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Effect of Host substrate (*Vicia Faba* L.) on the growth of *Sclerotium Rolfsii* Sacc.

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Abstract

Sclerotium rolfsii Sacc. [teleomorph *Athelia rolfsii* (Curzi) C.C. Tu & Kimbr.] is a soil borne fungus which is particularly severe on leguminous crops. Broad bean (*Vicia faba* L.) is one of the major pulse crops in Manipur using both as vegetable and pulse grain. Wilt disease which is a complex disease caused by *Sclerotium rolfsii*, causes great reduction in the annual production of the broad bean. As the legumes are the important source of vegetable protein, protection of these crops from diseases is also veryimportant. The effect of substrate extract on the radial growth of *S. rolfsii* was performed using Czapek-dox agar medium amended with leaf, stem and root extracts of *Vicia faba* at concentrations of 1, 10, 20 and 50% (v/v). It was observed that the leaf extract (1% and 10% concentration) was stimulatory on the radial growth of *S. rolfsii* during early incubation period (12-24 hrs.) and inhibitory effect was observed during late incubation period (36-60 hrs.). Highest concentration 50% of leaf extract gave the highest inhibitory effect was found during 36 hrs. incubation period. It was also observed that both stem and root extract of *Vicia faba* gave significant inhibition on the radial growth of *S. rolfsii*.

***S**clerotium rolfsii* primarily attacks host stems, although it may infect any part of a plant under favourable environmental conditions including roots, fruits, petioles, leaves and flowers. The recorded history of *Sclerotium rolfsii* Sacc. dates back 1892 when Dr. Rolfs[12] describes the disease tomato blight caused by the fungus. In 1911 Saccardo gave the present scientific name to this fungus. The fungus is a facultative parasite that grows well on plant debris found in moist soils of fairly low fertility[15]. The organism has two distinct ecological phases, the mycelial phase and the sclerotial phase. The mycelial phase is also referred to as the growth phase or pathogenic phase and the sclerotial phase enables the fungus to survive adverse periods. The factors that check the development of mycelial phase favour the production of sclerotia[2]. Paparu *et al.*[9] also reported that mycelial growth rate and the number of sclerotia produced by *S. rolfsii* on artificial media varied among agroecological zones but not within a zone. The teleomorph of *S. rolfsii* (*Athelia rolfsii*) is

rarely observed on hosts or in culture.

S. rolfsii causes losses of yield of various economic crop plants like sugar beets, tomato, onion and many other leguminous plants etc. Control of this fungus, a problem with many crop plants, has been attempted by fertilizer and other chemical treatment, rotation, and tillage method. Tisdale[14] suggested changes in time of planting as a control measure, because he found that rice sown later than usual grows vigorously and escapes much infection by *S. rolfsii*. Organic plant debris matter enhances the sclerotial formation of *S. rolfsii*[5]. Patil[10] reported that plant extracts may have control potential. The phytoncides of the citrus skin and pulp have different effects on the growth of the same or different species of *Fusasium*. The phytoncides from different parts of the citrus fruits have different degrees of fungicidal effect[1]. There are many other reports on the toxic substances present in plant tissues.

Sclerotium rolfsii Sacc.; a soil borne fungus with a broad host range of more than 600 species[11], is the causal agent of southern blight disease in a wide variety of crops. Southern blight disease caused by *S. rolfsii* is an emergent disease of common bean (*Phaseolus vulgaris* L.) in the country Uganda and is currently the most important soil borne disease of common bean in the country[8]. *S. rolfsii* produces abundant hyphae and sclerotia[7] as asexual resting structures that facilitate pathogen survival in the absence of the host. In 2015 Mahadevakumar *et al.*,[6] also reported southern blight and leaf spot of common bean disease caused by *S. rolfsii* in India.

Broad bean (*Vicia faba* L.) is one of the major pulse crops in Manipur using both as vegetable and pulse grain. Wilt disease, which is a complex disease caused by *S. rolfsii*, causes great reduction in the annual production of the broad bean. As the legumes are the important source of vegetable protein in India, protection of these crops from disease is also very important. With these views, the present work was planned to study the effect of host substrate on the growth of *S. rolfsii*.

The materials utilised and the methods adopted during the course of investigation are presented below:

Test Organism:

As already mentioned the test fungus used for the study was a strain of *Sclerotium rolfsii* Sacc. (*Vicia faba* isolate) which is pathogenic to broad bean.

The fungus *S. rolfsii* was isolated on Czapek-dox agar (0.5g Mg SO_4 $7H_2O$+1.0 g KCl + 2.0g $NaNO_3$ + 1.0 g KH_2PO_4 + 0.066 g Fe SO_4 + 0.5 g yeast extract + 30.0 g sucrose + 17.0 g Agar + 1000 ml distilled water) from infected collar of *Vicia faba* using standard mycological techniques. The culture was maintained on the same medium.

