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Efficacy of hot water *Tamarix aphylla* (L. 1761) leaf extract on three stored grain insects under laboratory conditions

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Abstract

The purpose of the study was to determine the effects of a hot aqueous extract derived from Athel tree *T. aphylla* leaves on the biological performance of three insect species: the beetle, saw beetle, and red rusty flour beetle in a laboratory setting. These insects are serious pests that are regularly found in major storage areas around the world. The purpose of the study was to develop alternative strategies to limit the use of chemicals that can harm human health. The outcomes from the study assessing the impact of the aqueous extract on the khapra beetle (*Trogoderma granarium*) revealed significant effects in reducing the number of insect larvae. Specifically, plant extract at 10% was the most effective and resulting in 7.67 deceased insects compared to the control treatment. In contrast, using 2.50% exhibited least effectiveness with insect mortality of only one insect. Regarding the impact of the plant extract on the saw beetle (*Oryzaephilus surinamensis*), notable consequences were observed in reducing the population of treated insects. Out of 20 insects, the 10% plant extract had the highest decease insects rate showing mortality of 7.17 and indicating the most effective results. On the other hand, at the preliminary concentration of 2.50%, resulted in 3.50 mortality compared to the control treatment with mortality of only one insect. In case the red rusty flour beetle (*Tribolium castaneum*), the highest concentration of 10% was revealed to be the most effective in lowering the population of treated insects, resulting in 4.67 mortality. The concentration of 2.5%, on the other hand, had the lowest reduce rate, with 1.67 mortality. The study examined the percentage of germination in wheat seeds treated with the studied extract through different concentrations. The results showed that there were no negative effects on the treated wheat seeds compared to the control treatment. Particularly, when utilizing 5% and 10% concentrations, the germination rates were recorded at 90%, which did not indicate a significant difference compared to the control treatment that achieved a germination rate of 93%.

Keywords: *Trogoderma granarium*, **storage insects, pest management, Botanicals, Athel**

Introduction

Wheat is a staple grain and cereal crop that is important to the global economy. Wheat productivity declines can be ascribed to a variety of reasons, both living (biotic) and non-living (abiotic), with insects being recognized as one of the contributing elements (Hussain, Asrar et al. 2022). Insect infestation is considered as one of the most main factors causing damage and loss in stored grains, with the Khapra beetle (*Trogoderma granarium*) and *Oryzaephilus* on stored grains becoming the most significant pests that rapidly spread and are capable of causing large losses (GISD 2015). All grain storage in Iraq, including other developing nations, are infested with Khapra beetles, which generate annual losses of up to 20% (Phillips and Thorne 2010). The saw-toothed grain beetle (*Oryzaephilus*) is a common insect in Iraq, and it can be identified in practically all grains and other products that are stored. Furthermore, the red flour beetle (*Tribolium castaneum*) is a major pest of stored products (Ali et al., 2014), and these pests not only consume large amounts of grains and food products but also contaminate them with their bodies, shed skins, and feces, resulting in a foul odor in the food products and lowering their market value and quality. To control these pests, chemical pesticides are utilized, which are always effective however have negative side effects due to residues and their effects (Debnath et al., 2011). To decrease or eliminate excessive chemical use and minimize its detrimental effects on humans, animals, and the environment, the emphasis has turned to finding ecologically friendly and safe alternatives (Jayas and White, 2003; Jian and Jayas, 2012). As a result, the search for suitable replacements has been continuing for decades and continues to the present. Nature is regarded as the key source for managing pest problems and protecting crops through the employment of diverse plant-origin insecticides, biological enemies, and pathogenic agents (Tavares et al., 2021; Mohammed et al., 2022). Plant extracts are considered as a rich source of natural, effective chemical compounds that are effective against one or more pests and may have several effects on pests. The majority of these have been shown to act as feeding inhibitors or oviposition deterrents for a variety of agricultural pests (da Silva and Ricci, 2020; Heinz-Castro et al., 2021).

