BIOLOGIA PLANTARUM 34 (1-2): 143-147, 1992

Effect of essential oils from some higher plants against fungi causing damping-off disease

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Abstract

A screening of leaves of 25 taxa of angiosperms was made for their volatile toxicity against damping-off fungi. The volatile substances from *Hyptis suaveolens* and *Ocimum canum* were toxic against *Pythium aphanidermatum, P. debaryanum* and *Rhizoctonia solani.* The fungitoxicity of the leaves persisted for 15 d of storage. The volatile substances from the leaves of *O. canum* were thermostable, while those from *H. suaveolens* were thermolabile. The essential oils exhibited strong potency against the pathogens tested, non-phytotoxic nature to the host plants and superiority over commonly used synthetic fungicides *Agrosan G.N.* and *Captan.* The findings indicate the possibility to use these essential oils as potential natural fungicides in management of damping-off pathogens.

Introduction

Damping-off disease of seedlings is very common in agricultural and forest soils, in tropical as well as temperate climates and in almost every greenhouse and nursery bed. In these diseases the young seedlings are killed before they reach the soil surface (pre-emergence phase) or the newly emerged seedlings are collapsed and die after infection at ground level (post-emergence phase). Most common pathogens which are responsible for such diseases are several species of *Pythium* and *Rhizoctonia.* For the control of these diseases several synthetic chemicals are used, but most of them render severe side effects in the forms of carcinogenicity, teratogenicity and residual toxicities (*e.g.* Bajaj and Ghosh 1975). Several of the synthetic fungicides are reported to cause adverse effects to the treated soil ecosystems because of their pollutive and non-biodegradable nature. Higher plants in tropics are reservoir of different secondary metabolites and provide an inexhaustable source of useful chemicals which may be used for their different biological properties (*e.g.* Sbragia 1975, Swaminathan 1978). Moreover, because of their lower phytotoxicity, higher systemicity and biodegradable nature (Fawcett and Spencer

Received 11 December 1990, *accepted* 15 August 1991.
Acknowledgements: Authors are thankful to Prof. Y.B.K. Chowdhury, Head of the Department of Botany, Banaras Hindu University, Varanasi for providing laboratory facilities.

1970, Beye 1978), these higher plant products constitute an alternative and indigenous source of plant fungitoxicants. The present paper comprises the effects of some volatile compounds from higher plants as potential fungicides against *Pythium aphanidermatum, P. debaryanum* and *Rhizoctonia solani* which cause damping-off seedlings.

Material and methods

Screening of leaves of higher plants for volatile fungitoxicity: Fresh leaves (10 g) of *Ocimum canum* and *Hyptis suaveolens* were macerated with 10 ml sterilized water and filtered through cheese cloth. The volatile toxicity of the filtrate was tested against the test fungi by the inverted Petri plate technique of Bocher (1955). The fungitoxicity was calculated following Dixit *et al.* (1976), and recorded in term of percentage of mycelial inhibition of test fungi.

Effect of storage and temperature on toxicity of leaves: Freshly collected *Ocimum canum* and *Hyptis suaveolens* leaves, which showed high fungitoxicity, were air dried and stored at room temperature (27 - 37 °C). Fungitoxicity of the stored leaves was tested at 5, 10 and 15 d intervals by the same inverted Petri plate method.

To study the effect of temperature three 10-g samples of freshly collected leaves of *O. canum* and *H. suaveolens* were incubated at 60, 80 and 100 °C for 3 h and fungitoxicity of the treated samples was tested.

Isolation of the volatile antifungal substances: The volatile antifungal oils from the fresh leaves of both plants were extracted by hydrodistillation in Clevenger's apparatus (Langenau 1948) and were finally collected in the form of essential oils. The minimum inhibitory concentration of the oils against the fungi was tested by the poisoned food technique of Grover and Moore (1962) at 100, 500, 1000 and 2000 µl l^{-1} with respect to the potato dextrose agar medium. The fungistat and fungicidal nature of toxicity of the oils was tested following Garber and Hauston (1959). The comparison of efficacy with usual synthetic fungicides *viz.* Agrosan G.N. and Captan was also done by poisoned food technique.

Phytotoxic studies with oils: The phytotoxicity of oils was studied with respect to seed germination and seedling growth of *Vigna radiata* - the host plant following Dikshit *et al.* (1979). The seeds of *V. radiata* were soaked separately in fungitoxic concentration of oils (500 µl l^{-1} in *Ocimum* oil and 300 µl l^{-1} in *Hyptis* oil) in distilled water and percentage of germination and seedling lengths were compared with the control.

All experiments were kept in triplicate and were repeated twice.

Results

During screening of leaves of angiospermic taxa, the leaf extracts of *Hyptis suaveolens* and *Ocimum canum* exhibited strong toxicity against the test fungi inhibiting completely their mycelial growth.

Table 1. Volatile fungitoxicity in leaves of some higher plants against damping-off fungi.

