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# Alcoholic extracts of *Vitex agnus-castus* and *Populus euphratica* in controlling *Hyalomma anatolicum* parasitizing livestock

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**ABSTRACT:** The study was conducted from the beginning of April 2022 to the end of December 2022 in Kirkuk governorate, Al-Huweija district. The study examined the alcoholic extracts efficacy of *Vitex agnus-castus* and *Populus euphratica* in controlling the hard tick *Hyalomma anatolicum* using the dipping method. All treated larvae died within 48 hours of treatment. The alcoholic extract of *P. euphratica* was more effective in killing tick nymphs at a 90 mg $mL^{-1}$ concentration during the nymphal stage. The mortality rate was 100%. The lowest mortality rate was 67.7% at a concentration of 30 mg $mL^{-1}$ after 96 hours of treatment. As for the *V. agnus-castus* alcoholic extract, the highest mortality rate at a concentration of 90 mg $mL^{-1}$ was 93.33%, while the lowest was 66.66% after 96 hours of treatment. The alcoholic extract of the combination achieved a mortality rate of 93.33% at a concentration of 90 mg $mL^{-1}$. The lowest concentration of 30 mg $mL^{-1}$ resulted in a mortality rate of 46.66% after 96 hours of treatment.
**Keywords***:* hard tick; biological control; combination of natural products.

# Extratos alcoólicos de *Vitex agnus-castus* e *Populus euphratica* no controle de *Hyalomma anatolicum*

**RESUMO:** O estudo foi realizado desde o início de abril de 2022 até o final de dezembro de 2022 na província de Kirkuk, distrito de Al-Huweija. O estudo examinou a eficácia dos extratos alcoólicos de *Vitex agnus-castus* e *Populus euphratica* no controle do carrapato duro *Hyalomma anatolicum* usando o método de imersão. Todas as larvas tratadas morreram dentro de 48 horas após o tratamento. O extrato alcoólico de P. euphratica foi mais eficaz em matar ninfas de carrapatos na concentração de 90 mg/ml durante a fase ninfal. A taxa de mortalidade foi de 100%. A menor taxa de mortalidade foi de 67,7% na concentração de 30 mg $mL^{-1}$ após 96 horas de tratamento. Quanto ao extrato alcoólico de *V. agnus-castus*, a maior taxa de mortalidade na concentração de 90 mg $mL^{-1}$foi de 93,33%, enquanto a menor foi de 66,66% após 96 horas de tratamento. O extrato alcoólico da combinação atingiu taxa de mortalidade de 93,33% na concentração de 90 mg $mL^{-1}$. A concentração mais baixa de 30 mg $mL^{-1}$resultou numa taxa de mortalidade de 46,66% após 96 horas de tratamento.
**Palavras-chave:** carrapato duro; controle biológico; combinação de produtos naturais.

## 1. INTRODUCTION

Ticks are ectoparasites that infest birds, mammals, and reptiles. There are about 900 species, of which approximately 10% transmit germs (JONGEJAN; UILENBERG, 2004). Ticks have become one of the biggest problems in recent years, causing harm to humans and animals. They feed forcibly on blood by attaching to the surface of the host, as their survival and reproduction depend on blood. Ticks are of great importance due to their direct impact on livestock from several aspects, including economic and veterinary, because they transmit many blood pathogens and toxins that cause decreased production (GARCIA et al., 2012). Also, the animal's health condition will deteriorate infestation by external parasites causes discomfort, irritation, itching, weakness and emaciation in animals (HINKLE; CORRIGAN, 2020).

Despite the use of chemical pesticides, which are widespread and used for treatment, there has been a recent resurgence in the use of medicinal plants as natural alternatives to chemicals (HARBORNE, 1984). The attention to plant-based pesticides began in the 1930s and continued until the 1950s. Their use declined due to the emergence of fast-acting chemical pesticides. However, interest in plant extracts has returned in recent years due to the impact of chemical pesticides (GRAINGE et al., 1985). *Vitex agnus-castus* is a plant that contains many active ingredients, most importantly glycosides, flavonoids, terpenes, carbohydrates, phenols, saponins, and about 1.5% volatile oil. It also contains only 0.5% of essential oils; limonene is the most important (CHRISTIE; WALKER, 1997).

