Egyptian J. Desert Res., **64**, 1-16 (2014)

EXPLOITING *MATRICARIA CHAMOMILLA* FLOWERS ALLELOPATHIC CONSTITUENTS FOR CONTROL5LING ASSOCIATED WEEDS TO WHEAT CROP

Balah, Mohamed A.
Plant Protection Department, Desert Research Center, 1 Mathaf El-Matareya St., El-Matareya 11753, Cairo, Egypt
Email: mbaziz1974@gmail.com

Experiments were conducted to examine the allelopathic effect of chamomile (*Matricaria chamomilla* L. Rydb.) flowers on germination and early growth of some weeds (*Lolium multiflorum* Lam., *Avena fatua* L. *and Phalaris paradoxa* L. associated to wheat (*Triticum aestivum* L.) crops with various concentrations (10, 20, 30, 40, and 50 g l^{-1}) of aqueous extracts. Aqueous effect caused pronounced pre and post emergence inhibitory potential on *A. fatua, L. multiflorum* than *T. aestivum* that was proportional to the concentration of the extract. *Via* activity, acetylated flavonoids isolated from chloroform extracts were apigenin-7-O-(4", 6"-diacetyl glycoside) and apigenin 7-O-(4–acetyl glycoside), which caused reduction in the total weed fresh weights by 77.0 and 65.4% for *A. fatua* and 79.3 and 67.0% for *L. multiflorum*, respectively. The purified apigenin, matricolone, herniarin and coumarin reduced total biomass by 53.1, 59.1, 56.0 and 51.4% for *A. fatua* and 54.86, 58.61, 56.1 and 52.0% for *L. multiflorum*, respectively, compared to its control. The discovery of acetylated flavonoids allelopathic properties from chamomile flowers may contribute to effective and environmental friendly weeds control approaches in wheat crops.

Keywords: chamomile, allelopathy, flavonoids, sesquiterpenes, emergence activity

Chamomile (*Matricaria chamomilla* L. Rydb.) is a rich source of natural products, details on its chemical constituents of essential oil and plant parts as well as their pharmacological properties were investigated (Singh et al. 2011). Chamomile was chosen for investigation because it is widely used as a traditional medicine and belongs to a major group of cultivated medicinal plant in Egypt. Phenolic compounds including coumarins, herniarin, and umbelliferone, as well as the phenyl propanoids, chlorogenic acid and caffeic acid were reported (Pietta et al. 1987, Fonseca et al. 2008 and Kovacik and Repcak 2008). In addition to other compounds from

chamomile include common flavonoids and flavonols such as apigenin, luteolin, quercetin, rutin and naringen were identified by Redaelli et al. (1980 and 1982); Scalia et al. (1999), Bottcher et al. (2000), Fonseca and Tavares (2004) and Svehlikova et al. (2004), while some terpenes were identified by Zaiter et al. (2008) and Bottcher et al. (2000).

Chamomile extracts are used as anti-inflammatory, antiemetic, bactericide, fungicide and spasmolytic (Bisset 1994). Bisabolol was with the better growth inhibitions in *Matricaria chamomilla* essential oil, which is also the main compound, followed by the fraction containing espathulenol, bisabolol oxide and another with polyenes (Demarque et al. 2012). Characterizing new and previously identified phytochemicals with phytotoxic properties contributes to development of new weed management strategies that minimize adverse effects on the environment by replacing or reducing existing herbicide regimens. Thus, much interest has been centered on the possible incorporation of natural products as safe, selective, and low cost-effective herbicides. Natural products may offer novel molecular target sites and mechanisms of action for new herbicides (Duke et al. 2000a). The environmental half-life of many natural compounds is shorter, less toxic to the environment than many synthetic herbicides (Duke et al. 2000b), thus a large number of natural products are being tested as possible bioherbicides. For the biological importance of *Matricaria chamomilla,* its herbicidal activity was investigated.

The objective of the present work was to identify *Matricaria chamomilla* allelochemicals responsible for phytotoxic effects against several economically important weeds in wheat crops such as *Avena fatua* and *Lolium multiflorum*, as well as *Phalaris paradoxa*. Dose-response relationships between phytotoxin concentration and the plant growth parameters were evaluated. Purified active components were identified by spectroscopic methods, while the herbicidal activity was confirmed using commercial standards when possible.

MATERIALS AND METHODS

Chamomile (*Matricaria chamomilla* L. Rydb.) flowers were collected from the North Sinai, Egypt and identified according to Täckholm (1974). The specimen has been deposited in Desert Research Center. Seeds of wheat (Giza 193) were obtained from Agriculture Research Center and all weed seeds were obtained from wheat field at El Farafra Oasis, New Valley Governorate, Egypt.

1. Aqueous Extraction of Chamomile and Bioassay

Ten grams of air-dried ground flower tissues of chamomile were extracted with 100 ml distilled water on a rotary shaker for four hours at 25°C. The mixture was filtered through two layers of cheesecloth to remove

debris, and subsequently through Whatman no. 4 paper. The aqueous extract diluted with distilled water to 0.0, 10, 20, 30, 40 and 50 mg ml^{-1} concentrations. Ten seeds of each weed species (*T. aestivum, A. fatua, L. multiflorum, P. monosplensis* and *P. paradoxa*) were surface sterilized and placed on a filter paper in each of four Petri dishes (11 cm in diameter), and each treatment received ten ml of each concentration of the extract per dish. Distilled water was used as a control. Petri dishes were incubated in the room temperature at 25°C. The EC_{50} values that provided 50% reduction in plant germination percentage, the stem and root lengths of the seedlings were recorded after seven days of incubation.

