

Original Research Article

<https://doi.org/10.20546/ijcmas.2018.701.252>

Efficacy of Plant Oils and Oil Cakes against *Rhizoctonia solani* Kuhn Causing Collar Rot and Web Blight of Cowpea under *in vitro* Conditions

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ABSTRACT

Keywords

Antifungal, Bio-fumigant, Plant oils, Oilcakes, *Rhizoctonia solani*

Article Info

Accepted:
16 December 2017
Available Online:
10 January 2018

An attempt was made to evaluate the *in vitro* antifungal and bio-fumigant nature of four plant oils and oil cakes on suppression of mycelial growth of *R. solani*, the incitant of collar rot and web blight of cowpea. The plant oils viz., neem oil, lemongrass oil, mahua oil and tea tree oil at 5% concentration and the oilcakes such as groundnut oil cake (source: *Arachis hypogaea* (Linn.), neem cake (source: *Azadirachta indica* A. Juss.), mahua cake (source: *Madhuca longifolia*) and mustard oil cake (source: *Brassica juncea* L.) at 10% concentration were evaluated for their antifungal action by poisoned food technique and highest inhibition (100%) was obtained on incorporation of lemongrass oil, tea tree oil and mustard oil cake into the PDA medium. This was followed by groundnut oil cake and neem cake with a suppression of 79.62% and 76.29%, respectively. In the experiment to evaluate the bio-fumigant nature of tested plant oils and oilcakes, 100% suppression was noticed with lemongrass oil, tea tree oil and mustard oil cake and were statistically superior to all other treatments. Biofumigation with ground nut cake and neem cake also recorded lower values of 5.56% and 3.00%, respectively.

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp], an important leguminous crop of Kerala, is cultivated in the uplands and in rice fallows. The fresh pods are consumed as vegetable while the dried seeds are also utilized in cooking. All the above ground parts provide a good source of animal fodder. The semi-erect bush cowpea spread over the ground as a mulch and provide a protective cover from soil erosion and also suppress weed growth. Soil fertility restoring capacity makes it an essential component of almost all cropping

systems. The cultivation of the crop is affected by collar rot and web blight caused by *Rhizoctonia solani* Kuhn in all its growth stages which results in severe crop and yield loss (Lakshmanan *et al.*, 1979). Under congenial climatic conditions, collar rot symptom of the disease is more prevalent than web blight symptoms. It is often difficult to control ubiquitous soil and root-inhabiting pathogens like *R. solani* that survive saprophytically in soil organic matter and exist for long periods in the absence of a host plant in the form of sclerotia, except with the elaborate and repeated use of fungicides

(Upamanyu *et al.*, 2002). The detrimental effect of these chemicals as well as the huge cost involved, necessitate the disease to be managed by cheaper and environment friendly methods. Biofumigation of soil is an emerging ecofriendly means of disinfecting soil. It refers to the suppression of soil-borne pests and pathogens by biocidal compounds such as isothiocyanates (ITCs), terpenoids and other volatile compounds which are released through hydrolysis of incorporated plant tissues or seed meal extracts or oilcakes (Kirkegaard *et al.*, 1993). This study was intended to evaluate the antifungal and bio-fumigant efficacy of various plant oils/oilcakes on collar rot pathogen *R. solani* under *in vitro* conditions.

Materials and Methods

Cowpea plants showing typical collar rot symptom were collected from the Instructional Farm of College of Agriculture, Vellayani, Thiruvananthapuram, Kerala state during *Kharif* 2016. The infected portions were first washed in running water and cut into small bits, surface sterilized with 0.1% mercuric chloride (HgCl_2) solution for one min. followed by two successive washings in sterile water, and then transferred into sterile petri plates containing potato dextrose agar (PDA) medium under aseptic conditions. These petri plates were sealed with parafilm and incubated at room temperature ($28 \pm 1^\circ\text{C}$) for the development of fungal growth. The culture thus obtained was purified by hyphal tip method on PDA slants. The pathogenicity tests were performed and the causal agent was identified based on morphological and cultural characteristics.

Four oils *viz.*, neem oil (source: seeds of *Azadirachta indica* A. Juss.), lemongrass oil (source: leaves of *Cymbopogon flexuosus* [Nee ex Steudel] J.F. Watson), mahua oil (source: seeds of *Madhuca longifolia* (J.