*Populus Euphratica* tree belongs to the Salicaceae family, which includes more than 50 species naturally distributed in the northern hemisphere and eastern and central Asia. This is the native habitat of this plant. *Populus Euphratica* is one of the economic plants that is easy to cultivate and widespread

on the banks of the Tigris, Euphrates and their tributaries, as well as the valleys in mountainous areas and islands. The best way to propagate these trees is vegetative reproduction, taking advantage of their fast growth (Wei et al,2015). *P. euphratica* is rich in glycosides, rhamaoglucoside, rutionside, isoquercitrin, quercetin, aplgenin, phenols, ferulic acid, ellagic acid, comari acid, chloragenic acid, cinamic acid, and salicinic acid (AL-GHANAM, 2021).

The study aims to compare the extract of *V.agnus* castus and *P.euphratica* and their combination in controlling the larval and nymphal stages of the hard tick (*Hyalomma anatolicum*).

## 2. MATERIAL AND METHODS

### 2.1. Collection and breeding hard ticks (*Hyalomma anatolicum*)

Ticks were collected from Kirkuk governorate, Al-Huweija district, where engorged females were collected. They were placed in 2.5 cm diameter and 5 cm high plastic containers. The lids were covered with cloth and tied tightly, then transported to the parasitology laboratory at the College of Science - Tikrit University and diagnosed by Dr Saeed Maher Lafta according to the taxonomic key (WALKER et al., 2003). Ticks were placed in suitable conditions for egg laying. The egg-laying period lasted 12 days and 3-4 days until ovulation. The eggs were left under suitable 25-28°C temperature and 70-80% humidity. They took 12 days to hatch. After 24 hours of hatching, some were used to control and demonstrate the effect of the extracts on the larval stage. The rest were starved for ten days, then transferred to the host, 2.5 kg laboratory rabbits (*Oryctolagus cuniculus*), to take a blood meal. This process took about four days, then fell to the ground and transformed into nymphs after about ten days. The nymphs remained inactive for five days, reattached to the rabbits, and stayed attached for six days before dropping off again. The fed nymphs were collected and placed in containers, taking 20-24 days to transform into adults (KAPLAN; TIMMENS, 1972). The laboratory rabbits were placed in 50×50×90 cm glass containers, and a plastic collar was placed around the rabbit's neck so as not to impede tick feeding (WATTS et al.,1972).

Table 1. Plants extracts used in the control.
Tabela 1. Extratos vegetais utilizados no controle.

| Material name | Scientific Name | Part used | Purpose |
|---|---|---|---|
| Vitex agnus-castus | *V.agnus castus* | Leaves | Control |
| Euphrates poplar | *Populus euphratica* | Leaves | Control |

### 2.2. *V. agnus* castus taxonomy

Kingdom: Plantae
Division: Tracheophytes
Class: Dicotyledonas
Order: Lamiales
Family: Lamiaceae
Genus: *Vitex*
Species: *agnus–castus* (Feryal; Basima 2019).

### 2.3. *Populus euphratica* taxonomy

Kingdom: Plantae
Order: Malpighiales
Family: Salicaceae
Genus: *Populus*
Species: *P. euphratica* (Heptner; Sludskij ,1992).

Figure 1. *V. agnus castus* leaves (Verkaik et al., 2017).
Figura 1. Folhas de *V. agnus castus* (Verkaik et al., 2017).

