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Utilization of Phenolic Compounds Extracted from Agro-Industrial Wastes as Natural Herbicides

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Abstract

Phenolic compounds are considered one of the most important secondary metabolites categories in plant that involved in allelopathic effect. Allelopathy is a chemical interaction between plants and could be defined as the stimulatory or inhibitory effects of a plant on the growth of another plant through the release of secondary compounds. The phenolic compounds, in orange juice peel waste (ORPW), olive oil mill waste (OLMW) and mango leaves waste (MLW), were extracted, then identified and determined by HPLC. The allelopathic effect of ORPW, OLMW and MLW aqueous extracts, at successive concentrations, was examined on germination and growth of two weed species (*Phalaris minor* Retz. and *Malva parviflora* L.) in petri dishes and pots bioassays. The main detected phenolic compounds were ferulic, chrysin, sinapic, *p*-hydroxybenzoic, quercetin and rosmarinic in ORPW extract, vanillic, cateachin, rutin, protocatechuic and gallic in MLW extract, and gallic, *p*-coumaric, protocatechuic, *p*-hydroxybenzoic, vanillic and ferulic in OLMW extract. The aqueous extracts of ORPW recorded the highest concentration of phenolic compounds and were the most effective in inhibition the germination and growth of both weed species followed by MLW and OLMW in petri dishes and pots trails. It can be concluded that the aqueous extracts of ORPW and MLW wastes have high contents of phenolic acids and could be utilized in controlling weeds as natural herbicides.

Keywords: Phalaris minor Retz.; *Malva parviflora* L.; HPLC; Phenolic Compounds; Allelopathy; Weeds

1. Introduction

Weed management is considered a serious problem within the agricultural production systems. Weeds interfere and compete with growing crops for nutrient, water uptake, sunlight and space [1, 2].

Application of synthetic herbicide is the most common method for weed control in agricultural production since hand weeding is expensive and time consuming. The continuous use of the synthetic herbicides produces resistant species of weeds in addition to reducing crop quality and environmental contamination [3]. Hence, there is a growing interest in using natural products for weed control in crop production and reducing the application of synthetic herbicides.

Allelopathy is defined as the ability of plants to inhibit or to stimulate growth of other plants by releasing chemical compounds (allelochemicals) in the environment by leaching, exudation, volatilization or decomposition [4, 5]. Allelopathic properties of plants could be utilized in weed control through different methods such as: crop rotation, intercropping, and allelopathic mulches as well as using isolated allelochemicals as natural herbicides to reduce the impact of synthetic chemicals on the environment [6].

Phenolic compounds are considered one of the most important secondary metabolites categories in plant that involved in allelopathic effect. Phenolic compounds are biosynthesized by the shikimate

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pathway and are abundant in plants [7]. Phenolic allelochemical structures and modes of action are diverse and can be used as natural herbicide products [8]. These natural products are considered low in cost and eco-friendly. Application of allelopathic wastes in the form of aqueous extracts, which are rich in phenolic content, could be effective in weed control [9].

The disposal of agro-industrial wastes is now becoming a serious issue. The gradual accumulation or incorrect disposal of these wastes may cause environmental problems [10]. Recent studies reported that some agro-industrial wastes, such as orange peel, olive oil mill wastes and mango leaves, have allelopathic effect and could be utilized in weed control as natural products [11, 12, 13, 14].

Orange tree (*Citrus* × *sinensis* L.) is belonging to citrus species, family Rutaceae. Orange juice production is considered an important agro-industrial economic sector that consequently produces a large amount of Orange Peel Waste (ORPW) [15]. The solid waste mass created during the industrial processing amounts to approximately half of the orange weight [16]. ORPW is composed of seeds (0 - 9%), peels (60 - 75%) and membrane residues (23 - 33%) [17].

