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Effect of Organic Manures on Chemical Profile of Essential Oil of *Artemisia annua* L. cv. CIM Arogya Cultivated in Tarai Region of Uttarakhand

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ABSTRACT: In the present study we studied the effect of organic manures on essential oil content (%) and chemical profile of essential oil. GC-MS were used to screening of the chemical composition of the essential oil hydro-distilled from full flowering stage of the new cultivar variety CIM Arogya of *Artemisia annua*. More than forty compounds were identified, representing 92.5% of the total oil. The main bioactive compounds identified in all the analyzed samples were 1-8 Cineol, Camphor and (3E, 5E)-2, 6-Dimethylcota-3, 5, 7 trien-2-ol in all treatments. Content (%) of bioactive compounds and chemical composition affect by organic manure.

Keywords: Camphor, CIM Arogya, Farm Yard manure, Organic manure, Poultry manure, Vermicompost.

INTRODUCTION

Genus Artemisia is the one of the largest genera of the family Asteraceae. *Artemisia annua* L. (2n=18), also known as quinghao, annual sagewort, annual wormwood, sweet annie, mugwort, and sweet sagewort in Chinese and indian medicine system, is an open-pollinated and qualitative short day plant species of great medicinal and economic importance " (Klayman, 1985; Mucciarelli and Maffei**,** 2002; Wright, 2002; Hsu 2006; Efferth 2007). In Uttrakhand it is also known as "Paanti**.** *Artemisia annua* is native of temperate Asia (Pellicer *et al.,* 2007; Nibret & Wink 2010). In India, it was introduced by CIMAP in 1986, and the adapted for cultivation initially in Kashmir Velly. Artemisinin and similar chemicals, as well as essential oil, are isolated from the foliage (leaves, stem, and flowers) of genetically-bred *A. annua* cultivars (Woerdenbag *et al.,* 1994). Artemisinin and its equivalents, as well as the volatile componds of the essential oil, are produced in glandular trichomes on *A. annua* leaf. Artemisinin is sesquiterpene endo-peroxide lactone with a wide range of therapeutic activities, including antimalarial action. This chemical and its derivatives, such as artesunate, artemether, and dihydro-artemisinin, are used in combination with other medications to treat drug-resistant malaria, a treatment known as artemisinin combination therapy (ACT) (WHO 2015; Kumar *et al.,* 2015; Menard and Dondorp 2017). Artemisinin has also been found to be effective against metabolic disorders (asthma, rheumatoid arthritis, Crohn's disease, diabetes, and fatty liver obesity), cancers (of the breast, pancreas, gall bladder, kidney and colon, as well as leukaemia), viral infections (hepatitis B and C, cytomegalovirus, herpes, and others), fungal and bacterial diseases and leishmaniasis, toxiplasmo- sis, acanthamoebiasis and schistosomiasis trypanosomiasis (Elfawal *et al.,* 2012). The essential oil of *A. annua* contains a variety of volatile compounds which is used in the cosmetics, food, and pharmaceutical industries. Essential oils from *A. annua* have been shown to have antibacterial, antifungal, antiprotozoal, anti-inflammatory, and vasorelaxant, as well as antioxidant, anti-inflammatory and radical scavenging activity (Bilia *et al.*, 2014; Pandey *et al.*, 2016; Pandey and Singh 2017).

The chemical composition of essential oil of *A. annua* plant has been studied thoroughly and hundreds of bioactive components have been identified up to date (Brown, 2010). Camphor, Artemisia ketone, 1, 8-cineole and germacrene D, are commonly found as the major bioactive components (Ahmad & Mishra 1994; Tellez *et al.,* 1999; Malik *et al.,* 2009; Brown 2010).

It is generally recognized that variability of chemical composition of essential oil of *A. annua* depends on plant growth stage and geographical origin (Verma *et al.,* 2011; Lenardis *et al.,* 2011; Bhakuni *et al.,* 2002; Brown, 2010; Holm *et al.*, 1998). Thus, there is still a substantial research interest in the assay of chemical composition and biological activity of essential oil of *A. annua* from different plant development stage and geographical origin. The aim of the present study was

to reveal the effect of organic manures on essential oil content (%) and chemical profile of the essential oil of *A. annua* L. cv. CIM Arogya cultivated in Tarai region of Uttarakhand, at the full flowering stage.

MATERIAL AND METHODS

A. Field trials

Plants of *A. annua* cv. CIM Arogya were raised in the experimental field of G. B. Pant University, Pantanagar, Uttarakhand, (29°N, 79.5°E) and the altitude of 243.83 meter above mean sea level during 2018-19.The soil in the experimental field was course in texture, with a pH of 7.1, electrical conductivity of 0.11 ds m1, nitrogen of 281.35 kg ha^{-1}, phosphorus of 29.23 kg ha^{-1}, and potassium of 309.34 kg ha^{-1}.

