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Insecticidal effects of flower, fruit growth stages and leaf extracts of *Melia azedarach* on *Aphis fabae* nymphs

Mahsa Khabir[1], Kamal Ahmadi[1]✉, Mohammad Shojaaddini[2]

1 Department of Plant Protection, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

2 College of Agriculture, Technical and Vocational University, Tehran, Iran.

✉Corresponding author: kahmadi@mail.uk.ac.ir

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Abstract

Considering the low risk of botanical pesticides for humans and the environment, and their broad-spectrum pest control capabilities, it seems beneficial and necessary to consider these compounds in integrated pest management. Different plant parts produce different amounts of secondary metabolites that can have insecticidal properties. Therefore, by evaluating the effectiveness of substances extracted from different parts of plants, it is possible to open a window to identify important effective substances. This study investigated the insecticidal activity of *Melia azedarach* L. extracts (flowers, fruit growth stages, and leaves) against *Aphis fabae* Scopoli using different solvents (polar protic, dipolar aprotic, and non-polar). The highest mortality percentage among methanolic extracts was found in the flower extract (77.74±5.75%), in n-hexane extracts it was in the ripe fruit extract (76.18±4.09%), and in acetonic extracts it was in the flower extract (66.48±5.91%). Results indicated that all three plant parts' methanolic extracts showed the most potent insecticidal effects.

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Introduction

Uncontrolled use of pesticides in the long term can cause problems for humans and the environment. The presence of remaining pesticides in the environment and human body may lead to irreparable damage. Insect resistance to pesticides is a kind of problem that arises from uncontrolled use of pesticides. Despite the increasing public awareness about the harm of pesticides, and the growing trend to using non-chemical methods in order to control plant diseases, weeds and agricultural pests, the use of chemical pesticides is still a prevailing method in controlling these unexpected harms (Sarkar et al., 2021). Because of biologically active compounds, plants show advanced defense mechanisms against pests (War et al., 2012). Nowadays, the scientists are searching these secondary metabolites to find alternative combinations replacing commonly used chemical pesticides.

Increasing concerns about the dangers of synthetic pesticides on the environment and human health has become a major concern in pest management. Accordingly, the search for safer alternatives like biological products seems essential and necessary (Isman, 2006). Due to the high feeding, honeydew secretion and portability of viral diseases in plants, aphids are one of the most important economic pests that have spread throughout the world. They attack a wide range of agricultural products and ornamental plants (Clements et al., 2000). Black bean aphid, *Aphis fabae* Scopoli is one of the most important pests of agricultural products around the world (Völkl & Stechmann, 1998) that has a wide host range of over 200 species worldwide and 50 species in Iran (Hodjat, 1986). *Melia azedarach* Linnaeus belonging to the *Meliaceae* family is also known as Persian lilac, Chinaberry, Umbrella tree and white cedar. This tree, native to Australia and Southeast Asia, is also grown in Iran's northern regions and other provinces as an ornamental plant (Ghahreman, 1986). *Melia azedarach* has significant insecticidal potential (Schmutterer, 1989; Lee et al., 1991; Schmutterer, 1997; Valladares et al., 1997). Derivatives of *M. azedarach* tree have been detected as insecticides. In a study, the effect of methanolic extract of *Azadirachta indica* A. Juss. and *M. azedarach* on laying and hatching eggs of *Earias vittella* (Fabricius) was evaluated (Gajmer et al., 2002). Also antifeedant effects of aqueous extract of flower, fruits, stems and leaf of *M. azedarach* were studied on *Diabrotca speciosa* (Germar) (Ventura & Ito, 2000). In addition, it has been reported that *M. azedarach* leaf extract influences the survival rate of *Epilachna paenulata* (Germar) larvae as well as the adults (Kraus et al., 1986). In another study, the effect of M. azedarach leaf extract on growth of Epilachna varivesti was determined (Zhu & Ermel, 1991). An effect of extracts from *M. azedarach* leaves and unripe fruit on mortality of greenhouse whitefly adults has been demonstrated (Nardo et al., 1997). Effects of extracts from different growth parts of *M. azedarach* including green and ripe fruits, leaves and stems has been demonstrated on the whitefly mortality (De Souza & Vendramin, 2001). It was also reported that the aqueous extract of ripe fruit of *M. azedarach*, influences on various species of aphids (Defago et al., 2009). In another study, the impact of *M. azedarach* leaf extract on the fatality, larval period and growth speed of *Spodoptera frugiperda* (J.E. Smith) was studied (McMillian et al., 1969; De Brito et al., 2004). Also, chemical composition of methanol extract of the leaves of *M. azedarach* L. was investigated (Sen & Batra, 2012). Solvent extraction is a common technique used in both industrial applications and in the laboratory. In one study, the effect of solvents and extraction techniques, on the antioxidant activity of extracts of some medicinal plants was investigated (Sultana et al., 2009). A Solvent is a liquid substance capable to dissolve other substances (solutes) without chemical changes (Reichardt, 2003). The Ability of a substance to dissolve another substance is determined by compatibility of their molecular structures. There are three main types of solvents based on their polarity: Polar protic, dipolar aprotic and non-polar solvents such as methanol, acetone and n-hexane, respectively. Polar protic molecule consists of a polar group OH and a non-polar tail. The structure may be represented by a formula R-OH. Polar protic solvents dissolve other substances with polar protic molecular structure. Polar protic solvents are miscible with water (hydrophilic). Dipolar aprotic molecules possess a large bond dipole moment (a measure of polarity of a molecule chemical bond). They do not contain OH group. Electric charge in the molecules of non-polar solvents is evenly distributed; therefore the molecules have low dielectric constant. Non-polar solvents are hydrophobic (immiscible with water). Non-polar solvents are lipophilic as they dissolve non-polar substances (Reichardt, 2003).

