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Sublethal effects on reproduction and biomarkers by spinosad and indoxacarb in cockroaches *Blattella germanica*

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Abstract

The present project investigated effects of two insecticides, the naturalyte spinosad and the oxadiazine indoxacarb, against adults of the cosmopolitan pest insect *Blattella germanica* (L.). At 6 days after topical treatment on newly moulted adults, the median lethal dose (LD_{50}) was 429 ng/insect for spinosad and 51 ng/insect for indoxacarb, indicating that indoxacarb was more active than spinosad. Both treatments showed a cessation of feeding, followed by paralysis and death. In continuation, surviving adults showed a reduced reproduction with a significant reduction in numbers of oocytes, in basal oocyte volume, and in numbers of laid and hatched eggs per ootheca. In parallel, we determined the biomarkers acetylcholinesterase (AChE), lactate deshydrogenase (LDH), glutathione *S*-transferase (GST) and glutathione (GSH) upon treatment. For both spinosad and indoxacarb, AChE and GSH were reduced, while LDH and GST were increased. The data are discussed in relation to insecticide use, sublethal effects, chemical stress and metabolism.

Key words: insecticides, spinosad, indoxacarb, neurotoxicity, reproduction, fecundity, chemical stress, metabolism.

Introduction

Organophosphate (OP), carbamate and pyrethroid insecticides, and gel bait formulations of newer insecticides such as fipronil and imidacloprid, have been widely used to control cockroaches as the important and cosmopolitan German cockroach *Blattella germanica* (L.). However, many cockroaches have developed resistance to these insecticide groups, so that insecticide resistance is now a huge practical problem, challenging the control of economically and medically important insect pests (Dong *et al*., 1998; Wang *et al*., 2004). Therefore, safer and more selective insecticides with new modes of action and a benign ecotoxicological profile are urgently needed to reduce resistance development. Thus, new potent chemistries with low ecotoxicological risks such as spinosad and indoxacarb have been developed (Wing *et al*., 2000).

Spinosad is naturally derived from the fermentation of the actinomycete *Saccharopolyspora spinosa* Mertz et Yao, comprising two macrocyclic lactones: spinosyn A and spinosyn D (Mertz and Yao, 1990; Sparks *et al*., 1995). Interestingly, spinosad has a new mode of action, primarily targeting binding sites on the nicotinic acetylcholine receptors (nAChRs), and in addition, it has secondary effects on gamma-aminobutyric acid (GABA) neurotransmission (Salgado, 1998; Salgado *et al*., 1998). For practice, spinosad is reported to act both by contact and ingestion. It has a strong insecticidal activity, particularly against Lepidoptera and Diptera, and it shows low levels of mammalian toxicity and relatively low hazards to non-target insects and aquatic invertebrates (Thompson and Sparks, 2002; Pineda *et al*., 2004; Sarfraz *et al*., 2005; Gao *et al*., 2007; Wang *et al*., 2004; 2005; 2009; Besard *et al*., 2011; Biondi *et al*., 2012). Combined with its biodegradability, selectivity and lack of cross-resistance, this new mode of action makes it useful in IPM and anti-resistance applications.

Indoxacarb is an oxadiazine pesticide and its main mode of action is blockage of the nerve sodium channels (Lahm *et al*., 2001). Moreover, mammals convert indoxacarb into non-toxic metabolites, which contributes to its selective toxicity to insect pests (Wing *et al*., 2000). Indoxacarb exhibits insecticidal activity against a wide range of the pest insects with no or low adverse effects on numerous non-target insects (Dinter and Wiles, 2000; N'Guessan *et al*., 2007; Habbachi *et al*., 2009; Gondhalekar *et al*., 2011; Mahmoudvand *et al*., 2011). Therefore, this compound could constitute as reduced-risk insecticide useful in IPM programs. Finally, several studies demonstrated the successful use of indoxacarb in controlling insects resistant to carbamates, OPs and pyrethroids (Chai and Lee, 2010).

The objective of the present research was to determine the insecticidal potency of these two biorational insecticides when applied topically against adults of *B. germanica*. In addition to toxicity, the sublethal effects on reproduction in surviving adults were determined. Here, the numbers of oocytes per ovary, size of the basal oocyte, preoviposition period, fecundity and egg fertility were measured. Finally, we investigated the metabolic effects as response to an exposure to spinosad and indoxacarb with a selection of biomarkers as acetylcholinesterase (AChE), lactate deshydrogenase (LDH), gluthatione *S*-transferase (GST) and glutathione (GSH).

Materials and methods

Insects

Colonies of *B. germanica* were reared in plastic boxes (30 × 30 × 30 cm) at 27 ± 1 °C under a 12 h light:12 h dark regime and 70 ± 1% relative humidity, and fed with dog food pellets and water *ad libitum* as described previ-

ously (Habes *et al*., 2006). Newly moulted males and females were separated according to sexual morphologic characters (body morphology, posterior abdomen, and styli) and they were used in the insect bioassays.

Insecticides

The commercial formulation Success 480 SC (Suspension Concentrate) containing spinosad at 480 g/L was obtained from Dow Agrosciences, Indianapolis, IN, USA). For indoxacarb, we used the commercial 30 WG formulation (Water Granule, 30% active ingredient) as obtained from DuPont, Wilmington, DE, USA.

Treatment of insects and insect toxicity bioassay

The insecticides were treated separately by topical application at 3 µl solution per insect on newly moulted male and female adults as collected from the insect colony. Different stock solutions of spinosad were prepared in acetone and of indoxacarb in distilled water, so that insects were treated with different doses of 180, 360, 720 and 1440 ng spinosad per insect and of 45, 60, 75 and 90 ng indoxacarb per insect. Per dose, 3 replicates were done each consisting of 20 insects. The experiment was done separately for males and females. At daily basis up to 6 days after treatment, we scored the insects for abnormalities as cessation of feeding, tremors and paralysis and mortality. The mortality percentages were corrected for control mortality (<20%) with Abbott's formula and then analysed by probit analysis to calculate the lethal doses (LD_{50} and LD_{90}) and lethal times (LT_{50} and LT_{90}) with their corresponding 95% fiducial limits (95%FL) as described before (Kilani-Morakchi *et al*., 2009).

Ovarian parameters

An extra series of newly moulted females of *B. germanica* was treated topically with spinosad and indoxacarb at their respective LD_{50} and LD_{90} as determined above: spinosad was dosed at 429 and 1539 ng/insect, and indoxacarb at 51 and 87 ng/insect. Adult females from control and treated series were sampled at different ages (0, 2, 4 and 6 days) during the first gonadotrophic cycle and their ovaries dissected out. The numbers of oocytes in each paired ovary were scored together with the volume of the basal oocyte (Lambreas *et al.*, 1991).

Reproductive events

Newly moulted females of *B. germanica* were treated topically as described above with 3 µl of a solution of spinosad or indoxacarb per insect. Spinosad and indoxacarb were dosed at their respective LD_{50} and LD_{90} as above. In these experiments the males were not treated. The duration of the preoviposition period, incubation period and embryonic development of ootheca of the first gonadotrophic cycle were recorded (Taibi *et al.*, 2003). The fecundity (numbers of eggs laid per female during the first gonadotrophic cycle) and the egg hatchability (percentage of neonates that emerged from eggs) were determined in each series (4 to 8 repeats per dose).

