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The Effect of Some Chemicals on The Viability of *Lymnaea* spp. snails and Metacercariae as Control Measures of Zoonotic Fascioliasis in Qualyobia Province, Egypt

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ABSTRACT

Fasciola spp. is one of the most causes of liver diseases in the world. Infection with *Fasciola* is important from a veterinary and medical standpoint, particularly in areas with high densities of sheep and cattle production. The infection by fascioliasis takes place through ingestion of metacercariae attached to vegetables or drinking of contaminated water. Prevention of human fascioliasis could depend on cleaning of the leafy salads from the metacercariae and control of *Lymnaea* snails. The aim of the present study was to determine the effect of NaOCl on *Fasciola* intermediate snail host (*Lymnaea)*, as method of control *Fasciola* spp. and the effect of some acids as well as KMno4 on encysted metacercariae to find the best method**(s)** to clear the salads from metacercariae, while keeping the leaves as fresh as possible so that they become safe and acceptable for human consumption. It was observed that washing of vegetables in running water for 10 minutes could detach some of the metacercariae. The intermediate hosts (*Lymnaea* spp. snails) were exposed to a range of NaOCl concentrations, which resulted in an increase cercariae mortality and caused damage to specific parts of the snail's foot. The duration and concentration of the exposure also had an impact on the outcome. It was found that citric acid at the concentration of (10 mg/L), acetic acid (120 ml/L,) and KMn04 **(**24 mg/L) detached all metacercariae after 10 minutes of exposure**.** They were recommended as vegetable leaves as they were not softened and remained fresh.

1. INTRODUCTION

Fascioliasis is a prominent illness that affects both humans and animals and results in both serious health issues and significant economic losses (Alajmi, 2019). It is caused by *Fasciola hepatica* and *Fasciola gigantica*. The distribution of two species can overlap clinically in many parts of Africa and Asia (Degheidy and Al-Malki, 2012; Ashrafi *et al*, 2014; Phalee *et al*, 2015).

Fasciola has a complicated lifecycle with intermediate hosts like snails and final hosts like humans. *Lymnaea trancatula* and *Lymnaea natalensis* are the common intermediate snails for *F. hepatica* and *F. gigantica*, respectively. It is frequently present with animals grazing in wet and swampy areas (Javid et al., 2011).

The distribution of infection follows the spreading of the intermediate snails that is essential for maintaining *Fasciola* in an endemic area. The snail infection is significant because it increases *Fasciola* production by releasing a lot of cercariae from a single miracidium. The free swimming cercariae disperse a few meters from their snails usually by following water currents. They encyst into metacercariae that hang onto aquatic plants or stay in the water (Rondelaud et al., 2020).

Adult *Fasciola* flukes can be found in the biliary tree of final hosts like sheep, cattle, and human beings. the parasite eggs go with the bile to the duodenum and is left in the stool. After 2-3 weeks, the miracidium that has cilia is formed inside the eggs under ideal temperature and humidity conditions. Each egg releases a fully-fledged miracidium, which then swims around in search of *Lymnaea* snails. The miracidium loses its cilia after entering the snail's body, forms a sporocyst, and then generates a redia. The final larval stage is produced by the redia, which also creates the cercariae, that has a long tail for swimming. Within 4-7 weeks of infection, the fully grown cercariae leave the snail. They freely move through the water, settling on diverse objects for anywhere between a few minutes and two hours, typically leaves of aquatic plants above or below the surface. Each cercaria then loses its tail and encysts to form a metacercaria, which infects the final victims practically instantly. Following the consumption of infectious metacercaria, humans and other final hosts contract the disease (Mas-Coma et al., 2014).

Mature metacercariae have a diameter of 0.215–0.256 mm, are white when laid and are virtually instantly infectious to the ultimate host. Because they contain quinine, the cyst gradually turns yellow after a day or two and darkens as they harden. Metacercaria can live for a long time in the

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environment and passively infect animals and humans that eat contaminated plants (Hussein et al., 2010).

Fascioliasis has serious impacts on livestock, as it may cause decrease in growth rate, lower meat, and milk production. It may also lead to other complications such as anemia, decreased fertility, abortion in late stages of pregnancy, or even mortality. It was estimated that fascioliasis can cost the US economy about $3 billion annually (Jaja et al, 2017).

