedbv F solari

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

Table 2: Efficiency of Seed coating with some Plant powders agents on controlling damping-off of cantaloupe cvs., incitedby F moniliforme

Gy I. montujorme.											
Treatment	Percentage of seedling infection (PSI)										
	Varieties										
	CREDO F1			IS	I 54139 F1	L		TID			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	111	
F. moniliforme + R. reatam	20	12.5	32.5	25	15	40	30	17.5	47.5	40.00	
F. moniliforme + C. trifurcatum	22.5	15	37.5	27.5	17.5	45	32.5	20	52.5	45.00	
F. moniliforme + P. dactylifera	20	15	35	25	17.5	42.5	30	20	50	42.50	
Control (1) (F. moniliforme	25	17.5	42.5	32.5	17.5	50	35	20	55	49.17	

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alone)										
Control (2) (Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	2.5	0.83
L.S.D	1.54	1.27	2.11	1.87	1.38	1.79	1.61	1.23	1.92	

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

Table 3: Efficiency of Seed coating with some Plant powders agents on controlling damping-off of cantaloupe cvs., incited by *F. semitectum*.

	Percentage of seedling infection (PSI)											
Treatment	Varieties											
	CREDO F1			IS	I 54139 F	l		ANANAS	TID			
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	IIP		
F. semitectum + R. reatam	15	10	25	22.5	15	37.5	30	17.5	47.5	36.67		
F. semitectum + C. trifurcatum	22.5	12.5	35	27.5	17.5	45	32.5	20	52.5	44.17		
F. semitectum + P. dactylifera	17.5	12.5	30	25	15	40	30	20	50	40.00		
Control (1) (F. semitectum alone)	22.5	17.5	40	27.5	20	47.5	35	20	55	47.50		
Control (2) (Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.00		
L.S.D	1.44	1.69	2.63	1.63	1.40	2.05	1.88	1.71	2.27			

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

 Table 4: Efficiency of Seed coating with some Plant powders agents on controlling damping-off of cantaloupe cvs., incited by *M. phaseolina*

	Percentage of seedling infection (PSI)												
Treatment		Varieties											
	CREDO F1			I	SI 54139 H	F1		TID					
	PRD	PTD	TIP	PRD	PTD	TIP	PRD	PTD	TIP	1112			
M. phaseolina + R. reatam	20	15	35	25	20	45	32.5	22.5	55	45.00			
M. phaseolina + C. trifurcatum	22.5	20	42.5	32.5	20	52.5	35	25	60	51.67			
M. phaseolina + P. dactylifera	22.5	17.5	40	30	20	50	30	25	55	48.33			
Control (1) (<i>M. phaseolina</i> alone)	25	20	45	35	22.5	57.5	40	25	65	55.83			
Control (2) (Untreated)	0.0	0.0	0.0	0.0	0.0	0.0	2.5	2.5	5	1.67			
L.S.D	1.13	1.54	1.77	1.31	1.67	1.86	1.22	1.48	2.12				

PRD = Pre emergence damping-off, PTD = Post emergence damping-off, TIP = Total infection percentage, PSI = Percentage of seedling infection.

2. <u>F. moniliforme (Table 2)</u>: According to data shown in Table (2), the following could be concluded:

- 1) All seed treatments with the tested different plant powders were significant in decreasing PRD incidence, however, *R. raetam* and *P. dactylifera* were the most effective among them, since reduction rates were equal values (20%, 23.07% and 14.28%, respectively, CREDO F1, ISI 54139 F1 and ANANAS cvs.. The least efficiency rates among the tested plant extracts was that of *C. trifurcatum* in all cantaloupe cultivars.
- 2) Seed treatments with the *C. trifurcatum* or *P. dactylifera*, all the obtained PTD values, were insignificant, compared with control, except in CREDO F1 cv. However, pronounced and significant PTD values were recorded throughout the experiment in all *R. raetam* cantaloupe treatments, however, the best controlling results were detected in *R. raetam* treatments (12.5% to 28.57% less than control).
- 3) TIP values were significantly reduced as a result of seed treatment with the tested different plant powders in all cantaloupe cultivars. However, the highest disease

suppression rates were detected in *R. raetam* treatments (23.53%, 20% and 13.65% in CREDO F1, ISI 54139 F1 and ANANAS cvs., compared with control, respectively). *C. trifurcatum* and *P. dactylifera* were also efficient but at rates lower than those of *R. raetam*. The least effective plant extract was *C. trifurcatum*. This was true for all tested cultivars.

