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Inactivation of *Toxocara vitulorum* eggs by Ammonia in combination with solar energy

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ABSTRACT

Eggs of intestinal parasitic helminthes *Toxocara* spp. are one of the most resistant pathogens to all ways of water disinfection at waste water treatment plants. In this study inactivation of (*Toxocara vitulorum*) eggs by ammonia and solar energy in laboratory conditions has been indicated. *Toxocara vitulorum* eggs were used as an alternative model for *Ascaris lumbricoides*. Eggs in samples with ammonia different concentrations (0%, 1%, and 2%) and pH values were duplicated and the results suggested that the presence of ammonia at (2%) concentration allowed 70% inactivation of eggs. The applicability of solar energy for disinfection of eggs using one-side blackened PET bottles were continuously exposed to direct sun light for 7 hours with water temperature in the range of 50 – 65.5 °C that was enough for destroying 95 % of eggs. Complete destruction of the eggs was achieved at 65.5°C.The influence of solar energy against the viability of eggs in water using Diamidino-2-Phenylindole (DAPI) and Propidium Iodide (PI) vital stains was found to be highly significant ($P < 0.001$). The combined effect of ammonia and solar energy on samples containing eggs with temperature 55°C within 21 days of storage within experiment operated in lab . The effect of ammonia and solar energy on viability of eggs using scanning electron microscope , (DAPI); and Propidium Iodide (PI) vital stain were evaluated. The results showed that 100 % inactivation of eggs could be possible in samples, supporting results have an important implication for ecological sanitation and sustainable development technologies in water and waste water treatment plants. The low cost and operational simplicity of this study make it affordable and usable especially in rural areas where other sources of energy like electricity and gas are not easily available.

INTRODUCTION

Toxocara sp. is one of the most important soil-borne helminthes. It causes Toxocariasis , the most common parasite disease that infects almost 1.4 billion people worldwide (CDC, 2018). Ascariasis and Toxocariasis is a disease causing physical and mental impairment, including cognitive and social impairment, increased susceptibility to infection, reduced responsiveness to vaccination, malnutrition (Crompton *et al*., 2001) , and It is further considered to be associated with blindness and asthma (PAHO, 2018). *Toxocara* eggs can survive for several months or even

years in soil and water and remain viable. The eggs that are highly resistant to desiccation, chemicals such as strong acids, strong bases and chlorine with aerobic and anaerobic environmental resistance, and survive in soil for up to seven years (Johnson *et al*., 2010).

High temperature and pH changes are common methods used to inactivate *Toxocara* eggs, although the temperature affects eggs only when they are higher than 40 ° C at long exposure (Jamil *et al*., 2009). Also alkaline stabilization medium used for inactivating *Toxocara* eggs with time consuming to achieve a high level of inactivation (> 95 percent) ranging from two hours to 180 days (Brewste *et al*., 2003). So that the need for an alternative technology to inactivate those eggs is urgent issue (Rijsberman and Zwane, 2012).

The viability assessment of eggs depends on determining the accuracy of their morphological structure during microscope examination or observing the develop-pment of embryos during incubation in their eggs. However, due to both time consuming and expensive cost, these conventional methods are insufficient (Acar *et al*., 2016). The most important element in the viability assessment test is the subjectivity of the new detection and evaluation methods of analysis (Bowman *et al*., 2003). Therefore, an effective alternative way of assessing viability is needed, which will be more efficient, simple, fast and useful for all the studied samples.

Using Propidium iodide and DAPI staining process to detect viability and vitalit y of *Toxocara* eggs is very appropriate and useful for this study (Gaspard *et al*., 1995). Disinfection using different concentrations of ammonia is one of the choices for an eco-friendly agent to inactivate *Toxocara* eggs. Ammonia is naturally present in wastewater resulting from urea hydrolysis and degeneration of protein and nitrogen compounds (Pecson and Nelson, 2005). Alkaline therapy increases sludge pH and present as ammonification. The concentrations of NH3, which dominate at high pH (pKa) 9.3 at 25°C, are more lipophilic than NH4+ and can be transported and diffused more easily through biological membranes. Ammonia has been reported to cause pH changes in a variety of organisms, including pathogenic wastewater (Jimenez, 2007).

Ammonia sanitization is a simple method of technology that requires only a sou rce of ammonia and an airtight storage system, so that the unloaded ammonia does not pass to the atmosphere. Because the human *Ascaris lumbricoides* has difficultygetting egg, so that Ghiglietti *et al*., (1995) was indicated that, the effect of ammonia inactivation on *Ascaris* eggs was studied using *Ascaris suum* eggs as a model organism, as it was shown to be similarly inactivated to *Ascaris lumbricoides* eggs.

Results from an earlier study (Mohamed *et al*., 2014) have also showed that the influence and potential of using solar energy to disinfect, naturally, polluted raw water has been used to destroy encyclical protozoa (*Giardia and Entamoeba*) thro ugh its thermal and UV effect, thereby improving drinking water quality in Egypt.

