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Antifungal Activity of Some Extracts Against Some Plant Pathogenic Fungi

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Abstract: *In vitro* studies were carried out to determine the antifungal activity of five plant extracts viz., *Allium sativum*, *Cymogopogon proxims*, *Carum carvi*, *Azadirchia indica* and *Eugenia caryophyllus* extracted with either Cold Distilled Water (CDW), Boiling Distilled Water (BDW) or Cold Ethanol (CET) as well two culture filtrates of *Trichoderma* antagonistic fungi against *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea* and *Rhizoctonia solani*. The results revealed that plant extracts especially those extracted with CDW had strong antifungal activity with significant inhibition on the growth of the three tested fungi. In addition, the inhibitory magnitude of the tested plant extracts to the tested pathogen fungi was proportional to the applied concentrations. The most effective plant extracts were *Allium sativum*, *Carum carvi* and *Eugenia caryophyllus*. Also, the study showed that the culture filtrates of the antagonistic fungus, *T. harzianum* was more efficiency than *T. viride* to decrease the growth of tested fungi, but with levels less than plants extracted CDW or benomyl. Findings in this study confirmed that plant extracts can be used as natural fungicides to control pathogen fungi to reduce the dependence on the synthetic fungicides.

Key words: Plant extract, fungi, benomyl, antifungal activity, inhibition

INTRODUCTION

The inappropriate use of agrochemicals especially fungicides which found to pose more of carcinogenic risk than insecticides and herbicides together[1] may give rise to undesirable side effects[2]. Additionally, resistance by pathogen to fungicides has rendered certain fungicides infective. There may be a need to develop new management systems to reduce the dependence on the synthetic pesticides. Now days, plant extracts as natural products are widely used to control pests[3-5]. Plant extracts and essential oils show antifungal activity against a wide rang of fungi[6-9]. In this respect, *Aristea ecklonnii* and *Agapathus inapertus* were found to have antifungal activity against *Botrytis cinerea*, *Fusarium oxysporum*, *Rhizoctonia solani*[10]. Also, garlic and neem have been recommended against *Pesteloxia palmarum*[5,11].

Biological control is another way to avoid the environment pollution to minimize the intensive use of pesticides[12]. *Trichoderma* spp. were commercially applied as biological control agents against fungal pathogen, where *T. harzianum* was found to be effective against the growth of *F. culmorum*[13], while the dual culture of *T. harzianum* and *T. viride* were most effective in reducing the mycelial growth of *F. oxysporum* and *Pythium aphanidermatum*[14].

The present study was undertaken to evaluate the efficacies of five plants namely garlic (*Allium sativum*), half-bar (*Cymbopogon proximus*), craway (*Carum carvi*), neem (*Azadirachia indica*) and carnation (*Eugenia caryophllus*) extracted with either cold distilled water, boiling distilled water or cold ethanol and two antagonistic *Trichoderma* spp. namely *T. harzianum* and *T. viride* compared to benomyl as fungicide against *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea* and *Rhizoctonia solani*.

MATERIALS AND METHODS

Isolation and purification of the causal organisms: The causal pathogens of tomato wilt disease (*Fusarium oxysporum* f. sp. *lycopersici*), tomato gray mould (*Botrytis cinerea*) and potato black scurf (*Rhizoctonia solani*) were isolated from naturally infected tomato and potato plants cultivated in the Farm of the Faculty of Agriculture and Veterinary Medicine, Al-Qassem University, Saudi Arabia and purified in pure cultures. The isolates were maintained on Potato Dextrose Agar (PDA).

Preparation of plant extracts: The bulp of garlic, leaf of half-bar, fruit of craway, seed of neem and flower of carnation were obtained from the local market and immediately kept in refrigerator until starting the experiments. The tested plants were ground to fine powder and then extracted by macerating 200 g of each plant in 1000 mL of either sterilized Cold Distilled Water (CDW) for 24 h, Boiling Distilled Water (BDW) for 1 h or

Cold Ethanol 90% (CET) for 1 h. The extract was filtered-sterilized by passing it through bacterial filter (Seitz). Alcohol extract was evaporated under vacuum to 10 mL and then completed to 1000 mL with sterilized DW. These extracts were set as original concentrations (20%). Dilutions of 0.5, 1 and 2% of the tested extracts were prepared by adding 25, 50 and 100 mL of the original concentration to 1000 mL of warm PDA, respectively. The plant extracts were thoroughly mixed with the medium after autoclaving, then 20 mL of the medium was poured in each 9 cm sterilized petridishes. The medium without extracts saved as control. Mycelial discs were prepared using a cork borer (5 mm diameter) from the tip of 5 days old culture of the three tested fungi and then placed at the center of petridishes after solidification of PDA. Each treatment was replicated three times. Plates were incubated in an incubator at 28°C for the control reach to full growth. Fungal growth was measured by averaging the two diameters taken at right angles for each colony. Percentage Inhibition (%I) of the fungal growth were calculated according to the following formula:

