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Antifungal Potential of Spices and Medicinal Herb against Selected Phytopathogenic Soil-Borne Fungi

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ABSTRACT: Indian spices and botanicals are popular for their aromatic and therapeutic properties. Spices possess anti-oxidant, anti-spasmodic and immune boosting potential. Some botanicals besides having medicinal importance also flavor Indian cuisines. These can also be utilized for the management of soil-borne phytopathogens like *Sclerotinia sclerotiorum, Rhizoctonia solani* and *Sclerotium rolfsii*. Since overuse of synthetic pesticides against phytopathogens had caused detrimental effect on human health and on the entire ecosystem. Hence; present study focuses on the selection of seven botanicals such as *Allium sativum* (Garlic), *Myristica fragrans* (Nutmeg), *Piper nigrum* (Black pepper), *Trachyspermum ammi* (Ajwain), *Terminalia chebula* (Myrobalan), *Trigonella foenumgraecum* (Fenugreek) and *Zingiber officinale* (Ginger) for eco-friendly disease management. The selected botanicals were investigated for their *in vitro* antifungal potential against three deadly soil-borne sclerotia fungi. The efficacies of aqueous extracts of selected spices were compared with two known synthetic fungicides *viz*., Carbendazim and Mancozeb. At 5% concentration; the aqueous extracts of *M. fragrans, P. nigrum, T. chebula* and *T. ammi* showed strong antifungal efficacy by inhibiting 90% mycelial growth of *R. solani* and *S. rolfsii*. Similarly; *A. sativum* and *T. ammi* extracts inhibited 100% mycelia growth of *S. sclerotiorum* at 5% concentration. Henceforth, the present finding confirms the presence of fungitoxic component in the selected botanicals which might got solubilize into their aqueous extracts. As compared to synthetic pesticides; use of such spices and herb through crop rotation would increase the soil quality. Even their crude extract spray would lessen the financial burden on farmers. Transformation of the active principle to nano particle would open up the way towards sustainable disease management strategy.

Keywords: Botanicals, Eco-friendly, antifungal, *R. solani, S. rolfsii, S. sclerotiorum.*

INTRODUCTION

Sclerotial fungi are ubiquitous, devastating soil-borne phytopathogens causing numerous diseases in mono and dicotyledonous crop plants. Among the sclerotial fungi, *S. sclerotiorum*, *R. solani* and *Sclerotium rolfsii* are major soil-borne phytopatogens prevailing in the locality. *S. sclerotiorum* is characterized by the formation of black sclerotia with white fuzzy mycelial growth (Mehta, 2009). Previous reports had showed that approx 60% loss of rapeseed-mustard was caused due to "*Sclerotinia* rot"; which led to the discouragement of agriculturists for these crops in several Indian states (Yadav *et al*., 2012). It spends 90% of the lifecycle as sclerotia and could withstand the extremity of temperature, pH and moisture. Similarly; *Rhizoctonia solani* (Teleomorph: *Thanatephorus cucumeris*); causing collar rot or damping off like symptoms in infected plants produces visible brown and compact sclerotia around the infected region (Sharma and Sohli 1980). Even *R. solani* infestation on rice, caused 50% yield loss in rice growing states of India (Chahal *et al*., 2003). Likewise; *S. rolfsii* Sacc. (Curzi) Tu & Kimbr., a corticoid and strong phytopathogen cause yearly yield loss of 27-60% to agricultural crops and horticultural plants (Masum *et al*., 2017). In last few decades; in order to manage disease outbreak; the extensive use of synthetic fungicides had added fuel towards ecological disturbances, man-made pollution & several health disorders in humans. Taking these perspective in mind and pacing towards more potent, eco-friendly alternative as botanicals; six spices commonly used for culinary purposes were selected *viz. A. sativum* (Liliaceae), *M. fragrans* (Myristicaceae), *P. nigrum* (Piperaceae), *T. ammi* (Apiaceae), *T. foenumgraecum* (Fabaceae), *Z. officinale* (Zingiberaceae) and a

medicinal herb *T. chebula* (Combretaceae). The aqueous extracts of these botanicals were screened for their biological efficacy against three disease causing fungi: *S. sclerotiorum, R. solani and S. rolfsii* and their effects were compared with two known synthetic fungicides *i.e.,* Carbendazim & Mancozeb.

