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RESEARCH ARTICLE

# Efficacy of Two Natural Materials Against Some Invertebrate Pests as a Safe Alternative to Pesticides and Their Bio-Safeties on Mammalian

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The continuous use of pesticides can cause many serious problems in the environmental system. So, it is very necessary to go back to nature again and look for safe alternatives to chemical pesticides. During this research, we evaluated the toxicity of two types of natural substances (wood vinegar and seaweed extract) against three types of zoological pests, two-spotted spider mites (*Tetranychus urticae*) and two land snails (*Monacha cartusiana* and *Eobania vermiculata*). Results indicated that wood vinegar and seaweed extract had negative effects on eggs, immature stages and adults of *T. urticae*. Their mortalities were significantly increased with increasing of the applied dose of wood vinegar and seaweed extract. The highest mortalities of eggs, immature stages and adults of *T. urticae* were 82.9, 71.8 and 98.4% and were 75.3, 62.9 and 83.9% after 72 h from the application of wood vinegar and seaweed extract, respectively. Data showed also a positive relationship between the mortality percentages of snails and the concentration. The highest mortality rates for *M. cartusiana* and *E. vermiculata* (73.91 and 60.87%) and (91.67 and 83.33%) were recorded due to the use of wood vinegar and seaweed extract at the concentration of 25% individually. The values of $LC_{50}$ and $LC_{90}$ were calculated and the results confirmed that the seaweed extract was more toxic for the tested land snails than wood vinegar. The higher values of $LC_{50}$ of wood vinegar and seaweed extract (21.67% and 11.87%) were selected for the biosafety tests in mammals by using *Rattus norvegicus albinus* males. It was clear that both wood vinegar and seaweed extract had no harmful effects on mammals according to the non- significant differences in liver and kidney functions (ALP, AST, ALT, Creatinine and urea in the blood samples) between treated and untreated rats.

**Keywords:** *Tetranychus urticae*, *Monacha cartusiana*, *Eobania vermiculata*, Wood Vinegar, Seaweed-Extract, *Rattus norvegicus albinus*.

## 1. INTRODUCTION

Plant arthropods such as mites can use the plants as a food source, causing generous damage and yield loss in many crops. The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is a disreputable phytophagous belonging to the family Tetranychidae [1]. Moreover, it is one of the most destructive and resistant arthropod pests in the world and can cause a dangerous threat for agricultural crops under field and greenhouse conditions because of its short life cycle, high offspring production, and ability to develop pesticide resistance [2–4]. The land snails, *Monacha cartusiana* and *Eobania vermiculata* are invertebrate pests that appear at many governorates of Egypt, especially in Northeast of Delta [5]. These pests caused much damage to different types of plants that appear on different parts of plants, starting from the roots below the surface of the soil to the stems, leaves and fruits. These pests belong to the mollusk tribe and describe the gastropods. Many different types of pesticides have been used

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in recent years to reduce the number and appearance of these pests and thus reduce their harm [6, 7]. The use of synthetic pesticides is a common and effective practice to control mites and snails in the past but it is responsible for many harmful impacts on the target pests and human health and leads to a clear imbalance in the ecological system through increasing the environmental pollution [7, 8]. Moreover, it can be responsible for the extinction or reduction in the numbers of natural enemies, which have effective roles in biological management in recent years [9–11]. So, it is imperative to find alternative natural materials to pesticides without injurious effects on biodiversity and ecological balance. Among these natural substances, wood vinegar and marine algae extract gain concentrated attention by many scientists. Wood vinegar is a brown-dark liquid obtained as a secondary material from biochar production by the pyrolysis process (carbonization) of agricultural wastes in airless conditions [12–14]. Wood vinegar is mainly composed of organic compounds such as organic acids, phenols, aldehydes, alcohols, esters, ketones, furan and pyran derivatives, hydrocarbons, and nitrogen compounds [14–16]. It has been used in many useful aspects in agriculture in terms of growth and improvement of some productive qualities of some plants [17, 18]. Also, wood vinegar has been tested as a dermatological antifungal and has been used to control many fungal, insect and animal pests, and it has shown excellent results in reducing the population of these pests [19–22]. Some studies have indicated that the use of wood vinegar in controlling various pests at certain concentrations does not have any negative impact on the environment, humans and other beneficial animals in the soil. Moreover, it has increased the beneficial biological activity within the agricultural soil [23]. The efficiency of wood vinegar in controlling the pests was strongly depended upon their phenolic compound contents [24]. The seaweed extract of marine macro-algae is a natural organic material and can be used in fertilization because it contains many nutrients that have an effective role in seed germination and improving the productive qualities of some plants [25]. Marine algae have a large and effective role in the ecological balance and have been used for years in many areas beneficial to humans and other living organisms, where marine algae extract has been used in the field of medicines and biological materials, as it is a good store for many stimulants [26]. Marine algae extract has been used against some internal parasitic worms of some animals, and it has shown great effectiveness without any side effects [27]. These marine algae are available on many Egyptian coasts and have been used as antimicrobial agents in foodstuffs, they have also been used as a stimulant to increase plant resistance and increase productivity [28]. Marine algae extract gave very good results in controlling many agricultural insect and animal pests without causing any environmental pollution or appearance of any negative effects on other living organisms [29–31].

