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Bioefficacy of Plant Extracts on Collar Rot Disease (*Sclerotium rolfsii* Sacc.) of Soybean

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Abstract

Among different production constraints in soybean cultivation, the most serious being is diseases. In Assam and other North Eastern states, collar rot caused by *Sclerotium rolfsii* Sacc. has been found to be a major disease causing plant death and low productivity as rainfall has played a significant role in the establishment of progression of collar rot in soybean. Therefore, the study was undertaken to evaluate the bioefficacy of some commonly available plant extracts against *Sclerotium rolfsii* Sacc. The aqueous plant extracts of commonly available six plant species were evaluated *in vitro* by poisoned food technique against their inhibitory effect on the mycelial growth of *Sclerotium rolfsii* Sacc. *Allium sativum* was found to be most effective with growth inhibition of 89.77% followed by *Chromolaena odorata* (86.00%), *Allamanda cathertica* (83.66%), *Laurus nobilis* (72.11%), *Ageratum conyzoides* (54.88%) and *Aegle marmelos* (50.66%).

Keywords: *Allium sativum*, plant extract, *Sclerotium rolfsii*

1. Introduction

Soybean (*Glycine max*) is most important source of vegetable oil in India that occupies 35-65% of total oilseed crop in the country. It is known as world's most important crop due to its high protein (35–45%) and oil content (15-25%). Due to high protein content, soybean is known as "poor man's meat". Seeds of soybean also contain about 33% carbohydrates, up to 16.6% of which are soluble sugars (Hou et al., 2009). Among the grain legumes, it has the greatest potential of producing the cheapest source of food protein (Rao and Reddy, 2010). Frequent soybean protein consumption lowers the cholesterol levels and also reduces the risk of coronary heart disease (FDA, 1999; Henkel, 2000). Moreover, it improves the glucose tolerance in some diabetic patients (Messina, 1999).

The crop is known to be attacked by many fungal, bacterial and viral pathogens causing substantial yield loss. Among the soil-borne fungal diseases, collar rot disease of soybean caused by *Sclerotium rolfsii* Sacc. is a potential threat to soybean production and is of considerable economic significance. The sclerotia produced by this pathogen remains in soil year after year (Chet and Henis, 1972; Punja, 1985). This disease is often found in soybean plants both dry land, rainfed and tidal with attack intensity of 5-55% (Marlina et al., 2020). *S. rolfsii* Sacc. attack on soybean plants causes damage to roots, stems, leaves and fruits.

Various ways have been carried out to control collar rot disease in soybean. Use of synthetic pesticides is most common. But synthetic pesticides imbalances our ecology, interferes our food chain and causes many abnormalities to the environment as it usually takes a long time to degrade completely (Damalas and Eleftherohorinos, 2011). Therefore, plant based pesticides appear to be one of the better alternatives for the control of plant diseases, as they are known to have minimal environmental problems and less danger to consumers in contrast to synthetic pesticides (Varma and Dubey, 1999). Plant extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties (Bhatnagar et al. 1990; Tewari, 1995; Fawzi et al., 2009). These botanical pesticides are important because they reduce crop losses; they are eco-friendly, bio-degradable and are cheap and affordable. Several researchers have shown that extracts of many plants may control different fungal plants pathogens. Some also have reported various plant extracts to have antimicrobial activity against *S. rolfsii* Sacc. infecting different crops, with positive outcomes (Jabbar Sab et al., 2014; Sacchi Sneha et al., 2016; Wavare et al., 2017).

Therefore, the present study has been done to explore the antimicrobial activities of different plant extracts against *Sclerotium rolfsii* Sacc. causing collar rot disease of soybean under laboratory condition.

2. Materials and Methods

2.1. Source of fungal pathogen, *Sclerotium rolfsii* Sacc

Soybean plants showing typical symptoms of collar rot were collected from the ICAR farm of Assam Agricultural University, Jorhat, Assam (India) (Latitute-26°45′ N, Longitue-94°12′ E, Altitude-87m with an elevation of 116m above mean sea level) and isolation of the pathogen was done after ascertaining their presence in the infected stem under the microscope. *Sclerotium rolfsii* Sacc. infection occurs at or just below the soil surface. Yellowing or wilting of plants is the first symptom. Leaves turn brown, dry and often cling to dead stem. Numerous tan to brown spherical sclerotia form on infected plant material (Figure 1, A-B) (Borah, 2019; Borah and Saikia, 2019; Belkar et al., 2013; Deb and Dutta, 1991). Thus, identification of the fungal pathogen was done and the pure culture of the fungus was maintained (Figure 1, C-D).

