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Synergistic Effect of 2,4-D Herbicide and Copper Sulphates in Control the Snail *Bulinus Truncatus* by Using Bioassay

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Abstract

The current study was designed to examine the synergistic effects of 2,4-D and copper sulfates in controlling *Bulinus truncatus* snail (the vector of urinary Schistosomiasis). Freshwater snails *B. truncatus* (Audouin, 1827) were exposed to copper sulphates ($CuSO_4$) and the herbicide (2,4-D). The mortality was assessed for 24, 48, 72 and 96 hours of exposure time. The toxicity of $CuSO_4$ showed to be more than the toxicity of 2,4-D to *B. truncatus* snail. Such, the toxicity of both substances (2,4-D and $CuSO_4$) appeared to be more toxic than each substance toxicity alone. A lethal concentration means of $CuSO_4$ (LC50) was 0.44 ppm, while the 2,4-D was 0.38 ppm. In addition, the mixture of 2,4-D and $CuSO_4$ (1ml+2g) was 0.22 ppm while the mixture (2ml+1g) was 0.26ppm. The study was concluded the synergistic toxicity of a mixture of two substances used. Also, mixing the both substances was produced more toxic effect than the prober substances alone. Protection of the environment must be taken in considerations when use these materials to kill the snails. The results of this study showed that the mortality rates were increased with increasing of concentrations used in treatment. The study was improved that the mixture of 2,4-D and $CuSO_4$ has synergistic toxicity when mixed together.

1. Introduction

Urinary Schistosomiasis is a worldwide health problem especially in tropical areas such as Iraq. In Iraq, Balladur distract is a famous with rice agriculture. The source of irrigation water for rice, palm trees, and other agricultural fields is Al-Bzania River. Balladur distract is a focus of Schistosomiasis with a percent of 18%, as the reports of the health region associations and many related studies. In the last two decades, Schistosomiasis cases frequency was increased especially in primary school. Al-Bzania River is the main cause of Schistosomiasis prevalence, in addition to many authorities' factors that lead to increase the frequency of schistosomiasis prevalence such as using the river water as a waste place whereas it specialized to palms irrigation. A natural factor as the prevalent of *Bulinus truncatus* snails is a cause of Schistosomiasis [1].

Gastropods are useful biological observing of metal pollution. Also, it has position in food chains [2]. *B. truncatus* (Mollusca: Gastropod) is the middle host of Schistosomiasis in Iraq [3].

The biological control of diseases studies is a complex operation, so it needs more studies in details and pig efforts to do it. Some studies are concentrating on drugs by chemical control. While, others concentrating on disease agent eradication before entering the host body though biological control, but the best methods lead to cut-off the life cycle of the disease cause via controlling of the middle host [4, 5].

Chemical control of drugs has some disadvantages as side effects; unable to select the effect, and it represents the treatment but not prevention. Biological control summarized by trying to cut-off the life cycle of the disease cause or via controlling the middle host of the disease cause. Therefore, the biological control is better than the chemical control. The materials that used in biological control must be studied with details to protect the environment and living communities [6].

Heavy metals considered as a main pollutant for environment especially the aquatic environment. The increased levels of heavy metals in water lead to bioaccumulation of these heavy metals in animal tissues that caused pathological effects [7, 8]. These increased levels often found in river sediments; as a result of industrial discharge, industrial effluents discharge and oil pollution in aquatic media [9]. Hence, there it needs to more advanced knowledge for heavy metal pollution assessment.

Copper sulfate ($CuSO_4$) (or cupric sulfates) is an inorganic compound. Different toxic effects have been reported on aquatic organisms such as fish, trout, crab, shimp, oyster, mussels and other invertebrates [10]. Documental biological activities of CuSO4 are a pesticide, fungicides, and molluscicide [10]. Other studies determined the toxicity of $CuSO_4$ to animals (such as rat) with LD50 300 mg/kg orally), the toxicity of $CuSO_4$ due to their content of heavy metals that concerned as a major problem in wastewater treatment [11]. A previous study referred that $CuSO_4$ was used as a molluscicide to control *Biomphalaria alxandry* snail (the middle host of Schistosoma mansoni) in Sudan and Egypt, *B. truncatus* (the middle host of Schistosoma heamatobium) and *Lymnaea caillaudi* (the middle host of Faciola hepatica) in Iraq [10, 12].

The 2,4-Dichlorophenoxy acetic acid (2,4-D), also called hedonal, trinoxol, and chloroacetanilide according to the International Union of Pure and Applied Chemistry (IUPAC). It is a common herbicide structured ($C_8H_6Cl_2O_3$) described as an eclectic pesticide, and it is a moderately hazardous as classified in Class II by WHO. It is widely used for agricultural purposes, especially for the broad and narrow leaf weeds. In addition, it is considered as a pesticide [13]. The common concentration that used in agriculture is 5ml/l. The pesticide spraying in the fields may include a residual concentration as an environment contaminant [14]. Pesticide residuals might be mixed with other substances that have the ability to produce more toxic substances than the proper substances [13]. Even the half-life of 2,4-D degradation is low (6.2 days), it was used with other substances such copper sulfates to get synergistic effect to produce a more toxic substance than 2,4-D to may obtain a double interest for controlling the snails and eradication of weeds in the same time [15, 16].

