

Bioefficacy of lion's ear and African basil extracts in management of adult two-spotted spider mite on french beans

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

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: kennedyobuya@yahoo.com

Abstract

Methanol extracts of Lion's ear, *Leonotis nepetifolia* L. (LN) and African basil, *Ocimum gratissimum* L. (OG), were evaluated for their toxic and ovicidal effects against the female adult two-spotted spider mite, *Tetranychus urticae* Koch. Laboratory bioassays were conducted at Egerton University using five concentrations (0.0, 1.5, 3.0, 6.0 and 12.0 % w/v) of each plant extract and methanol and Abamectin (0.6 ml/L) as negative and positive controls, respectively, arranged in completely randomized design replicated four times. Data on adult mite mortality and number of eggs laid were collected 24, 48 and 72 hours after treatment (HAT) and subjected to analysis of variance (ANOVA) and means separated using Tukey's HSD test ($P < 0.05$). Preliminary results showed concentration- and exposure time-dependent increase in efficacy of LN and OG extracts against adult mite. At 12% w/v and 72 HAT, LN and OG extracts produced 93.8 and 96.4% mortality of adult TSSM, respectively. Similarly, both extracts produced 100% reduction in the number of eggs laid by the female adult TSSM. Synthetic acaricide, abamectin had 43.5 and 45.3% mortality, 34.4 and 62.9% reduction in number of eggs laid respectively. These findings demonstrate the potential use of *L. nepetifolia* and *O. gratissimum* extracts for the management of two-spotted spider mite on French beans.

Key words: *Leonotis nepetifolia*, *Ocimum gratissimum*, *Tetranychus urticae*

Résumé

Les extraits de méthanol de l'oreille du lion, *Leonotis nepetifolia* L. (LN) et le basilic africain, *Ocimum gratissimum* L. (OG), ont été évalués pour leurs effets toxiques et ovicidaux contre des tetranyques femelles adultes à deux taches, *Tetranychus urticae* de Koch. Des essais biologiques en laboratoire ont été réalisés à l'Université Egerton en utilisant cinq concentrations (0,0, 1,5, 3,0, 6,0 et 12,0% en poids / volume) pour chaque extrait de plante, de méthanol et de l'abamectine (0,6 ml / L) en tant que contrôles négatifs et positifs, respectivement, disposés complètement dans des conceptions aléatoire répété quatre fois. Les données sur la mortalité des tetranyques adultes et le nombre d'œufs pondus ont été recueillies 24, 48 et 72 heures après le traitement (HAT), et soumis à une analyse de variance (ANOVA) et des moyens séparés par le test HSD de Turquie ($P < 0,05$). Les résultats préliminaires ont montré l'augmentation de la concentration et de l'exposition à la dépendance

au temps dans l'efficacité des extraits LN et OG contre les acariens adultes. À 12% p / v et 72 HAT, les extraits LN et OG ont produit 93,8 et 96,4% de mortalité d'adultes TSSM, respectivement. De même, les deux extraits ont produit la réduction de 100% du nombre d'œufs pondus par la femelle adulte TDP. L'acaricide synthétique abamectine avait 43,5 % et 45,3% de mortalité, 34,4, et 62,9% de réduction du nombre d'œufs pondus, respectivement. Ces résultats démontrent l'utilisation potentielle des extraits de *L. nepetifolia* et d'*O. gratissimum* pour la gestion de deux tetranyques tachetés sur les haricots verts.

Mots clés: *Leonotis nepetifolia*, *Ocimum gratissimum*, *Tetranychus urticae*

Introduction

French bean, *Phaseolus vulgaris* L., is a major vegetable export crop and income earner for small holder farmers who constitute more than 80% of total French bean production in Kenya. Although annual exports from fresh vegetables account for 35-45 % of foreign exchange in Kenya, the area under French bean has recently decreased attributed to low yields of French bean in smallholder farmers' fields. Two-spotted spider mite, *Tetranychus urticae* Koch, is one of the major pests that have contributed to the limitation in French beans production. The available synthetic acaricides used have caused serious problems such as unacceptable pesticide residues in food and lethal effects on non-target organisms. These negative effects have resulted in an increasing interest for natural plant-based pesticides which are assumed to be safer than the synthetic chemicals. Studies have pointed to numerous plant species possessing potential pest controlling properties. *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. have been studied for various biological activities such as antifungal, antibacterial and antioxidant activities. However limited information exists for usage as crop protectants under laboratory and field conditions, hence the objective of the study was to determine their toxic and ovicidal effects against adult *T. urticae* Koch and possibility of using the extracts to manage the pest.

