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Research Article

Comparison of the toxicity of the pesticide imidacloprid and the bioactivity of *Artemisia herba-alba* alcohol extract against *Tribolium castaneum*

Nagham Kareem Jaber
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq
Alaa Abdulrahim Khalaf
Department of Biology, College of Medicine, Aliraqia University, Baghdad, Iraq
Maan Abdul Azeez Shafeeq*
Department of Biology, College of Science, Mustansiriyah University, Baghdad, Iraq

*Corresponding author. E-mail: maanalsalihi@uomustansiriyah.edu.iq

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Abstract
The red flour beetle, *Tribolium castaneum*, is a serious pest of grain products that have been stored. As worries about the toxicity of conventional insecticides like Imidacloprid grow, alternate pest control methods are becoming necessary. The present study aimed to assess the insecticidal effectiveness of an alcoholic ethanol extract of *Artemisia herba-alba* against *T. castaneum*. During a 10-day observation period, various concentrations of the extract (4%,8%,12%) were tried. For second-stage larvae, fifth-stage larvae, and adult beetles, both direct and indirect treatments (spraying and mixing the extract with food) were evaluated. This one was compared to Imidacloprid's insecticidal ability. The findings showed that the alcoholic extract of A. *herba-alba* had a considerable insecticidal effect; under direct exposure, mortality rates for the second larval instar, fifth larval instar, and adults were 75%, 70%, and 82.8%, respectively. Indirect treatment resulted in even higher mortality rates, reaching 91.8%, 92.4%, and 91.4% for the same developmental stages. In comparison, Imidacloprid produced higher mortality rates of 95.4%, 98.2%, and 98.8% for direct application, and 96.4%, 94.4%, and 96% for indirect application. Even though the efficacy of Imidacloprid is higher, our results suggest that alcoholic extract of A. herba-alba as a natural replacement for *T. castaneum* populations. Further investigation is necessary to clarify the potential applications and underlying mechanisms of action of this herbal extract in pest management.

Keywords: *Artemisia herba-alba,* Imidacloprid, *Tribolium castaneum,* Toxicity

INTRODUCTION

Grains are an important food for humans, as they give us energy. However, they are often subjected to infestations by specific species of beetles, which primarily feed on seeds and cause significant qualitative and quantitative losses, subsequently diminishing their nutritional and market value. Over the years, chemical pesticides have been used to protect stored grains against these pests, which adversely affect environmental balance. This study aims to assess alternative pest management strategies that are environmentally friendly while maintaining the integrity of grain storage, thereby addressing the challenges posed by these beetle infestations (Mohapatra and Giri,2015; Golob,1999; Denux and Zagatti, 2010; Tapondjou et al., 2002; Isman, 2006; Pavela, 2015). The use of chemical pesticides has significantly decreased in recent years in favor of natural, eco-friendly alternatives (Sharma et al., 2020). This study's primary focus is on using plant extracts to control insect pests and reduce the damage they do to crops.

The potential applications of organic and natural plant extracts and secondary metabolites produced by microorganisms—such as phenols, terpenes, and alkaloids—in pest management have been extensively studied (Singh et al., 2023). Since 1950, research has established that approximately 247 plant families possess insecticidal properties. Notably, the therapeutic qualities of *Artemisia herba-alba* and its proven ability to eliminate *Acanthoscelides obtectus* insects have garnered significant interest.

In addition, experiments have shown that extracts from different *Artemisia* species can successfully remove *Culex pipiens molestus* larvae (Al-Mansour et al.,2022). The efficiency of natural and organic plant extracts, as well as secondary chemicals made by microorganisms, including phenols, terpenes, and alkaloids, in controlling pests, has been shown in numerous research. It has been demonstrated that about 247 plant families have insecticidal qualities since 1950. The effectiveness of these natural solutions for managing pests has been empirically demonstrated in references (Lahlou, 2004; Issakul, 2007; Evans, 1997; Derwich et al., 2009; Al-Myah et al., 2011). The present study aimed to assess the efficacy of an alcoholic extract of *A. herba-alba* in inhibiting *T. castaneum*, a common pest of stored grain. This natural extract's efficacy was compared with Imidacloprid, a synthetic pesticide. The study assessed several elements, including the death rates of *T. castaneum, which was* subjected to different treatments.

