



Comparative studies of nanoparticles and ethanol extract of rosemary plant on some biochemical aspects of the American cockroach, *Periplaneta americana* (L.) (Dictyoptera: Blattidae).

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ABSTRACT

The American cockroach, *Periplaneta americana* (L.) is considered one of the most important medical and veterinary insects among 3500 species in the world, control methods have been developed for these insects using some plant extracts and green nanoparticles of some medical plants such as rosemary (*Rosmarinus officinalis* L.). The present work was conducted at the Economic Entomology & Agricultural Zoology Department, Faculty of Agriculture, Menoufia University during 2020-2021 under laboratory conditions. The objective of the present study was to investigate the toxicity and biochemical effects of the ethanol extract and nanoparticles (NPS) of rosemary on the nymphs and adults of the American cockroach. The results demonstrated that the LC₅₀ values of nanoparticles of rosemary extract were the most toxic for the two stages than the rosemary ethanol extract. LC₅₀ values for nanoparticles extract were 118.22 and 112.33 ppm when contact and feeding technique were applied for nymphs, respectively, while for adults were 127.41 and 121.12 ppm when contact and feeding technique were applied, respectively. Additionally, these compounds had biochemical effects, which generally led to a decrease in the total content of protein and an increase in the total content of carbohydrates and lipids. Moreover, the activity of the acetylcholinesterase enzyme was increased. In general, the treatment with feeding was more effective than contact treatment.

Key words: Rosemary, ethanol extract, rosemary Ag nanoparticles, *Periplaneta americana*, biochemical aspects

INTRODUCTION

The American cockroach *Periplaneta americana* (L.) is considered one of the worldwide distributed insects which has medical and veterinary importance among 3500 species existing in the world. *P. americana* is more common in restaurants, grocery stores and bakeries as well as other sites where food, warmth, dark and damp cracks and moisture exist; they can develop to enormous numbers. American cockroach may be become a pest in homes after being introduced in cartons, grocery bags and containers since it can transmit many pathogens to the humans and the domestic

animals, as it lives in human living spaces and interact with humans directly and feeds on his foods (Fakoorziba *et al.*, 2010).

The effective methods of combating the medical and veterinary pests that deal with humans directly must be safe for both human and the environment. One of these effective methods is the use of some plant extracts, which have an effective impact against many insect pests, such as the extract of neem plant, which has been used since ancient times, also some other plants such as rosemary. Natural plant extracts play an increasing role as alternatives to synthetic

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pesticides due to the increase of health hazards, negative effects on non-target organisms, and environmental pollution (Sharma *et al.*, 2006). There are more than 2400 plant species belonging to 189 plant families, which have rich sources of organic compounds that have some biological and physiological effects on different insect pests (Rao *et al.*, 2005). Different species from over 60 plant families have been identified as an important natural source of insecticides (Prakesh & Rao, 1997, Rouhani *et al.*, 2012 and Sangeetha *et al.*, 2017).

Recently, the nanoparticles technology is widely applied in various fields, especially agricultural and industrial activities (Stadler *et al.*, 2010; Athanassiou *et al.*, 2018; Said, 2017; Said and Abdelaal, 2020). In agriculture, nanoparticle technology can be applied as a new biopesticides or new bioagents which using natural products containing effective compounds against different insect pests (Worrall *et al.*, 2009; Oliveira *et al.*, 2019; Jameel *et al.*, 2020 and Monteiro *et al.*, 2021). Several attempts have made to synthesize the green silver nanoparticles (AgNPs) using plant extracts such as *lantana camara* (Hikmat *et al.*, 2018) and ginger extracts which help to produce a new insecticides or insect repellents (Owolade *et al.*, 2008; Chinnamuthu & Boopathi, 2009 and Kulkarni *et al.*, 2012). These compounds increase the effectiveness in the control process, where the effect of nanoparticles is more than the natural compounds, as the nanoparticles compounds are more penetrating into the body of the pest (Owolade *et al.*, 2008 and Athanassiou *et al.*, 2018). The main objective of the present study was to investigate the toxicity and biochemical effects of rosemary ethanol extract and rosemary nanoparticles (NPs),

on the nymphs and the adults of *P. americana*.

