Journal of Biological Control, 34(3): 215-222, 2020, DOI: 10.18311/jbc/2020/25599

Research Article

Factors influencing the incidence of basal stem rot and blight disease caused by *Sclerotium rolfsii* in vegetable cowpea and its management using botanicals

P.T. SRI[1], A. SAJEENA[2*], J.M. JOHNSON[1], J. JOHN[2] and N.S. RADHIKA[1]

[1]*Department of Plant Pathology, College of Agriculture (Kerala Agricultural University), Vellayani, Thiruvananthapuram – 695522, Kerala, India*

[2]*Integrated Farming System Research Station (Kerala Agricultural University), Karamana, Thiruvananthapuram – 695002, Kerala, India*

**Corresponding author Email: sajeenamanjima@gmail.com*

ABSTRACT: Basal stem rot and blight disease incited by *Sclerotium rolfsii* resulted in significant crop loss in vegetable cowpea. Among the different levels of pH tested under *in vitro* conditions, pH 6.0 was revealed to be optimum for the mycelial growth, whereas pH 7.0 supported maximum sclerotia formation. Soil moisture of 35 to 50 per cent was ideal for early disease expression and establishment. A minimum level of two per cent fungal inoculum multiplied in sand oats medium resulted in complete disease incidence. Among the botanicals tested *in vitro*, garlic bulb extract (1%) and garlic creeper leaf extract (5%) revealed cent per cent inhibition of mycelial growth and sclerotia formation of the fungus. Both the extracts also inhibited the mycelial regeneration from sclerotia. Thus, garlic bulb and garlic creeper were revealed to be potent botanicals which can be used as effective alternatives to soil fumigants for the management of *S. rolfsii*.

KEY WORDS: Basal stem rot and blight, garlic bulb, garlic creeper, *Sclerotium rolfsii*

(Article chronicle: Received: 04-07-2020; Revised: 19-09-2020; Accepted: 22-09-2020)

INTRODUCTION

Sclerotium rolfsii is a saprophytic soil borne fungal pathogen having a wide host range (Punja, 1985; Okabe *et al*, 2001; Billah *et al*., 2017). High levels of variability, wide host range and facultative parasitic ability make it a fungus of great relevance. The survival rate of the fungus is also very high, as it can survive as sclerotia in the soil up to and even, above seven years period (Billah *et al*., 2017). *S. rolfsii* has been identified as a major soil borne fungus causing southern blight of cowpea worldwide resulting in 53.4 per cent yield loss (Sharma *et al*., 2002). Moreover, the fungus can cause wilting or stem rot or root rot resulting in plant death and complete yield loss. However, in Kerala, it was not identified as a major pathogen of vegetable cowpea, until recently. Thus, it is of utmost importance to undertake detailed study of the pathogen and the disease incited by it in vegetable cowpea. The soil borne nature and facultative saprophytic ability of the fungus necessitates studies for its management. Hence, the present study was undertaken to analyse the various factors affecting the survival rate of *S. rolfsii* and its management using botanicals under *in vitro* conditions.

MATERIALS AND METHODS

Vegetable cowpea plant samples exhibiting characteristic symptoms of basal stem rot and blight disease were collected and the fungus was isolated as per the standard procedures described by Rangaswami (1958). The fungal isolate was subcultured on PDA slants. Soil inoculation method as described by Pande *et al*. (1994) was followed to perform the pathogenicity test. The identification of the fungus was done based on the morphological characters of the reisolated fungus.

Standardisation of optimum pH for pathogen growth

The optimum pH which can favour the maximum mycelial growth and production of sclerotia of *S. rolfsii* was standardised. PDA media with different levels of pH *viz*., 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 were prepared by adjusting the pH with HCl and NaOH. The calibration of pH was made using a pH metre. 15 ml molten PDA medium having different levels of pH were poured separately into Petri plates and allowed to solidify. Seven day old, five mm mycelial discs of *S. rolfsii* were placed on the medium. The inoculated plates

were incubated at room temperature (28±1˚C). The mycelial growth (diameter in cm) was recorded at every 24 h interval up to four days. The number of sclerotia produced per plate was recorded at 15 DAI (Tanimu, 2018).

Standardisation of optimum moisture percentage for pathogen growth

The standardization of moisture percentage for the optimum growth of the fungus was performed as per the methodology described by Maiti and Sen (1988). Sterilized soil was filled in plastic cups (400 ml capacity). The inoculum of the fungus multiplied on sand oats medium was added into the soil and different levels of moisture *viz*., 35, 45, 50, 60, 70 and 80 per cent were maintained by adding the required quantity of sterile, distilled water at every 24 h interval. The moisture percentage at each level was calibrated using a soil moisture meter. Two week old vegetable cowpea seedlings (var. Gitika) were transplanted into the soil maintained at each level of moisture percentage. Each treatment was replicated thrice. The observations on the initiation of basal stem rot and blight disease symptoms in the seedlings were recorded at each moisture percentage level.