MATERIALS AND METHODS

Insect rearing

The *Tribolium castaneum* adults were kept on a synthetic diet that was mostly composed of a mixture of wheat, groats, and yeast powder in a weight ratio of (1:1:13(.The insects were raised in optimal conditions of 26 ± 2 °C temperature, 12 hours of light and dark photoperiod, and 60 ± 5% relative humidity. The insect colony was being continuously maintained fresh after each generation to get different ages of the insect to perform the experiment on the insect.
After that, adult insects that were between 0 and 24 days old were taken out of this controlled setting for experimental. In order to do tests on *T. castaneum,* its adults and larvae were recognized using taxonomic keys of (Bouchard et al., 2023).

Extraction and analysis of plant

Plant material was bought from local markets and dried (leaves and stem) for 8 days, then samples were ground with fine powder with the help of an electric grinder, then 20 grams of *A. herba□alba* powder was extracted using Soxhlet apparatus with 200 ml 80% ethanol for 24 hours at 78°C. The product was filtered through filter paper (Whatman No. 4). The extract was concentrated under vacuum using a rotary evaporator (50°C) and stored at 4°C for further analyses. The pH of the plant extract was determined by suspending 10 g of the powdered material in 50 mL of distilled water (DW) for 10 minutes, followed by filtration (Shihata et al., 1951). Analysis and identification of the chemical compounds present in the extract were performed using high-performance liquid chromatography (HPLC) using a C18-ODS column (250 mm × 4.6 mm, 5 µm) and a SYKAM HPLC system (Germany). A sample volume of 100 µL was put into the system for chromatography. The mobile stage consisted of two solvents: solvent A, which contained 0.01% trifluoroacetic acid and 95% acetonitrile, and solvent B, which contained 5% acetonitrile and 0.01% trifluoroacetic acid, with a flow rate set at 1 mL per minute. The gradient software was created as described below: 10% of solvent A from 0 to 5 minutes, increasing to 25% solvent A from 5 to 7 minutes, then progressing to 40% solvent A from 7 to 13 minutes, and finally returning to the initial conditions. The UV-visible detector was calibrated to identify phenolic compounds at a wavelength of 278 nm (Ngamsuk et al., 2019).

Source of Imidacloprid

An insecticide called "Modesta" was purchased from local markets. It contained 35% imidacloprid. Three different concentrations of the insecticide were prepared: 4, 8, and 12 ml/L. Each of the above concentrations was diluted with one liter of distilled water and shaken well for 15 minutes (Bonmatin et al., 2015). The three concentrations (4%,8%,12%) were then applied to 100 ml hand sprayers to prepare them for use.

Determination of the direct effect of the *Artemisia herba-alba* extract on the adult and larval instars

Three replicates of each concentration, and a control group, were added to five pairs of adult insects on Petri plates (9×1.6cm). Using a 100 mL sprayer (3mL), adults were treated with alcoholic extract. the plates were then incubated at 28 ± 2 °C and 70% relative humidity under controlled circumstances. Observations and data were obtained at regular intervals between the second, fourth, sixth, eighth-, and tenth days following therapy. The second and fifth larval instars were treated in the same way.

Determination of the direct effect of Imidacloprid on the mortality of adults and larval instars of *Tribolium castaneum*

Insects of flour beetles were collected and placed in a Petri dish lined with filter paper. Then, using a 100mL hand sprayer, the beetles were sprayed with different concentrations (4%,8%,12%) of Imidacloprid, every concentration was replicated three times, along with a control treatment. Observations were made after 2,4,6,8, and10 days of incubation at 28°C and 70% humidity. The second and fifth larval instars were treated in the same way.

Determination of the indirect impact of the *Artemisia herba-alba* extract on adult mortality and larval instars of *Tribolium castaneum*

One mL of the extract (4%,6%, and 12%) was combined with five grams of wheat, and three replicates were made for each concentration to evaluate the extract's indirect impact on the larval instars and adults. Insects were exposed to the treated diet after this mixture was prepared and then incubated on Petri plates at 28 °C and 70% relative humidity under close observation. To assess the effect of the extract on the larvae, observations were made at intervals of 2, 4, 6, 8, and 10 days after treatment.

