



Bio-Insecticidal Efficacy of *Aegle Marmelos* Against *Myzus Persicae*

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Article History	Abstract
Received: 06 June 2023 Revised: 05 Sept 2023 Accepted: 11 Aug 2023	<p><i>Myzus persicae</i> (Green peach aphids) has wide range of distribution in different plant hosts. As a pest it causes adverse effect to crops both directly and indirectly by transmitting different viruses and also by producing honey dew. <i>Myzus persicae</i> transmits almost 100 different viral diseases of plants on thirty different plant families which include some major crops like, sugar cane, tobacco, paddy, citrus, sugar beet etc. <i>Myzus persicae</i> is cosmopolitan in distribution. This insect pest causes damage to wide range of economically important crops and their control is usually dependent upon chemicals, intensive use of which may develop resistance in them. During this experiment botanical pesticide is used as natural control measure and tested its efficacy against Green peach aphids at different concentrations. The plant used is <i>Aegle marmelos</i>. Plant parts such as stem and leaves were used to prepare Boiling and Soxhlet extract. Each extract of plant was individually tested against the aphids' nymph which gave maximum mortality of about 97.7% results at the concentration of 500 ppm. The use of this botanical pesticide helps to eradicate the crop pests by an eco-friendly manner.</p>
CC License CC-BY-NC-SA 4.0	<p>Keywords: <i>Myzus Persicae</i>, <i>Aegle Marmelos</i>, Botanical Pesticide, Boiling Method, Soxhlet Method</p>

1. Introduction

Oryza sativa (Rice) is one of the most important food crops globally. Highest production of rice occurs in China. China is the second largest country in rice production. Beside China Paddy also gets cultivated in different countries like India, Japan, Indonesia, and other parts of Asia. In country different regions have different seasons of rice cultivations.. The rice can be cultivated in any kind of soil provided the humidity should be very high. Majority of the time the soil required for the rice cultivation is red soil, Black soil and red sandy soil etc⁷.

Different species of Aphids are found near irrigated rice, the aphids belong to 17 genera of 37 species are considered. Aphids namely *Aphis craccivora* Koch along with *Myzus persicae* are considered as rice aphids¹¹. *Myzus persicae* is considered as one of the most important aphid pests globally. This pest has the ability to transmit 100 different plant viruses and beside that it can also affect different parts of the plant directly. The control of this aphid pest is mostly dependent upon chemical methods which lead to the development of resistance².

The recent impact due to the increasing use of synthetic pesticides on non-targeted organisms generated the higher demand for alternative products with lesser environmental effect. This demand moreover led to formulate the plant based Organic compounds as the source of bio pesticides. The essential oils were extracted from the Alaskan yellow Cedars, (*Cupressus nootkatensis*) have significant level of activity against imported fire ant workers. Further analysis revealed that ants were repelled by nootka oils. The test was performed to analyse the effect of the bio-oil where the ants were treated with nootka oils prior to that the control was established in the Laboratory. In addition, nootka

oil could be beneficial in situations where ant suppression rather than entire quarantine elimination is the management goal¹.

Aegle marmelos belongs to the family rutceae commonly known as Bael. It is a slender, moderate sized aromatic tree. This plant is effective against different diseases as it shows antiulcer, antibacterial, and antifungal activities. In India *Aegle marmelos* is considered as one of the most important medicinal plants. It is mostly cultivated in north India, but also found as a wild plant in south and central India. *Aegle marmelos* are rich in beta carotene, Vitamin B, Vitamin C along with numerous ranges of bio-active substances extracted from it. In India, due to the traditional practices *A. marmelos* is believed to elicit therapeutic properties. Bael is listed as only member of specific monotypic genus *Aegle marmelos*. The plant is physically characterised as the deciduous shrub, with the size of the tree ranging from small to medium accordingly. The tree does not form the messy crown of leaves because of the slender dispersion. The leaves are trifoliolate, alternate, and each leaflet is approximately 5-14 x 2-6 cm, ovate with the pointed and tapering ends. During the initial development leaves appear as pale green or pinkish, hairy but after maturation the leaves become more pigmented with smooth texture. Individual leaf comprises of 4-12 pairs of veins in the leaves that are accumulated at the peripheral part of leaves⁴.

