

Full Length Research Paper

## Laboratory assessment of insecticidal properties of *Tagetes minuta* crude extracts against *Brevicoryne brassicae* on cabbage

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The cabbage aphid, *Brevicoryne brassicae* is one of the most serious insect pests of cabbage in Lesotho. Chemical insecticides for efficient control of this pest are often unaffordable for resource-poor farmers. As a first step in a search for effective pest management techniques that can be affordable and easily available to these farmers, the aphicidal activities of *Tagetes minuta* crude extracts were evaluated against the cabbage aphid. A comparison was made on lethal and sub-lethal effects of *T. minuta* crude extracts from acetone, methanol, water and a mixture of acetone/methanol/water (7:7:1, v:v). The solvent system that produced the most toxic extract was the mixture, followed by methanol and water, whereas acetone produced the least toxic extract. The sub-lethal effect on cabbage aphid fecundity was well described by linear regression equations that showed significant reduction in fecundity as the concentration of crude extracts increased. The extract from the mixture solvent system had the strongest effect on fecundity.

**Key words:** *Brevicoryne brassicae*, cabbage aphid, *Tagetes minuta*, crude extracts, lethal concentration, sub-lethal, fecundity.

### INTRODUCTION

Cabbage is the most popular vegetable grown and consumed in Lesotho. Its popularity causes the national demand to be far greater than the local production, leading to a significant deficit that has to be met by imports from the neighbouring South Africa. Although, the optimum seasons for growing cabbage are those characterized by moist cool conditions, it can easily be grown around the year in Lesotho. The nutrition capacity of 100 g of cabbage provides 5.8 g of carbohydrates, 2.5 g dietary fibre, 1.3 g protein, 0 g fat, 36.6 mg vitamin C (44% of daily requirement), 76 mg vitamin K (72% of daily requirement) (USFDA, 2006). The simplicity of cabbage production and its nutrition capacity make it a vitally important vegetable especially in poverty-prone countries

like Lesotho.

Production of cabbage is however negatively affected by various insect pests with cabbage aphid, *Brevicoryne brassicae* L., being one of the most serious pest species throughout all the cabbage-growing areas of the world (Annecke and Moran, 1982; Bhatia and Verma, 1994; Munthali, 2009). Cabbage aphids feed by sucking sap from plants and reproduce very rapidly to produce heavy infestations that, in young plants, cause leaves to curl inward and become chlorotic, resulting in stunted growth (McKinley, 1992). Heavy infestations on mature plants reduce the market value due to not only the aphid presence but also the accumulations of their exuviae, honeydew and the sooty mould that grows on honeydew

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(McKinley, 1992; Ellis et al., 1998). Cabbage aphids further cause problems through their ability to vector viruses, like Tulip Mosaic Virus, that cause many diseases in crucifers (Chivasa et al., 2002).

Pest control in cabbage by small-scale farmers is still heavily dependent on chemical insecticides even though their use is associated with many undesirable and sometimes lethal consequences. Improper and widespread use of chemical insecticides can cause underground and surface water pollution (Schottler et al., 1994; Schultz et al., 2001; Dalvie et al., 2003). Excessive use of insecticides also induces resistance development in target pests as well as killing beneficial organisms such as pollinators (especially bees) and natural enemies (insect parasitoids and predators) (Pedigo and Rice, 2006). The greatest concern with use of chemical insecticides in vegetable production is their potential poisonous effects on human health through dietary exposure (USFDA, 2006; McKone et al., 2007; Lu et al., 2008, 2010; Łozowicka et al., 2012). There is therefore a need for alternatives to chemical insecticides that can be cheaper and easily accessible to small-scale farmers, as well as being safe to humans and the environment.

Many botanical insecticides derived from commonly available plants effectively meet these criteria of affordability and accessibility to small-scale farmers as well as human- and environmental-safety (Isman, 2006, 2008). Botanical insecticides are generally considered to have low toxicity to mammals; with some, like the neem-based azadirachtin, being non-toxic to mammals, fish and pollinators (Isman, 2006; Naumann and Isman, 1996; Wan et al., 1996). Even those botanical insecticides that have some level of toxicity to non-target organisms, their tendency to be rapidly degraded by sunlight renders them non-persistent and as such less likely to have significant impact through residual effect as is often the case with synthetic chemical insecticides (Isman, 2006). An example of a botanical insecticide that is highly poisonous to natural enemies and pollinators is an essential oil made from purified terpenoid constituents; but its volatility results in limited persistence in the field and thus less impact on non-target species (Isman, 2000). In their pure forms, other botanicals (e.g. rotenone) are as poisonous to mammals as synthetic insecticides but the concentration levels used in the formulated products are much less toxic (Isman, 2006).

