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SCHISTOSOMIASIS VECTOR CONTROL USING *CUCUMIS MELO* PLANT EXTRACTS WITH BIOASSAY EXPERIMENT

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Abstract

The aim of this study is to get a plant extracts to use it as molluscicides to control the snail vector of Schistosomiasis and finely control the disease. Laboratory study was performed to compare the molluscicidal activity of leaves and stems extracts of *Cucumis melo* against *Bulinus truncatus* snail. The snail *B. truncatus* was exposed to a serial concentrations of leaves and stems extracts (4000ppm, 5000ppm) in this work. Different effects of the extracts to the snail *B. truncatus* were recorded. These effects includes death, escaping and imbalance of snail behavior. 96hr-LD50 values of leaves extracts were calculated for the doses 4000 and 5000ppm as (76 and 37%) respectively while for stems were (105 and 47%) respectively. We found that the snail *B. truncatus* was more susceptible to leaves than stems extracts. The Molluscicidal activity of *C. melo* extracts depended on increase of extract concentrations and time of exposure ($p>0.05$).

Key words : Schistosomiasis, control, snails.

Introduction

Schistosomiasis is human infection results with penetration of the cercariae of Schistosoma spp. through the skin. Iraq is one of many countries that suffer from a health problem of Schistosomiasis. Some studies suggest that the cases of Schistosomiasis are increased through the last two decades especially in primary school children. Using of the irrigation water as a west place, washing and swimming may cause of Schistosomiasis. Presenting of *B. truncatus* snails is a limited factor of Schistosomiasis (Chen *et al*., 2016; WHO, 2017).

Extracts from some plants like *Tetrapleura tetraptera, Phytolacca dodecandra* and *Euphorbia splendens* was reported as toxic substances against the snails. Also, extractes from plants like *Agave americana, Vaccaria pyramidata* and *Jatrapha gossypifolia* was toxic against the snail *L. luteol* too (Rawani, Ghosh and Chandra, 2014).

Molluscicides of plant origin have an importance because it is natural products, ecologically safety and cultivable. A large number of plants which possess natural molluscicidal activity have been identified. The plant phytochemicals derived from plant resources can be used as an alternative to the synthetic molluscicides (Dias, Marçal Jr and Glasser, 1995).

Cucumis melo (Family: Cucurbitaceae) known as muskmelon, cantaloupe and honeydew. Melon is a long trailing yearly vine from the cucumber family. It grows in sandy areas and near river banks. It is a good source of appetite, weight loss, urinary tract infections, constipation, acidity, and ulcers(Ismail, Chan, Mariod and Ismail, 2010).

The strategy of Schistosomiasis control includes control of snail populations in lakes and rivers using chemical molluscicides. Recently, the common substance Niclosamide (Bayluscide, Bayer Leverkusen, Germany) is the only commercially available molluscicide recommended by the World Health Organization (WHO). This molluscicide is commonly used in Schistosomiasis Control Programs (WHO, 1992).

Schistosomiasis control is depending on a treatment of population at-risk, safe water, sanitation, hygiene education and snail control. The strategy of WHO about Schistosomiasis control is focused on using the drug (Praziquantel) to reducing disease. The groups targeted must consist of: children, adults at risk in endemic areas, people with occupations involving contact with infested water. The use of plant molluscicides might be one of the

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best ways for the control of Schistosomiasis infections in the third world (Agi, 1996).

The aim of this study is to detect suitable substances can useful to control the snail of *B. truncatus* with safety effect to the environment.

Materials and Methods

Collection of animal samples

Samples of snail, *Bulinus truncatus* were collected through the period (June to August 2015) weekly from Al-Rasheed district (30km) south of Baghdad. Collection site was near the street number 37 (linked between Al-Rasheed districts and Tigris river) with abscissae (33 8 32.83 North, 44 25 37.20 East). The samples were collected from one small irrigation canal beside the main canal commonly called (Muhyii River). Zooplankton net and steel spoon were used in the collection of the snails. Snails were placed in 5 L plastic containers with the amount of river water. The snails were fed with the extracts of leaves of *Alfa alfa* plant 10ml per 50L daily. The collected snails were isolated, identified according to stander keys of snails then they are acclimatized to laboratory conditions (T 25± 3) before testing for two days. Snails were cultivated in the laboratory according to the method of McClelland as described by Benelli *et al*. (2015).