Material and Methods

Insects rearing

To investigate the effect of different extracts of flower, fruit growth stages and leaf of *M. azedarach* in insect control, *A. fabae* was used. Aphids were collected from the experimental teaching greenhouse of Shahid Bahonar University of Kerman, Kerman, Iran (30°19'51.33" N, 56°55'41.72" E, 1776 H) and reared in the laboratory. Aphids were reared on broad bean leaves (*Vicia faba* Linnaeus) in special rearing containers (plastic containers with a diameter of 6 cm and a height of 4 cm, containing 0.7% agar gel). The agar gel was used to retain moisture and extend the life of the host plant leaves. In all containers, there were typically 10-15 adult aphids for mass rearing and container opening were covered with special lace (glassy silk), to allow air circulation. Then the containers were kept inside a growth chamber at temperature 25±1 ° C, relative humidity of 50±10%, and 16L:8D. By aphid reproduction and population increase, the number of adults were transferred to new dishes containing agar gel and new leaves of the host plant.

To obtain the same-aged aphids, 200 adult aphids from the initial colony were isolated and maintained in a separate colony. After 24 hours, the adults were removed and returned to the main colony. The produced nymphs being 1-2 days-old were used to perform some tests. To obtain 3-4 days-old nymphs, 1-2 days-old nymphs were kept in a separate colony and after 48 hours, the nymphs reached the age of 3-4 days-old and were used for the experiments.

Preparation of plant samples

To investigate the effects at different stages of development, various plant parts of *M. azedarach* were collected from their natural habitat Mahan (30°9'70" N, 57°9'30" E, 1794 H) These included flowers, small green fruits (3 mm diameter), large green fruits (7 mm diameter), ripe fruits, and leaves. The Plant material was transferred to the laboratory and dried under appropriate conditions (shade and ventilation). After complete drying of plant parts, these parts were powdered using the electric stainless steel mill.

