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Simmondsin as natural alternative fungicide in squash root rot disease

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Abstract

Jojoba (*Simmondsia chinensis*) meal was extracted with acetone, isopropanol, boiling distilled water and methanol to obtain simmondsin. Simmondsin was tested against Squash root rot disease at four concentrations (10, 20, 30 and 40 mg/ml) using the radial mycelia growth under greenhouse and field conditions. *Rhizoctonia solani* (Kuhn) and *Fusarium solani* (Mart.) were isolated from naturally infected squash roots collected from different localities in Qalubia Governorate. Evaluation simmondsin extracts revealed that acetone was the most effective as it prevented the myceial growth of *F. solani* and *R. solani* at 40 mg/ml, followed by isopropanol, boiling water and methanol in vitro. All extracts even at concentration 10 mg/ml had little effect and failed to produce a considerable reduction in growth of the tested fungi. Squash Eskandrany seeds were treated with simmondsin extracts before sowing in artificially infested soil with *F. solani* and *R. solani* (pot experiments) or soil naturally infected (epidemic soil) by the tested pathogenic fungi (field experiments) resulted in significant reduction in both damping- off and dead plant (resulted from infection by root-rot disease) compared with untreated seeds. Also, both acetone and isopropanol extract were more efficient in reducing infection by damping-off and root-rot than boiling distilled water and methanol. While, squash seeds treated with tested simmondsin extract significantly increased fruit yield/plot compared with untreated seeds. Also, all tested simmondsin extracts significant increased plant survival.

Key words: Simmondsin, antifungal, plant extract, squash, root rot, *Fusarium solani*, *Rhizoctonia solani.*

Introduction

Squash *(Cucurbita pepo)* is one of the most important vegetable crops in Egypt. Squash is subjected to attack by numerous pathogenic fungi, wherever the crop is grown. Root rot caused by *Fusarium solani* and *Rhizoctonia solani* is considered among the most deleterious diseases, which cause great losses in many parts of the world, including Slovenia (Celar, 2000) in Italy (Fantino et al., 1989) and in Egypt (Madkour et al., 1983; Abdel-el-Rehim et al., 1987). In Saudi Arabia, Nawar-Lobna (2007) reported that isolations from diseased squash roots revealed the presence of *Alternaria tenuis, Aspergillus niger, Fusarium oxysporum, F. solani* and *Rhizoctonia solani*. *Fusarium solani* and *Rhizoctonia solani* were more frequent than any of the other fungi. In Sharkia governorate Egypt, squash (*Cucurbita pepo* L.) suffer from root rot disease that causes substantial economic loss. *Rhizoctonia solani* and *Fusarium solani* were detected as the main causes of squash root rot (Abbas-Entsar, 2010).

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The use of synthetic fungicides can cause toxic residues in treated crops because they take a long time to be degraded completely (Ishii, 2001). In addition, there is a risk of pathogenic microorganisms developing resistance; this is the other disadvantage of synthetic fungicides usage (Brent & Hollomon, 1998). Bergeron et al., (1995) stated that the potential use of plant extracts with antifungal properties for control of phytopathogens has been demonstrated under laboratory, greenhouse and field conditions. Jojoba is an oil seed shrub that grows naturally on arid lands in the southwest of the United States and in Mexico.Several thousand acres have been planted in Egypt as a basis for a new economic crop (FAO, 2003). Jojoba meal contains approximately 15% of simmondsins which a group of glucosides (Elliger et al., 1973; Van Boven et al., 2000). Eight glucoside compounds (simmondsin and seven simmondsin derivatives) have been extracted and identified from jojoba seeds (Bellirou et al., 2005)**.** The extracted simmondsin compounds and simmondsin 2′-ferulate showed moderate to high antifungal activity against four plant pathogenic fungi. This is the first study on the insecticidal, antifeedant and antifungal activities of these glucosides (Abbassy et al., 2007).

