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ALLELOPATHIC EFFECT OF CARAWAY ESSENTIAL OIL AND ITS ALCOHOL EXTRACT ON THE GROWTH OF THE FIELD DODDER GROWTH ON THE LUCERNE

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Abstract

Pot trial was carried out at the plastic house of the Center for Desert Studies in the summer season of 2018 to test the allelopathic effect of the essential oil and the alcohol extract of caraway fruits on dodder growth lucerne. Trial consisted of two factors: extract type, represented by essential oil, extract of alcohol and the second concentration factor, with 0, 10% and 15% of the essential oil and the extract alcohol, the factors were completely distributed within with three replicates (CRD). Results showed the superiority of the alcohol extract in the rate of killing the dodder after 3, 6 and 9 after of spraying (36.12, 53.89 and 66.02%) and the re-growth lucerne after 9 and 16 after of spraying (12.00 and 16.1%) and the number of branches after 33 after of spraying (4.67 plant^{-1} branch) and number of leaves after 33 days of spraying (8.89 leaf^{-1}) and plant height (8.00 cm), sequentially However, the 10% concentration increased the number of leaves and plant height after 33 after of spraying (8.33 leaf plant^{-1} and 8.17 cm), respectively. GC / MS analysis showed that there were 6 compounds in the essential oil and d-limonene and d-carvone were present (45.00 and 28.22%). and 21 in the non-fatty layer isolated from the alcohol extract. 9,12-octadecadienoic acid, 1,4-benzenedi carboxylic acid and octadecanoic ethyl ester were highest ratio of 29.73% and 21.55% 12.44%, respectively. While the number of compounds separated in the fatty layer isolated from the alcohol extract was 42 compounds, which contained compound 9, 12-octadecadienoic acid (z, z) and nonacosane by 47.05 and 9.75%, respectively.

Keywords : Carway essential oil and alcoholic extract

Introduction

Field dodder *Cuscuta campestris* L. is a flowering parasitic plants feeding on other plants. It belongs to the family Cuscutaceae (Jorda *et al*., 2001). Field dodder relies on host plants to take food and water, causing direct damage while indirect damage causes transfer viruses (Habib, 1984). Which cause various economic losses that may lead to total loss of yield (Press and Phoenix, 2005 and Ali 2010). The difficulty of controlling field dodder is its rapid growth, and keep its seeds alive for long periods of time and resistance to harsh environmental conditions and production of large quantities of seeds (Nickrent and Musselman, 2004), It has been shown in previous studies until the present, there is no universally acceptable, effective, selective, practically and economically selective method for controlling that parasitic plant for many reasons, including the close correlation bond between parasite and host plant, extensive range of parasites and parasite production has a huge number of seeds, One plant of them produces more than 1600 seeds which can survive in the soil for decades (Sandler, 2010). There is an urgent need to discover and use products found in nature to kill pests that harm agriculture and public health (Duke and Rimando *et al*., 2002). Plant extracts were used against weeds are safe because they do not leave harmful and toxic aspects of the environment. Alternatives to manufactured compounds Allelochmicals, secondary metabolisms, metabolized during the process of metabolism of carbohydrates, fats and amino acids and turn into the pathway of acid or acid Shikimic, and the compounds are allelopathic secondary metabolites built within the plant during the period of growth and include compounds phenols, flavonoids and tannins alkaloids, turpines, resins, cyanides, sulphides, mustard oils, amino acids, multiple peptides and others (Einhllig, 1996; Duke *et al*., 2000). Allelopathic compounds are released from plants by leeching or decomposition of fallen plant parts on the ground, root exudation or volatile substances from aromatic plants. These volatile oils are one of the main components of a number of higher plants that could be obtained by steam distillation, Aromatic and volatile essential oils help to attract pollinators and nutritional interactions, it can also test the toxic effect against plants of these compounds as pesticides (Shreeya *et al*., 2006).

Caraway is one of the plant species whose seeds contain 3-7% volatile oil, the main composites Carvone 50-60% and Limonene 40%. These compounds make up 95% of the Caraway oil (Spaldone, 1986; Kallio *et al*., 1994; Wichtl, 1994 and Sedlakove *et al*., 2003 and Almehemdi *et al*., 2011). These compounds possess pesticide properties both against insects Zubaidi *et al* (2013) found that the fruit extracts of caraway were effective in reducing the life of the potato tuber moth due to its active components, especially carvone and limonene compounds, or against other plant species. In another study, Silva *et al*., (2007) found that the treatment of potato tubers with B-cyclodextrin and solid carvone compounds inhibited or prevented potatoes from being grown. Sanly *et al*. (2010) found that the seeds of the crushed coriander inhibited the tubing of the tubers for 105 days. The growth in potato tubers was more effective at the 15^{o}C temperature. Thus, this trial was carried out with the aim of identifying the allelopathic properties of the alcoholic extract of the caraway fruits and their spread oil in inhibiting the growth of field dodder growing on the Lucerne.

