

The Effect of Lion's Ear (*Leonotis nepetifolia*) and African Basil (*Ocimum gratissimum*) Plant Extracts on Two-Spotted Spider Mites (*Tetranychus urticae*) for Improved Yield and Quality of French Beans

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Abstract

An experiment to evaluate the bio-control potential of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts against two-spotted spider mites on French beans was conducted in the field. Five plant extract concentrations (1.5%, 3.0%, 6.0% and 12.0% w/v) were applied with water and Abamectin 0.6 ml/L as controls. Mite counts were done before and after treatment application and expressed as corrected percent efficacy. The impact of the mites on the French beans was evaluated by recording percent leaf reduction and quality and quantity by number of pods, pod length, diameter and yield. There was a dose dependent response in percent mite and leaf reduction, number of pods and yield. Treatments applied at 12% w/v indicated higher mite reduction (82.75%) for *L. nepetifolia* and 69.06% for *O. gratissimum* compared to abamectin (65.76%). The lowest percent leaf reduction of 1.71% for *L. nepetifolia* 0.39% for *O. gratissimum* and abamectin (20.46%) was also at 12% w/v. Similarly, the highest number of pod (61.00) for *L. nepetifolia*, 48.67 for *O. gratissimum* compared to 28.33 abamectin and yield (0.88 kg) for *L. nepetifolia* and 0.90 kg for *O. gratissimum* was also recorded at 12% w/v compared to 0.36 kg for abamectin. There were no significant differences in pod diameter and pod length between the extracts concentrations and abamectin. The study demonstrated the efficacy of *L. nepetifolia* and *O. gratissimum* in managing two-spotted spider mite and subsequent increase in French bean yield under field conditions.

Keywords

Leonotis nepetifolia, *Ocimum gratissimum*, *Tetranychus urticae*, French

1. Introduction

French bean (*Phaseolus vulgaris* L.) is among the most important horticultural crops grown and consumed worldwide. It is grown for its tender pods and shelled green or dry beans. The crop is a rich source of important nutritional elements such as flavonoids, vitamin A, dietary fibres, potassium, folate, iron, magnesium, thiamine, riboflavin, copper, calcium, phosphorus, Omega-3 fatty acids and niacin [1]. French bean production in Kenya is mainly undertaken by smallholder farmers who constitute approximately 80% of all growers. Production of this crop is however often undermined by major pests like spider mites, bean fly, white flies and aphids [2].

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari; Tetranychidae), is one of the most important pests that attack French beans and other crops worldwide [3]. The pest feeds on the plants through piercing and sucking cell contents. The resulting symptoms include tiny yellow or white speckles and bronzing of leaves [4] [5]. Severe mite infestations cause premature defoliation leading to reduced sugar content and drastic reduction in the crop yield [6] [7] and quality due to increased risk of pod damage from ultraviolet rays. The rapid developmental rate, short generation time and high net reproductive rate of *T. urticae* allow them to achieve damaging population levels very quickly when growth conditions are good, resulting in an equally rapid decline of host plant quality [8].

Most of the smallholder farmers heavily rely on synthetic pesticides to manage the two spotted spider mite pest on French beans. Chemical pesticides used for spider mite suppression are usually weak acaricides and often do not perform well. Some of the active ingredients that have been used by smallholder farmers with little success include abamectin, spiromesifen, dicofol and chlorfenapyr [9] [10]. Synthetic acaricides have also caused serious problems such as pesticide resistance, environmental contamination, unacceptable pesticide residues in food and lethal effects on non-target organisms [11] [12]. Results from a study by [13] demonstrated variable levels of abamectin resistance in *T. urticae* populations. These negative effects have resulted in the increasing interest for natural plant-based pesticides which might be safer, biodegradable and have shown low pest resistance [14].

Several studies have evaluated the potential of natural plant extracts to protect crops from insect and mite pest species such as whiteflies and spider mites [15]. Plant species have been found to contain natural deterrents which are toxic to various insect and mite pests [16] but safe to mammals. The extracts of *Satureja hortensis* L. (Lamiaceae) were found to be toxic to TSSM [17]. Similarly the extracts from neem (Meliaceae), some species of solanaceae, *Capparis aegyptia* (Capparaceae), *Nerium oleander* L. (Apocynaceae) and *Alianthus altissima* L.

