

## ORIGINAL ARTICLE

# Comparision of Antiparasitic Effects of *Allium sativum* and *Triclabendazole* on Eggs and Miracidium of *Fasciola hepatica* In-vitro

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## ABSTRACT

**Key words:**

*In-vitro*, *Allium sativum*, *Triclabendazole*, *Miracidium*, *Fasciola hepatica*

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**Background:** Fascioliasis is a parasitic disease of animals (in cattle and sheep, but also in pigs, donkeys, etc.) that can be transmitted to humans or other definitive host. The infection is acquired by eating aquatic or semi-aquatic plants that have the metacercaria of the worm attached to their stem or leaves, raw or poorly cooked. Eggs derived from their uteri were also appendaged. Diagnostic morphological traits of *F. hepatica* eggs. Three dimensional models of *F. hepatica* eggs were created to demonstrate the eggs features. Since fecal examination remains gold standard in diagnosing fasciolosis in humans and animals worldwide, our findings may contribute to improved diagnostics. *Fasciola* is a type of leaf shaped parasitic worms and Anti parasitic effects of *Allium sativum* and *Triclabendazole* on the eggs and Miracidium of *Fasciola hepatica* were done in vitro. Experiments were done with different concentrations of garlic alcoholic extract after the complete hatching of *Fasciola* eggs and the release of miracidium. *Fasciola* eggs were incubated for two weeks in suitable laboratory conditions to stimulate hatching. **Objectives:** Fascioliasis is a zoonotic and food-borne parasitic diseases that caused by *Fasciola hepatica*. Garlic (*Allium sativum*) is an important and valuable medical plant which is compatible due to its medical properties and effects. *Allium sativum* is used as a liver protector, anti-inflammatory, anti-bacterial and anti-parasitic agent. **Methodology:** Anti parasitic effects of *Allium sativum* and *Triclabendazole* on the eggs and Miracidium of *Fasciola hepatica* were done invitro. Experiments were done with different concentrations of garlic alcoholic extract after the complete hatching of *Fasciola* eggs and the release of miracidium. *Fasciola* eggs were incubated for two weeks in laboratory conditions under suitable pH, light and temperature conditions to stimulate hatching. Then 10 microliters of non-chlorinated water containing 20 miracidia were added to the wells and 50 microliters of each extract at the rate of 2, 5, and 10µg/ml were added to the wells. We used dechlorinated water and *Triclabendazole* as negative and positive control groups. **Results:** The results showed that the hydroalcoholic extract of *Allium sativum* reduced the survival time and also the movement speed of Miracidium *fasciola* and finally stopped it. The concentration of 10 µg/ml of hydroalcoholic extract of *Allium sativum* destroyed Miracidia in  $107 \pm 2$  seconds ( $P < 0.001$ ). But at a concentration of 2 µg/ml, this time was  $493 \pm 2$  seconds ( $P < 0.001$ ). At a concentration of 10 µg/ml, the speed of miracidia reaching  $0.07 \pm 0.02$  (mm/s) was determined ( $P < 0.001$ ). **Conclusion:** The use of garlic plant extract (*Allium sativum*) by inhibiting the development of *Fasciola hepatica* eggs interrupts the life cycle of this parasite and can reduce the cases of contamination among livestock and humans. On the other hand, due to the fact that this extract is a natural product, it seems to have minimal negative effects on the ecosystem.

## INTRODUCTION

Fascioliasis is a zoonotic and food-borne parasitic diseases that caused by *Fasciola hepatica*. The disease caused by *Fasciola hepatica* is called fascioliasis or dystomiasis. This parasite is observed in most parts of the world, especially in areas where sheep, cattle and buffalo are raised. *Fasciola hepatica* is a helminth

parasite in the shape of tree leaves, 20 to 30 mm long and 8 to 13 mm wide. The life cycle of *Fasciola hepatica* is indirect and adult *Fasciola hepatica* lives inside the bile ducts and gall bladder. The eggs of *Fasciola hepatica* are excreted with feces, and if these eggs reach the water, after 9 to 15 days, the embryo inside the egg or miracidium is formed, and in order to continue living, it must enter the body of the

intermediary host, which is a freshwater snail (*Lymnaea stagnalis*). In the body of the snail, after the developmental stages of sporocyst and redia, it turns into cercaria. After leaving the body of the snails, the cercariae turn into metacercariae on the plants, and if the metacercariae are eaten by the final host along with the plants, they pass through the intestinal wall in the duodenum and reach the liver tissue through Glysson's capsule or lymph, and then Matures from 2 to 4 months<sup>1</sup>.

