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Efficacy of five plant extracts against Black bean aphid, *Aphis fabae* Scopoli (Hemiptera: Aphididae)

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ABSTRACT

The efficacy of five selected plant extracts: Basil (*Ocimum basilicum)*, Cloves (*Syzygium aromaticum*), Tarragon (*Artemisia dracunculus*), Mint (*Mentha* sp.), and Dill (*Anethum graveolens*) against *Aphis fabae* on both nymph and adult stages was evaluated under laboratory conditions. Four concentrations were used 10000, 20000, 50000, and 100000 ppm. The results showed that the maximum mortality percentage of the nymph stage of *A. fabae* was achieved by basil, tarragon, and clove aqueous extracts at concentrations of 10000 ppm after 72 hours of treatment. Conversely, basil, clove, and tarragon extracts recorded the highest mortality rate of adults under the same experimental conditions. The positive control (Acetamiprid 20%) produced the highest mortality rate after 72 hours of exposure while using distilled water as the negative control resulted in a minimal mortality rate. The LC_{50} values indicate that basil extract exhibited the highest toxicity for nymphs at 1664.177 ppm after 72 hours, while for adults, tarragon extract was the most toxic at 720.553 ppm at 72 hours.

1.Introduction

Botanical pesticides effectively manage various crop pests. They are affordable, biodegrade easily, employ diverse modes of action are derived from readily available sources, and exhibit low toxicity to non-target organisms (Yousuf e*t al.,* 2021). The varied modes of action result from the diverse phytochemical compositions found in different plants (Lengai *et al.,* 2020). One of the largest families among flowering plants is the family of Lamiaceae, which contains approximately 250 genera and more than 7000 species distributed worldwide (Mesquita *et al.,* 2019). The *Mentha* genus includes many species rich in botanical compounds such as phenolics, flavonoids and essential oils which have been used to repel insects (Mamadalieva *et al.,* 2020). *Ocimum* genus known as sweet basil belongs to the Lamiaceae family and consists of more than 200 chemical components present as hydrocarbons, oxygenated monoterpene, sesquiterpene hydrocarbons, oxygenated sesquiterpene, triterpene, and flavonoids which work as antibacterial and antifungal, antiviral and insecticidal (Marwat *et al*., 2011). Tarragon, *Artemisia dracunculus* L. (Asteraceae), a perennia1 herb used as botanical extracts and essential oils to control specific aphid species (Gospodarek *et al.*, 2022). Clove (*Syzygium aromaticum*) is an aromatic plant rich in compounds like eugenol, widely used as a natural pesticide. Its essential oil has antimicrobial, antioxidant, and insecticidal properties, making it valuable in health and various industries(Haro-González *et al.,* 2021) Dill, scientifically named *Anethum graveolens* L. comes from the Umbelliferon family. The essentia1 oil extracts derived from the *A. graveolens* plant can be used as insecticidal, antifungal, antibacterial and antioxidant agents (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 (Ahmed and Majeed, 2016). These side effects have prompted research into alternative strategies for aphid management, such as utilizing plant extracts and natural compounds (El-Wakeil *et al.,* 2013). *Aphis fabae* Scopoli (Hemiptera: Aphididae) is a pest that feeds on various crops. It causes plant death and reduces both yield quantity and quality. Infested fields can suffer losses exceeding 50% (Hansen *et al.*, 2008). Adults and nymphs pierce leaves, stems, flowers, and young pods, leading to yellowing, withering, and eventual plant death. Additionally, it serves as a vector for over 30 plant viruses, infecting crops such as tomatoes, peas, potatoes, beets, cucurbits, and crucifers (Blackman *et al.,* 2007). This study evaluated the toxic impact of five selected botanical extracts against both nymphs and adults of *A. fabae.*

2. Materials and methods

2.1. Collection and rearing of insects

The study was conducted at the Plant Protection Department laboratories at the College of Agricultural Engineering Sciences /Salahaddin University/ Kurdistan region-Iraq. *A. fabae* individuals were collected from *Aguadulce* faba bean cultivars specifically planted for this purpose at the Grdarasha field station in Erbil Governorate during 2023. A continuous culture was maintained for laboratory experiments. One generation was raised under laboratory conditions before their use (Abdel-Rahman *et al*., 2019).

