Effect of some essential oils on *Rhizoctonia solani* Kuhn infecting flue - cured virginia tobacco

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ABSTRACT

The effect of 12 essential oils *viz*, pepper oil (*Piper nigrum* L.), nutmeg oil (*Myristica fragrans* Houtt.), turmeric oil (*Curcuma longa* L.), capsicum oil (*Capsicum annum* L.), coriander oil (*Coriandrum sativum* L.), fennel oil (Sweet) ((*Ocimum gratissimum* L.), fennel oil (Bitter) (*Foeniculum vulgare ssp piperitum.* Mill.), clove oil *(Syzygium aromaticum* L.), tulsi oil (*Ocimum sanctum* L.), cinnamon oil (*Cinnamomum zeylanicum* Breyne.), mustard oil (*Brassica juncea* L.) and eucalyptus oil (*Eucalyptus citriodora* Hook) were tested for fungicidal properties against *Rhizoctonia solani* - the causal agent of sore shin disease of tobacco by poisoned food technique. The minimum inhibitory concentration varied between 500 - 2000 ppm. Essential oil of cinnamon was found most effective, as it recorded complete inhibition of the pathogen at 500 ppm. Clove oil showed mycelial inhibition at 1000 ppm. Fennel and nutmeg oil were effective at 2000 ppm.

Key words : Essential oils, *Rhizoctonia solani*, tobacco.

INTRODUCTION

Flue Cured Virginia (FCV) tobacco is one of the remunerative rain-fed commercial crops in Karnataka. It is grown in Karnataka light soils (KLS) in around seventy thousand hectares of land in the southern transitional belt, extending from Mysore district up to Shimoga district. The four taluks of Mysore districts *viz*, H .D. Kote, Hunsur, Periyapatna and K.R. Nagar alone produce 80 to 90 million Kgs of tobacco of which more than 70% is exported annually (Anonymous, 2005). Apart from smoking and chewing purposes, tobacco has medicinal properties, insecticidal properties and also used in synthesis of pharmaceutical products including vaccine production (Bhattacharjee, 2004; Hammond, *et al.*, 2004). In recent years sore shin disease is found to be causing damage in tobacco nurseries caused by *Rhizoctonia solani* Kuhn. This disease was recorded for the first time in KLS nurseries during the nursery survey conducted in 2005 (Anonymous, 2006). The pathogen is gaining importance due to severe damage to seedling in isolated pockets as well as in tray nurseries. Hence, the farmers suffer from transplant shortage for taking up timely planting in the zone. Various chemicals are effective fungicides against *R. solani* (Csinos and Stephenson 1999; Agrois, 2005; Nene and Thapliyal, 1987; Domsch, 1962). Different methods have been used to control *R. solani* such as cultural practices, solarisation and chemical control (Baker and Cook, 1979; Dubey, 2001). The conventional synthetic chemicals have raised ecological problems due to their high cost as well as adverse effect on environment and may induce resistance in the pathogen (Rathmell, 1984). Keeping these facts in mind attempts have been made to control this fungus by natural extracts especially by essential oils namely pepper oil (*Piper nigrum* L.), nutmeg oil (*Myristica fragrans* Houtt.), turmeric oil (*Curcuma longa* L.), capsicum oil (*Capsicum annum* L.), coriander oil (*Coriandrum sativum* L.), fennel oil (Sweet) (*Ocimum gratissimum* L.), fennel oil (Bitter) (*Foeniculum vulgare ssp. piperitum* Mill.), clove oil *(Syzygium aromaticum* L.), tulsi oil (*Ocimum sanctum* L.), cinnamon oil (*Cinnamomum zeylanicum* Breyne.), mustard oil (*Brassica juncea* L.) and eucalyptus oil (*Eucalyptus citriodora* Hook). Many volatile oils of many plants species posses significant biological activity against agriculturally important microbes and insect pests (Singh and Pant, 2001). Essential oils represent very complex mixture of compounds mainly monoterpenes and sesquiterpenes (Letessier *et al.*, 2001). Essential oils are known to posses a variety of biological properties including antimicrobial activity (Dubey *et al.*, 2000). There are reports on the screening of esential oils against many phytopathogenic fungi (Duhey, 2001). In the present study an attempt study an attempt has been made to evaluate fungitoxic properties of some oils for the successful, safe and ecofriendly control of sore shin pathogen in *in vitro* conditions

