Journal of Oleo Science
Copyright ©2015 by Japan Oil Chemists' Society
doi : 10.5650/jos.ess15078
J. Oleo Sci. **64**, (8) 881-893 (2015)

JOURNAL OF OLEO SCIENCE
JOS

Sitophilus granarius L. (Coleoptera) Toxicity and Biological Activities of the Essential Oils of *Tanacetum macrophyllum* (Waldst. & Kit.) Schultz Bip.

Kaan Polatoğlu[1, 2*] , Ömer Cem Karakoç[3], Betül Demirci[4], Nezhun Gören[2] and Kemal Hüsnü Can Başer[5]

[1] *Department of Analytical Chemistry, Faculty of Pharmacy, İstanbul Kemerburgaz University, 34217 İstanbul, Turkey.*
[2] *Yıldız Technical University, Faculty of Science & Letters, Department of Molecular Biology and Genetics, 34210, İstanbul, Turkey.*
[3] *Çankırı Karatekin University, Yapraklı Vocational School, Department of Crop and Animal Protection, 18100, Çankırı, Turkey.*
[4] *Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy, 26470, Eskişehir, Turkey.*
[5] *Department of Botany and Microbiology, Faculty of Science, King Saud University, 11451 Riyadh, Saudi Arabia.*

Abstract: Insecticides of the natural origin are an important alternative to the synthetic insecticides that are being employed for the preserving stored products. The volatiles obtained from *T. cinerariifolium* (=*Pyrethrum cinerariifolium*) is being used for many types of insecticidal applications; however there is a very little information on the insecticidal activity of the essential oils of other *Tanacetum* species. The main purpose of the present study is to determine the chemical composition of *T. macrophyllum* (Waldst. & Kit.) Schultz Bip. essential oils and evaluate their insecticidal activity against *S. granarius* as well as its other beneficial biological activities. Highest contact toxicity was observed in the leaf oil of (88.93%) against *S. granarius*. The flower oil showed considerable fumigant toxicity against L. minor at 10 mg/mL application concentration (61.86 %) when compared with other samples at the same concentration. The highest DPPH (2,2-Diphenyl-1-picrylhydrazyl) scavenging activity (47.7%) and phosphomolybdenum reducing activity was observed also for the flower oil of *T. macrophyllum* at 10 mg/mL concentration. The essential oils were analyzed by GC, GC/MS. The flower and leaf oils were characterized with γ-eudesmol 21.5%, (*E*)-sesquilavandulol 20.3%, copaborneol 8.5% and copaborneol 14.1%, 1,8-cineole 11%, bornyl acetate 9.6%, borneol 6.3% respectively. AHC analysis of the qualitative and quantitative data obtained from the essential oil composition of the *T. macrophyllum* essential oil from the present research and previous reports pointed out that two different chemotypes could be proposed with current findings which are *p*-methyl benzyl alcohol/ cadinene and eudesmane chemotypes.

Key words: *Tanacetum macrophyllum*, essential oil, *Sitophilus granarius* toxicity, HPTLC-PRAP activity, chemotype

1 INTRODUCTION

The synthetic insecticides that are being employed for the protection of stored products namely methyl bromide, phosphine are very toxic compounds and they produce serious damage to the environment including the depletion of the ozone layer, soil and water contamination etc. The use of these fumigants were banned in the United States, European Union and other developed countries.

Increasing food demand of the world and environmental concerns of the societies produce the urge to find new nature friendly alternatives of pest management for the stored products. Additionally the new regulations like the European Pesticide Regulation (EC) No. 1107/2009 supports the use of pesticides that are not harmful or less harmful to health and environment. New regulations like this is expected to provide additional attention to the biopesticides[1)]. In the last two decades there is a growing interest on the insecticides obtained from the natural sources for stored product protection. Especially the essential oils and their components are being the focus of many studies due to their volatility therefore the potential ease of their application. The essential oils and their components were proposed to be considered as natural, environmentally friendly and relatively safe alternatives to the

*Correspondence to: Dr. Kaan POLATOĞLU, Department of Analytical Chemistry, Faculty of Pharmacy, İstanbul Kemerburgaz University, 34217 İstanbul, Turkey
E-mail: kaanpolatoglu@gmail.com / kaan.polatoglu@kemerburgaz.edu.tr
Accepted May 7, 2015 (received for review April 2, 2015)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
http://www.jstage.jst.go.jp/browse/jos/ http://mc.manusriptcentral.com/jjocs

synthetic fumigants[2−5].

There are many examples of natural insecticides that are employed in pest management. *Tanacetum cinerariifolium* (Syn. = *Pyrethrum cinerariifolium*) is one of the best known natural insecticide sources that is cultivated for its pyrethrin content which is being used as a general purpose insecticide. Pyrethrins is class of irregular monoterpenes that acts on the nervous system of the insects and cause toxicity[3, 6]. Previous investigations on the insecticidal activity of natural substances from the genus *Tanacetum* reported insecticidal activity of *T. parthenium*[7], *T. cadmeum* ssp. *cadmeum* extracts against *Spodoptera littoralis*[8], *T. vulgare* extracts against *Leptinotarsa decemlifineata*[9] and *T. abrotanifolium* extracts against *S. granarius* and *S. oryzae*[10]. However the insecticidal activities of *Tanacetum* essential oils against any insect species were not covered very well in the literature. There are only a couple of reports that presents the insecticidal activities of *T. vulgare* essential oil against moths and mites[11−13].

The previous investigations on the essential oils of *Tanacetum* species reported beneficial biological activities including antimicrobial[14−18], anticoagulant, antifibrinolytic[19], herbicidal[20] and anticancer[21] activities. Especially *Tanacetum* essential oils are reported to have considerable antimicrobial activity against pathogens that could be found on wheat[14−16]. The subject of the present study, *T. macrophyllum* is a tall (60-100 cm) perennial herb growing near streams or in pastures under shaded grounds at 800-2300 m altitude. In Turkey this species naturally grows northeast Anatolia[22]. Essential oil composition of many *Tanacetum* species was covered very well in the literature as well as the essential oil composition of *T. macrophyllum*. Previously four different studies on the essential oil composition of *T. macrophyllum* reports the main components as β-eudesmol 89.5%[23], β-eudesmol 21.4% and *cis*-chrysanthenol 12%[24]; isobornyl acetate 9.5%, borneol 9.1%, 1,8-cineole 8,6% and γ-eudesmol 6.2%[25] and δ-cadienene (11.2-8%), γ-cadinene (8.1-4.5%) and unusual compound *p*-methyl benzyl alcohol (34.1-41.5%)[26]. According to the previous results the chemical composition of the *T. macrophyllum* essential oil show variation depending on the geographical origin. Usually *Tanacetum* essential oils have 1,8 cineole, camphor, borneol, thujones[27−34] and rarely carvone, pinene, irregular monoterpenes[16, 35−37] could be encountered as the main components.

The known beneficial uses, activities and the previous reports regarding the insecticidal activity of *Tanacetum* species prompted us to investigate the essential oil composition and insecticidal activities of the species from this genus. We suspect other members of this genus also have essential oils or volatiles that have the insecticidal activity. The purpose of this study was to evaluate the insecticidal activity of *T. macrophyllum* essential oils against *S. granarius*. Additionally other beneficial biological activities such as radical scavenging, antioxidant and phytotoxic activities were investigated. The chemical composition of the essential oils is reported and compared with the previous results.

