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BIOACTIVITY OF *LEONOTIS NEPETIFOLIA* AND *OCIMUM GRATISSIMUM* EXTRACTS IN MANAGEMENT OF *TETRANYCHUS URTICAE* KOCH ON FRENCH BEANS

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ABSTRACT
Methanol extracts of Lion's ear (*Leonotis nepetifolia* L.) (LN) and African basil (*Ocimum gratissimum* L.) (OG) were evaluated in the laboratory at Egerton University for lethal concentrations (LC_{50}), toxic, oviposition inhibition and repellent effects against the adult female two-spotted spider mite, *Tetranychus urticae* Koch. Laboratory bioassays were conducted in a completely randomized design replicated four times using five concentrations (0.0, 1.5, 3.0, 6.0 and 12.0 % w/v) with methanol and Abamectin (0.6 ml/L) as negative and positive controls, respectively. Data on mite mortality, oviposition inhibition and repellence indices were recorded 24, 48 and 72 h after treatment and subjected to analysis of variance and means separated using Tukey's HSD test ($P< 0.05$). The results showed concentration- and exposure time-dependent increase in efficacy of both extracts against the mites. At 12.0% and 72 h, LN and OG produced 93.8 and 96.4% mortality of mites, respectively. Similarly, both extracts produced 100% oviposition inhibition. The extracts also significantly repelled the mites over the exposure periods. Synthetic acaricide, abamectin produced 43.5 and 34.4 % mortality and oviposition inhibition, respectively, compared to the extracts. According to LC_{50} values, LN had the highest percent mortality whereas OG showed highest inhibition and repellence at 24, 48, and 72h after treatment.These findings demonstrate the potential use of LN and OG extracts for the management of two-spotted spider mite on French beans.

KEY WORDS: Toxicity, Oviposition, mortality, French beans, Repellence.

INTRODUCTION

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is one of the major pests that have contributed to the limitation in French beans production[1]. It is not only a major pest causing reduction in French bean yields, but has also been listed as a quarantine pest and hence a leading cause of Kenya's fresh produce rejections in European markets[2]. It attacks over 300 host plant species including vegetables, fruits and ornamental plants[3]. The direct damage includes defoliation, leaf burning and even plant death. Indirect effects of feeding by this spider mite include reduced photosynthesis, transpiration which can lead to yellow to white discoloration of leaves(bronzing) causing loss of yield and quality of host plants[4,5]. Most smallholder farmers rely on synthetic pesticides (acaricides) to manage this pest. However, the available synthetic acaricides used have caused serious problems such as pesticide resistance[5], environmental contamination, unacceptable pesticide residues in food and lethal effects on non-target organisms[6]. Chemical pesticides remain suspended in the atmosphere, on the ground, in waterways and affect humans and beneficial organisms such as predatory mites[7]. These negative effects have resulted in an increasing interest for natural plant-based pesticides which are safer, easily available and easily biodegradable and with no resistance than synthetic chemicals[8]. Studies have also pointed to numerous plant species possessing potential pest controlling properties. These plant species have been found to contain natural deterrents to help them remain healthy. These deterrents are toxic to various insect and mite pests[9]. The extracts of *Satoreja hortensis* L. (Lamiaceae) have been reported to be toxic to TSSM [10]. Similarly the extracts from neem (Meliaceae), some species of solanaceae, *Capparis aegyptia* (Capparaceae), *Nerium orleander* L. (Apocynaceae) and *Alianthus altissima* L. (Simaroubaceae) have been found to be effective against TSSM[11,15]. The antifungal, antibacterial and antioxidant activities of *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. have also been confirmed in human and animal health[16]. However, limited information exists for usage as crop protectants, hence the objectives of the study was to determine their toxic, oviposition inhibition and repellent effects against adult female *Tetranychus urticae* Koch under laboratory conditions and possibility of using the extracts to manage the pest in the field.

MATERIALS & METHODS

Preparation of test plant materials

The studies were conducted at Biotechnology laboratory, Egerton University. Test plants, Lion's ear (*Leonotis nepetifolia* L.) and African basil (*Ocimum gratissimum* L.) were collected from the wild in Bahati district, Kenya in 2013. Composite of fresh leaves and tender stems were

shade dried in well ventilated room at 18-28 ºC for two weeks and further oven dried at 35 ºC for 48 h[17]. Dried leaves were ground into fine powder using an electric laboratory hammer mill, and subjected to methanol (100 % AR) extraction at a rate of 200gL^{-1}. The extracts were kept in air tight containers refrigerated at 4ºC for use in the bioassays.

