Efficacy of some essential oils against *Phomopsis azadirachtae* - the incitant of die-back of neem

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ABSTRACT

Phomopsis azadirachtae causes die-back of neem. This disease has become a major devastating disease of neem in India resulting in 100% loss of fruits. To develop an eco-friendly biocontrol strategy for the control of this pathogen five essential oils *viz*., eucalyptus oil, fennel oil, pepper oil, coriander oil, nutmeg oil, and two oleoresins namely, capsicum oleoresin and turmeric oleoresin were tested *in vitro* against this fungus. Nutmeg oil, coriander oil and turmeric oleoresin were very effective against *P. azadirachtae* and can be used to manage this fungus.

INTRODUCTION

Neem (*Azadirachta indica* A. Juss.) (Meliaceae) is well-known for its biomedical properties. It is an evergreen, eco-friendly, native tree of Indian sub-continent. The neem tree inspite of having antimicrobial properties is infected by various pathogens belonging to bacteria and fungi. The most destructive pathogen of neem is *Phomopsis azadirachtae* Sateesh, Bhat and Devaki, a deutero mycetous fungus, which causes die-back disease (Sateesh *et al.,* 1997; Girish and Shankara Bhat, 2008). The chief symptoms of the disease are twig blight, inflorescence blight and fruit rot. The disease results in almost 100% loss of fruit production because of which, neem seeds used as a raw material in the preparation of biopesticides, medicines and various industrial products are not obtained.

Effective management of plant diseases is absolutely essential since plants are important to us both economically and aesthetically. Fungal diseases of plants are primarily controlled by the application of fungicides (Maloy, 1993). Die-back of neem can be managed by spraying Bavistin 50 W.P. (Sateesh, 1998). It is not an eco-friendly approach to use synthetic fungicides as they are reported to have carcinogenic, teratogenic, oncogenic and genotoxic properties (Carter *et al*., 1984; Dalvi and Whittiker, 1995). According to Rathmell (1984), the synthetic fungicides are biohazardous and adversely affect the components of ecosystem. Further the constant use results in development of resistance in the pathogens against these fungicides (Brent, 1995). Thus the use of eco-friendly alternative approaches for the management of plant diseases has attracted attention (Ezhilan *et al*., 1994; Anandraj and Leela, 1996). A good number of plant derived natural products are reported to be antifungal in nature (Mishra and Tewari, 1990; Bisht and Khulbe, 1995). Green plants, which are reservoirs of various defense chemicals can provide systemic, non-phytotoxic, easily biodegradable and host metabolism stimulatory pesticides (Dubey and Tripathi, 1987). Essential oils have been used by several workers for controlling fungi, bacteria, viruses and insect pests (Singh and Upadhyay, 1993; Singh, 1996; Dubey *et al*., 2000). The antimicrobial properties of essential oils invariably depend on the chemical nature of the constituents present in them (Nidiry, 1998). Essential oils represent very complex mixtures of aromatic compounds mainly monoterpines and sesquiterpines. In the present study, five essential oils of aromatic plants (Table 1) have been screened for their antifungal activity against the die-back pathogen of neem. Two oleoresins (complex mixture of essential oils and resins) were also screened for their antifungal efficacy. The non-toxic, non-pollutive and biodegradable nature of these essential oils prompted us to exploit these volatile natural products of higher plants against *P. azadirachtae*.

MATERIAL AND METHODS

Five essential oils such as eucalyptus oil, fennel oil, pepper oil, nutmeg oil, coriander oil and two oleoresins namely, capsicum oleoresin and turmeric oleoresin (Messrs Flavours and Essences Company, Mysore) were tested against *P. azadirachtae*. The evaluation of antifungal effects of essential oils on growth of *P. azadirachtae* was carried out by poisoned-food technique (Dhingra and Sinclair, 1995). Essential oils were separately dissolved in acetone (100 mg oil in 1.0 ml of acetone). The Czapek Dox Agar (CDA) containing 500, 1000, 2000, 3000, 4000 and 5000 ppm concentrations of each oil was prepared. The CDA medium with acetone without any oil (5000 ppm) served as control. The oils were added to CDA medium