Olive tree (*Olea europaea* L.), a species from the Oleaceae family, is growing widely in the east Mediterranean region. The olive's fruit has a major agricultural importance as the source of olive oil. Olive Oil Mill Waste (OLMW) is a by-product of olive oil production. OLMW is the solid residue that remains after olive oil extraction [18].

Mango tree (*Mangifera indica* L.) is an important fruit crop in Egypt. Mango leaves waste (MLW) is a farm residue which could be utilize in weed control as aqueous extract or soil mulch [19]. Several studies reported that ORPW, OLMW and MLW contain high value substances including phenolic compounds [20, 21, 22], which make these wastes important as allelopathic materials.

The aim of the present work was to identify and determine the phenolic compounds in orange peel, olive oil mill and mango leave wastes that could be responsible of their allelopathic effect. It also aimed to assess the allelopathic effect of these wastes on weed control in petri dishes, and pots bioassays.

2. Experimental

2.1. Preparation of aqueous extracts

Orange peel waste was obtained from El-Marwa Food Industries, Juhayna Group, Sixth of October City, Egypt. Olive oil mill waste was obtained from El-Heba Farm, Cairo-Alexandria Desert Road, Egypt. Mango leaves were obtained from the Agriculture Experimental Station of the National Research Centre, El-Nubaria, Egypt.

The examined agro-industrial wastes were checked for defects, insect damage, disease, colour change and other defects, to ensure the quality of the final product. After that, wastes were air dried at room temperature for two weeks. Then, they were grounded in electric mill until become fine powder. A known weight of each waste powder was added to 1000 ml distilled water to obtain the required concentration of the aqueous extract (10, 20, 30 and 40% w/v) for each waste material. Aqueous extracts were left for 4 hours on a shaker at room temperature, and kept in the refrigerator for 48 hours, then they were filtered by Whatman No.3 filter paper.

2.2. Phenolic compounds identification and determination by HPLC

Quantification of phenolic compounds was performed by High Performance Liquid Chromatography (HPLC) according to Kim et al. [23] using Agilent Technologies 1100 series liquid chromatography equipped with an auto sampler and a diode-array detector. The analytical column was a Eclipse XDB-C18 (150 X 4.6 µm; 5 µm) with a C18 guard column (Phenomenex, Torrance, CA).

Phenolic acids were extracted according to El-Mergawi et al. [24]. Approximately 20 ml of each extract was added to 15 ml of 4 N NaOH, shaken for 2 h in the dark with a shaker and acidified with 6 N HCl to reduce pH to 2. Samples were centrifuged at 3000 rpm, and the supernatant was decanted into separatory funnel. The supernatant was extracted with ethyl acetate (3 x 50 ml) with shaking for 10 s, and the mixture was left to settle for 5 min between extractions. The ethyl acetate fraction was dried by adding anhydrous sodium sulfate and concentrated using rotary evaporator at 40°C to dryness. The residue was resolubilized in 3 ml of methanol and filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection.

The mobile phase consisted of acetonitrile (solvent A) and 2% acetic acid in water (v/v) (solvent B). The

flow rate was kept at 0.8 ml/min for a total run time of 60 min and the gradient program was as follows: 100% B to 85% B in 30 min, 85% B to 50% B in 20 min, 50% B to 0% B in 5 min and 0% B to 100% B in 5 min. The injection volume was 10 µl and peaks were monitored simultaneously at 280, 320 and 360 nm. Peaks were identified by congruent retention times and UV spectra and compared with those of the standards. Standard phenolic compounds (gallic, protocatechuic, p-hydroxybenzoic, gentisic, cateachin, chlorogenic, syringic, ferrulic, caffeic, vanillic, sinapic, rutin, rosmarinic, apigenin-7-glucoside, qurecetin, apigenin, kaempferol, chrysin, p-coumaric and cinnamic) were purchased from Sigma Aldrich.