2.2. Preparation of Plant Extracts

Table 1 shows that five selected plants were obtained from the local markets in Sulaymaniyah and Erbil cities. The plants were cleaned with tap water, dried under shade, and pulverized using an electric mil1 (DAMA1 - China). A 100000ppm concentrated solution of the plant extracts in the water was made by combining 100 grams of powder for each (seeds or leaves) with 1000m1 of distilled water. The Magnetic Stirrer (INTLLAB, China) was used to mix it with a Stir Bar at 3000 rpm. After overnight soaking, the muslin cloth was used to filter the solution and kept in the fridge and stored in the refrigerator till they were used(Irié-N'guessan *et al*., 2011, Abdulhay, 2012, Khidr and Khalil, 2024). In addition, the study was extended to evaluate different concentrations of 5%, 2%, and 1% of the extracts, which correspond to 50000, 20000, and 10000 ppm, respectively.

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

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

2.3. Extracts bioactivity assay

The insecticidal activity of five plant extracts on *A. fabae* was investigated under laboratory conditions at the Plant Protection Department. The toxicity of plant extracts was assessed on both the nymph and adult stages of aphids with a total sample of 240 individuals. Three replicates each included ten nymphs or adults. were sprayed with four concentrations 100000, 50000, 20000, and 10000 ppm in Petri dishes containing faba bean leaves with filter paper which maintains the moisture. The dishes were arranged in a completely random design (CRD) system and covered with muslin cloth.

Acetamiprid 20% chemical pesticide at a rate of 0.35g/liter was used as a positive control and the distilled water was used as the negative control. All experiments were conducted under controlled conditions: room ‘temperature at 25±2 ℃, RH at 65±3%, and 14:10 (L:D).

The mortality rate and lethal concentrations (LC_{50}) values of both nymph and adult stages of *A. fabae* were evaluated at 24, 48, and 72 hours.(Sadeghi *et al*., 2009, Sayed *et al.,* 2020).

2.4.Statistical analysis

To assess the effect of various botanical extracts on the mortality rate of *A. fabae*, statistical analysis was conducted. A multi-factor ANOVA (Analysis of Variance) in Statgraphics Centurion XV was used to analyze the data, followed by Fisher’s LSD test to determine significant differences in mortality rate means at P ≤ 0.05. Abbott used his algorithm (Abbott, 1925) to adjust mortality data to determine LC50 values and associated statistics. The maximum likelihood program of Probit analysis in SPSS software version 20 was then applied to the mortality data.

3.Results

3.1. Nymph stages of *A. fabae* mortality rate responds to treatment with plant extracts

Results obtained in Figure 1 show that the type of botanical extracts used in the laboratory test has a considerable impact on the mortality rate of the nymph stage of *A. Fabae* ($F_{(6,197)}$ = 19.07, P < 0.000). Among the five species studied, basi1 extract demonstrated the highest toxicity, resulting in a 70.8 % mortality rate, followed by tarragon and clove with mortality rates of 63.2% and 59.1%, respectively, and there was significant variance between them and both synthetic chemical insecticides (Acetamiprid 20%) positive control and the distilled water negative control which had maximum and minimum MR at 88.3% and 7.1%, respectively (Figure1-a).

Moreover, the effectiveness of toxic botanical extracts in the nymph stage depended on the concentration used ($F_{(5,\ 197)}$ = 6.45, P <0.000, Fig.1b). Among the four concentrations, the plant extract with 100000 ppm was the most toxic, resulting in a 56.9% mortality rate, followed by 20000,50000 and 10000 ppm with mortality rate of 49.9.4%, 45.5.4%, and 43%, respectively. However, these concentrations were not significantly different from each other. Additionally, the concentrations of botanical extracts showed significant differences with both positive control and negative control (Figure 1-b).

The mortality rate of *A. fabae* varied significantly not only depending on the concentration of the plant extract but also on the duration of exposure to the plant extracts ($F_{(2,195)}$ = 42.12, P <0.000, Figure 1-c). Consequently, peak mortality rate (74.4%) was observed at the 72 hours mark, although it did not differ significantly from 48 hours while the lowest (23%) occurred at 24 hrs.

(Figure1-c).

Similarly, the interaction between the botanical extracts and the duration of immature stage exposure significantly influenced nymph survival. The three most toxic extracts (basil, tarragon, and c1ove) resulted in a maximum nymph mortality rate at 72 hours after treatment, while the chemical insecticide caused 100% mortality within 24 hours of exposure (Figure 2-b). Additionally, the concentration applied affects the effectiveness of botanical extracts on the immature stage of *A. fabae*. (Figure 2-a).