MATERIALS AND METHODS

The tested plant and the study area

This study was carried out at Economic Entomology & Agricultural Zoology Department, Faculty of Agriculture, Menoufia University, Egypt. Leaves of rosemary (*Rosemaryinus officinalis* L.) were collected from the farm of the Faculty of Agriculture in Shebin El-Koum. Plants were identified by the specialist in the faculty farm. An ethanolic extract of rosemary, as well as the nanoparticles of the extract were prepared as follows:

Preparation of rosemary ethanol extract

Five hundred grams of dried leaves of plant were used and placed in 1 liter of absolute ethanol. The mixture was then kept in shaker incubator for 24 h at controlled room temperature then filtered by filter paper and centrifuged at 3000 rpm. The filtrate was placed in Frees Drier to evaporate the ethanol from it. The dried extract was kept in sterile universal flask then put in the refrigerator for later usage (Dorman, *et al.*, 2003)

Nanoparticles of rosemary ethanol extract:

Preparation of 1 ml of silver nitrate solution

Sixteen milligrams (16 mg) of silver nitrate (Blulx laboratories (P) Ltd. 99.9% AgNO₃, MW = 168.87 g/mol) were weighed and transferred into 500 mL flask. The silver nitrate was dissolved slowly in the flask containing distilled deionized water. After all solid has dissolved, more water was slowly added to obtain a volume of 1000 mL. The prepared 1 mL silver nitrate solution was stored at 4°C in amber colored bottle (Rajkumar and Malathi, 2015).

Synthesis of rosemary ethanol extract AgNPs

One gram from the dray ethanol extract prepared in the preceding step was put in

conical flask and dissolved in 100 mL distilled water, pH was adjusted at 12 using 0.01N sodium hydroxide. This system was kept under magnetic stirring, when the temperature reaches 80 °C, 1mL 0.1N silver nitrate was added, and keep under magnetic stirring for 30 min, (EL-Bisi *et al.* , 2013)

Characterization technique of silver nanoparticles Ultraviolet-visible (UV-vis) spectra

UV-vis spectra was used to the formation of silver colloids, UV-vis spectra have been proved to be quite sensitive to the formation of silver colloids where AgNPs exhibit highly absorption peak due to the surface Plasmon excitation which describes the excitation of conductive electrons in a metal.

Transmission Electron Microscopy (TEM)

Shape and size Characterization of AgNPs were obtained using TEM; JEOLJEM-1200. Specimens for TEM measurements were prepared by using a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the prepared AgNPs was determined from the diameter of 100 nanoparticles found in several chosen areas in enlarged microphotographs.

Preparation of tested compounds concentrations

To study the activity of ethanol extract and Ag NPs of rosemary extract against *P. americana* nymphs and adults. Five serial concentrations were prepared in distilled water as follow; (250, 200, 150, 100, 50 and 25 ppm) for rosemary ethanol extract and (200,150,100, 50, and 25 ppm) for rosemary Ag NPs.

Insect rearing

The American cockroach was reared at the Economic Entomology & Agricultural Zoology Department, Faculty of

Agriculture, Menoufia University during 2020-2021, under the laboratory conditions in glass cages (60x35x40cm) at $27 \pm 1^\circ\text{C}$ under a 12 h dark regime and $70 \pm 1\%$ relative humidity. Cages were covered with mesh screen with cloth sleeve opening at the top. The insects were fed with wetted biscuit and cotton soaked with water. Small plastic tubes were put inside cages serve as shelters. The cages were regularly cleaned and insect excrements were removed.

Insects Treatment

To study the effect of tested compounds, the previous concentrations were applied on both nymphs (third instar) and adults of American cockroaches, then the mortality percentages were determined. Nymphs and adults were treated with previous compounds by contact and feeding technique. In contact test, ten nymphs were kept in Petri dish (10 cm in diameter) containing filter paper which was sprayed with different concentrations of tested compounds by 2 mL of each concentration, then left for 24 h, after that the insects were transferred to glass cages containing its food, the same method was followed with adults. In feeding test, ten starved insects of each (nymphs or adults) were kept in glass cages containing 10 g of its food mixed finely with 2 mL of different concentrations and allow the insect to feed on treated food for 24 h, then the insects were transferred to glass cages containing untreated food. Percentages of dead and live insect stages were counted after 24 h for both contact and feeding techniques. Each treatment was replicated three times and the LC₅₀ of tested compounds were calculated by using propane software.