B. Sowing and Transplantation

Seeds of *A. annua* cv. CIM Arogya collected from the Central Institute of Medicinal and Aromatic Plant (CIMAP) at Lucknow, India. Seeds were sown in 4m × 1m nursery bed in late December 2018 and seedlings were transplanted into the field in early March 2019. The plot was 34.20 m2 (5.70m × 6 m) in size, with a plant to plant distance of 45 cm and a row to row distance of 50 cm. The plants were irrigated till the requirement.

Treatments: T1: Control (without manure), T2: 10 ton ha^{-1} Farm yard manure, T3: 5 ton ha^{-1} Vermicompost, T4: 5 ton ha^{-1} Poultry manure, T5: 10 ton ha^{-1} Farm yard manure + 5 ton ha^{-1} Vermicompost + 5 ton ha^{-1} Poultry manure, T6: Chemical fertilizers (NPK).

Farm yard manure, poultry manure and vermicompost were used as the source of organic manure and Urea, murate of potash and diammonium phosphate, were used as the source of nitrogen, potassium and phosphorus, respectively. Nitrogen fertilizer 150 kg ha^{-1} were applied in four equal splits, one at the time of transplantation and 2^{nd}, 3^{rd} and 4^{th} at continuous in 30 days, respectively. While Potassium and Phosphorus fertilizers (50 kg ha^{-1} each) were applied at the time of transplantation. Organic manure was applied at the time of seedling transplantation.

The experimental design was organized in a randomized block design (RBD), of which each treatment have three replicates. The plants were collected at full flowering stage.

C. Isolation of Essential oils

The fresh flowers of the 6 different treatments (200 g. each) were hydro-distilled for 4 hrs. separately in a Clevenger–type apparatus in 500 ml water. The resulting data (%) were calculated as volume of essential oils per 200 gm. of flower fresh weight basis. The essential oils were collected, dried over anhydrous sodium sulphate and stored in a refrigerator at 4°C until analyzed.

D. Gas chromatography- Mass spectrometry (GC-MS)

GC-MS analysis of essential oil of six different treatments (Organic manures and NPK) was carried out an GC-MS QP2010 Plus (Gas Chromatograph Mass spectrometer, SHIMADZU, USA.) with Rxi ®-5Sil MS capillary Gas Chromatography column (5% phenyl and 95% dimethyl polysiloxane) with ID: 0.25mm, dimension: 30 mts, and df: 0.25µm. In the gas chromatography part, conditions were; helium as a carrier gas at flow rate of mobile phase was set at 1.21 ml/min, injected temperature (oven temperature) was 80ºC upraised to 250ºC at 5ºC/min. The injected volume was 1µl volume.

RESULTS AND DISCUSSION

A. Essential Oil Content

The essential oil content (%) from the flower of *A. annua* L. cv. CIM Arogya in 6 different treatments are presented in Table 1. The oil content (%) was varies from treatment to treatment. Maximum oil content (%) was found in T8 application of 10 tonne ha^{-1} farmyard manure+ 5 tonne ha^{-1} vermicompost+ 5 tonne ha^{-1} poultry manure (1.4%), followed by T12 (0.98 %) and minimum in T1 (0.64%).

Different scientist, have used chemical fertilizers to increase the *A. annua* essential oil. An rise in the oil content (%) was also observed by Singh (2000), i.e. 84.6, 76.26 and 60.42 kg/ha with 100, 50 and 0 kg/ha of nitrogen.

Table 1: Impact of Organic manures on Essential oil content (%) in *A. annua* L. cv. CIM Arogya

Sr. No.	Treatments	Essential Oil Content (%)
1.	T1	0.64
2.	T2	0.71
3.	T3	0.68
4.	T4	0.72
5.	T5	1.4
6.	T6	0.98

A. Characterization of Bioactive constituents by Gas Chromatography- Mass Spectrometry (GC-MS)

In the present study, impact of organic manure on essential oil composition of *A. annua* cv. CIM Arogya were examined by GC-MS and the bioactive compound identified with the NIST library WILLEY and mass spectrum. Identified bioactive compounds are presented in Tables 2-7. GC-MS analysis of essential oil of *A. annua* L. cv. CIM Arogya revealed the presence of major bioactive compounds (>2.0 % quantity) such as, 1-8 Cineol (Eucalyptol), Camphor, (3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol, 1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-, 1, 5- Heptadien-4-one, 3, 3, 6-trimethyl, Sabinene, Camphene, Cymene <para->, 1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-, 1, 5-Heptadien-4-one, 3, 3, 6- trimethyl-, Myrcene, 3-CYCLOHEXEN-1-OL, and 4-METHYL-1-(1-METHYLETHYL).

