Int.J.Curr.Microbiol.App.Sci (2021) *10*(03): 180-187

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 10 Number 03 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1003.025

In vitro Evaluation of Selected Bio-agents, Neem Oil and Amendments against Stem Rot (*Sclerotium rolfsii* Sacc.) Disease of Groundnut (*Arachis hypogea* L.) Waldron and Kohler

Rellu Salome* and Sunil Zacharia

Department of Plant Pathology, SHUATS, Allahabad, U.P

**Corresponding author*

Keywords

Groundnut, *Sclerotium rolfsii*, Bio-agents, Neem oil, Organic amendments, *Pseudomonas fluorescens*, *Bacillus subtilis*

Article Info

Accepted:
04 February 2021
Available Online:
10 March 2021

A B S T R A C T

Groundnut (*Arachis hypogea* L.) is one of the important economic oilseed crops of the world. Groundnut productivity is obstacled by several diseases, among which stem rot caused by *Sclerotium rolfsii* is one of the major constraints. Fungicidal sprays for the management of the disease may not be economically viable and feasible. Hence, the present *in vitro* study was conducted with aim to know the antifungal activity of three bio-agents (*Trichoderma viride, Pseudomonas fluorescens and Bacillus subtilis*), Neem oil and amendments (FYM and Neem cake) against *Sclerotium rolfsii* causing Stem rot in groundnut. The study was conducted in Plant Pathology laboratory, SHUATS during 2020. Among the treatments, *Trichoderma viride* was most effective in inhibiting percent growth of *Sclerotium rolfsii* (64.62 %) and FYM recorded highest inhibition (51.58 %) at the 15 % concentration.

Introduction

Groundnut (*Arachis hypogea* L.) is one of the important economic oilseed crops of the world. It is commonly called peanut, goober pea, jack nut, manila nut and monkey nut (Rathnakumar *et al.*, 2013). It is known as king of oilseeds (Aycock, R. 1966). In world groundnut is grown in an area of about 21.7m.ha with a production of 38.6 tones (FAOSTAT 2011).Oilseed crops have a specific place in Indian agriculture because edible oil is the next to food grain in Indian diet. The Groundnut seed contains moisture (5.52%), lipid (46.22%), calcium (0.087%), Phosphorous (0.29%), carbohydrate (21.26%), ash (2.57%) and energy (601.85%), saturated fatty acid (10.44%) and unsaturated fatty acid (33.51%) (Ingale and Shivastava, 2011).

Groundnut productivity is obstacled by bacterial, fungal and viral diseases among which stem rot caused by *S. rolfsii* is one of

the major constraints that occurs at the lower portion of the stem. *S. rolfsii* is a necrotrophic soil borne plant pathogen which firstly attacks host plants like groundnut at the soil line and rapidly moves to the root and destroys plant tissues with pectolytic enzymes and oxalic acid. Sclerotia are disseminated through the movement of infested soil and infected transplants. *Sclerotium rolfsii* occurs worldwide but is most important in tropical and subtropical regions. Stem rot is a persistent soil-borne disease throughout India and its incidence is increasing even at maturity stage of the groundnut crop. Though *S. rolfsii* survives both on seed and in soil, a greater threat is posed by soil-borne inoculums (Kumar *et al.,* 2013).Most of the first symptom associated with *S. rolfsii* are usually yellowing and wilting of leaves following stem rot infections. *S. rolfsii* forms brownish Sclerotia that can survive for long periods in the soil and retain their viability by tolerating biological and chemical degradation due to the presence of melanin in the outer membrane (Chet, 1975). Whitish mycelium of *Sclerotium rolfsii* was seen around the effected plants at or near the surface of soil, imparting a 'White Washed' appearance to the base of the infected plant. In some case the seeds from the diseased pods shows a characteristic bluish-grey discoloration known as 'blue damage' Anahosur (2001). Sclerotia may be spherical or irregular in shape and at maturity resemble the mustard seed (Barnett and Hunter, 1972). Sclerotial size was reported to be varied from 0.1 mm to 3.0 mm (Ansari and Agnihotri, 2000).

