

Bio Efficacy of Aqueous Crude Fruit Sap Extract of *Solanum incanum* against Green Peach Aphids *Myzus persicae* Sulzer (Homoptera: Aphididae)

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Abstract

Conventional insecticides are expensive and arguably associated with various severe adverse side effects hence the need to develop botanical pesticides that are effective as alternative. Though *S. incanum* fruits sap has been used by the local farmers to control aphids, literature show no scientifically investigated report of its effectiveness and the mode of its insecticidal action. This study was therefore designed to evaluate anti-aphids properties of crude fruit sap extract of *S. incanum*. A total of 180 kales were planted in the Plant Transformation Laboratory of Kenyatta University. They were divided into six groups with 10 plant stands each. Each group was subjected to routine spray with 10, 25, 50 and 75% *S. incanum* extract. Dimethoate was utilized as reference insecticide while the last group was not sprayed. The number of live and dead aphids was counted for two weeks after every spray in each group with an interval of one day to determine the deterrent and insecticidal activity. The extract at different tested concentrations showed some level of insecticidal and deterrent activities against green peach aphid. Further, phytochemical screening results showed that the crude fruits sap extract of *S. incanum* have phytochemicals associated with insecticidal and deterrent activity. The study has established that the crude fruits sap extract of *S. incanum* are effective in managing insects' pest.

Keywords: *Solanum incanum*; Dimethoate; Aphids; Phytochemical

Introduction

Aphids are small, soft-bodied insects mostly ranging from 1.5 to 3.5 mm in length [1]. They feed on plants with their piercing and sucking mouthparts. Beside the mechanical damage caused by aphids, they also serve as the largest group of vectors of plant viruses [2]. The damage is further compounded by fouling of the host plant with honeydew excreted from the anus. Apart from having an influence on predators and parasitoids, the honeydew serves as a substrate for the growth of fungal complexes that cause sooty mould. In addition to reducing the photosynthetic ability of plants, sooty mould reduces a plant's aesthetic market value [3].

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphididae) is a worldwide aphid species which are responsible for important economic losses [4]. It is feeding on more than 50 plant families [1], causing many losses to agro industrial crops (including potato, sugar beet and tobacco), vegetables (including kales, cabbage, and spinach), horticultural crops (including plants of Brassicaceae, Solanaceae and Cucurbitaceae families) and stone fruits (peach, apricot, and cherry, among others) and it is presently categorized as one of the most important agricultural pests worldwide [4].

Various methods have been attempted in the control of *Myzus persicae* which include using a strong jet of water from a hose which dislodges aphids, but it has not been practical for the soft stemmed annuals [5], use of ash mixed with water in vegetable garden, intercropping of plants with lemon grass and onions but this has not been practical in large scale production and the use of both hard and soft chemicals.

The protection of plants from insect damage is currently dependent on synthetic pesticides [6]. The repeated use of synthetic insecticides for insect pests and vectors control has disrupted natural biological control systems. It has also resulted in the development of resistance, undesirable effects on non-target organisms and fostered

environmental and human health concern, which initiated a search for alternative control measures [7].

Botanical insecticides such as pyrethrum, nicotine and rotenone, among others, have been extensively used until recently for insect control. It has been reported that many compounds with insecticidal potential have been isolated from the genus Piper – Piperide, isolated from *Piper nigrum* (black piper) has been found to be just as active against adjuki bean weevils as the pyrethroids [8]. It has also been reported that essential oils of leaf and bark of some plants demonstrated high larvicidal and insecticidal activity against insect pests [9]. Limonoids such as azadirachtin and gedurin present in species from the Meliaceae and Rutaceae are recognized for their toxic effects on insects and are used in several insecticide formulations in many parts of the world [10].

Solanum incanum is a perennial, wild shrub like herb that belongs to family Solanaceae, which grows in many regions of Africa, Middle East and Far East Asia. It is an erect or spreading perennial shrub with leaves and stem occasionally having small prickles. The fruits are small berries of 2-3 cm in diameter and yellowish orange or brown in color when ripe [11]. It is common as a weed around houses, in overgrazed

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grass land and on road sides. It is also found at forest edges and in bush land and grasslands, from sea-level up to 2500 m altitude [12].

Despite the extensive use of the fruits sap extract of *S. incanum* by the local farmers to control aphids, there is no scientific documentation that shows its effectiveness and the mode of insecticidal action. Therefore, this study was carried out to evaluate bio efficacy of aqueous crude fruit sap extract of *S. incanum*.

