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The Effect of Isolated Bacteria against Adult Stages of *Periplaneta americana* (Blattodea: Blattidae) and *Aedes aegypti* (Diptera: Culicidae) using Spraying Methods as a Biological Control

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Abstract

Medical entomology involves the study of medically important insects, such as cockroaches and mosquitoes, which have a dangerous role as transmitters for deadly diseases, such as Malaria, Leishmaniasis, and Dengue fever, which are responsible for many deaths among humans. Huge concern about the use of chemicals insecticides encourages the development of alternative methods for insect control, and due to the harmful effects of these chemicals, new strategies are being developed to replace or reduce the use of synthesized insecticides. Therefore, chitinolytic enzymes produced by microorganisms have a significant effect as biocontrol agents and will be more critical than synthetic pesticides for control. This study was primarily aimed to study the impact of various isolated bacteria using chitinolytic and spraying assays against adult stages of *Periplaneta americana* and *Aedes aegypti* as biological controls. Eight species of bacteria were isolated, and only *Chryseomonas luteola* was used against adult insects because of its high chitinolytic activity by spraying assay. Our results showed that the LC_{50} values of *C. luteola* against *P. americana* were 22.04% and 17.21% after 24 and 48 h, respectively. For *A. aegypti* adult stages, LC_{50} values of *C. luteola* were 2.78% and 2.12% after 24 and 48 h, respectively. Based on the results of this investigation, it is reasonable to say that using microbial insecticides may be an effective strategy to control the adult stages of *P. americana* and *A. aegypti*.

Keywords: Insecticides, *Periplaneta americana, Aedes aegypti,* Chitinolytic Activity, Biological Control

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INTRODUCTION

Medical entomology involves the study of medically important insects, such as cockroaches and mosquitoes, which have a dangerous role as transmitters for deadly diseases, such as Malaria, Leishmaniasis, and Dengue fever, which are responsible for many deaths among humans. As these diseases increase in communities in developing countries, the environmental contamination caused by increasing the use of synthesized insecticides and alternative methods for controlling insect populations have become important research subjects to increase the use of biological or ecological control methods to limit the destructive impacts of insect populations.[1,2] Numerous pathogens, including bacteria, fungi, nematodes, and viruses that are antagonistic to insects, are used as biological insecticides to control them by dissolving and digesting chitin in the cell wall by making different hydrolytic enzymes, the most important of which are chitinases.[3,4] Because the cuticle of insects is composed of a vast amount of chitin, it was assumed that chitinase produced by these microorganisms could be involved in insect control by causing severe damage and even death, thereby exhibiting insecticidal activity.[5] Recently, much attention has been paid to the production of chitinases for insect control, which has also received widespread attention for its biotechnological applications in certain insects.[6,7] Many researchers have investigated the degrading effect of chitinase enzymes produced by bacterial species on insect chitin (aerobic and anaerobic bacteria), which can be found in a wide range of habitats;[5] these chitinolytic bacteria can be found in soil, marine, lake, or chitinous wastes, such as industrial shrimp waste.[8] Cody[9] pointed out that chitinases are the constituents of several bacterial species, including *Aeromonas, Serratia, Vibrio, Streptomyces,* and *Bacillus.* Biological insecticides and their toxins can be utilized in the form of conventional insecticidal sprays, dust, liquid drenches, liquid concentrates, wettable powders, and granules.[10] Paulitz et al.[11] found that *Streptomyces, Bacillus*, and *Pseudomonas* species could be used as insecticides. The effect of chitinase enzymes on insect growth has been investigated and lead to death if insects are in contact with chitinases.[12,13] Chitinases are one of the most significant biocatalysts with the potential to dissolve chitin in several phytopathogenic fungi and the integument of insects.[14] Microbial chitinases have become promising candidates for controlling plant pests. These enzymes can be used directly as biocontrol agents and in combination with chemical pesticides or other biopesticides.[15] In 1978, Brandt et al.[16] demonstrated that chitinases destroy the peritrophic matrix in *Orgyia pseudotsugata*, and this effect was also observed *in vivo* in *Spodoptera littoralis* and *Escherichia coli* expressing the endochitinase ChiAII from *Serratiamarescens*.[17] For many decades, *Bacillus thuringiensis* has been a well-known biological insecticide, and many strains express chitinase.[18] Hollensteiner et al.[19] and Prasanna et al.[20] found that *Brevibacillus laterosporus* possesses chitinase enzymes with hydrolytic effects. Based on previous research, many agricultural fields have been ruined due to insects, which unfortunately depend on the use of chemical pesticides to reduce insect abundance as a consequence.[21] A recent study by Sharawi et al.[22] found that isolated bacteria can be used as biological control agents against the ootheca of *P. americana* due to the pathogenic action of their toxins through the cuticle of the egg case. Huge concerns about the use of these chemicals encourages the development of alternative methods for insect control, and because of the harmful effects of the traditionally used chemicals, new strategies are being developed to replace or reduce synthesized insecticides.[23,12] The use of chitinase as a biocontrol agent is an attractive and environmentally safe strategy.[24] In this context, chitinolytic enzymes produced by microorganisms, such as bacteria, have a significant effect as biological control agents and are more effective than synthetic pesticides.[5] This study aimed to determine the effect of isolated bacteria from soil samples against adult stages of *Periplaneta americana* and *Aedes aegypti* as biological controls using a spraying method.