Many studies have been conducted to evaluate the effect of wood vinegar or seaweed extract on various pests but their potential effects on spider mites or land snails have not been documented well. So, the main objective of this research is to test two natural and eco-friendly materials (wood vinegar and seaweed extract) as alternatives to pesticides to control some agriculture invertebrate pests (spider mites and snails).

## 2. MATERIALS AND METHODS

### 2.1. Wood Vinegar Production

Rice straw (RS) and peanut residues (PR) were collected from the Agronomy farm of the Faculty of Agriculture, Benha University, Egypt. Both RS and PR were mixed, air-dried for a week to control the water content within 5% and then crushed into small pieces (1–2 cm). The dried RS and PR were charred at 400–450 °C for 6 h in the reactor by slow pyrolysis [32]. Wood vinegar was simultaneously collected during the biochar production using a circulating water condensation system. The hot steam condensed into liquid was collected. It is called raw wood vinegar and is necessary to pure before using. The wood vinegar was characterized with pH (3.25), K (9.29 mg $kg^{-1}$), Ca (13.8 mg $kg^{-1}$), Mg (2.47 mg $kg^{-1}$) and Fe (4563 mg $kg^{-1}$).

### 2.2. Seaweed Extract

The seaweed extract (Alga 600) was purchased from the Techno-green Company, Heliopolis, and Cairo, Egypt). The seaweed contained a mixture of *Laminaria* spp., *Ascophyllum nodosum*, and *Sargassum* sp. The tested seaweed extract was characterized with pH (8.96), N (0.5–0.8%), K (17–19%), Ca (0.6–1.8%), Mg (0.04%), Fe (0.15–0.3%), S (1–1.5%), and alginic acids (11–14%). The used natural materials were shown in Figure 1.

### 2.3. Mite Rearing

*T. urticae* was taken from a vegetable field at Faculty of Agriculturist, Benha University, Egypt. It was raised and mass maintained on the leaves of the common bean,


**Fig. 1.** The natural materials.

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*Phaseolus vulgaris*, which were placed on dampening cotton in Petri-dishes (12 cm in diameter) and the cotton was moistened daily under laboratory conditions of 25 °C, 60% RH, and a photoperiod of 16:8 h. (L:D).

### 2.4. Toxicity Bioassays Against Immature Stages and Adults of *T. urticae*

Five concentrations of wood vinegar or sea-weed extract (0 = control, 0.5, 1, 1.5, 2 and 2.5%) were applied with four replicates. Leaf disks (3 cm diameter) of common bean were submerged in each concentration of either wood vinegar or sea-weed extract for 30 s and allowed to air dry for 1 h at room temperature. Afterward, twenty immature stages or young females of *T. urticae* were put on each leaf disk and then placed on saturated cotton wool (0.5 cm thick) in a plastic petri-dish with an aeration hole (1 cm diameter). To prevent *T. urticae* from escaping, a ring of Vaseline was placed around the leaf disks. The mortalities of *T. urticae* (immature stages and adults) were observed under a dissecting microscope at three exposure times (24, 48 and 72 h).

### 2.5. Toxicity Bioassays Against Eggs of *T. urticae*

Bioassays were used to test the acaricidal activity of wood vinegar or seaweed extract against the egg stage. Thirty females of *T. urticae* were allowed to ovipositor on the dorsal side of common bean leaf disks. The adults were removed after 24 h and fifteen fresh eggs were transferred by a fine brush on each experimental leaf disk and then sprayed with the chosen concentrations of wood vinegar or seaweed extract (0 = control, 0.5, 1, 1.5, 2 and 2.5%). In the control treatment (0%), the eggs were sprayed with distilled water. All experiments were conducted under laboratory conditions at $25\pm1$ °C, $60\pm10$% RH and 16:8 h L: D photoperiod. The egg hatching was recorded at three exposure times (24, 48, and 72 h.) for all the tested treatments.

