Journal of
Plant Pathology & Microbiology
ISSN: 2157-7471

Abu Irmaileh BE et al., J Plant Pathol Microbiol 2017, 8:8
DOI: 10.4172/2157-7471.1000417

Research Article Open Access

Antifungal Activity of the Stinkwort (*Inula graveolens*) Extracts

Barakat E Abu Irmaileh[1]*, Nida' M Salem[1], Amal MF Al Aboudi[2], Musa H Abu Zarqa[2] and Amany O Abdeen[3]

[1]School of Agriculture, Department of Plant Protection, The University of Jordan, Amman, Jordan
[2]School of Science, Department of Chemistry, The University of Jordan, Amman, Jordan
[3]Royal Scientific Society, Amman, Jordan

Abstract

Bioactive guided fractionation was carried out to determine the antifungal activity of the stinkwort weed, *Inula graveolens*, on various phytopathogenic fungi species. Dried shoots were extracted and partitioned in various solvent systems. The aqueous methanol extract (AqMeOH) inhibited the growth of several soil-borne fungi; *Alternaria* sp., *Fusarium* spp. and *Rhizoctonia* sp. in *in-vitro* plate assay. Fractionation of AqMeOH extract by column chromatography in dichloromethane/AqMeOH yielded twenty-two fractions of which fractions #2, 3 and 4 showed antifungal activity. Further fractionation of the combined fractions #2, 3 and 4 by column chromatography in solvent system benzene/ethyl acetate on fine silica columns, yielded ten sub fractions of which sub fraction #2 has the most antifungal activity on all fungi in the experiment. The fungitoxicity of sub fraction #2 in terms of percentage inhibition of mycelia growth of all tested-fusaria strains after ten days of incubation was higher compared to that obtained by the fungicide hymexazol (Tachigaren ® 70 WP). The AqMeOH extract significantly reduced browning length at the crown area, a fusarium-known symptom in *in-vivo* experiments. Furthermore, the growth of *Fusarium*-treated cucumber plants was enhanced by drenching with AqMeOH extract. The activity of the extract was comparable to the activity of the fungicide hymexazol in improving plant health, and surpassed the fungicide activity in preventing the discoloration of the crown vascular tissue of cucumber at comparable concentrations.

Keywords: Stinkwort; *Inula graveolens*; *Fusarium;* Cucumber; Antifungal activity

Introduction

Modern agricultural pest management practices rely significantly on the use of synthetic pesticides. The ability to control pests efficiently has been one of the decisive components of conventional agriculture for increasing production. In spite of the large number of commercial pesticides, efforts to discover new active compounds, that are environmentally safer, has kept scientists searching for natural products from all sorts of living organisms, especially members of the plant kingdom.

Natural products have historically been a valuable source of many biologically active ingredients, used either as crude preparations, pure compounds, or as structural leads for the discovery and development of natural product-based pesticides [1]. Large number of biologically active compounds are derived from plants, microbes, and animal natural products with wide range of structural diversity that arose from co-evolution between competing organisms [2]. A wide variety of organic compounds released from plants and microbes demonstrated toxic activity against other organisms that could be utilized and further investigated as potential bio-pesticides. Well-known biologically active natural products are the allelochemicals which are mostly secondary metabolites emanated, washed off from the foliage or secreted in the root zone of the growing plants [3-6]. Allelochemicals can naturally help or hinder the growth of the receiving living species.

Allelopathic interference is one of the important mechanisms for the successful establishment of invasive species [7], which might be evaluated for potential biological activity. Many growth inhibitors of natural origin have been identified in recent years [8-10] and it became clear that their action is an important feature in characterizing the interrelationships among organisms [11]. These compounds influence patterns in vegetation communities, plant succession, and seed preservation, germination of fungal spores, nitrogen cycle, mutualistic associations, crop productivity and plant defense [12].

In our search for natural compounds that may have the potential use as biopesticides, we tested the antifungal activities of extracts obtained from the stinkwort (*Inula graveolens* (L). Desf. Synonyms: *Dittrichia graveolens* (L.) Greuter; *Erigeron graveolens* L.). This herbaceous plant is an invasive spreading strong-smelling summer annual. It invades waste areas, roadsides and fields in Jordan. One stinkwort plant can produce up to 30,000 seeds, creating areas blanketed with the weed, eliminating open spaces and pasturelands. It is believed that the invasive nature of the plant could be related to its ability to produce secondary metabolites that inhibit the growth of other organisms [13]. Many plant growth inhibitors were isolated and identified from the stinkwort [14]. Allelochemicals are known to suppress or eliminate competing species near the invasive plant [15-17]. The antifungal activity of the obtained fractions was compared with the suppressive activity of the fungicide hymexazol (3-hydroxy-5-methylisoxazol), sold as Tachigaren ® 70WP. Hymexazol is a systemic fungicide that has activity against many fungi such as; *Aphanomyces cochlioides*, *Pythium* and some *Rhizoctonia* species [18,19].