More than 40 bioactive compounds were identified in the T1 (control). Camphor (27.89%), Eucalyptol (25.39%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.97%) are recorded as the main components. The rest of the compounds identified in T1 are presented in Table 2. In the Oil of T2 50 bioactive compounds (%) are identified out of which Eucalyptol (23.84%), Camphor (19.68%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.63%), sabinene (4.43%) are the

dominant comparison to other compounds. The rest of the compounds identified in T4 are presented in Table 3. In T3 53 bioactive compounds (%) were identified. Eucalyptol (34.18%), Camphor (11.23%) and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (8.14%), 1, 5, 7-Octatriene-3-ol, 2, 6-dimethyl-(5.70%) and sabinene (4.77%) are the dominant components. The rest of the compounds identified in T5 are listed in Table 4.

In T4 48 compounds were identified. Major bioactive compounds are Eucalyptol (35.57%), Camphor (21.15%), sabinene (4.56%) and (3E, 5E)-2, 6-Dimethylcota-3, 5, 7 trien-2-ol (3.56%). The rest of the compounds identified in T7 are presented in Table 5. In T5 (FYM+ Vermicompost + Poultry manure) more than 45 bioactive compounds were identified. The most abundant compound were Eucalyptol (33.20%), Camphor (24.11%), (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol (5.29%) and sabinene (4.60%). The rest of the compounds identified in T5 are presented in Table 6.

In T6 (NPK) 50 bioactive compounds were identified of which the most abundant compounds were Eucalyptol (29.80%), Camphor (26.00%) and (3E, 5E)-2, 6-Dimethylcota-3, 5, 7 trien-2-ol (5.60%), camphene (4.03%) and 1, 5, 7-Octatriene-3-ol, 2, 6-dimethyl-(4.01%). The rest of the bioactive compounds identified in T6 are presented in Table 7.

Table 2: List of Bioactive compounds present in Treatment 1.

Peak#	R.Time	Area	Area%	Name
1.	6.198	3594323	0.26	TRICYCLO[2.2.1.0(2, 6)]HEPTANE, 1, 7, 7-TRIMETHYL-
2.	6.576	30094228	2.16	Pinene <alpha->
3.	7.146	57364483	4.12	Camphene
4.	7.992	55334091	3.97	Sabinene
5.	8.110	10657250	0.77	Pinene <beta->
6.	8.638	28962799	2.08	7-METHYL-3-METHYLENE-1, 6-OCTADIENE
7.	9.700	8119821	0.58	Terpinene <alpha->
8.	10.533	353567546	25.39	Eucalyptol
9.	11.433	14576367	1.05	Terpinene <gamma->
10.	12.017	13651251	0.98	Sabinene hydrate <cis->
11.	12.573	4248931	0.31	Terpinolene
12.	13.440	5186393	0.37	Butanoic acid, 2-methyl-, 2-methylbutyl ester
13.	13.980	83153704	5.97	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
14.	14.212	6408640	0.46	Chrysanthenone
15.	14.778	9421033	0.68	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-
16.	15.721	388409160	27.89	Camphor
17.	15.817	5438182	0.39	Chrysanthemol <trans->
18.	15.940	1796785	0.13	Bicyclo[3.1.0]hexan-2-one, 5-(1-methylethyl)-
19.	16.164	60234118	4.32	1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-
20.	16.319	4337930	0.31	5-OCTEN-2-YN-4-OL
21.	16.598	22376185	1.61	Terpineol <delta->
22.	16.989	42095386	3.02	3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLET
23.	17.350	1777091	0.13	trans-p-mentha-1(7), 8-dien-2-ol
24.	17.524	1049959	0.08	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6, 6-dimethyl-
25.	17.671	25858007	1.86	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)
26.	18.808	7236072	0.52	2-Cyclohexen-1-ol, 2-methyl-5-(1-methylethenyl)-, cis-
27.	19.183	2739993	0.20	Butanoate <2-methyl-, (3Z)-hexenyl->
28.	21.591	9958938	0.72	Lavandulyl acetate
29.	25.270	2737148	0.20	Copaene <alpha->
30.	25.903	4152977	0.30	BUTANOICACID, 3-METHYL-, PHENYLMETHYLES
31.	27.096	29986837	2.15	Caryophyllene <(E)->
32.	28.528	2226346	0.16	Humulene <alpha->
33.	28.717	25446345	1.83	Farnesene <(E)-, beta->
34.	29.662	35076958	2.52	1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-
35.	29.920	3393958	0.24	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-meth
36.	30.202	8153646	0.59	Bicyclogermacrene
37.	31.167	1610038	0.12	Isogermacrene D
38.	33.167	2078084	0.15	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-
39.	33.443	2704200	0.19	Spathulenol
40.	33.547	2620683	0.19	Caryophyllene oxide
41.	33.731	1463336	0.11	Ledene oxide-(II)
42.	35.087	5619274	0.40	(3R, 3aS, 6S, 7R)-3, 6, 8, 8-Tetramethyloctahydro-1H-3a, 7-me
43.	35.295	2071528	0.15	TRICYCLO[8.6.0.0(2, 9)]HEXADECA-8, 16, HEAD, TAIL-
44.	35.544	1745570	0.13	Selina-3, 11-dien-6-alpha-ol
45.	35.909	1435441	0.10	Perilla alcohol tiglate
46.	36.667	2574899	0.18	Intermedeol