The management of groundnut stem rot disease is most difficult because of long term survival and wide host range of the pathogen. The management of this disease relies upon the use of resistant varieties, seed treatment with fungicides and prolonged crop rotation. However, the existence of *S. rolfsii* isolates with various virulence potentials might lead to the breakdown of resistance (Sarma *et al.,* 2002; Saraswathi and Madhuri, 2014). Further, the use of fungicides is discouraged due to ground water pollution, residues on food crops, effect on non-target organism and development of resistance to the chemical fungicides besides their high cost (Bonanaomi *et al.,* 2007). *Trichodermaspp*. are widely used in agriculture as bio-pesticides, bio-protectants, bio-stimulants, and bio-fertilizers on a wide variety of plants (Harman and Kubicek,1998). Addition of organic amendments to soil exerted favourable effect on disease reduction due to its suppressive nature (Adiver, 2003). The organic amendments not only increasing the activity of bio-agents but also acts as source of nutrients to crop plant. Neem cake enriches the soil natural nutrients and also provide more and more nitrates to the plants, which in turn includes the nitrogen metabolism. Bio-control agents, oils and soil amendments in the form of green manures, farm yard manures, compost and oil cakes are known to improve crop productivity and suppress certain soil-borne diseases (Sivaprakasam, 1991). Several studies have shown the organic amendments can be very effective in managing diseases caused by *S.rolfsi* (Blum and Rodriguez-Kabana, 2004).Essential Oils, amendments and bio agents are eco-friendly and less toxic to environment, thus use of Plant oils, amendments and bio agents for management of fungal and viral diseases is becoming more and more popular and has become a very interesting area of research.

Materials and Methods

Isolation and identification

Small pieces of tissues about 3mm from infected collar region with some healthy where cut with sterile scalpel. Then the pieces surface sterilized with one percent solution

for 30 sec. The tissue pieces were subsequently washed in 3 changes of sterile distilled water to climate excess sodium hypochlorite and then pieces were transferred to PDA plated petri dishes plates were incubated at 28± 2°C and were observed periodically for growth of the fungus. The culture so obtained was stored in refrigerator at 4°C were cultured periodically once in a month for further studies. The culture was purified by single hyphal tip method and maintained throughout the present investigation by periodical transfer into PDA. The pathogen was identified as *Sclerotium rolfsii* based on its mycelia and sclerotial characters (Barnett and Hunter, 1972).

Mass multiplication

Mass cultures of *Sclerotium rolfsii* were prepared using wheat grains in 1000 ml conical flaks. Wheat grains are soaked overnight in two per cent sucrose solution, air dried to remove excess moisture then autoclaved, to which 5 discs (5mm diameter) from three days old culture of test pathogen was added and thoroughly shaken. These inoculated conical flasks were incubated for 14 days at 28 ±2°C. The inoculated flasks were shaken periodically to allow the uniform growth and maintain utilization of substrate by the pathogen.

Dual culture technique

The antagonists and pathogen were inoculated by dual culture technique on PDA medium. Sterilized cork borer was used to cut 5 mm diameter disc from the actively growing pathogen and fungal antagonist *Trichoderma viride* and placed on PDA at opposite sides. Whereas, sterilized inoculation loop was used to take bacterial antagonists and streaked on PDA at one end and other end is inoculated with pathogen. After transferring the pathogen and antagonists, all the plates were incubated at 25 ± 2°C temperature in inverted position and measurements were taken after 96 hrs. At the end of incubation period, radial colony growth (mm) was measured in each treatment and percent growth inhibition was calculated for each treatment Dickinson (1976).

$PIRG=R_1-R_2/R_1 \times 100$

Where,

R_1=Radius of *Sclerotium rolfsii* colony in control plate

R_2=Radius of *Sclerotium rolfsii* colony in dual culture plate

Poisoned food technique

Aqueous extracts of neem cake and Farm Yard Manure (FYM) were prepared according to the procedure of Dhingani *et al., (*2013). Forty grams of each organic amendments was suspended in 150 ml of sterilized distilled water in a conical flask. Shaken the flask every day for mixing and dissolution of the content. After 15 days, the extract was stained through muslin cloth and then filtered using Whatman's filter paper No. 41 into 250ml conical flasks which were sterilized in autoclave at 121°C for 20 minutes. Autoclaved extracts were tested at concentrations of 5%, 10% and 15% and neem oil and carbendazim test according to Dhingra and Sinclair (1993) at different concentrations (1%, 3% and 5% and 0.1%, 0.2% and 0.5% respectively). Different quantities of the neem oil and carbendazim were mixed with the PDA medium before pouring. Each treatment was replicated 3 times. One treatment only with PDA medium was served as control. After, solidification of the medium, mycelial plug from 7dayold culture cut with a cork borer (5 mm) and placed at the centre of each Petri plate. The

