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Biocontrol of *Macrophomina phaseolina* causing root rot of sesame by four derivatives of Citronella oil

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Macrophomina phaseolina, a soil borne pathogen has a wide host range causing root rot, stem rot, collar rot and seedling blight disease of various crop plants. Here attempts have been made to control root rot of sesame, caused by *Macrophomina phaseolina* by four derivatives of citronella oil, namely citronella top, citronellel, terpinoide and geraniol. *In vitro* studies, the poisoned food technique for plant oils revealed that different concentrations and days after inoculation (DAI) of four derivatives showed significant differences in radial growth, as well as in inhibition percentage. The interaction between DAI and concentration was also statistically significant. In all the cases oils had static effect on the growth of the pathogen. Citronellel (Conc. 0.3%), citronella Top (Conc. 0.18%), geraniol (Conc. 0.15%) and terpinoide (Conc. 0.2%) were selected for pot trial. *In vivo* studies revealed that all the oils reduced the root rot of sesame significantly, when applied as seed soaking. Maximum reduction of mortality percentage (28.71 %%) compared to control was showed by geraniol followed by citronella top (20.63%), terpinoide (20.19%) and citronellel (16.83%). Considering the treatments performed in the experiment, geraniol oil showed the most effective antagonism against root rot disease of sesame caused by *Macrophomina phaseolina.*

Key words: Sesame, *Macrophomina phaseolina*, citronella top, citronellel, terpinoide, geraniol, inhibition percentage, mortality percentage

INTRODUCTION

Root rot, caused by *Macrophomina phaseolina* is the most important and destructive disease. This disease occurs at all stages of plant growth causing root rot, stem rot, seedling rot, seed rot depending on the growth stages. Among theses, root rot is the most destructive phase. The fungus attacks the seedling just after the emergence of the plant. As the fungus attacks the root system, roots become brown and discoloured. The affected plant can not up take the nutrients and water properly. Within few days, the infected plants die.

The fungus has two different morphological growth stages. *Macrophomina phaseolina* (Tassi) Goid. is the pycnidial stage , where as the sclerotial stage of the fungus is *Rhizoctonia bataticola* (Taub.) Butl. The primary source of inoculum is mainly soil in the form of sclerotia and infected seed in the form of mycelia. Cloudy weather and high atmospheric humidity (85%) and temperature around 40ºC are congenial for the disease incidence. The pathogen has a wide host range including jute, potato, cotton, tobacco, soybean, mulberry, brinjal, black gram, bengal gram etc. and remains active all the year around (Rangaswami and Mahadevan, 1999).

The biological control of soil borne pathogens has been gaining importance. Because the use of large quantities of chemical pesticides is contaminating the environment and enrichment of more resistant pathogens. Some compounds of plant origin have recently proved their effectiveness as fungitoxicant because of their low phytotoxicity, more systemicity, easy biodegradableness and

stimulation in host metabolism (Sarma *et al.*, 1999). Among them crude plant extract like plant oil is one of the important one. There are so many reports of antagonistic action of plant oils on growth of plant pathogenic fungi.

Antifungal activities of lemongrass oil (*Cymbopogon citratus*) is well documented (Ashrafuzzaman *et al.*, 1989).Recent studies provide evidence that another essential oil like citronella (*Cymbopogon winterianus*) and lemongrass oil (*Cymbopogon citratus*) has antifungal action against *Macrophomina phaseolina* (Thakare *et al.*, 2003). Essential oil of *Lippia alba* (Dwivedi *et al.*, 1990), *Cymbopogon gouriana* (Bashar, 1991), *Coriari nepalensis* (Chatterjee *et al.*, 1996), oils from *Aloe barbadensis , Datura stramonium, Zingiber officinale, Murraya koenigii, Azadirachta indica , Mentha piperita* and *Brassica juncea* extracts (Pankaj *et al.*,2003) has antifungal properties against *Macrophomina phaseolina* and other plant pathogenic fungi. The extracts of onion and leek contain factors that inhibit the polygalacturonases(PGs) produced *in vitro* by *Macrophomina phaseolina* and also the inhibition factors are destroyed by heat treatment, but unaffected by the presence of protease (Favaron *et al.*,1993).Essential oil of curry leaf is composed of monoterpene hydrocarbon(16%) and sesquiterpene hydrocarbon(80%), with alpha-caryophyllene, alpha-gurjunene, alpha-elemene and alpha – phellandrene as the major components and has fungitoxicity against *Macrophomina phaseolina* (Ray and Srivastava, 2006).

