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The insecticidal effect of diatomaceous earth against adults and nymphs of *Blattella germanica*

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PEER REVIEW

Peer reviewer

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Comments

This is a valuable study in which authors have tried to evaluate the insecticidal effect of DE, one of the probable alternatives for chemical insecticides against German cockroach (*B. germanica*). Surely the results of this study in combination with other studies will be a an important step in the field of finding suitable and less-hazardous candidate for control of stored products/urban pests like German cockroach (*B. germanica*).
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ABSTRACT

Objective: To evaluate the insecticidal effect of diatomaceous earth (DE) against adults and nymphs of *Blattella germanica*.
Methods: This cross sectional study has been done on the laboratory strain of German cockroaches. Two stages, nymph and adult, were exposed to six dose rates of the DE, 2.5, 5, 10, 15, 20 and 25 g/m^2, at 24, 48 and 72 h exposure period. Mortality (number of dead cockroaches) was assessed after 24 h. Other exposed specimens were transferred to the beakers contained food and water for counting the retard mortality rate after 1 week.
Results: Increasing in dose rates of DE increased mortality rate, so that the lowest and highest mortality rates were observed in 2.5 and 25 g/m^2, respectively. The results of the statistical analysis showed no significant difference in the lethality of 50% of DE plus water on the German cockroach nymphs.
Conclusions: Due to the resistance of German cockroach against organochloride, organophosphorus, carbamate and pyrethriodes insecticides, it is suggested to use DE for insect's control.

KEYWORDS
Diatomaceous earth, German cockroach, Insecticides, Insecticidal effect, Nymph, Adult

1. Introduction

Cockroaches belong to Kingdom Animalia, Phylum Arthropoda, Class Insecta and Order Blattaria. German cockroaches, *Blattella germanica* (*B. germanica*) (L.), are the most common roaches in houses and restaurants[1].

German cockroach has a worldwide distribution and commonly found in tropical and subtropical regions. German cockroach is a relatively active species, moving around readily within structures. They move from one location to another and can pass through very small openings. They are also regularly carried from place to place in such things as bagged potatoes and onions, beverage cartons, food cans, other food packages and

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the folds of clothing. Cockroaches reproduce quickly. A female may produce four to six ootheca during her lifetime, each containing 30 to 40 eggs. Eggs hatch in 28 to 30 d, and nymphs develop in 40 to 125 d. Female roaches live about 200 d, with males living not quite as long[2].

German cockroach lives in human habitations where water, food and its waste, suitable temperature, darkness and humidity exist, such as the kitchens of houses, hotels and hospitals. The roaches feed on all available substances such as human's food and its waste, starchy substances, dried blood, stool, *etc*., and they play a major role in transmission of pathogens. They can also transmit pathogens mechanically, so they can carry various diseases[3], especially different forms of gastroenteritis including food poisoning, dysentery, and diarrhea. They transmit 33 species of bacteria such as shigella, salmonella, *Escherichia coli*[4], six species of parasitic worms like nematodes, and viruses such as polio to humans. They also involve in exacerbation of asthma and allergies because of their presence on waste deposits and foods simultaneously[3]. So they can be introduced as an important public health problem.

Resistance in German cockroach and other insects due to uncontrolled insecticides is one of the most important subjects in many countries that made many problems in vector control. In Iran, German cockroach's resistance to a different types of insecticides, phosphate groups (diazinon and actillic), carbamate (Ficam) and pyrethroid (permethrin and limbdo-cyhalothrin), has been reported[5,6]. Hence, using a combination of these pesticides in a greater dose, to counteract the resistance, can cause resistance to a larger proportion of pesticides. In recent years, other methods such as baits containing boric acid and gel bait insecticides have also been used. Boric acid is relatively safe for humans but can cause skin irritations. It is also harmful for plants[7]. These kinds of baits make bait shyness and should be replaced with other substances regularly. Other dusts based on silica aerogel or diatomaceous earth (DE) applied in the same way as boric acid. These two recent methods act more slowly by rubbing off the protective wax layers on the cockroach's body (cuticle), thus causing death by dehydration[7].

DE is used in dry and wettable powder formulations as inert fillers[3]. A DE dust has been mostly used in stored products[8] and grain beetles [9].