Effect of tested compounds on Biochemical Aspects of American Cockroach

The nymphal and adult stages were treated with LC₅₀ of rosemary ethanol

extract and extract nanoparticles to study their effects on biochemical aspects of *P. americana* (L.) after 24 h from treatment i.e., total protein, total carbohydrates, total lipids, Acetylcholinesterase (AChE) and polyphenol oxidase. Total proteins were determined by the method of (Bradford, 1976), total carbohydrates were estimated in acid extract of sample by the phenol-sulphuric acid reaction of (Dubois *et al.*, 1956) and total carbohydrates were extracted and prepared for assay according to (Crompton and Birt, 1967). Total lipids were estimated by the method of (Knight *et al.*, 1972) and AChE (acetylcholinesterase) activity was measured according to the method described by (Simpson *et al.*, 1964)

Statistical analysis

Each treatment was replicated 3 times. Statistically analyzed values were expressed as mean \pm SE of replication and Student's t-test was applied to locate significant ($P \leq 0.05$) differences between treated and control groups, (Snedecor, 1952, and Duncans, 1957).

RESULTS

Synthesis of rosemary ethanol extract (AgNPs)

The appearance of brown color is an indicator for the successive formation of AgNPs because of the excitation of surface Plasmon vibrations in nanoparticles of silver. It was a quick interaction as demonstrated by the immediate color change on blending the solution of silver nitrate and ethanol extract. This color change demonstrates performing of redox reaction, whereby ions of Ag^+ are reduced to Ag^0 by the extract components, which are oxidized to different species (Halawani, 2017).

Formation of Ag nanoparticles was investigated by UV-Vis spectroscopy to ensure the successful synthesis using Lambda 25 UV-Visible spectrometer, Perkin Elmer, Inc. Characteristic absorption peak of Ag-NPs in the UV-Vis spectra was around 450 nm which was generated due to the surface Plasmon resonance (SPR) of Ag-NPs (Fig., 1) (Liz-Marzan, 2006).

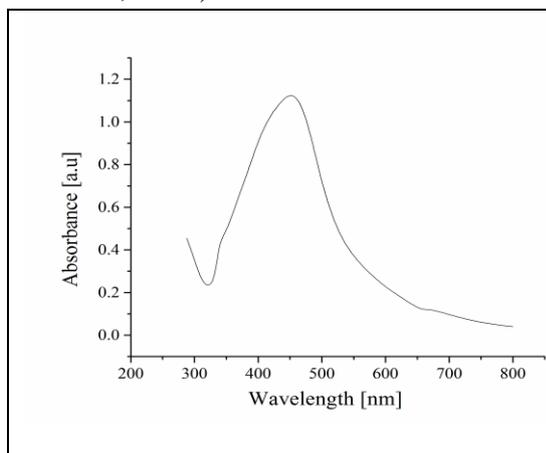


Fig. 1: UV-Visible spectrum of the prepared Ag NPs rosemary (ethanol extract)

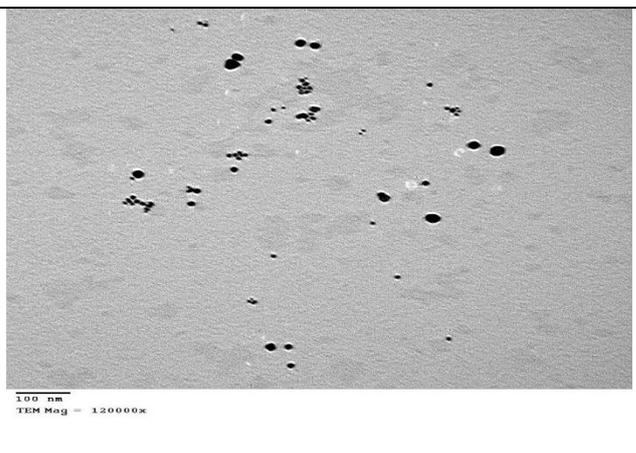


Fig.2: TEM image of the prepared Ag NPs particles in the polymer matrix

Transmission electron microscopy (TEM) was utilized to elucidate shape and size of the prepared Ag nanoparticles (this investigation was made by using JEOL JEM 10 \times 10 (Electron microscope-Japan). It was

noted from TEM images in fig.2. That the prepared Ag nanoparticles have a spherical shape and well dispersed in the polymer matrix with average particle size of 12 nm.