Materials and Methods

Sample collection and preparation of the plant material

Solanum incanum fruits used for this study were collected from their natural habitats after information from local farmers. They were collected with bioconservation aspects in mind from Mbeere North sub-county, Embu County. The specimen was taken for taxonomic authentication by an acknowledged botanist and a voucher specimen was deposited at Kenyatta University herbarium. A traditional medical practitioner provided information on the plant and the precise location where it grows.

Extraction procedure

The fruits were washed thoroughly with distilled water to remove any dirt and debris. The ripen fruits of *S. incanum* was weighed and sliced to separate the pericarp from seeds and sap. The seeds and sap collected from 1 kg of fresh ripe fruits of *S. incanum* (400 ml) was diluted with 100 ml distilled water and sieved to remove the seeds and the sap extract collected in a beaker. The resulting sap extract was then put in 50 ml falcon tubes and stored at 4°C for later use in bioassay.

Preparation of extract concentrations

This study employed different bioassay dose of the aqueous crude fruits sap extract of *S. incanum*. These doses include 75%, 50%, 25% and 10% crude extract. They were sprayed on kales plants to determine the optimal dose that has insecticidal and deterrent activities against green peach aphids. The 75% dose was prepared by diluting 75 ml crude fruits extract of *S. incanum* with 25 ml of distilled water; 50% dose was prepared by diluting 50 ml of crude fruits extract of *S. incanum* with 50 ml of distilled water; 25% dose was prepared by diluting 25 ml of the extract with 75 ml of distilled water; and 10% dose was prepared by diluting 10 ml of the extract with 90 ml of distilled water.

Preparation of plants

Seeds of kales were purchased from local agrovet shops. Five seeds were planted in each of the 90 pots and arranged in a Completely Randomized Design in the plant transformation laboratory of Kenyatta University. Upon germination, they were thinned to two seedlings per pot thereby totaling to 180 plant stands, which were used in the study.

This thinning enables the plants to be strong and achieve considerable maturation heights capable of infestation by aphids.

Experimental design

The kales were divided into six groups with 10 plants each. Group one was subjected to routine spray with 75% dose of crude fruits sap extract of *S. incanum*; group two was sprayed with 50% dose extract; group three was sprayed with 25% dose extract; group four was sprayed with 10% dose extract. Group five was sprayed with dimethoate and group six was sprayed with water. The experimental was conducted in triplicate.

Determination of deterrent and insecticidal activity

To determine the deterrent properties of the aqueous crude fruits sap extract of *S. incanum*, the number of live aphids was counted in each group for two weeks after every spray. The insecticidal properties of extracts were determined by counting the number of dead aphids for two weeks after every spray in each group. The data obtained was compared with the results of the negative and normal controls.

Qualitative phytochemical screening

The crude fruit sap extract of *S. incanum* was subjected to qualitative phytochemical screening to identify presence or absence of selected chemical constituents using methods of analysis as described in refs. [13,14]. Standard screening tests for detecting the presence of different chemical constituents were employed. Secondary metabolites tested included alkaloids, saponins, steroids, cardiac glycosides, terpenoids, flavonoids, phenolics, and tannins.

Data management and statistical analysis

Analysis of the data was done using SAS statistical software. The results were expressed as mean \pm standard error of mean (SEM) for analysis. Statistical significance of difference among groups were analysed using One-Way Analysis of Variance (ANOVA) followed by Tukey's tests to separate the means and obtain the specific significant differences among the different groups. The values of $P \leq 0.05$ were considered to be significant.

Results

Insecticidal activity of crude fruits sap extract of *S. incanum* against green peach aphids

Generally, crude fruit sap extract of *S. incanum* showed insecticidal activity against green peach aphids for all the extract concentrations tested. This was indicated by the mean number of dead green peach aphids counted in the experimental and control groups after spraying. As Table 1 shows, different concentrations of crude fruits sap extract

