Evaluation of five plant extract on *Aphis fabae* Scopoli (Hemiptera: Aphididae)and its predator, *Coccinella septempunctata* L.(Coleoptera: Coccinellidae), under laboratory conditions

Research project

Submitted to the department of Plant Protection in partial fulfillment of the requirements for the degree of bachelors in Agricultural Engineering science

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بسم الله الرحمن الرحيم

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ۝ خَلَقَ الْإِنسَانَ مِنْ عَلَقٍ ۝ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ۝

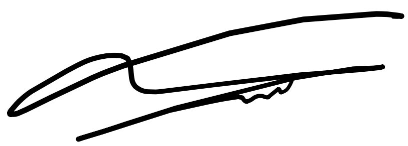
الَّذِي عَلَّمَ بِالْقَلَمِ ۝ عَلَّمَ الْإِنسَانَ مَا لَمْ يَعْلَمْ

صدق الله العظيم

سورة العلق، الآية (1-5)

Certificate

This research project has been written under my supervision and has been submitted for the award of the BSc. degree in Agriculture Engineering Science – Plant Protection with my approval as a supervisor



Signature Name: Lecturer Dr. Gona Sirwan Sharif

Date: 23.11.2023

Dedication

I would like to dedicate this thesis to my beloved supervisor Dr. Gona Sirwan Sharife, who taught me confidence in myself.

Brwa Sami Abdullah

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I would like to express my appreciation and gratitude to my supervisor, Dr. Gona Sirwan Sharif, for her invaluable advice, enormous assistance, and guidance during this article review. Thanks are also due to all the employees of the Plant Protection Department.

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1.Introduction

Plants produce secondary metabolites and chemical substances to protect themselves from the attack of pests and pathogens (Silva, 2008; Hussein *et al*., 2019). Furthermore, an increasing demand for innovative active substances and pest control products, which aim to mitigate the adverse effects of chemical insecticides on the environment and human health (Rodriguez, 2019). Thus, thousands of plant extracts have been evaluated as alternatives to chemical insecticides. The use of botanical insecticides can cause mortality, infertile adults, slow growth, and a decrease in the egg viability of insects. However, these botanical extracts are generally less harmful to the environment, low cost, and their use as food ingredients indicate their low toxicity to humans (Da Silva, 2017; Bedini *et al*., 2020)

Lamiaceae Is among the largest families of flowering plants with about 250 genera and over 7,000 species distributed around the world (Mesquita,*et al* ., 2019).Numerous species of *Mentha* genus are rich in several botanical compounds such a essential oils, phenolics, and flavonoids. have conventionally been used to repulse insects. Also, the *Ocimum* genus belongs to the Lamiaceae , it was known to comprise at least 200 species and numerous varieties which have been recently reclassified into 64 species (Tchoumbougnang, *et al*., 2003; Muralidharan and Dhananjayan,2004). Tarragon, scientifically known as *Artemisia dracunculus* L., is a perennial herb belonging to the Asteraceae (daisy) family. Extracts and essential oils derived from this plant find application in the management of specific aphid species (Gospodarek *et al*.,2022). *Syzygium aromaticum*, also known as clove, is a dried flower bud belonging to the Myrtaceae family that is indigenous to the Maluku islands in Indonesia but has recently been farmed in different places worldwide (Cortés-Rojas, *et al*., 2014; Batiha, *et al*., 2019). *Anethum graveolens* L. commonly known as dill belonging to the family Umbelliferon. The essential oil and extracts obtained from the dill plant exhibit considerable potential in terms of antibacterial, antifungal, antioxidant, insecticidal (Chahal *et al*., 2017).

The widespread application of synthetic chemical pesticides for aphid control at a large scale has given rise to environmental risks and adverse impacts on beneficial organisms, particularly natural enemies (El-Wakeil *et al* ., 2013). These side effects have stimulated research on alternative strategies of aphid management. Alternative methods include using plant extracts and natural compounds (Ghodke,*et al*., 2013).The black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae), is a major polyphagous pest of agricultural crops. The pest can cause to death of plants and causes reductions in the yield quantity and quality. Field losses infested by this pest can reach above 50% (Hansen, 2008). This insect pierce leaves, stems, flowers and young pods during adulthood and nymphs and leads to yellowing plants, withering and dying. Furthermore, it is a vector of more than 30 plant viruses, such as those of tomato and peas, potato, beets, cucurbits, crucifers etc (Blackman and Eastop, 2007).