The objectives of this study were to evaluate the antifungal activities of simmondsins extracted with different methods from jojoba meal on growth of two plant pathogenic fungi **(***Fusarium solani* and *Rhizoctonia solani***)** under laboratory, greenhouse and field conditions.

Materials and methods

Isolation, purification and identification of the pathogen: Naturally infected squash plants showing root rot symptoms were collected from different field in many locations in Egypt. The infected parts were cut into small pieces. These pieces were surface sterilized with sodium hypochlorite (1% NaOCl) solution for 2 minutes followed by washing with sterilized water. These sterilized pieces were transferred into potato dextrose agar (PDA) medium in 9 cm diameter Petri dishes and incubated at 25±2˚C for 5 days. Hyphal tips of the growing fungi were cut off and transferred into PDA to obtain pure cultures (Dhings & Sinclair, 1985**)**. Subcultures of purified fungi were maintained on PDA slants to be used in this study. The pure culture identified according to their morphological characters and microscopically features (Barnett and Hunter, 1981; Nelson et al., 1983).

Determination of Chemical composition of jojoba meal: Jojoba meal by-product was obtained from the Egyptian Natural Oil Co. Cairo, Egypt. Moisture, total protein, ash and fiber were determined according to the methods of AOAC, (1995)**.** Total carbohydrates were determined according to Bernfeld, (1955) and Miller, (1959). Simmondsin content in defatted jojoba meal was determined according to Van Boven et al., (1993). Total phenols were determined according to Gutfinger, (1981). Phytic acid was determined according to Latta and Eskin, (1980).

Extraction of simmondsin: Four solvents were used to extraction of simmondsin. The following methods were used to extraction of simmondsin from jojoba meal:

- Extraction with acetone: the deoiled meal was extracted with acetone for 12h by means of soxhlet apparatus according to Verbiscar et al., (1980).
- Extraction with isopropanol: Jojoba meal was extracted with isopropanol-water (7: 3) according to Medina et al., (1988).
- Extraction with boiling water: Jojoba meal was extracted with boiling water according to Verbiscar et al., (1980).
- Extraction with Methanol: Jojoba meal was extracted with Methanol according to Verbiscar et al., (1980).

Pure simmondsin was prepared as described by Van Boven et al., (1993). A typical extraction procedure was as follows:

The ground jojoba seeds (100 g) were magnetically stirred during 2 h with water (500 ml) at 90^0C in a round-bottomed flask provided with a reflux condenser. The supernatant was filtered and the oil was removed and weighed. The filtrate was dried and the residue was taken up in methanol (30 ml). After filtration in order to eliminate insoluble products, the solvent was evaporated and the brown residue obtained was weighted.

Effect of different concentration of of extracted simmondsin with some solvents *in vitro*: The reduction of mycelial growth of the tested fungi was assessed on plates containing potato dextrose agar (PDA) amended with extracts of simmondsin at the concentrations of 10, 20, 30 and 40 mg/ml according to the following procedure: Four Petri dishes (9 cm diameter) containing PDA amended with simmondsin extracts of each concentration were centrally inoculated with mycelial disk (6 mm) of each isolate taken from 7-day old culture to determine the average mycelial growth for each isolate on each concentration. Plates containing PDA medium without extract were used as control. The plates were incubated at 25°C. The linear growth of each of *Fusarium solani* and *Rhizoctonia solani* was measured when the pathogenic fungi completely covered petri dishes (Cobb et al., 1968**)**. The colony diameter was measured as the average of two perpendicular diameters. Percentage reduction of mycelial growth was calculated using the formula according to Tohamy et al., (2002)**.**

$$\% \text{ Reduction} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Greenhouse experiments: This experiment was carried out to study the efficacy of some extracts on the incidence of pre- and post-emergence damping-off diseases of squash Eskandarani cv. Pots (25 cm) in diameter were sterilized by immersing in 5% formalin solution for 15 min and then left to dry before use. Each pot was filled by 3 kg sandy loam soil sterilized by 5% formalin solution. All treatments consisted of three replicates, each replicate contained 5 pots. Healthy seeds of Eskandarani squash variety were soaking in different concentrations (10, 20, 30 and 40 mg/ml) for 15 min in extract and left to dry at room temperature. The check treatment

was treated with the fungicide Topsin-M 70. The soil was treated with different doses of simmondsin and fungicide after 10 days from sowing as soil treatments.