Beef liver as human food is a good source of protein and provides many vitamins as B12, vitamin A, riboflavin, and copper. Previous study was performed in Abu Simbel abattoir, Aswan Governorate, Egypt for a period of one year from 22 December 2018 to 21 December 2019 in which 81452 male cattle were slaughtered and inspected for fascioliasis. Total number of condemned livers due to *Fasciola* infection was 6516/81452 (7.99%) with total economic losses was 152718$ (Rassol et al., 2020).

Destroying the intermediate host during the fluke's life cycle is one strategy to reduce the prevalence of fascioliasis; nevertheless, artificial molluscicides used to limit snail numbers may disrupt the ecological system and cause a return of mollusc populations (Wang et al., 2006).

The most practical source of active oxygen is sodium hypochlorite (NaOCl), which is electrochemically produced from aqueous sodium chloride solutions. It is non-toxic, may be used safely in drinking water, is quickly excreted, and can easily pass-through cell membranes due to its small size and low molecular mass (Khan et al., 2008). Solutions of NaOCl, is a well-known and potent disinfectant, work effectively to reduce the ability a range of microorganisms such as viruses, bacteria, and fungi to spread infection (Peeters et al., 2008).

Numerous chemical agents, including citric acid and acetic acid have been recommended for the prevention and control of fascioliasis along with potassium permanganate and the effectiveness of these agents depends on the concentration of the agent and the duration of exposure. When utilizing these chemicals, the parasites may be separated from the vegetables but are not always destroyed, and in general, the parasites appear to be highly resistant (FAO 2020).

Acetic acid and citric acid, two types of acidic disinfectants, work by rupturing the bonds between nucleic acids and precipitating proteins. Additionally, they alter the pH of the environment making it detrimental to many microorganisms (Maris, 1995).

A simple and cheap method to control the infection is by decontamination of raw vegetables from metacercariae, which act as a prophylactic measure. Washing of raw vegetables vigorously with safe running water may decrease the infection although it does not eliminate it (WHO, 1999). Chemical disinfectants can be used to decontaminate the surface of raw vegetables, followed by washing in running water. The disinfectants employed must be safe, do not affect color or flavor of vegetables and effective in complete elimination of infection (Gilbert, 1970).

Potassium permanganate possesses oxidizing properties, which in turn confer disinfecting and deodorizing properties (Reynolds, 1993). It dissociates in water to release the permanganate ion (MnO_4^-) and manganese dioxide (MnO_2) along with liberation of elemental oxygen molecules. Its primary effect then, is powerfully oxidative. The permanganate ion is the toxic active agent that affects a variety of microorganisms including bacteria, fungi, viruses, and parasites. It may kill the organisms by destroying the cell wall via oxidation (Webber and Posselt, 1972).

The goal of the current study is to examine the effect of NaOCl on *Fasciola* intermediate snail host (*Lymnaea)*, as method of control *Fasciola* spp. and the effect of some acids as well as KMno4 on encysted metacercariae to find the best method(s) to clear the salads from metacercariae, while keeping the leaves as fresh as possible so that they become safe and acceptable for human consumption.

2. MATERIAL AND METHODS

2.1. Collection of snails.

Lymnaea snails (*Lymnaea natalensis* and *Lymnaea trancatula*) were collected from the canal at Al seed, Shebeen El Quenater, Egypt (Figure- 1). Snails were manually captured in a sweeping net, where they lost their grip on the plants and fell to the net's bottom. *Lymnaea* snails were present in areas with stagnant water or water that was flowing slowly in places where the livestock was present. The snails were maintained in plastic containers with water that came from the same place and transported to the Lab of Zoonoses department, faculty of veterinary medicine, Benha University at the same day. *Lymnaea* snails were fed on lettuce once or twice a week and the water in the containers was changed every 24 to 48 hours. *Lymnaea* snails were distinguish morphologically based on the color, number of whorls and shell diameter according to (Burch, 1980). They were examined for infection with *Fasciola* by shedding and crushing technique (Madsen and Monrad, 1981). Cercariae were collected and allowed to encyst on the lettuce and Arugula used as salad (Hodasi, 1972) 24 hours later, some chemicals were used to clear salads from the metacercariae.