Table 3: Bioactive compounds present in Treatment 2.

Peak#	R.Time	Area	Area%	Name
1.	6.190	1920477	0.18	Tricyclo[2.2.1.0(2, 6)]heptane, 1, 7, 7-trimethyl-
2.	6.333	1562181	0.15	Thujene <alpha->
3.	6.566	25966400	2.42	Pinene <alpha->
4.	6.930	1359802	0.13	BENZENE, (2-METHYLPROPYL)-
5.	7.118	38264179	3.57	Camphene
6.	7.975	47495415	4.43	Sabinene
7.	8.097	8323435	0.78	Pinene oxide <beta->
8.	8.610	29406351	2.74	CINEOLE <DEHYDRO-1, 8-> DB5-408
9.	9.694	7809029	0.73	Terpinene <alpha->
10.	10.512	339154353	31.66	Eucalyptol
11.	11.417	11569729	1.08	Terpinene <gamma->
12.	11.995	10004568	0.93	Sabinene hydrate <cis->
13.	12.566	1743026	0.16	Terpinolene
14.	12.941	1511120	0.14	Epoxymyrcene<6, 7->
15.	13.422	14692542	1.37	Butyrate <3-methylbutyl-, 2-methyl->
16.	13.748	60335792	5.63	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
17.	14.194	6391573	0.60	Bicyclo[3.1.1]hept-2-en-6-one, 2, 7, 7-trimethyl-
18.	14.544	5845585	0.55	Mentha-2, 8-dien-1-ol <trans-, p->
19.	15.542	252876326	23.60	Camphor
20.	15.719	2068932	0.19	Camphene hydrate
21.	15.864	1909697	0.18	BICYCLO[3.1.0]HEXAN-2-ONE, 5-(1-METHYLETHYL
22.	16.068	40376111	3.77	1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-
23.	16.249	2568283	0.24	Chrysanthenol <cis->
24.	16.521	10849837	1.01	Cyclohexanemethanol, .alpha., .alpha.-dimethyl-4-methylen
25.	16.921	26371375	2.46	3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLET
26.	17.467	705158	0.07	BICYCLO[3.1.1]HEPT-2-ENE-2-CARBOXALDEHYDE,
27.	17.619	19682201	1.84	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)
28.	18.812	2897765	0.27	Carveol <trans->
29.	19.168	3131537	0.29	Butanoate <2-methyl-, (3Z)-hexenyl->
30.	19.410	3112120	0.29	Butanoic acid, 2-methyl-, hexyl ester
31.	21.574	5733619	0.54	Lavandulyl acetate
32.	25.256	1763227	0.16	Copaene <alpha->
33.	25.902	2539768	0.24	PENTANOIC ACID, PHENYLMETHYL ESTER
34.	27.057	16588627	1.55	Caryophyllene <(E)->
35.	28.511	1147212	0.11	Humulene <alpha->
36.	28.685	15516022	1.45	Farnesene <(E)-, beta->
37.	29.625	22869385	2.13	1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-
38.	29.901	3663415	0.34	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-meth
39.	30.176	3098357	0.29	Bicyclogermacrene
40.	31.156	1124670	0.10	Alloaromadendrene
41.	33.159	1372983	0.13	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-
42.	33.432	1043684	0.10	Spathulenol
43.	33.531	1794121	0.17	Caryophyllene oxide
44.	33.890	2652263	0.25	Propanedinitrile, dicyclohexyl-
45.	35.075	1218092	0.11	(3R, 3aS, 6S, 7R)-3, 6, 8, 8-Tetramethyloctahydro-1H-3a, 7-met
46.	35.286	1533698	0.14	Cedroxyde
47.	35.533	1598096	0.15	Selina-3, 11-dien-6-alpha-ol
48.	35.902	2296194	0.21	Carvyl angelate, cis-
49.	36.392	2113918	0.20	Allopregnan-3.alpha.-ol-20-one
50.	36.658	1788905	0.17	Intermedeol

Table 4: Bioactive compounds present in Treatment 3.