Sterilized soil in each pot was infested with the tested fungi *Fusarium solani* and *Rhizoctonia. solani*. The infestation was carried out at the rate of 4% of the soil weight. Control treatment was infested with the pathogen only. After infestation, all pots were irrigated twice at 3 days intervals before sowing to enhance fungal growth. Two surfaces sterilized seed of Eskandarani squash variety were sown in each pot. Percentages of pre- and post-emergence damping-off and survived plants were recorded after 15 and 25 days from sowing, respectively.

Field experiments: The experiments were carried out in a field naturally infested with the causal organisms of damping off and root rot disease of squash on the farm at El-Dear, Qalyobyia Gov., during two successive grown seasons (2012 and 2013). The experiments were designed to study the effect of the following treatments with extracts on the extent infection of Eskandarani squash cv. The experimental design was a complete randomized block with three replicates. The experimental unit area was 50 m^2 (5×10m). Each unit included five rows; was 10m in length and 100cm width. Squash seeds were planted at a rate of 20 seeds within each row. All seeds were taken from healthy plants and selected carefully, seeds dipped in each extract at the rate 10, 20, 30 and 40 mg/ml for 15 minutes before planting and other treated with fungicide (Topsin-M 70) as check, other treatment is a soil drench by different concentrations of the extract and fungicide was carried out after 15 days from planting. All agriculture practices, i.e. irrigation, fertilization as well as weeds and pests control, were applied according to the standard recommendations of the Ministry of Agriculture. Percentages of infected plants were calculated after 25 days from sowing. Also, fruit yield (kg/plot) was weighted at each harvesting time and the average was calculated.

Chemical characterization in Squash: Proximate composition was evaluated with the following methodology. Reducing sugars were determined by Lane-Eynon General Volumetric Method (AOAC, 1975). Pectins were determined by the method reported by the (AOAC, 1975). Total fiber were determined according to Prosky et al., (1988) and AOAC Method (AOAC, 1995). Total carotenoids were determined by method of the AOAC, (1995). Phenolic compounds from fruits were extracted with acetone (80%) and quantified using the reactive of Folin-Ciocalteu, following the recommendations of Xu and Chang, (2007) and Garrido et al., (2007). Simmondsin were determined according to Van Boven et al., (1993).

Statistical analysis: The data were statistically analyzed by analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, inc, 1996). Means were separated by least significant difference (L.S.D.) Test at $P \leq 0.05$ level.

Results

Chemical composition of jojoba meal: The chemical composition of jojoba meal is given in Table (1). The results showed that moisture content was 8.08%. Crude protein was 29.89%, total carbohydrates 38.68%, crude fiber 9.20% and ash content 3.11%. These results confirm the view that defatted meal is considered to be an excellent source of protein. Simmondsin level after defatted meal is 4.13%. The high toxicant level may also toxic to pathogenic fungi. Results indicate that the total phenolic compounds content of meal was 3.62% and phytic acid was 3.29%.

Table 1: Chemical composition of jojoba meal.

Components	Percentage of components
Protein	29.89
Total carbohydrates	38.68
Moisture	8.08
Ash	3.11
Fiber	9.20
Phytic acid	3.29
Phenols	3.62
Simmondsin	4.13

Effect of different concentration of four solvent of jojoba meal against squash root rots fungi *f. solani* and *R. solani*: Effect of different concentration (10, 20, 30 and 40mg/ml) of four solvent of jojoba meal against squash root rot fungi *Fusarium solani* and *Rhizoctonia solani* under laboratory conditions was shown in Table (2). The reduction percentages of the mycelial growth of the tested fungi were studied at four concentrations (10, 20, 30 and 40 mg/ml) of the simmondsin extracts from jojoba meal compared with control treatment. The results showed significant effects among the extract methods and on the concentrations (Table 2).