%I = (growth in control-growth in treatment/growth in control) x 100

Preparation of *Trichoderma* extract and benomyl: Two fungal antagonistic fungi namely, *Trichoderma harzianum* and *T. viridi* were grown on potato dextrose liquid medium. The flasks were autoclaved, then inoculated with 5 mm agar discs of the two fungi. Inoculated flasks were incubated at 28°C for three weeks. The grown culture were filtered through sterilized filter paper followed by filtration through bacterial filter. These extracts were set as original concentration. Dilutions of 0.5, 1 and 2% of the fungi were prepared as previously described.

Benomyl (Benlate, 50% WP, Du Pont, France) was tested at 12.5, 25 and 50 ppm against the three tested fungi.

Statistical analysis: The data were calculated as mean±SD and analyzed using analysis of variance technique (ANOVA). Probability of 0.05 or less was considered significant according to Duncan's Multiple Range.

RESULTS

The *in vitro* antifungal properties of plants extracted by either CDW, BDW and CET and two antagonistic fungi were compared with the benomyl against *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea* and *Rhizoctonia solani* using radial growth technique (Table 1-3). In general, plants extracted with CDW were most potencies to inhibit the growth of the three tested fungi, followed by plants that extracted with CET, while plants extracted with BDW were non-effective against the fungi except carnation at all the tested concentrations (0.5, 1 and 2%) and half-bar at concentration of 2% were found to be effective. In addition, the percentages of growth inhibition produced by either plants extracted with CDW or CET were significantly different from control values and the inhibition was dose-dependent in most cases.

Table 1 show that the highest growth inhibition of *Fusarium oxysporum* was obtained with CDW tested at concentration ranging from 0.5-2%, where the corresponding percentages of inhibition ranging from 68.9-94.4%, followed by carnation extracted with CET (41.1-83.3%), craway extracted with CDW (48.9-62.2%), half-bar extracted with CDW (36.7-58.9%), neem extracted with CDW (33.3-51.1%) and carnation extracted with CDW (27.8-53.3%).

Results in Table 2 show that the highest growth inhibition of *R. solani* was recorded when craway extracted with CDW and tested at either 0.5, 1 and 2%, where the corresponding percentage of growth inhibition was 94.4%, followed by garlic extracted with CDW (85.6-94.4%), carnation extracted with BDW (80-90%), carnation extracted with CET (51.1-94.4%), half-bar extracted with CDW (54.4-86.7%) and carnation extracted with CDW (46.7-66.71%). On the other hand, all plants extracted with BDW were non-effective against *R. solani* except carnation at all the tested concentration and half-bar at concentration of 2% affect the growth of *R. solani*.

Table 3 show that the growth of *B. cinerea* was highly inhibited by all the tested concentrations of craway, half-bar, neem, carnation and garlic extracted with CDW where the corresponding percentages of growth inhibition were 94.4, 87.8-94.4, 87.8-94.4, 83.3-94.4 and 76.7-94.4%, respectively, followed by carnation extracted with BDW (75.6-94.4%). On contrary, carnation extracted with CET inhibited the growth of *R. solani* by 38.9 and 94.4% at concentration of 1 and 2%, respectively.

The effect of culture filtrates of *T. harzianum* and *T. viride* on the radial growth of the three tested fungi were evaluated (Table 1-3). The results shows that *T. harzianum* was more potent than *T. viride* against the three tested fungi, where the percentages of growth inhibition at the three tested concentrations (0.5-2%) were ranging from 17.4-50, 43.3-60 and 15.6-62.2% against *F. oxysporum*, *R. solani* and *B. cinerea*, respectively. Also the present data showed that either *T. harzianum* or *T. viride* were less potent to affect the growth of all the

Table 1: Effect of plant extracts and *Trichoderma* culture filtrates on the linear growth of *Fusarium oxysporum* f. sp. *lycopersici*

