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RESEARCH ARTICLE

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Allelopathic effects of essential oil from *Eucalyptus grandis* × *E. urophylla* on pathogenic fungi and pest insects

Abstract This study on the allelopathic effects and chemical components of the essential oil from *Eucalyptus grandis* × *E. urophylla* shows that the leaf oil emulsion of *E. grandis* × *E. urophylla* can inhibit the proliferation of pathogenic fungi *Fusarium oxysporum*, *Pyriculerie grisea*, *Glorosprium musa rum* and *Phytophthora capsici.* Pupation and feeding of the pest insects *Spodopteralitura* Fabricius and *Helicoverpa armigera* Hubner are shown to be affected with restraining effects which increase with the increasing levels of oil concentration. A GC/MS analysis of the leaf oil indicated that the main components, with a relative content of $\geqslant$3%, were allo-ocimene (43.22%), α-pinene (13.63%), γ-terpinene (5.49%), (E)-3,7-dimethyl-2,6-octadien-1-ol (3.58%), β-fenchyl alcohol (4.58%), and 2-amino-3,5-dicyano-6-(4-methoxyphenoxy)-pyridine (3.67%). Terpenes played an important role in the inhibitory effects of *E. grandis* × *E. urophylla* essential oil on pathogenic fungi and pest insects. Poor biodiversity of eucalyptus plantations is a function of allelopathy.

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1 Introduction

Eucalyptus grandis × *E. urophylla* is a hybrid eucalyptus species with great hybrid heterosis, crossed by artificial pollination. It has been successfully introduced to tropical and subtropical regions of southern China and has become a major plantation species. In the Hainan Province, a large proportion of the area set aside for plantations is devoted to *E. grandis* × *E. urophylla* and is widely used as a short-rotation industrial timber species under an intensive forest management. The production practice has shown that the introduction and planting of *E. grandis* × *E. urophylla* over large areas produces considerable economic benefits. At the same time, this monoculture leads to poor biodiversity in the plantations. Allelopathy is a quite common phenomenon in eucalyptus plantations (Del Moral and Muller, 1969, 1970; Cao and Luo, 1996; Zeng and Li, 1997; Gilanisyed et al., 2002; Chen et al., 2003). From the point of view of chemical ecology, investigators have focused on the weakened biodiversity of the plantations and research results show that the water extract of branches and leaves of *E. grandis* × *E. urophylla* react biologically with other plants (Liao et al., 2000; Zhao et al., 2000). The leaves of *E. grandis* × *E. urophylla* are rich in volatile oil (Tian et al., 2006). During the last ten years, a number of investigators have become interested in the allelopathy of some species of *Eucalyptus* (Zhang, 1997; Yan and Tan, 1998; Chen et al., 2003). However, most studies concentrate on the allelopathic effects of the essential oils from eucalyptus. Little attention has been paid to the allelopathic effects and allelo-chemicals of the essential oil from *E. grandis* × *E. urophylla*, especially their effect on pathogenic fungi and pest insects. Therefore, a study of the allelopathic effects of the essential oil from eucalyptus, by analyzing its chemical components, should be important for the recognition of the chemical relationship between *E. grandis* × *E. urophylla* and other life-forms in plantations and to lay a primary and experimental basis for the exploitation and utilization of such eco-friendly practices as botanical pesticides.

2 Materials and methods

2.1 Materials

Fresh leaves of *Eucalyptus urophylla* were collected from the eastern forest farm of Wenchang Island in Hainan Province. The pathogenic fungi *Pyriculerie grisea*, *Phytophthora capsici*, *Glorosprium musarum* and *Fusarium oxysporum* were supplied by the Environment and Plant Protection Institute of the Chinese Academy of Agricultural Sciences. The insect pests *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hubner were artificially cultivated indoors for two generations.

2.2 Methods

2.2.1 Extraction of essential oil

Samples of fresh leaves were cut into small pieces (about 2 cm). The light yellow volatile oil was extracted by aqueous vapor distillation and then dried with anhydrous sodium sulfate. The extraction rate (essential oil/fresh weight) was approximately 0.47%.

2.2.2 Preparation of the reagent

The preparation of the PDA medium, the beef extract-peptone medium, the oat medium and the carrot medium followed the protocol by Zu (1993).

Stock solutions for antifungal assays were prepared by dissolving 2.5 g essential oil, with two dips of Tween-80 (produced by the Guangzhou Chemical Regent Factory), in 50 mL distilled H_2O. Appropriate volumes of the stock solutions (16, 8, 4, 2 and 1 mL) were diluted in 50 mL deionized water to obtain a range of concentrations (16000, 8000, 4000, 2000 and 1000 mg/L).

