Scientific J. Flowers & Ornamental Plants
www.ssfop.com/journal
ISSN: 2356-7864

EFFICACY OF ESSENTIAL OILS FROM THREE MEDICINAL AND AROMATIC PLANTS IN CONTROLLING WILT DISEASE OF ROSELLE (*HIBISCUS SABDARIFFA* L.)

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Scientific J. Flowers & Ornamental Plants, 3(2):95-105 (2016).

Received:
17/5/2016

Revised by:
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ABSTRACT: Roselle (*Hibiscus sabdariffa* L.) is one of the most important medicinal plants. Root rot and wilt diseases of roselle attack either seedlings or adult plants. In this study, the isolation and identification of the pathogen associated with root rot and wilt symptoms of roselle in Assiut were studied during the seasons 2012-2014. The efficacy of essential oils from three plants i.e., sweet basil (*Ocimum basilicum* var. *basilicum* L.), marjoram (*Majorana hortensis* L.) and peppermint (*Mentha piperita* L.), at three concentrations (0.5, 1 and 2%) were tested against the most aggressive *Fusarium* isolate *in vitro*, as well as evaluation *in vivo*. Results showed that all tested isolates, *Fusarium moniliform*, *F. solani* and three isolates of *F. oxysporum* (No. 1, 2 and 3) were pathogenic to roselle plants while the highest pathogenicity was caused by *F. oxysporum* No. 3 followed by No. 1. *In vitro*, the three tested oils were found to have an inhibitory effect against the growth of the pathogenic fungus, the highest inhibition was observed at 2% of marjoram and peppermint (2.23 and 2.00 cm inhibition zone, respectively).Under open greenhouse conditions, results of seed coating with the three essential oils under study suppressed infection with pathogenic *F. oxysporum*. Marjoram and peppermint were the most effective oils in reducing disease incidence, 0.5% concentration demonstrate the best protection of roselle seeds against *F. oxysporum*. Growth characteristics ie., plant height, No. branches and fruits/plant, fresh and dry weights of sepals were significantly higher at all treatments. Also, acidity and total anthocyanin increased significantly. Marjoram and peppermint oil at 0.5% and basil oil at 2% were the most effective concentrations in decreasing disease incidence in addition to increasing the quantity and quality of roselle yield.

Key words: Roselle, pathogenicity, *Fusarium*, anthocyanin, acidity.

INTRODUCTION

Hibiscus sabdariffa L., commonly known as karkadeh, is a summer growing famous medicinal plant belongs to family Malvaceae. In Egypt, roselle is cultivated mainly in Upper Egypt. One of the most serious obstacles that limit roselle production in many growing areas is wilt and root rot diseases. The most frequent pathogenic soil-borne fungi associated with rotted roots were *Fusarium oxysporum*, *F. solani*, *Rhizoctonia solani* and *F. aquiseti* (Ploetz *et al.*, 2007). According to Ploetz (2000) among the species of *Fusarium oxysporum*, there are pathogenic and nonpathogenic isolates that cannot be distinguished morphologically. *Fusarium oxysporum*, *F. solani* and *M.*