Peak#	R.Time	Area	Area%	Name
1.	6.190	658861	0.06	Tricyclo[2.2.1.0(2, 6)]heptane, 1, 7, 7-trimethyl-
2.	6.331	2325429	0.21	Thujene <alpha->
3.	6.555	13045695	1.19	Pinene <alpha->
4.	6.925	1319720	0.12	2, 4(10)-THUJADIEN
5.	7.096	16322115	1.48	Camphene
6.	7.980	52445848	4.77	Sabinene
7.	8.101	11362441	1.03	Pinene <beta->
8.	8.649	49312705	4.48	Myrcene
9.	9.702	10484769	0.95	Terpinene <alpha->
10.	10.534	376000581	34.18	Eucalyptol
11.	11.425	14107895	1.28	Terpinene <gamma->
12.	11.985	8824127	0.80	Sabinene hydrate <cis->
13.	12.565	2113823	0.19	Terpinolene
14.	12.917	3506706	0.32	Epoxymyrcene<6, 7->
15.	13.423	20614734	1.87	Butanoic acid, 2-methyl-, 2-methylbutyl ester
16.	13.656	89576456	8.14	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
17.	14.183	6534965	0.59	Chrysanthenone

18.	14.484	7627010	0.69	3-THUJANOL
19.	15.390	123561348	11.23	Camphor
20.	15.712	978859	0.09	Ethanone, 1-[2-methyl-5-(1-methylethenyl)cyclopentyl]-, (
21.	15.843	2787738	0.25	BICYCLO[3.1.0]HEXAN-2-ONE, 5-(1-METHYLETHYL
22.	16.112	62647353	5.70	1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-
23.	16.266	2855340	0.26	Verbenol <trans->
24.	16.538	12543751	1.14	Cyclohexanemethanol, .alpha., .alpha.-dimethyl-4-methylen
25.	16.930	36405689	3.31	3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLETH
26.	17.480	884173	0.08	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6, 6-dimethyl-
27.	17.641	37235451	3.39	3-Cyclohexene-1-methanol, .alpha., .alpha., 4-trimethyl-, (R
28.	18.398	718604	0.07	TRANS-3(10)-CAREN-2-OL
29.	18.809	3267723	0.30	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
30.	19.158	998662	0.09	Butanoate <2-methyl-, (3Z)-hexenyl->
31.	21.092	2764390	0.25	Bicyclo[3.1.1]hept-3-ene, 4, 6, 6-trimethyl-2-vinyloxy-
32.	21.325	1792847	0.16	5-(Furan-3-yl)-2-methylpent-1-en-3-ol
33.	21.576	9263416	0.84	Lavandulyl acetate
34.	25.252	1810641	0.16	Copaene <alpha->
35.	25.891	5023595	0.46	PENTANOIC ACID, PHENYLMETHYL ESTER
36.	27.064	20044084	1.82	Caryophyllene <(E)->
37.	28.512	1434856	0.13	Humulene <alpha->
38.	28.692	19108456	1.74	Farnesene <(E)-, beta->
39.	29.632	25637761	2.33	1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-
40.	29.904	5346385	0.49	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-meth
41.	30.182	3736642	0.34	Bicyclogermacrene
42.	33.156	1340411	0.12	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-
43.	33.433	1651467	0.15	Spathulenol
44.	33.533	2352869	0.21	Caryophyllene oxide
45.	33.708	1006632	0.09	1, 4A, 7, 7-TETRAMETHYLDECAHYDROCYCLOPROPA
46.	33.887	3153229	0.29	Propanedinitrile, dicyclohexyl-
47.	35.078	2936537	0.27	(3R, 3aS, 6S, 7R)-3, 6, 8, 8-Tetramethyloctahydro-1H-3a, 7-met
48.	35.283	4015713	0.37	Longifolenaldehyde
49.	35.534	3453586	0.31	Selina-3, 11-dien-6-alpha-ol
50.	35.895	3547965	0.32	Carvyl tiglate, cis-
51.	36.369	4276862	0.39	Allopregnan-3.alpha.-ol-20-one
52.	36.660	3775549	0.34	Intermedeol
53.	37.519	1392115	0.13	(1R, 7S, E)-7-Isopropyl-4, 10-dimethylenecyclodec-5-enol

Table 5: Bioactive compounds present in Treatment 4.