Two grams of essential oil, emulsified by two dips of Tween-80, was dissolved in 50:50 (v/v) acetone/water to concentrations of 2500, 1000, 500, 250, 100, 50 mg/L and used for insecticidal assays.

Preparation of carbendazim solution: 0.15 g bavistin with 50% wettable powder was dissolved in 100 mL deionized water to 1.5 g/L.

2.2.3 Determination of the allelopathic effect of essential oil on fungi and insects

1) Antifungal assay

The antifungal activity of the essential oil was tested by using a growth rate method (Mu, 1994). The mycelial net growth and the antifungal rate were calculated as follows:

Mycelial net growth = Colony diameter − Fungal disc diameter.

Antifungal rate = $[(GC - GT)/GC] \times 100\%$

where GC is the mycelial net growth of the control and GT that of the treatments.

2) Antifeed assay

The antifeeding activity of the essential oil was tested using a leaf disc method. Feeding restraint, expressed as a per cent, was determined from the formula by Huang (1993):

Antifeed index (AFI) = $[(C - T)/C] \times 100\%$

where C is the eaten area of leaf discs in the control and T is the eaten area of leaf discs in the treatment.

2.2.4 Identification of the allelo-chemicals

The samples were tested by a GC system (Thermo Quest TRACE GC20000) equipped with a selective mass detector (Thermo Quest TRACEMS). A sample solution of 1 μL was injected into a DB-17 capillary column (30.00 mm × 0.25 mm × 0.10 μm). The injection and interface temperatures were 280 and 260°C. Initially, the column temperature was 80°C and then increased to 280°C at a rate of 10°C/min and held at this temperature for 6 min. Helium was used as the propellant with a flow rate of 1 mL/min with a splitless mode. The pressure of the propellant was 100 kPa.

MS conditions: EI (Electron impact) was 70 eV, the source temperature 220°C, the emission current 300 μA, the detector pressure 350 V and the mass scanning ranged from 35 to 350 amu.

3 Results

3.1 Antifungal activity of the leaf oil emulsion from *E. grandis* × *E. urophylla*

Each of the different treatment concentrations of the leaf oil emulsion had certain restraining effects on the four test fungi, as shown in Table 1. The effect of the restraints increased with increasing concentrations. The diameters of the fungal colonies of the treatment groups were all significantly smaller than that of the control at the 200 μg/mL or higher concentrations. At the maximum concentration of 1600 μg/mL, the leaf oil emulsion had a strong restraining effect on the fungi *Pyriculerie grisea*, *Phytophthora capsici*, *Glorosprium musarum* and *Fusarium oxysporum* with antifungal rates of 79.24%, 92.18%, 75.27% and 58.08%, respectively. Moreover, when the treatment concentration was one level higher in each colony, the colony diameters became significantly lower. These results show that antifungal activities are a function of concentration.

Table 1 Restrained activity of essential oil of *E. grandis* × *E. urophylla* on pathogenic fungi

concentration/ μg·mL^{-1}	*Fusarium oxysporum*		*Pyriculerie grisea*		*Glorosprium musarum*		*Phytophthora capsici*	
	average diameter/cm	antifungal rate/%	average diameter/cm	antifungal rate/%	average diameter/cm	antifungal rate/%	average diameter/cm	antifungal rate/%
bavistin	0.218fF	93.02	0fF	100	0.106gF	96.67	0gF	100
1600	1.31eE	58.08	0.364eE	79.24	0.787fE	75.27	0.226fE	92.18
800	1.653dD	47.17	1.159dD	33.88	1.073eD	66.28	0.567eD	80.39
400	2.299cC	26.38	1.438cC	17.97	1.271dD	60.01	2.383dC	17.57
200	2.811bB	9.99	1.683bB	3.99	1.954cC	38.59	2.679cB	7.33
100	3.074aA	1.57	1.696bAB	3.25	2.556Bb	19.67	2.692bAB	6.88
H_2O	3.123aA		1.753aA		3.182aA		2.891aA	

Note: Significant differences at $p = 0.05$ are represented by small letters, significant differences at $p = 0.01$ by capital letters. Same as below

3.2 Restraining effect of leaf oil emulsion on insects

3.2.1 Antifeed activity of the leaf oil emulsion from *E. grandis* × *E. urophylla*

The leaf oil emulsion had an antifeeding effect on the tested vegetable pest insects with its restraining effect increasing at higher concentrations (Tables 2 and 3). Table 2 shows that the amounts of food consumed by the two pest insects were smaller or very much smaller than that of the control at concentrations of 250 and 500 μg/mL, respectively. When the concentrations were higher than 250 μg/mL, the antifeed index was much higher than that of the control. The highest antifeeding activity was observed at 2500 μg/mL where the antifeeding percentage was 56.26% for *Spodoptera litura* Fabricius and 86.6% for *Helicoverpa armigera* Hubner. Furthermore, the differences in the antifeed index were extremely large between the 50 and 250 μg/mL concentrations and between 250 and 1000 μg/mL. We can conclude that the antifeeding activity of the leaf oil emulsion also clearly depends on concentration.