Standardization of inoculum level of the fungus for disease development

Soil was sterilized in an autoclave at 121˚C temperature and 15 psi pressure for 15-20 minutes for two consecutive days. The sterilized soil was filled in plastic cups of 300 g capacity. Different concentrations (1, 2, 3, 5, 7, 9 and 11 per cent) of the inoculum of *S. rolfsii* multiplied on sand oats medium for two weeks were added as a layer on the top of soil taken in each cup. The inoculum levels were prepared as per the methodology described by Yaqub (2005). One gram of the inoculum was added to 100 g soil to prepare one per cent inoculum amended soil. The same procedure was followed to prepare all other concentrations (2, 3, 5, 7, 9 and 11 per cent). Cups filled with sterilized soil alone without any fungal inoculum was maintained as the control. The cups were incubated at room temperature (28± 1˚C) for one day. After 24 h, two week old, vegetable cowpea seedlings (var. Gitika) were transplanted into the cups. The number of days taken for the seedlings to exhibit the disease symptoms was recorded separately in each treatment.

Screening of botanicals for biofumigation potential

To test the effect of selected botanicals, mustard, cabbage, garlic creeper, castor, cauliflower, combination of garlic and onion bulbs, garlic bulbs and onion bulb separately were tested for their inhibition potential against *S. rolfsii* by paired plate technique (Prasad *et al*., 2018). Fresh plant samples (mustard, cabbage, garlic creeper, castor, cauliflower, combination of garlic and onion bulbs, garlic bulbs and onion bulb separately) were collected and washed with tap water to remove dirt. 100 g of the samples were weighed and macerated with equal amount of sterile distilled water (w/v basis) as per the methodology given by (Charron and Sams, 1999). Two equal sized, bottom lids of Petri plates were selected. 15 ml of PDA medium was poured in the upper plate and allowed to solidify. To assess the biofumigation potential against fungal mycelium, seven day old, five mm mycelial discs of *S. rolfsii* was placed on the PDA medium whereas, to assess the inhibition of regeneration of mycelia from sclerotia, a single sclerotium was placed on the upper lid. 1, 5, 10, 15, 20 and 25 g of the macerated plant samples were kept separately in the lower plate. The plates were closed and sealed at the joining portion with parafilm to prevent the escape of any fumes. The untreated control contained the mycelial disc of the fungus and sclerotia inoculated on PDA separately on the upper lid and the lower lid without any macerated plant samples. All the plates were incubated at room temperature (28±1˚C). The mycelial growth (diameter in cm) of *S. rolfsii* was measured in all the plates, when there was complete mycelial growth in untreated control plates. The percentage suppression of mycelial growth was calculated.

RESULTS AND DISCUSSION

Basal stem rot and blight disease was manifested as wilting, yellowing of leaves, defoliation and stem shredding. The disease appeared to have white coloured, fan or thread like mycelial growth at the collar region of vegetable cowpea plants. When soil inoculation of vegetable cowpea seedlings (var. Gitika) was undertaken using *S. rolfsii*, water soaked lesions were observed initially at the collar region on third DAI. Gradually, the lesions increased in size and resulted in collar rot. There was yellowing of the leaves which later got defoliated. Ultimately, the seedlings completely wilted on six DA I (Plate 1).

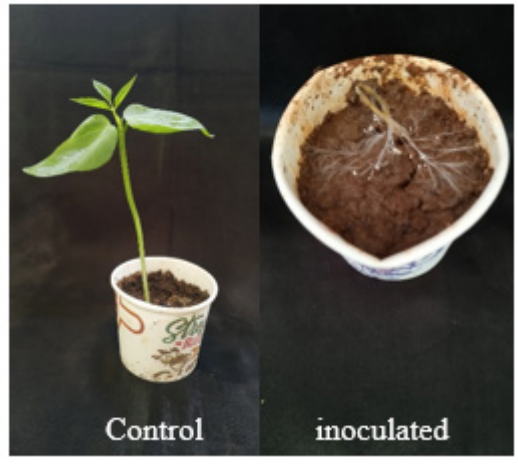

Plate 1. Artificial inoculation of *S. rolfsii* on stem base and symptom expression

The fungus completed its mycelial growth on PDA medium within three DAI. The mycelia appeared to be pure white in colour with conspicuous thread like growth on PDA medium. The initiation of sclerotia started on four DAI which appeared as smooth, round and dark brown in colour. Their arrangement was dense towards rim with excellent degree of formation. Characteristic clamp connections were also observed under microscope at 1000X magnification (Plate 2).

