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FUNGICIDAL EFFECT OF VOLATILE OILS FROM *EUCALYPTUS CITRIODORA* AND ITS MAJOR CONSTITUENT CITRONELLAL

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ABSTRACT

A study was undertaken to explore the effect of volatile oils from *Eucalyptus citriodora* and its major constituent citronellal against two well-known rice pathogens, *Rhizoctonia solani* and *Helminthosporium oryzae*. The radial growth and dry weight of both the test fungi were drastically reduced in response to the volatile oils. A complete inhibition of *R. solani* and *H. oryzae* was observed at 10 and 20 ppm, respectively. Citronellal alone was found to be more effective than eucalypt oils. Based on the study, it was concluded that eucalypt volatile oils have potential for the suppression of phytopathogenic fungi.

Keywords: eucalypt oils, fungicidal activity, *Rhizoctonia solani*, *Helminthosporium oryzae*, radial growth.

INTRODUCTION

Pathogenic fungi alone cause nearly 20% reduction in the yield of major food and cash crops (Agrios 2000). Since World War II, traditional agricultural practices have been replaced by the use of synthetic chemicals for the management of plant pathogens, pests and weeds. This has, no doubt, increased crop production but with some deterioration of environmental quality and human health (Cutler & Cutler 1999). In addition to the target pathogen, pesticides may also kill various beneficial organisms and their toxic forms can persist in soil (Hayes & Laws 1991). The increasing incidence of resistance among pathogens towards synthetic chemicals is also a cause for serious concern. Because of these problems there is a need to find alternatives to synthetic pesticides.

Among the various alternatives, natural plant products that are biodegradable and ecofriendly are catching the attention of scientists worldwide. Such products from higher plants and microbes are relatively broad-spectrum, bio-efficacious, economical, and environmentally safe and can be ideal candidates for use as agrochemicals (Macias et al. 1997; Cutler 1999). Among these, essential oils from a number of plants have been reported to show activity against a wide array of plant pathogenic fungi (Rice 1995). These are relatively safe to the user and the environment (Wilson et al. 1997). However, no work has been carried out to explore the fungicidal activity of the volatile oil from *Eucalyptus citriodora*, which is well known for its weed suppressing and insecticidal properties (Kohli et al. 1998; Isman 2000). This study explores the effect of eucalypt oil and its major constituent, the monoterpene citronellal, against two fungal pathogens of rice viz. *Rhizoctonia solani* and *Helminthosporium oryzae*.

MATERIAL AND METHODS

Cultures of test fungi *R. solani* and *H. oryzae* were procured from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute (IARI), New Delhi, India and maintained on a suitable medium until used. Citronellal was procured from Sigma Chemical Co., St. Louis, USA. Fresh mature leaves of *Eucalyptus citriodora*

Hook. were collected from 27-year-old trees growing in the campus of Panjab University, Chandigarh, India. The volatile oil was extracted by hydrodistillation.

The crude oil and its major monoterpene citronellal were tested at different concentrations for antifungal activity against both the test fungi by the food poison technique (Grover & Moore 1962). For this, 20 µl of volatile oil or monoterpene was mixed with Tween-80 (0.05%) and diluted with 20 ml of distilled water to make a 20 ppm stock solution. This was further diluted with distilled water to give concentrations of 0.5, 1, 2.5, 5, 7.5, 10, and 15 ppm. An aliquot (1 ml) of each concentration was then added to 20 ml of PDA (potato dextrose agar) for *R. solani* and CMA (corn meal agar) for *H. oryzae*, and mixed thoroughly. Thereafter, a mycelial disc of approximately 5 mm diameter, cut from the periphery of a 8-day old culture, was inoculated in the centre of each Petri plate. Tween-80 (0.05%) mixed with distilled water instead of oils served as the control. For each treatment three replicates were maintained in a completely randomised design. These Petri plates were then incubated at 25±2°C and observations were recorded on the 8th day for *R. solani* and 9th day for *H. oryzae*. Fungitoxicity was calculated as per the method given by Pandey et al. (1982) and the MICs (minimum inhibitory concentrations) were determined. To determine minimum fungicidal concentration (MFC), the culture discs were inoculated onto the test medium containing different concentrations of oils. After 72 h, the culture discs were transferred into fresh medium and incubated further for 72 h. If there was no growth, this indicated fungicidal activity (Bindu et al. 1998).

Further, to determine the effect of these volatile oils on the dry weight of the test fungi, 1 ml of each treatment concentration was added to 20 ml of the PD broth medium in 100 ml Erlenmeyer flask and inoculated with a 5 mm disc of test fungi. The flasks containing medium with 1 ml of Tween-80 (0.05%) in distilled water served as control. After 8 days (for *R. solani*) and 9 days (for *H. oryzae*) dry weight of mycelia were determined. The entire experiment was repeated and results are presented as means subjected to one-way ANOVA followed by separation of means at P<0.05.