The aim of this research was to determine the inhibitory activity of various extracts of the stinkwort on the growth of several economically phytopathogenic fungi. Experiments were carried out in the laboratory and greenhouse.

Materials and Methods

Preparation of plant extracts and fractions

Flowering stinkwort shoots were collected from the vicinity of

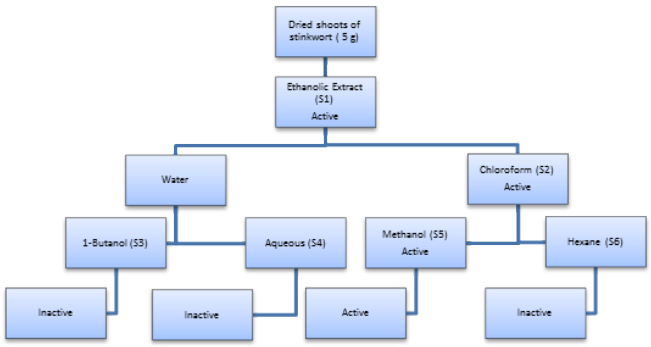
***Corresponding author:** Barakat E Abu Irmaileh, School of Agriculture, Department of Plant Protection, The University of Jordan, Amman-11942, Jordan, Tel: 96265355000; E-mail: barakat@ju.edu.jo

Received July 26, 2017; **Accepted** August 12, 2017; **Published** August 18, 2017

Citation: Abu Irmaileh BE, Salem NM, Al Aboudi AMF, Abu Zarqa MH, Abdeen AO (2017) Antifungal Activity of the Stinkwort (Inula graveolens) Extracts. J Plant Pathol Microbiol 8: 417. doi: 10.4172/2157-7471.1000417

Jordan University, Amman, Jordan. The flowering shoots were air dried in shade inside the greenhouse for over three weeks. The dried material was ground to give fine powder suitable for extraction. Aqueous extracts were prepared by soaking 5 g of dried shoots of stinkwort in one liter of distilled water for two weeks, and then filtered to obtain a clear aqueous extract.

Also, five kilograms of the dried shoots were extracted three times each with 20 L of 96% ethanol for one week with agitation once or twice daily. Ethanol was evaporated to dryness under reduced pressure to give the crude ethanolic extract (S1). The ethanolic extract was partitioned between chloroform and water (3:2). The chloroform extract (S2) was further partitioned between aqueous methanol (AqMeOH) (S5) and hexane (S6), both were evaporated to dryness. The water fraction was further partitioned between water (S4) and 1-butanol (S3) as shown in Scheme 1. All extracts were evaporated and dried under fume hood to get solidified materials and evaluated for their antifungal activity. Solvents were used from commercial sources and were distilled before use.



Scheme 1: Fractionation of the ethanolic extract of *Inula graveolens*.

The active AqMeOH extract (137.8 g) was furhter fractionated using column chromatography in increased polarities of benzene:ethylacetate solvent system as an eluent. The 22 fractions thus obtained were evaluated for their antifungi activity. The active fractions #2, 3 and 4 were combined and chromatographed on a fine silica column in solvent system benzene/ethyl acetate to give seven subfractions, of which fraction #2 (0.4 g) was the most active against tested fungi.

In-vitro experiments

Testing antimicrobial activity of various extracts: A preliminary experiment was set to test the antimicrobial activity in 9 cm diameter petri dishes containing 25 ml medium of potato dextrose agar (PDA). Tap water and room air were used as source of inoculum. A weight of 0.1 g of each of the dried extract material was dissolved in 1 ml absolute ethanol then added to 99 ml tap water to get a solution of 1000 ppm concentration. Open petri dishes were exposed to room air for one minute prior to treatments. One ml of each extract solution was spread over the surface of agar media in each petri dish. A blank treatment composed of 1 ml tap water was also spread over the surface of agar media for comparison. Petri dishes were then covered and incubated at 25°C for two weeks. Microbial growth was then recorded as either fungi or bacteria. Each treatment was replicated five times (Figure 1).

