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Isolation and *in vitro* evaluation of biocontrol agents, fungicides and essential oils against stem blight of tomato caused by *Sclerotium rolfsii* (Curzi) C.C Tu & Kimber

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Abstract
Tomato is subjected to several diseases at all the stages of its development from nursery to the consumption of tomatoes. Among all the diseases infecting tomato, the stem blight of tomato is the most severe threat for the tomato industry and also for foreign exchange earnings. This disease is incited by the number of pathogens but the major causal organism is *Sclerotium rolfsii* (Curzi) C.C Tu & Kimber. Three antagonistic fungi of *Trichoderma* species were tested against *Sclerotium rolfsii* under *in vitro* conditions by using the dual culture method the results revealed that *Trichoderma harzianum* has most promising antagonist against *S.rolfsii*. *In vitro* evaluation of fungicides were tested hexaconazole and mancozeb was the most efficacious fungicide to inhibiting the mycelial growth of *Sclerotium rolfsii*, while essential oils i.e palmarosa (*Cymbopogon martini* oil) and thyme (*Thymus vulgaris*) oils were provided significantly high inhibitory responses against *Sclerotium rolfsii*.

Keywords: *Sclerotium rolfsii*, mycelium, biocontrol agents, fungicides and essential oils

Introduction
Tomato *(Lycopersicon esculentum* L.) is an essential crop of vegetables that is rich in nutrients and cultivated in a warm climate all the worldwide, (Sahana *et al*, 2017) [17, 18]. Tomato is grown in nearly every country in the world. Tomato is one of the most studied, cultivated dicotyledonous plants. Tomato plants have been used at molecular levels and as a model species for gene mapping, gene characterization (e.g. plant pathogen resistance genes) and gene transfer approaches. It is also useful to study other plant traits such as fruit ripening, hormonal function and vitamin biosynthesis (Gebhardt *et al*, 1991; Chetelat and Ji, 2006; Ji and Scott, 2006) with several fungal, bacterial, nematode and viral diseases. The tomatoes contain a large amount of water, vitamins and minerals, a low amount of protein and fat, and some carbohydrate. Tomato is considered "protective food" because of its nutritional benefits and year-round production throughout the world (Rajandra Prasad *et al*, 2017). The disease caused by *Sclerotium rolfsii*, soil-borne fungi that cause foot rot or collar rot in tomatoes, has become more serious among plant pathogenic fungi. It is known to be pathogenic to nearly 500 species of plants. The disease is also referred to as Sclerotium blight, Sclerotium wilt, Southern blight, Southern stem rot and white mold, which cause 55-95 percent crop mortality at the seedling stage under conducive conditions (Sahana *et al*, 2017) [17, 18], The *S. rolfsii* Sacc is a serious soil-borne plant pathogen which is widely spread across the tropics, subtropics and warmer regions of the temperate zone of the world, (Mahato *et al*, 2018) [9]. In India, it is common in almost all states and causes economic losses in many crops, (Natedara Chanutsa *et al*, 2014) [14]. Numerous reports from tropical and subtropical regions of the world, along with a large number of hosts, suggest that economic losses are significant every year due to *S.rolfsii* infection, (Sahana *et al*, 2017) [17, 18]. Soil-borne fungal pathogens, such as *Pythium* sp, *Rhizoctonia solani* and *Sclerotium rolfsii*, infect the tomato crop which causes disease damping and is becoming a potential threat to its production. *Sclerotium rolfsii*, a member of the agaricomycetes class, is one of the most crucial soil-borne plant pathogens and causes significant losses in crop production. This fungus-like organism is a non-specialized parasite with a wide range of hosts. Young tissues and plants are more deeply infected and affected by this pathogen. It causes collar rot disease in several plants, including tomatoes it affects the pre and post-emergence plants in nursery beds and pots, (Rajandra Prasad *et al*, 2017). *S.rolfsii* has become a major limiting factor and a challenge for both scientists and farmers.

Many approaches have been used to manage this disease, such as cultural practices, (Sahana *et al*, 2017) [17, 18].

Materials and Methods

Isolation of *Sclerotium rolfsii*

In the present investigation, the diseased samples were collected from different localities of Dehradun, Haridwar and Rishikesh districts of Uttarakhand during the 2019 cropping season. Small tissue from infected stem or roots (5 mm) along with healthy tissue were cut with a sterile scalpel. The tissues were surface sterilized with 0.1% $HgCl_2$ for 30 sec. The tissues were subsequently washed in three changes of sterile distilled water to eliminate mercury ions. The surface-sterilized tissues were transferred on to PDA and incubated at 25 ± 2 °C in BOD incubator and growth was observed periodically.