Toxicological evaluations of tested compounds against nymphs and adults of American cockroach

Toxicity levels for tested compounds against nymphs and adults of *P. americana* were estimated in the presented study at 24 h passed from all treatment. Data summarized in Table (1) recorded that the LC₅₀ values of nanoparticles of rosemary extract were considered to be the most toxic for the two stages than rosemary ethanol extract. LC₅₀ values for nanoparticles extract were 118.22 and 112.33 ppm when contact and feeding technique were applied for nymphs, respectively. The estimation of LC₅₀ values for adults were 127.41 and 121.12 ppm when contact and feeding technique were applied. On the other hand LC₅₀ values for ethanol extract were 131.66 and 122.51 ppm for nymphs when contact and feeding technique were applied, but the LC₅₀ values were 133.14 and 126.55 ppm when contact and feeding technique were applied for adults. Previous results show that the tested compounds have a toxic effect on the tested insect stages and rosemary nanoparticles were more toxic than ethanol extract. The results in table (2&3) indicate that the tested compounds have biochemical effects on both nymphs and adult insects. The total content of protein and carbohydrate, lipids, as well as the activity of the acetylcholinesterase enzyme are affected.

As for the nymphs, the results in Table (2) indicate that the ethanol extract of the rosemary plant led to a decrease in the total protein content compared to the control in both treatment methods, and the averages of total protein content were 9.51 and 9.01 and 10.31 (mg/g.b.wt) for contact, feeding and control, respectively. Similar results appeared with the total carbohydrate content, where the treatment led to a

significant decrease when treated by feeding method, this decrease was slight but not significant in the case of contact treatment, the averages were 16.42, 22.41 and 16.71 (mg/g.b.wt) for contact, feeding and control, respectively. On the contrary, the treatment of nymphs with ethanol extract led to a significant increase in the total lipids content when treated with both methods of treatment compared to the control, the averages were 12.63, 14.22 and 11.44 (mg/g.b.wt) for contact, feeding and control, respectively. On the same approach the activity of acetylcholinesterase was significantly increased compared to the control, the averages were 278, 298 and 180 ($\mu\text{g AchBr}/\text{min /g.b.wt}$) for contact, feeding and control, respectively.

Regarding the adults, the results in the table (2) indicate a significant increase in the total protein content when treated with both methods of treatment, the averages were 16.66, 19.71 and 15.22 (mg/g.b.wt) for contact, feeding and control, respectively. Similar result was observed with the total carbohydrate content as the averages were 17.32, 24.41 and 16.40 (mg/g.b.wt) for contact, feeding and control, respectively. The treatment of adults also led to a significant increase in the total lipids content when treated with feeding compared to control, while there was a slight, non-significant, decrease when treated with contact compared to control as the averages were 14.22, 17.86 and 14.31 (mg/g.b.wt) for contact, feeding and control, respectively. There was a significant increase in acetylcholinesterase when treating adults with both methods of treatment, the averages were 223.51, 253.48 and 211.5 ($\mu\text{g AchBr}/\text{min /g.b.wt}$) for contact, feeding and control, respectively. Similar observations were obtained when treating the nymphs with both methods of treatment.

Table 1: Toxicological evaluation of ethanol extract and nanoparticles of Rosemary extract against American cockroach nymphs and adults after 24 h under laboratory conditions.

Tested compounds	Treated stages	technique	LC ₅₀ (ppm) (95% confidence limits)	Slope
Ethanol extract	Nymphs	contact	131.66	1.74±0.31
		feeding	122.51	1.88±0.53
	Adults	contact	133,14	1.91±0.41
		feeding	126.55	1.65±0.22
Rosemary extract Nanoparticles	Nymphs	contact	118.22	1.34±0.63
		feeding	112.33	1.45±0.28
	Adults	contact	127.41	1.91±0.19
		feeding	121.12	1.83±0.22

Table 2: Biochemical aspects of nymphs and adults treated by LC₅₀ concentration with rosemary ethanol extract using contact and feeding technique.

