

# Scientific validation of plant extracts used by farmers in the management of bean flower thrips on French beans in Kenya

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## Abstract

Bean flower thrips (*Megalurothrips usitatus*) is a major French bean (*Phaseolus vulgaris* L.) pest. Small-scale farmers manage the pest using mixed plant extracts although their efficacy has not been scientifically validated. We evaluated the efficacy of mixed plant extracts comprising; *Capsicum frutescens*, *Allium sativum*, *Lantana camara*, *Tagetes minuta* and *Azadirachta indica*, against *M. usitatus* under laboratory and greenhouse. We identified and quantified the secondary metabolites associated with insecticidal activity using spectrophotometry and liquid chromatography-mass spectrometry (LC-MS). The plant combinations included PE1 (*C. frutescens* + *A. sativum* + *L. camara* + *T. minuta* extracts infused for 14 days), PE2 (same as PE1 but infused for 24 h) and PE + N (the five plant extracts infused for 24 h) in distilled water. We used an organic commercial botanical (Pyneem) as a positive control and distilled water as a negative control. Pyneem and PE + N induced the highest mortality at 88% and 77%, respectively, in the laboratory, and 68% and 71%, respectively, in the greenhouse. Phenolics, terpenoids and organosulfur compounds were identified in PE + N and individual plant extracts in varied quantities. These compounds were significantly higher ( $p < 0.001$ ) in PE + N compared to individual plant extracts. The study showed that PE + N efficiently manages bean flower thrips, and mixing different plant extracts amplifies the secondary metabolites' abundance. The use of mixed plant extracts could be incorporated into integrated pest management strategies for thrips management in legumes. The specific compounds identified in PE + N should be investigated further to understand their modes of action against the pest.

## KEYWORDS

amplified effect, efficacy, mortality, plant extracts, secondary metabolites

## 1 | INTRODUCTION

French bean (*Phaseolus vulgaris* L.) production holds an important economic role globally. In Kenya, it is mainly produced for export specifically to the EU market, with Britain being the major market (Fulano et al., 2021; Kariuki et al., 2012). It accounts for about 60% of all vegetables exported from Kenya, with small-scale farmers contributing over 80% of its production. Despite the good and promising lucrative markets, French bean production faces many challenges, including insect pests such as bean flower thrips (Lengai et al., 2020; Nyasani et al., 2012). The pest infests during all growing stages, leading to reduced yield and low-quality produce (Gupta & Singh, 2021). The yield losses of about 21%–83% result from direct feeding causing flower abortion, malformed pods and leaves with white specs on the legumes (Niassy et al., 2019; Reitz et al., 2020). In addition, bean flower thrips can spread phytopathogenic fungi and bacteria (Moritz et al., 2013). This prompts farmers to use synthetic pesticides to manage the pests (Nkechi et al., 2018).

Indiscriminate use of synthetic pesticides can lead to contamination and detrimental effects on human and environmental health (Khan et al., 2023; Tang et al., 2021). Harmful concentrations of organophosphate pesticides such as chlorpyrifos and neonicotinoids such as imidacloprid have been detected in soil and water bodies (George et al., 2014; Kampermann et al., 2024). Furthermore, the bioaccumulation of toxins in food webs has been documented to have hazardous impacts on human life (Fuhrmann et al., 2021; Nkechi et al., 2018; Tolera, 2020). The World Health Organization (WHO) reported that pesticides cause about 200,000 deaths worldwide each year (Belmain et al., 2013). Moreover, the overuse of synthetic pesticides could lead to the development of pesticide-resistant pests, aggravating the need for stronger active ingredients, hence fuelling the harmful cycle. Thus, there is a great need to develop ecologically sound pest management strategies such as the use of plant extracts.

The use of plant extracts for pest management has grown over the last two decades (Dar et al., 2021; Isman, 2020; Pavela, 2016; Phambala et al., 2020). Plant extracts are easily biodegradable, pose minimal risks to human and environmental health and are readily available at farm levels (Ngegba et al., 2022; Shai et al., 2023). Moreover, plants possess diverse secondary metabolites such as phenolic compounds, terpenoids, glycosides, alkaloids and organosulfur compounds (Aneklaphakij et al., 2021; Lengai et al., 2020) which play a key role in the defence mechanisms against insect herbivory and pathogenic microbial activities (Pang et al., 2021). These secondary metabolites have different modes of action including interference with feeding behaviour, toxic effects, reproduction or altering insect pest growth and development (Lengai et al., 2020). However, despite the plant extracts being able to degrade faster and safer than synthetic pesticides, their acceptance, and adoption by small-scale farmers for pest management is still very low. This has been attributed to inadequate data on secondary metabolites and a lack of proof of their efficacy against crop pests (Ngegba

et al., 2022). Therefore, in this study, we aimed to evaluate the efficacy of different plant extracts against *M. usitatus* and analysis of the secondary metabolites profile in the selected plant extracts associated with insecticidal activities. We hypothesized that mixing the different plant extracts would have an effect on *M. usitatus* and change the composition of secondary metabolites.