In vitro screening of *Trichoderma* species

Three antagonistic fungi of *Trichoderma* species i.e *T.harzianum*, *T.viride* and *T.asperillum* were tested against *Sclerotium rolfsii* under *in-vitro* conditions through dual culture technique. The data regarding % growth inhibition by the different antagonists of *Trichoderma* species. For this experiment PDA plates were used. Each Petri dish was divided into two halves, the first half was inoculated with disk (0.6 cm in diameter) of the tested antagonist fungus and the second half was inoculated with a similar disk of the pathogenic fungus. Plates inoculated only with the pathogenic fungi acted as a control. Each treatment was replicated ten times. All Petri dishes were incubated at 25 ± 2°C in the BOD incubator and observed daily. After 5 days of incubation, the pathogenic fungi almost covered the surface of the medium in the control treatment, the percentage of inhibition (I%) was calculated according to Vincent (1947).

In vitro evaluation of fungicides against *Sclerotium rolfsii*

The relative efficacy of five fungicides including systemic and non-systemic fungicides were evaluated under *in vitro* conditions hexaconazole, Cabrio top, difenoconazole, mancozeb, chlorothalonil at four different concentration levels i.e. 250, 500, 750 and 1000 ppm by using poisoned food technique (Nene and Thapliyal, 1979). From the stock double strength potato dextrose agar medium, different lots each containing 50ml double strength potato dextrose medium in a conical flask (150 ml) were sterilized at 15 psi (1.05 kg/cm2) pressure at 121.6^{0}C for 20 minutes. Simultaneously, concentrations of different fungicides were also prepared in an equal amount (50 ml) of sterilized distilled water to get the desired concentration of fungicides after mixing the fungicide solutions in the double strength media. Fungicides solution were added separately to equal quantities of double strength PDA medium aseptically before pouring in Petri plates. The culture discs (6 mm) cut from the margin of 7 days old vigorously growing culture of the test pathogen were placed in the center of each Petri plate.

In vitro evaluation of essential oils against *Sclerotium rolfsii*

The relative efficacy of five different phyto-essential oils were evaluated under *in vitro* conditions Palmarosa (*Cymbopogon martini*), Thyme (*Thymus vulgaris*), Menthol (*Mentha species*), Karanja (*Pongamia pinnata*) and Lemongrass (*Cymbopogon citrates*) at four different concentration levels i.e. 0.5, 1.0, 1.5 and 2.0 (%) by using poisoned food technique (Nene and Thapliyal, 1979). From the stock double strength potato dextrose agar medium, different lots each containing 50ml double strength potato dextrose agar medium in a conical flask (150ml) were sterilized at 15 psi (1.05 kg/cm2) pressure at 121.6 ^{0}C for 20 minutes. Simultaneously, concentrations of different essential oils were also prepared in an equal amount (50ml) of sterilized distilled water to get the desired concentration of essential oils after mixing the essential oil solution s in the double strength media.

Results and Discussion

The sensitivity of *Sclerotium rolfsii* to different fungal antagonist

Three antagonistic fungi of *Trichoderma* species were tested against *Sclerotium rolfsii* under *in vitro* conditions through dual culture technique. The data regarding the percent growth inhibition by the different antagonists of *Trichoderma* species. The study revealed variable inhibition responses of different antagonists against *Sclerotium rolfsii.* An antagonist of *Trichoderma* species were found more inhibitory against the test pathogen. Irrespective of different *Trichoderma* sp. were screened against the growth of *S. rolfsii*, *Trichoderma harzianum* is most efficacious providing percent growth inhibition followed by *Trichoderma viride* and was recorded minimum in *Trichoderma asperellum.*

Table 1: *In-vitro* evaluation of % Inhibition of different biocontrol agents against stem blight of tomato caused by *Sclerotium rolfsii* (Curzi) C.C Tu & Kimber

***Trichoderma* species**	**% of Inhibition**	
	Day 6th (144 hrs)	**Day 18th (432 hrs)**
Trichoderma harzinum	50.812	56.926
Tichoderma viride	39.715	39.715
Trichoderma asperillum	33.874	33.874
C.D.	0.026	0.026
SE(m)	0.009	0.009