2. Post Emergence Stage Bioassay of Chamomile Aqueous Extract

Plastic pots (25 cm diameter) were filled with sand soil and sown with ten seeds of *A. fatua, L. multiflorum* and *T. aestivum* in the greenhouse during February and March, 2013. Pots were watered two times weekly until the end of experiment. The sprays were done at 3-4 leaves stage of plant age with a glass sprayer at 10 ml of liquid solution per pot of extracts concentration at 5, 10, 20 g l^{-1}. Control pots were similarly sprayed with distilled water (Heisey, 1996). The sprayed pots were arranged in a randomized complete block in the greenhouse. Data for survival of seedlings were recorded in four replicates. Total fresh and dry weights of five plants were recorded two weeks after spraying. The reduction (R%) was calculated as follows: R% = $C-T/C\ x100$ Where the growth trait value is C, in control and T in treatments.

3. Organic Extraction of Chamomile

Air dried flowers were ground and 250 grams were macerated with ethanol: water (3:1) for 24 hours, followed by shaking for five hours on a shaker at room temperature. After filtration, the solution was collected and exposed to indirect heat of 40^{o}C to evaporate ethanol. Water solutions were successively extracted by partitioning with diethyl ether, chloroform and ethyl acetate with equal volumes for three times. The solvent was evaporated with a rotary evaporator and 50 milligrams of the resulting crude extracts were dissolved in ten ml of 70% ethanol for the bioassay. The most active extracts (chloroform) were fractionated by columns chromatography (120 g), which were added to the top of the column, and eluted in a successive system with increasing polarity using 100 ml of each of hexane, hexane: chloroform (1:1), chloroform, chloroform: ethyl acetate (1:1), ethyl acetate, and methanol. Twelve fractions with 50 ml volume were collected from each solvent system, then evaporated to dryness and re-dissolved in five ml of 70% ethanol to be used in the bioassay. Control treatment was treated with the same volume of 70% ethanol without extracts. Selected active fractions were further purified using TLC and HPLC system equipped with an auto

sampler and UV detector at 254 and 280 nm. Samples were run on an analytical C_{18} column (5 µm, 4.6-150 mm) using gradient elution. Mobile phase consisted of 0.1% (v/v) acetic acid in water (Solution A)-MeOH (Solution B) using the following linear gradient: 10 to 90% of Solution B over 40 min. The flow rate of the mobile phase was 0.7 ml min^{-1} and the injection volume was 20 µl.

4. Pre-emergence Activity and Phytotoxicity Bioassay

Organic extracts were tested in a similar way as the aqueous extracts with the following modifications: extracts diluted to 0, 500, 1000 and 2000 µg ml^{-1} and added to a filter paper disc (Whatman no. 1) in nine cm Petri dishes moistened with five ml distilled water, then ten seeds were sown. Samples were incubated at room temperature with a photoperiod of 16 hours and 8 hours dark. Control treatment was sown on filter paper moistened with distilled water without extracts. After seven days of incubation, the EC_{50} values that provided 50% reduction in germination percentage, the length of roots and shoots of plant seedlings were measured.

5. Phytotoxicity Assays for Seedlings in Liquid Media

Seeds of *A. fatua, L. multiflorum* and *T. aestivum* were sterilized using sodium hypochlorite (0.3% v/v) for 10-12 min and washed four times in sterile double-distilled water. Seeds were placed on static Murashige and Skoog (MS) basal media (Murashige and Skoog 1962) and allowed to germinate for seven days until roots and shoots emerged. Seven-day-old seedlings were transferred to tissue culture tubes containing five ml of liquid MS medium where the roots were submerged. Seedlings were treated with diethyl ether, chloroform and ethyl acetate extracts. Each extract was dissolved in ten ml of ethanol and diluted with water to a concentration series of 0, 50, 100, 200, 400, and 800 µg ml^{-1}. Control treatment was treated with the same volume of ethanol without extracts. The pH distribution in most liquid media treatment with and without extracts ranged from 5.5 to 5.8. Plant cultures were maintained on incubators with a photoperiod of 16 hours light and 8 hours dark at 25°C. The reduction (R%) was calculated of the total biomass of each seedling after seven days from treatment.

6. Statistical Analysis

All experiments were designed with a randomized complete block design with four replicates, repeated independently at least twice and data were statistically analyzed by ANOVA, according to Snedecor and Cochran (1990) and treatment means were compared by LSD test at 5% level of probability.

7. Chemical Identification and Structure Elucidation

The isolated compounds structures were deduced using ^{1}H and ^{13}C NMR spectra data obtained from JEOL instrument, operating at 500 MHz. The spectra were run in CD_3OD; chemical shifts (d) are given in ppm and the coupling constant (J) in hertz (Hz), using TMS as an internal standard. Chromatographically, pure materials were dissolved in pure methanol and subjected to ultraviolet spectrophotometric investigation using UV-VIS spectrophotometers thermo (Nicolet evolution 300). ESIMS was measured with a Thermo Finnigan LCQ instrument system with a Fision VG Autospec apparatus.

