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Insecticidal Effects of *Schinus molle* against Woolly Aphids (*Eriosoma lanigerum*), Green Peach Aphids (*Myzus persicae*) and Citrus Aphids (*Aphis citricidus*) Under Laboratory Settings

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ABSTRACT

Eriosoma lanigerum, *Myzus persicae* and *Aphis citricidus* cause significant damage to ornamental and economically important plants. They are controlled by synthetic products, some with undesirable effects. *Schinus molle* (Anacardiaceae) is reported to have insecticidal properties against various pest insects. This study investigated the total phenolic content of *S. molle* leaf extracts and their effects against *E. lanigerum*, *M. persicae* and *A. citricidus* using direct application and contact assays. The total phenolic content was determined at 13.65 ± 0.75 mg GAE/g. Mortalities of *E. lanigerum* were recorded at 40, 65, 79, 86, and 95%, respectively. The determined LC_{50} and standard error of LC_{50} for direct and contact application were 0.047 w/v%, 0.047 ± 0.006 and 0.056w/v%, 0.056± 0.011, for *M. persicae*, respectively, whereas, for *A. citricidus* were 0.051w/v%, 0.051 ± 0.006 and 0.059w/v%, 0.059± 0.011, respectively. Mortalities of aphids exposed to *S. molle* leaf extracts were concentration dependent and direct application was more effective compared to contact. Therefore, *S. molle* can be concluded to have phenolic compounds and insecticidal properties against the evaluated aphids.

INTRODUCTION

Aphids are soft-bodied, sap-sucking insects belonging to the family Aphidae within the order Hemiptera (Singh and Singh, 2021). According to a study conducted by Favret *et al*. (2019), there are 5,558 recognized aphid species in 703 genera, arranged within 30 subfamilies in the world. Of these, 450 species of aphids are regarded as pests and are extremely difficult to control as most have complex life cycles and reproduce asexually through parthenogenesis (Le Trionnaire *et al*., 2008). Pest aphids significantly impact horticultural industries and household gardens as they destroy various economically important crops, grain and ornamental plants (Feldmann and Vogler, 2021). These pest aphids also serve as vectors for plant viruses and mold. They stunt plant growth and cause yellowing, wilting, curling and darkening of leaves (Agrios, 2008).

Eriosoma lanigerum (Hausmann, 1802) is an example of a polyphagous aphid that is known to feed on various plant species but particularly favors apple trees (Potter and Redmond, 2013). The feeding activity of *E. lanigerum* causes galls that destroy the growth

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of buds and disrupt the translocation of water and minerals (Adhikari, 2022). These galls impair the growth of plants and in severe cases, they push trees up from the ground (Urban, 2003). *Myzus persicae* (Sulzer, 1776) and *Aphis citricidus* (Kirkaldy, 1907) are polyphagous aphid species that feed on economically important plants such as cotton, cocoa, eggplants, cucumber, potato, sugar beet and citrus trees (Ebert and Cartwright, 1997; Ali, 2023). *Aphis citricidus* though polyphagous prefers citrus trees and it helps spread the Citrus tristeza virus, a cosmopolitan, economically important virus that annually kills millions of citrus trees (Sharma *et al*., 2021) *Myzus persicae* is known to infest more than 700 species of plants and is responsible for the spread of plant viruses such as turnip virus, yellow virus and potato virus A and Y (Hemming *et al*., 2022).

Aphid infestations are controlled through biological methods and the use of synthetic insecticides (Mateos Fernández *et al*., 2022). Insects such as *Aphelinus mali* (Haldeman, 1851) and lady bugs are natural enemies of *E. lanigerum* and *M. persicae* and are commonly deployed as biological control measures in areas infested with these pests (Bale *et al*., 2008). Synthetic insecticides are also used to control these pests. These products are usually made from organophosphates, vamidothions, triazamate and chlorpyrifos (Nicholas *et al*., 2003). These control measures have many limitations and disadvantages, for example biological control insects such as *A. mali* are affected by adverse climatic conditions and the lack of parasitism with woolly aphids (Boivin *et al*., 2012). The improper application of synthetic insecticides, on the other hand, harm helpful non-targeted organisms, accumulate in the flesh of fruits and they also run off into various natural habitations (Ore *et al*., 2023).