2 EXPERIMENTAL

2.1 Chemicals & Reagents

In GC, GC/MS analysis GC grade *n*-hexane (SupraSolv®, Merck Darmstadt, Germany) and C7-C40 *n*-alkane standard (Supelco, Sigma-Aldrich, St. Louis MO, USA) were used. In insecticidal activity assays, DPPH scavenging, PRAP, Phytotoxicity and AChE inhibition assays, acetone, *n*-hexane and methanol (EMSURE®, Merck Darmstadt, Germany) solvents were used. In DPPH scavenging tests 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), butylated hydroxytoluene (BHT), (±)α-tocopherol (Sigma-Aldrich, St. Louis MO, USA) and Precoated Silica gel 60 F254 0.2 mm aluminium TLC plates (Merck, Darmstadt, Germany) were used. In PRAP assay butylated hydroxytoluene (BHT), (±) α-tocopherol (Alfa aesar, Karlsruhe, Germany), phosphomolybdic acid (Sigma-Aldrich, St. Louis MO, USA) and Precoated Silica gel 60 F254 0.2 mm aluminium TLC plates (Merck, Darmstadt, Germany) were used. In phytotoxic activity tests E-medium was prepared using the following substances $Ca(NO_3)_2.4H_2O$, KNO_3, KH_2PO_4, $FeCl_3.6H_2O$, $MgSO_4.7H_2O$, $ZnSO_4.7H_2O$, $Na_2MoO_4.2H_2O$, $CuSO_4.5H_2O$, $MnCl_2.4H_2O$, HCl, H_3BO_3, EDTA and tartaric acid (Merck, Darmstadt, Germany). Acetyl cholinesterase, acetylcholine iodide, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), Trizma base and galanthamine hydrobromide (Sigma-Aldrich, St. Louis MO, USA) were used in AChE inhibition assay.

2.2 Plant Material

Plant materials were collected during the flowering period. *Tanacetum macrophyllum* was collected on 05 July 2006 from Şavşat – Artvin at 2031 m altitude. Voucher specimens have been deposited in the Herbarium of the Faculty of Science, Istanbul University Turkey (Voucher no. ISTE 83749). Plant materials were identified by Dr. Kerim Alpınar.

2.3 Isolation of the Essential Oil

Flowers and leaves (100 g each) of the air dried plants were separately subjected to hydro distillation for 4 h using a Clevenger- type apparatus to produce essential oils. *T. macrophyllum* afforded a green colored oil from flowers in 0.45% (A) and the leaf oil was light-green colored obtained in 0.36% (B) yield (v/w). Oils were preserved in amber vials under −20℃ until the day they were analyzed.

2.4 Gas Chromatography-Mass Spectrometry Analysis

The essential oil analyses were done simultaneously by

gas chromatography (GC) and gas chromatography–mass spectrometry (GC/MS) systems. The GC–MS analyses were done with an Agilent 5975 GC-MSD system with Innowax FSC column (60 m × 0.25 mm, 0.25 μm film thickness) and helium as a carrier gas (0.8 mL/min). The oven temperature was programmed to 60℃ for 10 min and raised to 220℃ at a rate of 4℃/min. The temperature kept constant at 220℃ for 10 min and then raised to 240℃ at a rate of 1℃/min. The injector temperature was set at 250℃. Split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range m/z 35 to 450. The GC analyses were done with Agilent 6890N GC system. The FID detector temperature was set to 300℃ and same operational conditions applied to a duplicate of the same column used in GC/MS analyses. The simultaneous auto injection was done to obtain the same retention times. The relative percentage amounts of the separated compounds were calculated from the integration of the peaks in FID chromatograms (Table 1). Identification of essential oil components was done by comparison of their retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, MassFinder 3 Library)[38, 39] and in-house "Baser Library of Essential Oil Constituents" built up by genuine compounds and components of known oils, as well as MS literature data[40] was used for identification.

2.5 Insecticidal Contact Toxicity Assay

Stock cultures of *Sitophilus granarius* were obtained from the Çankırı Karatekin University, Faculty of Science, Department of Biology. In order to obtain single aged insect population, 1/3 of 5 L glass jars were filled with clean wheat than male and female insects were introduced. After 48 hours of incubation, male and female insects were removed from the jars. The infested grains were incubated at 27 ± 2℃ in a dark climate chamber for 45 days and single aged insects were obtained. The oils were diluted with acetone to obtain 20% (v/v) stock solution. Stock solutions prepared from each oil sample (1 μL) were applied to insects with a micro applicator (Hamilton, Bonaduz, GR, Switzerland). The acetone was also applied at the same volume as the blank in the control. The stock solutions of the essential oils were applied topically to the dorsal surface of the thorax of the insects[41, 42]. After the sample application the insects were transferred to the 65 mL tubes which were filled with 10 g of wheat. The insects were incubated at 27 ± 2℃ in a climatic chamber and the mortality was recorded every 24 hours for 3 days. In each replicate 10 insects were used and each treatment was replicated three times and the whole experiment was repeated three different times. A randomized block design was employed including sample treatments and blank controls. The results were translated into arcsine values and transformed to percentage mortalities. The resulting values were evaluated by analysis of variance at the 5% significance level. The data obtained by analysis of variance were compared using Tukey's multiple comparison test for differences.

2.6 Fumigant Insecticidal Toxicity Assay

The fumigant toxicity of *T. macrophyllum* essential oils against *Sitophilus granarius* were tested according to our previous protocol[43]. Glass tubes (10 mL) with tight caps were filled with 5 g sterile wheat and 10 insects were placed. Filter paper discs that have 10 mm diameter were prepared from Whatman filter paper N° 1 and these were placed on the tube caps with a needle. Stock solutions of the essential oils were prepared with acetone 10% (v/v) and 10 μL of the stock solution of the oil was impregnated on the filter paper disc with a micropipette. In order to evaporate acetone caps were left open for five minutes then closed. Tubes were incubated at 25℃ in dark for 24 h in climate controlled cabinet. After 24 h number of mortality was recorded. The experiment was performed according to random block pattern. Each experiment was done with three replications and all experiment was replicated three times.

2.7 Fumigant Phytotoxic Activity Assay

The fumigant toxicity of *Tanacetum* essential oils against *Lemna minor* were tested according to the protocol described by[44] with a modification for essential oils. Petri dishes (d: 10 cm; h: 1.5 cm) were filled with 40 mL of E-medium and ten plants of *Lemna minor* which contain three fronds were placed in petri dishes. Filter papers with a size 10 mm × 10 mm were prepared from Whatman filter paper N° 1 and these were placed on the lids of petri dishes with a transparent adhesive tape. Stock solutions of the essential oils (10 mg/mL) were prepared with *n*-hexane. 50 μL of the essential oils with 10, 5, 1 mg/mL concentrations prepared from stock solution and negative control (*n*-hexane) were applied to different petri dishes and the lids are sealed with an adhesive tape. Petri dishes were incubated in climate control cabinet at 30℃, 56 ± 10 relative humidity and 9000 lux light intensity for seven days. After an incubation the number of fronds was counted in every petri dish. Percent phytotoxicity was calculated according to Phytotoxic activity% = [(No. fronds Control – No. fronds Sample)/ No. fronds Control] × 100. The results of the experiments were given as mean ± standard deviation of three parallel experiments. The results were subjected to ANOVA analysis and multiple comparison with Tukey's test differences of results were given with different letters with 95.00% confidence level.