2.3. petri dishes experiment

Laboratory experiment was carried out at the laboratory of Botany Department, National Research Centre, Dokki, Cairo, Egypt. The aim of this experiment was to evaluate the allelopathic effect of aqueous extracts of orange peel, olive oil mill and mango leave wastes on germination and seedling growth of two weed species (*Phalaris minor* Retz., as a narrow-leaved-weed and *Malva parviflora* L., as a broad-leaved weed). Experiments were carried out in sterilized petri dishes 5 cm diameter. Filter paper was put in each petri dish as a seed bed. In each petri dish, 5 ml of extract concentration was added, kept in dark at room temperature. Each petri dish contained 10 seeds. The experiments were performed as factorial with two factors arranged in completely randomized design. The first factor was the aqueous extract of three agro-industrial waste sources (orange peel, olive oil mill and mango leave wastes), and the second factor was five concentrations of each extract (0, 10, 20, 30 and 40% w/v). Each treatment had three replicates. The experiment was repeated twice. The following data was recorded after 14 days: germination percentage, plumule length, radical length and fresh weight.

2.4. Greenhouse experiment (soil-filled pots)

The experiment was carried out at the greenhouse of Botany Department, National Research Centre, Dokki, Cairo, Egypt. This experiment was conducted to evaluate the effect of the aqueous extracts of orange peel, olive oil mill and mango leave wastes on growth of two weed species (*Phalaris minor* Retz. as a narrow-leaved-weed and *Malva parviflora* L. as a broad-leaved weed) under greenhouse conditions. Experiments were carried out in pots 30 cm diameter; pots were filled with the same volume of soil mixture (1 sand: 1 clay). Each pot was planted with 30 seeds of weeds. The pots were maintained under greenhouse condition and watered with tap water as required. Foliar spraying of treatments was applied at the rate of 500 L/ha after weed germination.

There was difficulty in the filtration of the highest concentration of the aqueous extracts. Oversize particles of the waste solid matter accumulated on top of the filter paper, blocked the filter pores and prevented the fluid phase i.e the aqueous extract from crossing the filter paper. Thus, it was difficult to obtain the required amounts of ORPW, MLW and OLPW extracts at 40% concentration that could be enough for pots trial application. Therefore, the highest concentration from all aqueous extracts used in this experiment was 30%.

The experiments were performed as factorial with two factors arranged in completely randomized design. The first factor was aqueous extract of three agro-industrial waste sources (orange peel, olive oil mill and mango leave wastes), and the second factor was four concentrations of each extract (0, 10, 20 and 30 w/v). Each treatment had three replicates. The experiment was repeated twice. After 14 days from treatment application, the following data was recorded: plant fresh weight, shoot length, root length and weed density.

2.5. Statistical analysis

Data for current study were statistically analyzed using Mstat and significant differences were calculated using Duncan multiple range test at p = 5% level. Combined analysis was made for the two growing seasons since the results of the two seasons followed a similar trend [25].

3. Results and discussion

3.1. Phenolic constituents

Twenty components of phenolics were identified and determined by HPLC, in the aqueous extracts of the examined agro-industrial wastes i.e., orange juice peel waste (ORPW), mango leaves waste (MLW) and olive oil mill waste (OLMW), as presented in Table 1.

The highest concentration of phenolic compounds was detected in the aqueous extract of ORPW, whereas MLW extract ranked the second order. The lowest content of phenolic compounds was found in OLMW extract (Table 1).

The aqueous extract of ORPW 30% (w/v) was characterized by high amounts of ferulic (68.81 mg/ 100 ml), chrysin (32.87), sinapic (30.19), *p*-

hydroxybenzoic (25.72), quercetin (23.34) and rosmarinic (15.12). It also contained moderate amounts of chlorogenic (6.64), apigenin-7-glucoside (4.06), *p*-coumaric (3.48), caffeic (2.37), vanillic (2.37) and protocatechuic acid (1.16). Some other phenolic compounds were detected in lower concentrations such as gallic (0.80), syringic (0.59), kaempferol (0.55), rutin (0.44) and cinnamic acids (0.24).