Table 3 presents a comparison of the antifeeding effect of essential eucalyptus oil between *Spodoptera litura* Fabricius and *Helicoverpa armigera* Hubner. From the values of AFC_{50} (concentration causing a 50% feeding restraint), it is seen that the first insect species was the more sensitive to essential oil. With an increase in treatment time, the AFC_{50} gradually decreased while the sensitivity of the pest insects rose. An analysis of the regression of the antifeed index on the natural logarithm of levels of concentration at three different time periods reveals a high correlation between these two variables. This implies a statistically significant level of correlation between the antifeed index and the concentration of leaf oil emulsion.

Table 2 Antifeeding activity of essential oil of *E. grandis* × *E. urophylla* on insects

concentration of oil emulsion/μg·mL^{-1}	*Spodoptera litura* Fabricius		*Helicoverpa armigera* Hubner	
	eaten area/cm^2·larvae^{-1}	antifeed index/%	eaten area/cm^2·larvae^{-1}	antifeed index/%
2500	5.04eB	56.26aA	1.6eC	86.60aA
1000	6.4deB	44.48aA	3.4dC	71.58aA
500	8.45cB	26.77bB	7.46cB	37.61bB
250	9.73bA	15.63bB	10.19bA	14.80bB
100	10.79abA	6.42cC	10.95abA	8.40cC
50	11.07aA	3.98cC	11.56aA	3.30cC
CK	11.54aA		11.95aA	

Table 3 Antifeeding effects of essential oil of *E. grandis* × *E. urophylla* on insects

insects	time/h	linear regression function	correlation coefficient r	F inspection value	AFC_{50}/μg·mL^{-1}
Spodoptera litura Fabricius	24	$y = 1.1284x + 1.2277$	0.9947^{**}	377.102^{**}	2203.190
	48	$y = 1.1158x + 1.2975$	0.9943^{**}	345.250^{**}	2080.880
	72	$y = 1.1922x + 1.2277$	0.9952^{**}	412.523^{**}	1639.884
Helicoverpa armigera Hubner	24	$y = 1.6093x + 0.2941$	0.9826^{**}	111.832^{**}	839.829
	48	$y = 1.6827x + 0.1708$	0.9829^{**}	114.216^{**}	741.158
	72	$y = 1.7973x + 0.0345$	0.9855^{**}	134.731^{**}	632.625

Note: linear regression function $y = ax + b$, where y represents the antifeed index and x the natural logarithm of levels of concentration; ** represents statistical significance at a probability level of 0.01

Table 4 Restraining effect of essential oil of *E. grandis* × *E. urophylla* at different concentrations on insect pupation

concentration of oil emulsion/μg·mL^{-1}	*Spodoptera litura* Fabricius		*Helicoverpa armigera* Hubner	
	pupation rate/%	revised pupation rate/%	pupation rate/%	revised pupation rate/%
2500	40.00	45.28bB	23.33	27.45bC
1000	43.33	49.05bB	35.00	41.18bB
500	53.33	60.38aA	51.67	60.79aA
250	63.33	71.70aA	68.33	80.39aA
100	78.33	88.68aA	76.67	90.20aA
50	85.00	96.23aA	83.33	98.04aA
CK	88.33		85.00	

3.2.2 Insecticidal effect of the essential oil of *E. grandis* × *E. urophylla*

Table 4 indicates that leaf oil emulsion had a strong insecticidal effect on our test insects. The higher the concentration, the lower the pupation rate. At the maximum concentration of 2500 μg/mL, the revised pupation rate of *Spodoptera litura* Fabricius was 45.28% and that of *Helicoverpa armigera* Hubner was 27.45%, which were much lower than the revised pupation rate at a concentration of 500 μg/mL or less. The results suggest that, although the essential oil of *E. grandis* × *E. urophylla* had no direct lethal effect on the test insects, they exerted a strong restraining effect on pupation.