2.8 HPTLC-DPPH Scavenging Activity Assay

The DPPH scavenging activity of the essential oils was determined with our previous HPTLC-DPPH radical protocol[14]. Stock solutions of the extracts (5 and 10 mg/mL),

Table 1 Composition (%) of Essential Oils obtained from *T. macrophyllum*.

Compound	RRI	A	B	Identification
Tricyclene	1014	tr	0.1	MS
α-Pinene	1032	0.4	1.2	t_R, MS
α-Thujene	1035	0.1	0.2	MS
Camphene	1076	0.4	2.1	t_R, MS
Hexanal	1093	tr	tr	t_R, MS
β-Pinene	1118	0.1	0.4	t_R, MS
Sabinene	1132	0.3	1.7	t_R, MS
Thuja-2,4(10)-diene	1135	tr	tr	MS
Myrcene	1174	0.1	0.1	t_R, MS
α-Phellandrene	1176	tr	−	t_R, MS
α-Terpinene	1188	0.3	0.2	t_R, MS
Dehydro 1,8-cineole	1195	tr	0.1	MS
Limonene	1203	0.1	0.3	t_R, MS
1,8-Cineole	1213	2.3	**11**	t_R, MS
(*Z*)-3-Hexanal	1225	−	0.1	MS
Isochrysanthenone	1234	0.1	0.1	MS
2-Pentylfuran	1244	tr	tr	MS
γ-Terpinene	1255	0.2	0.4	t_R, MS
p-Cymene	1280	0.5	0.9	t_R, MS
1,3,5-Trimethyl benzene	1355	0.2	0.4	MS
1,2,3-Trimethyl benzene	1355	0.2	0.2	MS
Nonanal	1400	tr	tr	t_R, MS
α-Thujone	1430	0.1	0.1	MS
Filifolene	1445	tr	−	MS
β-Thujone	1451	3.5	**9**	MS
trans-Sabinene hydrate	1474	0.1	0.6	MS
α-Amorphene	1481	1	2.2	MS
Cyclosativene	1492	0.3	0.7	MS
α-Copaene	1497	0.1	1.3	t_R, MS
Chrysanthenone	1522	0.2	0.5	MS
Camphor	1532	2.3	4.2	t_R, MS
Benzaldehyde	1541	tr	tr	t_R, MS
α-Gurjunene	1544	tr	−	MS
cis-α-Bergamotene	1545	tr	−	MS
Linalool	1553	1.8	0.1	t_R, MS
cis-Sabinene hydrate	1556	0.1	0.4	MS
trans-*p*-Menth-2-en-1-ol	1571	0.1	0.1	MS
cis-Chrysanthenyl acetate	1582	0.1	−	MS
Bornyl acetate	1591	1.2	9.6	t_R, MS
Thymol methyl ether	1604	tr	−	t_R, MS
Terpinen-4-ol	1611	0.8	1.5	t_R, MS
β-Caryophyllene	1612	1.2	0.5	t_R, MS
cis-*p*-Menth-2-en-1ol	1638	0.1	0.2	MS
cis-Verbenyl acetate	1645	−	0.1	MS
Alloaromadendrane	1661	0.3	0.5	MS
trans-Pinocarvyl acetate	1661	0.2	0.3	MS
Phenyl acetaldehyde	1663	tr	−	t_R, MS
(*Z*)-β-Farnesene	1668	0.1	−	MS
Sesquisabinene	1669	0.3	0.1	MS
δ-Terpineol	1682	−	0.3	MS
trans-Verbenol	1683	0.4	0.7	t_R, MS

Table 1 Continued.

Compound	RRI	A	B	Identification
α-Humulene	1687	0.2	−	t_R, MS
Selin-4(11)-diene	1688	0.1	−	MS
γ-Curcumene	1704	0.1	−	MS
α-Terpineol	1706	0.8	1.8	t_R, MS
Borneol	1719	1.2	6.3	t_R, MS
Germacrene D	1726	0.1	−	MS
Neryl acetate	1733	0.6	−	t_R, MS
α-Muurolene	1740	0.2	0.2	MS
(Z)-γ-Bisabolene	1754	tr	−	MS
β-Curcumene	1755	0.1	−	MS
cis-Piperitol	1758	tr	−	MS
Naphthalene	1763	0.3	0.2	t_R, MS
Geranyl acetate	1765	0.1	−	t_R, MS
δ-Cadinene	1773	0.5	0.5	MS
β-Sesquiphellandrene	1783	0.2	−	MS
ar-Curcumene	1786	0.3	0.2	MS
Cumin aldehyde	1802	tr	−	t_R, MS
Myrtenol	1804	tr	0.1	MS
p-Cymen-8-ol	1864	0.1	−	t_R, MS
epi-Cubebol	1900	−	0.2	MS
α-Calacorene	1941	0.1	0.1	MS
1,5-Epoxy salvial-4(14)-ene	1945	0.1	0.4	MS
Cubebol	1957	0.1	−	MS
Caryophyllene oxide	2008	4	−	t_R, MS
Methyl eugenol	2030	0.1	−	t_R, MS
Salvial-4(14)en-1one	2037	0.1	−	MS
Ledol	2057	0.3	0.5	MS
Humulene epoxide II	2071	0.2	−	MS
Caryophylla-2(12), 6(13)-dien-5-one	2074	0.8	0.8	MS
Cubenol	2080	−	tr	MS
1-*epi*-cubenol	2088	−	0.1	MS
β-Oplopenone	2092	−	tr	MS
Elemol	2096	0.4	−	t_R, MS
cis-Sesquisabinene hydrate	2096	0.7	0.1	MS
(*E*)-Sesquilavandulyl acetate	2100	1.1	−	MS
α-Guaiol	2103	0.1	−	MS
Cumin alcohol	2113	−	0.1	t_R, MS
Spathulenol	2144	−	0.6	MS
Hexahydrofarnesyl acetone	2153	−	0.1	MS
Muurola-4,10(14)-dien-1-ol	2161	0.2	0.6	MS
β-Bisabolol	2170	0.1	−	t_R, MS
(*E*)-Sesquilavandulol	2183	**20.3**	−	MS
γ-Eudesmol	2185	**21.5**	3.2	MS
Eugenol	2186	0.2	−	t_R, MS
Copaborneol	2210	**8.5**	**14.1**	MS
ar-Turmerol	2214	0.3	0.2	MS
α-Bisabolol	2232	1.5	0.2	t_R, MS
α-Eudesmol	2250	1.4	0.3	MS
β-Eudesmol	2257	1.4	0.6	MS
Selin-11-en-4α-ol	2273	1.5	1.2	MS
Decanoic acid	2298	0.4	−	t_R, MS

Table 1 Continued.