Preparation of fungi cultures: Pure fungi cultures which were previously isolated from different crop plants and identified morphologically and molecularly {personal communications} were used in this research (Table 1). Fungi isolated from beans and grapes were identified only to the genus rank.

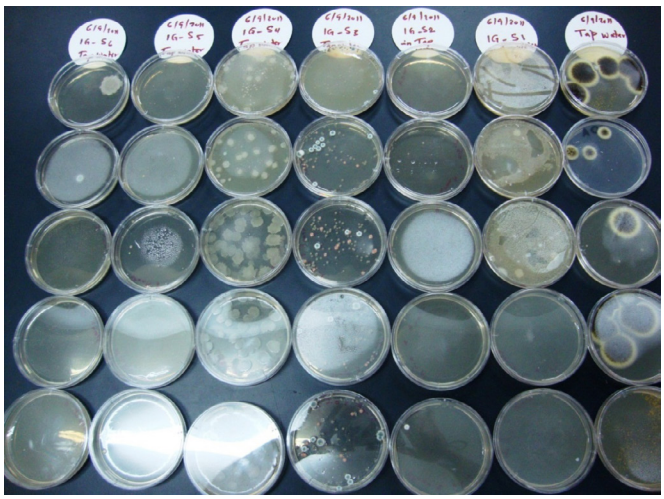


Figure 1: Microbial growth in various extracts; ethanolic extract (S1), chloroform (S2), 1-butanol (S3), water (S4), aqueous AqMeOH (S5), and hexane (S6), and the blank.

Number	Fungus species	Infested plants
1	*Alternaria* sp.	Tomato
2	*Fusarium* sp.	Beans
3	*Fusarium oxysporum*	Cucumber
4	*Fusarium proliferatum*	Cowpea
5	*Fusarium* sp.	Grapes
6	*Fusarium proliferatum*	Date palm
7	*Fusarium oxysporum* f.sp. *Lycopersici*	Tomato
8	*Rhizoctonia* sp.	Tomato

Table 1: Fungal cultures used in this research.

Testing the antifungal activity of AqMeOH extract: Antifungal activity assay against the phytopathogenic fungi listed in Table 1 was carried out. A stock solution of AqMeOH extract was prepared by dissolving 2.5 g of the extract in 10 ml absolute ethanol then added to 990 ml distilled water. A 100 µl of either the blank solution or from the AqMeOH extract were spread on the surface of the media in petri dishes containing 25 ml PDA. The final concentration of the extract in the petri dish was calculated to be 100 ppm/1 ml agar. A 4-mm disc from each fungus was placed in the middle of each PDA plate, and then incubated at 25°C. The diameter of the fungal growth was measured at 15 days after treatment. Each treatment with each fungus was replicated 4 times. A blank treatment was also included. Percent of fungal growth inhibition was calculated according to the following equation:

Percent of fungal growth reduction=((fungal growth diameter of the blank-fungal growth diameter of treatment) fungal growth diameter of blank) × 100%) [3].

Testing the activity of AqMeOH extract on growth of *Fusarium oxysporum* isolated from cucumber (FOXYC) plants: Since the growth of *F. oxysporum* isolated from cucumber plants (FOXYC) was most inhibited by the AqMeOH extract in the previous experiment, this experiment was carried out to test the antifungal activity of the AqMeOH extract at 40 ppm, 100 ppm, 200 ppm and 400 ppm against a blank solution in 9-cm diameter PDA plates. The experiment was carried out with four replications. Fungal growth diameter readings were performed three times 5 days, 10 days and 15 days after treatment. The experiment was repeated at three different times. The results of three experiments were pooled and statistically analyzed with the SAS

program (Version 7, Statistical Analysis System, 1998) for complete randomized design (CRD) arrangement.

Bio guided activity of active fractions obtained from AqMeOH

The AqMeOH extract (S5) was fractionated using column chromatography in solvent system, 3.1 dichloromethane/AqMeOH to 22 fractions. Each fraction was tested for its antifungal activity against FOXYC. Fractions #F2, F3 and F4 showed antifungal activity. Subsequently, the experiment was carried out on the active fractions in 9-cm diameter PDA plates in four replications. Fungal growth diameter readings were taken at 4 days, 7 days and 10 days after treatment. The antifungal activity of each treatment was compared with the fungicidal activity of hymexazol at 25 ppm and 50 ppm. Blank treatments were also included.