S. rolfsii is studied to produce several enzymes and oxalic acid, which were directly correlated with its ability to disintegrate the plant tissues at stem portion. These enzymes and oxalic acid together disintegrate the host outer cell layer. The entry of the fungus into the cortex region resulted in stem girdling and plant death (Kwon and Park, 2002).

Standardisation of optimum pH for pathogen growth

Among the different levels of pH tested, the fungus completed its mycelial growth (9 cm) within three DAI in PDA medium of pH 6.0. The maximum number of sclerotia (245) was recorded in the media of pH 7 at 15 DAI. The study revealed that the mycelial growth as well as the production of sclerotia were comparatively less in the alkaline pH range (7.5 to 8.5) (Table 1). It was also observed that at a soil pH of above 8, the disease incidence was significantly lower than that at acidic soil. Zape *et al.* (2013) opined that the optimum pH level for the production of mycelium and sclerotia of *S. rolfsii* ranged from 5.5 to 7.5. Recently, Sravani and Chandra (2020) reported that maximum mycelial growth was recorded at pH 6, proving that the fungus required slightly acidic condition for its mycelial growth and sclerotial production, which is in concurrence with the results of our study.

Standardisation of optimum moisture percentage for pathogen growth

The study revealed that under high moisture percentage (60 to 80%), more number of days (10 to 12 days) were taken by the fungus to develop basal stem rot and blight disease symptoms

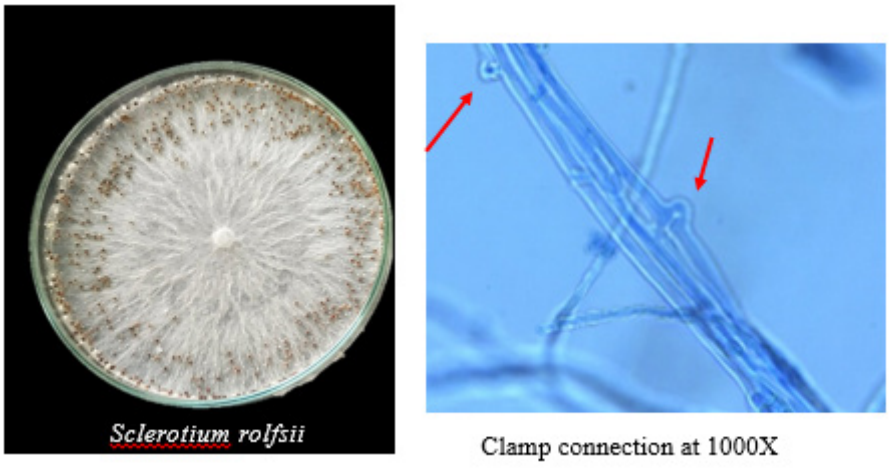

Plate 2. Identification of *S. rolfsii* based on mycelia, sclerotia and clamp

Table 1. Effect of pH on mycelial growth and formation of sclerotia of *S. rolfsii*

pH	Mycelial growth (Diameter in cm)*			Nature of mycelial growth	No. of sclerotia	Colour of sclerotia
	1 day	2 day	3 day			
5.0	1.50±0.058^{d}	3.90±0.635^{bc}	8.00±0.577^{cd}	Fluffy growth	105.67±2.963^{b}	Reddish brown
5.5	2.00±0.115^{c}	4.20±0.115^{b}	8.80±0.115^{ab}	Sparse growth	80.67±2.186^{c}	Brilliant brown
6.0	2.50±0.173^{b}	5.10±0.058^{a}	9.00±0.058^{a}	Fluffy growth	60.33±2.404^{d}	Reddish brown
6.5	2.90±0.058^{a}	4.50±0.058^{ab}	8.90±0.058^{ab}	Suppressed growth	101.67±2.728^{b}	Brilliant brown
7.0	1.50±0.058^{d}	3.20±0.115^{cd}	7.60±0.058^{d}	Fluffy growth	245.67±8.090^{a}	Light cream
7.5	2.00±0.115^{c}	4.30±0.058^{b}	8.10±0.115c^{d}	Fluffy growth	81.67±2.728^{c}	Brilliant brown
8.0	1.10±0.058^{e}	2.90±0.115^{d}	7.50±0.058^{d}	Sparse fluffy growth	85.67±2.963^{c}	Reddish brown
8.5	1.70±0.115c^{d}	4.00±0.115^{b}	8.30±0.058^{bc}	Fluffy growth	74.33±2.963^{c}	Honey colour
CD (0.05)	0.309	0.730	0.656	-	11.575	-
SEm±	0.102	0.242	0.217	-	3.828	-

