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EFFICACY OF SOME ESSENTIAL OILS AGAINST STEM ROT OF GROUNDNUT (*ARACHIS HYPOGAEA* L.) CAUSED BY *SCLEROTIUM ROLFSII* (SACC.)

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ABSTRACT
The essential oils, like-Lemon grass, Citronella and its derivatives showed effective against stem rot of groundnut caused by *Sclerotium rolfsii* in *in vitro* as well as *in vivo* condition. The efficacy of these oils against the *Sclerotium rolfsii* was assessed with the inhibition percentage at very low concentration (0.0125% to 0.15%) under *in vitro* condition. Under in vivo condition, the seeds were soaked with the above oils with the concentration of 0.05% and 0.1% for 30-40 minutes before sowing to show the efficacy on disease mortality percentage. The result showed that all essential oils recorded the better performance, but derivatives of Citronella oil, Geraniol and Terpinoid were most effective under *in vitro* condition and *in vivo* condition. Highest disease control was recorded in treatment with terpinoid where 9.1% disease mortality was found. So, the applications of essential oils are possible by farmers for sustainable crop production through eco-friendly management that can be incorporated in Integrated Disease Management (IDM) system.

INTRODUCTION

Groundnut (*Arachis hypogaea* L) suffers from various fungal diseases out of which Sclerotial rot or Stem rot of groundnut caused by *Sclerotium rolfsii* is the most devastating one which causes severe losses, reducing yield by 10-25% (Mehan *et al*., 1995). Control efforts have often met with limited success partially due to the extensive host range, prolific growth rate and ability to produce large numbers of sclerotia that may remain viable in soil for several years. To avoid the indiscriminate use of fungicides which caused ecological hazards, environmental pollution, high residual toxicity, new emphasis should be given to use different essential oil which are commonly available, biodegradable and safe for use in agricultural fields. Very little work was done for the *in vitro* control of most dreaded disease of groundnut with the use of some essential oils and their derivatives (Sharma *et al*., 2009; Abdalla *et al*., 2009 and El-Batal and Fathy, 2011). The antifungal action of lemongrass oil on some soil borne plant pathogens, like *Rhizoctonia solani*, *Sclerotium rolfsii* and *Sclerotiana sclerotirum* were reported by Handique and Singh,1990. Kole *et al*. (1993) found that the essential oils of Citronella exhibited antifungal activities against *Alternaria solani*, *Sclerotium rolfsii* and *Fusarium oxysporum* where growth was completely inhibited by the oil while the other fungi were inhibited to varying degrees. For the management of stem rot of groundnut no suitable fungicide is available so essential oil and its derivatives may be tried as reported by many workers (Kole *et al*., 1993; Sharma *et al*., 2009; Abdalla *et al*., 2009 and El-Batal and Fathy, 2011).

Keeping in view, the present investigation was carried out to find out the efficacy of some essential oils of *Cymbopogon citrates* (Lemongrass) and *C. winterianus* (Citronella) with its four derivatives against the important soil-borne phytopathogenic fungi, *Sclerotium rolfsii* under *in vitro* and *in vivo* condition.

MATERIALS AND METHODS

Preparation of oil extracts

The oils were extracted from the two aromatic grasses like citronella (*Cymbopogon winterianus*) and lemongrass (*Cymbopogon citrates*) by hydrodistillation process (Chakraborty, 2005).Citronella has four derivatives namely Citronella Tops, Citronellal, Geraniol and Aldehyde which were used for determination of efficacy against *S.rolfsii*.

Preparation of different concentrated oil extracts

All the oils and its derivatives were prepared by separately dissolved in mixing 99.8 ml of oil + 0.2 ml of Tween-80 (0.2%) to prepare the stock solution (100%). The different concentrations of oils (0.15, 0.10, 0.05, 0.025, 0.0125%) were prepared by mixing the oils in a conical flask with PDA medium as follows:

0.15%	=	0.15 mL of oil in 99.85 ml PDA medium
0.10%	=	0.10 mL of oil in 99.9 ml PDA medium
0.05%	=	0.05 mL of oil in 99.95 ml PDA medium
0.025%	=	0.025 mL of oil in 99.975 ml PDA medium

0.0125% = 0.0125 ml of oil in 99.9875 ml PDA medium

Effect of oils against *S. rolfsii* in *in vitro* condition

All the petriplates containing above different oil containing media were inoculated separately with the test fungi (*Sclerotium rolfsii*) with the help of the sterilized inoculating needle under laminar air flow and kept in a B.O.D. Incubator at 28 ^{0}C ± 1 for proper growth of the fungi.

