

## ACUTE AND CHRONIC EFFECTS OF SPINOSAD ON BUMBLE BEES, *Bombus terrestris* L. UNDER LABORATORY CONDITIONS

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### ABSTRACT

Under laboratory conditions, the acute toxicity of spinosad on adult workers of bumble bee *Bombus terrestris* L. was investigated through 96 hrs post-treatment by using three different exposure methods; orally, wet and dry contact. The results indicated that within 24 hrs, the 1/1 and 1/10 of maximum field recommended concentration (MFRC) of spinosad caused 100 % and 12-65 % mortality, respectively the three methods. While, the mortality in the control insects did not exceed 5 %. The highest LC<sub>50</sub> value was detected in dry contact method at 6 hrs, the LC<sub>50</sub>=1046.15 ppm (2.62 MFRC). However, the lowest one in oral method was detected after 96 hrs, the LC<sub>50</sub> values was 5.03 ppm (0.01 MFRC). At concentration 0.01 MFRC with dry contact, LT<sub>50</sub> was 73 days but in the oral method had 14 days. The chronic effects of 1/100, 1/1000, 1/10000 of MFRC using the same three exposure methods were evaluated. The 1/100 of MFRC in oral method caused significant mortality in worker through 8 weeks. Moreover, reduction in survival of workers, drones produced, male delay emergency, and numbers of dead larvae. However, no significant differences were found between the control and the other treatment 1/1000 and 1/10000 MFRC using oral assay. In dry contact method, the concentrations from 1/1000 to 1/10 of MFRC spinosad had no negative effects. In wet contact method the concentrations 1/100 and 1/1000 had no negative effect, but the concentration 1/10 caused decreasing survival workers. Our results suggested that spinosad was highly toxic in wet contact method, although more safe in dry contact method to bumble bees under worse case laboratory condition with MFRC.

**Keywords:** *Bombus terrestris*, spinosad, acute and chronic effects, pesticides.

### INTRODUCTION

The bumble bee *B. terrestris* L. is one of the most important pollinators of wild flowers and glasshouse crops sweet peppers, tomatoes and eggplants and in fruit production (Goulson, 2003). It is the main species has wide distribution in all Europe, costal North Africa, and in West and Central Asia (Velthuis and Doorn, 2006). Populations of wild bees may be declining in agriculture areas, likely due to habitat loss, decreased plant diversity and increased pesticide use (Johansen and Mayer, 1990).

Spinosad is a microbial bioinsecticide made from a mixture of spinosyn A and D, two of the main metabolites formed fermentation of the actinomycete bacterium, *Saccharopolyspora spinosa* Mertz & Yao. Spinosyns

are broad-spectrum insecticides, with activity against Diptera, Lepidoptera, Hymenoptera, Siphonaptera and Thysanoptera (Salgado, 1998 and Sparks *et al.*, 1998). Spinosad registered in 37 countries for use on 150 crops as of 2001 (Cleveland *et al.*, 2001).

Owing to lack of data with respect to the effects of insecticide especially new group against bumble bees, frequently data obtained for honey bee (*Apis mellifera* L.) are used; however two bee species have several biological and morphological differences (Thompson and Hunt, 1999). Therefore, studies to evaluate the acute and chronic effects of spinosad on bumble bees are necessary.

The purpose of this investigation was to assess the acute toxicity of spinosad on bumble bees (*B. terrestris* L.) using three laboratory stranded methods (orally by drinking sugar solution, wet contact and dry contact) of testing pesticides on bumblebees (OECD, 1998 a & b; Sterk *et al.*, 1995). The worker mortality is the end point through four days post treatments in acute toxicity. Because, mortality is obviously not only the end point to consider and there is a growing interest in the development of chronic effects. We tested by the same three exposing routes the effect of lowest concentration that have no acute toxicity on workers on work survival, number of drones produced and their delay emergency, dead larvae ejected and sugar solution consumed.