MATERIALS AND METHODS

Bacterial isolation from soil samples

One g of soil sample was mixed with 2 ml of distilled water in a Falcon tube and shaken for

2 min. The supernatant (100 µL) was spread using a spreader on nutrient agar after many dilutions. In nutrient broth, a single colony was selected and inoculated into 50 mL nutrient broth solution and placed in a shaking incubator for two days at 28°C. The sample was centrifuged and filtered through a mini-pore (0.2 mm). Bacterial isolates were identified using an Analytical Profile Index test (API-20E test strip).

Adulticidal bioassays of bacterial isolates against *A. aegypti*

Lab strains of adult *A. aegypti* were collected from the Dengue Mosquito Research Station at King Abdulaziz University, Jeddah (Saudi Arabia). To determine the toxicity of isolated bacteria against *A. aegypti*, five concentrations were prepared (0.5%, 1%, 1.5%, 2%, and 3%) using sterilized water. A commercial hand-sparing product and cylindrical cages (12 cm long and 80 cm in diameter) made of a wire screen were used in this study. The control group was maintained without exposure to isolated bacteria. Twenty adults were used for a single replicate, and the experiment was repeated three times. Mortality was recorded after 24 and 48 h of exposure.

Adulticidal bioassays of bacterial isolates against *P. americana*

The field strain of *P. americana* was used in this study and collected from dark, damp places like sewers in Jeddah Province, Saudi Arabia, using food jars surrounded with a dark cloth as traps. Jars from the upper inside surface (3 cm) were lightly grassed with petroleum jelly to prevent cockroaches from escaping, and a piece of bread soaked in a small amount of juice was placed in the collecting jars to attract cockroaches.[25] The traps were placed in sewers, and cockroaches were collected every two days and maintained in glass containers. The collected adults were separated in glass containers (30 × 60 × 30 cm). The containers were glued 2 cm from the top with petroleum jelly to prevent cockroaches from escaping and supplied with water, dry dog pellets, and cardboard as shelter.The cultures were maintained in the laboratory at 25 ± 3 °C and 75 ± 5% Relative humidity (R.H).After two weeks, adult cockroaches were used in the experiments. Five concentrations of isolated bacteria were prepared using sterilized water (5%, 10%, 15%, 20%, and 30%). Water alone wasused for the control group. Spraying bioassays were performed according to Baggio et al.[26] with some modifications to the

