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Combined Activity of Spinosyns A, D, J and L On Two Stored Product Insects

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ABSTRACT

To evaluate the combined activity of spinosad (spinosyns A and D) and spinetoram (spinosyns J and L) against the adult of two stored grain insects, *Sitophilus oryzae* and *Tribolium castaneum*, laboratory bioassays were conducted for each insecticide alone at five concentration levels, and at four levels for the binary mixture. The results indicated that the values of Co-toxicity Factor were affected by the mixture concentration level, the exposure interval and the insect susceptibility. They ranged from +344.4 (a potentiation effect) at the highest mixture concentration level on *S. oryzae*, after one day exposure time, to -14.29 (an additive effect) at the lowest mixture concentration level, which gave the least adult mortality of *T. castaneum* after 14 days exposure time. It was concluded that the activity of the spinosad and spinetoram mixture will increase at high concentrations, for short exposure times and against susceptible insects.

INTRODUCTION

Several grain protectants, particularly organophosphorus (OP), and also carbamate and pyrethroid insecticides, were registered for application in raw grain commodities, such as wheat, rice, barley, and maize (Arthur, 1996). Spinosad is a novel compound that has been evaluated with success for direct application on the grains, which is based on fermentation products of the actinomycetes *Saccharopolyspor aspinosa* (Thompson *et al*. 1997). These fermentation products are bacterial metabolites, which belong to a group known as ‘‘spinosyns,’’ while spinosad is based on spinosyns A and D (Hertlein *et al*. 2011). More recently, a new member of the spinosyn group, spinetoram, has been commercially introduced in various crops (Sparks *et al*. 2008, 2012 and Jones *et al*. 2010). Spinetoram is based on two secondary metabolites, spinosyn J and L, and has been proved very effective against a wide range of pests, in several crops, often more effective than spinosad (Sparks *et al*. 2008; Jones *et al*. 2010; Dripps *et al*. 2011 and Yee and Alston, 2012). Trials with spinosad, in many parts of the world, clearly suggested that spinosad is quite effective against several major insect species (Thompson *et al*. 1997; Fang *et al*. 2002a, b; Fang and Subramanyam, 2003; Toews *et al*. 2003; Toews and Subramanyam, 2003; Athanassiou *et al*. 2008a, b; Chintzoglou *et al*. 2008a, b; Getchell and Subramanyam, 2008; Kavallieratos *et al*. 2010; Subramanyam *et al*. 2007, 2012 and Pozidimetaxa and Athanassiou, 2013).

Also, laboratory and field trials have shown that spinosad, a commercial insecticide based on the fermentation products of a bacterium, to be an effective grain protestant at the labelled rate of 1 mg (a. i.) /kg of grain based on laboratory and field studies in the United States, Kenya, and Australia (Subramanyam, 2006). After a thorough experimentation, in 2013, spinosad became commercially available in the USA as a grain protectant. Only six insect species have shown resistance to spinosad or spinetoram in the field, whereas there are no reports for stored product insects (Sparks *et al*. 2012).

Moreover, initial evaluation tests clearly indicated that spinetoram was indeed a promising grain protectant, despite the fact that there is no commercially available formulation for this purpose (Vassilakos and Athanassiou, 2012a, b, 2013 and Athanassiou and Kavallieratos, 2014). Both insecticides have the same mode of action targeting the nicotinic and gamma aminobutyric acid (GABA) receptors (Salgado, 1998; Orr *et al*. 2009 and Dripps *et al*. 2011; Hertlein *et al*. 2011 and Sparks *et al*. 2012). The potential for combined actions should be determined for a wide range of exposure levels, and the possibility of interaction effects of would depend on the number and types of insecticides being used, their mechanisms of action, the exposure conditions and duration (Cassee *et al*. 1998; Danish Environmental Protection Agency, Danish Veterinary and Food Administration, 2003; Crofton *et al*. 2005; Moser *et al*. 2006; Gennings *et al*. 2007; VKM, 2008 and Vassilakos *et al*. 2012).