## 2 | MATERIALS AND METHODS

We carried out the experiments at the International Centre of Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya (01.22317°S, 36.89653°E, 1617m altitude above sea level). The laboratory settings for the efficacy trials were controlled to a temperature of  $24 \pm 1^\circ\text{C}$  and relative humidity of  $65 \pm 5\%$  whereas the greenhouse settings were uncontrolled. Temperature and relative humidity were monitored using Cole-Parmer monitors (Model WZ-37803-98), from Cole-Parmer, an Antylia Scientific company in the United Kingdom.

### 2.1 | Evaluating the efficacy of plant extracts against bean flower thrips

#### 2.1.1 | Cultivation of French beans

The French bean plants (*Phaseolus vulgaris* L.) were raised in perforated plastic pots measuring 10 cm in diameter and 12 cm in height filled with 1 kg of autoclaved red loam soil mixed with poultry manure at a ratio of 3:1. Thereafter, we planted the French bean seeds at the rate of four seeds per pot and kept in the greenhouse. The seedlings were watered using distilled water once per day and maintained in the greenhouse to ensure the continuous supply of host plants for thrips colony maintenance and bioassay.

#### 2.1.2 | Establishment of thrips colony

Thrips were collected from farmers' fields growing French beans in Kandara sub-county, Murang'a County in Kenya (0°48'61"S and 36°59'61"E, 1754m altitude above the sea level) to establish colonies in the laboratories at *icipe*. We collected the thrips using the leaf-beating method as described by Bacci et al. (2008) and transferred them into plastic jars containing fresh French bean pods. The specimens were then transported to the laboratory in a cooler box lined with wet cotton wool to provide relative humidity. Thrips-infested pods and flowers were transferred to jars with bean pods, covered with a netting material of mesh size 4760  $\mu\text{m}$  for feeding and oviposition. The laboratory temperatures were maintained at  $27 \pm 2^\circ\text{C}$  and a relative humidity of  $60 \pm 5\%$  with a light regime of 12L:12D photoperiod. The insect cultures were maintained for at least three generations before setting up the efficacy experiments. The food substrates (French bean pods) were changed with fresh ones regularly after 48h. In addition, the insect cultures were

periodically replenished with fresh field cultures to maintain the vigour of the stock.

### 2.1.3 | Sources of the plant materials

We collected all the plant materials (*T.minuta*, *L.camara*, *A.sativum*, *C.frutescens* and neem cake powder) from *icipe* (01.22317°S, 36.89653°E, 1617m altitude above sea level). A commercial botanical, Pyeneem® was acquired from Juanco Ltd., Nairobi Kenya.

### 2.1.4 | Preparations of different aqueous plant extracts

The first mixture (PE1) comprised 400 g of fresh leaves of *Tagetes minuta* and *Lantana camara* shredded into small pieces and put into a 5-L bucket. In addition, 200g of *Capsicum frutescens* and *Allium sativum* was crushed into a fine paste. The paste was mixed into the shredded pieces of *T.minuta* and *L.camara* leaves. Fifty millilitres of molasses and 2 L of distilled water were added to the mixture. The mixture was covered and kept well to infuse for 14 days. The PE1 plant extract formulation was presumed to have a concentration of 100% (0.60g/mL) as the standard treatment. Thereafter, four more concentrations were prepared from the stock solution (0.60g/mL) as defined above through serial dilutions at the rate of 50% (0.30g/mL), 75% (0.45g/mL), 125% (0.75g/mL) and 150% (0.90g/mL) giving a total of five concentrations replicated four times. After 14 days, the mixture was sieved through a fine netting material. Ten millilitres of Teepol® which is a liquid soap was added to the extract to act as an adjuvant.

We prepared a second set of plant extracts as described above using the same components except that molasses was not added. The plants were infused for 24 h (PE2). This was carried out to evaluate the effect of infusing the plant extracts over different time regimes. The concentration was presumed to be at 100% (0.60g/mL) and was treated as the standard treatment. Thereafter, four more concentrations were prepared from the stock solution (0.6g/mL) as defined above through serial dilutions at the rate of 50% (0.3g/mL), 75% (0.45g/mL), 125% (0.75g/mL) and 150% (0.9g/mL) giving a total of five concentrations replicated four times.