2. Material And Methods

The plant like *Aegle marmelos* has been selected for Organic Compound extraction.

Collection of plant materials

Plants like *Aegle marmelos*, commonly known as Beal and was used for Organic compound extraction. Collection of these plants was done from different destinations such as LPU campus and Phagwara. Different plants parts were used for preparation of extracts such as leaves and stem. Leaves of the plants are washed off in the running water to remove extra dust and contamination.

Boiling Metho

In boiling method, 10 gm of leaves were weighed with the help of weighing machine and dissolved in 100 ml distilled water. The mixture was allowed to boil at 70°C for 10-15 minutes in Water Bath. After boiling, the extract was kept for 10 minutes to cool down. Filter out the extract with the help of Whatman filter paper and preserve the sample into the air tight container in refrigerator until use. The extracts for both the plants are prepared by the same methods⁶.

Soxhlet Method

Soxhlet apparatus was used to prepare extract of different plant parts such as stem and leaves. The technique employs the generation of extract by the use of organic solvent like methanol and ethanol. For preparation of *Aegle marmelos* extract. 70% methanol was chosen as a solvent. 30 gm of dried leaves (sun dried and were weighed and adjusted properly in the soxhlet apparatus, further 200ml of methanol was added in the soxhlet as a solvent⁹.

Collection of Aphids nymph: Aphids nymph were collected from the paddy field inside LPU campus. The nymphal stages of aphids were collected with the help of brush and forceps, and eventually kept inside beakers until the setup was established.

Identification of Aphids Nymph: Aphids nymph were identified by application of standard Identification keys.

Experimental Setup and treatment to nymph: Aphids were treated with the extracts of *Aegle marmelos* obtained through various plant parts. The triplicate setup of different concentrations was selected for the aphid treatment. Beakers were used to perform the treatment process. Each beaker was subjected with 15 nymphs and small amount of leaf as a feed for nymphs. Different concentrations like 200ppm, 300ppm, 400ppm, 500ppm of extracts were made and tested to each triplicate set using spray bottle. Evaluation of nymphal mortality was done after 3 hours, 6 hours, 12 hours, 24 hours, 48 hours, and 72 hours.

3. Results and Discussion

Organic compound extraction from the plant like *Aegle marmelos* is meant to eradicate the pest of paddy. The plant-based compound referred as botanical pesticides is used to control the pests in crop. The plant parts such as stem and leaves contribute in extract formulation. Extract is prepared from leaves dried in sun and through stem. Both boiling and Soxhlet extract is prepared, methanol was used as a solvent for Soxhlet extraction process. *Myzus persicae* being the major pest of paddy is tested with the formulated plant-based pesticides in Laboratory conditions.

The selected concentrations of the extract for treatment were 200ppm, 300ppm, 400ppm and 500ppm. The nymph of green peach aphid was taken and treated in triplicate manner in laboratory conditions. The different concentration verified contrasting results in each set. Once the experimental setup was made, nymphal mortality was counted after 3hours, 6 hours, 12hours, 24hours, 48hours, and 72hours.

The extracts were prepared by two methods i.) Boiling method ii.) Soxhlet Method

Boiling Method

Boiling method is extensively used to prepare natural and eco-friendly compounds. The plant parts such as leaves and stem were used to prepare boiling extract. The extraction process uses distilled water as a solvent. Leaves and stem of *Aegle marmelos* was taken and made extract out of it. The boiling extracts were also made from Sun_dried leaves the first and foremost step for preparation of sundried extract is drying the freshly collected leaves in sun for 4-5 days. Once this procedure is done the leaves are grinded into powdered form for extract preparation. The sun-dried leaves extract of *Aegle marmelos* shows maximum mortality of 82.2% (Table 3) and the maximum mortality rate is 97.7% when treated with stem extracts after 72hrs of time interval (Table 1).

Soxhlet Method

Soxhlet method is used to prepare various plant parts extracts i.e stem and leaves. Methanol was used as a solvent to carry out the overall extraction process. Likewise Stem of *Aegle marmelos* was also used for Extraction process. The maximum mortality rate observed by treating the Aphids nymph with *Aegle marmelos* stem extract was 88.86% after 72 hours at concentration of 500ppm (Table 2). Sun dried extracts were prepared by using the leaves of *Aegle marmelos*. The freshly collected leaves were dried under sunlight for 4-5 days before introducing in the extraction process. The nymphal death count after introducing the *Aegle marmelos* sun dried extract for 72 hours of treatment was 93.33% (Table4).

