

## Efficacy of *Melia azadarach* and *Mentha piperita* Plant Extracts Against Cabbage Aphid, *Brevicoryne brassicae* (Homoptera: Aphididae)

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**Abstract:** Cabbage is one of the important vegetable crops in Ethiopia. The cabbage aphid *Brevicoryne brassicae* become a key pest which affects the quality of cabbage. The resource poor small scale farmers in Gondar highlands are unable to afford the cost of imported chemical pesticides. In order to find out alternative strategies to farmer's laboratory studies were conducted to check the efficacy of solvent extract of *Melia azadarach* leaves and seeds and *Mentha piperita* leaves against aphid. Three concentrations of methanol and aqueous extracts such as 0.25, 0.5 and 1% were tested against aphid by using choice and no choice method. In no choice method methanol extract of *Melia* seeds treatment showed minimum number ( $1.33 \pm 0.57$ ) of aphids at 0.25% concentration. Higher concentration (1%) of *Mentha* leaves and lower concentration (0.25%) of *Melia* seed treatment recorded only  $0.6 \pm 0.57$  aphids. Irrespective of concentration and period of exposure wide fluctuation was observed in choice method. Among the three extracts tested, *M. piperita* leaf extract and *M. azadarach* seed extract showed promising result. These two plants are easily growing in Ethiopian highlands and it is found to be suitable for resource poor farmers to control aphid menace in cabbage.

**Key words:** Cabbage • Aphid • Plant extracts • Solvents • Choice method • No choice method

### INTRODUCTION

Chemical pesticides have played a significant role in increasing the agricultural production and in the protection of crops from the damage caused by the insect pests and pathogens. It has been estimated that hardly 0.1% of the agrochemical used in crop protection reach the target pests and the remaining 99.9% enter the environment and cause hazards to non-target organisms [1]. Due to higher dose and repeated application of pesticides, every year one million people suffer from pesticide poisoning [1]. Many neurotoxic insecticides are damaging the environment and/or pose a threat to public via food residues, ground water contamination, or accidental exposure [2].

Botanical insecticides [3, 4] are eco-friendly and environmentally safer alternative methods for crop protection. The evaluation of plant extracts for their deleterious effects on insects is one of the approaches used for the search of novel botanical insecticides [5-8].

During the last one-decade ample emphasis has been placed on the use of biopesticides because of less disruption to the environment [9]. The morphogenetic changes in *Dysdercus cingulatus* was reported by the treatment of *Catharanthus roseus*, *Parthenium hysterophorus* and *Nephrolepis exaltata* [10]. The extracts of young plants, old leaves, inflorescence, stem and roots of *Parthenium hysterophorus* showed ovicidal activity against eggs of *Spodoptera litura* [11].

Hexane and methanol extract of *Jatropha podagrica* root, bark exhibited potent antifeedant activity against maize and sorghum stem borer, *Chilo partellus* [12]. The methanolic neem seed extract fed to the larvae of *S. litura* inhibited feeding at 0.3% concentration and the feeding was further reduced at 0.5% of neem extract [13]. Acetone extracts of *Cassia fistula* exhibited ovicidal activity against the eggs of *Trogoderma granarium* [14]. *Annona squamosa* leaves showed antifeedant activity against *Crypsiptera coclesalis* [15]. Plant extracts of *Artemisia vulgaris*, *Ageratum conyzoides*, *Vitex trifolia*,

*Corcus calamus* and neem all exhibited insecticidal activity against cotton aphid, *Aphis gossypii* [16]. Tripathi *et al.* [17] reported that essential oil of *Aegle marmelos* leaf reduced growth rate and feeding consumption of *Helicoverpa armigera* and *Spilarctia obliqua*.

The highlands of in and around Gondar University are colonized by resource poor farming community. In order to find out safer alternative pest control strategies a laboratory study was undertaken to check the efficacy of *Melia azadarach* and *Mentha piperita* plant extracts against aphids. Aphids are polyphagous pest of many plant species causes damage to the plants by direct sucking of xylem sap from the stem or leaves of plants. In addition to mechanical and physiological damage, they are also responsible for the transmission of varieties of viral diseases [18].

## MATERIALS AND METHODS

*Melia azadarach* and *Mentha piperita* plants were collected from GTZ area (Gesellschaft Technische Zusammenarbeit- German Agency for Technical Co-operation) and Maraki campus of Gondar University, Gondar, Ethiopia. Those plant materials were thoroughly washed with water and dried by using oven under a temperature of 37°C. After complete drying the plant materials were powdered by using electric blender and sieved through kitchen strainer and used for solvent extraction. 100 gm of powdered plant material was added individually in to a conical flask containing 500ml of solvent such as water and methanol. Both are polar solvents, farmers can prepare water extract easily for pest control programme and the methanol was selected for comparison.