(Simaroubaceae) have also been found to be effective against TSSM [18] [19]. Antifungal, antibacterial and antioxidant activities of *L. nepetifolia* and *O. gratissimum* have also been confirmed [20] [21].

Further studies by [22] have demonstrated insecticidal, antifungal and antibacterial activity of *L. nepetifolia* and *O. gratissimum* extracts. Phytochemical examination of these plants indicated the presence of different diterpenoids and other bioactive compounds [23] [24]. Water extracts of *O. gratissimum* have been reported to inhibit egg hatch and resulted in juvenile mortality of root-knot nematode (*Meloidogyne incognita*) while improving grain yields of cowpea [25]. [26] reported highest mortality and repellence of *Callosobruchus maculatus* treated with ethanolic extracts from *O. gratissimum* leaves under laboratory conditions. They attributed the insecticidal activity to the presence of secondary metabolites such as saponins, flavonoids, alkaloids, phenolics and terpenes. Similar results with ethanolic extracts of *O. gratissimum* leaves against bean weevil have been documented [27] [28].

This study therefore sought to determine the miticidal activity of *L. nepetifolia* and *O. gratissimum* plants extracts against *T. urticae* and their subsequent influence on the quality and yield of French bean under field conditions.

2. Materials and Methods

2.1. Study Site

Field experiments to evaluate the miticidal activity of *L. nepetifolia* and *O. gratissimum* methanolic crude plant extracts on the two spotted spider mite (*T. urticae*) were conducted at Horticulture Research and Teaching Farm of Egerton University situated in Nakuru county Kenya during the 2014 and 2015 growing seasons. The farm lies at a latitude of 0°30'S, longitude 36°30'E and an altitude of 2238m. The experimental site receives an annual rainfall of 1013 mm and the dominant soil type is mollic andosols.

2.2. Preparation of Plant Extracts

Composite fresh leaves and tender stems of *L. nepetifolia* L. *O. gratissimum* were collected from fallow fields at Egerton University and the surrounding. The plant materials were dried in well ventilated room at 18°C - 28°C for two weeks. The dried leaves were ground into fine powder using an electric laboratory hammer mill, and subjected to methanol (100% AR) extraction at a rate of 200 gL⁻¹. The extracts were kept in air tight containers refrigerated at 4°C for use in the bioassays.

2.3. Mass Rearing of Two-Spotted Spider Mites

The two spotted mites were obtained from infested leaves of French bean plants which had not been sprayed with any acaricide. Rearing was done on 2 - 3 week old beans which were maintained in the greenhouse (25°C ± 1°C) and RH 65 ± 5 for use in the bioassays

2.4. Field Bioassay

The plant extracts were evaluated at different concentrations (1.5%, 3%, 6%, 12% w/v) in a Randomised Complete Block Design (RCBD) replicated three times. A synthetic acaricide (Abamectin 0.6 ml/L DW) was used as positive control and water as negative control. Experimental plots measuring 2 m × 2 m with a spacing of 0.5 m between and 0.2 within the rows were planted with certified French bean seeds (variety Teresa) according to recommendations for commercial French bean production. To avoid natural infestation, all plants in each plot were covered with nylon mesh size 0.4 by 0.5 mm and thread thickness of 0.1 mm at the primary leaf stage.

Twenty adult spider mites from greenhouse cultures were randomly introduced onto each bean plant at 21 days after planting using a fine hair brush. The plant extracts were applied as spray solutions using a hand held sprayer 14 days after mite infestation. A repeat treatment application was done after another 14 days. Six plants from the two middle rows in each plot were randomly selected and tagged for data collection.