RESULTS AND DISCUSSION

1. Effect of Chamomile Aqueous Extraction on Weeds at Pre-emergence Stage

As shown in table 1, aqueous extract of chamomile flowers showed EC_{50} values ranged from 19.0 to 17.2 (germination), 15.0 to 14.9 (root length) and 17.5 to 13.0 mg ml^{-1} (shoot length) for *A. fatua* and *L. multiflorum*, respectively. The obtained EC_{50} displayed a high level of sensitivity to chamomile alleochemicals, and an application rate needed to reach 50% reduction was 13.3 and 16.2 mg ml^{-1} (germination), 9.20 and 13.0 mg ml^{-1} (root length), 17.5 and 17.8 mg ml^{-1} (shoot length) of *P. paradoxa* and *P. monosplensis*, respectively. As for the crops (*T. aestivum*), EC_{50} reached 48.7 (germination), 37.0 (root length) and 40.0 mg ml^{-1} (shoot length) of the inhibitory effect of chamomile aqueous extracts, which were proportional to the concentrations applied. Especially, the highest concentration (50 mg ml^{-1}) completely inhibited and suppressed seed germination of all weeds; *A. fatua, L. multiflorum, P. monosplensis and P. paradoxa* and significantly reduced *T. aestivum* growth parameters.

Table (1). Quantitative evaluation of allelopathic aqueous extracts of chamomile flowers on plants growth parameters.

Plant species	**EC_{50} (mg ml^{-1})**		
	Germination	**Root length**	**Shoot length**
T. aestivum	48.70±3.552	37.00±4.964	40.00±4.800
A. fatua	19.00±4.455	15.00±3.617	17.50±3.589
L. multiflorum	17.20±2.309	14.90±2.528	13.00±3.754
P. monosplensis	16.20±1.732	13.00±4.538	17.80±2.873
P. paradoxa	13.30±1.341	9.20±1.618	17.50±4.671

2. Effect of Chamomile Aqueous Extract on Weeds at Post Emergence Stage

This experiment was conducted in pots in the greenhouse during February and March, 2013. Chamomile water extracts were tested for their post emergence abilities with (0. 5, 10, 20 g dry wt. l^{-1}) on *A. fatua* and *L. multiflorum* and *T. aestivum,* 30 days after emergence. Thereafter, it is noteworthy that treatments of chamomile aqueous extract at 20 g l^{-1} significantly inhibited *A. fatua* and *L. multiflorum* fresh weight by 85.72 and 84.83% and dry weight by 84.27 and 87.93%, respectively over the control treatment. On the other hand, this concentration reduced the total biomass dry weight of *T. aestivum* significantly by 29.97% over its control. Furthermore, applying chamomile aqueous extract at 10 g l^{-1} showed significant decrease by 56.0 and 73.45% of *A. fatua* and *L. multiflorum* dry weight, respectively than the control. These results suggested that one of the possible mechanisms by which chamomile aqueous extract carry natural herbicides action is due to suppress weeds growth at 10 g l^{-1}. However, it has no obviously significant effect on wheat seedling (Fig. 1).

3. Liquid Media Bioassay with Weed Seedlings

The assess of diethyl ether, chloroform and ethyl acetate extracts conducted at six concentrations (0, 50, 100, 200, 400, and 800 µg ml^{-1}) were evaluated against *A. fatua, L. multiflorum* and *T. aestivum* seedlings for seven days under laboratory conditions. Results of this experiment (Fig. 2) revealed that both chloroform and ethyl acetate extracts remarkably inhibited weeds total biomass as compared with controls. The EC_{50} values of both chloroform and ethyl acetate extracts were 212.0 and 268 µg ml^{-1} (*A. fatua*), 204.5 and 263 µg ml^{-1} (*L. multiflorum*) and 278 and 315 µg ml^{-1} (*T. aestivum*) (Table 2). At higher dose (800 µg ml^{-1}), growth of weeds was significantly suppressed as compared with control. By using this assay, toxicity and dose response relationship of chloroform extracts was much better in inhibiting weeds than ethyl acetate extracts regardless of EC_{50} values. Comparing EC_{50}values revealed that *L. multiflorum* was more sensitive to chamomile extracts than *A. fatua*. Furthermore, lower negative effect observed in *T. aestivum* total biomass fresh weight than associated weeds with EC_{50} estimated by 278 µg ml^{-1} (chloroform) and 315 µg ml^{-1} (ethyl acetate) extracts, respectively, as compared with control.

4. Filter Paper Bioassay for Organic Extracts with Weed and Crop Species

Sterilized seeds of *A. fatua* and *L. multiflorum* as well as *T. aestivum* were placed on filter paper to treat with 0, 250, 500 and 1000 µg ml^{-1} concentrations of chamomile diethyl ether, chloroform and ethyl acetate extracts. Results in table 3 show that the EC_{50} of chloroform was 180, 170, 140 µg ml^{-1} for germination, root and shoot length of *L. multiflorum*,