Preparation of plant extracts

Considering that secondary metabolites of plants are different in terms of polarity, solvents with different polarity have different efficiency in extracting each of them. Three solvents with purities of 95-99.9% were used for extraction: methanol (polar protic), acetone (dipolar aprotic) and n-hexane (non-polar). At each fruit growth stage, flower and leaf of the plant extraction, 70 g of powdered plant parts were poured into in a 500 ml flask. According to the type and goal the experiments, aforementioned solvents were added to sample to reach 150 ml. After one hour of stirring, the flask lids were sealed with Parafilm® to prevent solvent evaporation. To prevent flask from direct light exposure, they were covered with aluminum foil. The remaining mixture and herbal extracts were kept in a refrigerator (4 °C) for 24 hours. After this period, the extracts were separated by the filter paper Whatman N ° 91.

Bioassay tests

The effects of flower, fruit growth stages and leaf of M. azedarach extracts, on the mortality percentage of 1-2 days and 3-4 days old nymphs of *A. fabae* were studied. To determine the effect of the solvent used in the insecticidal potential in different parts of *M. azedarach*, extracts from methanol, acetone and n-hexane were used. Each experimental unit consisted of a 6 cm diameter plastic petri dish, containing a 1 cm thick layer of 0.7% agar gel, on which a new bean leaf was placed. The aphids as biotest were transferred to the leaves of the host plant before the experiment and were completely adapted to the recent environment. For each experiment, the concentrations of 50 µg/mL of each of the above extracts were prepared. After extracting the plant derived chemicals, the dry matter concentration of each extract was calculated. Preparation of the desired concentration (50 µg/mL) was done by diluting or concentrating the extract according to the initial concentration. In three separate treatments, three solvents methanol, acetone and n-hexane were sprayed alone. In the control treatment, no stimulant was sprayed on the aphids. Spraying was done with a laboratory reagent sprayer, glass with rubber bulb and homogeneously and with the same volume (5 mL/10 Petri dish). Treated aphids were kept in a growth chamber at 25 ± 1°C, 50 ± 10% relative humidity, and 16 hours light and 8 hours darkness. After 48 hours, the number of dead insects in each treatment, was counted. Mortality was determined if the legs and antennae were stimulated by brush, no reaction was observed. For each extract at least 10 replications were carried out.

Analysis of the data

In order to affirm the basic assumptions of the data to be analyzed, they were first tested for the normal distribution and the homogeneity of variance using the Bartlett test (Köhler et al., 2002). The data that had not conformed to the assumptions of normal distribution were transformed to conform to the assumptions, using the Box-Cox formula:

$Y = X2 – 1/\lambda$ if $\lambda \neq 0$,

$Y = \ln X$ if $\lambda = 0$,

Where: Y – the transformed value, X – the untransformed value, and $0 < \lambda < 1$ (Anonymous, 1996). Statistical analyses of experiments performed in this study, were performed using Stat-plus (Version 5.8.4 2009) took place. For comparisons of in vitro data, One-Way ANOVA with Fisher LSD test was used.

Results

The effect of methanolic, acetonic, and n-hexane extracts of flower, small green fruit, big green fruit, ripe fruit and leaf of *M. azedarach* on the mortality of 1-2 days-old nymphs of *A. fabae* are depicted in Fig. 1. According to the findings, the extracts in the order of effectiveness are ripe fruit methanolic extract (59.76±5.69%), ripe fruit acetonic extract (59.29±6.82%), leaf methanolic extract (57.17±5.16%) and flower methanolic extract (54.72±5.56%). Moreover, from among the extracts taken using the mentioned solvents, n-hexane flower extract with two methanolic and acetonic flower extracts; small green fruit and big green fruit methanolic extracts with acetonic extract and also n-hexane leaf extract with two acetonic and methanolic extracts regarding the pesticidal effect on 1-2 days-old nymphs showed significant differences among each other ($P \geq 0.01$). While there is not any significant difference among ripe fruit methanolic, acetonic, and n-hexane extracts and also between different solvents regarding causing mortality percentage.

