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Determination the Toxicity of some Organic Acids and Fertilizers against the Land Snail *Eobania vermiculata*

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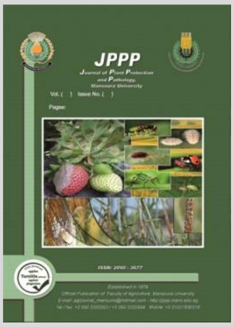


ABSTRACT

The toxic influence of boric, salicylic and sulfonic organic acids and the ammonium hydroxide, copper sulfate, and urea as fertilizers were evaluated against the adult snails of *Eobania vermiculata* for 21 days by spraying technique under the laboratory conditions. Organic acids and fertilizers were decomposing in the soil accelerated than pesticides; four recommended concentrations (0.5, 1, 1.5 and 2%) were opted for each acid and fertilizer and selected as low concentrations to avoid any noxious impact against plants. Moreover, efficacy of the binary mixing of organic acids with fertilizers at LC_{25} against the adult snail individuals was also investigated. The utmost mean mortality of snails was remarked at the concentration of 2% of all tested compounds, which attained 52.00, 82.66 and 83.33% for boric, salicylic and sulfonic acids while it was 95.33, 94.00, and 50.00 for ammonium hydroxide, copper sulfate, and urea, consecutively. Based on LC_{50}, the efficiency of tested compounds could be arranged in descending order sequentially to ammonium hydroxide, copper sulfate, sulfonic acid, salicylic acid, boric acid and urea. The binary mixture of sulfonic acid with urea and salicylic acid with ammonium hydroxide achieved the highest molluscicidal effect against snails in comparison with the other tested mixtures, accomplished 100% mortality of snails. On the contrary, the binary mixing of salicylic acid with copper sulfate achieved the lowest mean mortality which it was only 12.66%.

Keywords: *Eobania vermiculata*, Toxic effect, Organic acids, Fertilizers.

INTRODUCTION

Land snails involved several numerous differentiated lineages of terrestrial gastropods, belonging to the second-largest phylum after arthropods in terms of the number of species. The chocolate band snail, *Eobania vermiculata* is a circum-Mediterranean species; it is deemed a graver snail and disadvantage creatures to the environment (Gad *et al*., 2019). It eventuates in diversities of habitats usually in vegetables, garden, vineyards and ornamental plants (Khafaji *et al*., 2016; Ali *et al*., 2020). Many control methods have been used for control harmful land snails including chemical control, it is considered one of the most common and successful methods. But, in recent years the offensive effects of pesticides on human and environmental health have become known in addition to concern for their acute and chronic toxicity and their potential as carcinogens, teratogens and mutagens (Mansour, 2005). So, other methods were resorted for use as alternative control methods. These methods characterized by many advantages such as cheapness, easy degradable in the soil and safety. Related to these aspects, acetylsalicylic acid and tannic acid represent potent molluscicides for control *E. vermiculata* snails. Acetylsalicylic acid has significant necrotic impact on the mucous gland tissues of snails and thus it is negatively affected on the mucus synthesis which is very important for the snail life (Mobarak, 2008). Moreover, fertilizer represent other new effective molluscicides with different mode of action and considered safe to the environment when applied by low rates (recommended concentrations) and concurrently it's addition to the soil improve plant growth and yield (Elisabeth, 1997). Urea and ammonium nitrate induced observed molluscicidal influence against *E. vermiculata* and *Monacha cartusiana* snails in the laboratory and field (Hend, 2013). On the other hand, the combining of two molluscicides is more potent than when each one is used alone. The molluscicides when combined with each other they act synergistically by increasing or prolonging their effects (Oparaeke *et al*., 2005).

The present study is established to evaluate the toxicity of the organic acids (boric, salicylic and sulfonic) and the fertilizers (ammonium hydroxide, copper sulfate and urea) separately and as binary mixtures against *E. vermiculata* snail under laboratory conditions.