## **MATERIALS AND METHODS**

### **Chemicals**

Commercial Tracer<sup>®</sup> insecticide (480 g L<sup>-1</sup> spinosad, suspension concentrate) was provided from Dow AgroSciences Co., Belgium. We followed the recommendation of the company using the rate for field assays in cotton (120 g AI ha<sup>-1</sup>). In this case, maximum field rate were transformed to maximum field concentration taking into consideration a normal spraying volume of 300 liter ha<sup>-1</sup> (when applied on cotton). The MFRC value is equal 0.04 % and 400 ppm. Triton X-100 (100 % purity, Sigma-Aldrich, USA) was used as surfactant in dry and wet contact methods.

### **Insects**

The workers were supplied from colonies held at Biobest NV, Belgium, Westerlo. In all experiments, artificial nests were used, each containing five *B. terrestris* workers. The nests were produced in-house and made of transparent plastic (15 cm wide, 15 cm deep, 10 cm high). At the centre of the nest there was the brood feeding area. Under the nest a container with 500 mL sugar/water was provided. The dominant worker started to produce eggs that developed into males after 1 week. The nests were kept under standardized laboratory conditions in the dark at 28 ±2 °C and 60±10 % relative humidity (RH), and sugar/water and commercial pollen were provided as food (Mommaerts *et al.*, 2006).

### **Bioassay of acute toxicity**

Adult workers were exposed via contact and oral methods according to Sterk *et al.*, 1995. Serial concentrations of spinosad (1/1, 1/10, 1/100, 1/1000,

1/10000 of MFRC) were prepared using commercial sugar solution in dilutions, moreover, for a negative control, we used blank sugar water. Imidacloprid insecticide at its MFRC (200 ppm) was used as positive control.

In the first experiment, adult worker bees had 2-3 days old were distributed as 5 workers in each nest box and kept with supplying by blank sugar solution and pollen under the lab conditions for 1 week before exposing to spinosad orally. For each concentration, four nests were treated each contains five workers. Mortality percentages were recorded after 6, 24, 48, 72, 96 hrs.

In the second experiment, adult workers had 4-7 days old when exposed to wet contact and dry contact of the serial concentrations of spinosad, 1/1, 1/10, 1/100, 1/1000 MFRC. Four liters of spinosad solution were prepared for each concentration using distilled water. To each replicate, we put small droplet of Triton X-100 on the glass plate surface (dimensions: 9 cm long × 7 cm wide cm × 0.3 cm thin). The detergent droplet was loosed gently to cover all the surface of the plate. Then this glass plate was dipped on spinosad solution for 5 seconds with keeping the side that have triton on the top side and put gently on paper on the desk with keeping also the triton side on the top. In wet contact, we keep 5 workers in small cylindrical plastic cup; (dimensions: 9 cm height × 4 cm wide, with 5 air hole (dimensions: 1 cm, were covered with microfiber)) that put on the wetted glass plate. Then each plastic cup covered by plastic cylinder to keep workers on glass plate.

In dry contact method, the same procedures that used in wet contact method were used, except we leave the glass treated plate for 4 hours to dry under laboratory conditions then the workers were exposed to this treated plate.

In both wet and dry contact methods, each nest was supplied by blank sugar solution during exposure time. After 24 hours exposure, the treated workers were transferred to a transparent plastic box (15 cm wide, 15 cm length, 10 cm height), with supplying by sugar solution and pollen. The worker mortalities were recorded through 4 days after exposing. With the same procedures and under the same conditions, distilled water without spinosad was used as control.

The mortality data were corrected by Abbott's formula (Abbott, 1925) and subjected to probit analysis according to Finny, 1971 with using SPSS version 16 (probit analysis). The  $LC_{50}$  values at different times and  $LT_{50}$  values in different concentrations were determined

#### **Determination of chronic effects**

The nests that have no mortality with low concentrations of spinosad in different methods of treatment in acute toxicity tests were kept under laboratory conditions and followed for 8 weeks post treatment.

In oral sugar/water treatment, each nest was supplied by about 250 ml of sugar/water mixed with spinosad for 8 weeks and pollens which changed 3 times per week.

