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Research Article

INVITRO BIO-ASSAY OF SOME BOTANICALS AGAINST SOME IMPORTANT SOIL-BORNE FUNGI

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ABSTRACT

Sclerotinia sclerotiorum causing white mold disease, *Fusarium solani* and *Rhizoctonia solani* causing root rot/wilt complex and *Sclerotium rolfsii* causing collar rot are economically important soil-borne pathogens of various crops having wide host range. Eight different botanical extracts were evaluated for their antifungal effects against the four above-mentioned fungi under laboratory conditions using food poison technique. Five percent crude extracts of garlic clove (*Allium sativum*), rhizome of bojo (*Acorus calamus*), leaves of castor (*Ricinus communis*), asuro (*Justicia adhatoda*), sambucus (*Sambucus hookeri*), neem (*Azadirachta indica*), bougainvillea (*Bougainvillea glabra*), and dried fruits of timur/Sichuan pepper (*Zanthoxylum armatum*) were included in the experiment. Three PDA plates for each treatment were inoculated with each pathogen and incubated at room temperature (24-25°C). Efficacy of the treatments on inhibition of radial growth of the pathogen colony was assessed 96 hours (4 days) after inoculation and compared with control without botanical extract. Significant inhibitory effect on radial growth of *S. sclerotiorum* was found with garlic (70% inhibition) followed by sambucus (22%) and asuro (12%). It was also observed that asuro treated plates had least sclerotia formation of the pathogen followed by sambucus. Bojo was found effective in inhibiting the radial growth of *R. solani* by 14% and *S. rolfsii* by 54% whereas, castor extract was found effective against *F. solani* with 21% inhibition in colony growth. Hence, these botanicals could have the potential in managing the diseases incited by the test pathogens, however, firstly their efficacy in the field conditions needs to be verified.

Key words: Botanical, colony growth, inhibition, soil-borne

INTRODUCTION

Soil-borne diseases are one of the major limitations to crop production, particularly for vegetables. They are often difficult to control, even with conventional strategies. Many soil-

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borne fungi persist in soil for long periods because they produce resistant survival structures such as melanized hyphae, chlamydospores, oospores, and sclerotia. They often survive on host plant debris, soil organic matter, or as free-living organisms. *Rhizoctonia solani*, *Sclerotinia sclerotiorum,Fusarium solani* and *Sclerotium rolfsii* are economically important soil-borne fungal pathogens having wide host range. *R. solani* causes damping-off, stem rot and root rot on various crops (Sneh *et al.*, 1991). *S. sclerotiorum* causes white rot, stalk rot and wilting on different host plants (Bolton *et al.*, 2006), and is known to attack over 400 species of host plants worldwide in many different soil types and environmental conditions (Boland and Hall, 1994). Traditionally, the fungal diseases of plants are controlled by using synthetic fungicides.

Indiscriminate use of synthetic fungicides is not only expensive, but also hazardous to the environment. On the other hand, it may result into development of resistance in the pathogens. To overcome these problems, some alternative control methods are needed to be employed. Recently, in different parts of the world, attention has been paid towards exploitation of plant products as novel chemo therapeutants in plant protection. Now a days, the antifungal action of plant extracts has gained much attention and are being used against many plant pathogenic fungi (Swami and Alane, 2013). The plants serve as ecofriendly and economic biocontrol agents.

Antifungal and antibacterial properties have been shown in a number of plants, such as onion bulb extract, leaf and flower extract of dhatura, mentha, and many more and those curing the diseases like root rot, powdery mildew, rusts etc (Ghosh, 2000; Gurjar *et al*., 2012). Botanicals such as *A. sativum* (garlic) and *eucalyptus* were found effective to reduce mustard diseases like Alternaria blight, white rust and Sclerotinia rot (Yadav, 2009). However, neem (*Azadirachta indica, A. juss*) products and pyrethroids are well established commercially as botanical pesticides. Garlic (*Allium sativum*), eucalyptus (*Eucalyptus globulus*), turmeric (*Curcuma longa*), tobacco (*Nicotiana tabacum*), and ginger (*Zingiber officinale*) are the commonly used plant extracts in plant disease control (Gurjar *et al.*, 2012). Hence, botanicals could be one of the sustainable components of integrated disease management in agriculture because, they are eco-friendly, easily bio-degradable, cheaper and can be used in organic farming (Gurjar *et al.*, 2012).

Keeping this in view, a study was conducted to find the effectiveness of some plant extracts against economically important four soil-borne fungi: *Sclerotinia sclerotiorum* (white rot fungus), *Rhizoctonia solani*, (wire stem and root rot fungus), *Fusarium solani* (root rot fungus) and *Sclerotium rolfsii* (collar rot fungus).