Materials and Methods

The seeds of lucerne crop were planted locally in pots, 25 cm in the plastic house of the Center for Desert Studies, Anbar University in mid-May 2018, in a soil composed of 50%: 25%: 25% (sand:clay:silt). Twenty-five seeds from lucerne plant were planted for each sandain, the Hamoul seeds collected and diagnosed at the Center for Desert Studies / Anbar University by Dr. Ali Almehemdi. Five seeds were laid for each pots.

Collection and Configuration of Samples

Caraway fruits were collected from the field. and then cleaned the samples of dust and impairs then dried fruits in the shade in lab at the room temperature and after the full drying, it is ground by an electric mill of the type of El-Arabi for the preparation of extracts.

Preparation of Extract

Essential oil Extraction

The process of extracting oil from the fruits was applied according to the method of Clevenger (Almehemdi, 2011). Extract the essential oil from the grind drying matter to the fruit, with the use of a Clevenger device connected with a flask to a 1 liter, the weight of the 100 g of dried fruits is crushed with an electric grinder and then placed in a special flask with the addition of 500 ml of distilled water. Distillation process was carried out by continuously for two and a half hours for each sample until the amount of essential oil was extracted from the sample. water and oil layers were formed. These layers were separated by the separation via bath section in the oil collection tube. Bottom layer represent the water and the upper is essential oil because it is lighter than water. After separating the oily layer, each oil sample was placed in sealed, bottles. The amount of oil per transaction was weighed by a sensitive balance (1260MP-Sartorius), and the bottles were kept at a temperature of 4^{o} until use. distillation process was repeated several times until a suitable amount of essential oil was obtained

Chemical extraction

Weight 120 g of crushed dried fruits. Add 300 ml of ethanol alcohol, 200 ml of chloroform alcohol and 100 ml of distilled water for 15 minutes and mixing it in thermal magnetic stirrer for 48 temperature of 45-50^{o} C intermittently and then put the solution in the centrifuge at 3000 cycles, 1 minute for 30 minutes, the filter was neglected and the filtrate was repeated three times to ensure the disposal of the sediment and was nominated using the filter paper Whatman No.1 and dry the leachate using water bath at 60°C and put the extract in sterile bottles and stored in the refrigerator for at 5 °C until use. This process was repeated to the extent required.

Analysis of essential oil components

GC / MS method

The essential oil components were analyzed in the labs of the Ibn Al-Bitar Research Center of the Industrial Research and Development Authority of the Ministry of Industry and Minerals using a gas chromatography / mass spectroscopy device, 5 microliters of methanolic extract were injected with a fine silicone rubber syringe that was quickly healed after the injection. The temperature of the thermal source of the device reaches 350 °C while the sample is evaporated at 150 °C. And the Helium gas, an inert gas, is used to carry the material to represent the mobile phase to push the material to the column, which varies from one device to another. In this study , the length of the column was contains different materials at the Stationary phase, material with a lower molecular weight than the column is driven first, followed by materials with a larger molecular weight. And then move all materials to the detector after bombardment with a fixed voltage of 70, V to diagnose the compounds to the elements, leaving an electronic signal to detect the compound, the greater the concentration of the compound and the greater the signal. Then its molecular weight is calculated by the mass / charge ratio (Z / M where Z is fixed) (70, V) obtained from the graph. Then draw the chart with the calculator connected to device the GC-MS, which is called the scheme of inhalation where the y axis represented the signal intensity to determine the element in the injection sample and the x-axis represented retention time. The identification of the compounds was compared with the compounds stored in the computer library bound with GC-MS device.

Trial Application

Trial included two factors: extract type, extracting essential oil by distillation method, and second preparation of alcohol extract. Use two concentrations of essential oil and alcohol extract, 10and 15%. Sodium dodecyl sulphate (2%) was added to all treatments to break surface tension, including comparison. Treatments were sprayed to the point of complete wetness with concentrations prepared from the extract and oil. Data were taken every three days, starting on the day of spraying until the trial was over. Trial was designed according to the design of Completely Randomized Design (CRD) with three replicates for each treatment. (0-100% kill scale), re-growth of lucerne plant (0 without 100% growth), lengths and number of new branches and number of new leaves as well as diagnosis of active substances.