The present work was devoted to investigate the antifungal properties of four derivatives of citronella oil namely, citronellel, citronella top, geraniol and terpinoide against *Macrophomina phaseolina* causing root rot of sesame.

MATERIALS AND METHODS

The whole experimental work was carried out in the laboratory of Department of Plant Pathology, Bidhan Chandra Krishi Viswavidyalaya, (B.C.K.V.) Mohanpur, Nadia, West Bengal. *Macrophomina phaseolina* (Tassi) Goid, the test pathogen, was isolated from naturally infected jute seedling from the Instructional Farm, Jaguli, B.C.K.V.,Nadia,West Bengal and identified in the laboratory. After conferming pathogenecity, the pathogen was maintained on PDA at 4 ± 1º C by keeping them in the refrigerator and sub-cultured at every 15 days interval. Sesame seeds of local variety were collected from the Instructional Farm, Jaguli, B.C.K.V. The leaves of citronella were collected from the centre for Aromatic and Medicinal plants, Central Regional Research Farm, Gayeshpur, Nadia. Then four derivatives (citronellel, citronella top, geraniol and terpinoide) were extracted by hydro-distillation process.

Antagonistic Study in vitro : To prepare the aqueous solution oils needed a sticker Tween-80.The oils were separately dissolved by mixing 99.8 ml of oil + 0.2 ml of Tween- 80 to prepare the stock solution (100%).The different concentrations of oils used in the experiment were as followes :
citronellel - (0.15,0.2,0.25,0.3,0.35 0.4)%
citronella top-(0.1,0.15,0.18,0.2,0.23 0.25)%
geraniol- (0.1,0.12,0.15,0.18,0.2 0.25)%
terpinoide- (0.1,0.15,0.18,0.2,0.23 0.25)%
According to the poisoned food technique these concentrations were prepared by mixing the oils with sterilized PDA medium under laminar air flow chamber. Then the PDA medium was poured into the petri plates, and allowed to gel. All the Petri plates were inoculated separately with the 5mm disc of test fungi and kept in B.O.D. incubator at 28 ± 1º C for proper growth of the fungi. The radial growth of the fungus was measured by a standard millimeter (mm) scale at 5 days after inoculation(DAI) and 10DAI. The percentage of inhibition of fungal growth was calculated by using the following formula,

Per cent of Inhibition (radial growth) = $(T_0 - T_1 / T_0) \times 100\%$
Where, T_0 = growth in control
T_1 = growth in treatment

Antagonistic Study in vivo: Garden soil and farm yard manure in 5:1 ratio were mixed and sterilized; and after that, filled in flat earthen pots of 30 cm diameter. 20 days old culture of *Macrophomina phaseolina* grown on sand-maize meal medium was thoroughly mixed with the soil of the pot @ 200 g/ pot. In case of plant oils, the concentration that showed 50% inhibition (ED_{50}) in *in vitro* condition at 10DAI were selected for pot trial and prepared by

dissolving in sterile water along with emulsifier. Seeds were sterilized by 10% sodium hypochlorite. Treatments were given by soaking the seeds for 30min. in different oils (0.2% citronellel, 0.17% citronella top, 0.17% geraniol and 0.19% terpinoide), or in water (control) and then dried in shade for 2 hours. Then the seeds were sown in the pot. Irrigation of all pots was carried out routinely using a constant amount of tap water per pot. Pots were placed under natural condition of daily light and darkness. Percent mortality was recorded from 10 days after sowing (10DAS) upto 30 DAS. To calculate the percent mortality of root rot of sesame in the pot, the following formula was followed

$$\text{Per cent mortality} = \frac{\text{No. of infected piants}}{\text{Total No. of Plants}} \times 100\%$$

Radial growth, inhibition percentage and mortality percentage were statistically analyzed according to complete randomized block design (CRD) to find out the critical difference (CD) of different concentrations and the co-efficient of variation regarding the experiment.