A third set of plant extracts just like the plant extracts infused for 24 h above was prepared except that 100g of neem cake powder was incorporated into the mixture (PE+N). This was done to assess if neem improved the efficacy. This was presumed to be at 100% (0.65) of the standard treatment. Four more concentrations were prepared from the standard stock (0.65g/mL) through serial dilutions at the rate of 50% (0.33g/mL), 75% (0.49g/mL), 125% (0.81g/mL) and 150% (0.98g/mL) giving a total of five concentrations replicated four times. We used a commercial botanical, Pyeneem® that contained Pyrethrins 10g/L and Azadirachtin 10g/L as a positive control and distilled water as a negative control.

### 2.1.5 | Evaluation of plant extracts against bean flower thrips in the laboratory and screenhouse

In the laboratory setting, four French bean pods were placed in a jar and infested with 80 two-day-old adult thrips. The thrips were allowed to settle for 24 h before treatment application. The infested pods were sprayed once per jar with 5 mL of the plant extract mixture and controls (treatments) in the respective concentrations using a hand sprayer at a flow rate of three sprays. Mortality data for thrips were recorded daily after 24 h post-treatment for 7 days. We replicated the experiment four times per treatment using different batches of plant extracts. A complete randomized design was used for the experiments.

To validate the results obtained in the lab, we repeated the experiment in the screenhouse using a similar method as described above except temperatures and relative humidity were not controlled as opposed to the laboratory settings. However, Cole-Parmer monitors were used to record the temperature and the relative humidity throughout the experimental period.

## 2.2 | Evaluating plant extracts' secondary metabolites

### 2.2.1 | Plant extract preparations

We prepared the plant extracts from five locally available plants: *Tagetes minuta*, *Lantana camara*, *Capsicum frutescens*, *Allium sativum* and *Azadirachta indica* (Table 1). Thereafter, the plant materials were infused in 500-mL distilled water for 24 h separately. In addition, a mixture of all the plant extracts plus neem powder (PE+N) was prepared and infused for 24 h. The plant extracts were sieved to obtain filtrates for secondary metabolite analysis.

### 2.2.2 | Qualitative analysis of secondary metabolites in the plant extracts

We conducted the phytochemical screening tests for flavonoid, phenolic, cardiac glycoside, terpenoid, tannin and saponin to determine selected secondary metabolites before quantitative analysis using the protocol described by Gul et al. (2017), Panchal and Parvez (2019), Khanal (2021) and Mlozi et al. (2022).

The test for flavonoids in the plant extracts was carried out using an alkaline reagent test. Two millilitres of different aqueous plant extract solutions was mixed with 2% sodium hydroxide separately. Production of a yellow colour, which turned colourless after adding two drops each at 200 µL of dilute 0.1 M hydrochloric acid indicated the presence of flavonoids. The test was carried out by drawing 2 mL of each plant extract sample into 15-mL falcon tubes. Thereafter, two drops of approximately 200 µL of ferric chloride were added to the mixture. The formation of blue-black or blue-green colour indicated the presence of tannins (Khanal, 2021). Terpenoids were

TABLE 1 Preparation of the plant extract samples for the analysis of the secondary metabolites.

Plant sample	Product	Amount of product (g)	Preparation method
<i>Tagetes minuta</i>	Young leaves	100	Cutting (0.5 cm pieces)
<i>Lantana camara</i>	Young leaves	100	Cutting (0.5 cm pieces)
<i>Capsicum frutescens</i>	Mature red fruit	50	Crushing
<i>Allium sativum</i>	Bulbs	50	Crushing
<i>Azadirachta indica</i>	Seed powder	25	–
Plant extracts (PE + N)	Combination of all plant extracts above (similar preparation)		

tested in different plant extract samples by picking 2 mL of plant extract into a 25-mL falcon tube and adding 2-mL chloroform analytical grade (Sigma Aldrich). This was followed by slowly adding 2 mL of concentrated  $\text{H}_2\text{SO}_4$  and heating the mixture for 2 min in a water bath. The presence of a brown-reddish colour indicated positive results for terpenoids.