Statistical Analysis

Data were subjected to statistical analysis using the Genstat statistical analysis program. The averages were compared by applying a two-way variance analysis using the least significant difference of LSD ($P > 0.05$)

Results and Discussion

Dodder Controlling (3d after Spraying)

Table (1) shows the effect of the essential oil and the alcoholic extract of the seeds of caraway plant and its concentrations in the rate of killing of field dodder after three days of spraying. There were significant differences between the essential oil and the alcohol extract in the ratio of controlling of field dodder 3 d after spray. (36.12%), while the essential oil (22.23%) and the concentration (15%) gave the highest kill (50.00%) followed by the concentration of 10% (37.50). However, the 0% concentration gave the lowest killing.

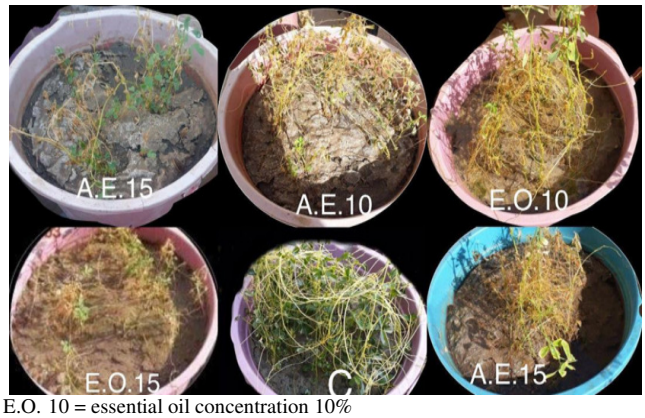
Significant differences were also observed between alcohol extract methods, as the combination of the interaction of alcohol extract X15% and the Killing extracts showed the highest kill reached (70.00%) compared to the comparison combination which gave the lowest of killing (0.04%), followed by the interaction of alcohol extract X 10% (38.33%), (15.10%), followed by the mixture of essential oil X15% (30.00). The superiority of the alcohol extract may be attributed to the presence of high concentration oxygen compounds such as Carvone and Limonene. (Synowiec *et al*., 2016) that the essential oil of caraway was very effective in inhibition weed namely Amaranth and yellow peroxide because it contains a compound Oxygenation by 64.1 93.3% and that the addition of fatty acid methyl ester may increase the eternal properties of the compounds in the essential oil and the alcoholic extract (Synowiec *et al*., 2017) and that the high concentration of these extracts from the essential oil may increase the eternal properties of weed.

Field Dodder Controlling (9d after spraying)

Table (2) shows the effect of the essential oil and the alcoholic extract of the fruits of caraway plant and its concentrations on the dodder controlling after nine days of spraying. Table shows significant differences between the two types of extract and the levels of extract and the interaction between them. Alcohol extract gave the highest average of controlling of 66.02% essential oil gave a controlling of 57.78%. Table also showed a significant increase as 15% of matter was sprayed on dodder plant. Highest mortality rate was 96.33% followed by 10%

concentration of 89.33% compared to the comparison treatment.

There was also a significant superiority of interaction combinations among the extraction methods and concentration. interaction of the alcohol extract X15% was achieved highest controlling rate reached 99.33% compared to control, followed by interaction of alcohol extract X10% of 98.67%. interaction of essential oil X15% gave 93.33 In comparison to the concentration treatment, which gave the lowest controlling of 00.00, followed by interaction of the essential oil X10% 80.00, the inhibitory effect of the alcohol extract of the caraway fruit or the essential oil may be duty to alcohol extract or the essential oil be curtained phenolic and hydrocarbon compounds such as carvone and limonene, which prevent the growth of parts as in potatoes whish were prevented to be sprouting potatoes (Sanly *et al*., 2010). It may interfere with some of the enzymes (Thippcowam *et al*., 2013) and may inhibit the growth of the root and the feather (Marichali *et al*., 2011) and may interfere with ATP and ADP energy levels and reduce their levels (Stolarska and Wieczorak, 2015).



