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Evaluation of Effectivity of Phytoextracts on Radial Growth of *Sclerotinia sclerotiorum*

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A B S T R A C T

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is the most damaging disease and has been recorded in rapeseed-mustard cultivating nations of the world. It is more common and severe in temperate and sub-tropical regions during cool and wet seasons, although it may also be found in some semi-arid regions where conditions seem unfavourable for disease development. Botanical extracts are used as fungicides to control fungal growth and detection of new antifungal compounds which have no side effects on the environment or animal health. The evaluation of effectiveness of different phytoextracts were carried out through food poisoning techniques. Application of phytoextracts through food poisoning technique significantly reduced the myceliogenic growth of *Sclerotinia sclerotiorum*. *Allium sativum* registered maximum percent of mycelial inhibition of *S. sclerotiorum*, followed by *Citrus lemon*, *A. indica*, *M. koenigii* and *A. cepa*. Concentration wise, greater myceliogenic inhibition was observed in 15% fallowed by 10% and 5% phytoextract containing petri plates. Absolute mycelial inhibition was observed in *A. sativum* (15 and 10 % concentrations) treated plates. On the other hand, least efficacy of the phytoextract was observed in 5% *A. cepa* treated plate. The current *in vitro* studies of botanicals mycelial growth of *S. sclerotiorum* revealed that phytoextracts are an important component in crop disease management.

Keywords

Sclerotinia sclerotiorum, phytoextracts, radial growth, pathogen

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Introduction

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib.) de Bary is the most damaging disease and has been recorded in rapeseed-mustard cultivating nations of the world (Sharma 2014). It is more common and severe in temperate and sub-tropical regions during cool and wet seasons, although it may also be found in some semi-arid regions where conditions seem unfavourable for disease development. Disease outbreaks in drier areas occur in irrigated fields because irrigation provides favourable conditions for disease development (Sharma, 2014).

In India, *Sclerotinia* stem rot was first reported by Shaw and Ajreker (1915) from Pusa (Bihar). Later on, the occurrence of this disease has also been observed in other parts of the country (Roy and Saikia 1976). This disease is more severe in high land areas in north-eastern states and certain areas of Rajasthan (Shivpuri *et al.,* 2000). During the eighties and nineties, this disease was of minor importance but over the years due to the adoption of newer crop husbandry practices and lack of host resistance, it has become a serious threat to rapeseed-mustard cultivation (Kolte, 1985).

In Raya *(B. juncea*) growing areas of Rajasthan and Haryana states where farmers perform mono-cropping and cultivate the crops under irrigated conditions. This pathogen become very destructive and results some time total failure to crop yield. Sclerotinia stem rot is a serious problem in major rapeseed-mustard growing areas of the country, such as Uttar Pradesh, Rajasthan, Haryana, Punjab, Madhya Pradesh, West Bengal, Bihar, and Assam (Ghasolia *et al.,* 2004; Sharma *et al.,* 2015;). This disease has now attained next position to Alternaria blight in terms of its economic importance (Parveen *et al.,* 2007). The yield losses vary considerably with the growth stage of the plant and the number of plants infected. If plants are infected at the early flowering stage produce little or no seed, but those infected at the late flowering stage results in no yield (Kolte, 1985).

Generally, the yield losses due to stem rot in rapeseed-mustard have been recorded from 40 – 80 % in different growing regions of the country, and as high as up to 100 % in severe outbreaks in Raya growing areas of Rajasthan and Haryana (Sharma *et al.,* 2001; Singh *et al.,* 2003; Ghasolia *et al.,* 2004).

S. sclerotiorum is a necrotrophic pathogen and infects the plant tissues severely and appears to be a non-specific pathogen, producing white rot or mold or soft rot symptoms on different plant parts such as leaf, stem, root, and fruit (Sharma *et al.,* 2015). The disease is monocyclic and symptoms usually appear 4-6 weeks after sowing or at the post-flowering stage. Sudden drooping of leaves followed by drying of plants are characteristic features of the disease. Initial symptoms include the appearance of elongated water-soaked lesions at the base of the stem which usually expands rapidly, becomes bleached, necrotic, and subsequently develops patches of fluffy white mycelium (Sanogo and Puppala, 2007).

When the stem is completely girdled and covered by a cottony mycelium growth, it breaks from where it shows rotting and drying (Bolton *et al.,* 2006). However, infection is restricted to a smaller area of pith, resulting in slow stunting of the plant and premature ripening.

The fungus initially produces white-coloured small melanized resting mycelial aggregates on the collar or crown region of the stem, later turning into black colour hardened sclerotia. When such infected stem of the host is split large cavities are lined by fluffy mycelium and numerous black-coloured sclerotia are observed (Tripathi *et al.,* 2017).