phaseolina are the causal pathogens of wilt and root rot diseases in roselle (*Hibiscus sabdariffa* L.), endanger roselle production wherever this crop is cultivated extensively (Hassan *et al*., 2014). Boulanger *et al.* (1984) found that *F. oxysporum* causes infection to the young plants of *H. sabdariffa*, the pathogens can damage the crop. Although synthetic fungicides are often the first line of defense against fungal diseases, the global current trend has converted to use safer and environmentally friendly alternative methods to control these organisms. The use of plant extracts and essential oils as an alternative treatment has also been discussed as a promising practice in controlling diseases (Fernando *et al*., 2013). Essential oils are aromatic substances which are obtained from various plant parts by steam distillation; many of which exhibit antifungal (Chee and Lee, 2007) activity. El-Mougy *et al*. (2007) studied the essential oils from four plants, i.e. geranium, rosa, lemon and mint and reported that seed coating at concentration of 1% clearly demonstrated a good protection of emerged bean seeds against invasion of *R. solani* and *F. oxysporum* f. sp. *Phaseoli*. *Ocimum basilicum* L. is one of the most important species amongst the member of the Ocimum genus belonging to family Lamiaceae, it has antibacterial, antifungal, insecticidal and hepatoprotective activity (Holm, 1999). A number of studies report on the antifungal action of basil essential oil (Kocic *et al*., 2011; Soliman and Badeaa, 2002 and Doube *et al*., 1989). Dried peppermint typically has 0.3-0.4% of volatile oil containing menthol, menthone, menthyl acetate, menthofuran 1.8-cineol and also contains small amounts of many additional compounds (Leung, 1980). Peppermint oil showed antifungal activity against *Fusarium sp*. by agar well diffusion method (Aqil *et al*., 2000). The genus *Majorana hortensis* Moench belonging to the family Lamiaceae is an important aromatic medicinal herb, the volatile oil commercially known as the oil of sweet marjoram (Tejavathi and Podma, 2013). The herb of Marjoram contains 1-2% of essential oil, Terpinen-4-ol, borneol (Biondi *et al*., 1993). The objectives of The present study was to investigate the efficacy of essential oils of peppermint, sweet basil and marjoram against *Fusarium oxysporum* (causal pathogens from wilted roselle plants) *in vitro*. Application of essential oils as seed treatment against wilt and root rot incidence of roselle was also evaluated under open greenhouse conditions.

MATERIALS AND METHODS

This work was carried out on pots in open greenhouse and laboratory of Plant Pathology Department, Faculty of Agriculture, Assiut University during the seasons 2012, 2013 and 2014.

Source, isolation and identification of the causal pathogen(s):

Naturally infected roselle plants that showed root rot and wilt diseases symptoms were collected from roselle plants in the farm of Arab-El-Awamer Research Station, Assiut Governorate, Egypt. The infected plant parts were cut into small pieces, surface sterilized with 5% of sodium hypochlorite, washed several times with sterilized distilled water and then dried between sterilized filter papers. The sterilized pieces were transferred aseptically into Potato Dextrose Agar (PDA) media. The inoculated plates were incubated at temperature (25±1°C), then examined after 7 days for fungal growth. The fungal colonies were purified using single conidial spore method (Booth, 1985). Identification of the isolated fungi was done (Mycology Center, Assiut University, Assiut, Egypt) as described by Booth, 1985. The obtained isolates were maintained on PDA slants and stored in refrigerator at 4°C for further study.

Pathogenicity test:

Seeds of roselle Sabahya 17 cultivar were obtained from Medicinal and Aromatic plants, Horticultural Research Institute, Agriculture Research Center, Ministry of Agriculture, Egypt. Inoculum of five tested isolates of fungi belonging to *Fusarium* genus were prepared by inoculating sterilized milk bottles 0.5 L containing Barley medium

(75 g Barley + 25 pure sand + 2 g sucrose + 0.1g yeast extract + 100ml water) Abd-El-Moneem (1996), with the fungal isolates separately and incubated at 28 ℃ for two weeks. Sterilization of soil and pots (25 cm in diameter) were carried out by 5% formalin solution for 15 minutes and left to dry for two weeks. Soil was covered with polyethylene sheet for 7 days to retain the gas. The soil was not planted until all traces of formaldehyde disappeared (after 2 weeks). Soil inoculation was performed by mixing of 2% of the inocula with the soil in each pot (100 g/5 kg soil) and then irrigated directly.

Sterilized non-inoculated barely grains were used as the control treatment. Seed disinfestations was carried out by dipping the seeds in 0.1% sodium hypochlorite for 2 min, then washed with sterile water. Each pot was seeded with 5 seeds and 3 pots were used for each isolate as replicates. Percentage of infected plants with Pre-emargance damping off, Post-emargance damping off and wilt were recorded after 15, 30 and 90 days from sowing, respectively.