The first objective of this research was therefore to determine the conditions under which ammonia and solar energy inactivate *Toxocara vitilorum* eggs, which is often used as a model for the *Ascaris lumbricoides* human species. The second goal of the current research was to assess the effectiveness of solar energy and ammonia combination together on *Toxocara vitilorum* viability and vitality of eggs.

MATERIALS AND METHODS

Eggs collection:

T. vitulorum eggs were collected from female nematode taken from the intestines of slaughtered calves. *T. vitulorum* eggs were extracted , washed, and

counted, by the procedure that was described by (Bowman *et al*., 2003) using a solution containing 250,000 eggs/ml, as determined by microscopic examination of aliquot samples. Eggs samples was then diluted using PBS buffer or by using deionized sterilized distilled water and stored in membrane filter nylon bags for experimental use. *T. vitulorum* eggs have been used to be demonstrated to behave similarly to *Ascaris lumbricoides* eggs in inactivation studies and are commonly used as a surrogate, So that it is used as an alternative model (Ghiglietti *et al*.,1995). The experimental eggs sampling steps were started after permission from the Institutional Animal Ethical Committee, Menoufia University, Egypt (approval ID: MUFS/S/PA/1/2015).

Experiment set-up :

This study was conducted in the laboratories of the Department of Zoology, Faculty of science , Menoufia university , and in the central laboratory of the Holding Company of Water and Wastewater , Egypt during July and August 2018.

Samples with different ammonia concentrations :

At this study pH was adjusted using analytical-grade calcium hydroxide, and ammonia and urea supplementation different concentration was achieved using granular NH4Cl 1% (wt/wt) urea amendment (≈2,300 mg/liter) 2% urea amendment (≈4,600 mg/liter).

In this study, urea was degraded and converted to ammonia using the enzyme urease prior to experimental use. Inactivation research that used laboratory chemicals, enzymes, moisture, and temperature manipulation to achieve completely inactivation of eggs . This study was designed with a longer period (4 weeks) in order to observe the inactivation that can be achieved in a time frame due to the change in pH values. Ammonia was shown to be toxic to pathogens as NH_3 gas, which is the dominant nitrogen species at pH values above the NH_4^+/NH_3 pK_a of 9.30.

Three groups of samples containing *T. vitulorum* eggs mixed with different concentration of ammonia (Zero % , 1 % and 2 %) were placed in duplicates at four different temperatures (25°C, 35°C, 45°C, and 55°C) in the dark for a period of 4 weeks. During these experiment , samples were detected in duplicates at times: 1, 3, 7, 14, and 21 days.

After incubation each sample was left to be adjusted with the room temperature before opening. Ammonia and pH were measured using an ammonia electrode (Ion Selective, Ammonia Gas-Sensing Combination Electrode, Fisher Scientific), a pH electrode (Accu Cap pH Combination Electrode, Fisher Scientific), and a meter (Accumet Excel Dual Channel pH/Ion/Conductivity/DO Meter XL 60, Fisher Scientific). Moisture was detected using the oven drying method , and temperature was monitored daily during the treatment and incubation period.

T. vitulorum eggs removed from the membrane filter nylon bags containers and were washed twice in distilled water and placed for incubation in a tube with 25 ml of sulfuric acid (0.1 N) for 21 days. After incubation, the membrane filter nylon bags containing eggs were Re-opened directly into a Petri dish with 2 ml of 0.1N sulfuric acid. A sample (50 µl) was taken to examine viability of eggs that was observed microscopically . A minimum of 100 eggs were observed from each sample, and larval stages were used to calculate final viability.

Influence of solar energy:

The method of treatment for solar energy disinfection based on the synergetic effect of both temperature and UV-A radiation (wavelength: 320-400 nm). Longer wavelength radiation (> 400 nm) does not efficiently eliminate eggs and UV-

B (280-320 nm) is transmitted only through special PET plates and only reaches ground level at low intensity.

PET (Polyethyleneterephtalate) has a good transmittance of UV-A and is therefore ready for use. This study uses PET plates for the treatment of solar disinfection. Applying black surface-based PET plates in the bottom will raise the water temperature to 60 - 70 ° C and may help the *T. vitulorum* efficiency removal of eggs by increasing water temperature and transmission of UV. So that *T. vitulorum* is completely damaged by the germicidal effect of both temperature and UV radiation from solar energy.

To study the effect of solar energy on *T. vitulorum* eggs that used as an alternative model of *Ascaris* eggs , three sets were used under direct sunlight. Polyethylene terephthalate (PET) containers were used in this study, one was painted with black color on one side, the second was non painted and the third one had an open at the top One-side blacked two-liter volume bottles were continually exposed to direct sunlight for about seven hours, maintaining water temperature in the range of 50-65.5 ° C. Another set of bottles with an ambient temperature of 40 ° C were painted on one side black facing south next to those placed vertically in positions at 65.5 ° C.

The initial sample temperature was measured, then temperatures were recorded hourly for seven hours, one PET container painted black on one side was taken hourly for viability testing, and the remaining containers were analyzed after seven hours. One of the PET containers was used as a control (without being exposed to the sun), and kept in no direct sunlight for seven hours.