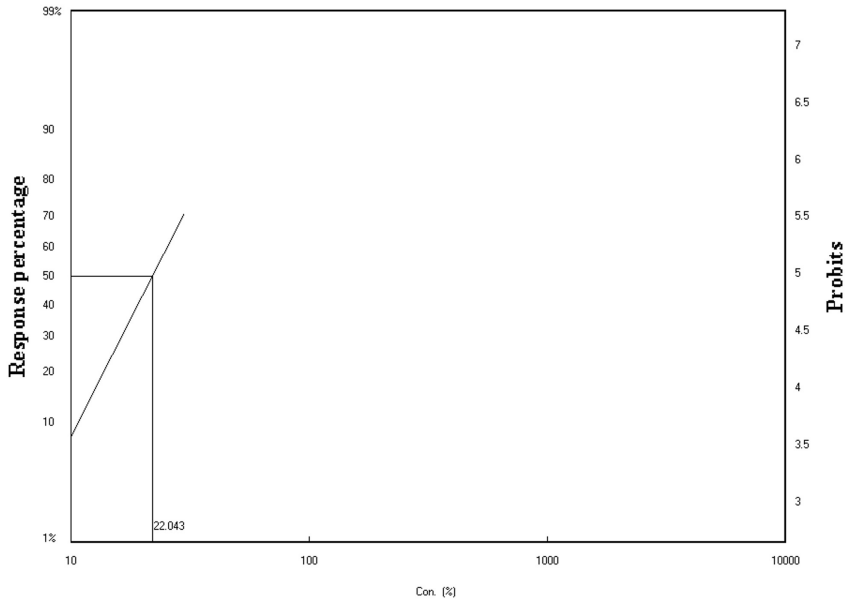

Figure 1. Laboratory toxicity line of *C. luteola* against adult stages of *P. americana* after 24 h of exposure

adult stages of cockroaches. Spraying bioassays were conductedusing plastic boxes, and 1 mL of each concentrationwas applied using a hand sprayer. Ten adult insectswere used as a single replicate, and the experimentwas repeated three times. Mortality was recordedafter 24 and 48 h of exposure.

Statistical analysis

The experimental design was randomized, and the mortality percentage was calculated using SAS. Determination of lethal concentrations (LC_{50}) was performed by probit analysis using LDP line software with the lower and upper confidence limits, the inclination of the toxicity line, and chi-square.[27]

RESULTS AND DISCUSSION

In the present study, different types of bacteria were isolated from soil samples

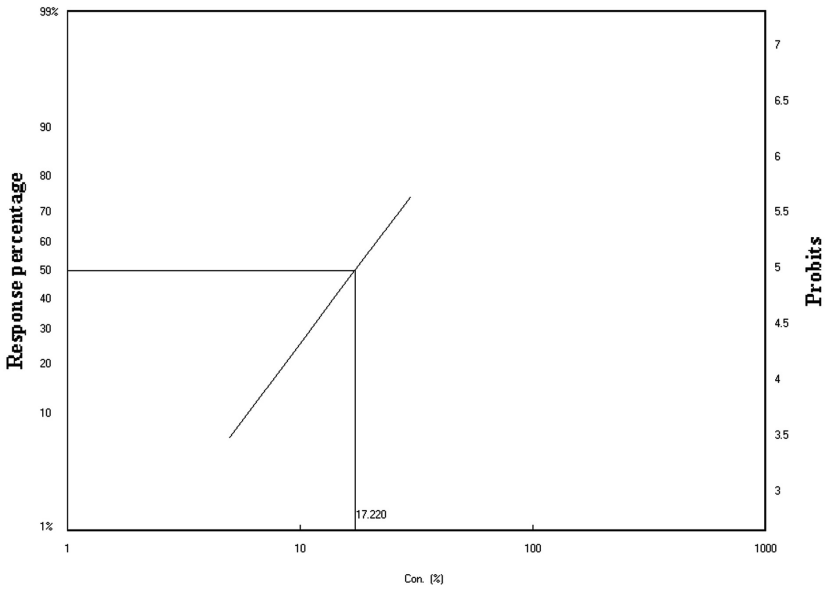

Figure 2. Laboratory toxicity line of *C. luteola* against adult stages of *P. americana* after 48 h of exposure