The saponin test was carried out by vigorously shaking 2 mL of the plant extract and the development of a stable froth/foam indicated the presence of saponins in the sample. The test for alkaloids was done using 2 mL of each plant extract sample and adding 5 mL, 1.5% HCl (v/v) followed by Meyer's reagent into the mixture. The appearance of turbidity or change to yellowish colour indicated the presence of alkaloids (Shaikh & Patil, 2020). Salkowski's test was carried out to determine the presence of cardiac glycosides. Two millilitres of different plant extract samples was treated with 2-mL chloroform, followed by slowly adding 2 mL of concentrated  $\text{H}_2\text{SO}_4$  to the mixture. The presence of a reddish colour indicated the presence of cardiac glycosides (Panchal & Parvez, 2019).

### 2.2.3 | Analysis of the total flavonoid and phenols in the plant extracts using spectrophotometry

#### Total flavonoid content

The measurement of the total flavonoid content of the samples was based on the aluminium chloride colorimetric assay following the published protocol (Mokaya et al., 2020), with minor modifications. One millilitre of the plant extracts was mixed with 6.4 mL of water after which 0.3 mL of 5%  $\text{NaNO}_2$  was added into it. After 5 min, 0.3 mL of 10%  $\text{AlCl}_3$  was added and left for 1 min before adding 2 mL of 1 M NaOH. The absorbance was measured against the blank (the mixture minus the sample, which was substituted with water) at 510 nm. Quercetin was used to generate a calibration curve (0–100  $\mu\text{g/mL}$ ), and total flavonoids were expressed as  $\mu\text{g/mL}$  quercetin equivalents.

#### Total phenol content

Calorimetrically, we determined the total phenols content following the approach described by Mokaya et al. (2020) with slight adjustments. One millilitre of the extracts was mixed with 5 mL of 0.2 N Folin–Ciocalteu reagent and left for 5 min, after which 4 mL of 75 g/L

sodium carbonate was added. The mixture was incubated at room temperature for 2 h, before the absorbance was read at 760 nm against the blank (all reagents except the sample, which was substituted with water) using a Jenway 6850 UV/Vis spectrophotometer (Kobian). Gallic acid was used as a standard to yield the calibration curve (0–65  $\mu\text{g/mL}$ ). The total phenols content was expressed as  $\mu\text{g/mL}$ .

### 2.2.4 | Qualitative analysis of secondary metabolites using liquid chromatography–mass spectrometry

All the samples were centrifuged at 4000 rpm for 5 min at room temperature. Using a micropipette, 200  $\mu\text{L}$  of the supernatant was transferred into 2-mL clear glass vials (Supelco, Bellefonte, PA, USA) each containing 800- $\mu\text{L}$  distilled water, and immediately analysed using LC–MS. All the analyses were carried out in triplicates using the same batch of plant materials. The chromatographic separation was achieved on an Agilent system 1100 series (MA, USA) using ZORBAX SB-C18, 4.6  $\times$  250 mm, 3.5  $\mu\text{m}$  column, operated at 40°C. Mobile phases used were made up of water (A) and acetonitrile (B) each with 0.01% formic. The utilized gradient was: 0–8 min, 10% B; 8–14 min, 10–100% B; 14–19 min, 100% B; 19–21 min, 100–10% B; 21–25 min, 10% B. The flow rate was held constant at 0.5  $\text{mL min}^{-1}$  while the injection volume was 10  $\mu\text{L}$ . The LC was interfaced with a mass spectrometer. The mass spectrometer was operated on ESI-positive mode and data were acquired from a mass range of  $m/z$  50–600 at 40 eV cone voltage.

### 2.3 | Data management and analysis

We analysed the generated data sets using R software (version 4.2.2) (R Core Team, 2023). The profiled secondary metabolites were tentatively identified by comparing mass spectral data to an online library and where available confirmed using authentic standards. The data were subjected to analysis of variance (ANOVA). We subjected the mortality data to normality tests using the Shapiro test (Shapiro & Wilk, 1965). The total mortality data were calculated as the mean cumulative mortality data over 7 days for

each concentration in different treatments corrected using Abbott's formula (Abbott, 1925; Kerns et al., 2022) as follows:

$$\text{Mortality correction} = \frac{\% \text{ Mortality of thrips after 7 days} - \% \text{ Control mortality}}{(100 - \text{Control mortality} \times 100)}$$

Since the mortality data were abnormally distributed, we arcsine transformed it and thereafter analysed using analysis of variance (ANOVA). The means for secondary metabolites and mean cumulative mortality were compared and separated using the least significant difference (LSD) test at a 0.05 significance level (Searle et al., 1980) in the R program (R Core Team, 2023).

