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In vitro evaluation of some essential oil and *Trichoderma viride* against Stem rot (*Sclerotium rolfsii*) of Groundnut

Amrita Subhadarshini, Shashi Tiwari and Kondakari Mahanthi

Abstract
Three essential oils and one bio-agent were investigated in order to control groundnut plants against *Sclerotium rolfsii* causing stem rot. The study was conducted in plant pathology laboratory, SHUATS during 2019. The essential oils i.e. Neem oil, Eucalyptus oil, Clove oil, bio-agent (i.e. *Trichoderma viride)* and their combinations were evaluated at 5% concentrations and studied the radial growth of the pathogen. The complete inhibition was obtained with clove oil + *T. viride*, Clove oil, Eucalyptus oil+ *Trichoderma viride*, Eucalyptus oil at all selected concentration. *Trichoderma viride* was also screened against *S. rolfsii* following dual plate culture technique.

Keywords: Neem oil, Eucalyptus oil, Clove oil, *Trichoderma viride* and *Sclerotium rolfsii*.

1. Introduction
Groundnut (*Arachishypogaea* L.) is an important oil seed crop. It also known as Peanut, Monkeynut, Goobernut. Groundnut contains on an average 40.1 per cent of fat and 25.3 per cent of protein and is a rich source of calcium, iron and vitamin B complex like thiamine, riboflavin, niacin and vitamin A. The plant is a central, upright stem that may stand up to 18 inches tall which bears numerous branches with compound leaves. Groundnut productivity obstacled by bacteria, pest and fungal diseases among which stem rot caused by *S. rolfsii* is one of the major constraints occurring at the lower portion of the stem. *S. rolfsii* is a saproproph fungus with living plants. It firstly attack host plants like groundnut at the soil line and rapidly moves to the root as it destroys plant tissues with pectolytic enzymes and oxalic acid. *S. rolfsii* grow at 70% soil moister of field capacity and at temperature range between 25-30 ^{o}C.

White mycelium of *Sclerotium rolfsii* was seen around the affected plants at or near the soil surface, imparting a 'white washed' appearance to the base of the affected plants. The infected area of the collar portion was shredded and mycelium quickly produced abundant spherical sclerotia on the collar portion of the affected plant. The sclerotia were initially white and turned light brown to dark brown in colour towards maturity.

The mycelium of the pathogen is septated and hyaline with conspicuous branching at acute angles. The well develop mycelium had cord-like strands. The hyphae have clamps in the form of forks and hooks or H-like connections (Aycock, 1966). The young growing mycelial mass is snow white with a silky – cluster. Sclerotia are at first white, becoming light brown to dark brown at maturity. They are sub-spherical, the surface being finely wrinkled or pitted, sometimes flattened, normally 0.5-1.5 mm in diameter.

In recent years, farmers have been using chemical fungicides in the field against soil borne fungus which is more advantageous, but chemicals being hazardous to both environment and human health in long term biological control of the pathogen is in rage these days. Biological control of pathogen neither creates environmental pollution nor human health.

2. Materials and Methods
2.1. Sample collection:
From the groundnut field the infected plant was selected. The infected plant parts and rhizospheric region and non-rhizospheric were carried out in a polythene bag and preserved for the further use.

2.2. Isolation and identification
The infected plant parts were cut in to small pieces (2-4 mm length) and sterilized with 0.5% mercuric chloride solution for 30 seconds, then washed with distilled water. The small pieces

were placed in potato dextrose agar plates and incubated in room temperature for 6-7 days. The fungal mycelium was observed after 7 days and pure culture was maintained in PDA slants. The fungus was identified by its morphological characters.

2.3. Evaluation of botanicals
Three botanicals viz. Neem oil, *Eucalyptus* oil, Clove oil at 5% concentration were evaluated. Redial growth of *S. rolfsii* were recorded applying poison food techniques (Nene and Thapliyal, 1993) using Potato dextrose agar (PDA) as basal culture media.

2.3.1. Poison food technique
The principle involved in this techniques was to make the nutrient medium toxic with a fungitoxicant and allow the text fungi to grow on it and study the mycelial inhibition.100 ml of PDA was taken in 250 ml flask and the botanicals were added at 5% concentration and sterilized. Later this PDA was poured in petriplates and inoculated with 5 mm discs of test fungal culture which were cut using cork borer.