The following formulae were used to determine the disease criteria:

$$\text{Pre-emargance-damping off \%} = \frac{\text{Number of non-emerged seedlings}}{\text{Number of sown seeds}} \times 100$$

$$\text{Post-emargance-damping off \%} = \frac{\text{Number of diseased seedlings}}{\text{Total number of seedlings}} \times 100$$

$$\text{Wilt \%} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

Effect of different concentrations of essential oils on the growth of pathogenic fungus:

***In vitro*:**

The antifungal activities of essential oils of three plants, sweet basil (*Ocimum basilicum* var *basilicum* L.), marjoram (*Majorana hortensis* L.*)* and peppermint *(Mentha piperita* L.) at three concentrations (0.5, 1 and 2%) were tested against the most aggressive *Fusarium sp.* isolate using the disc diffusion method (Sahin *et al*., 2004). Three replicates were used for each treatment. The clear inhibition zones of the fungal growth produced by different treatments were measured. Discs which were immersed in sterile distilled water were used as control.

All tested essential oils were obtained through hydrodistillation of marjoram, peppermint and basil plants, grown in experimental farm of Agricultural Research station, Arab-El-Awamer, Assiut Governorate.

In vivo

1- Effect of essential oils on incidence of root-rot and wilt diseases of roselle:

The effect of three concentrations (0.5, 1, and 2%) of essential oils from plants of *Ocimum basilicum*, *Mentha pipereta* and *Majorana hortensis* against root-rot and wilt diseases of rosell was evaluated under open greenhouse conditions as seed treatments.

Disinfected roselle seeds were soaked for 5 min in the tested concentrations of the above mentioned oils, 1% Tween 20 were added to the essential oil solutions (May *et al*., 2000) to obtain an aqueous emulsion. Treated and untreated seeds were sown in earthen pots (30 cm diam.) in artificially infested soil with inocula of the most aggressive *F. oxysporum* f.sp. Five seeds were sown in each pot, and three pots were used as replicates for each treatment. Seeds soaked in water were sown in control pots. Percentages of root rot and wilt incidence of roselle at pre- and post-emergence stages were estimated 30 and 90 days from planting date, respectively. Pre-emergence (%) was based on the number of un-emerged seeds in relation to the number of sown seeds, while Post-emergence (%) was based on the number of plants showing disease symptoms in relation to the number of emerged seedlings.The following formulae were used to determine the disease criteria.

$$\text{Root-rot \%} = \frac{\text{Number of diseased seedlings}}{\text{Total number of seedlings}} \times 100$$

$$\text{Wilt \%} = \frac{\text{Number of wilted plants}}{\text{Total number of plants}} \times 100$$

2- Effect of the three concentrations of essential oils of plants on growth and yield of roselle plants:

At harvest time, plant samples (5 plants each) were taken from each pots to determine the following traits; plant height (cm), number of branches/plant, number of fruits/plant, fresh weight of fruits (g)/plant, fresh and dry weights of sepals (g)/plant, total anthocyanin content and the acidity % were determined in dry sepals.

Chemical analysis:

The extraction of the total anthocyanin pigments of the dry sepals were done by using ethyl alcohol according to the method described by Tribor and Francis (1968) and the total anthocyanin content(mg/100g) was determined according to the method of Fuleki and Francis (1968), developed by Due and Francis (1973).

Dry sepals also were used to prepare extract in which total acids were determined by titrating 100 ml of 10% solution against 0.1N NaOH to pH 8.1. Total acids were expressed as citric according to the equation shown by Ruck (1963).

$$\text{Total acidity \%} = \frac{1/10 \times \text{eq. wt. of citric acid} \times \text{normality of NaOH} \times \text{titre}}{\text{wt. of sample}}$$

All data were statistically analysed according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The causal pathogen of root-rot and wilt of roselle:

Five isolates of fungi belonging to the genus *Fusarium* were isolated from naturally infected plants of roselle that showed root rot and wilt diseases symptoms. Isolates were identified as (*F. oxysporum* No. 1, 2 and 3, *F. solani* and *F. moniliform*). All the isolates were tested in a pathogenicity test and induced severe lesions were further identified and included in overall pathogenicity tested. The symptoms of pre- and post-emergence damping off and wilt that observed during the pathogenicity test were close to those observed in natural symptoms. Testing the pathogenic capability of the isolated fungi (Table, 1) indicated that all tested isolates were pathogenic to roselle plants with different rates, such results are in accordance with that reported by Ansari1 *et al.* (2013). Our results showed that the highest percentage of pre-emergence damping off caused by *F. oxysporum* No. 2 followed by isolate No. 1 (40% and 26.67%, respectively), while the highest percentage of post-emergence damping off and root rot/wilt were caused by isolate *F. oxysporum* No. 3 (80% and 93.33%), followed by the isolate *F. oxysporum* No. 1 (53% and 73.3% respectively).