Konig)) and tea tree oil (source: leaves of *Melaleuca alternifolia* (Maiden & Betche)) each at 5 per cent concentration, was tested for their antifungal activity on *R. solani* under *in vitro* conditions. Each oil (5 mL) was filtered using sterilized bacterial proof filters, mixed with 0.1 mL emulsifier *viz.*, Polysorbate (Tween 80) and added into 250 ml conical flask containing 95 mL of sterilized PDA.

Under *in vitro* conditions, studies were carried out on the fungicidal action of four oilcakes/seed meals *viz.*, groundnut oil cake (source: *Arachis hypogaea* (Linn.)), neem cake (source: *Azadirachta indica* A. Juss.), mahua cake (source: *Madhuca longifolia*) and mustard oil cake (source: *Brassica juncea* L.) over the pathogen *R. solani* at 10% concentration. The extract was prepared based on the method given by Bhadrasree (2007). The oil cakes were dried at room temperature for 3-4 days, broken into small pieces, ground well with mortar and pestle and soaked in sterilized water in the ratio 1:2 (W/V). After 24 h, the hydrated cake was squeezed through four folds of sterilized muslin cloth and cake extract collected and centrifuged at 5000 rpm for 10 min. The supernatant was filtered through bacterial proof filters and decanted into a 250 mL conical flask.

The antifungal nature of different plant oils and oil cakes over *R. solani* was tested by poisoned food technique (Nene and Thapliyal, 1993). Mycelial discs (5 mm diameter) was taken from 7 day old culture of *R. solani* and placed at the centre of petri plate containing PDA amended with plant oils and oil cake extract. The un-amended medium poured into the sterile petri plate with pathogen at the centre served as the control. The inoculated plates were incubated at $28 \pm 1^\circ\text{C}$. Observation on colony diameter was recorded when the control plates were fully covered by *R. solani*. Percentage inhibition in colony growth was recorded as per Vincent (1947).

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

Where,

C = Colony diameter growth in control

T = Colony diameter growth in treatment

The bio-fumigant nature of plants, plant oils and oil cakes against *R. solani* under *in vitro* conditions was studied by Paired plate technique (Prasad *et al.*, 2016). The bases of two equal sized sterilized petri plates were taken. Sterilized filter paper disc dipped in 5% plant oil/ 10% concentrate extract of oil cake was placed at the centre of the one kept at the bottom. The sterile molten PDA was poured into the other lid and this was inoculated with 5 mm mycelial disc cut from 7 day old *R. solani* culture grown on PDA and was inverted over the other plate containing filter paper disc dipped in plant oil/ concentrate extract of oil cake. The adjoining portions of two petri plates were sealed with parafilm. The paired plates with upper inverted plate carrying PDA and inoculated pathogen alone served as control. Percentage suppression of the pathogen over control was calculated as already mentioned.

Results and Discussion

Under *in vitro* evaluation for the antifungal nature of plant oils, lemongrass oil and tea tree oil were found to be the most effective with 100 per cent suppression of mycelial growth of the pathogen (Table 1). Mahua oil and neem oil afforded suppression of 40.74 and 16.29 per cent. Out of the four oil cakes tested for their inhibitory effect on mycelial growth of *R. solani*, mustard oil cake induced the maximum suppression (100 per cent) (Table 2). This was followed by groundnut cake and neem cake with a suppression of 79.62 and 76.29 per cent, respectively.

Apart from antifungal action, when used as a bio-fumigant lemongrass oil and tea tree oil (5%) also gave 100% suppression (Table 3). But neem oil and mahua oil did not have any inhibitory bio-fumigant effect on the collar rot pathogen. It was observed that among the oil cakes, only mustard oil cake gave effective bio-fumigant action on pathogen. The suppression of pathogen in treatments with groundnut and neem cake recorded lower values of 5.56 per cent and 3.00 per cent, respectively (Table 4).

The results of the present study indicated that certain plant oils and oil cakes tested could effectively inhibit *R. solani* when incorporated into media or when used as a bio-fumigant under *in vitro* conditions and these could be explored further through evaluation under *in vivo* conditions for the management of collar rot and web blight of cowpea.

Plants synthesize secondary metabolites and some of them as well as their derivatives have antimicrobial activity such as alkaloids, flavonoids, isoflavonoids, tannins, coumarins, glucosides, terpenes and phenolic compounds. An attempt was made to evaluate the *in vitro* antifungal and bio-fumigant nature of four plant oils and four oil cakes on suppression of mycelial growth of *R. solani*. Mahadevan (1982) highlighted the use of plant oils in the management of *R. solani* incited diseases.