The aqueous extract of MLW 30% (w/v) contained major amounts of *p*-hydroxybenzoic (33.35 mg/100 ml), vanillic (24.55), cateachin (15.45), rutin (14.89), protocatechuic (11.46) and gallic acid (9.22), as presented in Table 1. It also contained moderate concentration of p-coumaric (4.94), apigenin-7-glucoside (3.75), ferulic (1.74) and quercetin (1.38). Some other minor phenolic compounds were detected such as cinnamic (0.81), kaempferol (0.55), syringic (0.32), caffeic (0.31), rosmarinic (0.22), chlorogenic (0.18) and Sinapic acid (0.13).

OLMW aqueous extract 30% (w/v) was characterized by moderate amounts of gallic (4.15 mg/ 100 ml), *p*-coumaric (4.15), protocatechuic (3.16), p-hydroxybenzoic (2.03), vanillic (1.73) and ferulic acid (1.01), as showed in Table 1. It also contained lower concentration of rosmarinic (0.98), rutin (0.57), quercetin (0.55), syringic (0.21), kaempferol (0.14), cinnamic (0.09), apigenin-7-glucoside (0.09), sinapic (0.07), chlorogenic (0.07) and caffeic acids (0.02).

Similar results were found by [26] who determined phenolic and flavonoid compounds of orange peel powder by HPLC and reported the presence of catechin (12.5 mg/g), caffeic acid (3.6 mg/g), naringin (5.7 mg/g), epicatechin (6.1 mg/g), rutin (17.9 mg/g), quercetin (14.0 mg/g), kaempferol (3.8 mg/g) and luteolin (5.8 mg/g). Lesage-Meessen et al. [27] examined the qualitative and quantitative HPLC analyses of phenolic compounds in the extracts of olive oil wastes and found that the amounts of p-coumaric, caffeic, ferulic and vanillic acids ranged from 2 to 30 mg/100 g of waste dry weight. Saleem et al. [28] identified the presence of the following phenolic compounds: hydroxyl benzaldehyde, m-coumaric, p-coumaric, 4-hydroxy benzoic, vanillic, caffeic, gallic and protocatechuic acids through HPLC analyses of mango leaves.

Table 1 Phenolic compounds in aqueous extracts from agro-industrial wastes (mg/100 ml) [a]

Phenolic compound	Orange peel AE[b] 30%	Mango leaves AE 30%	Olive oil mill AE 30%
Gallic	0.80	9.22	4.15
Protocatechuic	1.16	11.46	3.16
p-hydroxybenzoic	25.72	33.35	2.03
Gentisic	ND [c]	ND	ND
Cateachin	ND	15.45	ND
Chlorogenic	6.64	0.18	0.07
Caffeic	2.37	0.31	0.02
Syringic	0.59	0.32	0.21
Vanillic	2.37	24.55	1.73
Ferulic	68.81	1.74	1.01
Sinapic	30.19	0.13	0.07
p-coumaric	3.48	4.94	4.15
Rutin	0.44	14.89	0.57
Rosmarinic	15.12	0.22	0.98
Apigenin-7-glucoside	4.06	3.57	0.09
Cinnamic	0.24	0.81	0.09
Qurecetin	23.34	1.38	0.55
Apigenin	ND	ND	ND
Kaempferol	0.55	0.48	0.14
Chrysin	32.87	ND	ND

[a] mg phenolic compound per each 100 ml of the aqueous extract, [b] AE: aqueous extract, [c] ND: non detected.

According to HPLC results in the current study, the aqueous extracts of orange peel, mango leaves, and olive oil mill wastes contains high concentrations of phenolic acids and could be utilized as allelopathic extracts or natural herbicides.

3.2. Weed germination and seedling growth inhibition in petri dishes experiment

The aqueous extracts of the three agro-Industrial waste sources *i.e.*, ORPW, OLMW and MLW at different concentrations and their interaction showed significant inhibitory effects on seed germination, radicle length, plumule length and seedling fresh weight of tested weed species in petri dishes, compared to control treatment (Table 2 & 3).

