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Susceptibility of Field Populations of *Blattella germanica* (Blattaria: Blattellidae) to Spinosad

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Abstract: The German cockroach is an important household insect pest worldwide and acts as a mechanical vector and reservoir for pathogenic agents. The aim of this study was to examine the basic laboratory toxicity of *Blattella germanica* to spinosad. The M, T, A22, AZAR4, BOOSTAN7 and ABAN21 strains were collected from field populations of six infested kitchen student dormitories and the SAMAN strain was collected from a residential area after insecticide spraying control failure in Tehran, Iran. Technical grade spinosad was delivered in 0.5 μL acetone to the first abdominal sternum of briefly CO_2-anesthetize adult male cockroaches by topical application bioassay. Treated males monitored for mortality. Mortality data from the replicates was assessed by probit analysis. The average LD_{50} of susceptible strain was 494.3, 148.8 and 55.1 ng per insect after 24, 48 and 72 h, respectively. The LD_{50} of spinosad decreased with time in the field population strains. All German cockroach strains showed a similar susceptibility or lower tolerance (1.6-folds) for spinosad compared with the susceptible laboratory strain and the steep slopes of dose-response curves indicated that the field population of these German cockroach strains was homogenous in response to spinosad. These results indicated that the spinosad was relatively slow-acting in topical application bioassay, with LD_{50} values decreasing until 72 h and becoming stable thereafter. The effectiveness of spinosad against susceptible and the field population German cockroach strains in laboratory condition showed that spinosad probably could be useful for the control of the German cockroach.

Key words: *Blattella germanica*, field populations, spinosad, susceptibility

INTRODUCTION

Spinosad is the first member of the Naturalyte class of insecticides developed by Dow Agro Sciences (Sparks *et al*., 1995) and relatively new insecticide that was commercially introduced in 1997. It is effective with a high level of activity against many insects, including agricultural, urban and medical pests and low environmental and human risk (Thompson *et al*., 2000; BCPC, 2004). Spinosad is derived from the bacterium *Saccharopolyspora spinosa* and it consists of a mixture of 50-95% spinosyn A and 5-50% spinosyn D (Sparks *et al*., 1995). This insecticide acts primarily through activation of the nicotinic acetylcholine receptor but at a different site from that of nicotine itself or another compound known as imidacloprid. A secondary site of attack involves the GABA receptors (Salgado, 1997) but here also at a different site from one activated by abamectin (Thompson *et al*., 2000). These facts suggest that changes in the target sites of these receptors because of the development of resistance to other insecticides (i.e., *ace, kdr,* or *Rdl*) would not cause cross-resistance to spinosad (Shono and Scott, 2003; Hsu and Feng, 2006).

The German cockroach, *Blattella germanica* (L.), is an important household insect pest worldwide. In addition to its economic importance, the German cockroach is a significant health hazard and acts as a mechanical vector and reservoir for pathogenic agents (Weber, 1984; Rust *et al*., 1995; Ramirez, 1989; Pridgeon *et al*., 2002). Increased incidents of asthma caused by German cockroach allergens are associated with relatively high cockroach infestations and have added emphasis to the need for the control of this insect pest (Roberts, 1996).

Efforts to control this pest often include the use of insecticides. However in laboratory experiments, this species has been shown to develop resistance and cross-resistance to different classes of insecticides, including carbamate-, organophosphate- and pyrethroid-based compounds (Cochran, 1989; Scott *et al*., 1990; Atkinson *et al*., 1991; Valles and Yu, 1996; Dong *et al*., 1998; Valles, 1999; Valles *et al*., 2000; Wei *et al*., 2001; Ladonni, 2000, 2001; Nasirian *et al*., 2006a-c; Hsu and

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Feng, 2006; Limoee *et al*., 2006, 2007; Nasirian *et al*., 2009; Nasirian, 2010; Paksa *et al*., 2011). Generally, in Iran so far insecticide resistance to permethrin, cypermethrin, cyflutherin, deltamethrin, coopex, Actelic, Ficam, diazinon and lambdacyhalothrin have been reported but in some studies the susceptibility to pirimphos methyl, lambdacyhalothrin, diazinon, fenitrothion and propoxor have been reported (Nasirian, 2010).

