Chemical composition and antifungal activity of the essential oil from *Lippia javanica* (Verbenaceae)

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Abstract. GC–MS was used in the investigation of the essential oils chemical composition isolated from the leaves of *Lippia javanica*. A total of 22 components were identified accounting for 76.85% of the oil composition. The oil is characterized by Monoterpenes as shown by the high percentage of Artemisia ketone (49.52%), m-tert-Butylphenol (8.73%), Linalool (4.43%), beta-myrecene (3.13%), Targetone (2.85%) and Isopiperitenone (2.13%). Sesquiterpenes constituted only 6.06% of the total oil. The oil showed inhibitory effects on the visible growth of *Fusarium gramenearum* while there was no observable activity of the oil against *Fusarium moniliforme*. The extent of inhibition of visible fungal growth was dependent on the oil concentration. The oil's Minimum Inhibitory Concentration (MIC) value was found to be 27.07 mg/ml. The results obtained shows that the essential oil from *L. Javanica* can be used to inhibit the growth of phytopathogenic fungi that causes maize diseases in the farms.

Keywords: Antifungal, essential oil, *Fusarium gramenearum*, *Lippia javanica*.

INTRODUCTION

Harvest losses in maize due to pathogenic fungi are approximated at around 12% in the developing countries (Clara et al., 2014). Fungal infection in crops not only causes reduction in quality of the yields but also causes significant economic losses. The infestation contaminates the grains with fungal secondary metabolites known as mycotoxin (Munkvold, 2003). The presence of mycotoxin in food has been documented to cause problems in health because they are capable of causing diseases both to man and animals (Bryden, 2007). Among the most economic important fungi is the *Fusarium* species that is responsible for the pathogenic maize diseases in the field (Munkvold et al., 1997). The species cause GLS, root rot, ear and kernel rot affect growth, quality and the development of the infected maize crops (CIMMYT, 2004).

Prevention and management of fungi have faced a lot of challenges. The problems include both environmental and health due to their non- biodegradability and overuse. According to Koul et al. (2008), 2.5 million tons

of pesticides are estimated to be used on crops annually, and the consequent damage reaches $100 billion annually. The damages are attributed to the high toxicity of synthetic pesticides and their non-biodegradable properties. Moreover, their residue in the crops, soils, and water resources affects human's health directly and other organisms like fish in the water bodies (Kock-Schulmeyer et al., 2013). Therefore, there is greater need to search for immediate alternatives that are highly selective in the mode of action and are biodegradable. It will solve the long-term challenges of toxicity to humans. In addition, techniques should be developed to ensure that the pesticides usage is reduced, but the crop yield should be maintained or even improved to curb the economic losses. Natural products are presented as an immediate and excellent alternative to synthetic pesticides as a way of solving the limitations to the environment and human health (Matasyoh et al., 2007). The move toward using natural products and the continuing need for research on new crop protection tools has generated new research

targeting compounds with novel modes of action. The target natural compounds include materials like plant extracts, toxins, hormones and pheromones. These materials have the characteristics of inhibiting microbial growth.

According to Souza et al. (2005), through research some plants have been shown to contain compounds known as essential oils that have the ability to inhibit the microbial growth. In the present study, *Lippia javanica* was selected for the screening of antifungal activity. *L. javanica* belong to genus Lippia, which is under the Verbenaceae family. The family contains approximately 200 herbs, shrubs and small trees that are often of an aromatic nature (Viljoen et al., 2005). They are distributed throughout in South and Central America, Tropical and Southern Africa (Stafford et al., 2008). Traditional healers have been using the plant in treating minor ailments (Savithramma et al., 2011).

MATERIAL AND METHODS

Sample collection

Fresh leaves of *L. javanica* were collected from the Botanical Garden of Egerton University. The sample was identified by a taxonomist from the biological sciences lab, Egerton University, Nakuru Kenya where the voucher specimen was deposited.