Eggs viability detection :

Samples were prepared for staining methods using Propidium iodide and DAPI staining process to detect viability of eggs after the treatment process with ammonia different concentration and solar energy

Morphological observation :

T. vitulorum eggs morphology were examined first with a light microscope, second with a fluorescent microscope and finally with scanning electron microscope SEM at different stages of control and treatment groups . Measuring of eggs viability and eggs vitality was carried out.

Staining methods observation :

According to Boulos *et al*. (1999), samples with about 25000 eggs/1 ml of each sample of different treated groups containing live and dead eggs *T. Vitulorum* were prepared and ready to be stained with DAPI staining kits : die A and B from the standard ready to use kits od DAPI stain were mixed well and added to distilled water to make stock solution of the stain (4 ml of stock mixture of dyes / added to 1 ml of eggs samples suspended in distilled water), Then eggs were isolated by filtration system and its color after staining process was evaluated using a fluorescence microscope. , and color intensity of dead and live *T. Vitulorum* eggs were visually assessed , then all detected eggs were counted.

The viability of eggs isolated from adult female nematode *T. vitulorum*, and the effectiveness of inactivation were assessed during the incubation at 28.5 °C. After 3 weeks of incubation, embryonic development in most of non-inactivated eggs of nematodes was observed.

Scanning Electron Microscope observation :

Prior to SEM , preserved egg samples in glutraldehyde were placed on the aluminum stub using a double sticky carbon tape , samples were dehydrated in ethanol series (50% , 70% , 80% , 90% , 95% and finally washed in 100% absolute

ethanol) . Dehydrated eggs samples were mounted on SEM with double adhesive tape, then completely dry the sample in air drying oven at 65°C to remove ethanol and coated with gold in an ion coater for 21 minutes. The golden coated stubs were observed using a SEM (Joel , JSM , ISI DS- 5800) at an accelerating voltage 15 kV.

RESULTS

The results of this investigation study recommended that inactivation of *T. vitulorum* eggs by various ammonia focuses with exceptionally low moisture substance would be conceivable in mix with solar energy that oriented vitality in the wake of including 1% ammonia and having the appropriate physical and chemical conditions that present at low temperature rate of 28°C, with an underlying initial pH estimation of 8.3, and low moisture level of 29.5% .

Ammonia-free (zero percent concentrations) samples never reached 100 percent *T. vitulorum* inactivation of eggs at 28 ° C. The inactivation of eggs in samples exposed to concentration of 2% ammonia and temperature above 28°C was faster; whereas eggs were totally inactivated after 21 days, 7 days and 24 hours of storage at 35 ° C, 45 ° C and 55 ° C, respectively (Table 1) .

All ammonia and solar exposed samples vitality accomplished 100% inactivation following 14 days at (28°C), 3 days at (35°C), and 24 hours at (55°C) of capacity storage, and these results can be clarified in (Table 2) .

Table 1: Effect of Ammonia concentrations on viability of *T. vitulorum* eggs

The concentration of ammonia	**Ammonia value**	**pH value**	**Percentage of egg viability**
Zero %	140.72 ppm	8.5	**97 %**
1 %	630.30 ppm	8.97	**55 %**
2 %	**1150.45 ppm**	**9.33**	**5 %**

Table 2 : Effect of Ammonia and Temperature on viability *of T. vitulorum* eggs

Temperature	***Viable eggs Toxocara vitulorum number***			
	Zero ammonia %	1% ammonia	2% ammonia	**PH**
	Control samples	Treated Samples	Treated Samples	**Values**
25 ° C	140	97	85	**8.3**
35 ° C	140	60	53	**8.75**
45 ° C	140	45	40	**8.97**
55 ° C	**140**	**15**	**10**	**9.33**

Table 3: Logistic Regression Analysis of *T. vitulorum* eggs inactivation by Temperature and Ammonia using Minitab 16 statistical package.

Analyzed parameters	**Parameter Estimated Values**	**Regression Ratio**	**Ammonia concentration %**	***P* – Value**
Temperature	0.6099	1.855	2.505	**0.0001**
Ammonia	3.2565	25.999	4.055	**0.0006**
Storage Time	**0.1677**	**1.189**	**1.288**	**0.0003**

A triplicated database consequences result of this investigation was made in Microsoft Excel 2010 to perform statistical analysis and imported into logistic regression analysis utilizing programmed Minitab 16. The inactivation logistic regression model created with the data mean collected in the experiment worked well within the parameters used in this study for both ammonia and solar energy disinfection (Table 3).

Results demonstrated that, data analysis have a significant difference ($p< 0.0001$) between inactivation levels in ammonia-exposed samples (1% and 2%) and ammonia-free samples as could be seen in in Figure (1). However, data showed no significant difference (p=1.000) in the inactivation of samples exposed to different concentrations of 1% ammonia and 2% ammonia (Figure 1).

