Turkish Journal of Botany

Volume 32 | Number 2 Article 7

1-1-2008

Physicochemical Properties and Fungitoxicity of the Essential Oil of Citrus medica L. against Groundnut Storage Fungi

EMMANUEL PETER ESSIEN

JOSEPH PETER ESSIEN

BASIL NSE ITA

GODWIN ASUKWO EBONG

Turk J Bot
32 (2008) 161-164

Research Note

Physicochemical Properties and Fungitoxicity of the Essential Oil of *Citrus medica* L. against Groundnut Storage Fungi

Emmanuel Peter ESSIEN[1], Joseph Peter ESSIEN[2], Basil Nse ITA[3], Godwin Asukwo EBONG[3,*]

[1]Department of Botany, University of Uyo, P. M. B 1017, Uyo-NIGERIA

[2]Department of Microbiology, University of Uyo, P.M.B 1017, Uyo-NIGERIA

[3]Department of Chemistry, University of Uyo, P.M.B 1017, Uyo-NIGERIA

Received: 21.02.2006
Accepted: 12.12.2007

Abstract: The in vitro antifungal effect of the essential oil of *Citrus medica* L. on storage fungi of *Arachis hypogea* L. stored for 6 months was evaluated using the disc diffusion agar method. The oil exhibited a wide spectrum of fungitoxicity, inhibiting all 14 fungus species tested. Thus, the oil can be exploited as a fumigant against storage fungi for the preservation of stored legume seeds due to its wide range of activity, non-phytotoxicity, and long-term persistence of fungitoxicity.

Key Words: *Citrus medica*, essential oil, fungitoxicity, storage fungi, groundnut

Introduction

Legume seed deterioration during storage is a major constraint to profitable grain legume crop production in Nigeria and in some other semi-humid tropic regions, since it causes considerable loss. Hal and Harman (1991) estimated post harvest loss due to mould attacks to be approximately 30%-80% in grain legumes in semi-arid Africa. This figure is alarming considering the value of grain legumes as a major source of plant protein on the continent. Plant products have recently proven their usefulness in providing less phytotoxic, more systemic, and easily biodegradable fungicides, in contrast to many synthetic fungicides that have many adverse effects (Jadhav & Jadhav, 1984; Hal & Harman, 1991). The alternative choice may be the use of botanical fungicides that are easily biodegradable and safe, with minimal environmental impact and danger to consumers (Fawecett & Spencer, 1970). The anti-fungal activity of higher plants has long been thought to be an important factor for disease resistance and control against a wide range of fungi that infect crops (Kurucheve & Padmavathi, 1997; Nwachukwu & Umechurupa, 2001; Ramezani et al., 2002; Oluma & Garba, 2004).

Several studies have shown that natural products are capable of fungitoxic activity against a good number of micro-organisms (Akgul & Kivanc, 1898; Chausaria & Kher, 1978; Dubey & Dwivedi, 1991; Oluma & Elaigwu, 2006). Extracts of plants have also exhibited marked effects on spore germination (Singh et al., 1983; Singh & Dwivedi, 1990). Oluma and Garba (2004) found that the crude extract of *Eucalyptus globulus* Labill. and *Ocimum gratissimum* L. inhibited spore germination and reduced radial growth of *Pythium aphanidermatum* Edson by 44.5%-100%, with *E. globulus* being potent. Radial growth and dry weight of 2 well known rice pathogens, *Helminthosporium oryzae* Link ex Fr. and *Rhizoctonia solani* DC, have been drastically reduced by the volatile oils of *Eucalyptus citriodora* Hook and its major constituent, citronella (Ramezani et al., 2002).

The anti-fungal effects of the essential oil of *Cedrus deodara* Roxb. ex Lambert G. Don, as well as some of its active components, against storage moulds of *Capsicum annuum* L. have been previously investigated (Essien & Essien, 2000). The present study focused on the potential use and efficacy of the volatile fraction of *Citrus medica* L. leaves against storage fungi of groundnut.

* E-mail: jomato652003@yahoo.com

Materials and Methods

Fresh *Citrus medica* L. leaves were collected from the University of Uyo Botanical Farm and authenticated by a taxonomist in the Department of Botany, University of Uyo, Uyo. The leaves were thoroughly washed in distilled water and cut into smaller pieces. Each 100-g sample was hydro-distilled for 4 h using a Clevenger-type apparatus (Mechkovski & Akerele, 1992). The separated oil was dried over anhydrous sodium sulphate (Sigma-Aldrich, USA). Samples were stored in a refrigerator (6 ºC) until used. Some physicochemical properties of the oil were determined by the method of Langenau (1970) in order to characterise the oil. The fungitoxic spectrum and effect of some physical factors (temperature and storage) on the viscosity of the oil was determined by the hanging drop technique (Pereira, 1983). The viscosity of the oil was determined using a Hakes rotoviscometer, following the method of Dixit et al. (1978).