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MATERIALS AND METHODS

Twelve essential oils such as pepper oil, nutmeg oil, turmeric oil, capsicum oil, coriander oil, fennel oil (Sweet), fennel oil (Bitter) ,clove oil, tulsi oil, cinnamon oil, mustard oil and eucalyptus oil (Messrs, NKCA Pharmacy Ltd, Mysore) were tested against *R. solani*. Essential oils were assessed for fungitoxicity by poison food technique (Dhingra and Sinclair, 1995). Essential oils were separately dissolved in acetone (100 µl of oil in 1.0 ml of acetone). The Czapek Dox Agar (CDA) containing 500, 1000, 2000, 3000, 4000 and 5000 ppm concentration of each oil was prepared. The CDA medium with acetone without any oil (5000 ppm) served as control. The oil amended medium was poured into sterile 90 mm diameter petriplates (15 ml per plate). The mycelial disc (5 mm) obtained from the margin of seven - day - old culture was inoculated at the centre of the petriplate to both control and essential oil amended CDA medium. The petriplates were incubated at $28^{o}C \pm 2^{O}C$ for seven days. The experiment was replicated three times. The diameter of the fungal colonies and growth characteristics in each petridish were recorded. The fungicidal activity was expressed as percentage of mycelial growth inhibition with respect to the control was computed using Srivatsava and Singh (2001) method.

RESULTS AND DISCUSSION

Among essential oils, cinnamon oil completely inhibited the mycelial growth of *R. solani* at 500 ppm concentration. Whereas, clove oil and coriander oil showed 100% inhibition at 1000 ppm. Nutmeg oil and Fennel oil was effective at 2000 ppm (Table. 1). Pepper oil and Turmeric oil showed moderate growth at 4000 ppm. But did not totally inhibit the mycelial growth even at 5000 ppm. While other essential oils namely Capsicum oil, Tulsi oil, Fennel (Sweet), Mustard oil and Eucalyptus oil were non toxic to *R. solani*. Singh and Pandey (1998) have reported that oil of Eucalyptus was effective against *R. solani* causing Leaf spots in Sugar cane at 3000 ppm, but in the present study the oil did not inhibit the mycelial growth at 5000 ppm. Mustard oil was not effective against *R. solani* at 5000 ppm. Above this concentration the essential oils were reported to be phytotoxic (Chaijuckam *et al.*, 2010). However, Dhingra *et al.* (2004) have reported that this oil completely inhibited *R. solani* causing seedling damping off and seedling blight in nursery at 50000 ppm. Since this concentration of essential oils is reported to be phytotoxic, attempts were not done to test the minimum inhibitory concentration during the present investigation. Girish and Bhat, (2008) have reported that nutmeg oil completely inhibited the growth of *Phomopsis azadirachtae* at 2000 ppm, and the same concentration was found to be effective against *R. solani*. Piyo *et al*. (2009) have reported the antifungal activity of essential oils from basil and fennel against *R. solani*. The antimicrobial properties of essential oils have been known for a long time and they show antifungal activity against a wide range of fungi (Kishore *et al.*, 1988; Jeyalakshmi and Seltharam, 1997; Guddewar, *et al.*, 1999; Singatwadia and Ketewa, 2001; Nidiry, 1998; Vaijayanthimala, *et al.*, 2001). The strength of antifungal properties of essential oils depends on the plant and fungal species, concentration of the testing oil and testing condition (Piyo *et al*., 2009).

In all the effective oils namely, cinnamon oil, clove oil, coriander oil, fennel oil and nutmeg oil completely suppressed the formation of sclerotia at 500 ppm. In addition to this, pepper oil also showed inhibition of sclerotia formation at 500 ppm. However, capsicum oil, turmeric oil, mustard oil and tulsi oil suppressed the sclerotia formation at 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm respectively. Previous reports are not available regarding the effect of essential oils on sclerotia formation. The present work has revealed the effective concentration required to suppress the sclerotia formation.