Fractions #2, 3, and 4 which showed antifungal activity against FOXYC in the previous experiment were combined and eluted in solvent system benzene/ethyl acetate on fine silica columns (230-400 Mesh, Merck). Seven sub-fractions were obtained. Each sub-fraction was evaluated for its antifungal activity against several fungi. Sub fractions 1 and 2 (sf1 and sf2) showed antifungal activity. Sub-fraction 2 was obtained in enough quantity for carrying out further testing. The antifungal activity of sf2 was compared with the activity of the fungicide hymexazol, both at 50 ppm. The experiment was carried out in 9-cm diameter PDA plates in four replications. Blank treatments were also included. Fungal growth diameter was measured at different days after treatment for almost three weeks. The percent of fungal growth reduction was then calculated as previously described and then plotted against days after treatment.

Furthermore, the antifungal activity of sf #2 on different species was studied in 9-cm diameter PDA plates in four replications. The antifungal activity of each treatment was compared with the fungicidal activity of hymexazol at 50 ppm. Blank treatments were also included. Fungal growth diameter was measured at 4 days, 7 days and 14 days after treatment. The percent of fungal growth inhibition was calculated as previously described.

In vivo potential ability of AqMeOH extract to control FOXYC in greenhouse

The roots of one-month old cucumber seedlings (*Cucumis sativus* L. cv. Beit alpha) were immersed for five minutes in cucumber *Fusarium* culture media containing spores at 8×10^6. Then, seedlings were planted in pots containing peat moss (planting media). The treatments included the following treatments in 10 replications each (one seedling is one replicate):

1. Cucumber seedlings soaked in water, planted then drenched with 40 ml water.

2. Cucumber seedlings soaked in fungal medium, then drenched with 40 ml water.

3. Cucumber seedlings soaked in fungal medium, then drenched with 40 ml hymexazol liquid formulation at a label recommended drenching rates of the fungicide Tachigaren calculated to equal 1400 ppm active ingredient.

4. Cucumber seedlings soaked in water, then drenched with 40 ml extract 1000 ppm.

5. Cucumber seedlings soaked in fungal medium, then drenched with 40 ml extract 1000 ppm.

6. Cucumber seedlings soaked in fungal medium, then drenched with 40 ml extract 500 ppm.

7. Cucumber seedlings soaked in fungal medium, then drenched with 40 ml extract 250 ppm.

The treatments were arranged in CRD in the greenhouse. The following parameters were measured three months after planting; plant shoots height, shoot dry weight, and root dry weights per plant. Browning length in the conductive tissues was measured after making a longitudinal cut in the crown area.

Statistical analysis

All experiments were statistically analyzed. Analysis of variance was conducted with the SAS program (Version 7, Statistical Analysis System, 1998) for CRD arrangement, and the means were separated by LSD0.05 according to GLM procedure (Statistical Analysis System (SAS), 1998). If the treatment mean is significantly different from the blank mean, the values of percent reduction in fungal growth were noted by a superscript $^{(S)}$. T test was also performed to detect significance of percent of mycelial growth reduction by the subfraction 2 and hymexazol.

Results

Antimicrobial activity of various extracts

The antifungal activity of the various extracts of the stinkwort plant was traced. Petri dishes containing PDA medium treated with tap water (source of inoculum) showed several types of fungal and bacterial colonies; mainly *Aspergillus niger* and *Fusarium* species, besides many un-identified bacterial colonies (Figure 1). The antifungal activity was most evident in the crude ethanolic extract (S1), followed by chloroform extract (S2) obtained from S1, then in the AqMeOH extract (S5) obtained from S2. Butanol and water extracts had no inhibitory activity.

Antifungal activity of AqMeOH extract

This result of the antifungal effect of the AqMeOH at 100 ppm indicated that the growth of the following fungi was significantly reduced; *Alternaria* sp., *Fusarium* sp. isolated from beans, *Fusarium oxysporum* isolated from cucumber (FOXYC), *Fusarium proliferatum* and *F. oxysporum* f. sp. *Lycopersici*. The percent reduction of fungal growth was marked by the super script(^{s}) as ANOVA showed significant mean differences between the treatments and their corresponding blank mean (Table 2). The growth of other fungi was reduced but not significantly. It is also noted that the antifungal effect of the extract was different on different fusaria species. *Fusarium* isolated from cowpea, tomato and grapes were least affected compared to FOXYC. Percent of fungal growth reduction was calculated (Table 2). FOXYC was most sensitive, as its growth was reduced the most (Table 2). Since FOXYC was most affected, it was selected for further experimentation.