Plant and plant-based insecticides are reported to be safer and effective solutions for controlling pest insect infestations with minimal negative effects (Ahmed *et al*., 2022). *Schinus molle* (Linnaeus, 1753) is an ornamental tree that is native to the southern parts of South America. The plant is an evergreen tree with imparipinnate leaves and has green round small edible fruits that turn red when ripe (Murray *et al*., 2012). The plant is used for firewood and charcoal, and also used as timber. The tree is used by indigenous people as medicine to treat wounds and it has antiviral, anticancer and antimicrobial properties (Eryigit *et al*., 2017). The tree also has insecticidal repelling properties and has been reported to be effective on *Trogodema granarium*, *Tribolium castaneum*, *Bactericera cockerelli* and *Sitophilus zeamais* (Abdel-Sattar *et al*., 2010). However, a thorough scientific literature review search provides little information on the insecticidal potential of *S. molle* against *E. lanigerum*, *M. persicae* and *A. citricidus* a research gap which the current study aimed to address.

MATERIALS AND METHODS

Collection of *Schinus molle* and *Eriosoma lanigerum*:

Schinus molle leaves were collected from gardens located within the premises of Sefako Makgatho Health Sciences University (SMU), Pretoria, South Africa. The plant samples were collected in March 2024 and verified for taxonomical confirmation at the South African National Biodiversity Institute (SANBI) within the Botanical Gardens in Pretoria. White stinkwood (*Celtis africana*) leaves infested with *Eriosoma lanigerum* colonies were collected at Pretoria gardens and transported to the Department of Biology and Environmental Sciences laboratory at SMU in sealed containers.

***Myzus persicae* and *Aphis citricidus* Collection and Rearing:**

Last stage larvae of *Myzus persicae* colonies were collected from Pretoria gardens on tamarillo (*Solanum betaceum*) leaves and transported to the SMU laboratory in sealed containers. *Myzus persicae* were reared by inoculating five separate spinach plants with ten aphid specimens for a period of 15 days in a controlled environment of 25ºC and a 16:8 photoperiod. The same procedure was followed for the culturing of *Aphis citricidus*.

Chemicals:

All chemicals were of the highest purity (≥90). Sodium bicarbonate, gallic acid and Folin-Ciocalteu reagent were all purchased from Merk, USA.

Preparation of *Schinus molle* Extracts:

The leaves of *S. molle* were extracted using the maceration technique following the slightly modified guidelines of Koffi *et al*. (2010). The leaves were thoroughly washed with distilled water, air dried for a period of seven days, and on the eighth day, they were ground into powder using an electrical blender. The powdered leaf samples were sieved through a metal sieve with 0.125 mm openings, and fine materials were separated from the course and weighed at 100 g. The weighted fine powered leaf samples were transferred into a volumetric flask filled with 1000 ml of 80% ethanol and the mixture was transferred to an orbital shaker and rotated for a period of 24 hrs at 120 rpm. The leaf extracts mixture was then filtered through 11 µm filter paper (Whatman No. 1). The filtrates were evaporated in a water bath at a temperature of 40ºC and the remaining substrates were stored in dry clean sealed containers.

Quantitative Phenols Test of *S. molle* Leaf Extracts:

The total phenolic content within *S. molle* leaf extracts was determined using the Folin-Ciocalteu reagent following the guidelines of Madhu *et al*. (2016) with slight modifications. Concentrations of 20, 30, 40, 50, 60, and 80 $mg.ml^{-1}$ of standard gallic acid were each measured in triplicate using the spectrophotometer, and their absorbance was used to plot a standard curve. The standard curve showed a linear regression of $y = 0.004x + 0.487$, $R^2 = 0.9503$ (Fig. 1) and was used to calculate the total phenolic content, expressed as mg of gallic acid per gram of extract (GAE/g).

