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Phytotoxic and Insecticidal Properties of Essential Oils and Extracts of Four *Achillea* Species

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Abstract: The essential oils and hexane extracts of four *Achillea* species were analyzed by GC/MS. *Achillea biserrata* Bieb., *Achillea wilhelmsii* C. Koch and *Achillea biebersteinii* Afan oils contained mainly 1,8-cineole (38.1-14.4%), camphor (46.6-23.6%) and borneol (11.7-2.9%,). *Achillea coarctata* Poir. oil had more different composition, with its main components being viridiflorol (37.7%), α-cadinol (8.9%) and cubenol (6.1%). The hexane extracts of *A. wilhelmsii*, *A. coarctata* and *A. biebersteinii* had high percentages of camphor (44.7%, 16.2% and 18.0%, respectively) and 1,8-cineole (19.5%, 30.8% and 165.1%, respectively), whereas the most abundant components in *A. biserrata* extract were ethyl oleate (13.1%), *n*-nonadecane (11.3%), and *n*-eicosane (11.3%). Herbicidal activities of the oils and hexane, acetone and methanol extracts of four *Achillea* species were assessed against six weed species and germinations, root and shoot growths of weed species were significantly inhibited by both the oils and extracts. In the pest toxicity assay on *Leptinotarsa decemlineata*, the oils showed toxic effect against the pest. According to the present results, *Achillea* species could be used as alternative bio-insecticides and bio-herbicides.

Keywords: *Achillea*; essential oil; extract; phytotoxic; insecticidal; *Leptinotarsa decemlineata*.

1. Introduction

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The genus *Achillea* (Compositae or Asteraceae) contains a large number of species widely distributed throughout the world and has been used since ancient times. Popular indications of the several species of this genus include treatment of wounds, bleedings, headache, inflammation, pains, spasmodic diseases, flatulence and dyspepsia. Phytochemical investigations of *Achillea* species have revealed that many components from this genus are highly bioactive [1]. This genus comprises flavored species which produce intense essential oils. The volatile oils of *Achillea* contain monoterpenes as the most representative metabolites. However, there are reports on high levels of sesquiterpenes compared with monoterpenes [2,3]. It has been reported that the major volatiles of *Achillea* species as being 1,8-cineole, camphor, terpinen-4-ol, β-eudesmol, α-thujone, β-thujone, piperitone, chamazulene, borneol, bornyl acetate, *p*-cymene, α-pinene, β-pinene and β-caryophyllene [2,4-7].

Plants have been suggested as alternative agents in controlling insect pests or weeds because some are selective, usually biodegradable to nontoxic products and have few or no harmful effects on non-target organisms and the environment [8-10]. Environmental constraints of crop production systems have stimulated interest in alternative weed management strategies. Indeed, the continued use of synthetic herbicides may potentially threaten sustainable agricultural production and has resulted in serious ecological and environmental problems, such as the increased incidence of resistance in weeds to important herbicides and increased environmental pollution and health hazards [11].

The use of plants and plant-derived products to control pests in the developing world is highly common [12-14]. They may provide potential alternatives to currently used insect-control agents because they constitute a rich source of bioactive chemicals [9]. There is an increasing interest in developing safe alternatives that have the potential to replace the toxic chemicals used in pest control. Natural products are known to have a range of useful biological properties against insect pests [14,15]. Recently, research on essential oils has received increasing attention for assessing its insecticidal efficacy and many essential oils prepared from herbs are known to possess insecticidal activities [16-19].

There is an urgent need to develop safer, more environmentally friendly and efficient alternatives that have the potential to replace synthetic pesticides and herbicides and are convenient to use [20]. Although a lot of information is available on the medicinal and biological properties of *Achillea* species, little is known about their herbicidal and insecticidal properties. The objectives of this research were to: a) determine the chemical compositions of essential oils and *n*-hexane extracts of four Turkish *Achillea* species, *A. biserrata*, *A. wilhelmsii*, *A. coarctata*, *A.biebersteinii,* and b) examine the herbicidal and the insecticidal effects of their oils and *n*-hexane, acetone and methanol extracts.

2. Materials and Methods

2.1. Plant materials,extraction and isolation of essential oils

Achillea biebersteinii Afan., *Achillea coarctata* Poir. and *Achillea wilhelmsii* C. Koch were collected at the flowering stage from Erzurum region (latitude 39° 91′ 00" N, longitude 41° 28′ 00" E, temperature 20-22 °C, altitude about 1950 m, loam soil) in 2009, while *Achillea biserrata* Bieb. was collected at the flowering stage from Samsun, Kocadağ region, Turkey (latitude 40° 44′ 07" N, longitude 34° 28′ 26" E, temperature 21°C, altitude about 1000-1200 m, limy soil) in 2009. The identification of plant materials were made by Dr. Tülay Aytas Akcin, in Department of Biology, Ondokuz Mayıs University, Samsun, Turkey. The voucher specimens of *A. biebersteinii* (ATA-9869), *A. coarctata* (ATA-9870) and *A. wilhelmsii* (ATA-9867) have been deposited in the herbarium of Ataturk University, Erzurum-Turkey, while the voucher specimen (OMUB 6443) of *A. biserrata* has been deposited at the Herbarium of Department of Biology, Ondokuz Mayıs University, Samsun-Turkey. Collected plant materials were dried in shadow and ground in a grinder. The dried plant samples (500 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hours. Hydrodistillation of *A. biebersteinii, A. biserrata, A. coarctata* and *A. wilhelmsii* yielded 0.63% (w/w),

0.56 (w/w), 0.60 and 0.85 (w/w), respectively the essential oils. The yields were based on dry materials of plant samples.

In order to prepare the *n*-hexane extracts, the dried and powdered flowers (about 0.100–0.400 mm) of *A. biebersteinii, A. biserrata, A. coarctata and A. wilhelmsii* (each one 100g) were extracted with *n*-hexane (750 mL x 4) at room temperature. The extracts were filtered using Whatman filter paper (No.1) and then concentrated under reduced pressure at 40 °C using a rotary evaporator. The extracts (2.06g, 2.54g, 1.94g and 2.25g, respectively) were stored in a freezer at 4 ºC until further tests.