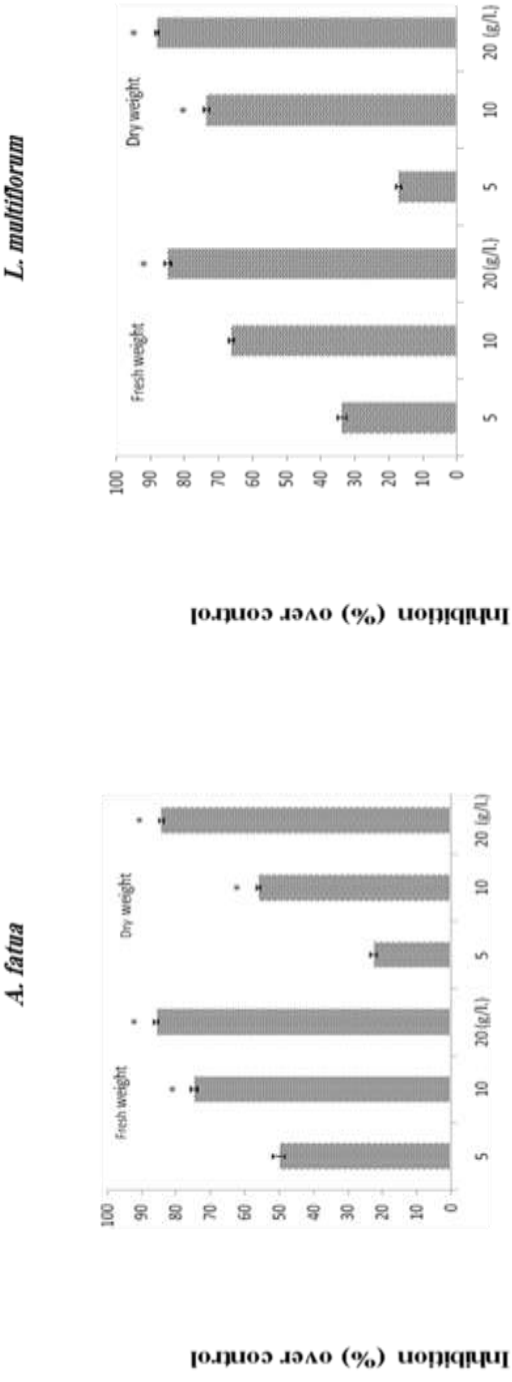

Fig. (1). Post emergence activity of *M. chamomilla* flowers water extracts on fresh and dry weights of total biomass of narrow leaf weeds (gm).

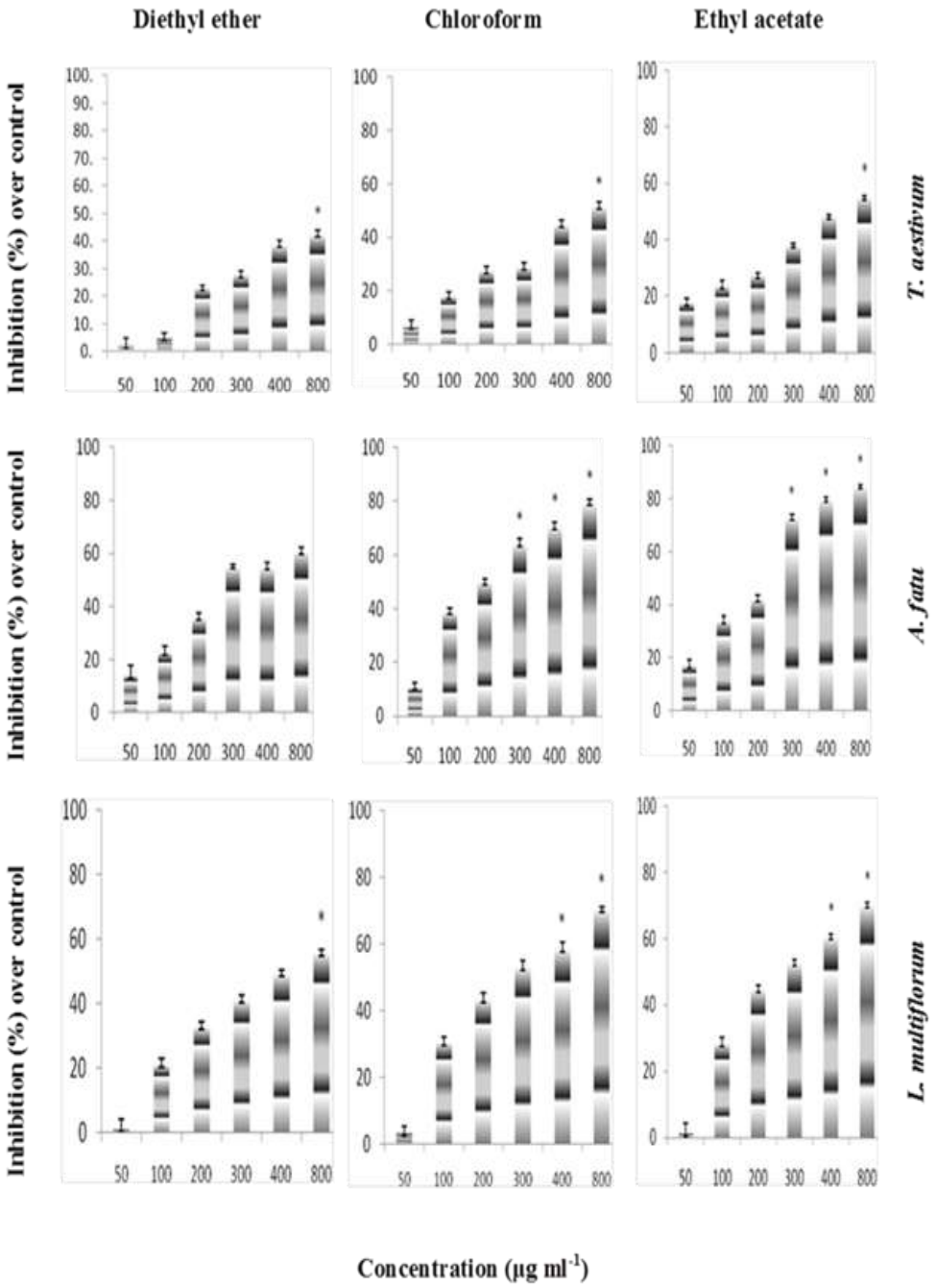

Fig. (2). Activity of diethyl ether, chloroform, and ethyl acetate extracts of chamomile flowers against tested plants.

respectively. While it showed 190, 210 and 180 μg ml^{-1} for germination, root and shoot length of *A. fatua*, respectively. On the other hand, chamomile chloroform extracts resulted EC_{50} values reaching 190 μg ml^{-1} (shoot lengths), 250 μg ml^{-1} (root length), and 260 μg ml^{-1} (germination) of *T. aestivum*. This activity results followed with a moderate effect from ethyl acetate extracts. Furthermore, the replacement of the diethyl ether chamomile extracts showed a slight effect toward the tested plant. These results proved that the presence of the chloroform extract was one of the essential extracts for the recognition of bioactive constituents as a better inhibitor against the tested weeds.