Days	10%	25%	50%	75%	DMT	Control
Day 1	99.7 \pm 22.5 ^a	99.7 \pm 28.2 ^a	127.7 \pm 41.9 ^a	143.7 \pm 20.8 ^a	169.0 \pm 9.1 ^a	11.7 \pm 6.6 ^b
Day 3	88.7 \pm 12.0 ^a	56.3 \pm 18.7 ^a	90.0 \pm 24.6 ^a	105.3 \pm 7.3 ^a	52.7 \pm 22.8 ^a	17.3 \pm 2.3 ^b
Day 5	60.7 \pm 18.9 ^a	71.3 \pm 18.5 ^a	58.3 \pm 12.0 ^a	68.3 \pm 9.0 ^a	75.0 \pm 63.0 ^a	8.0 \pm 1.0 ^b
Day 7	46.3 \pm 23.8 ^a	47.0 \pm 11.5 ^a	61.3 \pm 23.6 ^a	53.3 \pm 1.9 ^a	29.3 \pm 11.3 ^a	5.3 \pm 1.5 ^a
Day 9	23.0 \pm 8.6 ^a	40.3 \pm 10.7 ^a	42.0 \pm 22.4 ^a	32.0 \pm 4.0 ^a	3.3 \pm 2.0 ^b	3.7 \pm 3.7 ^b
Day 11	20.0 \pm 6.0 ^a	19.3 \pm 4.7 ^a	16.7 \pm 4.9 ^a	21.0 \pm 3.5 ^a	0.0 \pm 0.0 ^b	3.3 \pm 3.3 ^b
Day 13	12.3 \pm 3.9 ^a	9.7 \pm 0.9 ^{ab}	14.3 \pm 1.8 ^a	8.3 \pm 2.7 ^{ab}	0.0 \pm 0.0 ^b	6.7 \pm 4.2 ^{ab}

- Values are expressed as Mean \pm SEM of three replicates. Values with the same alphabet within the same row are not significantly different by ANOVA followed by Tukey's post hoc test ($p < 0.05$).
- DMT – Dimethoate™

Table 1: Insecticidal activity of crude fruits sap extract of *S. incanum* against green peach aphids.

of *S. incanum* and dimethoate induced significantly higher green peach aphids mortalities than the control (untreated group) on day one and day three after spraying ($P < 0.05$). However, no significant differences in the mortality of green peach aphids was detected in the different concentrations of the crude fruits sap extract of *S. incanum*, dimethoate and the control groups on five and seven days after spraying ($P > 0.05$; Table 1).

Nine and eleven days after spraying, different concentrations of crude fruit sap extract of *S. incanum* showed significantly higher mortalities than dimethoate and control ($P > 0.05$; Table 1). The order of the insecticidal activity of the four different concentrations was $75 > 50 > 25 > 10\%$. On the other hand, different concentrations of the extract and dimethoate exhibited significantly higher percentage mortalities of the green peach aphids on days one and three after spraying ($P < 0.05$; Figure 1). However, no significant differences in the mortality of the green peach aphids was observed in the different concentrations of the extract, dimethoate and the control on day five after spraying ($P > 0.05$; Figure 1).

Similarly, the different concentrations of crude fruits sap extract of *S. incanum* showed significantly higher percentage mortalities of green peach aphids than the reference insecticide and the control nine and eleven days after spraying ($P < 0.05$; Figure 1). However on thirteenth day after spraying, the different concentrations of the extract were significantly higher than dimethoate (Figure 1). The activities of the

crude fruit sap extract of *S. incanum* decreased proportionately from first to thirteenth day after spraying.

Deterrent activity of crude fruit sap extract of *S. incanum* against green peach aphids

Field tests were carried out to evaluate the deterrent activity of *S. incanum* crude fruits sap extract against green peach aphids. Deterrent activity was determined by subtracting the number of live aphids from the total number of aphids counted after spraying. Generally, all the concentrations of the crude fruits sap extract of *S. incanum* showed some deterrent activity against green peach aphids. The deterrent activity of crude fruits sap extract of *S. incanum* was not significantly different from dimethoate and control on day one after spraying. However, the deterrent activity of crude fruits sap extract of *S. incanum* and dimethoate were significantly lower than that of the control (untreated group) on day three after spraying ($P > 0.05$; Table 2).

