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Controlling *Anagallis arvensis* and *Malva parviflora* Associated Wheat Using Some Essential Oils. I- Using *Citrus sinensis* Peel Oil

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Abstract

In this study, allelopathic effects of essential oil of *Citrus sinensis* peel were investigated against the two broad leaf weeds *Anagalis arvensis* and *Malva parviflora* that infested with wheat (*Triticum aestivum* L.) in pot experiment. This investigation was carried out in the greenhouse of National Research Centre, Dokki Egypt in the two successive winter seasons, 2017/2018 and 2018/2019. The pots were sprayed with *Citrus sinensis* peel essential oil at concentrations,1, 2, 3, 4 and 5%. The results revealed inhibitory effects of essential oil on the two weeds *Anagalis arvensis* and *Malva parviflora* with more inhibitory action on *Anagalis arvensis.* The essential oil of *Citrus sinensis* peel showed a high inhibitory effect against the two weeds at high concentrations. On the other hand, weed growth inhibitions were accompanied by an increase in wheat growth as well as yield and yield components. The results suggested using *Citrus sinensis* peel essential oil as a bioherbicide.

1. Introduction

Citrus and its products are of high economic value, due to their multiple uses in many industries such as cosmetics, medical and food industries [1].It has been suggested to use citrus waste for various applications such as flavonoids and fibber pectin [2]. Orange peel essential oil is produced from citrus juice extract in the food industry. Essential oil plant can possess allelochemical activities against the growth of other organisms [3,4]. Weeds in wheat caused severe competition for important nutrients, moisture and space thus reducing wheat yield [5]. Therefore, weed management is a strategy for increasing crop production. In general, weed control methods are mechanical, chemical and biological. The continuous use of chemical compounds i.e. herbicides caused harmful effects on the environment as well as increasing resistance of weeds to the herbicides. So, producing a safe environment beside controlling weed safely must be taken in consideration through replacing with natural compounds called bioherbicides [6] . These natural compounds are called allelochemicals produced as secondary metabolites from plants a phenomenon that is called allelopathy. These allelochemicals are plant extracts and essential oils [7,8].

In general, weed suppression by essential oils has been documented by Several workers added to that, essential oil peel of several Citrus species can inhibit the germination and growth of other species[9-11]. The orange peel essential oil posses' strong activity against the germination and initial growth of several species [12]. This led to dual purposes; one of them is getting rid of a large amount of waste every year. So, this causes significant economic and environmental disposal of waste, and the accumulation of organic materials. The other is their uses in weed suppression. El-Sawi *et al*. (2019) [13] reported that the essential oil of *Citrus. sinensis* peel has an allelopathic effect on germination and seedling growth of *Heliantus annus*, *Portulaca oleracea*, *Lupinus albus* and *Malva parviflora* (1-3%). The authors added that *C. sinensis* peel essential oil at 3% inhibited 100% of germination and growth of *Lupinus albus*.

In previous studies that was carried out in the laboratory of National Research Centre (Egypt), Citrus oils peel shown inhibitory activity against germination and seedling growth of *Heliantus annus*, *Portulaca oleracea*,

Lupinus albus and *Malva parviflora* (El-Sawi *et al*., 2019) [13]. In consistence with the previous work, further studies must be applied under greenhouse conditions. Consequently, the current study aims to further applications of Citrus oils peel (*C. sinensis*) to control weeds associated wheat in pot experiment.

2. Materials and methods

2.1. Plant material and isolation of essential oils

Fruits of Citrus sinensis L (cultivar Balady orange), were obtained from the farms of the Egyptian Ministry of Agriculture. They were peeled and dried in a shady place. The essential oils of C. sinensis extracted by hydro-distillation using a Clevenger-type [14] apparatus for 4h. The essential oil was subjected to GC/MS.

2.2. Chemical analysis

2.2.1. Gas chromatography

GC analysis was performed using a Shimadzu GC- 9A gas chromatograph equipped with a DB5 fused silica column (30m×0.25mmi.d., film thickness 0.25µm). The oven temperature was held at 40°C for 5 min and then programmed until 250°C at a rate of 4°C/min. Injector and detector (FID) temperature were 260°C; helium was used as a carrier gas with a linear velocity of 32cm/s.

2.2.2. Gas chromatography/mass spectrometry

The gas - chromatograph apparatus was used. A capillary DB5 (methyl‑ silicone containing 5% phenyl groups) column (30m×0.25mm i.d.) was used. Temperature program: 2min at 60°C, 60–100°C (2°C/ min) and 100–250°C (5°C/min). Helium was used as the carrier gas at a flow rate of 1.0 ml/min. Injection volume: 1.0µL at a 1:50 split. A mass spectrometer (EI-MS 70eV) was used by using a spectral range of m/z 40–350.