Currently, the fights against German cockroaches are considered as a serious problem. Despite all the losses would be caused by use of chemical insecticides and although much research to be going in the field of alternative methods for pest control. Insecticides are still considered the most appropriate tool in the management of pests and disease vectors. Even in advanced combat integration systems, application of the insecticides is the most important keys in the cockroach combat programs, especially German cockroach (Alali *et al*., 1998; Nasirian, 2010).

Attentive to resistance of *B. germanica* to current consuming insecticides and ever-increasing add to intensity of it, the researchers have been considering the susceptibility level of *B. germanica* and efficiency estimation to new insecticides for control of this pest. The new insecticidal compounds such as spinosad acting at novel sites on the nicotinic acetylcholine receptor which are probably not affected by resistance can be used for pest control especially *B. germanica* (Nasirian, 2010).

When using any new insecticide such as spinosad, it is important to understand the basic susceptibility of organisms such as *B. germanica,* to monitor the development of any resistance and to determine whether any cross-resistance to other insecticides occurs. Because spinosad has not previously been used for control purposes in commercial for German cockroach infestation control in Iran, it is an appropriate time to investigate these issues. The present work examined the basic susceptibility of *B. germanica* to spinosad.

MATERIALS AND METHODS

Insects: The toxicity of spinosad was examined in German cockroach. Eight *B. germanica* strains were used in this study. The susceptible strain SUS has been held at the Cockroach Insectary of the Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences without exposure to any insecticide for >30 year and was used in bioassays for comparison (Ladonni, 2001). The M, T, A22, AZAR4, BOOSTAN7 and ABAN21 strains were collected from field populations of six infested kitchen student dormitories and the SAMAN strain were collected from a residential area after insecticide spraying control failure in Tehran, Iran and reared at insectary. The all strains were permethrin, cypermethrin, cyfluthrin, DDT resistant and the SAMAN strain was fipronil very low resistant (2.4-fold) after collecting and rearing in the insectary (Nasirian *et al*., 2006a-c; Limoee *et al*., 2006, 2007; Nasirian *et al*., 2009).

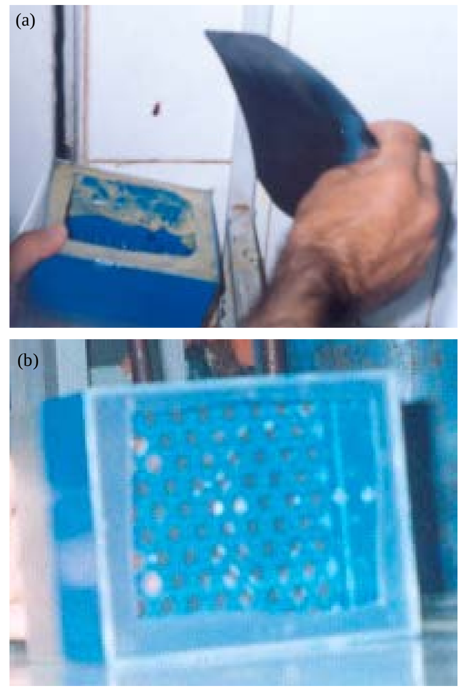

Fig. 1(a-b): (a) Cockroach collecting approach with a piece of a radiology film and apparatus; (b) Lateral view of apparatus

Cockroaches collecting and rearing: Cockroaches were collected in the last hours of the night with a piece of a radiology film (10×10 cm) for the flexibility nature and a flash light (Fig. 1a) and transferred to an apparatus by hand catch (Fig. 1b). The apparatus manufactured from two parts, the upper inside surface of the upper part (5 cm) was lightly greased with petroleum jelly to prevent cockroaches from escaping (Fig. 1a), after collecting cockroaches and in the insectary the lower part separated from the upper part and cockroaches transferred to glass rearing jars to prevent cockroaches from greasing (Nasirian, 2006; Nasirian *et al*., 2009, 2006c).