A CDC report referred to 2,4-D toxicity that studied in different animals such as hamster with the concentration (500 mg/kg orally), dog (100 mg/kg orally), rat (699 mg/kg orally) and mouse (347 mg/kg orally) [17].

The synthetic pesticides are effect on the water bodies though their high toxicity, bioaccumulation, long-term persistence, and their widespread using in the world [11, 18]. However, there are many restarted rules to reduce the eco-toxicological hazard of the artificial pesticides. One of the most important rules is using a specific pesticide for the targeted organism without any eco-toxicological risks for other organisms [19].

The bioassay (biological assay, biological assessment or biological standardization) is a type of scientific experiments that involves the use of living organisms to determine the biological activity of the substances. In addition, it may be used for other purposes such as measuring different biological effects, developing new drugs, monitoring the environmental pollutants, and determining the level of the particular constitution of the mixtures that may cause harmful effects on the organisms or the environment [20].

Previous studies reported that the extract of *Euphorbia splendens*, *Phytolacca dodecandra*, and *Tetrapleura tetraptera* were found to be toxic to the gastropods. On the other hand, other studies reported that the N-butanol

extracts of *Agave americanam*, *Sapindus trifoliatusm*, *Balanites agyptica*, *Jatrapha gossypifolia*, and *Vaccaria pyramidata* were found to be toxic to the eggs of *Lutorea luteol* snail [21].

The present work was reached that the Thymus vulgaris leaves extracts contains chemical substances make it suitable to snail control [5]. The aim of this work is to evaluate the possibility of interest form the synergistic effects of copper sulfate and 2,4-D herbicide to control the snail *Bulinus truncatus*.

2. Methodology

2.1. Study Area

The area of the study was in Balladur at Diyala province, it is a village consist of rural and town. The center of the village is beside the main street between Baquba (City Center) and Himreen hills (North-West of Diyala province). The village contains educational and health associations. Also, the rural areas are containing palm trees and agricultural fields. In addition, Al-Bzania River is an irrigation canal with a length of 10km (5km up the town called Al-Sadrania and 5km down the town called Al-Bzania).

2.2. Experimental Samples

The freshwater snails' *B. truncatus* (L) were collected from the Al-Bzania River in the district of Balladur, Diyala province (Iraq). Zooplankton net was used to collect the snails in nylon bags. The snails' collection was beginning from June to October 2016. Snails were put in plastic container with a proper amount of river water. In the laboratory, the snails were acclimatized to laboratory conditions (Temp. 25 ºC ± 3) for a week before the testing. *Alfa alfa* extract (10ml/ 50L/ 24h.) and ventilation were provided to the snails. The snails were isolated, identified and classified according to stander keys of snails [22]. Such, McClelland method was followed for snails cultivating [7]. The characterization of selected snails for the experimentation were 20-25 mm of shell height and 2.6-3.6 g of weight. The mortality rate was studied against the both toxicants alone and within the mixture.

2.3. Treatments

Copper Sulphates and herbicide were used to control the snail of *B. truncatus* in the current study. Four groups of concentrations were prepared depending on stock solutions (v/v and w/v) as:

S1 (substance1) =1g of copper sulfates
S2 (substance2) = 1ml of 2,4-D herbicide
S3 (substance3) = S1+2S2
S4 (substance4) =2S1+S2

From these stock solutions, series dilution was made as 0.1, 0.2, 0.3, 0.4 and 0.5ppm. Twelve snails were tested in each replicate and the average of these experiments was calculated [23].

Calculation of safe concentration
C = 48 h. LC50 × 0.3/ S
S = 24h LC50 /48h. LC50
C: Safe concentration
LC50: Median lethal concentration [24]

2.4. Statistical Analysis

The regression analysis that depended on the probit units was used to calculate LC50s. Probit unit method was chosen because it includes calculated the normal death may occur in control. Probit analysis is a conversion of the percentages of death into probit units from special tables and graphics the units with Log of concentrations.
By selecting the Logarithm of concentration that corresponded the probit unit in 5 and turned it into an anti-logarithm, it could get the concentration that kill a half number of exposed organisms that called LC50. This analysis is expressed how death in control events.

LC50s values were recorded for each exposure period (24, 48, 72, 96 hours) in all concentrations. Comparison for each exposure period and concentrations was tabulated.

3. Results and Discussion

Toxicity of herbicide $CuSO_4$ (1ml/L) was relatively evaluated for mortality and in the targeted organs of *B. truncatus*. Copper sulfate (1g/L) showed a higher toxicity than 2,4-D. The highest mortality rate was recorded at a 0.5 ppm that was 78% after 96h of exposure. Also, the concentrations that have not mortality recorded considered as safe concentrations (Table 1).