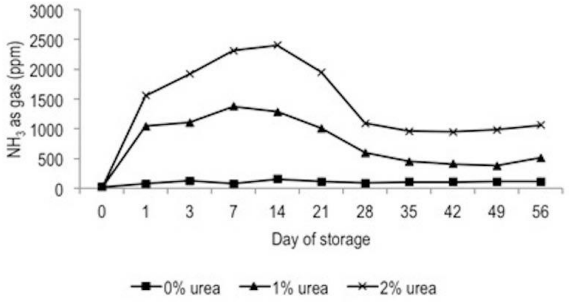


Fig. 1: Average variations in ammonia concentration in samples during storage for 56 days.

The results demonstrated that if there should arise an occurrence of temperature gatherings, a huge distinction ($p < 0.001$) in inactivation happened between control bunch tests (Zero% ammonia) and treated gathering tests (1% or 2% ammonia) was shown, at 55°C. At the most elevated temperature (55°C), the majority of the samples accomplished a 100% inactivation of eggs under 24 hours, without of ammonia expansion. It is seen that *T. vitulorum* inactivation is predominantly affected by high temperature values, warmth and impact of sunlight based vitality

***T. Vitulorum* eggs infectivity.**

Results exhibited that solar energy oriented vitality sanitization framework is a suitable procedure in waste water and water treatment innovation for use as a crisis against pathogenic *T. Vitulorum* eggs. These examinations were rehashed multiple times and the normal information were utilized for investigation as could be found in (Tables 4 and 5).

Table 4: Solar energy influence in killing *T. vitulorum* eggs During July 2018.

Time intervals	**Temperature degree °C**	**Viable numbers of *Toxocara vitulorum***	**Percentage of eggs viability**
9:00 am	26.9	140	100
9:30 am	30.3	125	95
10: 00 am	33.5	115	92
10: 30 am	37.2	100	81
11: 00 am	42.5	85	80
11: 30 am	43.5	70	75
12: 00 pm	47.5	55	70
12: 30 pm	48.5	40	60
13: 00 pm	51.5	25	48
13: 30 pm	52	17	30
14: 00 pm	55.3	13	25
14: 30 pm	61.3	7	10
15: 00 pm	65.3	Zero	Zero
15: 30 pm	65.3	Zero	Zero
16 : 00 pm	59.9	Zero	Zero
16: 30 pm	59.5	Zero	Zero
17: 00 pm	58.5	Zero	Zero

Table 5: Sunlight effect on water temperature samples containing *T. vitulorum* eggs during July 2018

Time Interval	Group (1) No direct sun light samples.	Group (2) Direct sun light exposed samples	Group (3) Direct sun light with one side black samples
	Mean values	**Mean values**	**Mean values**
10: 00 am	25.5 º C (25 – 27)	29.8 º C (28 – 3 0)	33.5 º C (30 – 35)
10: 30 am	25 º C (25 – 26)	29.5 º C (27 – 30)	37.2 º C (33 – 35)
11: 00 am	26.9 º C (26.5 – 27.5)	32.7 º C (32 – 35)	42.5 º C (42 – 44)
11: 30 am	27.3 º C (26 – 29)	33.5 (33 – 35)	43.5 º C (42 – 44)
12: 00 pm	28.4 (26.5 – 30)	38.62 º C (38 – 42)	47.5 º C (45 – 48)
12: 30 pm	30.3 º C (30 – 32)	40.5 º C (40 – 42)	48.55 º C (48 – 49)
13.00 pm	31.23 º C (31 – 32)	45 º C (45 – 45)	50.46 º C (50 – 51)
13: 30 pm	29.5 º C (30 – 31)	45 (45 – 45)	51.5 º C (50 – 53)
14: 00 pm	28.69 º C (28.5 – 29)	48.15 º C (48 – 49)	55.5 º C (55 – 56)
14 : 30 pm	28.5 º C (28 – 28.5)	48.5 º C (46 – 4 7)	61.3 º C (57 – 62)
15 : 00 pm	26.69 º C (26 – 27)	40.38 º C (40 – 41)	65.3 º C (57 – 65)
15 :30 pm	25.5 º C (26 – 26)	39.5 º C (41 – 46)	58.5 º C (56 – 58)
16 : 00 pm	25.9 º C (25 – 26.5)	38.1 º C (36 – 40)	57.3 º C (50 – 58)

The results have been demonstrated that utilizing of two liters PET containers containing tainted examples with *T. vitulorum* with no immediate daylight (33.5 °C), just 10% of *Toxocara* eggs separately were decimated and become not infective.

Then again, if there should be an occurrence of two liters PET containers, with direct daylight and no darkened side (48.5 °C) the results demonstrated that when the temperature raised to 48.5 °C, it appeared just 30% of *T. vitulorum* were pulverized and rendered non infective, while 70% of these eggs were as yet feasible.