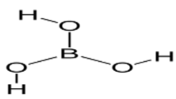
MATERIALS AND METHODS

Tested compounds:

A- Organic acids

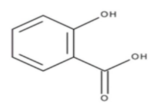
*** Boric acid (99%)**

- Chemical structure (IUPAC): boron oxide hydroxide
- Structure formula:



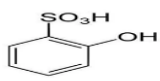
*** Salicylic acid (95%)**

- Chemical structure (IUPAC): 2-hydroxybenzoic acid
-Structure formula:



*** Sulfonic acid (85%)**

- Chemical structure (IUPAC): Hydroxysulfane dioxide
- Structure formula:



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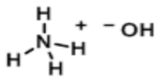
B- Organic fertilizers

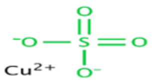
*** Ammonium- hydroxide (33% N)**

- Chemical structure (IUPAC): Ammonium hydroxide
- Structure formula:



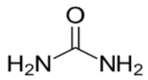
*** Copper sulfate (100%)**

- Chemical structure (IUPAC): Copper sulfate
- Structure formula:



*** Urea (46% N)**

- Chemical structure (IUPAC): carbamide Carbonyldiamide
- Structure formula:



* The tested fertilizers and organic acids were purchased from El-Gomhouria Company, Egypt.

Experimental animals

Adult individuals of the chocolate band snail, *Eobania vermiculata* were collected from navel orange trees (*Citrus sinensis*) in Inshas village, Belbies district, Sharkia governorate, Egypt. Snail individuals were gathered and transported to the laboratory of Harmful Animals Research Department, Plant Protection Research Institute, Agricultural Research Center. They kept in a glass container contains humid clay soil and covered with cloth netting fixed with rubber band to prevent snails from escaping. The snails were supplied daily with fresh leaves of cabbage for 14 days for acclimatization.

Preparation of tested compounds

Four concentrations (0.5, 1.0, 1.5 and 2.0%) for each of boric, salicylic, sulfonic acids and ammonium hydroxide, copper sulfate and urea fertilizers were prepared by dissolving the amount of each tested compound in water required to get the wanted concentration (Hend, 2013).

Bioassay of tested compounds against snails

The toxic effect of each tested organic acid and fertilizer at the concentrations of 0.5, 1.0, 1.5 and 2.0% was bio assayed separately against adult snails of *E. vermiculata* by spray method under laboratory conditions. Plastic boxes (3/4 kg capacity), where each box contained humid clay soil. Ten adult snail individuals and ten discs of cabbage leaves were placed on the surface of soil in each box. Each concentration of each tested compound was sprayed on both soil and cabbage discs by using a hand sprayer. Three repetitions were designed for each concentration and other three control repetitions were prepared in the same way but without any treatment. All tested and control boxes were covered with muslin cloth and fixed with rubber band to avoid snails escaping. Dead snails were removed daily and the mortality percentages were recorded for 21 days according to Ghamry (1997) and corrected by Abbott (1925). The lethal concentrations LC_{25} and LC_{50} of each tested compound were determined on the 7th day of experiment using Bio Stat (2007) (professional Build 3200). The toxicity index and relative toxicity were also calculated according to Sun (1950).

Toxicity index = [LC_{50} of the most effective compound / LC_{50} of the other compound] ×100

Relative toxicity = LC_{50} of the least effective compound / LC_{50} of the other compound

Efficacy of the binary mixtures against snails

The molluscicidal potency of the binary mixture of each of boric, salicylic and sulfonic acids at LC_{25} value with the LC_{25} of ammonium hydroxide, copper sulfate and urea fertilizers separately against adults of *E. vermiculata* was investigated. Ten adult snails were introduced in plastic boxes, each box contained humid clay soil and ten discs of cabbage leaves were placed on the soil surface in each box. The tested binary mixtures were sprayed directly on the soil and cabbage discs. Three replicates were prepared for each mixture and other three untreated ones were prepared as a control. All boxes were covered with muslin cloth and fixed with rubber band. Mortality percentages of snails were recorded daily for 21 days, and corrected by Abbott's formula (Abbott, 1925).

Joint action of the binary mixtures against snails

The joint action in the binary mixtures between organic acids and fertilizers against *E. vermiculata* snail after 21 days of the experiment was expressed as the Co-Toxicity factor (C.F.) estimated according to Mansour *et al.* (1966) as follows:

$$\textbf{C.F.} = \frac{\textbf{Observed mortality \% - Expected mortality \%}}{\textbf{Expected mortality \%}} \times 100$$

Where: **the observed mortality was the visualized efficacy of the two compounds applied in a mixture, and the expected mortality was the sum of the efficacy of each compound used individually. It was indicated potentiation if the Co-Toxicity factor was positive factor ≥ 20. While, negative factor ≤ 20 considered antagonism and intermediate values between – 20 and + 20 means an additive effect.**

Data analysis

All data were subjected to the statistical analysis, and the treatment means were compared by the least significant difference (LSD) test at $P \leq 0.05$ levels using the statistical analysis computer program software (Co Stat, 2005).