The conical flask was tightly covered with aluminum foil and the materials in the flask were mixed for about 20hrs by using reciprocating shaker. The solutions was filtered through Whatman no.1 filter paper and the filtrates transferred to the petriplates and kept in 37°C in an oven for complete solvents evaporation. The crude extracts were collected from the petriplate and 1% stock solution was prepared. From the stock solution 0.5% and 0.25% concentration were prepared by serial dilution and tested against aphid by using choice and no choice method.

**Aphid Maintenance:** Aphids were collected together with the infested leaves from the farmer's field near Dashen brewery, Gondar, Ethiopia. Every day fresh tender cabbage leaves were provided for Aphid in the laboratory till the completion of experiment.

The experiments were conducted in the biology laboratory Maraki campus. In no choice method one larger tender cabbage leaf was treated with plant extract separately and the petiole was tied with cotton soaked with water to avoid early drying of the leaves. The untreated leaf was used as a control for the comparison of botanical effects. In the choice method one leaf was treated and the other was kept as such. Both the leaves were kept in a petriplates above the filter paper and 10 healthy aphids were introduced individually in choice and no choice method. After 24 and 48hr exposure period number of aphids in treated and untreated leaf was recorded at different concentrations. The experiment was replicated in three times and the data collected from the experiment was subjected to statistical analysis by using Microsoft excel to find out mean and standard deviation. Two-way analysis of variance was applied to find out statistical significance.

## RESULTS AND DISCUSSION

Naturally many plants are having unpalatable substances which play a defensive role such as antifeedant, oviposition deterrent, insecticidal, ovicidal and insect growth regulators (IGRs) against insect pests [19]. In the no choice experiment number of aphids observed in 0.25% concentration of *Melia* seed methanol extract was 1.33 (i.e., 13.3%). The number of aphid population on 0.25% concentration of methanol extract of *Melia* leaf was 1.66 (i.e., 16.6%) which is on par with the result of 1% concentration of *Mentha* leaves (Table 1). In control 100% aphid population was observed in the leaf (data was not included because all the insects are attached to the leaf). The variation noted within the treatment and the plant materials tested are statistical not significant ( $p > 0.05$ ).

Water extract of *Melia* seed treatment the number aphid aggregated on the leaf was 0.66 (6.6%) at lower concentration (0.25%) which is one par with the result of *Mentha* leaf extract at higher concentration (1%) (Table 2). Results confirmed that the deterrent properties of the plant extracts repel the insect on treated surface. Moreover, most of the time insects were moving around the petri plates and some of the dead insects were also observed in the petriplates. The mortality of insects was one way related to the toxic odour of the extract enter in to the spiracle and block the oxygen supply; due to suffocation the insects are died. Secondly the insects avoid treated leaf foliage for longer time without food under starvation insects may be died.

Table 1: Mean number of aphids on leaf treated with methanol extract in no choice method

Plant parts tested	24hr exposure			48hr exposure		
	0.25%	0.5%	1.0%	0.25%	0.5%	1.0%
<i>Mentha</i>	3.0±1.0	1.33±0.57	1.66±0.57	2.66±0.57	3.33±1.15	3.0±1.0
<i>Melia</i> leaves	1.66±0.57	1.66±1.0	2.0±1.0	2.33±1.15	3.66±0.57	4.0±1.0
<i>Melia</i> Seeds	1.33±0.57	1.66±0.57	3.0±1.0	3.33±0.57	3.66±1.52	4.66±0.57

Values are Mean±Standard deviation of three replications; within the column and rows are statistically not significant ( $p>0.05$ ) two way ANOVA.

Table 2: Mean number of aphids on leaf treated with water extract in no choice method

Plant parts tested	24hr			48hr		
	0.25%	0.5%	1.0%	0.25%	0.5%	1.0%
<i>Mentha</i>	1.33±0.57	6.0±1.0	0.6±0.57	3.66±0.57	6.6±1.52	1.66±0.57
<i>Melia</i> leaves	1.33±0.57	3.0±1.0	2.66±2.08	4.66±1.15	5.33±0.57	4.66±0.57
<i>Melia</i> Seeds	0.66±0.57	2.66±1.15	1.66±0.57	4.33±0.57	5.66±1.15	6.0±2.0

Values are Mean±Standard deviation of three replications; within the column and rows are statistically not significant ( $p>0.05$ ) two way ANOVA.