While, the 2,4-D herbicide toxicity (1ml/L) was more toxic to the exposed snails than the $CuSO_4$. The highest rate of mortality was recorded at a 0.5ppm that was 64% after 72 h of exposure. Also, the 96 h of exposure to 2,4-D pesticide, the mortality rate was increased to 37% at a 0.5ppm (Table 2).

In addition, the toxicity of $CuSO_4$ and 2,4-D (2g+1m, respectively) mixture and the mortality of the targeted freshwater snail *B. truncatus* was comparatively assessed. The mixture results showed that the highest mortality rate was recorded at a 0.5ppm that was 100% after 96h of exposure. Also, the mixture was more toxic than each substance alone (Table 3).

A reverse concentration of CuSO4 and 2,4-D was assessed by mixing 1g of $CuSO_4$ with 2ml of 2,4-D herbicide. The highest rate of mortality was at a 0.5ppm concentration that was 99% after 96h of exposure. Also, the mixture toxicity was more toxic than substances alone (Table 4).

In compression the toxicity of the both mixtures, the 2g+1m of $CuSO_4$ and 2,4-D was more toxic than the 1g+2ml $CuSO_4$ and 2,4-D (Tables 5 and 6).

The $CuSO_4$ LC50 mean for 96h of exposure time was 0.30 ppm, while the 2,4-D LC50 mean of was 0.44 ppm. So, the present results verified that $CuSO_4$ was more toxic than 2,4-D (Table 5). Furthermore, the LC50 mean of 2g+1ml of $CuSO_4$ and 2,4-D for 96h of exposure time was 0.22ppm. While, the LC50 mean of 1g+2ml of $CuSO_4$ and 2,4-D was 0.26ppm. Therefore, the doubling concentration of $CuSO_4$ is more toxic than the doubling concentration of 2,4-D to the snail *B. truncatus* (Table 6).

Table (1). Mortality percent of *B. truncatus* during intoxication of $CuSO_4$ at different time intervals.

Concentration ppm	**Mortality (%) of snail exposed to $CuSO_4$**			
	24h	48h	72h	96h
Control	**3**	**3**	**3**	**3**
0.1	**8**	**8**	**8**	**8**
0.2	**17**	**17**	**17**	**17**
0.3	**30**	**30**	**30**	**35**
0.4	**47**	**47**	**47**	**58**
0.5	**64**	**64**	**64**	**78**

Table (2). Mortality percent of *B. truncatus* during intoxication of 2,4-D at different time intervals.

Concentration ppm	**Mortality (%) of snail exposed to 2,4-D**			
	24h	48h	72h	96h
Control	**0**	**0**	**2**	**2**
0.1	**1**	**1**	**5**	**5**
0.2	**4**	**4**	**10**	**10**
0.3	**9**	**10**	**17**	**17**
0.4	**18**	**22**	**26**	**26**
0.5	**31**	**36**	**37**	**37**

Table (3). Mortality percent of *B. truncatus* during intoxication mixed of $CuSO_4$ and 2,4-D (2g+1ml) at different time intervals.

Concentration ppm	Mortality (%) of snail exposed to $CuSO_4$ and 2,4-D (2g+1ml)			
	24h	48h	72h	96h
Control	**2**	**2**	**2**	**3**
0.1	**5**	**5**	**7**	**19**
0.2	**35**	**35**	**45**	**55**
0.3	**80**	**80**	**88**	**88**
0.4	**98**	**98**	**99**	**99**
0.5	**100**	**100**	**100**	**100**

Table (4). Mortality percent of *B. truncatus* during intoxication mixed of $CuSO_4$ and 2,4-D (1g+2ml) at different time intervals.

Concentration ppm	Mortality (%) of snail exposed to $CuSO_4$ and 2,4-D (1g+2ml)			
	24h	48h	72h	96h
Control	**2**	**2**	**2**	**3**
0.1	**5**	**5**	**7**	**19**
0.2	**35**	**35**	**45**	**55**
0.3	**80**	**80**	**88**	**87**
0.4	**98**	**98**	**98**	**99**
0.5	**99**	**99**	**99**	**99**

Table (5). Comparative data of the LC50 values of heavy metal, copper sulphates and pesticide 2,4-D for *B. truncates.*

Time of Exposure (h)	Type of LC50	LC50 of $CuSO_4$ (ppm)	Mean of LC50	LC50 of 2,4-D (ppm)	Mean of LC50
24	Calculated	**0.38**		**0.52**	
48	Calculated	**0.36**		**0.47**	
72	Calculated	**0.28**	**0.30**	**0.39**	**0.44**
96	Calculated	**0.19**		**0.38**	

Table (6). Comparative data of the LC50 values of heavy metal copper sulphates and pesticide 2,4-D for *B. truncates.*

