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Ecofriendly management of stem rot of groundnut (*Arachis hypogaea* L.) caused by *Sclerotium rolfsii* Sacc.

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Abstract
Present investigations were carried out to study *in vitro* efficacy of antagonists and aqueous extracts of botanicals against *S. rolfsii* Sacc inciting stem rot of groundnut. Six fungal, two bacterial antagonists and aqueous extracts of the 8 botanicals were evaluated against *S. rolfsii* Sacc *in vitro*. The results revealed that all the eight antagonists caused significant inhibition of mycelial growth of the test fungus. However, maximum inhibition of mycelial growth was obtained with *B. subtilis* which was most effective with least linear mycelial growth (35.32 mm) with highest mycelial inhibition (60.76%) of the test pathogen and significantly superior over all the bioagents tested. The second and third best antagonists found were *T. Asperellum* (41.47 mm and 53.93%) and *T. harzianum* (53.93 mm and 50.00%) and *T. koningii* was found least effective with 86.40 mm linear mycelial growth and 4.00 percent mycelial inhibition, respectively. All the evaluated botanicals (each @ 15 and 20%) were found antifungal to *S. rolfsii* Sacc. However, on the basis of highest average mycelial growth inhibition recorded at 15% conc., *A. sativum* (90.89%), followed by *O. sanctum* (83.61%), *A. racemosus* (73.94%), *Vitex* spp. (45.56%) and *A. cepa* (35.78%). The botanicals *A. indica* (1.86%) and *C. longa* (1.78%) were found comparatively less effective. At 20% conc., *A. sativum* (93.81%), followed by *O. sanctum* (90.77%), *A. racemosus* (82.94%), *Vitex* spp. (56.22%) and *A. cepa* (53.66%). The botanicals *A. indica* (25.32%) and *C. longa* (25.00%) were found comparatively less effective.

Keywords: *In vitro*, groundnut, *Sclerotium rolfsii* Sacc, bioagents, botanicals

Introduction
Groundnut (*Arachis hypogaea* L.) is an important oilseed crop commonly cultivated worldwide in tropical and subtropical regions. It is called as 'king' of oilseeds on account of its diversified uses. It is one of the most important food and cash crops of our country. Groundnut being a legume crop, it fixes a large amount of nitrogen and improves the fertility status of the soil. Developing countries account for more than 80% of groundnut area in the world. The production is confined mainly to Asian and African countries. Asia accounts for about 50% of the global area and 60% of production. India accounts for about 25% of global area and contributes 19% to world groundnut production. In India, stem rot occurs in all groundnut growing states, particularly more severe in Gujarat, Maharashtra, Madhya Pradesh, Odisha and Tamil Nadu, where approximately over 50,000 ha of groundnut fields are infected with *S. rolfsii*. Latur, Raichur, Dharwad, Junagadh and Hanumangarh have been identified as 'hot spots' for the diseases.

About 27% or more yield loss due to this disease has been reported from India (Chohan, 1974) [2]. Mayee and Datar (1988) [5] have reported yield losses of over 25% in Maharashtra. The indirect losses such as reduction in dry weight and oil content are also reported. The initial symptoms are partial or complete wilting of the stem or branches that are in contact with the infected soil. Several bio-agents were found to have antagonistic effect on *Sclerotium rolfsii* Sacc.

Materials and Methods
The isolation of the pathogen was done in two ways

a. **Direct isolation:** A pointed needle duly sterilized was used and the fungus growth / sclerotia from infected stem was directly transferred in to plates containing PDA media under aseptic condition and plates were incubated at 26 ± 2 °C for optimum growth.
b. **Tissue isolation method:** Repeated isolations were carried out from groundnut plant showing typical stem rot symptoms. After washing thoroughly with tap water, the infected stem part was cut in to small bits, surface sterilized with 0.1% $HgCl_2$ (1 g/lit.) for 1 minute

followed by three subsequent washing with sterilized distilled water in aseptic condition. The sterilized pieces then transferred aseptically under laminar airflow on sterilized Petri plates containing 20 ml Potato Dextrose Agar (PDA) medium. The Petri plates were incubated in Biological Oxygen Demand (B.O.D.) incubated at 26 ± 2 °C temperature for optimum growth.