Previous research has shown that many plant extracts are effective against insects, and they are frequently utilized in pest control due to their environmentally friendly nature, plant origin, and rapid environmental degradation, as well as their non-toxicity to humans, animals, and natural predators (Yadi et al., 2018; Benelli et al., 2018). *T. aphylla* is a member of the Tamaricaceae family, which was formerly distributed in Asia, North Africa, and Southeast Europe. Because of its chemical contents, including terpenoids, tannins, flavonoids, polyphenols, glycosides, amino acids, and essential oil, it has various traditional therapeutic benefits in several countries (Jasiem et al., 2019). The objective of this study was to investigate how effective *T. aphylla* leaf extract was at controlling the Khapra beetle, saw-toothed grain beetle, and red flour beetle. Ghitheeth (2023) reported that boiling *T. aphylla* leaf extract effectively reduced the number of green peach aphids on cucumber plants. He gave diagnostic data for the phenolic and alkaloid chemicals in *T. aphylla* extract, demonstrating that all concentrations employed resulted in the highest rates of mortality for adults and nymphs, reaching (100, 100%) after 6 days of treatment.

Materials and Methods:

Insect collection and rearing:

The study involved conducting laboratory experiments to examine the impact of an aqueous extract derived from *Tamarix aphylla* leaves on three insect species: the khapra beetle (*Trogoderma granarium*), the saw beetle (*Oryzaephilus surinamensis*), and the red rusty flour beetle (*Tribolium castaneum*). The aim was to evaluate the effects of the *T. aphylla* leaf extract on these insects and assess its potential as a pest control agent. On August 1, 2023, insect pests were collected from contaminated grains at University of Al-Kufa - Plant Protection Department laboratories and local markets in Al-Kufa city. The samples were transferred in 1000 ml glass bottles for inspection under a dissecting microscope to identify the sorts of insects employed in the studies. In order to secure a continuous and uncontaminated supply of insects for utilization as a source in the conducted experiments, artificial infestation was conducted on clean and sterilized wheat seeds, placed in plastic boxes 30cm* 20cm (2 boxes with 1000 g of wheat per box). The insects were subjected to standard growth conditions, encompassing a temperature maintained at 25 ± 2 °C and a relative humidity of 65 ± 5% (Al-Naimi, 2007).

Preparation of hot-water extract from *T. aphylla* leaves

Retrospectively, the process of preparing a solution effective against insects like *Trogoderma granarium*, *Oryzaephilus*, and *Tribolium castaneum* was conducted. Leaves were sourced from orchards and agricultural fields located in the Najaf province. Adhering to the procedure outlined by Harborne (1984), 10 grams of plant powder were introduced into a 500 ml glass beaker. The mixture underwent drying, after which 200 ml of hot sterile distilled water, heated to its boiling point, were introduced while continuously stirring for a duration of 30 minutes. Subsequently, the solution was allowed to settle for an additional 30 minutes to enable the plant particles to settle at the bottom. The resultant solution was subsequently filtered through two layers of gauze and subjected to centrifugation at a speed of 300 rpm for 10 minutes, resulting in the acquisition of a clear solution. This solution was then subjected to drying at a temperature of 50°C. For each specific weight category, namely 2.5, 5.0, and 10.0 grams of the dry extract, they were dissolved in distilled water until reaching a final volume of 100 ml, obtaining concentrations of 2.5%, 5.0%, and 10.0%, correspondingly.

Influence of hot-water leaf extract of *Tamarix aphylla* on *Trogoderma granarium*, *Oryzaephilus surinamensis*, and *Tribolium castaneum*.

The impact of hot-water leaf extract of *T. aphylla* was investigated on the khapra beetle, saw beetle, and red rusty flour beetle in the Insect Laboratory at Department of Plant Protection/Collage of Agriculture, University of Kufa. Three concentrations of the aqueous extract (2.50%, 5.00%, and 10.0%) were utilized, with three replicates for each concentration and insect species. Sterile Petri dishes with perforated tops were arranged to facilitate air circulation and respiration for the insects. Within each dish, 50 pre-treated sterilized wheat seeds were positioned on filter paper and subsequently treated with 1 ml of the water extract using a 5 ml hand sprayer for each replicate. Following the drying of the seeds, 20 insects were introduced into each dish for every replicate. The control group received spraying with sterile water exclusively. Data were recorded after 1, 2, 3 and 4 days after treatment by counting the number of dead insects for each treatment. To clarify, the identical procedure was actually applied to all the tested insects, with differences based on the specific insect types: khapra beetle larvae, sawfly beetle adults, and red rusty flour beetle adults. Furthermore, all experimental dishes were maintained in an incubator with a temperature range of 25°C (±5°C) and a relative humidity range of 60% (±10%).