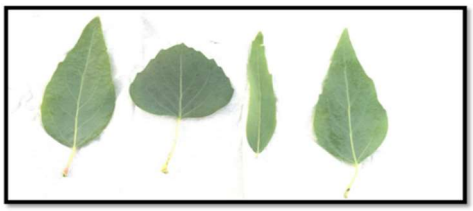

Figure 2. *Populus euphratica* leaves(Al-Ghanam, 2021)
Figura 2. Folhas de *Populus euphratica* (Al-Ghanam, 2021)

### 2.4. Collection of the plants used in the study

Leaves of *Vitex agnus-castus* and *Populus euphratica* were collected from areas close to the Tigris River in Al-Alam district, Salah al-Din governorate. A suitable amount was collected, washed with water to remove dirt and impurities, then placed on cotton cloth and left to dry. They were kept in a room and continuously turned over until completely dry. The leaves were then ground using an electric grinder into a fine powder, placed in a tightly sealed plastic jar and refrigerated until extract preparation. (RIOSE; ANDERSON, 1987).



### 2.5. Preparation of the alcoholic extract

A previously prepared *Vitex agnus-castus* and *Populus euphratica* extracts, 9gm, dissolved in 11 mL of ethanol alcohol, then completed to 100 ml of ethanol to get 90 mg/ml, then other concentrations of 70, 50, and 30 mg $mL^{-1}$ were then prepared by serial dilution using the formula C1V1=C2V2 (AL-RUBAIE, 1999: AL-SALAMI, 1998).

### 2.6. Preparation of the alcoholic extract of the combination

4.5 gm of *Populus euphratica* and 4.5 gm of *Vitex agnus-castus* were mixed; 11 mL of ethanol was added and then completed to 100 ml to get the 90 mg $mL^{-1}$ concentration. Other concentrations of 70, 50 and 30 mg $mL^{-1}$ were prepared by serial dilution using the formula C1V1=C2V2 and the efficacy of the combination was tested at all tick stages (AL-RUBAIE, 1999: AL-SALAMI,1998).

### 2.7. Testing the effect of the extract on the larval stage

The concentrations were prepared in advance, and the sensitivity of these extracts was tested on the larval stage. 5 larvae were taken for each replicate, with three replicates for each concentration, and ten larvae were dipped only in alcohol as a control. The larvae were thoroughly dipped in the extract and then transferred to 150 mL plastic containers which were quickly covered as the larvae moved very fast. Results were taken after 24, 48, 72 and 96 hours of treatment (FERNANDES et al., 2005). The results were corrected according to Abbott's formula (ABBOTT, 1925).

### 2.8. Testing the effect of the extract on the nymphal stage

The concentrations were prepared in advance, and the sensitivity of these extracts was tested during the nymphal stage. 5 nymphs were taken for each replicate, with three replicates for each concentration, and ten nymphs were dipped only in alcohol as a control. The nymphs were thoroughly dipped in the extract and then transferred to 150 ml plastic containers covered with organdy fabric. Results were taken after 24, 48, 72 and 96 hours of treatment (FERNANDES et al., 2005). The results were corrected according to Abbott's formula (ABBOTT, 1925).

## 3. RESULTS

### 3.1. Efficacy of plant extracts in controlling the Ticks' larval stage

Table 2 shows that all treated larvae were killed after 24 hours with the plant extracts by 100% mortality. The killing is attributed to the substances contained in the extract, which close the respiratory tracts as the materials are viscous, and part of the extract consists of fatty materials that prevent respiration.

### 3.2. The effectiveness of the *Populus euphratica* extract in controlling the nymphal stage of ticks

Table 3 shows that the highest mortality rate was at a concentration of 90 mg $mL^{-1}$, where the mortality rate was 100% after 96 hours of treatment, while the concentration of 30 mg $mL^{-1}$ had a mortality rate of 66.7% after 96 hours of treatment, which is the lowest. The concentration and time had a direct effect on the mortality rate. The main reason for the death of the nymphs is the prevention of respiration because the *Populus euphratica* extract contains viscous and fatty substances that hinder respiration.

### 3.3. The efficacy of *Vitex agnus-castus* plant extract on controlling the ticks' nymphal stage

Table 4 shows that the highest mortality rate was 93.33% at the concentration of 90 mg/ml after 96 hours of treatment, while the concentration of 30 mg/ml recorded the lowest mortality rate, 66.66%, after 96 hours of treatment. The concentration and time had a direct effect on the mortality rate.