Compound	RRI	A	B	Identification
Tricosane	2300	tr	−	t_R, MS
Caryophylladienol I	2316	−	0.2	MS
Caryophylladienol II	2324	0.2	0.8	MS
Eudesma-4(15),7-dien-1β-ol	2369	0.1	0.1	MS
1-Hexadecanol	2384	0.1	−	MS
Caryophyllenol II	2392	0.1	0.3	MS
Pentacosane	2500	0.3	−	t_R, MS
γ-Costol	2533	0.3	0.1	MS
Phytol	2622	−	0.5	MS
Tetradecanoic acid	2670	−	tr	t_R, MS
Nonacosane	2900	tr	−	t_R, MS
Hexadecanoic acid	2931	0.7	0.8	t_R, MS
Monoterpene Hydrocarbons		2.5	7.4	
Oxygenated Monoterpenes		16.2	47.2	
Sesquiterpene Hydrocarbons		5.2	6.3	
Oxygenated Sesquiterpenes		64.9	24.9	
Others		2.9	2.2	
Total		91.7	88.0	

RRI: Relative Retention Indices; tr: Trace (<0.1%); A: *Tanacetum macrophyllum* – Flower Oil; B: *Tanacetum macrophyllum* – Leaf Oil; Identification method: t_R, identification based on the retention times (t_R) of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

positive controls (±) α-tocopherol, BHT (Aldrich, St. Louis MO, USA) (1,5 and 10 mg/mL) and DPPH (0.1mM) (Aldrich, St. Louis MO, USA) were prepared with CH_3OH. 200 μL of the sample solutions were mixed with 1000 μL of DPPH solution as well as positive controls and sample free blank controls in 1.5 mL Eppendorf tubes and vortexed for 2 minutes. After incubating all the samples and controls for 1 h in the dark at room temperature, 2 μL of them were applied on an aluminum 60 F254 TLC Plate (Merck Darmstadt, Germany) with 5 mm band length by the help of the TLC applicator system (CAMAG, Muttenz, Switzerland). After application of samples and controls on the TLC; plates were scanned at 517 nm with a TLC Scanner (CAMAG, Muttenz, Switzerland) and absorbance of the bands were detected. The percentage of DPPH scavenging activity was calculated according to DPPH Scav. Act.% = [(AControl - ASample)/ AControl] × 100 formula.

2.9 HPTLC-PRAP (Phosphomolybdenum Reducing Antioxidant Power) Assay

The antioxidant activity of the *T. macrophyllum* essential oils was determined with a new HPTLC-PRAP protocol based on a previous spectrophotometric protocol[45]. Stock solutions of 10% (w/v) phosphomolybdic acid (Aldrich, St. Louis MO, USA), 10, 5, 1 mg/mL essential oils, positive controls: α-topocherol (Alfa aesar, Karlsruhe, Germany) and BHT (Alfa aesar, Karlsruhe, Germany) were prepared. As a negative control essential oil free methanol was used. Phosphomolybdic acid solution (200 μL) and samples (200 μL) were mixed then incubated at 80℃ for 30 minutes. After all of the samples and controls were cooled to room temperature they were applied (2 μL – 5 mm) on silica gel TLC plate (Precoated Silica gel 60 F254 0.2 mm aluminium TLC plate Merck, Darmstadt, Germany) with the help of TLC applicator (Linomat 5 – CAMAG, Muttenz, Switzerland). TLC plate was scanned at 600 nm with a TLC-Scanner (TLC-Scanner 3 – CAMAG, Muttenz, Switzerland). Absorbances of the each spot were detected and increased absorbance of the reaction meant increased reducing power of the compounds when compared to blank control.

2.10 Agglomerative Hierarchical Cluster Analysis

Essential oil composition data of *Tanacetum macrophyllum*[23−26] reported in the literature and the present work was used in the agglomerative hierarchical cluster (AHC) analysis. The data set was prepared based on the qualitative and quantitative properties of the oils reported in the literature and from the GC, GC/MS analysis performed in this research. AHC analysis was performed using XLSTAT – 2013.5.01 program trial version (Addinsoft, New York-U.S.A.). AHC analysis was done using Pearson's dissimilarity method and unweighted pair-group average as aggregation criterion. Dendrograms were obtained showing dissimilarity of the analyzed oils within the range 0-1.

2.11 Statistical Analysis

Analysis of variance (ANOVA) was applied on the data obtained from the HPTLC-DPPH and HPTLC-PRAP assay followed by the post hoc "Tukey's test". The results of this analysis are given as the mean ± standard deviation followed by different letters which indicate compared results have significant differences $p<0.05$. Statistical analysis was performed with XLSTAT-2012.6.02 trial version (Addinsoft, NewYork, USA).

3 RESULTS

The composition of the flower and leaf oils of *Tanacetum macrophyllum* is presented in Table 1. A hundred and one, and seventy nine compounds were detected representing 91.7% (A) and 88.2% (B) of *Tanacetum macrophyllum* flower and leaf oils, respectively. Flower oil was dominated by oxygenated sesquiterpenes (64.9%) unlike leaf oil which was dominated by oxygenated monoterpenes (47.2%) and sesquiterpenes (24.9%). Flower oil was characterized with γ-eudesmol 21.5%, (*E*)-sesquilavandulol 20.3% and copaborneol 8.5%. Leaf oil was rich in copaborneol 14.1%, 1,8-cineole 11%, bornyl acetate 9.6% and borneol 6.3%. Borneol derivatives and 1,8-cineole were also present in flower oil but with small percentages.

T. macrophyllum flower (A) and leaf (B) oils were investigated for their contact toxicity against S. granarius. The insecticidal activity of the oils was given in Table 2. The highest contact toxicity of the investigated oils against *S. granarius* in 0.2 μL/mL application dose after 24 hours was observed for B (88.93%). The activity observed for B was higher than the other oil (A: 50.00%) when compared at the same applied concentration. *T. macrophyllum* flower (A) and leaf (B) oils were also investigated for their fumigant toxicity against *S. granarius*. None of the oils produced any toxic activity after 48 hours of application. In the fumigant toxicity tests 10 μL of oil (in acetone 10% v/v) were applied however this quantity was not able to produce any toxic effect on *S. granarius*. *T. macrophyllum* flower (A) oil was also investigated for fumigant phytotoxicity against *Lemna minor* at three different concentrations. The highest activity was observed for oil A (61.86%) at 10 mg/mL concentration. The fumigant phytotoxicity of the investigated oil was concentration dependent. The fumigant phytotoxicity of the oil was given in Table 3.

Table 2 Contact toxicity of essential oils obtained from *T. macrophyllum* against *Sitophilus granarius* after 24-hour*.

Treatment	% Mortality ± SE
Control	0.00 ± 0.00d[1]
A	50.00 ± 0.00c
B	88.93 ± 0.09a

*Results were given as mean of three parallel experiments ± standard error.

Table 3 The fumigant phytotoxic activity of essential oils obtained from *T. macrophyllum* against *Lemna minor**.

Concentration	A
10 mg/mL	61.86 ± 5.99
5 mg/mL	21.19 ± 3.60
1 mg/mL	16.10 ± 5.99

*Results were given as mean of three parallel experiments ± standard deviation.

