

Available online at [GSC Online Press Directory](#)

GSC Biological and Pharmaceutical Sciences

e-ISSN: 2581-3250, CODEN (USA): GBPSC2

Journal homepage: <https://www.gsconlinepress.com/journals/gscbps>

(RESEARCH ARTICLE)



## Laboratory evaluation of toxicity of spinosad tablets and tracer 48 SC insecticides against different stages of American cockroaches (*Periplaneta americana* L.), in Jeddah governorate

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Publication history: Received on 20 December 2018; revised on 14 January 2019; accepted on 18 January 2019

Article DOI: <https://doi.org/10.30574/gscbps.2019.6.1.0163>

### Abstract

*Periplaneta americana* is an important household insect pest worldwide and acts as a mechanical vector and reservoir for pathogenic agents. Fermented insecticides are biopesticide, derived from fermentation by the soil-dwelling actinomycete. The aim of this study was to test the susceptibility of Spinosad tablets and Tracer 48 SC against *P. americana* adults and nymphs using different concentrations. Bioassays were done by feeding and contact toxicity methods. Mortality was recorded after 48 hours of exposure. Mortality data from the replicates was assessed by probit analysis. All tested insects showed high susceptibility for spinosad compared with the control. The effectiveness of fermented insecticides against susceptible different stages of *P. americana* showed that these formulations can be strongly effective for the control of *P. americana*.

**Keywords:** Spinosad; Tracer; Insecticides; Mortality; *P. americana*; Toxicity

### 1. Introduction

American cockroaches (*Periplaneta americana*) (Linnaeus), order Dictyoptera, suborder Blattaria is an important insects in medical [1], they are the most notorious pests, found in kitchens [2]. It is one of the largest common cockroach species [3]. Out of 500, 30 species are considered as household pest [4]. A number of cockroaches pests live in/or around homes, and they are omnivorous scavengers [1]. They survive in warm weather with high moisture conditions as well as in unfavorable environments for humans [5].

*P. americana* can spread bacteria, fungi, and other pathogenic microorganisms from infected areas [6], and cause allergies to human [7]. They play important role in the transmission of different diseases by mechanical and biological ways [8, 9]. *P. americana* spends most of its time in sewage, sewer pipe which usually contains high density of pathogens [10]. Also, they feed on garbage and they have large opportunities to disseminate human pathogen [11, 12]. In addition, their nocturnal and filthy habits of eating their feces make them ideal carriers of numerous pathogenic microbes [13]. Cockroaches spread pathogens through their cuticle [14], because their nymphal cuticles go through ecdysis [15]. Therefore, they transfer pathogens in different ways such transmission routes may occur among populations with infected individuals such as vertical transmission which occurs when an infected mother passes on the pathogen or disease to her progeny [16]. All of these pathogens used as dangerous organisms targeting animal or human populations.

A numerous pathogens such as bacteria, fungi and molds, helminths, protozoans and viruses, harmful to humans being are carried by cockroaches as well as they present in their faeces [12,17]. More than 100 species of bacteria have been

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isolated from domestic cockroaches [18], out of which, a few could be potential mechanical transmitters of pathogenic bacteria [19]. In addition more than 40 pathogenic and nonpathogenic bacterial species have been isolated from cockroaches. Moreover, 70 species of Gram positive and negative bacteria belonging to 37 genera were isolated from the surface and fecal pellets; including: *Actinomyces randingae*, *Alcaligenes faecalis*, *Arthrobacter cumminnsii*, *Aureobacterium* spp., *Bacillus* spp., *Brevibacterium* spp., *Burkholderia vietnamiensis*, *Buttiauxella* sp., *Citrobacter* sp., *Corynebacterium* spp., *Enterobacter* spp., *Erwinia* sp., *Escherichia coli*, *Hafnia* sp., *Kigali* sp., *Klebsiella* spp., *Kluyvera* sp., *Kauri rosea*, *Leuconostoc* sp., *Micro bacterium* spp., *Micrococcus* sp., *Proteus* spp., *Providencia ruttier*, *Pseudomonas* spp., *Rhodococcus australis*, *Rhodococcus rhodochrous*, *Salmonella typhimurium*, *Serratia* spp., *Shigella* sp., *Spingobacterium thalpophilum*, *Staphylococcus* spp., *Stenotrophomonas maltophilum*, *Streptococcus* sp., *Tsukamurella inchnonensis*, *Vibrio metschnikovii*, *Xanthomonas* spp., *Entamoeba histolytica*, *Escherichia coli*, *Klebsiella pneumoniae*, *Mycobacterium leprae*, *Shigella dysenteriae*, and *Salmonella* sp., including *Salmonella typhi* and *Salmonella typhimurium*, *Serratia species*, *Staphylococcus aureus* and *Aeromonas* sp. [14].

