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ANALYSIS AND ANTIVIRAL ACTIVITY OF ESSENTIAL OIL OF EUCALYPTUS GOMPHOCEPHALA.DC.

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ABSTRACT

The essential oil of the leaves of *Eucalyptus gomphocephala DC. (Myrtaceae)* was isolated and subjected to GC/MS analysis. Its constituents were identified by comparison of their retention times and mass spectra with literature records. Citronellal was found to be the dominant component representing about 81% of the oil. The antiviral activity of the oil was studied against two important plant viruses namely, cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) using the local lesions assay. The oil was found to have a significant inhibition of the infectivity of both viruses in concentrations as little as 100 ppm.

INTRODUCTION

Certain plant viruses are known to infect many plant crops, vegetables, fruits and also some trees in most cultivated areas around the world including Egypt. Among these important viruses are the cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV).They are reported to infect about 191 and 199 plant species respectively (Francki *et al.*, 1979) and (Zaitlin *et al.*, 1975), which may cause reduction in the yield of many food crops. The use of synthetic chemicals for the management of plant pathogens has no doubt increased crop production but with some deterioration of environmental quality and human health (Culter and Culter, 1999). Because of these problems, the seek for biologically active natural products as alternatives, has been the target of many scientists allover the world. Such products can be ideal candidates as agrochemicals being bioactive, economic and safe to the user and environment.(Macias *et al.*, 1997). Among these natural products are essential oils of a number of plants which have been reported to show activity against a wide array of plant pathogens (Rice, 1995). Oil of Eucalyptus has been traditionally used in medicine as antiseptic and in respiratory tract infections (Trease and Evans, 1983).Recent studies have been found dealing with the antifungal activity of oil of *Eucalyptus citriodora* (Ramazani *et al.*, 2002) and the antimicrobial activity of oil of *Eucalyptus globulous* (Trividi *et al.*, 2004). No reports were found dealing with biological activity and the constituents of the essential oil of *Eucalyptus gomphocepha DC.* This prompted us to carry out the analysis and test the antiviral activity of this oil against CMV and TMV viruses which infect many important crops.

MATERIALS AND METHODS

The plant material: Fresh leaves were collected from trees growing near Nakita (Mansoura district) on April 2004, and was identified by Dr. Ali Abdul-Aati Hammuda, Dept. of Flora Research in the Horticulture Research Institute in Cairo, and Mrs. Treeza Labib the agriculture engineer in Orman Garden in Giza.

The essential oil: The essential oil was prepared by hydro-distillation of 150 g. of fresh leaves of *Eucalyptus gomphocephalaDC.* using Clevenger type apparatus for oils lighter than water. The leaves yielded 1.6 % (v/w) of faint yellow oil, having a lemon odor. The oil is readily soluble in alcohol, with specific gravity of 0.71. The oil was subjected to GC/MS analysis and its components were identified by matching their relative retention times (taking cineole peak as the reference) in conjunction with a small set of discriminating MS ions against a computer library file of large number of data obtained under identical experimental conditions (Adams, 1995). The results are recorded in Table (I).

Table (I): Constituents of the Essential Oil of *Eucalyptus gomphocephala.*

No.	Compound	Rel. Ret. Time*	Parent Peak	Base peak	Major peaks	Conc. (%)
1	Isocitronellene	0.535	138	41	55, 83, 67, 111, 109.	1.337
2	A-Pinene	0.711	136	41	93, 91, 79, 105.	0.481
3	β-Pinene	0.839	136	41	93, 69, 77, 53.	2.260
4	Myrecene	0.906	136	41	93, 69, 51, 67.	0.136
5	Cineole	1.000	154	43	81, 55, 111, 67.	2.600
6	Unidentified	1.103	n.d.**	41	53, 69, 82, 91, 136.	0.267
7	Rose oxide (Cis)	1.250	154	41	139, 69, 55, 83.	0.254
8	Myrecenol	1.269	154	41	59, 79, 67, 93.	0.267
9	Rose oxide (Trans)	1.288	154	41	139, 55, 69, 99	0.077
10	Citronellal	1.535	154	41	69, 95, 55, 121, 111.	81.036
11	Citronellol	1.642	156	41	69, 81, 55, 95, 123.	7.311
12	Unidentified	1.686	n.d.**	41	59, 67, 85, 109, 156.	0.301
13	Citronellyl acetate	1.852	198	43	67, 81, 95, 55, 123, 138.	1.651
14	Unidentified	1.954	n.d.**	41	69, 95, 123, 184, 109.	0.072
15	B-Caryophy llene	2.003	204	41	91, 79, 56, 69, 105, 133, 147, 189.	0.987
16	Unidentified	2.380	n.d.**	41	57, 77, 91, 121, 169, 226.	0.230

* Relative retention time using Cineole as reference compound. ** *n.d.= not detected.*

GC/MS Analysis: was carried out on Finningan Mat SSQ7000 mass spectrometer directly coupled to a Varian 3400 gas chromatograph equipped with DB-5 (0.25mm i.d.x 30m, 0.25 coating thickness, fused silica capillary column, using helium as the carrier gas at flow rate of 1.017 ml/min. Injector temperature, 220°C, transfer line, 250°C, oven temperature, programmed, 50 to 250°C at 3°C/min.Sample size, 0.1ul (10% solution).EI/MS were recorded at 70 ev.