Peak#	R.Time	Area	Area%	Name
1.	6.189	1827798	0.17	Tricyclo[2.2.1.0(2, 6)]heptane, 1, 7, 7-trimethyl-
2.	6.332	1976647	0.19	Thujene <alpha->
3.	6.561	22231957	2.10	Pinene <alpha->
4.	6.928	2092294	0.20	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-
5.	7.113	35827121	3.38	Camphene
6.	7.974	48398964	4.56	Sabinene
7.	8.097	9049282	0.85	Pinene <beta->
8.	8.604	28762222	2.71	CINEOLE <DEHYDRO-1, 8-> DB5-408
9.	9.701	9659640	0.91	Terpinene <alpha->
10.	10.538	377431647	35.57	Eucalyptol
11.	11.424	15204341	1.43	Terpinene <gamma->
12.	11.992	6344783	0.60	Sabinene hydrate <cis->
13.	12.566	1958315	0.18	Terpinolene
14.	13.418	13280674	1.25	Butanoic acid, 2-methyl-, 2-methylbutyl ester
15.	13.679	37782974	3.56	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
16.	14.209	9359380	0.88	Sabina ketone<dehydro->
17.	14.510	3861975	0.36	2-Cyclohexen-1-ol, 1-methyl-4-(1-methylethyl)-, trans-
18.	15.504	224338835	21.15	Camphor
19.	15.712	1892822	0.18	2, 2-Dimethylocta-3, 4-dienal
20.	15.844	2866679	0.27	BICYCLO[3.1.0]HEXAN-2-ONE, 5-(1-METHYLETHYL
21.	16.040	29567247	2.79	1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-
22.	16.234	4687352	0.44	Chrysanthenol <cis->
23.	16.512	8629721	0.81	Cyclohexanemethanol, .alpha., .alpha.-dimethyl-4-methylen
24.	16.921	32223166	3.04	3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLETH
25.	17.470	1061490	0.10	BICYCLO[3.1.1]HEPT-2-ENE-2-CARBOXALDEHYDE,
26.	17.614	20419809	1.92	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)
27.	18.389	1032590	0.10	TRANS-3(10)-CAREN-2-OL
28.	18.820	2507831	0.24	Carveol <trans->
29.	19.167	2307943	0.22	Butanoate <2-methyl-, (3Z)-hexenyl->
30.	19.410	2053628	0.19	Butanoic acid, 2-methyl-, hexyl ester
31.	21.571	6205756	0.58	Lavandulyl acetate
32.	25.253	1921539	0.18	Copaene <alpha->
33.	25.901	2895521	0.27	9, 12, 15-Octadecatrienoicacid, phenylmethylester, (Z, Z, Z)

34.	27.062	20814433	1.96	Caryophyllene <(E)->
35.	28.510	1461769	0.14	Humulene <alpha->
36.	28.689	18276880	1.72	Farnesene <(E)-, beta->
37.	29.631	26202041	2.47	1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-
38.	29.901	2995082	0.28	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-meth
39.	30.180	4190083	0.39	Bicyclogermacrene
40.	31.158	1259540	0.12	Isogermacrene D
41.	33.427	1416167	0.13	Spathulenol
42.	33.532	1972181	0.19	Caryophyllene oxide
43.	33.889	2722659	0.26	Propanedinitrile, dicyclohexyl-
44.	35.077	2426520	0.23	(3R, 3aS, 6S, 7R)-3, 6, 8, 8-Tetramethyloctahydro-1H-3a, 7-met
45.	35.285	1909099	0.18	TRICYCLO[8.6.0.0E2, 9]HEXADECAN-8, 16, KOPF, SCH
46.	35.531	1452490	0.14	Selina-3, 11-dien-6-alpha-ol
47.	35.901	1985558	0.19	Carvyl tiglate, cis-
48.	36.663	2201670	0.21	Pogostol

Table 6: bioactive compounds presents in Treatment 5.