However in control, wet and dry contact, the nests were supplied with blank sugar/water and pollen for the same period. The number of life and dead bumble of workers, the amount of brood and brood care, egg hatching, the number of dead, the number of males produced in each nest as biological

end points of effects on reproduction and larvae growth were determined weekly.

Each treatment started with four nests each contains five workers. For different methods of exposures, means  $\pm$  SEM were analyzed by one-way ANOVA and separated by a Tukey HSD- Post Hoc test ( $P= 0.01$ ) using SPSS 16 software for Windows.

## RESULTS AND DISCUSSIONS

### Acute toxicity of spinosad on adult worker of bumble bees

In tables 1&2. Spinosad was clearly harmful to worker bumble bees when administrated orally or by contact. The  $LC_{50}$  values in oral assay of spinosad against bumble workers were < 400, 27.4, 12.45, 8.03 and 5.03 ppm after 6, 24, 48, 72 and 96 hrs post feeding, respectively. These values recorded 110.84, 23.75, 10.31, 9.27 and 9.27 ppm in wet contact assay. However in exposing bumble bees worker to different dry residue concentrations of spinosad, the corresponding values were 1046.15, 86.75, 46.95, 46.95, and 46.95 ppm, respectively.

$LT_{50}$  values of spinosad by 1/1 of MFRC were < 6, 9.27 and 7.89 hours in oral, wet contact and dry contact methods, respectively. These values with 1/10 of MFRC were 17.66, 25.88 and 209.32, respectively. With 1/100 MFRC the corresponding values reach 348.26, 703.09 and 1770.54 hours, respectively (table2).

The impact of spinosad on bees has been studied in some laboratories over last couple years. The effects depend on species, application method and biological end point in the most cases (Scott-Dupree *et al.*, 2009; Morandin *et al.*, 2005; Miles, 2003).

Table 1: The  $LC_{50}$  values of spinosad on *Bombus terrestris* with three different exposure methods in different time post treatments under worse case laboratory conditions.

Time (Hours)	$LC_{50}(95\%FL)^a$ oral sugar solution		$LC_{50}(95\%FL)$ wet contact		$LC_{50}(95\%FL)$ dry contact	
	ppm	Ratio of MFRC	ppm	Ratio of MFRC	ppm	Ratio of MFRC
6	< 400	< 1	110.84 (75.21-169.37)	0.28	1046.15 (-) <sup>b</sup>	2.62
24	27.4 (18.73-38.53)	0.07	23.75 (-) <sup>b</sup>	0.06	86.75 (66.18-163.24)	0.22
48	12.45 (6.14-18.68)	0.03	10.31 (6.79-14.68)	0.03	46.95 (-) <sup>b</sup>	0.12
72	8.03 (2.73-12.85)	0.02	9.27 (6.13-13.12)	0.02	46.95 (-) <sup>b</sup>	0.12
96	5.03 (4.54-6.11)	0.01	9.27 (6.13-13.12)	0.02	46.95 (-) <sup>b</sup>	0.12

<sup>a</sup>  $LC_{50}$ / MFRC, both expressed in ppm

<sup>b</sup> the values not determined

**Table 2: The  $LT_{50}$  values of spinosad on *Bombus terrestris* with three different exposure methods in different concentrations under worse case laboratory conditions.**

Concentration		$LT_{50}(95\%FL)$ oral sugar solution	$LT_{50}(95\%FL)$ wet contact	$LT_{50}(95\%FL)$ dry contact
ppm	Ratio of MFRC	Hours	Hours	Hours
4	0.01	348.26 (294.07-423.20)	703.09 (235.70-5559121.22)	1770.54 (-) <sup>a</sup>
40	0.1	17.66 (8.83-24.53)	25.88 (8.79-45.79)	209.32 (-) <sup>a</sup>
400	1.0	< 6 (-) <sup>a</sup>	7.96 (2.30-14.17)	7.89 (-) <sup>a</sup>

<sup>a</sup> the values not determined

Feeding workers of spinosad orally is one of the continuous exposure experienced a worse-case situation. Our present results showed that spinosad with MFRC and 1/10 MFRC with oral had high acute toxicity against bumble bees workers within 96 hours post treatment. While 1/100 MFRC spinosad had no toxic within this period. All tested workers died after feeding on contaminated sugar 1/1 MFRC of spinosad for few hours (less than 6).