Effect of *T. aphylla* leaf water extract on wheat seed germination rates

The germination percentage of wheat seeds was determined by subjecting them to different concentrations of a plant extract (2.50%, 5.00%, 10.0%). The seeds were soaked in the extract for five minutes to evaluate their germination rate under the extract's influence at the specified concentrations. Afterwards, the seeds were removed and placed in sterilized Petri dishes containing moist cotton soaked in the same plant extract. This was done to ensure sufficient moisture for seed germination. Each experimental condition was replicated three times, with ten seeds in each replicate. The Petri dishes were then placed in an incubator with a temperature range of (5±25°C). After a period of seven days, the germination percentage of all treated seeds was calculated. Simultaneously, a control group was included, consisting of ten seeds soaked in sterilized water only.

Statistical Analysis

The laboratory experiments were organized utilizing a completely randomized design (C.R.D). The experiment data were analyzed using GenStat program (VSN International Gen Sat12.1, 2009) software. Where, means were compared among treatments using least significant difference L.S.D. ($P \leq 0.05$) (Al-Rawi and Khalafallah 2000).

Results and Discussion

The Effect of hot-water leaf extract of *T. aphylla* on the khapra *T. granarium* beetle larvae

The results presented in Table 1 pertaining to the quantity of deceased Khabra beetle larvae under various concentrations indicate substantial differences among the examined concentrations. Notably, the 10% concentration had the most noteworthy reduction in larval numbers, resulting in 6.8 deceased larvae. In comparison, the other concentrations exposed lower effectiveness, with the lowest observed in the control group and the 2.5% concentration, showing 0 and 1 deceased larvae, respectively. Moreover, varying time intervals produced distinct mortality rates. The four-day period exhibited the highest mortality rate at 2.92 deceased larvae, signifying a significant contrast with the one-day time interval, which recorded 1.75 deceased larvae. Regarding the most effective combinations between the concentrations and time intervals, the treatment with a 10% concentration demonstrated the greatest reduction in insect larvae during the warmest days, with 7.67 deceased larvae. This result showed significant differences in comparison to all other interactions, except for the same concentration within the two-day and three-day time intervals, which exposed 5.67 and 6.67 deceased larvae, respectively.

Table1. Effect of hot-water leaf extract of *T. aphylla* on the khapra *T. granarium* beetle larvae

Mortality of Insect Larvae After treatment					
Concentration %	**1 Day**	**2 Days**	**3 Days**	**4 Days**	**Concentration rate**
2.50%	0.67	0.67	1.33	1.33	1.0
5%	2.0	2.67	2.67	3.67	2.75
10%	4.33	5.67	6.67	7.67	6.8
Comparison	0.0	0.0	0.0	0.0	0.0
Average effect days	1.75	2.42	2.75	2.92	
		concentrations		1.961	
L.S.D. ($P \leq 0.05$)		days		1.961	
		interaction		3.923	

The effect of hot-water leaf extract of *T. aphylla* on saw beetles ***O. surinamensis***

The results in table no. 2 from the study on the mortality of adult sawfly beetles revealed significant impacts on these insects when different concentrations of hot aqueous *T. aphylla* leaf extracts were employed. Specifically, the 10% concentration resulted in the highest mortality rate among the insects, with a recorded count of 7.17 deceased insects. This figure significantly differed from all other concentrations. In contrast, the comparison treatment exhibited the least effect, with only one dead insect. Different time periods yielded varying death percentages; notably, the five-day time frame produced the most significant reduction in adult insects, with 5.0 deceased insects, significantly surpassing the one-day time period's 2.83 deceased insects. Furthermore, the interaction between the concentration levels and time periods demonstrated elevated mortality rates. For instance, the combination of the 10% concentration with a four-day period exposed the highest number of deceased insects at 9.33, significantly differing from all other interactions except for the same concentration with the two and three-day time periods.