The fungal hyphae developing from the infected tissues were sub-cultured aseptically on PDA media containing in Petri plates. Thus, pure culture was obtained by hyphal tip method and microscopically examined for identification and it was further purified by using single sclerotial body. The culture was maintained on PDA for further investigations.

Bio control agents

Pure cultures of biocontrol agents *viz*., *T. asperellum, T. harzianum, T. hamatum, T. koningii, T. longibrachiatum, Aspergillus niger, Bacillus subtilis and Pseudomonas fluorescens* were obtained from the Spawn Production cum Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture, VNMKV, Parbhani.

Table 1: Details plant extracts to be used

Sr. No.	Name of Plant	Botanical Name	Plant part used
1	Nirgudi	*Vitex* spp.	Leaves
2	Onion	*Allium cepa*	Bulb
3	Garlic	*Allium sativum*	Clove
4	Turmeric	*Curcuma longa*	Leaves
5	Tulsi	*Ocimum sanctum*	Leaves
6	Ginger	*Zingiber officinealis*	Rhizome
7	Neem	*Azadirachta indica*	Leaves
8	Shatavari	*Asparagus racemosus*	Leaves
9	Control (Untreated)	----	----

Dual culture technique

In dual culture technique, 20 ml of sterilized and cooled potato dextrose agar was poured into each sterile petri plates. Fungal antagonists were evaluated by inoculating the test pathogen at one side of petri plate and the antagonist inoculated at exactly opposite side of the same plate by leaving 3 cm gap. For this, actively growing cultures were used. In case of bacterial antagonist evaluation, two mycelia discs of pathogen were inoculated and bacterial antagonist were streaked in the center of the plate. Each treatment was replicated three times. After required period of incubation *i.e*., in the control plate growth reached 90 mm diameter, the radial growth of pathogen was measured.

Fungal antagonists

T_1 - *Trichoderma asperellum*
T_2 - *T. harzianum*
T_3 - *T. hamatum*
T_4 - *T. koningii*
T_5 - *T. longibrachiatum*
T_6 - *Aspergillus niger*

Bacterial antagonists

T_7 - *Bacillus subtilis*
T_8 - *Pseudomonas fluorescens*
T_9 - Control

Observations on radial mycelial growth of the fungal pathogen and biocontrol agents were measured and percent inhibition of the test fungus was calculated by applying formula given by Arora and Upadhyay (1978).

Colony growth in

$$\text{Percent inhibition (PI)} = \frac{\text{Colony growth in Control plate - intersecting plate}}{\text{Colony growth in control plate}} \text{ x } 100$$

***In vitro* evaluation of botanicals**

Several plant species reported to exhibit antifungal and therapeutic properties against fungal pathogens. Those botanicals reported earlier effective against many pathogens and which are locally available were collected from the farms of College of Agriculture, VNMKV, Parbhani and adjoining fields. Following locally available eight plant species / botanicals were used for *in vitro* studies against *Sclerotium rolfsii* Sacc. (Stem rot pathogen of groundnut) by applying poisoned food technique (Nene and Thapliyal, 1993) [12] and using PDA as basal medium. All the plant extracts were evaluated @ 15% and 20%.

Preparation of plant extracts

Hundred grams of fresh healthy plant parts (leaves / bulbs / rhizome) collected from field / market were washed with distilled water and air-dried and crushed in 100 ml of distilled water (w/v). The crushed product was filtered through double layer muslin cloth and further filtrated through Whatsman No. 1 filter paper using funnel and volumetric flasks (100 ml capacity). The prepared solution gave 100% concentration, which was further diluted to required concentrations of 15 and 20 percent. The extracts were tested against *S rolfsii* Sacc. On the cultural media using poison food technique (Nene and Thapliyal, 1993) [12] under *in vitro* condition.

An appropriate quantity of each plant extract (100%) was separately mixed thoroughly with autoclaved and cooled (40 ºC) PDA medium in conical flasks (250 ml cap.) to obtain desired concentrations (15 and 20%). The PDA medium amended separately with plant extract was then poured (20 ml/plate) into sterile glass Petri plates (90 mm dia.) and allowed to solidify at room temperature. Upon solidification of PDA, all the treatment and control plates were aseptically inoculated by placing in the center a 5 mm mycelial disc obtained from a week old actively growing pure culture of *S rolfsii* Sacc. Plates containing plain PDA without any plant extract and inoculated with mycelial disc of the test fungus served as untreated control. For each test plant extract and their respective concentrations, three plates/ replication were maintained. All these plates were then incubated at 26 + 2 ºC temperature for a week or till the untreated control plates were fully covered with mycelial growth of the test fungus.