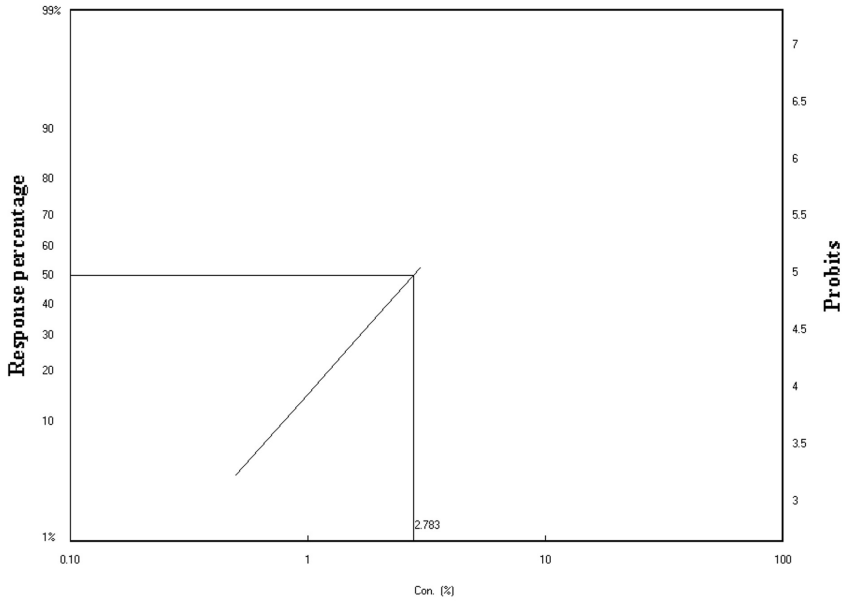

Figure 3. Laboratory toxicity line of C. luteola against adult stages of A. aegypti after 24 h of exposure

and tested against adult stages of *P. americana* and *A. aegypti* as biological controls after 24 and 48 h using the spraying method. Eight species of bacteria were isolated (*Enterobacter agglomerans, Bacillus subtills, Xantho maltophilia, Enterobacter amnigenus, Chryseomonas luteola,* and *Pseudomonas* spp.). In this study, *C. luteola* was chosen because of its high chitinolytic activity. The mortality percentage of *C. luteola* filtrate against the adult stages of *P. americana* and *A. aegypti* was used. Our findings are in agreement with those of Paulsen et al.[28] who also found that 11 types of isolated bacteria cause chitin degradation. Many studies have described the chitinase activity of bacterial species (*Enterobacteragglomerans, Bacillus subtills, Vibrio, Aeromonas, Pseudoalteromonas, Chitiniphilus, Nocardiopsis,* and *Burkholderia*).[29] The mode of action of chitinolytic bacteria depends on the production of chitinase and hydrolysis of chitin to produce monomers via enzymatic reactions.[30] The highest concentration (30%) caused higher mortality in P. americana after 24 (70%) and 48 h (80%) (Table 1). As shown in Figures 1 and 2, the lethal concentration (LC_{50}) was determined (22.04%, 17.21%) after 24 and 48 h, respectively. Our findings agree with many studies. Lacey et al.[31] pointed out that some bacterial species were developed as insect pest control, such as Bt sub-species, Lysini bacillus sphaericus, Paenibacillus spp., and Serratia entomophila. Bt sub-species kurstaki was the most used insect pest control in agricultural fields, and Bt sub-species israelensis and *L. sphaericus* were used as medical pest control. Bt toxins have minimal negative environmental impacts and hold more than 50% of the market share. Lacey et al. [31] found that bacteria and fungi such as Beauveria bassiana have a long history as biological control agents for various pests. Schnepf et al.[32] investigated that toxins present in Bt var. israelensis were used to control cockroaches. Payne et al.[33] reported that some bacterial species could be an effective agent in controlling cockroaches and reported that isolated Bt induced cockroach mortality.

Table 2 shows that the highest concentration (3%) caused higher mortality of *A. aegypti* after 48 (55%) and 72 h (65%). As shown in Figures 3 and 4, the lethal concentration (LC_{50}) was determined (2.78%, 2.12%) after 24 and 48 h, respectively. For many decades, many bacteria have been used for controlling mosquitos. For example, Wolbachia spp. has been used as a population control method for the *Aedes* mosquito.[34] Recently, Silva-Filha et al.[35] investigated the microbial insecticide *Bacillus thuringiensis* var.