2.2.3. Identification of essential oil components

The chemical compositions of C. sinensis peel essential oil were identified based on the database of mass spectra from the MS library (NBS75 and Wiely138 database) my. e. The obtained data were confirmed by injecting authentic samples of diff erent components in GC–MS under the same conditions and in comparison,with the data obtained from the literature [15, 16].

2.3. Pot experiment

The essential oil isolated from C. sinensis peel was dissolved in distilled water with the help of ethanol. The concentrations of *C. sinensis* peel oil was prepared at1, 2, 3, 4 and 5% (v/v).

Pot experiments were conducted in the greenhouse of the National Research Centre, Egypt for two winter seasons 2017/2018 and 2018/2019. Wheat cv. Giza168 was obtained from the Agricultural Research Centre, Egypt. The pots, 30 cm in diameter and 30 cm in height, contained equal amounts of sieved soil (2: 1 v/v clay and sand). Wheat grains were selected for uniformity by choosing those of equal size and with the same colour. Wheat grains were sown 2 cm deep (8 grains in each pot) and allowed to germinate. All pots (except weed free treatment) were infested with the same weight of weed (0.03 g) of both Anagalis arvensis and Malva parviflora seeds and mixed thoroughly at a depth of 2 cm in the soil. Wheat grains and both weeds were sown at the same time. The cultivated wheat grains were thinned two weeks after sowing so that three homogeneous seedlings were left per pot. Irrigation and routine fertilizers were carried out. The experiment consisted of seven treatments including: two untreated controls, wheat only, wheat with *A. arvensis* and *M. parviflora* (unweeded treatment). The other five treatments were C. sinensis peel oil at concentrations 1,2,3,4 and 5% (v/v). Each treatment was represented by 6 pots. The pots were distributed in a complete randomized design. Different concentrations (1-5%) of C. sinensis peel oil was sprayed on the pots contained wheat plants and the two weed species at the rate of 50 ml /pot. The treatments were applied two times during two weeks starting from two weeks old plants. The data were taken at 40 days after sowing and at harvest.

Weeds and Wheat data

Weeds

In each season, weed samples were taken from each of the three pots at the vegetative stage and at heading (all weed samples in each pot were pulled up). The fresh weights of A. arvensis and M. parviflora were recorded then were oven dried at 60°C for determination of dry weight (g/pot).

Wheat data

Three plants in each pot were taken for recording, plant height, number of leaves, as well as fresh and dry weight (g/plant) were recorded 40 days after sowing. At harvest, spike length, number of spikelets/spike, grain yield (g/plant) and 1000- grain weight (g) were determined.

2.4. Statistical analysis

The obtained data were subjected to analysis of variance (ANOVA) by using completely randomized design and the Least Significant Diff erence (LSD) at the 5 % probability level were calculated (Snedecor and Cochran, 1980) [17].

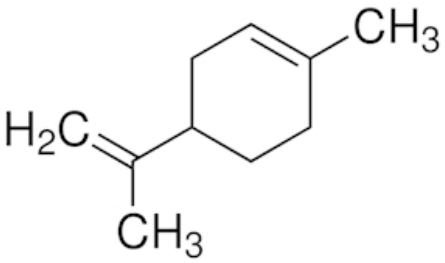
3. Results and discussion

3.1. Essential oil percentage

The oil percentage of orange peel recorded 0.73% (v/ w) based on dry weight of C. sinensis peel.

3.2. Essential oil constituents

Thirteen components have been identified that represented nearly 99.77% (Table 1). Essential oil of the Balady orange peel was characterized by high concentration of D-limonene.Also terpinene and myrcene compounds were detected in significant amounts. Some other minor components were found, such as, α−pinene, sabinene, 3-carene, terpinolene, terpinen4 ol, terpineol, decanal, cis carveol, linalyl acetate, e-citral, valencene and linalyl acetate.The monoterpenes' hydrocarbons group was founded as the main class of C. sinensis peel essential oil.



Scheme : D-limonene

Table1. Components of peel oil extracted from sweet orange (*Citrus sinensis*).

Compounds	Kovat index	%	Compound class
α-Pinene	939	1.70	MH
Sabinene	976	0.44	MH
Myrcene	991	4.42	MH
3-Carene	1011	0.55	MH
D-Limonene	1031	**86.02**	MH
γ-Terpinene	1062	0.95	MH
α-Terpinolene	1088	0.16	MH
Linalool	1098	0.23	OM
Terpinen-4-ol	1177	2.24	OM
α−Terpineol	1189	0.82	OM
Decanal	1204	1.55	VC
Linalyl acetate	1257	0.23	VC
Valencene	1491	0.46	SH
Total (all)		99.77	
Total Groups			
MH		94.24	
OM		3.29	
SH		0.46	
VC		1.78	

3.3. Weed and Wheat growth

3.31. Weed growth

The results in Table 2 revealed that *C. seninsi* oil peel at all concentrations (1-5%) suppressed significantly both fresh and dry weights of the two broad leaf weeds, *A. arvensis* and *M. parviflora* as compared to their corresponding controls. The inhibition in weed growth was concentration dependent and persistent during the experimental period. Weed growth inhibitions in both weed realized the maximum level by spraying the pots with *C. se*ninsi oil peel at 5%.

