

Fungitoxic Potential of *Ocimum sanctum* Essential Oil Based Formulated Product in Management of Collar-Rot Disease of a Rice Based Crop, Groundnut

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ABSTRACT

Keeping in view the hazardous effects of synthetic pesticides on environment, this study aimed at developing a suitable formulation from *Ocimum sanctum* essential oil with an adjuvant, coded, "A+". The formulated product, Oscilene-eo, bioassayed under *in-vitro* condition against *Aspergillusniger* Van Tieghem. causing collar-rot disease in rice based crop, groundnut exhibited complete inhibition of mycelial growth at 0.1 percent. Seed soaking treatment both under laboratory and greenhouse revealed that Oscilene-eo (the formulated product) exhibited significant infection reduction even at the lowest tested concentration (0.01%) compared to essential oil or A+ alone and Inoculated control. The product was found at par with standard fungicide carbendazim both in reduction of disease and yield at 0.1% concentration under field conditions.

Key words: Adjuvant, Oscilene-eo, *Aspergillusniger* Van Tieghem, mycelial growth, seed soaking treatment, greenhouse test, field experiment.

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INTRODUCTION

The groundnut (*Arachishypogaea* L.) is an important oilseed crop of the semi-arid tropics [1] [2] that ranks thirteenth in importance among world crops [3]. Groundnut is a staple food in a number of developing countries much valued for its protein content and as source of income for small farm holder [4]. It is also a good source of edible oil for humans, as well as a nutritive feed supplement for livestock [5] [6]. This crop in India has suffered, serious losses due to collar-rot disease caused by multiple pathogen complex mainly *Aspergillusniger*, *Aspergillusflavus*, *Sclerotiumrolfsii*, *Thielaviopsisbasicola*, *Rhizoctoniasolani* and *Pythiumaphanidermatum* perennating in soil and seed.

MATERIALS AND METHODS

A. Preparation of Essential oil (EO)

Fresh leaves from *O. sanctum* weighing 1kg were washed thoroughly, loaded in Clevenger's apparatus and sterilized distilled water was added to it (1:1w/v). Essential oil (5 ml) was collected and moisture from the oil was separated utilizing differential freezing point principle [7]. The remaining moisture was removed by addition of sodium sulphate

(Na₂SO₄) and pure essential oil was decanted in a clean sterilized glass vial. The Essential oil was diluted from 100% to 10%, 1%, 0.1%, 0.01% and / or 0.001% and utilized during course of studies.

B. Preparation of Formulated product (Oscilene-eo)

The formulating agent (FA), a surfactant coded A+ was similarly successively diluted as stated above from 100% to 10%, 1%, 0.1%, 0.01% and / or 0.001%. Each of these dilutions were combined with serially diluted EO (1:1v/v) and treated as Oscilene-eo which was subsequently used during the course of investigation[8].

C. Isolation and maintenance of *Aspergillusniger* Van Tieghem

Infected tissue of groundnut cultivar AK12-24 were separated, surface sterilized with 0.1% sodium hypochlorite for 30 s, washed thoroughly with sterilized distilled water thrice and dried on sterilized blotting paper before transferring it to previously prepared PDA medium as per method described by Booth [9] aseptically in petri plates. After 96 h of incubation at 28 ±20 °C white mycelial growth developed around the infected tissue. The colony that gradually turned black in color after sporulation and was examined under microscope and identified as *A. niger*. Pure cultures of the fungus were obtained separately by subsequent sub-culturing on PDA slopes. After 5 days of incubation at 28 ±20°C these were stored at 40°C separately. Pathogenicity of the isolate was confirmed through Koch's postulates.