Human fascioliasis infection has been reported from 70 countries around the world, which has infected 2.5 million people, and 180 million people are at risk of fascioliasis. Livestock contamination is mostly seen in cattle and sheep, which causes liver contamination, weight loss, and milk production in infected cattle, and ultimately causes significant economic losses<sup>2</sup>. Eradication of *Fasciola hepatica* is almost impossible. Indiscriminate treatment of *Fasciola* with the use of anthelmintic drugs such as Triclabendazole has caused drug resistance in some parts of the world, which is considered a serious problem. Therefore, at present, destroying or inactivating the eggs and miracidium of *Fasciola hepatica* is considered a control method of this parasite. For this purpose, methods such as heating and chemicals can be used. These methods cause destruction the egg wall prevents the formation of the cell mass and embryo (miracidium) inside the parasite egg, thus making the egg inactive. Asian countries have a high rank in terms of plant richness and biodiversity<sup>3</sup>.

Garlic is an aromatic medicinal plant that has been used in conventional medicine for thousands of years, and its scientific name is *Allium sativum*. This plant is native to Central Asia and South Asia<sup>4</sup>. Garlic has antibacterial and anti-parasitic properties, and is also considered a strong antioxidant<sup>5</sup>. Garlic is one of the oldest medicinal plants that has been used as a food and medicine all over the world since ancient times. This plant has many health properties and has countless benefits for health, slimming and digestion<sup>6</sup>.

Garlic contains allicin, fiber, vitamin C, minerals, and antioxidant compounds that help lower blood pressure, improve muscle cramps, strengthen the immune system, reduce the risk of cardiovascular disease, and even improve digestive function and the digestive system. Garlic is one of the native plants of Central Asia and the Middle East and is known as an important food and medicinal product. Garlic, due to its taste and properties, has been used as a natural substance and herbal medicine throughout history. Its antibacterial, anti-fungal and antiviral properties have made it play a significant role beneficent the body's immune system, lowering blood pressure, inducing hair growth, reducing inflammation and even reducing the risk of some diseases such as cardiovascular disease. Garlic is also known to be a powerful antioxidant and

can be helpful in protecting cells from oxidative damage<sup>7</sup>.

Medicinal plants are facing a lot of attention due to their great diversity and unique properties they have in human health. Herbal medicine is one of the methods that has been able to prove its ability to improve many health problems since thousands of years ago. It is necessary to get to know the complete list of medicinal plants that have a significant reparative and healing role and can be used as a low-risk and effective way to maintain health<sup>8,9</sup>. When a plant is called medicinal, at least one of its parts, such as root, stem, flower or leaf, contains medicinal and therapeutic properties. Herbal medicine is a common and popular method that is used for various therapeutic purposes. About the use and method of using various types of medicinal plants, various and numerous researches and investigations have been done in order to use these natural materials effectively and purposefully<sup>10,11</sup>.

In this study, we aim to investigate the effect of the alcoholic extract of garlic on the developmental stages of *Fasciola* eggs, from the formation of the cell mass to the exit of the embryo (miracidium) from the egg, in an in-vitro environment in Al-Diwaniyah city of Iraq.

#### **Study Area:**

Al-Diwaniyah is one of the cities of Al-Qadisiyah province, which is located in the country of Iraq. This city is located 200 kilometers south of Baghdad and near the Al-Diwaniyah River, which is one of the branches of the Euphrates River, which passes through the city of Al-Diwaniyah. Also, this city is one of the most fertile agricultural areas in Iraq for the cultivation of rice and date palm, for this reason, the population of this city has increased day by day and reached its current state.

## **METHODOLOGY**

#### **Fasciola egg collection:**

Sheep livers infected with *Fasciola hepatica* were obtained from the slaughterhouse. Then *Fasciola* worms were completely drained from the infected bile ducts. Worms were placed in a plate containing physiological serum and washed several times to separate from liver secretions. The uterus of these worms was also cut under the microscope and after straining the existing suspension of worm eggs, it was completely emptied. These eggs were washed 20 times with chlorine-free water. Then, a suspension containing 2000 eggs per milliliter of chlorine-free water was prepared from *Fasciola* eggs and kept at 4°C until the test.

#### **Preparing the alcoholic extract of garlic:**

Maceration method was used to prepare alcoholic extract of garlic plant. The procedure is as follows: 10 grams of dried garlic plant was added to 100 ml of 96% ethanol and incubated for 48 hours at room temperature. Then we filtered the obtained solution and incubated the

solution at room temperature until the complete evaporation of alcohol. In this way, the solution was dried and a powder was obtained which was the extract of the garlic plant. The said powder was kept at 4 degrees Celsius until use.