The seven-spotted lady beetle (Ladybird), *Coccinella septempunctata* Linnaeus (Coleoptera: Coccinellidae), is considered a generalist predator. Its larvae and adults actively prey on various species of soft-bodied insect pests, such as the green peach aphid, *Myzus persicae* (Sulzer), the cotton aphid, Aphis gossypii Glover, and the bird cherry-oat aphid (Darwish, 2019). This study was carried out to evaluate five different botanical extracts against the black bean aphid *Aphis fabae*, and its biological control agent, the ladybird beetle, *Coccinella undecimpunctata* L., under laboratory conditions.

2.Literature Review and Background

2-1 Botanicals as biopesticides

The development and promotion of eco-friendly bio-pesticides which only attack the target pest and harmless to beneficial biota are being stressed all over the world. Plants have evolved over some 400 million years and they have developed a number of protective genetically acquired inbuilt mechanisms, such as repellency and insecticidal action etc to protect themselves from pest attack. As such, plant products are regarded as an effective substitute for chemical pesticides. Botanicals or their derivatives of plant origins have good capability to regulate and control of harmful pests. Plants are known to provide a vast reservoir of biologically active chemical constituents (Saxena *et al*.,2014).

However, not more than 10% of these have so far been examined in detail for their biological activity against human diseases (Nitya Nand, 1977) and much less against plant diseases. The earliest mention of poisonous plants or those with pest control properties is found in ancient Indian literatures. Democritus tried plant extract for controlling plant diseases as early as in 470 earliest mention of poisonous plants or those with pest control properties is found in ancient Indian literatures. Democritus tried plant extract for controlling plant diseases as early as in 470 BC (Sherville, 1960). Pest control through pesticides of plant origin has a long history and farmers have used pesticides prepared from seeds of resistant plants. Thus, a large number of different plant species contain natural insecticidal materials. Some of these have been used by man as insecticides since very early times. But many of them can not be profitably extracted.

However, several of these extracts have provided valuable contact insecticides which possess the advantage that their use does not appear to result in the emergence of resistant insect strains in the same degrees as the application of synthetic insecticides do (Saxena *et al*.,2014). .

1. Mint *Mentha* sp.

The genus *Mentha* (mint) belongs to the Lamiaceae family, which includes 25 to 30 species (Mamadalieva, 2020). Chemical analyses of Mentha species have yielded a number of important phytocompounds belonging to different classes, such as organic acids, flavonoids, sterols, alkaloids, lignans, hydrocarbons, fatty acids, tocopherols, proteins, free sugars, etc. Moreover, the main compounds in mints are essential oils, phenolics, and flavonoids. These compounds can also serve as valuable ingredients in the creation of natural repellents for use in organic farming (Ciesielska et al. 2011).

2. Basil (*Ocimum basilicum* L.)

is one of the most famous, annual or perennial herb belonging to the family Lamiaccae, there is some confusion in the literature about the exact number of species of the genus *Ocimum*. Therefore, the genus is still being studied by researchers (Meyers,2003). According to (Tchoumbougnang *et al*2003; Muralidharan and Dhananjayan,2004) , the genus *Ocimum* (basil) was known to comprise at least 200 species and numerous varieties which have been recently reclassified into 64 species.

The chemical composition of basil essential oil has been investigated since the 1930s15 and by now more than 200 chemical components have been identified which revealed a huge diversity in the constituents of its oil from many regions of the world (Lawrance,1988).

3.Tarragon (*Artemisia dracunculus* L.)

Tarragon, *A dracunculus* is a perennial, herbaceous plant that is native to a wide area of the Northern Hemisphere, from Eastern Europe across central and eastern Asia to India, western North America, and south to northern Mexico (Fernández-Lizarazo *et al.*,2011). As the research conducted so far has shown,

extracts and essential oils from this plant are used in the control of some pests, who reviewed the results of 95 plants that have been shown to have insecticidal properties, ethanol and/or water extracts of these plants are environmentally friendly, and the solvents are easily available such as Tarragon water extract has strong aphicidal potential against *A. fabae* (Gospodarek *et al*., 2022).

4.Clove (*Syzygium armaticum* L.)