Effect of substrate extract on radial growth of *S. rolfsii* :

Fresh and healthy plant parts (leaf, stem and roots) of *Vicia faba* were collected from the field and brought to laboratory. After washing them thoroughly with tap water and further rinsed with distilled water, the extract

was prepared by grinding the leaves, stems and roots separately with distilled water at the ratio 1:1 (W/V) using a grinder. The pulps were then squeezed though a clean muslin cloth and then the extracts were sterilized by filtering through sterile sintered glass filter (Borosil; G-5) and the extracts thus obtained were transferred to sterilized conical flasks in inoculation chamber. The extract was stored at 0^0C if the study was not done in proper time, but not more than 24 hrs. Examination of radial growth of *S. rolfsii* was performed using Czapeck-dox agar medium amended with leaf, stem and root extracts at concentrations of 1, 10, 20 and 50% (V/V). Control sets were maintained by adding appropriate amount of sterile distilled water in the culture medium. Amendments with either extracts or water were prepared in such a way that all ingredients, except extracts, per unit volume of the medium were uniform in all concentrations and control sets. For every treatment the medium was poured in three replicate plates. One culture block (4mm diameter) cut from the margin of pregrown colonies of the test fungus was placed at the centre at each plate having 15 ml solidified medium with or without substrate extracts. The plates were incubated at 25°C and the colony diamter of the fungal colonies were recorded at an interval of 12 hrs for a total period of 60 hrs.

The percent inhibition or stimulation of colony radial growth was determined by the formula

$100 \times (r.\ 1 - r.2) / r.1$ (after Fokkema[4])

Where, r.1 = diameter of control colony

r.2 = diameter of substrate extract treated colony.

Characterization of test organism:

Sclerotium rolfsii is a soil borne fungal plant pathogen which is particularly severe on leguminous crops. Mycelium is white to tan coloured, densely floccose, not ropy, and bearing numerous pinkish buff to olive brown, to clove brown, globose sclerotia, 0.8 to 2.5 mm in diameter. The fungus grew well and produced sclerotia on Czapek's medium after around 15 days of growth. Sclerotia production was inhibited when grown on Martin's medium.

Effect of substrate extract on radial growth of *S. rolfsii* :

The effect of substrate extract on radial growth of *S. rolfsii* was performed using Czapek-dox agar medium amended with leaf, stem and root extracts of *Vicia faba* at concentrations of 1, 10, 20 and 50% (V/V). The results of the experiment re summarised in Table-1 and are shown in Fig. 1.

It was observed that the leaf extract (1% and 10% concentration) was stimulatory on the radial growth of *S. rolfsii* during early incubation period (12-24 hrs.) and inhibitory effect was observed during late incubation period (36-60 hrs.). Leaf extract (1% concentration) gave 2.913 and 8.569 percent stimulation at 24 and 12 hrs. incubation period respectively and 2.753 – 5.689 percent inhibition during late incubation period (36-60 hrs). Leaf extract (10% concentration) gave 2.828 – 2.913 percent stimulation during early incubation period (12-24 hrs.) and 7.322 percent inhibition at 36 hrs incubation period and 2.66 – 5.176 percent inhibition at (48-60 hrs.) incubation period. Higher concentration

(20 and 50%) of leaf extract gave inhibitory effect on the radial growth of *S. rolfsii* during 12-60 hrs. incubation period. Leaf extract (20% conc.) gave 5.741 – 12.827 percent inhibition and the highest inhibitory effect was found during 36 hrs. incubation period. Highest concentration (50%) of leaf extract gave the highest inhibitory effect (6.731 – 16.515 percent) was found during 36 hrs. incubation period.

From the results, it was also observed that both stem and root extract of *Vicia faba* gave significant inhibition on the radial growth of *S. rolfsii* in which the magnitude of inhibition was increased as the substrate extract concentration increased but there was no complete inhibition at any concentration of stem and root extract. The stem extract gave the highest inhibitory effect at 36 hrs. incubation period in all concentrations and it gave no inhibitory or stimulatory effect at 12 hrs. incubation period in 1% concentration. Root extract (1% and 50% concentration) gave the highest inhibitory effect at 24 and 12 hrs. incubation period. Both 10% and 20% concentrations gave the highest inhibitory effect at 36 hrs. incubation period. However, in colonies of *S. rolfsii* grown in extract amended medium, much thicker hypyhal growths were observed in comparison to the control colonies. *S. rolfsii* is a fast growing fungus so the plates were overgrown during incubation before the last readings could be taken.