Isolation of essential oils

The freshly cut leaves of *L. javanica* were subjected to hydrodistillation in a Clevenger-type apparatus for 3 h. The oil obtained was dried using anhydrous Na_2SO_4. The oil was stored in a sealed vial at 4°C.

GC, GC-MS analysis

The extracted essential oil was first diluted with methyl-t-butyl ether (MTBE) (1:100) and analyzed on Agilent GC-MSD apparatus that was outfitted with a Rtx-5SIL MS ('Restek') (30 mm × 0.25 mm, 0.25 μm film thickness) . The GC column temperature was maintained at 50°C for 2 min, and then programmed at a temperature of 260°C at the rate of 5°C/min and was held at 260°C for 10 min. Helium was used as the carrier gas (at 0.8 ml/min). The samples were injected in split mode at a ratio of 1:10 to 1:100. The temperature was maintained at 250°C while the transfer line was kept at the temperature of 280°C. The operation of MS was done in the electron impact ionization (EI) mode set at 70 eV, in *m/z* that ranged from 42 to 350. Compound identification was achieved by comparing the mass spectra and retention indices with ones from the literature (Adams, 2007). It was also supplemented by Wiley and QuadLib 1607 GC-MS libraries. The relative proportions of the essential oil

constituents are expressed as percentages obtained by peak area normalization, all relative response factors being taken as one.

Fungal assays

The paper disc diffusion inhibition test method was used to screen for antifungal activity of the essential oil according to the method by Souza et al. (2005). Approximately one hundred microlitres of mould suspension were uniformly and carefully spread on potato dextrose agar (PDA) media sterilised with 7.5% sodium chloride and 133 mg streptomycin sulphate (for 1 L of media) in Petri dishes. The sterilized sensitivity discs were soaked with 10 μl of the essential oil extract and carefully placed at the centres of the inoculated and sterilized culture plates. The diameters of inhibition were measured from the third day after the appearance of observable fungal growth and continuously measured for fourteen days. Measurements of the diameters of the observed inhibition zones were recorded. All measurements were recorded nearest to a millimetre (mm). For positive control, Nystatin discs (100 μg) were used, and dimethyl sulfoxide (DMSO) was used as the negative control. All the cultured plates were kept at 25°C throughout.

Determination of the minimum inhibition concentration of the oil

Approximately one millilitre of fungi suspension was dropped and uniformly spread on sterilized PDA media in Petri dishes. The serial dilutions of the oil were done using 100% DMSO. The oil was diluted to the following serial geometric dilutions: 100, 75, 50, 25, 12.5, 6.25 and 3.13%. Sterilized Whatman filter paper discs (No. 1, 6 mm in diameter) were soaked with 10 μl of the each of the oil concentrations and placed at the centre of the inoculated culture plates and then incubated 14 days. All assays were done in three replicates. The minimum inhibitory concentration was obtained from the least concentration of the oil made (3.13%).

Statistical analysis

Values of inhibition zones were analyzed using Microsoft Office Excel 2007 to derive the values of means and standard deviations.

RESULTS AND DISCUSSION

The essential oil extracted was analysed using gas chromatography-mass spectrometry (GC-MS) and screened for activity. The oil showed activity against *F.*

Table 1. Chemical composition of *L .javanica.*

S/N	R.T (min)	Compound name	% Concentration	Detection method
1	8.45	β – Myrcene	3.13	GC/MS
2	9.18	Menthatriene	0.51	GC/MS
3	10.12	Tagetone	2.85	GC/MS
4	11.23	Bicyclo[3.1.0]hexane,6-ethylene-	0.44	GC/MS
5	11.46	Linalool	4.43	GC/MS
6	12.67	Camphor	0.90	GC/MS
7	13.11	Artemisia Ketone A21	49.52	GC/MS
8	15.21	Phenol, M-tert butyl (Ocimenone)	8.73	GC/MS
9	16.28	Isopiperitenone	2.13	GC/MS
10	20.06	Z-caryophyllene	1.99	GC/MS
11	20.91	4,7,10-Cycloundecatriene,1,1,4,8-etramethyl-, cis, cis, cis-	0.92	GC/MS
12	24.05	Caryophellene oxide	1.31	GC/MS