Mass rearing of two-spotted spider mite

The local strain of *T. urticae* Koch was obtained from infested leaves of French beans which had not been sprayed with any acaricide. Rearing was done on 2-3 week old beans which were maintained in the greenhouse (25±1ºC and RH 65±5%) at Horticulture research and experimentation farm of Egerton University. Individual mites were then collected and transferred for bioassay studies using a fine hair brush. Laboratory bioassays were as follows:

Contact toxicity

Leaf dipping method was employed with modifications [18]. Bean leaf discs (3 cm diameter) were cut and dipped into prepared five extract concentrations (0.0, 1.5, 3.0, 6.0 and 12.0% w/v) for 30 seconds and allowed to dry under room temperature. Methanol and abamectin were used as negative and positive controls, respectively. Twenty (N_T) adult female mites were introduced onto each leaf disc placed in petri dishes with wet cotton wool. The test was arranged in completely randomised design (CRD) with four replicates. The numbers of dead (N_D) mites were recorded at 24, 48 and 72 hours (h) intervals. Percent mortality was corrected using Abbott's formula [19].

Oviposition inhibition test

The leaf disc method with modifications was used to determine the oviposition inhibition activity of female adult TSSM[20]. 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 then counted 24, 48 and 72 h and oviposition inhibition rate calculated using [20] formula.

Repellence test

Repellence bioassay experiment was carried out according to[21] with modifications. French bean leaf discs (4 cm diameter) were used by immersing half of the disc into the treatments for 30 seconds and dried at room temperature for 30 minutes then the other half immersed in the control groups. Each disc was immersed in such a manner that permitted a free area of 0.5 cm between the two halves where 20 adult TSSM were initially released. The leaf discs were placed on wet cotton wool in petri dishes arranged in CRD with 4 replications. The mites were then left to move freely and counting of mites present on each half disc done 24, 48 and 72 h after treatment. Percent repellence was computed according to the formula of [22]. The LC_{50} value of LM, and OG leaf extracts was predicted by Probit analysis for adult TSSM at 24, 48 and 72 hours post-treatment.

Statistical analysis

The data were subjected to analysis of variance using SAS [23] statistical program and the means separated using Tukey's HSD test whenever ANOVA showed significant difference.

RESULTS & DISCUSSION

For the past decades the acaricidal and insecticidal properties of the plant extracts have been widely tested against phytophagous pests such as spider mites. The laboratory results in this study showed concentration- and exposure time-dependent increase in efficacy of *Leonotis nepetifolia* (LN) and *Ocimum gratissimum* (OG) extracts against the adult female two-spotted spider mite (TSSM). At 12% w/v and 72 h, LN and OG extracts produced 93.8 and 96.4% mortality of adult TSSM, respectively. According to LC_{50} values, toxicity of pesticides to adult TSSM indicated that LN had highest mortality (0.48%) compared to OG (1.15%) after 72 hours (Table 1).

TABLE 1: Mortality of TSSM adults at different concentration of *L. nepetifolia* and *O. gratissimum* extracts with time on treated French bean leaf discs

	Percent Mortality					
	24 h		48 h		72 h	
Rate (% w/v)	LN	OG	LN	OG	LN	OG
0.0	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
1.5	43.8 ± 3.9^{bc}	22.4 ± 1.9^{c}	60.8 ± 6.7^{bc}	51.86±2.80^{c}	72.4 ± 6.4^{bc}	63.45±2.34^{c}
3.0	61.7 ± 4.3^{b}	34.0 ± 3.79^{c}	47.4 ± 3.7^{b}	53.64±2.13^{c}	72.4 ± 5.8^{b}	63.45±3.42^{c}
6.0	54.5 ± 4.4^{b}	61.7 ± 3.0^{b}	61.7 ± 3.0^{b}	81.28±1.32^{b}	71.5 ± 5.5^{b}	86.63±1.53^{b}
12.0	68.8 ± 3.6^{a}	83.1 ± 2.6^{a}	93.8 ± 3.4^{a}	94.65 ± 7.2^{a}	93.8 ± 2.7^{a}	96.43±9.50^{a}
Methanol	3.3 ± 1.2^{d}	3.3 ± 1.2	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}
Abamectin (0.6 ml L^{-1})	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}
LC_{50}	1.77	4.19	1.18	1.80	0.48	1.15

Means within columns followed by the same letters are not significant different ($P \leq 0.05$) using Tukey's HSD test.

Additionally at 72 h of exposure the level of mortality by the extract concentrations was more than 60 % higher than the synthetic acaricide. Similarly, both extracts produced 100% reduction in the number of eggs laid by adult female TSSM (Table 2). This could be attributed to the accumulating toxic compounds in the plant extracts coupled with longer period of exposure of the mites to lethal dose as they move and feed on the treated leaf discs [24]. The increased toxicity with concentration is also in agreement with the findings of the previous studies[18].

From the simple logistic regression analysis the two plant leaf extracts significantly increased the death rate in the spider mite population compared to controls. When the TSSM were exposed to the leaf extracts, dose dependent mortalities were greater than 90 % at 72 h.