### 3 | RESULTS

#### 3.1 | The efficacy of different plant extracts against bean flower thrips

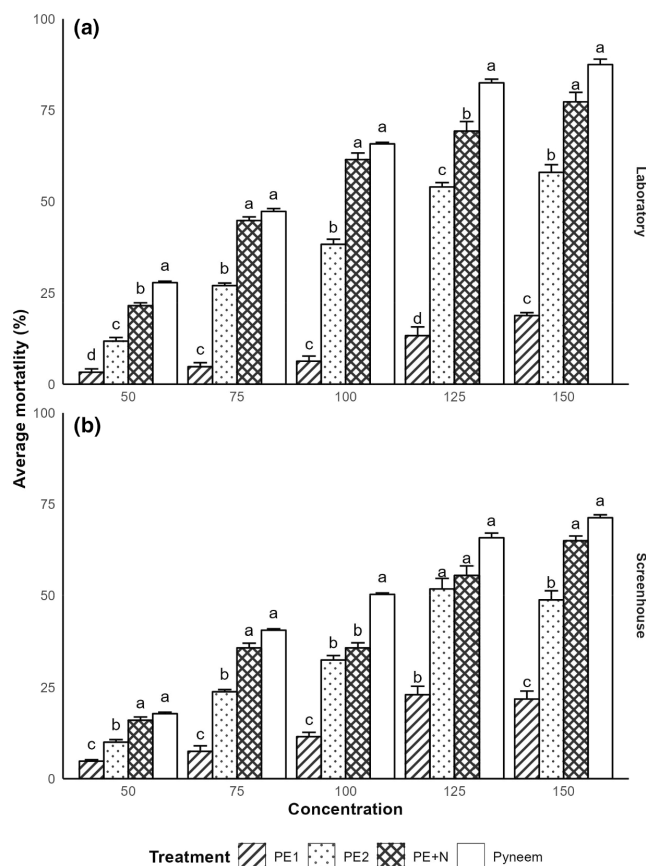
Results from the laboratory showed that the mean per cent mortality of bean flower thrips induced by the plant extract combined with neem (PE+N) was significantly higher ( $p < 0.05$ ) compared to the mean per cent mortality induced by the individual plant extracts (Figure 1). Only the PE+N (64%) and the individual plant extract from *Azadirachtin indica* (54%) induced a mean per cent mortality of over 50%. The comparison of the plant extract mixtures showed that the mean per cent mortality of bean flower thrips increased with higher concentrations. The mortality induced by the PE+N was comparable with that of the positive control treatment (Pyneem®) at concentrations of 75%, 100% and 150%. In contrast, PE1 did not induce mortality above 50% at any concentration (Figure 1a).

Under greenhouse conditions, there were significant differences within different concentrations ( $p < 0.05$ ) (Figure 1b). The mean per cent mortality induced by the PE+N was not significantly different from the mean per cent mortality induced by Pyneem® under various concentration levels, except at 100%. However, at all concentration levels, the mean per cent mortality induced by Pyneem®, PE+N and PE2 was significantly higher than the mortality induced by PE1 which did not induce mortality above 50% at any defined concentration (Figure 1b).

#### 3.2 | Plant extracts' secondary metabolites

##### 3.2.1 | Qualitative analysis of secondary metabolites in the plant extracts

The secondary metabolites screening of mixed plant extracts revealed the presence (+) of alkaloids, flavonoids, tannin, saponin, phenolic, cardiac glycoside and terpenoid (Table 2). Alkaloids and saponin were present in all tested plant extracts except in *A. indica*. Flavonoids were present in all tested plant extracts, except in *A. sativum*. Terpenoids were absent from the three plant species, *L. camara*, *T. minuta* and *C. frutescens*.



**FIGURE 1** The effect of different treatments on the mortality of bean flower thrips (a) in the laboratory and (b) in the greenhouse. Bars denote means  $\pm$  standard errors, and means followed by the same letters are not significantly different at  $p < 0.05$ .

##### 3.2.2 | Determination of total flavonoid and phenol contents in plant extracts

The total flavonoid content ranged between 0.46 and 59.14  $\mu\text{g/mL}$  in quercetin equivalent (QE), while the total phenols content ranged between 9.07 and 27.42  $\mu\text{g/mL}$  in gallic acid equivalent (GAE) (Table 3). In both total flavonoid and total phenols, there were significant differences among the selected plant extracts.