2.2. Effect of sodium hypochlorite (NaOCl) on Lymnaea spp. snail (Taha et al., 2014).

NaOCl solution 5.25 %, a commercial bleaching solution Clorox®, was employed. The test solutions were made from a stock solution of 100 part per million (ppm) that was diluted to 10, 20, 30, 40, 50, 60, 70 and 80 parts per million for snails with contact periods of 15, 30, 60, 90 and 120 minutes. The snails were then watched after 1, 2, 3, and 4 days until death occurred. Ten snails were incubated at each concentration. If a snail did not move or react to any disturbance, it was regarded as dead. The control group contained ten snails were incubated in de-chlorinated water (distilled water). The dead snail was crushed to see cercaria, redia, sporocyst and miracidium.

2.3. Effect of citric, acetic acid, potassium permanganate and running water on metacercariae on raw vegetables: (El-Sayad et al., 1997 and EL- Zawawy et al., 2003).

Different concentrations of citric, acetic acid, and potassium permanganate were prepared in beakers of one-liter capacity and left at laboratory temperature (22-26°C).

a-Citric acid crystals ($C_6H_8O_7$), (El-Gomhoria Co., Cairo, Egypt).

The following concentrations were prepared:

2 mg/L, 4 mg/L, 6 mg/L, 8 mg/L, 10mg/L. Calculated the amount of lemon juice that equal to each concentration of citric acid according to (Kristina et al., 2008).

b-Acetic acid (Vinegar 5% $C_2H_4O_2$), (commercial vinegar solution available in the market).

The following concentrations were prepared:

40, 60, 80, 100 and 120 ml/L.

c-Potassium permanganate (KMnO4 1/8000), (El-Nasr Pharmaceutical Chemical CO.).
The following concentrations were prepared:
4mg/L, 8mg/L, 12mg/L, 16mg/L, 20 mg/L and 24 mg/L.
The plants (lettuce and Arugula) upon which metacercariae were formed after 24 hours contact with snails. These plants were dipped in each concentration and left for 5 or 10 minutes. After exposure, the leaves were washed and any metacercariae remaining adherent were counted and tested for viability.

d- Effect of the running water on metacercariae.
The metacercariae which were allowed to encyst on the various plants were counted. The plants were washed by running water for a period of 5 or 10 minutes. The remaining metacercariae were then counted and the proportion of detached metacercariae were calculated.

Testing for viability of metacercariae.
The metacercariae which collected after washing were tested for viability. They were stained with natural red and examined by the light microscope. If they were alive, the typical formation of excretory granules became visible and slight active movement could be observed within cysts. This movement was activated by placing the slides on a hot stage at 38°C. Dead cysts appeared as diffuse dark masses without any typical structure (Wikerhouser, 1960).

3. RESULTS

3.1. Effect of different concentrations of sodium hypochlorite on viability of Lymnaea spp. snail.
Our result in (Table 1) revealed that 10 ppm (part per million) of NaOCL has lethal effect after 3 days of incubation and it reached to 9/10. None of the treated snails were lived after 4 days; also, the cercariae inside them were dead. Concentrations of 20, 30, 40 and 50 ppm produced fatal effects after 30 minutes of incubation ranging from 3/10 to 7/10. None of the treated snails were survived after 3 days at concentrations of 40 and 50 ppm, and after 4 days at concentrations of 20 and 30 ppm the cercariae inside them were also dead. The dead snails showed severe damage on the foot surface that could be seen with the naked eye. Concentrations of 60 and 70 ppm had lethal effect 8/10 after 30 minutes and none of the treated snails were survived after 24hrs, however, at concentration of 80 ppm all snails were dead (10/10).

Table 1 Effect of various concentrations of sodium hypochlorite on mortality of *Lymnaea* spp. (Ten snails in each group).

Time of exposure / concentration / mortality	Group1 10ppm	Group2 20ppm	Group3 30ppm	Group4 40ppm	Group5 50ppm	Group6 60ppm	Group7 70ppm	Group8 80ppm
15 min	0	0	0	0	0	0	0	10
30 min	0	3	4	7	7	8	8	10
60 min	0	4	5	7	8	8	8	10
90 min	0	6	5	7	8	8	8	10
120 min	0	6	5	7	8	9	9	10
24hrs	0	6	7	7	9	10	10	10
3 days	9	9	9	10	10	10	10	10
4 days	10	10	10	10	10	10	10	10

Figure 1 *Lymnaea natalensis* on the left and *Lymnaea trancatula* on the right.