Mycoflora analysis of the seeds was performed after 6 months of storage, by both agar plate and blotter methods (Agarwal & Singh, 1974). Pure cultures of fungi growing on the seeds were isolated, purified, and identified based on their cultural, morphological, and biochemical properties, as described by Samson et al., (1984). The fungistatic and anti-fungal nature of the essential oil was determined using the disc diffusion agar method of Akgul and Kivanc (1989). Spore suspensions of 10^5-10^7 cells/ml, counted with a haemocytometer, were made. About 10 ml of Sabouraud dextrose agar (SDA) was poured into petri dishes and allowed to solidify. A micropipette was used to introduce 0.1 ml of the spore or conidia suspension onto the agar plate before spreading with a glass rod under sterile conditions. Sterilized Whatman No AA 2017006 paper discs (6 mm) were soaked in 3 concentrations (500 ppm, 1000 ppm, and 2000 ppm) of the essential oil being assayed for 6 h. Three of the soaked discs were placed on a fungal spore or conidia seeded plate with the help of sterile forceps. Three replicate were produced for each fungus. All the plates containing the discs were then incubated at 25 ºC. The zone of inhibition was measured after 48 h of incubation. In addition, phytotoxicity of the oil to seed germination and early seedling growth were evaluated according to the method of Dixit et al. (1978).

Results and Discussion

The volatile anti-fungal fraction (essential oil) from the *Citrus medica* leaves was collected with 0.5% recovery. The physicochemical properties of the oil are presented in Table 1. The viscosity of the oil increased with increasing length of storage, but not significantly. The results of the fungitoxicity test showed that the essential oil of *Citrus medica* exhibited a wide spectrum of fungitoxicity, inhibiting all 14 isolated fungus species (Table 2). The minimum inhibitory concentration (MIC) of the oil was 500 ppm at which it exhibited a fungistatic nature, but was fungicidal at higher concentrations (1000-2000 ppm). Mean inhibition of spore germination was between 65.5% ± 1.02% and 98.5% ± 1.63%. This could partly be attributed to the acidic nature of the essential oil, in addition to other bioactive constituents of the oil. This result agrees with the findings of Essien and Essien (2000), who reported that the essential oil of *Cedrus deodara* exerted strong anti-fungal action against the storage moulds of *Capsicum annuum* at all the concentrations tested and was more efficacious than other tested synthetic fumigants. The spore germination inhibition rate at 500 ppm of the oil was slightly lower for *Aspergillus ruber* Minch Fr. and *Penicillium citrimum* Thom, respectively, compared to other species. Differences in the inhibitory effect of various plant extracts on storage fungi have been reported (Oluma & Elaigwu, 2006). These differences may be attributed more to variation in the intrinsic properties of the seeds than the individual fungal species adaptability to the toxic nature of the oil (Nwachukwu and Umechurupa, 2001).

Table 1. Physicochemical properties of the essential oil of *Citrus madica* L.

Parameters	Values
Specific gravity (kg/m^3)	0.8884
Specific rotation (rad/kg)	21.58
Refractive index	1.4770
Acid value (P^H)	1.99
Saponification value (Mg/g)	31.16
Carbonyl percentage (%)	30.40
Phenolic content (%)	ND
Viscosity (Ns/m^2)	0.052

ND: Not detected.
Values are the mean of 3 measurements.

Table 2. Fungitoxic spectrum of the essential oil of *Citrus medica* L. on isolated fungi of groundnut.

	Spore germination inhibition rate (%)		
	2000 ppm	1000 ppm	500 ppm
Aspergillus fumigant	100	100	90.5 ± 1.51
Aspergillus flavus	100	100	87.6 ± 1.42
Aspergillus niger	100	100	82.7 ± 1.40
Aspergillus ruber	100	100	65.8 ± 1.01
Aspergillus nidulans	100	100	98.5 ± 1.63
Alternaria alternata	100	100	75.0 ± 1.38
Lasiodiplodia theobromae	100	100	95.0 ± 1.52
Clasdosporium cladosporioides	100	100	72.1 ± 1.35
Curvularia lunata	100	100	70.5 ± 1.32
Fusarium solani	100	100	85.8 ± 1.42
Mucor racemosus	100	100	98.5 ± 1.63
Penicillium citrimum	100	100	65.5 ± 1.02
Penicillium rubrum	100	100	66.5 ± 1.20
Rhizopus nigricans	100	100	80.5 ± 1.40