moniliforme while there was no observable activity against *F. moniloforme*. The screening results for antifungal activity of the oil are shown in Table 1. GC and GC–MS analyses of the essential oil led to the identification of the chemical components, shown in Table 2 together with their retention indices and their area percentages. Twelve compounds that constituted 76.85% of the total oil composition were identified. The oil was dominated by monoterpenes hydrocarbons that accounted for 70.79% of the oil composition. The high percentages of Artemisia ketone (49.52%), m-tert-Butylphenol (8.73%), Linalool (4.43%), beta-myrecene (3.13%), Targetone (2.85%) and Isopiperitenone (2.13%) proved that the *L. javanica* essential oil clearly belongs to the monoterpenoid chemotype. In contrast, the content of Sesquiterpenes constituted only 6.06% of the total oil composition. Main representatives of sesquiterpenes were trans-caryophyllene (2.0%) and caryophyllene oxide (1.3%). According to Viljoen et al. (2005), there has been a lot of research on chemical constituents of the essential oil from the *Lippia* species. The results showed great variations due to ecological variations and population or chemotypic races. Certain studies have mentioned that *L. javanica* displays chemical variation, but most of these studies have mentioned myrcenone as a major component (Lukwa et al., 2009).

According to Chagonda et al. (2000), *L. javanica* samples collected from three locations in Zimbabwe showed high amounts of limonene. Viljoen et al. (2005) showed greater variations of the major oil constituent among the samples collected from different locations in Mozambique. Myrcenone, myrcene and α-phellandrene that were observed to be major compounds in five samples, were not detected in appreciable quantities in one of the samples. Monoterpenes have been shown to be dominating the essential oils. (Figure 1)

Antifungal activity of oil

The oil showed activity against *F gramenearum*. There was no observable activity of the oil against *F. moniliforme*. Inhibition zone for different concentrations were recorded over fourteen days. Results are tabulated in Table 2.

The results tabulated demonstrate that essential oils from *L. javanica* interfere with the growth of *F. gramenearum*. The bioassays were carried out at concentrations of 0.87, 0.65, 0.43, 0.22, 0.11, 0.054 and 0.027 mg/ml (essential oil/ml). For positive control in antifungal assays, commercial Nystatin at a concentration of 100 μg/disc was tested. In general, the oil is active against the *F. gramenearum*. From the data in Table 2, the oil showed the variation of inhibition effects on the visible growth of the fungi with the variation of concentration in comparison with control. The maximum antifungal activity was recorded from the concentration of 0.87 g/ml, and the least activity was recorded for the least concentration of 0.027 g/ml.

After the third day, the inhibition zone for the 0.87 g/ml concentration was larger (25.00 mm) as compared to that of the Nystatin (16.00 mm). The activity of Nystatin was comparable to the oil concentration of 0.435 g/ml (16.00 mm). It was also observed that the inhibition zones reduced with increasing time. It implies that the activity of both the oil concentration was reducing as time increases.

By the seventh day, the inhibition zone of the 0.87 g/ml concentration had reduced to 16.00 mm; while that of Nystatin was at 12.33 mm. Comparing the inhibition of Nystatin to that of 0.435 g/ml concentration of the oil that had the same concentration with Nystatin after three days, their values are 12.33 and 6.00 mm, respectively. The observations point out the fact that the activity of the

Table 2. Inhibition zones (mm) of *L. javanica* oil on *F. gramenearum* for fourteen days after inoculation.