#### Anti-parasitic effects of garlic alcoholic extract:

Experiments were done with different concentrations of garlic alcoholic extract after the complete hatching of *Fasciola* eggs and the release of miracidium. *Fasciola* eggs were incubated for two weeks in laboratory conditions under suitable pH, light and temperature conditions to stimulate (spur) hatching. Then 10 microliters of non-chlorinated water containing 20 miracidia were added to the wells and 50 microliters of

each extract at the rate of 2, 5, and 10 µg/mL were added to the wells. We used dechlorinated water and triclabendazole as negative and positive control groups. Each treatment was performed in three replicates for each positive and negative experimental group. Evaluation of the effect of each plant extract on the movement speed of Miracidium *Fasciola hepatica* was analyzed under a light microscope connected to a digital camera at 5-minute intervals of time (10, 15, 20, and 25) minutes at 40\* magnification. In each assessment, the activity of antiparasitics of plant extracts was measured by comparing the ratio of live to dead and immobile dead miracidia and using the following formula:

$$\text{The effect rate} = \frac{\text{Number of miracidiums in control group} - \text{Number of miracidiums in tested group}}{\text{Number of miracidiums in control group}} \times 100$$

In the present research, the survival time and movement speed of miracidium (mm/second) and then the hatching speed prior and after the treatment were calculated as the effectiveness of the extracts.

#### Calculation of the movement speed of miracidium by specially software:

In this study, special computer software was used to evaluate the effect of medicinal plant extracts on the movement speed of miracidium *fasciola*. This software can track the parasites and the movement speed and calculate the distance<sup>11,19</sup>.

#### Statistical analysis:

The data obtained from the eggs and Miracidium *fasciola* were analyzed by T-test. SPSS version 18 statistical program was used in data analysis.

## RESULTS

this study, the antifasciolocidal activity of the hydroalcoholic extract of *Allium sativum* in the survival time and the speed of movement of Miracidium *Fasciola hepatica* in different concentrations (2, 5, and 10 µg/ml) compared to the positive (triclabendazole) and negative (chlorine-free water) control groups were examined 1 -10 minutes after the treatment.

The results of this study showed that the hydroalcoholic extract of *Allium sativum* reduced the

survival time and also the movement speed of Miracidium *fasciola* and finally stopped it.

This antiparasitic inhibitory effect of hydroethanolic extract was proportional to the dose of the extract and strongly depended on the effect time of the extract ( P < 0.001). No killed parasites were shown in the negative control groups Fig 1,2 .

A significant positive relationship was detected between the concentration of *Allium sativum* extract and its lethality, as well as the duration of treatment and the mortality rate of miracidia (P<0.001). A comparison of the process of parasite control by *Allium sativum* extract was done using the antiparasitic drug triclabendazole with a concentration of 2 micrograms per ml (P< 0.001).

As the concentration of *Allium sativum* extract increased, the same amount of survival time and movement speed of miracidia was decreased.

The concentration of 10 micrograms/ml of hydroalcoholic extract of *Allium sativum* destroyed Miracidia in 107 ± 2 seconds. But at a concentration of 2 µg/ml, this time was 493 ± 2 seconds ( Table 1). By increasing the concentration of plant extract, the speed of parasite movement decreased significantly. Therefore, at a concentration of 10 µg/ml, the speed of miracidia reaching 0.07 ± 0.02 (mm/s) was determined (Table 2).

**Table 1: Comparison the effect of *Allium sativum* with Triclabendazole on the survival rate of Miracidium**

Rate of Concentration (Microgram/ml)	Type of Extracts	Survival time (second)		
		Max.	Min.	Mean±SD
2	<i>Allium sativum</i>	496	493	493±2
	Triclabendazole (positive control)	58	54	56±3.42
5	<i>Allium sativum</i>	297	293	294±5.23
	Triclabendazole (positive control)	0.00	0.00	0.00
10	<i>Allium sativum</i>	114	104	107±2
	Triclabendazole (positive. control)	0.00	0.00	0.00
Negative control	-	-	-	-
Groups (P -value)	<0.001	-	<0.001	-

**Table 2: Comparing the effect of hydroalcoholic extract of *Allium sativum* with Triclabendazole on the movement speed of Miracidium *Fasciola hepatica*.**

Rate of Concentration (Microgram/ml)	Type of Extracts	Survival time (second)		
		Max.	Min.	Mean $\pm$ SD
2	<i>Allium sativum</i>	0.76	0.06	0.75 $\pm$ 0.01
	Triclabendazole (positive control)	0.29	0.27	0.23 $\pm$ 0.03
5	<i>Allium sativum</i>	0.66	0.64	0.65 $\pm$ 0.01
	Triclabendazole (positive control)	0.06	0.04	0.05 $\pm$ 0.01
10	<i>Allium sativum</i>	0.08	0.06	0.07 $\pm$ 0.02
	Triclabendazole (positive control)	0.02	0.01	0.01 $\pm$ 0.04
Negative control	-	-	1.03	-
Groups (P-value)	<0.001	-	<0.001	-

**Fig. 1:** Miracidium of *Fasciola hepatica* before the effect of *Allium sativum* (Normal).**Fig. 2:** Miracidium of *F. hepatica* after the effect of *Allium sativum* (It has degenerated).