However, when the workers were exposed to dry and wet contact with the same concentration, the mortality ratios were 17.5 and 35 %, respectively. The mortalities reached 100 % within 24 % in both methods. Within 24 hours, the 1/0 MFRC was also caused 65, 12, 60 % mortality in workers, in oral, dry contact and wet contact, respectively. (Mortality data not published).

Dry contact exposure in our results was the safest method to workers, while the wet contact had the most risk method. Based on the  $LC_{50}$  values, the dry contact method is the safest one than the rest of tested methods. Dry contact exposure was more safe than wet contact by  $\approx 9, 3.7, 4.6, 5$  and 5 times, and more than oral sugar drinking by  $< 2.6, \approx 4, 4, 6$ , and  $\approx 9$  times after 6, 24, 48, 72, and 96 hours post treatment, respectively. The highest  $LT_{50}$  value was with dry contact method, while the shortest time was with wet contact of spinosad. With consider the  $LT_{50}$  values; dry contact method was safer on workers by 2.5, 8 times in 4, 40 mg  $Al L^{-1}$  tested concentrations than wet contact method, respectively. On the same tested concentrations, dry contact showed less toxicity on workers by 5,  $\approx 12$  times than oral sugar drinking method.

Wet residues are more easily taken via ingestion or penetration through the cuticle of the insect. Spinosad dried product is less toxic to honey bee than wet product (Edwards *et al.* 2003). Spinosad is primarily a stomach poison with some contact activity. Dry residue of MFRC spinosad was weakly toxic on workers, causing 17.5% mortality after exposing for 6 hours continuously. However in honey bees, the mortality reached 50 % after exposure to dry residue of spinosad for only 3 hours (Edwards *et al.* 2003). The honey bees totally differed from bumble bees in susceptibility to spinosad. We are in agreement with the following statement: Honey bees are

indicator species may not adequately reflect the risk posed by insecticides to wild bees because of their unique biology and differential susceptibility (Scott-Dupree *et al.*, 2009).

Dry residues of spinosad had repellent effect on honey bees (Miles, 2003). Dry and wet contact of MFRC spinosad concentrations were also highly toxic within 24 hours, while 1/10 MFRC after 96 hour, it was highly toxic in wet contact and weakly toxic with dry contact.

Spinosad was highly toxic to honeybee workers when administrated orally or topically, or by direct contact (Miles, 2003; Bailey *et al.*, 2005). In realistic field conditions, it is impossible for bumble bees to expose continuously exposed for 24 hrs to the residue of spinosad. Bumble bees were generally more tolerant to direct contact applications (Scott-Dupree *et al.*, 2009).

The exposing of bumble bees to spinosad solution is very risky. Especially, pesticide applications were usually done in the early morning or late evening. Therefore, spinosad insecticide may be a greater threat to foraging bumble bees, which fly at lower temperature than honey bees. In the field, bumble bees foragers can be exposed to spinosad by honeydew, nectar, pollen, or directly contact to spray. To minimize bumble bees these threaten, we recommended use spinosad with caution and with the recommendation label. Spraying application of it should be restricted to night-time or when the crops are not in bloom.