After collecting all cockroaches were maintained and colonized at 27±2°C, 60±10% RH and a photoperiod of

12: 12 (L: D) h in the insectary at the School of Public Health, Tehran University of Medical Sciences. Each strain was kept in separate labeled glass rearing jars of the same size (4 L). Cockroaches were provided with rodent diet, a cotton plugged water vial and a cardboard as a harborage.

Chemicals and reagents: Spinosad (technical grade, 90.4% (W/W)) was supplied by Dow AgroSciences (Indianapolis, IN), CO_2 as an anesthetic and acetone as a solvent were used.

Topical application bioassay: Technical grade spinosad (90.4% (AI); Dow AgroSciences), was delivered in 0.5 μL acetone to the first abdominal sternum of briefly CO_2-anesthetize adult male cockroaches by topical application using a micro applicator (Hamilton, Reno, NV) equipped with a 1.0 mL hypodermic glass syringe fitted with a 27-gauge needle (Scott *et al*., 1990). Individual cockroaches used for experiments were in all cases adult males <1 month old and the age of cockroaches were established by the separating of the female with egg capsule from the colony and the body shape freshness. Adult male cockroaches were treated with 5-6 dose concentration of insecticide giving > 0 and < 100% mortality and each concentration was replicated 3-6 times (10 cockroaches for each replicate). Control groups (10 cockroaches for each replicate) received acetone alone and was negligible (<1%) mortality. Treated males were placed in plastic Petri dishes (150 by 25 mm), provided with food and water and monitored for mortality for 24, 48 and 72 h under the same temperature and photoperiod as the colony. If insects on their backs were unable to right themselves when prodded, they were considered dead.

Statistical analysis: Mortality data from the replicates were pooled and the time exposure mortality was assessed by probit analysis (Finney, 1972), with a SPSS package (SPSS 11.5 version for windows) on an IBM computer.

RESULTS

Toxicity of spinosad against susceptible german cockroach strain: Spinosad was relatively slow-acting in topical application bioassays, with LD_{50} values decreasing until 72 h, which was used for determination of spinosad toxicity (Fig. 2a-c). The average LD_{50} of susceptible strain was 494.3, 148.8 and 55.1 ng per insect after 24, 48 and 72 h, respectively and the average LD_{90} was 873.0, 482.7 and 97.4 ng per insect after 24, 48 and 72 h, respectively (Table 1-3).