Table (2). Chloroform and ethyl acetate extracts of chamomile flowers EC_{50} values in total biomass fresh weight.

Plant species	**EC_{50} (μg ml^{-1})**	
	Chloroform	**Ethyl acetate**
T. aestivum	278.0±1.620	315±1.641
A. fatua	212.0±2.245	268.0±2.414
L. multiflorum	204.5±1.130	263.0±1.023

Table (3). Quantitative evaluation of organic extracts of chamomile flowers on plant growth parameters though pre-emergence activity.

Extracts	**Plant species**	**EC_{50}**		
		Germination	**Root length**	**Shoot length**
Diethyl ether	*T. aestivum*	630.0±7.591	400.0±6.725	350.0±8.074
	A. fatua	600.0±7.08	500.0±4.133	420.0±3.753
	L. multiflorum	480.0±5.00	420.0±5.722	390.0±9.371
Chloroform	*T. aestivum*	260.0±3.365	250.0±7.151	190.0±6.443
	A. fatua	190.0±7.074	210.0±6.671	180.0±7.083
	L. multiflorum	180.0±9.160	170.0±5.00	140.0±5.710
Ethyl acetate	*T. aestivum*	600.0±5.425	475.0±4.655	380.0±5.625
	A. fatua	270.0±3.162	260.0±6.208	240.0±2.516
	L. multiflorum	250.0±8.151	243.0±8.617	210.0±4.315

5. Characterization of Purified Phytotoxic Compounds from Chloroform Extracts

The structures of the most active chloroform fractions were elucidated by comprehensive spectroscopic analysis and also directed to comparison with the respective published data and with the commercial

product when it is possible (Fig. 3). Two active acylated flavonoids were detected in chamomile flowers and pre identified by their molecular ions; 86 equivalent mass units (amu) for diacetyl and 42 (amu) for mono acetyl larger than those of their corresponding parent peak (apigenin) and attached glycoside:

The first active compound had molecular weight of 516, this compound had UV (MeOH) λ_{max} that reached 296.8 nm; m/z (M-1): 539 (M-Na), 499.1 (15%), 359.2.3 (5%), 269.2 (100%), 199.1 (15%) and 143.3 (8%). The ^{1}H NMR: 6.6 (s, H-3″), 6.4 (d, J=1.5, H-2″), 6.5 (d, J=1.5, H-6″), 6.8 (d, H-8″), 3.2 (s, H-5″), 7.8 (d, J=5.5,H-2″), 6.9 (d, J=5.5,H-5″), 4.83 (s, H-4″), 4.6 (s, H-3″), 4.5 (s, H-6″), 3.2 (d, J=10) 2.3 (s, COCH3), 2.1(s,COCH3). ^{13}CNMR:165.5 (C-2), 104.3 (C-3), 183.2 (C-4), 158.7 (C-5), 100.3 (C-6), 164.9 (C-7), 95.6 (C-8), 162.0 (C-9), 105.1 (C-10), 222.7 (C-1′), 129.8 (C-2′), 117.3 (C-3′), 161.8 (C-4′), 117.3 (C-5′), 126.3 (C-6′), 169.6, 169.3 (acetyl), 20.5 (acetyl), 98.3 (C-1"), 76.0 (C-2"), 74.2 (C-3"), 69.7 (C-4"), 76.3 (C-5"), 59.8 (C-6"). It could be concluded that it is apigenin-7-O-(4", 6"-diacetyl glycoside).

The second active compound had λ_{max} thatreached 335.0 nm (MeOH) and molecular weight of 474.2 m/z $(M+1)^{+}$ 475.2, 439.4, 293.2, 201.2 ^{1}HNMR: 6.6 (s, H-3), 6.5 (d, J=1.5, H-6), 6.8 (d, H-8), 7.9 (d, J=8.0, H-2″), 6.9 (d, J=8.0, H-3″), 6.9 (d, J=8.9,H-5″), 7.9 (d, J=80, H-6″), 3.5 (m, H-3″), 3.4 (d, J=90, H-4″), 3.8 (d, J=4.5, H-5″), 4.2 (d, J=11.5, H-6a″), 4.4 (d, J=11.5,H-6″), 2.1 ($COCH_3$).^{13}CNMR: 166.9 (C-2), 104.1 (C-3), 1 84.2 (C-4), 163.3 (C-5), 100.6 (C-6), 164.6 (C-7), 96.3 (C-8), 158.8 (C-9), 106.7 (C-10), 122.9 (C-1″), 129.8 (C-2″), 117.2 (C-3″), 163.3 (C-4″), 1 17.2 (C-5″), 119.8 (C-6″), Glucose, 101.3 (C-1″), 74.5 (C-2″), 77.6 (C-3″), 71.3 (C-4″), 75.9 (C-5″), 64.9 (C-6″), 172.6 ($COCH_3$). It is an acylated flavonoids compound and identified as apigenin7-O-(4″–acetyl glycoside).