plates were incubated at 25 ± 2 ^{0}C at 12 hrs /12 hrs cycles of lightness and darkness for 5 days. After full mycelial growth in control plates, radial colony growth (cm) was measured in each treatment and per cent growth inhibition was calculated for each treatment by following the formula (Vincent, 1947)

$$I = \frac{(C-T)}{C} \times 100$$

Where,

C = Growth of mycelium in control
T = Growth of mycelium in treatment

Results and Discussion

In vitro evaluation is done by dual culture and poisoned food technique and the data on the mycelial growth influenced by bio-agent are presented in table 1. Among the bio-agents (*Trichoderma viride*, *Pseudomonas fluorescens and Bacillus subtilis*) against *Sclerotium rolfsii*, maximum growth inhibition was observed in *Trichoderma viride* (64.62 %) as compared to control (85.40 mm). FYM 15% was found most effective in maximum mycelial growth inhibition percent of *Sclerotium rolfsii* (51.58 %) as compared to control (86.44 mm) (Fig. 1–6).

In vitro efficacy of bio-agents against *Sclerotium rolfsii*-Dual culture technique

All the three bio-agents (*Trichoderma viride*, *Pseudomonas fluorescens* and *Bacillus subtilis*) inhibited the growth of*Sclerotium rolfsii* when applied in 1:1 ratio. Among the three bio-agents, *T. viride*at 1:1 ratio was most effective and resulted in maximum mycelial growth inhibition of *Sclerotium rolfsii* (64.62 %) after 96 hrs of inoculation, which was superior among the treatments. It was followed by *Pseudomonas fluorescens*at 1:1 ratio suppressing radial growth of 51.56 % after 96 hrs and 44.62 % reduction was found in *Bacillus subtilis* at 1:1 ratio after 96 hrs of inoculation. Maximum average mycelial growth inhibition percent of the treatments *Trichoderma viride* which was (64.62%) proved statistically significant. Next effective treatment was *Pseudomonas fluorescens* (51.56 %) and followed by *Bacillus subtilis* (44.62 %) as compared to control at 96 hrs of inoculation (Table 2).

Table.1 Antagonistic effect of fungal and bacterial bio-agents on mycelial growth (mm) and percentage inhibition of *Sclerotium rolfsii*

Treatments	Radial growth of pathogen (mm)				Inhibition of pathogen (%)			
	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
T_1 = Control (*Sclerotium rolfsii*)	34.52	51.88	76.65	88.37	----	-----	----	---
T_2 = *Sclerotium rolfsii* + *Trichoderma viride*	19.70	26.02	30.04	31.26	42.91	49.84	60.81	64.62
T_3 = *Sclerotium rolfsii* + *Pseudomonas florescence*	23.42	33.07	40.14	42.80	32.15	36.25	47.63	51.56
T_4 = *Sclerotium rolfsii* + *Bacillus subtilis*	24.62	39.10	46.50	28.6	46.06	24.65	39.33	44.62
SEd (±)	0.732	1.663	3.45	1.42	----	-----	----	---
CD (0.05%)	1.688	3.835	7.96	3.27	----	-----	----	---

Table.2 *In vitro* efficacy of FYM, Neem cake and Neem oil treatments against radial growth and percent inhibition of *Sclerotium rolfsii*at different hour interval

Treatments	Radial growth of pathogen (mm)				Inhibition % of Pathogen			
	12 hrs	24 hrs	48 hrs	72 hrs	12 hrs	24 hrs	48 hrs	72 hrs
T_1 = FYM at 5 %	14.30	27.76	34.28	44.28	13.85	21.05	35.71	48.77
T_2 = FYM at 10 %	13.10	25.76	33.05	43.21	21.08	27.23	38.03	50.00
T_3 = FYM at 15 %	11.88	20.86	30.85	41.85	28.41	41.09	42.15	51.58
T_4 = *Neem* seed cake at 5 %	15.10	29.15	33.21	45.11	9.00	17.67	37.71	47.80
T_5 = *Neem* seed cake at 10 %	14.16	27.60	34.16	44.16	14.66	22.06	35.93	48.90
T_6 = *Neem* seed cake at 15 %	13.05	25.69	33.20	43.20	21.38	27.45	37.74	50.00
T_7 = *Neem* oil at 1 %	14.98	28.88	34.98	44.98	9.74	18.42	34.40	47.96
T_8 = *Neem* oil at 3 %	13.24	25.80	33.66	43.66	20.22	27.13	36.87	49.48
T_9 = *Neem* oil at 5 %	12.10	23.43	32.28	42.28	27.09	33.82	39.46	51.08
T_{10} = Carbendazim 50WP at 0.1 %	15.00	28.90	35.03	45.03	9.63	18.37	34.31	47.90
T_{11} = Carbendazim 50WP at 0.2 %	14.02	25.80	34.03	44.03	15.54	27.13	36.18	49.06
T_{12} = Carbendazim 50WP at 0.3 %	13.03	23.64	33.05	43.05	21.48	33.22	38.03	50.19
T_{13} = Control (*Sclerotium rolfsii*)	16.60	35.41	53.33	86.44	--------	--------	--------	--------
SEd (±)	1.27	2.59	1.36	1.24	--------	--------	--------	--------
CD (0.05%)	2.62	5.34	2.80	2.55	--------	--------	--------	--------