Days	Essential oil concentration (g/ml) *10^{-2}								
	87	65.25	43.5	21.75	10.88	5.44	2.72	Nystatin	Control
3	25.00 ± 1.00	20.33 ± 1.53	16.00 ± 2.00	12.17 ± 1.26	8.33 ± 2.31	5.67 ± 2.52	3.33 ± 1.53	16.00 ± 1.00	0.00 ± 00
4	23.00 ± 1.00	17.00 ± 2.65	14.17 ± 2.08	10.00 ± 2.60	5.33 ± 1.53	4.67 ± 2.89	2.33 ± 1.15	15.33 ± 0.75	0.00 ± 00
5	22.00 ± 1.00	15.83 ± 2.25	10.33 ± 1.53	8.33 ± 1.61	4.00 ± 1.00	2.00 ± 1.00	0.67 ± 0.58	14.00 ± 0.69	0.00 ± 00
6	19.00 ± 1.00	13.67 ± 2.08	7.67 ± 0.58	5.17 ± 0.58	3.17 ± 1.04	1.67 ± 3.21	0.33 ± 0.58	13.33 ± 0.68	0.00 ± 00
7	16.00 ± 1.00	12.33 ± 2.08	6.00 ± 1.00	6.00 ± 1.00	2.83 ± 1.04	1.33 ± 0.58	0.17 ± 0.29	12.33 ± 0.66	0.00 ± 00
8	13.33 ± 0.58	10.67 ± 1.53	5.00 ± 1.00	5.00 ± 1.00	2.33 ± 0.58	0.67 ± 0.58	0.00 ± 00	11.00 ± 0.54	0.00 ± 00
9	10.67 ± 0.58	8.67 ± 0.58	4.67 ± 1.15	4.67 ± 1.15	1.50 ± 0.50	0.67 ± 0.58	0.00 ± 00	11.00 ± 0.54	0.00 ± 00
10	9.33 ± 0.58	6.33 ± 0.58	4.00 ± 1.00	4.00 ± 1.00	1.00 ± 00	0.50 ± 0.50	0.00 ± 00	11.00 ± 0.54	0.00 ± 00
11	8.00 ± 1.15	5.00 ± 1.00	3.67 ± 0.58	3.67 ± 0.58	0.67 ± 0.58	0.50 ± 0.50	0.00 ± 00	11.00 ± 0.54	0.00 ± 00
12	7.67 ± 0.58	4.00 ± 3.33	3.00 ± 1.00	3.00 ± 1.00	0.33 ± 0.58	0.33 ± 0.29	0.00 ± 00	11.00 ± 0.54	0.00 ± 00
13	7.33 ± 1.53	3.33 ± .058	2.67 ± 1.53	2.67 ± 1.53	0.33 ± 0.58	0.17 ± 0.29	0.00 ± 00	11.00 ± 0.54	0.00 ± 00
14	7.000 ± 1.73	2.67 ± .058	2.33 ± 1.15	2.33 ±1.15	0.33 ± 0.58	0.00 ± 00	0.00 ± 00	10.67 ± 0.49	0.00 ± 00
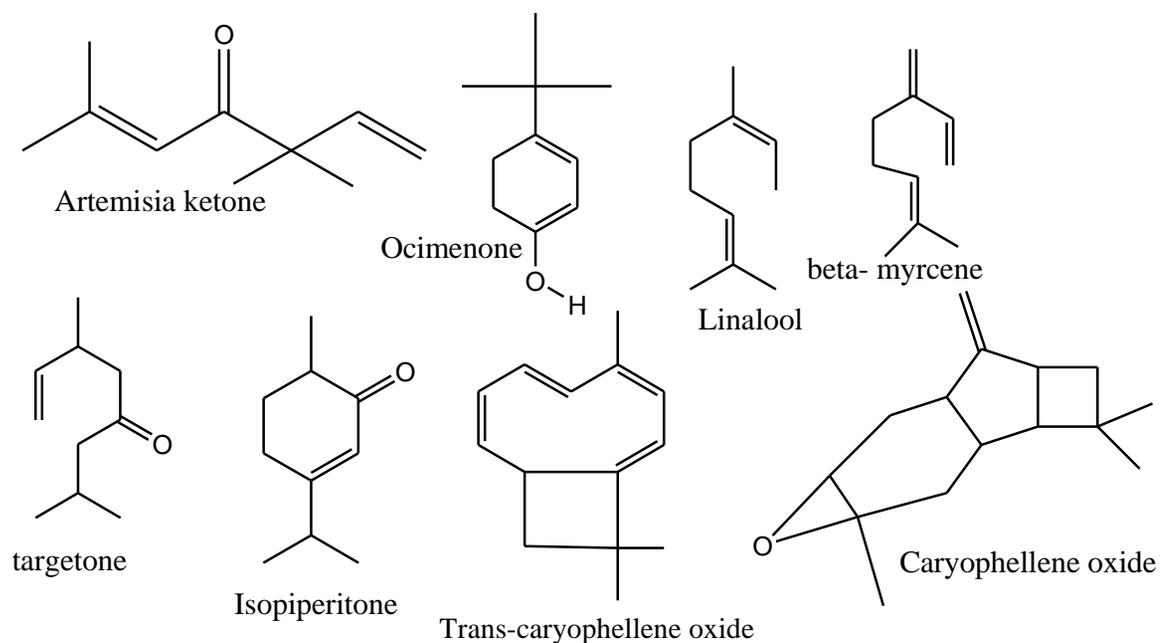
Figure 1. Structures of some of the major constituents of the oil.