## **Chronic effects of spinosad on bumble bees**

### **Survival of workers**

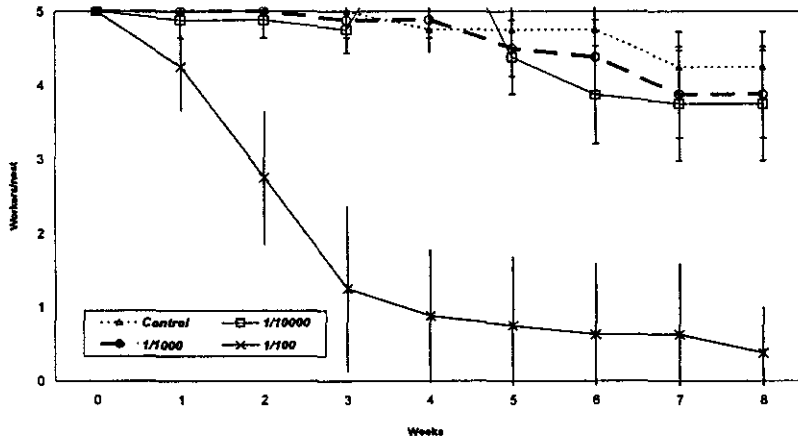
Figure 1 shows the number of survival workers with continuous feeding on sugar contaminated by spinosad orally with different concentrations. During the first two weeks of the test, 100 % survival rate was observed in the control and in 1/1000 MFRC populations. Whereas with 1/100, the mortality increased sharply from week 1 to week 3. Then it takes steady from week 4 to the end of the experiment. From week 3, the mortality increased gradually in the control, 1/10000 and 1/1000. The mortality evolved similarly in control, 1/10000 and 1/1000 from week 3 to week 5. Then from 6 - 8, there was slight difference in mortality within those treatments. At the end of 8 weeks, the total number of live workers in control, 1/10000, 1/1000, and 1/100 were  $16 \pm 0.50$ ,  $13 \pm 0.82$ ,  $14 \pm 0.59$  and  $1 \pm 0.53$ , respectively. These means represent 80 %, 65 %, 70 % and 5 %, respectively of tested workers.

In dry contact assay, no significant differences in survival workers among control, 1/1000, 1/100, and 1/10 MFRC (figure2) from the week1 to week 8 were found. The percent of survival workers in these treatments were 91 %, 65 %, 90 and 87 %, for control, 1/1000, 1/100, and 1/10 MFRC, respectively. However in wet contact assay, the survival workers were 91, 95, 50, and zero % for the same corresponding concentrations (figure3).

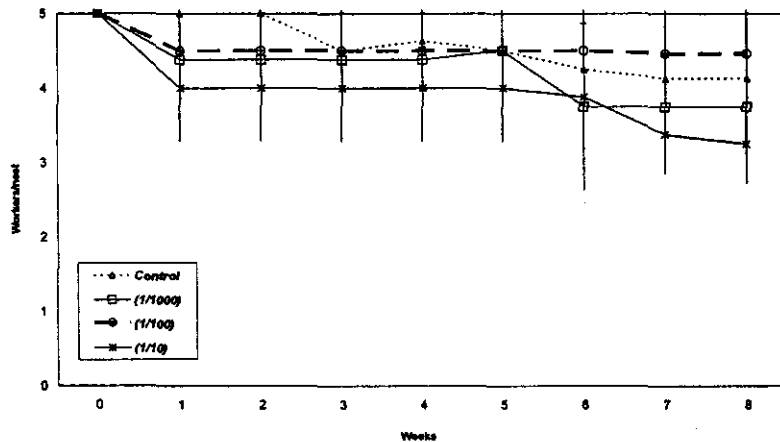
### **Brood production**

Figure 4 shows the mean accumulative number of males produced as biological endpoint of reproduction in treated nests. Oral treatment of spinosad at 1/1000 and 1/10000 MFRC on the mean numbers of worker produced did not cause any effect on reproduction after 8 weeks. The

number of males produced did not differ significantly ( $p = 0.01$ ) compared with the controls. In contrast with 1/100 MFRC spinosad, male production had negative effect. No significant differences were found in mean of drones produced/nest with the workers exposed to 1/1000, 1/100, 1/10, and control using dry contact exposure after 7 weeks (figure 5). Also the data in figure 6 showed that no negative effect of wet contact of spinosad on male production with 1/1000, 1/100, control concentrations within 7 weeks post-treatment. However, with the same wet condition, the 1/10 MFRC of spinosad did not any male production.