D. Bioassay test

a) Poisoned food technique

Oscilene-eo was combined with molten PDA media separately so as to get the final concentration of 1%, 0.1% , 0.01% and 0.001%. The extract mixed media was poured into the petri plates aseptically and inoculated after 4 days allowing ethanol to be evaporated from the media meanwhile, as also in control plate .The contaminated plates were removed. Actively growing mycelia of *A. niger* was cut with a sterile cork-borer and inoculated separately in the center of each such petri plates aseptically. All such plates were incubated at 28±2°C for seven days. Appropriate controls were maintained keeping three replications in each case and the experiment was repeated thrice. Due to patches of growth in *A. niger*, grown patches were drawn on transparent polythene sheet on cultured petri plates and actual growth was computed through graph paper. No mycelial growth was accorded numerical value 0.2 cm², for the purpose of statistical analysis.

b) Groundnut seed soaking effect in *O. sanctum* (Krishna) essential oil extract, adjuvant A+ and their formulation on infection of *A. niger* pathogen under *in-vitro* condition.

Healthy seeds of groundnut of collar-rot susceptible cultivar AK12-24 were soaked in 1%, 0.1% and 0.01 % *O. sanctum* essential oil extracts, adjuvant A+ and formulated product, Oscilene-eo (EOA+) separately for 12 h. Such seeds were then inoculated with *A. niger* by pin-prick method. Three sets of control were maintained viz. i) seed soaked in sterilized double distilled water for 12 h and inoculated thereafter similarly with the test pathogen to serve as inoculated control. ii) Seeds were only pin-pricked without inoculum after soaking in the sterilized distilled water to serve as un-inoculated control and iii) seeds soaked in standard fungicide carbendazim at 0.1% for the same period and inoculated thereafter with the test pathogen to serve as standard control. All such seeds were incubated at 28 ± 1 °C for five days in petri plates. There were three replications in each case and the experiment was repeated thrice. Observation on percent seedling infection

was recorded on 5th day of incubation. Data were transformed to angular values and analysed statistically.

c) Dose response relationship studies of formulated product of EO under *in -vivo* conditions against collar-rot disease in groundnut crop.

Green house experiment (Seed soaking treatment)

Groundnut seeds of collar-rot susceptible cultivar AK 12-24, soaked in 1 %, 0.1% and 0.01% EO, adjuvant, A+ and Oscilene-eo, EOA+ for 12 h separately, and these were inoculated with *A. niger* pathogen by pin-prick method. Seeds soaked in standard fungicide, carbendazim @ 0.1 % a.i. and inoculated with the test pathogen served as standard check. Rest of the experiment was conducted as per method stated earlier in the text. The experiment was conducted thrice, during dry seasons, 2011-2013 keeping three replications each time. Observation on percent plant infected with collar - rot disease was recorded thirty days after sowing in the pots. Data were transformed to angular values and analysed statistically.

Field experiment (Seed soaking + Spraying treatment)

For evaluation of seed soaking + spraying effect, groundnut seeds of collar-rot susceptible cultivar AK12-24 were soaked in 0.1 % EO, adjuvant coded A+ and Oscilene-eo (EOA+) for 12 h separately, and these were inoculated with *A. niger* pathogen by pin prick method. Appropriate controls were maintained and the experiment was conducted as per method described earlier in the text during dry seasons, 2011-2013 keeping three replications each time. Observations on percent plant infections with collar-rot were recorded 50th day of sowing. Data were transformed to angular values and analysed statistically.

RESULTS

A) Poisoned food technique

Table 1.Fungitoxicity of Oscilene-eo against *A. niger* mycelial growth

Concentration (%)	Treatments		
	Oscilene-eo	EO	A+
	Mycelial growth (cm ²)		
1	0.2	0.2	0.2
0.1	0.2	0.2	22.90
0.01	8.52	21.26	63.6
0.001	31.37	42.92	63.6
Control	63.6	63.6	63.6

C.D.at P=0.05=2.60 for *A.niger*.Oscilene-eo (formulated product), EO (*O. sanctum* leaves essential oil), A+ (an adjuvant).Area was calculated through graph paper as stated earlier in the text.Complete inhibition is represented by 0.2cm².