### 2.6. Preparing of Land Snails

This experiment was conducted in the Plant Protection Department, Faculty of Agriculture, Benha University. Adults of two types of land snails (*Monacha cartusiana* and *Eobania vermiculata*) were collected from different plants and brought to the laboratory. The snails were cleaned well with running water, and healthy individuals of the same size were selected. The snails were placed in large plastic dishes; each dish was containing moist clay soil, and was covered with a light breathable cloth. Fresh lettuce leaves were continuously served as a food source for individuals. The food source was withheld from the snails seven days before the start of the experiment.

### 2.7. Toxicity Bioassays Against Land Snails

Five concentrations of wood vinegar or marine algae extract (5, 10, 15, 20, and 25%) were prepared by using distilled water to test their toxic effects on *M. cartusiana* and *E. vermiculata*. For each concentration, four replicates were performed, and each replicate contained six adult snails. The tested concentrations were sprayed on fresh lettuce leaves and then left to dry and presented to the tested snails. The numbers of dead and live individuals were calculated daily for seven consecutive days.

The percent of cumulative mortalities and corrected mortalities of all the experimental animals were calculated during all experiment days using Abbott's formula [33].

$$\% \text{ Mortality} = (a - b/a) \times 100$$

Where:

a = the initial numbers of individuals placed in each treatment.

b = the numbers of individuals still alive per treatment.

Corrected % mortality = (b-a /100 – a) × 100

Where:

b = % mortality in treatment.

a = % mortality in control.

### 2.8. Mammalian Biosafety Experiment

This work was carried out in the Plant Protection Department, Faculty of Agriculture, Benha University, Egypt. The laboratory of animal research at Faculty of Veterinary Medicine, Benha University, Egypt, provided the healthy adult males of the white albino rat strain, *Rattus norvegicus* albinus (Berk). The rat was about 150 g in weight. The rats were kept in metal cages and fed a diet that included 21 percent protein, 4.59 percent fat, 4.20 percent fibre, and water. The animals were acclimatized to laboratory conditions for two weeks before the experiment began. The high doses of $LC_{50}$ values 21.67 and 11.87% of wood vinegar and seaweed extract were chosen. In this trial, 21.67 ml and 11.87 ml of wood vinegar or seaweed extract were mixed with 78.33 and 88.13 g of *R. norvegicus albinus* feed, respectively. Rats were fed on this mixture for one month before the biochemical tests. Levels of Alkaline Phosphatase (ALP), Aspartate amino transferase (AST), Alanine Transaminase (ALT), Creatinine (CREA) and Urea were measured in the rat's blood. Three replicates were used for each concentration plus to the control, and each replicate had three adult male of *R. norvegicus albinus*. Blood samples were drawn from the individual's hearts directly to serum tubes, and then placed under a centrifugal force of 3000 rounds per minute (rpm) for up to 15 consecutive minutes. The samples were kept at a very low temperature(−20 °C) according to the method of Raoofi et al. [34]. The serum levels of AST, ALT, ALP, creatinine and urea were determined spectrophotometrically as described by Madaki et al. [35]. All biochemical parameters were carried out in Dr. Mahmoud Abou El. Makarem laboratory, Toukh, Qalubia Governorate, Egypt.

### 2.9. Statistical Analysis

The statistical analysis was carried out using two-way ANOVA using SPSS ver. 25. Data were arranged as a complete randomization design according to Steel et al. [36]. Multiple comparisons were carried out applying Duncan test. The significance level was set at $p < 0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Sensitivity of Mites to Wood Vinegar and Marine Algae Extract

The use of wood vinegar and seaweed extract at the used concentrations (0.5, 1, 1.5, 2 and 2.5%) caused marked mortalities in eggs, immature stages and adults of *T. urticae*, Table I.

The results proved that the negative effects become more noticeable with increasing of their applied doses at all chosen contact times (24, 48 and 72 hours). The increase of the contact time between *T. urticae* and wood vinegar or seaweed extract led to considerable drops in its eggs, immature stages and adults. The highest reductions were recorded due to using 2.5% of wood vinegar or seaweed extract after 72 h. The toxic effect of wood vinegar on eggs, immature stages and adults of *T. urticae* was higher than that of seaweed extract. The eggs of *T. urticae* decreased by 26.5–18.9%, 35.7–24.5% and 48.3–31.7% and by 63.2–54.2%, 82.9–71.7% and 93.4–75.3% after contact times of 24, 48 and 72 hours when the dose of wood vinegar and seaweed extract changed from 0.5% to 2.5%. Reductions of immature stages by 22.4–15.3%, 30.8–20.4% and 36.7–26.5% were recorded under 0.5%, and by 51.9–45.7%, 71.8–60.4% and 77.4–62.9% under 2.5% of wood vinegar or seaweed extract at contact times of 24, 48 and 72 hours, respectively. The highest decreases in adults of *T. urticae* after contact times of 24, 48 and 72 hours were 91.3, 95.5 and 98.4% and were 75.9, 80.2 and 83.9%, respectively, when wood vinegar or seaweed extract was applied at a concentration of 2.5%.