Table 2. Effects means of plant extracts, exposure duration, and their interaction on the larval stage of *Hyalomma anatolicum* (%).
Tabela 2. Efeitos médios dos extratos vegetais, duração da exposição e sua interação na fase larval de *Hyalomma anatolicum* (%).

| Extracts name | Concentrations (mg $mL^{-1}$) | 24 | 48 | Control |
|---|---|---|---|---|
| *Populs euphratica* | 90 | 100 a | 100 a | 0% |
| | 70 | 100 a | 100 a | |
| | 50 | 100 a | 100 a | |
| | 30 | 100 a | 100 a | |
| *Vitex agnus* | 90 | 100 a | 100 a | 0% |
| | 70 | 100 a | 100 a | |
| | 50 | 100 a | 100 a | |
| | 30 | 100 a | 100 a | |
| Combination | 90 | 100 a | 100 a | 0% |
| | 70 | 100 a | 100 a | |
| | 50 | 100 a | 100 a | |
| | 30 | 100 a | 100 a | |

The values followed by the same letter within the interactions and rates individually do not differ significantly according to Duncan's test at the probability level (P≤0.05).



Table 3. Effect means of *Populus Euphratica* extract, exposure duration, and interaction on the nymphal stage of *Hyalomma anatolicum* (%).
Tabela 3. Médias de efeito do extrato de *Populus Euphratica*, tempo de exposição e interação no estágio ninfal de *Hyalomma anatolicum* (%).

| Time (hour) Concentration (mg $mL^{-1}$) | 24 | 48 | 72 | 96 | Concentration effect rate |
|---|---|---|---|---|---|
| 90 | 60 f | 73.3 d | 86.7 b | 100 a | 80 a |
| 70 | 60 f | 66.7 e | 80 c | 100 a | 76.67 b |
| 50 | 40 h | 60 f | 66.7 e | 66.7 e | 58.33 c |
| 30 | 40 h | 53.3 g | 60 f | 66.7 e | 55 d |
| Control | 0 i | 0 i | 0 i | 0 i | |
| Rate of time effect | 40 d | 50.66 c | 58.66 b | 66.66 a | |

The values followed by the same letter within the interactions and rates individually do not differ significantly according to Duncan's test at the probability level (P≤0.05).

Table 4. Effect means of *Vitex agnus-castus* extract, exposure duration, and interaction on the hard tick nymphal stage _*Hyalomma anatolicum* (%).
Tabela 4. Médias de efeito do extrato de *Vitex agnus-castus*, tempo de exposição e interação no estágio ninfal de *Hyalomma anatolicum* (%).

| Time (hour) Concentration (mg $mL^{-1}$) | 24 | 48 | 72 | 96 | Concentration effect rate |
|---|---|---|---|---|---|
| 90 | 40 f | 53.33 e | 73.33 c | 93.33 a | 65 a |
| 70 | 40 f | 40 f | 66.66 d | 86.66 b | 58.33 b |
| 50 | 33.33 g | 40 f | 53.33 e | 73.33 c | 50 c |
| 30 | 20 i | 26.66 h | 33.33 g | 66.66 d | 36.66 d |
| Control | 0 j | 0 j | 0 j | 0 j | |
| Rate of time effect | 26.67 d | 32 c | 45.33 b | 64 a | |

The values followed by the same letter within the interactions and rates individually do not differ significantly according to Duncan's test at the probability level (P≤0.05).

### 3.4. The combination efficacy in controlling the nymphal stage of ticks

Table 5 illustrates that the highest mortality rate was 93.33% at a concentration of 90 mg/ml after 96 hours of treatment, while the concentration of 30 mg $mL^{-1}$ had the lowest mortality rate of 46.66% after 96 hours of treatment. The concentration and time had a direct effect on the mortality rate.