Schinus molle leaf extracts of 100 mg were dissolved in 100 ml of distilled water, and 0.1 ml of the mixture was added to a 100 ml volumetric flask containing 75 ml of distilled water. Thereafter, 0.5 ml Folin-Ciocalteu reagent and 1 ml of sodium bicarbonate were added to the mixture. The aliquot was taken to the spectrophotometer, and the absorbance was measured at 760 nm. The estimation of the phenolic content in the leaf extract was carried out in triplicate.

Insecticidal Effects of Leaf Extracts of *Schinus molle* on *Eriosoma lanigerum:*

Insecticidal effects of *S. molle* leaf extracts against *E. lanigerum* were conducted following Potter's tower direct application method Potter (1952) and the guidelines of Ma *et al*. (2021) and Šarac *et al*. (2024) with slight modifications. Five *C. africana* leaves, each infested with similar *E. lanigerum* colonies, were placed into six separate containers sealed with cotton mesh for aeration.

The varying containers were treated with 1 ml of specific aqueous concentrations of *S. molle* leaf extracts of 0.04, 0.05, 0.06, 0.07, and 0.08 w/v%, applied as a fine mist using separate spray bottles. The sixth container served as a negative control and received only distilled water. After 24 hours of exposure, the aphid colonies on leaf samples were removed from the containers and observed under a light Nikon dissection microscope. The exposed aphids were then after, gently brushed with a fine camel-hair brush, and those that exhibited no signs of movement were considered dead. *E. lanigerum* mortalities were recorded as percentages, and each experimental trial was carried out in triplicate.

Insecticidal Effects of Leaf Extracts of *Schinus molle* on *Myzus persicae and Aphis citricidus* Using the Direct Application Method:

Spinach leaves and stalks infested with apterous *M. persicae* were harvested from the laboratory-maintained stock. Ten unsexed aphids were randomly selected underneath a Nikon dissection light microscope and transferred into six separate petri-dishes using a fine camel-hair brush. The first five petri dishes were treated with various aqueous *S. molle* leaf extract concentrations of 0.04, 0.05, 0.06, 0.07, and 0.08 w/v%, respectively. The sixth petri dish served as a negative control and received only distilled water. The concentrations were applied as 1 ml fine spray mist using separate spray bottles following Potter's tower application method (Potter, 1952), as well as the guidelines of Ma *et al*. (2021) with slight modifications. After 24 hours of exposure, aphid mortality was assessed under the Nikon dissection light microscope. The aphids were gently brushed with a fine camel-hair brush and those that displayed no signs of movement were considered dead. The procedure was carried out in triplicate and repeated for *A. citricidus* using the same protocol.

Insecticidal Effects of Leaf Extracts of *Schinus molle* on *Myzus persicae and Aphis citricidus* Using Contact Assay Method:

The insecticidal activity of *S. molle* leaf extracts against *M. persicae* was also assayed using the contact assay method following guidelines of Hyder *et al*. (2022) with slight modification. Serial concentrations of aqueous *S. molle* leaf extracts of 0.04, 0.05, 0.06, 0.07, and 0.08 w/v%, and negative control of distilled water at a volume of 5 ml were impregnated onto six separate filter papers. The filter papers were then evaporated by air drying for 15 minutes and placed into separate petri dishes. Subsequently, ten apterous *M. persicae* were transferred into the experimental petri dishes and each experimental trial was carried out in triplicate and tested for 24 hours. Post experimental trials termination, the aphids were observed for mortality records under a light Nikon dissection microscope. The same procedure was similarly repeated for the insecticidal activity of *S. molle* leaf extracts against *A. citricidu*s.

Data Analysis:

The total phenolic content within *Schinus molle* leaf extracts was analyzed using standard deviation and represented as means ± standard error of mean. Aphid mortality percentages were recorded 24 hours post-treatment and corrected using Abbott's formula (**Abbott, 1925**). The median lethal concentration (LC_{50}) values were calculated using probit analysis according to the method of Finney and Stevens (1948). The final mortality data for *Myzus persicae* and *Aphis citricidus* exposed to *S. molle* leaf extracts were reported as means ± standard error of the mean.