Peak#	R.Time	Area	Area%	Name
1.	6.190	1949668	0.18	Tricyclo[2.2.1.0(2, 6)]heptane, 1, 7, 7-trimethyl-
2.	6.333	1618437	0.15	Thujene <alpha->
3.	6.562	22597881	2.11	Pinene <alpha->
4.	6.929	1858290	0.17	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-
5.	7.115	38069925	3.56	Camphene
6.	7.975	49249052	4.60	Sabinene
7.	8.095	7425839	0.69	Pinene <beta->
8.	8.595	22174127	2.07	CINEOLE <DEHYDRO-1, 8-> DB5-408
9.	9.697	8176765	0.76	Terpinene <alpha->
10.	10.514	355082367	33.20	Eucalyptol
11.	11.423	14221955	1.33	Terpinene <gamma->
12.	11.999	5596009	0.52	Sabinene hydrate <cis->
13.	12.567	1903654	0.18	Terpinolene
14.	13.422	8862847	0.83	Butanoic acid, 2-methyl-, 2-methylbutyl ester
15.	13.733	56561218	5.29	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
16.	14.196	4882774	0.46	Bicyclo[3.1.1]hept-2-en-6-one, 2, 7, 7-trimethyl-
17.	14.556	2715063	0.25	Menth-2-en-1-ol <cis-, para->
18.	15.539	257875964	24.11	Camphor
19.	15.700	1624416	0.15	Camphene hydrate
20.	15.857	1461373	0.14	BICYCLO[3.1.0]HEXAN-2-ONE, 5-(1-METHYLETHYL
21.	16.072	39787817	3.72	1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-
22.	16.253	4882144	0.46	Chrysanthenol <cis->
23.	16.526	13481349	1.26	Cyclohexanemethanol, .alpha., .alpha.-dimethyl-4-methylen
24.	16.932	38142022	3.57	3-CYCLOHEXEN-1-OL, 4-METHYL-1-(1-METHYLET
25.	17.627	22567292	2.11	(+)-ALPHA-TERPINEOL (P-MENTH-1-EN-8-OL)
26.	18.393	1711961	0.16	TRANS-3(10)-CAREN-2-OL
27.	18.825	2585098	0.24	trans-Carveol
28.	19.168	1967076	0.18	Butanoate <2-methyl-, (3Z)-hexenyl->
29.	21.573	6201472	0.58	Lavandulyl acetate
30.	25.256	1645138	0.15	Copaene <alpha->
31.	25.907	1329696	0.12	PENTANOIC ACID, PHENYLMETHYL ESTER
32.	27.049	13615900	1.27	Caryophyllene <(E)->
33.	28.513	930198	0.09	Humulene <alpha->
34.	28.688	18045668	1.69	Farnesene <(E)-, beta->
35.	29.621	20447354	1.91	1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-
36.	29.900	2559233	0.24	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-meth
37.	30.179	3070044	0.29	Bicyclogermacrene
38.	31.158	882347	0.08	Alloaromadendrene
39.	33.158	1329356	0.12	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-
40.	33.428	1695535	0.16	Spathulenol
41.	33.530	1376430	0.13	Caryophyllene oxide
42.	33.891	1522311	0.14	PROPANEDINITRILE, DICYCLOHEXYL-
43.	35.067	1727384	0.16	(3R, 3aS, 6S, 7R)-3, 6, 8, 8-Tetramethyloctahydro-1H-3a, 7-me
44.	35.275	480109	0.04	Cedroxyde
45.	35.902	763389	0.07	Propanedinitrile, dicyclohexyl-
46.	36.660	1814983	0.17	Pogostol
47.	37.541	1039792	0.10	(1R, 7S, E)-7-Isopropyl-4, 10-dimethylenecyclodec-5-enol

Table 7: Bioactive compounds present in Treatment 6.

Peak#	R.Time	Area	Area%	Name
1.	6.186	2467259	0.22	Tricyclo[2.2.1.0(2, 6)]heptane, 1, 7, 7-trimethyl-
2.	6.330	1444234	0.13	Thujene <alpha->
3.	6.558	22710591	2.06	Pinene <alpha->
4.	6.926	1897180	0.17	2, 4(10)-THUJADIEN
5.	7.118	44447644	4.03	Camphene
6.	7.963	41915256	3.80	Sabinene
7.	8.088	5933049	0.54	Pinene <beta->
8.	8.632	29577133	2.68	Myrcene
9.	9.683	8006003	0.73	Terpinene <alpha->
10.	10.488	328987810	29.80	Eucalyptol
11.	11.412	12159971	1.10	Terpinene <gamma->
12.	11.993	7032574	0.64	4-THUJANOL, STEREOISOMER
13.	12.564	2368843	0.21	Terpinolene
14.	13.419	7119650	0.64	1-Heptanol, 2-propyl-
15.	13.769	61880985	5.60	(3E, 5E)-2, 6-Dimethylocta-3, 5, 7-trien-2-ol
16.	14.235	7125913	0.65	Sabina ketone<dehydro->
17.	14.570	4091193	0.37	Mentha-2, 8-dien-1-ol <trans-, p->
18.	15.564	287026893	26.00	Camphor
19.	15.722	2630527	0.24	Cyclopentanol, 1, 2-dimethyl-3-(1-methylethenyl)-, [1R-(1.
20.	15.859	2341224	0.21	BICYCLO[3.1.0]HEXAN-2-ONE, 5-(1-METHYLETHYL
21.	16.067	44276547	4.01	1, 5, 7-Octatrien-3-ol, 2, 6-dimethyl-
22.	16.247	3220097	0.29	Chrysanthenol <cis->
23.	16.519	8365141	0.76	Cyclohexanemethanol, .alpha., .alpha.-dimethyl-4-methylen
24.	16.919	28271717	2.56	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
25.	17.467	662538	0.06	Bicyclo[3.1.1]hept-2-ene-2-carboxaldehyde, 6, 6-dimethyl-
26.	17.616	17891636	1.62	.alpha.-Terpineol
27.	18.817	2839861	0.26	Carveol <trans->
28.	19.166	3047375	0.28	Butanoate <2-methyl-, (3Z)-hexenyl->
29.	19.406	1496334	0.14	Butanoic acid, 2-methyl-, hexyl ester
30.	21.568	5523432	0.50	Lavandulyl acetate
31.	25.250	2231324	0.20	Copaene <alpha->
32.	25.894	2336957	0.21	PENTANOIC ACID, PHENYLMETHYL ESTER
33.	27.059	20901127	1.89	Caryophyllene <(E)->
34.	28.492	1161464	0.11	Humulene <alpha->
35.	28.696	25300517	2.29	Farnesene <(E)-, beta->
36.	29.631	27839259	2.52	1, 6-CYCLODECADIENE, 1-METHYL-5-METHYLENE-
37.	29.898	2669471	0.24	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-meth
38.	30.176	5224643	0.47	Bicyclogermacrene
39.	31.151	1093600	0.10	Isogermacrene D
40.	33.153	886365	0.08	Naphthalene, 1, 2, 3, 4, 4a, 5, 6, 8a-octahydro-4a, 8-dimethyl-2-
41.	33.422	2073445	0.19	Spathulenol
42.	33.528	2312666	0.21	Caryophyllene oxide
43.	33.692	1323822	0.12	Acetoxyelemol<8-alpha->
44.	33.887	1436605	0.13	Propanedinitrile, dicyclohexyl-
45.	35.071	2528001	0.23	(3R, 3aS, 6S, 7R)-3, 6, 8, 8-Tetramethyloctahydro-1H-3a, 7-met
46.	35.267	1187914	0.11	Cedroxyde
47.	35.528	1601209	0.15	Selina-3, 11-dien-6-alpha-ol
48.	35.897	1501832	0.14	Carvyl angelate, cis-
49.	36.645	1732949	0.16	Intermedeol
50.	37.507	2002459	0.18	(1R, 7S, E)-7-Isopropyl-4, 10-dimethylenecyclodec-5-enol