2.3.2. Dual Culture
Dual culture technique was used to study the antagonism of *T. viride* in combination with the above botanicals at same concentrations. Discs of 5mm diameter fungal mycelium for both *T. viride* and *S. rolfsii* were cut and placed in petriplates containing different botanical treated PDA. Plates with only mycelia discs of text pathogen served as control. Every treatment had 3 replications and all operations were conducted aseptic condition under laminar air flow chamber. The percent inhibition of the pathogen was calculated by the following formula.

$$\text{Percent inhibition} = \frac{\text{Control} - \text{Treatment}}{\text{Control}} \times 100$$

Fig 1: Mycellial growth on plant

Fig 2: Microscopic view of *S. rolfsii*

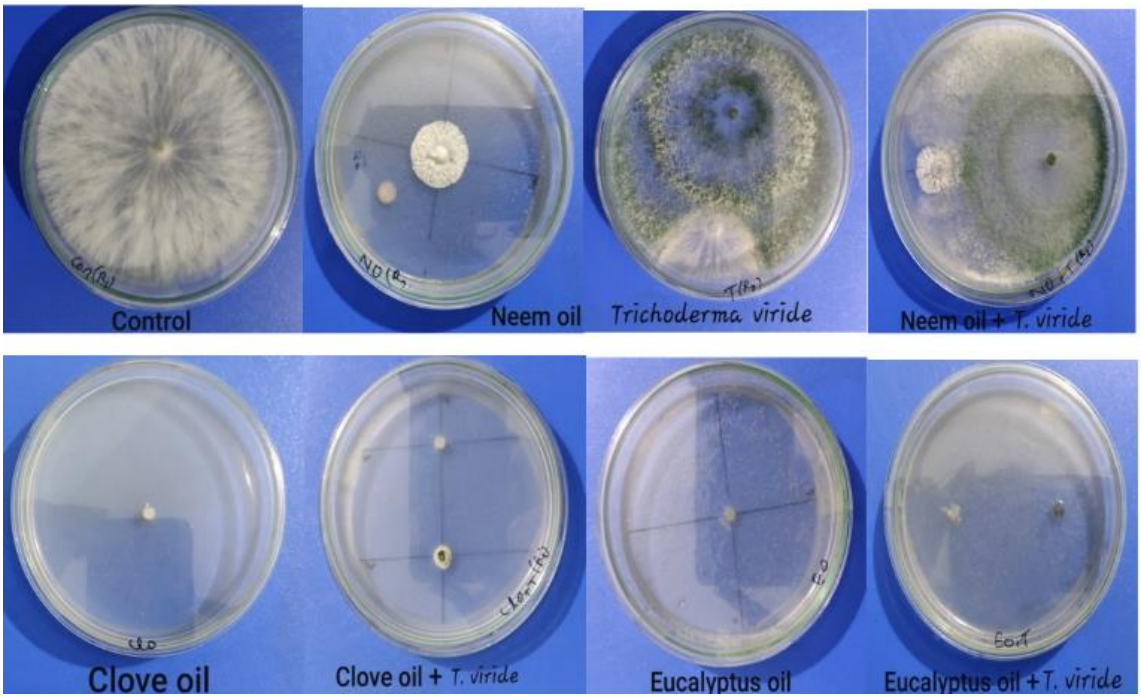

Plate 1: Effect of treatments against the *S. rolfsii In vitro* condition

3. Result and Discussion
We were tested the dual culture method to find out the antagonistic activity against the *S. rolfsii in vitro* condition and it was observed that *T. viride* was the most effective bio-agent. The most effective combination were found that Eucalyptus oil+ *T. viride*, Eucalyptus oil, clove oil + *T. viride* and clove oil of 94.45% of inhibition followed by Neem oil+ *T. viride* and Neem oil.

After 48 hrs inoculation the minimum mycelial growth were recorded in treatment T_2 Eucalyptus oil, T_3 Clove oil, T_6 Clove oil + *Trichoderma viride* and T_7 Eucalyptus oil + *Trichoderma viride* which was 0 mm followed by T_5 Neem

oil + *Trichoderma viride* (5.85 mm). The maximum mycelial growth was recorded in T_0 control (23.16 mm).

After 72 hrs inoculation the minimum mycelial growth were recorded in treatment T_2 Eucalyptus oil, T_3 Clove oil, T_6 Clove oil + *Trichoderma viride* and T_7 Eucalyptus oil + *Trichoderma viride* which was 0 mm followed by T_5 Neem oil + *Trichoderma viride* (11.21 mm). The maximum mycelial growth was recorded in T_0 control (39.64 mm).

After 96 hrs inoculation the minimum mycelial growth were recorded in treatment T_2 Eucalyptus oil, T_3 Clove oil, T_6 Clove oil + *Trichoderma viride* and T_7 Eucalyptus oil + *Trichoderma viride* which was 0 mm followed by T_5 Neem oil + *Trichoderma viride* (13.42 mm). The maximum mycelial growth was recorded in T_0 control (65.07 mm).

After 120 hrs inoculation the minimum mycelial growth were recorded in treatment T_2 Eucalyptus oil, T_3 Clove oil, T_6 Clove oil + *Trichoderma viride* and T_7 Eucalyptus oil + *Trichoderma viride* which was 0 mm followed by T_5 Neem + *Trichoderma viride* (15.71 mm). The maximum mycelial growth was recorded in T_0 control (90 mm).

