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Research Article
Non-volatile Chemical Composition and Botanical Extracts from *Lippia javanica* (Burm. F) Spreng. In Control of Cowpea Aphids

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Abstract

Background and Objective: Pesticidal plants have become increasingly popular among small scale farmers in Kenya. Little is known about the efficacy of the non-volatile bioactive compounds of this species. There exist gaps such as the chemical compounds responsible for pest repellency and the right concentration of the botanical extracts. This study, therefore, determined the non-volatile bioactive compounds and evaluated the efficacy of three concentrations of *L. javanica* complex in control of aphids of *Vigna unguiculata*. **Materials and Methods:** Crushed powder of *L. javanica* leaves which were collected from different localities were used in the preparation of crude extracts, which were then screened for the presence of the bioactive compounds. Three prepared concentrations of 10, 5 and 1% were sprayed on cowpea plants planted on four blocks which had been replicated four times. Cowpea aphids were enumerated according to the categorical indices prior to and after spraying. Synthetic pesticide atom (Osho) and water plus soap were used as positive and negative controls, respectively. **Results:** *Lippia javanica* was found to be rich in a variety of non-volatile compounds namely: phenols, flavonoids, tannins, alkaloids, cardiac glycosides and terpenoids that perhaps exhibit pesticidal effects on cowpea aphids. However, phenolic glycosides, resins and polyuronides were absent. Application of the extracts irrespective of concentrations significantly suppressed aphid population at $p \leq 0.05$. It was notable that aphid suppression increased with increasing concentration. Consequently, the mixture of *L. javanica* extracts at 10% outperformed 1 and 5% extract concentration. In addition, the 10% extract concentration favorably competed with the synthetic insecticide (Atom, Osho Syngenta). **Conclusion:** The findings from this study showed that *Lippia javanica* possessed a wide range of phytochemical compounds; also, the 10% botanical extract was found to be comparable to the synthetic pesticide already in use for control of cowpea aphids.

Key words: Cowpea aphids, efficacy, *Lippia javanica*, pesticidal, phytochemical composition, *Vigna unguiculata*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lippia javanica is a multi-branched woody shrub of the tribe verbenae of the Verbenaceae family. It is a strongly fragrant medicinal indigenous plant that naturally occurs in eastern and southern tropical Africa[1]. Its essential oil composition have been investigated widely. Viljoen *et al.*[2] reported that myrcenone, myrcene and (E)-and (Z)-tagetenone as among the major constituents of the essential oils of *L. javanica*. Other components were found to be caryophyllene, linalool and p-cymene[2]. The essential compounds were found to vary sharply even with the samples taken from the same location. Geranial and neral chemotypes were found to occur in Tanzania[2]. However, most research on the chemical composition of *L. javanica* focuses on volatile essential oils. The non-volatile phytochemical compounds have been partially evaluated. Even though Maroyi[3] determined non-volatile secondary metabolites such as alkaloids, amino acids, flavonoids, iridoids and triterpenes, it is worth to note that a wide range of other non-volatile compounds were neglected. Previous studies on *L. javanica* showed it to be acaricidal used by smallholder farmers against cattle ticks[4]. Accordingly, it has been used as a cheap supplement in controlling cattle ticks in southern Africa. Other applications of the extracts include management of microbial infections like coughs or colds in humans. It also exhibited wound healing properties thus used to treat skin infections. According to Maroyi[3] the previously mentioned non-volatile compounds which were isolated from *L. javanica* and volatile oils are responsible for antimicrobial properties. Bio-active compounds are believed to act with synergic effect to deter microbes and pests. Pests, therefore, find it difficult to develop resistance to them, thus they should be further evaluated. For instance, the right concentration for the botanical extracts to be used as natural pesticides is unknown. Plants with pesticidal properties have been investigated for centuries as alternatives to synthetics. However, little progress has been made to develop new effective products[5]. Although research on pesticidal plants is increasing, it is failing to address gaps in our knowledge that constrain their adoption[6]. Studies concerning pesticidal efficacy on *L. javanica* have been partially addressed while the findings are unpublished. In the southern Africa region, *L. javanica* is known to demonstrate variation in efficacy. There was a need to investigate if any variation in terms of chemical compounds and efficacy of the different concentrations on aphids existed among *L. javanica* complex species. Extracts from this aforementioned species complex perhaps may play a significant role as pest repellants.