Mortality rate in flower, ripe fruit and leaf methanolic extracts, compared with mortality rate in control, methanol, small green fruit and big green fruit methanolic extracts grow were significantly greater ($P \geq 0.00001$); regarding the mortality rate in flower, ripe fruit and leaf methanolic extracts, significant differences were not observed. The control and acetone mortality rate reflect a significant difference with mortality rate of flower, small green fruit, big green fruit, ripe fruit and leaf acetonic extracts ($P \geq 0.00001$). Based on the results, there was a significant difference between mortality rates in control, n-hexane and leaf n-hexane extract with flower, small green fruit, big green fruit and ripe fruit n-hexane extracts (P≥0.009).

Insecticidal effect of methanolic, acetonic, and n-hexane extracts of flower, small green fruit, big green fruit, ripe fruit and leaf of *M. azedarach* on 3-4 days-old nymphs of *A. fabae* is shown in Fig. 2. Based on the results the extracts causing more mortalities are methanolic flower extract (77.74±5.75%), ripe fruit n-hexane extract (76.18±4.09%), leaf methanolic extract (73.74±2.97%) and flower acetonic extract (66.48±5.91%). Moreover, between the extracts taken by mentioned solvents, n-hexane flower extract with methanolic and acetonic flower extracts regarding the percentage of mortality on 3-4 days-old nymphs, no significant difference was revealed. This fact is also true for big green fruit, ripe fruit, and leaf n-hexane extracts. Furthermore, small green fruit acetonic extract showed a significant difference with two methanolic and n-hexane extracts regarding the mortality effect on 3-4 days-old nymphs (P≥0.03). Also, considering the mortality rate caused by different solvents, acetonic solvent showed the lowest effect and showed a significant difference with methanolic and n-hexane solvents. (P≥0.00001).

Mortality percent in two methanolic extracts (flower and leaf), compared to small green fruit, big green fruit and ripe fruit methanolic extracts, were significantly higher (P≥0.002). There is a significant difference in terms of insecticidal between small green fruit acetonic extract with all acetonic extracts (P≥0.00001), and also there is a significant difference between flower acetonic extract and all acetonic extracts except leaf acetonic extract (P≥0.01). Based on the results, there are significant differences in mortality rates between control and n-hexane with all other n-hexane extracts (P≥0.02) and between ripe fruit n-hexane extract with all other n-hexane extracts (P≥0.00001).

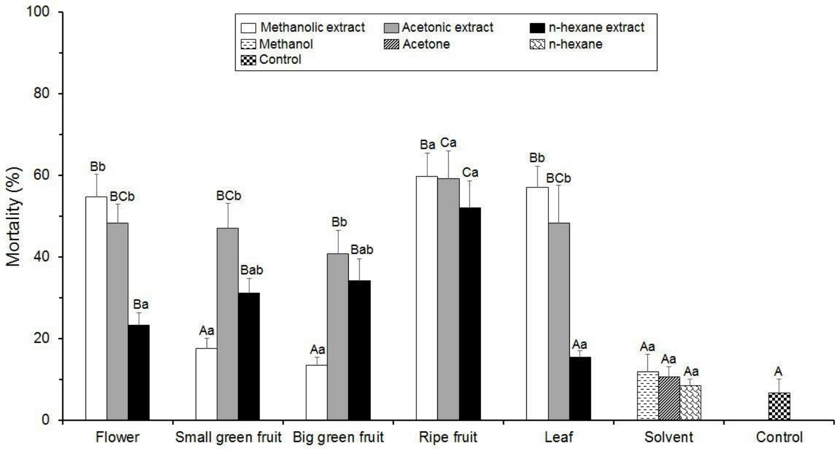

Fig. 1. Insecticidal effects of methanolic, acetonic and n-hexane extracts of flower, different fruit growth stages and leaf of *Melia azedarach* on 1-2 days-old of *Aphis fabae* nymphs mortality in laboratory conditions. Different small letters show a significant difference between different solvents in the same plant part, and different capital letters show a significant difference between different plant parts in the same solvent.