RESULTS AND DISCUSSION

Radial growth and biomass of both *R. solani* and *H. oryzae* were significantly reduced in response to various concentrations of eucalypt oil ranging from 0.5 to 20 ppm except in the case of radial growth of *R. solani* at lower concentrations. A similar effect was observed when the test pathogenic fungi were treated with constituent monoterpene citronellal (Table 1). Greater suppression of test fungi was observed in response to treatment with monoterpene citronellal than eucalypt oil. In the case of *R. solani* no fungal growth was observed at concentrations of eucalypt oil beyond 7.5 ppm, whereas in the case of *H. oryzae* decreased growth was observed up to 15 ppm after which there was complete inhibition. On the other hand, after treatment with citronellal, no radial growth was observed at 15 ppm and at 10 ppm biomass was too little to be measured (Table 1).

Based on these experiments, the MICs in response to treatment with citronellal and eucalypt oil were calculated to be 5 and 7.5 ppm, respectively, for *R. solani* and 2.5 and 0.5, respectively, for *H. oryzae*. MFC for radial growth was calculated to be 17.5 and 22.5 for citronellal and eucalypt oil, respectively, in the case of *H. oryzae* and 12.5 for both citronellal and eucalypt oil in the case of *R. solani* (Table 2).

From the above results it is evident that volatile eucalypt oil and monoterpene citronellal exert fungitoxic effects. Likewise, essential volatile oils from a number of plant species such as *Cymbopogon martinii* (Singh et al. 1980), *Hyptis suaveolens* (Pandey et al. 1982), *Ocimum gratissimum* (Dubey et al. 2000), *Azadirachta indica* (Amadioha 2000) and other plants (Rice 1995) have been reported to be fungitoxic. Singh et al. (1980) tested antifungal properties of essential oils from several species of *Cymbopogon* and *Trachyspermum ammi* and found that these exhibited strong fungitoxicity and a wide range of activity. Singh & Upadhyay (1991) observed that cumaldehyde, a main

TABLE 1: Effect of eucalypt oil (E) and citronellal (C) on the radial growth and dry weight of *R. solani* and *H. oryzae*. Different letters within a column represent a significant difference at $P < 0.05$.

Concentration (ppm)	Treatment	Radial Growth (mm)		Dry weight (mg)	
		<i>R. solani</i>	<i>H. oryzae</i>	<i>R. solani</i>	<i>H. oryzae</i>
0 (Control)	-	9.00 a	9.00 a	169.0 a	206. a
0.5	E	9.00 a	7.50 b	86.0 b	90.2 c
	C	9.00 a	9.00 a	68.1 d	98.0 b
1.0	E	9.00 a	7.10 c	74.6 c	57.9 d
	C	9.00 a	9.00 a	29.3 f	97.0 b
2.5	E	9.00 a	6.90 cd	47.6 e	50.2 e
	C	9.00 a	8.10 a	9.9 h	86.0 c
5.0	E	9.00 a	6.10 d	14.8 g	37.7 f
	C	7.27 b	7.10 c	8.0 h	47.0 e
7.5	E	5.10 c	3.60 e	0	29.2 g
	C	1.17 d	3.80 e	0	33.7 f
10	E	0	3.00 f	0	16.1 h
	C	0	2.90 f	0	0
15	E	0	1.20 g	0	0
	C	0	0	0	0
20	E	0	0	0	0
	C	0	0	0	0

constituent of essential oils from *Cuminum cyminum*, exhibited strong fungitoxicity against *Aspergillus* sp. The pathogen suppressing activity of crucifers has even been correlated with the amount of volatile components present in its different genotypes (Olivier et al. 1999).

Based on the present study, it could be concluded that volatile oils from *E. citriodora* and its major constituent monoterpene citronellal, possess fungitoxic activities worth exploiting for the biomanagement of plant diseases. If eucalypt oils exhibit the same fungitoxic effect under field conditions, these can serve as natural fungicides or at least templates for the synthesis of novel fungicides. However, further studies are needed to test their thermostability, stability to storage or autoclaving and also their phytotoxicity towards host plants. Moreover, since the present study investigated the fungitoxic effect of eucalypt volatile oils against only two phytopathogenic fungi, it is difficult to say whether they possess a wide or narrow range of toxicity.

TABLE 2: Values of MIC and MFC for *R. solani* and *H. oryzae* in response to treatment of eucalypt oil and citronellal. Different letters within a column represent significant difference at $P < 0.05$.

Parameter	Treatment	MIC		MFC	
		<i>R. solani</i>	<i>H. oryzae</i>	<i>R. solani</i>	<i>H. oryzae</i>
Radial Growth	E	7.50 a	0.50 b	12.50 a	22.50 a
	C	5.00 b	2.50 a	12.50 a	17.50 b
Dry Weight	E	0.50 c	0.50 b	10.00 b	17.50 b
	C	0.50 c	0.50 b	10.00 b	12.50 c

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