Results of this study are in agreement with those reported by (Hassan *et al*., 2014 and Boulanger *et al.*, 1984).

Effect of different concentrations of essential oils on the growth of pathogenic fungus:

***In vitro*:**

The antifungal activities of three concentrations (0.5, 1 and 2%) of the three essential oils of *Majorana hortensis*, *Mentha piperita* and *Ocimum basilicum* were tested against aggressive isolate of *F. oxysporum* No. 3 and their effects were assessed by measuring the diameter of zone of inhibition (cm).

The results in Table (2) revealed that all used essential oils were effective against the tested *Fusarium* isolate at the highest concentration. The best results appeared after treatment with marjoram oil, while all tested concentrations (0.5, 1 and 2%) of the essential oil showed strong antifungal effect (0.7 cm, 2.07 cm and 2.33 cm inhibition

Table 1. Pathogenic capability of *F. spp.* isoltes on roselle (sabahya17 cultivar).

Isolates number	% of infected plants		
	Pre-emergence damping-off	Post-emergence damping-off	Wilt
Control	6.66	0.00	6.67
F. moniliform	20.00	46.00	60.00
***F. oxysporum* No. 1**	26.67	53.00	73.33
***F. oxysporum* No. 2**	40.00	20.00	26.66
***F. oxysporum* No. 3**	20.00	80.00	93.33
F. solani	13.33	26.66	40.00
L.S.D. at 5%	15.82	20.67	24.57

Data recorded after 15, 30 and 90 days from sowing date for root-rot and wilt, respectively.

Table 2. Effect of different concentrations (0.5, 1 and 2%) of the three essential oils expressed as diameter of inhibition zone (cm) against *Fusarium oxysporum.*

Treatments	Concentrations %	Zone (cm) of inhibition
Control	0.00	0.00
Marjoram essential oil	0.50	0.70
	1.00	2.07
	2.00	2.33
Peppermint essential oil	0.50	0.87
	1.00	1.83
	2.00	2.00
Basil essential oil	0.50	0.00
	1.00	0.00
	2.00	0.33

zones, respectively). The different concentrations of peppermint essential oil resulted in delay the growth of the tested fungus with different inhibitory effect on growth rate, and the inhibition zone of the growth was increased with increasing the concentrations. In case of sweet basil oil, the highest applied concentration (2%) was minimal antifungal for the tested *F. oxysporum* comparing to other treatments.

Our results were similar to the findings of Baraka *et al.* (2011), they showed that marjoram plant extract was the most effective against soil borne pathogenic fungi followed by basil, and marjoram was moderate effective at high concentration 100%. Also, Peppermint essential oil showed antifungal activity against *A. niger, Alternaria alternate* and *Fusarium sp.* by agar well diffusion method (Aqil *et al*., 2000). In other hand Soliman and Badeaa (2002) found that the oil from *O. basilicum* significantly reduced the growth of pathogenic fungi including *F. Verticillioides*. Kocic *et al.* (2011) also showed a strong antifungal activity of basil extract towards *Fusarium* spp. (*F. oxysporum, F. proliferatum* and other species) isolated from cakes, and their growth was completely inhibited at extract concentration of 1.5 ml/100 ml.

In vivo:

1- Effect of essential oils on incidence of root-rot and wilt diseases of roselle:

Results in Table (3) showed that the tested oils reduced disease severity of root rot and wilt diseases on roselle plants.

Table 3. Effect of essential oils on incidence of root-rot and wilt diseases of roselle plants.