Fungus species	Percent of growth inhibition [a]
Alternaria sp.	34^s
Fusarium sp. from bean	15^s
FOXYC	50^s
Fusarium sp. from cowpea	8
Fusarium sp. from grapes	1
Fusarium proliferatum	19^s
Fusarium oxysporum f.sp. *Lycopersici*	20^s

[a] Numbers carrying (^{s}) are significantly different from their respective blank values according to mean separation using LSD 0.05

Table 2: Percent of inhibition of fungal growth by 100 ppm AqMeOH.

Effect of AqMeOH extract on growth of FOXYC

The results of various concentrations of AqMeOH extract indicated that the mycelia growth was reduced as AqMeOH extract concentration was increased almost linearly (Table 3).

Readings (days after treatment)	Average diameter (mm) of Mycelia growth of FOXYC in AqMeOH extract concentration in ppm[a]				
	0 (Blank)	40	100	200	400
5	24.25[a]	21[a]	17.25[b]	14.25[cd]	10.5[d]
10	53.5[e]	42[f]	27[gh]	29[gh]	21.5[h]
15	90[i]	90[i]	83.3[i]	85[i]	69.3[k]
[a]Analysis of growth averages of three experiments. Means carrying same letters within the same raw are not significantly different using LSD 0.05. Analysis was performed per reading.					

Table 3: Mycelia growth of FOXYC under various concentrations of AqMeOH, during two weeks of incubation at 25°C.

There was no significant reduction of fungal growth at concentrations less than 100 ppm of the extract during the first 5 days. However, fungal growth was significantly reduced by 40 ppm and higher concentrations after 10 days of incubation. Significant reductions in mycelia growth of FOXYC was evident at concentrations ranging from 100 ppm to 400 ppm at ten days after treatment, as mycelia growth was about 50% of the growth in the blank treatment. Significant reduction of mycelia growth was obtained by the extract at 400 ppm after 15 days of incubation.

Bio guided activity of the active fractions obtained from AqMeOH in solvent system, 3.1 dichloromethane/AqMeOH on the growth of FOXYC

Partitioning AqMeOH extract in solvent system, 3.1 dichloromethane/AqMeOH yielded 22 fractions of which 2nd, 3rd and 4th fractions (F2, F3 and F4) showed significant antifungal activity.

The results indicated that the mycelia growth was significantly reduced by both hymexazol and by F2, F3, and F4 for seven days after treatment at 25 ppm and 50 ppm concentrations (Table 4). However, hymexazol activity continued to reduce the mycelia growth for the longer period, specifically with the higher concentration.

Number	Treatment	Fungal growth diameter (mm) (Days after treatment) *		
		4	7	10
1	Blank	41[a]	79.25[a]	85[a]
2	Hymexazol 50 ppm	23.75[e]	47.25[f]	73[b]
3	Hymexazol 25 ppm	28.5[d]	54.25[e]	66[c]
4	F2 at 50 ppm	32.5[c]	66.25[c]	85[a]
5	F2 at 25 ppm	34.75[b]	69.75[b]	85[a]
6	F4 at 50 ppm	29.25[d]	62.5[d]	85[a]
7	F4 at 25 ppm	34.75[bc]	70.75[b]	85[a]
8	F3 at 25 ppm	34[bc]	69.75[b]	85[a]
9	F3 at 50 ppm	30[d]	62.75[d]	85[a]
*Means carrying same letters within the same column are not significantly different at P<0.05.				

Table 4: Growth diameter (mm) of FOXYC mycelia at 4 days, 7 days and 10 days after treatment.

Bio guided activity of subfraction #2 on different species

Partitioning of the combined fractions #2, 3, and 4 in benzene / ethyl acetate system yielded seven sub fractions of which sf #2 showed antifungal activity against FOXYC. The antifungal activity of this sub fraction was comparable, if not higher than the fungicidal activity of hymexazol (Figure 2) (Table 5). The percentage of mycelial growth reduction by sf #2 was higher. T test indicated significant reduction percentage of the mycelial growth by both treatments for most of the testing period (Table 5).