Details of experiment:

Design : CRD
Replications : Three
Treatments : Nine

Table 2: Details of Treatment

Sr. No.	Name of Plant	Botanical Name	Plant part used	Concentrations used (%)	
T_1	Nirgudi	*Vitex* spp.	Leaves	15	20
T_2	Onion	*Allium cepa*	Bulb	15	20
T_3	Garlic	*Allium sativum*	Clove	15	20
T_4	Turmeric	*Curcuma longa*	Leaves	15	20
T_5	Tulsi	*Ocimum sanctum*	Leaves	15	20
T_6	Ginger	*Zingiber officinale*	Rhizome	15	20
T_7	Neem	*Azadirachta indica*	Leaves	15	20
T_8	Shatavari	*Asparagus racemosus*	Leaves	15	20
T_9	Control (Untreated)	----	----	--	--

Observations on radial mycelial growth and sporulation of the test fungus were recorded at 24 hrs. interval and was continued till growth of the test pathogen in untreated control plate is fully covered.

Percent inhibition of test pathogen was recorded as per Vincent (1947) formula.

$$R = \{(C - T) / C\} \times 100$$

Where,
R = Percent inhibition
C = Radial growth of pathogen colony in control
T = Radial growth of pathogen colony in treatment

Results and Discussion

In vitro evaluation of antagonists

Results of the study revealed (Table 3 and PLATE I) that all the bioagents evaluated exhibited fungistatic / antifungal activity against *Sclerotium rolfsii* Sacc. And significantly inhibited its growth over untreated control. The antagonistic activity of fungal bioagents against *Sclerotium rolfsii* Sacc. Revealed that all the seven antagonistics caused significant inhibition of mycelial growth of the test fungus (Table-3). However, maximum inhibition of mycelial growth was obtained with *B. subtilis* which was found most effective with least linear mycelial growth (35.32 mm) with highest mycelial inhibition (60.76%) of the test pathogen and significantly superior over all the bioagents treatments. The second and third best antagonists found were *T. asperellum* and *T. harzianum* which recorded mycelial growth of 41.47 mm and 45.00 mm of the test pathogen, respectively and inhibition of 53.93 and 50.00 percent, respectively. These were followed by *A.* Niger (col. dia.: 49.35 mm and inhibition: 45.13%) and *Pseudomonas fluorescens* (col. dia.: 57.80 mm and inhibition: 35.78%). *T. longibrachiatum* and *Pseudomonas fluorescens* were found at par with each other. *Trichoderma koningii* was found least effective with 86.40 mm linear mycelial growth and 4.00 percent mycelial inhibition, respectively.

Table 3: *In vitro* efficacy of different bioagents against mycelial growth and inhibition of *Sclerotium rolfsii Sacc.*

Sr. No.	Treatments	Colony Dia.(mm)* of pathogen	% Inhibition*
T_1	*Trichoderma asperellum*	41.47	53.93 (47.25)
T_2	*T. harzianum*	45.00	50.00 (45.00)
T_3	*T. hamatum*	66.68	25.91 (30.60)
T_4	*T. koningii*	86.40	03.98 (11.51)
T_5	*T. longibrachiatum*	59.42	35.08 (36.32)
T_6	*Aspergillus niger*	49.35	45.17 (42.23)
T_7	*Bacillus subtilis*	35.32	60.76 (51.21)
T_8	*Pseudomonas fluorescens*	57.80	35.78 (36.74)
T_9	Control (untreated)	90.00	00.00 (00.00)
S.E. +		0.60	0.67
C.D. (P=0.01)		1.81	2.00

*Mean of three replications.
Figures in parenthesis are arc sine transformed value

T_1: *T. Asperellum* T_4: *T. koningii* T_7: *B. subtilis* T_2: *T. harzianum* T_5: *T. longibrachiatum* T_8: *P. fluorescens* T_3: *T. hamatum* T_6: *A. niger* T_9: Control untreated)

Plate 1: *In vitro* efficacy of different bioagents against mycelial growth and inhibition of *Sclerotium rolfsii* Sacc.