Biomarker assays for AChE, LDH, GST and GSH

As described by Habes *et al*. (2006), new moulted male adults of *B. germanica* (<8 h old) were treated topically with 3 µl of a solution of spinosad or indoxacarb per insect. Spinosad and indoxacarb were dosed at their respective LD_{50} and LD_{90} as above. Then, samples were collected at 24, 48 and 72 h after treatment. For AChE activity measurements, we used 3 pooled heads per repeat and 4-5 repeats per time interval; for LHD, GST and GSH activities, individual decapitated bodies were used per repeat and 4-5 repeats per time interval.

The AChE activity was measured according the protocol of Ellman as previously described (Habes *et al*., 2006). In brief, adult heads were homogenized in 1 ml of the following solution: 38 mg ethylene glycol tetracetic acid (EGTA), 1 ml Triton X-100%, 5.845 g NaCl and 80 ml Tris buffer (0.01 M, pH 7). After centrifugation (5000 rpm, 5 min), AChE activity was measured in aliquots (100 µl) of resulting supernatant added to 100 µl of 5-5'dithiobis-2-nitrobenzoic acid (DNTB) and 1 ml of Tris buffer (0.1 M, pH 7). After 5 min, 100 µl of acetylthiocholine were added. Measurements were conducted at 412 nm every 4 min for a period of 20 min.

The assay of LDH was conducted according to the method of Hill and Lévi (1954) using NAD (nicotinamide adenine dinucleotide) as substrate. The adult decapitated bodies were individually homogenized in 1 ml of Tris/HCl (0.1 M, pH 7.2). The homogenate was centrifuged (3000 rpm for 5 min) and then the supernatant recovered for use as enzyme source. The assay was performed with 50 µl of supernatant added to 675 µl of substrate buffer (0.2 M, pH 10) and 50 µl of NAD solution. The absorbance reading was done every minute for 5 min at 340 nm.

The assay of GST was carried out according to Habig *et al*. (1974) with use of GSH (5 mM) and 1-chloro-2-4-dinitrobenzoic acid (CDNB, 1 mM). Adult decapitated bodies were individually homogenized in 1 ml of buffer phosphate (0.1 M, pH 6). The homogenate was centrifuged (1300 rpm for 30 min) and the supernatant collected used for the enzymatic assay. Hereto, 200 µl of the resulting supernatant were added to 1.2 ml of the mixture GSH-CDNB in phosphate buffer (0.1 M, pH 7). Changes in absorbance were measured at 340 nm every minute for a period of 5 min.

The assay of GSH was conducted according to the method of Weckberker and Cory (1988). Adult decapitated bodies were individually homogenized in 1 ml of EDTA (0.02 M). The homogenate was then subjected to a deproteinization with sulfosalicylic acid (SSA) 0.25%. Then 0.8 ml homogenate was added to 0.2 ml of the mixture, this was vortexed and left for 15 min in an ice bath before centrifugation (1000 rpm for 5 min). The supernatant (0.5 ml) was supplemented with 1 ml of Tris-EDTA (0.02 M, pH 9.6) and 0.025 ml (5-5'-dithiobis-2-nitrobenzoic acid (DTNB, 0.01 M) and then left at room temperature for 5 min. The optical density was measured at 412 nm after 5 min.

Protein assay

All enzymatic activities were expressed as µmol per min and per mg protein and GSH as µmol per mg protein. Hereto, the protein amounts in the total homogenate were quantified with Coomassie brilliant blue G250 as reagent and bovine serum albumin (BSA) as

standard. The absorbance was read in a spectrophotometer at 595 nm.

Statistical analysis

Results are presented as means ± standard deviation (s.d). The homogeneity of variances was checked by a Levene's test. The significance between different series was tested using Student's *t*-test at 5% level. Data were subjected to one-way or two-way analysis of variance (ANOVA) followed by a *post-hoc* Tukey test HSD. All statistical analyses were performed using MINITAB software v14 (Penn State College, PA, USA) and $p < 0.05$ was considered statistically different.

Results

Insecticidal activity against adults

Typically, adults of *B. germanica* treated with spinosad and indoxacarb demonstrated a cessation of feeding and symptoms of tremors, followed by paralysis and death. The percentages of mortality after treatment of adult males and females were determined as function of different doses of spinosad (180, 360, 720, 1440 ng/insect) and indoxacarb (45, 60, 75, 90 ng/insect) and the time after treatment (3, 4, 5 and 6 days). After 6 days of treatment with the highest dose of spinosad (1440 ng/insect) and indoxacarb (90 ng/insect), the corrected mortality increased to 88 ± 4% in males and 89 ± 4% in females and 91 ± 4% in males and 91 ± 3% in females, respectively. ANOVA indicated that the two insecticides are toxic with a significant ($p < 0.001$) effect of dose, time and interaction dose-time.

As presented in table 1, the respective values of LD_{50} (ng/insect) for spinosad and indoxacarb against adults of *B. germanica* are 3420 and 237 at 3 days after topical treatment and these decreased to 429 and 51 ng/insect at day 6. The respective values of LD_{90} (ng/insect) for spinosad and indoxacarb are 70620 and 1917 at 3 days, and 1539 and 87 at 6 days (table 1). For spinosad, the respective LT_{50} and LT_{90} (days) are 6.1 and 10.2 at 180 ng/insect, and 4.1 and 6.3 at 1440 ng/insect; for indoxacarb, these are 6.0 and 9.2 at 45 ng/insect, and 4.6 and 6.4 at 90 ng/insect (table 2).

Effects on morphometry of ovaries

In the control females, the numbers of oocytes per paired ovary decreased at day 4 which is the moment that coincides with the beginning of ovulation (Kilani-Morakchi *et al*., 2009). As shown in table 3, topical application of spinosad and indoxacarb at their respective LD_{50} and LD_{90} caused a significant decrease ($p < 0.001$) in the numbers of oocytes at 2, 4 and 6 days. ANOVA indicated a significant ($p < 0.001$) effect of dose, time and interaction dose-time for the two insecticides. Comparison between spinosad and indoxacarb demonstrated a higher effect by indoxacarb ($p < 0.001$).

On the volume of the basal oocyte during the gonadotropic cycle, the controls showed an increase ($p < 0.05$) from 0.0060 ± 0.0006 mm^3 at day 0 to 0.0930 ± 0.0090 mm^3 at day 6 (table 4). Topical treatment of spinosad and indoxacarb at their respective LD_{50} and LD_{90} (at 6 days) reduced ($p < 0.001$) the basal oocyte volume at all tested ages. ANOVA indicated a significant ($p < 0.001$) effect of dose, time and interaction dose-time for the two tested insecticides. Comparison between both insecticides indicated that indoxacarb was more active ($p < 0.001$).

Effect on reproductive events

In the controls, the mean duration of the preoviposition period was 7.3 ± 0.6 days, while this was increased by 33-105% due to treatment with spinosad and indoxacarb (figure 1). With spinosad at LD_{50} and LD_{90} this was 9.7 ± 0.6 days and 12.3 ± 0.6 days, respectively; for indoxacarb these respective values were 12.3 ± 0.6 days and 15 ± 0 days. Comparison between the two insecticides indicated that indoxacarb was more active ($p = 0.005$).

