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ANTIFUNGAL EFFICACY OF AQUEOUS EXTRACTS OF SOME SPICES AGAINST SCLEROTIUM ROLFSII

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ABSTRACT
Botanicals are considered as better, safe, cost effective and ecofriendly alternatives rather than synthetic fungicides in the management of plant diseases in the era of modern agriculture. Among the botanicals, spices have richest source of secondary metabolites which have potential ethno botanical importance. In the present study, seven (7) spices viz. *Allium sativum* (Garlic), *Zingiber officinale* (Ginger), *Myristica fragrans* (Nutmeg), *Piper nigrum* (Black pepper), *Terminalia chebula* (Chebulic myrobalan), *Trachyospermum ammi* (Ajowan) and *Trigonella foenumgraecum* (Fenugreek) were selected to see the antifungal efficacy of their aqueous extracts (3, 4 and 5 %) against *Sclerotium rolfsii*, a dreaded soilborne, phytopathogenic fungi causing several diseases in monocotyledonous and dicotyledonous plants. Among the screened spices, aqueous extracts of garlic, ginger, nutmeg, black pepper, myrobalan and ajowan showed strong antifungal efficacy which inhibited 100% mycelial growth and sclerotial formation of *S. rolfsii* at 3, 4 and 5 % while fenugreek showed negative effect on mycelial growth. Mycelial dry weight showing negative effect of fenugreek extract at 3%, 4% and 5% were 1.37g, 1.23g and 1.23g as compared to control (1.24g).

INTRODUCTION

Introduction of high yielding cultivars pose threat to the soil as due to this indiscriminate use of synthetic fungicides had left soil degraded, affected microbial world, disturbed food chain and contaminated the environment. Pollution as well as heavy metal toxicity reduced the physical properties of the soil all around. To overcome the man made problems thousands of researchers initiated research to search over better alternatives to synthetic fungicides as these pose threat to human health. Among the alternatives of synthetic fungicides; botanical fungicides and biocides paid highest attention in the management of insect and pests diseases (Maurya *et al*., 2002, Kumar *et al*., 2012).

Sclerotium rolfsii Sacc.(Teliomorph: *Athelia rolfsii* (Curzi)- Tu and Kimbrough) is a dreaded Soil borne fungus responsible for Southern blight and causative agent of stem rot of *Glycine max* (Soyabean) had depleted several commercially valuable plants (Vivek *et al*., 2014, Punja, 1985, 1988, Sarma *et al*., 2002a, 2002b, Singh *et al*.,2003). It had perfect stage *Magnaporthe salvinni catt*. and devastative at suitable climatic conditions.(Venkateswarlu *et al*.,2013) The sclerotia produced by this pathogen remains in soil year after year (Chet and Henis, 1972, Punja , 1985). Several researches showed different plants tested for antimicrobial efficacy with positive outcome (Ram *et al*., 2012, Gurjar *et al*., 2012 and Sehajpal *et al*., 2009). Diversified plant material including spices possess chemical and bioactive constituents that were antagonistic to plant diseases (Kandasamy *et al*.,1974). Spices possess therapeutic properties due to presence of Phthalates, Polyacetylenes, limonoids, Phenolics, flavonoids, terpenoids and glycosides (Purthi, 1998).

Present experimental work was designed to find the potent ethnomedicinal plant; incorporated with antifungal properties and may further be exploited as natural alternatives to synthetic pesticides. To screen antifungal efficacy of 7 spices viz. *Allium sativum* (Liliaceae), *Zingiber officinale* (Zingiberaceae), *Myristica fragrans* (Myristicaceae), *Piper nigrum* (Piperaceae), *Terminalia chebula* (Combretaceae), *Trigonella foenumgraecum* (Fabaceae), *Trachyospermum ammi* (Apiaceae) against *S. rolfsii*; preparation of aqueous extracts were done *in vitro*.

MATERIALS AND METHODS

Isolation and purification of *S. rolfsii*

A southern blighted diseased plant of *Glycine max* (Fabaceae) commonly known as soyabean was collected from experimental research farm ICAR- RCER- RC, Plandu, Ranchi. The infected collar region was cut into small pieces with the help of sterile blade and washed thoroughly with sterilized distilled water to remove soil and surface contaminants and finally sterilized in 2% sodium hypochlorite for 2 min and again washed with sterile distilled water. The infected collar region of soybean was cut into 3-5mm in length and kept in moist chamber for induced mycelial growth. After 36-48 hrs the white mycelial growth was observed and then mycelial threads were transferred into Petri plates and culture were

purified with the help of hyphal tip isolation techniques and then after culture was maintained at 27 ± 2 ºC (Shukla and Dwivedi, 2012).