On the other hand, the interactions between different chemical components in a mixture may take place in the toxicokinetic phase (i.e. processes of uptake, distribution, metabolism and excretion) or in the toxicodynamic phase (i.e. effects of chemicals on the receptor, cellular target or organ) (VKM, 2008).

The purpose of this work is to make a direct comparison with spinosad and spinetoram as grain protectants and to evaluate the potential benefits of using the four spinosyns (A, D, J and L) in one single formulation.

MATERIALS AND METHODS

Insects

Laboratory strains of the rice weevil (*Sitophilus oryzae* L.) and the red flour beetle (*Tribolium castaneum* Herbst.) were used as an adult stage in these experiments. These insects were reared in glass jars (approx. 250 ml), each jar contained (about 200 g) wheat kernels (variety Shandaweel1) for *S. oryzae* or crushed wheat grains in case of *T. castaneum* and covered with muslin cloth and fixed with a rubber band. Insect cultures were kept under controlled conditions of 26±2°C and 55±5% RH in the rearing room of the laboratory.

Insecticides

1- Spinosad (a mixture of 50-95% of spinosyn A and 50-5% spinosyn D)

Spinosyn A: (2R, 3aS, 5aR, 5bS, 9S, 13S, 14R, 16aS, 16bR) - 2- (6-deoxy-2,3,4-tri-O-methyl- α- Lmannop-yranosyloxy) – 13 - (4- dimethylamino - 2, 3, 4, 6- tetradeoxy- β-Derythrop-yranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16bhexadecahydro- 14- methyl -1H-8 oxacyclododeca [b] as-indacene-7,15-dione

Spinosyn D: (2S, 3aR, 5aS, 5bS, 9S, 13S, 14R, 16aS, 16bR) - 2- (6-deoxy-2, 3, 4- tri- O- methyl- α-Lmannop-yranosyloxy) -13- (4-dimethylamino - 2, 3, 4, 6- tetradeoxy- β-Derythrop-yranosyloxy)-9-ethyl-2, 3, 3a, 5a, 5b, 6, 7, 9, 10, 11, 12, 13, 14, 15, 16a, 16b hexadecahydro-4,14-dimethyl- 1H-8-

oxacyclododeca [b] as-indacene-7,15-dione

2- Spinetoram (a mixture of 3'-O-ethyl-5,6-dihydro Spinosyn J and 3'-O-ethyl Spinosyn L)

3'-O-ethyl-5,6-dihydro Spinosyn J: (2R,3aR,5aR,5bS,9S,13S,14R,16aS,16b R) -2- [(6-deoxy-3-O-ethyl-2,4-di-O-methyl-α-Lmannopyranosyl) oxy] -13-[[(2R,5S,6R) -5- (dimethylamino) tetrahydro-6-methyl-2H-pyran-2-yl] oxy] -9-ethyl- 2,3,3a,4,5,5a,5b,6,9,10,11,12, 13,14,16a,16b -hexadecahydro-14-methyl- 1H-asindaceno [3, 2-d] oxacyclododecin-7,15-dione

3'-O-ethyl Spinosyn L: (2R,3aR,5aS,5bS,9S,13S,14R,16aS,16b S)- 2- [(6-deoxy-3-O-ethyl-2,4-di-O-methyl-α-Lmannopyranosyl) oxy]-13-[[(2R,5S,6R) -5- (dimethylamino) tetrahydro-6-methyl-2Hpyran-2-yl]oxy]-9-ethyl- 2,3,3a,5a,5b,6,9,10,11,12, 13,14,16a,16b tetradecahydro-4,14-dimethyl- 1H-asindaceno [3,2-d] oxacyclododecin-7,15-dione

The insecticide formulations were spinetoram Radiant (12% SC) and spinosad Spintor (24% SC) produced by Dow Agro- Sciences.

Bioassay tests

Spinetoram and spinosad were applied as solutions against *S. oryzae* adults in wheat kernels or in crushed wheat grains against *T. castaneum* at five insecticidal concentrations. Water solution (3ml of each insecticide) was added to (30g) wheat kernels or crushed wheat grains (in glass jars of approx. 250 ml) to give 10, 5, 2.5, 1.25 and 0.625 mg/kg of spinetoram and 1, 0.5, 0.25, 0.125 and 0.0625 mg/kg of spinosad. In the experiment of the binary mixture, four concentration levels were tested according to the following Table (1):

Table 1: Mixture levels and the concentration of each insecticide in the mixture.