Figure 2 Effects of different concentrations of sodium hypochlorite on *Lymnaea* snails.

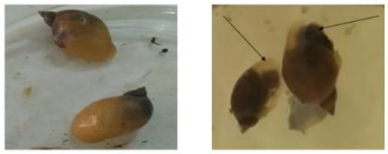

Figure 3 Arrows indicating to snails after treated by NaOCL, damages at the foot surface and death.

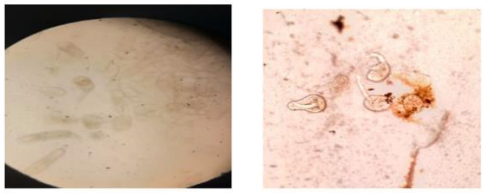

Figure 4 Cercariae after treated by NaOCL, 10X.

3.2. Effect of citric acid on detachment of metacercariae from raw vegetables.
Result in (Table 2) displayed that citric acid succeeded in detachment not killing of metacercariae when used in concentrations from 2 to 10mg/L for 5-10 minutes. All metacercariae were detached at concentration of 10mg/L for 10 minutes.

Table 2 Effect of citric acid on detachment of metacercariae from raw vegetables. (Twenty metacercariae in each concentration).

Conc. of citric acid (mg/L)	Mean proportion % of detached Metacercariae		Viability of cyst (Wikerhouser, 1960)
	5 minutes	10 minutes	The undetached and detached cyst take light pink color by natural red, without broken cyst wall, has internal structure and has motility
2mg= 42microlitter lemon juice	4.66	8.66	
4mg= 83microlitter lemon juice	9	12.66	
6mg= 125microlitter lemon juice	15.66	16.66	
8mg= 166.7microlitter lemon juice	17.66	19	
10mg=208microlitter lemon juice	19.33	20	

3.3. Effect of acetic acid on detachment of metacercariae from raw vegetables.

Commercial vinegar (acetic acid) was effective on detachment of *Fasciola* metacercariae when used in concentrations 40 to 120ml/L for 5-10 minutes. all metacercariae were detached at concentration 120ml/L for 5 and 10 minutes and complete clearance of the vegetables was obtained. However, acetic acid not effected on viability of metacercariae (Table 3).

Table 3 Effect of acetic acid on detachment of metacercariae from raw vegetables. (Twenty metacercariae in each concentration).

Conc. of vinegar (ml/L)	Mean proportion % of detached Metacercariae		Viability of cyst (Wikerhouser, 1960).
	5 minutes	10 minutes	The undetached and detached cyst take light pink color by natural red, without broken cyst wall, has internal structure and has motility.
40	13.33	14.66	
60	15.33	16.66	
80	17.33	18	
100	19.33	19.66	
120	20	20	

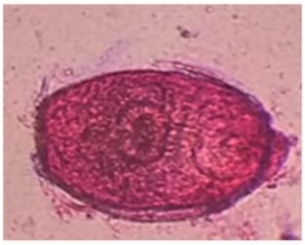

Figure 5 Live detached encysted metacercaria after treated with citric and acetic acid, 10X.

3.4. Effect of potassium permanganate on detachment of metacercariae from raw vegetables.

The present data noted that potassium permanganate has effect on detachment of *Fasciola* metacercariae in concentrations 4 to 24mg/L for 5-10 minutes. All metacercariae were detached at concentration 24mg/L for 10 minutes. However, 6.7% (2 from 30 metacercariae) of this detached metacercariae remained viable (have purple color and motility) while the remaining appeared dark masses without typical structure. At concentrations 4 to 20mg/L for 5 or 10 minutes, all the undetached and detached cyst take purple color without broken cyst wall and slight active movement (Table 4).

Table 4 Effect of potassium permanganate on detachment of metacercariae from raw vegetables. (Thirty metacercariae in each concentration).