Determination of the indirect effects of Imidacloprid on adults and larval instars of *Tribolium castaneum*

Five grams of the wheat and one mL of Imidacloprid were combined into three replicates for each concentration (4%,8%, and 12%). Then, this mixture was put in Petri plates for the experimental setting. 2nd and 5th larvae and adults were released onto the plates and incubated at a controlled temperature of 28 °C and 70% relative humidity. Results were recorded at intervals of 2, 4, 6, 8, and 10 days to assess the effects of the pesticide on larval development and survival.

Statistical analysis

The study data were statistically analyzed using a completely randomized design, a 10× 3experiment was implemented, and only one age group was used for each stage. The results of the analysis of variance (ANOVA) were clearly explained. (F value, P value). The results of the Duncan test and the significance were tested by correcting the mortality ratios according to the Abbott equation (1925).

Corrected % Mortal =Treatment % Mortality – Control / 100 – Control x100   Eq. 1

RESULTS

The results of the analysis of the alcoholic extract of *A. herba-alba* using high-performance liquid chromatography (HPLC) showed the presence of active compounds, as shown in Table 1 and Fig. 1.

Direct impact of *Artemisia herba-alba's* alcoholic extract on adults and larvae of *Tribolium castaneum*

The results indicated a direct impact of the *A. herba-alba's* alcoholic extract on death rates of different life stages of *T. castaneum* (Table 2). Specifically, the alcoholic extract of *A. herba-alba* caused the highest mortality rate of second-instar larvae at a concentration of 12%, with mortality of 75% after 10 days of treatment. The maximum mortality rate for fifth instar larvae was also observed at the same concentration of 12%, reaching 70% after 10 days. Additionally, the highest mortality rate for adult *T. castaneum* was recorded at a concentration of 12%, resulting in 82.8% mortality after 10 days of exposure to the alcoholic extract of *A. herba -alba* where the effect increased with the increase in concentrations and the increase in the treatment period.

Direct effect of Imidacloprid on *Tribolium castaneum* in different periods

The direct application of imidacloprid pesticide to various life stages of *Tribolium castaneum* demonstrated significant toxicity, as illustrated in Table 3. The highest mortality rate among second-instar larvae was 95.4% at a concentration of 12% after 10 days of treatment. For fifth-instar larvae, the peak mortality rate reached 98.2%, also at a concentration of 12% after 10 days. Additionally, adult *T. castaneum* exhibited a mortality rate of 98.8% following direct treatment with Imidacloprid at the same concentration 12% over the same duration, representing the highest efficacy compared to other concentrations tested.

Indirect effect of the alcoholic extract of *Artemisia herba-alba* on *Tribolium castaneum* in (2,4,6,8and10 days)

The results showed that of the alcoholic extract on death rates of various life stages of *Tribolium castaneum*, as summarized in Table 4. Notably, the alcoholic extract of *A. herba-alba* achieved the highest mortality rate of second-instar larvae at a concentration of 12%, resulting in a kill rate of 91.8% after 10 days of treatment. In comparison, the highest mortality rate in fifth instar larvae was also observed at a concentration of 12%, with a mortality rate of 92.4% after the same duration. Additionally, the highest mortality rate for adult *T. castaneum* was noted at a concentration of 12%, yielding a kill rate of 91.4% after 10 days of exposure to the alcoholic extract from *A. herba-alba*.