Table 1: Mortality rate of Green peach Aphids (*Myzus persicae*) nymph at different interval of time by introducing different concentration of stem extract of *Aegle marmelos* (Boiling method)

Concentration (ppm)	Mortality (Percent)					
	3 hour	6 hour	12 hour	24 hour	48 hour	72 hour
200	2.2±0.58	6.66±1.00	28.66±0.58	42.2±0.58	64.4±0.58	82.2±0.58
300	4.4±0.58	6.66±0.00	31.06±0.58	42.2±0.58	77.7±1.15	84.0±0.58
400	4.4±0.58	9.0±0.58	37.06±0.58	48.86±0.58	80.0±0.00	84.0±0.58
500	6.66±1.00	13.0±1.00	42.2±1.15	53.33±0.00	82.2±0.58	97.7±0.58
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

% Percent, ± SE

Fatal percentage of the nymph increases from 82.2%, 84%, 84%, 97% with the increase in the extract concentration from 200ppm <300ppm, <400ppm and <500ppm. The mortality check was done till 72 hours of treatment. Thus maximum death rate was 97.7% observed in 72 hours with the exposed concentration of 500ppm. In absence of any extract, all nymph were alive in control sets. (Table 1)

Table 2: Mortality rate of Green peach Aphids (*Myzus persicae*) nymph at different interval of time by introducing different concentration of stem extract of *Aegle marmelos* (Soxhlet method)

Concentration (ppm)	Mortality (Percent)					
	3 hour	6 hour	12 hour	24 hour	48 hour	72 hour
200	0.00±0.00	4.4±1.15	15.53±0.58	26.66±0.00	53.33±1.00	66.66±1.00
300	2.2±0.58	6.66±0.00	17.73±1.15	31.06±0.58	56.0±1.15	71.06±1.52
400	4.4±0.58	10.6±0.58	20.0±1.00	33.33±1.00	60.0±1.00	73.73±0.58
500	9.0±0.58	24.0±1.00	24.0±0.58	37.06±0.58	64.4±0.58	88.86±0.58
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

% percent, ± SE

The overall mortality rate observed after treating the nymph with methanolic stem extract of *Aegle marmelos*. Increase in mortality rate was observed as per the concentration increased. Therefore the highest mortality rate observed was 88.86% at the concentration of 500ppm after 72 hours of the treatment. Death count of nymph increased from 66%, 71%, 73%, 88% with increasing concentration of <200ppm<300ppm<400ppm<500ppm. The mortality rate was 0.00% in control sets. (Table 2).

Table 3: Mortality rate of Green peach Aphids (*Myzus persicae*) nymph at different interval of time by introducing different concentration of sun dried leaves extract of *Aegle marmelos* (Boiling method)

Concentration (ppm)	Mortality (Percent)					
	3 hour	6 hour	12 hour	24 hour	48 hour	72 hour
200	0.00±0.00	4.4±0.58	10.6±0.58	15.53±0.58	24.0±1.15	51.0±1.15
300	2.2±0.58	4.4±0.58	13.0±1.00	17.73±0.58	26.6±1.00	68.0±1.15
400	4.4±0.58	6.6±0.00	17.7±0.58	22.0±0.58	33.3±0.00	75.5±2.08
500	4.4±0.58	6.6±0.00	20.0±0.00	26.66±0.00	37.06±0.58	82.2±0.58
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

% Percent, ±SE

The above mentioned table (Table 3) denotes the maximum mortality rate up to 82.2% while subjecting the nymph with Sun dried extract of *Aegle marmelos*. Concentration increased from 200ppm<300ppm<400ppm<500ppm gave the results about 51%, 68%, 75%, 82.2% after 72 hours of treatment. No nymphal death was observed in control sets.

Table 4: Mortality rate of Green peach Aphids (*Myzus persicae*) nymph at different interval of time by introducing different concentration of sun dried leaves extract of *Aegle marmelos* (Soxhlet method).