MATERIALS AND METHODS

Collection and processing of the *L. javanica* plant materials: Fresh aerial parts of *L. javanica* plant were collected in the floral areas of Kenya where it is known to occur in June/July 2016 growing season. The plant materials were stored in dark bags and transported to Maseno University botany laboratory. Immediately, the *L. javanica* materials were cleaned and air-dried under the shade and then crushed into a fine powder using Kika Werke M20 grinder.

Qualitative test for the phytochemical compounds: The extracts were screened for presence of non-volatile phytochemical constituents using standard methods as reported by Nalubenga *et al.*[7]. To test for the presence of alkaloids, Mayer's reagent was used while cardiac glycosides were detected by Keller-Killiani test. Whereas the foam test detected presence of saponins. Shibata's reaction was used to confirm presence of flavonoids. Tannins were detected using Braemer's test. Coumarins were tested by evaporating ether extract to dryness and dissolving the residue by heating in 2 mL of water and they were confirmed if 0.5 mL of 10% ammonia solution was added and a blue or green fluorescence under ultraviolet light developed. To detect for the presence of polyuronides, about 2 mL of aqueous extract were added dropwise to 10 mL of ethanol. Formation of a thick precipitate indicated presence of polyuronides. The formed precipitate was separated off and washed away with ethanol and stained with methylene blue and occurrence of violet color further denoted presence of polyuronides.

Study site for the field experiment: Pesticidal efficacy study was undertaken in Makueni County (Nzouni, village). The study was conducted from November 2015 to February, 2016 growing season on Mrs. Patricia Mukai's farm (S 02 00.877 E 37.862569, 920 m above sea level). The soil type was a mixture of red sandy and clay black cotton soils[8].

Plant materials collection and processing: Fresh leaves of *L. javanica* were collected from different localities around Kenya where the species occurs naturally. Voucher specimens and Geographical Positioning Systems (GPS) coordinates were lodged at the East Africa Herbarium Nairobi National Museum. Leaves were dried under shade for a week and then ground into fine powder using a grinder.

Field preparation and cowpea planting: The field was disc harrowed and ridged prior to planting. The common cowpea (*Vigna unguiculata*) seeds used for planting were of the variety Katumani KVU 27-1. They were obtained directly from the breeder at Kenya Agricultural and Livestock Research Organization (KALRO). The seeds were planted at a spacing of 50 cm between rows and 20 cm within rows in 3×4 m plots which were 1 m apart. Three seeds were planted per hole and then thinned to two plants one week after germination. The experimental layout was a randomized complete block design and the treatments were replicated on 4 blocks, all within the same field location.

Preparation of the three concentrations of the botanical extracts and field treatments: To determine effective pesticidal concentration three different botanical extracts 10, 5 and 1% w/v were prepared. As extraction was carried out in water, a second variable of 0.1% soap was added. The extracts were kept in sealed plastic buckets to extract at ambient temperature (20±5°C) overnight. Thus, there were 5 treatments 1, 5 and 10% plus positive and negative controls. Each was replicated four times, thus giving 20 blocks. Extracts were kept in 10 L buckets with tight lids in the shade and filtered through a fine cloth to remove all plant materials that may inadvertently clog the sprayer. The positive control in the trial was a synthetic pesticide Atom (Osho, Syngenta). The number of aphids before the treatments were counted and found to have an average of eight. All treatments were sprayed throughout the growing season at an interval of 7 days starting one week after cowpea plant emergence.