Table 2. Effect of different levels of soil moisture on symptom development by *S. rolfsii*

Sl. No.	Soil moisture percentage (%)	Days for symptom initiation
1	35	4.67 ± 0.33^{e}
2	45	6.33 ± 0.33^{d}
3	50	7.67 ± 0.33^{c}
4	60	11.67 ± 0.33^{a}
5	70	10.67 ± 0.33^{ab}
6	80	10.33 ± 0.33^{b}
CD (0.05)		1.038
SEm±		0.333

in vegetable cowpea seedlings. Thus, it can be inferred that the disease development and symptom expression were favoured in the moisture range from 35 to 50 per cent at which the disease occurred within a short period of 5 to 8 days (Table 2). Beute *et al*. (1981) reported that the mycelia of *S. rolfsii* got destroyed rapidly in moist soil, but were observed to survive for a period of six months in dry conditions of soil. Tarafdar *et al*. (2018) revealed that the survival of the fungus decreased with increase in soil moisture content and was high in well-drained as well as sandy soils. However, the authors opined that the disease incidence had a direct relation with soil moisture.

Standardization of inoculum level of the fungus for disease development

Among the different inoculum levels (1, 2, 3, 5, 7, 9, 11%) of *S. rolfsii* inoculated into the soil for symptom expression, the maximum number of days (6) was taken for the disease to exhibit in the seedlings inoculated with one per cent concentration of the inoculum. Three days were taken for symptom expression in the seedlings at all other levels of the inoculum (2 to 11%), the least being at 2 per cent and hence this (2%) inoculum level was selected for further studies (Table 3; Plate 3). Sugha *et al*. (1993) also reported inoculum

Table 3. Effect of different levels of inoculum of *S. rolfsii* on symptom development

Sl. No.	Inoculum level of *S. rolfsii* (%) (w/w)	Days for symptom development
1	1	6
2	2	3
3	3	3
4	5	3
5	7	3
6	9	3
7	11	3