S. aromaticum belongs to the Myrtaceae family, which has more than 3000 species and 130–150 genera, such as the myrtle, eucalyptus, clove, and guava families. Clove is an aromatic flower cultivated in Madagascar, Sri Lanka, Indonesia, and China (Guan *et al.,* 2007; Golmakani, *et al*., 2017; Tunç and Koca, 2019). Several reports suggest that *S. aromaticum* contains a high amount of phenolic compounds with several biological activities, including antibacterial, antifungal, insecticidal, and antioxidant properties (Tunç and Koca, 2019; Guan *et al.,* 2007; Golmakani, *et al*., 2017 ; Hatami, *et al*., 2019)

5.Dill (*Anethum graveolens* L.)

Anethum graveolens commonly known as dill belonging to the family Umbelliferon, is one of the most useful essential oil-bearing spices as well as medicinal herb. Dill is cultivated throughout the world as a medicinal plant. Dill seeds are used as a flavoring agent. Essential oil can be extracted from various parts of a plant and chiefly consisted of dill carvone, limonene, dill apiol and α-phellandrene. The essential oil and extracts of dill plant possess promising antibacterial, antifungal, antioxidant, insecticidal, anti-inflammatory, antidiabetic, antispasmodic, hypolipidemic activities etc. (Chahal *et al* 2017).

2.2-Black bean aphid, *Aphis fabae* Scopoli

2.2.1-Classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Hemiptera

Suborder: Sternorrhyncha

Family: Aphididae

Genus: *Aphis*

Species: *A. fabae*

2.2.2- Distribution

The black bean aphid may have originated in Europe and Asia, but it is now one of the most widely distributed species of aphids (Esmaeili-Vardanjani *et al*., 2013). It is found throughout temperate areas of Western Europe, Asia, and North America and in the cooler parts of Africa, the Middle East, and South America (Carvalho *etal*., 2015; Meradsi, Laamari, 2018).

2.2.3-Host plants

The black bean aphid can feed on a wide variety of host plants. Its primary hosts on which the eggs overwinter are shrubs such as the spindle tree (Euonymus europaeus), *Viburnum* species, or the mock-orange (*Philadelphus* species). Its secondary hosts, on which it spends the summer, include a number of crops including sugar beets, spinach, beans, runner beans, celery, potatoes, sunflowers, carrots, artichokes, tobacco, and tomatoes. It colonises more than 200 different species of cultivated and wild plants. Among the latter, it shows a preference for poppies (*Papave*r species), burdock (*Arctium tomentonum*), fat-hen (*Chenopodium album*), saltbush (*Atriplex rosea*), chamomile (*Matricaria chamomilla*), thistles (*Cirsium arvense*), and docks (*Rumex* spp.), (Capinera, 2001).

2.2.4- Life cycle

In temperate areas, black bean aphid overwinters as an egg on one of its primary hosts. Initially green, the eggs soon turn shiny black. In warmer areas, the aphids reproduce continuously without producing eggs. The nymphs are dark yet display four pairs of transverse white bars on the dorsal surface of the abdomen. The durations for instars 1–4 are 2 days, 1.5 days, and 2.5 days, respectively ,when reared at about 20°C. however, reported only three instars, with durations of about 2.3, 3.0, and 2.5 days. Total nymphal development time requires 5–10 days at 28–17° C, respectively. Black Bean aphid adult is dark olive-green to dull-black in color. The body length is 1.8–2.4 mm in females, with males only slightly smaller. The appendages tend to be black, but the tibiae may be pale, in part. The wings are transparent. Reproduction commences soon after attainment of the adult stage, usually a period of about 3–6 days. Both alate and apterous females reproduce. (Figure 2). The adult produces about 85–90 nymphs during her reproductive period, which is estimated at 20–25 days. Most offspring are produced in the first 5–10 days of the reproductive period. Reproduction increases with temperatures, up to a threshold of about 24°C, and then decreases. The reproductive period is followed by a post-reproductive period of about seven days (Frazer, 1972). Apterous females give birth to more, and larger, nymphs than alatae. (Figure 1).