RESULTS AND DISCUSSION

Effect of oils under in vitro condition: In this experiment, six different concentrations of four different oils and the untreated control were taken for comparison. Each treatment has three replications. The mean data of the three replications of each treatment are presented below.

All the four derivatives showed different radial growth and inhibition percentage at different concentrations and their difference was statistically significant. It was also observed that with increase in the concentration there was a significant decrease in radial growth and as a result the inhibition percentage was significantly increased.

The effect of citronellel oil against *Macrophomina phaseolina* (Table 1) showed that 0.3% concentration

Table 1 : Effect of citronellel and citronella top on inhibition percentage of *Macrophomina phaseolina* in different days after inoculation(DAI) under *in vitro* condition.

Inhibition percentage(%) of the fungus in different oils					
Citronellel			Citronella top		
Conc. of oil	5 DAI	10 DAI	Conc. of oil	5 DAI	10 DAI
0.15	16.29 (23.61)	—	0.1	31.11 (33.89)	—
0.2	54.82 (47.77)	—	0.15	100 (88.35)	28.82 (31.96)
0.25	70.38 (57.53)	24.44 (29.08)	0.18	100 (88.35)	55.56 (48.21)
0.3	99.27 (87.27)	56.29 (48.62)	0.2	100 (88.35)	88.89 (70.78)
0.35	100 (88.35)	68.89 (56.13)	0.23	100 (88.35)	100 (88.35)
0.4	100 (88.35)	100 (88.35)	0.25	100 (88.35)	100 (88.35)
Control	—	—	Control	—	—
	SEm(±)	CD at 5%		SEm(±)	CD at 5%
DAI	1.43	8.68		0.39	2.37
Conc.	1.52	4.43		0.79	2.30
DAI× Conc.	2.15	6.27		1.11	3.25
CV%		8.39			3.32
ED_{50}	0.17	0.2		0.14	0.17

Figures in the parenthesis are average angular transformed value.
Conc. = Concentration of oil

Table 2 : Effect of geraniol and terpinoide on inhibition percentage of M*crophomina phseolina* in different days after inoculation(DAI) under *in vitro* condition.

Inhibition percentage(%) of the fungus in different oils					
Geraniol			Terpinoide		
Conc. of oil	5 DAI	10 DAI	Conc. of Oil	5 DAI	10 DAI
	21.48	—		42.96	—
0.1	27.16		0.1	40.93	
	59.26	32.59		54.82	1.49
0.12	50.38	34.61	0.15	47.77	5.15
	100	61.48		60.73	27.4
0.15	88.35	51.66	0.18	51.21	31.51
	100	75.56		93.33	67.4
0.18	88.35	60.39	0.2	75.18	55.21
	100	100		100	91.11
0.2	88.35	88.35	0.23	88.35	73.02
	100	100		100	100
0.25	88.35	88.35	0.25	88.35	88.35
Control	—	—	Control	—	—
	SEm(±)	CD at 5%		SEm(±)	CD at 5%
DAI	0.51	3.08		0.68	4.11
Conc.	1.28	3.72		1.18	3.45
DAI× Conc.	1.80	5.27		1.67	4.88
		5.73			6.25
	0.13	0.17		0.12	0.19

Figures in the parenthesis are average angular transformed value.
Conc. = Concentration of oil

inhibited the fungal growth by 93.27 to 56.29% up to 10 DAI. With the increase in concentration, the inhibition percentage was increased and was 100 to 68.89%, and 100% up to 10 DAI at 0.35 and 0.4% concentration respectively. Among all the concentrations 0.3% was the least but most effective that inhibited the fungus more than 50% up to 10 DAI. The calculated ED_{50} value was 0.17 at 5DAI and 0.2 at 10 DAI.

The effect of citronella top oil against *Macrophomina phaseolina* (Table 1) showed that 0.18% concentration inhibited the fungal growth by 100 to 55.56% up to 10 DAI. With the increase in concentration, the inhibition percentage was increased and was 100 to 88.89%, 100% and 100% up to 10 DAI at 0.2, 0.23 and 0.25% concentration respectively. Among all the concentrations 0.18% was the least but most effective that inhibited the fungus more than 50% up to 10 DAI. The calculated ED_{50} value was 0.14 at 5DAI and 0.17 at 10 DAI.