The third toxic constitute was provisionally identified based on the analysis of retention time (Rt = 39.4 min) beside mass and NMR data. This compound had UV (MeOH) (λ_{max}) reached 337.9 nm and have molecular weight of 270 m/z $(M+1)^{+}$: 272 (18%), 271 (100%), 243.04 (10%), 153.019 (96%), 145.03 (8%), 119.04 (22%), 69.03 (8%). ^{1}H NMR: 7.8 (d, J=2.2,H-6), 6.9 (d, J=5.5,H-3′), 6.56 (s, H-5), 6.42 (s,H-3), 6.18 (s, H-8), 4.83 (s, H-1), 3.28 (d, J=4, H-4). ^{13}CNMR: 165.5 (C-2), 104.3 (C-3), 183.2 (C-4), 158.7(C-5), 100.3(C-6),164.9 (C-7), 95.6 (C-8), 162.0 (C-9), 105.1 (C-10), 222.7 (C-1′), 129.8 (C-2′), 117.3 (C-3′), 161.8 (C-4′), 117.3 (C-5′), 126.3 (C-6), it could identified as apigenin.

The fourth compound have UV (MeOH) (λ_{max}) reached 310.0 nm and molecular weight of 176.1 with m/z $(M+1)^{+}$: 177.1, 133.1, 121.2, ^{1}H - NMR: 7.8 (d, J = 9.4, H-8), 7.4 (d, J = 8.5 Hz, H-3), 6.81 (d, J = 2.4, H-5), 6.8 (d, J = 8.5, 2.4, H-4), 6.25 (d, J = 9.5, H-6), 3.87 (S, 3H) ppm. ^{13}C NMR: 162.8 (C=O), 161.2 (C), 155.9 (C), 143.4 (CH), 128.8 (CH), 128.8 (CH),

113.1 (CH), 112.6 (CH), 112.5 (C), 100.9 (CH), 55.8 (CH_3) ppm. It could be herniarin (7-methoxycoumarin).

The fifth compound was identified by its molecular ion peak (M^+) at m/e 146 (76%) and a base peak at m/z 118 (100%) by the loss of 28 mass units equivalent, this is equivalent to carbon monoxide. It was additionally and provisionally identified based on the analysis of retention time (RT= 25.0 min) with stranded chemicals as a coumarin.

The sixth compound have UV absorbance (λmax) that reached 266 nm in MeOH and molecular weight of 326 with m/z 327.3 (60%), 319.4 (100%), 279 (30%), 267 (21%), 219.2 (9%). ^{1}HNM: 3.58 (d, J=11.7, H-1), 1.62 (m, H-2), 4.81 (d, J=12.5), 1.7 (d, J=11.4, H-5), 1.65 (m, H-6), 1.46 (d, J=12.9, H-7), 1.91 (d, J=12.9, H-8), 2.0 (d, J=12.9, H-9), 2.29 (d, J=12.6), 1.23 (d, J=6.9, H-12), 1.01 (S, H-13),1.37 (S, H-14), 2.10 (S, H-15). ^{13}CNMR: 75.7 (C-1), 75.9 (C-3), 73.4 (C-4), 54.0 (C-5), 79.8 (C-6), 23.3 (C-8), 39.2 (C-9), 42.1 (C-10), 40.4 (C-11), 177.8 (C-12), 12.4 (C-13), 13.7 (C-14), 18.9 (C-15), 21.4 ($COCH_3$). It could be matricolone (3β-Acetoxy-1 β,4 α -dihydroxy-c5,6 β,7 α,11 β H-10 β α-methyl)-eudesman-12, 6 α-olide).

The obtained results indicate the presence of apigenin-7-O-(4", 6"-diacetyl glycoside), apigenin 7-O-(4"–acetyl glycoside), apigenin, matricolone, herniarin and coumarin have been isolated from active chloroform extracts, which were purified from chamomile at 50 µg ml^{-1}. These extracts reduced total biomass by 77.0, 65.4, 53.1, 59.1, 56.0 and 51.4% (*A. fatua*) and 79.3, 67.0, 54.86, 58.61, 56.1 and 52.0% (*L. multiflorum*), respectively, over their control (Fig. 3).

Summarizing this study, effects of chamomile and its major components on suppression of weeds associated with wheat crop was elucidated. The results proved that chamomile flowers have pre and post emergence potential against *A. fatua, L. multiflorum, P. monosplensis* and *P. paradoxa* weeds and have a slight effect in *T. aestivum*, except in the higher concentrations. It means there was little selectivity of chamomile allelopathic activity in wheat specially when sprayed as post emergence application during 3-4 leave stage. The toxicity of aqueous and organic extracts increased with increasing concentration to all weeds and the crop to prove that the most allelochemicals were presented in water extracts. In the same issue, chloroform extracts of chamomile flower had clear herbicidal activity than ethyl acetate and diethyl ether. Both of *P. monosplensis* and *P. paradoxa* are the most sensitive weeds to chamomile allelochemicals, whereas *L. multiflorum* is more sensitive than *A. fatua* for both aqueous and organic extracts. As for the host crop *T. aestivum*, it appeared more tolerant to chamomile allelochemicals. This result is supported by Itani et al. (2013) and Matysiak et al. (2014) who determined the allelopathic activity of *Matricaria* sp.