Indirect effect of Imidacloprid on *Tribolium castaneum* in different periods (in days)

The indirect application of imidacloprid pesticide at various developmental stages of *Tribolium castaneum* demonstrated notable toxicity, as summarized in Table 5. The highest mortality rate observed among second-instar larvae was 96.4% at a concentration of 12% following a 10-day treatment period. Similarly, fifth-instar larvae exhibited a mortality rate of 94.4% under the same concentration and treatment duration. Additional-

Table 1. The primary chemical makeup of *Artemisia herba -alba's* alcoholic extract

No	Name (ppm)	Sample
1	Caffeic acid	19.32
2	Quercetin	22.66
3	Catechin	12.90
4	Hispidulin	33.15
5	Eupalitin	25.32
6	Ferulic acid	9.04

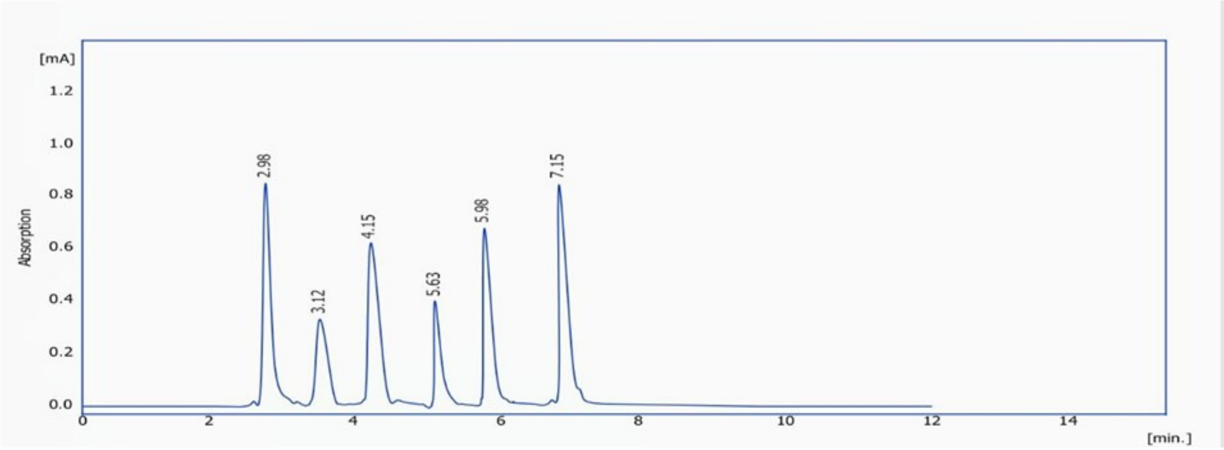

Fig. 1. *Showing chromatography analysis of the alcoholic extract of Artemisia herba-alba*

ly, the adult stage of *T. castaneum* subjected to indirect treatment with Imidacloprid for 10 days showed a maximum mortality rate of 96% at the 12% concentration, which was the highest compared to other tested concentrations.

DISCUSSION

The alcoholic extract from the wormwood plant, *A. herba-alba*, was analyzed using High-Performance Liquid Chromatography (HPLC), which identified several bioactive compounds. These included flavonoids and phenols such as ferulic acid, hispidulin, catechin, caffeic acid, and quercetin. The findings align with the extract's demonstrated ability to cause mortality in *Tribolium castaneum* at various growth stages. The larval and adult stages exhibited significant susceptibility after direct and indirect exposure to the extract. The growth-inhibiting effects observed in bacteria, fungi, and insects are likely attributed to the identified phenolic and flavonoid compounds, underscoring the potential of *A. herba-alba* as a natural pesticide. These findings align with previous studies that have shown flavonoids such as caffeic acid, chlorogenic acid, and protocatechuic acid negatively affect the development and survival of *Helicoverpa armigera* (Punia et al., 2023). Additionally, the flavonoid quercetin has demonstrated lethal effects on various pest species, including the grasshopper *Oedaleus asiaticus*, *Helicoverpa zea*, and *Heliothis virescens*, at a specific concentration of 22.66 ppm. It has also been proven to effectively reduce *Spodoptera eridania* larval populations (Cui et al., 2019).