**Fig. 1: Survival of *Bumbus terrestris* workers in relation to the spinosad level concentrations in sugar solution through 8 weeks feeding.**



**Fig. 2: Survival of *Bumbus terrestris* workers which exposed contacts to dry concentrations of spinosad for 24 hours after 8 weeks post treatments.**

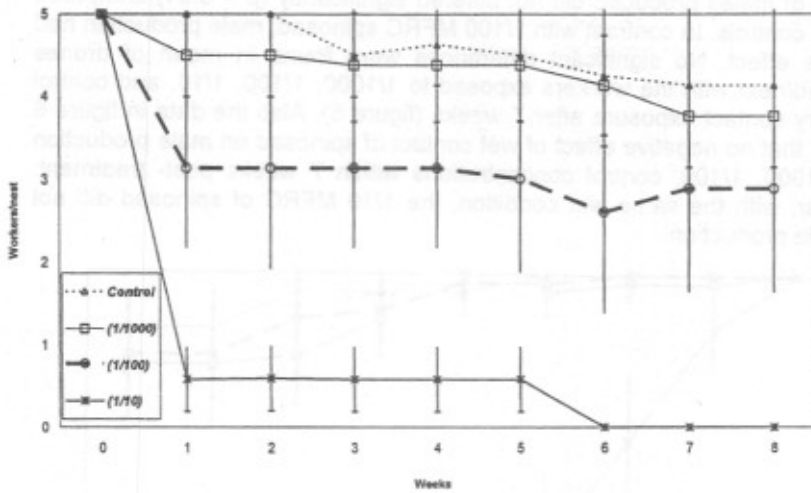


Fig. 3: Survival of *Bumbus terrestris* workers which exposed contacts to wet concentrations of spinosad for 24 hours after 8 weeks post treatments.

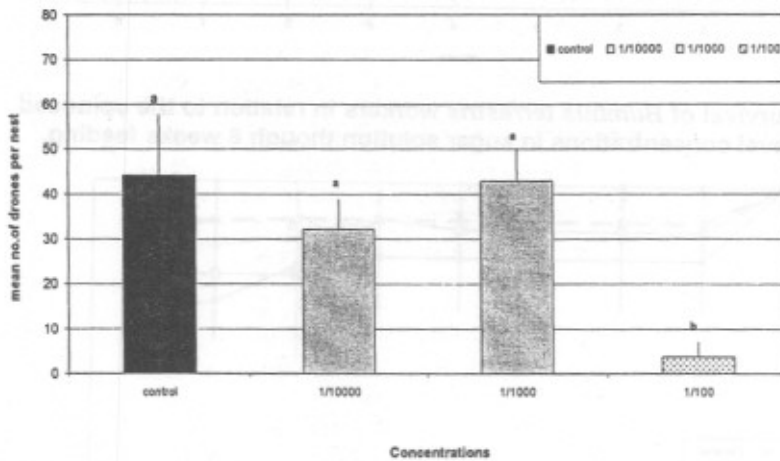


Fig. 4: Effect of different concentrations spinosad (1/100, 1/1000, 1/10000 MFRC and control) on number of drones produced 8 weeks post oral sugar solution drinking. Means  $\pm$  SEM followed by a different letter (a-b) are significantly different (Tukey-HSD post hoc with  $p = 0.01$ ).



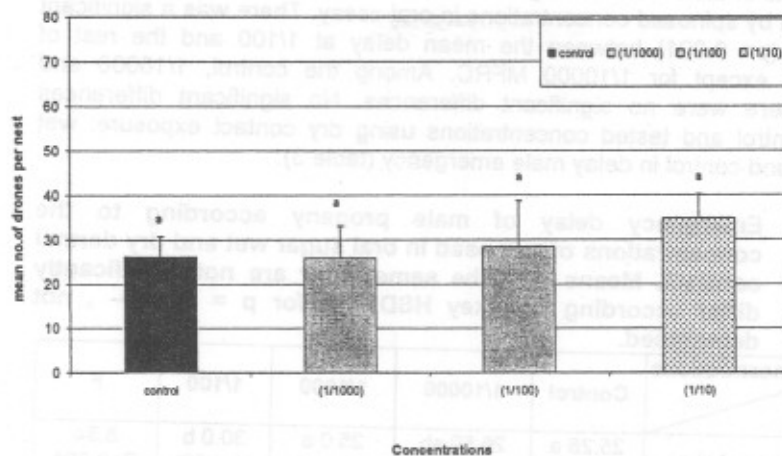


Fig. 5: Effect of exposed contacts to dry concentrations of spinosad for 24 hours on number of drones produced 8 weeks post from workers. Means  $\pm$  SEM followed by a different letter (a-b) are significantly different (Tukey-HSD post hoc with  $p=0.01$ ).