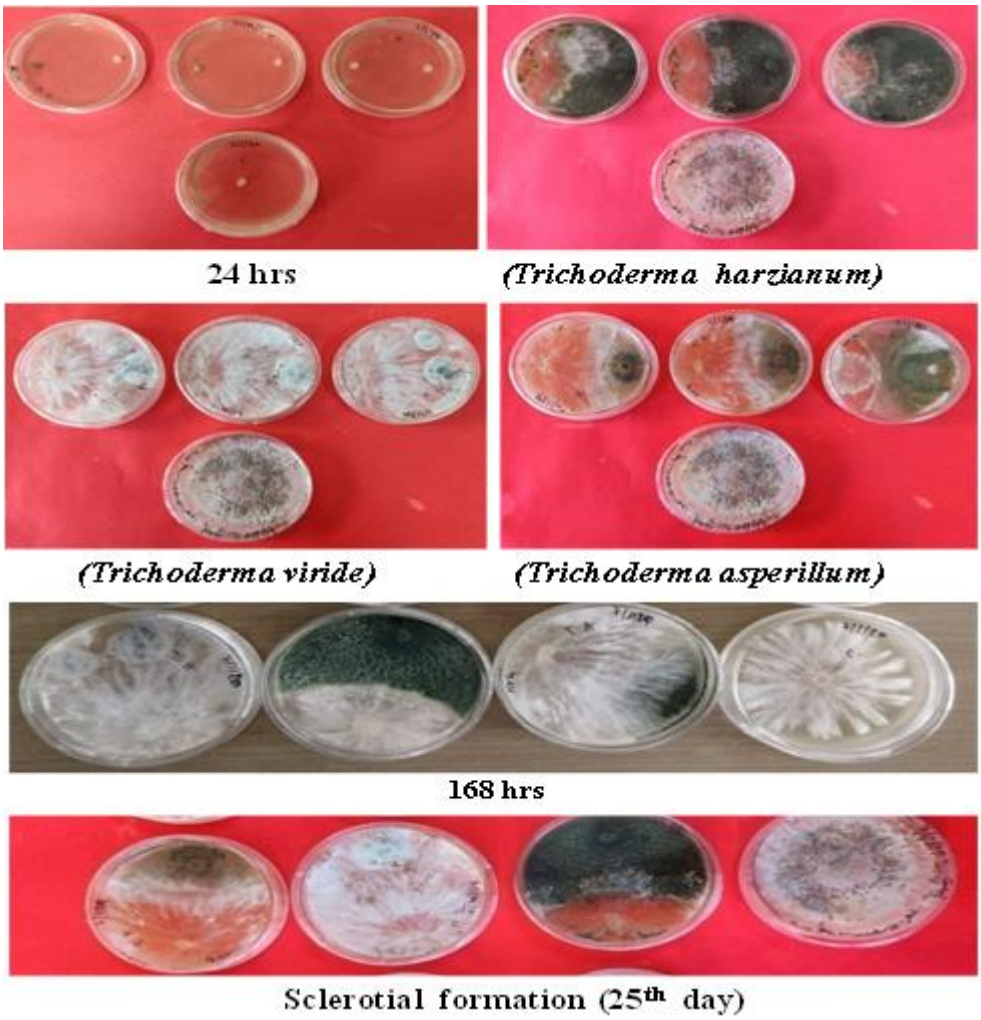

Plate 1: Sensitivity of *Sclerotium rolfsii* to different fungal antagonist (Duel Culture Techique)

High efficacies of *Trichoderma harzianum* has been recorded against *Sclerotium rolfsii* by Divya Bharathi and Benagi (2018) [8]. While evaluating different antagonists of *Trichoderma* species, Vineela *et al.* (2017) [21] also reported *Trichoderma harzianum* as the most promising antagonist against the mycelial growth of *S. rolfsii*. Nagaraja *et al.* (2012) [10], reported *Trichoderma harzianum* is most effective against *S. rolfsii* followed by *Trichoderma asperillum*. Mohammad *et al.* (2016) [12] recorded *Trichoderma harzianum* as the potent antagonist against mycelial growth of *Sclerotium rolfsii* causing stem blight of tomato.