E.O. 10 = essential oil concentration 10%
E.O.15 = essential oil concentration 15%
A.E.10 = Alcoholic extract Concentrated 10%
A.E.15 = Alcoholic extract Concentrated 15%

Fig. 1 : Effect of the essential oil and alcohol extract in the growth of the field dodder on lucerne

Re-growth of Lucerne (9 d after spray)

Table (3) shows the effect of the extraction type and the concentrations of the extract and the interaction between them in the growth of the lucerne plant after nine days of spraying. Table showed significant differences between the levels of the factors. The alcohol extract of the fruits gave highest percentage of re-growth of 12.00% while essential oil gave 0.67%. Table also showed that greater concentration 15% increase re-growth of the Lucerne plant which gave 12.5%. Followed by 10% concentration of 6.50 in relative to the control treatment.

It was also shown from table that there were significant differences between interaction combinations. combined interaction of the alcohol extract X15% gave highest re-growth of 25.00%. However, the interaction of the alcohol extract X10% and X10% gave 11.00.

Re growth of the Lucerne (16 d after spraying)

Table (4) indicates the effect of the type of extract and the concentrations of the extract and the interaction between them on percentage of re-growth of the lucerne plant after sixteen days of spraying, it is clear from the table there are significant differences between the levels of factors gave the extract of alcohol caused highest re growth of 16.1% compared to the essential oil which gave 0.7%. Table also showed concentration 15% increase ratio of re growth, which gave the highest growth of 15.8% followed by 10% concentration, which gave a 9.3% re growth compared to the control.

Results showed that there were significant differences between the interaction combinations. Combined interaction of alcohol extract X15% achieved highest re growth average of 31.7%, followed by alcohol extract X10% 16.7%.

Lucerne Re Branches (33 d after spray)

Table showed that there were significant differences between the two types of extract and the levels of the extract concentrations and the interaction between them. Alcoholic extract gave the highest number of new branches with 4.67% branch per plant while the essential oil gave the lowest average number of new branches of 0.45%.

It is noted from the same table that the concentration 15% produced highest number of new branches of 5.00 branches per plant, while control treatment was 0.00. additionally, Table shows the superiority of interaction of alcohol extract X15%, gave with the biggest number of branches of 10.00 branches per plant, but control gave the lowest number of branches.

Lucerne Re Leaves (33 d after spray)

Table (6) shows the number of new leaves of lucerne plant after thirty three days of spray with the extract of alcohol and essential oil. Table shows significant differences between the two types of extract and the levels of the extract concentrations and the interaction between them. Alcoholic extract achieved highest number of re- leaves 8.89 leaves per plant, while the essential oil gave the lowest average number of new leaves of 1.67. leaves plants it also noticed that15% increase number of new leaves, which reached 7.50 leaves plant^{-1} in comparison to the lowest ratio of control.

Table also showed the superiority of interaction of alcohol extract X15% as it gave the largest number of new leaves amounted to 15.00 leaves plant^{-1} followed by a combination of alcohol extract x10% gave 11.67 leaves of plant, but the control gave the lowest number of leaves amounted to 0.0 leaves plant $^{-1.}$

Lengths of Re branches (33 d after spray)

Table 7 indicates the effect of the extraction type and the concentration of the extract and interaction between them on length of the new branches after thirty three days of spraying. The table showed significant differences between the levels of the factors. The alcohol extract achieved the highest ratio of the longest Lucerne plant at 8.00cm, while essential gave lower average length of branches, which amounted to 1.78cm, also noted from table the superiority of the concentration of 10% on length of new branches of 8.17cm, followed by the concentration of 15% 6.50 cm, compared to the control which gave 0.00.

Table also showed the superiority of interaction of alcohol extract X15% , which resulted in highest length of the branches of the Lucerne plant with a weight of 13.00cm, followed by its combined with 10%, which gave 11.00cm, compared to control.

GC / MS analysis

Analysis of essential oil components

Table 9 indicates that GC\MS technique separate of 6 compounds from the components of the essential oil represented by D-limonene compounds by 45.00%, followed by d-carvone with 28.22%, caryophylene oxide by 12.76% and caryophylene by 8.43% and 1, z, 5, E-7-Dodecatriene by 3.66%. While the compound 3-Buten-2-one gave the lowest level of 1.93%.

Analysis of fatty layer components:

It is noticed from the Table (11) that there are 42 compound separated by GC/MS in the fatty layer isolated from the alcoholic extract, the highest concentration of 9,12-Octadecadienoic acid (z, z) by %47.05 followed by the compound Nonacosane (9.75%) and n-hexadecanoic acid (8.67), 9-Octadecenoic acid (z), 2,3-dihy (2.73%) and Eicosan (10-heptyl-10-octyl), by 2.19%. Carvone (-1.34%), while Benzo (h} quinolone, 2,4-dimethyl, gave the lowest percentage of 0.33.