Sampling of aphid infestation: All assessments were carried out the day before treatments were to be sprayed following the modified method[6]. The target insect pests to be evaluated were cowpea aphids. Three inner rows from each plot were selected for sampling. Due to often very high numbers, a categorical index was used to assess aphid abundance according to the modified method[6]:

- 0 = None
- 1 = A few scattered individuals
- 2 = A few isolated colonies
- 3 = Several isolated colonies
- 4 = Large isolated colonies
- 5 = Large continuous colonies

The severity or degree of infestation in each infested plant was assessed by scoring the extent of damage using scoring grades[6]. After spraying with all the extracts, a similar procedure of sampling was conducted again the following day in the morning. The difference was taken to be the number of aphids suppressed where it was recorded:

- 0 = No damage
- 1 = Damage up to 25%
- 2 = Damage from 26-50%
- 3 = Damage from 51-75%
- 4 = Damage more than 75%

Data analysis: Differences among treatments in the number of aphids and severity of damage were assessed by one-way analysis of variance (ANOVA). Means were separated by Least Significant Difference (LSD) test at 5% probability level. Analyses were performed in STATA data analytical package.

RESULTS

Phytochemical composition of *L. javanica*: The study revealed that the extracts from the leaves of *L. javanica* had a variety of phytochemical groups (Table 1). Out of the 13

Table 1: Results for phytochemical screening of bioactive compounds in the *L. javanica* for 10 accessions of *L. javanica*

Samples/test	0019	0020	0025	0029	0035	0036	0037A	0037B	0038	0039	Observation
Alkaline reagent test	++	++	++	++	++	++	++	++	++	++	Pink scarlet color
Flavonoids	++	++	++	++	++	++	++	++	++	++	Yellow color
Flavones	+	+	+	+	+	+	+	+	+	+	Orange color
Terpenoids	++	++	++	++	++	++	++	++	++	++	Greyish color
Phenols	++	++	++	++	++	++	++	++	++	++	Blue color
Phenolic glycosides	-	-	-	-	-	-	-	-	-	-	Color persists
Cardiac glycosides	++	++	++	++	++	++	++	++	++	++	Yellow color
Saponins	++	++	++	++	++	++	++	++	++	++	Stable foam
Alkaloids	++	++	++	++	++	++	++	++	++	++	Turbidity formation
Resins	-	-	-	-	-	-	-	-	-	-	No purple color
Anthraquinones	+	+	+	+	+	+	+	+	+	+	Violet-pink color
Coumarins and anthocyanins	+	+	+	+	+	+	+	+	+	+	Pink in acidic medium
Tannins	+	+	+	+	+	+	+	+	+	+	Black color
Polyuronides	-	-	-	-	-	-	-	-	-	-	Color persists

++: Highly present, +: Fairly present and -: Absence of phytochemical. Sondu (0019; 0020), Kedong (0025; 0029), Nyahururu (0035; 0036), Maralal (0037A; 0037B), Narok (0038; 0039)

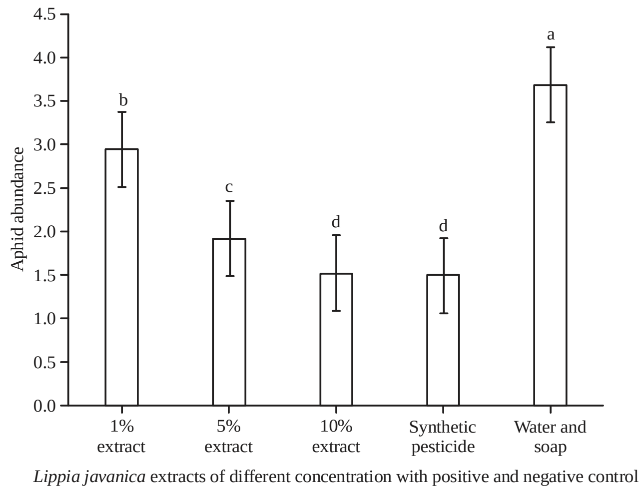

Fig. 1: Aphid abundance (number of aphids repelled) of cowpea aphids repelled after being sprayed with extracts of three conc. 1, 5 and 10% of *L. javanica* plant and positive/negative controls treatments after 10 weeks of treatment