The dried and powdered flowers (about 0.100–0.400 mm) *of A. biebersteinii, A. biserrata, A. coarctata* and *A. wilhelmsii* (each one 50g) were individually extracted with acetone and methanol (250 mL x 4) at room temperature to prepare the acetone and methanol extracts. The extracts were filtered using Whatman filter paper (No.1) and then concentrated under reduced pressure and temperature using a rotary evaporator. The extracts were stored in a freezer at 4 ºC until further tests.

2.2. GC and GC/MS analysis

GC analysis of the essential oils and *n*-hexane extracts were performed using a Thermofinnigan Trace GC/A1300, (EI) equipped with a SGE/BPX5 MS capillary column (30 m x 0.25 mm i.d., 0.25 μm). Helium was the carrier gas at a flow rate of 1 mL/min. Injector and detector temperature were set at 220°C and 250°C, respectively. The oven temperature was programmed from 50°C to 150°C at 3°C/min, held isothermal for 10 minutes and finally raised to 250°C at 10°C /min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μL were injected manually in splitless mode. The quantitative data of the oils and the hexane extracts was obtained from FID area percentage (Table 1).

GC-MS analysis of the essential oils and *n*-hexane extracts was performed using a Thermofinnigan Trace GC/Trace DSQ /A1300, (EI Quadrapole) equipped with a SGE-BPX5 MS fused silica capillary column (30 m x 0.25 mm i.d., film thickness 0.25 μm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Carrier gas was helium at a flow rate of 1 mL/min. Injector and MS transfer line temperatures were set at 220 °C and 290 °C, respectively. The oven temperature was programmed from 50°C to 150°C at 3°C/min, then held isothermal for 10 min and finally raised to 250°C at 10°C /min. Diluted samples (1/100, v/v, in methylene chloride) of 1.0 μL were injected manually in splitless mode.

The identification of individual compounds was achieved based on comparison of their relative retention times with those of authentic samples on SGE-BPX5 capillary column, and by matching of their mass spectra of peaks with those obtained from authentic samples and/or the Wiley 7N and TRLIB libraries spectra and published data [21], and by comparison of the retention index of the components with published data [21].

2.3. Seed germination and seedling growth experiments

The seeds of *Amaranthus retroflexus* L.,*Chondrilla juncea* L., *Chenopodium album* L., *Lactuca serriola* L., *Rumex crispus* L. and *Taraxacum officinale* F.H.Wigg. were collected in Erzurum region (Turkey) in October 2007. Empty and undeveloped seeds were discarded by floating in tap water. To avoid possible inhibition caused by toxins from fungi or bacteria, the seeds were surface-sterilized with 15% sodium hypochlorite for 20 min [22] and then rinsed with distilled water.

To determine the contact herbicidal effects of the oils and the extracts, the oil and the extracts were dissolved in dimethylsulphoxide (DMSO)-water solution (1%, v/v). The final concentration of the treatments was 1.0 mg/mL. The emulsions (10 mL) were transferred to Petri dish (9 cm diameter) placed on the bottom two layers of filter paper (10 mg/Petri dishes). Afterwards, 50 seeds of *A. retroflexus, C. juncea, C. album, L. serriola, R. crispus* and *T. officinale* were placed on the filter paper [22,23]. Petri dishes were sealed with adhesive tape to prevent escaping of volatile compounds and were kept at 23 ± 2°C on a growth chamber supply with 12 h of fluorescent light and humidity of 80% [24]. After 10 days, the number of germinated seeds was determined and seedling lengths (roots and aerial parts) were measured. Germination was measured as the percentage of seeds from which a radicle

emerges. 2,4-D (2,4-dichlorophenoxyacetic acid), isooctyl ester is a common systemic herbicide used in the control of broadleaf weeds. Therefore, 2,4-D, isooctyl ester (10 mg/Petri dishes) was used as positive control. Petri dishes containing 10 mL DMSO-water solution (1%, v/v), without essential oil and the extracts solutions were used as negative control. The treatments were arranged in a completely randomized design with three replications including controls.

2.4. Insect material and bioasays

The adults of *L. decemlineata* were collected from potato fields of the Eastern Anatolia (Erzurum) and reared in laboratory at 25 ± 1 °C and 64 ± 5, relative humidity. The adults and larvae obtained from laboratory cultures were stored in separate insect cages containing appropriate potato leaves. Tests were also carried out under the same condition and in the same laboratory.

Glass Petri dishes (9 cm wide x 1.5 cm deep, corresponding to 120 mL volume) were used as exposure chambers to test the toxicity of the essential oils against the adults of *L. decemlineata*. A filter paper was placed on bottom of the petri dishes (9cm x 1.5 cm deep) and 10 adults of the insect were placed on this filter paper, containing appropriate amount of potato leaves. The solutions of the essential oils at different concentrations in DMSO-distilled water (1:9) were prepared and 1 mL of these solutions, containing 5, 10 and 20 µL of the essential oils (corresponding to 41.7 µL/L air, 83.3 µL/L air and 166.7 µL/L air, respectively) of *A. biebersteinii*, *A. coarctata* and *A. wilhelmsii* were pulverized using an atomizer. The petri dishes covered with a lid and transferred into an incubator, and then kept under standard conditions of 25ºC, 64 ± 5 relative humidity and 16:8 (light: dark) photoperiod four days. Lambda-cyhalothrin (0.5 mg/mL), a mixture of isomers of pyrethroid insecticides, cyhalothrin was used as positive control in the same conditions. After exposure, the mortality of the adults was conducted at 12, 24, 48 and 72 h. Control treatments without the essential oils were treated in the same way. The treatments were arranged in completely randomized design with three replications including controls.

2.5. Statistical analysis

In order to determine whether there is a statistically significant difference among the obtained results for herbicidal and insecticidal activity assays, variance analyses were carried out using the SPSS 10.0 software package. Differences between means were tested by Duncan's Multiple Range testand values with $p< 0.05$ were considered significantly different.