MATERIALS AND METHODS

Collection of pathogen isolates

Isolates of *S. sclerotiorum, Rhizoctonia solani, Fusarium solani* and *Sclerotium rolfsii* were isolated from diseased plants of eggplant, cauliflower, tomato and bean, respectively. Pieces of infected plant tissue were surface sterilized in 1% sodium hypochlorite (NaOCl) solution by dipping for 1-2 minutes and dried well under laminar flow. The tissue pieces were placed on 2% water agar plates and incubated at 24-25^{o}C for 24 to 48 hours depending on the growth. Typically growing colonies of the such pathogens were observed under stereo and compound microscope for identification. Purification of those fungi was carried using disc from actively growing point on potato dextrose agar (PDA) plates. Incubation of those plates was done at 24-25 $^{\circ}$C for 4-5 days.

Testing of botanicals

Fungitoxicity of different botanicals mentioned to having antibiotic or antifungal properties was studied by poisoned food technique (Nene and Thapliyal, 1979). After thorough washing with clean water, aqueous extracts of six different plant species were prepared by blending water and leaves of castor (*Ricinus communis*), bojo (*Acorus calamus*), asuro (*Justicia adhatoda*), sambucus (*Sambucus hookeri*), neem (*Azadirachta indica*), bougainvillea (*Bougainvillea glabra*), and cloves of garlic (*Allium sativum*) at the ratio of 1:1 individually. Driedfruits of timur (*Zanthoxylum armatum*) at the ratio of 1:10 were boiled for 10 minutes. These concentrate extracts were filtered through double layer muslin cloth and autoclaved for 10 minutes at 115^{o}C. Amendment of these botanical extracts in PDAwas done separately to give 5% concentration at luke warm stage and mixed well before pouring onto Petriplates. PDA plates without any botanical amendment were used as control.

A small disc of 0.5 cm size of the actively growing pure culture of the test fungus grown on PDA for seven days was cut with a sterile cork borer and transferred aseptically at the centre of the PDA plate containing agar medium and plant extract. The plates were incubated at 24-25^{o}C for 96 hours. Three plates per treatment for each pathogen were used as replications. The plates were arranged in completely randomized design. The colony diameter was measured from two sides and average diameter was noted at 48, 72 and 96 hours after inoculation. In case of *S. sclerotiorum*, total number of sclerotia produced was noted 16 days after inoculation. Finally, percent reduction or inhibition in radial colony diameter was calculated. Efficacy of the treatments or the botanicals was assessed by comparing with the growth in control. Percent inhibition was calculated by using the formula given below. The data were analysed using MSTAT-C.

$$R/I\% = \frac{RG_c - RG_t}{RG_c} \times 100$$

where,

R/I = Reduction/inhibition
RG_c= Radial growth in control
RG_t= Radial growth in treatment

RESULTS AND DISCUSSION

Results presented in table 1 and 2 show the response of four challenging soil-borne fungal pathogens to the inhibitory effect of tested botanical extracts. The efficacy was found to be significant in the inhibition of radial growth of the tested fungi. Significant reduction in mean colony diameter of *S. sclerotiorum* in garlic extract by 57% and 82% was observed during the year 2013 and 2014, respectively (Table 1). Pooled inhibition or reduction in mean colony growth was 70%. Similar result was found with garlic extract by Yadav (2009). Effects of bojo and timur were not found significant; however, reduction in mean colony diameter was only 10% in bojo. Among the treatments, garlic extract was found to be the most effective against *R. solani, F. solani and S. rolfsii* and mean colony diameter reduction were found 70%, 27% and 100%, respectively (Table 2). Sehajpal *et al*. (2009) also showed the strong fungitoxic activity of garlic extract against *R. solani*. Bojo was also effective in reducing colony diameter of *R. solani* by 14% and *S. rolfsii* by 54% . With *F. solani*, effect of castor extract was observed to be significant with 21% reduction in colony diameter compared to the control. Other test treatments were not much effective. The colony growth of *F.solani* was very slow, even in control plates.

Significant differences were found in number of sclerotia formation of *S. sclerotiorum* in test botanicals-amended media (Figure 1). Number of sclerotia formation was highly reduced in asuro treatment with mean number of sclerotia being 16 compared to control with 48 sclerotia. Asuro was not significantly effective to check the mycelial growth of *S. sclerotiorum* (Table 1). Effect of sambucus was also obvious to some extent in significantly reduced sclerotia production. Mycelial growth of *R. solani* was observed to be stimulated by asuro and sambucus in which the radial growth of the colony were increased by 7% and 4% (in the first year) and 13% and 40% (in the second year test, data not shown) over control (Table 2).