These results confirmed that both wood vinegar and seaweed extract could be used as a good strategy to control *T. urticae.* It was shown by Pangnakorn et al. [37] that wood vinegar succeeded to control mosquito larvae (*Culex quinquefasciatus* Say) and increased the mortality by 16.25, 30, 35.75 and 82.5% after 48 h, and by 22.5, 40, 47.5 and 96.25% after 72 h from its application by dropping method at concentrations of 0.5, 1, 1.5 and 2%, respectively. Yamauchi et al. [38] examined the effects of wood vinegar on red mites (*Dermanyssus gallinae*, poultry red mite chicken mite), its safety test for chicken, and egg production. They found that wood vinegar could be used as a useful natural substance to exterminate red mites without any marked reduction in egg production. It was noticed by Pangnakorn and Chuenchooklin [39] that numbers of the citrus leaf miner (*Phyllocnistis citrella)* declined from 83 and 81 to 23.5 and 34.15, while numbers of thrips (*Scirtothrips dorsalis*) were decreased 79.5 and 72.15 to 17.8 and 34.65 after 1 and 15 days from wood vinegar application. They also reported that the marked ability of wood vinegar solution to control this insect could result from its acidity and the presence of some chemical compounds such as formaldehyde, propionic acid and phenol. Hossain et al. [40] investigated the insecticidal action of wood vinegars imitative by the fast pyrolysis process of lignin, cellulose, and hemicellulose at 450 and 550 °C, and indicated that both of them had toxic effects on *Acyrthosiphon pisum*. Oramahi et al. [41] reported that the presence of phenolic in wood vinegars could be initially responsible for their insecticidal concerts against *Coptotermes formosanus*, especially at the high chosen concentrations (7.5 and 10%).

For the seaweed extract, Asharaja and Sahayaraj [42] evaluated the competence of seaweed extracts (*Sargassum swartzii* and *Padina pavonica*) at different concentrations (100, 200, 300, 400 and 500 ppm) in controlling cotton leaf miner *Dysdercus cingulatus*. They indicated

**Table I.** Effect of wood vinegar and seaweed extract against *T. urticae* under laboratory conditions.

| | | Mortality of *Tetranychus urticae* stages (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Treatments | | Egg | | | Immature | | | Adult | | |
| | Con. | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h | 24 h | 48 h | 72 h |
| | 0 | – | – | – | – | – | – | – | – | – |
| Wood vinegar | 0.5% | 26.5 e | 35.7 f | 48.3 f | 22.4 f | 30.8 e | 36.7 f | 41.3 f | 53.6 f | 60.7 e |
| | 1.0% | 38.4 d | 51.3 d | 66.5 d | 27.3 e | 38.1 d | 49.5 e | 50.7 e | 62.9 e | 74.5 d |
| | 1.5% | 47.6 c | 59.2 c | 79.3 c | 31.6 d | 45.9 c | 57.2 d | 63.1 d | 76.3 c | 85.0 c |
| | 2% | 56.5 b | 71.4 b | 85.3 b | 43.7 b | 60.1 b | 69.6 b | 80.3 b | 86.7 b | 93.9 b |
| | 2.5% | 63.2 a | 82.9 a | 93.4 a | 51.9 a | 71.8 a | 77.4 a | 91.3 a | 95.5 a | 98.4 a |
| Sea-weed extract | 0.5% | 18.9 f | 24.5 g | 31.7 g | 15.3 g | 20.4 f | 26.5 g | 37.4 f | 49.6 f | 54.2 f |
| | 1.0% | 27.3 e | 35.1 f | 45.6 f | 24.3 f | 29.5 e | 37.3 f | 43.1 f | 55.8 f | 63.7 e |
| | 1.5% | 38.6 d | 43.3 e | 58.1 e | 33.9 d | 37.1 d | 49.6 e | 51.9 e | 63.7 e | 72.6 d |
| | 2% | 42.7 c | 56.8 c | 64.9 d | 36.8 c | 48.2 c | 54.3 d | 68.1 c | 71.5 d | 79.8 c |
| | 2.5% | 54.2 b | 71.7 b | 75.3 c | 45.7 b | 60.4 b | 62.9 c | 75.9 b | 80.2 c | 83.9 c |