Table 3 Mean number of aphids on leaf treated with methanol extract after 24hr exposure period in choice method

Plants parts tested	Concentration of plant extracts tested					
	0.25%		0.5%		1.0%	
	Treated	Un Treated	Treated	Un Treated	Treated	Un Treated
<i>Mentha</i> leaves	2.0±1.0	8.0±1.0	3.0±1.0	7.0±1.0	2.66±0.57	7.33±0.57
<i>Melia</i> leaves	3.0±1.73	7.0±1.73	3.66±0.57	6.33±0.57	3.33±0.57	6.66±0.57
<i>Melia</i> seeds	4.0±1.0	6.0±1.0	3.33±0.57	6.66±0.57	2.33±1.15	7.66±1.15

Values are Mean±Standard deviation of three replications; within the concentration results were statistically significant ( $p<0.05$ ) two way ANOVA.

Table 4: Mean number of aphids on leaf treated with methanol extracts after 48hr exposure in choice method

Plants parts tested	Concentration of plant extracts tested					
	0.25%		0.5%		1.0%	
	Treated	Un Treated	Treated	Un Treated	Treated	Un Treated
<i>Mentha</i> leaves	2.66±0.57	7.33±0.57	3.0±1.0	7.0±1.0	2.66±0.57	7.33±0.57
<i>Melia</i> leaves	2.33±0.57	7.66±0.57	2.33±0.57	7.66±0.57	3.0±1.0	7.0±1.0
<i>Melia</i> seeds	3.0±1.0	7.0±1.0	3.0±1.0	7.0±1.0	1.33±0.57	8.6±0.57

Values are Mean±Standard deviation of three replications; within the concentration results were statistically significant ( $p<0.05$ ) two way ANOVA.

Table 5 Mean number of aphids on leaf treated water extracts after 24hr exposure in choice method

Plants parts tested	Concentration of plant extracts tested					
	0.25%		0.5%		1.0%	
	Treated	Un Treated	Treated	Un Treated	Treated	Un Treated
<i>Mentha</i> leaves	1.66±0.57	8.33±0.5	3.0±2.0	7.0±2.0	2.0±1.73	8.0±1.73
<i>Melia</i> leaves	3.0±1.73	7.0±1.73	4.0±1.0	6.0±1.0	1.66±1.15	8.33±1.15
<i>Melia</i> seeds	3.66±1.52	6.33±1.5	3.33±0.57	6.66±0.57	2.33±1.15	7.66±1.15

Values are Mean±Standard deviation of three replications; within the column and rows are statistically not significant ( $p>0.05$ ) two way ANOVA.

Table 6 Mean number of aphids on leaf treated with water extracts after 48hr exposure in choice method

Plants parts tested	Concentration of plant extracts tested					
	0.25%		0.5%		1.0%	
	Treated	Un Treated	Treated	Un Treated	Treated	Un Treated
<i>Mentha</i> leaves	2.0±1.0	8.0±1.0	2.66±1.15	7.33±1.15	1.66±1.5	8.33±1.52
<i>Melia</i> leaves	3.0±1.0	7.0±1.0	3.66±0.57	6.33±0.57	2.33±0.57	7.33±0.57
<i>Melia</i> seeds	2.66±1.15	7.33±1.5	3.33±0.57	6.66±0.57	1.66±0.57	8.33±0.57

Values are Mean±Standard deviation of three replications; within the column and rows are statistically not significant ( $p>0.05$ ) two way ANOVA

The present results are in confirmation of with earlier findings such as terpenoids, alkaloids like nicotine from tobacco and pyrethrum and Cinerin from pyrethrum inflorescence play an important defensive role against insect pests and other herbivores (Ishaaya [20]. Potential antifeedant properties of meliatoxin A2 and Meliatoxin B1 isolated from *Melia azadarach* var *Australasia* was confirmed by MacLeod *et al.* [21]. Bohnenstengel *et al.* [22] also reported three new meliacarpin derivatives namely 1,3-dicinnamoyl-11-hydroxymeliacarpin, 1-cinnamoyl-3-methacrylyl-11-dihydroxy -liacarpin and 1-cinnamoyl-3-acetyl-11-hydroxymeliacarpin isolated from the leaves of *Melia azadarach* showed insecticidal and growth disrupting activity against *S. littoralis*.