oil reduces faster as compared to that of Nystatin.

After fourteen days, the inhibition zone of the 0.87 g/ml concentration of the oil had reduced to 7.00 mm while that of Nystatin only reduced to 10.67 mm. The activity of Nystatin is slightly stable when compared to that of the oil. According to Birkett et al. (2008), the volatility nature of the essential oil is the characteristic feature for easy oxidation. Therefore, the oil loses its activity with time. This feature is evident with the inhibition zone reducing with increasing time. From Table 2, comparing the oil concentration of 0.87 g/ml to that of Nystatin, it shows that after three days, the oil was very active with 25.00 mm inhibition zone compared to that of Nystatin with 16.00 mm. After seven days, inhibition zone of the oil had reduced to 16.00 mm while that of Nystatin only reduced to 12.33 mm. By the fourteenth day, the inhibition zone of the oil had further reduced to 7.00 mm while that of Nystatin slightly reduced to 10.67 mm. The difference in the observed activity of the oil and Nystatin could be the attributed to the different components which synergistically active.

The activity of the oils was increased with concentration. The antifungal mechanisms of the chemical constituents are unknown but may be related to their general properties of destroying the development of fungi's cells wall and cells membranes (Isman and Machial, 2006). In addition, the mode of antimicrobial action of the oil may also be due to the inhibition of respiration (Koschier and Sedy, 2001). The other speculation on the enhanced efficacy of the oil is due to differential permeability as a result of molecular actions that have been prompted by adhesive activities of the oil molecules (Lukwa, 1994).

The antifungal activity of the oil cannot be easily attributed to a single constituent since the essential oil is a very complex mixture (Koul and Walia, 2009). Major and minor compounds might be responsible for the observed antimicrobial activity exhibited. The possible synergistic and antagonistic effect of compounds in the oil should be taken into consideration. Tripathi et al. (2009) argued that the structural modifications common to natural monoterpenoids may lead to improved biological activity. In addition, their biological activities are related to position and nature of the functional groups and molecular configurations of the oil constituents rather than its volatility and molecular size (Kumbhar and Dewang, 2001). The other speculation on the enhanced efficacy of the oil is due to differential permeability as a result of molecular actions that have been prompted by adhesive activities of the oil molecules (Lukwa, 1994). However, according to Bakkali et al. (2008), it is possible that the activity of the major components is modulated by other minor molecules in the mixture.

The activity observed could be attributed to the presence of β-caryophyllene oxide. According to Cakir et al. (2004), β-caryophyllene oxide exhibited a pronounced inhibition effect (range 33–85%) for the growth of all agricultural pathogenic fungi. From Cakir et al., (2004) study, Targetone was the major constituent of the oil. There is no literature of the pure compound being tested against *F. g*ramenearum fungi.