Table 1 : killing of field dodder plant 3d after spray with alcohol extract and essential oil.

Concentration / Extraction method	**0**	**10**	**15**	**Average**	**L.S.D. 0.05**
Distillation (essential oil)	0.00	36.67	30.00	22.23	2.877
Alcoholic extract	0.00	38.33	70.77	36.12	
L.S.D to overlap (0.05)	4.982				
Average	0.00	37.50	50.00		
L.S.D (0.05)	3.523				

Table 2 : Controlling of field dodder plant after 9d of spray with alcohol extract and essential oil.

Concentration / Extraction Method	**0**	**10**	**15**	**Average**	**L.S.D 0.05**
Distillation (essential oil)	0.00	80.00	93.33	57.78	1.456
Alcoholic extract	0.00	98.67	99.33	66.02	
L.S.D to overlap (0.05)	2.522				
Average	0.00	89.33	96.33		
L.S.D (0.05)	1.784				

Table 3 : Re-growth of Lucerne plant after 9 days of spray with alcohol extract and essential oil.

Concentration / Extraction Method	**0**	**10**	**15**	**Average**	**L.S.D 0.05**
Distillation (essential oil)	00.0	2.00	0.00	0.67	2.293
Alcoholic extract	00.0	11.00	25.00	12.00	
L.S.D to overlap (0.05)	3.972				
Average	0.00	6.50	12.50		
L.S.D (0.05)	2.809				

Table 4 : Re growth of lucerne plant after sixteen days of spraying with alcohol extract and essential oil

Concentration / Extraction Method	**0**	**10**	**15**	**Average**	**L.S.D 0.05**
Distillation (essential oil)	0.00	2.0	0.00	0.7	4.50
Alcoholic extract	0.00	16.7	31.7	16.1	
L.S.D to overlap (0.05)	7.80				
Average	0.00	9.3	15.8		
L.S.D (0.05)	5.52				

Table 5 : Effect of spraying with extract and essential oil in the number of new branches (33 d after spray)

Concentration / Extraction Method	**0**	**10**	**15**	**Average**	**L.S.D 0.05**
Distillation (essential oil)	0.00	1.33	0.01	0.45	1.896
Alcoholic extract	0.00	4.00	10.00	4.67	
L.S.D to overlap (0.05)	3.284				
Average	0.00	2.67	5.00		
L.S.D (0.05)	2.322				

Table 6 : Number of lucerne Re leaves after thirty-three days of spraying with alcohol extract and essential oil

Concentration / Extraction Method	0	10	15	Average	L.S.D 0.05
Distillation (essential oil)	0.00	5.00	0.00	1.67	2.025
Alcoholic extract	0.00	11.67	15.00	8.89	
L.S.D to overlap (0.05)	3.508				
Average	0.00	8.33	7.50		
L.S.D (0.05)	2.481				

Table 7 : The length of Re branches of lucerne 33 d after spray with alcohol extract and essential oil

Concentration / Extraction Method	0	10	15	Average	L.S.D 0.05
Distillation (essential oil)	0.00	5.33	0.00	1.78	0.709
Alcoholic extract	0.00	11.00	13.00	8.00	
L.S.D to overlap (0.05)	1.228				
Average	0.00	8.17	6.50		
L.S.D (0.05)	0.868				

Table 9 : Secondary compounds separated from essential oil by GC/MS

S. No.	Phytochemical compounds	RT	Area %	Qual
1	D-Limonene	4.260	45.00	99
2	1,z,5,E-7-Dodecatriene	4.901	3.66	83
3	Caryophy11ene	5.993	8.43	99
4	D-Carvone	7.161	28.22	96
5	Caryophy11ene oxide	10.296	12.76	86
6	3-Buten-2-one, 4- (2,5,5-trimethyl-3-oxatricyclo{5.1.0.0 (2,4) } oct-4-y1)-	10.507	1.93	53

Table 10 : Secondary compounds separated from the non- fatty layer isolated from the alcoholic extract