*Note*: Con. = Concentrations. Different letters (a–g) within the same column indicated significant differences between the treatments at $p < 0.05$.

that the used seaweed extracts were negatively affected several biological parameters of *D. cingulatus* could be applied as efficient bio-insecticides. The ethanolic extracts of three macro-algal types (*Laurencia johnstonii*, *Sargassum horridum* and *Caulerpa sertularioides*) caused significant insecticidal and revolting actions against adults of *Diaphorina citri*. Moreover, the chemical structure of these algae contained alkaloids, terpenes, tannins, flavonoids, saponins, anthraquinones and phenols could be responsible for their insecticidal and repellent activity. The existence of the phenolic compounds such as quercetin, kaempferol, and resorcinol in *Arthrospira platensis* could illustrate its insecticidal action against cotton leaf-worm *Spodoptera littoralis* [43]. Results of Rashwan and Morsi [44] showed the negative effects of two algae *Fucus vesiculosus* and *Spirulina platensi* as natural alternative pesticides against *Bruchidius incarnatus* stages. Great noteworthy changes were recorded in the mortality percentages between two algae in comparison to the control treatment. The mortalities of its larvae and adults after 10 days were 32–29.7, 39–32.8 and 43–36.3% and 53–68.2, 67–72.3 and 89–81.2% due to the application of 0.25, 0.35 and 0.50 g *F. vesiculosus* or *S. platensi*, respectively. They explained the efficient effect of *F. vesiculosus* and *S. platensi* against *B. incarnatus* with the presence of some biological components such as phenolic substances, fatty acids and terpenoids, which might be responsible for their insecticidal activities. Asimakis et al. [45] reported that marine macro algae extracts were safer than synthetic products and exhibited insecticidal/acaricidal activity as eco-friendly methods in the integrated pest management.

### 3.2. Sensitivity of *M. cartusiana* and *E. vermiculata* to Wood Vinegar

The sensitivity of *M. cartusiana* and *E. vermiculata* adults to wood vinegar during seven consecutive days with five different concentrations (5, 10, 15, 20, and 25%) was presented in Table II.

The mortality percentages were 12.50, 16.67, 20.83% for *M. cartusiana* during the second day of treatment for the higher concentrations (15, 20 and 25%), while it was

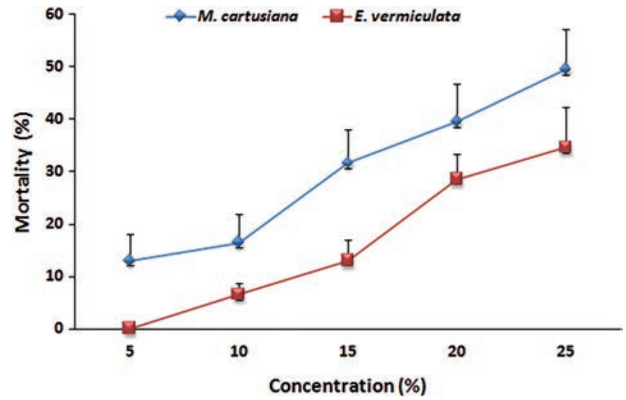


**Fig. 2.** Mean effect of wood vinegar on *M. cartusiana* and *E. vermiculata* under laboratory conditions.

only 4.35 and 4.17% with the lower concentrations (5 and 10%) during the fifth and third days, respectively. Death rates gradually increased for all five concentrations, reaching 21.74, 34.78, 56.52, 65.22 and 73.91% on the seventh day.

As for *E. vermiculata*, no deaths occurred during all experiment days when it was treated with a concentration of 5%, while a slight death rate of 4.35% was appeared on the sixth day at 10% of wood vinegar. At the higher concentrations (15, 20 and 25%) of wood vinegar, the mortality percentages were 4.35, 16.67 and 8.33% on the fourth, third and second days, respectively. These mortality rates reached their maximum on the seventh day of treatment with 8.69, 21.74, 43.47 and 60.87% for concentrations 10, 15, 20, 25% of wood vinegar, respectively.

From the mortalities mean presented in Table II and Figure 2, data confirmed that *M. cartusiana* was more sensitive to wood vinegar than *E. vermiculata* snail, especially at its high concentrations. These means were 13.04, 16.48, 31.64, 39.52 and 49.58% for *M. cartusiana*, while they were 0.00, 6.52, 13.04, 28.55 and 34.57% only for *E. vermiculata* under the tested concentrations.