1-8 cineole was found to be the major bioactive compound in the oil of different treatments except in T1 (control). In T1 main bioactive compound is camphor. *A. annua* plants growing in India, camphor (10.5-44.4%) was found to be the dominant bioactive compounds of essential oil (Woerdenbag *et al.* 1994; Juteau *et al*., 2002; Padalia *et al.* 2011; Verma *et al.* 2011; Rana *et al*., 2013). Artemisia ketone absent in all six treatments.In this analysis chemical nature of compounds are monoterpene hydrocarbon, oxygenated monoterpene, monoterpene alcohol, sesqui-terpene hydrocarbon, oxygenated sesqui-terpene and oxygenated di-terpene.Various biochemical compounds in *A. annua* essential oil have been identified.

Artemisia ketone (52.9%), 1.8- cineole (8.4%) and camphor (6.0%) were the major components *A. annua* compounds of the essential oil (Jain *et al*., 2002). Soylu *et al.,* (2005) recorded that chemical profile of *A. annua* essential oil was rich in camphor (31.7%), Eucalyptol (10.1%), caryophyllene oxide (7.1%), α-copaene (3.4%) and camphene (3.3%). Tzenkova *et al.,* (2010) reported that *A. annua* growing wild in Bulgaria, the main bioactive component of essential oil was found to be α-caryophillene (24.73%), α- cuvebene (13.53%), Artemisia ketone (8.45%), α-selinene (8.21%), α-copaene (7.24%), and camphor (3.61%). Significant quantitative variations in the content percentage of different bioactive constituents in essential oils from *A. annua* have been verified. The deviation between chemical profile of the essential oil is commonly influenced by the harvesting period, the pH of soils, fertilizer, and the geographic location, subspecies or

chemotype, choice of plant part or extraction method, and the choice and stage of drying conditions (Bilia *et al.,* 2014).

CONCLUSION

In conclusions our results showed that the different treatment (organic manures) also affect the content (%) and chemical profile of essential oil (%) obtained from *A. annua* cv. CIM Arogya. The essential oil content was low in T1 (0.64%), but significantly increasing in the other treatment (0.71%, 0.68%, 0.72%, 1.4% and 0.98 % in T2, T3, T4, T5 and T6 respectively). Major bioactive constituients 1.8-cineole (Eucalyptol), camphor and (3E, 5E)-2, 6- Dimethylcota-3, 5, 7 trien-2-ol) amounts were diverse in different treatments. On the basis of chemical profiles and odors of the oils, it is suggested that the cultivars *A. annua* L. cv. CIM Arogya of may be of importance for differential use in pharmaceuticals, aromatherapy, perfumery and cosmetics.

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Conflict of Interest. None.

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