Preparation of extracts and evaluation of molluscicidal activity

Aquatic extracts of *C. melo* leaves and stem were prepared, concentrated, dried, shade, shredded in a hand mill (Estrella®, model 41B) and an electric mill (Moulinex®) then sifted through a mesh (number 30) to obtain a fine powder left in a cool dry place. The W.H.O. method (II) for testing for molluscicides was followed; exposure and recovery periods were 24 hours in all the tests. Three replicates and average number were maddened per test. Susceptibility of snails, compare extracts potency, lethal concentrations, and their 95% confidence limits were determined by probit analysis (Wang *et al*., 2016).

Stock solutions

Leaf and stem of *C. melo* powder (50 and 100g) were macerated in 1L of distilled water for 24 hr to getting stock solutions. The macerate was filtered through cotton gauzes in a plastic funnel to getting crude extracts.

Bioassays and acute toxicity

Bioassays were conducted to evaluate the effect of extracts by different values of LD_{50}. These parameters were determined for each exposure period (96, 72, 48, 24 hours) in all concentrations (zero, which represents control) (Anderson and Lydy, 2002). Acute toxicity tests were conducted to the leaves and stem extracts of *C. melo* separately. The exposed ten of snails for 96 hours were treated without any food. Mortality was accounted at the end of each 24-hour exposure. Dead snails were removed and recorded at 24, 48, 72, 96 hr. after each application. The end point of individuals was identified when there was no movement, no response to stimulation by glass rod and lack of the ability to adhere. Mortality numbers were corrected by correction equation below:

□=□–□/□–1

Where,

p = proportion of mortality for a given dose

The c = proportion of mortality for a zero dose (natural mortality).

Recorded results were compared with the control group and percent of mortality was estimated with respect to the total population (WGMEE, 2007; Seber, 1982).

Statistical analysis

Regression analysis depending on the Probit units was used to calculate LD50 by using the provider of SPSS v. 21 programs. By selection, the Log of concentration that contrast probit unit in 5 and turn it into inverse logarithm or applying of regression equations tabled can get LD50. Relationships between Logarithm of concentrations and probit units in different periods of exposure were recorded and plotted (Al – Obaidi *et al.*, 2013).

Results and Discussion

Mortality rates

The results of this study found that the total mortality number of *B. truncatus* snail exposed to extracts of *C. melo* leaves 4000ppm for 96-hr was 49 of 720. Mortality was increased depending on concentrations and period of exposure (table 1). The results of this study found that the total mortality number of *B. truncatus* snail exposed to extracts of *C. melo* leaves (5000ppm) for 96-hr was 107 of 720. Mortality was increased depending on concentrations and period of exposure (table 2). The results of this study found that the total mortality number of *B. truncatus* snail exposed to extracts of *C. melo* stems (4000ppm) for 96-hr was 43 of 720. Mortality was increased depending on concentrations and period of exposure (table 3). The results of this study found that the total mortality number of *B. truncatus* snail exposed to extracts of *C. melo* stems (5000ppm) for 96-hr was

Table 1 : Numbers of *B. truncatus* mortality exposed to *C. melo* leaves extracts (4000ppm) for 96 hr.

Total	Cumulative number of mortality			Stock solution of leaves 4000ppm		
	96hr (N=30)	72hr (N=30)	48hr (N=30)	24hr (N=30)	Stock sol. /ml	DW
0/120	0	0	0	0	0	(100)Con
3/120	2	1	0	0	10	90
5/120	3	1	1	0	20	80
8/120	4	2	2	0	30	70
13/120	7	3	2	1	40	60
20/120	11	5	3	1	50	50
49/720	**27/180**	**12/180**	**8/180**	**2/180**	**Total**	

Table 2 : Numbers of *B. truncatus* mortality exposed to *C. melo* leaves extracts (5000ppm) for 96 hr.