DE, a powdery substance, has been made from the fossilized remains of unicellular algae known as a diatom. This powder contains 1% crystalline silica. According to WHO guideline, DE needs to be less than 2% of crystalline silica in order to be considered safe. DE mode of action for insects control is mechanical. DE sharp edge makes mechanical abrasions on the thin wax covering of insects. This coating cover prevents water loss from the cuticle surface. DE also absorbs the oil easily, so it is effective at absorbing protective waxy covering. Finally death occurs due to rapid water loss and desiccation[10,11]. DE is totally organic and natural with no harm to the environment. It is non-toxic to fish, aquatic animals, birds and other wildlife. It can be used easily without presence of any residue in foods or environment. These properties make it suitable for using as an insecticide[12]. DE can be used as a natural pesticide replacement for internal parasite control in poultry[13]. It was introduced by the US Environmental Protection Agency as a harmless substance. In this study, the effect of DE was evaluated on mortality rate of various stages of German cockroach, one of the main public health problems in Iran.

2. Materials and methods

2.1. B. germanica

Specimens were collected from the kitchens of restaurants, hospitals and houses in Noor city by hand catch.

2.2. Rearing the specimens

The collected German cockroaches were reared in the insectarium of Department of Medical Entomology, Tehran University of Medical Sciences. For this purpose, some collected encapsulated females were transferred to insectarium and reared in laboratory condition of (26±2) °C, (50±5)% relative humidity, photoperiod 12/12 L:D.

2.3. Exposure with DE

The tests were done on F3 generation with DE powder (Hudson Industrial Group Inc. - Markham, ON L3R 3K2, Canada). Nymphs stage two and males (females and males have the same resistance to insecticides, males were chosen for this study) were used for treating after 96-100 h after hatching. They were also treated after each molting.

We put a filter paper in test container with five cockroaches of any stages (nymph stage one to six and adult male). After 2 h, when they were injured, treatment was started by 2.5, 5, 10, 15, 20 and 30 g/m^2 of DE[5,6,10] in 24, 48 and 72 h of exposure period. Another test was also done with the same quantities of DE powder with water (50 mL). This mixture (DE plus water) was tested after 24 h, when the

containers were dried completely. After counting the initial death in defined times, the survived cockroaches were followed for calculating the delayed death after 1 week[6]. We considered 24 h as the first contact time in this study.

Data analyzing was done according to Kruskal-Wallis test and probit statistical software.

3. Results

3.1. The effects of DE on the nymphal stage of German cockroach

Mortality rates after 24, 48 and 72 h contact time with 2.5, 5, 10, 15 and 20 g/m^2 concentrations of DE against different ages of German cockroaches, were 33.3%-81.1% and the average of the delayed mortality rate after 1 week was 72.2% (Figure 1). Increasing the concentration of DE up to 25 g/m^2 led to 66.7%-100% mortality rate of nymphal stages. This indicated the higher efficacy of DE against German cockroach nymphs in concentration of 25 g/m^2.

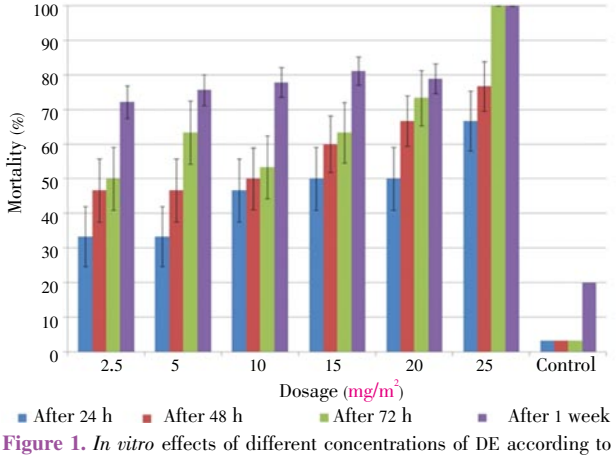

Figure 1. *In vitro* effects of different concentrations of DE according to the exposure time against *B. germanica* (*n*=570).

The difference between mortality rate in concentration of 25 g/m^2 after 72 h contact and the delayed mortality rate, compared to the other concentrations, was statistically significant (P<0.001).

But the mortality rate in the periods of 24 and 48 h was not significant for all other concentrations (Figure 1). Values of the lethal concentrations of 50% and 90% (LC_{50} and LC_{90}) of DE in contact periods of 24, 48 and 72 h were calculated by regression analysis and Probit Finney method. In the contact period of 24 h, the quantities of the LC_{50} and LC_{90} were 4.238 0 g/m^2 and 58.070 3 g/m^2, respectively. In the contact period of 48 h, the quantities were 5.214 8 g/m^2 and 302.737 9 g/m^2, respectively. While in the contact period of 72 h, the quantities were 12.903 4 g/m^2 and 626.094 2 g/m^2 (Figure 2).