Table 5. Mean of effects of the combination, exposure duration, and their interaction on the nymphal stage of hard ticks *Hyalomma anatolicum* (%).
Tabela 5. Média dos efeitos da combinação, duração da exposição e sua interação na fase ninfal dos carrapatos duros *Hyalomma anatolicum* (%).

| Time (hour) Concentration (mg mL$^{-1}$) | 24 | 48 | 72 | 96 | Concentration effect rate |
|---|---|---|---|---|---|
| 90 | 33.33 h | 60 d | 66.66 c | 93.33 a | 63.33 a |
| 70 | 33.33 h | 53.33 e | 60 d | 86.66 b | 58.33 b |
| 50 | 26.66 i | 33.33 h | 40 g | 60 d | 40 c |
| 30 | 20 j | 40 g | 40 g | 46.66 f | 36.67 d |
| Control | 0 k | 0 k | 0 k | 0 k | |
| Rate of time effect | 22.66 d | 37.33 c | 41.33 b | 57.33 a | |

The values followed by the same letter within the interactions and rates individually do not differ significantly according to Duncan's test at the probability level (P≤0.05).

## 4. DISCUSSION

### 4.1. Efficacy of plant extracts in controlling the Ticks' larval stage

The results obtained in this study agree with those reached by Wathah (2013), where all larval stages of the tick *Hyalomma schulzei* Olinev treated with datura plant extract were killed after 24 hours at all concentrations used, reaching mortality of 90%.

The results also concur with those indicated by Al-Yasiri (2011) in his study of the *Rhipicephalus turanicus* Pomerantzev tick, where he used the ethyl extract of castor seeds at concentrations of 80, 60, 40 and 20 mg mL$^{-1}$. All larval stages were killed after 48 hours of treatment except at 20 mg mL$^{-1}$, where the mortality rate was 71.8%. The results are close to those of Ricarte et al. (2020), where the essential oils present in high concentrations in the *Vitex agnus-castus* were highly effective against the third instar larvae of the mosquito *A. aegypti*, with mortality rates between 0% and 100% after 24 hours of treatment.

The results conform with Ribei et al. (2007) in his study of the crude ethanol extract of *H. polyanthemum* against *B. microplus* tick larvae, leading to 100% mortality at a 50 mg mL$^{-1}$ concentration. The results concur with Fernandes et al. (2008) in their study of *R. sanguineus* ticks, where 99% of tick larvae were killed using the ethanol extract of *Magonia pubescens*. The results are consistent with the study conducted by Ashor (2012), where all unfed and fed larvae of the tick *R. turancuis* treated with extracts of *C. colocynthis* seeds and *Clerodendron inerme* were killed.

The study results concur with those of Yousif (2022), where three extracts were used, including rosemary leaves (*Rosmarinus officinalis*), jujube fruits (*Zizyphus lotus*), and fenugreek seeds (*Trigonella foenum* graecum) against red flour beetle larvae at concentrations of 20-10-5-2.5 mg mL$^{-1}$. The fenugreek and rosemary ethanol extracts recorded 100% mortality at 20 mg mL$^{-1}$, and the jujube extract caused 100% mortality in flour beetle larvae at 20 mg mL$^{-1}$.

It is referred by Wigglesworth (1972) and Rockstein (1978) that the cause of larval mortality is the binding of active compounds to the fatty materials in the digestive system, leading to their excretion without benefiting from them. Similarly, Abdulhameed; Abdulmajeed (1988) pointed out that the cause of larval mortality is the arrival of the toxic material to the midgut of the tick, which damages the epithelial layer and, in turn, hinders the secretion of digestive enzymes, ultimately causing larval death.

### 4.2. The effectiveness of the *Populus euphratica* extract in controlling the nymphal stage of ticks

The current results conformed to the results of Matov (2007), where ethyl extract of the *T. vollagaris* plant greatly affected tick nymphs, leading to their death by 90%. Moreover, the study's results agreed with the results of Al-Yasiri (2011) on *R. turanicus* ticks, where the plant extract of colocynth seeds was used with ethyl acetate and ethyl alcohol and hexane. Ethyl was superior to ethyl and hexane in killing fed and unfed tick nymphs.

Furthermore, the results are consistent with the study Ashor (2012) results, where the *Clerodendron inerme* extract of leaves was used, which led to the killing of all unfed nymphs at 100%, and the mortality rate for fed nymphs was 81.04% at a concentration of 60 mg mL$^{-1}$.