Time of Exposure (h)	Type of LC50	LC50 of $CuSO_4$+2,4-D (2g+1ml) ppm	Mean of LC50	LC50 of $CuSO_4$+2,4-D (1g+2ml) ppm	Mean of LC50
24	Calculated	**0.33**		**0.36**	
48	Calculated	**0.29**		**0.29**	
72	Calculated	**0.18**	**0.22**	**0.27**	**0.26**
96	Calculated	**0.11**		**0.12**	

There was a strong correlation between $CuSO_4$ concentrations and the exposure snails' responses that observed at 24h and 48h compared to 72h and 96h of exposure time (R2=0.9288 vs. R2=0.9108 and 0.9241, respectively) (Figure 1). The same findings approximately appeared when 2,4-D herbicide was used and mortality was assessed at *B. truncatus* for 24, 48 and 72h of exposure time with a correlation factor 0.9499 (Figures 2). Such, a highly dose-response correlation between the tested substances and snails was observed at 96h of exposure (the correlation factor was 0.964) (Figures 2). In addition, the correlation factor was 0.922 for 24 and 48h of exposure time for 2,4-D and $CuSO_4$ (1ml+2g) mixture (Figure 3). Such, the correlation factors of 72 and 96h of exposure were 0.9142 and 0.9242, respectively when the snails exposed to 1ml+2g of 2,4-D herbicide and $CuSO_4$ mixture (Figure 3). While, a high correlation factor value was detected at 24h of exposure time to the mixture of 2ml 2,4-D and 1g $CuSO_4$ (R2=0.8836) (Figure 4). The same results detected between 2ml 2,4-D and 1g $CuSO_4$, the snails' response at 48 and 72h of exposure was (R2=0.0073). Such, the results observed a weak correlation between the mixture and snails' responses at 96h of exposure time (R2=0.0589) (Figure 4).

According to regression equations and correlation factors, it was found that these substances caused the death to *B. truncatus* with the dose and time- dependent. Although the used substances were toxic, but it appeared as safe concentrations according to the equations. The safe concentration that calculated of $CuSO_4$ was 0.10ppm and 0.12ppm for 2,4-D, 0.070 for 1ml of 2,4-D and 2g $CuSO_4$ mixture and 0.076 for 2ml of 2,4-D and 1g $CuSO_4$ mixture (Table 7).

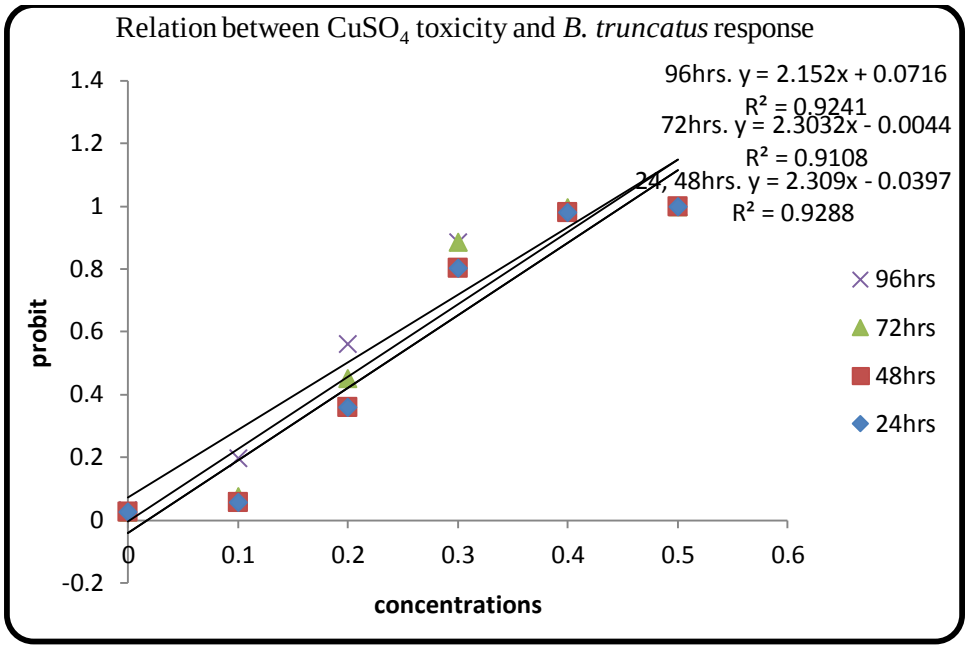

Figure (1). Regression equation of $CuSO_4$ and the exposed freshwater snail *B. truncates*.