In vitro* evaluation of fungicides against *Sclerotium rolfsii:
Five fungicides among which there are three systemic and two non-systemic fungicides were tested against *Sclerotium rolfsii* under *in vitro* conditions through poison food technique. The data regarding the percent growth inhibition by different fungicides at different concentration levels 250, 500, 750 and 1000 ppm. Systemic fungicides were found more inhibitory as compared to non-systemic fungicides even at equal levels of concentration against the pathogen. The data regarding the percent growth inhibition by five fungicides (three systemic and two non-systemic) are as follows, Among the systemic fungicide, hexaconazolel produced significant growth inhibition (8.53%) at the concentration level of 250 ppm and (93.44%) at the concentration level of 1000 ppm. Difenoconazole and cabroitop provided statistically similar inhibition at higher (1000 ppm) dosage level proved next efficacious. Among the non-systemic fungicide, mancozeb produced significant growth inhibition (6.35%) at the concentration level of 250 ppm and (93.44%) at the concentration level of 1000 ppm. Chlorothalonil provided statistically similar inhibition at different dosage levels proved next efficacious.

Table 3: *In-vitro* evaluation of different systemic and non-systemic fungicides against stem blight of tomato caused by *Sclerotium rolfsii* (Curzi) C.C Tu & Kimber.

concentrations	% of Inhibition				
	Chlorothalonil	Difenconazol	Hexaconazol	Cabriotop	Mancozeb
250PPM	5.30	5.65	8.53	4.70	6.35
500PPM	27.09	26.48	29.71	25.75	28.59
750PPM	78.80	49.59	78.49	46.78	78.67
1000PPM	92.92	92.38	93.44	92.21	93.44
C.D.	0.39	0.43	0.31	0.26	0.33
SE(m)	0.13	0.14	0.10	0.09	0.11

