



Asian Journal of
Plant Pathology

ISSN 1819-1541



Academic
Journals Inc.

www.academicjournals.com

Antifungal Activity of Methanol Extracts of *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. against Ascochyta Blight (*Phoma exigua*) on French Bean

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

¹Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Egerton, Kenya

²Department of Biological Sciences, Egerton University, P.O. Box 536-20115, Egerton, Kenya

Corresponding Author: J.O. Ogendo, Department of Crops, Horticulture and Soils, Egerton University, P.O. Box 536-20115, Egerton, Kenya

ABSTRACT

The potential of crude plant extracts as antimicrobial agents has been demonstrated both in animal and plant health. The objective of this study was to evaluate the fungicidal efficacy of methanol extracts of *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. against Ascochyta blight (*Phoma exigua*) pathogen *in vitro*. Composite leaves and tender stems were shade-dried, ground to fine powder using electronic hammer mill and extracted using methanol. Two-fold serial dilution was performed to obtain final concentrations of 40.0, 20.0, 10.0, 5.0, 2.5, 1.25, 0.63, 0.31 and 0.16 mg mL⁻¹ (w/v) used in the laboratory bioassays against the pathogen. The experiment was performed according to Kirby-Bauer disc diffusion method in a Completely Randomised Design (CRD) in triplicate. The extracts showed antifungal activity at all the concentrations tested with Minimum Inhibitory Concentration (MIC) of 1.25 mg mL⁻¹ for both *L. nepetifolia* and *O. gratissimum*. There was plant extract concentration-dependent increase in the diameter of zones of inhibition. The average diameter of zones of inhibition for the two plant extracts at 40.0 mg mL⁻¹ were comparable to the synthetic fungicide (Carbendazim) used as positive control. These results showed that the two plant extracts possess antifungal activity against the pathogen and can be used as natural alternatives to the synthetic fungicides in the management of Ascochyta blight on French bean.

Key words: Ascochyta blight, *Leonotis nepetifolia*, *Ocimum gratissimum*, *Phoma exigua*

INTRODUCTION

French bean (*Phaseolus vulgaris* L.) is an important export horticultural crop accounting for 60 and 21% of all vegetable and horticultural exports in Kenya (HCDA., 2010), thus contributing to 35-40% of foreign export exchange annually. The crop is grown as a cash crop by both large and small holder farmers thereby creating employment opportunities for the rural communities especially to women and youth. In the recent years, there has been a decline in French bean production due to problems caused by biotic stresses (Monda *et al.*, 2003). The crop is prone to attacks by several diseases, amongst which Ascochyta blight (*Phoma exigua*) is emerging as an economically important disease that has resulted in decline in French bean production in the recent past. For long time now, research has been directed at other diseases considered to be important in major French bean producing regions. However, with the increase in incidence of Ascochyta blight in French bean production areas, there is need to refocus the studies. The pathogen can stay on the

seed for over two years and thus can be spread from overwintering debris and infected seeds (Hanson *et al.*, 1993). Seed treatments and foliar sprays with various synthetic fungicides have been suggested for control of the pathogen (Gossen *et al.*, 2011). The synthetic fungicides are known to cause more harm to the environment than benefits and therefore there is need for an alternatives approach to controlling the disease.

Secondary plant metabolites or bioactive compounds from plants with fungicidal properties are an option in the management of pathogens (Adjou *et al.*, 2012). Previous studies of several species plant from the Labiatae family have demonstrated the antifungal activities of the plants against plant pathogens (Dikbas *et al.*, 2008; Pinto *et al.*, 2010). This shows the possibility of new control agents to be developed from them. Amongst some of the widely studied and promising botanical control agents are *Ocimum gratissimum* (OG) and *Leonotis nepetifolia* (LN) (Ayanwuyi *et al.*, 2009; Imran *et al.*, 2012; Veerabadran *et al.*, 2013). Studies have shown that *O. gratissimum* and *L. nepetifolia* have antimicrobial properties *in vitro* (Ayanwuyi *et al.*, 2009; Pinto *et al.*, 2010). This study sought to evaluate the potential of OG and LN extracts to inhibit mycelia growth of *Phoma exigua in vitro*, with the aim of promoting them for adoption by the smallholder farmer for the management of Ascochyta blight in French bean.

MATERIALS AND METHODS

Collection of test plants and preparation of extracts: The plant materials used in this study were composite fresh parts (leaves, succulent stems and fruits) of OG and LN. These plants are natural weed species and were abundantly found in undisturbed and/or in cultivated fields and along road reserves. The plants were collected from fallow fields at Egerton University and its surrounding environment and identified by a Taxonomist. Samples were deposited in the Biotechnology Laboratory, Egerton University.