RESULTS

The total phenolic content within *Schinus molle* leaf extracts was determined at 13.65 ± 0.75 mg GAE/g (Table 1).

Table 1. Total phenolic content of *Schinus molle* leaf extracts.

Sample	Total phenolic content
***S. molle* leaves**	13.65 ± 0.75

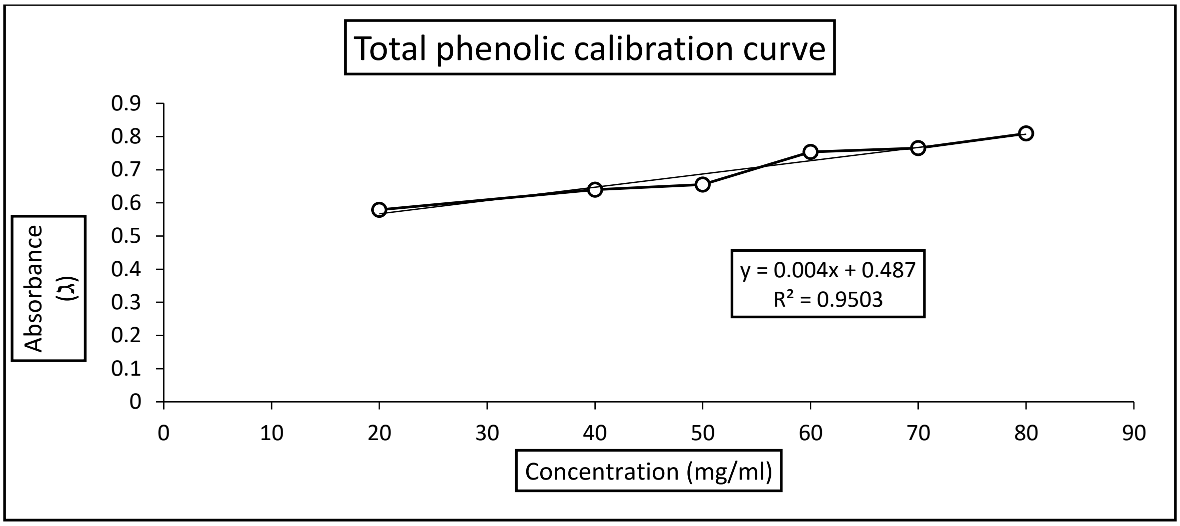

Fig. 1. Standard calibration curve for estimation of total phenolic content for standard gallic acid (GAE/mg).

The mortality percentages of *Eriosoma lanigerum*, *Myzus persicae* and *Aphis citricidus* exposed to *S. molle* leaf extracts increased with increasing concentrations. Additionally, *M. persicae* and *A. citricidus* were more susceptible to *S. molle* leaf extracts compared to *E. lanigerum* (Tables 2, 3 & 4). The direct application method applied against *M. persicae* and *A. citricidus* using various *S. molle* leaf extracts yielded higher mortality percentages compared to the contact assay method (Tables 3 & 4).

The mortality percentages of *E. lanigerum* exposed to *S. molle* leaf extracts were recorded at 40, 65, 79, 86, and 95%, respectively (Table 2). The *S. molle* leaf extract concentration of 0.08% deployed against *M. persicae*, *A. citricidus* and *E. lanigerum* using the direct application method yielded 100, 100 and 95% mortality, respectively.Whereas the same concentration exposed to *M. persicae* and *A. citricidus* using the contact assay method yielded 80% mortality for both aphid species (Tables 3 & 4). *E. lanigerum*, *M. persicae* and *A. citricidus* in all of the control experimental trials survived for over 24 hrs (Tables 2, 3 & 4).