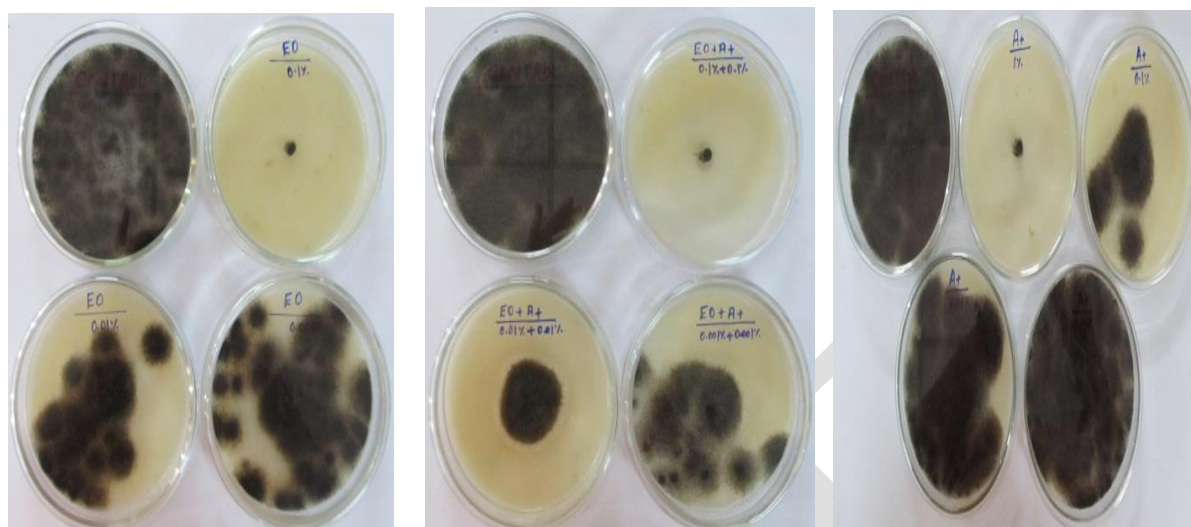


Fig. 1:Fungitoxic effect of Oscilene-eo against mycelial growth of *A. niger*

Complete mycelial growth inhibition ($0.2 \text{ cm}^2 \pm 2.60$) was exhibited by EOA+ and EO alone at 0.1% concentration in *A. niger*. EOA+ displayed significantly reduced mycelial growth ($31.37 \text{ cm}^2 \pm 2.60$) at 0.001% when compared with either EO or A+ ($63.60 \text{ cm}^2 \pm 2.60$) tested alone (Table 1).

B) Groundnut seed soaking effect in *O. sanctum* (Krishna) essential oil extract, adjuvant A+ and their formulation on infection of *A. niger* pathogen under *in-vitro* condition.

EOA+ significantly reduced the disease [$25.4\% \pm 3.4$] compared to EO and A+ at 0.01% concentration and found to be at par with carbendazim [$6.2\% \pm 3.4$] at 0.1% concentration. EOA+ and EO exhibited phytotoxicity at 1% concentration (Table2).

Table 2. Groundnut seed soaking effect in *O. sanctum* leaves EO and Oscilene-eo under *in-vitro* condition

Treatment	Concentration (%)				
	1	0.1	0.01	IC	UC
	Infection (%)				
EOA+	0.1*(1.81)	5.2(13.18)	25.4 (30.26)	80(63.44)	0.1 (1.81)
EO	0.1*(1.81)	16.2(23.73)	33.3 (35.24)	80(63.44)	0.1 (1.81)
A+	14.3(22.22)	26.7(31.11)	46.6(42.71)	80(63.44)	0.1 (1.81)
Carbendazim	-	6.2 (14.42)	-	80(63.44)	0.1 (1.81)

C.D. at $P = 0.05 = 3.4$. Data in parentheses represents transformed angular values.* represents phytotoxicity. EOA+ (formulated product), EO (*O. sanctum* leaves essential oil), A+ (an adjuvant) IC= Inoculated control (seeds inoculated with collar-rot infection).UC = Un-inoculated control.