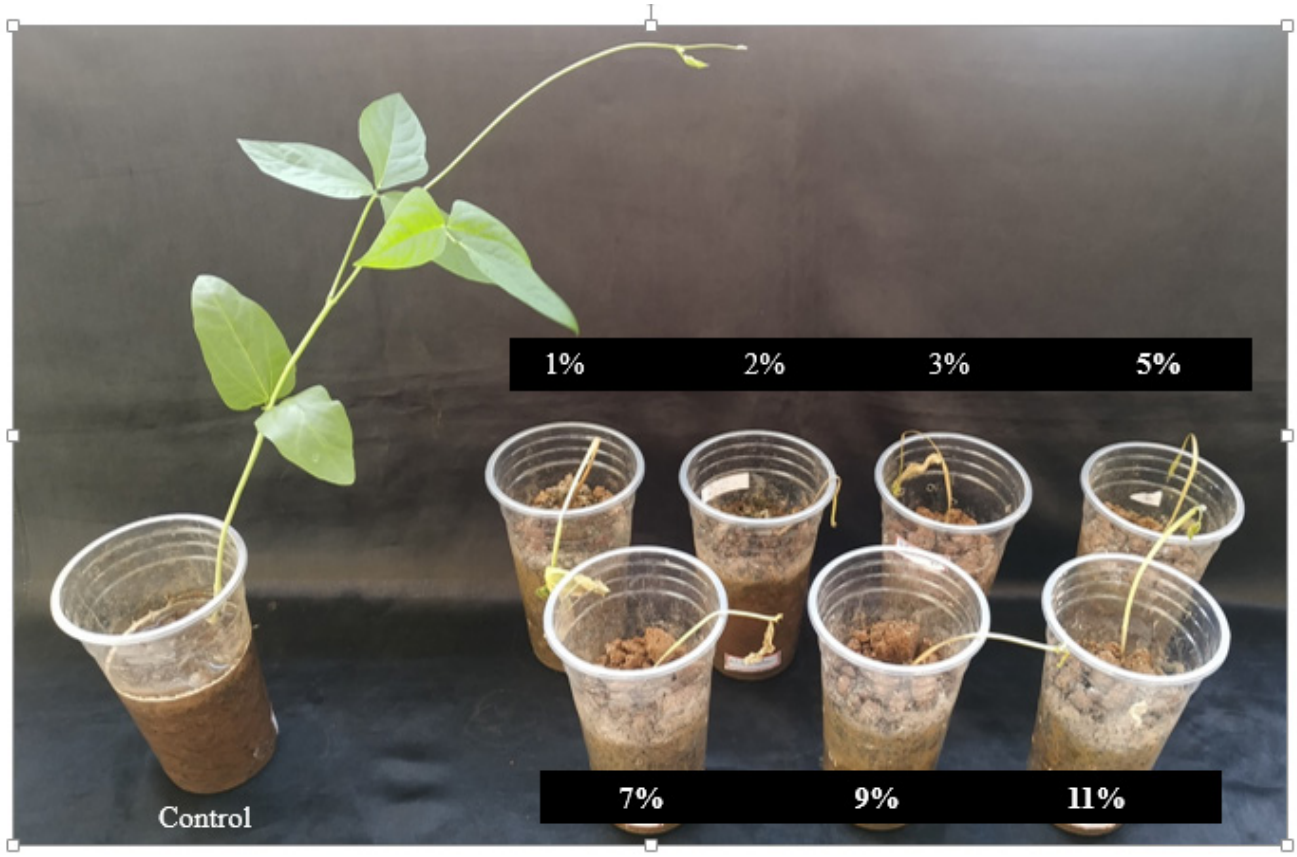

Plate 3. Effect of inoculum levels on disease incidence in vegetable cowpea

load and disease incidence by *S. rolfsii* in chickpea was directly proportional. Thiribhuvanamala *et al*. (2000) tested the effect of 3 inoculum levels *i.e.,* 5, 10 and 15 sclerotia of *S. rolfsii* in tomato and reported increase in inoculum load directly results in increased disease incidence of 72.5, 87.5 and 97.5 respectively. Muthukumar and Venkatesh (2013) is of the opinion that, five per cent fungal inoculum produced the maximum collar rot incidence in pepper mint followed by 4 per cent inoculum load of *S. rolfsii*. The disease incidence is positively correlated with the inoculum level.

a. Paired plate technique

Plate 4. Evaluation of biofumigation potential of plant extracts against *S. rolfsii*

Screening of botanicals for biofumigation potential

The biofumigation potential of the plant extracts at different concentrations (1, 5, 10, 15, 20 and 25 g) was tested against the mycelia of *S. rolfsii* (Plate 4).

Among all the plant extracts tested at different concentrations, garlic extract (1g) and the combination of garlic and onion extracts (1g) resulted in complete inhibition of the mycelial growth. However, onion extract (1g) when tested individually resulted only in 52.55 per cent inhibition of the mycelial growth. Hence, it can be concluded that the effect of the combination of garlic and onion extract (1g) is due to the biofumigation potential of garlic bulbs alone (1g). The next best effective treatments were garlic creeper (5g) and onion (5g) which also resulted in the complete mycelial inhibition. However, onion at 15 g revealed a reduction in inhibiting the mycelial growth and hence, cannot be considered as an effective biofumigant against *S. rolfsii*. The inhibition of sclerotial formation from mycelia was also analysed and it was found that mustard, garlic creeper, the combination of onion and garlic as well as garlic alone at

Table 4. Effect of biofumigation potential of plant extracts on *in vitro* mycelial inhibition

Treatmentsnts	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)*					
	1	5	10	15	20	25
	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
Mustard	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	53.70 (47.10±0.56)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	93.70 (75.52±1.19)^{b}
Cabbage	0.00 (0.00±0.00)^{d}	39.25 (38.78±0.22)^{b}	72.96 (58.65±0.62)^{b}	92.22 (73.80±0.69)^{c}	90.37 (71.90±0.36)c	94.81 (76.92±1.31)^{b}
Garlic creeper	66.29 (54.49±0.22)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
Castor	0.00 (0.00±0.00)^{d}	9.62 (18.02±0.97)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
Cauliflower	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	32.22 (34.57±0.68)^{d}	100.00 (90.00±0.00)$_{a}$	93.70 (75.45±0.44)b	93.33 (75.04±0.74)^{b}
Onion and garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)$_{a}$	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
Garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
Onion	52.59 (35.78±11.43)^{c}	95.18 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	95.18 (77.41±1.29)^{b}	100.00 (90.00±0.00)a	100.00 (90.00±0.00)^{a}
Control	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{e}	0.00 (0.000±0.00)^{d}	0.00 (0.00±0.00)d	0.00 (0.00±0.00)^{c}
CD (0.05)	11.413	0.992	1.084	1.462	0.571	1.916
SEm±	3.812	0.331	0.362	0.488	0.191	0.640

one per cent completely inhibited the sclerotial formation (Table 4: Plate 5).

The biofumigation potential of the plant extracts at different concentrations (1, 5, 10, 15, 20 and 25g) was tested against the mycelial regeneration from sclerotia. Among all the extracts tested at different concentrations, garlic extract (1g), onion (1g) and the combination of garlic and onion extracts (1g) (Table 5; Plates 5) resulted in complete inhibition of sclerotia followed by garlic creeper (5g). The combination of onion and garlic as well as garlic alone at one per cent completely inhibited sclerotial formation. Thus, from this study, garlic (1g) followed by garlic creeper (5g) were selected as the best effective biofumigants for inhibiting the mycelia as well as sclerotia of *S. rolfsii*.