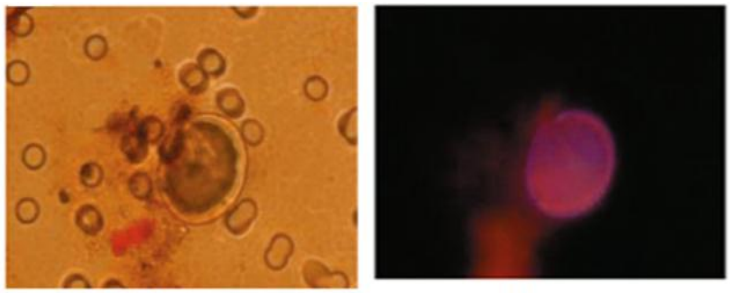
At the same time, uncovering two liters PET jugs with direct daylight and one side dark (65.5 °C) in tests debased with eggs of *T. vitulorum* showed 98% of eggs were devastated and become non infective (Tables 4 and 5).

These results were affirmed by utilizing DAPI fluorescent as an imperative stain that separates among reasonable and crushed dead eggs.

Staining method to assets viability of *T. vitulorum* eggs :

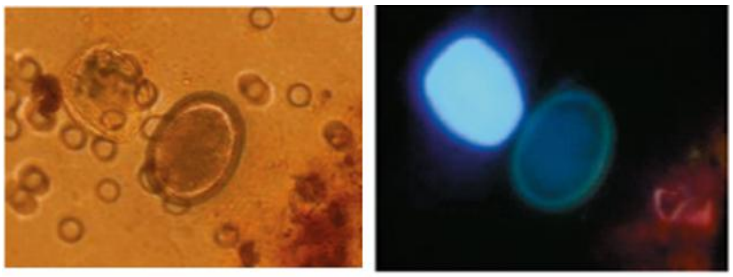
In the wake of recoloring by DAPI pack of live and dead *Toxocara vitulorum* eggs suspended in refined water, the distinctions in the capacity to retain the fluorescent colors by eggs were watched. After the recoloring of live eggs, 93% of *Toxocara* eggs were splendid green-hued(Figs. 2 -10).

Subsequent to recoloring of inactivated eggs, 95% of *Toxocara* eggs were red-hued. The rest of the eggs were not recolored and there was no advancement of the developing life in any of the inactivated eggs. Staining of live and dead eggs suspended in water (Figs. 2 -10).



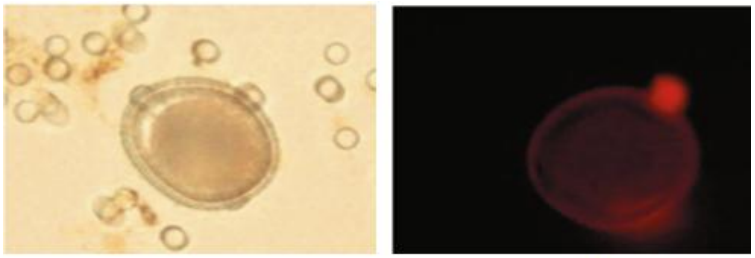
A B

Fig. 2: (A) photomicrograph of live egg of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains(100 X).
(B): Photomicrograph of dead egg of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains (100 X).



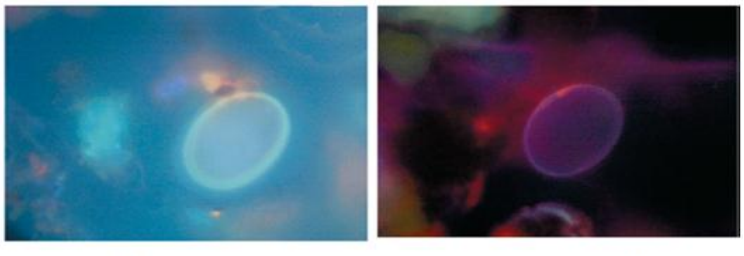
A B

Fig. 3: (A) Photomicrograph of live egg of *Toxocara vitulorum* in distilled water, Stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains, (100 X).
(B) Photomicrograph of live egg of *Toxocara vitulorum* in distilled water, Stained b 4, 6 Diamidino-2-Phenylindole (DAPI) Flouregenic vital stains (100 X).



A B

Fig. 4: (A) Photomicrograph of live egg of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains (100 X).
(B) Photomicrograph of dead egg of *Toxocara vitulorum* In distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stain (100 X).



A B

Fig. 5: (A) Photomicrograph of live egg of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains after exposure of ammonia (1%) concentrations. (100 X).
(B) Photomicrograph of dead egg of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2-Phenylindole (DAPI) Flourogenic vital stains after exposure of ammonia (2%) concentrations. (100 X).