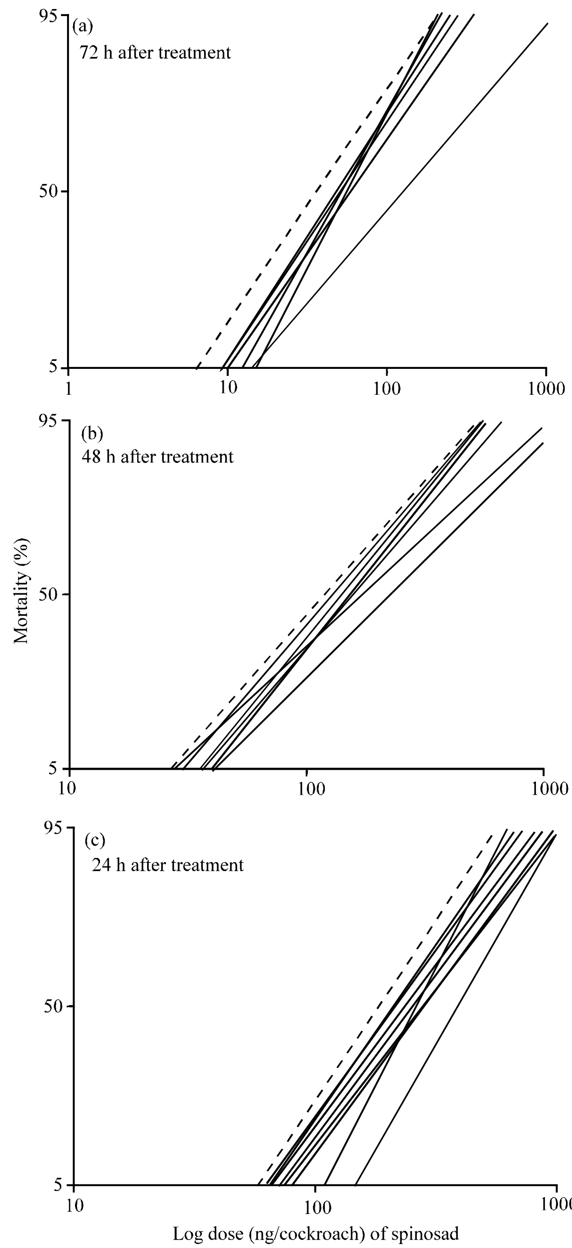

Fig. 2(a-c): Probit-regression lines of spinosad on mortality of different German cochroach strains. Solid lines indicate field population strains and broken line show susceptible laboratory strain

Toxicity of spinosad against field population german cockroach strains: All German cockroach strains showed a similar susceptibility or lower tolerance (1.6-folds) for spinosad compared with the susceptible laboratory strain (Table 1-3) and the steep slopes of dose-response curves indicated that the field population of these German

Table 1: Toxicity of spinosad topically applied to different *Blattella germanica* strains monitored for mortality for 24 h after treatment

	24 h after treatment									
Strains	n^a	Intercept±SE	Slope±SE	χ^2	df	p-value	LD_{50}^b (CI)	LD_{90}^b (CI)	RR_{50}^c	RR_{90}^c
SUS	200	-1.67±0.20	0.003±0.0005	6.56	3	0.09	494.32 (347.06-647.55)	872.98 (686.99-1123.30)	-	-
BOOSTAN7	240	-0.54±0.16	0.003±0.0005	27.09	4	0.004	496.01 (239.52-657.75)	895.90 (704.42-1180.71)	1.003	1.03
M	240	-1.42±0.18	0.003±0.0005	4.94	4	0.18	504.22 (402.12-584.63)	902.72 (746.81-1198.17)	1.02	1.03
T	200	-4.96±0.86	0.009±0.0016	0.41	3	0.98	542.08 (506.38-583.13)	882.16 (730.70-1077.11)	1.10	1.01
SAMAN	200	-3.10±0.60	0.004±0.0011	1.68	3	0.43	793.44 (661.94-1048.82)	1395.19 (1177.78-1539.84)	1.60	1.60
A22	200	-1.56±0.25	0.003±0.0006	3.01	3	0.22	599.93 (498.14-716.44)	1092.45 (858.47-1389.47)	1.21	1.25
AZAR4	240	-2.42±0.30	0.007±0.0010	0.68	4	0.71	497.49 (323.05-632.77)	877.27 (684.16-1101.15)	1.01	1.005
ABAN21	200	-1.58±0.21	0.002±0.0005	1.98	3	0.58	661.62 (445.69-892.96)	1059.18 (902.26-1208.12)	1.34	1.21

[a]Numbers of cockroach treated. [b]LD_{50}, LD_{90} values in nanograms per insect (95% CI). [c]Resistance ratio: LD_{50} of feral strain/LD_{50} of susceptible strain

Table 2: Toxicity of spinosad topically applied to different *Blattella germanica* strains monitored for mortality for 48 h after treatment