##### 3.2.3 | Quantitative analysis of secondary metabolites using liquid chromatography–mass spectrometry

We identified a total of 24 major secondary metabolite profiles in the aqueous plant extracts using LC–MS (Table 4). Polyphenols were predominant among these secondary metabolites from the plant extracts. Flavonoids, phenolic acids and coumarins emerged as the primary constituents within the spectrum of polyphenols. The various subgroups such as flavones, flavonols, flavan-3-ols and flavanones were discerned across all the plant extracts. Different types of flavonol such as quercetin, rutin and kaempferol were present in all plant extracts in different concentrations. In the



TABLE 2 Secondary metabolites identified in the various aqueous plant extracts.

Secondary metabolites	Aqueous plant extract samples					
	<i>L.camara</i>	<i>T.minuta</i>	<i>A.indica</i>	<i>C.frutescens</i>	<i>A.sativum</i>	PE + N
Alkaloid	✓	✓	–	✓	✓	✓
Flavonoid	✓	✓	✓	✓	–	✓
Tannin	✓	✓	–	✓	–	✓
Saponin	✓	✓	–	✓	✓	✓
Phenolic	✓	✓	–	–	–	✓
Cardiac glycoside	✓	✓	–	–	–	✓
Terpenoid	–	–	✓	–	✓	✓

Note: ✓, presence; –, absence.

TABLE 3 Secondary metabolites content of the extracts.

Plant sample	TF (µg/mL QE)	TP (µg/mL GAE)
<i>C.frutescens</i>	16.43 ± 0.40 <sup>a</sup>	27.42 ± 1.71 <sup>a</sup>
<i>T.minuta</i>	13.04 ± 0.41 <sup>e</sup>	23.44 ± 4.69 <sup>a</sup>
<i>A.sativum</i>	0.46 ± 0.02 <sup>b</sup>	13.44 ± 0.10 <sup>b</sup>
<i>L.camara</i>	59.14 ± 2.04 <sup>c</sup>	25.68 ± 4.76 <sup>a</sup>
<i>A.indica</i>	1.10 ± 0.02 <sup>b</sup>	11.51 ± 0.13 <sup>b</sup>
PE + N	43.22 ± 1.34 <sup>d</sup>	26.34 ± 1.92 <sup>a</sup>
p-Value	2.00E–16	<0.001

Note: Different superscript letters within the same column signify significant differences between the groups.

Abbreviations: TF QE, total flavonoid in quercetin equivalent; TP GAE, total phenols in gallic acid equivalent.

plant extracts combined with neem (PE + N), the concentration of flavonol such as rutin (175.11) and kaempferol (327.12) was higher compared to their concentration in individual plant extracts. Phenolic acids such as hydroxycinnamic and hydroxybenzoic acids were also present in all the plant extracts. However, their concentrations were higher in PE + N compared to each plant extract just like in the case of flavonol. Terpenes such as lantanilic acid were identified in *L.camara* extract and PE + N. The concentration of lantanilic acid in PE + N was as high than in *L.camara*. S-allyl-cysteine, which is an organosulfur, was not detected in the plant extracts except in *A.sativum* (Table 4).

## 4 | DISCUSSION

Plant extracts combined with neem infused for 24 h (PE + N) were more efficacious against bean flower thrips in French beans compared to the other tested mixed plant extracts. Moreover, the mortality induced by PE + N on flower thrips was comparable to that of Pyreneem® in both laboratory and screenhouse setups. We attributed the efficacy of PE + N against bean flower thrips to the bioactive compounds; Pyrethrin and Azadirachtin. Our findings corroborate with the documentation of Mpumi et al. (2016) about the insecticidal effects on crop pests of these bioactive

compounds. The plant extract infused for 14 days (PE1) induced mortality of below 50% in all concentrations. The prolonged time for infusion (14 days) could have led to the biodegradation of the major insecticide-active compounds. Thus, the preparation method for the different plant extracts could have played a key role in their efficacy against the flower bean thrips management on French beans. However, we did not assess the effect of individually isolated secondary metabolites against the pest to determine their mode of action.

The various secondary metabolites analysis of the plant extracts showed the presence of various bioactive compounds associated with insecticidal effects. For example, tannins which are bitter polyphenols reduce feeding performance in phytophagous insects (El-Aswad et al., 2023), by interfering and acting on peripheral sensilla (Isman, 2002; Rao et al., 2017). Further components like saponins, which are glycosylated triterpenes have also shown insecticidal effects against insect pests (Chaieb, 2010; Qasim et al., 2020; Roopashree & Naik, 2019). It has been demonstrated that they interact with cholesterol molecules in the prothoracic glands of the insect, by inhibiting the production of ecdysteroid which is a hormone responsible for moulting, thus interfering with the growth and development of the insects (Ellen et al., 2007; Singh and Kaur, 2018). Saponins have also been reported to interfere with the peripheral nervous system of insects causing them not to feed, leading to starvation and death of insects (Ukorioje & Otoyayor, 2020). The findings of our study also concur with previous studies of Palma-Tenango et al. (2017) and Lamara et al. (2020).