Concentration (ppm)	Mortality (Percent)					
	3 hour	6 hour	12 hour	24 hour	48 hour	72 hour
200	0.00±0.00	4.4±0.58	10.6±0.58	28.66±1.15	56.0±0.58	80.0±0.00
300	2.2±0.58	4.4±0.58	10.6±0.58	33.0±0.00	60.0±1.00	80.0±1.00
400	9.0±0.58	10.6±0.58	17.73±0.58	35.53±0.58	62.2±0.58	84.0±0.58
500	6.66±1.00	10.6±1.15	17.73±1.15	40.0±0.00	64.4±0.58	93.33±1.00
Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

% Percent, ±SE

The above mentioned table (Table 4) denotes the maximum mortality rate up to 93.33% while subjecting the nymph with Sun dried extract of *Aegle marmelos*. Concentration increased from 200ppm<300ppm<400ppm<500ppm gave the results about 80%, 80%, 84%, 93.33% after 72 hours of treatment. No nymphal death was observed in control sets.

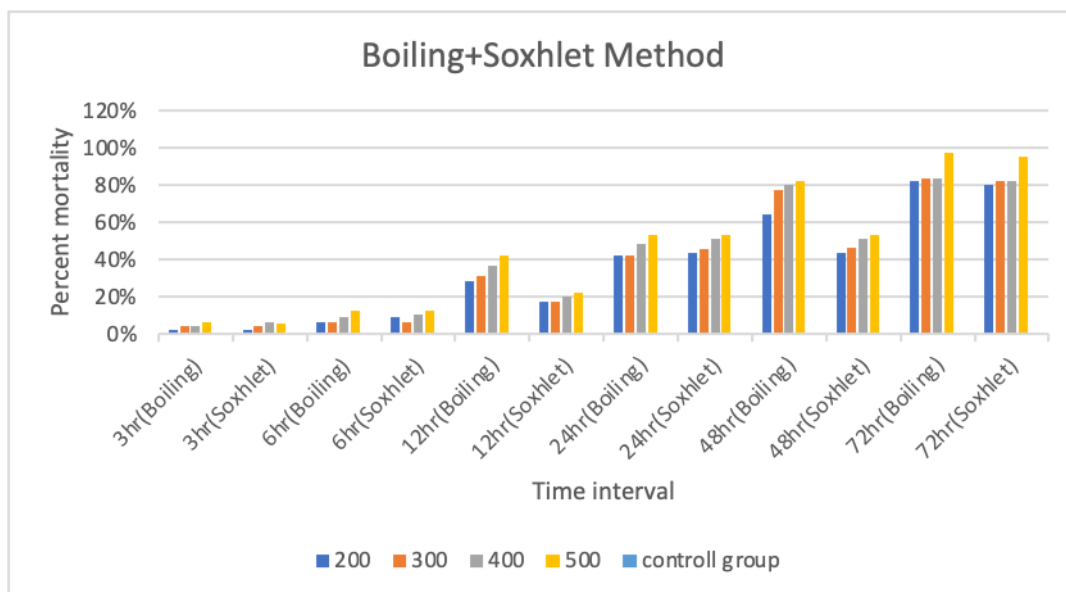


Figure 1: Comparison of different concentrations of Boiling and Soxhlet method of *Aegle marmelos* stem against green peach Aphids (*Myzus persicae*) nymph at different interval of time

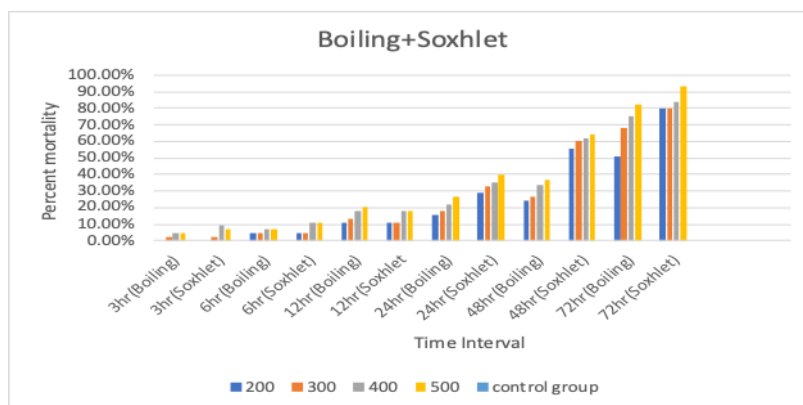


Figure 2: Comparison of different concentrations of Boiling and Soxhlet method of *Aegle marmelos* leaves against green peach Aphids (*Myzus persicae*) nymph at different interval of time

