

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.811.072>

First Report on Application of Clove Oil in Combination with *Trichoderma viride* against Stem Rot of Groundnut from Prayagraj (U.P) India

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ABSTRACT

Groundnut is one of the most important economic oilseed crops of the world. It belongs to the family leguminosae and mostly infected by several fungal, bacterial and viral diseases among which Stem rot caused by *Sclerotium rolfsii* is considered as one of the most destructive diseases in the groundnut growing areas. An experiment was conducted to check efficacy of few selected essential oils and *Trichoderma viride* against *Sclerotium rolfsii* on field conditions. Three types of essential oils viz., Neem oil, Clove oil, Eucalyptus oil and one bio-agent viz. *Trichoderma viride* were selected @ 5 percent concentration as seed treatment. The other three treatments were the combination of each selected oil with the taken bio-agent viz. Neem oil @ 5 % + *Trichoderma viride* @ 5 %, Eucalyptus oil @ 5 % + *Trichoderma viride* @ 5 % and Clove oil @ 5 % + *Trichoderma viride* @ 5 %. An untreated replication served as control. Readings for disease incidence, growth parameters were taken at desired no of days. On field conditions, among all the treatments Clove oil @ 5 % + *Trichoderma viride* @ 5 % showed the most significant results followed by Eucalyptus oil @ 5 % + *Trichoderma viride* @ 5 % and Clove oil @ 5 %. The lowest inhibition was seen in control plot. Subsequently benefit cost ratio was also most desirable with Clove oil @ 5 % + *Trichoderma viride* @ 5 %.

Keywords

Groundnut, Neem oil, Eucalyptus oil, Clove oil, *Trichoderma viride*

Article Info

Accepted:
07 October 2019
Available Online:
10 November 2019

Introduction

Groundnut (*Arachis hypogaea* L.) is the world's fourth most important source of edible oil and important source of protein. It contains rich sources of edible oil (43-55%) and protein (25%-28%). 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). Stem rot incited by *Sclerotium rolfsii* is one of the major production constraints of Groundnut majorly in tropical and subtropical countries. Stem rot causes pod yield losses of 10-25% but under severe diseased conditions yield losses can range up to 80% (Rodriguez-

kabana *et al.*, 1975). *Sclerotium rolfsii* has wide geographic diversity and commonly found in the tropics, subtropics and other warm temperate regions especially the Southern United States, Central and South America, West Indies, Southern European countries bordering the Mediterranean, Africa, India, Japan, Philippines and Hawaii (Aycok, 1966). The fungus survives as sclerotia in soil and plant debris and remains viable for 2 to 3 years. High temperature 30°C and high soil moisture (77 %) encourages the disease.

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 Stem rot fungus also attacked the roots, pegs and pods of groundnut. Pods on damaged pegs were shed before harvest. Diseased pods turned dark brown and some of the pods disintegrated in the soil. Severely infected pods were completely covered with a white fungal growth and decayed. In some cases the seeds from the diseased pods showed a characteristic bluish-gray discoloration known as 'blue damage' Anahosur (2001).

Spray of chemical fungicides possesses serious threat to beneficial organisms of ecosystem. Among the available alternate disease management options, biological control seems to be the most promising. *Trichoderma* spp. are effective in controlling soil/seed borne diseases in many crop plants (Kubicek *et al.*, 2001), including Groundnut (Podile and Kishore, 2002). Essential oils are eco-friendly and less toxic to environment, thus use of plant oils 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

Study site

The present study was carried out in the Department of plant pathology site located at central field of SHUATS, Prayagraj, Uttar Pradesh, during 2018. The experiment was done during *Kharif* season using Randomized Block Design having 8 treatments including a control and 3 replications each with a plot size of 2×1.5m.

Seed treatment

Groundnut seeds were taken in separate conical flasks and then three types of essential oils viz., Neem oil, Clove oil, Eucalyptus oil and one bio-agent viz. *T. viride* were taken in 5% concentrations accordingly and poured. For combinations, oil and *T. viride* were taken 5% each. The flasks were shaken by hand for 5 minutes until the seeds were saturated. In control, seeds were planted with no treatments (Hashem *et al.*, 2010).

Isolation and identification of pathogen

The pathogen *Sclerotium rolfsii* was isolated from the infected Groundnut plants showing typical symptoms of the disease (Fig.1 and 2). The part of collar or stem region showing typical symptoms of the disease was cut into small pieces. These pieces were surface sterilized with 0.1 % mercuric chloride solution for 30 seconds. These were then washed thoroughly in sterile water thrice to remove traces of mercuric chloride and then transferred aseptically to sterilized potato dextrose agar (PDA) plates. They were incubated at 27±1°C and checked on 24 hour basis for the growth of the fungus. Later, the

bit of fungal growth was transferred to PDA slants. The pure culture of the fungus was obtained by further growing the culture and following hyphal tip culture under aseptic conditions (Rangaswamy, 1972). The pathogen was confirmed by observing the morphological features.