## DISCUSSION

Fasciolosis in ruminants is a zoonotic disease, and the cause of this parasitic disease is a trematoda worm that transmitted through food, and two liver flukes, *Fasciola hepatica* and *Fasciola gigantica*, cause fasciolosis in the liver and other related organs<sup>12,13</sup>.

Currently, the treatment of fasciola is based on the use of chemical anthelmintic drugs, which can cause drug resistance and Side effects in humans and also if it

enters the environment, it causes destruction and pollution of the environment<sup>14-16</sup>. The results of previous studies have shown that anti-parasitic drug treatment methods are ineffective for controlling fasciolosis, and animals that are intrinsically and naturally resistant to different types of fasciola should be selected for keeping and breeding<sup>17-21</sup>. Nowadays, due to the high veterinary costs, the limited number of anti-parasitic chemical compounds available in the market, drug resistance, drug retention in milk and related toxicity, it is justified to examine the properties of selected traditional medicinal plants<sup>22-25</sup>.

The results of this study showed that the hydroalcoholic extract of *Allium sativum* reduced the survival time and also the movement speed of Miracidium fasciola and finally stopped it. As the concentration of *Allium sativum* extract increased, the same amount of survival time and movement speed was reached or decreased. So far, few studies about the effect of Physical, chemical and plant extracts factors were done on *Fasciola* eggs. For example: Moazeni et al<sup>26</sup>. with changes on the temperature of water containing *Fasciola hepatica* eggs in different time intervals they concluded that by increasing the temperature up to 50°C and increasing the exposure time, the mortality of *Fasciola hepatica* eggs also increases. From the studies using plant extracts on eggs *Fasciola* done can be referred to the study of moazeni<sup>27</sup>. In his study Ginger extract was used on *Fasciola hepatica* eggs. Results showed that after 14 days exposure of *Fasciola hepatica* eggs with concentrations of 5, 10, 25 and 50 mg/liter of this extract of ginger plant, Miracidium was not formed in eggs. Moreno et al<sup>28</sup>. investigated the effect of the extract of lemon on the metacercars of *Fasciola hepatica*. Their results showed that the metacercaria of *Fasciola hepatica* after 72 hours exposure to the extract of lemon becomes inactive.

The history of herbal medicine is equal to the creation of man; this means that since the beginning of human existence and the beginning of life on earth, human life has been tied to plants. Historical studies show that medicinal plants are one of the best methods

in the treatment of various diseases, which are associated with the least harm and complications.

The benefits of using herbal medicines include: Reducing side effects due to being natural, having self-healing properties and targeting the source of pain and disease, helping to improve the overall health of the body, being affordable.

The benefits of using chemical drugs include: Easy access, the effect of each of the chemical drugs on several diseases, high durability, and quick effect on the disease. Chemical drugs effective on fascioliasis include: Triclabendazole, Bithionol, Dihydroemetine, Hexyl resorcinol, Praziquantel, and Metronidazole<sup>29</sup>.

There is evidence that medicinal plants have been used for medical and therapeutic purposes about 60,000 years ago. The burial site of a Neanderthal man was discovered in 1960, which was buried with eight species of plants, some of which are still used for medicinal purposes today. Hippocrates, 460-380 BC, known as the "Father of Medicine", classified plants into the main categories hot and cold, wet and dry, and created a diagnostic system using plants. The number of effective medicinal plants he researched was between 300 and 400 species<sup>30</sup>.

Our study shows that *Allium sativum* extract has an effect on the eggs and miracidium of *Fasciola*. So we can use *Allium sativum* extract as a potential effective agent in controlling *Fasciola hepatica* in nature.

## CONCLUSION

The use of garlic plant extract (*Allium sativum*) by inhibiting the development of *Fasciola hepatica* eggs interrupts the life cycle of this parasite and can reduce the cases of contamination among livestock and humans. On the other hand, due to the fact that this extract is a natural product, it seems to have minimal negative effects on the ecosystem.

### Recommendations for the control and prevention of fasciolosis:

Accurate control of fasciolosis depends on eradicating the disease in herbivores. Although it is possible to treat infected animals, this is not practical for wild herbivores. Despite these problems, the following are recommended: Informing the public about the disease and its transmission methods. Refraining from consuming raw wild aquatic plants as well as local vegetables and cooking or drying them before consumption. Washing vegetables with a solution of 24 mg of potassium permanganate for 10 minutes. Sanitary disposal of feces of ruminant animals in villages. Refraining from eating raw liver and giving enough heat before consumption.

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