The plant substrate extracts are known to contain a number of fungal growth inhibitory toxic substances such as saponin like compounds[13], aromatic substances such as polyphenols, phenols glucosides, flavonoids, anthocyanins, aromatic amino acids and coumarin derivatives. The inhibitory effect of *V. Faba* (leaf, stem and root) extract on the radial growth of the test fungus may be attributed to anti-fungal substances which may be present in the host extract. Although, the effect of different parts of *V. faba* has not been reported, Sonoda[13] found that the growth of *S. rolfsii* was inhibited *in vitro* on potato dextrose agar amended with hot water extracts of plant parts containing saponin like compounds.

Not only in case of fungal pathogens, there are reports on inhibitory effect of plant extracts on viral pathagens also. Patil[10] reported that *Gomphrena* juice, cucumber leaf juice, rose leaf juice and diluted milk reduced the infection of Pinto bean (*Phaseolus vulgaris*) by tobacco mosaic virus. Extracts may have control potential. Batikyan and Gasparyan[1] found that the phytoncides of the citrus skin and pulp have different effects on the growth of the same or different species of *Fusarium*. The phytoncides from different parts of the citrus fruits have different degrees of fungicidal effect. El-Hissy[3] studied the antifungal substances present in *Helianthus annuus, Chrysanthemum coronarium, Nigella sativa* and *Datura innoxia*.

However, thicker hyphal growths were observed on extract treated medium in comparison in control plates irrespective of inhibition in colony diameter. Thus, the smaller colony diameter in the presence of higher concentration of host extract may also be explained in confining the colony diameter as nutrients for mycelial growth are present in concentrated form. The faster growth in colony diameter in distilled water treated

Table 1: The effect of plant substrate extract on radial growth of *S. rolfsii*

Treatments	Incubation period (hr)	Colony diameter (mm)	Percent inhibition of radial growth
1	2	3	4
(A) Control	0	4.00 (± 0)	0
	12	11.67 (± 0.577)	0
	24	23.00 (± 1.000)	0
	36	36.33 (± 0.577)	0
	48	50.00 (± 2.645)	0
	60	64.33 (± 1.527)	0
(B) 1% Leaf	0	4.00 (± 0)	0
	12	12.67 (± 0.577)	- 8.569**
	24	23.67 (± 1.155)	- 2.913
	36	35.33 (± 1.155)	2.753
	48	48.00 (± 2.000)	4.000
	60	60.67 (± 1.527)	5.689
(C) 10% Leaf	0	4.00 (± 0)	0
	12	12.00 (± 0)	- 2.828
	24	23.67 (± 0.577)	- 2.913
	36	33.67 (± 0.577)	7.322
	48	48.67 (±0.577)	2.660
	60	61.00 (± 1.000)	5.176
(D) 20% Leaf	0	4.00 (± 0)	0
	12	11.00 (± 0)	5.741
	24	21.00 (± 0)	8.696
	36	31.67 (± 0.577)	12.827
	48	46.67 (± 0.577)	6.660
	60	58.00 (± 1.00)	9.840
(E) 50% Leaf	0	4.00 (± 0)	0
	12	7.67 (± 0.577)	11.482
	24	15.67 (± 0)	8.696
	36	15.67 (± 0.577)	16.515
	48	39.67 (± 0.577)	7.340
	60	52.33 (± 1.000)	6.731

(F) 1% Stem	0	4.00 (± 0)	0
	12	11.67 (± 0.577)	0
	24	22.67 (± 0.577)	1.435
	36	33.67 (± 0.577)	7.322
	48	47.67 (± 0.577)	4.660
	60	60.00 (± 1.000)	6.731
(G) 10% Stem	0	4.00 (± 0)	0
	12	10.67 (± 0.577)	8.569
	24	20.33 (± 0.577)	11.609
	36	30.67 (± 0.577)	15.579
	48	46.00 (± 1.000)	8.000
	60	59.34 (± 0.577)	7.757
(H) 20% Stem	0	4.00 (± 0)	0
	12	10.00 (± 1.000)	14.310
	24	19.67 (± 0.577)	14.478
	36	29.33 (± 1.527)	19.268
	48	43.33 (± 0.577)	13.340
	60	55.67 (± 1.155)	13.462
(I) 50% Stem	0	4.00 (± 0)	0
	12	7.67 (±0.577)	34.276
	24	15.67 (± 0.577)	31.870
	36	15.67 (± 0.577)	56.868
	48	39.67 (± 0.577)	20.660
	60	52.33 (± 0.577)	18.654
(J) 1% Root	0	4.00 (± 0)	0
	12	11.33 (± 0.577)	2.913
	24	22.00 (± 0)	4.348
	36	35.00 (± 0)	3.661
	48	49.33 (± 0.577)	1.340
	60	63.00 (± 1.732)	2.067
(K) 10% Root	0	4.00 (± 0)	0
	12	10.00 (± 1.000)	5.741
	24	20.00 (± 1.000)	13.043
	36	29.33 (± 1.155)	19.268