Results and Discussion

Effects of treatments on plant growth parameters

Observations were recorded at 60,75, 90 DAS for disease incidence and for growth parameters the observations were recorded at 15,30,45,60 DAS. Growth parameters included plant height and branches. Of all the treatments, T₆ (clove oil+ *T.viride*) showed the maximum growth in height and more number of branches followed by T₇ (Eucalyptus oil + *T.viride*) and T₃(clove oil).

At 60 DAS maximum plant height was recorded in treatment T₆ (47.86) followed by T₇ (46.93), T₃ (44.6), T₁(42.26), T₅ (42.06), T₄(39), T₂(38) and T₀(37.53). The maximum number of branches were recorded in treatment T₆ (10.8) followed by T₇ (10.13), T₃ (9), T₁ (8.6), T₅ (7.06), T₄ (6.33), T₂(5.66) and T₀(5.53).

Similar findings were reported by Rama Yalla Reddy (2002) and he found that integrated use of *T. viride*, *P. fluorescens*, commercial Neem product Niwaar and thiram for seed treatment of Groundnut improved pod yield, increase the growth parameters and controlled soil mycoflora viz., *A. niger*, *Alternaria* spp., *Curvularia* sp., *Fusarium* spp., *Drechslera* spp., *Penicillium* spp., *R. stolonifer*, *Rhizoctonia* spp., *S. rolfsii* and *Verticillium* spp. Kishore *et al.*, (2007) tested Clove oil, cinnamon oil, and five essential oil components (citral, eugenol, geraniol, limonene, and linalool) against growth

inhibition of 14 phyto-pathogenic fungi. Citral completely inhibited the growth of *Alternaria alternata*, *Aspergillus flavus*, *Curvularia lunata*, *Fusarium moniliforme*, *F. pallidoroseum*, and *Phoma sorghina* in paper disc agar diffusion assays (Fig. 3 and Table 1).

Effect of treatments on disease incidence

The table 2 indicates the disease incidence in 60, 75 and 90 DAS. The minimum disease incidence recorded at 90 DAS were with the treatment Clove oil + *Trichoderma viride* T₆ (4.66%) followed by Eucalyptus oil + *Trichoderma viride* T₇ (7%), Clove oil T₃ (8.33%), Neem oil + *Trichoderma viride* T₅ (13.66%), *Trichoderma viride* T₄ (17.33%), Eucalyptus oil T₂ (19.33%), Neem oil T₁ (21%) and Control T₀ (41.33%). Suryawanshi *et al.*, (2007) showed anti-fungal activity of fresh leaf extracts (10%) of all six plant species and effectively inhibited mycelial growth of *Sclerotium rolfsii* Sacc. inciting collar rot in Pigeonpea.

However, leaf extracts of *Azardira chtaindica*, *Eucalyptus* spp. *Annona squamosa* and *Ricinus communis* recorded maximum inhibition of mycelial growth of the test fungus by 43.6, 42.3 and 27.6% over control respectively. Similarly, in pot culture experiment leaf extracts (20%) of all six plant species were found significantly superior over control and there by recording maximum seed germination (75 to 88%) minimum pre-emergence (11 to 25%) and post-emergence (11 to 33%) seedling mortality and increased final plants and (48 to 78%) (Fig. 4).

B:C ratio

Benefit-cost analysis (BCA) is a technique for evaluating a project or investment by comparing the economic benefits of an activity with the economic costs of the activity. The maximum yield was recorded in

treatment T₆ (10.67q/ha), T₇ (10.27q/ha), T₃ (7.83q/ha), T₂ (6.99q/ha), T₀ (6.06q/ha) (Fig. 5 (9.33 q/ha), T₁ (9.27q/ha), T₅ (8.67q/ha), T₄ and Table 3).