Fig.1 Mycelial growth on leaves

Fig.2 Pure culture and mycelium of *Sclerotium rolfsii*

Fig.3 Mass multiplication of *Sclerotium rolfsii* on wheat grains

Fig.4 *In vitro* efficacy of fungal and bacterial bio-agents against *Sclerotium rolfsii*

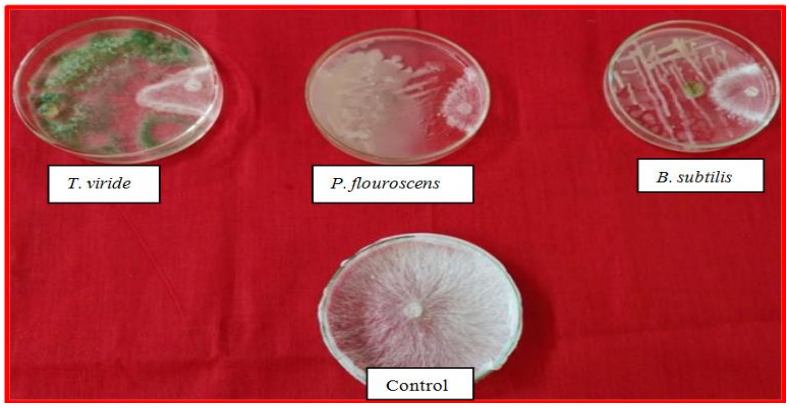


Fig.5 *In vitro* efficacy of FYM, Neem cake and Neem oil against *Sclerotium rolfsii*

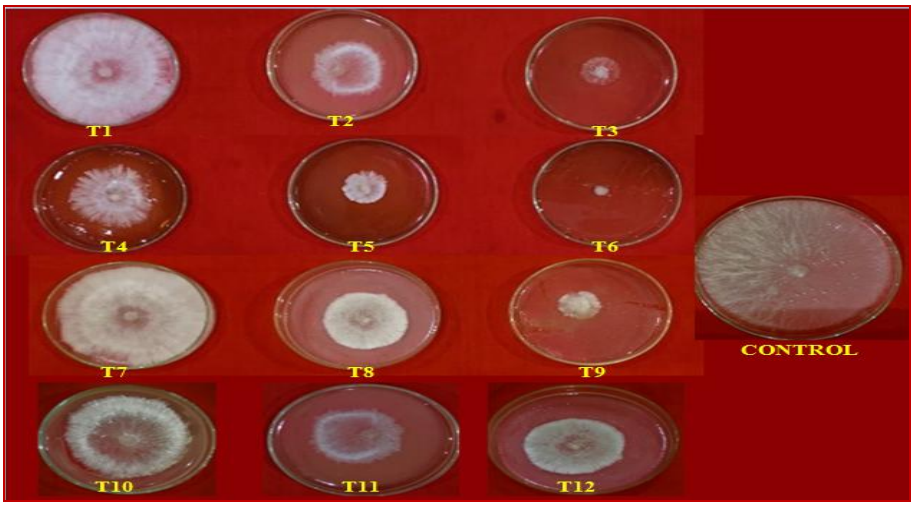
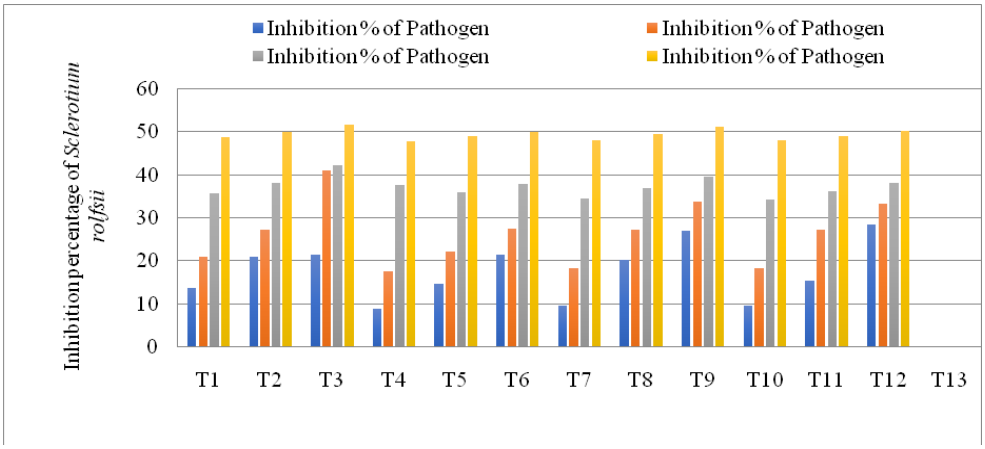