Conc. of Pot. permanganate (mg/L)	Mean proportion % of detached Metacercariae		Viability of cyst (Wikerhouser, 1960).
	5 minutes	10 minutes	The undetached and detached cyst take purple color, without broken cyst wall, has internal structure and slight active movement.
4	9.66	15.66	
8	13.66	19.33	
12	17.66	22.66	
16	18.66	26.66	
20	22.66	28.66	
24	28.33	30	At 10 minutes, 2 from 30 (6.7%) cyst have purple color and has motility while the remaining appeared dark masses without typical structure.

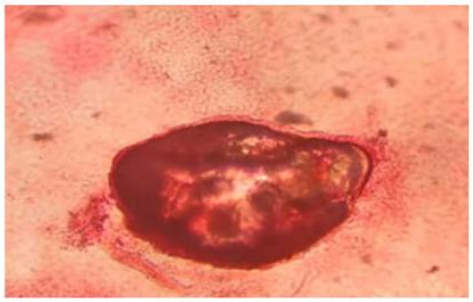

Figure 6 Dead detached encysted metacercaria after treated with KMnO4 for 10 minutes at 24mg/L that appeared dark masses without typical structure and broken cyst wall, 10X.

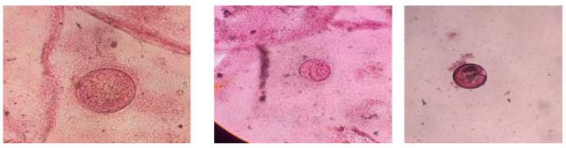

Figure 7 Live detached encysted metacercariae after treated with KMnO4 that appeared with purple color, without broken cyst wall, 10X.

3.5. Effect of running water on detachment of metacercariae from lettuce and Arugula.

Washing of raw vegetables (Lettuce and Arugula) with running water for a period 5 to 10 minutes succeed for detachment of some metacercariae but not considered a proper method. In which after 5 minutes, (1.33%) and (3%) metacercariae detached in Arugula and lettuce respectively, while 2.66% and 5.33% detached after 10 minutes (Table 5).

Table 5 Effect of running water on detachment of metacercariae from lettuce and Arugula. (Ten metacercariae used for each time of exposure).

Plants	Mean proportion % of detached Metacercariae	
	5 minutes	10 minutes
Arugula	(1+1+2=4/3) 1.33	2.66
Lettuce	(3+3+3=9/3) 3	5.33

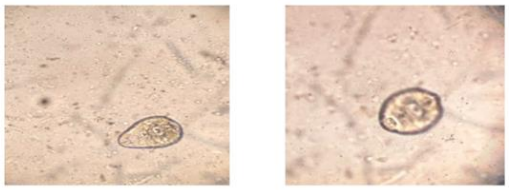

Figure 8 Detached encysted metacercariae from lettuce and Arugula after washing with running water by using light microscope, 10X.

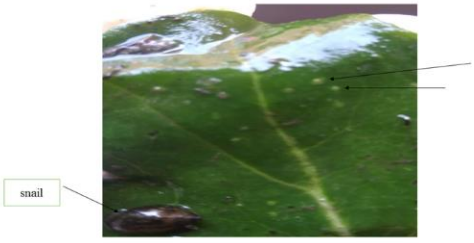

Figure 9 Arrows indicating to mature metacercariae without any treatment by chemicals that appeared white when was deposited after 1-2 days, progressively turns yellow in color due to the presence of quinine and darkens as it hardens.

4. DISCUSSION

Improper food hygiene and food habits are the two factors responsible for food-transmitted diseases. It was recommended those vegetables that are not boiled should only be eaten raw where there is no risk of contamination and then only after proper washing with running water. Controlling of Fascioliasis occurred by broke the life cycle by killing its infective stage and control its snails.

The mortality rate of *Lymnaea* snails with damage at the foot surface increases when NaOCL is used to treat *Lymnaea* snails in order to lower the risk of fascioliasis. While most of the sodium hypochlorite in water hydrolyzes into sodium hydroxide and hypochlorous acid, the remaining sodium hypochlorite entirely dissociates into the sodium cation Na+ and the hypochlorite anion CIO-. Hypochlorous acid acts as a solvent when in contact with organic tissue, creating chlorine that reacts with the amino group of proteins to create chloramines, which disrupt cellular metabolism. Furthermore, sodium hypochlorite reduces the surface tension of the residual solution by acting as an organic and fat solvent that breaks down fatty acids into fatty acid salts (soap) and glycerol (alcohol), as well as other fatty acids. Additionally, sodium hypochlorite reduces pH by neutralizing amino acids, resulting in the formation of water and salt and the outflow of hydroxyl ions (Yohana and Mashauri 2008).