Different botanical extracts have been studied by many researchers for the control of root rot fungi. The efficacy of the promising botanicals could be affected by the concentrations used as well as extraction methods. Considering the possible complications associating with chemical extraction methods, simple crude aqueous extraction technique was adopted in the present experiment for easy use by the farmers. The use of locally available plant extracts with antifungal properties would not only provide a potent tool for control of particular diseases, but also could act as the alternatives to conventional fungicides. However, most of their studies are limited *in-vitro* or under laboratory conditions.

Mokhtar *et al.* (2014) found eucalyptus leaves extract effective to reduce growth of *R. solani* and *F. solani* significantly. Azadirachtin and neem oil were effective in inhibiting the growth of *S. rolfsii, R. solani* and *Fusarium* sp. (Sangeetha and Jahagirdar, 2013) where as in the present study neem extract stimulated the growth of *R. solani* instead of inhibiting the growth. It was also not found effective against *S. sclerotiorum*, contradicting with the results of Mello *et al*.(2005). However, it reduced the growth of *F. solani* by 13% but was not significant compared to control. Enespa and Dwivedi (2014) reported 64% and 74% inhibition of growth of *F. solani* by 25% and 50% concentration of leaf extract of neem, respectively. As for the asuro on *S.sclerotiorum*, it was not effective in inhibiting the hyphal growth but significantly reduced number of sclerotia production. This indicates that its use could be helpful in reducing the inoculum density in the field to manage Sclerotinia disease. Number of botanical extracts such as drek, onion, tulsi, bael and paanchphooli were effective in inhibiting mycelial growth of *R. solani* (Dutta and Kalha, 2011). In the present study, only 13% reduction of the colony growth of *S. rolfsii* was found, whereas, 80-86% inhibition in colony growth was reported by Singh *et al.,* 2007). Since we used the botanicals at only 5% concentration, the higher percent inhibition may be achieved with the use of higher concentration of the botanicals.

CONCLUSION

Based on the results obtained, garlic could be used to manage the diseases caused by *Sclerotinia sclerotiorum, Fusarium solani, Sclerotium rolfsii and Rhizoctonia solani*. Meanwhile, asuro and sambucus extracts may be used against *S. sclerotiorum*, bojo (Acorus) rhizome extract against *R. solani* and *S. rolfsii* and, castor leaf extract against *F. solani*. These botanicals can be used to minimize diseases caused by above-mentioned fungi as an alternative to pesticides and as an environmentally safe measure. However, the present study was limited in *in-vitro* conditions, their efficacy must be verified under field conditions before taking it to the farmers.

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Table 1. *In-vitro* effects of different botanical extracts (5% concentration) on the growth of *Sclerotinia sclerotiorum*

Botanical extracts	*S. sclerotiorum*			
	2013		2014	
	Mean colony diameter (cm)	Reduction percent in mean colony diameter	Mean colony diameter (cm)	Reduction percent in mean colony diameter
Garlic (*Allium sativum*)	3.4 c	56.96	1.4 c	82.5
Castor (*Ricinus communis*)	7.9 a	0.00	8.0 a	0.00
Bojo (*Achorus calamus*)	7.1 b	10.12	8.0 a	0.00
Asuro (*Justicia adhatoda*)	7.9 a	0.00	6.03 ab	24.62
Sambucus (*Sambucus hookeri*)	7.7 ab	13.88	5.63 b	29.62
Timur (*Zanthoxyllum armatum*)	7.1 b	10.12	7.33 ab	8.37
Neem (*Azadirachta indica*)	-	-	8.0 a	0.00
Bougainvillea (*Bougainvillea glabra*)	-	-	8.00 a	0.00
Control	7.9 a	-	8.00 a	
CV %	5.25		15.76	
P value	<0.0001		<0.0001	

Mean followed by the same letter are not significantly different by DMRT at P=0.05

Table 2. ***In-vitro*** **effects of different botanical extracts (5% concentration) on the growth of** ***Sclerotium rolfsii, Fusarium solani*** **and** ***Rhizoctonia solani***