The degree of inhibition depends on waste source, weed species response and extract concentrations. Seeds of Phalaris minor Retz. were more sensitive to the allelopathic effects of the aqueous extracts than *Malva parviflora* L. seeds (Tables 2 & 3). Aqueous

extracts of ORPW were, significantly, the most effective in weed inhibition followed by MLW and OLMW (Table 2). The inhibition in weed germination, radicle length, plumule length and seedling fresh weight was, significantly increased, by increasing of aqueous extract concentration (Table 2). All concentrations of ORPW extract inhibited germination and growth of P. minor and M. parviflora seeds by 100%, relative to control (0% treatment) as showed in Table 3. MLW and OLMW at 40% treatments inhibited germination and growth of *P. minor* by 100%, as compared to control (Table 3). MLV 40% treatment significantly decreased germination, radicle length, plumule length and seedling fresh weight of *M. parviflora* by 83.3, 80.6, 86.8 and 72.3%, respectively, compared to control (Table 3). Whereas, OLMW 40% treatment significantly reduced germination, radicle length, plumule length and seedling fresh weight of *M. parviflora* by 76.7, 67.2, 60.5 and 57.0%, respectively, compared to control (Table 3).

Similar results were found by [29] who found that aqueous methanol extracts of orange peel waste inhibited the growth of the roots and shoots of alfalfa, cress, crabgrass, lettuce, timothy, and ryegrass seedling in petri dishes. The methanol extracts of mango leave inhibited seedling growth of garden cress, radish, rapeseed, foxtail fescue and crabgrass, and the inhibitory effects increased with the increasing the extract concentration [11]. [30] also reported that application of OLMW aqueous extracts at different concentration decreased cheeseweed germination in petri dishes and the suppression of cheeseweed germination was rising with the increasing of extract concentration.

It can be noticed that the efficacy of the aqueous extract on inhibition the germination and growth of both weed species was related to phenolic acids content in each aqueous extract (Tables 1 & 2). The highest concentration of phenolic compounds was detected in the aqueous extract of ORPW, followed by MLW and OLMW extracts (Table 1). Consequently, the aqueous extracts of ORPW recorded the highest efficacy in weed inhibition followed by MLW and OLMW (Table 2). These results agree with [31] who reported a significant correlation between weed inhibition of allelopathic extracts and its total phenolic content.

There are several allelopathic mechanisms of phenolic compounds such as: changes in cell membrane permeability. Politycka [32] reported that benzoic and cinnamic acids derivatives increased membrane permeability of cucumber (*Cucumis sativus*) seedlings. Another mechanism of phenolic allelopathic effect is through inhibition of cell division, elongation, and submicroscopic structure. Coumarin significantly inhibited the root elongation and reduced cellular activity and the amount of Golgi body of lettuce (*Lactuca sativa* L.) seedlings [33]. Patterson [34] reported that caffeic acid, coumaric, ferulic, cinnamic and vanillic acids significantly inhibited the photosynthetic products in soybean (Glycine max). Benzoic and cinnamic acids significantly decreased leaf transpiration, stomatal conductance, and the intercellular CO_2 concentrations [35]. Phenolic could also affect enzyme functions and activities. Rice [36] reported that chlorogenic, and caffeic acids can inhibit activities of phosphorylase, while cinnamic acid and its derivatives can inhibit the hydrolysis activities of ATPase. Caffeic acid induced generation of reactive oxygen species and resulted in a significant change in the activities of peroxidase in mung bean hypocotyl cuttings [37]. Ferulic acid significantly reduced the activities of hydrolase, maltase, phospholipase and protease [38]. Benzoic acid compounds could affect the decomposition process of plant endogenous hormones e. g indole acetic acid and gibberellin [39]. Some phenolics (*i.e.*, ferulic acid and cinnamic acid) can also inhibit protein synthesis [8].