The objective of this study was to evaluate the insecticidal effectiveness of crude extracts of *Tagetes minuta* L. (Asteraceae) against cabbage aphids, *B. brassicae*. *T. minuta* is a plant species that is now cosmopolitan and grows easily in disturbed areas (both agricultural and non-agricultural) as a weed. The plant species was selected on the basis of the survey results that showed its wide application by local farmers in Lesotho as a concoction constituent in combination with *Aloe*, onion and hot pepper for the control of various pest insects (Mekbib et al., 2011). It is widely known to have

pesticidal properties due to the numerous secondary compounds it possesses (Soule, 1993).

## MATERIALS AND METHODS

### Collection and preparation of plants for extraction

Plant specimens of *T. minuta* at post-flowering growth stage were collected from Roma campus of the National University of Lesotho and areas around Roma in brown paper bags on the 15<sup>th</sup> and 16<sup>th</sup> February, 2012. They were then transported to the Biochemistry Laboratory at the National University of Lesotho for processing. Fresh leaves were washed thoroughly in water and dried in the oven at 35°C for three days. Dried leaves were pulverized to powder using a mortar and pestle and then stored at room temperature until needed.

### Preparation of crude extracts

The following solvents were used in the preparation of crude extracts: (i) 99.5% acetone, (ii) 99.5% methanol, (iii) distilled water, and (iv) a mixture of acetone/methanol/water (7:7:1, v:v). The powdered material was weighed and extracted in each of the above solvent in the ratio of 1:20 v:v. Each extraction suspension was mixed with a vortex mixer (VM-300 (Axiom, Germany)) then placed on a rotary shaker for 1 h at 170 rpm followed by centrifuging in a micro-centrifuge (Hettich zentrugen D-78532 (Tuttlingen, Germany) at 6000 rpm for 10 min. The residue from each solvent - plant mixture was subjected to two additional extraction cycles by adding 20 ml of a solvent. Supernatants from the extractions from each solvent were combined and concentrated to 1 ml under vacuum before being mixed with sterile distilled water to a volume of 10 ml. The extracts were sterilized by using a hypodermic syringe driven filter paper (0.22 µm pore size). These extract samples were then either used immediately or kept in the refrigerator at 4 ± 1°C until further use.

### Preparation of plant extract dilutions

Each plant extract was serially diluted with distilled water to obtain the following extract concentrations: 0% (distilled water alone), 10% (1:9 v:v), 20% (2:8 v:v), 30% (3:7 v:v), 40% (4:6 v:v), 50% (5:5 v:v), 60% (6:4 v:v), 70% (7:3 v:v) 80% (8:2 v:v), 90% (9:1 v:v) and 100% (plant extract stock solution). Each dilution was prepared on the day of the experimental trial.

### Cabbage aphid rearing

Cabbage aphids, *B. brassicae*, rearing was started with aphids collected from several home gardens planted with cabbage in Botha-Bothe township, Lesotho in April, 2012. The aphids were then transferred to cabbage plants growing on a mixture of three parts soil, one part sand, one part manure and one part vermiculite in 500 ml plastic plant pots in the greenhouse. Aphids were transferred to new 5 - 8 leaf stage cabbage plants at every 10 days.

### Aphid insecticidal bioassay

An aphid-dip bioassay developed by Chandrasena et al. (2011) was slightly modified for use in this study as described below. A 3 by 3 by 3 cm container constructed from the metal mesh cut out from a tea strainer (mesh size 0.5 mm) was used as a holding cell for

**Table 1.** Proportions of aphids that died as a function of an increase in concentration of *T. minuta* leaf extracts from different solvents.