Slusarenko *et al*. (2008) made a detailed study on the antimicrobial properties of garlic (*Allium sativum*). Garlic tissue substrates have alliin (S-allyl- L-cysteine sulphoxide). When garlic tissues get disrupted, the enzyme *viz*., alliin lyase will act on alliin to produce the volatile antimicrobial, membrane permeable substance *viz*., alliciin (diallyl thiosulphinate), which takes part in thiol disulphide exchange reactions with the free thiol groups of fungal

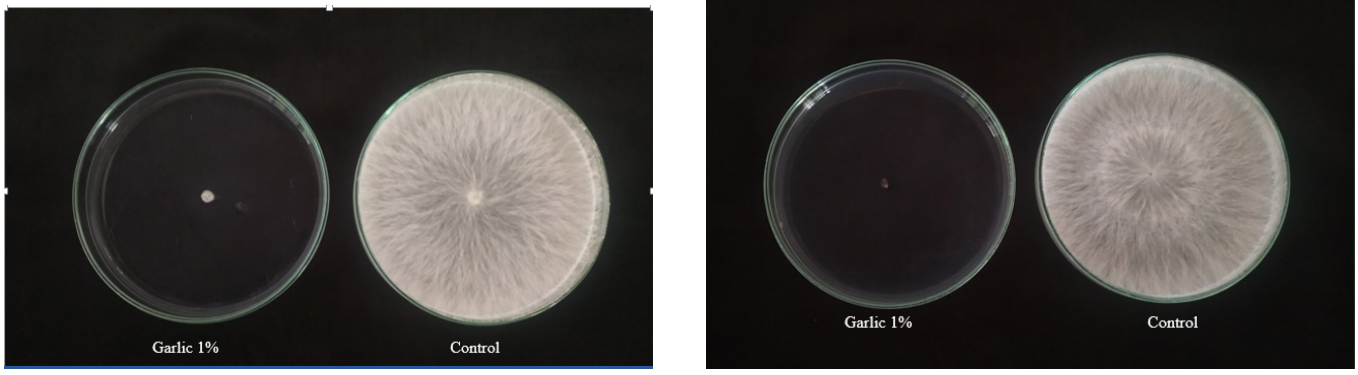

Plate 5. Effect of garlic bulb extract (1%) on mycelia and sclerotia of *S. rolfsii*

Table 5. Effect of biofumigation potential of plant extracts on mycelial regeneration from sclerotia

Treatments	Quantity of macerated tissue of selected plants for biofumigation potential against *S. rolfsii* (g)					
	1	5	10	15	20	25
	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)	PI (%)
Mustard	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	32.22 (34.57±0.68)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	91.85 (73.50±1.45)^{c}
Cabbage	0.00 (0.00±0.00)^{d}	27.77 (31.76±1.44)^{b}	44.44 (41.79±0.98)^{b}	100.00 (90.00±0.00)^{a}	96.21 (78.06±1.85)^{b}	96.29 (79.05±1.47)^{b}
Garlic creeper	54.07 (47.32±0.21)^{b}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
Castor	0.00 (0.00±0.00)^{d}	4.07 (11.45±1.50)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	90.37 (71.90±0.36)^{c}	100.00 (90.00±0.00)^{a}
Cauliflower	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	42.59 (40.72±0.57)^{b}	100.00 (90.00±0.00)^{a}	97.03 (80.09±0.65)^{b}
Onion and garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
Garlic	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
Onion	40.36 (39.41±1.51)^{c}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}	100.00 (90.00±0.00)^{a}
Control	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{c}	0.00 (0.00±0.00)^{d}	0.00 (0.00±0.00)^{d}
CD (0.05)	1.520	2.078	1.192	0.565	1.877	2.161
SEm±	0.508	0.694	0.398	0.189	0.627	0.722