Polyphenols such as flavonoids have also been reported to inhibit the enzymatic activity of glutathione S-transferase Noppera-bo (Nobo) which is responsible for the biosynthesis of ecdysone hormone during immature stages of insects, thus interfering with their growth and development. Rutin, luteolin and quercetin interfere with the moulting and reproduction processes of several insects by inhibiting the formation of ecdysone and juvenile hormones (Oberdörster et al., 2001). Quercetin, rutin and naringin were also found to be effective in controlling the nymph and adult stages of aphids (Goławska et al., 2014; Pereira et al., 2024). In addition, phenolic acids such as vanillic acid and 4-hydroxybenzoic acid have been shown in previous studies to inhibit acetylcholinesterase in rice weevils (Singh et al., 2021) and could, thus, cause similar effects on the treated thrips.

**TABLE 4** Major secondary metabolites identified in the aqueous plant extracts using LC-MS and their abundance.

No.	Name of the compound	Type treatment	Retention time	Abundance
1	Lantanilic acid	PE + N	37.7	749.62 ± 6.65 <sup>a</sup>
		<i>Lantana camara</i>	28.9	59.70 ± 1.12 <sup>b</sup>
2	(-)-Epicatechin	<i>Azadirachta indica</i>	5.8	14.20 ± 0.38 <sup>a</sup>
3	(+)-Catechin	<i>Azadirachta indica</i>	5.8	18.36 ± 0.13 <sup>a</sup>
4	4-Hydroxybenzoic acid	<i>Capsicum frutescens</i>	5	26.99 ± 2.70 <sup>b</sup>
		PE + N	5.1	769.77 ± 11.27 <sup>a</sup>
5	Apigenin	<i>Tagetes minuta</i>	36.3	19.54 ± 0.08 <sup>a</sup>
6	Caffeic acid	<i>Lantana camara</i>	5	29.41 ± 2.27 <sup>c</sup>
		<i>Capsicum frutescens</i>	5.1	56.82 ± 6.06 <sup>b</sup>
		PE + N	5	176.78 ± 0.78 <sup>a</sup>
7	Gallic acid	<i>Tagetes minuta</i>	26.9	20.84 ± 0.13 <sup>d</sup>
		<i>Allium sativum</i>	27.71	67.65 ± 1.08 <sup>a</sup>
		<i>Lantana camara</i>	27.8	52.06 ± 0.74 <sup>b</sup>
		<i>Azadirachta indica</i>	27.7	10.68 ± 0.28 <sup>e</sup>
		<i>Capsicum frutescens</i>	12	28.29 ± 1.48 <sup>c</sup>
8	Kaempferol	<i>Tagetes minuta</i>	36.4	16.31 ± 0.14 <sup>b</sup>
		<i>Lantana camara</i>	36.1	16.49 ± 0.13 <sup>b</sup>
		PE + N	36.3	327.12 ± 3.10 <sup>a</sup>
9	Kaempferol 3-glucoside	<i>Azadirachta indica</i>	38.6	2.12 ± 0.10 <sup>a</sup>
10	Kaempferol 7-O-glucoside	<i>Tagetes minuta</i>	33.4	82.65 ± 0.66 <sup>a</sup>
11	Luteolin	<i>Capsicum frutescens</i>	27.8	62.26 ± 7.23 <sup>a</sup>
		<i>Tagetes minuta</i>	28.4	38.80 ± 1.42 <sup>b</sup>
12	Margolonone	<i>Azadirachta indica</i>	36.8	3.30 ± 0.01 <sup>a</sup>
13	Naringenin	<i>Tagetes minuta</i>	35.7	20.27 ± 0.13 <sup>a</sup>
14	Naringin	<i>Tagetes minuta</i>	37.1	6.61 ± 0.05 <sup>a</sup>
15	Nimbandiol	<i>Azadirachta indica</i>	29.4	2.93 ± 0.05 <sup>a</sup>
16	Nimbidiol	<i>Azadirachta indica</i>	11.4	7.39 ± 0.14 <sup>a</sup>
17	Oleuropein	<i>Capsicum frutescens</i>	35.9	47.13 ± 5.54 <sup>a</sup>
18	p-Coumaric acid	<i>Lantana camara</i>	5.1	191.01 ± 0.77 <sup>a</sup>
19	Pyrogallol	<i>Allium sativum</i>	13.42	59.32 ± 2.51 <sup>a</sup>
20	Quercetin	<i>Tagetes minuta</i>	27.5	86.82 ± 1.41 <sup>a</sup>
		<i>Allium sativum</i>	33.33	26.43 ± 0.97 <sup>b</sup>
		<i>Azadirachta indica</i>	33.9	20.21 ± 0.18 <sup>c</sup>
		<i>Capsicum frutescens</i>	33.5	5.87 ± 0.39 <sup>d</sup>
21	Rutin	<i>Allium sativum</i>	40.5	13.99 ± 0.38 <sup>c</sup>
		<i>Lantana camara</i>	40.5	153.31 ± 1.26 <sup>b</sup>
		<i>Azadirachta indica</i>	40.5	1.37 ± 0.02 <sup>d</sup>
		<i>Capsicum frutescens</i>	40.5	14.06 ± 0.83 <sup>c</sup>
		PE + N	38.3	175.11 ± 0.96 <sup>a</sup>
22	S-allyl-cysteine	<i>Allium sativum</i>	5.02	15.43 ± 0.14 <sup>a</sup>
23	S-allyl-sulfoxide	<i>Allium sativum</i>	4.94	5.25 ± 0.25 <sup>a</sup>
24	Vanillin	<i>Capsicum frutescens</i>	5	50.05 ± 1.54 <sup>a</sup>