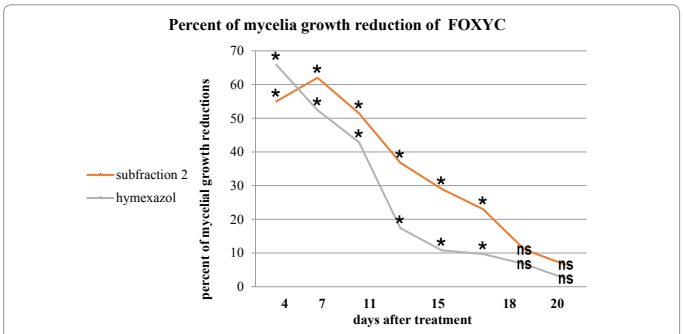


Figure 2: Percent of mycelia growth reduction of FOXYC by sub fraction 2 compared with hymexazol. Percent reduction mean carrying* is significantly different from its corresponding blank treatment mean, ns denotes that there was no significant difference between the treatment and the blank mean.

Percent of mycelial growth reduction of FOXYC*	Days after treatment							
	4	7	9	11	14	16	18	20
Hymexazol	65.9 (-75.7)	52.4 (-22.0)	42.9 (-38.1)	17.4 (-14.3)	10.9 (-10.8)	9.7 (-7.0)	6.7 (-2.4)	2.4 (-1.0)
Sub fraction 2	55.1 (-8.5)	60.2 (-8.6)	51.5 (-9.4)	36.8 (-3.6)	29.2 (-2.6)	23.2 (-2.5)	10.6 (-1.7)	6.5 (-1.7)
*(T value at p<0.05)								

Table 5: Percent of mycelial growth reduction of FOXYC.

Antifungal activity of subfraction #2 on different fungi species

The antifungal activity of the sf #2 was significant on certain fungi species and it was comparable, or more active than the fungicidal activity of hymexazol during two weeks of incubation. Most *Fusarium* spp. and *Rhizoctonia* were affected by this sub fraction. However, *Alternaria* sp. was less affected as no significant reduction was observed in its growth after 14 days of treatment (Table 6).

Fungus	Days after treatment	Percent of fungal growth inhibition (mm)*	
		Subfraction #2	Hymexazol
Fusarium proliferatum	4	59.0[(s)]	61.5[(s)]
	7	52.4[(s)]	17.4[(s)]
	14	25.0[(s)]	6.7
Fusarium sp. from bean	4	54.5[(s)]	63.5[(s)]
	7	51.8[(s)]	19.1[(s)]
	14	23.9[(s)]	0
Fusarium sp. from grapes	4	57.5[(s)]	60.3[(s)]
	7	51.8[(s)]	20.6[(s)]
	14	23.8[(s)]	7.9
Fusarium sp. from cowpea	4	44.9[(s)]	42.1[(s)]
	7	36.2[(s)]	5.0
	14	7.6	0
Alternaria sp.	4	21.4[(s)]	54.7[(s)]
	7	34.1	24.3
	14	33.0	-16.4
Rhizoctonia sp.	4	49.5[(s)]	20.7[(s)]
	7	37[(s)]	23.5[(s)]
	14	36.5[(s)]	14.4
*Values carrying the mark [(s)] indicate that the percent inhibition of fungal growth is significantly different from their perspective blanks.			

Table 6: Antifungal activity of sub fraction #2 as compared with the activity of hymexazol, both at 50 ppm on different fungi species.

In vivo potential potential ability of AqMeOH extract to control FOXYC in a greenhouse

Symptoms of *Fusarium*-infected plants appear as browning of the conductive tissues at the crown area. Stem browning at the crown area near soil surface was evident in fungal inoculated plants (Figure 3).

Figure 3: Browning of the vascular tissue of the crown area in FOXYC- infected cucumber plants.

Browning within the conductive tissues was significantly the longest among all treatments in fungal infected plants (Table 7). Drenching the infected plants with the fungicide hymexazol significantly reduced the browning length. However, drenching fungal infected cucumber plants with the AqMeOH extract at concentrations ranging from 1000 ppm to 250 ppm significantly reduced the browning at the crown area much more than the fungicide; and browning appeared lightly if present in most plants. However, drenching diseased plants with lower concentration of AqMeOH extract, light browning appeared in small areas at the crown area. Root dry weight showed variable improvement in treated plants with AqMeOH extract. Cucumber shoot height, shoot and root dry weights and fruit weight values were the highest in plants drenched with AqMeOH extract.