2.5. TSSM Population Reduction

The population of the mites was assessed three days before treatment application by counting the number of adult mites from the underside of leaves from the six tagged plants in each plot. A second mite population count was done at 72 h after the second treatment application. The corrected percent efficacy of the plant extracts was then calculated according to Sun-shepard formula [29]

$$\% \text{ corrected efficacy} = \frac{\% \text{ change in treated} - \% \text{ change in control}}{100 - \% \text{ change in control}} \quad (1)$$

2.6. Percent Leaf Reduction

This was done by counting the number of leaves on the six tagged plants in each plot before treatment and after the second treatment application. The change in number of leaves constituted the damage by TSSM which was calculated as follows

$$\% \text{ Leaf reduction} = \frac{\% \text{ change in treated} - \% \text{ change in control}}{100 - \text{change in control}} \quad (2)$$

2.7. Number of Pods, Pod Length, Diameter and Yield

This was done by hand plucking all immature green pods from the two middle rows. The number of pods was counted before measuring their pod length and diameter using a ruler and a veneer calliper respectively. The pods were then weighed when still fresh using an electric weighing balance.

2.8. Data Analysis

All data collected was subjected to analysis of variance (ANOVA) at $P \leq 0.05$ using SAS statistical package [30].

3. Results

3.1. Two Spotted Spider Mite Population Reduction

Results of the field bioassays indicated a dose dependent percent mite population reduction expressed as corrected percent efficacy during the two seasons (Table 1). The highest efficacy of 82.75% for *L. nepetifolia* and 69.06% for *O. gratissimum* plant extracts occurred at 12% w/v concentration in 2014 and 78.0% for *L. nepetifolia* and 77.56% for *O. gratissimum* in 2015. The synthetic acaricide (Abamectin 0.6 ml/L) showed a corrected percent efficacy of 65.76% and 69.05% in 2014 and 2015 respectively. Abamectin however showed higher percentage efficacy when compared with the lower plant extract concentrations of 0.0%, 1.5%, 3.0% and 6.0% w/v during both growing seasons.

3.2. Percent Leaf Reduction

The plant extracts concentrations 12% w/v, 6% w/v and 3% w/v showed significantly lower percent leaf reduction 1.71, 4.88% and 5.92% respectively for *L. nepetifolia* and 0.39%, 3.5% and 19.86% for *O. gratissimum* compared to abamectin (20.46%) in 2014 (Table 2). The trend was the same in 2015 with *L. nepetifolia* showing 0.58%, 5.09% and 21.72% leaf reduction at concentration levels of 12% w/v, 6% w/v and 3% w/v respectively and *O. gratissimum* showed 12.49%, 13.86% and 33.58%. Abamectin on the other hand showed a percent leaf reduction of 23.94% in 2015.

3.3. Number of Pods, Pod Diameter, Pod Length and Pod Yield

A higher number of pods was recorded in the plots that were treated with plant extracts compared to the synthetic acaricide (abamectin). *L. nepetifolia* plant extract recorded 61.00, 48.33 pods at concentrations levels of 12% and 6% w/v followed by *O. gratissimum* plant extract which recorded 48.67 and 35.00 pods while abamectin recorded 28.33 pods during 2014 growing season (Table 3). The trend was similar in 2015 where *L. nepetifolia* plant extract recorded 41.00

Table 1. Mite population reduction (corrected % efficacy) at different plant extracts concentrations in season 1 and 2.

Rate (%w/v)	Corrected percent efficacy			
	Season 1		Season 2	
	<i>L. nepetifolia</i>	<i>O. gratissimum</i>	<i>L. nepetifolia</i>	<i>O. gratissimum</i>
Control	-14.16 ± 1.35*	-1.42 ± 2.37*	-20.00 ± 2.35*	-2.34 ± 1.47*
1.5	30.98 ± 0.00	40.50 ± 8.92	31.63 ± 9.12	44.17 ± 6.13
3.0	62.22 ± 7.90	45.19 ± 2.00	45.72 ± 7.30	46.34 ± 1.45
6.0	53.82 ± 5.77	57.57 ± 5.75	69.61 ± 6.06	57.85 ± 7.51
12.0	82.75 ± 6.13	69.06 ± 4.03	78.63 ± 10.38	77.56 ± 1.85
Abamectin (0.6 ml/L)	65.76 ± 3.47	65.76 ± 3.47	69.05 ± 9.30	69.05 ± 9.29

*Means in a column whose standard error values do not overlap are significantly different at $P \leq 0.05$.

Table 2. Percent French bean leaf reduction at different plant extracts concentrations in season 1 and season 2.