RESULTS AND DISCUSSION

Efficiency of the tested organic acids against *Eobania vermiculata* snails after indicated days under laboratory conditions

The toxic influence of boric, salicylic and sulfonic acids individually against adult snails of *E. vermiculata* after 21-days exposure period was summarized in Table 1. All tested organic acids attained their highest molluscicidal toxic effect against snails at the highest concentration of 2%. On the contrary, as their concentrations decrease, the mortality rate decreased. In general, sulfonic acid recorded the highest mortality of 70% at the highest tested concentration of 2% after only one day of exposure, then slightly increased to 80% on the third day of the experiment and then increased to 93.33% on the 14th day of the investigation. Although, the mortality rate (53.33%) was achieved by salicylic acid at the concentration of 2% on the first day of exposure was less than that attained by sulfonic acid, where the effect of salicylic acid at the same concentration on the seventh day of the experiment was stronger than any other tested acid, as it completely killed all snail individuals with a mortality of 100%.

After one day of the treatment with salicylic acid at 0.5% concentration, visible swelling of the soft body of snail individuals was observed and extended out the shell (Photo 1). Boric acid had the least effective acid against the tested snail species, where it recorded only 20% mortality after one day of exposure and then exhibited its highest molluscicidal impact by recording 70% mortality on the 14th day of the investigation. No mortalities were achieved in the control throughout the trial period where all tested acids showed highly significant differences in snail's mortality compared to the untreated control. These results were strongly corroborated by Hend (2013) who showed that salicylic acid caused 56.66, 86.66 and 96.66% mortality of *E. vermiculata* adult snails which were treated with 0.5, 1 and 1.5% concentrations consecutively after 21 days of spraying, respectively. While, the highest tested concentration (2%) attained 100% mortality of the snails after the same experimental period.

Table 1. Accretive mortality of *Eobania vermiculata* snails caused by tested organic acids

Organic acids	Conc. (%)	Mean of mortality % after indicated days					General mean
		1	3	7	14	21	
Boric	0.5	0.00^{f}	0.00^{f}	13.33^{f}	20.00^{e}	20.00^{f}	10.66^{e}
	1.0	10.00^{ef}	16.66^{e}	20.00^{f}	20.00^{e}	33.33^{e}	20.00^{e}
	1.5	13.33^{e}	20.00d^{e}	46.66^{de}	46.66^{d}	46.66^{d}	34.66^{d}
	2.0	20.00^{de}	36.66^{c}	63.33^{c}	70.00^{c}	70.00^{c}	52.00^{bc}
Salicylic	0.5	26.66^{cd}	30.00^{cd}	36.66^{e}	40.00^{d}	40.00^{de}	34.66^{d}
	1.0	33.33^{c}	36.66^{c}	50.00^{d}	50.00^{d}	50.00^{d}	44.00^{cd}
	1.5	36.66^{c}	66.66^{b}	66.66^{c}	70.00^{c}	70.00^{c}	62.00^{b}
	2.0	53.33^{b}	60.00^{b}	100.00^{a}	100.00^{a}	100.00^{a}	82.66^{a}
Sulfonic	0.5	30.00^{cd}	36.66^{c}	40.00^{de}	40.00^{d}	40.00^{de}	37.33^{d}
	1.0	36.66^{c}	40.00^{c}	40.00^{de}	50.00^{d}	50.00^{d}	43.33^{cd}
	1.5	63.33^{ab}	70.00^{ab}	70.00^{bc}	86.66^{b}	86.66^{b}	75.33^{a}
	2.0	70.00^{a}	80.00^{a}	80.00^{b}	93.33^{ab}	93.33^{ab}	83.33^{a}
Control		0.00^{f}	0.00^{f}	0.00^{g}	0.00^{f}	0.00^{g}	0.00^{f}
P		.0000***	.0000***	.0000***	.0000***	.0000***	.0000***
LSD $_{0.05}$		1.17	1.20	1.11	1.23	1.26	1.00