	48	42.67 (± 0.577)	14.660
	60	55.67 (± 0.577)	13.462
(L) 20% Root	0	4.00 (± 0)	0
	12	10.00 (± 1.000)	14.310
	24	19.00 (± 0)	17.391
	36	27.67 (± 0.577)	23.837
	48	40.00 (± 0)	20.00
	60	53.33 (± 0.577)	17.099
(M) 50% Root	0	4.00 (± 0)	0
	12	8.00 (± 0)	31.448
	24	16.67 (± 0.577)	27.522
	36	25.33 (± 1.155)	30.278
	48	39.00 (± 2.000)	22.000
	60	51.33 (± 1.155)	20.208

* The values are the mean of three replicates in each case. For calculation of percent inhibition of radial growth, control is taken to be standard. Figures in the parentheses are the values of standard deviation.
** Values preceded by (-) are percent stimulation in radial growth.

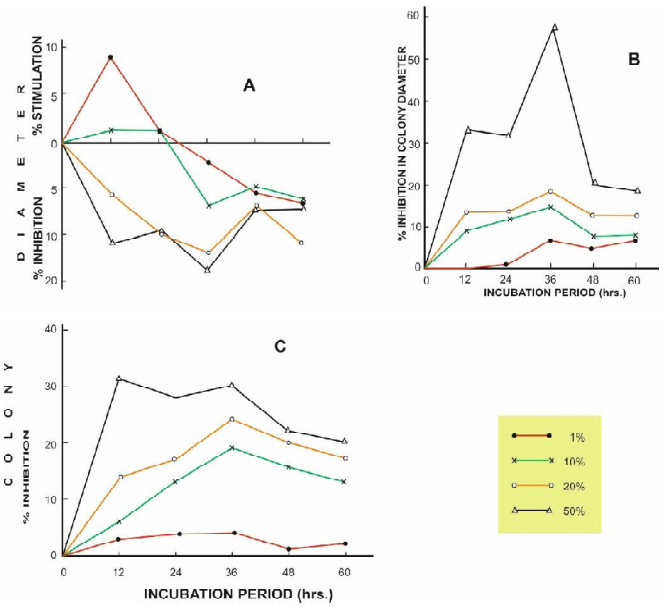

Fig. 1 : Effect of substrate (different parts of *Vicia faba* L.) extracts on the radial growth of *Sclerotium rolfsii*.
A. Leaf extract, B. Stem extract, C. Root extract

medium (control) may be a result of faster extension of hyphae in search of more nutrients in the zone of fresh medium around the colony.

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References :

1. Batikyan, S.G. and M.A. Gasparyan (1975). *Biol. Zharm 28*(7) : 58 – 64.
2. Boyle, L.W. (1961). *Phytopathology. 51:* 117 – 119.
3. El-hissy, F.T. (1974). *Egypt J. Bot. 17*(1): 93 – 96.
4. Fokkema, N. J. (1973). *Physiological Plant Pathology 3:* 195 – 205.
5. Garren, K.H. (1959). *Virginia Agr. Expt. Sta. Tech. Bull. 144:* 29.
6. Mahadevakumar, S., G.S. Tejaswini, G.R. Janardhana and Y. Vandana (2015). *Plant Dis. 99 :* 1280. https://doi.org/10.1094/PDIS-01-15-0125-PDN Link, ISI Google Scholar.
7. Mullen, J. (2001). Southern blight, southern stem blight, white mold. Plant Health Instruct. doi.org/10.1094/PHI-1-2001-0104-01 Google scholar.
8. Paparu, P., A. Acur,, F. Kato, C. Acam, J. Nakibuule, S. Musoka, S. Nikalubo and C. Mukankusi (2018). *J. Exp. Agric. 54:*888-900. https://doi.org/10.1017/S0014479717000461 Crossref, ISI, Google Scholar.
9. Paparu, P., A. Acur, F. Kato, C. Acam, J. Nakibuule, A. Nkuboye, S. Musoke and C. Mukankusi (2020). *Plant disease. 104*(8): 2130-2137. https:dci.org/10.1094/PDIS-10-19-2144-RE.
10. Patil, P. L. (1973). *Indian J. Microbiol. 13*(3) : 161 – 162.
11. Rivard, C. L., S. O' Connell, M.M. Peet and F.J. Louws (2010). *Plant Dis. 94:* 1015 – 1021, doi : 10.1094/PDIS-94-8-1015.
12. Rolfs, P.H. (1892). Tomato blight – some hints. *Fla. Agric. Exp. Stn. Bull.* 18 Google Scholar.
13. Sonoda, R.M. (1978). *Soil Crop Sci. Soc. Fla. Proc. 37:* 47-50.
14. Tisdale, W. H. (1921) *J. Agr. Research. 21 :* 649-658.
15. Watkins, G. M. (1961) *Phytopathology. 51 :* 100 – 113.