Data of $LC_{50}$ and $LC_{90}$ values of wood vinegar on the tested snails were presented in Table III. $LC_{50}$ and $LC_{90}$ values reached 12.14 and 53.77% for *M. cartusiana*, while

**Table II.** Effect of wood vinegar on *M. cartusiana* and *E. vermiculata* under laboratory conditions.

| | | Cumulative mortality % within seven days | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Snails | Con. (%) | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Mean |
| *M. cartusiana* | 5 | – | – | – | – | 4.35 | 13.04 | 21.74 | 13.04 |
| | 10 | – | – | 4.17 | 8.69 | 13.04 | 21.74 | 34.78 | 16.48 |
| | 15 | – | 12.50 | 20.83 | 26.09 | 34.78 | 39.13 | 56.52 | 31.64 |
| | 20 | – | 16.67 | 29.17 | 30.43 | 43.47 | 52.18 | 65.22 | 39.52 |
| | 25 | – | 20.83 | 37.50 | 47.82 | 56.52 | 60.87 | 73.91 | 49.57 |
| *E. vermiculata* | 5 | – | – | – | – | – | – | – | 0.00 |
| | 10 | – | – | – | – | – | 4.35 | 8.69 | 6.520 |
| | 15 | – | – | – | 4.35 | 8.69 | 17.39 | 21.74 | 13.04 |
| | 20 | – | – | 16.67 | 21.74 | 26.09 | 34.78 | 43.47 | 28.55 |
| | 25 | – | 8.33 | 20.83 | 30.43 | 39.13 | 47.82 | 60.87 | 34.57 |
| Control | | – | – | – | 4.17 | 4.17 | 4.17 | 4.17 | 4.17 |

**Table III.** $LC_{50}$ and $LC_{90}$ values of wood vinegar against *M. cartusiana* and *E. vermiculata.*

| Snails | P. Value | $LC_{50}$ % | $LC_{90}$ % | Lower 95% | Upper 95% | Slope | R |
|---|---|---|---|---|---|---|---|
| *M. cartusiana* | 0.9211 | 12.14 | 53.77 | 42.96 | 521.65 | 1.9828 | 0.9858 |
| *E. vermiculata* | 0.7789 | 21.67 | 55.03 | 44.14 | 244.31 | 3.6159 | 0.9802 |

*Note*: R = Correlation coefficient of the regression line.

they became 21.67 and 55.03% for *E. vermiculata* after seven consecutive days. This could confirm that the wood vinegar compound was more toxic to *M. cartusiana* than *E. vermiculata*.

It is clear from the above-mentioned results that the wood vinegar had a marked toxic influence on some invertebrate pests such as *M. cartusiana* and *E. vermiculata*, which established its potential ability in combating them. These results are in agreement with Hagner et al. [46], who proved that wood vinegar had a high effect in controlling some invertebrate pests such as terrestrial mollusk, and also it could be used as a repellent material against *Arianta arbustorum* snail with significant results. Koraag et al. [47] also showed that wood vinegar had a powerful lethal effect on mollusks pests such as *Oncomelania hupensis lindo ensis* snail, the vector of *schistosomiasis* disease.

### 3.3. Sensitivity of *M. cartusiana* and *E. vermiculata* to Seaweed Extracts

The effect of seaweed extract on *M. cartusiana* and *E. vermiculata* under laboratory conditions during seven consecutive days was shown in Table IV.

The results confirmed the sensitivity of *M. cartusiana* to marine algae extract on the first day for all tested concentrations (5, 10, 15, 20, and 25%) with mortalities of 4.17, 8.33, 16.67, 20.83 and 20.83%, respectively. Mortality percentages were gradually enlarged by increasing the tested concentrations of seaweed extract on all the experiment days until they reached their peaks on the seventh day. These results indicated the existence of a positive relationship between the concentration and the mortality percentage. The highest effect of marine algae extracts occurred during the seventh day post treatment with 83.33 and 91.67% mortality for 20 and 25% concentrations.

The mortality percentages for *E. vermiculata* were 8.33 and 4.17% due to the of 5 and 10% of seaweed extract during the fourth and third days compared to the higher concentrations (15, 20 and 25%) of seaweed extract, which caused mortality by 8.33, 8.33 and 12.50% after one day only from the treatment. On the other hand, the highest concentrations of seaweed extract (20 and 25%) triggered the highest mortality percentages (75.00 and 83.33%) during the seventh day post treatment.