Concentration	Solvent							
	Acetone		Methanol		Water		Mixture	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
0.0	8	0	20	0	12	0	16	0
10	16	22.3	20	22.5	20	22.4	24	22.6
20	20	23.6	24	25.1	28	23.7	24	25.9
30	28	26.5	36	30.2	36	26.8	32	31.8
40	28	30.6	32	36.4	40	31.2	36	38.8
50	28	35.4	44	43.0	40	36.3	48	45.9
60	40	40.5	48	49.3	44	41.5	56	52.5
70	44	45.5	48	55.1	44	46.6	56	58.5
80	52	50.3	56	60.4	48	51.4	60	63.8
90	52	54.7	60	65.0	48	56.0	64	68.4
100	68	58.8	84	69.1	60	60.1	80	72.3

aphids during the insecticidal testing of plant extracts. Individual groups of five wingless adults from the greenhouse aphid colony were placed in the metal mesh container and were dipped for 10 s in distilled water (control), in the different plant extract stock solutions or in their dilutions. Each group of aphids was then transferred to a fresh-cut cabbage leaf square (2 x 2 cm) on moist filter paper in a Petri dish (90 mm diameter, 15 mm depth). The Petri dishes were placed in the growth chamber at 22°C and a photoperiod of 16:8 (L:D) h for 48 h. Multiple metal mesh containers were used, each assigned to a particular treatment so as to avoid cross contamination.

After 48 h aphids were classified as dead or alive and then counted. Aphids were considered dead when they did not move after multiple proddings with a fine-haired paint-brush. Number of newborn nymphs (progeny) per group (treatment) was also determined as a way of assessing the sub-lethal effects of plant extracts on aphid fecundity (reproduction). Enumeration of aphids was performed using a colony count magnifying glass.

#### Data analysis

Extract concentration- mortality curves for all bioassays were estimated using Probit analysis in the SPSS software (version 11.0.1) (SPSS Inc., 2001). Extract concentrations, and their 95% confidence limits, required to kill a given percentage (for example, 50% or LC<sub>50</sub>) of the cabbage aphids were estimated using the Probit regression. We developed a probit model that used the number of dead aphids as a response variable, the total number of aphids subjected to an extract concentration as a total observations variable, the type of solvent used in leaf extraction as a factor, coded 1 for acetone, 2 for methanol, 3 for water and 4 for the mixture. We used solvent concentration as a covariate. A single analysis was therefore executed for all solvent extracts, with the assumption of similar or common probit regression slopes checked with the test of parallelism. Pearson's Chi-square test was used to determine the fit of the statistical model (Finney, 1971). Data from all bioassays were corrected using Abbott's formula (Abbotts, 1925). Estimates of the relative median toxicity, defined as the ratio of LC<sub>50</sub>s between two solvent extracts, was determined for the six pairs of the solvent extracts. This means that a relative median toxicity of 1.0 implies that the two solvents extracts are equivalent in the aphid mortality response they cause. The 95% confidence

interval for each relative median toxicity was determined and used for testing significant differences in aphid mortality responses caused by the different solvent extracts.

Regression analysis was performed to establish whether the relationship between extract concentration and aphid fecundity can be described adequately by a linear regression. Estimates of the regression model parameters were obtained from the SPSS software (version 11.0.1) (SPSS Inc., 2001).

## RESULTS

### Cabbage aphid mortality

The Chi square statistic for the parallelism test was not significant ( $\chi^2 = 1.86$ ,  $df = 3$ ,  $P = 0.60$ ), indicating that the slope of the probit regression is the same for each solvent extraction. This result therefore justified our combined probit regression analysis instead of separate analyses for each solvent extraction. On the basis of the proportion of dead aphids sampled at different extract concentrations of each solvent (Table 1), probit analysis calculated expected proportions of dead aphids at various extract concentrations from the linear regression equation:

$$E_d = \alpha + \beta \log E_c$$

where  $E_d$  is the expected proportion of dead aphids expressed in probits,  $E_c$  is extract concentration,  $\alpha$  is the intercept and  $\beta$  is the slope of regression line. Both  $\alpha$  and  $\beta$  were estimated by the linear regression after probit transformation of the proportions of dead aphids. The estimates of  $\alpha \pm \text{SEM}$  for extracts of acetone, methanol, water and the mixture of all three solvents were  $-5.93 \pm 1.53$ ,  $-5.60 \pm 1.15$ ,  $-5.89 \pm 1.58$  and  $-5.49 \pm 1.49$ , respectively. The estimate of the common  $\beta \pm \text{SEM}$  was

**Table 2.** LC<sub>50</sub>s and LC<sub>99</sub>s together with their 95% confidence limits of the *T. minuta* leaf extracts from different solvents on cabbage aphid, *B. brassicae*.