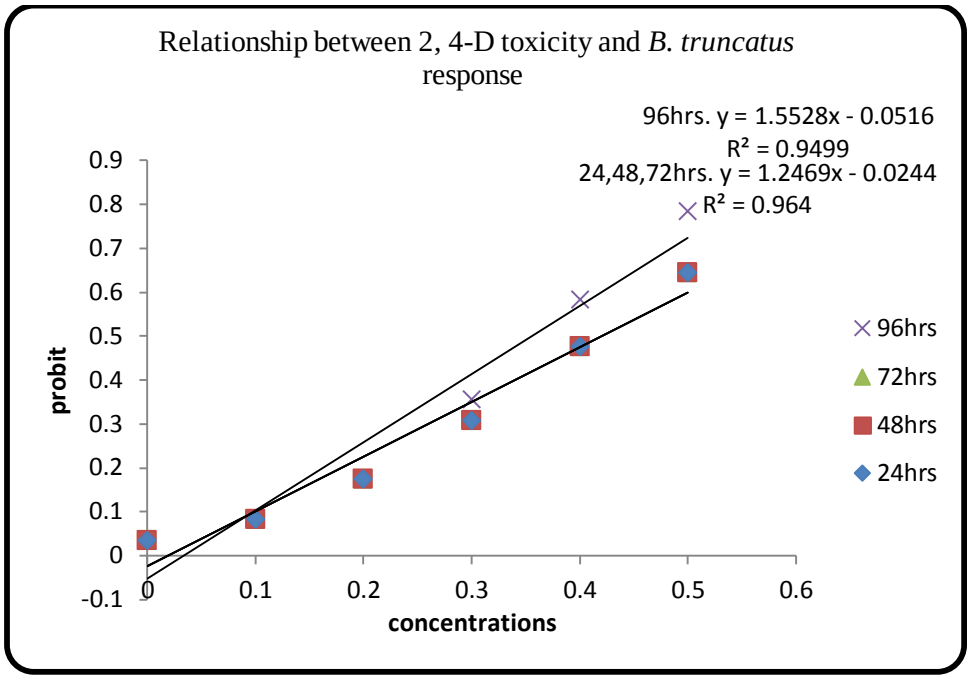

Figure (2). Regression equation of 2,4-D herbicide and the exposed *B. truncatus* snail.

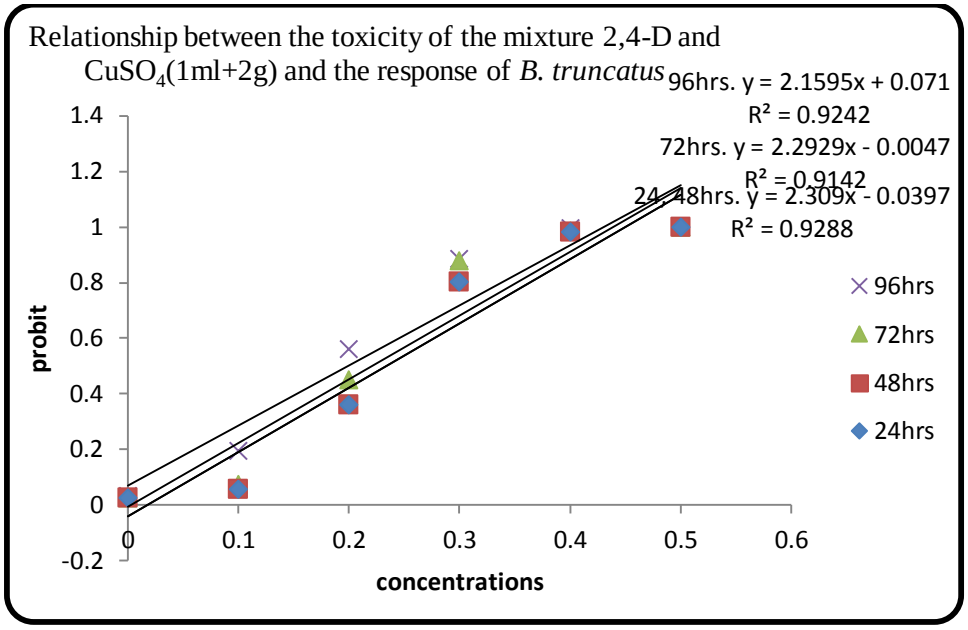

Figure (3). Regression equation of 1ml 2,4-D and 2g $CuSO_4$ mixture and the exposed *B. truncatus* snail.