On the other hand, acetic acid had no any lethal influence against the tested snail species at the concentrations of neither 0.5 nor 1% until the end of trial. It caused only 26.66 and 43.33% mortality of snails at the other concentrations of 1.5 and 2% on the 14th day of treatment and these rates remained constant until the end of the investigation, respectively. The strong toxic effect of acetylsalicylic acid against *E. vermiculata* snail may be due to its influence on the mucous gland, which may attributed to its acidic characteristics and that later leads to the death of the snail individuals (Barakat *et al*., 2006). Moreover, Khidr (2019) reported that adult snails of *Monacha obstructa* were more sensitive to acetic acid than *E. vermiculata* snails and by increasing the tested concentrations of acetic acid, he noticed that the mortality rate of both snail species increases. Acetic acid recorded 25, 35, 40, 50 and 65% mortality of *E. vermiculata* snails at the tested concentrations of 4, 6, 8, 10 and 12% respectively after 72 hrs of exposure. Whereas, the same acid after the same experimental period caused 90, 92.5% mortality of *M. obstructa* snails at the concentrations of 4 and 6% consecutively and completely killed all snail individuals at 8, 10 and 12% concentrations. At the same trend, Capinera (2008) published that boric acid considered an important toxicant against the terrestrial harmful snails, and the concentrated citric acid also had the ability to kill land snails (Mitchell, 2002). Also, Keller *et al*. (2002) demonstrated that linoleic acid showed a significant lethal impact against *Biomphalaria glabrata* snails at the concentration of 50 µg / ml.

Photo 1. Dead adult snails of *E. vermiculata* treated with 0.5% of salicylic acid, after one day showing swelling and extending a part of their soft bodies out the shell.

Efficacy of the tested fertilizers against *Eobania vermiculata* snails after indicated days under laboratory conditions

The molluscicidal toxicity of ammonium hydroxide, copper sulfate and urea fertilizers against *E. vermiculata* snails was evaluated. As shown in Table 2., ammonium hydroxide caused the highest mortality percentage of 76.66% mortality on the first day of investigation at the tested concentration of 2%, then increased to 100% mortality of snails on the third day of the experiment. While, copper sulfate attained 70% mortality at the highest concentration of 2% after one day of exposure and also completely killed all snails by reaching 100% mortality on the third day of the trial.

Table 2. Accretive mortality of *Eobania vermiculata* snails caused by tested fertilizers

Fertilizers	Conc. (%)	Mean of mortality % after indicated days					General mean
		1	3	7	14	21	
Ammonium hydroxide	0.5	0.00^{e}	20.00^{d}	20.00^{f}	20.00^{e}	26.66^{e}	17.33^{e}
	1.0	20.00^{bcd}	50.00^{c}	50.00^{de}	50.00^{cd}	50.00^{cd}	44.00^{cd}
	1.5	60.00^{a}	73.33^{b}	73.33^{b}	73.33^{b}	73.33^{b}	70.66^{b}
	2.0	76.66^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	95.33^{a}
Copper sulfate	0.5	0.00^{e}	20.00^{d}	20.00^{f}	20.00^{e}	20.00^{e}	16.00^{e}
	1.0	33.33^{b}	46.66^{c}	60.00^{cd}	60.00^{bcd}	60.00^{bcd}	52.00^{c}
	1.5	36.66^{b}	83.33^{b}	100.00^{a}	100.00^{a}	100.00^{a}	84.00^{a}
	2.0	70.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	94.00^{a}
Urea	0.5	0.00^{e}	0.00^{e}	0.00^{g}	10.00^{ef}	20.00^{e}	6.00^{ef}
	1.0	10.00^{de}	16.66^{d}	20.00^{f}	20.00^{e}	20.00^{e}	17.33^{e}
	1.5	13.33^{cde}	20.00^{d}	46.66^{e}	46.66^{d}	46.66^{d}	34.66^{d}
	2.0	30.00^{bc}	30.00^{d}	63.33^{bc}	63.33^{bc}	63.33^{bc}	50.00^{c}
Control		0.00^{e}	0.00^{e}	0.00^{g}	0.00^{f}	0.00^{f}	0.00^{f}
P		.0000***	.0000***	.0000***	.0000***	.0000***	.0000***
LSD $_{0.05}$		2.00	1.56	1.32	1.40	1.34	1.25

After one day of treatment with ammonium hydroxide at a concentration of 1%, *E. vermiculata* slowly retracted their soft bodies into the shell and released excess of mucus (Photo 2A). Furthermore, after the same exposure period and at the same concentration (1%) of copper sulfate, the snails withdrawing into their shell and released clear mucus mixed with copper sulfate and the foot of some treated snails extended out the shell (Photo 2B). The lowest molluscicidal influence was achieved by urea causing only 30% mortality at the concentration of 2% on the first day of the experiment, then the mortality significantly increased to 63.33% on the seventh day of the investigation and after that no further increase in effect occurred until the end of the trial. On the other hand, no mortality has been occurred in the control. All the tested fertilizers showed a high significant difference in the means of snail mortality compared to the untreated snails.