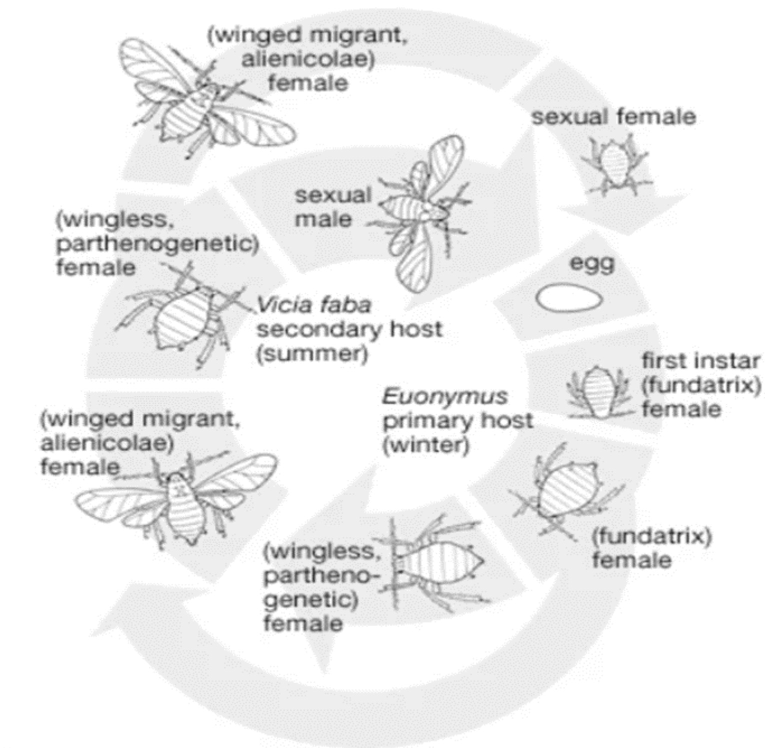

Figure 1: Life cycle of black bean aphid

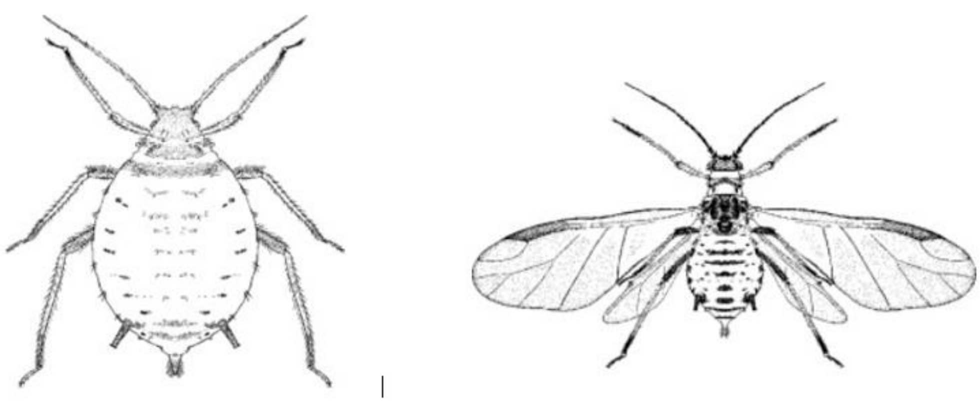

Figure 2: Adult female bean aphid, wingless and winged form

2.2.5-Damage

The black bean aphid is a major pest of sugar beet, bean, and celery crops, with large numbers of aphids cause stunting of the plants. Beans suffer damage to flowers and pods which may not develop properly. Early-sown crops may avoid significant damage if they have already flowered before the number of aphids builds up in the spring. Celery can be heavily infested. The plants are stunted by the removal of sap, the stems are distorted, harmful viruses are transmitted, and aphid residues may contaminate the crop. As a result of infestation by this aphid, leaves of sugar beet become swollen, roll, and cease developing. The roots grow poorly and the sugar content is reduced. In some other plants, the leaves do not become distorted, but growth is affected and flowers abort due to the action of the toxic saliva injected by the aphid to improve the flow of sap (Webster *et al.*, 2008)

To obtain enough protein, aphids need to suck large volumes of sap. The excess sugary fluid, honeydew, is secreted by the aphids. It adheres to plants, where it promotes growth of sooty molds. These are unsightly, reduce the surface area of the plant available for photosynthesis and may reduce the value of the crop. These aphids are also the vectors of about 30 plant viruses, mostly of the nonpersistent variety. The aphids may not be the original source of infection but are instrumental in spreading the virus through the crop. Various chemical treatments are available to kill the aphids and organic growers can use a solution of soft soap (Webster *et al.*, 2008).

2.3-The seven spotted ladybeetle, *Coccinella septempunctata* L.