Treatment	Browning length at crown (mm)*	Shoot height (cm)	Shoot dry weight (g)	Root dry weight (g)
Blank (untreated plants)	0.2^{c}	43.1^{b}	2.5^{ab}	0.75^{c}
FOXYC treated plants	22.3^{a}	46.5^{b}	2.0^{ab}	0.78^{bc}
FOXYC+hymexazol drench	11.2^{b}	41.7^{b}	2.1^{ab}	0.75^{c}
AqMeOH 1000 ppm drench	0.4^{c}	59.4^{a}	1.9^{b}	1.1^{ab}
FOXYC+AqMeOH 1000 ppm drench	0.4^{c}	51.3^{ab}	2.1^{ab}	0.8^{bc}
FOXYC+AqMeOH 500 ppm drench	3.6^{bc}	47.2^{b}	2.6^{a}	1.2^{a}
FOXYC+AqMeOH 250 ppm drench	2.7^{bc}	47.2^{b}	2.4^{ab}	0.8^{bc}
*Means carrying same letters within the same column are not significantly different at P<0.05.				

Table 7: Shoot height, shoot and root dry weights, fruit weight per plant and browning length of potted cucumber plants in different treatments with the fungus FOXYC.

Discussion

Some biologically active natural compounds are produced as a mean of defense mechanism against stress or attack from other organisms. Several hundred different phytochemicals released from plants via various chemicals that are emanated, washed off from the foliage or secreted in the root zone [10], are known to affect the growth of the receiving species [20] which could be utilized as potential biocides [21].

In our search for natural compounds that may have the potential use as biopesticides, we found that extracts obtained from the stinkwort have potential antifungal activities, specifically against soil borne fungi; specifically, phytopathogenic fusarium species. Various species and corresponding strains of *Fusarium* are among the most widespread fungi in the world. However, they are highly variable because of their genetic makeup and changes in environment in which they grow causing morphological changes [22]. These pathogens affect a wide variety of hosts at any age. Phytopathogenic species of *Fusarium* are responsible for vascular wilt ex., f. sp. *cucurbitae,* which causes crown browning and rotting of fruit and root [23]. The fungus can be seed borne both internally and externally, and survive more than 1 to 2 years in seed [24]. Although plants may be affected at any stage of development, the most common expression in mature plants is marginal leaf yellowing progressing to a general yellowing of the older leaves. Vascular discoloration (browning lesions) is evident and is very diagnostic. Pre-emergence rot and damping-off can occur during propagation. Infection of older plants is more common usually leading to wilt of the entire plant. Planting fungicide-treated seed is effective in reducing the incidence of disease initiated from infected seed [25].

The directed research for the control of fusarium wilt is continuing, as no one control method proved to be sufficient. Crop rotation is not totally effective because chlamydospores survive so long in the soil, and the pathogen can survive in or on the roots of symptomless carrier plants [26]. Soil fumigation with a broad-spectrum biocide provides good initial control, but recolonization of the soil occurs very quickly. Liming the soil to pH 6.0-7.0, as well as reducing nitrogen levels in the soil, significantly reduces wilt. The most effective and practical means of controlling fusarium wilt is through the use of resistant varieties [27,28]. Fungicides as Aliette, Benlate, Carbendazim, Tachigarin, and Topsin-M were effective in controlling this phytopathogenic fungus.

Conclusion

In conclusion, the results of this research indicated that AqMeOH extract of *Inula graveolens* was effective in controlling Fusarium wilt in cucumber. The AqMeOH extract significantly reduced the fungal growth in petri dishes (Table 2). Comparably, AqMeOH extract was at least as effective as hymexazol for improving the plant health, and was more effective in reducing browning of the vascular system (Table 7). The bio guided activity testing of the active fractions obtained from AqMeOH extract in solvent system, 3.1 dichloromethane/AqMeOH resulted in obtaining antifungal fractions which were further fractionated in benzene/ethyl acetate and yielded the antifungal subfraction #2 against FOXYC besides a range of fungi (Table 6). This encouraging result necessitates that further experimentation to isolate and identify the active compound is needed.

Acknowledgements

This research was funded by the Deanship of Academic Research, The University of Jordan, Amman, Jordan. Authors would like to extend their gratitude to Miss Dania Al Muhaeisen for the technical assistance.

Conflicts of Interest

We the authors have not declared any conflict of interests.

References

1. Dayan FE (2002) Natural pesticides. In: Pimentel D, (ed). Encyclopedia of Pest Management. CRC Press, Boca Raton, Florida, USA. pp. 521- 525.

2. Hinkle T, Brunne RM, Müller H, Reichel F (1999) Statistical investigation into

the structural complementarily of natural products and synthetic compounds. AngewChem Inter (ed) 38: 643-647.

