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NOTE

Control of Blackpod Disease of Cocoa Using Leaf Extract of *Ocimum gratissimum* L.

ERNEST OWUSU-ANSAH*, F.K. OPPONG-BOACHIE† and R.T. AWUAH†
Department of Chemistry, University of Cape Coast, Cape Coast, Ghana
E-mail: eowusu_ansah@yahoo.com

The leaves of *Ocimum gratissimum* are characterized by strong aromatic odour and are used for the treatment of stomach disorders, sores and catarrh. Essential oil from the leaves, by steam distillation, gave four fractions upon column chromatography. Only the second fraction which is proven to be thymol by spectroscopic studies (IR and NMR) competed favourably in the suppression of the growth of *Phytophthora palmivora*, a pathogen that causes blackpod disease in cocoa pods with the crude essential oil in *in vivo* studies.

Key Words: Blackpod disease, Cocoa, *Ocimum gratissimum*, *Phytophthora palmivora*, Thymol.

Ocimum gratissimum L. is a shrub mostly found in the tropics. In Ghana, it is grown around houses and mainly used in the treatment of many diseases like diarrhoea, catarrh, eyesores, rheumatism, stomach disorders, lumbago cough and whooping cough[1].

Studies done using essential oil obtained from the leaves in Nigeria by Sofowora[2] and El-Said *et al.*[3] proved to have antibacterial activity. They concluded that the active compound for the control of organisms in this essential oil was thymol. Tripathi *et al.*[4] using *O. gratissimum* [clocium] leaves showed antifungal activity against *Alternaria alternata* [Wees ex Wallr], *Sclerotium rolfsi* [Sacc] and *Collectotrichium capsici* [Corda] fungi which attack stored seeds. The fungicidal property according to these researchers was probably due to the presence of eugenol, which they found to be the major constituent in the essential oil from the leaves in India.

Awuah[5] using the steam distillate from the Ghanaian *O. gratissimum* leaves found it to suppress the growth of *Phytophthora palmivora*, a fungus that causes black pod disease in cocoa. There was, therefore, the need to establish the active ingredient in the leaves of Ghanaian *O. gratissimum* that is responsible for the suppression of *P. palmivora* and therefore controlling the blackpod disease.

The present work reports on the antifungal activity of the essential oil using oats meal agar medium and dilution methods on fresh cocoa pods.

Plant materials: *O. gratissimum* leaves were collected from T.I .Ahmadiyya Secondary School, Prempeh College and Staff Quarters of Kwame Nkrumah

†Department of Chemistry, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

University of Science and Technology (KNUST), Kumasi. The leaves were identified at Herbarium of Biological Sciences Department, KNUST, Kumasi, Ghana.

Steam distillation: 6.3 kg fresh leaves of *O. gratissimum* were chopped into pieces and subjected to steam distillation. Diethyl ether was used to extract the crude oil and dried over anhydrous sodium sulphate. Diethyl ether was removed by simple distillation and the crude oil which was yellow in colour was obtained.

Thin layer chromatography (TLC): Microscope slides coated with Macherey Nagel's polygram silica gel ($0.25/UV_{254}$) were used for TLC. The dried *O. gratissimum* oil (0.5 mL) was dissolved in diethyl ether 5 mL and spotted on microscope slides with silica gel. The slides were developed using petroleum ether : toluene mixture (8 : 2 v/v). The separated spots on the developed chromatogram were viewed under iodine vapour and UV light (254 nm). Four separated spots in iodine vapour were identified and the R_f values calculated.

The dried oil (15.0 g) was put on a glass column packed with silica gel (60.120 mesh) (375 g) and eluted with petroleum ether : toluene mixture (8 : 2 v/v). Drops of the eluate from the column (5 mL) were collected into test tubes. The contents of the test tubes were subjected to TLC and eluted with the same R_f values as obtained previously were pooled together. Three fractions C_1, C_2 and C_3 were obtained in this manner. Each fraction was concentrated on Buchi rotary evaporator and finally dried in an oven at 60°C to a constant weight. A fourth fraction, C_4, was obtained when the column was eluted with ethanol.

Fungitoxicity Test: The *P. palmivora* isolate used for the test was maintained on oatmeal agar (OMA) in a refrigerator until needed. For use in a bioassay, subcultures were made on fresh OMA and a mycelium piece from the resulting colony placed in a well on a detached cocoa pod. The pod was incubated in a humidified transparent polythene bag on a laboratory beach for seven days. The mycelia bearing sporangia of the fungus from the resulting blackpod lesion were then employed as inoculum in the fungitoxicity test.