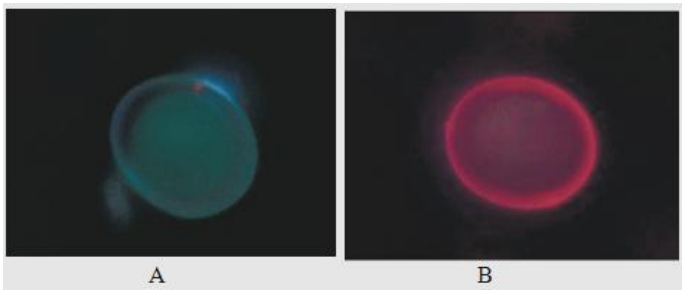
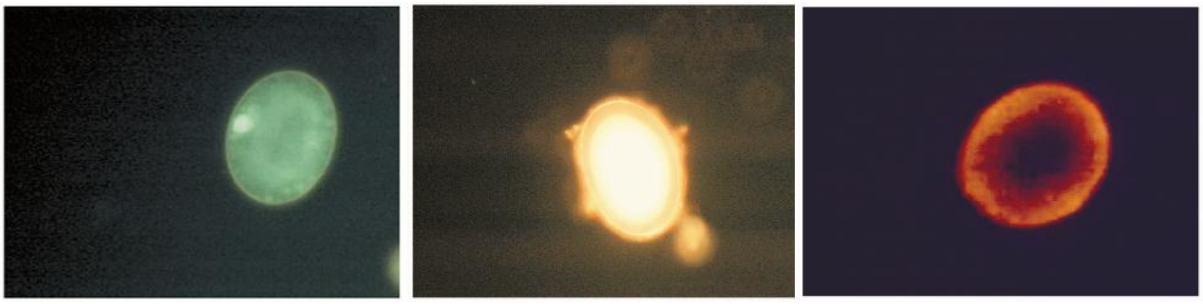


Fig. 6: (A) Photomicrograph of live egg of *Toxocara* vitulorum In distilled water, stained by 4, 6 Diamidino-2-Phenylindole (DAPI) Flourogenic vital stains after effect of ammonia exposure (1%) (100 X).

(B) Photomicrograph of dead egg of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains after effect of (100 X). Ammonia (2%)



A **B** **C**

Fig. 7: (A) Photomicrograph of live Eggs of *Toxocara vitulorum* in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains (100 X).

(B) Photomicrograph of dead Egg of *Toxocara vitulorum* inactivated at 61° C in distilled water, stained by 4, 6 Diamidino-2- Phenylindole (DAPI) Flourogenic vital stains after effect of solar energy (100 X).

(C) Photomicrograph of dead Eggs of *Toxocara vitulorum* Inactivated at 61° C in distilled water, stained by (DAPI) Flourogenic vital stains after effect of solar energy (100 X)

Fig. 8: Photomicrographs of (A) solar energy dead *Toxocara* vitulorum egg, using SODIS technique. Egg stained with PI (Propidium Iodide) showing red fluorescence (100 X)

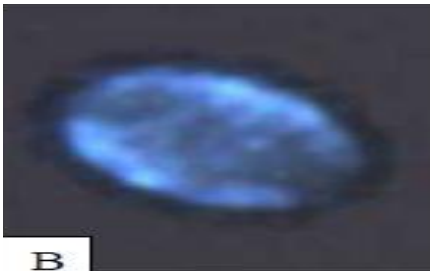


Fig. 9: Photomicrograph of (B) solar energy dead *Toxocara vitulorum* egg using SODIS technique. Egg stained with DAPI (4', 6-Diamidino-2-Phenylindole and PI stain showing blue fluorescence only inside the egg) (100 X)

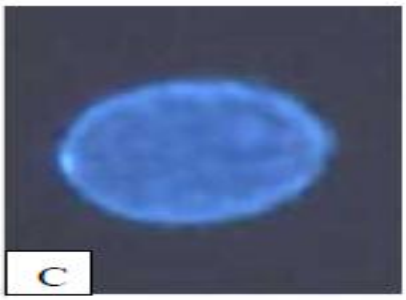


Fig. 10: Photomicrograph of (C) live *Toxocara vitulorum* egg stained with DAPI showing blue fluorescence all over the egg(100 X).

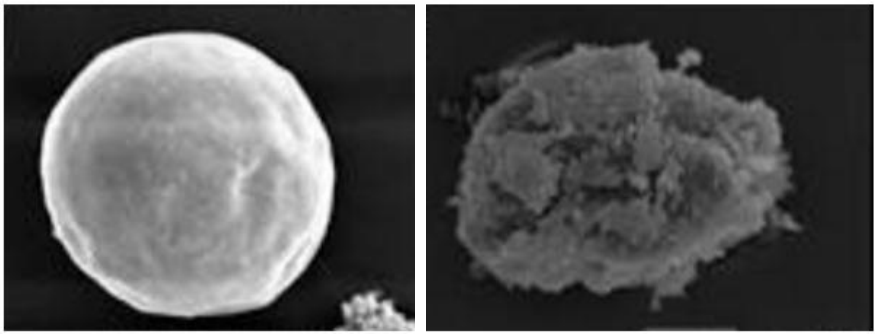


Fig. 11 : 1- Scan Electron photomicrograph of normal live *Toxocara vitulorum egg*
2- Scan Electron photomicrograph of dead *Toxocara vitulorum* egg after treatment of 2% ammonia concentration Scale bar (10µm).