Solvent	LC <sub>50</sub>	95% Confidence Limit	LC <sub>99</sub>	95% Confidence Limit
Acetone	105.94	81.92 - 150.03	660.20	328.74 - 5608.91
Methanol	81.53	61.25 - 103.18	508.07	271.15 - 3496.66
Water	102.55	74.84 - 137.46	639.05	333.38 - 4629.96
Mixture	74.77	55.81 - 92.97	465.92	251.88 - 3090.54

**Table 3.** Estimates of the relative median toxicity together with their 95% confidence limits of the *T. minuta* leaf extracts between different solvents on cabbage aphid, *B. brassicae*.

Solvent comparison	Relative median toxicity	95% Confidence limit	
		Lower	Upper
Acetone vs. Methanol	0.77	0.42	1.60
Acetone vs. Water	1.03	0.69	1.45
Acetone vs. Mixture	0.71	0.37	0.96
Methanol vs. Water	0.80	0.43	1.08
Methanol vs. Mixture	1.09	0.82	1.60
Water vs. Mixture	0.73	0.35	1.00

2.93 ± 0.78, which shows that the increase in the concentration of leaf extracts resulted in an increase in the percentage of aphids killed.

The probit analysis performs a goodness-of-fit test based on the Pearson Chi-square to examine how the data agree with predicted values. The Chi-square value obtained was 129.55 (*df* = 194, *P* = 0.99). This non-significant Chi-square statistic indicates a good fit between the data and the probit regression model. The solvent that produced the most toxic extract (one with lowest LC<sub>50</sub> and LC<sub>99</sub>) was the mixture (of acetone/methanol/water (7:7:1, v:v)) followed by methanol and water, whereas acetone produced the least toxic extract (one with the highest LC<sub>50</sub> and LC<sub>99</sub>) (Table 2). Estimates of the relative median toxicity between various paired solvent extracts (Table 3) show that *T. minuta* extracts from most of the solvents did not differ in their ability to kill cabbage aphids. This is evidenced by the confidence intervals that included or bracketed the value of 1.0. The only relative median toxicity that was significantly different from 1.0 was obtained between acetone extract and the extract obtained from the mixture of the three solvents (Table 3).

### Cabbage aphid fecundity

There was a significant negative relationship between aphid progeny and the concentration of *T. minuta* extracts from all the four solvents (Figure 1 and Table 4). The relationship was that an increase in concentration of

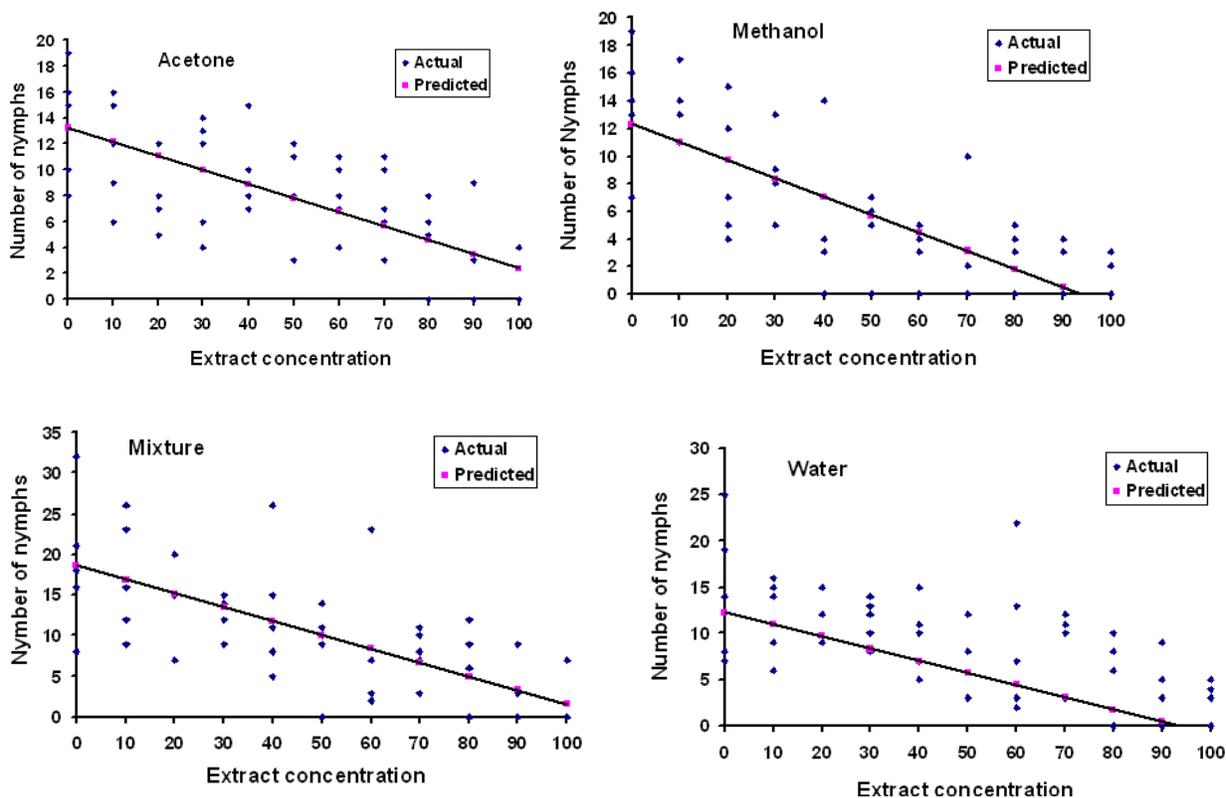
*T. minuta* extracts significantly reduced the fecundity of cabbage aphids (Figure 1). The regression equations explaining the relationship between aphid fecundity (*y*) and the extract concentration (*x*) for each of the extracts were:

$$\begin{aligned} \text{Acetone: } y &= 13.28 - 0.11x, (r^2 = 0.51) \\ \text{Methanol: } y &= 12.26 - 0.13x, (r^2 = 0.59) \\ \text{Water: } y &= 14.40 - 0.11x, (r^2 = 0.40) \\ \text{Mixture: } y &= 18.62 - 0.17x, (r^2 = 0.50). \end{aligned}$$

The y-intercepts of the above equations can be considered as estimates of the cabbage aphid fecundity after being subjected to approximately 10 s of water. It can also be estimated from the equations that the concentrations of *T. minuta* extract needed to completely arrest reproduction in cabbage aphids, that is, zero fecundity are 120% of the acetone extract, 94% of the methanol extract, 130% of the water extract and 110% of the extract obtained from the mixture of the three solvents.

### DISCUSSION

This study tested the effects of crude extracts of *T. minuta* obtained from the four extraction solvent systems of acetone, methanol, water and the acetone/methanol/water mixture. We specifically wanted to test the efficacy of *T. minuta* crude extracts as contact or topical insecticides against aphids as this mode of



**Figure 1.** Relationship between cabbage aphid fecundity and concentration of *T. minuta* crude extracts obtained from acetone, methanol, water and the mixture of acetone/methanol/water (7:7:1; v/v). The equations for the predicted relationship are given in the text.

**Table 4.** Analysis of variance results showing the amount of variation in cabbage aphid fecundity that is explained by regression on the concentration of *T. minuta* leaf extracts from different solvents.

Solvent	Sum of squares		Mean square <sup>a</sup>		F <sup>b</sup>
	Regression	Residual	Regression	Residual	
Acetone	656.73	641.45	656.73	12.10	54.26
Methanol	939.93	642.98	939.93	12.13	77.48
Mixture	1586.10	1564.01	1586.10	29.51	53.75
Water	645.85	1000.135	645.85	18.97	34.06

<sup>a</sup>n = 55; regression df = 1; residual df = 53 for all solvents; <sup>b</sup>All F-statistics are significant at P < 0.0001.

entry into aphid bodies is one that is most likely to operate under natural field conditions because of the piercing-sucking feeding habits of aphids. Piercing-sucking insects like aphids do not get poisoned by insecticides that work as stomach poisons unless the insecticides is systemic and therefore flows together with the fluid materials in the plant vascular system after being absorbed from soil (Pedigo and Rice, 2006).