Many parasites have been isolated from cockroaches e.g., cysts of *Entamoeba histolytica*, oocysts of *Cryptosporidium parvum*, *Cyclospora cayetanensis* and *Isoospora belli*, cysts of *Balantidium coli*, ova of *Ascaris lumbricoides*, *Anchylostoma deodunale*, *Enterobius vermicularis*, ovae *Trichuris trichura* and larvae of *Strongyloides stercoralis* [20].

A number of fungal species have been isolated from both the external body parts as well as faecal of cockroaches such as *Candida* spp., *Rhodotrula* spp., *Aspergillus* spp., *Fusarium* spp., *Penicillium* spp. and *Geotrichum* spp. were appeared on external surfaces of cockroaches. Other medically important mold, *Alternaria* spp., *Cladosporium* spp., *Trichoderma* spp., *Mucor* spp. and *Chrysosporium* spp. have been isolated from a few cockroaches, *Chrysosporium glabrata* and *Chrysosporium albicans* were the highest species isolated from cockroaches. *Chrysosporium parapsilosis* and *Chrysosporium guilliermondii* were present on the external surfaces in a few cockroaches. In addition, three species of *Aspergillus* have been identified via molecular characterization. *Aspergillus niger* was common and frequently isolated species from cockroaches. However, *A. fumigatus* and *A. flavus* were isolated from the external surface a few cockroaches. A total of 6 samples were found to carry two species of *Aspergillus* on their external surfaces [20, 21].

Helminths have also been reported in cockroaches collected from different areas [22], being widely implicated as reservoir hosts of medically important parasites [23, 24]. Pathogenic helminths included *Strongyloides stercoralis*, *Ascaris lumbricoides*, *Trichuris trichiura* and *Taenia* spp. [25]. The cockroaches are also implicated in the spread of at least some pathogens like hepatitis [12, 15, 26].

Protozoa types that were identified from cockroaches included *Cyclospora* spp., *Endolimax nana*, *Blastocystis hominis*, *Isoospora belli*, *Entamoeba histolytica*, *Cryptosporidium* spp., *Chilomastix mesnilli*, *Entamoeba coli*, *Balantidium coli* and *Iodamoeba butschlii* [25].

The control of *P. americana* can be done by applying insecticides to the hiding and resting places in the form of insecticidal dusts and residual sprays. Chemical control has been the most popular and effective method so far [27], but their control as insecticides is not a suitable because of several reasons; the most important of which is that they may develop resistance against certain frequently used insecticides [28]. Conventional or non-conventional insecticides were used against *P. americana* exhibited a high efficiency in controlling the insect pest [29].

Biological insecticides such as microbes, do not pose a disease risk to wildlife, humans, and other organisms not closely related to target insect [30, 31, 32]. Several new chemical substances with low mammalian toxicity have been evaluated for this purpose in several parts of the world, aiming to gradually replace the use of conventional insecticides, such as the organophosphorates (OPs) [33]. For instance, some pyrethroids have been successfully used as alternatives to OPs [34]. Spinosad, which is based on the metabolites from the actinomycete *Saccharopolyspora spinosa*, appears to be one of the most promising new grain protectants [35]. Spinosad has low mammalian toxicity and acts on the insects' nervous system, by ingestion or contact [34].