(Spore germination inhibition rate ± SD)

The oil retained up to 50% of its toxicity for 90 days. In addition to the presence of antioxidative compounds derived from plants (Mechkovski & Akerele, 1992), the ability of the essential oil to impart reduced growth conditions in substrates by retarding the availability of moisture to spoilage organisms has been reported by Nwachukwu & Umechurupa (2001). The oil did not exhibit any adverse effect on seed germination or early seedling growth of groundnut. It is proposed that due to its wide range of activity, non-phytotoxicity, and long-term persistence of fungitoxicity, the essential oil of *Citrus medica* can be exploited as a fungitoxicant against storage fungi for the preservation of legume seeds; however, further research should be directed towards elucidating the phytochemical properties of the essential oil of *Citrus medica* in order to identify the bioactive constituents of the oil, and to ascertain the appropriate concentration for higher efficacy and safety.

References

Agarwal VK & Singh OV (1974). Routine testing of crop seeds for *Fusarium monoiliforme* with a selective medium. *Seed Sci Res* 2:19-22.

Akgul A & Kivanc M (1989). Sensitivity of four foodborne moulds to essential oil from Turkish spice, herb and citrus peel. *J Sci Food Agric* 47:129-132.

Chaurasia SC & Kher A (1978). Inhibitory effects of essential oils of three medicinal plants against various fungi. *Indian Drugs Pharm Ind* 13:7-9.

Dixit SN, Tripathi SC & Tripathi NN (1978). Fungitoxicity of some seed extracts. *Natl Acad Sci Lett* 1:287-288.

Dubey RC & Dwivedi RS (1991). Fungitoxic properties of some plant extracts against vegetative growth and sclerotia of *Macrophomina phaselina*. *Indian Phytopathol* 44: 411-413.

Essien EP & Essien JP (2000). Control of fungal deterioration of two varieties of Capsicum annuum during storage by the essential oil of *Cedrus deodara*. *Nig J Natl pdts Med 4: 62-64.*

Fawcett CH & Spencer DM (1970). Plant chemotherapy with natural products. *A Rew Phytopathol* 8: 403-418.

Hal JS & Harman GE (1991). Protection of stored legume seeds against attack by storage fungi and weevils, mechanism of action of lipoidal and oil seed treatment. *Crop Protection* 10: 375-380.

Jadhav KD & Jadhav LD (1984). Use of some vegetable oils, plant extracts and synthetic products as protectants from pulse beetle *Callosobruchus masculates* in stored grain. *J Food Sci Tech* 14: 100-113.

Kurucheve V & Padmavathi R (1997). Fungitoxicity of selected plant products against *Phythium aphanidermatum*. *Indian Phytopathol* 50: 529-535.

Langenau EE (1970). *Essential oils*. Krieger Publishing Company, Huntington, New York, pp. 338-339.

Mechkovski A & Akerele CO (1992). Quality control methods for medicinal plant materials, WHO/PHARM/92.559. World Health Organization, Switzerland.

Nwachukwu EO & Umechurupa OI (2001). Anti-fungal activities of some leaf extracts on seed-borne fungi of African yam bean seeds, seed germination and seedling emergence. *J Appl Sci Magt* 5: 24-32.

Oluma HOA & Garba IU (2004). Screening of Eucalyptus globules and *Ocimum gratissimum* against *Phythium aphanidermatum*. *Nig J Plant Protection* 21: 109-114.

Pereira J (1983). The effectiveness of 6 vegetable oils as protectants of Cowpea and Bambara nuts against infestation by *Callosobruchus masculates* (F). *J Stored Pdts Res* 19: 57-62.

Ramezani H, Singh HP, Batish DR, KohlI RR & Dorgan JS (2002). Fungicidal effect of volatile oils from *Eucalyptus citriodora* and its major citronella. *New Zealand Plant Protection* 55: 57-62.

Samson RA, Hoekstra ES & Vanpoerschot C (1984). *Introduction to food-borne fungi*. Netherlands Central Bureau Voor Schimeculture, pp. 85-100.

Singh RK & Dwivedi RS (1990). Fungicidal properties of neem and babul gum against *Sclerotium rolfsii*. *Acta Botanica India* 18: 260-262.

Singh Y, Tripathi RD, Tripathi NN & Dixit SN (1983). The isolation and properties of fungitoxic principle from *Zinziber officinale*. *Indian Plant Pathology* 1: 89-96.