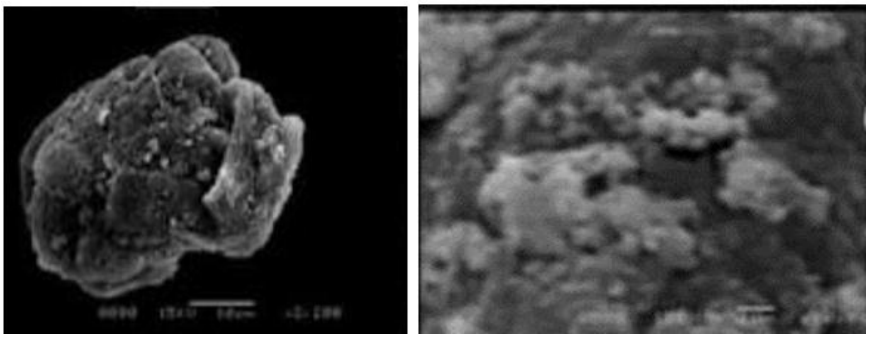


Fig. 12 : A and B - Scan Electron photomicrographs of dead *Toxocara vitulorum* egg after exposure to solar energy. Scale bar (10µm).

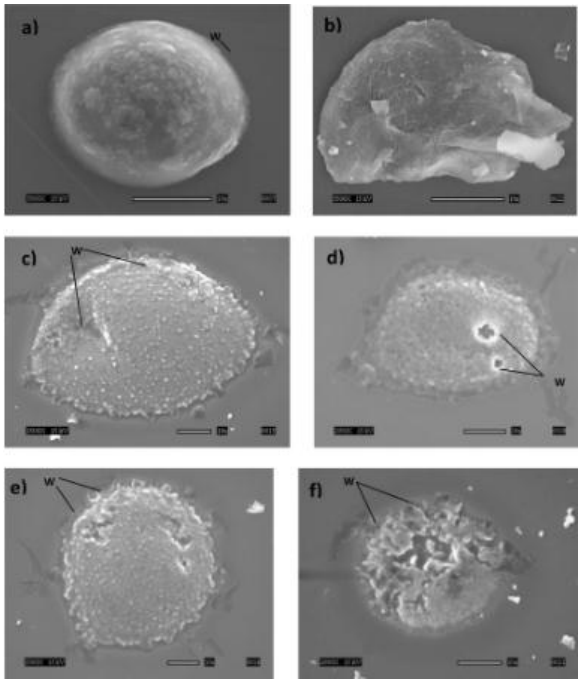


Fig. 13 : Scan electron photomicrographs of :

- **a-** normal live *T. vitulorum* egg at positive control group. Scale bar (10µm).
- **b-** dead *T. vitulorum* egg at ammonia concentration treatment group Scale bar (10µm).
- **c-** and **d -** *T. vitulorum* eggs showing little pores and semi destruction (w) on egg surface after treatment with 2% ammonia concentration . Scale bar (10µm).
- **e-** dead *T. vitulorum* egg showing moderate destruction(w) on egg surface after treatment process with solar energy Scale bar (10µm).
- **f-** dead *T. vitulorum* egg showing completely dead and destruction (w) of egg surface after treatment process effect of solar energy in combination with ammonia. Scale bar (10µm).

In the event of both recoloring method with DAPI and Propidium Iodide and scanning Electron microscope magnifying lens examination, the typical live eggs of *Toxocara vitulorum* demonstrated ordinary thick external layer film with the development of chitin-protein complex layer with individual parts granules encompassed by fat vacuoles. So it doesn't fully absorb vital colors. While in the case of dead inactivated eggs, it loses the normal morphology of the thick outer layer membrane and fully absorbs DAPI stains as showed in (Figures 2 -10) , under a fluorescent microscope, and in the case of scanning electron microscope observation eggs showing complete destruction of the egg surface after treatment with solar energy and ammonia as showed in (Figs. 11 -13) . All indicated and photomicrograph fully indicated the difference between live activated and dead inactivated *Toxocara vitulorum* eggs with DAPI & Electron microscope investigations.

DISCUSSION

In the present study, the results indicated that utilizing of two liters PET containers containing tainted samples with *T. vitulorum* with no immediate day sunlight (33.5°C), just 10% of *T. vitulorum* eggs separately were decimated and became not infective, while an occurrence of two liters PET containers, with direct day sunlight and no darkened side (48.5 °C), just 30% of the eggs become unviable.

The current results demonstrated that when the temperature raised to 48.5 °C, it was appeared just 30% of *T. vitulorum* were destroyed and rendered non infective, while 70% of these eggs were as yet feasible and viable. At the same time, uncovering two liters PET bottles with direct day sunlight and one side black (65.5 °C) in tests debased with eggs of *T. vitulorum* showed 100 % devastated eggs and became non infective, these findings in accordance with the investigations of Mohamed *et al*., (2015), who indicated that the influence and potential of solar energy to disinfect, naturally, polluted raw water with encyclical protozoa (*Giardia and Entamoeba* cysts) through its thermal and UV effect**.** So, that SODIS disinfection system can be used as a simple, environmentally sustainable, low-cost solution for contaminated water and wastewater with *T.vitulorum* and other parasitic nematodes.