Spinosad is microbes that can be fermented to produce an insecticide such as abemectins, a fermented product of *Streptomyces avermitilis* used in baits for household insect pests. The best-known home gardening product of this type is spinosad. It composed of spinosyns A and D. The fermented product is very toxic to caterpillar pests such as cabbageworm, cabbage looper, diamondback moth, armyworm, and cutworm, as well as fruit flies such as spotted wing drosophila. Spinosad can act on a susceptible insect's stomach and nervous system. It is primarily ingested by feeding insects but can have some efficacy when sprayed directly on insects. Affected pests cease feeding and undergo partial paralysis within minutes upon exposure to spinosad, but it may take up to two days for the insects to die [36]. It has low toxicity to many beneficial insects that prey on pests and is nontoxic to mammals and other vertebrates, with the

exception of some fish. Because it is selectively toxic for many pest species and relatively safe to non-target species, spinosad has become highly desirable as an organic insecticide. Spinosyn A affect because it is effect on the involuntary muscle contractions and tremors by widespread excitation of neurons in the central nervous system and caused excitation when applied directly to isolated insect ganglia at submicromolar concentrations. Prolonged spinosyn-induced hyperexcitation resulted in paralysis that was associated with neuromuscular fatigue [36].

Much of the work regarding insecticidal efficacy has been done on *B. germanica*, however, very little data is available with respect to *P. americana*. Therefore, keeping in view the work carried out by various researchers, the present work was designed to investigate the insecticidal efficacy of formulations of fermented insecticides against *P. americana*, and the susceptibility of different stages to these insecticides through laboratory bioassay using feeding and contact toxicity methods.

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## 2. Material and methods

### 2.1. Experimental insect

*P. americana* was collected from dark and damp places (sewers) from different areas in Jeddah province by using food jars surrounded by dark cloth as a trap [37]. The strains were stored in the lab and used in this study. Traps were placed into main sewers. Cockroaches were collected every two days and placed in glass containers (30 × 60 × 30 cm). Then, they were thus kept under the laboratory condition of 25 ± 3 °C and 75 ± 5% RH.

### 2.2. Chemicals

The present study was designed to investigate the insecticidal efficacy of two different fermented insecticides: Spinosad tablets and Tracer 48 SC. The choice of these formulations was based on the fact that those chemicals have not been tested against different stages of *P. americana* in Jeddah governorate so far. All chemicals were obtained from Machinery & Agricultural Materials Co., Ltd, Jeddah, Saudi Arabia.

Insecticides were tested against *P. americana* adult and nymphs by feeding and contact toxicity methods, different concentrations were prepared and mortality percentages were recorded after 48 h.

### 2.3. Feeding bioassay

Feeding bioassay was done according to [38], with some modifications against adults and nymphs. For the present study, Bait was improvised in the laboratory. Feeding bioassays were conducted with lab strains using previous plastic boxes coated with petroleum jelly 2 cm from the inside top to prevent the cockroaches from escaping. 1 gm of white flour, 1gm of powder milk, 1 gm of sugar were prepared manually and treated with different concentrations of insecticides and appropriate amount of water to make semisolid bait. A single pellet was large enough to be entirely eaten by adults or nymphs starved for 24 h. Treated pellets were dried in a fume hood for 15-20 min. A single pellet was then provided to adults and nymphs held in approximately 3-4 gm. Control insects received treated pellets only with water. Each replicate consisted of 30 insects and three replicates for each concentration. Mortality was assessed at 48 h.

### 2.4. Contact toxicity bioassay

Contact toxicity bioassay was done according to [39], with some modifications against adults and nymphs. Contact toxicity mixture was improvised in the laboratory. Contact bioassays were conducted with previous method. Liquid mixture was then conducting by spraying different concentrations of the insecticide from inside plastic box and make sure that the insecticide covered all the sides. Three plastic boxes with 30 cockroaches (adults and nymphs) were used for each concentration.