Radial growth of the fungus was measured by a standard millimeter (mm) scale after a definite interval of days after inoculation and this was continued until the control plate was fully covered with fungal growth.

The percent inhibition of the fungal growth over control was calculated by using the formula given by Vincent (1947).

$$I = \frac{C - T}{T} X\ 100$$

Where, I = percent inhibition

C = growth in control

T = growth in treatment

Effectiveness of essential oils against *S.rolfsii* in *in vivo* condition

Essential oil of different concentration diluted with the help of distilled water and the seeds were soaked in this suspension for 30-40 minutes. After that, treated seeds were dried in shade and sown in the pots. These oils were used at a concentration of 0.1 % and 0.05%. Seeds of groundnut soaking with distilled water were used as control for comparison with oils.

Garden soil and farmyard manure in 5:1 ratio were mixed and sterilized; and after that, filled in earthen pots of 30 cm diameter. Fifteen days old culture of *Sclerotium rolfsii* grown on sand-maize meal medium was thoroughly mixed with the soil of the pot @ 100 g/pot. Untreated groundnut seeds were sown and the development of typical disease symptom was studied. When the seedlings showed more than 50% infection (stem rot), it was taken as sick soil. The prepared sick pots were used for conducting the present experiments.

Treated groundnut seeds were sown and the development of typical disease symptom was studied. The percent mortality was recorded 10 and 28 days after sowing.

The percent mortality of stem rot or sclerotial rot of groundnut in the pot was calculated using the formula by Vernell and Hecloud (1975).

$$\text{Percent mortality} = \frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100\%$$

Statistical analysis

Complete Randomized Block Design (CRD) was followed for all the experiments. Statistical analysis was carried out in the following method described in Chandel (1978).

RESULTS AND DISCUSSION

Lemongrass oil against *Sclerotius rolfsii* under *in vitro* condition

The inhibition percentage of the growth of this pathogen at different concentration (Table 1) showed that with the increase in concentration, there was a significant inhibition in growth. Maximum inhibition (100%) was obtained at a concentration of 0.1 % at 3 DAI, 6 DAI and 10 DAI and at 0.05% and 0.025% concentrations at 3 DAI. Minimum inhibition (no inhibition) was recorded in control treatments in all DAI. With increase in concentration, there was a significant increase in inhibition percentage. The interaction between days after inoculation and concentration also showed a significant difference. It indicates that inhibition percentage in every concentration showed a significant decrease at every days after inoculation and it was due to volatile nature of this oil causes significant reduction.

Citronella oil and its derivatives on *Sclerotium rolfsii* under *in vitro* condition

The results (Table 1) showed that all the concentrations inhibited the radial growth significantly in comparison to untreated control. 100% inhibition of growth was recorded in all the concentrations at 3, 6 and 10 DAI, except in 0.1% concentration at 10 DAI, 0.05% concentration at 6 and 10 DAI and in 0.025% concentration at 6 and 10 DAI. Minimum inhibition (no inhibition) was recorded in control treatments in all DAI. Within the concentration inhibition percentage also showed a significant difference among themselves. With increase in concentration there was a significant increase in inhibition percentage. The interaction between days after inoculation and concentration also showed a significant difference among themselves. Due to volatile nature of this oil reduction of growth and inhibition percentage of the pathogen is gradually decreased significantly (Table 1).

All the concentrations of Citronellal also showed inhibition of mycelial growth in comparison to untreated control. After 3 DAI, all the concentrations inhibited 100% except 0.0125% concentration which showed 87.08% inhibition, but no inhibition at 6 DAI and 10 DAI as like as control. 0.15% concentration showed 100% inhibition upto 10 DAI, 0.025% concentration showed 100% inhibition at 3 DAI which reduced at 6 DAI (66.38%) and at 10 DAI (44.16%). More than 50% inhibition was observed at all observations upto 10 DAI in case of 0.15% to 0.05% concentration (Table 1). The interaction between days after inoculation and different concentrations showed a significant difference, and with increasing days after inoculation, there was significant decrease in inhibition percentage (Table 1).

