

In vitro* evaluation of *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. plant extracts against *Colletotrichum lindemuthianum

Ochola, S.O.¹, Ogendo, J.O.¹, Wagara, I.N.², Ogweni, J.O.¹, Nyaanga, J.G.¹ & Ogayo, K.O.¹

¹Department of Crops, Horticulture and Soils, Egerton Univ., P. O. Box 536, Egerton, Kenya

²Department of Biological Sciences, Egerton University, P. O. Box 536, Egerton, Kenya

Corresponding author: ocholaochieng@gmail.com

Abstract

The potential of crude plant extracts as antimicrobial agents has been demonstrated both in animal and plant health. Extracts with potential for control of plant pathogens such as *Colletotrichum lindemuthianum* (causal agent for bean anthracnose) which in many cases require chemical control are a necessary component in plant protection regimes. The objective of this study was to evaluate the efficacy of *Leonotis nepetifolia* L. (LN) and *Ocimum gratissimum* L. (OG) extracts to control *Colletotrichum lindemuthianum* *in vitro*. Composite plant parts were shade-dried, extracted using methanol and used for the exploratory laboratory bioassays. Preliminary results show that methanol extracts of LN and OG at concentration of 20.0 w/v produced inhibition results that were comparable to the synthetic fungicide, Rodazim 50SC at 20 ml/20l, with minimum inhibitory concentration (MIC) of 1.25mg/ml. These results will be used for levels bioassay studies of LN and OG extracts as potential antifungal agents against anthracnose.

Key words: Bioassays, *Colletotrichum lindemuthianum*, *Leonotis nepetifolia*, *Ocimum gratissimum*

Résumé

Le potentiel de la plante brute extrait en tant qu'agents antimicrobiens a été démontré à la fois en matière de santé animale et végétale. L'extrait à fort potentiel pour le contrôle des pathogènes de plantes telles que le *Colletotrichum lindemuthianum* (agent causal de l'anthracnose de haricots) qui dans de nombreux cas nécessitent un contrôle chimique, sont une composante nécessaire dans les régimes de protection des végétaux. L'objectif de cette étude était d'évaluer l'efficacité de *Leonotis nepetifolia* L. (LN) et *Ocimum gratissimum* L. (OG) extraits pour contrôler le *Colletotrichum lindemuthianum* *in vitro*. Les parties composant les plantes ont été séchées à l'ombre, extraits à l'aide de méthanol et utilisés pour les essais biologiques en laboratoire d'exploration. Les résultats préliminaires montrent que les extraits au méthanol de LN et OG, à une concentration de 20,0 p / v, ont produit des résultats d'inhibition qui étaient comparables au fongicide de synthèse, Rodazim 50SC à 20 ml/20l, avec la concentration minimale inhibitrice (CMI) de 1.25mg/ml. Ces résultats seront utilisés pour les niveaux d'études d'essais biologique d'extraits LN et OG comme agents antifongiques potentielles contre l'anthracnose.

Mots clés: essais biologiques, *Colletotrichum lindemuthianum*, *Leonotis nepetifolia*, *Ocimum gratissimum*

Introduction

French bean (*Phaseolus coccineus* L.) is an important export horticultural crop in Kenya. Production of French bean by smallholder farmers has been hampered by anthracnose, a fungal disease caused by *Colletotrichum lindemuthianum*. This pathogen adversely affects French bean production in Kenya causing 20 to 100 % reduction in crop yield depending on growth stage, plant part infected and cultivar (Fernandez *et al.*, 2000). Pod development stage is the most critically affected and is characterized by complete plant defoliation, stunting of plant growth and extensive yield loss if plants become diseased prior to or during pod fill. Although synthetic pesticides remain the main control agent against the pathogen, their continued use has elicited more problems than solving them. This has rekindled the search for alternative environmentally sound and low-risk control methods for anthracnose. Secondary plant metabolites or bioactive compounds with fungicidal properties has been shown to be an option in the management of pathogens (Adjou *et al.*, 2012). It has been demonstrated that if well harnessed and incorporated into existing control measures and quarantine procedures, plant extracts are potential bio-control agents of plant pathogens and phytosanitary treatments (Mendes *et al.*, 2010). Laboratory studies were conducted to evaluate the effects of LN and OG extracts against *Colletotrichum lindemuthianum*, the causal agent of anthracnose in French bean.