LC_{50} in the periods of 24 and 48 h were 4.238 0 g/m^2 and 5.214 8 g/m^2, which increased to 12.903 4 g/m^2 in the period of 72 h. This increasing process was shown clearly by drawing the regression lines of DE mortality rate against German cockroach nymphs (Figure 2).

3.2. The effects of DE on adult male German cockroach

DE in concentrations of 2.5, 5, 10, 15, 20 and 25 g/m^2 against adult male German cockroaches had mortality rates from 40% to 80%. LC_{50} and LC_{90} of DE against adult male German cockroaches were calculated by regression analysis and Probit Finney method and were 8.030 7 g/m^2 and 167.711 6 g/m^2, respectively.

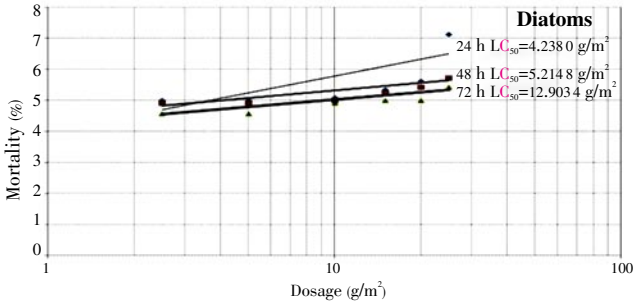

Figure 2. Regression line mortality of DE against *B. germanica* life cycle at 24, 48 and 72 h exposure time.

3.3. The effect of DE plus water on German cockroach nymphs

The mortality rates of DE plus water in these concentrations 2.5, 5, 10, 15, 20 and 25 g/m^2 against different stages of German cockroaches, in contact periods of 24, 48 and 72 h were 16.7%-80%, and the average delayed mortality rate after 1 week reached to 75% which was not statistically significant, except for the lowest concentration (Figure 3).

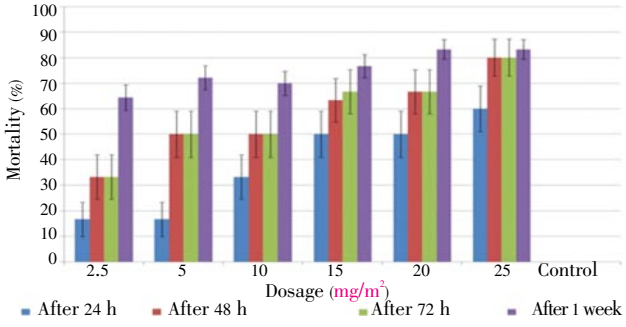

Figure 3. *In vitro* effects of different concentrations of DE dissolved in water according to the exposure time against *B. germanica* (*n*=570).

LC_{50} and LC_{90} of DE in contact periods of 24, 48 and 72 h were calculated by regression analysis and Probit Finney method, and the results indicated that in contact period of 24 h, the LC_{50} and LC_{90} were 20.035 8 g/m^2 and 175.590 4 g/m^2; and in contact period of 48 h were 5.917 3 g/m^2 and 91.027 9 g/m^2, respectively. The LC_{50} of DE plus water against German

cockroach nymphs showed a decreasing trend, in spite of increasing the time, while without water, there was an increasing trend of LC_{50}, parallel with increasing the time of contact (Figure 2). This situation was clearly shown by drawing the regression lines of DE mortality rate against German cockroach nymphs, in each contact times.

3.4. The effects of DE dissolve in water on adult male German cockroaches

The mortality rates of DE plus water against adult male German cockroach in concentrations of 2.5, 5, 10, 15, 20 and 25 g/m^2 were 33.3%-80% (Figure 4). LC_{50} and LC_{90} of DE against adult male German cockroaches were calculated by regression analysis and Probit Finney method, which were 7.409 3 g/m^2 and 91.206 3 g/m^2, respectively.