Table 1. Essential oils and oleoresins tested against *Phomopsis azadirachtae* for antifungal activity

Essential oil	Plant source	Family	Plant (s) part used
Eucalyptus oil	*Eucalyptus citriodora* Hook.	Myrtaceae	Bark and leaves
Fennel oil	*Foeniculum vulgare* Mill.	Apiaceae	Fruit
Pepper oil	*Piper nigrum* L.	Piperaceae	Fruit
Coriander oil	*Coriandrum sativum* L.	Apiaceae	Fruit
Nutmeg oil	*Myristica fragrans* Houtt.	Myristicaceae	Seed
Capsicum oleoresin	*Capsicum annuum* L.	Solanaceae	Fruit
Turmeric oleoresin	*Curcuma domestica* Val.	Zingiberaceae	Rhizome

before sterilization in one set and in another set, oils were added to CDA medium after sterilization under aseptic conditions. The oil amended medium was poured into sterile 90 mm diameter petri plates (20 ml / plate). The mycelial agar disc of *P. azadirachtae* (5 mm diameter) obtained from the margin of seven-day-old culture was inoculated at the centre of the petri plate to both control and essential oil amended CDA medium. The petri plates were incubated at 28 ± 2°C with 12 h photoperiod. The colony diameter was measured after 10 days of incubation and sporulation was observed after 15 days of incubation. The experiment was replicated three times. The per cent mycelial growth inhibition (PI) with respect to the control was computed from the formula (Srivatsava and Singh, 2001).

$$P = \frac{(C - T) \times 100}{C}$$

where C is the colony diameter of the control and T is that of the treated ones.

RESULTS AND DISCUSSION

Essential oils of nutmeg and coriander were effective in inhibiting the mycelial growth at lower concentrations in comparison to other oils. Nutmeg oil completely inhibited the mycelial growth at 2000 ppm concentration whereas coriander oil suppressed completely the mycelial growth of *P. azadirachtae* at 3000 ppm. Other oils showed moderate antifungal activity against *P. azadirachtae* at higher concentrations but did not totally inhibit the mycelial growth even at 5000 ppm (Table 2). Of the two oleoresins, turmeric oleoresin was more effective than capsicum oleoresin. It inhibited the fungal growth at a concentration of 4000 ppm.

The pycnidial formation and sporulation of *P. azadirachtae* was completely suppressed by nutmeg oil, coriander oil, and turmeric oleoresin at 2000 ppm, 3000 ppm and 4000 ppm respectively. At lesser concentration (1000 ppm of nutmeg oil and 2000 ppm of coriander oil) a few pycnidia that were devoid of conidial cirrhi were produced (Table 3).

Table 2. Effect of essential oils and oleoresins on the mycelial growth of *Phomopsis azadirachtae* (10-day-old culture) isolated from neem

Concentrations in ppm	Colony diameter (in cm)						
	Fennel oil	Fennel oil	Pepper oil	Coriander oil	Nutmeg oil	Capsicum oleoresin	Turmeric oleoresin
Control	8.50 ± 0.037^{g}	8.50 ± 0.037^{g}	8.50 ± 0.037^{g}	8.50 ± 0.037^{e}	8.50 ± 0.037^{d}	8.50 ± 0.037^{g}	8.50 ± 0.037^{f}
500	7.55 ± 0.039^{f}	7.33 ± 0.11^{f}	7.00 ± 0.04^{f}	5.55 ± 0.04^{d}	4.72 ± 0.16^{c}	7.88 ± 0.10^{f}	6.70 ± 0.19^{e}
1000	7.12 ± 0.047^{e}	6.47 ± 0.05^{e}	5.99 ± 0.09^{e}	4.54 ± 0.10^{c}	2.56 ± 0.33^{b}	7.41 ± 0.11^{e}	6.45 ± 0.12^{d}
2000	6.45 ± 0.034^{d}	5.66 ± 0.10^{d}	5.44 ± 0.10^{d}	2.68 ± 0.13^{b}	0.00 ± 0.00^{a}	7.00 ± 0.04^{d}	4.55 ± 0.09^{c}
3000	6.07 ± 0.032^{c}	4.15 ± 0.09^{c}	3.68 ± 0.11^{c}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	6.12 ± 0.11^{c}	3.12 ± 0.07^{b}
4000	5.65 ± 0.031^{b}	3.86 ± 0.04^{b}	3.35 ± 0.04^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.89 ± 0.07^{b}	0.00 ± 0.00^{a}
5000	4.57 ± 0.025^{a}	3.46 ± 0.11^{a}	3.02 ± 0.07^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.30 ± 0.05^{a}	0.00 ± 0.00^{a}