Table2. The effect of hot-water leaf extract of *T. aphylla* on saw beetles ***O. surinamensis***

Mortality of Insect Larvae After (Days)					
Concentration %	**1 Day**	**2 Days**	**3 Days**	**4 Days**	**Concentration rate**
2.50%	2.33	2.67	4.33	4.67	3.5
5%	3.0	4.33	4.33	6.33	4.5
10%	5.0	6.67	7.67	9.33	7.17
Comparison	1.0	1.0	1.0	1.0	1.0
Average effect days	2.83	3.75	3.92	5.0	

	concentrations	2.038
L.S.D.	days	2.038
	interaction	4.076

Effect of hot-water leaf extract of *T. aphylla* on the red rusty flour beetle *T. castaneum*

Finding in table (3) showed that differnt concentrations of hot-water *T. aphylla* extract differed in their effect on lowering the number of red rusty flour beetles. The 10% concentration resulted in the highest percentage of insect mortality, with 4.67 insect fatalities, which differed significantly from the control treatment. Although it was administered at a concentration of 2.5%, which resulted in 0 and 1.67 insect deaths, it did not differ significantly from the concentration of 5%. However, the mortality rates varied by time period, the four-day period had the highest mortality rate, which differed significantly from the one-day and two-day time periods. Whereas this did not differ from the previous three-day period, which recorded 2.33 insect mortality

Table3. Effect of hot-water leaf extract of *T. aphylla* on the red rusty flour beetle *T. castaneum*

Mortality of Insect Larvae After (Days)					
Concentration (%)	**1 Day**	**2 Days**	**3 Days**	**4 Days**	**Concentration rate**
2.50%	1.0	1.33	2.0	2.33	1.67
5%	2.67	3.33	3.67	5.67	3.67
10%	4.0	4.67	4.67	5.67	4.67
Comparison	0.0	0.0	0.0	0.0	0.0
Average effect days	2.08	2.17	2.33	4.42	
		concentrations		1.468	
L.S.D.		days		1.468	
		interaction		2.935	

The existence of various chemical components can be associated to the effect of the aqueous extract from *T. aphylla* leaves on the sawfly beetle, the red rusty flour beetle, and the red flour beetle, based on the results in Tables (1, 2, 3). These chemicals, which include flavonoids, terpenoids, tannins, alkaloids, glycosides, polyphenols, amino acids, and others, frequently have fatal, repellant, or deterring effects on insects (Ghitheeth, 2023). Barati et al. (2013) reported that extracts from two plant species, allium sativum and *Calotropis procera*, contained active compounds when tested against *Bemisia tabaci*. The effects of these compounds were compared to those of the pesticide pymetrozine. The results revealed significant effects on adult female survival and fertility, as well as a decrease in sexual reproduction rate, average egg incubation period, and rate of rise in these insects. Although plant extracts did not outperform chemical pesticides in terms of effectiveness, their potential for pest control remained clear. As a result, they are suitable substitutes for chemical pesticides in integrated pest management (IPM) systems. Bughio et al. (2017) reported on a study utilizing gas chromatography-mass spectrometry (GCMS) on water-distilled essential oils from *T. aphylla* flowers and foliage. The extract contained 31 chemical components, which they identified. Kim et al. (2003) also mentioned the possibility of applying different plant extracts to avoid stored grain pests. These extracts are high in beneficial chemical compounds, and many of them have little effect on non-target creatures while being effective against pests. They can thus be utilized in integrated pest management schemes. Hadi et al. (2015) investigated the effectiveness of *T. aphylla* leaf extract against the mosquito species *T. ramosissima*. Their findings demonstrated that the leaf extract successfully eradicated mosquitoes and had a substantial influence on the larval stage in the laboratory, with a mortality rate of 90% in a 20 mg/mL dosage. Furthermore, the extract reduced mosquito populations by 12.33% in field circumstances (Hadi et al., 2015).

The effect of different concentrations of hot aqueous extract of *T. aphylla* leaves on the percentage of wheat seed germination in vitro.