3. Results and Discussion

3.1. Chemical compositions of the oils and the n-hexane extracts

Chemical compositions of the essential oils and *n*-hexane extracts from aerial parts of *A. biserrata, A. wilhelmsii, A. coarctata* and *A. biebersteinii* were given in Table 1 and Table 2. As can be seen from these tables, considerable quantitative and qualitative differences or similarities in the compositions of these species were observed. The essential oils of *A. biserrata* and *A. wilhelmsii* had relatively similar composition, and they contained camphor and 1,8-cineole as main components. *A. biserrata* oil is composed of camphor (33.5%), 1,8-cineole (17.7%), borneol (11.7%), bornyl acetate (10.8%) and terpinen-4-ol (8.7%), whereas the main components of *A. wilhelmsii* oil were camphor (46.6%), 1,8-cineole (14.4%), *cis*-thujone (4.6%) and camphene (3.3%). Remarkably, the highest 1,8-cineole content (38.1%) was detected in *A. biebersteinii* oil, with other main components such as camphene (23.6%), borneol (5.9%), and α-terpineol (5.2%) (Table 2). Among all the essential oils, the best different composition was observed in *A. coarctata* and it contained viridiflorol (37.7%), γ–cadinol (8.9%), cubenol (6.1%), borneol (5.8%), caryophyllene oxide (3.9%) and camphor (3.4%) as main components (Tables 1 and 2).

Numerous studies on the essential oil compositions of *A. bibersteinii* and *A. wilhelmsii* have been published by various authors [6, 25-33]. These studies demonstrated that main constituents in *A. bibersteinii*, and *A. wilhelmsii* oils were 1,8-cineole and camphor, with other components such as borneol, α-pinene, *p*-cymene, limonene and piperitone. Usually, our results concerning these two species concur with the earlier publications. There were only two studies reporting on the essential oil composition of *A. biserrata* [34,35] and as can be seen in Table 1, our results regarding *A. biserrata* oil showed great similarity to these previous findings. In literature survey, we could find only a few reports of the essential oil composition of *A. coarctata*. These studies indicated that the most abundant compounds were 1,8-cineole and camphor, with other main constituents borneol and viridiflorol [32,36,37]. Compared to these previous studies, our *A. coarctata* sample showed considerable differences in oil composition. The observed difference may be due to climatic and geographical variations.

The results with respect to the chemical compositions of the hexane extracts of the *Achillea* species mentioned above were shown in Tables 1 and 2. As seen from these tables, there are important qualitative and quantitative differences among the extract compositions of the species investigated. The three *Achillea* species, *A. wilhelmsii*, *A. coarctata*, and *A. biebersteinii*, had high percentages of camphor and 1,8-cineole. The percentages of camphor and 1,8-cineole were 44.7 vs. 19.5% for *A. wilhelmsii*, 16.2 vs. 30.8% for *A. coarctata*, and 18.0 vs. 15.1% for *A. biebersteinii*. In contrast to the other species, in *A. biserrata* extract, the most abundant components were ethyl oleate (13.1%), *n*-nonadecane (11.3%), and *n*-eicosane (11.3%), respectively. Indeed, *A. biserrata* also contained camphor and 1.8-cineole but the percentages of these components were lower (6.7 vs. 6.03%) relative to the other three species. However, with the exception of the hexane extract of *A. wilhelmsii*, the hexane extracts of other *Achillea* species contain relatively high content of non-volatile compounds, besides volatile compounds as compared with the essential oils (Tables 1 and 2). To the best of our knowledge, the chemical composition of *n*-hexane extracts of these *Achillea* species mentioned above has not been reported until now. Hence, the present study appears to be the first report on the hexane extract composition of the species. These results show that less volatile compounds such as *n*-alkanes, fatty acids and fatty acid esters in plant samples can be extracted with *n*-hexane, besides volatiles. However, these compounds cannot be extractable with distillation methods.

Table 1. Chemical composition (%) of *n*-hexane extract of flowers and the oils of aerial parts of *A. biserrata* and *A. wilhelmsii*.

		A. biserrata		***A. wilhelmsii***		
RI[a]	**Components**	**Oil (%)**	**Extract (%)**	**Oil (%)**	**Extract (%)**	**Identification methods**
900	*n*-Nonane	–	–	–	3.1	GC, MS, RI
930	α-Thujene	–	–	t	0.4	GC, MS, RI
938	α-Pinene	–	–	t	6.4	GC, MS, RI
957	Camphene	1.0	t	3.3	5.9	GC, MS, RI
979	Sabinene	–	–	0.5	1.2	GC, MS, RI
999	Yomogi alcohol	–	–	1.7	–	GC, MS, RI
1000	*n*-Decane	–	–	–	5.8	GC, MS, RI
1023	α-Terpinene	0.9	t	–	–	GC, MS, RI
1034	*p*-Cymene	0.6	t	0.9	t	GC, MS, RI
1042	1,8-Cineole	17.7	6.0	14.4	19.5	GC, MS, RI
1067	γ-Terpinene	2.9	t	–	2.7	GC, MS, RI
1071	Artemisia ketone	–	–	t	0.3	GC, MS, RI
1084	Artemisia alcohol	–	–	t	3.7	GC, MS, RI
1106	Linalool	0.6	t	–	–	GC, MS, RI
1099	α-Pinene oxide	–	–	0.3	–	GC, MS, RI
1100	*n*-Undecane	–	–	–	1.3	GC, MS, RI
1114	*cis*-Thujone	–	–	4.6	0.2	GC, MS, RI
1130	*cis*-*p*-Menth-2-en-1-ol	0.4	–	–	–	GC, MS, RI
1134	α-Campholenal	–	–	2.2	t	GC, MS, RI
1153	Camphor	33.5	6.7	46.6	44.7	GC, MS, RI
1172	Borneol	11.7	2.0	2.9	1.1	GC, MS, RI
1178	Terpinen-4-ol	8.7	1.4	2.6	0.5	GC, MS, RI