In case of Geraniol, highest inhibition (100.00%) was observed at 0.15% to 005% concentration upto 10 DAI, and in 0.025% concentration at 3DAI only. More than 50% inhibition was observed at a concentration of 0.025% (73.89% to 78.89%). With increase in concentration, there was significant increase in inhibition percentage of mycelial growth. It was also observed that with the increase in age of medium, there was decrease in inhibition percentage (Table 1).

Terpinoid an aldehyde of Citronella oil derivatives also showed maximum inhibition at a concentration of 0.15% and 0.1% (100%) at 3 DAI to 10 DAI. At 0.05%, 0.025% and 0.0125% concentrations also showed the maximum inhibition at 3 DAI which was slightly reduced to 89.44% to 72.78% and 86.38% to 5.56% at 6 DAI and 10 DAI respectively (Table 1). Here also with increase in concentration there was a significant increase in inhibition percentage. It indicates that inhibition

Table 1: Efficacy of five essential oils on inhibition percentage of radial growth of *Sclerotium rolfsii* in different days after inoculation

Concentration	Lemongras oil			Citronella Tops			Citronellal		
	3 DAI*	6 DAI	10 DAI	3 DAI	6 DAI	10 DAI	3 DAI	6 DAI	10 DAI
0.15	-	-	-	-	-	-	100 (88.57)	100 (88.57)	100 (88.57)
0.1	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	94.44 (78.24)
0.05	100 (88.57)	88.88 (71.39)	80 (63.73)	100 (88.57)	100 (88.57)	86.11 (71.6)	100 (88.57)	84 (70.78)	64.22 (57.22)
0.025	100 (88.57)	77.78 (62.28)	63.61 (52.94)	100 (88.57)	86.67 (69.66)	66.67 (55.1)	100 (88.57)	66.38 (55.57)	44.16 (41.08)
0.0125	64.44 (58)	41.44 (40)	30.56 (29.32)	77.78 (62.38)	24.16 (21.98)	10.31 (13.55)	-	-	-
Control	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)
	DAI (D)	Con. (C)	D × C	DAI (D)	Con. (C)	D × C	DAI (D)	Con. (C)	D × C
SEm (±)	0.83	2.99	5.19	3.1	3.1	5.83	1.56	2.3	3.99
CD at 5%	5.05	8.58	14.89	18.85	9.67	16.73	9.48	6.6	11.45
CV %	20.21	21.46	18.82	22.35	20.45	20.35	16.56	18.39	14.32

Table 1: Cont.....

Concentration	Geraniol			Terpinoid (aldehyde)		
	3 DAI	6 DAI	10 DAI	3 DAI	6 DAI	10 DAI
0.15	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)
0.1	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)	100 (88.57)
0.05	100 (88.57)	100 (88.57)	90.56 (74.77)	100 (88.57)	89.44 (73.6)	81.94 (68.18)
0.025	100 (88.57)	78.89 (62.93)	73.89 (59.64)	100 (88.57)	86.38 (74.37)	59.44 (57.22)
0.0125	-	-	-	100 (88.57)	72.78 (58.95)	5.56 (11.69)
Control	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)
	DAI (D)	Con. (C)	D ´ C	DAI (D)	Con. (C)	D × C
SEm (±)	1.38	2.29	3.98	1.07	4.4	7.62
CD at 5%	8.39	6.57	9.72	6.5	12.63	21.87
CV %	16.54	18.37	12.1	22.78	20.59	23.41

*DAI = Days after inoculation; Figure in parentheses are angular transform value of percent inhibition

Table 2: Efficacy of five essential oils on percent mortality of *Sclerotium rolfsii in vivo* condition after 10 and 28 days after sowing

Oil treatment	Concentration	Percent mortality (%)		Percent decrease mortality over control (%)	
		10 DAS	28 DAS	10DAS	28 DAS
Lemon grass	0.1	27.51 (31.636)	26.39 (30.913)	66.24	70.00
	0.05	30.72 (33.662)	28.21 (32.086)	62.29	67.93
Citronella Tops	0.1	18.52 (25.486)	19.49 (26.202)	77.27	77.84
	0.05	30.66 (33.622)	30.69 (33.643)	62.37	65.11
Citronellal	0.1	15.19 (22.942)	18.84 (25.728)	81.35	78.59
	0.05	30.65 (33.615)	34.14 (35.754)	62.38	61.12
Geraniol	0.1	10.0 (18.43)	10.0 (18.43)	87.72	88.63
	0.05	12.43 (20.644)	12.43 (20.644)	84.36	85.87
Terpinoid	0.1	9.26 (17.72)	9.10 (17.56)	88.63	89.66
	0.05	12.74 (20.912)	13.21 (21.309)	84.36	84.98
Control	-	81.48 (64.514)	87.98 (69.712)	-	-
SEM±		4.05	4.12		
CD at 5%		11.83	12.03		
CV%		21.84	22.41		