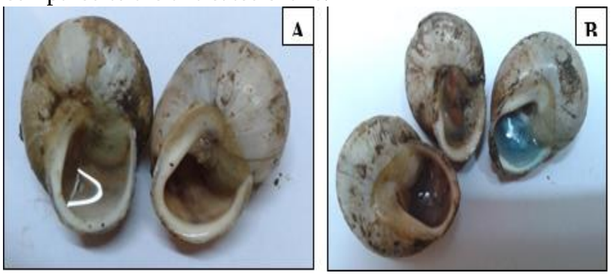


Photo (2 A, B). Effect of fertilizers on adult *E. vermiculata*. (A) dead adult *E. vermiculata* after treated with 1% ammonium hydroxide, after one day showing retraction of the soft bodies into the shell and mucus secretion. (B) dead adult *E. vermiculata* after treated with 1% copper sulfate, after one day the soft bodies were withdrawn into the shell with release mucus and foot extended.

These findings were in accordance with Hend (2013) indicated that urea and ammonium nitrate fertilizers have a significant molluscicidal efficiency against *E. vermiculata* snails, especially at the concentrations of 1.5 and 2% which gave 56.66 & 76.66 and 96.66 & 100% mortality of snails by both fertilizers respectively after 14 days of treatment. Related to this aspect, Nabih *et al*. (1993) stated that urea has a dangerous impact on *Biomphalaria alexandrina* snail; it caused increasing in the glycogen content of the snail and also decreases the haemolymph glucose which referred to accelerate the glycogen synthesis. Moreover, ammonium nitrate showed a negative impact on the metabolism of *Planorbarius purpura* snail (Stadnichenko and Kirichuk, 2000). In another study, Zidan *et al*. (1997) evaluated the toxic effect of ferrous sulfate, ammonium nitrate, ammonium sulfate and potassium sulfate against *Helix aspersa* snail. Ferrous sulfate was the most effective fertilizer against snails followed by ammonium nitrate, ammonium sulfate and potassium sulfate consecutively. Similarly, Mohmoud (1994) reported that ferrous sulfate has significant molluscicidal influence against the terrestrial slug, *Limax maximus* followed by ammonium nitrate and ammonium sulfate, respectively. The other fertilizer, NPK recorded 54.1% mortality of the apple snail, *Pomacea canaliculata* after 7 days of exposure (Cruz *et al*., 2001). On the other hand, copper ammonium carbonate and cinnamamide showed an irritant effect on the snail, *Oxyloma pfeifferi* and decreased the locomotion of the snail which negatively affected on the snail survival (Schuder *et al*., 2004).

Determination the lethal concentrations of tested organic acids and fertilizers against snails

The lethal concentrations of LC_{25} and LC_{50} of tested compounds were determined as indicated in Table 3. and Fig. 1. Sulfonic acid has the highest toxic influence against snails, thus it recorded the lowest LC_{25} and LC_{50} values, which were 0.33 and 0.84% consecutively. On the contrary, boric acid has the highest values of LC_{25} and LC_{50} which were 1.02 and 1.80%, respectively. The toxicity indexes were 97.67, 85.71, 84.00, 50.29 and 46.66% sequentially for copper sulfate, ammonium hydroxide, salicylic acid, urea and boric acid when compared with the most effective compound, sulfonic acid which recorded 100% toxicity index. Whereas, the relative toxicity of sulfonic acid, copper sulfate, ammonium hydroxide, salicylic acid and urea were 2.14, 2.09, 1.84, 1.80 and 1.07 folds, respectively compared with the least effective compound, boric acid.