Total	Cumulative number of mortality			Stock solution of leaves 5000ppm		
	96hr (N=30)	72hr (N=30)	48hr (N=30)	24hr (N=30)	Stock sol. /ml	DW
0/120	0	0	0	0	0	(100)Con
7/120	4	2	1	0	10	90
12/120	6	3	2	1	20	80
17/120	8	4	3	2	30	70
29/120	17	6	4	2	40	60
42/120	22	10	7	3	50	50
107/720	**57/180**	**25/180**	**17/180**	**8/180**	**Total**	

Table 3 : Numbers of *B. truncatus* mortality exposed to *C. melo* stems extracts (4000ppm) for 96 hr.

Total	Cumulative number of mortality			Stock solution of stems 4000ppm		
	96hr (N=30)	72hr (N=30)	48hr (N=30)	24hr (N=30)	Stock sol. /ml	DW
0/120	0	0	0	0	0	(100)Con
4/120	3	1	0	0	10	90
6/120	4	2	0	0	20	80
8/120	5	2	1	0	30	70
10/120	5	3	1	1	40	60
15/120	8	4	2	1	50	50
43/720	**25/180**	**12/180**	**4/180**	**2/180**	**Total**	

92 of 720. Mortality was increased depending on concentrations and period of exposure (table 4).

LD_{50} values

The median lethal concentration of mortality of *B. truncatus* exposed to the aquatic extracts of *C. melo* leaves and stem (40 & 50 g/L) were summarized below in (table 5).

The result of the recent study showed that the LD50 of leaves extracts were less than stem extracts. Also, LD50 values of 5000ppm leaves and stem extracts were less than of 4000ppm. Furthermore, LD50 values recorded after 96hr of exposure were less than which recorded after 24hr of exposure.

Dose-response correlations

The study found that the correlation factor of Dose-Response relationships was arranged (0.6857-0.9328). The relationships between *C. melo* extracts doses and *B. truncatus* snail responses were summarized below in (table 6).

Linear relationships between leaves *C. melo* extracts (4000ppm) and *B. truncatus* with equations and correlation factors were reported in (fig. 1). Linear relationships between leaves *C. melo* extracts (5000ppm) and *B. truncatus* with equations and correlation factors were reported in (fig. 2). Linear relationships between stems of *C. melo* extracts (4000ppm) and *B. truncatus* with equations and correlation factors were reported in (fig. 3). Linear relationships between stems of *C. melo*

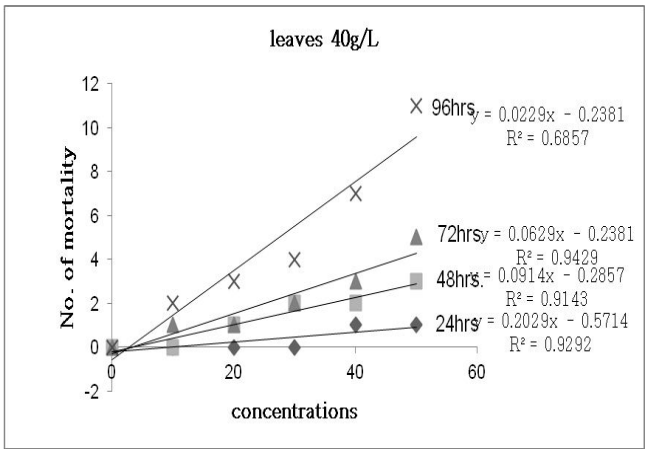

Fig. 1 : The relationship between concentrations of *C. melo* leaves extracts (4000ppm) and response of *B. truncatus* snail for 96hr. of exposure.