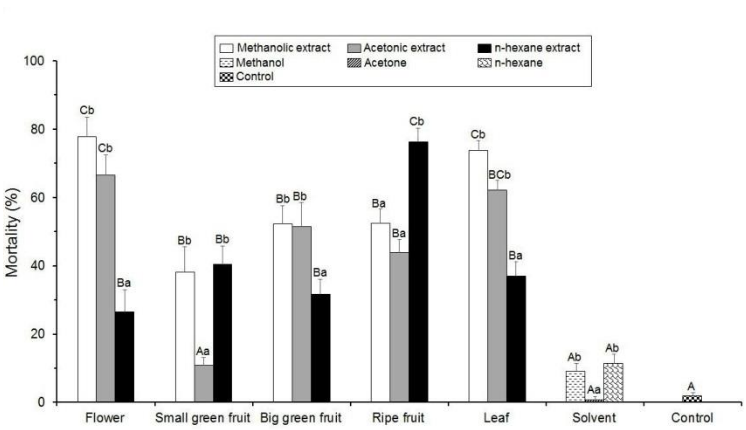

Fig. 2. Insecticidal effects of methanolic, acetonic and n-hexane extracts of flower, different fruit growth stages and leaf of *Melia azedarach* on 3-4 days-old of *Aphis fabae* nymphs mortality in laboratory conditions. Different small letters show a significant difference between different solvents in the same plant part, and different capital letters show a significant difference between different plant parts in the same solvent.

Discussion

The results of the effects of extracts derived from flower, fruit growth stages and leaf showed all plant extracts had insecticidal activity against black bean aphid. It should be noted that the insecticidal effects of extracts derived from various parts of the plant are different. The insecticidal activity of different plant extracts, depending on the extraction solvent and insect age (1-2 days-old or 3-4 days-old nymphs) differ. Different insecticidal effect rate of extracts from the same plant material using different solvents reveals that the reaction of insect facing materials taken by varied solvents is not the same. In other words, toxicity of the extract of one plant part in a solvent is different from that of the same plant part in a different solvent. The existence of this difference is possibly due to the fact that plant secondary metabolites with toxicity effect on the insect are different in terms of polarity. One study revealed that aqueous solvent (80% methanol, 80% ethanol) from plant materials exhibited higher antioxidant activity and phenolic content (Sultana et al., 2009). Some researches indicate that *M. azedarach* extracts has a significant impact on some insects. Due to its high limonoid content, *M. azedarach* has been shown to control pests and to possess pesticide properties (Huang et al., 1996). Limonoids, mono-, di-, sesqui-, and triterpenoids, coumarins, chromones, lignans, flavonoids and other phenolics were isolated from its certain species with wide-ranging uses in ethnomedicine (Mulholland et al., 2000; Srinivasan et al., 2014).As an instance in a research on the effect of *M. azedarach* leaf extract on *S. frugiperda*, it was proved that this extract significantly increases fatality and decreases both larval period and also larval growth speed (McMillian et al., 1969; De Brito et al., 2004). These results are in agreement with the mortality effect of leaf methanolic extracts on *A. fabae* in our observations. A GC-MS analysis of the methanolic *M. azedarach* leaf extract identified , 48 bioactive phytochemical compounds including Kampherol, Quercetin (Flavonoid), stigmasterol, β-sitosterol, Campesterol (phytosterols), Phytol (Diterpene), 3- Methyldecane, Heptadecane (alkane hydrocabon), hexadecanoic acid, Pentadecanoic acid (n-alkanoic acids), Beta-Carotene, tocopherol (vitamin-E) and squalene, 1-Eicosanol (tri-terpene), 3,7,11,15-Tetramethyl-2-hexadecen-1-ol (Terpene alcohol) (Sen & Batra, 2012). The results of the present investigation indicate that the extracted materials from all parts of M. azedarach have the insecticidal potential, therefore through the proper selection of plant parts and an appropriate solvent, it may be possible to increase the effect of these materials on pests.

Conflict of interest

The authors declare that there are no conflicts of interest present.

CRediT author statement

M. Khabir: Field and laboratory works & writing original draft preparation. **K. Ahmadi**: Supervision, methodology, writing, reviewing & editing. **M. Shojaaddini**: methodology, reviewing & editing.

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