Table 3. Determination of lethal concentrations of tested compounds

Tested compounds	LC_{25} (%)	LC_{50} (%)	Slop	Toxicity index	Relative toxicity
Boric acid	1.02	1.80	2.71 ± 0.36	46.66	1.00
Salicylic acid	0.40	1.00	1.74 ± 0.30	84.00	1.80
Sulfonic acid	0.33	0.84	1.65 ± 0.28	100	2.14
Ammonium hydroxide	0.71	0.98	3.60 ± 0.40	85.71	1.84
Copper sulfate	0.55	0.86	3.53 ± 0.41	97.67	2.09
Urea	1.09	1.67	3.64 ± 0.63	50.29	1.07

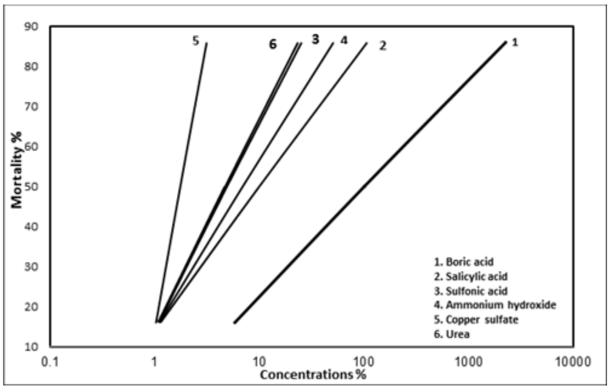


Fig. 1. Toxicity regression lines of tested compounds against *Eobania vermiculata* snails

In this regard, Mobarak (2008) recorded that the LC_{50} of acetylsalicylic acid against *E. vermiculata* and *M. obstructa* snails were 0.67 and 0.3 mg / cm^2, respectively. While, the LC_{50} values of acetic acid against both snails were 8.69 and 2.19% after 72 hrs of application consecutively (Khidr, 2019). In a related study, Mostafa *et al*. (2005) showed that the LC_{50} of copper sulfate against *B. alexandrina* snail was 0.42 ppm. Whereas, the LC_{50} and LC_{90} of ammonium chloride against the same snail species were 90 and 130 ppm, respectively (El-Sayed, 2001). The fertilizers; ammonium nitrate, ferrous sulfate, ammonium sulfate, potassium sulfate and calcium super phosphate were achieved 4.48, 2.48, 7.77, 15.33 and 9.81% as the LC_{50} values of these fertilizers sequentially against the land snail *H. aspersa* (Asran, 1994). On the other hand, in the treatment of *Pomacea paludisa* snail with nitrate, LC_{50} could not be determined due to low rates of mortality despite nitrate at concentrations more than 500 ppm (Norah *et al*., 2006).

Toxic potency of binary mixtures against snails

The toxic effect of mixing the LC_{25} of each of boric, salicylic and sulfonic acids with the LC_{25} of ammonium hydroxide, copper sulfate and urea against the snails of *E. vermiculata* was demonstrated in Table 4. The binary mixture of salicylic acid with ammonium hydroxide and sulfonic acid with urea were the most potent mixtures against snails, they caused 100% mortality after only one day of exposure. They were followed by the mixing of sulfonic acid with copper sulfate and salicylic acid with urea which caused 96.66 and 93.33% mortality of snails after the same period of exposure (one day), consecutively. On the other hand, the combination of boric acid with copper sulfate and sulfonic acid with ammonium hydroxide attained the same mortality rate 90% on the first day of treatment and this rate remained fixed for both mixtures until the end of the investigation. No mortality occurred until the third day of exposure to each of boric acid with ammonium hydroxide and salicylic acid with copper sulfate. After seven days of the experiment, the mixing of boric acid with ammonium hydroxide recorded 36.66% mortality of snails, then increased to 50% on the 14th day and then no more increase in effect occurred until the end of the trial. While in the case of salicylic acid and copper sulfate mixture, it gave 16.66% mortality of snails on the seventh day of the investigation, then this rate slightly increased to 23.33% mortality on the 14th day after which no further increase in mortality attained until the end of the experiment. All snails in the control were survived until the end of the investigation and high significant difference was recorded between the means of snail mortality caused by the tested binary mixtures in comparison with the control.