MATERIALS AND METHODS

The plant parts of *Dolichos lablab* (Hyacinth bean) infected with crown rot disease, *Vigna radiata* (Green gram) infested with pod rot disease and *Glycine max* (soybean) showing collar rot disease were collected from agricultural field of ICAR-RCER, Research centre, Palandu, Ranchi, Jharkhand. 5-10 mm square lesions containing diseased & healthy region from infected plants were cut into pieces & subsequently washed with sterilized distilled water (DW). Surface sterilization of infected plant parts and sclerotia with 2% $NaOCl_2$ solution for 1-2 mins were done followed by final washing with DW. These were then transferred aseptically in sterile moist chamber fitted with Whatmann filter paper for induced mycelial growth. After 48hrs, with the appearance of mycelial threads in the test fungi; these were then subjected for proper identification, culturing & purification at 24±2°C. After undergoing pathogenicity test for the selected fungi, the sub-culturing and further experimental works were performed. In the present study, five to seven days old fungal culture were used in bioassay.

Non-infected rhizome of ginger, bulb of garlic, seeds of nutmeg, ajwain, myrobalan, fenugreek & black pepper were brought from local market of Jharkhand. The collected materials were thoroughly washed under tap water and further by sterilized DW. The washed seeds of selected spice and medicinal herb were then kept for 24hrs at room temperature (24±2°C) for shade drying & later were finely ground in powder under sterilized mortar and pestle. Rhizome of ginger and bulb of garlic after thorough washing with tap water and distilled water were peeled and chopped. The chopped garlic was left for 1hr in presence of oxygen and for the production of antimicrobial compound allicin (Leontiev *et al*., 2018).

The macerated and crude plant materials were mixed in 100ml of PDA media in ascending order of their weight *viz.*1, 2, 3, 4 & 5g to make concentration of 1, 2, 3, 4 & 5% against the test fungi. It was then autoclaved at 121°C, under 15psi for 15min. From the fully grown fresh culture of *S. sclerotiorum, R. solani* and *S. rolfsii*; 5mm beads were cut & inoculated in various treatments including control. Moreover, two synthetic fungicides namely Carbendazim 50WP (500, 750 & 1000ppm) and Mancozeb 75WP (1500, 2000 & 2500ppm) were mixed in autoclaved PDA media and tested for comparison in three replicates. The observations on mycelia and sclerotial growth of the test fungi were recorded at each 24hrs interval. Percent growth inhibition on radial growth of test fungi were calculated using following formula (Okigbo and Nmeka 2005):

Growth inhibition (I)% = [(DC-DT)/DC] × 100

Where,

DC = Average diameter of fungal colony in control.
DT = Average diameter of fungal colony in treatment.

RESULTS AND DISCUSSIONS

Efficacy of aqueous extract of selected spices against *S. sclerotiorum.* Antifungal efficacy of the aqueous extracts of six spices and a medicinal herb showed varied sensitivity against the tested soil borne fungal pathogens. *T. chebula* showed >70% inhibition of *S. sclerotiorum* at all tested concentrations (Fig. 1 a & f). Moreover, *A. sativum* & *T. ammi* at 4-5% inhibited 100% growth of *S. sclerotiorum* (Fig. 1 d & e). In *Z. officinale* aqueous extract; there were full mycelia growth at 1-4% but no sclerotia formation was observed at 3-5% upto 120 hrs. At 5% efficacy of *Z. officinale* against *S. sclerotiorum* was 51.85% while *P. nigrum* was more than 50% effective at 3-5% (Fig. 1 c). Against *S. sclerotiorum*; *M. fragrans* at 5% was 70.3% effective but *T. foenumgraecum* failed in its efficacy at all test concentrations (Table 1a). Carbendazim 50 WP was 100% effective against *S. sclerotiorum* at all test concentrations *viz*., 500 ppm, 750 ppm and 1000 ppm. Similarly, Mancozeb 75 WP was strongly effective against *S. sclerotiorum* & *R. solani* at conc. 1500, 2000 and 2500 ppm (Table 2).