Furthermore, another study indicated that applying quercetin significantly increased larval mortality in the Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) (War and Sharma, 2014). The findings highlight the significant potential of incorporating flavonoid compounds into pest management strategies. An impressive study revealed that ferulic acid extracts from various cotton varieties achieved a remarkable 100% mortality rate in *Helicoverpa armigera* within just one week of treatment (Mesbah et al., 2007). Additionally, it has been shown that phenolic compounds, such as quercetin, gallic acid, and catechin, which are abundant in Indian sugarcane flour extract, not only prolong the prepupal stage but also increase mortality rates in *Spodop-*

Table 2. Direct effect of *Artemisia herba-alba's* alcoholic extract on *Tribolium castaneum* mortality (in days)

Treatments	Concentrations	Mortality periods/days					Average of Mortality
		2	4	6	8	10	
Second Larval Instar	4%	50 h	54 h	57 g	59 f	63 e	56.6 c
	8%	61 f	65 e	68 d	70 c	70 c	66.8 b
	12%	70 c	73 b	76 ab	77 b	79 a	75 a
Fifth Larval Instar	4%	40 h	45 g	48 f	49 f	53 e	47 c
	8%	57 e	66 d	67 c	70 b	73 b	66.6 b
	12%	64 d	68 c	70 b	72 b	76 a	70 a
Adult Stage	4%	54 g	57 h	59 g	62 f	65 f	59.4 c
	8%	55 g	59 g	63 f	64 f	70 e	62.2 b
	12%	75 d	79 c	83 b	88 a	89 a	82.8 a

Table 3. Direct effect of Imidacloprid on *Tribolium castaneum* mortality (in days)

Treatments	Concentrations	Mortality periods/days					Average of Mortality
		2	4	6	8	10	
Second Larval Instar	4%	50 f	54 ef	57 ef	67 e	70 d	59.6 c
	8%	80 cd	84 c	86 c	90 b	94 ab	86.8 b
	12%	90 b	94 ab	95 ab	99 a	99 a	95.4 a
Fifth Larval Instar	4%	58 d	59 d	60 de	65 de	69 d	67.6 c
	8%	86 c	88 c	90 bc	95 b	99 ab	91.6 b
	12%	96 ab	97 ab	98 ab	100 a	100 a	98.2 a
Adult Stage	4%	76 ef	79 e	85 d	90 c	90 c	84 c
	8%	93 b	96 ab	98 ab	100 a	100 a	97.4 b
	12%	95 ab	99 ab	100 a	100 a	100 a	98.8 a

Table 4. Indirect effect of the alcoholic extract of *Artemisia herba-alba* on *Tribolium castaneum* mortality (in days)

Treatments	Concentrations	Mortality periods/days					Average of Mortality
		2	4	6	8	10	
Second Larval Instar	4%	82 c	83 c	85 bc	85 bc	88 c	84.6 c
	8%	87 bc	88 b	88 b	90 ab	93 a	89.2 b
	12%	89 b	90 ab	90 ab	94 a	96 a	91.8 a
Fifth Larval Instar	4%	70 f	73 f	74 e	76 de	78 de	74.2 c
	8%	80 d	82 c	82 c	87 c	89 bc	84 b
	12%	90 b	91 ab	93 ab	93 ab	95 a	92.4 a
Adult Stage	4%	59 fg	63 f	66 e	67 e	70 d	65 c
	8%	80 cd	84 c	85 c	90 b	95 a	86.8 b
	12%	90 b	92 ab	92 ab	93 a	90 b	91.4 a

Table 5. Indirect effect of Imidacloprid on *Tribolium castoreum* mortality in days

Treatments	Concentrations	Mortality periods/days					Average of Mortality
		2	4	6	8	10	
Second Instar Larva	4%	90 bc	93 bc	93 Bc	95 b	95 b	93.2 c
	8%	90 bc	95 b	95 B	97 ab	99 a	95.2 b
	12%	90 bc	97 ab	97 Ab	99 a	99 a	96.4 a
Fifth Instar Larva	4%	72 e	77 e	80 D	84 cd	89 c	80.4 c
	8%	85 cd	88 c	98 A	100 a	100 a	94.2 b
	12%	90 b	93 b	95 Ab	95 ab	99 a	94.4 a
Adult Stage	4%	78 f	80 e	84 De	86 d	90 c	83.6 c
	8%	88 cd	88 cd	90 C	98 ab	99 a	92.6 b
	12%	90 c	94 b	97 Ab	99 a	100 a	96 a

tera frugiperda larvae (Marques et al., 2016). This evidence strongly supports the integration of these natural chemicals into pest control efforts for enhanced effectiveness.