### 2.5. Statistical analysis

This study was completely randomized design (CRD) in a factorial experiment. The data were statistically analyzed using analysis of variance (ANOVA). LC<sub>50</sub> and LC<sub>90</sub> were calculated according to Probit analysis program [40]. All Malformations were captured using digital camera.

### 3. Results

Feeding bioassay of Spinosad tablets was resulted in Table 1 and Fig. 1 against *P. americana* adults and nymphs after 48 h., of exposure periods and the results shows that low concentrations exhibited high mortality to adults and nymphs (100.00%) at the highest concentration of 5% and 3%, respectively. The nymphs were more sensitive to Spinosad tablets by LC<sub>50</sub>'s values (0.019%) followed by adults (0.065%) after 48 h., for contact toxicity bioassay with Spinosad tablets, there was positive correlation between mortality of Spinosad tablets concentrations and exposure intervals.

Mortality of adults and nymphs resulted after feeding bioassay with Tracer 48SC was summarized in Table 2 and Fig. 2. Mortality percentage were highly increased by increasing concentrations at all exposure intervals for adults and nymphs. After 48 h., Tracer 48SC gave high level of mortality to adults and nymphs (96.66%) at low concentrations. In the susceptibility level of nymphs and adults of *P. americana* after 48h., the nymphs were more sensitive to Tracer 48SC by LC<sub>50</sub> values (0.068%) than adults (0.097%) after 48h. For contact toxicity method, also there was positive correlation between mortality of Tracer 48SC concentrations and exposure intervals.

**Table 1** Susceptibility adults and nymphs of *P. americana* to Spinosad tablets using feeding and contact toxicity methods after 48h

Treatment	Concentration (%)	Mortality (%)	Parameters		X <sup>2</sup> *		Slope
			LC <sub>50</sub> (L-U)	LC <sub>90</sub> (L-U)	Calculated	Tabulated	
Adult Feeding	/ 0.01-5	26.66-96.66	0.065 (0.046-0.088)	1.921 (1.307-3.096)	2.456	11.1	0.873
Adult Contact	/ 0.0001-0.5	13.33-93.33	0.0018 (0.0013-0.0024)	0.062 (0.0405-0.1046)	3.897	12.6	0.832
Nymph Feeding	/ 0.01-1	43.33-96.66	0.019 (0.010-0.028)	0.698 (0.418-1.464)	5.232	9.5	0.816
Nymph Contact	/ 0.0001-0.1	26.66-96.66	0.001 (0.0004-0.0019)	0.063 (0.0322-0.2785)	11.84	11.1	0.709

LC<sub>50</sub>=lethal concentration that kill 50% of the treated insects, LC<sub>90</sub>= lethal concentration that kill 90% of the treated insects, U: upper limit, L: lower limit,

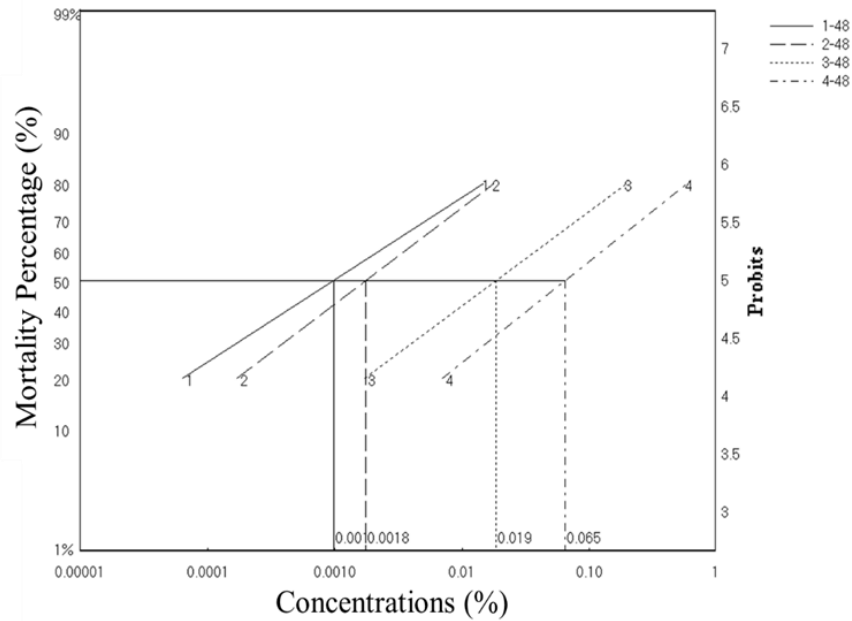
\* X<sup>2</sup>= Chi square, When tabulated (Chi)<sup>2</sup> larger than calculated at 0.05 level of significance indicates the homogeneity of results.