Rosemary ethanol extract						
Biochemical parameters	Treated nymphs (Mean±SE)				F value	P value
	Control	Contact	Feeding	F value		
Total protein (mg/g.b.wt)	10.31±0.51a	9.51±0.42b	9.01±0.41 b	21.51	0.0012**	
Total carbohydrate (mg/g.b.wt)	16.71±1.61b	16.42±0.1.66b	22.41±2.63 a	18.221	0.0003***	
Total lipids (mg/g.b.wt)	11.44±1.35c	12.63±2.73b	14.22±3.44 a	33.221	0.0004***	
AchE activity (µg AchBr/min /g.b.wt)	180±21.38c	278.11±22.75b	298.61±24.21a	128.42	0.0001***	
LC ₅₀ (ppm)	-	131.66	122.51	-	-	
	Treated adults (Mean±SE)				F	P
	Control	Contact	Feeding	F		
Total protein (mg/g.b.wt)	15.22±1.34c	16.66±2.61b	19.71±2.24a	11.541	0.0001***	
Total carbohydrate (mg/g.b.wt)	16.40±2.73c	17.32±2.81b	24.41±3.22a	27.810	0.0004***	
Total lipids (mg/g.b.wt)	14.31±1.72b	14.22±1.52b	17.86±2.51a	42.911	0.0013**	
AchE activity (µg AchBr/min /g.b.wt)	211.5±21.64c	223.51±22.61b	253.48±22.66a	52.471	0.0005***	
LC ₅₀ (ppm)	-	133.14	126.55	-	-	

In each row, means followed by the same letter are not significant at the 5% leve.

Table 3: Biochemical aspects of nymphs and adults treated by LC₅₀ concentration with rosemary nanoparticles using contact and feeding technique.

Rosemary nanoparticles					
Biochemical parameters	Treated nymphs (Mean ±SE)				
	Control	Contact	Feeding	F value	P value
Total protein (mg/g.b.wt)	10.31±1.73a	8.4±0.64c	6.33±0.23b	22.23	0.000***
Total carbohydrate (mg/g.b.wt)	16.71±2.11c	17.11±2.31b	31.21±11.61a	15.371	0.0001***
Total lipids (mg/g.b.wt)	11.44±0.74b	11.9±0.62b	17.32±1.23a	29.721	0.0021**
AchE activity (µg AchBr/min /g.b.wt)	180±19.82c	271±21.71b	316±24.66a	121.42	0.0003***
LC ₅₀ (ppm)	-	118.22	112.33	-	-
	Treated adults (Mean ±SE)				
	Control	Contact	Feeding	F value	P value
Total protein (mg/g.b.wt)	15.22±1.33c	17.31±2.53b	22.77±2.87a	12.223	0.0004***
Total carbohydrate (mg/g.b.wt)	16.4±2.64c	18.51±2.61b	28.91±3.51a	25.401	0.0001***
Total lipids (mg/g.b.wt)	14.31±2.81b	13.8±2.33b	19.64±2.82a	36.362	0.0031**
AchE activity (µg AchBr/min /g.b.wt)	211.5±13.71c	241±16.22b	278.64±22.71a	42.723	0.0001***
LC ₅₀ (ppm)	-	127.41	121.12	-	-

In each row, means followed by the same letter are not significant at the 5% level

The results in table (3) indicate the biochemical effects in both nymphs and adults when they were treated with rosemary nanoparticles by both feeding and contact techniques. For nymphs, the results indicate a significant decrease in the total protein content compared to the control, the averages were 8.41, 6.33 and 10.31 (mg/g.b.wt) for contact, feeding and control, respectively. Contrary, the total carbohydrate content was significantly increased compared to the control when treated with both techniques of treatment, the averages were 17.11, 31.21 and 16.71 (mg/g.b.wt) for contact, feeding and control, respectively. As for the total lipids content, there was a significant increase when treated

with feeding, but there was a slight, non-significant, decrease when treated with contact as the averages were 11.01, 17.32 and 11.44 (mg/g.b.wt) for contact, feeding and control, respectively. There was also a significant increase in the activity of acetylcholinesterase enzyme when treated with both feeding and contact, the averages were 271, 316 and 180 (µg AchBr/min /g.b.wt) for contact, feeding and control, respectively.

The previous results were in the same direction when the adults were treated with rosemary nanoparticles by both techniques of treatment. The total protein content was significantly increased, the averages were 17.31, 22.77 and 15.22(mg/g.b.wt) for

contact, feeding and control respectively. Also, there was a significant increase in the total carbohydrate content, where the averages were 18.51, 28.91 and 16.41 (mg/g.b.wt) for contact, feeding and control, respectively.