In the case of fungi *F. solani*, the highest effect was observed by acetone method. And no. significant differences were obtained in mycellial growth between the tested 30 and 40mg/ml concentrations; the reduction% is 100%. While significant differences were observed in all concentrations in the other extract methods except concentration 40 mg/ml in isopropanol method (100%). The lowest reduction was observed in methanol method, it was ranged from 34.4% to 83.3%. In this respect, the concentration 10 mg/ml in acetone method gave higher effect more than the other extracts, reached it 77.8%.

In the case of *R. solani*, the highest effect was observed in acetone. Significant differences were observed in mycellial growth between the all tested concentrations, and the reduction percentage ranged from 66.7% to 100%. Also, significant differences were observed in mycellial growth among all the other of extract methods. The reduction percentage ranged from 27.8% to 100% in all extract methods.The lowest effect was observed in methanol method. Wherever, the reduction percentage ranged from 27.8% to 76.7%. No significant differences were observed in concentration 40 mg/ml in acetone and isopropanol extract methods. The low concentration which gave high reduction was 10 mg/ml by acetone method; it was 66.7%.

Studying Pre and Post-emergence damping off seedling disease caused with the tested fungi under greenhouse conditions: Table (3) showed the effect of four solvent extracts on percentage of pre and post-emergence damping off disease caused by *F. solani* and *R. solani*. The infested soil with *F. Salami* has a significantly increased pre- and

post-emergence damping off of squash seedlings reduced survival rate (46.7%) compared Topsin-M 70 treatment (86.7). However, Acetone solvent extract was the most effective for decreasing percentage of pre- and post- emergence damping off, being 20 and 3.3%, followed by Isopropanol solvent extract being 20 and 6.7% on 40 mg/ml concentration respectively. On the other hand, Boiling water solvent extract showed the lowest effect being 30.0 and 67% on 40 mg/ml concentration, Acetone caused the highest percentages of plant survival (76.7%) compared with control (46.7%). Table (4) showed the effect of four extracts at the four concentrations on percentage of pre- and post-emergence damping off caused by *R. solani*. All concentrations of Acetone, Isopropanol, Methanol and Boiling water increased the percentage of healthy plants and total yield compared with control treatment during 2012 and 2013 seasons (Table 5 & 6). The highest effect was detected with Acetone treatment (40 mg/ml) 90.7 and 85.7%, followed by Isopropanol treatment (40 mg/ml) 85.7 and 84.3%. While, Methanol treatment (10 mg/ml) 78.0 and 72.3% recorded the lowest effect on healthy plants compared to control treatment 75.3 and 70.0% during the two growing seasons.Table (5 and 6) illustrated also that, Acetone, Isopropanol, Boiling water and Methanol treatments gave significant effects in increasing total fruit yields. Moreover, the increase of their concentration led to increase their effect on increasing total yields. The highest fruit yield in the two seasons was recorded with Acetone treatment (40 mg/ml) 82.3 and 78.7 kg/plot followed by Isopropanol treatment (40 mg/ml) 70.0 and 74.0 kg/plot. While, Methanol treatment (10 mg/ml) 67.0 and 61.7kg/plot recorded the lowest yield compared to control treatment 61.3 and 56.7kg/plot during the two growing seasons 2012 and 2013.

Table 2: The effect of simmondsin extracts with the four concentrations on liner growth and reduction percentage of the tested fungi.