Table 2. Insecticidal effects of leaf extracts of *Schinus molle* on *Eriosoma lanigerum.*

Sample no	Concentration	Mortality % of apterous *Eriosoma lanigerum*	
0	Negative control	Before	25
		After	25
		Mortality	0%
1	0.04%	Before	30
		After	12
		Mortality	40%
2	0.05%	Before	31
		After	20
		Mortality	65%
3	0.06%	Before	33
		After	26
		Mortality	79%
4	0.07%	Before	35
		After	30
		Mortality	86%
5	0.08%	Before	37
		After	35
		Mortality	95%

Table 3. Insecticidal effects of leaf extracts of *Schinus molle* on *Myzus persicae* and *Aphis citricidus* using the direct application method.

Sample no	Concentration	No of tested aphids	*Myzus persicae* Mortality %	*Aphis citricidus* Mortality %
0	0	10	0	0
1	0.04%	10	30%	20%
2	0.05%	10	50%	40%
3	0.06%	10	60%	60%
4	0.07%	10	80%	90%
5	0.08%	10	100%	100%

Table 4. Insecticidal effects of leaf extracts of *Schinus molle* on *Myzus persicae* and *Aphis citricidus* using the contact assay method.

Sample no	Concentration	No of tested aphids	*Myzus persicae* Mortality %	*Aphis citricidus* Mortality %
0	0	10	0%	0
1	0.04%	10	20%	20%
2	0.05%	10	40%	30%
3	0.06%	10	60%	40%
4	0.07%	10	70%	60%
5	0.08%	10	80%	80%

The LC_{50} of *S. molle* leaf extracts against *M. persicae* and *A. citricidus* were recorded at 0.047 and 0.051 w/v% for the direct application method, respectively. Whereas, for the contact assay method, it was recorded at 0.056 and 0.059 w/v% for *M. persicae* and *A. citricidus*, respectively. The standard error LC_{50} of the mean of *S. molle* leaf extracts against *M. persicae* using the direct application and contact assay methods was determined at 0.047 ± 0.006 and 0.056± 0.011, with a confidence interval of 0.041-0.053 and 0.045-0.067, respectively (Table 5). For *A. citricidus* it was determined at 0.051 ± 0.006 and 0.059± 0.011, with a confidence interval of 0.045-0.023 and 0.058-0.060 for direct application and contact assay methods, respectively (Table 6).

Table 5. Insecticidal effects of leaf extracts of *Schinus molle* on *Myzus persicae* using the contact assay and direct application method in terms of LC_{50} values and 95% confidence interval.

Method type	LC_{50}	Standard error of LC_{50}	Confidence interval
Direct application method	0.047	0.047 ± 0.006	0.041-0.053
Contact assay method	0.056	0.056± 0.011	0.045-0.067

Table 6. Insecticidal effects of leaf extracts of *Schinus molle* on *Aphis citricidus* using the contact assay and direct application method in terms of LC_{50} values and 95% confidence interval.

Method type	LC_{50}	Standard error of LC_{50}	Confidence interval
Direct application method	0.051	0.051 ± 0.006	0.045-0.023
Contact assay method	0.059	0.059± 0.011	0.058-0.060

DISCUSSION

The current study reports on the presence of phenolic content within *Schinus molle* leaf extracts. These findings support previous scientific studies that documented the presence of phenolic compound within various plant parts of *S. molle*. For instance, a South African study, by Bvenura and Kambizi (2022) investigated the composition of phenolic compounds in *S. molle* berries and reported three phenolic acids, a phenolic acid glucoside, and three phenolic acid esters. Another study by Lamboro *et al*. (2020) evaluated the phytochemical composition, characterization and antimicrobial activity of *S. molle* seed essential oils. The authors revealed that the seed essential oils of the plant, through preliminary screening contain various phytochemical constituents, including phenolic compounds.