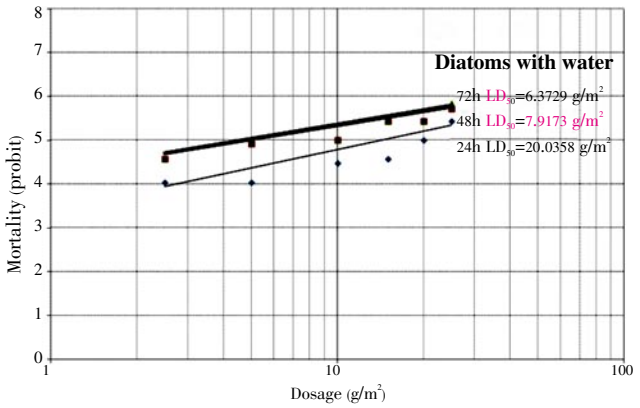

Figure 4. Regression line mortality rate of DE dissolved in water against B.germanica life cycle at 24,48 and 72 hours exposure time

4. Discussion

Uncontrolled use of chemical insecticides has irretrievable harms on beneficial arthropods, vertebrates, fish and aquatic invertebrates[13]. Resistance to insecticides has made many problems in pest control[6]. Various species of roaches are resistant to different type of pesticides. Replacement of pesticides with other methods is inevitable in some conditions. So, researching on the non-toxic methods in pest control is very important[13]. DE is one of the substances which can be used easily, due to its appropriate characteristics[14]. Particles of DE have small pores which are able to absorb the insect cuticle epithelium wax[10,11]. Hence, in contact with body surface of insects scratches, the waxy layer of cuticle and death occurs due to dehydration. DE is non-toxic for mammals and environment. It has been introduced by the US Environmental Protection Agency as a harmless substance[15]. So it can be suitable for insect control as an alternative for pesticides. DE effects were studied for the first time in Iran.

Mortality was reported in different treatment with concentrations of 2.5, 5, 10, 15, 20 and 25 g/m^2 in time periods of 24, 48 and 72 h. By increasing the exposure time, the mortality was increased too. Based on the exposure time, the lowest mortality rate was in 24 h, and the highest one was in 72 h. Also, the lowest mortality rate was in 2.5 g/m^2 concentration, while the highest was observed in 25 g/m^2. According to the Kruskal-Wallis test which was used for evaluating the significance of the mortality rate among different ages of nymphs (P<0.001), there is a significant difference between the number of dead and survived nymphs, but there is not any significant difference in the number of delayed deaths. Also, there is a significant difference among the number of the survived, the dead and the delayed dead, in different concentrations (P<0.001). The results of the study in different exposure time showed that there is a significant difference between the number of survived, dead and delayed dead of different nymphal stages (P=0.012). The results of the statistical analysis suggest that there is no significant difference in the LC_{50} of DE, sanitary talcum, industrial talcum and industrial talcum plus water on German cockroach nymphs, but mixing DE and water decreases the effectiveness of DE 10 times for LC_{50} and 67 times for upper confident limits (P<0.01).

There are most studies on DE effects on storage pests, but the insecticidal effects of DE on German cockroaches have been done for the first time.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Some species of cockroaches such as German cockroach (*B. germanica*), American cockroach (*Periplaneta*

americana) and oriental cockroach (*Blatta orientalis*) are important urban and medical pests. Using chemicals for their control remains as one of the main approaches. Emerging the resistance against wide range of insecticides and their adaptability to different conditions, compel the researchers to try to develop new control measures against them.

Research frontiers

This study has tried to determine the insecticidal effect of DE against different life stages of one of the most important urban pests, *B. germanica*.

Related reports

A few studies have been conducted before to evaluate the toxic effects of DE against some stored products and urban pests. All studies tried to clarify the different dimension of using DE even with some modifications for pest control, but because of the complexity of the life cycle of cockroaches and their relationship with domestic environment, more studies are needed in this field.

Innovations and breakthroughs

This is a well-designed study in which authors gathered useful data. The data have been analyzed properly using standard methods. Also the interpretation of the results has been carried out acceptably.

Applications

Theoretically the results of this study improve the knowledge of researchers who have focused on the control measures against cockroaches and tried to find suitable alternatives for chemical insecticides. Also as a step forward, the results of this study will be useful for the urban and health pest control sections.

Peer review

This is valuable study in which authors have tried to evaluate the insecticidal effect of DE, one of the probable alternatives for chemical insecticides against German cockroach (*B. germanica*). Surely the results of this study in combination with other studies will be a an important step in the field of finding suitable and less-hazardous candidate for control of stored products/urban pests like German cockroach (*B. germanica*).

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