Treatments	Rate of application (mg/ml)	*F. solani*		*R. solani*	
		Linear growth (cm)	Reduction (%)	Linear growth (cm)	Reduction (%)
Acetone	10	2.0	77.8	3.0	66.7
	20	1.7	81.1	2.4	73.3
	30	0.6	100.0	0.9	90.0
	40	0.6	100.0	0.6	100.0
Isopropanol	10	3.6	60.0	3.9	56.7
	20	2.1	76.7	2.9	67.8
	30	0.9	89.7	1.5	83.3
	40	0.6	100.0	0.6	100.0
Boiling water	10	4.0	55.6	4.4	51.1
	20	2.9	67.8	3.8	57.8
	30	1.6	82.2	2.2	75.6
	40	0.7	92.2	1.0	88.9
Methanol	10	5.9	34.4	6.5	27.8
	20	3.9	56.7	4.5	50.0
	30	2.5	72.2	3.0	66.7
	40	1.5	83.3	2.1	76.7
Control		9.0	0.0	9.0	0.0
LSD (5%):					
Treatments (T)		0.096		0.046	
Concentrations (C)		0.086		0.042	
T×C		N.S		N.S	

Table 3: Percentage of pre - and post-emergence damping off of simmondsin by four extract methods at four concentrations on *F. solani* under greenhouse conditions.

Treatments	Rate of application (mg/ml)	Pre-emergence (%)	Post-emergence (%)	Survival (%)
Acetone	10	26.7	10.0	63.3
	20	23.3	10.0	66.7
	30	23.3	6.7	70.0
	40	20.0	3.3	76.7
Isopropanol	10	26.7	13.3	60.0
	20	26.7	10.0	63.3
	30	23.3	10.0	66.7
	40	20.0	6.7	73.3
Boiling water	10	33.3	10.0	56.7
	20	30.0	10.0	60.0
	30	30.0	6.7	63.3
	40	26.7	6.7	66.7
Methanol	10	36.7	10.0	53.3
	20	33.3	10.0	56.7
	30	33.3	6.7	60.0
	40	30.0	6.7	63.3
Fungicide (Topsin-M-70)		13.3	0.0	86.7
Control		40.0	13.3	46.7
LSD (5%):				
Treatments (T)		5.67	4.22	3.62
Concentrations (C)		2.96	3.44	2.96
T×C		3,24	3.77	3.24

Table 4: Percentage of pre- and post-emergence damping off of simmondsin by four extracts methods at four concentrations on *R. solni* under greenhouse conditions.

Treatments	Rate of application (mg/ml)	Pre-emergence (%)	Post-emergence (%)	Survival (%)
Acetone	10	26.7	13.3	60.0
	20	23.3	13.3	63.3
	30	20.0	10.0	70.0
	40	20.0	6.7	73.3
Isopropanol	10	30.0	13.3	56.7
	20	30.0	10.0	60.0
	30	26.7	6.7	66.7
	40	23.3	6.7	70.0
Boiling water	10	33.3	13.3	53.3
	20	30.0	13.3	56.7
	30	26.7	10.0	63.3
	40	26.7	6.7	66.7
Methanol	10	36.7	13.3	50.0
	20	33.3	13.3	53.3
	30	33.3	10.0	56.7
	40	30.0	10.0	60.0
Fungicide (Topsin-M 70)		16.7	3.3	80.0
Control		40.0	16.7	43.3
LSD (5%):				
Treatments (T)		3.75	3.35	4.33
Concentrations (C)		3,06	2.74	3.53
T×C		3.35	2.30	3.87

Table 5: Effect of different concentrations of solvent simmondsin on squash root rots disease and yield during seasons 2012.

Treatments	Rate of application (mg/ml)	Dead plants (%)	Healthy plants (%)	Yield kg/plot
Acetone	10	18.7	81.3	73.0
	20	15.0	85.0	77.0
	30	12.7	87.3	80.0
	40	9.3	90.7	82.3
Isopropanol	10	20.0	80.0	70.7
	20	19.3	80.7	75.3
	30	17.3	82.7	76.7
	40	14.3	85.7	79.0
Boiling water	10	21.0	79.0	68.3
	20	19.7	80.3	69.7
	30	18.0	82.0	71.7
	40	16.7	83.3	75.0
Methanol	10	22.0	78.0	67.0
	20	20.7	79.3	68.0
	30	19.7	80.3	69.3
	40	17.7	82.3	70.7
Fungicide (Topsin-M-70)		5.7	94.3	91.7
Control		24.7	75.3	61.3
LSD (5%):				
Treatments (T)		2.92		3.75
Concentrations (C)		2.38		3.06
T×C		2.61		3.35

Table 6: Effect of different concentrations of solvent simmondsin on squash root rots disease and yield during seasons 2013.