C) Dose response relationship studies of formulated product of EO under *in -vivo* conditions against collar-rot disease in groundnut crop.

a) Green house experiment (Seed soaking treatment)

All treatments significantly reduced groundnut seedling infection compared to inoculated control ($75\% \pm 2.5$). EOA+ significantly reduced disease ($40.9\% \pm 2.5$) compared to other treatments at 0.01% concentration (Fig2) and found at par [$25.02\% \text{ (EOA+)} \pm 2.5$] with carbendazim ($26\% \pm 2.5$) at 0.1% concentration. EOA+ and EO exhibited phytotoxicity at 1% concentration. Un-inoculated control produced no infection ($0.1\% \pm 2.5$).

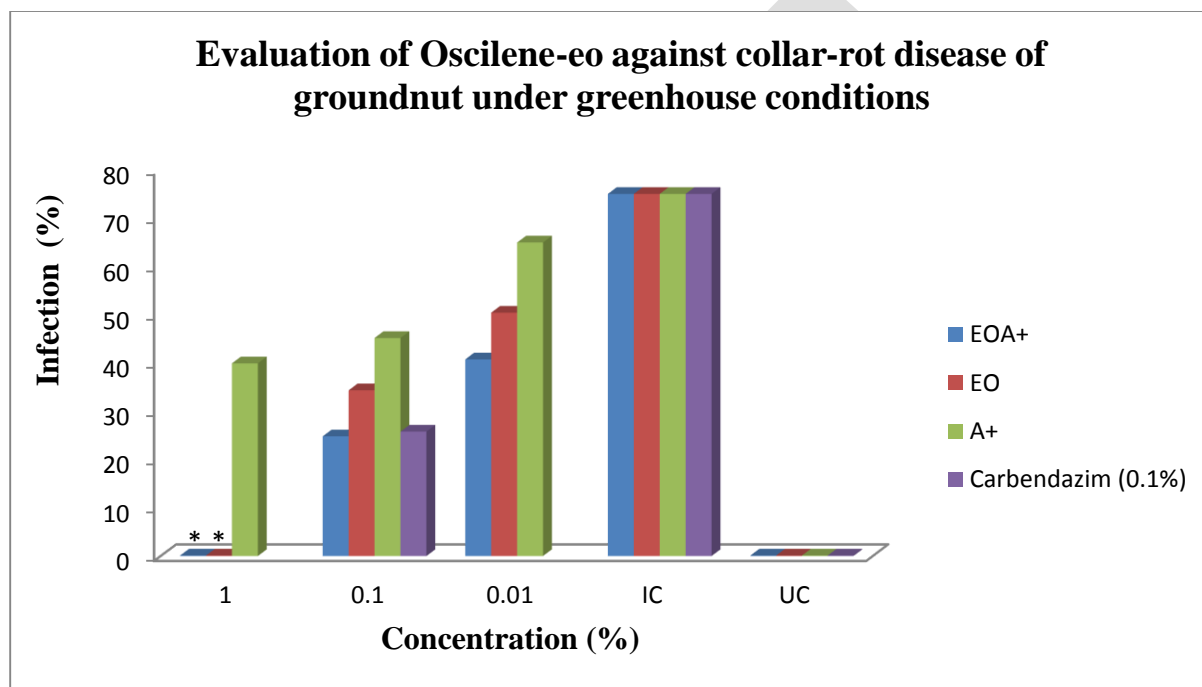


Fig. 2: C.D. at $P=0.05=2.5$. Data in parantheses represents angular values.*represents phytotoxicity. Data pooled for years 2011-2013. EOA+ (formulated product), EO (*O. sanctum* leaves essential oil), A+ (an adjuvant) IC= Inoculated control (seeds inoculated with collar-rot infection). UC = Un-inoculated control.

b) Field experiment (seed soaking+spraying treatment)

