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Phytochemical and Insecticidal Study of Essential Oil of *Anethum sowa* L.

MAZHAR MEHDI, AGA MANSOOR, S. JAMAL HAIDER ZAIDI
Department of Chemistry, Shia P.G. College, Lucknow, U.P. (India).

Abstract

The essential oil of seeds of *Anethum sowa* L. growing in India was obtained by hydro-distillation. It was analysed by GC-MS. Components were identified and R-(-)-carvone (38.899%), apiol (30.812%), limonene (15.938%) and trans-(+)-dihydrocarvone (10.999%) were the main components. The essential oil was found to be toxic to *Periplanata americana* L., *Musca domestica* L. and *Tribolium castaneum*. The mean mortality ranged from 25 to 100% for the different insects. In conclusion, the essential oil of *A.sowa* may have the potential to be developed as a natural insecticide against various insects. The oil obtained by the hydro-distillation method from the fresh leaves and stems of Anethum sowa L. (Dill) herb was analyzed by GC-MS. Twenty compounds were isolated and identified. The major constituents were apiole (25.39%), o-cymene (15.25%), α-thujene (14.84%) β–phellandrene (7.17%), 6,6-dimethyl-2-(3-oxobutyl) bicycle (3.1.1) heptan-3-one (6.90%), exo-2-hydroxycineol (5.03%), limonene (4.13%), 3-isopropyl-4-methyl-1-pentyn-3-ol (2.89%), myristicine (2.46%) and dihydroumbellulone (2.14%).

Keywords: *Anethum sowa* L., insecticidal activity; essential oil; R-(-)-carvone; apiol

Introduction

Natural products have proved to be excellent insecticides and pesticides after a great deal of research[1]. Synthetic insecticides may initiate various problems to our environment and health. These insecticides are neurotoxic to man as well as domestic animals. This fact leads us towards the application of natural insecticides such as essential oils as good alternative to chemical insecticides. Modern research has proved that natural insecticides are safe to use against different insects and pests and do not cause any environmental damage[2].

Anethum sowa, commonly known as dill, is an annual medicinal plant with tiny yellow flowers belonging to the plant family Umbelliferae. The plant grows in Pakistan, India, Afghanistan, Middle East, Russia, Iran and Egypt[3]. Its seeds have a strong spicy odor and are therefore used as a flavouring agent in the food industry. Dill seeds are commonly used for aches in the stomach and intestines, dyspepsia, bladder inflammation, liver diseases, cramps, and insomnia[4]. The essential oil and extracts of dill seeds have shown varying degrees of antimicrobial activities[4]. The literature survey points out that several studies have been done on chemical composition of the essential oil of dill seeds[5-10]. The present study was undertaken to investigate the chemical composition and insecticidal activity of the essential oil of the seeds of *A. sowa* against *Periplaneta americana* (Linnaeus) and *Musca domestica* L., important vector of diseases, and *Tribolium castaneum*, an insect causing serious damage to stored grain products.

MATERIALS AND METHODS

Plant Material: The seeds of *Anethum sowa* were purchased from local market of Varanasi (U.P.) India of the seeds were deposited at the Herbarium, Department of Botany, Banaras Hindu University, Varanasi (U.P.) India.

Extraction of the essential oil: The seeds were ground and submitted to hydrodistilltion using Clevenger-type apparatus for 6 hours. The extracted essential oil were dried over anhydrous sodium sulphate and stored in amber-coloured bottles in a refrigerator at 4ºC until use.

Gas Chromatography/Mass Spectrometry analysis: The essential oil was analyzed on a Hewlett-Packard 6890NGC-MS system (Agilent Technologies) coupled to a HP5973 mass-spectrometer. Separations were carried out using a DB-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness). The injector and detector temperatures were maintained at 220 and 280ºC, respectively. Column temperature was initially kept at 60ºC for 2 minutes and then gradually increased to 240ºC at the rate of 5ºC min^{-1} . Helium was used as carrier gas at a constant flow of 1.0 mL min^{-1} and an injection volume of 1 μL was employed. The MS scan parameters included electron impact ionization voltage of 70 eV, a mass range of 40-750 m/z and a scan interval of 0.5 s. Samples diluted in n-hexane were injected manually in the splittless mode. The identification of the components was based on comparison of their mass spectra with those of NIST3.0 Libraries provided with the computer-controlling GC-MS system as well as from the published literature.