Treatments	Concentrations (%)	Percentage of infected plants			
		Root-rot		Wilt	
		2013	2014	2013	2014
Control	0.00	36.67	40.00	90.00	93.33
Marjoram essential oil	0.50	6.67	10.00	0.00	0.00
	1.00	13.33	13.33	6.67	10.00
	2.00	20.00	20.00	13.33	16.67
Peppermint essential oil	0.50	13.33	16.67	13.33	13.33
	1.00	16.67	20.00	26.67	33.33
	2.00	20.00	23.33	40.00	40.00
Basil essential oil	0.50	20.00	23.33	40.00	43.33
	1.00	16.67	20.00	33.33	40.00
	2.00	10.00	13.33	20.00	26.67
L.S.D. at 5% (A) =		N.S	3.29	5.39	4.01
L.S.D. at 5% (B) =		4.64	3.80	6.23	4.60
L.S.D. at 5% (A×B) =		N.S	N.S	10.79	8.02

L.S.D. at 5% of Essential oils (A), Concentration (B) and Interaction (A×B).

Marjoram essential oil was the most effective against pathogenic fungus followed by peppermint oil under greenhouse conditions in the first and the second seasons, while basil essential oil was moderately effective. On other hand, all the tested concentrations of the studied oils significantly reduced the percentages of infected plants compared to control treatment. However marjoram essential oil at concentration 0.5% was the most effective in reducing infection caused by *F. oxysporum* to roselle plants recorded zero% of wiltted plants for the two cultivated seasons, Fig. 1 (A and B). According to the application of peppermint oil reduced the percentages of infected plants with *F. oxysporum* to 13% at 0.5% concentration, and severity of the disease increased with increasing the concentrations Fig. 2 (A and B). Basil essential oil was significantly decreased the percentage of rotted and wilted plants as compared to the control.

The protection increased by increasing the basil essential oil concentrations (23.33, 20 and 13.33% infected plants, respectively comparing to 40% for untreated control) Fig. 3 (A and B). These results agree with Baraka *et al*., (2011), they showed that under greenhouse conditions Marjoram, Garlic and Jojoba were the most effective as a biotic agents to reduce the disease incidence and disease severity. Similar results were also reported by Satya *et al.* (2005), they reported that application of essential oils of geranium, rosa, lemon and mint as seed coating and/or foliar spray reduced the incidence of root rot at both pre- and post-emergence stages under *in vivo* condition.

2- Effect of the three concentrations of essential oils of plants on growth and yield of roselle plants:

Data in Table (4) represented the effect of three essential oils of *Majorana hortensis*, *Mentha piperita* and *Ocimum basilicum* plants, on the plant height, number of branches/plant, number of fruits/plant and fresh weight of fruits (g)/plant, in the two cultivated seasons 2013 and 2014. Each of the tested oils was used at three different concentrations 0.5, 1.0 and 2.0%. The results showed that the effect of essential oils treatments on vegetative growth significantly

(A) **(B)**

Fig. 1. Effect of marjoram essential oil (A), at three concs. 0.5%, 1% and 2% (B) on *Fusarium oxysporum* infection of roselle plants compared with control.

(A) **(B)**

Fig. 2. Effect of peppermint essential oil (A), at three concs. 0.5%, 1% and 2% (B) on *Fusarium oxysporum* infection of roselle plants compared with control.

(A) **(B)**

Fig. 3. Effect of sweet basil essential oil (A), at three concs. 0.5%, 1% and 2% (B) on *Fusarium oxysporum* infection of roselle plants compared with control.

Table 4. Effect of three essential oils at different concentrations (0.5, 1 and 2%) on growth and yield of roselle plants.

Treatments	Conc. (%)	Plant height (cm)		No. of branches/plant		No. of fruits/plant		Fresh weight of fruits (g)/plant	
		2013	2014	2013	2014	2013	2014	2013	2014
Control	0.00	46.67	45.67	2.00	1.00	2.33	2.00	8.67	7.73
Marjoram essential oil	0.50	77.33	74.33	5.45	4.33	10.78	10.56	61.47	63.13
	1.00	70.00	71.00	4.33	4.11	7.78	7.78	44.73	45.40
	2.00	63.33	64.67	4.33	3.67	6.67	8.45	34.80	35.40
Peppermint essential oil	0.50	62.67	59.00	4.56	4.44	6.78	6.33	33.60	34.40
	1.00	56.00	51.67	4.67	3.00	5.00	5.11	22.40	21.83
	2.00	54.33	50.67	4.22	4.00	5.00	4.78	22.17	20.13
Basil essential oil	0.50	51.00	49.33	3.33	3.44	5.33	4.33	23.00	22.60
	1.00	56.67	51.67	3.45	3.44	5.67	5.57	31.60	32.87
	2.00	66.33	69.00	3.00	3.67	5.67	5.33	33.67	28.00
L.S.D. at 5% (A) =		3.86	2.69	0.57	0.33	0.48	0.38	1.72	1.87
L.S.D. at 5% (B) =		4.46	3.11	0.66	0.39	0.56	0.44	1.99	2.16
L.S.D. at 5% (A×B) =		7.72	5.39	0.95	0.68	0.97	0.76	3.45	3.75