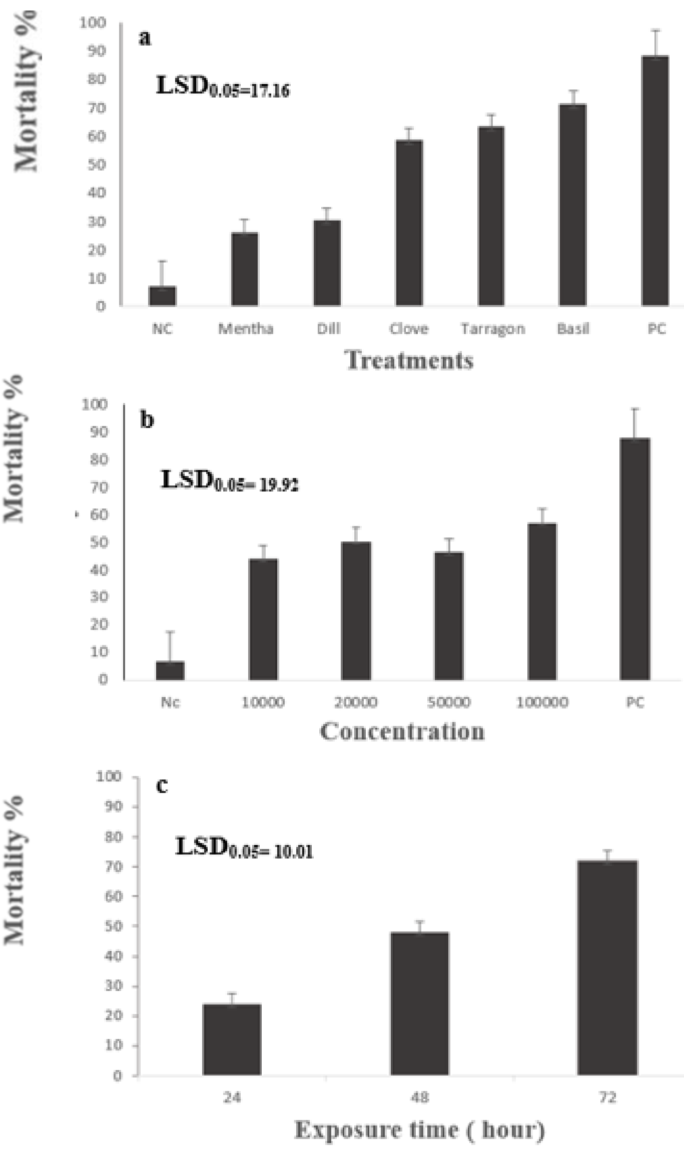

Figure 1. Mortality % of *A. fabae* nymph stages treated with (a) different plant extracts (b) different extract concentrations/plant (c) different exposure time (hours).