The mean incubation duration of the ootheca (*i.e.* time period between ootheca appearance and egg hatching) in the control series was 17 days and this period was not different over the different treatments with spinosad and indoxacarb ($p > 0.05$).

The numbers of eggs laid per female (fecundity) in the control series were 40 ± 2, while the treatments with spinosad and indoxacarb at LD_{50} and LD_{90} showed lower numbers of eggs laid per female ($p < 0.05$). The reductions varied between 37 and 63% over the controls. With the LD_{50} of spinosad, the numbers had de-

Table 1. Lethal doses of spinosad and indoxacarb when topically applied on newly moulted adults of *B. germanica.* The data are expressed as lethal doses LD_{50} and LD_{90} (ng/insect) together with the corresponding 95% fiducial limits (95%FL) as function of the exposure time (days).

Time (days)	Compound	Slope	LD_{50} (95%FL) (ng/insect)	LD_{90} (95%FL) (ng/insect)
3	Spinosad	7.50 ± 0.85	3420 (3080-4860)	70620 (73800-81966)
	Indoxacarb	4.70 ± 0.83	237 (216-342)	1917(1296-2838)
4	Spinosad	5.20 ± 0.40	2544 (1686-3843)	51498 (34104-62766)
	Indoxacarb	3.40 ± 0.11	489 (388-536)	669 (534-834)
5	Spinosad	3.70 ± 0.14	1332 (807-1599)	28965 (23010-32640)
	Indoxacarb	2.70 ± 0.17	93 (66-129)	354 (255-492)
6	Spinosad	2.70 ± 0.11	429 (342-537)	1539 (1230-1923)
	Indoxacarb	1.50 ± 0.20	51 (48-57)	87 (81-96)

Table 2. Lethal times of spinosad and indoxacarb when topically applied on newly moulted adults of *B. germanica*. The data are expressed as lethal times LT_{50} and LT_{90} (days) together with the corresponding 95% fiducial limits (95%FL) as function of the dose (ng/insect).

Treatment	Dose (ng/insect)	Slope	LT_{50} (95%FL) (days)	LT_{90} (95%FL) (days)
Spinosad	180	1.20 ± 0.12	6.10 (6.30-6.70)	10.20 (10.50-10.80)
	360	1.27 ± 0.14	5.70 (5.30-6.00)	9.70 (9.10-10.30)
	720	1.43 ± 0.12	4.80 (4.60-5.20)	7.60 (7.00-8.30)
	1440	1.53 ± 0.11	4.10 (3.70-4.40)	6.30 (5.60-6.90)
Indoxacarb	45	1.38 ± 0.17	6.00 (5.70-6.50)	9.20 (8.60-9.80)
	60	1.34 ± 0.04	5.50 (5.30-5.70)	8.10 (7.80-8.30)
	75	1.31 ± 0.09	5.00 (4.80-5.20)	7.10 (6.90-7.50)
	90	1.29 ± 0.14	4.60 (4.30-4.70)	6.40 (6.00-6.80)

Table 3. Number of oocytes per paired ovary in surviving adult females of *B. germanica* during the first 6 days following adult emergence (m ± s.d; n = 4-8).

Age (days)	Control	Spinosad		Indoxacarb	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}
0	53 ± 2 A				
2	65 ± 2 a B	45 ± 3 b A	38 ± 2 c A	42 ± 1 b A	37 ± 2 c A
4	58 ± 4 a C	36 ± 2 b B	29 ± 1 c B	32 ± 1 b B	26 ± 1 c B
6	44 ± 2 a D	27 ± 1 b C	21 ± 1 c C	22 ± 2 c C	16 ± 1 d C

Per column, different capital letters indicate a significant difference between ages of the same series.
Per row, different small letters indicate a significant difference between control and treated series of the same age.

Table 4. Volume of basal oocyte (mm^3) in surviving adult females of *B. germanica* during the first 6 days following adult emergence (m ± s.d; n = 4-8).

Age (days)	Control	Spinosad		Indoxacarb	
		LD_{50}	LD_{90}	LD_{50}	LD_{90}
0	0.0060 ± 0.0006 a A				
2	0.0097 ± 0.0004 a B	0.0027 ± 0.0005 b A	0.0016 ± 0.0002 c A	0.0019 ± 0.0001 b A	0.0008 ± 0.0001 c A
4	0.0240 ± 0.0010 a C	0.0058 ± 0.0010 b B	0.0026± 0.0006 c B	0.0030 ± 0.0003 b B	0.0014 ± 0.001 c B
6	0.0930 ± 0.0090 a D	0.0064 ± 0.0005 b B	0.0041 ± 0.0003 c C	0.0040 ± 0.0005 b C	0.0025 ± 0.0001 c C

Per column, different capital letters indicate a significant difference between ages of the same series.
Per row, different small letters indicate a significant difference between control and treated series of the same age.

creased to 25.3 ± 1.2, and then further to 20.3 ± 0.6 with the LD_{90}. With indoxacarb at LD_{50} this resulted in only 20.7 ± 1.2 eggs and at LD_{90} in even lower numbers of 15.0 ± 1.0. Indoxacarb was more active ($p = 0.003$) than spinosad.

Figure 1 shows that there were strong effects towards the fertility as the numbers of eggs hatched per female were reduced by 62-93% due to spinosad and indoxacarb. In the controls there were 38 ± 2 hatched eggs, while only 14.3 ± 0.6 with the LD_{50} and 6.7 ± 0.6 with the LD_{90} of spinosad ($p < 0.001$). With indoxacarb at its LD_{50} this was 9.7 ± 0.6 and at its LD_{90} only 2.7 ± 0.6 ($p < 0.001$). Taken together, the hatching percentage (fertility) in the control series was 93 ± 2% and this had decreased ($p < 0.001$) to 33 ± 3% with the LD_{90} of spinosad and to 18 ± 3% with the LD_{90} of indoxacarb ($p = 0.003$). The effects by indoxacarb were stronger than by spinosad ($p < 0.001$).

Effects on biomarkers of AChE, LDH, GST and GSH (figures 2 and 3)

In the controls, there was no significant change ($p > 0.05$) in AChE activity during the experiment of 3 days. With spinosad and indoxacarb at their LD_{50} and LD_{90} there was a significant ($p < 0.01$) decrease and this in a dose-dependent manner. Comparison indicated that indoxacarb was more active than spinosad ($p < 0.001$).

Data for the specific activity of LDH in control series demonstrated an increase in function of time ($p < 0.001$); the activity (in µmol/min/mg protein) increased from 65 ± 3 at 24 h over 86 ± 2 at 48 h to 107 ± 6 at 72 h. Treatment with spinosad and indoxacarb caused a significant ($p < 0.001$) induction. ANOVA showed a significant ($p < 0.001$) effect of dose and time and interaction dose-time. As for AChE, indoxacarb was more active ($p < 0.001$) than spinosad.