1185	*p*-Cymen-8-ol	–	–	0.9	–	GC, MS, RI
1190	α-Terpineol	2.4	1.6	1.8	0.2	GC, MS, RI
1200	*n*-Dodecane	–	–	–	1.3	GC, MS, RI
1217	*cis*-Carveol	–	–	0.3	–	GC, MS, RI
1274	(3*Z*)-Hexenyl angelate	–	–	0.6	–	MS, RI
1278	Bornyl acetate	10.8	1.6	t	–	GC, MS, RI
1289	Thymol	–	–	2.6	0.1	GC, MS, RI
1296	Carvacrol	–	–	1.9	t	GC, MS, RI
1313	Dihydrocarveol acetate	–	–	0.2	–	MS, RI
1357	9-Decenoic acid	–	–	0.4	–	MS, RI
1367	α-Yılangene	0.4	–	–	–	MS, RI
1373	α-Copaene	0.6	–	–	–	GC, MS, RI
1387	1-Tetradecene	–	0.6	–	–	MS, RI
1399	(*Z*)-Jasmone	–	–	2.4	t	GC, MS, RI
1400	*n*-Tetradecane	–	1.8	–	–	GC, MS, RI
1419	β-Caryophyllene	–	–	0.3	0.5	GC, MS, RI
1453	(*Z*)-β-Farnesene	0.8	t	0.3	t	GC, MS, RI
1460	α-Humulene	0.8	–	–	–	GC, MS, RI
1486	Germacrene D	0.8	–	–	–	GC, MS, RI
1500	*n*-Pentadecane	–	2.2	–	–	GC, MS, RI
1507	(*E*,*E*)-α-Farnesene	0.7	–	–	–	MS, RI
1536	δ-Cuprenene	t	1.4	–	–	MS, RI
1548	Elemicin	–	–	0.2	–	GC, MS, RI
1555	*trans*-Nerolidol	–	–	0.2	–	GC, MS, RI
1574	Spathulenol	1.0	1.0	3.2	–	GC, MS, RI
1579	Caryophyllene oxide	0.8	t	t	–	GC, MS, RI
1579	Caratol	–	–	0.3	–	MS, RI
1593	1-Hexadecene	–	2.9	–	–	MS, RI
1631	γ-Eudesmol	–		0.4	–	MS, RI
1651	β-Eudesmol	–		2.7	t	MS, RI
1790	*n*-Pentadecanol	–		0.5	–	GC, MS, RI
1844	(*Z*,*Z*)-Farnesyl acetone	–		0.8	–	GC, MS, RI
1849	*n*-Hexadecanol	–	1.1	–	–	MS, RI
1900	*n*-Nonadecane	–	11.3	–	–	GC, MS, RI
1908	Methyl palmitate	–		t	–	GC, MS, RI
1923	Isophytol	–	5.0	0.3	–	GC, MS, RI
1970	Linoleic acid	–	1.1	–	–	GC, MS, RI
1975	Ethyl oleate	–	13.1	–	–	GC, MS, RI
2000	*n*-Eicosane	–	11.3	–	–	GC, MS, RI
2012	Ethyl octadecanoate	–	3.5			GC, MS, RI
2100	*n*-Heneicosane	–	4.4	–	0.3	GC, MS, RI
2116	Oleic acid	–	3.0	–	–	GC, MS, RI
2200	*n*-Docosane	–	2.4	–	0.3	GC, MS, RI
2300	*n*-Tricosane	–	2.1	–	–	GC, MS, RI
2400	*n*-Tetracosane	–	4.7	–	–	GC, MS, RI
2500	*n*-Pentacosane	–	4.0	–	–	GC, MS, RI
Grouped components (%)						
Monoterpene hydrocarbons		5.4	t	4.7	16.6	
Oxygenated monoterpenes		85.8	19.3	83.0	70.3	
Sesquiterpene hydrocarbons		4.1	1.4	0.6	0.5	
Oxygenated sesquiterpenes		1.8	1.0	7.6	t	
Others		–	74.5	4.1	12.6	
Total		**97.1**	**96.2**	**100.0**	**100.0**	

[a] Calculated retention index relative to *n*-alkanes (C_8-C_{28}) on SGE-BPX5 capillary column.
GC: co-injection with standards; MS; tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data [21]; RI: identification based on comparison of retention index with those of published data [21].
t, trace (less than 0.1%).

Table 2. Chemical composition of *n*-hexane extracts and the oils of *A. coarctata* and *A. biebersteinii*.

		A. coarctata		***A. biebersteinii***		
RI[a]	**Components**	**Oil (%)**	**Extract (%)**	**Oil (%)**	**Extract (%)**	**Identification methods**
930	α-Thujene	–	2.9	–	–	GC, MS, RI
938	α-Pinene	–	6.2	1.0	–	GC, MS, RI
957	Camphene	t	4.7	2.4	–	GC, MS, RI
983	β-Pinene	–	2.0	1.1	–	GC, MS, RI