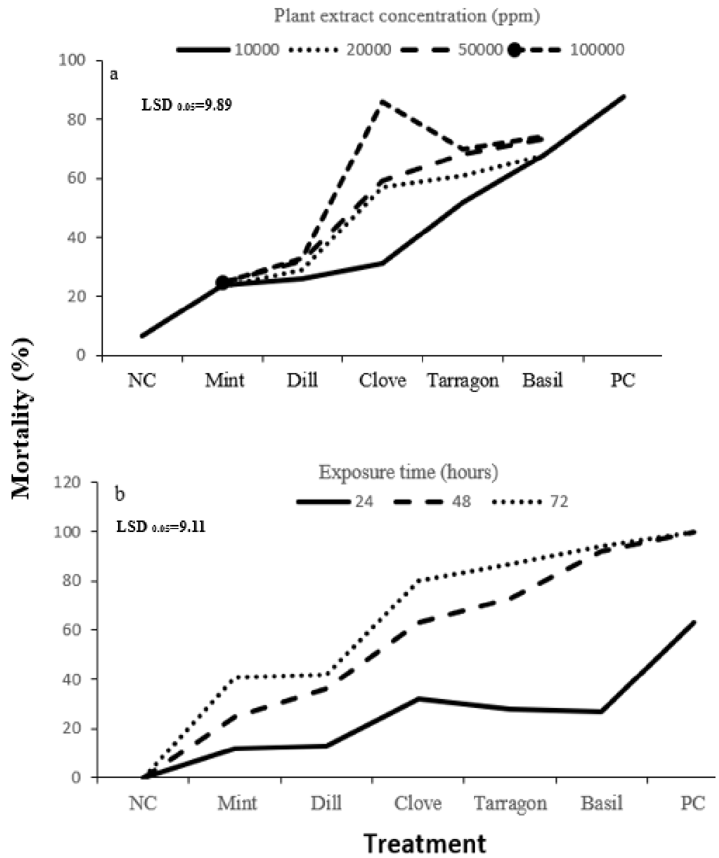

Figure 2. Mortality % of *A. fabae* nymph treated with different plant extract, (a) interaction between plant extract and concentration (b) interaction between plant extracts and exposure time.

3.2.Determination of lethal Concentration (LC50)

The toxicity of different botanical extracts was assessed by determining the LC_{50} values against the nymph stage of *A. fabae* (Table 2). The LC_{50} values indicate that, out of the five botanical insecticides studied, basil extract exhibited the highest toxicity at 1664.177 ppm after 72 hours while Dill displayed the lowest toxicity at 4786010625 ppm after 24 hours. Additionally, there was variability observed in the nymph stages of *A. fabae* accompanied by a steady decline in LC_{50} values.

Table 2. The LC50 average values (ppm) of plant extracts on nymph stages of *A. fabae* following different exposure periods.

Plant Extract	Period (hours)	LC50 (ppm)	Average
	24	114294230	
Basil	48	2620.858	38099504.97
	72	1664.058	
	24	82680.877	
Clove	48	19543.330	108807.03
	72	6582.821	
	24	800176.555	
Tarragon	48	2847.580	804708.31
	72	1684.177	
	24	4148174696.4	
Mint	48	459041.613	4148984820.68
	72	351082.662	
	24	4786010625.0	
Dill	48	61743603.180	4851358283.58
	72	3604055.402	

3.3. *A. fabae* adults mortality rate responds to treatment with plant extracts

Our result shows that *A. fabae* adults' mortality rate was significantly influenced by the variety of botanical extracts used in the laboratory conditions experiment ($F_{(6,197)} = 4.45$, $P < 0.000$). Basi1 extract recorded the highest toxicity, resulting in a 49.7 % mortality rate, followed by c1ove and tarragon with a mortality rate of 48% and 40%, respectively. There was no significant difference in mortality rate between c1ove and tarragon. Additionally, only basi1 and c1ove showed significant differences from the positive control but did not differ significantly from the negative control which had the highest and lowest mortality rates at (76.3 and 19.2 %), respectively (Figure 3-a).

Additionally, the effectiveness of plant extracts in the adult stage depended on the concentration applied ($F_{(5,197)} = 4.47$, $P < 0.0007$) (Figure 3-b). In the concentration of 100000 ppm, the extract had the most effect among the three other concentrations, resulting in a 49.7% mortality rate, followed by 50000, 20000, and 10000 ppm, with mortality rates of 39.9, 37.9, and 32.9%, respectively. While there is no considerable statistical variance between these toxic concentrations. Furthermore, the 100000 ppm concentrations of plant extracts showed a significant difference compared to positive and negative control (Figure 3-b).

The mortality rate of *A. fabae* adults varied significantly not only depending on the concentration of the plant extract but also on the duration of exposure to the plant extracts ($F_{(6,197)} = 96.67$, $P < 0.0000$) (Figure 3-c). Depending on the results, the highest mortality rate 67.5% happened at 72 hours of exposure while the lowest mortality rate 10.8% occurred at 24 hours of exposure (Figure 3-c). Similarly, the interaction between plant extracts and the duration of adult exposure significantly influenced adult survival. The two most toxic extracts (basil and clove) resulted in a maximum adult mortality rate at 72 hours after treatment (Figure 4-b). Moreover, the applied concentration affected the efficacy of plant extracts tested during the adult stage (Figure 4-a).