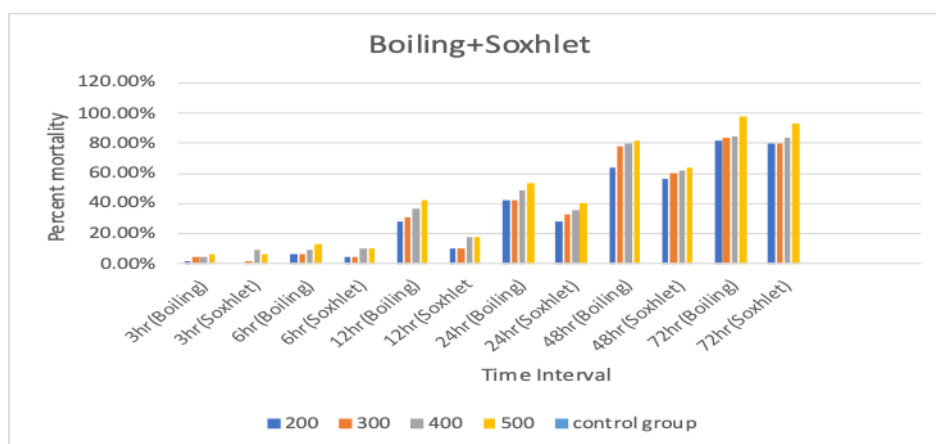


Figure 3: Comparison of the most effective extraction methods of *Aegle marmelos* i.e Stem (Boiling method) and Leaves(Soxhlet method) at various concentrations against green peach Aphids (*Myzus persicae*) nymph at different interval of time

Fig 1 is the graphical representation of mortality percentage of *Myzus persicae* after treatment with different concentrations of *A. marmelos* stem at different interval of time. The highest mortality was

observed to be 97.7% at a concentration of 500ppm when the stem extract was prepared through Boiling method, however, the mortality observed as 88.86 % at a concentration of 500ppm when the extracts were prepared through Soxhlet method and the aphids were exposed for the period of 72hrs.

Fig 2 represents that when sun dried leaves extract of *Aegle marmelos* was prepared by Boiling and Soxhlet method, the mortality of Aphids was observed as 93.3% when prepared through Soxhlet method, and it was observed as 82.2% when the extracts was prepared by boiling method at a concentration of 500ppm.

The 3rd Fig, is the representation of *Myzus persicae* when exposed to different concentration of both stem and leaves prepared by different methods, the highest mortality (97.7%) was observed when the stem extracts were prepared through boiling method was used against the Pests when applied for a period of 72hrs at a concentration of 500ppm and, and the highest mortality of aphids in case of leaves was observed as 93.3% when the leave extracts were prepared through Soxhlet method, so both the methods are effective in their own way. In case of stem boiling method appear to be more effective as compared to Soxhlet method, whereas in case of sun-dried leaves Soxhlet method seems to be more effective as compare to boiling method.

4. Conclusion

Myzus persicae being a polyphagous pest cause huge damage to different economically important crops including paddy, the control of this green peach aphid through chemical means leads to the development of resistance. *Aegle marmelos* as a botanical pesticide is the most efficient alternative to these chemicals. The extracts of different parts of *Aegle marmelos* like stem and leaves were used against the aphid at different concentrations prepared through boiling method and Soxhlet method. The highest mortality of pest was observed as 97.7% at a concentration of 500ppm, this percentage of mortality was observed when *Myzus persicae* was exposed to stem extracts of *Aegle marmelos* prepared by Boiling method at a concentration of 500ppm. From the present study, it is clear how efficient is the use of this botanical pesticide in controlling these crop pests. In future this method needs to be more utilized as compared to chemical method.

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