Mixture level (M)	Insecticide concentration (mg/kg)	
	Spinetoram (T)	Spinosad (D)
M_1	(T_1) = 5	(D_1) = 0.5
M_2	(T_2) = 2.5	(D_2) = 0.25
M_3	(T_3) = 1.25	(D_3) = 0.125
M_4	(T_4) = 0.625	(D_4) = 0.0625

In addition to (30 g) wheat kernels or crushed wheat grains, which served as controls, were treated with (3 ml) distilled water. The glass jars of treated wheat kernels or crushed wheat grains were manually shaken for 10 min to achieve an equal distribution of the insecticide in the entire grain mass. Batches (30 adult) insects of *S. oryzae* or *T. castaneum* (1-2 week-old) were introduced to different treatments. Every treatment was conducted in three replicates. Glass jars were covered with muslin cloth, fixed with rubber bands, and kept at 26±2ºC and 55±5% R.H. Mortality was recorded at 2, 3, 5, 7 and 14 days from the initial treatment.

Combination analysis

For the evaluation of the joint action between spinetoram and spinosad, the following equation was adopted as reported by Mansour *et al*. (1966):

Co-toxicity Factor = [(%Observed Mortality - %Expected Mortality) / %Expected Mortality] X100

This factor was used to classify the results into three categories. A positive factor of +20 or more meant potentiation effect, a negative factor of –20 or more meant antagonism, and any intermediate value, i.e. between +20 and –20 was considered an additive effect.

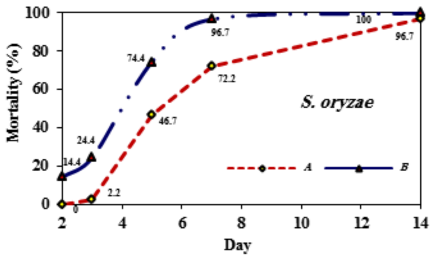
Statistical Analysis

The dosage mortality response was determined by probit analysis (Finney, 1971) using a computer program of Noack and Reichmuth (1978).

RESULTS

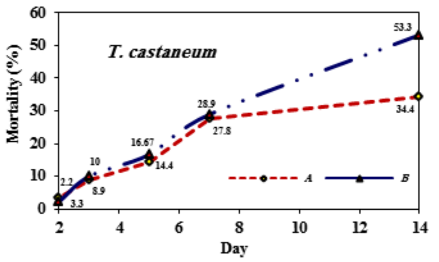
Figure 1 revealed that, after 14 days exposure time, spinetoram alone (at

10 mg/kg) gave complete mortality of the adults of *S. oryzae*, while the mortality was 96.7% by spinosad alone (at 1 mg/kg). Based on the LC_{50}s after 7 days exposure time, the activity of spinosad on *S. oryzae* adults was (LC_{50}= 0.65 mg/kg) significantly higher than spinetoram (LC_{50}= 2.5 mg/kg) (Table 2). Furthermore, the results also indicated that *S. oryzae* adults were more susceptible to each insecticide tested than *T. castaneum* adults, where the adult mortality of *T. castaneum* was low and reached 53.3% by spinetoram, and 34.4% by spinosad, at the highest concentrations used of spinetoram and spinosad, after 14 days exposure time (Fig. 2).



A: Mortality of spinosad alone (1 mg/kg)
B: Mortality of spinetoram alone (10 mg/kg)

Fig. 1: The single effect of spinetoram (10mg/kg) and spinosad (1mg/kg) on the adult mortality of *S. oryzae*



A: Mortality of spinosad alone (1 mg/kg)
B: Mortality of spinetoram alone (10 mg/kg)

Fig. 2: The single effect of spinetoram (10mg/kg) and spinosad (1mg/kg) alone on the adult mortality of *T. castaneum*

Table 2. Lethal concentrations and their 95% confidence limits of spinetoram and spinosad against the adult of *S. oryzae*.