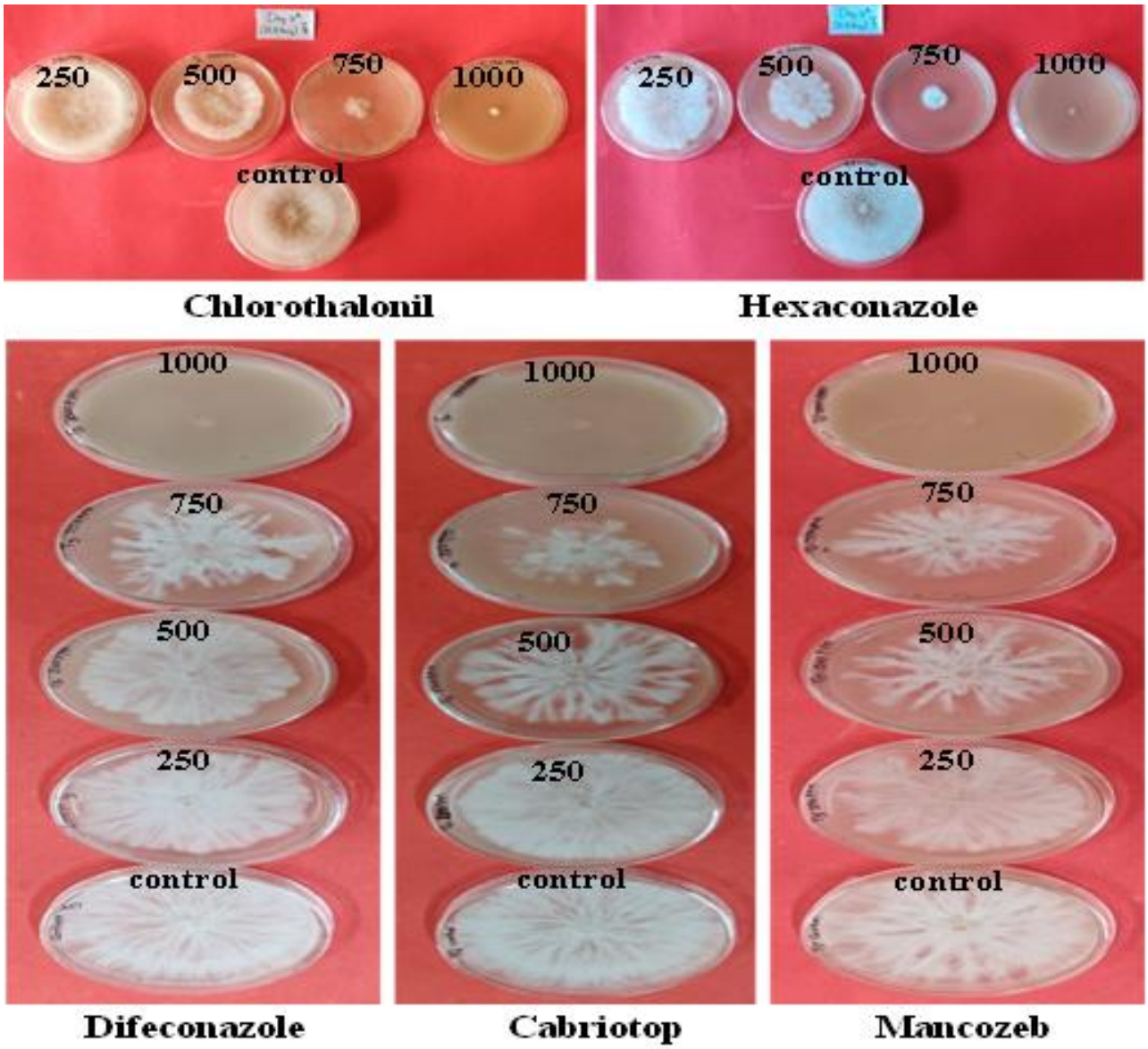

Plate 2: *In Vitro* effects of different fungicides against mycelial growth of *Sclerotium rolfsii* (7th day)

High efficacies of hexaconazole and mancozeb have also been recorded against *Sclerotium rolfsii* by Rakholiya (2015) [16]; Dinesh *et al.* (2018) [6, 7]; Soyal and Ratnoo (2018) [20] and Shirsole *et al.* (2019) [19]. While evaluating different fungicides Sharma and Dhruj (2018) reported that Cabrio top, difenoconazole and chlorothalonil as the most promising fungicides at higher concentrations against the mycelial growth of *Sclerotium rolfsii*. Similarly, Nagaraja *et al.* (2012) [10] and Das *et al.* (2014) reported hexaconazole as most effective against *Sclerotium rolfsii* followed by difenoconazole and Cabrio top. Ashis *et al.* (2014) [1] recorded chlorothalonil and mancozeb as potent fungicides against mycelial growth of *Sclerotium rolfsii*. *In vitro* studies (Rajendra Prasad *et al.*,2017; Vineela *et al.*,2017) [15, 21] also revealed hexaconazole, difenoconazole and mancozeb as a promising fungicide against mycelial growth of *Sclerotium rolfsii.*

In vitro* efficacy of different essential oils on mycelial growth of *Sclerotium rolfsii:

The usages of some essential oils can be an alternative to some chemicals for the management of some plant diseases. To find out the possibilities of replacing fungicides with the other eco-friendly products for the management of diseases, essential oils, ie. Palmarosa, Karanja, thyme, menthol and lemongrass oils were tested at different concentrations (0.5, 1.0, 1.5 and 2.0%) levels under *in vitro* studies against the mycelial growth of *Sclerotium rolfsii*. Irrespective of different concentration levels of essential oils tested against mycelial growth of *Sclerotium rolfsii*, Palmarosa (*Cymbopogon martini*) and Thyme (*Thymus vulgaris*) proved the most efficacious providing cent percent growth inhibition at 1.5 and 2.0 (%). Followed by Karanja oil and lemongrass oil also resulted in significant growth inhibition (5.26%) and (5.55%) of the pathogen at (0.5%). Menthol oil showed less inhibition percent (3.05%) against the mycelial growth of *Sclerotium rolfsii* at (0.5%).

Table 4: *In-vitro* evaluation of different essential oils against stem blight of tomato caused by *Sclerotium rolfsii* (Curzi) C.C Tu & Kimber.

concentrations	% of Inhibition				
	Palmarosa oil	Karanja oil	Menthol oil	Thyme oil	Lemon grass oil
0.5%	16.12	5.26	3.05	6.37	5.55
1%	26.12	11.95	10.82	18.61	16.66
1.5%	49.42	18.05	21.94	26.38	26.38
2%	72.76	38.21	34.15	42.77	34.15
C.D.	0.06	0.59	0.04	0.04	0.05
SE(m)	0.02	0.20	0.01	0.01	0.02