The results of this examination demonstrated that inactivation of *T. vitulorum* eggs by ammonia various fixations would be progressively viable in mix with the solar energy sunlight based vitality in the wake of including 2% ammonia and Adjust the suitable conditions that were available in a shut pilot scale framework : a base temperature of 25°C, an underlying pH of 8.5, and a minimum moisture level of 27.5% .

The inactivation of *T. vitulorum* eggs was quicker in tests presented to ammonia and with temperatures higher than 28°C. Tests without ammonia never achieved 100% inactivation at 28°C inside 21 days of capacity, while tests at 35°C, 45°C, and 55°C accomplished all out inactivation following 21 days, 7 days, and 24 hours of capacity, individually. All examples presented to 2% ammonia accomplished 100% inactivation following 21 days at (28°C), 3 days at (35°C), and 24 hours (45°C and 55°C) of capacity, with combination of solar energy influence.

Nordin *et al*. (2010) showed that comparative time ward and temperature inactivation results in the wake of including various fixations 1% and 2% ammonia of to human defecation. It watched viable eggs at 25°C, in fecal examples following 35 days of capacity. At 36°C, they watched 100% *Toxocara* eggs inactivation following 10 days of capacity with 1% ammonia and following 4 days of capacity

with 2% ammonia, and these were agree with the outcomes results shown in our investigation. In the present investigation with ammonia impact on *T. vitulorum* in blend with solar energy sun oriented vitality, 21 days, 3 days, and one day were required to accomplish 100% eggs inactivation treated with 1% or 2% ammonia concentration at 35°C, 45°C, and 55°C, separately. Totally inactivation of the parasite eggs happened quicker in the present investigation when contrasted with Nordin's examination; these distinctions could be credited to the lower moisture substance of the examples and the higher temperatures utilized in mix with solar energy sun oriented vitality influence. Likewise solar energy sunlight based purification framework (SODIS) utilizing PET compartments painted dark on one side can be utilized to sterilize water and waste water samples that containing *T. vitulorum* and pulverize their eggs too.

On account of examination of water and wastewater normally contaminated and sullied samples with parasitic nematode *T. vitulorum* eggs , the eggs are for the most part in the phase of cleavage and morula or blastula arrange. Under a light magnifying lens, the creating incipient organism appears as papules and the consequent perception of cell division is basically inconceivable. With the goal that the reasonability evaluation dependent on morphological qualities turns out to be troublesome (Johnson *et al*., 2010). With the goal that utilizing explicit colors, for example, DAPI stain is exceptionally expected to recognize live and dead eggs of parasitic nematode *T. vitulorum* eggs , because of the nearness of thick shells of eggs and furthermore different inhibitors in the examples containing eggs, for example, explicit framework and natural buildups that found in silt of the samples.

The displayed examination results demonstrated the value of DAPI recoloring staining unit to survey the practicality of *T. vitulorum* eggs happening in water and waste water tests . The consequences of recoloring by fluorescent DAPI pack of eggs suspended in water was the best. It was set up that inactivated dead *T. vitulorum* eggs recolored by DAPI under fluorescence microscopy sparkle red, and Live eggs green . The investigation has exhibited the high handiness of DAPI recoloring pack to evaluate the reasonability and imperativeness of *T. vitulorum*, eggs happening in water tests . This pack could be considered as a choice to tedious and emotional perception of eggs amid brooding delayed consequence of ammonia concentration with combination of solar energy.

CONCLUSION

Totally inactivation of *T. vitulorum* eggs were conceivable under Adjusted lab conditions when 2% Ammonia fixation in mix with the impact of sun oriented vitality solar energy effect that were incorporated into the analysis. These results examined that inactivation of *T. vitulorum* eggs by ammonia could happen in water and wastewater samples that were put away with a sunlight based vitality impact in the field if the reasonable physical and chemical conditions were met at the beginning stage of treatment: a shut pilot scale with a base temperature of 25°C, pH of 8.5, a moisture dampness dimension of 27.5%, and the expansion of 2% ammonia fixation to the test . The utilizing of sun oriented vitality solar energy effect alone could inactivate the parasite eggs, if temperatures of 65.5°C or higher were accomplished inside a shut pilot scale for in any event 7 hours. In the event that ammonia were added to the shut vault, with combination with solar energy effect the impact of eggs inactivation would be expanded and accomplish 100 % inactivation of *T. vitulorum* eggs.

So that, these procedures of both mix of solar energy effect and Ammonia can be connected in wastewater treatment plants to inactivate *Ascaris* and *Toxocara spp* eggs that can go through the treatment procedure and tainted human who eating yields sullied with flooded water plants, likewise ammonia salts can be utilized as great ooze manure clear of parasitic eggs and can be connected to developing products of the soil crops. This lead to the most extreme usage of all accessible water and wastewater assets to accomplish the objectives and the goals of sustainable development in developing countries and Egypt.

Conflict of Interest

The authors declare that there is no conflict of interest at this research paper.

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