Insecticide	LC_{50}^{A} (mg/kg)	Slope ±SE	R
Spinetoram	2.50 (1.36 - 4.60)	0.79±0.3	0.988
Spinosad	0.65 (0.38 – 0.98)	2.36±0.9	0.997

A: LC_{50}s were calculated after 7 days of insecticide treatment for 20 -80% insect mortality values SE: Standard error R: Correlation coefficient of regression line

Co-toxicity Factor was higher than +20 given a potentiation effect at the highest mixture concentration (M_1= spinetoram at 5mg/kg + spinosad at 0.5mg/kg), and for 2, 3 and 5 days exposure times with Co-toxicity Factors 344.4, 250 and 32.25, respectively. The potentiation effect was also recorded at the mixture concentration of M_2 (2.5mg/ kg spinetoram + 0.25mg/kg spinosad) for 2 and 3 days exposure times with Co-toxicity Factors 271.34 and 93.28, respectively. On the other hand, an additive effect was shown with the other exposure periods of M_1 and M_2, and at low mixture concentration levels (M_3 and M_4), as well as all treatments in case of *T. castaneum* which had an adult mortality (Table 3 and 4).

Table 3: The combined activity of spinetoram and spinosad on the adults of *S. oryzae.*

Parameter	Insecticide/ Concentration level	Days after insecticide treatment				
		2	3	5	7	14
Mortality % (mean ± SD)	T_1^a	10±0.0	1.9±75.89	1.9±48.89	1.9±58.89	100
	D_1^b	0	1.9±1.11	0.0±20.0	1.9±28.89	1.9±87.78
	OM^c (M_1^d)	44.44±1.9	3.3±70.0	1.9±91.11	100	100
	EM^e (M_1^d)	10	20	68.89	87.78	100
Co-toxicity Factor		344.4 (P)	250 (P)	32.25 (P)	13.92 (A)	0 (A)
Mortality % (mean ± SD)	T_2^f	7.78±1.9	0.0±16.67	1.9±42.22	1.9±51.11	100
	D_2^g	0	0	1.9±17.78	1.9±21.11	0.0±26.67
	OM^c (M_2^h)	28.89±1.9	16.67	1.9±67.78	3.3±80.0	100
	EM^e (M_2^h)	7.78	1.9±32.22	60	72.22	100
Co-toxicity Factor		271.34 (P	93.28 (P)	12.97 (A)	10.77 (A)	0 (A)
Mortality % (mean ± SD)	T_3^i	0	1.9±11.11	1.9±21.11	0.0±40.0	1.9±68.89
	D_3^j	0	0	1.9±2.11	0.0±3.33	0.0±16.67
	OM^c (M_3^k)	0	0.0±13.33	3.3±26.67	3.3±46.67	3.3±86.67
	EM^e (M_3^k)	0	11.11	23.22	43.33	85.56
Co-toxicity Factor		N	N	14.86 (A)	7.71 (A)	1.30 (A)
Mortality % (mean ± SD)	T_4^l	0	0	1.9±8.89	1.9±18.89	1.9±35.56
	D_4^m	0	0	1.9±1.11	1.9±2.22	1.9±7.78
	OM^c (M_4^n)	0	0	0.0±10.0	1.9±21.11	0.0±43.33
	EM^e (M_4^n)	0	0	10	21.11	43.34
Co-toxicity Factor		N	N	0(A)	0 (A)	0 (A)

a: Spinetoram at 5 mg/kg; b: Spinosad at 0.5 mg/kg; c: Observed mortality; d: T_1+ D_1; e: Expected mortality;
f: Spinetoram at 2.5 mg/kg; g: Spinosad at 0.25 mg/kg; h: T_2 + D_2; i: Spinetoram at 1.25 mg/kg;
j: Spinosad at 0.125 mg/kg; k: T_3 + D_3; l: Spinetoram at 0.625 mg/kg; m: Spinosad at 0.0625 mg/kg; n: T_4 + D_4;
N: No mortality recorded; SD: Standard deviation; P: A potentiation effect; A: An additive effect