***In vitro* evaluation of plant extracts/botanicals:** Aqueous extracts of eight botanicals were evaluated *in vitro* (each @ 15 and 20%) against *Sclerotium rolfsii Sacc*. and the results obtained on its mycelial growth and inhibition are presented in the Table 2 and PLATE-II AND III revealed that all the 8 botanicals extracts tested were fungi static/antifungal to *Sclerotium rolfsii* Sacc. which significantly reduced mycelial growth and increased its inhibition over untreated control.

Mycelial growth

The results at 15 percent concentration were revealed that (Table 2 and PLATE-II AND III) radial mycelial growth of the test pathogen was ranged from 8.20 mm (*A. sativum*) to 88.40 mm (*C. longa*), as against 90.00 mm in untreated control. However, significantly least mycelial growth was recorded with *A. sativum* extract (8.20 mm) which was found significantly superior over all the botanicals tested. This was followed by the botanicals *viz*., *O. sanctum* (14.75 mm), *A. racemosus* (23.45 mm), *Vitex* spp. (49.00 mm), *A. cepa* (57.80 mm), *Z. officinale* (85.00 mm). The botanicals *A. indica* (88.30mm) and *C. longa* (88.40 mm) were found least effective with maximum mycelial growth. The botanicals tested 20 percent conc. (Table 4 and PLATE-II AND III) were exhibits nearly similar trend of mycelial growth as that of at 15 percent conc. and it was ranged from 5.58 mm (*A. sativum*) to 67.50 mm (*C. longa*). However, significantly least mycelial growth was recorded with *A. sativum* extract (5.58 mm) which was found significantly superior over all the botanicals tested. This was followed by the botanicals *viz*., *O. sanctum* (8.30 mm), *A. racemosus* (15.25 mm), *A. cepa* (28.90 mm), *Vitex* spp. (39.40 mm), *Z. officinale* (45.60 mm) as against 90.00 mm in untreated control. The botanicals *A. indica* (67.20 mm) and *C. longa* (67.50 mm) were found least effective with maximum mycelial growth.

Percent Mycelial growth inhibition

Results obtained on percent mycelial growth inhibition (Table 4 and PLATE-II) of the test pathogen revealed that all the botanicals tested (@ 15 and 20% conc. each), significantly inhibited mycelial growth of the test pathogen over untreated control. Further, it was found that percent mycelial growth inhibition of the test pathogen was increased with increase in concentrations of the botanicals tested.

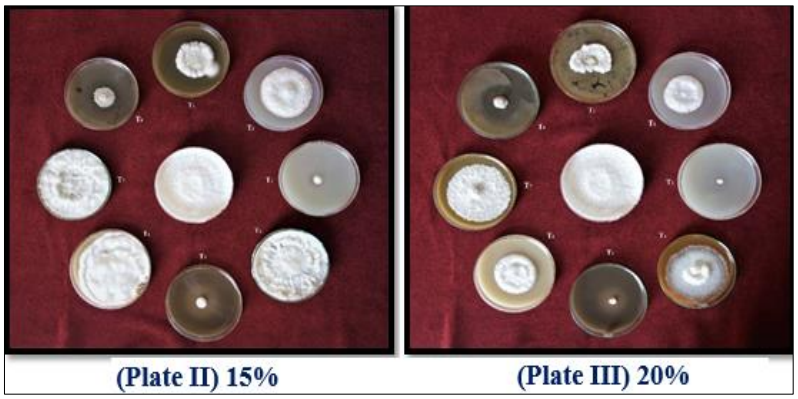
The results 15% conc. revealed that (Table 4 and PLATE-II AND III) mycelial growth inhibition of the test pathogen ranged from 1.78% (*C. longa*) to 90.89% (*A. sativum*). The botanical Garlic extract (*A. sativum*) was found significantly superior over all the botanicals tested. This was followed by the botanicals *viz*., *O. sanctum* (83.61%), *A. racemosus* (73.94%), *Vitex* spp. (45.56%) as against 90.00 mm in untreated control. *A. cepa* (35.78%), *Z. officinale* (6.67%). *A. indica* (1.86%) and *C. longa* (1.78%) were found least effective with less than 40% growth inhibition. botanicals tested at 20 percent conc. (Table 4 and PLATE-II AND III) exhibited nearly similar trend of mycelial growth as that of at 15 percent conc. and it was ranged from 25.00 (*C. longa*) to 93.81 (*A. sativum*) percent. However, significantly highest mycelial growth inhibition was recorded with *A. sativum* extract (93.81%) which was found significantly superior over all the botanicals tested. This was followed by the botanicals *viz*., *O. sanctum* (90.77%), *A. racemosus* (82.94%), *A. cepa* (53.66%), *Vitex* spp. (56.22%), *Z. officinale* (49.33%) as against 90.00 mm in untreated control. *A. indica* (25.32%) and *C. longa* (25.00%) were found least effective with less than 40% growth inhibition. Thus, on the basis of mycelial growth inhibition caused by the test botanicals and those found most antifungal against *S. rolfsii* were *A. sativum*, *O. sanctum*, *A. racemosus*, *Vitex* spp. and *A. cepa*.