Table 4. Accretive mortality of *Eobania vermiculata* snails caused by the binary mixtures of organic acids (LC_{25}) with fertilizers (LC_{25})

Binary mixtures	Mean of mortality % after indicated days					General mean
	1	3	7	14	21	
Boric acid + Ammonium hydroxide	0.00^{c}	0.00^{c}	36.66^{c}	50.00^{c}	50.00^{c}	27.33^{c}
Boric acid + Copper sulfate	90.00^{a}	90.00^{a}	90.00^{ab}	90.00^{ab}	90.00^{ab}	90.00^{a}
Boric acid + Urea	73.33^{b}	73.33^{b}	80.00^{b}	80.00^{b}	80.00^{b}	77.33^{b}
Salicylic acid + Ammonium hydroxide	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
Salicylic acid + Copper sulfate	0.00^{c}	0.00^{c}	16.66^{d}	23.33^{d}	23.33^{d}	12.66^{d}
Salicylic acid + Urea	93.33^{a}	93.33^{a}	93.33^{a}	93.33^{a}	93.33^{a}	93.33^{a}
Sulfonic acid + Ammonium hydroxide	90.00^{a}	90.00^{a}	90.00^{ab}	90.00^{ab}	90.00^{ab}	90.00^{a}
Sulfonic acid + Copper sulfate	96.66^{a}	96.66^{a}	96.66^{a}	100.00^{a}	100.00^{a}	98.00^{a}
Sulfonic acid + Urea	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
Control	0.00^{c}	0.00^{c}	0.00^{e}	0.00^{e}	0.00^{e}	0.00^{e}
P	.0000***	.0000***	.0000***	.0000***	.0000***	.0000***
$LSD_{0.05}$	1.07	1.07	1.12	1.16	1.16	1.03

These outcomes were supported by Godan (1983) published that the effectiveness of molluscicides against snails increases in a combination with other active ingredient. Similarly, Sallam and El-Wakeil (2012) reported that the combinations of salts with other different molluscicides in a bait formulation or by spraying represent an effective means for control exotic snails. Mixing of acetylsalicylic acid with methomyl was more effective against *E. vermiculata* and *M. obstructa* snails than the use of each compound individually (Kandil *et al*., 2014). In the same aspect, Rao and Singh (2002) confirmed that the combination of *Cedrus deodara* oil with *Allium sativum* induced a high lethal effect against the terrestrial snail, *Achatina fulica*. The dangerous impact of carbaryl and piperonyl mixture against *Lymnaea acuminata* snail may be due to decrease the glycogen, protein levels and alkaline phosphatase activity of the snail (Singh and Agarwal, 1989).

Joint action of tested compounds against *Eobania vermiculata* snails after 21 days mortality

The joint action between tested organic acids and fertilizers against *E. vermiculata* snails was investigated. As shown in Table 5. after 21 days of treatment, the binary mixing of boric acid with copper sulfate, boric acid with urea, salicylic acid with ammonium hydroxide, salicylic acid with urea and sulfonic acid with ammonium hydroxide showed a high potentiation influence against snails with co-toxicity factors of 92.88, 71.45, 72.41, 79.48 and 76.47%, respectively. Moreover, the combination of sulfonic acid with each of copper sulfate and urea were also achieved an observed potentiation impact against snails with the same co-toxicity factor 127.27%. One additive effect was recorded in the combination of boric acid with ammonium hydroxide with co-toxicity factor - 6.24%. On the other hand, the binary mixing of salicylic acid with copper sulfate induced an antagonistic influence against snails with a co-toxicity factor – 55.13%. These findings were in harmony with the earlier study reported by El-Fiki and Mohamed (1978) showed that the binary mixing of copper sulfate with each of Treflan and Preforan herbicides attained a potentiation toxicity against *B. alexandrina* snails. While, the combination of each tested herbicide with Niclosamide recorded an antagonistic activity against the same snail. Moreover, antagonistic action was achieved in the combination of copper sulfate with Diuron herbicide against the individuals of the same snail species (Zedan *et al*., 1990). On the other hand, the mixing of Zn, Cu, Pb and Cd showed an additive activity against the adults of *H. aspersa* snail (Laskowski and Hopkin, 1996). The binary mixing of Vinclozolin with benzo (a) pyrene or fluoxetine with bisphenol A exerts an additive influence against *Physa acuta* snail. The toxicity of these mixtures higher than the single exposures (Sanchez-Arquello *et al*., 2012). In the same trend, Barakat *et al*. (2006) demonstrated that the addition of tannic acid to abamectin caused significant potentiation impact recorded 100% (Co – toxicity factor) with 100% mortality of *M. obstructa* snails and 60% mortality of *E. vermiculata* snails with 20% Co – toxicity factor. Moreover, there is a potentiation activity was resulting from the combination of tannic acid with acetylsalicylic acid against both snails. This potentiation effect may be attributed to the fast reaction of acids with detoxification enzyme lead to the formation of a substrate enzyme complex which is stable and block the detoxifying enzyme. While, the binary combination of urea with mollutox gave an additive effect against *B. alexandrina* and *Lymnaea natalensis* snails. Additionally, the mixing of urea with copper sulfate caused great reduction in the growth and survival rate of the two snail species (Ragab and Shoukry, 2006).