According to El-shiekh et al. (2012), Targetone showed activity against *Sclerotium rolfsii* and *Rhizoctonia solani* fungi at the following concentrations; at 500 µg/ml concentration of linalool it showed a growth inhibition of 12.71%; the 2000 µg/ml gave 93.29%, and the 8000 µg/ml gave complete growth inhibition. In addition, Osée et al. (2004) reported that Tagetone was one of the major component in essential oil extracted from *L. javanica* and *Tagetes minuta* plants obtained from the Eastern Cape province of South Africa. It further reported that the oils showed remarkable activity against fungi and other microbes with that of *L. javanica* showing more activity. The *L. javanicca* essential oil having shown activity against fungi before might be due to the presence of Tagetone. However, no test of oil has been done before on any *Fusarium* species, the activity it has shown against *F. gramenearum* species might be attributed to the presence of Tagetone. Linalool could have contributed to the activity observed in the oil. Abad et al. (2007) reported that compound beta-Myrcene was a major constituent in the oil extracted from the plant *Haplophyllum tuberculatum* (Forsskal). This oil affected the mycelia growth of *Curvularia lunata* and *Fusarium oxysporium*. In the essential oil extracted from *H. hyssopifolium*, Myrcene concentration was reported to be 3.8% (Cakir et al., 2004).

This oil was found to be active against five agricultural *Fusarium* fungal pathogenic species (*F. sambucinum*, *F. oxysporum*, *F. solani*, *F. acuminatum* and *F. culmorum*), which originates from the soil (Cakir et al., 2004). Therefore, even though its activity against *F. gramenearum* has not been documented, its effects may not be ruled out due to its observed activity against some other *Fusarium* pathogens.

CONCLUSION

The antifungal activities shown by the *L. javanica* oil against *F. gramenearum* can help in the development of crop management strategies that are integrated for the control of maize phytopathogenic fungi. The effective, efficient, lesser toxic and environmental friendly drug against fungi is yet to be researched because of the numerous scientific cases of observed resistance against the existing synthetic antifungal drugs that are currently being used extensively. Therefore, the studies of essential oils and their constituent compounds have come into notice as scientists have shown them to be potent antifungal agents. No resistance or toxicological effects have been documented on the use of the essential oils. Therefore, they can be used as antifungal agent but also in synergism with the currently used

antifungal agents. The benefits include their ability to act through various modes towards different target microorganisms. Thus, essential oils are emerging as the reasonably efficient, effective antifungal agents that that can be integrated into crop management practices.

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REFERENCES

Abad MJ, Ansuategui M, Bermejo P (2007). Active antifungal substances from natural sources. Arkivoc, 7:116-145.
Bryden WL (2007). Mycotoxins in the food chain: human health implications. Asia Pac. J. Clin. Nutr. 16:95-101.
Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008). Biological effects of essential oils – A review. Food Chem. Toxicol. 46:446-475.
Birkett MA, Hassanali A, Hoglund S, Pettersson J, Pickett JA (2011). Repellent activity of catmint, *Nepeta cataria*, and iridoid nepetalactone isomers against Afro-tropical mosquitoes, ixodid ticks and red poultry mites. Phytochemistry 72:109-114.
Cakir A, Kordali S, Zengin H, Izumi S, Hirata T (2004). Composition and antifungal activity of essential oils isolated from *Hypericum hyssopifolium* and *Hypericum heterophyllum*. Flavour Fragr. J. 19(1):62-68.
Clara C, Matasyoh JC, Wagara IN, Nakavuma J (2014). Antifungal activity of flavonoids isolated from *Monanthotaxis littoralis* against mycotoxigenic fungi from maize. Am. J. Chem. Appl. 1(4):54-60.
Kock-Schulmeyer M, Villagrasa M, de Alda ML, Céspedes-Sánchez R, Ventura F, Barceló D (2013). Occurrence and behavior of pesticides in wastewater treatment plants and their environmental impact. Sci. Total Environ. 458:466-476.
Chagonda LS, Makanda CD, Chalchat JC (2000). Essential oils of wild and cultivated *Lippia javanica* (Spreng) and *Lippia oatesii* (Rolfe) from Zimbabwe. J. Essent. Oil Res. 12(1):1-6.
El-Shiekh YWA, El-Din NH, Shaymaa MAA, El-Din KAZ (2012). Antifungal activity of some naturally occurring compounds against economically important phytopathogenic fungi. Nat. Sci. 10:114-123.
International Maize and Wheat Improvement Center, (CIMMYT) (2004). Maize Diseases: A Guide for Field Identification. 4th edition. Mexico: D.F.: CIMMYT.
Isman MB, Machial CM (2006). Pesticides based on plant essential oils: from traditional practice to commercialization. Adv. Phytomed. 3:29-44.