Regarding the total lipids content, a significant increase was observed when treated with feeding technique, but decreased when treated with contact technique, the averages were 13.81, 19.64 and 14.31 (mg/g.b.wt) for contact, feeding and control, respectively. The activity of the acetylcholinesterase enzymes showed a significant increase compared to the control when treated with both treatment techniques, the averages were 241, 278 and 211.5 ($\mu\text{g AchBr}/\text{min /g.b.wt}$) for contact, feeding and control, respectively.

DISCUSSION

American cockroaches are one of the most medicinal and veterinary insects that can carry and transmit many pathogens to humans and animals, such as pathogenic bacteria, viruses and fungi, thus, their presence in houses, gardens, and hospitals is a health challenge. Therefore, there is an urgent need to combat this pest, taking into the consideration the safety of the materials used to humans, animals and the environment.

Recently, efforts are made to increase the efficiency of applied pesticides with the help of nanoparticles technology which is one of the novel methods that being explored and tested in the field of pest control, either in agricultural pests (Chinnamuthu and Boopathi, 2009; Debnath *et al.*, 2011; El-Bisi, *et al.*, 2013; Abd El-Zaher, 2017 and Athanassiou *et al.*, 2018), or in medical and veterinary pests such cockroaches, mosquitoes and flies (Abd El-

Raheem and Eldafrawy, 2016; Said, 2017 and Campos *et al.*, 2020). Furthermore, nanoparticles can be used as insecticide and herbicide (Bhattacharyya *et al.*, 2010).

The present study cleared that nanoparticles have entomotoxic effect against both nymphs and adults of *Periplaneta americana*, where the LC_{50} values of nanoparticles of rosemary extract was most toxic for the two stages than rosemary ethanol extract. Moreover, the biochemical aspects of *P. americana* were affected. Previous studies established by many authors confirmed this findings, Abd El-Raheem & Eldafrawy (2016) and Said (2017) showed that the mortality percentages of the German cockroach nymphs and adults were increased by increasing the concentrations of the silver nanoparticles under feeding and contact methods. El-Khodary *et al.*, (2020) Showed that the fumigation by the nanoemulsion of the German chamomile caused 44.44% mortality of *P. americana* at 10 mg/ liter after 72 h of application. Silva *et al.*, (2019) reported that the encapsulated nanoparticles of the essential oils of (*Piper aduncum L.* and *P. hispidinervum*) caused about 80% mortality in *Aedes aegypti*. The effect of the nanoparticles compound against insects could be attributed to that Nanoparticles act as effective repellents and insecticides for the agricultural and medical insects (Magro *et al.*, 2019 and Campos *et al.*, 2020).

Furthermore, silica nanoparticles and silver nanoparticles can suppress larval and adult stages of *Callosobruchus maculatus* as the mortality increased with increasing the concentrations of nanoparticles, while LC_{50} was decreased (Rouhani *et al.*, 2012). Also, Worrall *et al.*, (2009) found that coating nanoparticles loaded with garlic essential oil

has good potential in the control of stored product insects.

The current study also revealed that nanoparticles of rosemary extract was more effective when applied by feeding technique than contact. This may be due to nanoparticles can penetrate the alimentary canal faster than penetrating the insect's exoskeleton through contact method. The toxic action of nanoparticles may be due to the interaction between silver nanoparticles with various chemical compounds inside the insect's body, which inhibit the vital processes such as inhibiting the mechanism of insect's enzymes, defect in biological organs oxidative stress, alterations or damage in DNA, a decrease in membrane permeability and degradation of protein, which finally led to cell death. These suggestions are supported by (Benelli, 2016 A; Benelli 2016 B and Azarudeen et al., 2017).

Finally, according to these findings, nanoparticles of rosemary extract can be used effectively against *p. americana* either in nymphal and adult stage, as effective natural control and eco-friendly alternatives to the chemical pesticides.

Conclusion

The effect of the ethanol extract and nanoparticles (NPS) of rosemary on the nymphs and adults of the American cockroach was studied. The ethanol extract and nanoparticles compounds have a clear effect on these stages when treated with contact and feeding techniques, which makes these compounds have a good role in combating various medical and veterinary insects, such as the American cockroach. Further studies are needed to determine the potential use of such nanoparticle

compounds in the pest control programs away from the use of harmful pesticides.

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