Table 4: *In vitro* efficacy of different botanicals/plant extract against mycelial growth and inhibition of *Sclerotium rolfsii Sacc.*

Sr. No.	Treatments	Botanical Name	Col. dia. *(mm) at Conc.		% Inhibition*	
			15%	20%	15%	20%
T_1	Nirgudi	*Vitex* spp.	49.00	39.40	45.56 (42.45)	56.22 (48.57)
T_2	Onion	*Allium cepa*	57.80	28.90	35.78 (36.74)	53.66 (47.10)
T_3	Garlic	*Allium sativum*	8.20	5.58	90.89 (72.43)	93.81 (75.59)
T_4	Turmeric	*Curcuma longa*	88.40	67.50	1.78 (7.67)	25.00 (30.00)
T_5	Tulsi	*Ocimum sanctum*	14.75	8.30	83.61 (66.12)	90.77 (72.31)
T_6	Ginger	*Zingiber officinealis*	85.00	45.60	6.67 (14.97)	49.33 (44.62)
T_7	Neem	*Azadirachta indica*	88.30	67.20	1.86 (7.84)	25.32 (30.21)
T_8	Shatavari	*Asparagus racemosus*	23.45	15.25	73.94 (59.30)	82.94 (65.60)
T_9	Control (Untreated)	----	90	90	00 (00)	00 (00)
S.E.±			0.21	0.10	0.24	0.11
C.D. (P=0.05)			0.65	0.30	0.72	0.32

*Mean of three replications.

Figures in parenthesis are arc sine transformed value



T1	Nirgudi	T5	Tulsi
T2	Onion	T6	Ginger
T3	Garlic	T7	Neem
T4	Turmeric	T8	Shatavari
T9	Control (untreated)		

Plate 2: *In vitro* efficacy of plant extract/botanicals at 15% (Plate II) and 20% (Plate III) on growth and inhibition *Sclerotium rolfsii* Sacc.

Results of the present study on *S. rolfsii* Sacc. are in consonance with those reported earlier by several workers (Madhavi and Bhattiprolu, 2011; Kumar *et al.*, 2011; Sumi *et al.*, 2015; Rabeya *et al.*, 2016, Kuldhar and Suryawanshi 2017; Murthy *et al.*, 2018; Sarita, Shankar Soyal and RS Ratnoo 2018) [9, 8, 20, 15, 7, 11, 17]. Botanicals /plant extracts Garlic (*Allium sativum*), Onion (*Allium cepa*), Nirgudi (*Vitex spp.*), Turmeric *(Curcuma longa*), Tulsi (*Ocimum sanctum)*, Ginger (*Zingiber officinale*), Neem (*Azadirachta indica*), Shatavari (*Asparagus racemosus*) were reported earlier as antifungal / fungistatic against *S. rolfsii* Sacc. Causing disease on groundnut and many other crops by several workers (Sumi and Tiameren 2015; Suryawanshi 2015; Rabeya *et al*., (2016); Sneha 2016; Kuldhar and Suryawanshi (2017); Suranjit *et al*., 2018) [20, 22, 15, 18, 7, 21].

Conclusions

B. subtilis which was most effective with least linear mycelial growth (35.32 mm) with highest mycelial inhibition (60.76%) of the test pathogen and significantly superior over all the bioagents tested. The second and third best antagonists found were *T. Asperellum* (41.47 mm and 53.93%) and *T. harzianum* (53.93 mm and 50.00%) and *T. koningii* was found least effective with 86.40 mm linear mycelial growth and 4.00 percent mycelial inhibition, respectively.

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