Efficacy of aqueous extract of selected spices against *R. solani.* Through present study it was noted that on solid media *A. sativum, M. fragrans, T. ammi, T. chebula* & *P. nigrum* showed 100% inhibition of *R. solani* from 3-5% as compared to control which showed full mycelial growth and sclerotial formation. However, *M. fragrans, T. ammi, T. chebula* & *P. nigrum* were 100% effective against *R. solani* right from the initial concentration 1-5% (Table 1 c). Carbendazim 50 WP was 100% effective against *R. solani* at all test concentrations *viz*. 500 ppm, 750 ppm and 1000 ppm. Similarly, Mancozeb 75 WP was strongly effective against *R. solani* at conc. 1500, 2000 and 2500 ppm (Table 2). Ginger aqueous extract failed to inhibit radial growth of mycelia of *R. solani* at all test concentrations (Table 1 c). It also showed deformed sclerotia located haphazardly along with similar high oxalic acid secretion (oozing) unlike control (Fig. 1 g & h). Against fenugreek; at low concentration (1-3%) *R. solani* grew faster than control. After so much fluffy mycelial growth in fenugreek, delayed sclerotial formation (by 72hrs) in 4-5% compared to control was observed. However; it was noticeable to find fenugreek to be 68.1% effective at 5% conc. against *R. solani* (Fig.1.i). Maximum oxalic acid secretion (oozing) nearby the sclerotia were observed in control as compared to all the tested spices and the medicinal herb.

Efficacy of aqueous extract of selected spices against *S. rolfsii.* Against *S. rolfsii*; on PDA media, the aqueous extracts of botanicals *viz. M. fragrans, P. nigrum, T. chebula* and *T. ammi* were showing high fungitoxic property *i.e.* more than 70% inhibition right from initial concentration (Table 1 b). *T. foenumgraecum* failed to inhibit mycelia as well as sclerotial formation of *S. rolfsii* at lower conc. but at 5% conc. it was effective upto 52.2%. Also, as compared to control; the *S. rolfsii*

mycelia grew in spiral fashion containing the aqueous extract of *T. foenumgraecum* at all test concentrations (Fig. 1 k & l).

Antifungal efficacy of aqueous extracts of selected spices and the medicinal herb at different concentrations showed varying responses against selected soil borne sclerotial phytopathogens; Among sclerotial fungi; oozing in *R. solani* sclerotia was only produced in case of control and against ginger aqueous extracts *in vitro*. This oxalic acid exudate of *R. solani* in its sclerotial periphery certifies its pathogenesis factor and very similarly relates to the work of Singh *et al.* (2002); Molla *et al*. (2013). Previous researches also confirmed the integrated disease management efforts using waste products like cowurine, vermi-wash, botanicals and bio-control agents to combat the effect of *R. solani* and *S. sclerotiorum in vitro* (Jandaik *et al*., 2015; Singh *et al*., 2002). Present work goes in accordance with Sehajpal *et al.* (2009) who showed that garlic gave maximum fungitoxic effect against *R. solani*, at 1000 ppm. Similar effect of botanical extracts *viz. A. indica* and *O. sanctum* on *R. solani* was also reported by Gurjar *et al*. (2012).

The findings of high efficacy of garlic from 2-5% were in consonance with the work of Dutta *et al*. (2004), who reported that crude garlic aqueous extract could exhibit total sclerotial and mycelia inhibition in *R. solani*. Present research studies is comparable to the work done by Abdel-Kader *et al*. (2014) who concluded that chemical fungicides successfully controlled sclerotial germination in *Sclerotinia* spp. while Kumawat *et al*. (2018) also confirmed that Carbendazim 50 wp and Mancozeb 75 wp completely inhibited the growth of *S. sclerotiorum*. High efficacy of garlic at 3-5% and ginger at 5% concentration against *S. rolfsii* were also confirmed in the previous findings of Sneha *et al*. (2016).