Additionally, the results of the current study endorse existing scientific reports that document the abundance of phenolic compounds within the leaves of *S. molle*. A study by Volpini-Klein *et al*. (2020) evaluated the effects of the leaf and fruit extracts of *S. molle* on oxidative stability of some vegetable oils under accelerated oxidation. The authors determined the total phenolic content within the leaves and fruits of *S. molle* through quantitative analysis at 601.1 ± 0.6 and 127.9 ± 0.1 mg ±SD, respectively. The values reported by Volpini-Klein *et al*. (2020) are significantly higher than the 13.65 ± 0.75 mg GAE/g observed in the current study. The discrepancy between the two studies could be attributed to differences in extraction methods, Volpini-Klein *et al*. (2020) determined total phenolic content in essential oils whereas, the current study used ethanol extracts.

The current study also provides evidence that *S. molle* leaf extracts are effective against three aphid species namely, *E. lanigerum, M. persicae and A. citricidus.* Additionally, these findings according to thorough scientific literature research, are first to report on the insecticidal properties of *S. molle* against these specific aphid species. The results of the current study further endorse previous scientific studies that report on the insecticidal properties of *S. molle* against insects of the order Hemiptera. A study by Hussein *et al*. (2021), reported that *S. molle* leaf essential oils applied using the leaf dipping method were effective against *Aphis nerii*. The authors revealed that the plant's leaf essential oils achieved an LC_{50} against *A. nerii* at a concentration less than 1 mg/L.

In another study, Salas *et al*. (2022) tested *S. molle* essential oil as a biocide against the potato aphid (*Macrosiphum euphorbiae*) using the fumigation assay and contact assay. Their study revealed that the plant was effective against the aphid species. The authors determined the LD_{50} at 320.51 µl/L and 5.14 mg/l, using fumigation and contact assay, respectively. The current study determined LC_{50} of *S. molle* leaf extracts against *M. persicae* at 0.047 and 0.051 w/v%, and 0.056 and 0.059 w/v%, for *A. citricidus* through the direct and contact assay, respectively. These values are significantly lower than the LD_{50} and LC_{50} values reported by Hussein *et al*. (2021) and Salas *et al*. (2022). This may suggest that *S. molle* leaf extracts used against *E. lanigerum, M. persicae,* and *A. citricidus* are less effective than the essential oils used in the above-mentioned studies. This discrepancy could be attributed to the difference in examined aphid species and the usage of essential oils and aqueous solvent extracts.

The results of the current study reveal that the direct application method was more effective in inducing mortality in aphids compared to the contact assay method. The results of the current study support the findings of Lami *et al*. (2024), who investigated the effects of natural insecticides against green peach aphid (*Myzus persicae*) and its natural enemies *Propylea quotuordecimpuntata* and *Aphidus colemani*. The authors reported that the direct application method yielded a higher mortality percentage compared to the contact assay method. The insecticidal potency observed in the current study may be attributed to the presence of phenols in *S. molle*. Phenols are reported to have insecticidal by disrupting metabolic processes, physiological composition alterations and causing behavioral changes in aphids (Czerniewicz *et al*., 2017). The presence of phenols within *S. molle* may explain why the plant was effective against *E. lanigerum*, *M. persicae* and *A. citricidus*.

CONCLUSION

Therefore, it can be concluded that the leaves of *S. molle* have phenolic compounds and insecticidal properties against *E. lanigerum* and *M. persicae A. citricidus*. The plant has the potential to be a source of phenolic compounds, which can be deployed in medicinal and pharmaceutical fields. Additionally, the plant could potentially be used as a cheap and safe insecticide to control aphids however, the plant needs to be evaluated for its toxicological effects and biodegradability before field application.

Declarations

Ethical Approval: The current project was approved by the Sefako Makgatho Health Sciences University Research Ethics Committee, No. SMUREC/M/293/2019: PG. All applicable guidelines for the care and use of experimental animals were followed.

Competing Interests: The authors report no conflict of interest.

Authors' Contributions: M.C.M did the conceptualization, methodology, data collection, sample analysis, validation, data curation and writing of the initial draft. P.H.K also did the conceptualization, writing-review and editing. All authors read and approved the final manuscript.

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