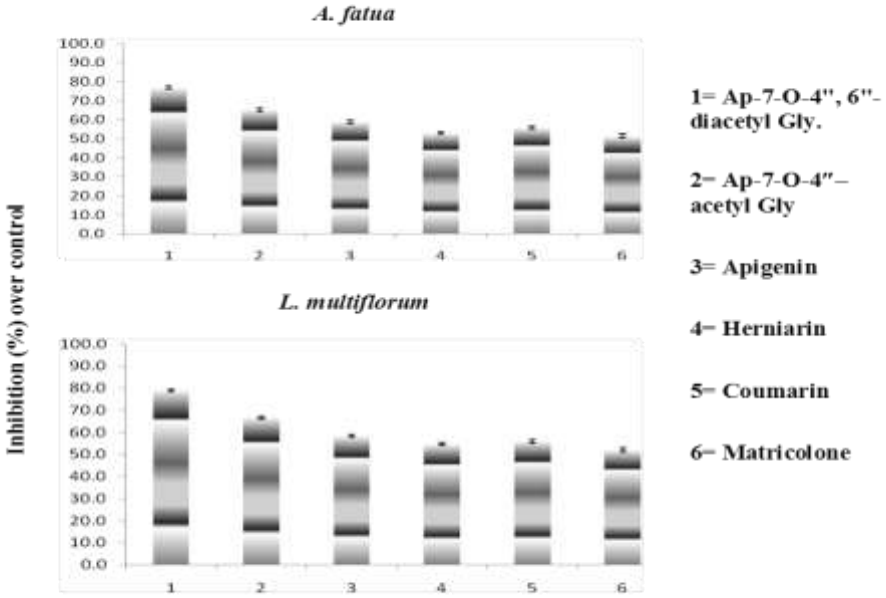

Fig. (3). Phytotoxicity of isolated compounds from *M. chamomilla* flowers.

The isolated flavnoides apigenin-7-O-(4", 6"-diacetyl glycoside), apigenin 7-O-(4"–acetyl glycoside) were also isolated from chamomile flower by Redaelli et al. (1980 and 1982) and Svehlıkova et al. (2004). Meanwhile, a flavonoid attached by mono and diacetyl with the major spectra is identified as apigenin, which is the major phytotoxic compound present in chamomile flowers, but the present data suggest that other compounds could also contribute to the phytotoxic activity of apigenin. The toxicity of acylated (mono and diacetyl) apigenin was higher than the toxicity of apigenin alone. However the natural apigenin attached with diacetyl isolated from chamomile was more toxic than mono acetyl apigenin glycoside suggesting that the attached mono and diacetyl improves their activity against *L. multiflorum* and *A. fatua*. The isolated coumarins in *M. chamomilla* are supported by previouse work of Redaelli et al. (1981) for herniarin (7-methoxycoumarin), umbelliferone (7-hydroxycoumarin), and other minor ones and terpens of Zaiter et al. (2008). Commercial standards of apigenin and coumarin at 50 µg ml^{-1} purchased from Sigma-Aldrich were tested against *L. multiflorum* and *A. fatua* seedling and total biomass was recorded. The phytotoxicity compared to the activity of the naturally isolated substances was slightly similar to the obtained results. The results of the present study are in harmony with Anya et al. (2005), Razavi et al. (2009) and Razavi et al. (2010) who confirmed that there are numerous reports on the allelopathic activity of coumarins. Cipollini et al. (2008) concluded that

leaves of fragrant honeysuckle (*Lonicera maackii* L.) contain phenolic compounds, including apigenin capable of having biological effects on other plants and insects. These findings suggest that chamomile contain potent allelopathic substances and associated weeds to wheat crop might be feasible be controlled by using these compounds, while further work on this issue is recommended to develop as new synthetic herbicide.

REFERENCES

Anya, A.L., M.M. Rubalcava, R.C. Ortega, C.G. Santana, P.N.S. Monterrubio, B.E.H. Bautista and R. Mata (2005). Allelochemicals from *Staurantus perforatus*, a rutaceae tree of the Yuctan Pensula, Mexico. Phytochemistry, 66: 487-494.

Bottcher, H., I. Gunther, R. Franke, and K. Warnstorff (2000). Physiological postharvest responses of Matricaria (*Matricaria recutita* L.) flowers. Postharvest Biology and Technology, 22: 39–51.

Bisset, N.G. (1994). Herbal Drugs and Phytopharmaceuticals. Boca Raton: CRC Press.

Cipollini, K., G. McClain and D. Cipollini (2008). Separating above-and below ground effects of *Alliaria petiolata* and *Lonicera maackii* on the performance of *Impatiens capensis*. Am. Midl. Nat., 160: 117-128.

Demarque, D.P., J.F. Sabóia, J.R. Fabri and C.A. Carollo (2012). Allelopathic activity of *Matricaria chamomilla* essential oil in bioautography test. Allelopathy Journal, 29 (1): 171-176.

Duke, S.O., F.E. Dayan and A.M. Rimando (2000a). In "Natural Products and Herbicide Discovery". Herbicides and Their Mechanisms of Action (Cobb, A.H. and Kirkwood, R.C., eds.). Academic Press, Sheffield, IL, p. 105-133.

Duke, S.O., F.E. Dayan, and J.G. Romagni (2000b). Natural products as sources for new mechanisms of herbicidal action. Crop Prot., 19: 583-589.

Fonseca, F.N. and M.F.M. Tavares (2004). Validation of a capillary electrophoresis method for the quantitative determination of free and total apigenin in extracts of *Chamomilla recutita*. Phytochemical Analysis, 15: 65-70.

Fonseca, F.N., M.F.M. Tavares and C. Horvath (2008). Capillary electrochromatography of selected phenolic compounds of *Chamomilla recutita.* Journal of Chromatography, 1154: 390–399.

Heisey, R.M. (1996). Identification of an allelopathic compound from *Ailanthus altissima* (Simaroubacea) and characterization of its herbicidal activity. American Journal of Botany, 82:192-200.