3. <u>*F. semitectum* (Table 3)</u>: Results presented in Table 3 show that:

- 1) All plant powders /cultivar treatments significantly suppressed PRD, incited by *F. semitectum* except two treatments e.i. CREDO F1or ISI 54139 F1 with *C. trifurcatum.* However, *P. dactylifera* significant with relatively lower effiency in disease suppression. The highest reduction in PRD values were observed in all *R. raetam* treatments, including CREDO F1, ISI 54139 F1 and ANANAS cvs. (33.33%, 18.18% and 14.24%, compared with control, respectively).
- 2) Values of PTD as affected by seed treatment with the tested different plant powders showed a great diversity

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among the tested cultivars. In cantaloupe, the highest PTD reduction rates were realized by *R. raetam* (42.86 in CREDO F1), followed by *C. trifurcatum* or P. *dactylifera* with CREDO F1 cv. (28.57%). *C. trifurcatum* or P. *dactylifera* treatments with ANANAS cv. were insignificant.

3) Generally, results showed that, TIP of *F. semitectum* were greatly and significantly reduced as a result of seed treatments with all the tested plant powders. However, the highest reduction values in TIP were recognized in *R. raetam* in all cantaloupe cvs. (37.5%, 21.05 and 13.65% in CREDO F1, ISI 54139 F1 and ANANAS cvs, respectively). The lowest reduction rates were realized by *C. trifurcatum*, which ranged 4.55% to 12.5%.

4. <u>M. phaseolina (Table 4):</u>Data in Table (4) reveal that:

- 1) All the tested plant powders treatments successfully reduced PRD incidence incited by *M. phaseolina*, however, *R. raetam* showed the highest PRD decreasing rates in ISI 54139 F1 cultivar (28.57%) and in both CREDO F1 and ANANAS cultivars (20.00% and 18.75%, respectively). *C. trifurcatum* was as effective as *P. dactylifera* / CREDO F1 cv. treatment. The least effective treatment was detected in *C. trifurcatum* treatment with ISI 54139 F1 cv.(7.14%). *P. dactylifera* / ANANAS cv. came next to *R. raetam*, (25%).
- 2) PTD values showed different trends in cantaloupe cultivars. In ANANAS cultivar, the only significant reduction was realized by *R. raetam* (10.00%), however, all the other plant extract / ISI 54139 F1 were significant, compared with control, whereas in CREDO F1 cultivar, the highest significant reduction were obtained in *R. raetam* (25%), since it highly suppressed disease incidence, followed by *P. dactylifera*, insignificant PTD value was produced *R. raetam* / CREDOF1cv.treatment.
- 3) Total infection percentages (TIP) expressed the highest reductions, compared with control, in *R. raetam* in all cultivars treatments (22.22%, 21.74% and 15.38% in CREDO F1, ISI 54139 F1 and ANANAS cvs, respectively). *P. dactylifera* treatments were also effective but still less efficient than *R. raetam*, since it reduced TIP incidence in CREDO F1, ISI 54139 F1 and ANANAS cvs, at 11.11%, 13.04% and 15.38%, respectively. *C. trifurcatum* was the least effective in all tested treatments, compared with control.

4. Discussion

Seed treatment with the tested plant powder significantly reduced TIP values incited by the tested seed-born pathogens. The highest reduction rate (37.5%) was detected in *R. raetam / F. semitictum /* CREDO cv. Treatment, whereas the least rate (4.55%) was realized in *C. trifurcatum F. semitictum* and *F. moniliforme* interactions in ANANAS cv.. The highest TIP reduction rates (33.33%, 23.53% and 22.22%) were observed in *R. raetam / F. solani*, *R. raetam / F. moniliforme* and *R. raetam / M. phaseolina* treatments, respectevily. The highest reduction rate (25.0%) was detected in *P. dactylifera / F. semitictum* and *F. solani* interactions in CREDO cv. Significant reduction in PRD values was detected as a result of seed treatment with the tested plant powder, attaining maximum rates in *R. raetam / F. solani* (43.75%), *F. semitictum* (33.33%), *M. phaseolina*

(28.57%) and *F. moniliforme* (23.07%), while in *P. dactylifera* treatment gave the highest reduction rates (37.5%, 26.32% and 25.0%) were observed in *F. solani* / CREDO cv., *F. solani* / ANANAS cv. and *M. phaseolina* / ANANAS cv. Significant reductions PTD in damping-off incidence in all cantaloupe cultivars were obtained due to treatment with most of the tested plant powder, but in some treatments were non effected, however, *R. raetam* was more efficient in reducing damping-off incided by *F. semitictum*, *F. moniliforme* and *F. solani* (42.86%, 28.57% and 27.27%, respectively compared with control).