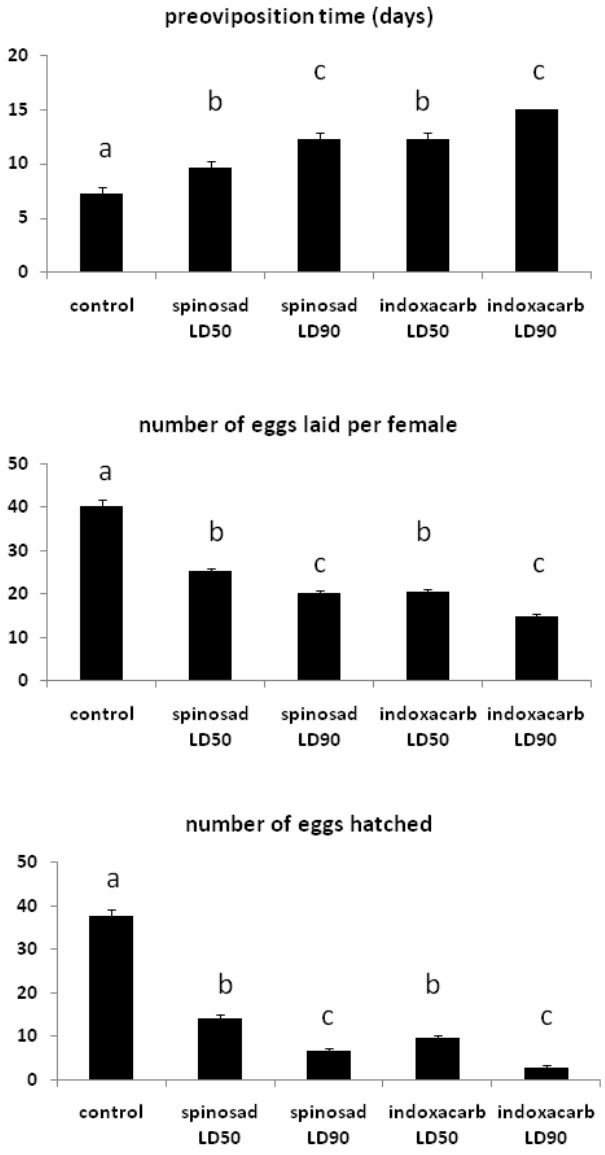

Figure 1. Effect of spinosad and indoxacarb when applied at their respective LD_{50} and LD_{90} on the reproductive events of adults of *B. germanica* as preoviposition period (days), fecundity (numbers of eggs laid per female) and fertility (numbers of eggs hatched). Data are expressed as m ± s.d, based on n = 4-8. Different letters indicate a significant difference between control and treated series of the same age.

In the control series, the specific activity of GST (in µmol/min/mg protein) increased to 43 ± 4 at 48 h ($p = 0.01$) and to 54 ± 2 at 72 h ($p = 0.009$). As for LDH, treatment with spinosad and indoxacarb caused an increase ($p < 0.001$) in the specific GST activity, and the effect was dependent in function of time, dose and the interaction dose-time ($p < 0.001$). The increases by indoxacarb were higher than with spinosad ($p < 0.001$).

The amounts of GSH (in µmol/mg protein) in the control series showed an increase in function of time with 0.20 ± 0.001 at 48 and 0.31 ± 0.001 at 72 h ($p < 0.001$). Data showed a significant ($p < 0.001$) reduction in GSH amounts in the series treated with both insecticides at their two doses over the whole experiment. Two-way ANOVA indicated a significant ($p < 0.001$) effect by dose, time and interaction dose-time. Indoxacarb was also more active than spinosad ($p < 0.001$).

Discussion and conclusions

Insecticidal toxicity against *B. germanica*

Typically, cockroaches intoxicated with spinosad and indoxacarb showed tremors followed by paralysis and insect death. For spinosad, these symptoms of poisoning agree with previous observations and can be explained by its action via binding with the insect nAChR (Wing *et al*., 1998). For indoxacarb, this oxadiazine insecticide is known to have an acute neurotoxicity by blockage of the sodium channels in the nervous system of insects, causing tremors, paralysis and death in a couple of hours as reported before in different insects (Wing *et al.*, 2005).

On the insecticidal potency, spinosad showed a lower potency against cockroaches as its LD_{50} was 429 ng/insect (corresponding with LC_{50} = 143.16 ppm) and this agrees with a previous report of Wei *et al.* (2001) calculating an LD_{50} of 500 ng/cockroach. On average, spinosad is about 50 times less toxic than other insecticides as fipronil (LD_{50} = 3.9 ng/cockroach), imidacloprid (96 ng/cockroach), deltamethrin (5.4 ng/cockroach) and permethrin (75 ng/cockroach). But it should be noted here that the insecticidal activity of spinosad largely depends on the insect species which may be related to the nAChR subunits in the insects (Rinkevich and Scott, 2012). Mosquitoes as *Glossina palpalis gambiensis* Vanderplank were found highly susceptible for spinosad (LC_{50} = 2.2 ppm) compared to deltamethrin (4.2 ppm) (De Deken *et al*., 2004). Spinosad was also effective against *Aedes albopictus* (Skuse) with an LC_{50} of 0.3 ppm (Bond *et al*., 2004). In Lepidoptera, spinosad caused significant mortality in *Lymantria dispar* (L.) with an LC_{50} of 8.7 ppm (Wanner *et al*., 2002), and another study demonstrated that spinosad is highly toxic against *Helicoverpa armigera* (Hubner) with an LC_{50} of 0.41 ppm (Wang *et al*., 2009). The high toxicity of spinosad by ingestion has also been reported in *Spodoptera frugiperda* (Guenee) (Méndez *et al.*, 2002). Finally, it is of interest for practice in resistance management that spinosad binds at the nAChR but at distinct sites on the receptor compared to imidacloprid (Blacquière *et al*., 2012; Rinkevich and Scott, 2012). For the second compound of this study, indoxacarb, the calculated LD_{50} (corresponding with LC_{50} = 17 ppm) indicated that this compound was more toxic than spinosad in *B. germanica*. Interesting for resistance management, Chai and Lee (2010) reported that German cockroaches resistant to organophosphates, carbamates, pyrethroids and fipronil were susceptible to indoxacarb. N'Guessan *et al*. (2007) showed larvicidal and adulticide activity against *Anopheles gambiae* (Meigen) resistant to pyrethroid insecticides. This compound was also found to be toxic in *Plutella xylostella* (L.) with an LC_{50} of 4.8 ppm (Mahmoudvand *et al*., 2011) and to *Ostrinia nubilalis* (Hubner) with an LC_{50} of 12.7 ppm (Alves *et al*., 2008).