All treatments significantly reduced disease in groundnut seedling compared with inoculated control ($75\% \pm 2.12$). EOA+ significantly reduced disease ($6\% \pm 2.12$) compared to EO and A+ alone and found at par with standard fungicide carbendazim ($8\% \pm 2.12$) at 0.1% concentration. Un-inoculated control produced no infection ($0.1\% \pm 2.12$). Highest seed yield was reported in EOA+ ($1184.41 \text{ kg ha}^{-1} \pm 2.12$) and found at par with carbendazim ($1183.50 \text{ kg ha}^{-1} \pm 2.12$). Inoculated control produced lowest yield ($99.45 \text{ kg ha}^{-1} \pm 2.12$) followed by surfactant, A+ ($321.3 \text{ kg ha}^{-1} \pm 2.12$).

Table3. Evaluation of Oscilene-eo (EOA+) and EO of *O. sanctum* against collar-rot disease of

Concentration (%)	Parameter		EOA+	EO	A+	Carbendazim
0.1	I (%)		6.0 (14.18)	15.34 (23.04)	33.0 (35.06)	8.0 (16.43)
	Y (kg ha ⁻¹)	Pod	1679.70	1079.20	530.6	1678.82
		Seed	1184.41	729.28	321.3	1183.50
Control (Inoculated)	I (%)		75 (60.0)	75 (60.0)	75 (60.0)	75 (60.0)
	Y (kg ha ⁻¹)	Pod	206.18	206.18	206.18	206.18
		Seed	99.45	99.45	99.45	99.45
Control (Un-inoculated)	I (%)		0.1 (1.81)	0.1 (1.81)	0.1 (1.81)	0.1 (1.81)
	Y (kg ha ⁻¹)	Pod	838.2	838.2	838.2	838.2
		Seed	576.9	576.9	576.9	576.9

C.D. at P= 0.05= 2.12. EOA+ (formulated product), EO (*O. sanctum* leaves essential oil), A+ (an adjuvant), I= Plant infection (%), Y= Yield (kg ha⁻¹). Data in parantheses represents transformed angular values. Data pooled for years 2011-2013