Highest radical scavenging activity was observed at 10 mg/mL concentration in the flower oil of *T. macrophyllum* (A) 47.7%. However the leaf oil (B) of this plant showed lower activity 29% at the same concentration. However all of the investigated oils produced low activities when compared to positive control α-tocopherol when compared at the same concentration. DPPH scavenging activities of the oils were concentration dependent. Previously described spectrophotometric PRAP protocol[45] was modified for HPTLC. Similar results were also obtained in phosphomolybdenum reducing antioxidant power assay when compared to DPPH scavenging assay. The highest activity was observed at 10 mg/mL concentration for the oil A. However both of the oils produced low activity when compared to positive controls butylated hydroxyl toluene (BHT) and α-tocopherol when compared at the same concentration. The PRAP activities of the oils were concentration dependent. The results of antioxidant activity assays were given in Table 4 and Table 5.

Agglomerative hierarchical cluster analysis was employed in order to define dissimilarities of the *T. macrophyllum* essential oil composition of the investigated samples and previously reported oils. The dendrogam obtained is given in Fig. 1. Dendrogram revealed that all of the compared oils presented dissimilarities. Highest dissimilarity is observed for the essential oils (Flower and leaf) previously reported to contain *p*-methyl benzyl alcohol and cadinene type sesquiterpenes as major components[26]. These oils separated from the rest of the oils with 0.51 dissimilarity level where dissimilarity ranged (0-1). The oils in this group (flower and leaf oils) separated from each other at the minimum amount of dissimilarity level (<0.01). The essential oils investigated in the present research (flower and leaf oils) formed a group together with the oil originating from Serbia[25] separating from the other oils at 0.33 dissimilarity level which is also the level of dissimilarity between flower and leaf oils. Previously reported studies of the essential oils of *T. macrophyllum* aerial parts originat-

Table 4 DPPH Scavenging activity (%) of essential oils obtained from *T. macrophyllum**.

Concentration	A	B	α-tocopherol
10 mg/mL	47.76 ± 1.78	29.03 ± 6.7	94.50 ± 0.79

*Results were given as mean of three parallel experiments ± standard deviation.

Table 5 Phosphomolybdenum reducing antioxidant power of essential oils obtained from *T. macrophyllum*[*,1].

Concentration	A	B	BHT[2]	α-Toc.[2]	Blank
10 mg/mL	353.27 ± 3.73	307.23 ± 4.19	668.23 ± 3.24	818.05 ± 1.65	36.05 ± 1.12
5 mg/mL	233.32 ± 3.75	249.50 ± 2.43	603.95 ± 1.42	796.12 ± 1.61	
1 mg/mL	74.00 ± 2.70	81.47 ± 0.94	321.90 ± 4.50	554.47 ± 1.66	

[1] Maximum peak height obtained from the spectral scanning of the TLC band at 600 nm; [2] Positive controls butylated hydroxyl toluene (BHT) and α-tocopherol (α-Toc.); * Results were given as mean of five parallel experiments ± standard deviation.

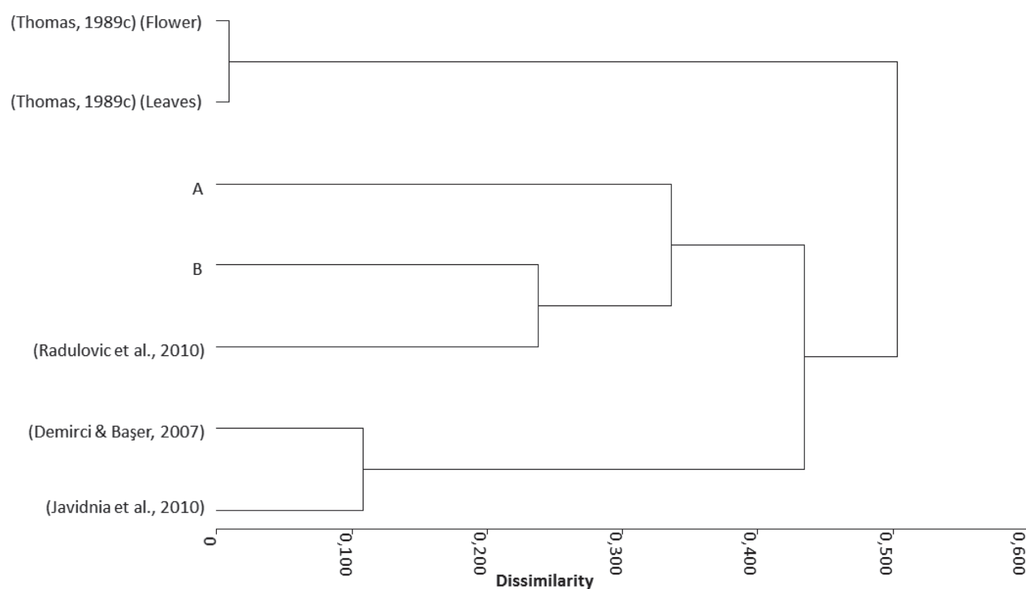

Fig. 1 Dendrogram Obtained from Agglomerative Hierarchical Cluster Analysis of Essential Oil Components of *T. macrophyllum*.

ing from Artvin – Turkey formed a group separating from the rest at 0.43 dissimilarity level. These oils from Artvin region separated from each other at 0.11 dissimilarity level[23, 24].

4 DISCUSSION

The essential oils of *Tanacetum* species usually does not contain high content of (*E*)-sesquilavandulol. Previously only in *T. argenteum* ssp. *flabellifolium* essential oil, this compound was reported to occur 16% as a major constituent[37]. Previous investigation on the *T. macrophyllum* from Yusufeli-Artvin/Turkey reported the main components of the essential oil as β-eudesmol (89.5%)[23]. Another report on the same plant obtained from Borcka-Artvin/Turkey have the essential oil composition as β-eudesmol (21.4%) and *cis*-chrysanthenol (12%). Borneol and derivatives were also present together with copaborneol, γ-eudesmol and (*E*)-sesquilavandulol however in small quantities[24]. Essential oil of *T. macrophyllum* from Bojanine/Serbia have isobornyl acetate 9.5%, borneol 9.1%, 1,8-cineole 8,6% and γ-eudesmol 6.2% main components[25]. In another report on *T. macrophyllum* essential oil presented the composition of flower and leaf oils as *p*-methyl benzyl alcohol (34.1%; 41.5%), γ-cadinene (8.1%;

Table 6 The comparison of major compounds previously reported from *T. macrophyllum* oil and the present study.

Compound	Flower Oil[26]	Leaf Oil[26]	Aerial Parts Oil[25]	Aerial Parts Oil[23]	Aerial Parts Oil[24]	A	B
α-Terpinene	4.3	1.5	–	–	tr[1]	0.3	0.2
1,8 Cineole	tr	tr	**8.6**	tr	2.5	2.3	**11**
β-Thujone	0.5	0.5	–		–	3.5	9
Camphor	2.6	2.3	**6.4**	tr	**5.8**	2.3	4.2
Bornyl acetate	0.1	0.2	–		4	1.2	**9.6**
Iso-bornyl acetate	–	–	**9.5**	0.2	–	–	–
p-Methyl benzyl alcohol	**34.1**	**41.5**	–	–	–	–	–
Borneol	0.5	0.4	**9.1**	–	4.8	1.2	**6.3**
Germacrene D	–	–	–	0.6	3.6	0.1	–
cis-Chrysanthenol	–	–	–	–	**12**	–	–
γ-Cadinene	8.1	4.5	–	–	0.2	–	–
δ-Cadinene	11.2	**8**	–	–	0.4	0.5	0.5
γ-Eudesmol	–	–	**6.2**	–	3.5	**21.5**	3.2
(*E*)-Sesquilavandulol	–	–	4.2	–	1.3	**20.3**	–
Copaborneol	–	–	–	–	**5.6**	**8.5**	**14.1**
β-Eudesmol	–	–	–	**89.5**	**21.4**	1.4	0.6

[1] Trace (<0.1%).