Literature summary

French bean production faces major constraints such as decline in acreage and yields in smallholder farmers' fields. This is attributed to abiotic and biotic factors (Monda *et al.*, 2003). Spider mite being one of the major pests that has caused yield reductions has been listed as quarantine pest hence a leading cause of Kenya's fresh produce rejection by European markets. Several strategies have been tested for spider mite management but with mixed results. Synthetic pesticides commonly used by farmers have proved otherwise as many negative effects have been reported (Jovanovic *et al.*, 2007). Therefore, natural plant-based pesticides seem to be the best alternatives for use by smallholder farmers. Lion's ear, *Leonotis nepetifolia* L., has been tested for antibacterial activity using its crude extracts (Narayan, 2012), but limited information exists for use as a crop protectant while African basil, *Ocimum gratissimum* L., has been widely used for control of insect pests especially storage insect pests (Asawalam *et al.*, 2006). Therefore exploration of the potential and utilization of these commonly available plants and their extracts against spider mites on French beans in relation to miticidal efficacy was tested under laboratory conditions.

Study description

Laboratory studies were conducted at Egerton University. Test plant extracts were prepared according to Asawalam *et al.* (2006) with modifications. TSSM used in this study was obtained from infested leaves of French beans grown inside a greenhouse. Laboratory bioassays were as follows: (i) *Contact toxicity*: A leaf dipping method was performed according to Erdogan *et al.* (2012) with modifications with extract concentration at five levels (0.0, 1.5, 3.0, 6.0 and 12.0% w/v) while methanol and abamectin as negative and positive controls, respectively. Bean leaf discs were cut into 3 cm diameter and dipped into prepared concentrations for 30 seconds then dried. Twenty (N_r) adult female mites were introduced onto the leaf discs placed in petri dishes with wet cotton wool. These were arranged in a completely randomized design (CRD) replicated four times. The numbers of dead (N_p) mites were recorded 24, 48 and 72 HAT. Percent mortality was corrected using Abbott's formula (Abbott, 1925). (ii) *Ovicidal test*: The leaf disc method according to Zhang *et al.* (2013) was used to determine the oviposition inhibition activity of female adult TSSM with modifications. Bean leaves were cut into 3 cm diameter discs and their backs carefully wiped with the extract concentrations and controls using swabs then left to dry under room temperature. Twenty female adult TSSM were introduced into petri dishes containing wet cotton wool treated with five rates of each plant extracts and arranged in CRD with four replications. The numbers of eggs in extract treated and control leaves were counted 24, 48 and 72 HAT and oviposition inhibition rate calculated using Zhang *et al.* (2013) formula. All data were subjected to multi-factorial ANOVA and mean separations done using Tukey's HSD ($P > 0.05$) test. Data were analysed using SAS (2011) statistical program.

Research application

Preliminary results showed concentration- and exposure time-dependent increase in efficacy of LN and OG extracts against the mite. At 12% w/v and 72 HAT, LN and OG extracts produced 93.8 and 96.4% mortality of adult TSSM, respectively. Similarly, both plant extracts produced 100% reduction in the number of eggs laid by female adult TSSM. The increased toxicity with concentration is in agreement with the findings of Erdogan *et al.* (2012). Differential efficacy of LN and OG extracts could be due to the varied quantity and quality of bioactive compounds responsible for toxicity in insects. The ability of these extracts to kill the mites and reduce egg laying makes these botanicals effective acaricides for use in managing this pest by resource poor smallholder farmers.

On-going studies include repellent studies of the plant extracts against TSSM on French beans under laboratory conditions. A field based experiment and a probit analysis to determine the dose response curves are on schedule. The on-going studies are expected to finish by end of July, 2014.

Expected outputs from this research are (i) Improved management of spider mites on French beans, (ii) Safe botanical products developed, (iii) A thesis leading to an award of Master of Science in Horticulture degree and (iv) Results of this research will be published in quality peer-reviewed scientific journals i.e. at least two research papers. The results will also be

Table 1. Mortality (mean±SE) of TSSM adults on *L. nepetifolia* extracts treated French bean leaf discs 72 HAT.