	Aqueous extract			Boiling water extract			Ethanolic extract		
Treatments	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%
Control	9.0±0.00a (0)	9.0±0.00i ((0)	9.0±0.00h (0)	9.0±0.00b (0)	9.0±0.00c (0)	9.0±0.00f (0)	9.0±0.00f (0)	9.0±0.00e (0)f	9.0±0.00e (0)
Garlic	2.7±0.12b (68.5%)	2.1±0.10b (76.7%)	0.5±0.00a (94.4%)	9.0±0.00b (0%)	9.0±0.00c (0%)	8.5±0.06c (7.8%)	8.0±0.00e (11.1%)	7.6±0.12e (15.6%)	7.3±0.06d (20%)
Craway	4.6±0.06c (49.3%)	3.6±0.12c (59.3%)	3.4±0.00b (62.2%)	9.0±0.00b (0%)	9.0±0.00c (0%)	8.4±0.06e (7.6%)	7.6±0.12d (15.6%)	6.6±0.06d (26.7%)	6.0±0.06c (33.3%)
Carnation	6.5±0.12f (26.30)	5.0±0.00f (44.40)	4.2±0.06d (52.90)	5.6±0.10a (37.80)	4.6±0.06a (52.20)	2.2±0.06a (75.20)	5.3±0.12a (41.10%	3.0±0.06a (66.70)	1.5±0.12a
Half-bar	5.7±0.06d 36.30	4.3±0.26d (52.20)	3.7±0.06c (58.90)	9.0±0.0b (0)	7.9±0.12b (12.60)	6.2±0.06b (31.50)	6.9±0.15b (23.30)	6.0±0.06b (33.30)	5.4±0.06b (40)
Neem	6.0±0.06e (32.90)	4.7±0.06e (47.80)	4.4±0.10e (51.10)	9.0±0.00b (0)	9.0±0.00c (0)	8.1±0.10c (10)	7.1±0.06c (21.10)	6.2±0.12c (31.10)	5.5±0.17b (38.90)
T. harzianum	7.4±0.06g (17.40)	6.8±0.06g (24.80)	4.5±0.06f (50)						
T. viride	9.0±0.00h (0)	8.5±0.06h (8.50)	8.0±0.06g (10.80)						
Benomyl	0.5±0.00a (94.4)*	0.5±0.00a (94.4)**	0.5±0.00a (94.4)***						

Table 2: Effect of plant extracts and *Trichoderma* culture filtrates on the linear growth of *Rizoctonia solani*

	Aqueous extract			Boiling water extract			Ethanolic extract		
Treatments	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%
Control	9.0±0.00h (0)	9.0±0.00g (0)	9.0±0.00g (0)	9.0±0.00b (0)	9.0±0.00b (0)	9.0±0.00d (0)	9.0±0.00e (0)	9.0±0.00f (0)	9.0±0.00f (0)
Garlic	1.3±0.0.06b (85.6)	1.1±0.06b (87.8)	0.5±0.00a (94.4)	9.0±0.00b (0)	9.0±0.00b (0)	9.0±0.00d (0)	9.0±0.00e (0)	6.9±0.12e (23.3)	5.9±0.12e (34.4)
Craway	0.5±0.00a (94.4)	0.5±0.00a (94.4)	0.5±0.00a (94.4)	9.0±0.00b (0)	9.0±0.00b (0)	9.0±0.00d (0)	7.0±0.10d (22.2)	5.8±0.06d (35.6)	4.6±0.06d (48.9)
Carnation	4.8±0.06e (46.7)	4.1±0.12e (54.4)	3.0±0.10d (66.7)	5.6±0.10a (37.8)	4.4±0.06a (51.1)	0.5±0.00a (94.4)	4.4±0.20a (51.1)	0.5±0.00a (94.4)	0.5±0.00c (94.4)
Half-bar	4.1±0.12d (54.4)	3.2±0.10d (64.4)	1.2±0.06c (86.7)	9.0±0.00b (0)	9.0±0.00b (0)	8.5±0.10c (55.6)	5.2±0.15b (42.2)	3.8±0.10b (57.8)	3.2±0.06b (64.4)
Neem	1.7±0.06c (80)	1.4±0.06c (84.4)	0.90±0.00b (90)	9.0±0.00b (0)	9.0±0.00b (0)	7.9±0.23b (12.2)	5.9±0.15c (34.4)	4.8±0.12c (46.7)	4.0±0.06c (55.6)
T. harzianum	5.1±0.12f (43.3)	4.0±0.06e (55.6)	3.6±0.06e (60)						
T. viride	8.4±0.06g (6.7)	7.9±0.12f (12.2)	7.4±0.17f (17.8)						
Benomyl	0.5±0.00a (94.4)*	0.5±0.00a (94.4)**	0.5±0.00a (94.4)***						

Table 3: Effect of plant extracts and *Trichoderma* culture filtrates on the linear growth of *Botrytis cinerera*