Different alphabet letters (a, b,c and d) indicate significant differences

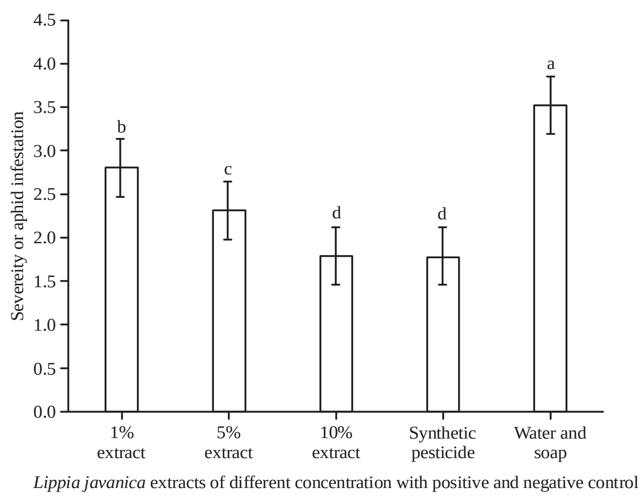

Fig. 2: Effect of the three conc. 1, 5 and 10% of *L. javanica* plant extracts and positive/negative controls treatments on severity or degree of aphid infestation (damage) after 10 weeks of treatment

Different alphabet letters (a, b,c and d) indicate significant differences

non-volatile phytochemical compounds screened, eleven were present. The three compound groups namely resins, phenolic glycosides and polyuronides were confirmed to be absent. Flavonoids, terpenoids, phenols, cardiac glycosides, saponins and alkaloids were present in high amounts (++). Flavones, anthraquinones, coumarins, anthocyanins and tannins occurred in fairly present amounts (+) irrespective of the locality.

Efficacy of the extracts of *L. javanica* in control of aphids of *V. unguiculata*: There were significant differences in efficacy of the extracts of *L. javanica* among the treatments where, the number of pests suppressed increased with increasing concentration of the treatments (Fig. 1 and 2) ($F = 184.543$ and $p<0.001$). However, there were no significant differences between the 10% extract level and the synthetic pesticide (positive control) ($F = 184.543$ and $p<0.001$) implying that the 10% concentration competed favorably with the synthetic pesticide (Atom, Osho from Syngenta). Also, it was notable that aphid numbers increased in the plots sprayed with water and soap solution (negative control).

Similarly, the severity or aphid infestation was highest where water and soap solution was applied. Aphid infestation decreased with increasing concentration of the extract. The results for analysis of variance (ANOVA) in the comparison of aphid infestation mean across the five treatment showed significant differences among the treatments ($F = 194.143$ and $p<0.001$). Aphid infestation was, however, least in the 10% and the synthetic pesticide (positive control), where there was no significant difference between the two treatments ($F = 194.143$ and $p<0.001$.

DISCUSSION

Lippia javanica was found to possess flavonoids, flavones, terpenoids, phenols, cardiac glycosides, saponins, alkaloids, anthraquinones, coumarins, anthocyanins and tannins. However, polyuronides, resins and phenolic glycosides were absent implying that the aforementioned species did not demonstrate dramatic variation in phytochemical compounds. Collection of the *L. javanica* materials in the same season and time was attributed to be the cause of lack of the variation. This highly aromatic plant has been reported to display great variations in phytochemical compounds[9]. Furthermore, Viljoen[9] noted that the dramatic variations in the essential and non-essential compounds was due to regional and different harvesting time. Nevertheless, Kamanula *et al.*[10] found that the qualitative phytochemical compounds did not vary among the different accessions of *L. javanica* which agrees with the results of this study. Surprisingly, phenolic glycosides were absent in this present study contrary to Endris *et al.*[11] findings. Also, essential oils were reported to vary with mycene, mycenone, linalool, caryophyllene and p-cymene among the chemotypes isolated from *L. javanica*[10,12]. A study by Sahreen *et al.*[13] found out that the non-essential greatly varied in *Rumex hastatus* L. a situation that was attributed to different harvesting time. It is worth noting that Endris *et al.*[11] also had similar findings in the *L. javanica* from Ethiopia. According to Nalubenga *et al.*[7], a number of plants contained chemical components that have been reported to be biologically active. These plants, therefore, have various parts such as; leaves, roots, rhizomes, stems, barks, flowers, fruits, grains or seeds, employed in the control or treatment of various disease conditions[14]. Leaves of *L. javanica* have a wide variety of the so-called classic nutrients, such as minerals, carbohydrates, proteins, fats and vitamins[11].