The sclerotia serves as overwintering or over summering structures of the pathogen and are germinated directly with new mycelial growth in presence of nutrients (myceliogenic germination) and are devoid of nutrients they germinate to form apothecia and liberate ascospores (Huang, and Kozub, 1991). Hyphae from myceliogenically germinated sclerotia infect the basal portion of the stem.

During the periods of high moisture and favourable temperature, the sclerotia are germinated carpogenically and produce ascospores which are germinated on the petals deposited on the leaves and act as a nutrient substrate for developing germ tube and infection. These spores act as the main source of primary inoculum and may travel a long-distance field for causing infection and epidemics (Adams and Ayers, 1979; Sun and Yang, 2000).

The devastating nature of *S. sclerotiorum* is also attributed to its prolonged survival, structure which is often facilitated by the production of vegetative sclerotia that provide primary inoculum for subsequent growing seasons (Saharan and Mehta, 2008; Sharma *et al.,* 2015). The mono-cropping of rapeseed mustard is reported to favour the disease severity in a specific geographical area, because sclerotia of the pathogen may survive for 4 to 8 years in soil under favourable conditions (Willets and Wong, 1980; Bardin and Huang, 2001).

Materials and Methods

All experiments were carried out under laboratory conditions in the Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh (U.P.) India.

Phytoextracts

Antifungal activity of five plant extracts such as Garlic extract (*Allium sativum*), Onion extract (*Allium cepa*), Neem extract (*Azadirachta indica*), Curry leaves (*Murraya koenigii***)**, lemon leaves (*Citrus lemon*) was evaluated against *S. sclerotiorum*, using poison food technique. The aforementioned plant parts were collected from AMU, Campus. About 100 gm of fresh plant leaves/rhizome/bulbs were taken and thoroughly washed in distilled water. Such plant parts were cut into small pieces and then ground in mortar and pestle by adding 100 ml of sterilized distilled water. The crude material was then filtered through double layer muslin cloth and then the filtrate was filtered through Whatman no. 1 filter paper. The plant extracts so prepared were heated at 400C for 5 minutes to avoid contamination (Jagnathan and Narsimhan, 1988). The requisite amount of plant extracts was incorporated into sterilized non-solidified potato dextrose agar medium (PDA) and shaken well to make it homogenous. Thereafter, 20 ml of amended medium was then poured into 90 mm Petri plates.

Sterilization of glassware

The sterilization of glassware (wrapped in butter paper/ brown paper) was done by autoclaving at 15 *psi* pressure for 30 minutes. Autoclaved glassware was dried in a hot air oven at 80°C for 45 to 60 minutes. All in vitro experiments were carried out under aseptic conditions.

Effect of phytoextracts on radial growth of *S. sclerotiorum*

The efficacy of five plant extracts was evaluated for their effectiveness in inhibiting the radial growth and sclerotial formation of *S. sclerotiorum* on potato dextrose agar medium. Each treatment was replicated thrice with a suitable check, wherein un-poisoned Petri plates (without plant extracts) were centrally inoculated with the test fungus. Each treatment was replicated thrice with a suitable check, wherein un-poisoned Petri plates (without nanoparticles, dual culture, and plant extracts) were centrally inoculated with the test fungus.

Per cent inhibition (T) = [(C-T) / C] × 100 Where, C = Colony growth diameter (mm) of fungus in check (in the unamended medium). T = Colony growth diameter (mm) of fungus in treatment (in fungicide amended medium).

Results and Discussion

This experiment revealed that plant extracts are important and may be used in sustainable management of plant pathogens. Application of phytoextracts through food poisoning technique significantly reduced the myceliogenic growth of *Sclerotinia sclerotiorum. Allium sativum* registered maximum percent of mycelial inhibition of *S. sclerotiorum*, followed by *Citrus lemon*, *A. indica*, *M. koenigii* and *A. cepa*. Concentration wise, greater myceliogenic inhibition was observed in 15% fallowed by 10% and 5% phytoextract containing petri plates. Absolute mycelial inhibition was observed in *A. sativum* (15 and 10 % concentrations) treated plates. On the other hand, least efficacy of the phytoextract was observed in 5% *A. cepa* treated plate (Table 2). The current *in vitro* studies of botanicals mycelial growth of *S. sclerotiorum* revealed that phytoextracts are an important component in crop disease management.