L.S.D. at 5% of Essential oils (A), Concentration (B) and Interaction (A×B).

higher in marjoram followed by peppermint treatment than basil essential oil and compared with untreated control. Statistically significant increase was recorded in the plant height, number of branches/plant, number of fruits/plant, fresh weight of fruits/plant, and compared to untreated control in the first and the second seasons, the highest results were recorded at 0.5% concentration of marjoram (77.33 cm, 5.45, 10.78 and 63.13 g, respectively) in the firest season and decreased with increasing the concentrations. It was concluded that the most of studied growth parameters have been affected significantly after treatment with peppermint at all concentrations in the two investigated seasons, the highest result was showed at the lowest tested concentration (0.5%). The observation showed moderat effective and reported the highest results at the highest studed concentration (2%) of basil treatment. Increasing in most of studied characters with increasing the concentrations of basil was observed .Our results were similar to the findings of Hegazi and El-Kot (2010), they studied the effects of essential oils of marjoram, clove, cinnamon, garlic, ginger and fennel plants on powdery mildow of Zinnia and they revealed that all tested essential oils significantly increased the most of studied growth and flowering parameters. Data in Table (5) represented the effect of three essential oils of marjoram, peppermint and basil plants on fresh and dry weights of sepals (g)/plant as well as acidity and anthocyanin content in dry sepals in the two cultivated seasons 2013 and 2014. In addition to suppress diseases incidence, all concentrations of the tested essential oils significantly increased yield of roselle (fresh and dry weights of sepals (g)/plant) as well as anthocyanin content and acidity in dry sepals of roselle plants compared to untreated control in the first and the second seasons. The best result was obtained at 0.5% conc. of marjoram (35.37 g and 6.83 g/plant 61.07 and 27.37, respectively comparing to 4.60 g, 1.03 g/plant, 29.9 and 17.84 for infected untreated control).

El-Hadad (2007) reported that oils of nigella, rocket, peppermint and clove tree were the most effective ones in decreasing all studied diseases and increasing the quantity of yield per palm tree.

Table 5. Effect of three essential oils at different concentrations (0.5, 1 and 2%) on yield, total anthocyanin content (mg/100g) and total acidity expressed as citric acid of dry sepals of roselle plants.

Treatments	Conc. (%)	Fresh weight of sepals (g)/plant		Dry weight of sepals (g)/plant		Anthocynin (mg/100g)		Acidity (%)	
		2013	2014	2013	2014	2013	2014	2013	2014
Control	0.00	5.10	4.60	1.13	1.03	30.29	29.9	17.46	17.84
Marjoram essential oil	0.50	30.67	35.37	7.87	6.83	60.46	61.07	26.81	27.37
	1.00	25.73	26.30	5.83	5.70	58.81	58.83	24.13	26.33
	2.00	19.40	17.43	5.13	4.30	53.60	54.99	23.31	24.11
Peppermint essential oil	0.50	22.45	20.82	4.83	4.50	54.23	57.03	22.15	24.31
	1.00	12.75	14.00	4.00	3.43	43.39	45.33	20.55	22.22
	2.00	11.72	13.70	3.03	2.93	40.59	42.91	18.36	20.38
Basil essential oil	0.50	13.32	13.63	3.10	2.57	37.29	38.05	18.70	20.23
	1.00	20.27	18.00	3.03	2.83	39.36	40.28	21.62	21.20
	2.00	25.45	22.43	4.63	5.00	42.12	45.22	23.23	22.56
L.S.D. at 5% (A) =		1.85	1.20	0.45	0.27	0.28	0.27	0.18	0.99
L.S.D. at 5% (B) =		2.13	1.39	0.52	0.31	0.32	0.31	0.20	1.15
L.S.D. at 5% (A×B) =		3.70	2.40	0.91	0.54	0.56	0.54	0.35	1.98

L.S.D. at 5% of Essential oils (A), Concentration (B) and Interaction (A×B).