Table 4: The combined activity of spinetoram and spinosad on the adults of *T. castaneum*

Parameter	Insecticide/ Concentration level	Days after insecticide treatment				
		2	3	5	7	14
Mortality % (mean ± SD)	T_1^a	0	0	1.9±1.11	1.9±8.89	1.9±21.11
	D_1^b	N	1.9±2.22	0.0±3.33	1.9±7.78	0.0±20.0
	OM^c (M_1^d)	0	1.9±2.22	4.44±1.9	1.9±18.89	1.9±46.67
	EM^e (M_1^d)	0	2.22	4.44	16.67	41.11
Co-toxicity Factor		N	0 (A)	0 (A)	13.32 (A)	13.53 (A)
Mortality % (mean ± SD)	T_2^f	0	0	0	1.9±1.11	1.9±11.11
	D_2^g	0	0	0	0.0±3.33	±1.97.78
	OM^c (M_2^h)	0	0	0	1.9±4.44	0.0±20.0
	EM^e (M_2^h)	0	0	0	4.44	18.89
Co-toxicity Factor		N	N	N	0 (A)	5.88 (A)
Mortality % (mean ± SD)	T_3^i	0	0	0	0	1.9±4.44
	D_3^j	0	0	0	0	0.0±3.33
	OM^c (M_3^k)	0	0	0	0	0.0±6.67
	EM^e (M_3^k)	0	0	0	0	7.77
Co-toxicity Factor		N	N	N	N	-14.29 (A)
Mortality % (mean ± SD)	T_4^l	0	0	0	0	0
	D_4^m	0	0	0	0	0
	OM^c (M_4^n)	0	0	0	0	0
	EM^e (M_4^n)	0	0	0	0	0
Co-toxicity Factor		N	N	N	N	N

a: Spinetoram at 5mg/kg; b: Spinosad at 0.5mg/kg; c: Observed mortality; d: T_1+D_1; e: Expected mortality;
f: Spinetoram at 2.5mg/kg; g: Spinosad at 0.25mg/kg; h: T_2 +D_2; i: Spinetoram at 1.25mg/kg;
j: Spinosad at 0.125mg/kg; k: T_3 +D_3; l: Spinetoram at 0.625mg/kg; m: Spinosad at 0.0625mg/kg; n: T_4 +D_4;
N: No mortality recorded; SD; Standard deviation; P: A potentiation effect; A: An additive effect.

DISCUSSION

There are several mixtures of insecticides on the market, which are registered either as grain protectants or for treatment of surfaces in storage and processing facilities (Arthur, 1996 and Athanassiou and Kavallieratos, 2014). The results clearly indicated that the susceptibility of the two insect species was greatly varied against each insecticide alone or as a binary mixture. Where spinosad was more toxic than spinetoram and *S. oryzae* adults were more susceptible to each insecticide tested than *T. castaneum* adults. Despite, in several investigations, spinetoram was very effective against a wide range of pests, in several crops, often more effective than spinosad (Sparks *et al.* 2008; Jones *et al.* 2010; Dripps *et al.* 2011 and Yeeand Alston, 2012).The insect susceptibility to both insecticides was also examined by Chintzoglou *et al.* (2008b), Sparks*et al.*(2008, 2012), Jones *et al.*(2010), Hertlein *et al.* (2011) and Vassilakos *et al.* (2012), and their resultsare in agreement withthe present observations. However, in a previous study, Athanassiou *et al.* (2008a, b) found that spinosad was very effective against *S. oryzae*. Fang *et al.* (2002a, b) reported also that spinosad was effective against *S. oryzae* and *T. castaneum* at 1 mg/kg. Furthermore, Vassilakos and Athanassiou, (2012a, b, 2013) concluded that spinetoram was equally and in some cases more effective than spinosad against major stored-product beetle species. Also, spinetoram was effective as a grain protectant, but its efficacy varies according to the target species, concentration and exposure interval (Thompson *et al.* 1997; Fang and Subramanyam, 2003; Toews *et al.* 2003; Toews and Subramanyam 2003 and Vassilakos *et al.* 2012).