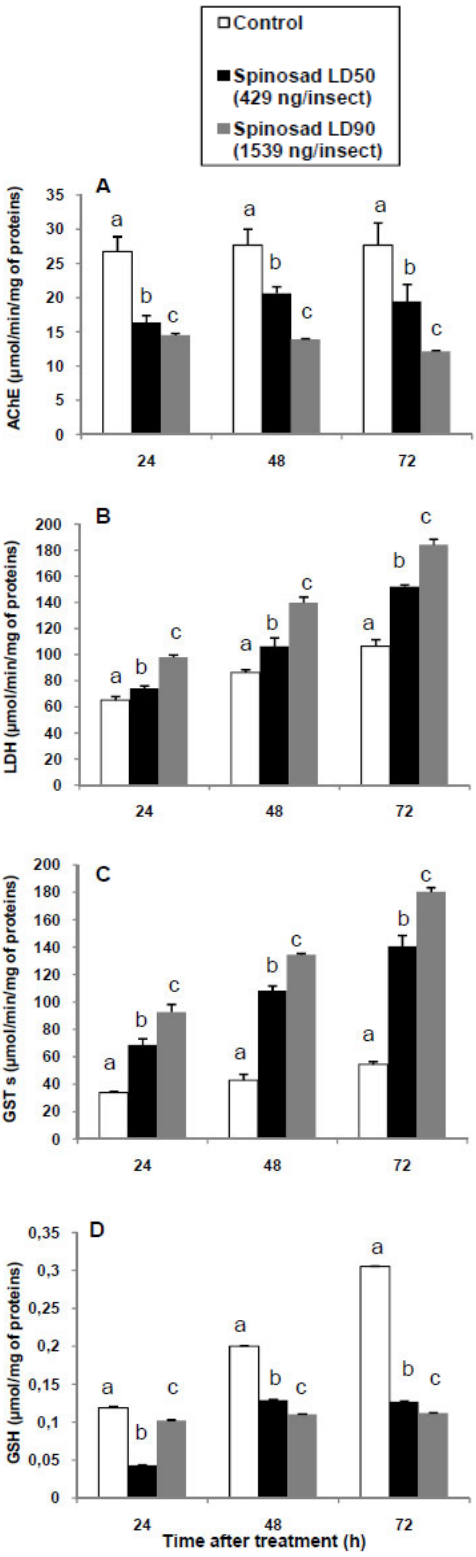

Figure 2. Effect of spinosad when applied at its respective LD_{50} and LD_{90} on four biomarkers in adults of *B. germanica* at 24, 48 and 72 h after topical treatment. Data are expressed as m ± s.d, based on n = 4-5. **A**: acetylcholinesterase (AChE) activity (µmol/min/mg protein); **B**: lactate deshydrogenase (LDH) activity (µmol/min/mg protein); **C**: glutathione *S*-transferase (GSTs) activity (µmol/min/mg protein); **D**: glutathione (GSH) amounts (µmol/mg protein). Different letters indicate a significant difference between control and treated series of the same age.

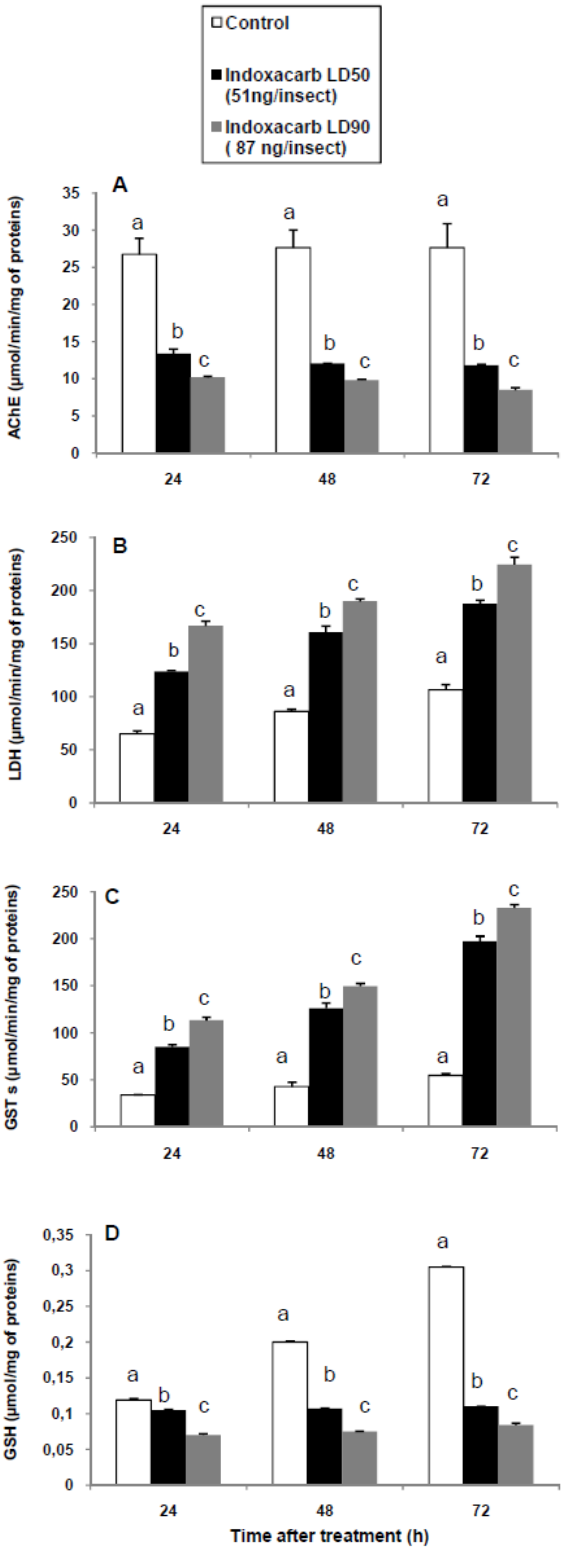

Figure 3. Effect of indoacarb when applied at its respective LD_{50} and LD_{90} on four biomarkers in adults of *B. germanica* at 24, 48 and 72 h after topical treatment. Data are expressed as m ± s.d, based on n = 4-5. **A**: acetylcholinesterase (AChE) activity (µmol/min/mg protein); **B**: lactate deshydrogenase (LDH) activity (µmol/min/mg protein); **C**: glutathione *S*-transferase (GSTs) activity (µmol/min/mg protein); **D**: glutathione (GSH) amounts (µmol/mg protein). Different letters indicate a significant difference between control and treated series of the same age.

Taken together, it can be concluded that the two insecticides tested exhibited insecticidal activity against *B. germanica*, and indoxacarb was the most potent. In addition, they have shown field insecticidal activity, environmental compatibility and safety to non-target organisms which makes them useful in IPM and anti-resistance programs.

Effects on reproductive parameters

In female insects, reproduction comprises a succession of interdependent steps from sex determination to oviposition (Gäde and Hoffmann, 2005). In untreated adults of *B. germanica*, the numbers of oocytes per pair of ovaries increased up to day 2 after adult emergence and decreased thereafter starting at day 4; the latter event coincides with the beginning of ovulation (Kilani-Morakchi *et al*., 2009). Treatment with spinosad and indoxacarb on newly emerged females of *B. germanica* disrupted the oocyte growth with a clear reduction in the numbers of oocytes per ovaries and the volume of the basal oocyte. In addition, also the preoviposition was longer upon treatment with spinosad and indoxacarb. While the preoviposition period in the controls took 7 days, which agrees with previous data of Ang and Yang (2011), this period was enlarged by 33-105% over the controls by spinosad and indoxacarb. However, the treatments had no effect on the duration of the oothecal incubation. But the two compounds had a strong effect on the total numbers of eggs per female and the numbers of eggs hatched. The fecundity was reduced by 37-50% with spinosad and by 49-63% with indoxacarb, the fertility by 68-82% and 74-93%, respectively. In agreement with our observations in *B. germanica*, Peterson *et al*. (1998) reported reproductive effects in the Lepidoptera *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.). Spinosad shortened adult longevity, reduced reproductive capacity, egg hatchability and egg production of *H. armigera* (Wang *et al*., 2009) and *P. xylostella* (Yin *et al*., 2009). Spinosad also had negative effects on survival and reproduction of *Daphnia pulex* (L.) and *Daphnia magna* Straus (Duchet *et al*., 2011). Finally, it is worth to mention that in the fruit fly *Ceratitis capitata* (Wiedemann), spinosad (2.1 ppm) did not pose an ovicidal effect on eggs but it reduced the survival of larvae hatched from treated eggs (Pineda *et al*., 2004).