S. No.	Phytochemical compounds	Relation time	Area %	Qual
1	Trichloromethane	4.328	2.30	80
2	D-Carvone	7.794	2.41	86
3	D-Carvone	7.892	0.13	90
4	Phensuximide	13.717	1.60	38
5	Hexadecanoic acid ethyle ester	15.714	6.64	93
6	9, 12-octadecadienoic acid (z, z)-	17.334	29.73	95
7	Octadecanoic acid, ethyle ester	17.545	12.44	93
8	γ-sitoterol	18.653	5.25	99
9	γ-sitoterol	18.886	0.55	96
10	Oleic acid	19.293	0.40	70
11	9-Octadecenal, (z)-	19.602	1.00	90
12	β-Amyrin	19.934	2.41	95
13	Oleic acid	20.047	0.50	91
14	Acetic acid, 4,4,6a,6b,8a,11,12,14,b-octamethyl-1,2,3,4,4a,5,6,6a,6b,7,8, 8a,9,10,11,12,12a,14b,-octadeca hydropicen-3-yl ester	20.544	4.03	96
15	Benzo {h} quinolone, 2,4- dimethyle-	20.695	1.12	83
16	Benzo {h} quinolone, 2,4- dimethyle-	20.815	3.05	51
17	Benzo {h} quinolone, 2,4- dimethyle-	20.951	1.04	83
18	Octadecene, 1-{3- (octadecyloxy) propoxy}-, (z)-	21.599	0.83	25
19	1,4-Benzenedi carboxylic acid, bis2-ethylexyl) ester	22.156	21.55	94
20	Supraene	22.812	0.57	62
21	N- (5-Ox0-tetrahydro-furan-2-ylmethyl) –acetamide	23.505	2.38	25

Table 11 : The compounds separated from the fatty layer isolated from the alcohol extract by GC/MS

S.No.	Phytochemical compounds	RT	Area %	Qual
1	Trichloromethane	4.403	0.66	96
2	(-) –Carvone	7.734	1.34	52
3	Trichloromethane	8.058	0.53	86
4	2,2,6-Trimethyl-1-1 (2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one	14.162	0.06	66
5	Aristolene epoxide	14.267	0.30	53
6	Butanone, 4- (2,2,6-trimethylcycleohexyl) -	14.410	0.45	42
7	13-Tetradece-11-yn-1-ol	14.606	0.37	35
8	Isooromadendrene epoxide	14.802	0.31	92
9	n-Hexadecanoic acid	15.721	8.67	99
10	Dodecanoic acid	16.422	0.38	64
11	8-Hexadecanoic, 14-methyl-m, (z) -	16.686	0.55	83
12	11-octadecenoic acid, methyl ester	16.754	0.71	98
13	Oleic acid	16.889	0.61	45
14	9,12-Octadecadienoic acid (z, z) -	17.492	47.05	98
15	Tricosane	18.449	0.60	96
16	Oleic acid	18.758	0.54	95
17	Oleic acid	19.060	0.17	95
18	Tetracosane	19.278	0.41	95
19	Oleic acid	19.353	0.87	91
20	Phenol, 2,2’ –methylenebis {16- (1,1-dimethylethyl) -4-methyl-	19.677	0.60	94
21	Amonafide	19.813	0.39	58
22	Nonadecane	20.077	0.75	92
23	Bis (2ethylhexyl) phthalate	20.552	2.24	97
24	Dodacahydropyrido {1,2-b} isoquinoli	20.710	0.46	45
25	1H-Indol, 5-methyl-2-phenyl-	20.838	1.30	50
26	Benzo {h} quinolone, 2,4-dimethyl	20.959	1.33	83
27	Cyclopropaneundecanal, 2-nonyl-	21.320	1.39	78
28	Tricosane	21.629	2.04	98
29	9-Octadecenoic acid (z) -, 2,3-dihy	21.900	2.73	95
30	Oleic acid	22.157	0.44	60
31	Nephthalene, 1- (1-decylundecyl) decahydro	22.247	1.75	64
32	Eicosane	22.503	0.66	92
33	Squalene	22.842	1.97	99
34	Nonacosane	23.588	9.75	99
35	Hexadecanoic acid 2- (actadecyloxy) -, tetradecyl ester	23.897	0.44	59
36	3- (5-cyano-4-methoxycarbonylmethyl-4,5-dimethyl-2-oxopyrrolidine-3-yl) –propionic acid methyl ester	24.086	0.85	83
37	3-Octyne, 2,2, 7-trimethyl-	24.153	1.29	81
38	Eicosan, 10-heptyl-10-octyle-	26.241	2.19	50
39	Stigmastan-3,5-diene	26.580	0.64	90
40	Decane, 1,10-diiodo-	26.715	0.80	23
41	2-Ethoxycarbonyl-3-methyl-4-azafluorenone, 2-fluorenylimime	27.175	0.66	78
42	Stigmasterol	29.797	0.81	95

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