The death means for *M. cartusiana* and *E. vermiculata* after seven days of treatment were shown in Table IV and Figure 3. They were 22.62, 26.79, 36.31, 48.21 and 63.69% for *M. cartusiana*, while they were 14.58, 18.33, 29.76, 41.67 and 51.19% for *E. vermiculata,* which confirmed that *E. vermiculata* had higher resistance to seaweed extract than *M. cartusiana*.

*M. cartusiana* was the most affected by seaweed extract as presented by the values of $LC_{50}$ and $LC_{90}$, which were 7.96 and 21.63% but were 11.87 and 36.22% for *E. vermiculata* after seven days of treatment, respectively, Table V.

It is possible to indicate that seaweed extract could be used as a natural substance in controlling of *M. cartusiana* and *E. vermiculata* because it caused high toxic effect and noticeable results in reduction of these snails population. Asharaja and Sahayaraj [42] studied the effect of some marine algae extracts against the cotton pest *Dysdercus cingulatus*, and they found that these substances led to a significant lessening in the population of this pest, and were responsible for a clear effect on fertility and hatchability, which significantly decreased when the incubation period increased. Rashwan and Hammad [43] evaluated the toxicity of three different concentrations (3, 5 and 7%) of blue and brown marine macro algae extracts against cotton Leaf worm *Spodoptera littoralis*. Their results showed significant effects for these natural substances on the eggs

**Table IV.** Efficiency of seaweed extracts against *M. cartusiana* and *E. vermiculata* under laboratory conditions.

| Snails | Con. (%) | Cumulative mortality % within seven days | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| *M. cartusiana* | 5 | 4.17 | 8.33 | 12.50 | 20.83 | 33.33 | 37.50 | 41.67 | 22.62 |
| | 10 | 8.33 | 12.50 | 16.67 | 25.00 | 37.50 | 41.67 | 45.83 | 26.79 |
| | 15 | 16.67 | 20.83 | 25.00 | 29.17 | 41.67 | 54.17 | 66.67 | 36.31 |
| | 20 | 20.83 | 33.33 | 41.67 | 45.83 | 50.00 | 62.50 | 83.33 | 48.21 |
| | 25 | 20.83 | 37.50 | 58.33 | 70.83 | 79.17 | 87.50 | 91.67 | 63.69 |
| *E. vermiculata* | 5 | – | – | – | 8.33 | 12.50 | 16.67 | 20.83 | 14.58 |
| | 10 | – | – | 4.17 | 12.5 | 16.67 | 25.00 | 33.33 | 18.33 |
| | 15 | 8.33 | 16.67 | 20.83 | 25.00 | 37.50 | 41.67 | 58.33 | 29.76 |
| | 20 | 8.33 | 25.00 | 37.50 | 41.67 | 50.00 | 54.17 | 75.00 | 41.67 |
| | 25 | 12.50 | 29.17 | 37.50 | 54.17 | 62.50 | 79.17 | 83.33 | 51.19 |
| Control | | – | – | – | – | – | – | – | – |

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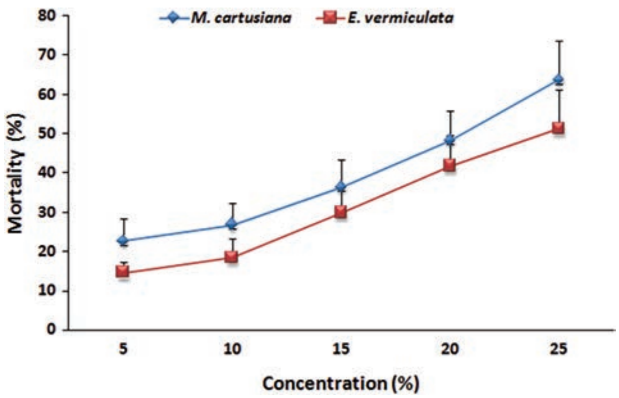


**Fig. 3.** Mean efficiency of seaweed extracts against *M. cartusiana* and *E. vermiculata* under laboratory conditions.

**Table V.** $LC_{50}$ and $LC_{90}$ values of seaweed extract against *M. cartusiana* and *E. vermiculata* snails.

| Snails | P. Value | $LC_{50}$ % | $LC_{90}$ % | Lower 95% | Upper 95% | Slope | R |
|---|---|---|---|---|---|---|---|
| *M. cartusiana* | 0.3159 | 7.96 | 21.63 | 28.784 | 155.6457 | 2.1486 | 0.9234 |
| *E. vermiculata* | 0.6964 | 11.87 | 36.22 | 32.7757 | 123.5104 | 2.6452 | 0.9753 |

*Note*: Different letters within the same column indicated significant differences between the treatments at $p < 0.05$.

hatching, which pointedly decreased, but the period of larvae and pupae was continued. Moreover, they caused a clear distortion in the fourth larval age and decreased the fertility of *S. littoralis*. Ibrahim et al. [30] proved that the application of red and brown marine algae extracts at concentrations of 0.5, 1 and 2% showed considerable negative effects on the population of root-knot nematode, *Meloidogyne incognita*.