The present data showed concentration of 10 ppm (part per million) of NaOCL has a lethal effect after 3 days of incubation and it reached to 9/10. None of the treated snails were lived after 4 days, the cercariae inside them were also dead. Concentrations of 20, 30, 40 and 50 ppm had lethal effect after 30 minutes of incubation ranging from 3/10 to 7/10. None of the treated snails were survived after 3 days at concentrations of 40 and 50 ppm and after 4 days in concentrations of 20 and 30 ppm also the cercariae inside them were dead. The dead snails showed severe damage on the foot surface that could be seen with the naked eye, this results similarly agreed with (Taha et al., 2014) that found concentrations of 20, 30, 40 and 50 ppm had lethal effects after 30 minutes of incubation from 3% to 6%. After 4 days, none of the treated snails were survived, also said by scanning electron microscope *Lymnaea* snails exhibit partial to extensive damage of the tegument, exfoliation, and disruption of the ciliary arrangement on the foot surface after exposure to different concentrations of NaOCl. In our study, concentrations of 60 and 70 ppm had lethal effect 8/10 after 30 minutes and none of the treated snails were survived after 24hrs, however, at concentration of 80 ppm all snails were dead (10/10).

Concerning the effect of running water on detachment of encysted metacercariae from lettuce and Arugula. Our result revealed that after 5 minutes, 1.33% and 3% metacercariae detached in Arugula and lettuce respectively, while 2.66% and 5.33% detached after 10 minutes. This indicates that washing of raw vegetables with running water for a period 5 to 10 minutes succeed for detachment of some metacercariae but not considered a proper method. These results in accordance with those obtained by (El-Sayed et al., 1997) who said washing of raw vegetables by running water for 10 minutes detached only 50% from the metacercariae.

Regarding the effect of citric acid on detachment of *Fasciola* metacercariae used in concentrations ranging from 2 to 10mg/L for 5-10 minutes. All metacercariae were detached at concentration 10mg/L for 10 minutes. The undetached and detached cyst take light pink color when stained by natural red, without broken cyst wall and all have motility indicate to the viability of metacercariae after treated with citric acid. Therefore, citric acid succeeded in detachment but not killing of metacercariae. This result run parallel to those attained by (El-Sayed et al., 1997) but our result disagreed with (Ali et al., 2008) who said that the citric acid at one to 10% for 15 or 30 minutes did not affect the *Fasciola*-encysted metacercariae, which easily infected the rabbits as indicated by recovery of eggs in stool, and flukes in the liver that caused marked pathological changes. Therefore, citric acid not effected on viability or infectivity of encysted metacercariae.

With respect to the effect of commercial vinegar (acetic acid) on detachment of *Fasciola* metacercariae, used in concentrations 40 to 120ml/L. All metacercariae were detached at concentration 120ml/L for 5 and 10 minutes and complete clearance of the vegetables obtained. However, acetic acid not effected on viability of metacercariae. These finding similar to those attained by (El-Sayed et al., 1997), however, (Ali et al., 2008) found that the acetic acid (5 to 10%) effect on the encysted metacercariae infectivity when immersed for 15 or 30 minutes.

The effect of potassium permanganate on detachment of *Fasciola* metacercariae, used in concentrations ranging from 4 to 24mg/L for 5-10 minutes. All metacercariae were detached at concentration 24mg/L for 10 minutes. However, (2 from 30 metacercariae) 6.7% of this detached metacercariae remained viable (have purple color and motility) while the remaining appeared as dark masses without typical structure. At concentrations 4 to 20mg/L for 5 or 10 minutes, all the undetached and detached cyst take purple color without broken cyst wall and slight active movement. The result in our study slightly like those obtained by (EL- Zawawy et al., 2003) who said that all metacercariae exposed to 10mg/L KMnO4 for 10 minutes were detached. However, (2 from 50) 4% of this detached metacercariae remained viable. However, (Ali et al., 2008) found that potassium permanganate at concentrations 10 to 40% for 15- or 30-minutes effect on the infectivity of *Fasciola* encysted metacercariae. Whereas the study carried out by (El-Sayed et al., 1997), all metacercariae exposed to KMnO4 at the same concentrations and times of exposure as used in the present study were dead, this might be referred to the difference in the strain of *Fasciola* used or development of resistance to potassium permanganate.