Fig.6 Graph showing *in vitro* efficacy of FYM, Neem cake and Neem oil against *Sclerotium rolfsii*



Similar finding was reported by Mahato and Mondal (2014) who found that *Trichoderma viride* and *Pseudomonas* showed highest inhibitory activity (68.28% and 74.25%) against *Sclerotium rolfsii* under *in vitro* conditions. Vardarajan *et al.,* (2005) found that the three isolates of *Trichoderma viride*, an isolate in each of *T. harzianum* and *Pseudomonas fluorescens* were inhibitory to the growth of *Sclerotium rolfsii* (Sacc.), the causal agent of stem rot of groundnut. The isolate *Tv1* of *T. viride* caused 69.40% inhibition of the mycelial growth of the pathogen followed by *P. fluorescens* resulting in 64.40% inhibition.

In vitro efficacy of FYM, Neem cake and Neem oil treatments against *Sclerotium rolfsii* –Poisoned food technique

Organic amendments were tested at different concentrations (FYM and *neem* seed cake at 5%, 10% and 15 % and neem oil at 2%, 3% and 5 %) and carbendazim at 0.1%, 0.2% and 0.3% against the mycelial growth of *Sclerotium rolfsiiin vitro*. The data revealed that the organic amendments *viz.,* FYM, neem seed cake and neem oil were found significant ($P<0.05$) in suppression of mycelial growth at higher concentration over untreated check. However, the suppression rate increased with the passage of time. The result reveals that FYM 15% was found most effective in maximum mycelial growth inhibition percent of *Sclerotium rolfsii* (28.41 %) at 12hrs, which gradually increased to 41.09%, 42.15% and 51.58 % at 24, 48 and 72 hrs, which was superior among the treatments. Next best treatment was neem oil 5% at 12, 24 and 48 hrs suppressing radial growth 27.09%, 33.82% and 39.40%. Minimum growth inhibition, 9.0%, 17.67%, 37.71% and 47.80% was found in neem seed cake (5%) at 12, 24, 48 and 72 hrs of interval.

Similar results were found by Vineela *et al.,* (2020) who reported that FYM 10 % was most effective in inhibiting the mycelial growth of *Sclerotium rolfsii* with per cent inhibition of 100 % showed *in vitro*. Senjaliya and Nathawat (2015) also reported that FYM at 10 & 20 % showed maximum per cent inhibition.

From present study it was concluded the use of bio-agents, amendments and oils provide an alternative to the use of synthetic pesticides with the advantage of minimizing the cost of cultivation and also avoid the health hazards. From the *in vitro* findings, it can be suggested that the combination of antagonists *Trichoderma viride* and amendment FYMcan be used against *Sclerotium rolfsii* under field condition.

References

Adiver, S. S. (2003). Influence of organic amendments and biological component on stem rot of groundnut, ISOR, *National Seminar: Stress Management in Oil Seeds*, January 28-30, 15-17.

Anahosur, K. H. (2001). Integrated management of potato *Sclerotium* wilt caused by *Sclerotium rolfsii*. *Indian phytopathology*. 54(2): 158-166.

Ansari, M. M. and Agnihotri, S.K. (2000). Morphological, Physiological and Pathological Variations Among *Sclerotium rolfsii* Isolates of Soyabean. *Indian Phytopathology*. *(*53): 65-67.

Aycock, R. (1966). Stem rots and other disease caused by *Sclerotium rolfsii*, North Carolina, Agricultural Experiment Station Technical Bulletin No. 174, p. 202.