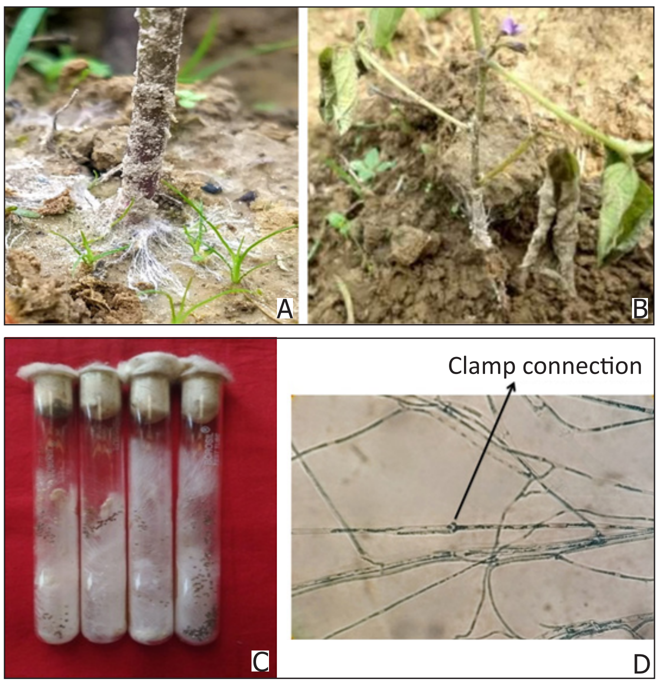

Figure 1: (A), (B) Infection of *Sclerotium rolfsii* Sacc. causing collar rot disease of soybean in Assam; (C) Isolation and pure culture of the fungus *Sclerotium rolfsii* Sacc. from the infected plants; (D) Microscopic observation of *Sclerotium rolfsii* Sacc. isolate showing mycelium of the fungus

2.2. Collection of botanicals

Different plant parts (fresh leaves, stem and bulbs) of six selected botanicals were collected from various localities of Jorhat, Assam (Latitute-26°45′ N, Longitue-94°12′ E, Altitude-87m with an elevation of 116 m above mean sea level) for the preparation of aqueous plant extracts. Different botanicals used in the investigation against *Sclerotium rolfsii* Sacc. Were *Aegle marmelos* (leaves), *Ageratum conyzoides* (leaves and stem), *Allamanda cathertica* (leaves), *Allium sativum* (bulb), *Chromolaena odorata* (leaves) and *Laurus nobilis* (leaves).

2.3. Preparation of aqueous plant extracts

Fresh leaves and other parts of healthy plants were collected and washed thoroughly in sterile distilled water. Hundred grams of washed plant parts was ground in pre-chilled mortar and pestle by adding equal amount (100 ml) of sterilized distilled water (1:1 w/v). After grinding, the extract was filtered through muslin cloth and finally the extracts were centrifuged at 10,000 rpm for 20 minutes in centrifuge (Remi C 24) at room temperature. The supernatant was taken as standard plant extract solution (100%). The method reported by Shekhawat and Prasad (1971) was followed with certain modifications. The plant extracts were further filtered through bacterial membrane filter (RanDisc, PVDF 0.22 µm) under aseptic condition and evaluated at 20% concentration (for preliminary screening) against the fungus by 'poisoned food technique' (Nene and Thapliyal, 2000).

2.4. Screening of different plant extracts in vitro against *S. rolfsii Sacc.*

The selected fifteen botanicals were screened for their antifungal activity against the pathogen at 20% concentration. For this, PDA medium was prepared in 250 ml Erlenmeyer flasks and sterilized. 20 ml of 100% aqueous plant extracts of each botanicals were aseptically added to 80 ml PDA in flasks so as to get the final concentration of 20% of the extracts in the medium. PDA without any extract served as control. The media was poured in 9 cm Petri plates at the rate of 20ml/plate. The fungal culture disc using a cork borer (5mm diameter) from the tip, obtained from a 7 days old culture were taken and inoculated in the centre of petri plates aseptically after solidification of the medium and incubated at 28±1ºC for 7 days. The diameter of the colony is measured when the mycelium fully covered the control plates and the inhibition of the mycelial growth of the other treatments were calculated by the formula given by Vincent (1927):

$I=(C-T)/C\times100$

Where, I = Inhibition of mycelial growth,

C = Growth in control,

T = Growth in treatment

3. Results and Discussion

The results revealed in Table 1 showed that all the plant extracts significantly inhibited the growth of the *Sclerotium rolfsii*.

Amongst all the six botanicals tested, maximum inhibition on mycelial growth of *S. rolfsii* was found in *Allium sativum* (89.77%) followed by extracts of *Chromolaena odorata* (86.00%), *Allamanda cathertica* (83.66%), *Laurus nobilis* (72.11%), *Ageratum conyzoides* (54.88%) and the least was shown by *Aegle marmelos* (50.66%). The results of the present investigation are analogous to the previous findings published by several workers.