The present experiment also shows that *Trichoderma viride* compatible with Neem oil, Eucalyptus oil and Clove oil.

Hence, from above experiment, it is clearly shown that *in vitro* conditions treatment T_2 Eucalyptus oil, T_3 Clove oil, T_6 Clove oil + *Trichoderma viride* and T_7 Eucalytus oil + *Trichoderma viride* show the maximum and equivalent inhibition which was similar to Thabet and Khalifa, 2018 where he proved that in *In vitro* conditions clove oil exhibited an inhibitory effect against the mycelial growth of soil borne pathogens. All tested concentrations significantly reduced mycelial linear growth of the tested fungi compared to respective controls. Bagwan, 2010 [2] also reported that maximum inhibition was given *Trichoderma viride* against soil borne disease of soyabean.

From the previous literature it was observed that the major drawback with the searchers was that they do not used the combinations which are used in this experiment. Therefore, combination of these treatments can be efficient for management stem rot of groundnut in field conditions.

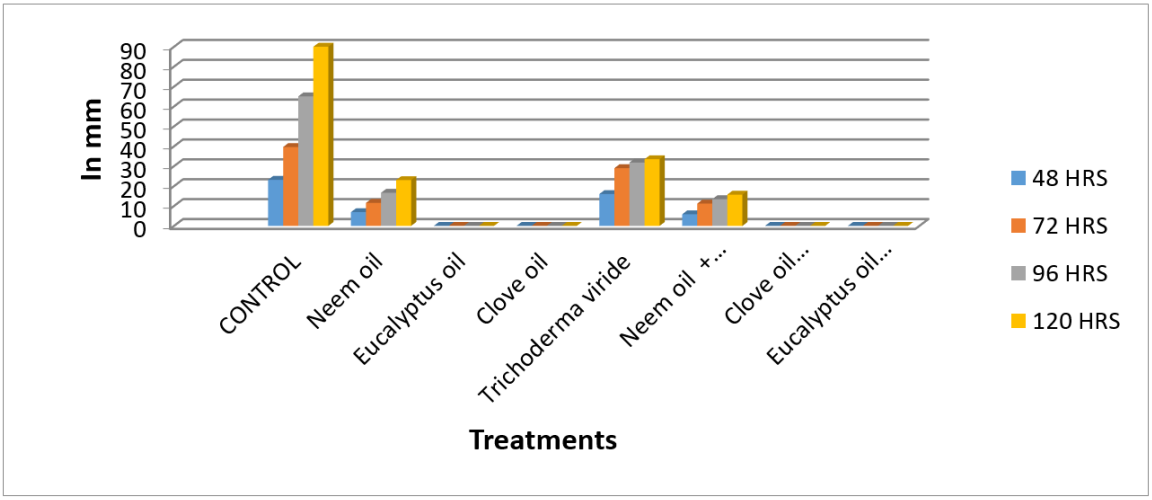

Fig 3: Mean colony diameter of *S. rolfsii* on culture media with different treatment

Table 1: Effect of treatments on Pathogen and bio-agent disease *in vitro* condition

Sl. No.	Treatments	Mean Colony Diameter (In mm)								Inhibition (%)
		48 HRS		72 HRS		96 HRS		120 HRS		
		S. rolfsii	*T. viride*	*S. rolfsii*	*T. viride*	*S. rolfsii*	*T. viride*	*S. rolfsii*	*T. viride*	
1	Control	23.16		39.64		65.07		90		0
2	Neem oil	6.92		11.57		16.64		23		74.45
3	Eucalyptus oil	0		0		0		0		100
4	Clove oil	0		0		0		0		100
5	*Trichoderma viride*	16	57.30	29	80.50	31.85	84.50	33.5	85.00	62.77
6	Neem oil + *Trichoderma viride*	5.85	43.00	11.21	65.00	13.42	80.00	15.71	85.10	82.54
7	Clove oil +*Trichoderma viride*	0	5.00	0	6.25	0	7.50	0	8.23	100
8	Eucalyptus oil +*Trichoderma viride*	0	5.00	0	5.50	0	6.25	0	7.00	100
F test		S		S		S		S		
SE d(+)		0.692		2.317		3.154		1.542		
CD(0.05)		1.465		4.918		6.683		2.312		

4. Conclusion

In the current study, the most promising findings include those obtained with Clove oil, Eucalyptus oil, Neem oil and bio-agent *Trichoderma viride* which were able to completely inhibit mycelial growth of pathogen evaluated. Clove oil and Eucalyptus oil have the anti-fungal properties which resulted that the maximum inhibition of the pathogen. Neem oil also encourage the growth of *Trichoderma viride*, these findings encourage others to pursue alternative compounds such as essential oils for disease control.

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