Rate (%w/v)	Percent Leaf Reduction			
	Season 1		Season 2	
	<i>L. nepetifolia</i>	<i>O. gratissimum</i>	<i>L. nepetifolia</i>	<i>O. gratissimum</i>
Control	48.49 ± 8.88*	48.49 ± 8.88*	53.63 ± 5.47*	53.63 ± 5.47*
1.5	14.69 ± 1.94	37.39 ± 9.49	30.31 ± 6.79	22.69 ± 5.27
3.0	5.92 ± 3.71	19.86 ± 5.51	21.72 ± 2.23	33.58 ± 2.58
6.0	4.88 ± 6.36	3.50 ± 4.89	5.09 ± 1.11	13.86 ± 7.77
12.0	1.71 ± 2.16	0.39 ± 0.67	0.58 ± 0.19	12.49 ± 1.02
Abamectin (0.6 ml/L)	20.46 ± 8.09	20.46 ± 8.09	23.94 ± 7.56	23.94 ± 7.56

*Means in a column whose standard error values do not overlap are significantly different at $P \leq 0.05$.

Table 3. Effects of *L. nepetifolia* (LN) and *O. gratissimum* (OG) plant extracts on number of pods, pod diameter (cm) pod length (cm) and pod yield (kg).

Treatment (% w/v)	Number of pods		Pod diameter		Pod length		Pod yield	
	LN	OG	LN	OG	LN	OG	LN	OG
SEASON 1								
Control	12.67 ± 1.15*	12.67 ± 1.15*	0.50 ± 0.10*	0.50 ± 0.10*	7.83 ± 1.86*	7.83 ± 1.86*	0.16 ± 0.04*	0.16 ± 0.04*
1.5	17.33 ± 4.16	19.33 ± 1.53	0.63 ± 0.11	0.66 ± 0.10	10.83 ± 1.0	11.03 ± 0.42	0.39 ± 0.06	0.37 ± 0.03
3.0	37.00 ± 6.08	22.33 ± 1.15	0.67 ± 0.06	0.67 ± 0.31	11.57 ± 0.0	11.07 ± 1.83	0.52 ± 0.03	0.48 ± 0.02
6.0	48.33 ± 4.66	35.00 ± 3.23	0.73 ± 0.06	0.67 ± 0.12	11.57 ± 0.7	12.17 ± 0.42	0.65 ± 0.02	0.53 ± 0.05
12.0	61.00 ± 8.19	48.67 ± 5.51	0.80 ± 0.10	0.77 ± 0.06	11.50 ± 0.2	12.60 ± 0.17	0.88 ± 0.02	0.90 ± 0.03
Abamectin	28.33 ± 2.58	28.33 ± 2.58	0.70 ± 0.17	0.70 ± 0.17	9.27 ± 1.44	9.27 ± 1.44	0.36 ± 0.02	0.36 ± 0.02
SEASON 2								
Control	11.33 ± 3.79*	11.33 ± 3.79*	0.50 ± 0.10*	0.53 ± 0.06*	11.33 ± 1.3*	0.16 ± 0.04*	0.13 ± 0.11*	0.13 ± 0.11*
1.5	17.00 ± 1.00	12.33 ± 4.73	0.57 ± 0.06	0.60 ± 0.10	13.70 ± 1.5	0.37 ± 0.03	0.16 ± 0.12	0.37 ± 0.20
3.0	22.33 ± 2.08	19.33 ± 4.16	0.60 ± 0.10	0.60 ± 0.00	13.63 ± 1.7	12.10 ± 1.23	0.35 ± 0.18	0.19 ± 0.02
6.0	27.67 ± 1.53	31.00 ± 1.00	0.60 ± 0.00	0.60 ± 0.00	14.97 ± 1.0	15.00 ± 0.53	0.35 ± 0.05	0.37 ± 0.13
12.0	41.00 ± 3.11	40.67 ± 2.52	0.63 ± 0.06	0.67 ± 0.06	14.23 ± 1.6	14.77 ± 1.42	0.76 ± 0.06	0.86 ± 0.20
Abamectin (0.6 ml/L)	18.00 ± 5.20	18.00 ± 5.20	0.63 ± 0.06	0.63 ± 0.06	12.60 ± 0.2	12.60 ± 0.72	0.25 ± 0.08	0.25 ± 0.08

*Means in a column whose standard error values do not overlap are significantly different at $P \leq 0.05$.

and 27.67 pods at concentrations levels of 12% and 6% w/v followed by *O. gratissimum* which recorded 40.67 and 31.00 pods while abamectin recorded 18.00 pods.