Figures followed by different superscript letters differ significantly when subjected to Tukey's HSD (Honestly Significant Differences) [á = 0.05]

Table 3. Effect of essential oils and oleoresins on pycnidia of *Phomopsis azadirachtae* isolated from neem

Concentrations in ppm	Number of pycnidia of *Phomopsis azadirachtae* (± S.E.)						
	Eucalyptus	Fennel oil	Pepper oil	Coriander oil	Nutmeg oil	Capsicum oleoresin	Turmeric oleoresin
Control	183.67 ± 1.34^{g}	183.67 ± 1.34^{g}	183.67 ± 1.34^{g}	183.67 ± 1.34^{e}	183.67 ± 1.34^{d}	183.67 ± 1.34^{g}	183.67 ± 1.34^{f}
500	159.33 ±1.50^{f}	148.83 ± 1.49^{f}	138.00 ±1.20^{f}	63.50 ± 1.35^{d}	56.33 ± 0.99^{c}	170.17 ±1.20^{f}	135.50 ± 1.22^{e}
1000	128.00 ± 1.66^{e}	99.50 ± 1.22^{e}	88.00 ± 1.41^{e}	48.00 ± 1.66 ^{c}	13.83 ± 1.05^{b}	142.67 ±1.50^{e}	86.33 ± 1.41^{d}
2000	103.50 ±1.35^{d}	73.50 ± 1.05^{d}	64.33 ± 1.30^{d}	13.33 ± 0.76^{b}	0.0 ± 0.0^{a}	127.33 ±1.17^{d}	46.67 ± 0.76^{c}
3000	82.17 ± 0.76^{c}	56.67 ± 1.34^{c}	37.17 ± 1.17^{c}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	84.00 ± 1.18^{c}	21.00 ± 1.05^{b}
4000	61.33 ± 0.99^{b}	35.83 ± 0.91^{b}	22.67 ± 1.18^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	67.50 ±0.99^{b}	0.0 ± 0.0^{a}
5000	43.17 ± 0.40 a	28.17 ± 1.20^{a}	15.67 ± 0.88^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	54.83 ±1.48^{a}	0.0 ± 0.0^{a}

Figures followed by different superscript letters differ significantly when subjected to Tukey's HSD (Honestly Significant Differences) [á = 0.05]

The use of many synthetic fungicides has been cautioned due to their pollutive effects, non-biodegradability and residual toxicities. Most of these fungicides have become a popular target of conservationists and are treated to be one of the most vital man-made pollutants (Khoshoo, 1980). Search is on for the development of plant disease control agents, which are non-toxic, biodegradable and eco-friendly. Essential oils show antifungal activity against a wide range of fungi (Kurita *et al.*, 1981; Srivatsava *et al*., 1993; Singh, 1997; Dubey *et al*., 2000). In the present study, two essential oils from nutmeg and coriander, and turmeric oleoresin have given promising results against *P. azadirachtae*. It is indicated as 100% inhibition of mycelial growth and sporulation by coriander oil and nutmeg oil at 3000 ppm and 2000 ppm respectively. Inhibition of sporulation is a significant effect as the spores are major infective propagules of phytopathogenic fungi (Agrios, 2004). Though, essential oils are often fungistatic rather than fungicidal (Jobling, 2000), the essential oils and oleoresin of present studies exhibited fungicidal activity against *P. azadirachtae*. These results confirm antimycotic properties of essential oils and oleoresins used in the present study. The volatility, ephemeral nature and biodegradability of these compounds of angiosperms will be advantageous if they are developed as pesticides. Further studies are required to know the exact mechanism of suppression of *P. azadirachtae* growth by these essential oils.

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