Table.1 Effect of treatments on plant height and number of branches

TREATMENTS	30 DAS		45DAS		60 DAS	
	HEIGHT (In cm)	BRANCHES	HEIGHT (In cm)	BRANCHES	HEIGHT (In cm)	BRANCHES
Control (T ₀)	16.93	3	30.93	4.43	37.53	5.53
Neem oil (T ₁)	19.73	5.86	34.66	7.40	42.26	8.6
Eucalyptus oil (T ₂)	18	3.06	31.4	4.46	38	5.66
Clove oil (T ₃)	19.93	6.06	36.2	7.73	44.6	9
<i>Trichoderma viride</i> (T ₄)	18.23	3.86	33.26	5.4	39	6.33
Neem oil + <i>Trichoderma viride</i> (T ₅)	18.6	4.4	33.86	5.93	42.06	7.06
Clove oil + <i>Trichoderma viride</i> (T ₆)	22.7	7.2	37.06	9.4	47.86	10.8
Eucalyptus oil + <i>Trichoderma viride</i> (T ₇)	21.93	6.2	36.93	8.6	46.93	10.13
F test	S	S	S	S	S	S
SE d (+)	1.262	0.548	0.503	0.341	0.673	0.314
CD (0.05)	2.711	1.178	1.083	0.736	1.439	0.665

Table.2 Effect of treatment on Stem rot disease incidence

Treatments	60 DAS (%)	75 DAS (%)	90 DAS (%)
Control (T ₀)	15.66	36	41.33
Neem oil (T ₁)	6.33	12.66	21
Eucalyptus oil (T ₂)	2	9	19.33
Clove oil (T ₃)	0.66	1.3%	8.33
<i>Trichoderma viride</i> (T ₄)	1.66	6	17.33
Neem oil + <i>Trichoderma viride</i> (T ₅)	1	5	13.66
Clove oil + <i>Trichoderma viride</i> (T ₆)	0.33	0.6	4.66
Eucalyptus oil + <i>Trichoderma viride</i> (T ₇)	0.33	1	7
F test	S	S	S
SE d(+)	1.033	1.089	1.645
CD(0.05)	2.211	2.334	3.533

Table.3 Effect of treatments on yield

Treatment	Yield of q/ha	Total cost Of yield (Rs.)	Total cost of cultivation	C:B ratio
T ₀	6.06	66,660/-	41,000/-	1:1.62
T ₁	9.27	1,01,970/-	42,000/-	1:2.42
T ₂	6.99	76,890/-	45000/-	1:1.70
T ₃	9.33	1,02,630/-	45,400/-	1:2.26
T ₄	7.83	86,130/-	41,280/-	1:2.08
T ₅	8.67	95,370/-	42,280/-	1:2.25
T ₆	10.67	1,17,370/-	45,680/-	1:2.56
T ₇	10.27	1,12,970/-	45,280/-	1:2.49