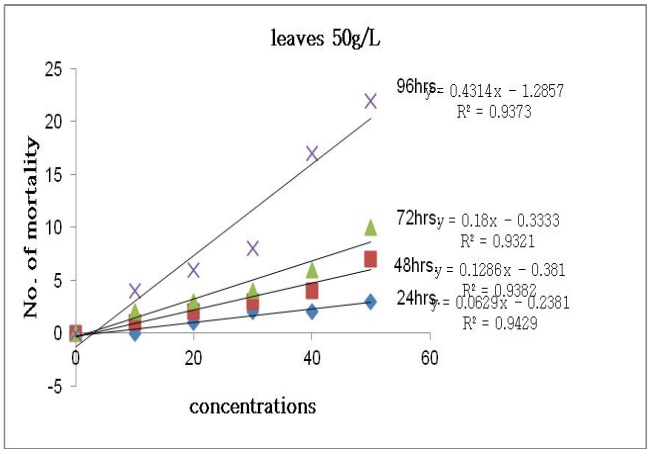

Fig. 2 : The relationship between concentrations of *C. melo* leaves extracts (5000ppm) and response of *B. truncatus* snail for 96hr. of exposure.

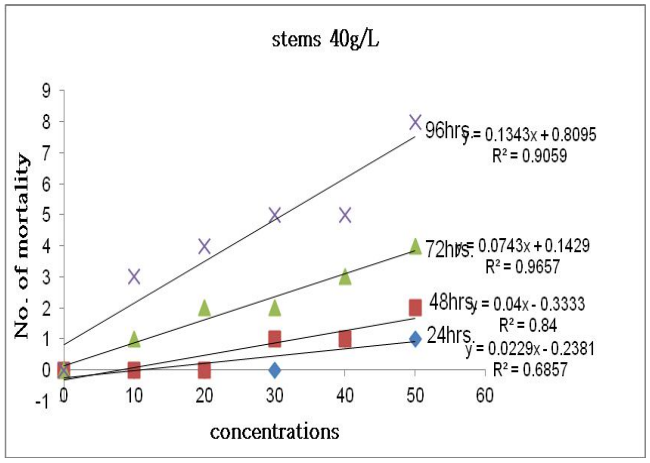

Fig. 3 : The relationship between concentrations of *C. melo* stem extracts (4000ppm) and response of *B. truncatus* snail for 96hr. of exposure.

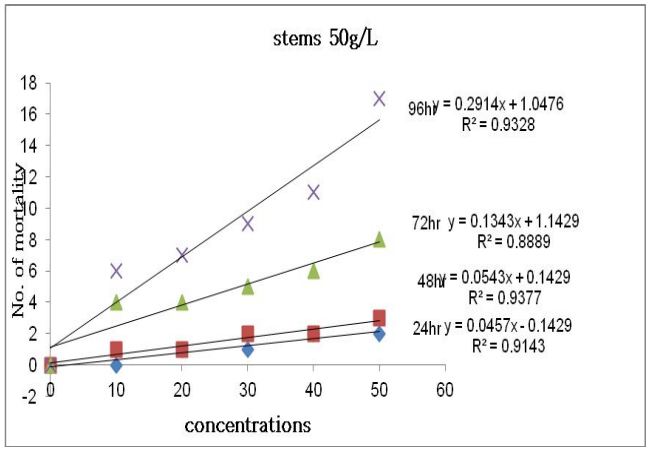

Fig. 4 : The relationship between the concentration of *C. melo* stem extracts (5000ppm) and response of *B. truncatus* snails for 96hr. of exposure.

extracts (5000ppm) and *B. truncatus* with equations and correlation factors were reported in (fig. 4).

The recent study noticed that no appearance of a dose-response relationship between the tested extracts and tested snails in the beginning of exposure (24hr). The absence of mortality may indicate that the effect in this period of exposure considered as NOEL appeared laterally. It's clear to be showed that there was a significant increase in the mortality rates of snails exposed to tested extracts compared to the control group. This finding agrees with finding which showed marked reduction in the survival rate of snails treated with concentrations of different plant extracts compared to control (Bakry, 2009; Goudreau, 1988; Moné *et al*., 2011).