Unfortunately, the results obtained in this study disagreed with those obtained by (Keyhan et al., 2006) who found that potassium permanganate was not affected on the viability of *Fasciola* metacercariae even at the very high doses of 300, 600 and 1200mg/L for 5 minutes.

The preceding results, potassium permanganate at 24mg/L considered the most useful tool to detach and kill metacercariae attached to the vegetables used for salads, in short time of 5 to 10 minutes, without softening the leaves or changing the color. However, more washing times were required to remove the purple color from the vegetables exposed to KMnO4 these agreed with (EL- Zawawy et al., 2003 and El- Sayed et al., 1997).

5. CONCLUSION

The Zoonotic fascioliasis is a risky parasitosis of worldwide distribution. To prevent human infection with

Fasciola spp. raw vegetables that are eaten or used in salad should be treated with potassium permanganate at 24mg/L for 10 minutes or citric acid at 10 mg/L for 10 minutes or acetic acid at 120ml/L for 5 or 10 minutes, which interfere with the proteins of the cyst wall causing the bond formed between the vegetables surface and the cyst wall during encystment to break down, thus allowing the metacercariae to separate from the vegetables. Moreover, it was noticed that using of NaOCL at concentration of 80 ppm after 15 minutes is effective for controlling of *Lymnaea* snail.