In choice experiment, at 24hr exposure period aphid population was minimum ( $2.0 \pm 1.0$ ) in *Mentha* leaf aqueous extract treatment compared to *Melia* seed and leaf extracts at lower concentration (0.25%) (Table 3). However, at 48hr exposure period lowest number ( $1.33 \pm 0.57$ ) of aphid aggregation was recorded in *Melia* seed extracts at 1% concentration compared to other extracts (Table 4). In water extract after 24hr exposure period minimum number ( $1.66 \pm 0.57$ ) of aphids was recorded at 0.25% concentration which is on par with *Melia* leaf extracts at 1% concentration (Table 5). Aphids exposed to aqueous extract of *Mentha* leaf and *Melia* seed at 1% concentration showed minimum number ( $1.66 \pm 0.57$ ) at 48hr exposure period (Table 6). In general most of the insects were present on untreated leaves due to the repellent activity of the plant extract. In the methanol extract within the concentration the results were statistically significant ( $p < 0.05$ ). Plant extracts tested unquestionably proved bioactivities against aphids. There are many scientific reports will support the bioactivity of plants extracts. Joshi *et al.* [23] reported that seed kernel of neem, *Azadirachta indica* was protected tobacco plants from the attack of *S. litura*. The methanol and hexane extract of neem seed kernel showed significant protection against damage caused by *S. litura* and also no oviposition in areas treated with methanol extract at 0.01% for 5 days as compared with 4 days for hexane extract [24]. Koshia and Ghelani [25] reported neem leaf and seed extracts and extracts of *Pongamia glabra* seeds were highly effective at 15% concentration against *S. litura*. Mohepatra *et al.* [26] reported methanol extracts of neem seed kernel at 1% concentration provided greatest protection (100%) of the cauliflower leaves against *S. litura*. Behera and Satapathy [27] reported neem seed kernel extract was the most effective causing

100% mortality 10days after treatment. Murugan *et al.* [28] reported that neem limonoids such as azadirachtin, salanin, deacetylgedunin, gedunin, 17-hydroxyazadiradione and deacetylnimbin showed antifeedant and growth inhibiting activity against *H. armigera*. In the present study *Melia azadarachta* also belongs to the same family they may have similar mode of action against pest.

## CONCLUSION

Two plants studied under the laboratory condition are showed promising results against aphid and also lot of scientific reports confirmed the bioactivity of the selected plant. Therefore, small scale farmers can prepare any one of the plant extracts to protect their cabbage crop against aphid infestation and also to guard their natural environment from dreadful toxic chemical pesticides used for pest control programme.

## REFERENCES

1. Bami, H.L., 1997. Pesticide use in India-Ten questions. *Chemical Weekly*, 4: 7-10.
2. Isman, B., O. Koul, A. Lucyzynski and J. Kaminski, 1990. Insecticidal and antifeedant bioactivities of neem oils and their relationship to Azadirachtin content, *J. Agri. and food Chemistry*, 38: 1407-1411.
3. Arnason, J.T., B.J.R. Philogene and P. Morand, 1989. Insecticides of plant origin, ACS symposium series 387, Amer. Chem. Soc., Washington, DC.
4. Isman, M.B., 1994. Growth inhibitory and antifeedant effects of azadirachtin on six noctuids of regional economic importance, *Pesticide Sci.*, 38: 57-63.
5. Jacobson, M., 1989. Botanical insecticides past, present and future. In: *Insecticidal of plant origin* (Eds. Philogene, B.J.R. and Morand, P.), *American Chemical Society Symposium Series* No.387. Washington, DC.
6. Schmutterer, H., 1992. Higher plants as sources of novel pesticides. In: *Insecticides: Mechanisms of action and resistance*. (Eds. Otto, D. and Weber, B.), Intercept Ltd, Antover.
7. Arnason, J.T., S. MacKinnon, A. Dust, B.J.R. Philogene, C. Hasbun and P. Sanchez, 1993. Insecticidal in tropical plants with non-neurotoxic modes of action. In: *Phytochemical Potential of Tropical Plants*. (Eds Downum, K.R., Romeo, J. and Stafford, H.), Plenum Press, New York pp: 107-131.