Mature green cocoa pods from KNUST Cocoa farm were cleaned with 75% aqueous ethanol. A cork borer (8 mm diameter) was used to bore 3 mm deep wells in the pods. Pieces of sporangia bearing mycelia were then transferred into the wells with sterilized spatula. An aliquot of each fraction, *viz.*, C_1, C_2, C_3, C_4 (0.1 mL) and the crude oil (unseparated oil) were injected into each infection court. This procedure was repeated using 0.075, 0.50 and 0.025 mL of each fraction and the unseparated oil. Each pod was then placed in a humidified transparent polythene bag and maintained on the laboratory bench. There were twelve replicate pods per extract. In fraction courts treated with sterilized distilled water served as the control. The diameter(s) of the lesions were measured daily for six days.

Fresh leaves of *O. gratissimum* (6.3 kg) yielded 22.9 g (0.36 %) of a crude yellowish oil upon steam distillation; the major component eluted with petroleum ether : toluene mixture (8 : 2 v/v) was the second component, C_2, whose yield was 52%. This component showed one spot on a TLC with R_f 0.34. Spectroscopic studies using infrared and NMR revealed the major component to be thymol.

The activity of the crude oil and its components towards the growths of *P. palmivora* as expressed by lesion development is summarized in Fig. 1.

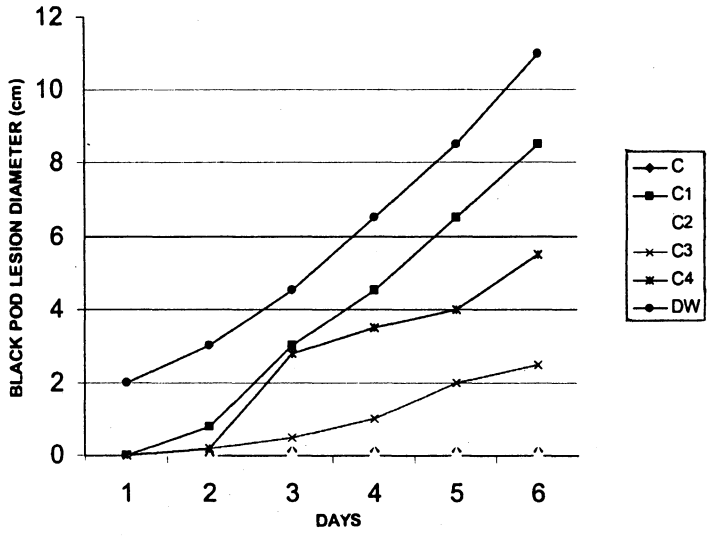

Fig. 1. Blackpod lesion development on detached cocoa pods treated with *O. gratissimum* oil and its components after inoculation with *P. palmivora*

Blackpod lesions developed on all inoculated detached cocoa pods treated with crude oil and its components and lesion sizes on such pods were significantly smaller than those associated with distilled water (control) (Fig. 1). The rates of growth of blackpod lesions on detached cocoa pods treated with the crude oil and component C_2 were the same during the period of the experiment. The crude essential oil and C_2 effectively suppressed lesion development over a d-day period while lesion development progressed with time for pod treated with components C_1, C_3 and C_4 as well as those treated with distilled water as control.

Inhibition of lesion development with crude essential oil corroborates an earlier work by Awuah[5] which demonstrated the efficacy of steam distillation from the leaves of *O. gratissimum* against *P. palmivora* and blackpod lesion development. In the present study, only component C_2 compared favourably with crude oil in suppressing blackpod lesion development. This shows that C_2 now proven to be thymol is not only the major component of the oil from *O. gratissimum* but also the main component of the oil that suppressed black pod lesion development caused by *P. palmivora* in Awuah's work[5]. This gives us the hope that blackpod disease in cocoa caused by *Phytophthora* sp. will be fully controlled by natural products.

REFERENCES

1. D. Abbiw, Useful Plants of Ghana, 2nd Edn., Intermediate Technology Publication, London, pp. 119, 137, 139, 141, 145, 182 (1990).
2. E.A. Sofowora, *Planta Medica*, **18**, 173 (1970).
3. F. El-Said, E.A. Sofowora, S.A.F. Malcom and A. Hofer, *Planta Medica*, **17**, 195 (1969).
4. R.D. Tripathi, R. Bonerji, M.L. Sharma, V.R. Balasubrahmanyam and S.K. Nigam, *Agric. Biol. Chem.*, **49**, 2277 (1985).
5. R.T. Awuah, *Ann. Appl. Biol.*, **124**, 173 (1994).

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