3. Aguilar-Mendez MA, Martin-Martinez ES, Ortega-Arroyo L, Cobiab-Portillo G, Sanchez-Espindola E (2011) Synthesis and characterization of silver nanoparticles: Effect on phytopathogen *Colletotrichum gloesporioides*. J Nanopart Res 13: 2525-2532.
4. Machado S (2007) Allelopathic potential of various plant species on downy brome: Implications for weed control in wheat production. Agron J 99: 127-132.
5. Willis RJ (2004) Justus Ludewig von Uslar, and the first book on allelopathy. Springer, Dordrecht, Netherland. p. 1.
6. Xuan TD, Shinkichi T, Khanh TD, Chung IM (2005) Biological control of weeds and plant pathogens in paddy rice by exploiting plant allelopathy: An overview. Crop Prot 24: 197-206.
7. Ridenour WM, Callaway RM (2001) The relative importance of allelopathy in interference: The effects of invasive weed on native bunchgrass. Oecologica 126: 444-450.
8. Cantrell CL, Dayan FE, Duke SO (2012) Natural products as sources for new pesticides. J Nat Prod 75: 1231-1242.
9. Dayan FE, Cantrell CL, Duke SO (2009) Natural products in crop protection. Bioorg Med Chem 17: 4022-4034.
10. Duke SO, Dayan FE, Rimando AM, Schrader KK, Aliotta G, et al. (2002) Chemicals from nature for Weed Manage Weed Sci 50:138-151.
11. Milbrath LR, Nechols JR (2014) Plant-mediated interactions: considerations for agent selection in weed biological control programs. Biol Control 72: 80-90.
12. Bais HP, Vepachedu R, Gilroy S, Callaway RM, Vivanco JM (2003) Allelopathy and exotic plant invasion: From molecules and genes to species interactions. Science 301: 1377- 1380.
13. Omezzine F, Ladhari A, Rinez A, Haouala R (2011) Allelopathic potential of *Inula graveolens* on crops and weeds. Allelopathy J 28: 63-76.
14. Abu Irmaileh BE, Al-Aboudi AMF, Abu Zarga MH, Awwadi, F, Haddad SF (2015) Selective phytotoxic activity of 2,3,11β,13-tetrahydroaromaticin and ilicic acid isolated from *Inula graveolens*. Nat Prod Res 29: 893-898.
15. Chengxu W, Mingxing Z, Xuhui C, Bo Q (2011) Review on allelopathy of exotic invasive plants. Procedia Eng. 18: 240-246.
16. Hierro JL, Callaway RM (2003) Allelopathy and exotic plant invasion. Plant Soil 256: 29-39.
17. Topçu G, Öksüz S, Shieh HL, Cordell GA, Pezzuto JM, et al. (1993) Cytotoxic and antibacterial sesquiterpenes from *Inula graveolens*. Phytochemistry 33: 407-410.
18. Haggag KHE, Nadia GE (2012) *In vitro* study on *Fusarium solani* and *Rhizoctonia solani* isolates causing the damping off and root rot diseases in tomatoes. Nat Sci 10: 16-25.
19. Windels CE (1989) Sugar beet Research and Extension Reports. 20: 143-148.
20. Taiz L, Zeiger E (2010) Plant physiology. (5th edn). Sinauer Associates Inc., Sunderland, Massachusetts, USA.
21. Duke SO, Charles LC, Meepagala KM, Wedge DE, Tabanca N, et al. (2010) Natural toxins for use in pest management. Toxins 2: 943-1962.
22. Nelson PE, Toussoun TA, Marsas WFU (1983) Fusarium species. An illustrated manual for identification. The Pennsylvania State University Press, University Park, Pennsylvania, USA. p. 193.
23. Zitter DL, Hopkins DL, Thomas CE (1996) Compendium of cucurbit Diseases. APS Press, St. Paul, MN, USA. p. 732.
24. Watt BA (2006) Fusarium rot of cucurbits. Fact sheet of insect and plant disease diagnostic Lab., Pest Management Office, University of Maine Cooperative Extension, USA.
25. Vakalounakis DJ (1985) A review of cucurbit diseases caused by soil-borne fungi. I. Vascular wilts. Agric Res 9: 225-264.
26. Vakalounakis DJ (1988) The genetic analysis of resistance to fusarium crown and root rot of tomato. Plant Path 37: 71-73.
27. Egelv DS, Martyn RD (2007) Fusarium wilt of watermelon and other cucurbits. The Plant Health Instructor, Purdue University, Indiana, USA.
28. Zhang S, Raza W, Yang X, Hu J, Huang Q, et al. (2008) Control of Fusarium wilt disease of cucumber plant with the application of a bioorganic fertilizer. Biol Fertil Soils 44: 1073-1080.