**Table 2** Susceptibility adults and nymphs of *P. americana* to Tracer 48SC using feeding and contact toxicity methods after 48h

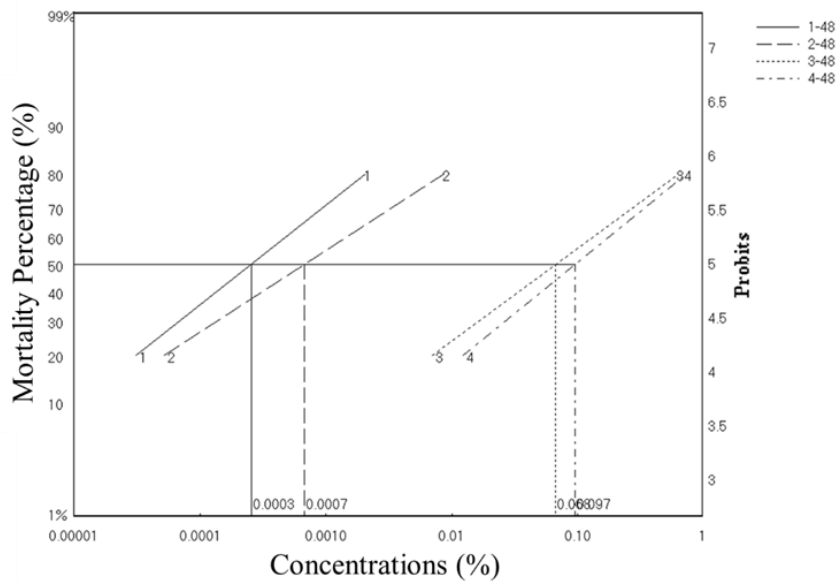
Treatment	Concentration (%)	Mortality (%)	Parameters		X <sup>2</sup> *		Slope
			LC <sub>50</sub> (L-U)	LC <sub>90</sub> (L-U)	Calculated	Tabulated	
Adult / Feeding	0.01-3	20-96.66	0.097 (0.0719-0.1282)	2.178 (1.4086-3.8346)	8.372	11.1	0.949
Adult / Contact	0.0001-0.1	26.66-96.66	0.0007 (0.0005-0.001)	0.034 (0.0216-0.0608)	1.35	12.6	0.756
Nymph Feeding	/ 0.01-3	23.33-96.66	0.068 (0.0476-0.0936)	2.128 (1.2976-4.1411)	2.365	9.5	0.858
Nymph Contact	/ 0.0001-0.01	40-96.66	0.0003 (0.0002-0.0004)	0.0064 (0.004-0.012)	4.792	9.5	0.919

LC<sub>50</sub>=lethal concentration that kill 50% of the treated insects, LC<sub>90</sub>= lethal concentration that kill 90% of the treated insects, U: upper limit, L: lower limit,

\* X<sup>2</sup>= Chi square, When tabulated (Chi)<sup>2</sup> larger than calculated at 0.05 level of significance indicates the homogeneity of results



**Figure 1** Susceptibility adults and nymphs of *P. americana* to Spinosad tablets using feeding and contact toxicity methods after 48 h