1000	*n*-Decane	–	12.7	–	–	GC, MS, RI
1023	α-Terpinene	–	2.2	0.6	–	GC, MS, RI
1034	*p*-Cymene	t	0.9	1.7	–	GC, MS, RI
1042	1,8-Cineole	2.0	30.8	38.1	15.1	GC, MS, RI
1055	(*E*)-β-Ocimene	t	0.6	–	–	GC, MS, RI
1067	γ-Terpinene	t	3.3	1.1	–	GC, MS, RI
1065	*cis*-Sabinene hydrate	–	–	–	1.1	GC, MS, RI
1084	Camphenilone	–	1.0	–	–	MS, RI
1100	*n*-Undecane	–	5.1	–	–	GC, MS, RI
1106	Linalool	–	–	0.7	1.5	GC, MS, RI
1114	*cis*-Thujone	–	2.5	–	–	GC, MS, RI
1130	*cis*-*p*-Menth-2-en-1-ol	t	–	t	–	GC, MS, RI
1134	α-Campholenal	–	–	1.6	0.1	MS, RI
1145	*trans*-Pinocarveol	–	2.0	–	–	GC, MS, RI
1150	*trans*-Verbenol	0.1	–	–	–	GC, MS, RI
1153	Camphor	3.4	16.2	23.6	18.0	GC, MS, RI
1162	Sabinaketone	–	–	1.5	–	MS, RI
1164	*cis*-Chrysanthenol	0.1	–			MS, RI
1172	Borneol	5.8	1.1	5.9	5.2	GC, MS, RI
1178	Terpinen-4-ol	0.4	t	3.3	–	GC, MS, RI
1185	*p*-Cymen-8-ol	–	–	t	0.1	GC, MS, RI
1190	α-Terpineol	1.0	0.9	5.2	0.6	GC, MS, RI
1211	*trans*-Carveol	–	–	t	–	GC, MS, RI
1217	*cis*-Carveol	–	–	–	1.2	GC, MS, RI
1242	Cuminaldehyde	–	–	0.5	–	GC, MS, RI
1254	Piperitone	–	–	0.4	1.0	GC, MS, RI
1278	Bornyl acetate	0.2	t	1.4	0.7	GC, MS, RI
1289	Thymol	0.5	–	1.0	1.7	GC, MS, RI
1296	Carvacrol	0.2	–	t	1.3	GC, MS, RI
1319	(*E*,*E*)-2,4-Decadienal	0.2	–	–	–	MS, RI
1346	α-Terpinyl acetate	0.5	–	–	–	GC, MS, RI
1332	*p*-Mentha-1,4-dien-7-ol	–	–	0.8	–	MS, RI
1357	Eugenol	–	–	t	–	GC, MS, RI
1365	2,3,5-Trimethyl benzaldehyde	0.5	–	-	–	MS, RI
1399	(*Z*)-Jasmone	–	–	0.3	–	GC, MS, RI
1419	β-Caryophyllene	0.1		0.4	–	GC, MS, RI
1453	(*Z*)-β-Farnesene	0.1	–	–	–	GC, MS, RI
1464	Alloaromadendrene	1.3	1.1	–	–	GC, MS, RI
1474	γ-Gurjunene	–	–	t	–	GC, MS, RI
1476	β-Chamigrene	–	–	0.3	–	MS, RI
1478	γ-Muurolene	0.3	–	–	–	GC, MS, RI
1486	Germacrene D	0.2	–	3.0	–	GC, MS, RI
1494	Viridiflorene	0.7	–	–	–	GC, MS, RI
1501	α-Muurolene	0.2	–	–	–	GC, MS, RI
1508	β-Bisabolene	0.1	–	–	–	MS, RI
1517	δ-Cadinene	1.3	–	–	–	MS, RI
1541	α-Calacorene	0.4	–	–	–	MS, RI
1551	Germacrene B	0.3	–	–	–	MS, RI
1564	Palustrol	1.7	–	–	–	MS, RI
1574	Spathulenol	1.9	–	0.3	0.2	MS, RI
1579	Caryophyllene oxide	3.9	1.3	0.3	0.4	GC, MS, RI
1595	Viridiflorol	37.7	1.2	–	–	GC, MS, RI
1605	Ledol	3.2	t	–	–	GC, MS, RI
1609	Humulene epoxide II	0.2	–	–	–	MS, RI
1612	Isolongifolan-7α-ol	0.4	–	–	–	MS, RI
1625	1-*epi*-Cubenol	1.3	–	–	–	MS, RI
1631	γ-Eudesmol	–	–	0.5	–	MS, RI
1634	Caryophylla-4(12), 8(13)-dien-5α-ol	0.4	–	–	–	MS, RI
1636	Caryophylla-4(12), 8(13)-dien-5β-ol	1.1	–	–	–	MS, RI
1642	Cubenol	6.1	t	–	–	MS, RI
1651	β-Eudesmol	-	–	1.0	–	MS, RI
1659	α-Cadinol	8.9	0.8	–	–	MS, RI
1672	β-Bisabolol	0.8	–	–	–	MS, RI
1682	*epi*-α-Bisabolol	0.4	–	–	–	MS, RI
1694	Eudesma-4(15),7-dien-1β-ol	1.0	–	–	–	MS, RI
1739	(2*Z*,6*E*)-Farnesol	0.2	–	–	–	GC, MS, RI
1844	(*Z*,*Z*)-Farnesyl acetone	0.8	–	0.6	–	MS, RI
1860	Benzyl salicylate	0.2	–	–	–	MS
1908	Methyl palmitate	0.6	–	–	–	GC, MS, RI
1923	*n*-Hexadecanoic acid	3.9	–	0.3	–	GC, MS, RI

1955	Methyl linoleate	1.1	–	0.3	10.2	GC, MS, RI
1970	Linoleic acid	–	–	–	16.4	GC, MS, RI
2000	*n*-Eicosane	0.4	–	0.2	2.7	GC, MS, RI
2100	*n*-Heneicosane	0.7	–	0.2	7.2	GC, MS, RI
2300	*n*-Tricosane	t	–	–	9.8	GC, MS, RI
Grouped components (%)						
Monoterpene hydrocarbons		t	22.8	7.9	–	
Oxygenated monoterpenes		14.2	54.5	84.0	47.6	
Sesquiterpene hydrocarbons		5.0	1.1	3.7	–	
Oxygenated sesquiterpenes		70.0	3.3	2.7	0.6	
Others		7.6	17.8	1.0	46.3	
Total		**96.8**	**99.5**	**99.3**	**94.5**	

[a]Calculated retention index relative to *n*-alkanes (C_8-C_{28}) on SGE-BPX5 capillary column.
GC: co-injection with standards; MS; tentatively identified based on computer matching of the mass spectra of peaks with Wiley 7N and TRLIB libraries and published data [21]; RI: identification based on comparison of retention index with those of published data [21].
t, trace (less than 0.1%).

3.2. Phytotoxic effects on weed species of the essential oils and the extracts of Achillea species

The phytotoxic responses to oils and hexane, acetone and methanol extracts of *A. biserrata, A. coarctata* and *A. wilhemsii* against six weed species, *A. retroflexus*, *C. juncea*, *C. album*, *R. crispus*, *L. serriola* and *T. officinale* were assessed in terms of germination, root growth and shoot growth (Tables 3, 4, and 5). In general, the oils of *Achillea* species were more phytotoxic to the weed species compared to their extracts, where the oils showed 100% germination inhibition in all weed species.

The efficacy of the essential oils and the extracts on weed species differed with *Achillea* species were assayed. As can be seen in these tables, the inhibition efficacy of the *Achillea* species on germination varied with the weed species tested; *A. retroflexus*, *C. juncea*, *C. album*, *R. crispus*, *L. serriola*, and *T. officinale*. The maximum efficacy of the oils and the extracts was observed against *A. retroflexus*. All extracts showed nearly similar effect on the germination and seedling growth of *A. retroflexus*. However, the results given in Table 3, 4, and 5 revealed that acetone and hexane extracts were more active in reducing germination of the weed species than methanol extracts. On the other hand, *A. coarctata* extracts were the most active against *A. retroflexus*, *C. juncea*, and *R. crispus* germination; *A. biserrata* extracts in *L. serriola*, and *A. biserrata* and *A. wilhelmsii* extracts in *T. officinale* were more active. Furthermore, acetone, hexane and methanol extracts of all three *Achillea* extracts, showed nearly identical inhibitory effect on the germination of *C. album* (Tables 3, 4, and 5).