The reduction in *A. arvensis* was higher than *M. parviflora.* At the end of the season, the growth of *A. arvensis* reached a maximum reduction by using 5% *C. seninsi* oil peel, it reduced to about 76.5% as compared to the control. The corresponding result in *M. parviflora* was about 57%.

Table 2. Effect of *Citrus senensi*s oils peels on the growth of the two broadleaved weed *Anagalis arvensis* and *Malva parviflora* associated wheat plants.

Treatments	Concentration (%)	Weed growth [weight (g / pot)]					
		40 days after sowing				At the end of the season	
		A. arvensis		*M. parviflora*		*A. arvensis*	*M. parviflora*
		Fresh weight	Dry weigh	Fresh weight	Dry weight	Dry weight	Dry weight
Wheat only	**0**	**0.00**	**0.000**	**0.00**	**0.000**	**0.00**	**0.00**
Wheat+*A. arvensis*+ *M. parviflora*	**0**	**5.61**	**1.770**	**9.95**	**1.877**	**46.00**	**51.33**
Wheat+*A. arvensis*+ *M. parviflora*	**1**	**4.85**	**1.322**	**7.18**	**1.381**	**34.77**	**44.17**
	2	**4.31**	**1.204**	**6.66**	**1.328**	**23.42**	**41.19**
	3	**3.94**	**0.878**	**4.73**	**0.949**	**16.67**	**37.24**
	4	**3.41**	**0.672**	**4.36**	**0.808**	**10.95**	**28.53**
	5	**2.53**	**0.599**	**3.04**	**0.749**	**10.83**	**22.08**
LSD at 5%		**0.17**	**0.023**	**0.18**	**0.032**	**1.61**	**2.08**

3.3.2. Wheat growth

The results in Table 3 reveal significant increases in different growth parameters in wheat plants 40 days after sowing with spraying *C. seninsis* peel oil at concentrations from 1 to 5% in comparison to unweeded control. Plant height, number of leaves as well as fresh and dry weight increased significantly by spraying the oil at all concentrations. However, the number of tillers increased significantly over unweeded control by using 4 and 5% C. seninsis peel oil. The maximum increase in dry weight that recorded about 99% over unweeded control was obtained by spraying the oil peel of *C. seninsis* at 5%.

3.3.3. Wheat yield

Significant increases in spike length, number of spikes/ plant as well as a number of spikelets/spike were obtained recording remarkable values over unweeded control with high concentrations of *C. seninsis* peel oil (3-5). The results of grain yield /plant as well as a weight of 1000 grains showed significant increases over unweeded control by using all concentrations.The maximum increase in grain yield/plant exceeded that of unweeded control by 69.43%. Weight of 1000 grains recorded corresponding results reached to 46.78% over unweeded control.

In general., the essential oils extracted from diff erent plants have allelopathic eff ects on diff erent species (Kaur et al., 2011 [18]; De Oliveira et al., 2015) [19].

The results of the current study showed that the two weed species, *A. arvensis* and *M. parviflora* were reduced significantly due to the allelopathic effect of *C. seninsis* peel oil. The results also indicated more growth inhibition achieved by the high concentrations (3-5%). The inhibition in *A. arvensis* growth was more than growth inhibition in *M. parviflora.* The inhibiting activity of Citrus oil peel that well documented by Sharma &Tripathi (2006) [20], Ali et al. (2007)[12] confirmed the obtained results (Table2). These results also coincided with that obtained by Ribeir and Lima (2012) [21] who reported that peel essential oils of C. sinensis L caused severe reduction in shoot and root length of *Euphorbia heterophylla* leading to complete death as well as reduction in *Ipomoea grandifolia* seedlings growth. The oil of *C. sinensis* peel was the most potent inhibitor for root and shoot growth of *Silybum marianum* (Saad, 2013)[22]. Moreover, the results of Erukainure *et al*., 2016 [23] confirmed the current results that orange peel extracts inhibited the growth of *Lemna minor*. The authors added that the phytotoxic potential increased with increasing concentrations. These obtained results confirmed the present data (Table 2). In addition, more confirming results are the recent findings of El-Sawi *et al*. (2019) [13] who reported severe reduction in germination and growth of Malva parviflora and Portulaca oleracea with the highest concentrations of orange oils peel (3%). The reduction in *A. arvensis* weed growth more than *M. parviflora* although exposed to the same essential oil may be attributed to the activity of the essential oil was selective (Shokouhian *et al*., 2016) [24].