4.5%), and δ-cadinene (11.2%; 8%) respectively[26]. It is interesting to have *p*-methyl benzyl alcohol in *T. macrophyllum* essential oils with very high amounts. Altough it was reported in the essential oils of many other plant species there is a high chance that this compound could be observed due to contamination in the sample. Antibacterial, anticoagulant and antifibrinolytic properties of these oils were also reported[18, 19]. Major differences can easily be seen on the main components of *T. macrophyllum* oils between previous reports and the present work. The differences of each essential oil main components reported in previous reports and presented in this work were compared in Table 6. Similarly all investigated *T. macrophyllum* oils contained high amounts of eudesmane sesquiterpenes. However in each report different sesquiterpenes were reported as the main components.

High contact toxicity of the oil B against the *S. granarius* could be explained by the chemical composition of the oils. The main components of the oil B were copaborneol, 1,8-cineole, copaborneol β-thujone, borneol and bornyl acetate. In the literature essential oils that contain 1,8 cineole[46, 47] and camphor[42] were reported to be toxic against *S. granarius*. Another report indicates high toxicity of pure 1,8 cineole and considerable toxicity of pure camphor against *S. granaries*[46]. The oil B contains 1,8 cineole and structurally very similar compounds to camphor, namely borneol and bornyl acetate in high quantities with the addition of tricyclic sesquiterpene copaborneol. The oil A does not contain 1,8 cineole (A: 2.3%), camphor (A: 2.3%), borneol (A: 1.2%) or bornyl acetate (A: 1.2%) in considerable amounts. However same oil (A) contains high amount of sesquiterpene γ-eudesmol (21.5%) and the irregular sesquiterpene (*E*)-sesquilavandullol (20.3%) which could be also associated with the observed activity. The investigated oils did not produce any fumigant insecticidal toxicity against *S. granarius*. This could be easily explained by the higher boiling points of all main components identified in the oils. Especially previously reported toxic compounds against *S. granarius* 1,8 cineole, camphor together with borneol observed in this research have very high boiling points (ranging between (176 - 213℃) therefore they have low volatility. Additionally sesquiterpenes such as copaborneol, γ-eudesmol and (*E*)-sesquilavandullol generally have higher boiling points than the monoterpenes. In our opinion this fact produces high contact toxicity results but no fumigant toxicity results against *S. granarius* for the oils at the studied concentrations and temperature. Probably at longer application time the oil will produce higher activity results due to the cumulative increase in the concentration of active compounds in the gas phase.

Phytotoxic activities of pure essential oil components such as borneol, 1,8 cineole and camphor were reported[48–50]. However there is no information in the literature regarding

the phytotoxic activities of pure sesquiterpenes such as (*E*)-sesquilavandulol, γ-eudesmol and copaborneol. The amounts of the borneol, 1,8 cineole and camphor are in the range 1.2-2.3% therefore we believe the observed activity is related to the major compounds namely (*E*)-sesquilavandulol, γ-eudesmol and copaborneol. These compounds have high boiling points therefore lower volatility than the monoterpenes. In the insecticidal activity assay (1 μL pure oil /65 mL (tube)) the higher amount of oil was used in unit area than the phytotoxic activity assay (0.55 μL pure oil /85 mL (petri dish) – 0.055 μL pure oil /85 mL (petri dish)). In the fumigant insecticidal activity assay the oil/compounds in the oil produced no toxicity; however in fumigant phytotoxic activity compounds produced considerable activity possibly due to higher assay temperature, light cycle, longer application time or high susceptibility of the *L. minor* to the oil/compounds in the oil.

The oils that contain major compounds that have alcohol and carboxylic acid groups presented higher DPPH scavenging and antioxidant activity as expected. Interestingly oils containing sesquiterpene alcohols such as (*E*)-sesquilavandulol and copaborneol as major compounds afforded highest activity. These compounds seem to be capable of forming stable carbocations or resonance structures after hydrogen donation to DPPH radical.

According to the obtained dendrogram three different groups of *T. macrophyllum* essential oils could be seen. However there are no previously reported seasonal variation data on *T. macrophyllum* essential oils in order to define the exact dissimilarity level for chemotype variation as previously explained[51]. Therefore the biosynthetic origins of the major compounds as well as their quantities in each of the oil were used in order to propose the possible chemotypes of this species according to the available data. In our opinion the groups obtained in the dendrogram roughly corresponds to β-eudesmol[23, 24], γ-eudesmol/(*E*)-sesquilavandullol (oils A,B and (Radulovic, et al. 2010)[25]) and δ-cadinene, *p*-methyl benzyl alcohol[26] chemotypes. The major compounds in each group have different biosynthetic origins or stereo centers than the compounds of other groups and the amount of major compounds are different in each group considerably.

5 CONCLUSION

The essential oil composition of *T. macrophyllum* presented differences in the results of previously reported essential oil compositions[23–26]. Differences were observed on the quantities of the major compounds identified in the present investigation and the previous reports. All of the major compounds observed in the compared oils exist in each of the oils except the previously reported compound *p*-methyl benzyl alcohol[26] which could be a contamination. From the comparison of *T. macrophyllum* oils it is obvious that these differences observed in different reports and present investigation points out different chemotypes. The differences in the oil composition between the present research and previous reports could be related to many factors such as different plant parts studied, collection time, climate, soil conditions, ecological factors, methods and instruments employed in the analysis. In order to provide a solid definition on the chemotypes of this species additional studies on the seasonal variation of the essential oil composition and DNA comparison of *T. macrophyllum* from different locations are necessary. The present investigation also revealed that *T. macrophyllum* afforded the oils which are rich in rarely observed compound (*E*)-sesquilavandulol for *Tanacetum* oils. The other main and minor compounds observed in the oils of these species are frequently observed in *Tanacetum* oils.

The oils obtained from *T. macrophyllum* also presented high *Sitophilus granarius* contact toxicity. The major components in the oils that afforded high activity contains interesting sesquiterpenes such as copaborneol, (*E*)-sesquilavandulol as well as compounds 1,8 cineole and camphor. Camphor and 1,8 cineole were already reported to have contact toxicity against *S. granarius*[42, 46, 47]. However none of the investigated oils produced any fumigant toxicity against *S. granarius*. This fact could be easily explained by the low volatility of the major compounds which are responsible for the contact toxicity. All of the major compounds identified in the oils have higher boiling points therefore with higher application doses in fumigant toxicity assay oils expected to produce higher activities. Additionally investigated *T. macrophyllum* flower oil also produces considerable fumigant phytotoxicity against *L. minor* at the applied dose of 50 μL at 10 mg/mL oil concentration. All of the oils were also investigated for radical scavenging and phosphomolybdenum reducing activities. However none of the oils afforded high activity when compared to positive controls at the same concentrations. The PRAP activity method employed in the evaluations is a new method for HPTLC. This new method involves densitometric measurement of the samples from the TLC plate with a TLC scanner which enables quantitative evaluation of the PRAP activity of extracts, essential oils and pure compounds. The method uses the TLC plate as a microplate and TLC scanner as a microplate reader. Therefore with this method quantitative PRAP evaluation of a number of samples on a single TLC plate is possible.