When studying the combined toxic effects of multiple chemical exposures, the main goal is to determine if an additive is the outcome of a combined action, or if interactions may occur. Interactions may remain constant over the total dose-span, or there may be dose-dependent variations. Critical, limiting steps in toxicokinetic and/or toxicodynamic pathways may become saturated or overwhelmed, and responses may be altered in a non-linear manner with increasing dose. This may affect metabolic processes as well as cellular defence and repair mechanisms. An increase in the exposure dose may e.g. shifts additive to synergism, toxic effects not seen without saturation of receptor or enzyme systems may appear or the metabolism of various chemical compounds may be modulated. It was observed that an additive was seen at lower doses of the mixture, whereas a greater-than-additive (synergistic) effect was seen at the three highest doses (Crofton *et al.* 2005; Moser *et al.* 2006 and Gennings *et al.* 2007).

Regarding the Co-toxicity Factor and the joint action between spinetoram and spinosad, it was obvious that the values of the Co-toxicity Factor were varied in *S. oryzae* adults according to the mixture concentration level and exposure interval. It was observed that, the values of Co-toxicity Factor decreased with the decrease in the mixture concentration level, the increase in the exposure interval and the decrease in the insect susceptibility. It is well established that both insecticides have the same mode of action targeting the nicotinic and gamma aminobutyric acid (GABA) receptors (Salgado, 1998; Orr *et al.* 2009; Dripps *et al.* 2011; Hertlein *et al.* 2011 and Sparks *et al.* 2012). The toxicological net-outcome of a toxicokinetic interaction depends on whether a higher or lower level of the biologically active species is achieved at the target site and/or whether the target site is exposed for a shorter or longer duration (Danish Environmental Protection Agency, Danish Veterinary and Food Administration, 2003). Thus, potentiation effects from mixtures could occur when exposures are above dose thresholds, due to both toxicodynamic and toxicokinetic interactions (VKM 2008).

Apparently, since both active ingredients interact with the same receptors, is it postulated that additive effect is unlikely to occur and also that resistance development to spinosad will automatically trigger the resistance development to spinetoram (Dripps *et al*. 2011). However, spinosyns are known to have a secondary mode of actions, distinct from currently known insecticidal sites, which merits additional investigation (Orr *et al*. 2009).

It was concluded that the potentiation and additive effects of mixtures could occur according to concentration thresholds, which affected by the concentration level, the exposure period and the insect species. This may be attributed to both toxicodynamic and toxicokinetic interactions.

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ARABIC SUMMERY

الفاعلية المشتركة لكل من إسبينوسين A و D وJ وL على حشرتين من حشرات المواد المخزونة

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لتقييم الفاعلية المشتركة لمبيد إسبينوساد (إسبينوسين A وD) ومبيد إسبينتورام (إسبينوسين J وL) على الحشرات الكاملة لكل من سوسة الأرز وخنفساء الدقيق الكستنائية تم إجراء تجارب التقييم الحيوى لكل مبيد منفرداً على خمس مستويات من التركيزات ثم كمخلوط لكلا المبيدين على أربع مستويات من التركيزات.

وقد أظهرت النتائج أن قيمة معامل السمية المشترك قد تأثرت بمستوى تركيز مخلوط المبيدين ومدة تعرض الحشرة للمخلوط وكذلك حساسية الحشرة لكلا المبيدين، حيث تراوحت قيم معامل السمية المشترك بين +344.4 (تأثير تقوية) على أعلى مستوى لتركيز المخلوط على حشرة سوسة الأرز بعد يوم من المعاملة إلى -14.29 (تأثير إضافى) على أقل مستوى لتركيز المخلوط والذى يعطى أقل نسبة موت لحشرة خنفساء الدقيق الكستنائية بعد 14يوم من المعاملة.

ويستنتج من ذلك أن فاعلية مخلوط مبيد إسبينوساد مع مبيد إسبينتورام سوف تزاداد بزيادة تركيز المخلوط أو عند المعاملة لفترات قصيرة أو بزيادة حساسية الحشرة المعاملة.