In most cases, phenolic compounds are existed as a mixture not as a single substance. Hence, the allelopathic effect of phenolic compounds is induced by the mixture effect not due to a single substance effect [40].

3.3. Weed density and weed growth inhibition in greenhouse experiment (soil-filled pots)

All extract sources and extract concentration as well as their combination significantly suppressed weed density, radicle length, plumule length and fresh weight of tested weed species in pots, as compared to control treatment (Table 4 & 5). Results of this experiment were in harmony with the laboratory experiment. The efficiency of weed suppression was also depended on extract source, weed species and extract concentration (Table 4 & 5). Growth of *P. Minor* weed was more affected by the foliar

Table 2 Effect of extract source and extract concentration on *Phalaris minor* Retz. and *Malva parviflora* L. germination, radicle length, plumule length and seedling fresh weight under laboratory conditions

Treatments	*Phalaris minor*				*Malva praviflora*			
	Weed ger.[e] (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (mg)	Weed ger. (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (mg)
Extract source								
ORPW[a]	19.3 c	2.4 c	2.2 c	10.6 c	20.0 c	1.3 c	0.7 c	7.0 c
MLW[b]	24.7 b	3.3 b	3.0 a	15.2 b	54.0 b	3.7 b	2.0 b	18.6 b
OLMW[c]	44.7 a	3.7 a	3.2 a	23.5 a	69.3 a	4.4 a	2.7 a	20.7 a
Extract conc.[d] (%)								
0	96.7 a	12.0 a	10.8 a	53.2 a	100.0 a	6.7 a	3.8 a	35.0 a
10	21.1 b	2.0 b	1.4 b	12.7 b	50.0 b	3.2 b	1.8 b	12.4 b
20	20.0 c	1.2 c	1.1 b	9.6 c	44.4 c	2.9 c	1.7 b	11.8 b
30	10.0 d	0.3 d	0.4 c	6.8 d	31.1 d	1.8 d	1.2 c	9.8 c
40	0.0 e	0.0 d	0.0 c	0.0 e	13.3 e	1.2 e	0.7 d	8.1 d
Extract source effect	***	***	***	***	***	***	***	***
Extract conc. effect	***	***	***	***	***	***	***	***
Source × Conc.	***	***	**	***	***	***	***	***

[a] orange juice peel waste; [b] mango leaves waste; [c] olive oil mill waste; [d] concentration; [e] germination. Different letters in the same column refer to statistically significant differences following Duncan's multiple range test at $P = 0.05$.

Table 3 Effect of interaction between extract source and extract concentration on Phalaris minor Retz. and Malva parviflora L. germination, radicle length, plumule length and seedling fresh weight under laboratory conditions

Treatments		*Phalaris minor*				*Malva praviflora*			
Extract source	Conc.[a] (%)	Weed ger.[b] (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (mg)	Weed ger. (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (mg)
	0	96.7 a	12.0 a	10.8 a	53.2 a	100.0 a	6.7 a	3.7 a	35.0 a
	10	0.0 g	0.0 f	0.0 d	0.0 g	0.0 i	0.0 g	0.0 e	0.0 h
ORPW [c]	20	0.0 g	0.0 f	0.0 d	0.0 g	0.0 i	0.0 g	0.0 e	0.0 h
	30	0.0 g	0.0 f	0.0 d	0.0 g	0.0 i	0.0 g	0.0 e	0.0 h
	40	0.0 g	0.0 f	0.0 d	0.0 g	0.0 i	0.0 g	0.0 e	0.0 h
	0	96.7 a	12.0 a	10.8 a	53.2 a	100.0 a	6.7 a	3.8 a	35.0 a
	10	10.0 e	2.4 c	1.9 b	11.3 e	60.0 d	4.0 c	2.4 c	18.3 bc
MLW [d]	20	10.0 e	1.7 d	1.4 bc	6.0 f	53.3 e	3.7 c	2.2 c	17.0 cd
	30	6.7 f	0.3 ef	0.7 cd	5.3 f	40.0 f	2.9 d	1.3 d	13.0 f
	40	0.0 g	0.0 f	0.0 d	0.0 g	16.7 h	1.3 f	0.5 e	9.7 g
	0	96.7 a	12.0 a	10.8 a	53.2 a	100.0 a	6.7 a	3.8 a	35.0 a
	10	53.3 b	3.7 b	2.3 b	26.7 b	90.0 b	5.5 b	3.1 b	19.0 b
OLMW [e]	20	50.0 c	1.9 cd	2.0 b	22..7 c	80.0 c	5.1 b	2.8 bc	18.3 bc
	30	23.3 d	0.7 e	0.6 cd	15.0 d	53.3 e	2.6 de	2.3 c	16.3 d
	40	0.0 g	0.0 f	0.0 d	0.0 g	23.3 g	2.2 e	1.5 d	14.7 e