Treatments	Rate of application (mg/ml)	Dead plants (%)	Healthy plants (%)	Yield kg/plot
Acetone	10	20.7	79.3	68.0
	20	18.3	81.7	71.7
	30	16.0	84.0	75.0
	40	14.3	85.7	78.7
Isopropanol	10	23.3	76.7	66.3
	20	20.3	79.7	70.0
	30	17.3	82.7	72.0
	40	15.7	84.3	74.0
Boiling water	10	25.3	74.7	63.3
	20	22.3	77.7	65.3
	30	20.7	79.3	66.7
	40	18.3	81.7	71.3
Methanol	10	27.7	72.3	61.7
	20	25.7	74.3	63.0
	30	24.0	76.0	65.7
	40	22.7	77.3	68.3
Fungicide (Topsin-M-70)		10.0	90.0	86.7
Control		30.0	70.0	56.7
LSD (5%):				
Treatments (T)		4.70		3.49
Concentrations (C)		3.83		2.85
T×C		4.20		3.12

Effect of simmondsin with higher level on the chemical composition of the squash fruits: The chemical components of the squash fruits Eskandarani cv. Were determined as moisture content, protein, oil, ash, fiber, pectin, reducing sugars, carotenoids, phenollics and simmondsin percentage and presented in Table (7). The squash fruits contained higher moisture content (86.73%). In this respect, squash fruits exhibited a good content of protein, pectin and phenolics as reach 2.54, 2.44 and 2.26%, respectively. While, the fruits had a low content of fiber, carotenoids, reducing sugar and ash as reach 1.96, 1.52, 1.19 and 1.14%, respectively. On the contrary, the fruits gave the lowest content of oil (0.24%), whatever the fruits does not contain any trace of simmondsin.

Table 7: Effect of simmondsin treatment with higher level on the chemical composition of the squash fruits.

Components	Percentage
Moisture	86.73
Protein	2.54
Oil	0.24
Ash	1.14
Fiber	1.96
Pectin	2.44
Reducing sugars	1.19
Carotenoids	1.52
Phenolics	2.26
Simmondsin	0.00

Discussion

Simmondsin is an extract of jojoba seeds), it was traditionally thought to be a toxic substance due to jojoba seeds, Simmondsin compounds showed moderate to high antifungal activity against four plant pathogenic fungi (Abbassy et al., 2007**)**.

Squash *(Cucurbita pepo)* is one of the most important vegetable crops in Egypt. Squash is subjected to attack by numerous pathogenic fungi during the growing season in the field. Root-rot caused by *Fusarium solani* and *Rhizoctonia solani* is considered among the most deleterious diseases, which caused significant losses in many places of the world (Madkour et al., 1983; Abdel-el-Rehim et al., 1987; Fantino et al., 1989; Celar, 2000; Nawar-Lobna 2007).

The effects of four concentrations of simmondsin, which obtained from jojoba meal by four solvents (Acetone, Isopropanol, Boiling water and Methanol) on the tested pathogenic fungi causing damping off in squash were evaluated for their inhibitory effect on the linear growth, *in vitro*. Also, these extracts were evaluated under greenhouse conditions for their efficiency in managing the artificial inoculation with the causal diseases. Moreover, different concentrations of simmondsin were applied under field conditions in alternations to manage the natural infection of the diseases.