**Figure 2** Susceptibility adults and nymphs of *P. americana* to Tracer 48SC using feeding and contact toxicity methods after 48 h

#### 4. Discussion

The modes of action of fermented insecticide have not been documented, but it kills a wide range and variety of insect pests when it ingested or topically applied to control [41], and they exhibited broad-spectrum activity against insect species in different orders, especially Lepidoptera and Diptera [42]. In our finding, spinosad tablets and Tracer showed high mortality for both adults and nymphs with continuous nervous effects. Similar to our results, [43], showed that the spinosad exhibits a high level of toxicity with a dose-response relationship, and the determined LC<sub>50</sub> revealed a neurotoxic activity of spinosad. The results match those observed in earlier studies by [44], who mentioned that

insecticidal spinosyns have potent effects on the function of the GABA receptors of small-diameter cockroach neurons and can elicit a small-amplitude Cl<sup>-</sup> current. In another study, [33], examined the insecticidal effect of spinosad, against adults of the lesser grain borer, *Rhyzopertha dominica*, the rice weevil, *Sitophilus oryzae*, the confused flour beetle, *Tribolium confusum* Jacquelin on wheat and the larger grain borer, *Prostephanus truncatus* on maize and he found that *R. dominica* and *P. truncatus* were very susceptible to spinosad, followed by *S. oryzae*, while *T. confusum* was the least susceptible. The insecticidal mode of action of fermented insecticides are not completely understood, but is considered unique in comparison with other insecticides. It has been demonstrated their interaction with g-aminobutyric acid receptors and nicotinic acetylcholine receptors, eventually leading to the disruption of neuronal activity and consequent insect paralysis and death [45]. Our selective insecticides have not been used before in the control of different stages of *P. americana* in Jeddah governorate, but other formulations from the same class have been tested against other insects in other areas. Spinosad or Success<sup>®</sup> is the first member of the fermented insecticides, and it was first introduced for control of diamondback moth (DBM), *Plutella xylostella*, in Asia [46]. Spinosad appeared to be effective against the pest on aubergines. In field tests on onion, lambda-cyhalothrin and fipronil were highly effective on *T. tabaci*. The effect of spinosad on thrips in cotton was studied by [47]. In other study, field trial on efficacy of spinosad against vegetable pests was conducted by [48], they found that the foliar application of spinosad can control thrips in leeks and salad onion as well as caterpillar pests in head and flowering brussels sprouts. [49], investigated the bioefficacy of eight different insecticides based on four active substances in 2002 in Slovenia and found that spinosad and abamectin exhibited the highest efficiency against *T. tabaci*. [50], evaluated some newer insecticides for control of pomegranate fruit borer at Mahatma Phule Krishi Vidyapeeth, Rahuri (MS), India, by spraying spinosad 45 SC and it was effective. [51], conducted a field experiment at Dharwad, Karnataka, India to evaluate the efficacy of different insecticides against sucking pests of okra and they found that spinosad 45 SC was the most effective against thrips.

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## 5. Conclusion

The present study revealed that Spinosad Tablets and Tracer 48SC were toxicity and can be used as a biological control against *P. americana* adults and nymphs in Jeddah governorate.

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## Compliance with ethical standards

### Acknowledgments

The authors express their sincere gratitude to the Dengue Mosquito Experimental Station (DMES), belonging to the Department of Biological Sciences, Faculty of Sciences, King Abdul-Aziz University, Jeddah, Saudi Arabia for providing necessary equipments and their nice cooperating throughout the research period.

### Disclosure of conflict of interest

It has to be declared that the authors of the study have no conflict of interest among them.

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### How to cite this article

Sharawi S, Mahyoub J and Assagaf A. (2019). Laboratory evaluation of toxicity of spinosad tablets and tracer 48 SC insecticides against different stages of American cockroaches (*Periplaneta americana* L.), in Jeddah governorate. GSC Biological and Pharmaceutical Sciences, 6(1), 21-29.

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