Fig.1 Effect of plant extracts on radial growth of *S. sclerotiorum.*

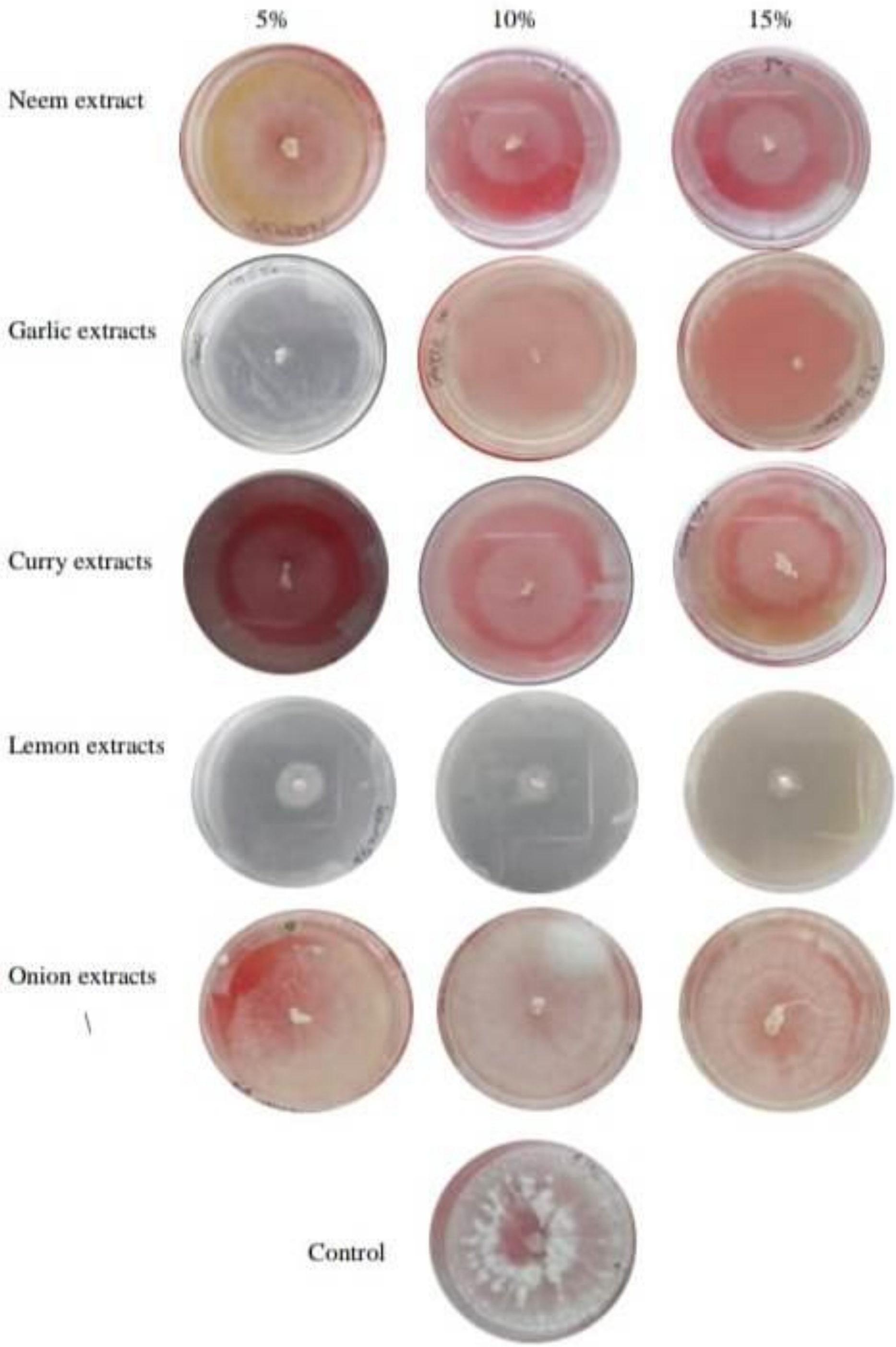

Table.1 Effect of phytoextract on radial growth of *Sclerotinia sclerotiorum.*

Phytoextracts	Concentrations					
	5%		10%		15%	
	Radial growth (mm)	% Inhibition	Radial growth (mm)	% Inhibition	Radial growth (mm)	% Inhibition
Azadirachta indica	55.5	38.33	27.4	69.55	16.7	81.44
Allium sativum	15	83.33	0	100	0	100
Allium cepa	72	20	65	27.77	60	33.33
Murraya konini	34	62.22	33	63.33	19.6	78.22
Citrus limon	22.5	75	20	77.77	12.5	86.11
control	90		90		90	
LSD P ≤0.05	6.75		4.32		3.85	

Mustard plants were found severely suffering from stem rot disease caused by *Sclerotinia sclerotiorum* which is a very devastating pathogen. The effectiveness of phytoextracts was also evaluated and it was observed that plant-based products may be used for the sustainable management of sclerotinia stem rot. The evaluation of effectiveness of different phytoextracts were carried out through food poisoning techniques. Among the phytoextracts, *Allium sativum* showed absolute inhibition in the myceliogenic growth of *S. sclerotiorum* followed by *Citrus lemon, Azadirachta indica, Murraya koenigii* and *Allium cepa.*

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