Plant materials

Bulb of Garlic, rhizome of ginger and seeds of fenugreek, ajowan, myrobalan, black pepper and nutmeg of particular varieties were purchased from local market of Jharkhand. The collected materials were brought and washed thoroughly with distilled water. The rinsed plant parts were shade dried for 24 hrs at room temperature 24 ± 2ºC (Avasthi *et al*., 2010). Peeled garlic and ginger and seeds of nutmeg, black pepper, ajowan, myrobalan and fenugreek were used after maceration in sterilized mortar and pestle. The macerated parts were used @ 3, 4 and 5% and the prepared aqueous broth were then autoclaved at 121ºC for 30 min (Kapadia *et al*.,2014) The experiments were designed in triplicates along with the control.

Antifungal activity of plant extracts

The autoclaved aqueous extracts of all the selected spices were inoculated with 5mm bead of *S. rolfsii*. After inoculation the growth and development of the fungus was recorded periodically after 24 hrs to check the antifungal efficacy. The aqueous extract of plant which showed full growth of *S. rolfsii* unlike control was measured for fresh and dry weight of the pathogen.

RESULTS AND DISCUSSION

In the present study, seven (7) spices viz. *Allium sativum* (Garlic), *Zingiber* officinalis (Ginger), *Myristica fragrans* (Nutmeg), *Piper nigrum* (Black pepper), *Terminalia chebula* (Chebulic myrobalan), *Trachyospermum ammi* (Ajowan) and *Trigonella foenumgraecum* (Fenugreek) were selected to see their antifungal efficacy of their aqueous extracts against *Sclerotium rolfsii*, a dreaded soilborne, phytopathogenic fungus causing several diseases in monocotyledonous and dicotyledonous plants. Among the selected and screened spices, aqueous extracts of garlic, ginger, nutmeg, black pepper, myrobalan and ajowan showed strong antifungal efficacy which inhibited 100% mycelial growth and sclerotial formation of *S. rolfsii* at 3, 4 and 5% while fenugreek showed negative effect on mycelial growth and sclerotial development. Fenugreek extract showing negative effect on the mycelial dry weight of *S. rolfsii* at 3%, 4% and 5% were 1.37g, 1.23g and 1.23g respectively similar to control (1.24g) (Table 1 and 2, Fig 1).

Results indicated that almost all the selected spices showed antifungal efficacy against the test pathogen except fenugreek and these antifungal efficacy might be due to the presence of water soluble secondary metabolites like phenolic acid, alkaloids, flavonoids and glycosides. Garlic and their constituents have wide range of medicinal properties against plant and human diseases (Singh *et al*., 1990). Singh *et al*., 2001 also reported that garlic and its constituents have potential to inhibit various human and plant pathogenic and saprophytic microorganisms. Similarly several reports indicated that the presence of essential oils in nutmeg and myrobalan might have inhibited the growth of plant pathogen (Fathima *et al*., 2009, Seema and Devaki, 2010, Sharma *et al*., 2011). Complete inhibition of the plant pathogens in the

Table 1: Qualitative behavior of selected spices on the growth and sclerotial formation in *Sclerotium rolfsii*

Treatments Control	Qualitative behavior on growth and sclerotial development of *S. rolfsii* Time interval (hrs)				
	24	48	72	96	120
Control	+	+	+	$+^{SI}$	$+^{SM}$
Garlic					
3%	-	-	-	-	-
4%	-	-	-	-	-
5%	-	-	-	-	-
Ginger					
3%	-	-	-	-	-
4%	-	-	-	-	-
5%	-	-	-	-	-
Nutmeg					
3%	-	-	-	-	-
4%	-	-	-	-	-
5%	-	-	-	-	-
Black pepper					
3%	-	-	-	-	-
4%	-	-	-	-	-
5%	-	-	-	-	-
Ajowan					
3%	-	-	-	-	-
4%	-	-	-	-	-
5%	-	-	-	-	-
Myrobalan					
3%	-	-	-	-	-
4%	-	-	-	-	-
5%	-	-	-	-	-
Fenugreek					
3%	+	+	+	$+^{SI}$	$+^{SM}$
4%	+	+	+	$+^{SI}$	$+^{SM}$
5%	+	+	+	$+^{SI}$	$+^{SM}$

Note: + = Mycelial growth; SI = Sclerotial initiation;SM = Sclerotial maturation; - = No growth

Table 2: Percent inhibition of aqueous extracts of selected spices on growth and sclerotial formation of *Sclerotium rolfsii*