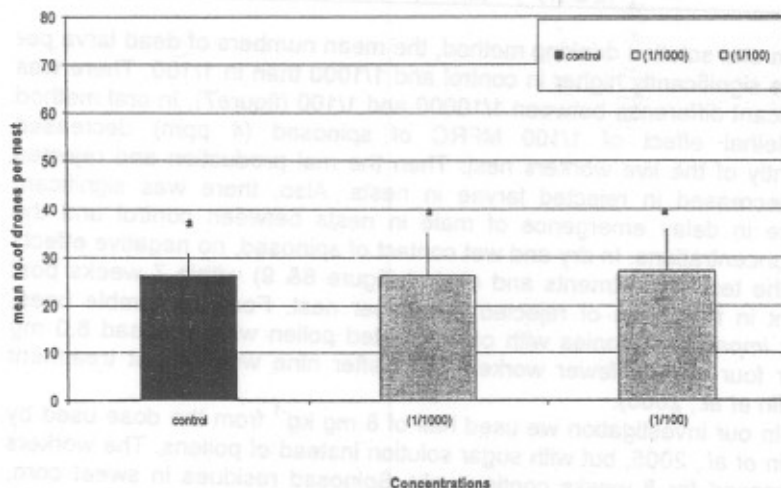


Fig. 6: Effect of exposed contacts to wet concentrations of spinosad for 24 hours on number of drones produced 8 weeks post from workers. Means  $\pm$  SEM followed by a different letter (a-b) are significantly different (Tukey-HSD post hoc with  $p=0.01$ ).

Data in table 3 is showing the delayed emergence (days) of first male as affected by spinosad concentrations in oral assay. There was a significant difference ( $p= 0.001$ ) between the mean delay at 1/100 and the rest of treatments except for 1/10000 MFRC. Among the control, 1/10000 and 1/1000, there were no significant differences. No significant differences among control and tested concentrations using dry contact exposure, wet exposure and control in delay male emergency (table 3).

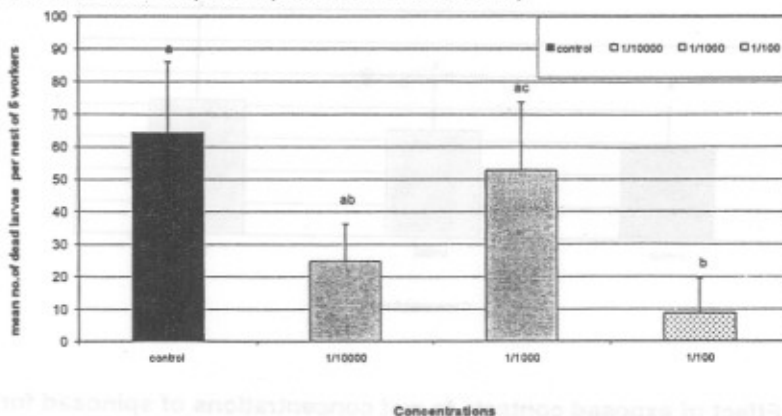
**Table 3: Emergency delay of male progeny according to the concentrations of spinosad in oral sugar wet and dry dermal contract. Means with the same letter are not significantly differ according to tukey HSD test for  $p = 0.01$ . -- , not determined.**

Concentrations		Control	1/10000	1/1000	1/100	F
Method						
Oral sugar solution		25.25 a ( $\pm 0.33$ )	26.50 ab ( $\pm 1.68$ )	25.0 a ( $\pm 0.33$ )	30.0 b ( $\pm 1.00$ )	8.34 P=0.001
Concentrations		Control	1/1000	1/100	1/10	F
Method						
Dry contact		32.00 a ( $\pm 2.97$ )	30.50a ( $\pm 2.79$ )	31.67 a ( $\pm 2.86$ )	26.00 a ( $\pm 0.32$ )	3.86 P=0.02
Wet contact		32.00 ac ( $\pm 2.97$ )	34.0 a ( $\pm 4.49$ )	29.67 a ( $\pm 2.48$ )	--	0.74 P=0.49