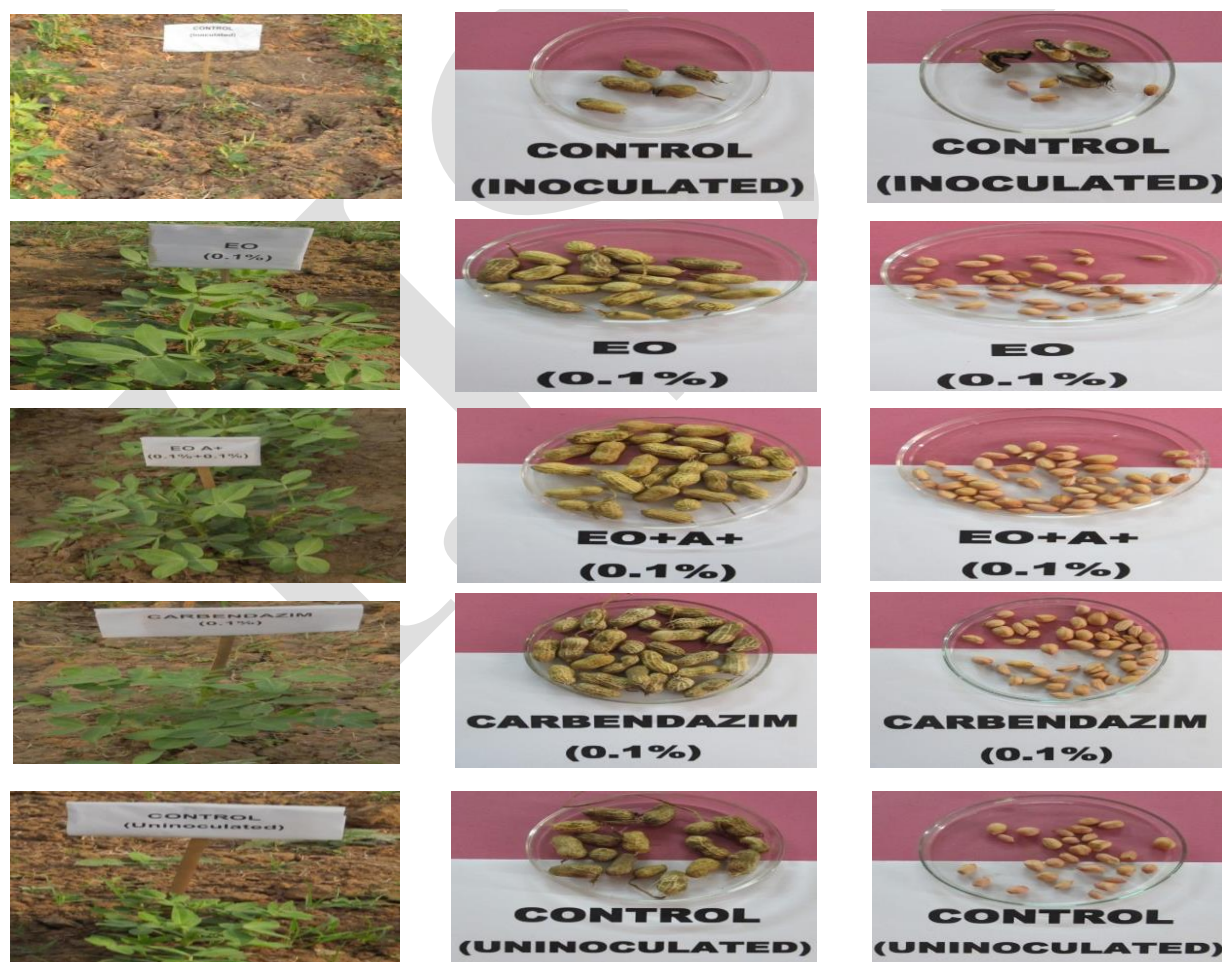


Fig.3: A rice based crop groundnut disease (collar-rot) management under field condition

CONCLUSION

Collar- rot of groundnut caused by *Aspergillusniger*(Van Teighem) is one of important seed and soil borne diseases. This disease was first reported from India by Jain and Nenra [10]. Infected seeds become black and did not germinate. After seed germination the collar region of the plant gets infected, as a result rotting in crown region of the plant is set in and then the seedlings are wilted. The loss due to this disease is reported 28 to 50% [11] [12].

Therefore to protect the yield loss from the collar-rot disease infection and wilting the antifungal bioactive protectants from plant origin have been explored in through recent past [13] specially reported antifungal activities from crude extracts of *Polygonum persicaria*, *Rumex hastatus*, *R. dentatus*, *R. nepalensis*, *Polygonum plebejum* and *Rheum australe* have been studied against *A. niger*.

The rapid decomposition of unformulated plant products if not utilized fresh, hinders its application as crop protectant, therefore the development of an effective formulated botanical product becomes necessary [14] [15]. In this study, a new product Oscilene-eo developed from *O. sanctum* essential oil extract was tested against economically important collar-rot disease of groundnut. At the lowest tested concentration, EOA+ was found superior to EO and A+ both under *in-vitro* and *in-vivo* conditions (Table 1, 2 and 3). Seed soaking experiment in laboratory, greenhouse and seed soaking + spraying experiment in field conditions revealed that the dosage at which synthetic fungicide, carbendazim was tested, EOA+ was found equally effective in controlling the collar-rot disease. Interestingly experiments conducted at Laboratory of Natural Plant Products, Central Rice Research Institute revealed that formulated product, Oscilene-eo (EOA+) at all tested concentrations was found to retain its fungitoxicity for a period of two years (i.e. 24 months) against the rice blast incitant *P. grisea* [8].

Hence the formulated product developed and tested as reported herewith possesses the potential to be deployed in collar-rot disease management strategy but can be recommended only after a large scale field trial.

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