Insects: Adult Indian cockro aches, house flies and *Tribolium castanum* were used in the bioassays. Indian cockroaches were reared in plastic cans at 28(±2ºC), 50(±10) % relative humidity on water and dog food. Adults of *Tribolium castanum* were obtained from PCSIR laboratories, BHU, Varanasi. They were reared on rice flour at 28(±2ºC), 50(±10) % relative humidity in darkness. The Musca domestica adults originated from Tollinton market in Varanasi, U.P. province, India and were kept in plastic cages (15cm×15cm×15cm). They were reared on sugar solution and liver of chicken at 28(±2ºC) and 50(±10)% relative humidity in 12hr light-12 hr dark photo-period.

Continuos exposure bioassays: Serial dilutions of essential oils were made in acetone. These bioassays were conducted by pipetting 0.5 ml of essential oil concentration (or 0.5 ml acetone in case of control) into a 9cm diameter glass Petri plate and by allowing the acetone to evaporate in fume hood for forty five minutes. Four cockroaches were used for each experiment. Mortality was assessed after 15 minutes, 30 minutes, 60 minutes, 120 minutes, 240 minutes, 6 hours, 12 hours and 24 hours.

Fumigant toxicity bioassays: Fumigant toxicity of the essential oil was determined by sealing four adult cockroaches or ten adult flies in a 120ml jar with a 1 cm diameter cotton ball treated with 100 µl of 100% essential oil. Essential oil was injected into the centre of each ball to allow its volatilization while preventing the insects from coming in contact with the oil. Mortality was recorded at 15 minutes, 30 minutes, 1 hour, 2 hours, 3 hours, 6 hours, 12 hours and 24 hours, respectively for the cockroaches and at 15 minutes, 30 minutes, 1 hour and 2 hours, respectively for the house flies. Control jar had insects and an untreated cotton ball. In case of *T. castaneum*, fumigant toxicity of the essential oil was determined by sealing ten adults in a 120ml jar with a 1cm diameter disc of filter paper treated with 100 µl of 100% essential oil. The disc was attached to the upper side of the jar. Mortality was recorded at 1 hour, 6 hours and 24 hours, respectively. Control jar had insects and an untreated filter paper. For each insect, the experiment is repeated thrice.

Statistical analysis of Data: Every experiment was performed in triplicate. The results were recorded as means ± standard deviation. Statistical analysis was performed using one-way analysis of variance (1-way ANOVA) using SPSS statistical package (SPSS 16) and the statistical significance was determined at $P < 0.05$.

RESULT AND DISCUSSION:

The essential oil was extracted from the seeds of *Anethum sowa* L. by hydrodistillation with a yield of 1.45% and was analyzed by GC-MS as shown in figure-1.

The composition of the essential oil is given in table-1 and mainly consists of R-(-)-carvone (38.89%), apiol (30.81%), limonene (15.93%) and trans-dihydrocarvone (10.99%) whereas gamma-terpinene (0.19%), m-cymene (0.29%), S-(+)-carvone (0.06%) and myristicin (0.86%) were present as minor components.

Ashraf *et al*.,[6] analyzed the dill seed essential oil by GLC and found the oil to be rich in carvone (52.25%), dill apiole (28.28%) and limonene (9.34%). Singh *et al*.,[7] reported carvone (55.2%), camphor (11.44%), limonene (16.6%) and dill apiole (14.4%) to be the key components present in the essential oil extracted from the seeds of *A. sowa* L.

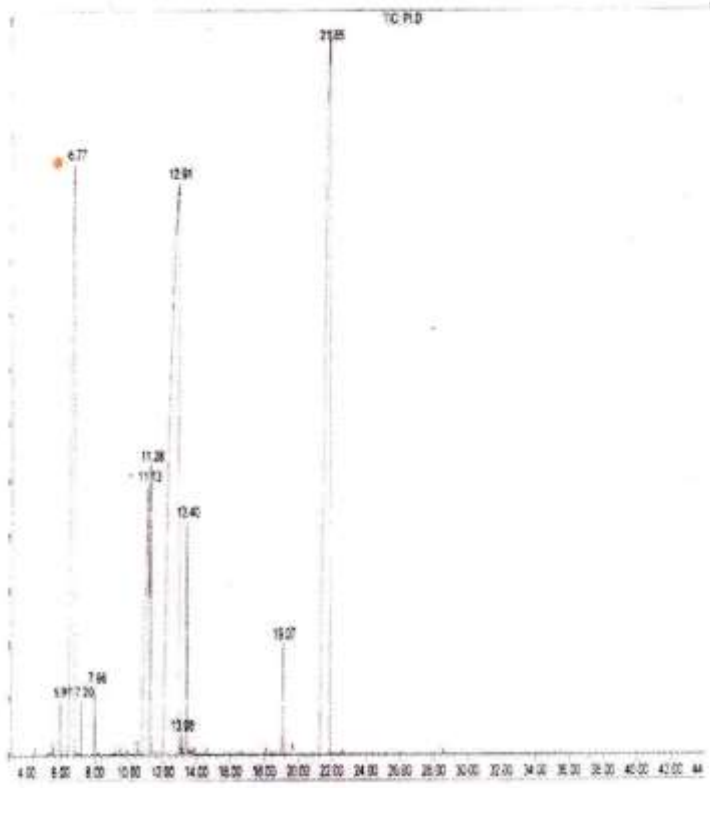

Fig.-1: GC-MS chromatogram of the essential oil of Anethum sowa L.