According to Maroyi[3], total phenolic compounds, tannins and minerals were isolated from *L. javanica*. Another study by Madzimure *et al.*[15] reported that alkaloids, flavonoids, iridoids, triterpenes and amino acids were confirmed to occur in *L. javanica*. As a matter of fact, alanine, asparagine and arginine were the amino acids detected[16]. Basic alkaloids, flavonoids, flavones were present in *L. javanica*[3]. Nevertheless, these compounds were found to vary in quantity. *Lippia javanica* leaf extracts chemical composition surprisingly varies within and between populations Madzimure *et al.*[15] owing to edaphic and climatic factors[17]. While phenolic glycosides were reported in high amounts in *L. javanica*, Dlamini[16] found out that they were completely absent. The fluctuation in some of the compounds was attributed to different times in harvesting, the maturity stage and season.

This study demonstrated that *L. javanica* possessed pesticidal effects on aphids that affect cowpea plants (Fig. 1). This scenario meant that the *L. javanica* was rich in compounds, which include alkaloids, flavonoids, flavones and terpenoids which were reported to exhibit anti-feedant and repellency and toxicity on insects. According to Stevenson *et al.*[5], the botanical pesticides of *L. javanica* acted in a synergistic effect to deter small bodied pests including rape spider mites and aphids. Several constituents in the volatile component have been identified in *L. javanica*. The major component camphor which has insecticidal properties[6]. Camphor occurred with other minor components including camphene, α-pinene, eucalyptol, Z and E α-terpineol, linalool, cymene, thymol, 2-carene, caryophyllene and α-cubebene and may account for the biological activity of plant species in this study[18]. Other potential biologically active components present in *L. javanica* were mono and sesquiterpenes i.e., perialdhyde in the essential oils, this compound is highly toxic through contact with insects. The efficacy of botanical insecticides on aphid pests was not fully effective. Some aphid pests had chances of survival perhaps through re-infection and behavioural mechanisms. Similar trends of the sought have been observed, like a study by Stevenson *et al.*[5] found out that rape aphid pests affecting tomato developed resistance against the botanical extract of *L. javanica* and *Vernonia amygdalina*. It is also possible that the active ingredients of pesticidal plants were quickly photodegraded upon their application and, therefore, reducing efficacy since they were sensitive to light[19]. Dissolving of the plant powder in the water overnight may have not achieved total extraction of all the pesticidal compounds. Especially non-polar compounds perhaps were not fully dissolved even though soap was used in the process. According to Mkenda *et al.*[6], the method of extracting the active ingredients also caused variations in concentration of the potent substances hence affecting efficacy. Chances may have been that the survival of the aphid population was caused by new infestation from neighboring cowpea crop areas[20]. The extracts of *L. javanica* were able to suppress the aphids' abundance and minimized cowpea damage below their economical threshold. The reduced number of aphids and mites was due to extracts' repellency, toxicity and anti-feedant effects since they contained alkaloids and other constituents with pesticidal properties[19]. These findings were in agreement with the findings of Mkenda *et al.*[6], who found out higher concentration of *L. javanica* botanical extract (20%) was comparable to the synthetic pesticides in reducing damage caused by foliage beetle. *Vernonia amygdalina* and *L. javanica* were the most effective plant species treatments to reduce damage caused by aphids[6].

CONCLUSION

Lippia javanica was found to be rich in phytochemical compounds such as alkaloids, tannins, flavonoids, flavones, cardiac glycosides, phenols, proteins, saponins and terpenoids, which may play a vital role in repelling aphids. These compounds make the species a potential pesticidal plant. Notably, phenolic glycosides were absent contrary to the findings of other researchers. There was no significant difference between the 10% extract concentration of *L. javanica* and synthetic pesticides treatments. Therefore, the 10% extract of the pesticidal *L. javanica* plant can be utilized as an alternative option for controlling field cowpea aphids instead of 5% which is relatively weak an 20% requires more powder and tiresome to prepare.

SIGNIFICANT STATEMENT

This study discovered that the 10% *L. javanica* botanical extract is the most suitable in controlling aphids. This study will help the researcher to uncover the critical areas of pesticidal plants use that many researchers were not able to explore. Thus a new theory on the right concentration of botanical extracts in controlling soft-bodied field pests on crops may be arrived at.

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