Fresh composite plant samples were shade-dried for three weeks to complete dryness and then ground to fine powder using electronic hammer mill. The resulting powders were weighed, placed in air-tight containers and stored in cool place until extraction. Sequential extractions were performed on 250 g of each plant powder by soaking them in 2 L of 100% methanol and left to stand for 24 h. The mixture was then filtered using Whatman filter paper No. 2. The filtrate was concentrated and methanol recovered using a Büchi Rotavapor (Model R-200, Switzerland). The concentrated plant extracts were transferred into 90 mm petri dishes and placed at room temperature to complete dryness. Stock solutions of the extracts were prepared by dissolving an appropriate amount of the plant extract in Dimethyl sulphoxide (DMSO) to yield a final concentration of 40 mg mL⁻¹. Two-fold serial dilution were conducted to obtain the final concentrations of 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, 10.0, 20.0 and 40.0 mg mL⁻¹ (w/v) that were used to determine the antifungal activities of the two plant extracts.

Isolation and culturing of the pathogen: Infected French bean pods were obtained from farmers' fields and used as source of initial inoculum of *Phoma exigua*. Small angular cuttings were made from the pods surface sterilized using 5% sodium hypochlorite (NaOCl) for two minutes. The sterilized pods were then rinsed three times in sterile distilled water, blot-dried in sterile blotting paper and placed on Potato Dextrose Agar (PDA) media. Streptomycin sulphate (0.1 g L⁻¹) was added to the media to suppress bacterial growth. The plates were sealed with laboratory parafilm and incubated in dark conditions at 21°C for 7 days. Further sub-culturing was conducted to obtain pure cultures of the pathogen that was used for the bioassays.

Antifungal bioassays: The antifungal bioassay of the extracts was done by adopting Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966) on PDA. An appropriate amount of PDA was autoclaved at 121°C for 15 min and allowed to cool to about 45°C. About 10 mL of the prepared PDA was aseptically poured into 90 mm petri dishes with pre-drawn diametrical lines on the bottom and allowed to solidify. Spore suspension (1 mL) of *Ascochyta* blight pathogen obtained from 7 days old culture was dispensed at the centre of the Petri dish and evenly spread all over the surface of the media using sterile glass rod.

Sterile 4 mm filter paper discs were impregnated with 10 µL of 40 mg mL⁻¹ extract of *O. gratissimum* and *L. nepetifolia*. This was repeated for the other remaining rates to obtain enough discs for the three replicates. The discs were left undisturbed in the laminar flow hood for three hours to allow for partial drying. The treated discs were then aseptically placed on the surface of the media inoculated with the pathogen. DMSO and carbendazim were used as negative and positive controls, respectively. The plates were sealed with laboratory parafilm and incubated in dark conditions at 21°C. The treatments were replicated three times in a Completely Randomized Design (CRD) and the experiment repeated three times. Antifungal activity of the extracts was determined by measuring the zones of inhibition (mm) 7 Days After Incubation (DAI). The extracts were considered to be active against the pathogen whenever zone of inhibition equivalent to or greater than 10.0 mm was recorded. The lowest concentration of the botanical extracts that resulted in inhibition zones equivalent to or greater than 10 mm was considered to be Minimum Inhibitory Concentration (MIC) for the individual plants.

Statistical analysis: The data collected was subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS., 2001) software. Treatment means were separated using Tukey's HSD test whenever ANOVA showed significant treatment effects.

RESULTS AND DISCUSSION

The antimicrobial activity of crude methanol extracts of *O. gratissimum* and *L. nepetifolia* are presented in Table 1. The extracts of the two plants exhibited antifungal activity through

Table 1: Zones of inhibition (mm) of methanol extracts of *Leonotis nepetifolia* and *Ocimum gratissimum* on *Phoma exigua*

Concentration (mg mL ⁻¹)	Inhibition zones of the plant extracts (mm)	
	<i>Ocimum gratissimum</i> ^{††}	<i>Leonotis nepetifolia</i> ^{††}
0.16	2.83±0.86 ^g	3.88±0.84 ^h
0.31	7.83±0.37 ^f	8.42±0.29 ^e
0.63	9.92±0.23 ^e	9.88±0.21 ^e
1.25	10.50±0.19 ^{de}	10.33±0.19 ^{ef}
2.50	11.33±0.36 ^{de}	10.67±0.28 ^{def}
5.00	11.83±0.47 ^{cd}	11.58±0.26 ^{de}
10.00	12.00±0.39 ^{cd}	11.92±0.26 ^{cd}
20.00	13.25±0.41 ^{bc}	12.25±0.35 ^c
40.00	14.08±0.26 ^b	14.13±0.20 ^b
DMSO	0.00±0.00 ^h	0.00±0.00 ⁱ
Carbendazim	18.67±0.26 ^a	18.67±0.26 ^a