Table 1: Antifungal efficacy of botanicals against radial growth of *S. sclerotiorum*, *S. rolfsii* and *R. solani.*

Botanicals extracts	Control	1%	2%	3%	4%	5%
		RG (Inhib%)	RG (Inhib%)	RG (Inhib%)	RG (Inhib%)	RG (Inhib%)
a. Inhibition of Mycelial growth of *Sclerotinia sclerotiorum*						
A. sativum	90.00 ± 0.0 (0)	90.00 ± 0.00 (0)	78.66 ± 3.17 (12.6)	42.66 ± 3.92 (52.6)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
M. fragrans	90.00 ± 0.0 (0)	46.00 ± 3.05 (48.8)	45.33 ± 1.20 (49.63)	43.66 ± 0.66 (51.4)	41.66 ± 2.02 (53.7)	41.0 ± 0.57 (54.4)
P. nigrum	90.00 ± 0.0 (0)	61.33 ± 0.9 (31.85)	55.00 ± 2.9 (38.8)	43.00 ± 1.0 (52.2)	27.66 ± 1.4 (69.2)	26.66 ± 4.40 (70.3)
T. chebula	90.00 ± 0.0 (0)	25.00 ± 2.9 (72.2)	23.30 ± 1.7 (74.1)	23.00 ± 2.5 (74.4)	21.66 ± 1.2 (75.9)	21.33 ± 0.88 (76.3)
T. ammi	90.00 ± 0.0 (0)	81.33 ± 2.0 (9.63)	76.33 ± 1.9 (15.1)	43.66 ± 1.8 (51.4)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
T. foenum-graecum	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	70.00 ± 2.88 (22.2)
Z. officinale	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	85.66 ± 1.2 (4.8)	59.33 ± 0.9 (34.0)	51.66 ± 1.9 (42.6)	43.33 ± 1.45 (51.85)
b. Inhibition of Mycelial growth of *S. rolfsii*						
A. sativum	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	50.6 ± 1.4 (43.7)	0.0 ± 0.0 (100)	0.0 ± 0.0 (100)
M. fragrans	90.00 ± 0.0 (0)	8.00 ± 1.73 (91.1)	6.66 ± 0.33 (92.6)	6.66 ± 0.33 (92.6)	6.33 ± 1.33 (92.9)	5.66 ± 0.66 (93.7)
P. nigrum	90.00 ± 0.0 (0)	16.00 ± 2.08 (82.2)	14.66 ± 1.45 (83.7)	12.33 ± 2.33 (86.3)	10.66 ± 3.48 (88.1)	8.00 ± 1.73 (91.1)
T. chebula	90.00 ± 0.0 (0)	24.00 ± 1.73 (73.3)	20.66 ± 0.66 (77.0)	16.00 ± 2.64 (82.2)	11.66 ± 5.69 (87.0)	9.66 ± 0.33 (89.2)
T. ammi	90.00 ± 0.0 (0)	23.66 ± 1.85 (73.7)	15.33 ± 2.02 (82.9)	10.33 ± 1.20 (88.5)	6.66 ± 1.66 (92.6)	6.33 ± 1.33 (92.9)
T. foenum-graecum	90.00 ± 0.0 (0)	82.33 ± 4.33 (8.5)	74.66 ± 2.40 (17)	74.33 ± 0.66 (17.4)	56.33 ± 0.88 (37.4)	43.00 ± 1.00 (52.2)
Z. officinale	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	90.00 ± 0.0 (0)	80.6 ± 4.0 (10.4)	74.0 ± 3.5 (17.2)	43.6 ± 3.4 (51.5)
c. Inhibition of Mycelial growth of *R. solani*						
A. sativum	90.00 ± 0.0 (0)	90.00 ± 0.00 (0)	25.33 ± 1.45 (71.8)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
M. fragrans	90.00 ± 0.0 (0)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
P. nigrum	90.00 ± 0.0 (0)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
T. chebula	90.00 ± 0.0 (0)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
T. ammi	90.00 ± 0.0 (0)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)	0.00 ± 0.00 (100)
T. foenum-graecum	90.00 ± 0.0 (0)	90.00 ± 0.00 (0)	90.00 ± 0.00 (0)	90.00 ± 0.00 (0)	86.33 ± 2.02 (4.4)	28.66 ± 1.20 (68.1)
Z. officinale	90.00 ± 0.0 (0)	90.00 ± 0.00 (0)	90.00 ± 0.00 (0)	90.00 ± 0.00 (0)	90.00 ± 0.00 (0)	90.00 ± 0.00 (0)

RG - Radial growth (Mean ± Standard Error), Inhib% - Percentage inhibition

Table 2: Radial growth of sclerotial fungi against synthetic fungicides.