The essential oils obtained from *T. macrophyllum* contain interesting sesquiterpenes copaborneol, γ-eudesmol and irregular sesquiterpene (*E*)-sesquilavandulol in high amounts. These compounds are highly suspected to be related to the observed insecticidal, phytotoxic, DPPH and PRAP activities of the oils. In conclusion our study verified that the volatile secondary metabolites of

other *Tanacetum* species could also provide high insecticidal activity similar to the pyrethrins obtained from *T. cinerariifolium*. The present research points out that the volatile secondary metabolites from the genus *Tanacetum* should be investigated further for the insecticidal activity.

ACKNOWLEDGMENTS

This research was financially supported by The Scientific and Technological Research Council of Turkey (TUBITAK-TOVAG 111O138). The results given in this article are partially presented in 45th International Symposium on Essential Oils.

References

1) Villaverde, J. J.; Sevilla-Morán, B.; Sandín-España, P.; López-Goti, C.; Alonso-Prados, J. L. Biopesticides in the framework of the European Pesticide Regulation (EC) No. 1107/2009. *Pest. Manag. Sci.* **70**, 2-5 (2014).
2) Isman, M. B. Plant essential oils for pest and disease management. *Crop Protect.* **19**, 603-608 (2000).
3) Isman, M. B. Botanical insecticides, deterrents, and repellents inmodern agriculture and an increasingly regulated world. *Annu. Rev. Entomol.* **51**, 45-66 (2006).
4) Isman, M. B. Perspective, Botanical insecticides: for richer, for poorer. *Pest. Manag. Sci.* **64**, 8-11 (2008).
5) Regnault-Roger, C. The potential of botanical essential oils for insect pest control. *Integrated Pest Management Review* **2**, 25-34 (1997).
6) Wandahwa, P.; Van Ranst, E.; Van Damme, P. *Pyrethrum* (*Chrysanthemum cinerariaefolium* Vis.) cultivation in West Kenya: origin, ecological conditions and management. *Ind. Crop Prod.* **5**, 307-322 (1996).
7) Pavela, R.; Sajfrtová, M.; Sovová, H.; Bárnet, M.; Karban, J. The insecticidal activity of *Tanacetum parthenium* (L.) Schultz Bip. extracts obtained by supercritical fluid extraction and Hydrodistillation. *Ind. Crop Prod.* **31**, 449-454 (2010).
8) Susurluk, H.; Çalışkan, Z.; Gürkan, O.; Kırmızıgül, S.; Gören, N. Antifeedant activity of some *Tanacetum* species and bioassay guided isolation of the secondary metabolites of *Tanacetum cadmeum* ssp. *cadmeum* (Compositae). *Ind. Crop Prod.* **26**, 220-228 (2007).
9) Erturk, O.; Uslu, U. Antifeedant, growth and toxic effects of some plant extracts on *Leptinotarsa decemfineata* (say.) (Coleoptera, Chrysomelidae). *Fresen. Environ. Bullet.* **16**, 601-607 (2007).
10) Alkan, M.; Gokce, A. Toxic and behavioural effects of *Tanacetum abrotanifolium* L. DRUCE (Asteraceae) stem and flower extracts on *Sitophilus granarius* and *Sitophilus oryzae* (Col., Curculionidae). *Türk Entomoloji Dergisi* **36**, 377-389 (2012).
11) Chiasson, H.; Belanger, A.; Bostanian, N.; Vincent, C.; Poliquin, A. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by three methods of extraction. *J. Econ. Entomol.* **94**, 167-171 (2001).
12) Gabel, B.; Thiery, D. Nonhost Plant Odor (*Tanacetum-vulgare*, ASTERACEA) Affects The Reproductive-Behavior of *Lobesia-botrana* DEN-ET-SCHIFF (LEPIDOPTERA, TORTRICIDAE). *J. Insect Behav.* **7**, 149-157 (1994).
13) Palsson, K.; Jaenson, T. G. T.; Baeckstrom, P.; Borg-Karlson, A. K. Thick Repellent Substances in the Essential Oil of *Tanacetum vulgare. J. Med. Entomol.* **45**, 88-93 (2008).
14) Polatoglu, K.; Demirci, F.; Demirci, B.; Gören, N.; Başer, K. H. C. Antibacterial Activity and the Variation of *Tanacetum parthenium* (L.) Schultz Bip. Essential Oils from Turkey. *J. Oleo Sci.* **59**, 177-184 (2010).
15) Polatoglu, K.; Demirci, F.; Demirci, B.; Gören, N.; Başer, K. H. C. Antimicrobial Activity and Essential Oil Composition of a New *T. argyrophyllum* (C. Koch) Tvzel var. *argyrophyllum* Chemotype. *J. Oleo Sci.* **59**, 307-313 (2010).
16) Polatoglu, K.; Demirci, F.; Demirci, B.; Gören, N.; Başer, K. H. C. Essential Oil Composition and Antibacterial Activity of *Tanacetum argenteum* (Lam.) Willd. ssp. *argenteum* and *T. densum* (Lab.) Schultz Bip. ssp. amani Heywood from Turkey. *J. Oleo Sci.* **59**, 361-367 (2010).
17) Salamci, E.; Kordali, S.; Kotan, R.; Cakir, A.; Kaja, Y. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum. Biochem. Syst. Ecol.* **35**, 569-581 (2007).
18) Thomas, O. O. Antibacterial Properties of the Leaf and Flower Oils of *Tanacetum macrophyllum. Fitoterapia* **60**, 327-328 (1989).
19) Thomas, O. O. Anticoagulant and Antifibrinolytic Properties of *Tanacetum macrophyllum. Fitoterapia* **60**, 329-330 (1989).
20) Salamci, E.; Kordali, S.; Kotan, R.; Cakir, A.; Kaja, Y. Chemical compositions, antimicrobial and herbicidal effects of essential oils isolated from Turkish *Tanacetum aucheranum* and *Tanacetum chiliophyllum* var. *chiliophyllum. Biochem. Syst. Ecol.* **35**, 569-581 (2007).
21) Verma, M.; Singh, S. K.; Bhushan, S.; Pal, H. C.; Kitchlu, S.; Koul, M. K.; Thappa, R. K.; Saxena, A. K. Induction of Mitochondrial-Dependent Apoptosis by an Essential Oil from *Tanacetum* gracile. Planta Medica 74, 515-520 (2008).