CONCLUSION

In the light of these results, it could be concluded that application of marjoram, peppermint and basil essential oils is applicable, have no harmful effect on the environment and cost-effective method for controlling soil borne diseases as well as improving plant growth and yield.

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فاعلية الزيوت الطيارة المستخلصة من ثلاث نباتات طبية و عطرية للتحكم فى مرض الذبول لنبات الكركديه

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أجرى هذا البحث بمزرعة كلية الزراعة، جامعة أسيوط خلال المواسم الزراعية ٢٠١٢، ٢٠١٣ و٢٠١٤ بهدف دراسة فاعلية ثلاثة من الزيوت الطيارة المستخلصة من ثلاث نباتات طبية وعطرية و هى البردقوش والنعناع والريحان فى مقاومة مرض الذبول الذى يصيب نبات الكركديه. تم عزل خمس عزلات من جنس الفيوزاريوم من نباتات الكركديه المنزرعة فى أسيوط و المصابة طبيعياً والتى ظهرت عليها اعراض الذبول كما تم عمل أختبار القدرة المرضية لهذه العزلات. أظهرت اختبارات القدرة المرضية أن العزلات الخمسة المختبرة و جميعها من جنس الفيوزاريوم قادرة على إحداث إصابة وأظهرت أعراض مرض الذبول بدرجات متفاوتة وكان أكثر الأنواع ضرراً فيوزاريوم أوكسبوريم رقم٣ حيث سجل أعلى حالة اصابة على نبات الكركدية. عند استخدام ثلاث تركيزات (٠,٥ ، ١ و ٢%) من الزيوت الثلاث المستخدمة فى المعمل، كان زيت البردقوش وزيت النعناع هما الاكثر فاعلية على عزلة الفيوزاريوم الاكثر ضرراً (فيوزاريوم اوكسبوريوم رقم ٣)، كما أظهرت النتائج أن زيادة الفاعلية للزيوت تتناسب تناسبا طرديا مع زيادة التركيز للزيوت الطيارة المستخدمة. تحت ظروف الصوبة المفتوحة أدت معاملة بذور نبات الكركديه بالزيوت الطيارة الثلاثة عند التركيزات المختلفة الى انخفاض شدة المرض بدرجات متفاوتة وسجلت معاملة البذور بزيت البردقوش أعلى نسبة انخفاض فى شدة المرض، كما ثبت أن تركيز ٠,٥% لزيوت البردقوش و النعناع هو الاكثر تأثيرا فى مقاومة المرض. أدت معاملة بذور الكركديه بالزيوت الثلاثة المستخدمة وزراعتها فى تربة معدية بالفطر المسبب للمرض الى زيادة معنوية فى معظم قياسات النمولنبات الكركديه وهى متوسطات اطوال النبات، عدد الفروع، عدد الثمار، الوزن الطازج للثمار، الوزن الطازج للسبلات وكذلك الوزن الجاف للسبلات وذلك للنبات الواحد بالمقارنة بالكنترول.

أظهرت النتائج ان معاملة البذور بالزيوت الثلاثة المستخدمة أدت الي زيادة معنوية فى درجة حموضة السبلات ومحتواها من صبغة الأنثوسيانين مقارنة بباقى المعاملات، كما أظهر تركيز ٠,٥% من الزيت الطيار لنباتي البردقوش والنعناع أعلى إنتاج وأيضاً أعلى محتوي من الأنثوسيانين و درجة الحموضة فى السبلات للنبات. بشكل عام أظهرت النتائج أن معاملة بذور الكركديه بالزيوت الطيارة المستخلصة من النباتات الثلاثة (البردقوش، النعناع والريحان) لها تأثير فعال فى مقاومة فطر فيوزاريوم اوكسبوريوم المسبب لمرض الذبول فى الكركديه و فى تحسين صفات النمو والانتاج وكذلك صفات الجودة المتمثلة فى محتوي الأنثوسيانين و درجة الحموضة في السبلات.