Figure (1) shows the results of different concentrations of hot aqueous *T. aphylla* leaf extract on the germination percentage of wheat seeds at all concentrations tested in the experiment (10%, 5.0%, 2.5%). The control treatment produced the highest germination percentage (93.3%) followed by the 5% and 10% extract treatments, each of which demonstrated a 90% germination rate. In comparison, the 2.5% concentration showed the lowest germination rate, which was 88.3%. These results imply that the plant extract used could be exploited to protect wheat seeds without altering their germination percentage. At the same time, they provide adequate protection against pests, particularly those evaluated in this study.

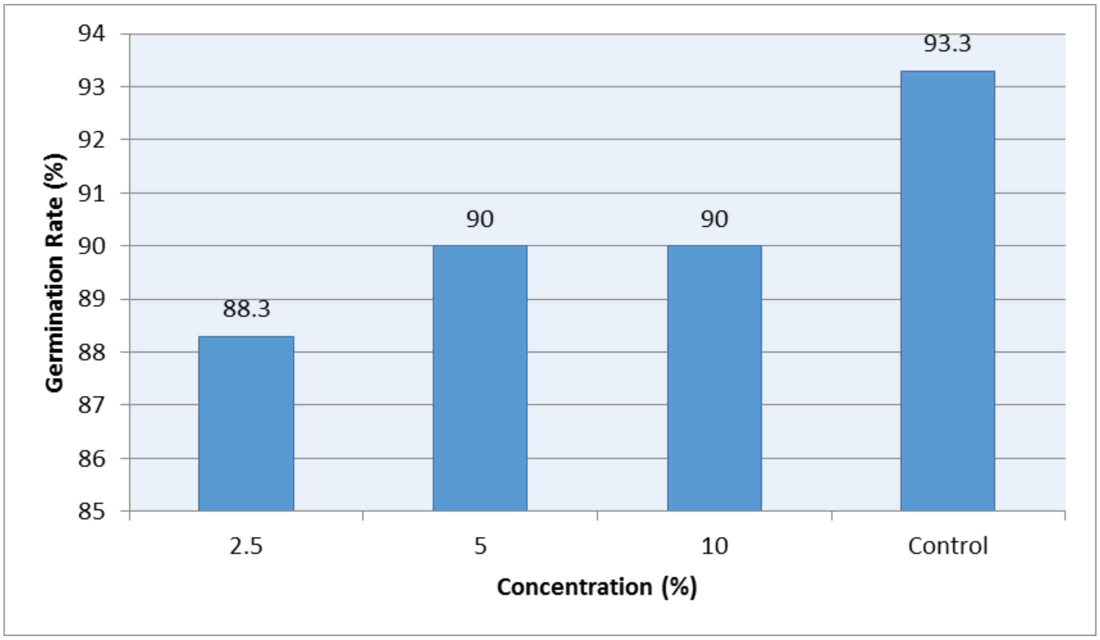

Figure1. Effect of different concentrations of hot-aqueous leaf extract of *T. aphylla* on *in vitro* wheat seed germination rate

According to the figure above, there were no significant differences between the experimental treatments, and there was no effect on the germination rates of wheat seeds after treatment with *T. aphylla* leaf extract. These results are consistent with Radwan and his colleagues (2019), who conducted laboratory studies to investigate the influence of an aqueous extract of *Calotropis procera* on the growth of wheat and barley. A plant chemical analysis of the *C. procera* aqueous extract was also performed to validate the presence of several biologically active components (alkaloids, phenols, flavonoids, saponins, steroids, and terpenoids). When compared to the control group, higher amounts of *C. procera* extract contributed to a small decrease in germination percentage, root length, and shoot length in wheat. The aqueous extract, on the other hand, had no effect on barley seed germination.

Conclusion

From the current study, we conclude that it is possible to use plant extracts to reduce the number of insects that infect grains. This study also indicates the possibility of studying other plant extracts on other storage insect pests. It was clear from the results obtained that there is a direct relationship between the percentage of death and the increase in the concentration of the plant extract, and the percentage of insect deaths increased with time for each treatment compared to the percentage recorded at the beginning of the application.

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