Table 1. Minimun inhibitory concentration (MIC) and percentage inhibition (PI) of growth of *Rhizoctonia solani* by some essential oils.

Essential oils	Percentage of growth inhibition				Minimum inhibitory concentration (MIC)* (in ppm)
	Control	500 ppm	1000 ppm	2000 ppm	
C. zeylanicum	00^{e}	100^{a}	100^{a}	100^{a}	500
S. aromaticum	00^{e}	11.48^{d}	100^{a}	100^{a}	1000
C. sativum	00^{e}	00^{e}	100^{a}	100^{a}	1000
F. vulgare	00^{e}	45.18^{b}	67.04^{c}	100^{a}	2000
M. fragrans	00^{e}	14.00^{c}	70^{b}	100^{a}	2000

*MIC data is given only to the most effective essential oils

Figures having the same letters are not significantly different according to Ducan's multiple range test (P<0. 05)

This concentration is important because the survival structure in *R. solani* is sclerotia (Gopalachari, 1984; Lucas, 1975; Agrios, 2005). Hence the concentration which suppresses the sclerotia formation should be considered in formulating the effective dosage for the control of *R. solani*. Several workers have identified the chemical compounds and showed that those fractions are very efficient in suppressing the growth of *R. solani*. Ozcan *et al*. (2006) have reported that fennel oil (bitter) consisted of monoterpenic hydrocarbons, oxygenated mono terpenes and sesquiterpenes which are very efficient in suppressing the growth of *Rhizoctonia solani*. Necha *et al*. (2009) have reported that carvacrol, geranol and trans - cinnamaldehye present in cinnamon have a high antifungal activity against *Fusarium oxysporum*. A clove oil formulation includes alcohol, esters, glycol ethers, mineral oil, methyl esters and hydrocarbon solvents which inhibits the soil borne fungal pathogen *R. solani* (Walter *et al*., 1996). The efficacy of the oils used in the current study may be due to synergistic action of different biomolecules present in them.

In the present study, five essential oils namely, Cinnamon, Clove, Coriander, Fennel (Bitter) and Nutmeg have shown promising results against *R. solani*. The results confirmed that these five essential oils have antifungal properties on both mycelial growth and sclerotia formation. The use of these essential oils is considered as eco-friendly approach for the control of plant diseases (Fathima *et al*, 2009; Manasi and Tewari, 1992). These most effective essential oils identified during the present investigation can be recommended for the control of nursery diseases like sore shin caused by *R. solani* and they can reduce the problem of pollution caused by the use of fungicides.

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Rhizoctonia solani KuÌˆhn is the causal pathogen of tobacco target spot, a serious fungal disease of tobacco that severely impairs yield and quality in northeast China. The objective of this study was to characterize isolates of R. solani from tobacco in China. Among 58 Rhizoctonia isolates examined, all of them were multinucleate.Â Y. Q. Zhao et al., "Characterization of Rhizoctonia solani AG-3 Isolates Causing Target Spot of Flue-Cured Tobacco in China", Advanced Materials Research, Vols. 726-731, pp. 4321-4325, 2013. Online since Flue-Cured Virginia (FCV) tobacco (Nicotiana tabacum L.) is the major commercial crop in light soils of southern transition zone of Karnataka called Karnataka Light Soil (KLS). Tobacco is cultivated in KLS in around 1.17 lakh ha extending from Mysore district up to Shimoga district. FCV tobacco nurseries in KLS are raised during pre-monsoon period (March-May). The seedlings are transplanted during the onset of southwest monsoon (May-June) (Devaki, 1991; Gopalachari, 1984; Shenoi and Nagarajan, 2000). During the pre-monsoon period the climatic conditions are congenial for the spread of several Rhizoctonia solani (teleomorph: Thanatephorus cucumeris) is a plant pathogenic fungus with a wide host range and worldwide distribution. It was discovered more than 100 years ago. R. solani frequently exists as thread-like growth on plants or in culture, and is considered a soil-borne pathogen. R. solani is best known to cause various plant diseases such as collar rot, root rot, damping off, and wire stem. R. solani attacks its hosts when they are in their early stages of development, such as seeds