The effect of geraniol oil against *Macrophomina phaseolina* (Table 2) showed that 0.15% concentration inhibited the fungal growth by 100 to 61.48% up to 10 DAI. With the increase in concentration, the inhibition percentage was increased and was 100 to 75.56%, 100% and 100% up to 10 DAI at 0.18, 0.2 and 0.25% concentration respectively. Among all the concentrations 0.15% was the least but most effective that inhibited the fungus more than 50% up to 10DAI. The calculated ED50 value was 0.13 at 5DAI and 0.17 at 10 DAI.

The effect of terpinoide oil against *Macrophomina phaseolina* (Table 2) showed that 0.2% concentration inhibited the fungal growth by 93.33 to 67.40% up to 10 DAI. With the increase in concentration, the inhibition percentage was increased and was 100 to 91.11%, and 100% up to 10 DAI at 0.23 and 0.25% concentration respectively. Among all the concentrations 0.2% was the least but most effective that inhibited the fungus more than 50% up to 10 DAI. The calculated ED_{50} value was 0.12 at 5DAI and 0.19 at 10 DAI.

The statistical analysis showed that different concentrations and DAI of oils showed significant differences in inhibition percentage. The interaction between days after inoculation (DAI) and concentration was also statistically significant. It indicated that the inhibition percentage in different concentrations was significantly differed with days after inoculation. It implied that the reduction of inhibition percentage from 5 DAI to 10 DAI was statistically differed among themselves. Also in all cases it was observed with the increase in concentration of oil, the inhibition percentage was increased. But, with the increase in the age of the fungus, the inhibition percentage was decreased. It indicates that the oils have static action against the pathogen.

Effect of oils under in vivo condition: In pot trial, different treatments showed different mortality percentage and their differences were statistically significant. Maximum mortality percentage (42.85%) was observed in case of treatment with citronellel oil, followed by terpinoide oil (41.12%), citronella top (40.89%)and minimum mortality percentage (36.73%) was observed in case of treatment with geraniol oil. Among all the treatments geraniol oil showed significant difference with citronellel, citronella top and terpinoide (Table 3). The values of percent decrease in mortality percentage showed that, maximum reduction of mortality percentage (28.71%) in contrast to control pot was exhibited by geraniol, followed by citronella top (20.63%), terpinoide (20.19%) and citronellel (16.83%).

So among the four derivatives of citronella oil, geraniol showed the most effective antagonism against root rot causing pathogen (*Macrophomina phseolina*) of sesame. On the other hand, citronella top, terpinoide and citronellel oil showed moderate efficacy regarding the antagonism against *Macrophomina phseolina* in *in vivo* condition.

All the four derivatives of citronella oil have antifungal properties against *Macrophomina phaseolina*. The present finding is in agreement with the finding of Srivastava *et al.* (1993) who reported the *in vitro* antifungal activities of palmarosa and eucalyptus oils, having predominantly geraniol and citronellel against *Aspergillus, Fusarium, Curvularia* and *Cladosporia*. The poisoned food study in *in vitro* condition proved the static action of four derivatives against the fungal growth, not the cidal effect. While, from the pot trial it is evident that among the four derivatives of citronella oil, geraniol has the highest efficacy to reduce the mortality percentage of root rot of sesame.

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Table 3 : Effect of seed soaking of plant oils on per cent mortality of root rot of sesame.

Treatment	Mortality percentage(%)	Per cent decrease in Mortality percentage(%)
Citronellel oil (0.2%)	46.25 (42.85)	24.51 (16.83)
Citronella top oil (0.17%)	42.87 (40.80)	30.03 (20.63)
Geranoil oil (0.17%)	36.35 (36.73)	40.67 (28.71)
Terpinoide oil (0.19%)	43.26 (41.12)	29.39 (20.19)
Control	61.27 (51.52)	
SEm(±)	1.1	
CD at 5%	3.49	
CV%	4.5	

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