For spinosad and indoxacarb, Galvan *et al*. (2005) reported that both compounds affected different life history parameters of adult ladybirds of *Harmonia axyridis* (Pallas). In these experiments, spinosad reduced the fertility of *H. axyridis* females, while indoxacarb reduced the fecundity without an effect on egg hatching but its effects on the overall reproductive capacity of *H. axyridis* were greater than for spinosad. Similarly, in *P. xylostella*, indoxacarb significantly reduced the fecundity (Mahmoudvand *et al*., 2011). Hence, it is of interest to mention that ingestion of low doses of spinosad also caused sublethal effects against the aggregation pheromone and the cuticular hydrocarbon profile in *B. germanica* (Habbachi *et al*., 2009), which resulted in social and sexual communication aberrations and finally in a loss of reproduction.

In summary, for the two tested compounds, spinosad and indoxacarb, it is suggested that the reduction in fecundity of the treated insects could be related to the indirect effects induced by the tested compounds, causing decreased food intake, disturbed somatic physiology or cytotoxic destruction of the ovaries/eggs. However, future research is needed to fully understand the molecular mechanisms behind the observed reproductive effects by spinosad and indoxacarb.

Effects on biomarkers

It is well recognized that biomarkers are useful tools for toxicologists and environmental scientists. They help to predict the toxicity and understand better the mode of action of chemicals and also to study environmental exposures and stress to potentially toxic compounds. In this project we have chosen to follow the enzyme activities of a selection of key enzymes. AChE (E.C.3.1.1.7.) is a serine protease that hydrolyzes the neurotransmitter ACh in the cholinergic nerves. It is found at mainly neuromuscular junctions and cholinergic brain synapses where it terminates the synaptic transmission. Indeed inhibition of AChE, resulting in over accumulation of ACh and prolonged electrical activity at nerve endings, comprises a key mechanism of toxicity for OP and carbamate pesticides. LDH (E.C.1.1.1.27) is a somewhat non-specific enzyme but present in a wide variety of tissues, and it is widely used as marker in toxicology to diagnose cell, organ and tissue damage and breakdown. As enzyme, it converts pyruvate to lactate, underlining its importance in glycolysis. For GSH, this tripeptide is known as antioxidant, preventing damage to important cellular components caused by reactive oxygen species such as free radicals and peroxides. It has been confirmed as a good indicator for oxidative stress; indeed the amounts of GSH within cells are often used as a measure of cellular toxicity. Finally, GSTs (E.C.2.5.1.18) compose an enzyme family of many cytosolic, mitochondrial and microsomal proteins. They are present in eukaryotes and in prokaryotes where they catalyze a variety of reactions and accept endogenous and xenobiotic substrates. GSTs contribute to the phase II biotransformation of xenobiotics as many pesticides; they conjugate these compounds with reduced GSH. Induction of GST activity is an indication of a detoxification process and is associated with pesticide resistance, and in addition, GSTs are well-known for their involvement in the mitigation of generalized oxidative stress.

In the current project, spinosad and indoxacarb significantly reduced the specific activity of AChE in *B. germanica*. Although these data were to our surprise because spinosad and indoxacarb are not AChE inhibitors as OP and carbamate insecticides, a similar reduction in AChE activity was also observed in *A. mellifera* upon treatment with spinosad (Rabea *et al*., 2009), in *B. germanica* by boric acid (Habes *et al*., 2006) and in Nilaparvata lugens (Stal) by azadirachtin (Senthil Nathan *et al*., 2008). To explain this effect, we hypothesize here that the impact of spinosad and indoxacarb against AChE is indirect. Indeed spinosad molecules bind on the nAChR in competition with ACh. Subsequently, when ACh cannot act because spinosad is bound on the nAChR, the postsynaptic potential and action potential are absent, and in turn the postsynaptic vesicles cannot liberate the neurotransmitter and

indirectly AChE is disrupted. Also for indoxacarb, this insecticide caused a reduction in AChE. It is known that this insecticide blocks the sodium channels and in turn this may negatively affect neurotransmission. In agreement, Gamil *et al*. (2011) reported that the toxic effect of indoxacarb causing the onset of paralysis and blockage of the action potential resulted in a decrease in AChE.

In this project with *B. germanica*, we believe that the increase in LDH activity in the control may be related to carbohydrate metabolism. For the treatment with spinosad and indoxacarb, however, the increase of LDH may here be explained by chemical stress induced by these insecticides. In contrast, for other insecticides as azadirachtin and spinetoram, treatment showed an inhibitory effect in the activity of LDH of *Cnaphalocrocis medinalis* (Guenee) (Senthil Nathan *et al*., 2006) and *S. littoralis* (Fahmy and Dahi, 2009). In conclusion, although we observed significant effects, we cannot make firm conclusions on the meaning of these. Future mechanistic investigations should indicate the importance, for instance, in relation to chemical stress and cell/tissue damage.

On the GST activities in *B. germanica*, spinosad and indoxacarb induced an increase with a significant dose effect. Similarly, Valles *et al*. (2000) described a strong correlation in *B. germanica* between the increase of GST activity and exposure/resistance to several pesticide groups. As reported by Gondhalekar and Sharf (2012), GSTs together with P450s are involved in insecticide resistance in the German cockroach. Evidently, the role of GSTs as detoxifying enzymes for pesticides, and particularly for spinosad and indoxacarb, has been confirmed over the last years by different authors and this for different insects (Sayyed *et al*., 2008; Wang *et al*., 2009; Pang *et al*., 2012; Reyes *et al*., 2012).

In our experiments, it was clear that the increase of GST activity was also correlated with a decrease in GSH amounts after treatment with spinosad and indoxacarb. Indeed GSH is known as a non-enzymatic oxidative stress parameter; GSTs conjugate xenobiotics with use of reduced GSH. Perez-Pertejo *et al.* (2008) reported a deficiency in GSH upon exposure to spinosad and this resulted in cellular damage and pathological disorders such as neurodegeneration. A decrease of GSH was also observed in larvae of *Galleria mellonella* (L.) treated with malathion (Büyükgüzel, 2009). Taken together, we believe that the increase in GST activity and the decrease in GSH amounts by spinosad and indoxacarb reveal an induction of oxidative stress and a stimulation of detoxification system, which were both more marked with indoxacarb.

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References

ALVES A. P., ALLGEIER W. J., SIEGFRIED B. D., 2008.- Effects of the synergist S,S,S-tributyl phosphorotrithioate on indoxacarb toxicity and metabolism in the European corn borer, *Ostrinia nubilalis* (Hübner).- *Pesticide Biochemistry and Physiology*, 90: 26-30.

ANG L. H., YANG C., 2011.- Absence of a fitness penalty in insecticide-resistant German cockroaches, *Blattella germanica* (L.) (Dictyoptera: Blattellidae).- *International Journal of Pest Management*, 57: 195-204.

BESARD L., MOMMAERTS V., ABDU-ALLA G., SMAGGHE G., 2011.- Lethal and sublethal side-effect assessment supports a more benign profile of spinetoram compared with spinosad in the bumblebee *Bombus terrestris*.- *Pest Management Science*, 67: 541-547.