It is well known that using the fungicides is considered as the shortest way to obtain efficient results of disease management. The use of synthetic fungicides can cause toxic residues in treated crops because they take a long time to be degraded completely (Ishii, 2001). In addition, there is a risk of pathogenic microorganisms developing resistance. The use of plant extracts which are not risky to the human health and the environment, for controlling plant diseases is a potentially powerful alternative method (Reddy et al., 2010; Yassin et al., 2013). Thus, simmondsin extracts are resumed as an alternative safety natural compound in this regard.

The obtained data revealed that the chemical composition of jojoba meal is Crude protein, total carbohydrates, crude fiber and ash content These results confirm the view that defatted meal is considered to be an excellent source of protein. Simmondsin level after defatted meal is high level; this high toxicant level may also toxic to pathogenic fungi. These results are in agreement with the results obtained by Hassan et al., (2003) and Abbassy et al., (2007).

In vitro experiment, using different solvent of simmondsin as natural antifungal against the tested damping off pathogens. Data revealed that all the tested concentrations caused significantly reduces the linear growth of *Fusarium solani* and *Rhizoctonia solani*. This reduction was gradually increased by increasing the incorporated concentration simmondsin to PDA medium. Similar results were obtained by Menghani et al., (2012) when simmondsins extracted by benzene, chloroform, ethyl acetate, methanol and distilled water. They were found that all the extraction methods of simmondsin were affected against selected bacteria and fungi. Also, Abbassy et al., (2007) tested the simmondsin which isolated from the jojoba plant against four plant pathogenic fungi (*Pythium debaryanum, Fusarium oxysporum, Rhizoctonia solani* and *Botrytis fabae*) which showed moderate to high antifungal activity against the tested plant pathogenic fungi. Baraka et al., (2011) reported that the three basil, marjoram and peppermint plant leaf extracts prepared with cold water were significantly inhibited the radial growth of all the tested fungi with inhibition varying from one extract to another. Marjoram extract gave the highest effect of percentage inhibition of radial growth and the lowest effect was obtained by peppermint.

Control of damping off and root rot diseases of squash using simmondsin extracts caused significant reduction to both damping off and root rot diseases either in the pot or field experiments compared to control. The obtained data show that the simmondsin extract was able to reduce pre- and post- emergence damping off caused by *F. solani* and *R. solani* and increased the healthy plants compared with control under greenhouse and field conditions. The role of plant extract to induce resistance against plant pathogens has been reported by different researchers. Similar results were obtained by Baraka et al., (2011) when the crude extracts were more effective in reducing the incidence of fungi than the aqueous extracts. This is an indication that dilution of the extracts reduced the toxic effects of the leaf extracts on the soil-borne fungi. Ghazanfar et al., (2011) found that the maximum disease reduction was observed by applying *Azadirachta indica* leaf extract, but *Datura metel* and *Allium sativum* extract were not effective. Guleria and kumar, (2006) found that aqueous leaf extract of Neem controlled Alternaria leaf spot of sesame. Antimicrobial activity and induction of systemic resistance by *Datura metal leaf* extract against *R. solani* and *Xanthomonas oryzae* pv. *oryzae* in rice has been investigated (Kagale et al., 2004) and it was found that foliar application of leaf extract effectively reduced the incidence of sheath blight and bacterial blight of rice. Satya et al., (2007) applied aqueous leaf extract of zimmu (*Allium sativum L*. x *Allium cepa L*.) to first and second leaves of cotton plants that induced systemic resistance in third and fourth leaves and reduced the number of lesions up to 73% after challenged infection with *Xanthomons campestris* pv. *malvacearum* compared with water treatment. It has been effectively used

against different plant pathogens, such as *Alternaria solani*, *Pseudomonas syringae* pv. *tomato*, *Xanthomons vesicatoria* and *Clavibacter michiganensis* of tomato and induce disease resistance (Balestra et al., 2009).

Squash is a healthy and functional vegetable because of its rich nutrients and bioactive compounds contents such as phenols, flavonoids, vitamins, amino acids, carbohydrates and minerals and its large amount of fiber **(**Tamer et al., 2010)**.**

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