8. Isman, M.B., 1995. Leads and prospects for the development of new insecticides. In: *Reviews of Pesticide Toxicology* (Eds. Roe, R.M and Kuhr, R.J.) 3: 1-20.
9. Koul, O., J.S. Multani, G. Singh and S. Wahab, 2002. Bioefficacy of toosendanin from *Melia dubia* (syn. *M. azedarach*) against gram pod-borer, *Helicoverpa armigera* (Hubner). *Current Sci.*, 83: 1387-1391.
10. Rajendran, B. and M. Gopalan, 1980. Juvenile hormone like activity of certain plant extracts on *Dysdercus cingulatus* Fabricius (Heteroptera: Pyrrhocoridae) *Indian J. Agri. Sci.*, 50: 781-784.
11. Gajendran, G. and M. Gopalan, 1981. Note on the ovicidal activity of *Parthenium hysterophorus* Linn. on the eggs of *Spodoptera litura* Fabricius. *Indian J. Agri. Sci.*, 51: 821-822.
12. Aiyelaagbe, O.O., E.K. Adesogan and O. Ekundayo, 1998. Antifeedant activity of *Jatropha podagrica* roots. *Fitoterapia*, 69: 175-176.
13. Kulkarni, N., 1999. Antifeedant and growth inhibitory effects of *Azadirachta indica* A. Juss, seeds against the larvae of *Spodoptera litura* (Fab.) (Noctuidae: Lepidoptera). *Nationa. Academic Sci. Lett.*, 22: 24-26.
14. Dwivedi, S.C. and M. Bajaj, 2001. Efficacy of botanicals as ovicides against *Trogoderma granarium*. *J. Advanced Zool.*, 22: 5-7.
15. Kulkarni, N., K.C. Joshi and P.K. Shukla, 2003. Antifeedant activity of *Annona squamosa* Linn. against *Crypsiptera coclesalis* Walker (Lepidoptera: Pyralidae). *Entomon*, 28: 389-392.
16. Devi, M.N., T.K. Singh and L. Chitra Devi, 2003. Efficacy of certain botanical insecticides against cotton aphid, *Aphis gossypii* Glover on brinjal. *Pestol.*, 28: 6-10.
17. Tripathi, A.K., V. Prajapati, A.A. Naqvi and P.S. Khunuja, 2003. Feeding deterrent, growth inhibitory and toxic effects of essential oil of *Aegle marmelos* leaf against three Lepidopteran insects. *J. Medicinal and. Aromatic Plant Sci.*, 25: 466-472.
18. Hill, D.S., 1983. *Agricultural Insect Pests of the Tropics and their Control*. Second Edition. Cambridge University Press, Cambridge.
19. Isman, M.B., P.J. Gunning and K.M. Spollen, 1997. Tropical timber species as sources of botanical insecticides", In: *Phytochemicals for pest control*. (Eds. Hedin, P.A., R.M. Hollingworth, E.P. Masler, J. Miyamoto and D.G. Thomson,). American Chemical Society Symposium Series, 658: 27-37.
20. Ishaaya, I., 1986. Nutritional and allelochemic insect-plant interactions relating to digestion and food intake: some examples. In: *Insect-Plant Interactions* (Eds). Miller, J.R. and T.A. Miller,). Springer-Verlag, New York. pp: 191-223.
21. Macleod, J.K., P.D.R. Moellar, T.F. Molinski and O. Koul, 1990. Antifeedant activity against *Sopdoptera litura* larvae and [<sup>13</sup>C] NMR spectral assignments of the meliatoxins. *J. Chemical Ecol.*, 16: 2511-2518.
22. Bohnenstengel, F.J., V. Wray, L. Write, R.P. Srivastava and P. Prokach, 1999. Insecticidal Meliascarpins (C-*seco* limonoids) from *Melia azadarach*. *Phytochemistry*, 50: 977-982.
23. Joshi, B., G. Ramaprasad and S.N. Rao, 1984. Neem seed kernel suspension as an antifeedant for *Spodoptera litura* in a planted fluecured virginia tobacco crop. *Phytoparasitica*, 12: 134-139.
24. Ayyangar, G.S.G. and P.J. Rao, 1989. Neem (*Azadirachta indica* A. Juss) extracts as larval repellents and ovipositional deterrents to *Spodoptera litura* (Fabr.). *Indian J. Entomol.*, 51: 121-124.
25. Koshiya, D.J. and D. Ghelani, 1993. Antifeedant activity of different plant derivatives against *Spodoptera litura* on groundnut. In: *Botanical pesticides in IPM*, Symposium Proceedings Rajamundry, India, pp: 270-182.
26. Mohapatra, S., S.K. Sawarkar, H.P. Patnaik and B. Senapati, 1995. Antifeedant activity of solvent extracts of neem seed kernel against *Spodoptera litura* F. and their persistency against sunlight through encapsulation. *International J. Pest Management*, 41: 154-156.
27. Behera, U.K. and C.R. Satapathy, 1996. Screening indigenous plant extracts on growth and development of *Spodoptera litura*. *Envir. Ecol.*, 15: 12-16.
28. Murugan, K., S. Jeyabalan, D. Senthilkumar and S. Sivaramakrishnan, 1998. Evaluation of certain tropical plant extracts for their antifeedant and toxic properties against *Spodoptera litura* (Fab.). *J. Insect Sci.*, 11: 186-187.