The inhibition of weed growth can be explained by the presence of different constituents in *C. seninsis* peel oil (Macías et al., 2007;[4] Saad and Abdelgaleil, 2014; [22] Mehmet *et al*., 2016).[10] In this respect, Vokou *et al*. (2003)[25] reported that the most active compounds against germination and seedling growth of *Lactuca sativa* that

belongs to terpinen-4-ol. These results coincided with those documented by De Almeida et al. (2010 [26]), Kotan et al. (2010) [27] as well as Ersilia *et al*. (2018) [28] who suggested that a high presence of oxygenated monoterpenes is linked to a potent phytotoxic activity of plant essential oils.

Consequently, the results of essential oil peel of C. sinensis (Table 1) are in confirmation with the finding of many workers that suggested the potent phytotoxic activity of plants essential oils is correlated to a high amount of oxygenated monoterpenes. In this respect, Other confirming results were obtained by Astani *et al*., 2009 [29]; De Almeida et al., 2010;[26] Saad, 2013 [30] and Ersilia *et al*., 2018 [28].

Growth inhibition of weeds (Table 2) was correlated with an increase in wheat growth and yield (Tables 3&4). Accordingly, plant height, number of leaves / plant as well as dry weight / plant increased over the unweeded control. In consistence, wheat yield and its components that represented by spike length, number of spiklets/spike, weight of grains/ plant and weight of 1000 grains were attained increases. Controlling *A. arvensis* and *M. parviflora* by *C. seninsis* oil peel (Table 2) reduced their competition with wheat. Consequently, increasing growth and yield (Tables 3&4). In general, a reduction in weed infestation increased crop yield (Kumar et al., 2013 [31]; Dey et al., 2015 [32]; Singh et al., 2016)[33].

Table 3. Effect of *Citrus senensis* oils peel on the growth wheat plants cv Giza 168 40 days after sowing.

Treatments	Concentration. (%)	Plant height (cm)	No. branches /plant	No. Leaves /plant	Fresh weigh (g/plant)	Dry weight (g/plant)
Wheat only	**0**	**34.00**	**3.50**	**13.00**	**1.058**	**0.129**
Wheat+*A. arvensis*+ *M. parviflora*	**0**	**23.00**	**2.66**	**8.00**	**0.585**	**0.089**
Wheat+*A. arvensis*+ *M. parviflora*	**1**	**29.67**	**2.66**	**9.00**	**0.766**	**0.091**
	2	**31.33**	**3.00**	**10.66**	**0.798**	**0.101**
	3	**32.33**	**3.00**	**12.66**	**0.817**	**0.123**
	4	**33.16**	**3.66**	**13.50**	**1.281**	**0.129**
	5	**35.00**	**3.66**	**14.17**	**1.356**	**0.177**
LSD at 5%	**2.95**	**0.56**	**0.87**	**0.176**	**0.033**	

Table 4. Effect of *Citrus senensis* oils peel on yield and yield components of wheat plants cv Giza 168.

Treatments	Concentration (percentage)	Spike length (	No. sp plant	No. Spik spike	Weight of gra plant (g)	Weight 1000grains (g)
Wheat only	**0**	**9.00**	**6.95**	**20.00**	**7.27**	**35.57**
Wheat+*A. arvensis* + *M. parviflora*	**0**	**8.00**	**5.03**	**16.50**	**4.58**	**28.47**
Wheat+*A. arvensi* *parviflora*	**1**	**8.50**	**6.10**	**18.50**	**5.48**	**33.46**
	2	**9.50**	**6.11**	**20.00**	**5.88**	**33.36**
	3	**10.50**	**6.86**	**22.00**	**6.47**	**37.99**
	4	**10.66**	**7.00**	**23.00**	**6.82**	**39.32**
	5	**10.73**	**7.00**	**23.00**	**7.76**	**41.79**
LSD at 5%		**0.66**	**0.14**	**0.95**	**0.79**	**1.37**

Conclusion

As part of our program to maximize the utilization of plant waste in order to resist weed growth and to preserve the environment, Orange peels were used in this study to achieve these objectives.The results reported an inhibitory action of essential oils on the two weeds *Anagalis arvensis* and *Malva parviflora* with more inhibitory action on *Anagalis arvensis.* The essential oil of *Citrus sinensis* peel showed a high inhibitory effect against the two weeds at high concentrations. On the other hand, weed growth inhibitions were accompanied by an increase in wheat growth as well as yield and yield components. The potent cytotoxic activity of plants essential oils is correlated to a high amount of limonene compound (monoterpenes compounds). The results suggested using *Citrus sinensis* peel essential oil as a bioherbicide.

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