BIONDI A., MOMMAERTS V., SMAGGHE G., VIÑUELA E., ZAPPALA L., DESNEUX N., 2012.- Non-target impact of spinosyns on beneficial arthropods, a review.- *Pest Management Science*, 68: 1523-1536.

BLACQUIÈRE T., SMAGGHE G., VAN GESTEL C. A. M., MOMMAERTS V., 2012.- Neonicotinoids in bees: a review on concentrations, side effects and risk assessment.- *Ecotoxicology*, 21: 973-992.

BOND J. G., MARINA C. F., WILLIAMS T., 2004.- The naturally derived insecticide spinosad is highly toxic to Aedes and Anopheles mosquito larvae.- *Medical and Veterinary Entomology*, 18: 50-56.

BÜYÜKGÜZEL E., 2009.- Evidence of oxidative and antioxidative responses by *Galleria mellonella* larvae to malathion.- *Journal of Economic Entomology*, 102: 152-159.

CHAI R. Y., LEE C. Y., 2010.- Insecticide resistance profiles and synergism in field populations of German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae) from Singapore.- *Journal of Economic Entomology*, 103: 460-471.

DE DEKEN R., SPEYBROECK N., GILLAIN G., SIGUE H., BATAWI K., BOSSCHE P. V. D., 2004.- The macrocyclic lactone, "spinosad", a promising insecticide for tsetse fly control.- *Journal of Medical Entomology*, 41: 814-818.

DINTER A., WILES J. A., 2000.- Safety of the new DuPont insecticide indoxacarb to beneficial arthropods: an overview.- *Bulletin OILB/SROP*, 23: 149-156.

DONG K., VALLES S. M., SCHARF M. E., ZEICHNER B., BENNETT G. W., 1998.- The knockdown resistance (kdr) mutation in pyrethroid-resistant German cockroaches.- *Pesticide Biochemistry and Physiology*, 60: 195-204.

DUCHET C., MITIEINAFUKU M., CAQUET T., LARROQUE M., FRANQUET E., LAGNEAU C., LAGADIC L., 2011.- Chitobiase activity as an indicator of altered survival, growth and reproduction in *Daphnia pulex* and *Daphnia magna* (Crustacea: Cladocera) exposed to spinosad and diflubenzuron.- *Ecotoxicology and Environmental Safety*, 74: 800-810.

FAHMY N. M., DAHI H. F., 2009.- Changes in detoxifying enzymes and carbohydrate metabolism associated with spinetoram in two field-collected strains of *Spodoptera littoralis* (Biosd.).- *Egyptian Academic Journal of Biological Sciences*, 1: 15-26.

GÄDE G., HOFFMANN K. H., 2005.- Neuropeptides regulating development and reproduction in insects.- *Physiological Entomology*, 30: 103-121.

GALVAN T. L., KOCH R. L., HUTCHISON W. D., 2005.- Effects of spinosad and indoxacarb on survival, development and reproduction of the multicolored Asian lady beetle (Coleoptera: Coccinellidae).- *Biological Control*, 34: 108-114.

GAMIL W. E., MARIY F. M., YOUSSEF L. A., ABDELHALIM S. M., 2011.- Effect of Indoxacarb on some biological and biochemical aspects of *Spodoptera littoralis* (Boisd.) larvae.- *Annals of Agricultural Science*, 56: 121-126.

GAO R., DONG J., ZHANG W., CHEN W. L., 2007.- Dietary risk assessment of spinosad in China.- *Regulatory Toxicology and Pharmacology*, 49: 31-42.

GONDHALEKAR A. D., SCHARF M. E., 2012.- Mechanisms underlying fipronil resistance in a multiresistant field strain of the German cockroach (Blattodea: Blattellidae).- *Journal of Medical Entomology*, 49: 122-131.

GONDHALEKAR A. D, SONG C., SCHARF M. E., 2011.- Development of strategies for monitoring indoxacarb and gel bait susceptibility in the German cockroach (Blattodea: Blattellidae).- *Pest Management Science*, 67: 262-270.

HABACHI W., BENSAFI H., ADJAMI Y., OUAKID M. L., FARINE J. P., Everaertz C., 2009.- Spinosad affects chemical communication in the German cockroach, *Blattella germanica* (L).- *Journal of Chemical Ecology*, 35: 1423-1426.

HABES D., KILANI-MORAKCHI S., ARIBI N., FARINE J. P., SOLTANI N., 2006.- Boric acid toxicity to the German cockroach, *Blattella germanica*: alterations in midgut structure, and acetylcholinesterase and glutathione *S*-transferase activity.- *Pesticide Biochemistry and Physiology*, 84: 17-24.

HABIG W. H., PABST M. J., JAKOBY W. B., 1974.- Glutathione S-transferases: the first enzymatic step in mercapturic acid formation.- *Journal of Biological Chemistry*, 249: 7130-7139.

HILL B. R., LÉVI C., 1954.- Elevation of a serum component in neoplastic disease.- *Cancer Research*, 14: 513-515.

KILANI-MORAKCHI S., ARIBI N., FARINE J. P., SMAGGHE G., SOLTANI N., 2009.- Halofenozide affects reproduction, sexual behaviour and cuticular hydrocarbon of the *Blattella germanica* (Dictyoptera: Blattellidae).- *Belgian Journal of Zoology*, 139: 147-155.

LAHM G. P., MCCANN S. F., HARRISON C. R., STEVENSON T. M., SHAPIRO R., 2001.- Evolution of the sodium channel blocking insecticides: the discovery of indoxacarb, pp. 20-34. In: *Agrochemical discovery insect, weed, and fungal control* (BAKER D. R., UMETSU N. K., Eds), Symposium Series 774. American Chemical Society, Washington, DC, USA.

LAMBREAS C. L., GALANTE F., MENA L., 1991.- Ovarian condition as an indicator of the phenology of *Bubas bubas* (Coleoptera: Scarabeidae).- *Annals of the Entomological Society of America*, 84: 190-194.

MAHMOUDVAND M., ABBASIPOUR H., GARJAN A. S., BANDANI A. R., 2011.- Sublethal effects of indoxacarb on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Yponomeutidae).- *Applied Entomology and Zoology*, 46: 75-80.

MÉNDEZ W. A., VALLE J., IBARRA J. E., CISNEROS J., PENAGOS D. I., WILLIAMS T., 2002.- Spinosad and nucleopolyhedrovirus mixtures for control of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) in maize.- *Biological Control*, 25: 195-206.

MERTZ F. P., YAO R. C., 1990.- *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar rum still.- *International Journal of Systematic Bacteriol*ogy, 40: 34-39.

N'GUESSAN R., CORBEL V., BONNET J., YATES A., ASIDI A., BOKO P., ODJO A., AKOGBÉTO M., ROWLAND M., 2007.- Evaluation of indoxacarb, an oxadiazine insecticide for the control of pyrethroid-resistant *Anopheles gambiae* (Diptera: Culicidae).- *Journal of Medical Entomology*, 44: 270-276.

PANG S., YOU W., DUAN L., SONG X., LI X., WANG C., 2012.- Resistance selection and mechanisms of oriental tobacco budworm (*Helicoverpa assulta* Guenee) to indoxacarb.- *Pesticide Biochemistry and Physiology*, 103: 219-223.