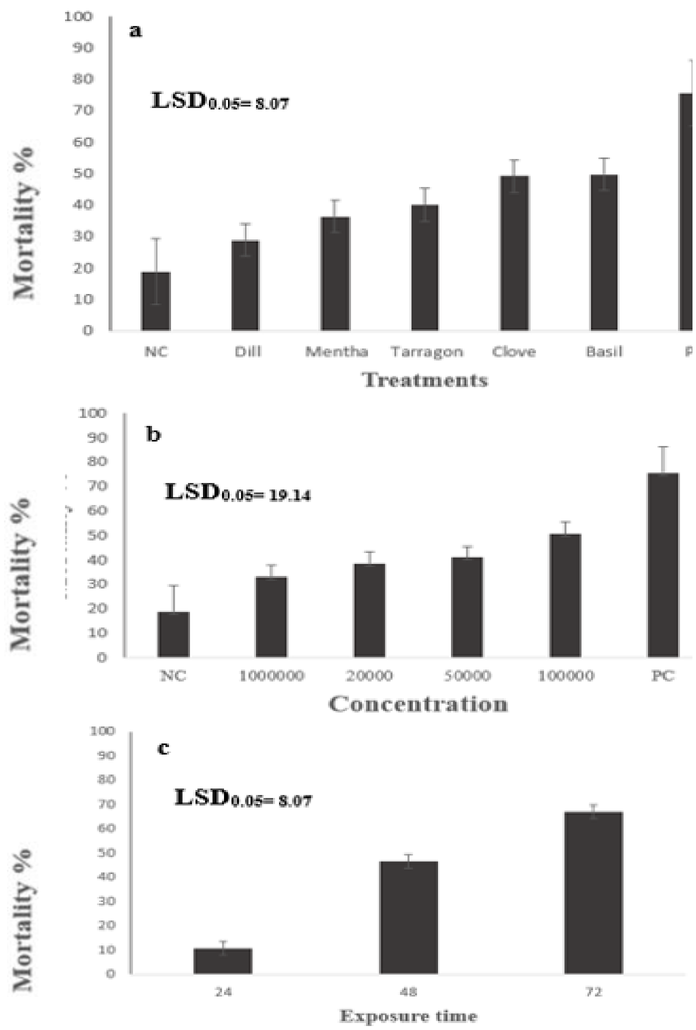

Figure 3. Mortality % of *A. fabae* adult stages treated with (a) different plant extracts (b) extract concentration/plant (c) exposure time (hours).

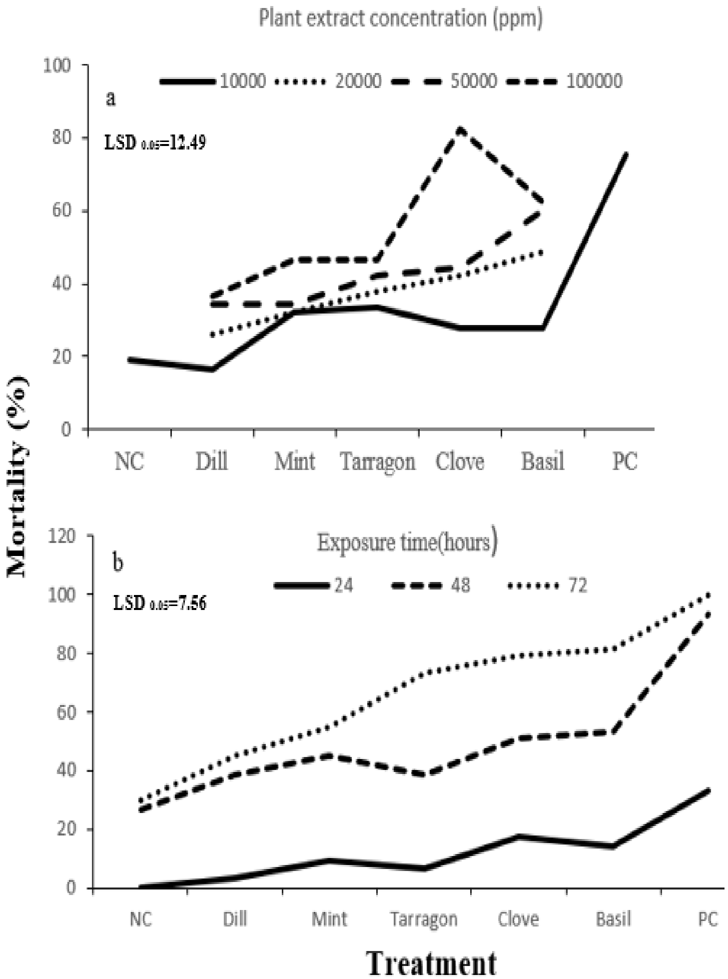

Figure 4. Mortality % of *A. fabae* adults treated with different plant extracts, (a) interaction between plant extract and concentration (b) interaction between plant extracts and exposure time.