Five and seven days after spraying, there were no statistically significant differences in the deterrent activities of the extract and the reference insecticide (Dimethoate) as compared to the untreated group ($P > 0.05$). Nine days after spraying, different concentrations of crude fruits sap extract of *S. incanum* extract and the control group showed significantly higher deterrent activity compared to dimethoate ($P < 0.05$; Table 2). However, the deterrent activities of the different concentrations of crude fruits sap extract of *S. incanum* and the

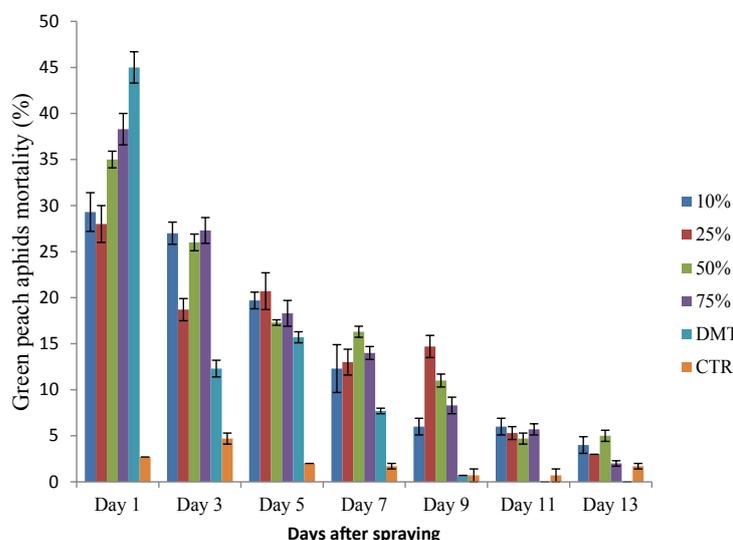


Figure 1: Effect of crude fruits sap extract of *S. incanum* on percentage mortality of green peach aphid.

Days	10%	25%	50%	75%	DMT	Control
Day 1	9.2 ± 2.7 ^a	13.3 ± 4.5 ^a	-8.6 ± 19.3 ^a	16.7 ± 5.7 ^a	1.7 ± 0.8 ^a	0.0 ± 0.0 ^a
Day 3	-0.7 ± 4.0 ^a	-12.7 ± 15.7 ^a	6.7 ± 31.2 ^a	27.7 ± 19.1 ^{ab}	-25.0 ± 3.8 ^a	61.0 ± 19.2 ^b
Day 5	25.0 ± 33.5 ^a	41.0 ± 42.1 ^a	-11.7 ± 19.8 ^a	-7.6 ± 27.7 ^a	-30.0 ± 19.7 ^a	45.3 ± 2.7 ^a
Day 7	20.0 ± 21.1 ^a	8.3 ± 12.3 ^a	36.0 ± 81.2 ^a	37.0 ± 24.0 ^a	-9.0 ± 10.5 ^b	33.7 ± 6.5 ^a
Day 9	15.0 ± 5.2 ^a	21.3 ± 15.7 ^a	9.0 ± 0.5 ^a	5.0 ± 7.2 ^a	0.0 ± 0.0 ^a	38.0 ± 11.2 ^b
Day 11	9.3 ± 3.8 ^a	2.7 ± 6.1 ^a	9.3 ± 4.8 ^a	0.3 ± 1.4 ^a	0.0 ± 0.0 ^a	31.3 ± 8.9 ^b
Day 13	-3.7 ± 2.6 ^a	-0.3 ± 2.7 ^a	-2.0 ± 6.4 ^a	-0.7 ± 0.6 ^a	0.0 ± 0.0 ^a	17.3 ± 4.0 ^b

- Values are expressed as Mean ± SEM of three replicates. Values with the same alphabet within the same row are not significantly different by ANOVA followed by Tukey's post hoc test ($p < 0.05$). Negative values indicate no deterrent activity.
- DMT – Dimethoate™,

Table 2: Deterrent activities of crude fruits sap extract of *S. incanum* against green peach aphids.

untreated group were significantly higher than dimethoate eleven and thirteen days after spraying. The results showed that 75% *S. incanum* extract concentration performed better than all other concentrations (Table 2). Dimethoate has no deterrent activity against green peach aphids.

Qualitative phytochemical screening

A number of compounds were found in the crude fruits sap extract of *S. incanum*. As Table 3 shows, the extract contained alkaloids, saponins, cardiac glycosides, terpenoids, flavonoids and tannins while steroids and phenolics were absent.

Discussion

The present study was designed to evaluate the insecticidal and deterrent activity of aqueous crude fruit sap extract of *S. incanum* against green peach aphids. After the first day of the test period, the crude fruits sap extract of *S. incanum* produced appreciable insecticidal activity against green peach aphids. The crude fruits sap extract of *S. incanum* at dose level 75% and 50% demonstrated the highest insecticidal activity of 144 and 128 mean number of dead aphids respectively (Table 1). Similar work carried out by Haile and Raja [15] demonstrated effective insecticidal activities of plant extracts of *Croton macrostachys* and *Schinus molle* against cabbage aphids *Brevicoryne brassicae*. In addition, related results were also observed by Abdalla et al. [16] who demonstrated insecticidal activity of neem extract (*Azadirachta indica*) under laboratory and field conditions against various insects.