	48 h after treatment									
Strains	n^a	Intercept±SE	Slope±SE	χ^2	df	p-value	LD_{50}^b (CI)	LD_{90}^b (CI)	RR_{50}^c	RR_{90}^c
SUS	200	-0.57±0.16	0.004±0.0005	24.88	3	0.002	148.76 (23.77-340.33)	482.67 (364.12-635.57)	-	-
BOOSTAN7	240	-2.44±0.32	0.025±0.0035	4.13	4	0.13	149.14 (57.77-262.35)	481.22 (308.09-626.31)	1.003	1.00
M	240	-1.68±0.17	0.007±0.0007	38.79	4	0.003	154.35 (36.88-331.04)	506.50 (356.89-668.99)	1.04	1.05
T	200	-0.59±0.21	0.003±0.0006	0.52	3	0.77	180.83 (81.80-287.48)	572.64 (474.95-664.85)	1.22	1.19
SAMAN	200	-0.83±0.21	0.002±0.0006	1.84	3	0.18	243.58 (108.87-398.48)	826.07 (672.48-979.84)	1.64	1.71
A22	200	-1.72±0.31	0.006±0.0009	0.34	3	0.56	186.74 (109.59-317.99)	555.86 (408.06-727.57)	1.26	1.15
AZAR4	240	-1.15±0.18	0.005±0.0007	12.80	4	0.005	163.44 (14.46-318.98)	491.34 (340.17-662.97)	1.10	1.02
ABAN21	200	-1.12±0.18	0.006±0.0007	16.54	3	0.001	196.82 (24.05-329.12)	590.91 (435.37-779.30)	1.32	1.22

[a]Numbers of cockroach treated. [b]LD_{50}, LD_{90} values in nanograms per insect (95% CI). [c]Resistance ratio: LD_{50} of feral strain/LD_{50} of susceptible strain

Table 3: Toxicity of spinosad topically applied to different *Blattella germanica* strains monitored for mortality for 72 h after treatment

	72 h after treatment									
Strains	n^a	Intercept±SE	Slope±SE	χ^2	df	p-value	LD_{50}^b (CI)	LD_{90}^b (CI)	RR_{50}^c	RR_{90}^c
SUS	200	-1.67±0.18	0.03±0.004	1.68	3	0.64	55.14 (47.73-64.28)	97.37 (84.70-116.71)	-	-
BOOSTAN7	240	-1.67±0.18	0.02±0.003	2.15	4	0.71	72.46 (62.59-75.43)	128.27 (110.38-146.67)	1.31	1.32
M	240	-1.61±0.16	0.02±0.002	1.42	4	0.84	69.14 (54.51-87.67)	115.95 (95.15-135.59)	1.25	1.19
T	200	-1.74±0.20	0.04±0.005	2.19	3	0.53	55.07 (38.92-73.03)	98.23 (77.44-125.49)	1.00	1.01
SAMAN	200	-1.53±0.21	0.03±0.004	0.11	3	0.95	91.16 (66.94-124.99)	161.33 (137.24-184.40)	1.65	1.66
A22	200	-1.85±0.20	0.03±0.004	0.76	3	0.86	58.97 (51.48-68.27)	99.77 (87.25-118.70)	1.07	1.02
AZAR4	240	-1.66±0.18	0.02±0.003	2.15	4	0.71	72.46 (62.59-85.43)	128.27 (110.38-156.67)	1.31	1.32
ABAN21	200	-1.75±0.21	0.03±0.003	16.90	3	0.001	65.41 (57.83-76.43)	113.26 (89.50-131.69)	1.19	1.16

[a]Numbers of cockroach treated. [b]LD_{50}, LD_{90} values in nanograms per insect (95% CI). [c]Resistance ratio: LD_{50} of feral strain/LD_{50} of susceptible strain

cockroach strains was homogenous in response to spinosad (Fig. 2). The LD_{50} of spinosad decreased with time in the field population strains. At the end of the bioassay at 72 h, LD_{50} varied 36-fold from 55.1 to 91.2 ng per insect (Table 1-3), which is 1.0- and 40-fold higher than the standard susceptible value of spinosad. These results indicated that the spinosad was relatively slow-acting in topical application bioassay, with LD_{50} values decreasing until 72 h and becoming stable thereafter.

DISCUSSION

These results are similar to the resistance ratios found in insecticide-resistant German cockroach strain, which ranged 1.3 in topical application bioassay (Wei *et al*., 2001). These results indicated that most populations of German cockroach were susceptible to spinosad and similar to earlier baseline values (Wei *et al*., 2001). In topical application bioassay spinosad was 57.4- and 7.8-fold less and more toxic to susceptible German cockroach strain compared with fipronil LD_{50} = 0.96 ng/cockroach and permethrin LD_{50} = 0.43 μg/cockroach, respectively (Nasirian *et al*., 2006a, c, 2009). The LD values of spinosad toxicity in topical application bioassay decreased 11.15, 30.10 and 37.03% from 24 to 72 h, 24 to 48 h and 48 to 72 h, respectively.