Table 1: *In vitro* Efficacy of bioproducts on collar rot disease (*Sclerotium rolfsii*) of soybean

Sl. No.	Treatments	Mycelial growth* (cm)	Inhibition over control (%)
1.	*Aegle marmelos*	4.44	50.66
2.	*Ageratum conyzoides*	4.06	54.88
3.	*Allamanda cathertica*	1.47	83.66
4.	*Allium sativum*	0.92	89.77
5.	*Chromolaena odorata*	1.26	86.00
6.	*Lauris nobilis*	2.51	72.11
7.	Control	9.00	-
	SEd±	0.080	-
	CD (p=0.05)	0.168	-

*After 7 days of incubation at 28±1°C, Mean of five replications

Karade and Sawant (1999) tested extracts of 12 medicinal plants against *Alternaria alternate* and observed 100% inhibition of spore germination by *Allium sativum* extract. Again, Kshirsagar et al. (2004) found that the crude extracts of garlic completely inhibited (100%) the mycelial growth of *Rhizoctonia solani* causing root rot of cotton. Of all the *Allium* species, garlic is the most important because of the presence of sulfur compounds like Allicin (diallyl-dithiosulfinate), diallyl disulphide (DADS), S-allylcysteine (SAC), diallyl trisulfide (DATS) etc. Durbin and Uchytil (1971) also reported that *A. sativum* extract contains allicin (antibiotic in nature) which can be used for the control of pathogenic bacteria and fungi.

The aqueous extracts of *Chromolaena odorata* was also used to control Onion bulb rot caused by *Aspergillus niger* at 75 g 100 ml^{-1} concentration. Allelopathic effect of leaf extract of *Chromolaena odorata* was reported by Yeni et al. (2010) against post-harvest and transit rot of tomato. Adeyemi et al. (2018) found that the minimum inhibitory concentration showed that 50 mg ml^{-1} and 25 mg ml^{-1} of cold water extract of C. *odorata* had fungicidal effect against *Phytophthora megakarya.* The inhibitory effect of the plant might be due to the presence of steroids, terpenoids, alkaloids, flavonoids, glucosinulate, amino acid, tannins, phenolic compounds and saponins.

Allamanda extract was found to be inhibitory against many disease causing plant pathogens like *Phomopsis vexans*, *Phytophthora capsici*, *Fusarium oxysporum*, *Rhizoctonia solani* (Panda et al., 1996; Khan, 1999; Jannat, 2006; Tania, 2007). Islam (2004) found 76-100% inhibition of mycelial growth of *Phomopsis vexans* by Garlic bulbs and Allamanda leaf extracts. Several researchers in India isolated and reported some antimicrobial compounds from Allamanda, like plumericin, isoplumericin, plumieride, long chain esters, etc. (Manogaran and Sulochana, 2005; Tiwari et al., 2002).

Fawzi et al. (2009) tested five plant extracts against pathogenic fungi *Alternaria alternata* and *Fusarium oxysporum in vitro* and found that *Lauris nobilis* can be successfully used for antifungal control. Bajwa et al. (2001) reported that the growth of *Aspergillus fumigatus* was significantly checked by aqueous root and shoot extracts of *Ageratum conyzoides*. Javed and Bashir (2012) also found that the aqueous and organic solvent extracts of *A. conyzoides* greatly reduced the biomass of *Fusarium solani*. Crude or refined extracts from *A. conyzoides* offer the possibility of biocontrol of plant pathogenic fungi (Iqbal et al., 2004). According to Tran et al. (2004), three phenolic compounds were identified in the leaf, stem and root of *A. conyzoides* including gallic acid, coumallic acid and protocatechuic acid and catechin was found only in the stem.

Zade et al. (2005) reported that the effectiveness of leaf extracts of *Aegle marmelos* at 50 and 100% concentrations in inhibiting the growth of uredospore germination of *Puccinia arachidis* in groundnut. The essential oil from the leaves of A. *marmelos* is known to exhibited antifungal properties (Renu et al., 1986; Rana et al., 1997).

4. Conclusion

In the present investigation, amongst all the six botanicals tested, *Allium sativum*, *Chromolaena odorata*, *Allamanda cathertica* and *Laurus nobilis* showed higher inhibition on mycelial growth of the fungus causing collar rot disease of soybean. All the four botanicals have strong toxic principles present in their extract which directly inhibit the growth of the fungus, as well as act as an outstandingly good model of biological control agent. These botanicals can be used as a possible substitute for synthetic fungicides.

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