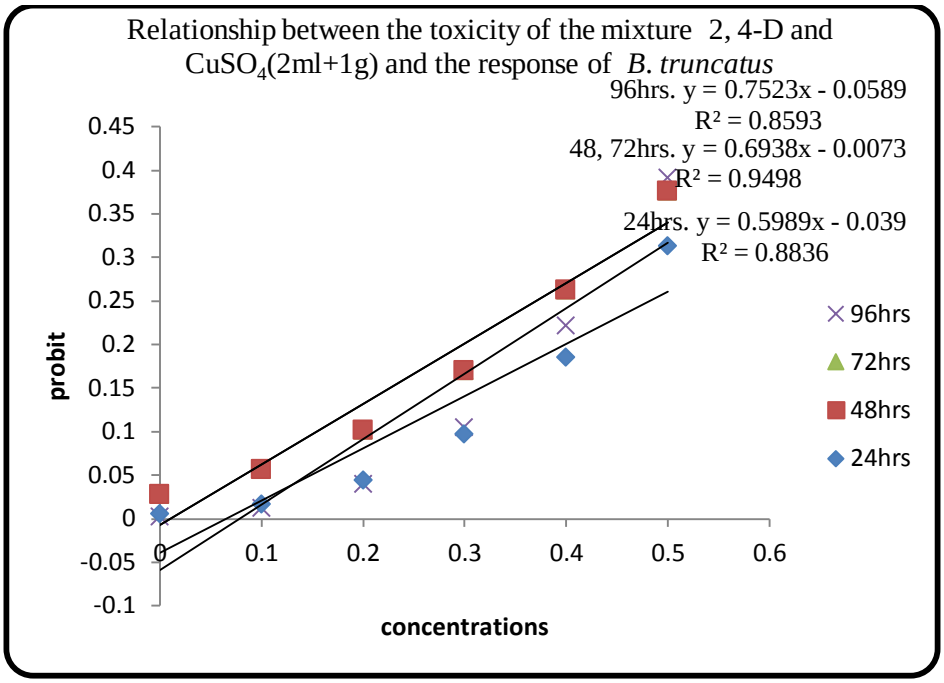

Figure (4). Regression equation of 2ml 2,4-D and 1g $CuSO_4$ mixture and exposed freshwater snail *B. truncates*.

Table (7). The safe concentration of $CuSO_4$, 2,4-D herbicide, 1ml+2g mixture and 2ml+1g mixture.

Exposure time (h)	Safe Concentrations(ppm)			
	$CuSO_4$	2,4-D	1ml 2,4-D+2g $CuSO_4$	2ml 2,4-D+1g $CuSO_4$
96	**0.10**	**0.12**	**0.070**	**0.076**

The recent results showed that the concentrations of $CuSO_4$ more than 0.10 ppm have a toxic impact at the snail. Also, 2,4-D herbicide concentrations more than 0.12 ppm have a toxic impact at the targeted snail. In addition, Mixing the two substances in small amounts (not exceed 2 ml/L for 2,4-D and 2g/L for $CuSO_4$) were appeared to be more toxic to the snail. The 96h LC50 (at 25 ºC) of 2,4-D was 0.38 ppm. While, the 96h LC50 (at 25 ºC) of $CuSO_4$ was 0.19 ppm. These results were disagreed with another study that limited the dose of $CuSO_4$ (at 20 ºC) (0.39 ppm). This difference is due to the Lab room temperature of the current study that was 25 ºC. Such, it is known that the toxicity of $CuSO_4$ is increased with increasing the Lab room temperature. However, these doses have to be taken carefully, and the factors might influence the toxicity of $CuSO_4$ such as the Lab room temperature, alkalinity, and the water hardness. Another study on the effect of $CuSO_4$ against *Biomphalaria glabrata* snail was limited the LC100 for 6h in the concentrations 1 and 0.1%, and LC50 for 6h in the concentration 0.01%. These results appeared to be different from the recent results; because the tested snails in the previous study were juveniles, while in the recent study were adults [25]. High percent of killing in appeared our work was obtained because of the chemical synergy between two substances. Each substance that used in this study was recorded as a toxic agent to the snails, and the mixture of the both subjects leaded to increase the toxicity. So, it can use them to kill the snails, especially the $CuSO_4$ was used as a molluscicide substance [26]. The 2,4-D herbicide concentration that used was 50 ml/ liter and the concentration of $CuSO_4$ that used to kill off the snails was 5 ppm. It is well known that the lethal dose of 96h LC50 (at 20 ºC) for pond snails was 0.39 mg/L or 0.39 ppm. This means that the concentration of 0.39 parts per million of $CuSO_4$ in our tank will kill half of the snails presented over a period of 96h if the temperature is kept constant at 20 ^{0}C (68 ^{0}F). These concentrations were highly compared to the used in the recent study. It can predict that the pesticide residuals in environments with low concentrations of $CuSO_4$ would achieve controlling the spread of snails. It must study the environmental damage that may occur though using such these substances. The toxicity substances arrangement according to the current study was: S3(1ml 2,4-D+2g $CuSO_4$) > S4(0.076)> S1(0.10)> S2(0.012). The heavy metals mechanism's effect to the snails was studied by many researchers that found the bioaccumulation and biomagnifications rate of heavy metals in the cells depended on its uptake and elimination in the tissue [12]. Accumulation of metals is more rapid