### 3.4. Effect of the Highest $LC_{50}$ Values of Wood Vinegar and Seaweed Extracts on Some Biochemical Parameters of *R. norvegicus albinus*

The effect of two highest levels of $LC_{50}$ of wood vinegar and seaweed extracts (21.67 and 11.87%) on some liver and kidney functions of *R. norvegicus albinus* by measuring levels of ALP, AST, ALT, Creatinine and Urea in its blood was shown in Table VI.

Data proved that there were no significant differences in the levels of ALP, AST, ALT, Creatinine and Urea between treated and untreated individuals with wood vinegar; whereas there were very low significant differences appeared between the treated and untreated *R. norvegicus albinus* treated with seaweed extracts, especially for the Creatinine and Urea levels.

**Table VI.** Effect of the highest $LC_{50}$ values of wood vinegar and seaweed extract on some biochemical parameters of *R. norvegicus albinus.*

| Tested materials | $LC_{50}$ values | ALP | AST | ALT | Creatinine | Urea |
|---|---|---|---|---|---|---|
| Wood vinegar | 21.67 | 84.00 a | 182.87 a | 21.50 a | 0.35 ab | 40.77 ab |
| Seaweed extracts | 11.87 | 85.56 a | 185.10 a | 22.86 a | 0.38 a | 44.74 a |
| Control | | 83.70 a | 183.57 a | 21.57 a | 0.34 ab | 40.33 ab |
| LSD at 0.05 | | 4.14 | 6.60 | 2.38 | 0.03 | 4.44 |

*Notes*: Different letters (a–b) within the same column indicated significant differences between the treatments at $p < 0.05$.

From the above-mentioned results, it is clear that both wood vinegar and seaweed extract were safe for mammals because they did not show any negative effects on the liver and kidneys functions, so they might be used as eco-friendly materials without expected harmful effects on humans or animals. These results are in agreement with Kumar et al. [48] indicated that methanol, ethanol and water extracts from the brown algae *Turbinaria conoides* were not toxic to Westar rats with weights ranging from 150 to 200 g. Ananthi et al. [49] confirmed the great importance of a marine brown alga, *Turbinaria ornata* as ahighly active Anti-inflammatory in the treatment of some diseases of rats because they contain many compounds with high activity in inhibiting signs of chronic and acute inflammation. Moreover, Wang et al. [50] suggested the possibility of using bamboo wood vinegar as a potential additive in animal production as an alternative to antibiotics, as it didn't show any harmful side effects for pigs, but rather greatly increased the protection of the tested animals against different types of pathogenic microbes. Bernardini et al. [51] pointed out that marine algae are very safe materials for humans, and they are effective as a chemical preventive agent against some types of cancers such as breast, cervical and colon cancer, when they helped prevent the spread of cancer cells.

## 4. CONCLUSION

The previously explained results showed the possibility of using both wood vinegar and seaweed extract in the control of two-spotted spider mites (*Tetranychus urticae*) and two land snails (*M. cartusiana* and *E. vermiculata*). The results showed marked effects for wood vinegar and seaweed extract substances in reducing the population of the chosen mite and snails. Increasing the applied concentrations of wood vinegar or seaweed extract and contact times led to noticeable reductions in populations of *T. urticae*, *M. cartusiana* and *E. vermiculata*. In the case of *T. urticae*, the wood vinegar was more toxic that seaweed extract on its eggs, immature stages and adults. The results also proved that *M. cartusiana* was more sensitive to the two tested substances than *E. vermiculata*. When the high levels of $LC_{50}$ values of wood vinegar and seaweed extract were tested on some liver and kidney functions of *R. norvegicus albinus* rats, it was found that these substances didn't cause harmful effects on mammals. So, it is possible to use and recommend them as eco-friendly and safe alternatives to pesticides in controlling some invertebrate pests.

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**Ethical Compliance**

The experimental study was conducted under the supervision of the ethics committee of animal experimental management at the Faculty of Agriculture, Benha University, Egypt.

**Conflicts of Interest**

There are no conflicts to declare.

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