TABLE 2: Oviposition inhibition of TSSM adults at different concentration of *L. nepetifolia*and *O. gratissimum* extracts with time on treated French bean leaf discs

Percent Inhibition						
	24 h		48 h		72 h	
Rate (% w/v)	LN	OG	LN	OG	LN	OG
0.0	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
1.5	24.31±1.0^{bc}	38.89±2.29^{c}	21.53±1.04^{bc}	53.47±1.57^{c}	72.4 ±6.4^{bc}	65.97±2.32^{c}
3.0	31.94±1.8^{b}	59.72±2.07^{c}	33.33±1.90^{b}	56.25±2.38^{c}	72.4 ± 5.8^{b}	68.75±2.23^{c}
6.0	52.08±3.2^{b}	84.03±1.03^{b}	53.47±3.42^{b}	90.28±6.96^{b}	71.5 ± 5.5^{b}	95.83±5.62^{b}
12.0	87.5± 2.1^{a}	93.1 ± 7.0^{a}	94.4± 8.9^{a}	97.2± 4.1^{a}	93.8 ± 2.7^{a}	100.0±0.0^{a}
Methanol	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}
Abamectin (0.6 ml L^{-1})	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}
LC_{50}	4.35	2.11	4.11	1.71	2.50	1.20

Means within columns followed by the same letters are not significant different ($P \leq 0.05$) using Tukey's HSD test.

The repellency test results showed that both LN and OG leaf extracts had significant effect on the activity of the TSSM (Table 3). The results showed spider mites moving towards either extract exposed side or non-treated side. Spider movement towards the non-treated side of the bean leaves and away from the extract exposed side could partly be due to toxic compounds found in the extracts [7].

TABLE 3: Repellency of TSSM adults at different concentration of *L. nepetifolia* and *O. gratissimum* extracts with time on treated French bean leaf discs.

Percent Repellence						
	24 h		48 h		72 h	
Rate (% w/v)	LN	OG	LN	OG	LN	OG
0.0	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
1.5	24.44±3.57^{bc}	31.1±3.71^{c}	36.67±3.82^{bc}	38.2±1.3^{c}	72.4 ±6.4^{bc}	42.9±3.8^{c}
3.0	32.78±4.13^{b}	39.5±2.1^{c}	47.78±3.28^{b}	46.3±3.4^{c}	72.4 ± 5.8^{b}	58.8±3.5^{c}
6.0	38.08±4.31^{b}	64.0±4.0^{b}	50.47±3.86^{b}	70.3±7.1^{b}	71.5 ± 5.5^{b}	75.8±5.6^{b}
12.0	70.5 ± 2.1^{a}	83.1±4.4^{a}	84.4 ± 5.9^{a}	97.2 ± 4.1^{a}	93.8 ± 2.7^{a}	100.0±0.0^{a}
Methanol	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}
Abamectin (0.6 ml L^{-1})	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}
LC_{50}	6.30	3.52	3.29	2.66	2.06	2.11

Means within columns followed by the same letters are not significant different ($P \leq 0.05$) using Tukey's HSD test.

Differential efficacy of LN and OG extracts can partly be attributed to the varied quantity and quality of chemical contents responsible for toxicity in insect and mite pests. The present study showed that the LC_{50} of OG had the highest inhibition both at 48 and 72 hours after treatment, this could have been due to the toxic effects on the female mites. Furthermore, the ovicidal activity of methanolic extracts of some plants were also evaluated by[25] and concluded that the leaf extracts had toxic effects on the female mites, while[9] also found that *Rosemarinus officinalis* extracts had a moderately toxic effect on the adult females of *T. urticae*. Thus the ability of these extracts to kill and repel the mites and reduce egg laying makes these botanicals to have a potential for the development of new preventative agents for the control of *T. urticae* Koch in smallholder French bean fields.

CONCLUSION

The efficacy of OG and LN extracts generally increased with the concentration and exposure time of treatments. At 72 h after treatment a 12 % extract was very effective against the adult female two-spotted spider mite as an acaricide, repellent and anti-oviposition agent compared with the synthetic acaricide, abamectin recommended for use against the spider mites. Lower concentrations of OG extracts (1.5% & 3.0% w/v) recorded insignificant differences across the 3 exposure times compared to LN extracts in case of mortality of TSSM. Although lower concentrations of both extracts had considerable acaricidal activity, repellent activity and anti-oviposition activity at different exposure times. The ability of these extracts to cause adult female TSSM mortality and also inhibit egg laying makes these botanicals effective acaricides for use in the control of TSSM by smallholder French bean farmers who are unable to meet the cost of buying expensive synthetic chemicals. These results are very promising and further evaluation of the bioactive components responsible for toxic, oviposition inhibition and repellent effects against this mite species should be conducted under field conditions.

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