The root length of weed species was affected by both the essential oils and the extracts. In general, the trend observed in root growth has been very similar to that of the germination. Root growth was completely inhibited by the essential oils in all weed species. The *Achillea* extracts had a smaller efficacy in inhibiting root growth relative to the oils (Tables 3, 4, and 5). Similar to germination results, the highest inhibiting effect in root growth within the weed species tested was noticed in *A. retroflexus*. *A.coarctata* extract was the most effective against seedling growths of *C. juncea, C. album* and *L. serriola*. The most inhibiting extract against root growth of *R. crispus* was the extracts obtained from *A. wilhelmsii*. On the other hand, the root growth in *T. officinale* was affected similarly by *A. biserrata, A. coarctata* and *A. wilhelmsii* extracts. These results implied that the efficacy of extract depended on the *Achillea* species and their oil compositions (Tables 3, 4, and 5).

Shoot growths of the weeds were also significantly affected by both the essential oils and extracts, but this effect was greater in the essential oils compared to the extracts. As can be seen in Tables 3, 4 and 5, all *Achillea* oils showed a complete inhibition on shoot growth. *A coarctata* extracts appeared to be the most effective as compared with other *Achillea* species. Very little differences in shoot growth of the weed species treated by *A.biserrata* and *A. wilhelmsii* extracts were observed. Also, the extracts studied showed more phytotoxic effect to shoot growth of *A. retroflexus* compared to the other weed species.

Table 3. Contact inhibitory effects of the essential oils and the extracts of three *Achillea* species on seed germination and seedling growth of *A. retroflexus* and *C. juncea.*

	Germination (%)		Root Growth (mm)		Seedling Growth (mm)	
	A.retroflexus	***C. juncea***	***A.retroflexus***	***C. juncea***	***A.retroflexus***	***C. juncea***
A. biserrata						
Essential oil	–	–	–	–	–	–
Hexane extract	–	28.0± 2.0e,f	–	9.4 ± 1.0a,b	–	2.4 ± 0.1a,b
Acetone extract	–	12.0 ± 4.2b,c,d	–	7.0 ± 1.0a,b	–	1.9 ± 0.1a,b
Methanol extract	6.7 ± 1.3a	8.0 ± 1.2a,b,c	10.1 ± 5.7a	11.2 ± 2.8a,b	11.3 ± 2.8b	2.6 ± 0.5a,b
A. coarctata						
Essential oil	–	–	–	–	–	–
Hexane extract	–	–	–	–	–	–
Acetone extract	–	12.7 ± 2.4b,c,d	–	11.3 ± 1.6a,b	–	2.2 ± 0.4a,b
Methanol extract	–	2.7 ± 0.7a,b	–	3.3 ± 0.5a	–	4.5± 2.2b
A. wilhelmsii						
Essential oil	–	–	–	–	–	–
Hexane extract	–	17.3 ± 3.5c,d	–	8.0 ± 1.0a,b	–	2.0 ± 0.1a,b
Acetone extract	–	11.3 ± 1.8b,c,d	–	9.7 ± 1.4a,b	–	2.4 ± 0.5a,b
Methanol extract	4.0 ± 2.0b	22.0 ± 9.5d,e	5.0 ± 0.6a	21.3 ± 1.8b	8.5± 1.0a,b	2.7 ± 0.6a,b
2,4-D	–	–	–	–	–	–
Control	**67.3 ± 12.8b**	**35.3 ± 4.8f**	**31.1 ± 1.1b**	**41.4± 3.3c**	**15.1 ± 0.6**	**4.5 ± 0.7b**

–: No germination or no growth
2,4-D: 2,4-dichlorophenoxyacetic acid, isooctyl ester
a,b,c,d,e,f: Means in the same column by the same letter are not significantly different Duncan's multiple range test ($p<0.05$).

Table 4. Contact inhibitory effects of the essential oils and the extracts of three *Achillea* species on seed germination and seedling growth of *C. album* and *R. crispus.*

	Germination (%)		Root Growth (mm)		Seedling Growth (mm)	
	C. album	***R. crispus***	***C. album***	***R. crispus***	***C. album***	***R. crispus***
A. biserrata						
Essential oil	–	–	–	–	–	–
Hexane extract	5.3 ± 1.8	83.3 ± 4.8d,e	13.1 ± 2.3d	19.9 ± 0.8c,d	9.6 ± 1.0d	6.2 ± 0.2b,c
Acetone extract	5.3 ± 2.7a	93.3 ± 1.8e	9.0 ± 2.2b,c,d	25.4 ± 0.7d,e	6.9 ± 1.3b,c,d	7.4 ± 0.2b,c
Methanol extract	10.7 ± 0.7a	94.7 ± 2.4e	12.6 ± 1.9d	33.7 ± 1.1e,f	8.9 ± 1.0d	9.0 ± 0.3c
A. coarctata						
Essential oil	–	–	–	–	–	–
Hexane extract	2.7 ± 0.7a	78.0 ± 3.1d	4.0 ± 0.9a,b,c	23.9 ± 1.5d,e	5.8 ± 0.5a,b,c	4.7 ± 0.2b
Acetone extract	8.7 ± 3.3a	17.3 ± 5.9b	2.5 ± 0.3a,b	44.0 ± 5.7f	5.1 ± 0.4a,b	4.8 ± 0.4b
Methanol extract	7.3 ± 1.8a	64.0 ± 9.5c	6.1 ± 1.0a,b,c,d	13.7 ± 0.8b,c,d	6.9 ± 1.1a,b,c,d	6.5 ± 0.2b,c
A. wilhelmsii						
Essential oil	–	–	–	–	–	–
Hexane extract	4.0 ± 2.3a	89.3 ± 1.3d,e	11.7 ± 2.9c,d	11.2 ± 0.7a,b,c	8.6 ± 1.9c,d	7.6 ± 0.3b,c
Acetone extract	2.7 ± 1.8a	83.3 ± 4.1d,e	3.0 ± 1.8a,b	3.9 ± 0.3a,b	5.0 ± 1.8a,b	6.2 ± 0.4b,c
Methanol extract	22.0 ± 5.1b	79.3 ± 4.7d	8.1 ± 0.6a,b,c,d	16.2 ± 1.0c,d	7.4 ± 0.3a,b,c,d	4.9 ± 0.4b
2,4-D	–	–	–	–	–	–
Control	36.0 ± 4.0c	85.3 ± 2.4d,e	32.8 ± 1.1e	24.6 ± 1.5d,e	6.3 ± 0.3e	7.1 ± 0.3b,c