6. REFERENCES

1. Alajmi, R. A., 2019. Molecular characterization of *Fasciola* flukes using mitochondrial28S rRNA gene in Naimi Saudi sheep. Saudi J. Biol. Sci. 26, 1:112-117.
2. Ali, A. A. H., Nahla, M. S., El-Motayam, M. and Ayman, T. A. M., 2008. Efficacy of five chemicals on *Fasciola gigantica* encysted metacercariae infectivity. J. Egypt. Soc. Parasitol, 38(3): 919-928.
3. Ashrafi, K., Bargues, M. D., O'Neill, S and Mas-Coma, S., 2014. Fascioliasis: a worldwide parasitic disease of importance in travel medicine. Travel Med. Infect. Dis, 12, 6:636-49.
4. Burch, J. B., 1980. A guide to the freshwater snails of the Philippines. Malacol Rev. 13: 121-143.
5. Degheidy, N. S. and Al-Malki, J. S., 2012. Epidemiological studies of fascioliasis in human and animals at Taif, Saudi Arabia. World Appl. Sci. J, 19:1099-104.
6. El-Sayad, M. H., Allam, A. F. and Ossman, M. M., 1997. Prevention of human Fascioliasis: A study on the role of acids detergents and potassium permenganate in clearing salads from metacercariae. J. Egypt. Soc. Parasitol., 27(1): 163-169.
7. El- Zawawy, A. L., Ali, S. M. and Allam, S. R., 2003. The effect of potassium permanganate and sodium dichloroisocyanurate on metacercariae of Fasciola gigantica. J. Egypt. Soc. Parasitol; 33:305-314.
8. FAO (Food and Agriculture Organization of the United Nations), 2020. Parasites in foods: An invisible threat. Food safety technical toolkit for Asia and the Pacific, 7. Bangkok. 17-20.
9. Gilbert, R. J., 1970. Comparison of materials used for cleaning equipment in retail food premises and of two methods for the enumeration of bacteria on cleaned equipment and work surfaces. J Hyg., 68: 221-232.
10. Hodasi, J. K., 1972. The output of cercariae of *Fasciola hepatica* by Lymnaea truncatula and the distribution of metacercariae on grass. Parasitology 64, 53-60.
11. Hussein, A. N., Hassan, I. M. and Khalifa, R. M., 2010. Development and hatching mechanism of *Fasciola* eggs, light and scanning electron microscopic studies. *Saudi J. Biol. Sci.*, 17, 247–251.
12. Jaja, I. F., Mushonga, B., Green, E. and Muchenje, V., 2017. Financial loss estimation of bovine fascioliasis in slaughtered cattle in South Africa. Parasit. Epidemiol. Control 2, 4:27-34.
13. Javid, A., Kuchai, J. A., Chishti, M. Z., Manal, M. and Zaki, S. A., 2011. Some Epidemiological Aspects of fascioliasis among Cattle of Ladakh. Global Veterinaria 7(4): 342-346.
14. Keyhan, A., Maria, A.V., Jafar, M., Abdolrasoul, S., Shahram, S. M., Patrica, C., Messaud, K., Maria, D. B. and Mas-Coma, S., 2006. Plant- borne human contamination by fascioliasis. Am. J. Trop. Med. Hyg., 75(2): 295-302.
15. Khan, A., Ullah, M. and Khan, M., 2008. Pathological effects of sodium hypochlorite administration through drinking water in male Japanese quails (*Coturnix japonica*), Human Exp. Toxicol., 27: 773–780.
16. Kristina, L., Penniston, M. D., Stephen, Y., Nakada, M. D., Ross, P. Holmes, P. h. D., Dean, G. and Assimos, M. D., 2008. Quantitative Assessment of Citric Acid in Lemon Juice, Lime Juice, and Commercially-Available Fruit Juice Products. J Endourol., 22(3): 567–570.
17. Madsen, H. and Monrad, J., 1981. A Method for laboratory maintenance of *Lymnaea* natalensis and for mass production of *Fasciola gigantica* metacercariae. The Journal of Parasitology., 67 (5): 735-737.
18. Maris, P., 1995. Modes of action of disinfectants. Rev. Sci. tech. Off. Int. Epiz, 14(1): 47-55.
19. Mas-Coma, S., Agramunt, V. H. and Valero, M. A., 2014. Neurological and ocular fascioliasis in humans. Adv. Parasitol. 84, 27-149.
20. Peeters, E., Nelis, H. J. and Coenve, T., 2008. Evaluation of the efficacy of dis-infection procedures against *Burkholderia cenocepacia* biofilms,J. Hosp. Infect.70 361–368.
21. Phalee, A., Wongsawad, C., Rojanapaibul, A. and Chai, J. Y., 2015. Experimental life history and biological characteristics of *Fasciola gigantica* (Digenea: Fasciolidae). Korean J. Parasitol., 53, 1:59-64.
22. Rassol, A. M. A., Ahmed, A. M., Sobhy, H. M., Abdelgayed, S. S. and Hekal, S. H. A., 2020. Effects of fascioliasis on the economic losses of beef liver at Abu Simbel Abattoir, Aswan Governorate, Egypt. Adv. Anim. Vet. Sci. 8(11): 1175-1179.
23. Reynolds, J. E. F., 1993. Disinfectants. Martindal, The Extropharmacopocia. Thirtieth ed. London: The Pharmacutical Press .,781-805.
24. Rondelaud, D., Vignoles, P. and Dreyfuss, G., 2020. *Fasciola hepatica*: the dispersal of cercariae shed by the snail *Galba truncatula*. *Parasite*. 27(17): 1-6.
25. Taha, A. H., El-Shaikh, K.A. and Al-Sadi, M. M., 2014. Effect of sodium hypochlorite on Fasciola gigantica eggs and the intermediate host, Lymnaea natalensis: A scanning electron microscopy study. Journal of Taibah University for Science, 8: 75–83.
26. Wang, H., Cai, W. M., Wang, W. X. and Yang, J. M., 2006. Molluscicidal activity of *Nerium indicum* Mill, *Pterocarya stenoptera* DC, and *Rumex japonicum* Houtt on *Oncomelania hupensis*, Biomed.Environ. Sci., 19: 245–248.
27. Webber, W. J.and Posselt, H. S., 1972. Disinfection. Physicochemical processes in water quality control. NewYork: John Wiley & Sons. 639.
28. Wikerhauser, T., 1960. A rapid method for determining the viability of *Fasciola hepatica* metacercariae. *Am. J. Vet. Res.*, (21): 895- 897.
29. WHO, 1999. Surface decontamination of fruits and vegetables eaten raw: a review. Geneva, WHO (WHO food safety programme document). 4-30.
30. Yohana, L. and Mashauri, D. A., 2008. Potential use of water guard as a disinfectant for domestic water use. Tanzania J. Eng. Tech., 2: 61-69.