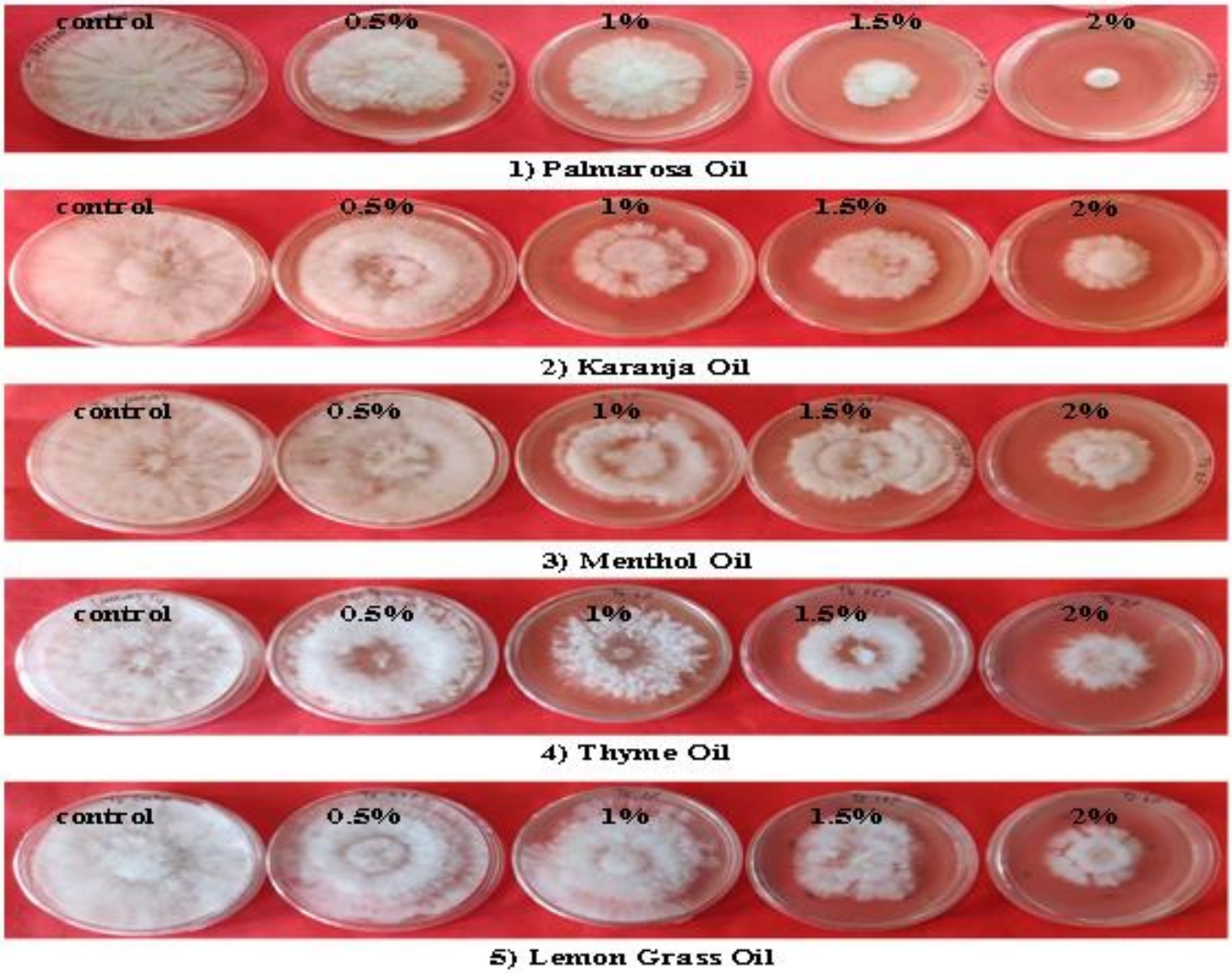

Plate 3: *In vitro* effects of essential oils against *Sclerotium rolfsii*

Essential oils are ecological safer, non-hazardous and non-polluting means of plant disease management. Maria Lima *et al.* (2015). reported that palmarosa (*Cymbopogon martini* oil) at 300 ppm inhibited the mycelia growth of *S. rolfsii* in 55% and also the number of sclerotial bodies. Awasthi *et al.* (2018) [2] reported that Citronella oil and palmarosa (*Cymbopogon martini* oil) @ 0.5%) showed an economically significant positive response in all respective parameters and was highly effective in inhibiting the growth of *S. rolfsii*. palmarosa (*Cymbopogon martini*) giving 100 percent of growth inhibition @ 10 percent followed by thyme (*Thymus vulgaris*). Mokhtar *et al.* (2011) [13], Investigated on mycelial growth of the tested fungi showed more sensitivity to high concentrations of thyme (*Thymus vulgaris*). Dasgupta, Barman and Muthu Kumar (2015) [5] also reported the efficacy of lemongrass (*Cymbopogon citrates)* against mycelial growth of *Sclerotium rolfsii.* Ashis *et al.* (2014) [1] found Karanja (*Pongamia pinnata*) as the next most effective against *S. rolfsii.* Marco *et al.* (2014) [11] revealed the efficacy of menthol oil against mycelial growth of *Sclerotium rolfsii*.

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