Plant derived essential oils are volatile aromatic concentrated hydrophobic oily liquids which are reported to contain non-phytotoxic compounds effective against several microorganisms including fungal pathogens (Chuang *et al.*, 2007). The antimicrobial nature of plant oils is generally due to their content of terpenes, which are phenolic in nature (Tabassum and Vidyasagar, 2013). In the present study, under *in vitro* evaluation for the antifungal nature of plant oils, lemongrass oil and tea tree oil were found

to be the most effective with 100 per cent suppression of mycelial growth of the pathogen. Mahua oil and neem oil afforded suppression of 40.74 and 16.29 per cent. Dhingra *et al.*, 2004 reported that mustard oil incorporated into the medium (50 $\mu\text{L L}^{-1}$) completely suppressed the mycelial growth of *R. solani* under *in vitro* conditions. The results obtained in our study was in conformity with

the observation of Handique and Singh (1990), who tested the antifungal activity of essential oil extracted from a mutant strain, Lm-81 of lemongrass (*Cymbopogon flexuosus*) for antifungal activity against three soilborne pathogens including *R. solani* and found 60% decrease in growth of *R. solani* at 100 ppm and total suppression of growth at 1000 ppm.

Table.1 Effect of the antifungal nature of plant oils against *R. solani* under *in vitro* conditions

Plant oils	Mycelial growth* (cm)	% Inhibition
O1 (Neem oil)	7.53	16.29 ^c (23.70)
O2 (Lemongrass oil)	0.00	100.00 ^a (89.04)
O3 (Mahua oil)	5.33	40.74 ^b (39.66)
O4 (Tea tree oil)	0.00	100.00 ^a (89.04)
Control	9.00	-
SE m (\pm)	-	0.76
CD (0.05)	-	3.02

Table.2 Effect of antifungal nature of oil cakes against *R. solani* under *in vitro* conditions

Oil cakes	Mycelial growth*(cm)	% Inhibition
OC1 (Groundnut oilcake)	1.83	79.62 ^b (63.18)
OC2 (Neem cake)	2.13	76.29 ^c (60.88)
OC3 (Mahua cake)	9.00	0.00 ^d (0.96)
OC4 (Mustard oilcake)	0.00	100.00 ^a (89.04)
Control	9.00	-
SE m (\pm)	-	0.39
CD (0.05)	-	1.56

Table.3 Bio-fumigant effect of plant oils against *R. solani* under *in vitro* conditions

Plant oils	Mycelial growth* (cm)	% Inhibition
O1 (Neem oil)	9.00	0.00 ^b (0.96)
O2 (Lemongrass oil)	0.00	100.00 ^a (89.04)
O3 (Mahua oil)	9.00	0.00 ^b (0.959)
O4 (Tea tree oil)	0.00	100.00 ^a (89.04)
Control	9.00	-
SE m (\pm)	-	1.12
CD(0.05)	-	1.20

Table.4 Bio-fumigant effect of oil cakes against *R. solani* under *in vitro* conditions

Oil cakes	Mycelial growth* (cm)	% Inhibition
OC1 (Groundnut cake)	8.50	5.56 ^b (13.57)
OC2 (Neem cake)	8.73	3.00 ^c (9.56)
OC3 (Mahua cake)	9.00	0.00 ^d (0.96)
OC4 (Mustard oilcake)	0.00	100.00 ^a (89.04)
Control	9.00	-
SE m (±)	-	0.82
CD(0.05)	-	3.28

*Mean of three replications.

Means followed by a common letter(s) are not significantly different by one-way ANOVA at P = 0.05)

Figures in the parenthesis are transformed

Plate.1 Effect of antifungal nature of plant oils against *R. solani* under *in vitro* conditions