percentage in every concentration showed a significant reduction at every day after inoculation and due to volatile nature of this oil.

The results thus showed that the different essential oils against *Sclerotium rolfsii* at different concentration produced different type of reaction in radial growth and inhibition percentage. In maximum cases, 0.15% and 0.1% concentration showed the 100% inhibition upto 10 DAI except lemongrass oil. More

than 50% inhibition was obtained by using 0.05% to 0.0125% concentration of all oil. One of this experiment also supported the experiments of Gangrade *et al.*,(1991) that the essential oils distilled from the foliage of *Cymbopogon martini var. motia*, inhibited the growth of *Aspergillus niger A. flavus, Fusarium oxysporum* and *Penicillium spp.* by 70-80%. Kole *et al.* (1993) also reported that essential oils of Citronealla exhibited 100% inhibition of growth of antifungal activities against *Alternaria solani, Sclerotium rolfsii* and *Fusarium oxysporum*. This result also supported the results of Handique and Singh (1990) that 100ppm of Lemongrass oil decreased the growth of *Rhizoctonia solani* by 67% and 40% in case of *Sclerotinia sclerotiorum* but ineffective against *Sclerotium rolfsii*. Where as, on 1000ppm it totally inhibited the growth of first two fungi and caused 80% inhibition of *Sclerotium rolfsii*. Bisht *et al*.(2013) also reported that *Curvularia* pathogen causing leaf spot of maize can be inhibited its growth through essential oils in *in vitro* condition.

From the above results, it could be concluded that with the increase in number of days, the inhibition percent reduced which showed the fungistatic effect. The essential oils like-Lemongrass and the different derivatives of Citronella oil eg-Citronella Tops, Geraniol, Citronellal and Terpinoid showed antifungal activity against *Sclerotium rolfsii*.

Percent decrease mortality over control

The data were collected at 10 DAS and 28 DAS (days after sowing). The oil and its derivatives with their respective doses reduced the Sclerotial rot of ground nut significantly in comparison to untreated control.

Maximum mortality (30.72%) was noticed in Lemongrass oil at a concentration of 0.05% after 10 DAS and the least mortality percent was noticed in case of Terpinoid (9.26%) at 0.1 % concentration after 10 DAS followed by Geraniol (10.0%) at 0.1 % concentration and 12.43% at 0.05% concentration after 10 DAS. (Table 2).

After 28 DAS, the mortality percent was also same as 10 DAS in case of all oils. But, in this case the maximum mortality percent was noticed in Citronellal (34.14%) at 0.05% concentration and the least mortality percent in case of Terpinoid (9.10%) at 0.1 % concentration. (Table 2).

In case of percent decrease mortality over control, Terpinoid showed the best result followed by Geraniol, Citronella!, Citronella Tops and Lemon grass. Terpinoid showed 88.63% and 84.36% decrease mortality at 0.1 % and 0.05% concentration at 10 DAS. But Lemon grass showed the minimum decrease in mortality percent (66.24% and 62.29%) at 0.1% and 0.05% concentration at 10 DAS.

From the above result, it can be concluded that, 0.1 % concentration of all the oils showed the good result than 0.05% concentration and Terpinoid is the most effective than the other essential oils under *in vivo* condition. These observations established the fact that essential oils in wet seed treatment at extremely low concentrations can provide strongly and lasting protection as dynamic defence (Dasgupta *et al.,* 1999). Similar type of results was also observed by Mahapatra and Das (2013) against Alternaria blight of Mustard in field condition.

So, the need based production and application of the effective oils are possible by farmers for sustainable crop production through eco-friendly management as there is no alternative for the management of this dreaded disease of groundnut. This management procedure can be incorporated in the Integrated Disease Management (IDM) as well as Integrated Pest Management (IPM).

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