3.3 Identification of the chemical components of the essential oil

The chemical constituents were separated and identified by gas chromatography-mass spectrometry (GC-MS) (Fig. 1). We made a comparison, by computer, of our mass spectra with those contained in the Willey mass spectral database, where the chemical components with matching indices above 800 were identified and the relative contents, in percent, determined by normalization. The results show that the main components with a relative amounts of ⩾3% were allo-ocimene (43.22%), α-pinene (13.63%), γ-terpinene (5.49%), (E)-3,7-dimethyl-2,6-octadien-1-ol (3.58%), β-fenchyl alcohol (4.58%) and 2-amino-3,5-dicyano-6-(4-methoxyphenoxy)-pyridine (3.67%). Among these components, the amount of terpenes, reportedly the allelo-chemicals, accounted for 63.31%. Combined with our biological measurements, we deduce that the terpenoids may be the main allelo-chemicals of the volatiles of oil from *E. grandis* × *E. urophylla*.

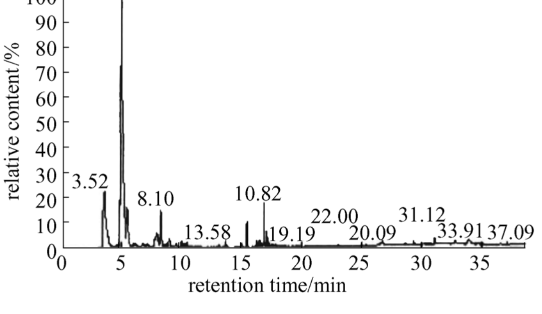

Fig. 1 GC chromatography of volatiles from *E. grandis* × *E. urophylla*

4 Discussion

The present results indicate that the essential oil of *E. grandis* × *E. urophylla* has significant insecticidal and antifeeding effects. The main chemicals were volatile terpenoid compounds whose allelopathic effects had been previously reported (Yu et al., 1999; Kong et al., 2001; Peng et al., 2002; Li, 2006). To a certain extent, these findings explain the generally poor biodiversity of *E. grandis* × *E. urophylla* plantations, which is consistent with the statement that multi-purpose ecological functions are suitable for dealing with the effect of allelo-chemicals (Kong, 1998). There were neither many insects nor pest insects in these eucalyptus plantations. This phenomenon may be directly related to the allelo-chemicals contained in eucalyptus that may exert some effect on insect herbivory and propagation. It may be for the same reason that these plantations in the tropics, although pest insects do occur, are less harmed by damaging insects compared with other crops. Fewer varieties and smaller amounts of microbes occur in these soils, as well. The allelo-chemicals contained in eucalyptus all belong to the category of terpenoid compounds, but the allelo-chemicals produced by different eucalyptus species are not the same, especially those with higher levels of allelo-chemicals. For example, the main allelo-chemicals of essential leaf oil from eucalyptus 12ABL are eudesmol and P cymene while those from *E. grandis* × *E. urophylla* are allo-ocimene and α-pinene (Chen et al., 2004). The difference among the species mentioned presents great difficulties in a quick determination of the main allelo-chemicals. However, it provides all-round opportunities to study the allelo-chemicals produced by various eucalyptus species. At the same time, it provides a broad area for potential research on botanical pesticides.

The volatile oil of *E. grandis* × *E. urophylla* and the ageratum conyzoides reported before, which are all terpenoid compounds, have similar allelopathic effects on fungi and insects. Allelopathic effects play an important role in agro-forestry ecosystems and the essential oil of *E. grandis* × *E. urophylla* exerts a significant restraint on the tested fungi, as shown in our study. Therefore, using the allelo-chemicals released by *E. grandis* × *E. urophylla* to manipulate the germination and growth of pathogenic microorganism in the soil or in the above-ground parts of plants is a good way for the biological control of plant diseases. However, further research should be carried out to confirm the field effect of the allelo-chemicals from *E. grandis* × *E. urophylla*. On the other hand, our study suggests that the essential oil of *E. grandis* × *E. urophylla* has a restraining effect on the feeding and pupation of tested vegetable pest insects and further comprehensive investigations of the effective components of this essential oil and its effect on other activities of noxious insect, such as molting, emergence, oviposition and so on, are highly recommended. Although the effect of the essential oil on the test pest insects is not lethal, it does affect the feeding and metamorphosis of insects. Our results on the insecticidal assay lays a basis for screening and isolation of the active substances and provides a reference in the search for new ways to control pests by exploiting and utilizing resources of the essential oil from *E. grandis* × *E. urophylla*.

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