[a]: concentration; [b]: germination; [c]: orange juice peel waste; [d]: mango leaves waste; [e]: olive oil mill waste. Different letters in the same column refer to statistically significant differences following Duncan's multiple range test at $P = 0.05$

Table 4 Effect of extract source and extract concentration on *Phalaris minor* Retz. and *Malva parviflora* L. density, radicle length, plumule length and seedling fresh weight under greenhouse conditions

Treatments	*Phalaris minor*				*Malva praviflora*			
	Weed density (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (g)	Weed density (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (g)
Extract source								
ORPW [a]	28.4 c	4.6 c	2.4 c	1.0 c	34.5 c	4.0 c	4.5 c	4.5 c
MLW [b]	35.2 b	5.6 b	2.9 b	1.2 b	46.0 b	9.8 b	5.0 b	5.8 b
OLMW [c]	55.4 a	7.1 a	3.8 a	2.4 a	54.3 a	11.0 a	5.5 a	7.5 a
Extract conc. [d] (%)								
0	73.4 a	9.1 a	4.9 a	3.1 a	76.0 a	13.8 a	10.1 a	11.1 a
10	40.0 b	6.4 b	3.0 b	1.5 b	48.8 b	8.4 b	3.7 b	5.8 b
20	26.3 c	4.4 c	2.2 c	0.9 c	32.4 c	5.9 c	3.3 bc	4.1 c
30	18.9 d	3.1 d	1.8 d	0.6 d	22.7 d	5.0 d	2.9 c	2.7 d

Extract source effect	***	***	***	***	***	***	**	***
Extract conc. effect	***	***	***	***	***	***	***	***
Source × Conc.	***	**	***	***	***	***	**	***

[a]: orange juice peel waste, b: mango leaves waste, c: olive oil mill waste, d: concentration. Different letters in the same column refer to statistically significant differences following Duncan's multiple range test at P = 0.05

Table 5 Effect of interaction between extract source and extract concentration on *Phalaris minor* Retz. and *Malva parviflora* L. density, radicle length, plumule length and seedling fresh weight under greenhouse conditions.