proteins. Chaturvedi *et al*. (1987) reported that the leaves of garlic creeper (*Adenocalymma alliaceum*) belonging to bignoniacea contained a volatile oil having antifungal potential against *Drechslera oryzae.* It was revealed to stimulate the nitrate reductase activity of the host plants, thereby preventing disease incidence. Zoghbi *et al*. (1984) suggested leaves of this plant as a good substitute for garlic. The methanolic extract of garlic creeper at 2.5 and 10.0 per cent inhibited the growth of *Pythium aphanidermatum* and *Macrophomina phaseolina* respectively (Girijashankar and Thayumanavan, 2005). Chloroform extracts of the leaves of garlic creeper were highly effective in inhibiting the mycelial growth and spore germination of *Colletotrichum gloeosporioides* (anthracnose) and *Botrytis theobromae* (stem end rot) in mango (Aswini *et al*., 2010). They also reported that thin layer chromatography of the leaf extracts revealed the presence of phenolic compounds *viz*., tannic acid and resorcinol, which had antimicrobial activity by reacting on sulph hydryl groups of pathogen enzymes. Besides, they were also reported to increase the activities of defence related enzymes such as peroxidase, poly phenol oxidase and phenyl alanine ammonia lyase. Jadesha *et al*. (2013) revealed that cold water and methanol extracts of leaves of garlic creeper and zimmu completely inhibited the growth of *Colletotrichum musae in vitro*.

CONCLUSION

Basal stem rot and blight was revealed to be a new and emerging soil borne disease of vegetable cowpea caused by *S. rolfsii*. The fungus was revealed to survive and incite the disease under acidic conditions and low moisture levels. Garlic bulb extract (1%) and garlic creeper leaf extract (2.5%) were effective in completely inhibiting both the mycelial growth and formation of sclerotia of the fungus. Future intense studies are required to identify the active fungicidal principles in the plant extracts and reveal the possibility of integration of the extracts with bio control agents for the management of the soil borne disease of the crop.

REFERENCES

Aswini D, Prabakar K, Rajendran L, Karthikeyan G, Raguchander T. 2010. Efficacy of new EC formulation derived from garlic creeper (*Adenocalymma alliaceum* Miers.) against anthracnose and stem end rot diseases of mango. *World J. Microbiol. Biotechnol*. Dec 19; 26: 1107–1116. https://doi.org/10.1007/s11274-009-0277-y

Beut MK, Rodriguez-Kabana R. 1981. Effects of soil moisture, temperature and field environment on survival of *Sclerotium rolfsii* in Alabama and North Carolina. *Phytopathol.* 71(12): 1293–1296.

Billah MK, Billai Md H, Mahamud HP, Parvez SM. 2017. Pathogenicity test of *Sclerotium rolfsii* on different host and its over wintering survival. *Int. Gen. Adv. Agric. Sci.* **2**(7): 1–6.

Charron C, Sams CE. 1999. Inhibition of *Pythium ultimum* and *Rhizoctonia solani* by shredded leaves of Brassica species. *J. Am. Soc. Hortic. Sci*. **124**(5): 462–467. https://doi.org/10.21273/JASHS.124.5.462

Chaturvedi R, Dikshit A, Dixit SN. 1987. *Adenocalymma alliacea*, a new source of a natural fungitoxicant. *Trop. Agric*. **64**: 150–155.

Chaurasia AK, Chaurasia S, Chaurasia S, Chaurasia S. 2014. *In vitro* efficacy of fungicides against the growth of foot-rot pathogen (*Sclerotium rolfsii* Sacc.) of brinjal. *Int. J. Curr. Microbiol. App. Sci*. **3**(12): 477–485. https://doi.org/10.3126/ijasbt.v3i1.12200

Girijashankar V, Thayumanavan B. 2005. Evaluation of *Lawsonia inermis* leaf extracts for their *in vitro* fungitoxicity against certain soilborne pathogens. *Indian J. Plant Prot.* **33**(1):111–114.

Gisi U, Binder H, Rimbach E. 1985. Synergistic interactions of fungicides with different modes of action. *Trans. Brit. Mycol. Soc.* **85**(2): 299–306. https://doi.org/10.1016/S0007-1536(85)80192-3

Jadesha G, Haller H, Noorulla H, Mondhe MK, Hubballi M, Prakasam V. 2013. Antifungal activity of zimmu and garlic creeper against *Colletotrichum musae* causing banana anthracnose disease. *J. Plant Dis. Sci.* **8**(1): 43–46.

Kwon JH, Park CS. 2002. Stem rot of tomato caused by *Sclerotium rolfsii* in Korea. *Mycobiol.* **30**(4): 244–246. https://doi.org/10.4489/MYCO.2002.30.4.244

Mahato A, Mondal B, Dhakre DS, Khatua DC. 2011. *In vitro* sensitivity of *Sclerotium rolfsii* towards some fungicides and botanicals. *Scholars Acad. J. Biosci*. **2**(7):467–471.

Maiti S, Sen C. 1988. Effect of moisture and temperature on the survival of sclerotia of *Sclerotium rolfsii* in soil. *J. Phytopathol.* **121**: 175–180. https://doi.org/10.1111/j.1439-0434.1988.tb00969.x

Muthukumar A, Venkatesh A. 2013. Occurrence, virulence, inoculum density and plant age of *Sclerotium rolfsii* Sacc. causing collar rot of peppermint. *J. Plant Pathol. Microbiol.* **4**: 211. https://doi.org/10.4172/2157-7471.1000211

Nene YL, Thapliyal PN. 1993. Fungicides in plant disease control. New York: International Science Publisher.