The aphicidal activity of the *T. minuta* extracts in our study is consistent with results from other studies, al-

though direct comparisons are difficult to make as no other study has evaluated the relationship between the extract concentrations and the percentage of aphids killed (dose-response assessment to estimate LC<sub>50</sub>s). Our results indicate that the mixture of methanol/acetone/water (7:7:1) solvent system produced the highest aphicidal activity (lowest LC<sub>50</sub>), followed by methanol extract whereas the acetone extract showed the least aphicidal activity (Table 2). Although, the water extract ranked third, its aphicidal activity was not signifi-

cantly different from that of the mixture extract (Table 3) and this was an encouraging outcome as it implies that the extract method that is employed by resource-poor farmers (water) is just as effective as the one derived from organic solvents. The efficiency of water extracts of *T. minuta* was also demonstrated by Ali et al. (2010) who found that an aqueous extract of *T. minuta* leaves at concentration of 1:2.5 g/ml (w/v) was quite effective, reducing mustard aphid, *Lipaphis erysimi* Kalt. by 96% when sprayed on Indian mustard plots.

Furthermore, *T. minuta* extracts have also been shown to significantly kill various kinds of insect pests such as stored product beetles (Weaver et al., 1994, 1997; Keita et al., 2000), mosquitoes (Philogene et al., 1985; Basabose et al., 1997; Perich et al., 1995) and armyworms (Rao et al., 2000; Aldana-Llanos et al., 2012). Most of these studies further identified chemical substances that appear as likely candidates for active compounds responsible for effecting insect mortality. Such chemicals include essential oils that affect insect pests negatively in several ways, that is, they have different modes of action. For example, Dunkel et al. (2010) found that *T. minuta* oil extracted by steam distillation of shoot extracts killed larvae of the sugarbeet root maggots, *Tetanops myopaeformis*, at higher concentrations whereas at lower concentrations, it prevented successful pupation from occurring. The third mode of action is repellency, cited in the recent Registration document for Tagetes Oil by the United States Environmental Agency as a biochemical pesticide whose active ingredient is intended for the control of mites, whiteflies, aphids, thrips, mealybugs, scales and psylla on a variety of food crops (USEPA, 2012). Since its mode of action is described as repellency, it follows that *T. minuta* plants have a potential to be used in a mixed- or inter-cropping system to reduce the above listed pests against crops. This potential has already been demonstrated in a study where white cabbage intercropped with another species of tagetes (*T. patula nana* L.) suffered significantly less cabbage aphid infestation when compared with a mono-cropped cabbage (Jankowska et al., 2009).

Our results also showed that *T. minuta* extracts have the fourth sub-lethal effect on cabbage aphids, that is, reduction in reproduction potential or fecundity (Figure 1 and Table 4). The reduction in fecundity increased linearly with an increase in concentration of each of our extracts (Figure 1). Our results are consistent with the findings of Tomova et al. (2005) who showed that fractionated tagetes oil volatiles reduced fecundities of three species of aphids (*Acyrtosiphon pisum* (Harris), *Myzus persicae* (Sulzer) and *Aulacorthum solani* (Kaltenbach)) in a dosage dependent manner. Other essential oils derived from different plant species have been shown to have the same negative effect on cabbage aphid fecundity (Işık and Görür, 2009).

Although, farmers in developing countries like Lesotho

at times receive subsidized synthetic pesticides, use of botanicals such as extracts of *Tagetes* spp. should be encouraged especially in the context of the integrated pest management principle that relies on several control methods. One of the shortcomings of botanical pesticides is their quick degradation which shortens their effectiveness in the field. Perhaps this problem will soon be overcome through recent technological advances such as nanotechnology that will allow future use of botanicals in conventional/commercial crop production systems (Khater, 2012). For example, botanical insecticides can be deployed through nanoencapsulation, which is a process through which a chemical is slowly but efficiently released to affect high insect control (Bhattacharyya et al., 2010).

## Conclusion

The current study demonstrates that the crude extract of *T. minuta* obtained using water as a solvent is as effective as crude extracts from organic solvent systems in killing cabbage aphids. Aqueous extracts of *T. minuta* also had reduced fecundity of cabbage aphids with the magnitude comparable to those obtained from organic solvents. These results augur well for the practical use of *T. minuta* as a source of effective and easily available botanical pesticide to resource-poor farmers against aphids in cabbage production. Based on the results from this study, we are planning to undertake a field-based experiment to evaluate *T. minuta* insecticidal activity against not just aphids but the whole insect herbivore complex of cabbage and other commonly grown vegetables in Lesotho. We also plan to study the insecticidal effects of whole plants of *T. minuta* when grown in an intercropping system with vegetables.

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