Treatments		*Phalaris minor*				*Malva praviflora*			
Extract source	Conc.[a] (%)	Weed density (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (g)	Weed density (%)	Radicle length (cm)	Plumule length (cm)	Fresh weight (g)
ORPW [b]	0	73.4 ab	9.1 a	4.9 a	3.1 a	76.0 a	13.8 a	10.0 a	11.1 a
	10	17.1 f	4.0 c	2.1 d	0.5 e	30.0 e	1.1 e	3.1 cd	3.2 f
	20	13.1 g	3.1 cd	1.5 de	0.2 f	22.1 f	0.7 e	2.9 d	2.4 g
	30	10.0 g	2.1 d	1.0 e	0.1 ef	10.0 g	0.5 e	2.0 e	1.3 h
MLW [c]	0	73.4 ab	9.1 a	4.9 a	3.1 a	76.0 a	13.8 a	10.1 a	11.1 a
	10	33.0 d	6.1 b	3.0 c	1.0 d	50.0 c	11.0 b	4.0 b	5.3 d
	20	22.7 e	4.0 c	2.0 d	0.5 e	30.0 e	8.1 c	3.0 d	3.7 e
	30	11.8 g	3.1 cd	1.5 de	0.1 f	28.0 e	6.3 d	2.9 d	2.9 f
OLMW [d]	0	73.4 ab	9.1 a	4.9 a	3.1 a	76.0 a	13.8 a	10.1 a	11.1 a
	10	70.0 b	9.0 a	4.0 b	3.0 a	66.3 b	13.0 a	3.9 b	8.8 b
	20	43.0 c	6.2 b	3.1 c	2.1 b	45.0 d	9.0 c	4.0 b	6.3 c
	30	35.0 d	4.0 c	3.0 c	1.5 c	30.0 e	8.2 c	3.8 bc	3.8 e

[a]: concentration; [b]: orange juice peel waste; [c]: mango leaves waste; [d]: olive oil mill waste. Different letters in the same column refer to statistically significant differences following Duncan's multiple range test at P = 0.05

application of allelopathic extracts than M. parviflora weed. ORPW treatments were the most effective in weed suppression, while MLW treatments came in the second order followed by OLMW (Table 4). Weed growth inhibition was increased, significantly, by increasing the extract concentration (Table 4).

The lowest values of weed density radicle length, plumule length and fresh weight was recorded in ORMW 30% treatment in both weed species, without significant difference from MLW 30% and ORPW 20% treatments in P. Minor weed (Table 5). Reduction in weed density, radicle length, plumule length and fresh weight of P. Minor weed ranged from 76.9 – 96.8% in ORPW 30% treatment, 65.9 – 96.8% in MLW 30% and 38.8 –56.1% in OLMW 30% treatment (Table 5), whereas reduction in the same parameters in M. parviflora weed ranged from 80.0 – 96.4% in ORPW 30% treatment, 54.3 – 73.9% in MLW 30% and 40.6 – 65.8% in OLMW 30% treatment respectively (Table 5).

These findings are compatible with [13] reported that orange peel waste significantly inhibited growth of canary grass and cheese weeds in pots experiment. [41] reported that mango leaves waste had allelopathic effects and significantly suppressed the shoot and root biomass of Parthenium hysterophorus L. in both petri dish and pot trails. [42] also indicated that olive oil mill wastes significantly decreased growth of four crop species: *Lepidium sativum*, *Lycopersicon esculentum*, *Lactuca sativa* and *Triticum sativum* in both laboratory and greenhouse bioassays.

In the current study, the allelopathic efficacy of the examined extracts on weed inhibition was more obvious in the petri dish bioassays (laboratory experiment) compared to the pot trails (greenhouse experiment). This agrees with results of Tubeileh et al. [29] and [43] who reported that the allelopathic effect on weeds in petri dish bioassays was higher than the pot experiments. The lower inhibitory effect in pot trails compared to petri dish bioassays can be attributed to extract loss and/or degradation by different abiotic and biotic factors e. g volatilization, photochemical decomposition, adsorbing by soil organic matter and clay, microbial decomposition, chemical breakdown and leaching. Furthermore, petri dishes bioassays are conducted in laboratory under more controlled conditions e. g temperature, humidity and light.

4. Conclusion

The aqueous extracts of orange juice peel, mango leaves, and olive oil mill wastes showed allelopathic effects against weeds in petri dishes, and pots trials. Application of the natural extracts could reduce the synthetic herbicides input in the agricultural systems. Orange juice peel followed by mango leaves wastes

showed the highest content of phenolic compounds, the most and weed control efficacy.

It can be concluded that the application of orange juice peel and mango leaves wastes in the form of aqueous extracts allelopathic effect due to its high content of phenolic acids and could be utilized in controlling weeds as alternative to synthetic herbicides.

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