Dill apiole and camphor which were the key components in the previous studies were not found in the present investigation. Our results are in good agreement with Jirovetz *et al*.,[5] who reported carvone (50.1%) and limonene (44.1%) as the major constituents of the *A. sowa* essential oil from Bulgaria. The contact toxicity bioassay of the essential oil against *P. americana* resulted in mortality which ranged from 25 to 100% during the first 3 hours for the various concentrations of the essential oil.

Table-1: Composition of essential oil of seeds of *Anethum sowa*

No.	Constituent[a]	RT[b] (min)	Percentage
1	E,E-2,6-dimethyl-3,5-octatetraene	5.963	0.583
2	Limonene	6.770	15.938
3	Gamma-terpinene	7.204	0.192
4	m-cymene	7.977	0.294
5	Trans-dihydrocarvone	11.124	10.999
6	R-(-)-carvone	12.909	38.899
7	S-(+)-carvone	13.081	0.064
8	1-methoxy-4-[1-propenyl]-benzene	13.401	1.354
9	Myristicin	19.078	0.864
10	Apiol	21.853	30.812

Compounds, identified on the basis of comparison with NIST(2005) MS database spectra and Adams(1995) libraries are listed in order of elution from a HP-5MS column.

RT, retention time on a HP-5MS column in minutes.

The mortality ranged from 25 to 100%during the first 12 hours in the fumigant toxicity bioassay of the essential oil against *P. americana*. In case of the house fly, mortality ranged from 33.3 to 70% during the first 3 hours, whereas the mortality ranged from 58.3 to 100% during 24 hours for the red flour beetle.

Table-2: Contact toxicity of the essential oil of *A. sowa* to *Peripalaneta Americana* (L.)

Essential oils	Conc. (%)	Mean mortality (%)							
		15 min	30 min	60 min	2 hr	3 hr	6 hr	12 hr	24 hr
A. sowa L.	5	0	0	25	75	100	100	100	100
	10	0	25	50	100	100	100	100	100
	100	50	100	100	100	100	100	100	100
Control	0	0	0	0	0	0	0	0	0

[a]Results are mean of three experiments, four insects per set

Table-3: Fumigant toxicity of the essential oil of *A. sowa* to various insects

Time	Mean mortality (%) of			
	P. americana	M. domestica	T. castaneum	Control
15 min	0	33.3	-	0
30 min	0	53.3	-	0
60 min	0	63.3	58.3	0
2 hrs	0	70.0	-	0

3 hrs	25	-	-	0
6 hrs	50	-	83.3	0
12 hrs	100	-	-	0
24 hrs	100	-	100	0

[a]Results are mean of three experiments,

Anethum sowa essential oil has shown good insecticidal activity due to appericiable concentration of carvone, limonene and trans-dihydrocarvone in its composition as shown in the table-1. Carvone was observed to be more toxic than bicyclic compounds such as verbinol and thujone[10]. Lee *et al.*,[11] reported the insecticidal activity of carvone against the house fly. Previously, Jang *et al.*,[12] studied the fumigant toxicity of carvone to the German cockroach with an LC_{50} value of 0.25 mg/cm^3. Carvone and dihydrocarvone were found to be toxic to larvae and adults of *T. castanum, S. oryzae* L. and *R. dominica* F by Tripathi *et al.*,[13]. Previously, the insecticidal activity of *A. sowa* essential oil against T.castanum with an EC_{50} value of 7.86μL was reported by Chaubel *et al.*,[14,15]. Limonene and sesquiterpenes posses weak to moderate toxicity to various insects[16,17].

CONCLUSION

Although, the insecticidal activity of essential oil is much less than that of synthetic insecticides such as dichlorvos, propuxor and permethrin, they are quite harmless. They are not toxic to mammals. Their volatility as well as their non-toxicity makes them an ideal candidate to be used in resteraunts, hospitals and schools against various insects. Most of the essential oils have pleasant smell and can be used to protect food from stored product insects such as *T. castanum* and maize weevil. There is a need to assess various essential oils for insecticidal activity against insects that cause diseases and damage food products.

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