Fig.1 Mycelial growth of *S. rolfsii*

Fig.2 Groundnut affected by stem rot



Fig.3 Effect of treatments in plant growth

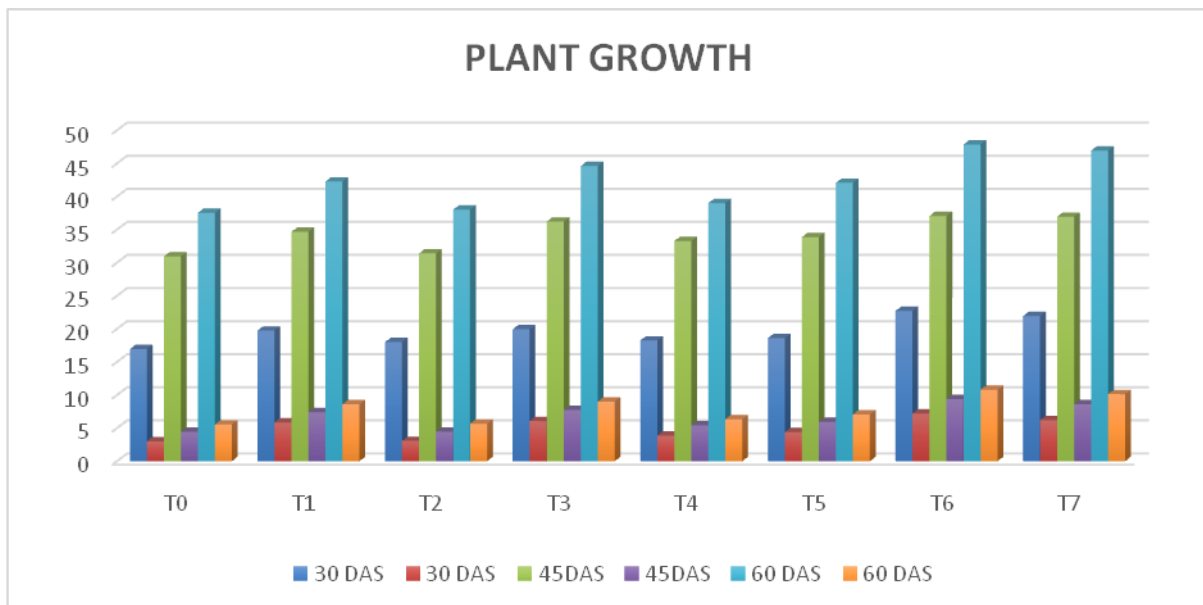


Fig.4 Disease incidence *in vivo* condition

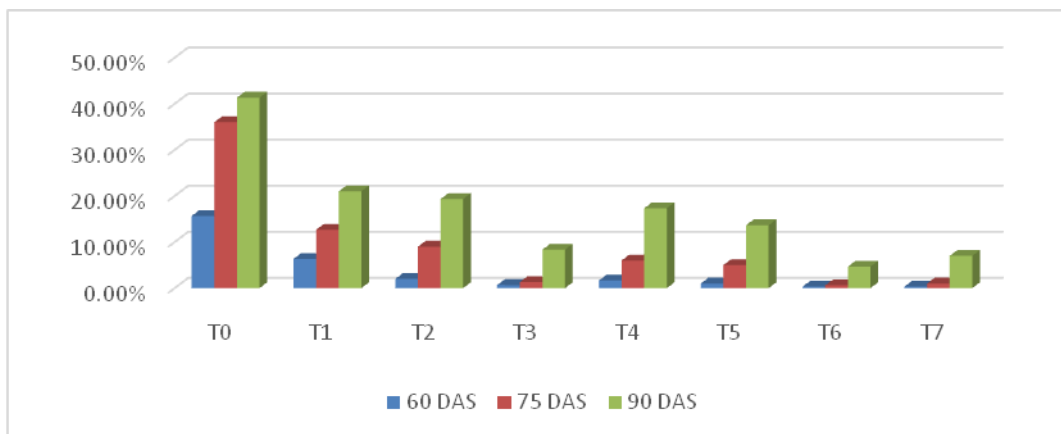
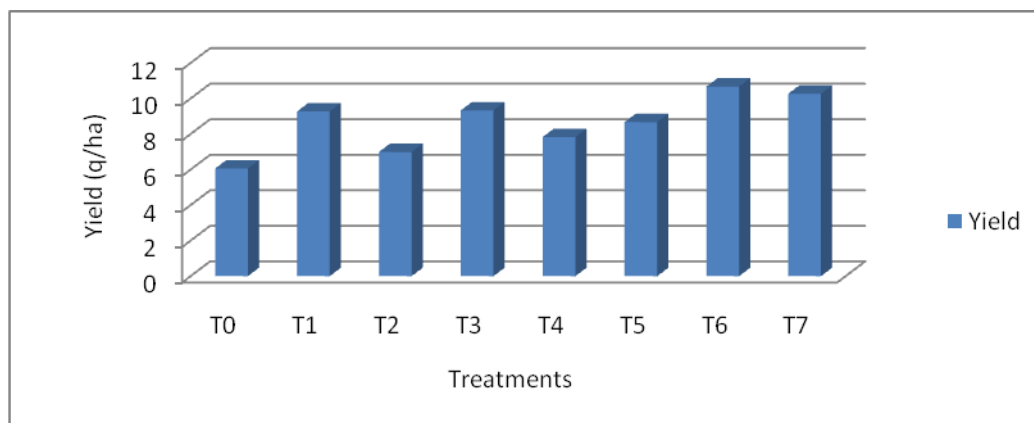


Fig.5 Effect of treatments on yield



Likewise the maximum B:C ratio was seen in the treatment T₆(1:2.56) followed by T₇ (1:2.49), T₁ (1:2.42) and T₃ (1:2.25).

The management of soil borne diseases by chemical methods may lead to hazardous effects on soil by making it loose fertility and also cause environmental pollution. So, better alternatives like essential oils and bio-agents must be encouraged as they are eco-friendly and cost effective in nature.

The present research concludes that seed treatment of Groundnut with Clove oil + *T. viride* @ 5% each was most effective in managing stem rot.

Subsequently the growth parameters were also good leading to a highest yield among the selected treatments. Thus, the treatment Clove oil + *T. viride* @ 5% can be reliable and cost effective management option against stem rot of Groundnut.

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How to cite this article:

Amrita Subhadarshini, Shashi Tiwari and Kondakari Mahanthi. 2019. First Report on Application of Clove Oil in Combination with *Trichoderma viride* against Stem Rot of Groundnut from Prayagraj (U.P) India. *Int.J.Curr.Microbiol.App.Sci*. 8(11): 589-595.
doi: <https://doi.org/10.20546/ijcmas.2019.811.072>