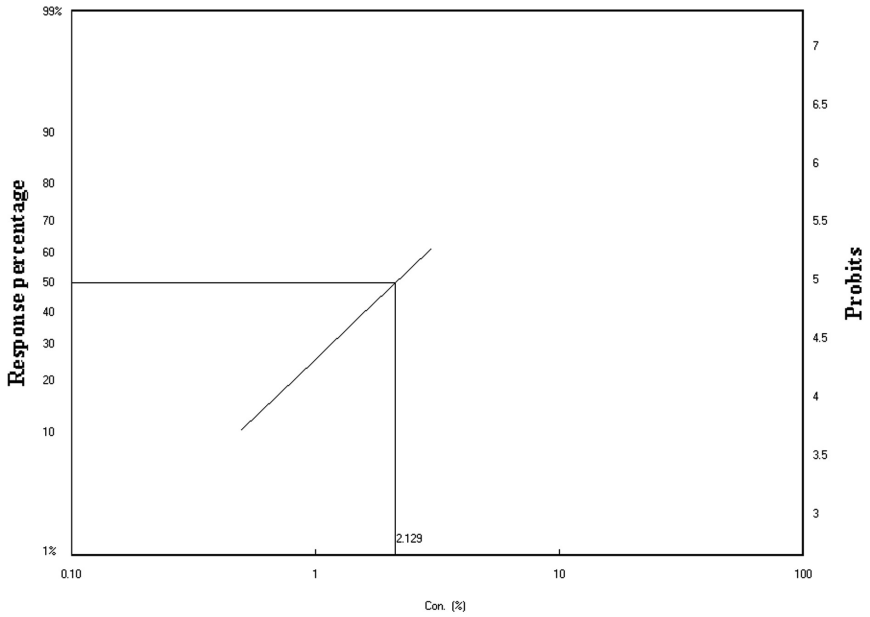

Figure 4. Laboratory toxicity line of *C. luteola* against adult stages of *A. aegypti* after 48 h of exposur

Table 1. Mortality effect of *C. luteola* against adult stages of *P. americana* after exposure times

Con. (%)	Exposure time	
	After 24 h.	After 48 h.
5	0 %	10 %
10	6.66 %	23.33 %
15	26. 66 %	36.66 %
20	43.33 %	56.66 %
30	70 %	80 %
Control	0 %	0 %
LC_{50}	22.04	17.21
(Lower limit – Upper limit)	20.37 - 24.20	15.57 - 19.22
Slope	4.11	2.77
Tubulated $(Chi)^2$	6	7.8
Calculated $(Chi)^2$	0.48	5.16

When tabulated $(Chi)^2$ larger than calculated at 0.05 level of significance indicates the homogeneity of results

Table 2. Mortality effect of *C. luteola* against adult stages of *A. aegypti* after exposure times

Con. (%)	Exposure time	
	After 24 h.	After 48 h.
0.5	6.66 %	15 %
1.0	10 %	18 %
1.5	25 %	35 %
2.0	38.33 %	50 %
3.0	55 %	65 %
Control	0 %	0 %
LC_{50}	2.78	2.12
(Lower limit – Upper limit)	2.38 - 3.45	1.84 - 2.56
Slope	2.33	2.00
Tubulated $(Chi)^2$	7.8	7.8
Calculated $(Chi)^2$	4.04	6.34

When tabulated $(Chi)_2$ larger than calculated at 0.05 level of significance indicates the homogeneity of results

israelensis (Bti), and *Lysinibacillus sphaericus* were active in controlling dipteran insects without harming non-target organisms. *Bti* was the first bacterium to be used against Diptera larvae.[36] *Lysinibacillus sphaericus* was also used against Culicidae larvae.[37] Therefore, insecticides composed of *Bti* are considered effective microbial insecticides for controlling mosquito larvae and black flies.[31] *Bti* and *L. sphaericus* showed high and selective larvicidal activity against some Diptera species, such as *Aedes, Anopheles, Culex,* and *Simulium*.[31]

CONCLUSION

This study shows that bacterial insecticides can be used for biological control by causing severe damage and even death of *P. americana* and *A. aegypti*. LC_{50} values of *C. luteola* against *P. americana* were 22.04% and 17.21% after 24 and 48 h, respectively. For adult stages of *A. aegypti*, LC_{50} values of *C. luteola* were 2.78% and 2.12% after 24 and 48 h, respectively. The use of bacterial insecticides may be an effective strategy to control the adult stages of *P. americana* and *A. aegypti*. However, biological control is only significant as a laboratory method. The current challenge is to use microbial pesticides in the environment to control targeted insects and limit their population.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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