–: No germination or no growth
2,4-D: 2,4-dichlorophenoxyacetic acid, isooctyl ester
a,b,c,d,e,f: Means in the same column by the same letter are not significantly different to Duncan's Multiple Range test ($p<0.05$).

Recently, it has been shown that monoterpenes and essential oils isolated from various plant species have potent herbicidal effects on weed germination and seedling growth of various weed species [22,23,30,38,39]. The herbicidal properties of camphor, 1,8-cineole, borneol, α-terpineol and terpinen-4-ol, which were found in the essential oils and the hexane extracts of *Achillea* species and the essential oils of some *Achillea* species rich in these compounds against *A. retroflexus, C. album and R. crispus* weeds have been reported [22,30]. In the present study, much of the variation in the germination, root and shoot growths of the weed species in response to the oils and extracts is likely to be due to these main constituents and high content of oxygenated monoterpenes of the *Achillea* species. On the other hand, in the observed response synergic and antagonistic effects of these oil constituents deserves

special attention. The essential oils from the *Achillea* species assayed contained camphor and 1,8-cineole as the main compounds. Compounds such as camphor and 1,8-cineole are known to inhibit cell mitosis, but whether or not this is a primary target site is still unknown for most of the allelochemicals cited [40]. Previous studies suggested that camphor and 1,8 cineole were phytotoxic for the germination root growth and shoot growth [41,42]. To date, various mechanisms have been proposed to account for the herbicidal activity in plant species [38]. Possible toxic mechanisms of plant species on weeds include a) accumulation of lipid globules in the cytoplasm, and reduced size of organelles such as mitochondria possibly due to DNA synthesis inhibition or membrane disruption [38]. b) structural breakdown and absence of intact organelles in response to volatility resulting in poor root growth [43], c) the inhibition of mitosis in the growing cells, as essential oil are reported to inhibit the sprout growth in potato by killing meristematic cells [44], d) essential oils and monoterpenes induce generation of reactive oxygen species resulting in lipid peroxidation and membrane disintegration [45,46, 47], and e) decrease in the amount of total chlorophyll content and the respiratory activity [47].

Table 5. Contact inhibitory effects of the essential oils and the extracts of three *Achillea* species on seed germination and seedling growth of *L. serriola* and *T. officinale.*

	Germination (%)		**Root Growth (mm)**		**Seedling Growth (mm)**	
	L. serriola	***T. officinale***	***L. serriola***	***T. officinale***	***L. serriola***	***T. officinale***
A. biserrata						
Essential oil	–	–	–	–	–	–
Hexane extract	7.3 ± 1.8a,b	4.7 ± 1.8a,b	15.4 ± 3.2a,b	5.3 ± 1.1a,b	2.6 ± 0.2b	3.1 ± 0.4b
Acetone extract	8.7 ± 0.7a,b,c	2.7 ± 0.7a,b	11.4 ± 1.9a,b	3.3 ± 0.8a	2.8 ± 0.3b	3.0 ± 0.8b
Methanol extract	22.0 ± 7.0d,e	11.3 ± 4.7a,b	14.8 ± 1.9a,b	13.2 ± 2.3a,b,c	2.7 ± 0.3b	3.1 ± 0.9b
A. coarctata						
Essential oil	–	–	–	–	–	–
Hexane extract	17.3 ± 2.7b,c,d,e	12.0 ± 1.1a,b	4.8 ± 0.5a,b	3.5 ± 0.5a	2.3 ± 0.2b	3.8 ± 0.9b
Acetone extract	30.0 ± 8.1e	14.7 ± 2.4b	10.0 ± 0.9a,b	13.3 ± 2.6	4.8 ± 0.7c	6.2 ± 1.2c
Methanol extract	21.3 ± 7.4c,d,e	10.0 ± 2.0a,b	6.2 ± 0.6a,b	13.5 ± 2.0a,b,c	3.5 ± 0.8	1.6 ± 0.2a,b
A. wilhelmsii						
Essential oil	–	–	–	–	–	–
Hexane extract	15.3 ± 2.7b,c,d	4.7 ± 1.8a,b	11.9 ± 1.1a,b	3.7 ± 0.6a	3.0 ± 0.2b	2.7 ± 0.5b
Acetone extract	19.3 ± 2.4b,c,d,e	6.0 ± 1.2a,b	9.4 ± 1.2a,b	8.6 ± 1.7a,b	2.5 ± 0.2b	3.3 ± 0.3b
Methanol extract	54.7 ± 5.2f	12.7 ± 2.9b	22.5 ± 1.6b,c	22.2 ± 3.1b,c	4.7 ± 0.7c	4.4 ± 1.5b
2,4-D	–	–	–	–	–	–
Control	82.0 ± 2.3g	33.3 ± 5.9c	36.3 ± 2.4d	26.8 ± 3.1c	5.4 ± 0.6c	7.9 ± 1.1c

–: No germination or no growth

2,4-D: 2,4-dichlorophenoxyacetic acid, isooctyl ester

a,b,c,d,e,f: Means in the same column by the same letter are not significantly different to Duncan's Multiple Range test ($p<0.05$).

The hexane extracts of *Achillea* species showed similar herbicidal properties against weed species as compared with their essential oils in the current study (Tables 3-5). However, as can be seen from Tables 3-5, in general hexane extracts of the plants were weak phytotoxic against some weeds. As seen in Tables 1 and 2, the hexane extracts are rich in less volatile compounds such as *n*-alkanes, fatty acids and fatty acids esters and these compounds comprise 72.15%, 46.38% of the total hexane extracts of *A. biserrata* and *A. biebersteinii*, respectively. Therefore, weak phytotoxic effects of the hexane extracts of the *Achillea* species can be attributed to high contents of the less volatile compounds and relatively low amount of oxygenated monoterpenes in the hexane extracts as compared to herbicidal effects of the essential oils tested.