On the other hand, P. dactylifera and C. trifurcatum were more efficient in controlling damping-off caused by F. semitictum, where reduction%, compared damping-off incidence, followed by P. dactylifera. This trend discussed above is similar to the observation on disease incidence by many scientists (AI-Dalmasini et. al, 2003; Ben Yephet and Nelson, 1999; Bennard and Clement, 1983; Benhamou et al, 1997; Bennard and Pesando, 1989; Boehm and Hoitink, 1992; Boehm et al., 1997; Bonsi et. al, 1995; Cariellon and Zanetti, 1979; Cotxarrera et. al, 2002; Craft and Nelson, 1996; Delaney et. al, 2001; Elad and Shteinberg, 1994; Erthart and Burian, 1997; Folman et. al, 2004; Fuchs and Larbi, 2004; Hoitink et. al, 1997; Kao and Ko, 1986; Verma and Kharmar, 2006). Also, several studies have been conducted to investigate the peculiar effects of plant extracts as fungicides, pesticides and as antibacterial agents on plant growth worldwide. The antifungal activity of Allium sativum was reported by Grewal (2006). The author, observed that essential oil of A. sativum on agar plate was active on Lenzites trabea, Lentinus lepideus and Polyporus versicolor. Similarly, the species antibacterial activity of ethanol extract was active on Escherichia coli, Salmonella typhosa, Shigella sonnei and Staphylococcus aureus. The author observed that water extract of the species was active on Bacillus, Klebsiella, Proteus vulgaris, S. styphosa, S. sonnei and S. aureus. Moslem and Kholie (2009) also reported the antifungal effects of neem leaf and seed extracts obtained by ethanol, on Fusarium oxysporum, Rhizoctionia solani, Alternaria solani and Sclerotinia sclerotiorum. The authors observed growth inhabitation of the tested fungi. It is important to note that bio-control agents induces the accumulation of enzymes such as Chitinnase, Peroxidase and Polyphenol oxidase which plays an important role in plant defense mechanisms against pathogens (Abd-EL-Khair and EI-Mougy, 2003). This study indicates that the application of the plant extracts increased the growth and reduced disease incidence in Casuarina equisetifolia. The increase in plant growth might be associated with secretion of auxin, gibberellins and cytokinnins and suppression of deleterious microorganism causing damping off (Gamliel and Katan, 1993). In addition to the direct antagonism and plant growth promotion, plant extracts from leaf of Azadirachta indica, seed of Azadirachta indica, bulb of Allium sativum, root of Zingiber officinale and leaf of Vernonia amygdalina increased the activities of various defense related enzymes and chemicals in response to infection by the pathogen. Many plants are endowed with defense mechanisms which can protect them against fungi (Dutta, 2003). Also, it has been reported that application of plant extracts triggers plants' latent defense mechanism in responses to infection by pathogen. Inducing a plants' own

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defense mechanism by prior application of a biological agent is a novel strategy in plant disease management. Aqueous plant extract is rich with nutrient and microorganism. It can stimulate growth, protect plants from disease and help suppress soil borne pathogen (Quarles, 2011). In this study, it could be stated that seedlings treated with the three plants powder: stem of R. raetam, seed of P. dactylifera and mixed of leaves, stems and roots of C. trifurcatum increased the activities of various defense-related enzymes which led to the synthesis of defense chemical in cantaloupe cvs. The result implies that the use of three plants powder inhibited the growth and development of damping off causing fungi. Therefore, new forest protection options involving the use of bio- fungicides need to be explored in order to ensure the success of any deforestation and reforestation programs. A great interest should be focused on biofungicides that are relatively cost effective and have minimal toxicity to both mammals and the environment. Unlike inorganic fungicides, the use of organic fungicides can cause no disruption to any stable food chain.

In conclusion, many reports revealed that, plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999; Harborne, 1998; Gottlieb *et al.*, 2002). Even though effective and efficient control of seed borne pathogenic fungi can be achieved by the use of synthetic fungicides, the same cannot be applied to grains for reasons of pesticide toxicity (Wodageneh and Wulp , 1997; Harris *et al.*, 2001). Thus, there is a need to search for alternative approaches to store seeds, grains/cereals for human consumption without toxicity problems that are ecofriendly and not capital intensive.

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