PÉREZ-PERTEJO Y., REGUERA R. M., ORDÓÑEZ D., BALAÑA-FOUCE R., 2008.- Alterations in the glutathione-redox balance induced by the bio-insecticide spinosad in CHO-K1 and Vero cells.- *Ecotoxicology and Environmental Safety*, 70: 251-258.

PETERSON G., HERZOG A., DURAN A., PILSNER F., MICINSKI S., LARSON L., NEAD-NYLANDER A., HUCKABA M., PORTEOUS J., 1998.- The ovicidal activity of Tracer Naturalyte insect control against heliothine species in conventional cotton, pp. 1209-1211. In: *Proceedings Beltwide Cotton Conference*. National Cotton Council San Diego, CA, USA.

PINEDA S., BUDIA F., SCHNEIDER M. I., GOBBI A., VIÑUELA E., VALLE J., DEL ESTAL P., 2004.- Effects of Two Biorational Insecticides, Spinosad and Methoxyfenozide, on *Spodoptera littoralis* (Lepidoptera: Noctuidae) under laboratory conditions.- *Journal of Economic Entomology*, 97: 1906-1911.

RABEA E. I., NASR H. M., BADAWY M. E., 2009.- Toxic effect and biochemical study of chlorfluazuron, oxymatrine, and spinosad on honey bees (*Apis mellifera*).- *Archives of Environmental Contamination and Toxicology*, 58: 722-732.

REYES M., ROCHA K., ALARCÓN L., SIEGWART M., SAUPHANOR B., 2012.- Metabolic mechanisms involved in the resistance of field populations of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) to spinosad.- *Pesticide Biochemistry and Physiology*, 102: 45-50.

RINKEVICH F. D., SCOTT J. G, 2012.- Reduction of dADAR activity affects the sensitivity of *Drosophila melanogaster* to spinosad and imidacloprid.- *Pesticide Biochemistry and Physiology*, 104: 163-169.

SALGADO V. L., 1998.- Studies on the mode of action of spinosad: Insect symptoms and physiological correlates.- *Pesticide Biochemistry and Physiology*, 60: 91-102.

SALGADO V. L., SHEETS J. J., WATSON G. B., SCHMIDT A. L., 1998.- Studies on the mode of action of spinosad: The internal effective concentration and the concentration dependence of neuronal excitation.- *Pesticide Biochemistry and Physiology*, 60: 103-110.

SARFRAZ M., DOSDALL L. M., KEDDIE B. A., 2005.- Spinosad: a promising tool for integrated pest management.- *Outlooks on Pest Management*, 16: 78-84.

SAYYED A.H., SAEED S., NOOR-UL-ANE M., CRICKMORE N., 2008.- Genetic, biochemical, and physiological characterization of spinosad resistance in *Plutella xylostella* (Lepidoptera: Plutellidae).- *Journal of Economic Entomology*, 101: 1658-1666.

SENTHIL NATHAN S., KALAIVANI K., MURUGAN K., 2006.- Effect of biopesticides on the lactate dehydrogenase (LDH) of the rice leaffolder, *Cnaphalocrocis medinalis* (Guenée) (Insecta: Lepidoptera: Pyralidae).- *Ecotoxicology and Environmental Safety*, 65: 102-107.

SENTHIL NATHAN S., YOUNG C. M., YUL S. H., HOON P. C., KALAIVANI K., DUK K. J., 2008.- Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens* (Stål).- *Ecotoxicology and Environmental Safety*, 70: 244-250.

SPARKS T. C., THOMPSON G. D., LARSON L. L., KIRST H. A., JANTZ O. K., WORDEN T. V., HERTLEIN M. B., BUSACCA J. D., 1995.- Biological characteristics of the spinosyns: a new and naturally derived insect control agent, pp. 903-907. In: *Proceedings of the Beltwide Cotton Conference*. National Cotton Council, San Antonio, Texas, USA.

TAIBI F., SMAGGHE G., AMRANI L., SOLTANI-MAZOUNI N., 2003.- Effect of ecdysone mimic, RH-0345 on reproduction of mealworm, *Tenebrio molitor*.- *Comparative Biochemistry and Physiology C*, 135: 257-267.

THOMPSON G. D., SPARKS T. C., 2002.- Spinosad: a green natural product for insect control, pp. 61-73. In: *Advancing Sustainability through Green Chemistry and Engineering* (LANKEY R. L., ANASTAS P. T., Eds), ACS Symposium Series 823. American Chemical Society, Washington, DC.

VALLES S. M., DONG K., BRENNER R., 2000.- Mechanisms responsible for cypermethrin resistance in a strain of German cockroach, *Blattella germanica*.- *Pesticide Biochemistry and Physiology*, 66: 195-205.

WANG C., SCHARF M. E., BENNETT G. W., 2004.- Behavioral and physiological resistance of the German cockroach to gel baits (Blattodea: Blattellidae).- *Journal of Economic Entomology*, 97: 2067-2072.

WANG X. Y., JARJEES E. A., MCGRAW B. K., BOKONON-GANTA A. H., MESSING R. H., JOHNSON M. W., 2005.- Effects of spinosad-based fruit fly bait GF-120 on tephritid fruit fly and aphid parasitoids.- *Biological Control*, 35: 155-162.

WANG D., GONG P., LI M., QIU X., WANG K., 2009.- Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae).- *Pest Management Science*, 65: 223-227.

WANNER K. W., HELSON B. V., HARRIS B. J., 2002.- Laboratory evaluation of two novel strategies to control first-instar gypsy moth larvae with spinosad applied to tree trunks.- *Pest Management Science*, 58: 817-24.

WECKBERKER G., CORY J. G., 1988.- Ribonucleotide reductase activity and growth of glutathione-depleted mouse lekemial 1210 cells *in vitro*.- *Cancer letters*, 40: 257-264.

WEI Y. A., APPEL G., MOAR W. J., LIU N., 2001.- Pyrethroid resistance and cross resistance in the German cockroach, *Blattella germanica* (L).- *Pest Management Science*, 57: 1055-1059.

WING K. D., SCHNEE M. E., SACHER M., CONNAIR M., 1998.- A novel oxadiazine insecticide is bioactivated in Lepidopteran larvae.- *Archives of Insect Biochemistry and Physiology*, 37: 91-103.

WING K. D., SACHER M., KAGAYA Y., TSURUBUCHI Y., MULDERIG L., CONNAIR M., SCHNEE M., 2000.- Bioactivation and mode of action of the oxadiazine indoxacarb in insects.- *Crop Protection*, 19: 537-545.

WING K. D., ANDALORO J. T., MCCANN S. F., SALGADO V. L., 2005.- Indoxacarb and the sodium channel blocker insecticides: chemistry, physiology and biology in insects, pp. 30-53. In: *Comprehensive insect science. Insect control. Vol 6* (GILBERT L. I., IATROU K., GILL S. S., Eds).- Elsevier B.V., Amsterdam, The Netherland.

YIN X. H., WU Q. J., LI X. F, ZHANG Y. J., XU B.Y., 2009.- Demographic changes in multigeneration *Plutella xylostella* (Lepidoptera: Plutellidae) after exposure to sublethal concentrations of spinosad.- *Journal of Economic Entomology*, 102: 357-365.

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