	Aqueous Extract			Boiling water extract			Ethanolic extract		
Treatments	0.5%	1%	2%	0.5%	1%	2%	0.5%	1%	2%
Control	9.0±0.00g (0)	9.0±0.00f (0)	9.0±0.00d (0)	9.0±0.00b (0)	9.0±0.00b (0)	9.0±0.00c (0)	9.0±0.00a (0)	9.0±0.00d (0)	9.0±0.00e (0)
Garlic	2.1±0.0.06d (76.7)	0.5±0.00a (94.4)	0.5±0.00a (94.4)	9.0±0.00b (0)	9.0±0.00b (0)	9.0±0.00c (0)	9.0±0.00a (0)	9.0±0.00d (0)	9.0±0.00e (0)
Craway	0.5±0.00a (94.4)	0.5±0.00a (94.4)	0.5±0.00a (94.4)	9.0±0.00b (0)	9.0±0.00b (0)	9.0±0.00c (0)	9.0±0.00a (0)	9.0±0.00d (0)	7.9±0.12d (12.2)
Carnation	1.5±0.06c (83.3)	0.9±0.06b (90)	0.5±0.00a (94.4)	2.2±0.06a (75.6)	1.5±0.06a (83.3)	0.5±0.00a (94.4)	9.0±0.00a (0)	5.5±0.12a (38.9)	0.5±0.00a (94.4)
Half-bar	1.1±0.06b (87.8)	1.0±0.06c (88.9)	0.5±0.00a (94.4)	9.0±0.00b (0)	9.0±0.00b (0)	8.6±0.32c (4.4)	9.0±0.00a (0)	7.5±0.06c (16.7)	6.6±0.15c (26.7)
Neem	1.1±0.06b (87.8)	0.5±0.00a (94.4)	0.5±0.00a (94.4)	9.0±0.00b (0)	9.0±0.00b (0)	8.3±0.26b (7.8)	9.0±0.00a (0)	7.0±0.06b (22.2)	6.1±0.10b (32.2)
T. harzianum	7.6±0.15e (15.6)	6.0±0.06e (33.3)	3.4±0.17b (62.2)						
T. viride	8.1±0.12f (10)	5.1±0.12d (43.3)	4.0±0.17c (55.6)						
Benomyl	2.2±0.06d (75.6)*	0.5±0.00a (94.4)**	0.5±0.00a (94.4)***						

Growth is expressed as cm. Values in parenthesis indicate percentage of growth inhibition. Data are expressed as mean±SD (n=3).
Means within the same column and followed by the same letter are not significantly different from each other according to Duncan's Multiple Range Test ($p \leq 0.05$). *, ** and *** mean that the concentrations of benomyl are 12.5, 25 and 50 ppm, respectively.

tested plants and benomyl. Benomyl inhibited the growth of all tested fungi by 94.4% at all the tested concentrations (12.5, 25 and 50 ppm) except, *B. cinerea* which was inhibited to 75.6% by 12.5 ppm.

DISCUSSION

The present investigation show that the different methods used to extract the plant appeared to have different effect on radial growth of the tested fungi. In this respect, the plant extract of the five tested plants especially those extracted with either CDW or CET were significantly different from the control in response of radial growth of the three tested fungi and the inhibition of fungi growth was dose dependent. Garlic and craway were the most effective to inhibit the growth of the tested fungi followed by carnation, half-bar and neem. The present results are in parallel with other studies, where garlic was the most potent among many plants against *Alteneria alternate* which cause blight of sunflower disease[15], leaf spot in betlnut caused by *Pestalotia palmarum*[5]. Although garlic was demonstrated early to have good antifungal activity and to be useful as post harvest treatment[1,9], it has not widely commercialized. Also, neem extract inhibited the germination of *Pestolata psidii*[11], *F. moniliforme* in sorghum[16] and suppressing *Cercospora arachidicola* of groundnut[17]. The bioactivity of neem extracts has been attributed to various compounds found in the seeds and leaf such as nimbin, nimbidin and salannin, but the most important of these compound is azadirachtin[3]. On the other hand, when carnation was extracted with boiling water, high level of antifungal activity was obtained. This may due to the hot water make the oil found in carnation to ooze-out the seeds. The crude boiled water and acetone extract of Allium sativum caused 100% inhibition of the mycelial growth of *R. solani*[18].

In addition, the culture filtrate of the antagonistic fungus, *T. harzianum* was found to be more efficiency than *T. viride* to decrease the growth of tested fungi, but with levels less than plants extracted cold distilled water or benomyl. Similar results were observed where *T. harzianum* decreased the growth of *F. oxysporium*, *Verticillium albo-atrum* and *F. culmorum*[12,19].

Findings in this study confirmed that plant extracts especially those extracted with cold water had strong antifungal activity with significant inhibition on the growth of *Fusarium oxysporum* f. sp. *lycopersici*, *Botrytis cinerea* and *Rhizoctonia solani*. In addition the inhibitory magnitude of the tested plant extract to the tested pathogen fungi was proportional to the applied concentrations. Because garlic was more efficiency, therefore it might be a promising material to control these fungi.

It can be concluded that to reduce the dependence on the synthetic fungicides as well as decrease the higher production costs, plant extract especially garlic and craway may be used as natural fungicides to control some pathogens.

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