In this study one strain (SAMAN), fipronil very low resistant (2.4-fold), permethrin (11.9-fold), cypermethrin (5.4-fold), cyfluthrin (2.9-fold) and DDT resistant strain (see material and methods), showed a 1.6-fold tolerant to spinosad. It should be concluded that this strain probably showed cross-resistance between spinosad and fipronil because the other strains (resistant to permethrin, cypermethrin, cyfluthrin and DDT) were susceptible to spinosad and fipronil. Because spinosad has a secondary site of attack that involves the GABA receptors, similar to fipronil mode of action. Some studies which confirmed this work showed that the P_{450} complex, which is involved in pyrethroid resistance, is not involved in spinosad. Furthermore, cross-resistance between spinosad and pyrethroids has not been detected in strains possessing enhanced detoxification systems (Sparks *et al*., 2001). More recently, no cross-resistance to spinosad has been found in pyrethroid-resistant *Musca domestica* L. (Liu and Yue, 2000), *B. germanica* (Wei *et al*., 2001) and *Culex quiquefasciatus* Say (Liu *et al*., 2004).

Like imidacloprid and fipronil, spinosad was relatively slow-acting against German cockroaches, with maximum toxicity at 72 h. Wei *et al*. (2001) reported that spinosad in a comprison among spinosad, permethrin and deltamethrin bioassay toxicity, had relatively low toxicity to German cockroaches, with an LD_{50} value of 500 ng per cockroach after 24 h treatment and monitoring for mortality (Wei *et al*., 2001). This study indicates that the response of cockroaches to spinosad differs from that of German cockroaches and house flies, where LD_{50} values for spinosad to German cockroaches 500 ng per cockroach after 24 h treatment and LD_{50} values for spinosad to house flies ranged from 24 (72 h) to 94 (24 h) ng per fly (Scott, 1998; Wei *et al*., 2001).

The results from resistant laboratory strains indicate that in German cockroach, cross-resistance to the pyrethroid class will not initially be of major concern for the use of spinosad for German cockroach control. Wei *et al*. (2001) in Apyr-R, a high level permethrin and deltamethrin resistant (resistance ratios of 97 and 480 for permethrin and deltamethrin, respectively), strain showed no cross-resistance to spinosad, although spinosad had relatively low toxicity to German cockroaches compared with permethrin and deltamethrin. This result further confirmed that the mode of action of spinosad to insects is unique. Absence of cross-resistance between permethrin and spinosad in the subject German cockroach populations could be a result of different modes of action and metabolism of these insecticides (Sparks *et al*., 2001). The effectiveness of spinosad against susceptible and permethrin-resistant German cockroach strains in laboratory condition, combined with the low mammalian toxicity of spinosad (LD_{50} oral in rat = 3,783-5,000 mg kg^{-1}) (Sparks *et al*., 2001), showed that spinosad probably could be useful for the control of permethrin resistant German cockroach.

CONCLUSION

This study demonstrates, for the first time, that the effectiveness of spinosad against susceptible and the field population German cockroach strains in laboratory condition probably could be useful for the control of the German cockroach especially in toxic bait formulations (Nasirian *et al*., 2006b; Nasirian, 2007, 2008).

Further studies are needed to clarify the effects and toxicity of this novel insecticide, spinosad, based on the data generated (LD_{50} of susceptible strain 494.3, 148.8 and 55.1 ng per insect after 24, 48 and 72 h, respectively) against German cockroach under field conditions.

Also are proposed further studies on toxicity of novel insecticides such as a vermectins acting at GABA, gama-aminobutyric acid and glutamate receptors in proximity to chloride channels, diacylhydrazines mimicking the action of ecdysone, indoxacarb acting at a novel site in sodium channels and thiamethoxam acting at novel sites on the nicotinic acetylcholine receptor, which are probably not affected by resistance that will be used for pest control specially against German cockroach, will be evaluated in Iran.

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