3.4.Determination of lethal Concentration (LC50)

The toxicity of different botanical extracts was assessed by determining their LC50 values on adult *A. fabae* (Table 3). The LC50 values revealed that among the five examined plant extracts, tarragon extract was the most toxic at 720.553 ppm at 72 hours, while mint was the least toxic at 48359883.30 ppm at 24 hours. Moreover, there was variability observed in *A. fabae* adults, accompanied by a steady decline in LC50 values.

Table 3. The LC50 average values (ppm) of plant extracts on *A. fabae* adults following different exposure periods.

Plant Extract	Period (hours)	LC_{50} (ppm)	Average
	24	2386991.861	807170.428
Basil	48	26912.415	
	72	7607.008	
	24	96337.114	43602.325
Clove	48	30381.994	
	72	4087.867	
	24	2937175.477	1019864.683
Tarragon	48	121698.02	
	72	720.553	
	24	48359883.30	16142236.629
Mint	48	55157.363	
	72	11669.224	
	24	349040.951	158269.010
Dill	48	78275.095	
	72	47490.985	

4.Discussion

The extended use of synthetic insecticides presents significant ecological and economic challenges in pest control due to their risk to the environment and humans. On the other hand, the increased development of a resistant generation of aphids has led our attention toward natural methods of pest control, including the use of botanical extracts, proposing possible alternatives to chemical pesticides. Chermenskaya *et al.* (2012) they demonstrated that extracts from certain toxic plants exhibit efficacy against various life stages of insect pests and are frequently employed for plant protection. Notably, our findings indicate that all tested plant extracts significantly enhanced the mortality rate of *A. fabae* at both nymph and adult stages when compared to Acetamiprid 20% (positive control) and distilled water (negative control). Among the plant extracts evaluated, Basil, Tarragon, and Clove exhibited the highest toxicity levels. The results agree with the findings of Yarou *et al.* (2020) and Yarou *et al.* (2021) which demonstrated the repellent effects of basil (*Ocimum* spp.) on various aphid species, including *Aphis fabae* Scopoli, *Aphis craccivora* Koch, *Acyrthosiphon pisum* Harris, and *Myzus persicae* Sulzer, highlighting its potential application in crop protection. The observed repellency is generally attributed to the volatile organic compounds present in *Ocimum basilicum* (Kergunteuil *et al.*, 2015).

Pinto and Agrò (2021) showed that essential oils (EOs) from Oregano, Clove, and Geranium significantly affected *Aphis gossypii,* the mortality rate of the aphids increased with both the concentration of the EOs and the duration of the treatment. In another investigation, potent insecticidal effects were observed against nymphs and wingless adults of the cotton aphid with essential oils extracted from *Lavandula spica* L., *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Juniperus communis* L., *Syzygium aromaticum* L., and *Origanum vulgare* L.(Atanasova *et al.,* 2018).The current discovery supports the findings of Gospodarek *et al*. (2022), who documented that Tarragon aqueous extract exhibits potent aphicidal properties against *Aphis fabae*, causing 84% mortality rate among nymphs with 30% fresh matter and 78% mortality rate among wingless females with 10% dry matter after 108 hours of exposure. The data in (figure1,3) showed that the mortality rate exhibited a significant effect based on concentrations and exposure time, with the maximum mortality rate of aphids occurring at a concentration of 100000 ppm after 72 hours. The present finding confirms Biniaś *et al.* (2017) data, which investigated the impact of water extracts of dried mint, *Mentha piperita* L. at concentrations of 10%, 5%, and 2% on black bean aphid, *Aphis fabae* Scop. survival. Only 10% of the extract proved effective, significantly increasing the aphid mortality rate on broad bean leaves within 48 hours.

5. Conclusions

In conclusion, the study revealed that basil, tarragon, and clove extracts exhibited significant toxicity against *A. fabae* nymph and adult stages at a concentration of 100000 ppm after 72 hours of treatment. These extracts caused the highest mortality rate among the tested plant materials.

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