Aqueous extracts of selected spices	Inhibition (%) on growth and sclerotial formation of *Sclerotium rolfsii*		
	3%	4%	5%
Control	0%	0%	0%
A.sativum	100%	100%	100%
M.fragrans	100%	100%	100%
P.nigrum	100%	100%	100%
T.chebula	100%	100%	100%
T. ammi	100%	100%	100%
T. foenumgraecum	0%	0%	0%
Z. officinale	100%	100%	100%

aqueous extract of ajowan and ginger were also reported by several workers (Bansod and Rai, 2008, Dwivedi and Sangeeta, 2015, Suleiman and Emua, 2009). Shukla and Dwivedi, (2012) reported that the active principle present in black pepper aqueous extract might be responsible for inhibition in mycelial growth and sclerotial formation in *S. rolfsii*. According to the result obtained from the present study the chemical constituents attributed to the respective plant extracts and their solubility in water might be responsible for antifungal efficacy against *S.rolfsii* whereas least efficacy of fenugreek against the test pathogen might be due to

insolublization of active component from plant extract into

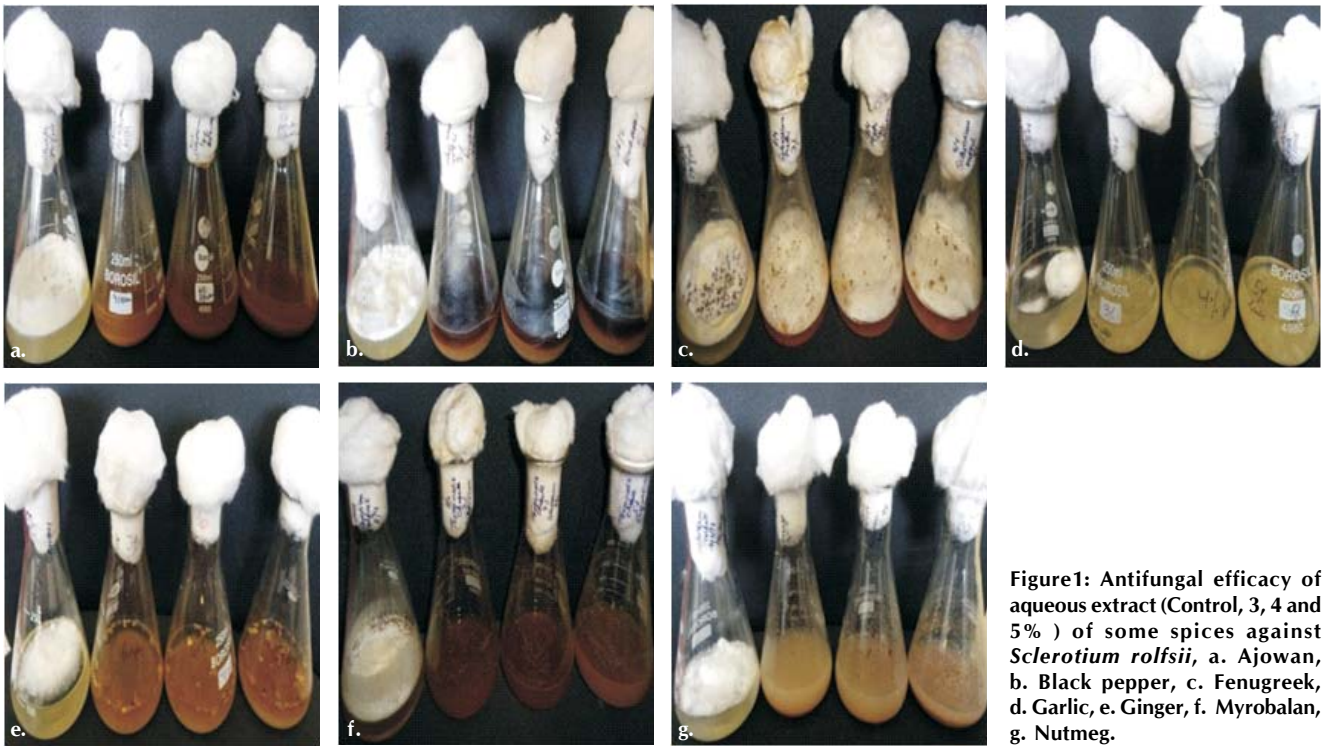

Figure1: Antifungal efficacy of aqueous extract (Control, 3, 4 and 5%) of some spices against *Sclerotium rolfsii*, a. Ajowan, b. Black pepper, c. Fenugreek, d. Garlic, e. Ginger, f. Myrobalan, g. Nutmeg.

water (Avasthi *et al.*, 2010, Farooq *et al.*, 2010).

In the present study of seven selected spices, six spices showed strong antifungal efficacy in term of mycelial growth and development of sclerotia in *S. rolfsii*. So plants like nutmeg, garlic, ajowan in their aqueous extracts could be exploited as natural fungicides. They could be planted along with the desired commercial crops or might be incorporated in crop rotation system to exploit their allelopathic effect against soilborne diseases caused by the *S. rolfsii* and other sclerotial and fungal pathogens. Conclusively the application of these plants would reduce the indiscriminate use and dependency on synthetic fungicides.

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