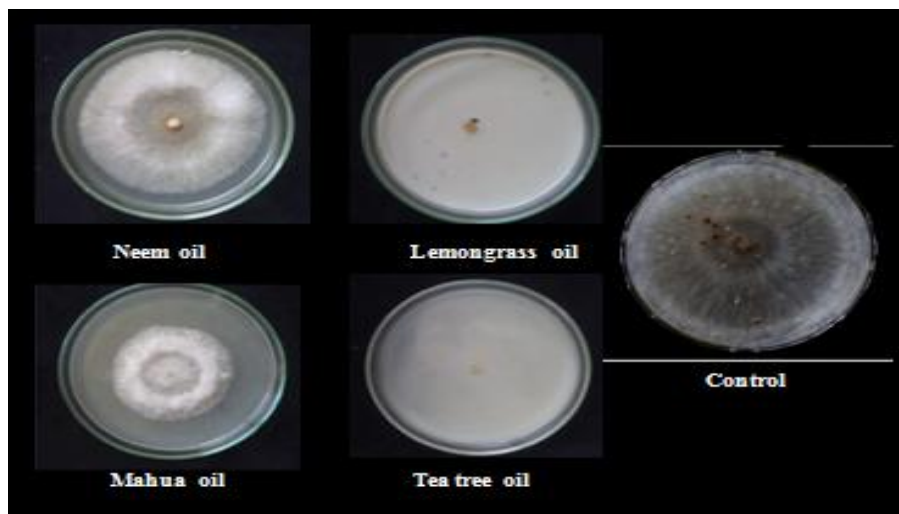


Plate.2 Evaluation of antifungal nature of oil cakes against *R. solani* under *in vitro* conditions



Plate.3 Evaluation of bio-fumigant nature of plant oils against *R. solani* under *in vitro* conditions



Plate.4 Evaluation of bio-fumigant nature of oil cakes against *R. solani* under *in vitro* conditions



Amini *et al.*, (2016) while studying the antifungal effect of plant based essential oil of *Cymbopogon citratus* and *Ocimum basilicum*

found that *C. citratus* was more toxic than essential oil from *O. basilicum*. Thobunluepop *et al.*, (2009) observed 100%

suppression of *R. solani* with incorporation of tea tree oil (2% v/v).

Apart from antifungal action, when used as a bio-fumigant lemongrass oil and tea tree oil (5%) also gave 100% suppression. Earlier workers also highlighted the bio-fumigant effect of essential oil on suppression of plant pathogens, suppression of *Ralstonia solanacearum* with palmarosa oil and lemongrass oil (Alves *et al.*, 2014), thymol (Momol *et al.*, 2000; Pradhanang *et al.*, 2003).

The seed meal/oil cakes very often inherit high amount of biochemical constituents, including fungitoxic biomolecules, originally present in the respective oil yielding seeds. Brown and Morra (2005) reported high concentration of glucosinolates in canola seed meal. Out of the four oil cakes tested for their inhibitory effect on mycelial growth of *R. solani*, mustard oil cake induced the maximum suppression (100 per cent). This was followed by groundnut cake and neem cake with a suppression of 79.62 and 76.29 per cent, respectively. Lenka and Pun (2014) investigated the *in vitro* antifungal action of oil cakes on *R. solani* and obtained promising results with mustard oilcake. However, they observed only mere suppression of the pathogen by treatment with neem cake. In a management study on the web bight disease of urd and mung bean caused by *R. solani*, Dubey and Patel (2000) observed maximum suppression of the pathogen with groundnut oil cake (10%).

In the present study, it was observed that among the oil cakes, only mustard oil cake gave effective bio-fumigant action on pathogen. Fayzalla *et al.*, (2009) explained the bio-fumigant effect of mustard seed meal in the management of soil-borne pathogens such as *R. solani*, *Macrophomina phaseolina* and *Sclerotium rolfsii* causing damping off, root

rot and wilt diseases of soybean under laboratory conditions and recorded 92.2% suppression of *R. solani* at a concentration of 25 mg/petridish.

Acknowledgement

All praises and thanks to the Almighty God, the most merciful and beneficent whose generous blessings showed upon me to perceive and pursue higher ideals of life. I express my sincere gratitude to my guide Dr. V.K. Girija, Professor and Head, Department of Plant Pathology, College of Agriculture, Vellayani for his expert guidance, practical suggestions, support and encouragement throughout the course of work.

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How to cite this article:

Aparna, K.P. and Girija, V.K. 2018. Efficacy of Plant Oils and Oil Cakes against *Rhizoctonia solani* Kuhn Causing Collar Rot and Web Blight of Cowpea under *in vitro* Conditions. *Int.J.Curr.Microbiol.App.Sci.* 7(01): 2091-2098. doi: <https://doi.org/10.20546/ijcmas.2018.701.252>