Ohazurike NC. 1996. Effect of some fungicides on extracellular enzymes of *Sclerotium rolfsii* Sacc. *Food* **40**(3):150–153. https://doi.org/10.1002/food.19960400312

Okabe I, Arakawa M, Matsumoto N. 2001. ITS polymorphism within a single strain of *Sclerotium rolfsii*. *Mycoscience* **42**(1): 107–113. https://doi.org/10.1007/BF02463983

Pande S, Rao JN, Reddy MV, Mc Donald D. 1994. Development of a greenhouse screening technique for stem rot resistance in groundnut. *Int. Arachis Newsl*. **14**: 23–24.

Prasad P, Kumar J, Pandey S. 2018. Investigating disease controlling ability of brassica volatiles and their compatibility with *Trichoderma harzianum*. In: *Proceedings of the National Academy of Sciences*, India Section B: Biological Sciences, **88**(3): 887–896. https://doi.org/10.1007/s40011-016-0829-5

Punja ZK. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. *Annu. Rev. Phytopathol.* **23**(1): 97–127. https://doi.org/10.1146/annurev.py.23.090185.000525

Rangaswami G. 1958. An agar block technique for isolating soil microorganisms with special reference to Pythiaceous fungi. *Sci. Cult*. **24**: 85.

Roy A, Bordoloi DK, Paul SR. 2013. Reaction of chilli (*Capsicum annum* L.) genotypes to fruit rot under field condition. *MPKV. Res. J*. **22**: 475–478

Sharma BK, Singh UP, Singh KP. 2002. Variability of Indian isolates of *Sclerotium rolfsii*. Mycologia **946**:1051–1058. https://doi.org/10.1080/15572536.2003.11833160

Slusarenko AJ, Patel A, Portz D. 2008. Control of plant diseases by natural products: Allicin from garlic as a case study. In Sustainable disease management in a European context. *Eur. J. Plant Pathol.* **121**: 313–322. https://doi.org/10.1007/s10658-007-9232-7

Sravani B, Chandra R. 2020. Influence of media, pH and temperature on the growth of *Sclerotium rolfsii* Sacc. causing collar rot of chickpea. *J. Pharmacogn Phytochem.* **9**(1): 174–178.

Sugha SK, Sharma BK, Tyagi, PD. 1993. Factors affecting development of collar rot of gram (*Cicer arietinum*) caused by *Sclerotium rolfsii*. *Indian J. Agri. Sci*. **63**: 382–385.

Tanimu MU, Mohammed IU, Muhammad A, Kwaifa NM. 2018. Response of Cowpea Varieties to Basal Stem Rot (*Sclerotium rolfsii*) Disease in southern guinea savanna, Nigeria. *Equity J. Sci. Technol.* **5**(1): 1–8. https://doi.org/10.19080/ARTOAJ.2018.15.555963

Tarafdar A, Rani TS, Chandran US, Ghosh R, Chobe DR, Sharma M. 2018. Exploring combined effect of abiotic (soil moisture) and biotic (*Sclerotium rolfsii* Sacc.) stress on collar rot development in chickpea. *Frontiers plant sci.* **9**: 1154. https://doi.org/10.3389/fpls.2018.01154

Thiribhuvanamala G, Rajeshwari E, Doraiswamy S, Doraiswamy S. 2000. Inoculum levels of *Sclerotium rolfsii* on the stem rot in tomato. *Madras Agri. J*. **86**: 334.

Vincent JM. 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature.* **159**(4051): 850–850. https://doi.org/10.1038/159850b0

Yaqub F, Shahzad S. 2005. Pathogencity of *Sclerotium rolfsii* on different crops and effect of inoculum density on colonization of mungbean and sunflower roots. *Pakist. J. Bot.* **37**(1):175–180.

Yaqub F, Shahzad, S. 2006. Effect of fungicides on *in vitro* growth of *Sclerotium rolfsii*. *Pakist. J. Bot.* **38**(3): 881.

Zape AS, Gade RM, Singh R. 2013. Physiological studies on different media, pH and temperature on *Sclerotium rolfsii* isolates of soybean. Scholarly *J. Agric. Sci*. **2**(6): 238–241.

Zoghbi MDGB, Ramos LS, Maia JGS, Da Silva ML, Luz AIR. 1984. Volatile sulfides of the Amazonian garlic bush. *J. Agric. food chem.* **32**(5): 1009–1010. https://doi.org/10.1021/jf00125a014