Itani, T., Y. Nakahata and H. Kato-Noguchi (2013). Allelopathic activity of some herb plant species. Int. J. Agric. Biol., 15: 1359–1362.

Kovacik, J. and M. Repcak (2008). Accumulation of coumarin-related compounds in leaves of *Matricaria chamomilla* related to sample processing. Food Chemistry, 111: 755–757.

Matysiak, K., S. kaczmarek and R. Kierzek (2014). Allelopathic effect of popular medicinal plants on *Fagopyrum esculentum* (Moench), *Papaver somniferum* (L.) and *Brassica napus var. oleifera* (L.). Journals of Medicinal Plant Research, 8: 1051-1059.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473–497.

Pietta, P., E. Manera, and P. Ceva (1987). Simultaneous isocratic high-performance liquid chromatographic determination of flavones and coumarins in *Matricaria chamomilla* extracts. Journal of Chromatography, 404: 279-281.

Razavi, S.M., S. Zahri, G. Zarrini, H. Nazemiyeh, S. Mohammadi and M.A. Abolghasemi (2009). A furanocoumarin from *Prangos uloptera*, biological effects. Nat. Prod. Res., 23: 1522-1527.

Razavi, S.M., G.H. Imanzadeh and M. Davari (2010). Coumarins from *Zosima absinthifolia* seeds, with allelopathic effects. Eur. Asia J. Biosci., 4: 17-22.

Redaelli, C., L. Formentini and E. Santaniello (1980). Apegenin 7-glucoside and its 2"-and 6"- acetates from ligulate flower of *Matricaria chamomilla.* Phytochemistry, 19: 985-986.

Redaelli, C., L. Formentini and E. Santaniello (1981). Reversed-phase high-performance liquid chromatography analysis of apigenin and its glucosides in flowers of *Matricaria chamomilla* and chamomille extracts. Planta Med., 42: 288–292.

Redaelli, C., L. Formentini and E. Santaniello (1982). Apegenin 7-glucoside diacetates in ligulate flower of *Matricaria chamomilla.* Phytochemistry, 21: 1828-4830.

Snedecor, G.W. and W.G. Cochran (1990). Statistical Methods 8th Ed. Iowa State Univ. Press, Ames, Iowa, U.S.A.

Svehlıkova, V., R.N. Bennett, F.A. Mellon, P.W. Needs, S. Piacente, P.A. Kroon and Y. Bao (2004). Isolation, identification and stability of acylated derivatives of apigenin 7-O-glucoside from chamomile (*Chamomilla recutita* [L.] Rauschert). Phytochemistry, 65: 2323–2332.

Scalia, S., L. Giuffreda and P. Pallado (1999). Analytical and preparative supercritical fluid extraction of chamomile flowers and its comparison with conventional methods. Journal of Pharmaceutical and Biomedical Analysis, 21: 549–558.

Singh, O., Z. Khanam, N. Misra and M.K. Srivastava (2011). Chamomile (*Matricaria chamomilla* L.): An overview. Phcog Rev., 5: 82-95.

Täckholm, V.D.Sc. (1974). In "Flora of Egypt". Published by Cairo University. Printed by Cooperative Printing Company Beirut, 1974.

Zaiter, L., M. Bouheroum, S. Benayache, F. Benayache, F. Leon, I. Brouard, J. Quintana, F. Estevez and J. Bermejo (2008). Sesquiterpene lactones and other constituents from *Matricaria chamomilla* L. Biochemical Systematic and Ecology, 35: 533-538.

إستغلال المكونات الأليلوباثية لأزهار المتريكاريا كاموميل فى مكافحة الحشائش المصاحبة لمحصول القمح

محمد عبد العزيز أبو الخير بلح
مركز بحوث الصحراء، المطرية، القاهرة، مصر

أجريت بعض التجارب لدراسة الكفاءة الأليلوباثية لأزهار نبات المتريكاريا كاموميل على إنبات ونمو بادرات بعض الحشائش الشائعة في زراعات القمح. حيث تم التقييم الحيوي على حشائش الصامة والعليق والفلارس وديل القط للمستلخص المائي للكاموميل بسلسلة من التركيزات. أوضحت النتائج أن المستخلص المائي كان أكثر كفاءة إبادية قبل وبعد الإنبات على حشائش الزمير والصامة عن بادرات القمح. بالإعتماد على الكفاءة الإبادية تم تنقية مستخلص الكلوروفورم وهو أعلى المستخلصات فاعلية وتم عزل بعض مركبات الفلافونيد المحتوية على مجموعة أستيل وتعريفها، ليتضح وجود مركبات االفلافونيد ابجنين٧-او-(٤ استيل جليكوسيد) وقد أحدثا إنخفاض في الكتلة الحيوية الكلية إلى ٧٧,٠ و٦٥,٤٪ للزمير و٧٩,٣ و٦٧,٠٪ للصامة على التوالي، عن الكنترول. بينما أحدث وجود الأبجنين منفرداً والمتركولون والهرنارين والكيومارين خفضاً في الكتلة الحيوية الكلية للزمير بنسبة ٥٣,١، ٥٩,١ ,٥٦,٠ و٥١,٤٪ و للصامة بنسبة ٥٤,٨٦ ، ٥٨,٦١، ٥٦,١ و٥٢,٠، على التوالي، مقارنة بالكنترول. إكتشاف الخصائص الفعالة على الحشائش للفلافونيد المرتبط بالأستيل من أزهار الكاموميل كمنتج طبيعي نباتي سوف يشكل نواة أساسية في الوصول إلى مبيدات طبيعية فعالة على الحشائش وصديقة للبيئة في برامج مكافحة الحشائش في زراعات القمح.