2.3.1-Classification

Kingdom: Animalia

Phylum: Arthropoda

Class: Insecta

Order: Coleoptera

Family: Coccinellidae

Genus: *Coccinella*

2.3.2- Distribution

The seven spotted ladybeetle, *C. septempunctata*, which is well-established in North America, has now spread across parts of five Canadian provinces and 34 contiguous eastern states in the United States (Schaefer et al., 1987). six species of ladybirds were recorded in , Iraq, representing six genera from different plant families(Najim *et al.*, 2022) .

2.3.3- Life cycle

Lady beetle is a holometabolous insect, due to the presence of eggs, larvae, pupae and adults' stages. Thus, the life cycle shows a complete metamorphosis. Life cycle was complete in 17 days (minimum) to 65 days (maximum) except in May and June months. (Chatterjee, 2006). Female lady beetles may lay from 20 to more than 1,000 eggs over a one-to-three-month period, commencing in spring or early summer. Eggs are usually deposited near prey such as aphids, often in small clusters in protected sites on leaves and stems. The eggs of many lady beetle species are small (about 1 mm; 1/25"), cream, yellow, or orange, and spindle shaped. Lady beetle larvae are dark and alligator-like with three pairs of prominent legs. Depending on the species and availability of prey, larvae grow from less than 1 mm (1/25") to about

1 cm (3/8") in length, typically through four larval instars, over a 20-to-30-day period. Large larvae may travel up to 12 m (about 40') in search of prey. The larvae of many species are gray or black with yellow or orange bands or spots (Gordon, 1985. : Hoffmann *et al*., 1993 :Ashraf *et al.*, 2010).

The last larval instar remains relatively inactive before attaching itself by the abdomen to a leaf or other surface to pupate. Pupae may be dark or yellow orange. The pupal stage may last from three to 12 days depending on the temperature and species.The adults emerge, mate, and search for prey or prepare for hibernation, depending on the availability of prey and time of year. Adults may live for a few months to over a year. The more common species typically have one to two generations per year. (Gordon, 1985. : Hoffmann *et al.*, 1993 :Ashraf *et al*., 2010).

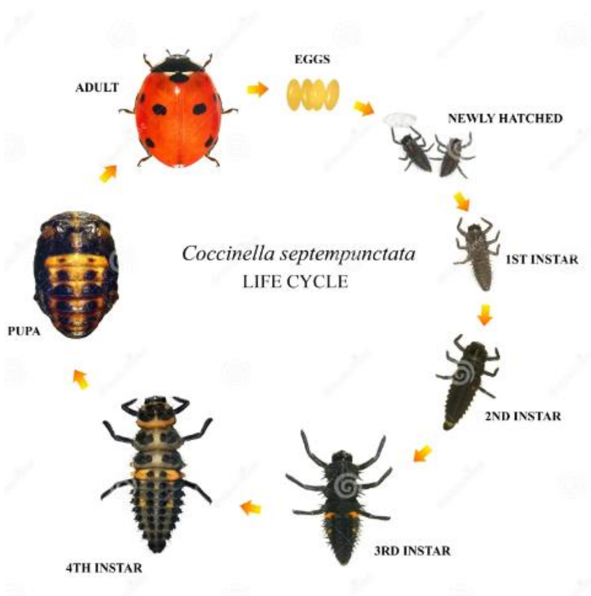

Figure 3: Life cycle of the seven spotted ladybeetle

2.4 - Management of Black bean aphid

Controlling black bean aphids can be managed through several methods, including cultural, mechanical, biological, and chemical approaches.

2.4.1-Biological Control

Natural enemies can be very important in the control of aphids, especially in gardens not sprayed with broadspectrum pesticides (organophosphates, carbamates, and pyrethroids) that kill natural enemy species as well as pests. Usually natural enemy populations do not appear in significant numbers until aphids begin to be numerous.

Among the most important natural enemies are various species of parasitic wasps that lay their eggs inside aphids. The skin of the parasitized aphid turns crusty and golden brown, a form called a mummy. The generation time of most parasites is quite short when the weather is warm, so once you begin to see mummies on your plants, the aphid population is likely to be reduced substantially within a week or two. Many predators also feed on aphids. The most well-known are lady beetle adults and larvae, lacewing larvae, and syrphid fly larvae. (Flint 2013).