Botanical extracts	*S. rolfsii*		*F. solani*		*R. solani*	
	Mean colony diameter (cm)	Reduction in mean colony diameter (%)	Mean colony diameter (cm)	Reduction in mean colony diameter (%)	Mean colony diameter (cm)	Reduction in mean colony diameter (%)
Garlic (*Allium sativum*)	0.00 c	100	2.2 c	26.66	2.0 b	69.69
Castor (*Ricinus communis*)	2.43 a	2.8	2.3 bc	21.21	5.7 c	13.63
Bojo (*Achorus calamus*)	1.13 b	54.5	2.9 ab	3.33	2.9 d	56.06
Asuro (*Justicia adhatoda*)	2.33 a	6.8	3.1 a	3.33	7.1 a	-7.04
Sambucus hookeri	2.43 a	2.8	2.8 abd	6.66	6.9 ab	-4.34
Timur (*Zanthoxyllum armatum*)	2.33a	6.8	2.6 abc	13.33	6.0 bc	9.09
Neem (*Azadirachta indica*)	2.17 a	13.2	4.27 ab	-14.47	-	-
Bougainvillea glabra	2.5 a	0.00	4.3 ab	-15.28	-	-
Control	2.5 a	-	3.73 ab		6.6 abc	-
CV %	10.62		12.67		9.87	
P value	<0.0001		0.0350		<0.0001	

Mean with the same letter are not significantly different at P=0.05% by DMRT

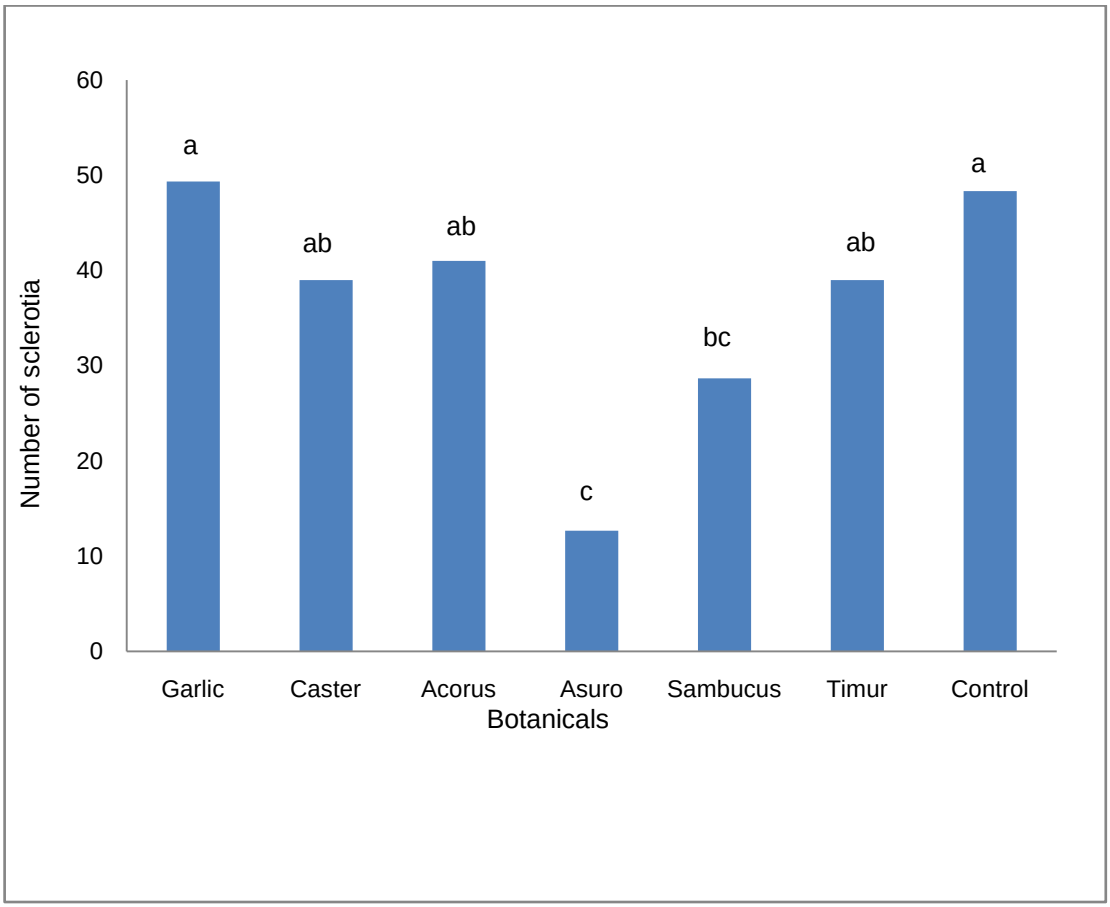

Fig. 1: Effect of different botanicals on number of sclerotia formation of *Sclerotinia sclerotiorum* (column followed by the same letter are not significantly different at p=0.05 by Duncan' multiple range test, CV=23.96%; P value=0.0023)