3.3.Toxicity of essential oils of Achillea species against L. decemlineata adults

In present study, the mortality percentages of potato Colorado beetle adults treated with the different exposure times and concentrations of the *A. biebersteinii, A. wilhelmsii, A. coarctata* essential oils, and an insecticide, lambda-cyhalothrin were also investigated and the results obtained were presented in Table 6 and Figures S1 and S2 (Supporting Information). The essential oils of *Achillea* species showed significantly toxic effect against the pest. Potato Colorado beetle was affected by both the concentrations and exposure times of the oils. The percentage of mortality increased with increasing

exposure time and the oil concentrations tested. The highest activity against potato Colorado beetle was observed in *A. biebersteinii*, followed by *A. wilhelmsii* and *A. coarctata*, respectively. As seen in Table 6, *A. biebersteinii* oil was very effective against adults of potato Colorado beetle, which showed high mortality percentages even at the lowest dose tested. This toxicity observed against the oils *Achillea* species could be caused by the presence in different proportions of 1,8-cineole, camphor, borneol and α-terpineol, terpinen-4-ol in these oils or could be the result of a synergistic effect of these compounds with the others present in the oils [48-52]. On the other hand, *A. coarctata* showed the lowest mortality percentages in the toxicity assay. As seen in Table 2, the ssential oil of *A.coarctata* have different chemical composition as compared with *A. biebersteinii* and *A. wilhelmsii* essential oils and these oil contain low amount of 1,8-cineole (2.0%), camphor (3.4%), borneol (5.8%), terpinen-4-ol (0.4%) and α-terpineol (1.0%), which potent insecticides. Therefore, the high toxic effects of the essential oils of *A. biebersteinii* and *A. wilhelmsii* can be attributed to the high content of these compounds as well as oxygenated monoterpenes. These results also verified the results of previous studies showing that oxygenated monoterpenes exhibited higher toxicity compared to sesquiterpenes against the larvae of Colorado potato beetles [50-54].

Colorado potato beetle is a destructive pest of potato plants and causes an important loss in the yield of the crop. Toxicity of essential oils against different pest species are well known [14, 49-57].) and the control of Potato Colorado beetle may be possible by using plant-derived products including essential oil and extracts. The toxicities of several sesquiterpenes against Colorado potato beetle has been previously reported [51,52]. However, *A. biebersteinii, A. wilhelmsii* and *A. coarctata* oils have been previously tested against Potato Colorado beetle [54,57]. So date, only two reports indicated that *A. biebersteinii* and *A. wilhelmsii* oils had a notable insecticidal effect on *Sitophilus granarius*, *Tribolum confusum*, and *Aedes aegypti* [56,58]. However, in the current study, mode of action of the tested oils was not studied.

Table 6. Toxicities of the essential oils of *A. biebersteinii*, *A. coarctata* and *A. wilhelmsii* against Colorado potato beetle *(L. decemlineata.*

Treatments		**% Mortality[a]**					
		Exposure time (h)					
Essential oils	**Dose (µl/Petri)**	**12**	**24**	**36**	**48**	**60**	**72**
A. biebersteinii	5	73.3 (15.8)*c	93.3 (12.4)*e	93.3 (12.4)*d	93.3 (12.4)*d	100.0 (0)*c	100.0 (0)*d
	10	93.3 (12.4)*de	100.0 (0)*e	100.0 (0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
	20	93.3 (12.4)*de	100. 0 (0)*e	100.0 (0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
A. coarctata	5	6.7 (86.5)a	26.7 (21.6)*b	30.0 (33.3)*bc	30.0 (33.3)*b	50.0 (0)*b	60.0 (0) *b
	10	23.3 (49.5)ab	30.0 (57.7)*bc	33.3 (34.6)*bc	33.3 (34.6)*b	53.3 (28.6)*b	63.3 (24.1)*b
	20	33.3 (17.3)*b	46.7 (44.6)*cd	46.7 (44.6)*c	50.0 (34.6)*c	63.3 (18.2)*b	73.3 (7.9)*c
A. wilhelmsii	5	23.3 (49.5)ab	53.3 (21.7)*d	86.7 (13.3)*d	90.0 (11.1)*d	96.7 (6.0)*c	100.0 (0)*d
	10	76.7 (27.2)*cd	90.0 (11.1)*e	96.7 (6.0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
	20	90.0 (11.1)*cde	93.3 (6.2)*e	96.7 (6.0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
Positive control (Lambda-cyhalothrin)	5	100.0 (0)*e	100.0 (0)*e	100.0 (0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
	10	100.0 (0)*e	100.0 (0)*e	100.0 (0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
	20	100.0 (0)*e	100.0 (0)*e	100.0 (0)*d	100.0 (0)*d	100.0 (0)*c	100.0 (0)*d
Control (non-treated)	-	6.7(86.5)a	6.7 (86.5)a	6.7 (86.5)a	6.7 (86.5)a	6.7 (86.5)a	6.7 (86.5)a

[a] Mean (RSD%) of three replicates, each set up with 20 adults. * Statistically different from control group according to LSD test. a,b,c,d,e: Values followed by different letters in the same column differ significantly at $p \leq 0.05$ according to Duncan's Multiple Range test.

The use of plant essential oils in insect control is an alternative pest control method for minimizing the noxious effects of synthetic chemicals on the environment [14,59,60]. The present

results suggested that the essential oils of the *Achillea* species investigated have notable toxic effects to the adults of *L. decemlineata*.

4. Conclusions

From the current study, it can be concluded that the *Achillea* species, particularly in their oils, show strong toxicity against weeds and potato Colorado beetle. Hence, the *Achillea* species could be useful for developing as a botanical herbicide or insecticide since their essential oils or extracts are considered to be less harmful than the majority of conventional herbicides and insecticides and poses fewer or lesser risks to human health and the environment. However, further studies under field conditions are necessary to evaluate the possible use of these essential oils and extracts.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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