Table 5. Joint action of organic acids and fertilizers as binary mixtures against *E. vermiculata* snails

Binary mixtures	Measurement	21 days Mortality	Co-toxicity factor
Boric acid + Ammonium hydroxide	Observed	50.00	- 6.24 d
	Expected	53.33	
Boric acid + Copper sulfate	Observed	90.00	92.88 p
	Expected	46.66	
Boric acid + Urea	Observed	80.00	71.45 p
	Expected	46.66	
Salicylic acid + Ammonium hydroxide	Observed	100.00	72.41 p
	Expected	58.00	
Salicylic acid + Copper sulfate	Observed	23.33	- 55.13 a
	Expected	52.00	
Salicylic acid + Urea	Observed	93.33	79.48 p
	Expected	52.00	
Sulfonic acid + Ammonium hydroxide	Observed	90.00	76.47 p
	Expected	51.00	
Sulfonic acid + Copper sulfate	Observed	100.00	127.27 p
	Expected	44.00	
Sulfonic acid + Urea	Observed	100.00	127.27 p
	Expected	44.00	

a = antagonism d = additive effect p = potentiation (synergism)

CONCLUSION

The results of this study shows that tested organic compounds could be used as successful molluscicides for controlling the terrestrial snail, *E. vermiculata*. Moreover, the binary mixing of these compounds especially salicylic acid with ammonium hydroxide and sulfonic acid with urea have a potent influence against the same snail species more than the use of each compound alone. These compounds are easily biodegradable and safe when used in the field by spraying at low rates. They could provide promising distinctive substitutes to the chemical pesticides in the control of the harmful snail, *E. vermiculata*.

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تقدير سميه بعض الأحماض العضويه و الأسمده ضد القوقع الأرضي إيوبانيا فيرميكيولاتا

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الملخص

أجريت هذه الدراسه بهدف إختبار التأثير القاتل للأحماض العضويه, حمض بوريك – سالسيلك و سالفونيك و الأسمده , هيدروكسيد أمونيوم – كبريتات نحاس و يوريا كلا على حدى ضد الأفراد البالغه لقوقع إيوبانيا فيرميكيولاتا عند التركيزات 0،5٪ و 1٪ و 1،5٪ و 2٪ تحت الظروف المعمليه. تم تحديد التركيز ربع مميت والنصف مميت من كل مركب و إختبار التأثير القاتل للمخاليط الثنائيه ما بين الأحماض العضويه و الأسمده عند التركيز الربع قاتل من كل مركب ضد الأفراد البالغه. أشارت النتائج إلى أن جميع المركبات عند أستخدامها بشكل فردي قد حققت أعلى تأثير قاتل للقواقع عند أعلى تركيز 2٪ كما حقق حمض السالفونيك أعلى تأثير ضد القواقع مقارنه بالأحماض الأخرى بينما كانت كبريتات النحاس هي الأعلى تأثيرا على القواقع مقارنه بباقي الأسمده. أما الخلط الثنائي بين المركبات المختبره أظهرت النتائج أن خليط حمض السالسيلك مع هيدروكسيد الأمونيوم و أيضا خليط حمض السالفونيك مع اليوريا قد حققا أعلى تأثير قاتل للقواقع بتسجيل 100٪ موت بعد يوم فقط من المعامله. بينما كان لخلط حمض السالسيلك مع كبريتات النحاس أقل تأثير قاتل حيث حقق 23،33٪ موت فقط بعد 21 يوم من التجربه. كما أوضحت النتائج أن جميع المخاليط الثنائيه قد سجلت تأثيرا تنشيطيا عاليا ضد القواقع خاصه خليط حمض السالفونيك مع كبريتات النحاس و خليط نفس الحمض مع اليوريا حيث حققا 127،27٪ كمعامل سميه بينما كان لخلط حمض البوريك مع هيدروكسيد الأمونيوم تأثيرا إضافيا كما تم تسجيل تأثيرا عكسيا واحدا لخلط حمض السالسيلك مع كبريتات النحاس مما يشيرإلى أن سميه المخاليط الثنائيه للمركبات المختبره ضد القواقع هي أعلى من سميه كل مركب عند إستخدامه بشكل فردي.