Treatment	Percent mortality (Mean±SE; n=24)		
	24 h	48 h	72 h
Methanol (control) 0.6 ml/L	20.67±10.78 ^d	23.42±10.20 ^d	27.75±26.05 ^d
Abamectin (control)	40.62±39.89 ^c	41.16±26.80 ^c	43.45±33.97 ^c
1.5 % w/v LN	43.83±21.94 ^{bc}	60.77±32.68 ^{bc}	72.36±23.90 ^{bc}
3.0 % w/v LN	61.66±13.27 ^b	47.40±22.56 ^b	72.36±14.75 ^b
6.0 % w/v LN	54.53±27.42 ^b	61.66±33.04 ^b	71.47±22.50 ^b
12.0 % w/v LN	68.80±27.55 ^a	93.76±15.44 ^a	93.76±10.66 ^a

Means within columns followed by the same letters are not significant ($\pm=0.05$) using Tukey's HSD test; LN- *Leonotis nepetifolia*; w/v- weight by volume.

Table 2. Mortality (mean±SE) of TSSM adults on *O. gratissimum* extracts treated French bean leaf discs 72 HAT.

Treatment	Percent mortality (Mean±SE; n=24)		
	24 h	48 h	72 h
Methanol (control)	22.42±20.23 ^d	30.17±25.46 ^d	38.25±20.26 ^d
0.6 ml/L Abamectin (control)	25.11±19.88 ^d	28.86±27.87 ^d	45.33±18.15 ^d
1.5 % w/v OG	22.44±15.89 ^c	51.86±26.80 ^c	63.45±24.34 ^c
3.0 % w/v OG	34.03±15.69 ^c	53.64±25.13 ^c	63.45±30.42 ^c
6.0 % w/v OG	61.66±33.04 ^b	81.28±18.32 ^b	86.63±16.53 ^b
12.0 % w/v OG	83.06±20.63 ^a	94.65±7.21 ^a	96.43±9.50 ^a

Means within columns followed by the same letters are not significant ($\pm=0.05$) using Tukey's HSD test; OG- *Ocimum gratissimum*; w/v- weight by volume.

Table 3. Oviposition inhibition (mean±SE) of TSSM adults on *L. nepetifolia* extracts treated French bean leaf discs 72 HAT.

Treatment	Percent inhibition (Mean±SE; n=24)		
	24 h	48 h	72 h
Methanol (control)	-34.72±37.07 ^d	-38.89±33.77 ^d	-12.50±42.42 ^d
0.6 ml/L Abamectin (control)	28.47±14.42 ^c	34.03±20.86 ^c	34.44±22.84 ^c
1.5 % w/v LN	24.31±12.03 ^{bc}	21.53±13.04 ^{bc}	37.50±19.30 ^{bc}
3.0 % w/v LN	31.94±15.01 ^b	33.33±21.90 ^b	47.22±28.94 ^b
6.0 % w/v LN	52.08±32.20 ^b	53.47±33.42 ^b	77.78±21.12 ^b
12.0 % w/v LN	87.50±12.05 ^a	94.44±8.94 ^a	100.00±0.00 ^a

Means within columns followed by the same letters are not significant ($\pm=0.05$) using Tukey's HSD test; LN- *Leonotis nepetifolia*; w/v- weight by volume.

Table 4. Oviposition inhibition (mean±SE) of TSSM adults on *O. gratissimum* extracts treated French bean leaf discs 72 HAT.

Treatment	Percent inhibition (Mean±SE; n=24)		
	24 h	48 h	72 h
Methanol (control)	9.03±29.18 ^d	13.19±33.42 ^d	38.19±32.27 ^d
0.6 ml/L Abamectin (control)	57.64±20.86 ^d	61.11±21.42 ^d	62.92±20.45 ^d
1.5 % w/v OG	38.89±22.29 ^c	53.47±17.57 ^c	65.97±22.32 ^c
3.0 % w/v OG	59.72±26.07 ^c	56.25±26.38 ^c	68.75±22.23 ^c
6.0 % w/v OG	84.03±12.03 ^b	90.28±6.96 ^b	95.83±5.62 ^b
12.0 % w/v OG	93.06±6.96 ^a	97.22±4.10 ^a	100.00±0.00 ^a

Means within columns followed by the same letters are not significant ($\pm = 0.05$) using Tukey's HSD test; OG- *Ocimum gratissimum*; w/v- weight by volume.

disseminated to target consumers of technology through national, regional and international conferences/seminars/workshops.

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