than its elimination, probably due to the presence of metal binding proteins in the tissues [24]. Another previous study reported that the copper can be deposited as insoluble granules intracellular as membrane-bound in the hepatopancreas of most terrestrial invertebrates [3, 20]. Also, another previous study demonstrated copper derivatives are highly toxic than lead to Helix aspera snail, and they reported that the higher toxicity of copper-induced because of its ability to the complex's formation with Anions. Also, they noted that the copper induced caused oxidation of quinone and hydroquinone in the target cell [23]. The researchers noted that copper, lead, and cadmium metals having a high bioaccumulation rate in the tissues of *B. truncatus* snails [22]. Also, some previous studies demonstrated the copper carbonate potential toxicity to *B. truncatus* snails dissociated with the biological and chemical reactions [24. Such, another previous study found that the copper was bioaccumulated in soft tissue of juvenile apple snail *Pomacea paludasa* (about 60% in the viscera and 40% in the foot), and in its shell copper was accumulated less than 4% of total copper concentration in the snail body [2]. The using of pesticides was contaminated the freshwater ecosystem and causes hazards to several non-targeted aquatic animals [8]. Therefore, many studies were determined the toxicity effect of the organic phosphorus pesticides such as cythion, zolone and rogor on freshwater snails (*Lymnaea acuminate*, *Thiara scabra* and *Thiara lineata*) [1]. It was proved that the toxicity effect of pesticides on *V. bengalensis*, they reported that, pesticides of organophosphorus were more toxic than chlorinated hydrocarbons pesticides [18]. Other researchers reported that the mortality effect of Cypermethin was less toxic than Copper sulphate toxicity to *Thiara tuberculatus* [27]. The snail's mortality occurred as a result of 2,4-D action which depends on its form. Some forms of 2,4-D ester can be very toxic to fish and other aquatic life; these aquatic animals are more sensitive to 2,4-D as water temperature rises. The aquatic invertebrates do not, in general, seem to be very sensitive to 2,4-D. But there are many Ecotoxic effects of 2,4-D on mollusks were noted includes behavior, growth, morphology accumulation, biochemistry, effects on cells, the effect of development, effects on enzymes, genetics, intoxication, physiology, population, and mortality [14]. However, the ACP was reported that there are insufficient data available to assess 2,4-D safety to aquatic life and there are needs to studies using 2,4-D in order to make a full assessment of its risks to wildlife.

4. Conclusions

The toxicity of $CuSO_4$ was more than the 2,4-D herbicide to *B. truncatus* snail. Also, mixing the both substances was produced more toxic effect than the prober substances alone. The study was concluded the synergistic toxicity of a mixture of two substances. Protection of the environment must be taken in considerations when use these materials to kill the snails. The results of this study showed that the mortality rates were increased with increasing of concentrations used in treatment.

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References

[1] M. Al-Obaidi, A. Abbas, A. Hanoon and K. Ibrahim, "Using of aquatic extracts of *salvia officinalis* to control the snail *Bulinus truncatus* the intermediate host of schistosomiasis in Iraq (part I)," *Basrah Journal of Veterinary Research* vol. 15, no 3, pp. 333-338, 2016.

[2] J. Hellawell, "Biological indicators of freshwater pollution and environmental management". ed. Springer Science & Business Media. 2012.

[3] K. J. Adamson, W. Tianfang, B. A. Rotgans, A. V. Kuballa, K. B. Storey, and S. F. Cummins. "Differential peptide expression in the central nervous system of the land snail *Theba pisana*, between active and aestivated," *Peptides*, vol. 80, pp. 61-71, 2016.

[4] A. Ahmed, N. Ismail, A. Osman, A. Mohamed, and M. Gharieb. "Bioaccumulation of heavy metals in freshwater snails in relation to lining of water courses in Egypt.*" Journal of Bioscience and Applied Research* vol. 2, no 8, pp. 561-573, 2016.

[5] M. Al-Obaidi, A. Abbas, A. Al-Azzauy, and A. Waael, "Survival rates of *Bulinus truncatus* as a way to determine the Molluscicidal activity of *Ricinus communis* extracts," *Iraqi Journal of Biotechnology*, vol. 14, no 2, pp. 32-53, 2015.

[6] F. Bakry, S. Ismail, and M. Abd El-Atti, "Glyphosate herbicide induces genotoxic effect and physiological disturbances in *Bulinus truncatus* snails," *Pesticide Biochemistry and Physiology*, vol. 123, pp. 24–30, 2015.

[7] K. Bianco, S. Otero, A. Oliver, D. Nahabedian and G. Kristoff, "Resistance in cholinesterase activity after an acute and sub chronic exposure to azinphos-methyl in the freshwater gastropod *Biomphalaria straminea*",