The deterrent activity of crude fruit sap extract of *S. incanum* may be associated to the odour of the plant extract. Mersha et al. [17] reports that the repellent activity of *Mentha piperita* (L.) against cabbage aphids was as a result of the odour of aromatic plant, *Mentha piperita*. The insecticidal and deterrent activities of crude fruit sap extract of *S. incanum* could be due to one or more groups of active principle(s) present in the extract. Qualitative phytochemical screening revealed that crude fruit sap extract of *S. incanum* contain alkaloids, saponins, cardiac glycosides, terpenoids, flavonoids and tannins. These phytochemicals are important for mediating interactions between plants and their biotic environment and do not have apparent function in physical or biochemical processes [18]. A number of these phytochemicals have been shown to have insecticidal and deterrent activities against various insects [7,19].

Alkaloids are cyclic organic compounds containing nitrogen in a negative oxidation state; they are widely distributed in plant kingdom [20] and constitute an interesting family synthesized and accumulated by several plants [21]. Alkaloids have previously been reported as the most important group of natural substances playing an important role

in the ecology of organisms which synthesize them. For example, it has been suggested that they constitute part of the plant defenses against phytophagous animals along with terpenoids, phenols, flavonoids and steroids [22-24].

Those compounds are insecticidal at low concentrations, their mechanism of action differ, but many affect acetylcholine receptors in the nervous system (for instance nicotine) or membrane sodium channels of nerves (for instance veratrin) [25]. A research conducted by Fatma and Bahia [18], reports that alkaloids interfere with neuroendocrine control by inactivating the acetylcholinesterase in treated larvae. A study conducted by Mukandiwa [26] reports that acetone leaf extracts of *Aloe zebrina*, *Clausenaanisata*, *Erythralyssystemon* and *Spirostachys africana*, induced anomalies in the blowfly such as paralysis, prolongation of the prepuparium stage and pupal malformations. These researchers argued that the plants probably contain compounds which interfere with the neuroendocrine control mechanisms in the blowfly.

The insecticidal and deterrent activity of crude fruit sap extract of *S. incanum* may also be attributed to the presence of saponins, which are associated with the alterations in the feeding behaviour, molting process, interaction with hormones that regulate the growth and causing death at different stages of development [27-29]. According to Marianna et al. [30], the insecticidal activity of saponins is attributed to their wide spectrum of action and its amplitude of physiological impacts.

Flavonoids have been previously shown to possess good insecticidal activities. For example Shripad et al. [31] reported insecticidal and antimicrobial activities of flavonoids isolated from *Ricinus communis* against *C. chinensis*. The insecticidal activity may be attributed to the synergic effects of phenolic compounds which include flavonoids [32,33]. Therefore, the presence of flavonoids in the crude fruits sap extract of *S. incanum* may be contributory to its insecticidal activity.

Conclusion

In conclusion, the present study has demonstrated the insecticidal and deterrent activities of crude fruit sap extract of *S. incanum*. The significant reduction in the number of green peach aphids from the first to thirteenth day of treatment with standard insecticides as well as different doses of the extracts, reflect that crude fruits sap extract of *S. incanum* is endowed with potent insecticidal activity. According to this study, it is possible to assume that *S. incanum* fruits sap extract may contain some components that are functionally or structurally similar to dimethoate.

Furthermore, the classes of phytochemicals in crude fruit sap extract of *S. incanum* have previously been observed to have insecticidal and deterrent activity. Therefore, the crude fruits sap extract of *S. incanum* might help in preventing insects pests and serve as good bio- resource for generating readily available botanical pesticide that is more effective and probably cheaper than the conventional insecticides. The present study, therefore, scientifically confirms and supports the traditional use of crude fruit sap extract of *S. incanum* for management of insects' pest.

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Test	Result
Alkaloids	+
Saponins	+
Steroids	-
Cardiac glycosides	+
Terpenoids	+
Flavonoids	+
Phenolics	-
Tannins	+

+: Present -: Absent

Table 3: Qualitative phytochemical screening of crude fruit sap extract of *S. incanum*.

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