Values are Means±SE of 3 replicates. Values within a column followed by the same letters are not significantly different (p≤0.05) by Tukey's HSD test

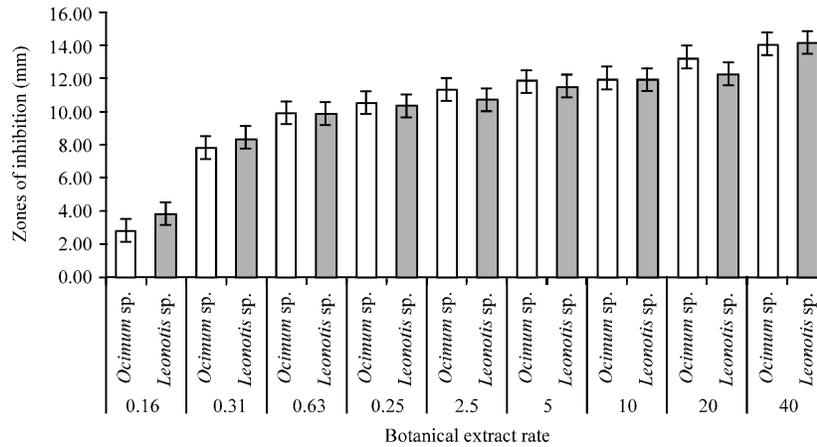


Fig. 1: Antifungal activity of *Ocimum gratissimum* (OG) and *Leonotis nepetifolia* (LN) methanol extracts against *Phoma exigua*

inhibition of mycelia growth of *P. exigua in vitro*. There was significant ($p < 0.05$) concentration-dependent antifungal activity of OG and LN extracts demonstrated by increased diameters of zones of inhibition (Table 1). An increase in the concentration of the extracts resulted in a progressive increase in diameter of the zones of inhibition (Fig. 1). Although the antifungal activity of the positive control (Carbendazim) was significantly ($p < 0.05$) different from the highest rates of the two extracts, their antifungal activities were still comparable. Minimum inhibitory concentration of 1.25 mg mL^{-1} was recorded for both plants tested. Generally, there was no significant ($p < 0.05$) species-dependent antifungal activity of the two plant extracts at all the rates tested (Fig. 1). The two botanicals showed the potential to be effective in controlling *Phoma exigua in vitro*.

The antibacterial and antifungal activity of plant extracts has been demonstrated in previous studies (Al-Reza *et al.*, 2010; Bajpai and Kang, 2012; Ngoci *et al.*, 2013). These properties may be partly attributed to the presence of phytochemicals or secondary metabolites that are active against most pathogens. Phytochemical analysis of the two test plants has shown that *O. gratissimum* contains alkaloids, resins, tannin, phenolics, glycosides, saponin and steroidal terpenes while *L. nepetifolia* contains alkaloid, phenolic, flavonoids, tannins, steroids, glycosides and saponins at different concentrations (Koche *et al.*, 2012; Imran *et al.*, 2012). These compounds are known to be produced by the plants as natural defensive mechanisms against pests and diseases and thus explain the activity of the two plants.

Therapeutic studies have confirmed the effectiveness of *L. nepetifolia* against both Gram negative and Gram positive bacteria (Ngoci *et al.*, 2013). *Leonotis nepetifolia* has been demonstrated to have antimicrobial activity against human diseases caused by bacterial and fungal pathogens. The potential antifungal activity of the plant extract against plant phytopathogens has been demonstrated in this study. The diameters of zones of inhibition obtained across all the rates of *L. nepetifolia* tested, contradicts those reported in previous studies. Ngoci *et al.* (2013) reported that *L. nepetifolia* resulted in zones of inhibition greater than 20 mm for both Gram positive and Gram negative bacteria. The variation could be attributed to the difference in pathogen being tested and the susceptibility of the pathogen to the active ingredients in the plant. The activity of

O. gratissimum against plant diseases has been reported in the several previous studies (Lemos *et al.*, 2005; Okigbo and Ogbonna, 2006; Pinto *et al.*, 2010). The findings of this study on the activity of *O. gratissimum* extract are in conformity with those obtained in the previous studies by other researchers (Pinto *et al.*, 2010; Koche *et al.*, 2012).