In addition, the study's results agreed with the study conducted by Yousif (2022), where *Rosmarinus officinalis* leaves were used against the pupal stage of *T. granarium*. The ethanolic extract and ethyl acetate had mortality rates of 33.3 and 26.7% at a concentration of 2.5 mg mL$^{-1}$, respectively, which is the lowest, and the highest pupal mortality rate was at a concentration of 20 mg mL$^{-1}$, where the mortality rate was 90 and 80%, respectively.

The cause of the nymphs' death is that the extracted substances affect the central nervous system and directly on the neural connections and cause toxicity within the tissues as a result of damage to cellular enzymes or as a result of deposition of extracted substances on the wall, which affects the spiracle and thus prevents gas exchange (HARBORNE,1984).

### 4.3. The efficacy of *Vitex agnus-castus* plant extract on controlling the ticks' nymphal stage

The study agreed with Matov's (2007), where the ethyl extract of *T. vollagaris* led to the death of tick nymphs by 90%. Also, the current study results are similar to the results of the Al-Yasiri (2011), where the ticks of the type *R. turanicus* are affected by colocynth seeds extract in which the ethyl acetate and ethyl alcohol and hexane were used. Ethyl was superior to ethyl and hexane in killing fed and unfed tick nymphs.

Moreover, the study results are consistent with the study of Ashor (2012), where the extract *Clerodendron inerme* leaves was used, which killed all unfed nymphs by 100% and the mortality rate for fed nymphs was 81.04% at a concentration of 60 mg mL$^{-1}$.

Furthermore, this study's results agreed with the Yousif (2022) results, where Rosmarinus officinalis leaves were used against the pupal stage of *T. granarium*. The ethanolic extract and ethyl acetate had a mortality rate of 33.3 and 26.7% at a concentration of 2.5 mg $mL^{-1}$, respectively, which is the lowest. The highest pupal mortality rate was at a concentration of 20 mg $mL^{-1}$; the mortality rate was 90 and 80%, respectively.

The nymphs' cause of death is attributed to the extracted substances affecting the central nervous system directly on the neural connections, which cause toxicity within the tissues as a result of damage to cellular enzymes or as a result of deposition of extracted substances on the wall that affects the spiracle and hence prevents gas exchange (Harborne,1984).

### 4.4. The combination efficacy in controlling the nymphal stage of ticks

The study results are consistent with the results of Matov (2007), in which the ethyl extract of *T. vollagaris* affected tick nymph mortality by 90%. Moreover, this study's results are similar to the study of Al-Yasiri (2011) that examined *R. turanicus* ticks, where colocynth seeds extract was used in which ethyl acetate and ethyl alcohol and hexane were used. Ethyl was superior to ethyl and hexane in killing fed and unfed tick nymphs.

Furthermore, the study's results are consistent with the results of the study of Ashor (2012), in which the extract of *Clerodendron inerme* leaves was used and killed all unfed nymphs by 100%; the mortality rate for fed nymphs was 81.04% at a concentration of 60 mg $mL^{-1}$.



In addition, the current study results agreed with the study of Yousif (2022), where *Rosmarinus officinalis* leaves were used against the *T. granarium* pupal stage. The ethanolic extract and ethyl acetate had a 33.3 mortality rate and 26.7% at a concentration of 2.5 mg $mL^{-1}$, respectively, the lowest. The highest pupal mortality rate was 90 and 80%, respectively, at a concentration of 20 mg $mL^{-1}$. The nymphs' cause of death is the extracted substances that affect the central nervous system and directly on the neural connections, which leads to toxicity within the tissues as a result of damage to cellular enzymes or as a result of deposition of extracted substances on the wall, which affects the spiracle and prevents gas exchange (HARBORNE, 1984).

## 5. CONCLUSIONS

An extract of the *Populus euphratic* leaves had the greatest effect on all stages of ticks.

The mortality rate of larval and nymphal stages was directly proportional to concentration and time, and the larval stage was the most affected in terms of mortality rate.

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