2.4.2 Chemical Control

The use of pesticides has increased many folds over the past few decades. According to (Alavanja,2009). approximately 2.54 billion tons of pesticides were used worldwide each year. The production of pesticides in India, which is one of the top pesticide manufacturer's countries, has increased by 20.5 folds from 1958 to 1999 (Aktar *et al*., 2009). The main reasons for using pesticides are to increase productivity and reduce yield losses by protecting crops from weeds, diseases, and insects (Mubushar *et al*., 2019). On the other hand, excessive use of pesticides results in health issues for both farmers and consumers (Lander *et al*., 2000).and environmental including water pollution (Ciglasch *et al*., 2008) (Hakeem *et al*., 2016), soil contamination (Islam *et al* ., 2006).and damage useful microorganisms, soil biomass, earthworms (van der Werf, 1996; Waichman *et al*., 2007).

If insecticides are needed, insecticidal soaps and oils are the best choices for most situations. Oils may include petroleum-based horticultural oils or plant-derived oils such as neem or canola oil. These products kill primarily by smothering the aphid, so thorough coverage of infested foliage is required.

Many other insecticides are available to control aphids in the home garden and landscape, including foliar- applied formulations of malathion, permethrin.

3. Materials and methods

3.1-Plant samples and Extracts

In October 2023, fresh leaves of four plant species and clove seeds (Syzygium aromaticum L.) were obtained from markets in the Erbil and Sulaymaniyah Governorates, Iraq, as indicated in Table 1. One kilogram of fresh leaves was collected from each plant species. All the materials were washed with tap water, dried in sunlight, and used for extraction.

Subsequently, the materials were dried in an oven at 60°C for 24 hours to achieve a constant weight. Afterward, 100 grams of dried materials for each sample were ground to a fine powder using an electric mixer for 5 minutes separately. Each sample was then soaked in one liter of water. The extracts were preserved in well-darkened, clean glass bottles labeled with tags at 25°C (room temperature) for twenty-four hours. All the mixtures were filtered using a filter to obtain a homogeneous substance, which was used for the experiment (Ahmed et al., 2019).

Table 1: The five plant materials used in the present study.

No.	Scientific Name	Common Name	Family Name
1	*Mentha* sp.	Mint	Lamiaceae
2	*Ocimum basilicum* L.	Basil	Lamiaceae
3	*Artemisia dracunculus* L.	Tarragon	Asteraceae
4	*Syzygium aromaticum* L.	Clove	Myrtaceae
5	*Anethum graveolens* L.	Dill	Umbelliferon

3.2- Insects

Aphis fabae will be collected from faba bean cultivars specifically planted for this purpose at the Grdarasha field station in Erbil Governorate, Iraq. A stock culture of *A. fabae* is being maintained on broad bean seedlings previously planted in pots, and it will be used for laboratory experiments. The larvae of the ladybird beetle, *Coccinella undecimpunctata*, will be gathered from the field, individually reared in 1 × 5 cm glass tubes, and supplied daily with an abundance of aphid nymphs until they pupate. One generation will be raised under laboratory conditions before their use.

3.3-Bioassay

The experiments consisted of seven treatments, comprising five plant materials, Acetamiprid insecticide used as a positive control, and water as negative control. The negative control involved the absence of pesticides or plant material solutions. Additionally, a treatment involving the application of a synthetic chemical (Acetamiprid at a rate of o.35g/ L according to the recommended label) was utilized as a comparative control to evaluate its efficacy in comparison with the five plant material extracts.

Each extract will be diluted to produce four concentrations (1%, 2%, 4%, and 10%). Then, 3 mL of each extract/concentration will be evenly sprayed using a fine sprayer machine (Thomas Scientific, USA) on a 300 cm2 area, which will hold 32 broad bean plant leaves divided into two groups (16 leaves in each group). In the first group, each leaf will host 5 aphid individuals, while in the second group, each leaf will accommodate 5 predator individuals (2nd larval instar) along with approximately 30 individual aphids as prey. The spray will be directly administered onto the plant leaves housing the tested insects.

Following the spray application, each leaf will be positioned in a Petri dish (100×15 mm) containing moistened cotton tissues to sustain humidity. These dishes will then be placed in a plant growth chamber set at 25 ±1 °C, 65 ± 3 RH, and with a light-dark cycle of 14:10 L:D. Each group will consist of four leaves considered as one replicate, totaling 20 individual aphids or predators per replicate. There will be a total of 3 replicates per concentration. After 24 hours, the aphid and predator. (Sadeghi, *et al.*, 2009: Sayed *et al*., 2020).

3.4-Statistical analysis

The estimation of lethal concentrations (LC50) will involve Log-probit analysis to correlate mortality with concentration. All statistical analyses will be performed using the SPSS software program, version 23.

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