Generally, there was an increase in the diameter of zones of inhibition with the increasing concentration rates of the two extracts. These results agree with Koche *et al.* (2012) who reported an increase in the activity of *O. gratissimum* with increase in the concentration of the extract against *E. coli* and *L. monocytogenes*. The increase in activity could be attributed to higher rates of diffusion due to increase in concentration of the active ingredients per unit volume of the solvent. This study has demonstrated the potential for the *L. nepetifolia* and *O. gratissimum* to be used as a natural substitute to the synthetic fungicides in managing Ascochyta blight. However, further evaluations of efficacy of the extracts control Ascochyta blight need to be conducted under field conditions.

ACKNOWLEDGMENT

The financial and institutional support from Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) and Egerton University, respectively, are herein acknowledged by the authors.

REFERENCES

- Adjou, E.S., E. Dahouenon-Ahoussi, R.G. Degnon, M.M. Soumanou and D.C.K. Sohounhloue, 2012. Bioefficacy of essential oil of *Lantana camara* from Benin against the growth of fungi and aflatoxin production. J. Resent Adv. Agric., 1: 112-121.
- Al-Reza, S.M., A. Rahman, Y. Ahmed and S.C. Kang, 2010. Inhibition of plant pathogens *in vitro* and *in vivo* with essential oil and organic extracts of *Cestrum nocturnum* L. Pestic. Biochem. Physiol., 96: 86-92.
- Ayanwuyi, L.O., A.H. Yaro and H.Y.S. Adamu, 2009. Studies on anticonvulsant activity of methanol capitulum extract of *Leonotis nepetifolia* Linn. Niger. J. Pharmaceut. Sci., 8: 73-79.
- Bajpai, V.K. and S.C. Kang, 2012. *In vitro* and *in vivo* inhibition of plant pathogenic fungi by essential oil and extracts of *Magnolia liliflora* Desr. J. Agric. Sci. Technol., 14: 845-856.
- Bauer, A.W., W.M. Kirby, J.C. Sherris and M. Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol., 45: 493-496.
- Dikbas, N., R. Kotan, F. Dadasoglu and F. Sahin, 2008. Control of *Aspergillus flavus* with essential oil and methanol extract of *Satureja hortensis*. Int. J. Food Microbiol., 124: 179-182.
- Gossen, B.D., S.F. Hwang, R.L. Conner and K.F. Chang, 2011. Managing the ascochyta blight complex on field pea in western Canada. Prairie Soils Crops J., 4: 135-141.
- HCDA., 2010. Horticultural crops production report. Horticultural Crops Development Authority (HCDA). <http://www.hcda.or.ke>.
- Hanson, P.M., M.A. Pastor-Corrales and J.L. Kornegay, 1993. Heritability and sources of Ascochyta blight resistance in common bean. Plant Dis., 77: 711-714.
- Imran, S., S.S. Suradkar and D.K. Koche, 2012. Phytochemical analysis of *Leonotis nepetifolia* (L.) R. Br., a wild medicinal plant of lamiaceae. Biosci. Discovery, 3: 196-197.
- Koche, D.K., P.S. Kokate, S.S. Suradkar and D.G. Bhadange, 2012. Preliminary phytochemistry and antibacterial activity of ethanolic extract of *Ocimum gratissimum* L. Biosci. Discovery, 3: 20-24.

- Lemos, J.D.A., X.S. Passos, O.D.F.L. Fernandes, J.R. de Paula and P.H. Ferri *et al.*, 2005. Antifungal activity from *Ocimum gratissimum* L. towards *Cryptococcus neoformans*. Mem. Inst. Oswaldo Cruz, 100: 55-58.
- Monda, E.O., S. Munene and A. Ndegua, 2003. French beans production constraints in Kenya. Afr. Crop Sci. Conf. Proc., 6: 683-687.
- Ngoci, N.S., M. Evalyne and E. Ng'ang'a, 2013. Screening for anti-bacterial activity and phytochemicals of *Leonotis nepetifolia* leaves methanol extract. J. Biotechnol. Sci., 1: 15-21.
- Okigbo, R.N. and U.O. Ogbonna, 2006. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot. Afr. J. Biotechnol., 5: 727-731.
- Pinto, J.M.A., E.A. Souza and D.F. Oliveira, 2010. Use of plant extracts in the control of common bean anthracnose. Crop Protect., 29: 838-842.
- SAS., 2001. Step-by-Step Programming with Base SAS® Software. SAS Institute Inc., Cary, NC.
- Veerabadran, U., A. Venkatraman, A. Souprayane, M. Narayanasamy and D. Perumal *et al.*, 2013. Evaluation of antioxidant potential of leaves of *Leonotis nepetifolia* and its inhibitory effect on MCF7 and Hep2 cancer cell lines. Asian Pac. J. Trop. Dis., 3: 103-110.