Koul O, Walia S, Dhaliwal GS (2008). Essential oils as green pesticides: potential and constraints. Biopestic. Int. 4(1):63-84.
Koul O, Walia S (2009). Comparing impacts of plant extracts and pure allelochemicals and implications for pest control. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutr. Nat. Resour. 4(049):1-30.
Koschier EH, Sedy KA (2001). Effects of plant volatiles on the feeding and oviposition of Thrips tabaci. Thrips and Tospoviruses pp. 185-187.
Kumbhar PP, Dewang PM (2001). Eco-friendly Pest Management Using Monoterpenoids. I. Antifungal Efficacy of Thymol Derivatives. J. Sci. Ind. Res. 60(8):645-648.
Lukwa N (1994). Do traditional mosquito repellent plants work as mosquito larvicides? The Cent. Afr. J. Med. 40(11):306-309.
Lukwa N, Molgaard P, Furu P, Bogh C (2009). *Lippia javanica* (Burm F) Spreng: its general constituents and bioactivity on mosquitoes. Trop. Biomed. 26(1):85-91.
Matasyoh LG, Matasyoh JC, Wachira FN, Kinyua MG, Muigai AWT, Mukiama TK (2007). Chemical composition and antimicrobial activity of the essential oil of *Ocimum gratissimum* L. growing in Eastern Kenya. Afr. J. Biotechnol. 6(6): ??-??
Munkvold GP (2003). Epidemiology of Fusarium diseases and their mycotoxins in maize ears. In Epidemiology of Mycotoxin Producing Fungi (pp. 705-713). Springer Netherlands.
Munkvold GP, McGee DC, Carlton WM (1997). Importance of different pathways for maize kernel infection by *Fusarium moniliforme*. Phytopathology 87(2):209-217.
Osée Muyima NY, Nziweni S, Mabinya LV (2004). Antimicrobial and antioxidative activities of *Tagetes minuta, Lippia javanica* and *Foeniculum vulgare* essential oils from the Eastern Cape Province of South Africa. J. Essent. Oil Bearing Plants 7(1):68-78.
Savithramma N, Rao ML, Suhrulatha D (2011). Screening of medicinal plants for secondary metabolites. Middle-East J. Sci. Res. 8(3):579-584.
Stafford GI, Pedersen ME, van Staden J, Jäger AK (2008). Review on plants with CNS-effects used in traditional South African medicine against mental diseases. J. Ethnopharmacol. 119(3):513-537.
Souza ELD, Lima EDO, Freire KRDL, Sousa CPD (2005). Inhibitory action of some essential oils and phytochemicals on the growth of various moulds isolated from foods. Braz. Archive. Biol. Technol. 48(2):245-250.
Tripathi AK, Upadhyay S, Bhuiyan M, Bhattacharya PR (2009). A review on prospects of essential oils as biopesticide in insect-pest management. J. Pharmacog. Phytother. 1(5):52-63.
Viljoen AM, Subramoney S, Van Vuuren SF, Başer KHC, Demirci B (2005). The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. J. Ethnopharmacol. 96(1):271-277.