Plant extracts applied at 12% concentration level also indicated the widest pod diameter of 0.80 cm and 0.77 cm for *L. nepetifolia* and *O. gratissimum* respectively in 2014. This was however not significantly different from abamectin which recorded a pod diameter of 0.70 cm. No significant differences in pod

diameter was observed between the plant extracts and abamectin in 2015.

Although *L. nepetifolia* plant extracts caused a significant increase in pod length compared to abamectin and the untreated control, there were no significant differences in pod length across the plant extracts applied either at low or a high concentrations in 2014 and 2015. *O. gratissimum* however recorded significantly longer pods at plant extract concentration levels of 6% and 12% w/v in 2014.

Results also showed significant differences in pod yields at different plant extract concentrations. The highest pod yield (0.88) was recorded at 12% w/v for *L. nepetifolia* and 0.90 for *O. gratissimum* in 2014 and 0.76 *L. nepetifolia* and 0.86 for *O. gratissimum* compared to 0.36 kg and 0.25 for abamectin in 2014 and 2015 respectively.

4. Discussion

The plant extracts from *L. nepetifolia* and *O. gratissimum* demonstrated biological potency against the two spotted spider mites. Leaves, stems and roots extract of *O. gratissimum* have been found to contain potent bioactive components (essential oils) made up of eugenol and other compounds such as diterpenes, coumarins, iridoids, saponins, condensed tannins, flavonoids, alkaloids and steroids that have antioxidant and insecticidal properties [31] [32]. Dried *O. gratissimum* leaves have also been reported to have insecticidal activity against cowpea bruchids (*Callosobruchus maculatus* F.) [33]. Phytochemical studies by [34] also identified and quantified fixed oil components from the leaves of *L. nepetifolia*. The current research did not however observe significant differences in the activity of *L. nepetifolia* and *O. gratissimum* on the two spotted spider mite population and subsequent pod yield. The results therefore suggesting that there may be similar compounds with similar bioactivities in the plant extracts.

The highest efficacy of the plant extracts in reducing TSSM populations was demonstrated at the highest plant extract concentrations of 12% w/v. This is consistent with studies by [35] who reported a concentration and exposure time-dependent increase in the efficacy of *L. nepetifolia* and *O. gratissimum* plant extracts against the TSSM under laboratory conditions. Similarly, this study observed the highest bean leaf reduction, pod numbers and pod yield in plots applied with the highest plant extract concentrations of 12%. Reduced mite infestations corresponded to lower mite damage and hence reduced percentage leaf reduction. Reduced leaf loss meant that more leaves were retained by the plant for photosynthesis and hence increased pod numbers and yield. A full canopy is required for French bean crop to fully intercept sunlight to produce sugars, fill pods and maximize yield [36] [37] [38]. The plant extracts did not however strongly influence the pod diameter and length which are important French bean quality parameters. All the treated plots recorded a pod length of about 9 - 12 cm representing size code number 4 according to the Asean standard for French beans [39].

This study has thus demonstrated the efficacy of *L. nepetifolia* and *O. gratissimum* in managing two-spotted spider mite and subsequent increase French bean yield under field conditions.

5. Conclusion

This study illustrates the potential biological potency of *L. nepetifolia* and *O. gratissimum* plant extracts against the two-spotted spider mite on French beans. Yields obtained from the fields where the plant extracts had been applied were comparable to or even better than yields from the fields where the Abamectin acaride had been applied. This research has however shown that although French bean pod yield increased with increase in plant extract concentrations, pod quality parameters (diameter and length) do not appear to significantly improve with increase in plant extract concentrations. The study also suggested that *L. nepetifolia* and *O. gratissimum* may contain similar compounds with similar bioactivities in their plant extracts.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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