Literature summary

French bean (*Phaseolus coccineus* L.) accounts for 60 and 21% of all export vegetables and horticultural exports in Kenya, respectively and generates 35-40% of foreign export exchange annually. Anthracnose is one of the most important diseases in French bean (Mendes *et al.*, 2010) and yield losses up to 100% can occur when infection occur at early stages under favourable. Frequent use of synthetic chemicals have been associated with development of pathogen resistance to the chemicals, environmental pollution, health risks and high costs to the smallholders, and reduced acceptance of bean exports in international markets. Bio-pesticides offer a viable alternative to synthetic agrochemicals as they are biodegradable and safe to higher animals and man (Bouda *et al.*, 2001). Plants from the Lamiaceae family have been used for centuries as folk medicine with plants in the genus *Leonotis* and *Ocimum* being recognized for their therapeutic potentials. Antimicrobial activity of *Ocimum gratissimum* and *Leonotis nepetifolia* has been demonstrated (Rajalakshmi *et al.*, 2013).

Study description

A laboratory study was conducted to evaluate the fungicidal effects of LN and OG methanol extracts against *C. lindemuthianum*. Composite fresh aerial parts of the two test plants were collected from fallow fields at Egerton University and the surrounding environment where they occur as natural weeds. The test plant samples were shade-dried for 2 weeks to constant weight, ground to fine powder using electric hammer mill and secondary metabolites extracted using 100% analytical reagent (AR) methanol in a Büchi Rotavapor (Model R-200, Switzerland). Resultant concentrated plant extracts were dried to complete dryness and dissolved in sterile distilled water in readiness for the bioassay.

The pathogen, *C. lindemuthianum*, was isolated from infected pods from farmers' fields in Subukia and cultured in PDA media. Disc diffusion method was used to test the efficacy of methanolic extracts of the two plants at six concentrations (0.0, 1.25, 2.5, 5.0, 10.0 and 20.0% w/v) and synthetic fungicide, Rodazim 50 SC (20 ml/20L), used as positive control. Data were collected on the length (mm) of the inhibition zones and subjected to analysis of variance (ANOVA) using SAS and JMP and treatment means separated using Tukey's HSD test.

Research application

Preliminary results showed that methanol extracts of LN and OG, at concentration of 20.0 w/v produced inhibition results that were comparable to the synthetic fungicide, Rodazim 50SC at 20 ml/20l (Table 1 a and b). The minimum inhibitory concentrations (MIC) for both LN and OG was 1.25mg/ml, and produced inhibition zones of 10.2 ± 0.27 and 10.4 ± 0.23 respectively.

Table 1a. Diameter of inhibition zones (mm) caused by *O. gratissimum* extract.

Treatment (% w/v)	Inhibition zone (mm)*
1. 0.0 OG (Distilled water)	No Inhibition
2. 1.25 OG	10.4 ± 0.23^c
3. 2.5 OG	11.5 ± 0.40^b
4. 5.0 OG	12.3 ± 0.47^b
5. 10.0 OG	12.5 ± 0.40^b
6. 20.0 OG	12.4 ± 0.50^b
7. Rodazim 50SC	18.7 ± 0.26^a

*Mean \pm standard error (n=8) values for diameter of inhibition zones at 5% level of significance; OG-refers to *Ocimum gratissimum* extract

Table 1b. Diameter of inhibition zones (mm) caused by *L. nepetifolia* extract.

Treatment (% w/v)	Inhibition zone (mm)*
1. 0.0 LN (Distilled water)	No Inhibition
2. 1.25 LN	10.2 ± 0.27^c
3. 2.5 LN	12.1 ± 0.36^b
4. 5.0 LN	11.2 ± 0.27^b
5. 10.0 LN	11.3 ± 0.38^b
6. 20.0 LN	12.1 ± 0.31^b
7. Rodazim 50SC (Carbendazim)	18.7 ± 0.26^a

*Mean \pm standard error (n=8) values for diameter of inhibition zones at 5% level of significance; LN-refers to *Leonotis nepetifolia* extract.

Ongoing research. Presented above were the results of exploratory/preliminary studies. In-depth laboratory and field (on-station at Egerton University and on-farm in Subukia District) studies on the bioefficacy of LN and OG extracts against anthracnose disease of French bean are currently being undertaken. The participatory on-farm evaluation is expected to increase acceptance / ownership and rate of adoption of indigenous technology by the smallholder French bean farmers.

Expected outputs. At the end of this study the following outputs are expected; (i) *In vitro* minimum inhibitory concentration (MIC) of LN and OG methanolic extracts against *Colletotrichum lindemuthianum* will be known, (ii) *In vivo* efficacy and MIC of the test extracts against *Colletotrichum lindemuthianum* will be determined, (iii) outstanding research findings disseminated to farmers, (iv) at least two manuscripts published in peer-reviewed scientific journals and (v) research outputs presented at national, regional and international conferences/seminars/workshops.

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