Plant species	Mycelial inhibition of the test fungi [%]		
	Pythium aphanidermatum	*Pythium debaryanum*	*Rhiyoctonia solani*
Adenocalyma allicea Miers.	56.25	62.24	61.11
Achyranthes aspera L.	80.85	68.66	86.11
Allamanda cathartica L.	73.33	52.76	29.41
Antigonon leptopus Hook & Arn.	69.33	69.23	46.67
Blumea membranacea Dc.	62.96	52.76	52.94
Cajanus cajan (L.) Druce	66.67	68.97	73.89
Calotropis procera (Ait.) R.Br.	73.98	74.58	52.22
Cestrum diurnum L.	100.0	100.0	85.55
Coccinia indica Wt. & Arn.	100.0	70.69	85.00
Croton bonplandianum Baill	92.31	88.89	69.44
Datura strumonium L.	53.19	55.22	72.22
Eclipta alba Hassk.	76.60	71.64	69.44
Eupatorium canabinum L.	68.89	60.63	47.06
Euphorbia hirta Lam.	80.00	83.89	50.00
Hyptis suaveolens Poi.	100.0	100.0	100.0
Lantana indica Roxb.	100.0	100.0	88.89
Lawsonia inermis Linn.	73.85	89.26	72.22
Malvastrum coromandelianum Garcke	92.31	82.22	72.22
Murraya paniculta (L.) Jacq.	76.00	80.00	72.22
Ocimum canum Sims.	100.0	100.0	100.0
Parthenium sp. Gray.	71.85	76.30	52.94
Rauwolfia serpentina (L.) Benth.	100.0	100.0	94.44
Ruelia tuberosa L.	85.53	73.33	60.56
Solanum nigrum L.	74.00	86.67	71.11
Tagetes erecta L.	100.0	100.0	75.00

The leaf extracts of *Cestrum diurnum, Lantana indica, Rauwolfia serpentina* and *Tagetes erecta* were effective only against *P. aphanidermatum* and *P. debaryanum*, while those of *Coccinia indica* were effective only against *P. aphanidermatum*. The rest of plants showed either poor or moderate activity (Table 1). The antifungal activity of *O. canum* leaves remained unchanged for the duration of 15 d; the maximum period taken into consideration. It also remained thermostable (leaves showed activity up to 100 °C). The leaves of *Hyptis suaveolens* were effective for 15 d against *P.aphanidermatum* and *P. debaryanum* but their fungitoxicity started to decline against *Rhizoctonia solani* after 5 d of storage. Similarly the fungitoxicity of

H. suaveolens leaf extracts against *P. debaryanum* and *R. solani* decreased after 3 h at 60 °C, however against *P. aphanidermatum* it was effective treatment upto 80 °C. The minimum inhibitory concentration of *O. canum* oil was found to be 100 µl l^{-1} against *P. aphanidermatum* and *P. debaryanum* but it was 500 µl l^{-1} against *R. solani.*

Table 2. Effect of temperature on fungitoxicity of leaves of *H. suaveolens.*

Leaves incubated at temperature [°C]	Mycelial inhibition of test fungi [%] *A. aphanidermatum*	*P. debaranum*	*R. solani*
60	100.00	84.62	73.33
80	100.00	81.54	66.67
100	92.31	76.92	60.00

Table 3. Minimum inhibitory concentration and nature of toxicity of *Ocimum canum* and *Hyptis suaveolens* oils.

Fungi	Inhibition of mycelial growth [%] *Ocimum* oil [µ l^{-1}]				*Hyptis* oil [µl l^{-1}]			
	100	500	1000	2000	100	500	1000	2000
P. aphanidermatum	100.00+	100.00*	100.00*	100.00*	62.50	100.00+	100.00+	100.00+
P. debaryanum	100.00+	100.00*	100.00*	100.00*	60.00	100.00+	100.00+	100.00+
R. solani	72.22	100.00*	100.00*	100.00*	42.22	61.11	67.77	68.89

+ denotes fungistatic nature, * denotes fungicidal nature

Table 4. Comparison of efficacy of essential oils of *Hyptis suaveolens* and *Ocimum canum* with synthetic fungicides.

	Minimum inhibitory concentration [µl l^{-1}] *P. aphanidermatum*	*P. debaryanum*	*R. solani*
Agrosan G.N.	1000	1000	5000
Captan	1000	1000	4000
H. suaveolens oil	300	300	4000
O. canum oil	100	100	500

The oil was fungistatic up to 200 µl l^{-1} against *P. aphanidermatum* and *P. debaryanum* and from 300 µl l^{-1} it was fungicidal. Against *R. solani*, however, it was fungicidal from the concentration 100 µl l^{-1}. The minimum inhibitory concentration of *H. suaveolens* oil was 300 µl l^{-1} against *P. aphanidermatum* and *P. debaryanum*,

however against *R. solani*, the strong toxicity was not recorded. This oil was fungistatic in nature.

During *in vitro* studies, both oils were more effective than Agrosan G.N. and Captan, which are frequently used for control of damping-off diseases (Table 4), becouse their minimum concentration inhibiting the mycelial growth of the test fungi was lower than that of synthetic fungicides. Moreover, the oils did not exhibit any adverse effect on germination and seedling growth of *Vigna radiata* and thus exhibited non-phytotoxic nature.

Discussion

The findings of the present study indicate that for the management of damping-off pathogens, the leaves of the fungitoxic plants (*H. suaveolens* and *O. canum*) which grow luxuriantly as weeds in tropical countries may be used. The persistence of fungitoxicity in the leaves for a long time indicated that even stored leaves may be used for soil amendments and isolated oils for seed treatments. The oils isolated from these plants exhibited superiority over the commonly used synthetic fungicides. Due to non-phytotoxicity, high fungitoxicity, and easily available and renewable sources the oils of *H. suaveolens* and *O. canum* may be used as biodegradable fungitoxicants in management of damping-off diseases of seedlings in nursery beds.

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