Ecotoxicology and Environmental Safety, vol. 109, pp. 85–92, 2014.
[8] T. Brock, M. Hammers-Wirtz, U. Hommen, T. Preuss, H. Ratte, I. Roessink *et al*. "The minimum detectable difference (MDD) and the interpretation of treatment-related effects of pesticides in experimental ecosystems." *Environmental Science and Pollution Research*, vol. 22, no 2, pp.1160-1174, 2015.
[9] J. Burger and M. Gochfeld, "Mercury and selenium levels in 19 species of saltwater fish from New Jersey as a function of species, size, and season", *Science of The Total Environment*, vol. 409, pp. 1418–29, 2011.
[10] Centers for Disease Control and Prevention (CDC) "Fourth national report on human exposure to environmental chemicals", Atlanta (GA): Centers for Disease Control and Prevention. 2015. [online]. Available: http://www.cdc.gov/ biomonitoring/pdf/ Fourth Report Updated Tables. [2015].
[11] B. Alan "Toxic metal uptake and essential metal regulation in terrestrial invertebrates a review," 1st Edition, CRC Press, [2020].
[12] Y. Chen, X. Huang, Y. Xiao, Y. Jiang, X. Shan, J. Zhang *et al*. "Spatial analysis of schistosomiasis in Hubei Province, China: a GIS-based analysis of schistosomiasis from 2009 to 2013". *PLoS One*, vol. 10, no 4, p. e0118362, 2015.
[13] World Health Organization, "The control of neglected zoonotic diseases: from advocacy to action: report of the fourth international meeting held at WHO Headquarters, Geneva, Switzerland, 19-20 November 2014." [2015].
[14] P. Wang and Y. Wu. "Applications of Metabonomics in Pesticide Toxicology", *Current Drug Metabolism*, vol. 16, pp. 191–199, 2015.
[15] R. De-Carvalho, M. Gomes-Carneiro, B. Geraldino, G. da, S. Lopes and F. Paumgartten, "Evaluation of the developmental toxicity of solvents, metals, drugs, and industrial chemicals using a freshwater snail (*Biomphalaria glabrata*) assay." *Journal of Toxicology and Environmental Health*, vol. 85, no 19, pp. 798-814, 2022.
[16] E. Gharedaashi, H. Nekoubin, M. Imanpoor, V. Taghizadeh "Effect of copper sulfate on the survival and growth performance of Caspian Sea kutum, *Rutilus frisii kutum*", *Springer Plus*, vol. 2, no 1, pp. 1-5, 2013.
[17] D. Saha, P. Azubuike, C. Panigrahi, N. Saha and C. Faggio, "Biochemical, physiological (haematological, oxygen-consumption rate) and behavioural effects of mercury exposures on the freshwater snail, *Bellamya bengalensis*," *Comparative Biochemistry and Physiology* Part C: *Toxicology & Pharmacology*, vol. 251, pp. 109-115, 2022.
[18] K. Gustafson, J. Belden and M. Bolek, "The effects of the herbicide atrazine on freshwater snails", *Ecotoxicology*, vol. 24, pp. 1183–1197, 2015.
[19] M. Carlos, T. Silva, T. Andreani and A. Silva, "Glyphosate vs. glyphosate-based herbicides exposure: a review on their toxicity", *Journal of Xenobiotics*, vol. 1, pp. 21-40, 2022.
[20] J-H. Lee, G. Birch, T. Cresswell, M. Johansen, M. Adams, and S. Simpson, "Dietary ingestion of fine sediments and microalgae represent the dominant route of exposure and metal accumulation for Sydney rock oyster (Saccostrea glomerata): a biokinetic model for zinc", *Aquatic Toxicology*, vol. 167, pp. 46-54, 2015.
[21] R. Henry, Č. Lucu, H. Onken and D. Weihauch, "Multiple functions of the crustacean gill: osmotic/ionic regulation, acid-base balance, ammonia excretion, and bioaccumulation of toxic metals", *Frontiers in Physiology*, vol. 3, pp. 1–33, 2012.
[22] C. Appleton and M. Nelson, "Locating bilharzia transmission sites in South Africa: guidelines for public health personnel", *Southern African Journal of Infectious Diseases*, vol. 3, pp. 78-85, 2015.
[23] R. Osborne, P. Gillis and R. Prosser, "Transgenerational effects of copper on a freshwater gastropod, *Planorbella pilsbryi*," *Freshwater Mollusk Biology and Conservation*, Vol. 23, no 1, pp. 42-54, 2020.
[24] S. Anzalone, N. Fuller, K. Huff, C. Fulton, G. Whitledge, J. Magnuson *et al*. "Pesticide residues in juvenile Chinook salmon and prey items of the Sacramento River watershed, California–A comparison of riverine and floodplain habitats," *Environmental Pollution*, vol. 303, p. 119102, 2022.
[25] R. Larba and N. Soltani "Use of the land snail *Helix aspersa* for monitoring heavy metal soil contamination in Northeast Algeria", *Environmental Monitoring Assessment*, vol. 186, pp. 4987–4995, 2014.
[26] P. Wang and Y. Wu, "Applications of Metabonomics in Pesticide Toxicology", *Current Drug Metabolism*, vol. 16, no 3, pp.191–199, 2015.
[27] S. Kamble and N. Kamble, "Acute Toxicity of Copper Sulphate and *Acacia sinuata* To the Freshwater Snail *Bellamya Bengalensis* (L) ", *International Journal of Toxicology and Applied Pharmacology*, vol. 3, no1, pp. 14-21, 2013.