22) Davis, P. H. Flora of Turkey and The East Aegean Islands. Vol. 5, University Press, Edinburgh, (1975).
23) Javidnia, K.; Gholami, M.; Firuzi, O.; Özer, H.; Kandemir, A. Antimicrobial and Antioxidant Activity and Chemical Composition of the Essential Oil of *Tanacetum macrophyllum* (Waldst. et Kit.) Schultz. *Bip. J. Essent. Oil Res.* 22, 186-188 (2010).
24) Demirci B.; Başer K. H. C. The Essential Oil Composition of *Tanacetum macrophyllum* (Waldst. et Kit.) Schultz. Bip. *J. Essent. Oil Res.* 19, 255-257 (2007).
25) Radulovic, N.; Blagojevic, P.; Skropeta, D.; Zarubica, A.; Zlatkovic, B.; Palić, R. Misidentification of tansy, *Tanacetum macrophyllum*, as yarrow, Achillea grandifolia: a health risk or benefit? *Nat. Prod. Commun.* 5, 121-127 (2010).
26) Thomas, O. O. Phytochemistry of the leaf and flower oils of *Tanacetum macrophyllum. Fitoterapia* 60, 323-326 (1989).
27) Afsharypuor, S.; Jahromy, M. M. Constituents of the essential oil of *Tanacetum lingulatum* (Boiss.) Bornm. *J. Essent. Oil Res.* 15, 74-76 (2003).
28) Başer, K. H. C.; Demirci, B.; Tabanca, N.; Özek, T.; Gören, N. Composition of the essential oils of *Tanacetum armenum* (DC.) Schultz. Bip., *T. balsamita* L., *T. chiliophyllum* (Fisch. & Mey.) Schultz Bip., var. *chiliophyllum* and *T. haradjani* (Rech. Fil.) Grierson and the enantiomeric distribution of camphor and carvone. *Flav. Fragr. J.* 16, 195-200 (2001).
29) Gören, N.; Demirci, B.; Başer, K. H. C. Composition of the essential oils of *Tanacetum* spp. from Turkey. *Flav. Fragr. J.* 16, 191-194 (2001).
30) Hendriks, H.; Bos, R.; Woerdenbag, H. J. The essential oil of *Tanacetum parthenium* (L.) Schultz-Bip. *Flav. Fragr. J.* 11, 367-371 (1996).
31) Mockute, D.; Judzentine, A. Composition of the essential oils of *Tanacetum vulgare* L. growing wild in Vilnius district (Lithuania). *J. Essent. Oil Res.* 16, 550-553 (2004).
32) Nori-Shargh, D.; Norouzi-Arasi, H.; Mirza, M.; Jaimand, K.; Mohammadi, S. Chemical composition of the essential oil of *Tanacetum polycephalum* (Schultz Bip. Ssp. *heterophyllum*). *Flav. Fragr. J.* 14, 112-120 (1999).
33) Polatoğlu, K.; Gören, N.; Başer, K. H. C.; Demirci, B. The essential oil composition of *Tanacetum densum* (Labill.) Heywood ssp. *sivasicum* Hub.-Mor. & Grierson from Turkey. *J. Essent. Oil Res.* 21, 1-3 (2009).
34) Polatoğlu K.; Gören N.; Başer K. H. C.; Demirci B. The Variation in the Essential Oil Composition of *Tanacetum cadmeum* (Boiss.) Heywood ssp *orientale* Grierson from Turkey. *J. Essent. Oil Res.* 21, 97-100 (2009).
35) Hassanpouraghdam, M. B.; Tabatabaie, S. J.; Nazemiyeh, H.; Vojodi L.; Aazami, M. A. Essential oil composition of hydroponically grown *Chrysanthemum balsamita* L. J. Essent. Oil Bear. Pl. 11, 6: 649-654 (2008).
36) Kaul, M. K.; Kitchlu, S.; Bakshi, S. K.; Bhan, M. K.; Thapa, R. K.; Agarwal, S. G. *Tanacetum gracile* Hook. F& T. a new source of lavandullol from Ladakh Himalaya (India). *Flav. Fragr. J.* 21, 690-692 (2006).
37) Tabanca, N.; Demirci, F.; Demirci, B.; Wedge, D. E.; Başer, K. H. C. Composition, enantiomeric distribution, and antimicrobial activity of *Tanacetum argenteum* subsp. *flabellifolium* essential oil. *J. Pharmaceut. Biomed.* 45, 714-719 (2007).
38) McLafferty, F. W.; Stauffer, D. B. *The Wiley/NBS Registry of Mass Spectral Data*. J Wiley and Sons, New York, (1989).
39) Koenig, W. A.; Joulain, D.; Hochmuth, D. H. *Terpenoids and Related Constituents of Essential Oils*. Library of MassFinder 3, Hamburg, (2004).
40) Joulain, D.; Koenig, W. A. *The Atlas of Spectra Data of Sesquiterpene Hydrocarbons*. EB-Verlag, Hamburg, (1998).
41) Abay, G.; Karakoç, Ö. C.; Tüfekçi, A. R.; Koldas¸ S.; Demirtas, I. Insecticidal activity of *Hypnum cupressiforme* (Bryophyta) against *Sitophilus granarius* (Coleoptera: Curculionidae). *J. Stored Prod. Res.* 51, 6-10 (2012).
42) Obeng-Ofori, D.; Reichmuth, C. H.; Bekele, A. J.; Hassanali, A. Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilimandscharicum*, against four stored product beetles. *Int. J. Pest Manage.* 44, 203-209 (1998).
43) Cam, H.; Karakoç, Ö. C.; Gökçe, A.; Telci, I.; Demirtaş, I. Farklı nane türlerine ait klonların uçucu yağlarının buğday biti [*Sitophilus granarius* L. (Coleoptera: Curculionidae)]' ne fumigant etkisi. *Türk Entomoloji Dergisi* 36, 255-263 (2012).
44) Einhellig, F. A.; Leather, G. R.; Hobbs, L. L. Use of *Lemna minor* as a bioassay in allelopathy. *J. Chem. Ecol.* 2, 65-72 (1985).
45) Falcioni, G.; Fedeli, D.; Tiano, L.; Calzuola, I.; Mancinelli, L.; Marsili, V.; Gianfranceschi, G. Antioxidant activity of wheat sprouts extracts in vitro: Inhibition of DNA oxidative damage. *J. Food Sci.* 67, 2918-2922 (2002).
46) Kordali, S.; Aslan, I.; Calmasur, O.; Cakir, A. Toxicity of Essential Oils Isolated from Three *Artemisia* species and some of their major components to granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). *Ind. Crop Prod.* 23, 162-170 (2006).
47) Obeng-Ofori, D.; Reichmuth, C. H.; Bekele, J.; Hassanali, A. Biological Activity of 1,8 cineole a Major Component of Essential Oil of *Ocimum kenyanse* (Ayobangira) Against Stored Product Beetles. *J. Appl. Entomol.* 121, 237-243 (1997).

48) De Martino, L.; Mancini, E.; De Almeda, L. F. R.; De Feo, V. The Antigerminative Activity of Twenty Seven Monoterpenes. *Molecules* 15, 6630-6637(2010).
49) Koitabashi, R.; Suzuki, T.; Kawazu, T.; Sakai, A.; Kuroiwa, H.; Kuroiwa, T. 1,8 Cineole Inhibits Root Growth and DNA Synthesis in the Root Apical Meristem of *Brassica campestris L. J. Plant Res.* 110, 1-6(1997).
50) Kordali, S.; Cakir, A.; Sutay, S. Inhibitory Effects of Monoterpenes on Seed Germination and Seedling Growth. *Zeitschrift für Naturforschung C* 62c, 207-214(2007).
51) Polatoglu, K. "Chemotypes"– A Fact that should not be Ignored in Natural Product Studies. *Natural Products J.* 3, 10-14(2013).