Barnett, H.H. and Hunter, B.B. (1972). Illustrated genera of imperfect fungi. *Burgess Publishing Company*, Minnesota.

Blum, L.E.B and Rodriguez-Kabana, R. (2004). Effect of soil organic amendments on sclerotial germination, mycelial growth and *Sclerotium rolfsii* induced diseases. *Fitopatalogia Brasileria*. 29: 66-74.

Bonanomi, G., Antignani, V., Pane, C and Scala, F. (2007). Suppression of soil

borne fungal diseases with organic amendments. *Journal of Plant Pathology*. 89(3): 311-324.

Chet, I. (1975). Ultrastructural basis of Sclerotial survival in soil. *Microbial Ecology*: 2: 194-200.

Dhingani, J. C., Solanky, K. U and Kansara, S. S. (2013). Management of root rot disease *Macrophomina phaseolina* (Tassi.) Goid of chickpea through botanicals and oil cakes. *The Bioscan*. 8(3): 739-742.

Dhingra, O. D. and Sinclair, J. B. (1993). Basic Plant Pathology Methods. CRC Press, Inc. of Boca Raton, Florida. Pp. 335.

Dickinson and Skidmore(1976).*Transactions of the British Mycological Society. (*66):57-60.

FAOSTAT (2011). Food and Agriculture Organisation of United Nations, Rome, Italy, 2011.

Harman, G. E. and Kubicek, C. P. (1998).*Trichoderma* and *Gliocadium* (vol, 2) Enzymes, *Biological control and Commercial Applications*. London: Taylor & Francis, London).

Ingale S. and ShrivastavaS. K. (2011). Nutritional study of new variety of groundnut (*Arachis hypogaea L.)* JL24 seeds. *African Journal of Food Science*, 8(5): 490 – 498.

Kumar, N., Dagla, M.C., Ahay, B.C., Jadon, K.S and Thirumalaisamy. (2013).*Sclerotium* stem rot: A threat to groundnut. *Indian Phytopathology*. 65(3): 238-243.

Mahato, A., Mondal, B., Dhakre, D. H, Khatua, D. C. (2014). *In vitro* sensitivity of *Sclerotium rolfsii* towards some fungicides and botanicals. *Scholars Academic Journal of Biosciences*. 2(7): 467-471.

Rathnakumar, A. L., Singh, R., Parmar, D. L. and Misra, J. B. (2013) Groundnut: a crop profile and compendium of varieties notified in India. Art India Press, Gujarat, India.

Saraswathi, M and Jaya Madhuri, R. (2014). Variation in oxalic acid production by groundnut isolates of *Sclerotium rolfsii.Bioresearch Bulletin.* 3(2): 1-3.

Sarma, B.K., Singh, U.P and Singh. K.P. (2002). Variability in Indian isolates of *Sclerotium rolfsii*. *Mycologia.* 94(6): 1051-1058.

Senjaliya, B.D and Nathawat, B.D.S. (2015). Evaluation of bioagents, phyto-extracts and organic amendment on stem rot of Groundnut, Annals of Plant Protection Science. 23(1):98-101.

Sivaprakasam, K. (1991). Soil amendement for crop disease management. In P. Vidyasekaran (ed.) – *Basic Research for crop disease management*. *Daya Publishing house*, Delhi. 306-315.

Vardarajan, K., Ambalavanam, S. and Sevugaperumal, N. (2005). Biological control of groundnut stem rot caused by *Sclerotium rolfsii* (Sacc.) *Archives of Phytopathology and Plant Protection*. 39 (3): 239-246.

Vincent, J, M. (1927). Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* 59: 850.

Vineela, D. R. S., Beura, S. K., Dhal, A and Swain, S. K. (2020). Efficacy of bio-agents, botanicals, organic amendments against groundnut pathogens *Sclerotium rolfsii* and *Aspergillus nigerin-vitro*. *Journal of Pharmacognosy and Phytochemistry*. 9(3): 1206-121.

How to cite this article:

Rellu Salome and Sunil Zacharia. 2021. *In vitro* Evaluation of Selected Bio-agents, Neem Oil and Amendments against Stem Rot (*Sclerotium rolfsii* Sacc.) Disease of Groundnut (*Arachis hypogea* L.) Waldron and Kohler. *Int.J.Curr.Microbiol.App.Sci.* 10(03): 180-187.
doi: https://doi.org/10.20546/ijcmas.2021.1003.025