From the results of the recent study, it was found that the used extracts which caused effect and death to snail of *B. truncatus* (melon contains a phenolic compounds as gallic acid, hydroxybenzoic acid, catechin, caffeic acid, vanillic acid, ellagitanins, quercetin-3-rutinoside, syringic acid, alkaloids and ellagic acid) also dose and time dependent. These results were agreed with applied study of water extract of *T. tetraptera* which used a concentration of 15, 20 and 25mg/liter in Nigeria (Adewunmi, 1991). In addition, these results were agreed with a histopathological study of *T. tetraptera* extract on *Bulinus* (Phyopsis) *globosus, Biomphalaria glabrata,* and *Physa waterlotti*. The effect of the extract on various snail tissues was found to be time and concentration dependent (Aladesanmi, 2007).

Some studies were suggested that the mechanism of activity of plant extracts to snails demonstrated by produced significant reductions in the glycogen and protein content and molluscicidal action on the carbohydrate metabolism of the snail (Aladesanmi, 2007; Mello-Silva, Vasconcellos, Pinheiro and Rodrigues, 2006). Mechanism of activity of plant extracts on the snails was included

Table 4 : Numbers of *B. truncatus* mortality exposed to *C. melo* stems extracts (5000ppm) for 96 hr.

Total	Cumulative number of mortality			Stock solution of stems 5000ppm		
	96hr (N=30)	72hr (N=30)	48hr (N=30)	24hr (N=30)	Stock sol. /ml	DW
0/120	0	0	0	0	0	(100)Con
11/120	6	4	1	0	10	90
13/120	7	4	1	1	20	80
17/120	9	5	2	1	30	70
21/120	11	6	2	2	40	60
30/120	17	8	3	2	50	50
92/720	**50/180**	**27/180**	**9/180**	**6/180**	**Total**	

Table 5 : Calculated LD_{50} values of *C. melo* leaves and stem extracts to snail *B. truncatus.*

LD_{50} values (%)				Time of exposure
Stems		Leaves		
5000ppm	4000ppm	5000ppm	4000ppm	
331	665	242	665	**24 hr.**
273	383	118	242	**48 hr.**
103	199	85	176	**72 hr.**
47	105	37	76	**96 hr.**

Table 6 : Correlation equations and correlation factors of Dose-Effect of *C. melo* to snail of *B. truncatus* for 96 hr. of exposure.

Extracts	Dose (ppm)	Exposure time	Correlation equations	Correlation factor R^2
Leaves	**4000**	24hr.	y=0.0229x-0.2381	0.6857
		48hr.	Y=0.0629x-0.2381	0.9429
		72hr.	Y=0.0914x-0.2857	0.9143
		96hr.	Y=0.2029x-0.5714	0.9292
	5000	24hr.	Y=0.0629x-0.2381	0.9429
		48hr.	Y=0.1286x-0.2381	0.9382
		72hr.	Y=0.18x-0.3333	0.9321
		96hr.	Y=0.4314x-1.2857	0.9373
Stems	**4000**	24hr.	Y=0.0229x-0.2381	0.6857
		48hr.	Y=0.04x-0.3333	0.84
		72hr.	Y=0.0743x+0.1429	0.9657
		96hr.	Y=0.1343x+0.8095	0.9059
	5000	24hr.	Y=0.0457x-0.1429	0.9143
		48hr.	Y=0.0543x+0.1429	0.9377
		72hr.	Y=0.1343x+1.1429	0.8889
		96hr.	Y=0.2914x+1.0476	0.9328

registration in many organs as kidney, hepatopancreas and gastrointestinal tract. Further effects of *T. tetraptera* extracts to *B. glabrata* and *Lymnaea columella* snail as growth and egg production were recorded in some studies (Adewunmi, Furu, & Madsen, 1989; Aladesanmi, 2007).

Conclusion

Both of *C. melo* leaves and stems extracts were effective to the snail of *B. truncatus*. The target snail was more sensitive to leaves than stems extracts. The target extracts can be able to use as molluscicides to the snail of *B. truncatus*. The author recommended that there are a necessary to make a field and applied work to discover the interest of the plant molluscicides.

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