Note: Different superscript letters within the same compound signify significant differences between the compounds. PE + N = mixture of *L. camara*, *C. frutescens*, *A. sativum*, *C. frutescens* and *A. indica*.

Mixing the various aqueous plant extracts had a multiplicative effect on bioactive compounds rather than additive in the case of kaempferol, lantanilic acid, rutin, caffeic acid and 4-hydroxybenzoic acid (Munyoki, 2023). The multiplicative and amplified effects of mixing plant extracts may enhance the efficacy of the bioactive compounds against insect pests and confer an advantage over the use of single plant extracts in the management of agricultural pests (Hossain et al., 2016; Oladipo-nee Ajayi et al., 2020; Williamson, 2001). However, it is important to carry out more studies to ascertain the mechanisms through which the secondary metabolites interacted resulting in the amplified effects. The diverse modes of action against agricultural pests exhibited by the various secondary metabolites make them a suitable candidate to be used in crop protection, especially in pest management. However, it is important to carry out more studies to ascertain their dose concentration regarding their insecticidal effect.

## 5 | CONCLUSION AND RECOMMENDATION

In this study, we demonstrate that the plant extracts incorporated with neem cake powder and infused for 24 h (PE + N) contained diverse and highly abundant secondary metabolites associated with insecticidal effects. We also established that the PE + N was effective against bean flower thrips, and thus can be used in the management of the pest. Combining different aqueous plant extracts resulted in amplified effect, amplifying the bioactive compounds' effectiveness beyond mere additive effects. This synergistic interaction holds promising potential for enhancing pest management capabilities using mixed plant extracts. Smallholder farmers stand to benefit from this approach as it surpasses the efficacy of using a single plant in extract preparation. Nonetheless, further investigations should be conducted to isolate and examine specific compounds, both individually and in combination, to gain a deeper understanding of their impact on insect behaviour. Furthermore, it is also important to validate these findings under field conditions in large-scale efficacy trials.

## AUTHOR CONTRIBUTIONS

**Nancy Mwende Munyoki:** Conceptualization; methodology; data curation; formal analysis; writing – original draft; writing – review and editing; investigation. **James Muthomi:** Supervision; writing – review and editing. **Dora Kilalo:** Supervision; writing – review and editing. **David Bautze:** Conceptualization; writing – review and editing; funding acquisition. **Milka Kiboi:** Writing – review and editing; resources; funding acquisition. **Edwin Mwangi:** Formal analysis; writing – review and editing. **Edward Karanja:** Supervision; investigation; writing – review and editing. **Felix Matheri:** Writing – review and editing. **Noah Adamtey:** Conceptualization; resources; project administration; funding acquisition. **Komivi S. Akutse:** Investigation; formal analysis; writing – review and editing. **Xavier Cheseto:** Data curation; formal analysis; methodology; validation; writing – review and editing.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no potential conflict of interest.

## DATA AVAILABILITY STATEMENT

The data included in this study are available at <http://doi.org/10.17632/h6jhc3ynff.1>.

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