Plant viruses: Cucumber mosaic virus (CMV) and Tobacco mosaic virus (TMV) employed in the experiments were previously isolated in the plant pathology department lab., faculty of Agriculture, Mansoura University. The viruses were maintained on *Nicotiana tabacum* CV Turkish which served as the virus source plant for each virus for subsequent inoculations. Identification of both tested viruses CMV ant TMV was based on indicator plants (Noordam,1973) and serological methods Ball,1974) namely; micro-precipitation test, double diffusion test and Rocket immuno-electrophoresis test.

Test plants for assay: *Chenopodium amaranticolor* and *Datura stramonium* were used as local lesion hosts for the CMV and the TMV respectively.

Virus inoculum: was freshly prepared by grinding infected leaves of *Tobacco* with 0.02M phosphate buffer (pH 7) in a 1:2 dilution ratio (w/v) in a mortar with a pestle. The leaves were dusted with 600 mesh carbrundum before inoculation by rubbing with fingers.Virus concentration was assisted by counting the developed local lesions per leaf.

The antiviral activity of the oil was tested against CMV and TMV viruses using the local lesions assay (Noordam,1973). 200 ul of the oil was mixed with tween-80
(0.05 %) and diluted with 200ml of distilled water to make 200 ppm stock solution. This was further diluted with distilled water to give concentrations of 25, 50 and 100 ppm. The test plants were sprayed with the different oil concentrations one hour before and one hour after virus inoculation. The lesions were counted on four different leaves on three different plants. Tween-80 (0.05%) mixed with distilled water served as the control. The results are recorded in Tables (II) & (III).

Table (II): Results of the Local Lesions Assay of Essential Oil of *Eucalyptus* on CMV virus using *Chenopodium amaranticolor* as Host Plant

Oil Conc.(ppm)	* No. of Lesions	% Inhibition
0 (control)	183	0
25	122	33
50	89	51.4
100	68	62.8

*** Mean of the number of lesions on four leaves (triplicate experiment).**

Table (III): Results of the Local Lesions Assay of Essential Oil of *Eucalyptus* on TMV virus using *Datura stramonium* as Host Plant.

Oil Conc. (ppm)	*No. of Lesions	% Inhibition
0 (control)	32	0
25	10	68.7
50	5	84.3
100	2	93.7

*** Mean of the number of lesions on four leaves (triplicate experiment)**

RESULTS AND DISCUSSION

The essential oil of *Eucalyptus gomphocephala DC.consists of* 16 components, of which 12 components representing about 99% of the oil were identified. The oil is rich in oxygenated components of which citronellal is the dominant one representing (81.04%) followed by Citronellol (7.31%), cineole (2.6%) and citronellyl acetate (1.65%). The hydrocarbon compounds are few in number and are represented by minor components such as β-pinene (2.26%), isocitronellene (1.34%), β-caryophyllene (0.98%) and α-pinene (0.48%).

Preliminary antiviral investigation of the isolated oil using the local lesions assay method showed that it has a good inhibitory effect on both the tested plant viruses CMV and TMV being more effective against TMV (93.7% inhibition at 100ppm concentration, table III). The results showed that increasing the concentration of the oil increases its inhibitory effect on both the tested viruses. Further work will be undertaken to assign the best conditions of the oil to perform the maximum effect to be used as a safe natural agrochemical to protect crop plants against the two important viruses CMV and TMV.

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تحليل الزيت الطيار لنبات الأيوكاليبتوس جمفوسيفالا و دراسةتأثيره على الفيروسات

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تم فصل الزيت الطيار لنبات الايوكاليبتوس جمفوسيفالا و تم تحليله باستخدام كروماتوجرافيا الغاز و مطياف الكتلة. وقد تم التعرف على مكوناته ووجد أن المكون الرئيسى للزيت هو السترونللال الذى يمثل ٨١% منه.

و قد تمت دراسة تأثيرالزيت على فيروسين يصيبان عدد كبير من المحاصيل الزراعية همافيروس موزايك الخيار و فيروس موزايك الدخان. وقد وجد أن للزيت تأثير مضاد للفيروسات و يخفض من نسبة الأصابة بها بنسبة كبيرة خاصة فيروس موزايك الدخان مما يشجع على استخدامه كمادة طبيعية مضادة للفيروسات ولا تمثل خطورة على صحة الانسان ولا تتسبب فى تلوث البيئة.