Mycelial growth inhibition							
Carbendazim				Mancozeb			
R. solani							
Control	500 ppm	750 ppm	1000 ppm	Control	1500 ppm	2000 ppm	2500 ppm
90.0 ± 0.0	0.0	0.0	0.0	90.0 ± 0	0.0	0.0	0.0
S. sclerotiorum							
90.0 ± 0.0	0.0	0.0	0.0	90.0 ± 0	0.0	0.0	0.0
S. rolfsii							
90.0 ± 0.0	0.0	0.0	0.0	90.0 ± 0	0.0	0.0	0.0

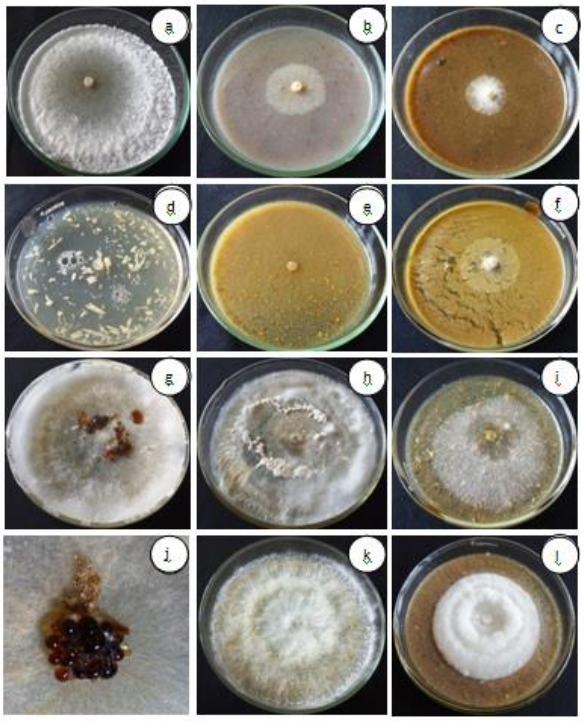

Fig. 1. Aqueous extracts of botanicals at 5% concentration showing Inhibition in radial growth of:
a. *S. sclerotiorum* in control b. Nutmeg against *S. sclerotiorum* c. Black pepper against *S. sclerotiorum* d. Garlic against *S. sclerotiorum* e. Ajwain against *S. sclerotiorum* f. Myrobalan against *S. sclerotiorum* g. *R. solani* in control h. Ginger against *R. solani* i. Fenugreek against *R. solani* j. Oozing in *R. solani* sclerotia k. *S. rolfsii* in control l. Fenugreek against *S. rolfsii*.

CONCLUSIONS

Previous works and the present study on spices and medicinal herb showed that the active principle present within them got exposed in their aqueous extracts (Dutta *et al*., 2004). The compound present within them showed high degree of antifungal properties against three selected deadly phytopathogens *viz. R. solani*, *S. rolfsii* and *S. sclerotiorum.* The efficacies of these botanicals had similar effect unlike the selected synthetic fungicides namely Carbendazim 50WP & Mancozeb 75WP. This experiment demonstrates that use of spices: the underground crop like garlic, ginger or above ground plants like fenugreek, black pepper and ajwain when introduced through crop rotation system to the field; might even show allelopathic effect against above soils borne sclerotial fungi. The antifungal compounds present within these spices and medicinal herbs when isolated and synthesized as bioformulations would pace up the disease management strategies against soil-borne phytopathogens.

FUTURE SCOPE

Synthetic pesticides had caused much harm to soil microflora and soil quality. Use of the crude extracts of such spices and herb for disease management would not only enhance the soil fertility but also cause minimal harm to the grazing cattle, birds and humans. Extracting the organic active principle of the selected botanical through chromatography method; converting these into bio-formulations or nano-particle would increase its efficiency to many fold against fungal phytopathogens.

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Conflict of Interest. None.

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