Research indicates that phenolic compounds play a significant role in pest management, demonstrating their effectiveness as insecticidal agents. In a study conducted by Vimaladevi et al., phenolic acids extracted from the seaweed *Chaetomorpha antennana* (Bory.) were found to have larvicidal effects on third-instar *Aedes aegypti* larvae (Vimaladevi et al., 2012). Additionally, another study revealed that when *Spodoptera litura* larvae were fed a synthetic diet containing phenolic compounds, particularly ferulic acid, their survival rate significantly decreased, leading to an increase in larval mortality (Punia et al., 2020; Punia et al., 2021). Based on these results, phenolic acids serve as effective biological controls for managing various insect pests. Caffeine has been observed to kill *T. castaneum* through both direct and indirect mechanisms, significantly altering the feeding behavior of both adult and larval insects.

Additionally, high levels of phenolic compounds in *Gossypium hirsutum* have been shown to exert antinutritional effects on *Spodoptera litura*, as noted by Rani and Pratyusha (Rani and Pratyusha, 2013). This suggests that phenolic compounds negatively impact pest development and survival. Rather than merely having an inhibitory effect, the toxic impact of consuming extracts rich in phenols and flavonoids is the primary reason for the harmful effects observed in both larvae and adults of *T. castaneum*. Flavonoids have a direct impact on intestinal function, which reduces the effectiveness of insects' digestion. This disruption in their digestive system ultimately leads to higher mortality rates among insects (Lindroth and Peterson,1988; Sakihama et al., 2002).

Research on the effects of Imidacloprid on both adult and larval stages of *T. castaneum* has shown that the toxicity of this pesticide increases significantly after a 10-day treatment period. The toxicity levels were influenced by the pesticide concentration and the exposure length. As a neonicotinoid, Imidacloprid is particularly effective in killing insects.

One factor contributing to the reported mortality rates in treated insects is the heightened neurotoxicity resulting from this pesticide's mechanism of action. Therefore, the effectiveness of Imidacloprid as an insecticidal agent is greatly affected by its concentration and the duration of its application (Elbert et al., 2023). The pesticide is primarily lethal because it targets the central nervous systems of insects. Imidacloprid disrupts the transmission of neurological impulses by inhibiting the brain's nicotinic acetylcholine receptors and interfering with nicotine circuits (Matsuda et al.,2020). This blockage leads to paralysis and eventually death in insects, as it blocks the transfer of acetylcholine and prevents nerve impulses from traveling between neurons. Imidacloprid has proven to be an effective pesticide, particularly against pest species such as *Tribolium castaneum* (Krishnan and Sehnal, 2006). Additional research indicates that Imidacloprid is lethal to *Anoplophora glabripennis* (Motschulsky) at all embryonic stages, with toxicity increasing upon repeated exposure (Houchat et al., 2020). In one study, mature Asian longhorn beetles exposed to Imidacloprid daily, whether through direct contact or oral ingestion, died within two to three weeks. After a five-day exposure period, all beetles perished within 15 days, leading to an 82–93% reduction in viable eggs. The pesticide's lethal and sublethal effects contributed to this decline in reproductive success (Ugine et al., 2011). Furthermore, another study focused on the Colorado potato beetle *Leptinotarsa decemlineata* found that combining Imidacloprid with thiamethoxam is a key component of a resistance management strategy. These findings underscore Imidacloprid's potent insecticidal capabilities and critical role in pest control plans (Alyokhin et al., 2007).

Conclusion

This study examined the effects of an alcoholic extract from *A. herba-alba* on adults and larvae of *T. castaneum*. The findings revealed that the death rate increased with higher concentrations of the extract (4%, 8%, and 12%), whether the treatment was applied directly or indirectly. At the higher dosages (12% and 8%), a 100% mortality rate was observed, showing that Imidacloprid is effective against *T. castaneum* under both treatment conditions. Based on these results, the alcoholic extract from the wormwood plant is recommended as a safer alternative to conventional pesticides. This approach effectively manages insect populations while prioritizing environmental preservation and human health.

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Conflict of interest

The authors declare that they have no conflict of interest.

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