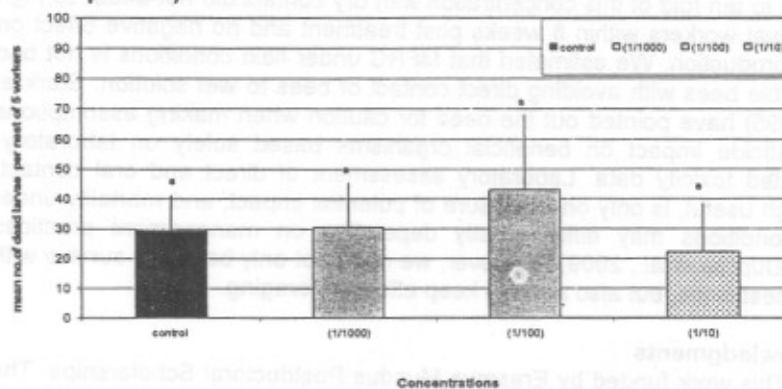
In oral solution drinking method, the mean numbers of dead larva per nest were significantly higher in control and 1/1000 than in 1/100. There was no significant difference between 1/10000 and 1/100 (figure7). In oral method the sublethal effect of 1/100 MFRC of spinosad (4 ppm) decreased significantly of the live workers nest. Then the mal production and rejected larvae decreased in rejected larvae in nests. Also, there was significant difference in delay emergence of male in nests between control and the tested concentrations. In dry and wet contact of spinosad, no negative effects among the tested treatments and control (figure 8& 9) within 7 weeks post treatment in the mean of rejected larvae per nest. Feeding bumble bees, *Bombus impatiens* colonies with contaminated pollen with spinosad 8.0 mg kg<sup>-1</sup> for four weeks, fewer workers lived after nine weeks post treatment (Morandin et al., 2005).

In our investigation we used half of 8 mg kg<sup>-1</sup> from the dose used by Morandin et al., 2005, but with sugar solution instead of pollens. The workers were exposed for 8 weeks continuously. Spinosad residues in sweet corn, with an application rate of 70 g Al ha<sup>-1</sup>(Success 480 g liter-1 SC formulation) were 0.27 mg kg<sup>-1</sup> in plant tissue (Bailey et al., 2005). Based on application rate and spinosad residues in plant, we assumed that spinosad residue should be around 0.46 mg kg<sup>-1</sup> after spray with MFRC (120 g Al ha<sup>-1</sup>) in sweet corn. In the present study, the concentration 1/1000 MFRC is equivalent to 0.4 mg Al ml<sup>-1</sup>. This concentration could be close to the residues of spinosad in spraying by MFRC. This concentration has no lethal or sublethal effects on

the parameters tested. In *Bombus impatiens*, there was no significant difference in number of workers or amount of brood at any time between colonies in the control and 0.2 mg Al mg Kg<sup>-1</sup> or 0.8 Al mg Kg<sup>-1</sup> spinosad concentrations in pollen (Morandin *et al.*, 2005).



**Fig. 7:** Effect of different concentrations spinosad (1/100, 1/1000, 1/10000 MFRC and control) on larval growth 8 weeks after oral sugar solution drinking. The scored numbers of dead larvae based on four replicates. Means  $\pm$  SEM followed by a different letter (a-b) are significantly different (Tukey-HSD post hoc with  $p=0.01$ ).



**Fig. 8:** Effect of exposed contacts to dry concentrations of spinosad for 24 hours on larval growth 8 weeks after oral sugar solution drinking. The scored numbers of dead larvae based on four replicates. Means  $\pm$  SEM followed by a different letter (a-b) are significantly different (Tukey-HSD post hoc with  $p=0.01$ ).

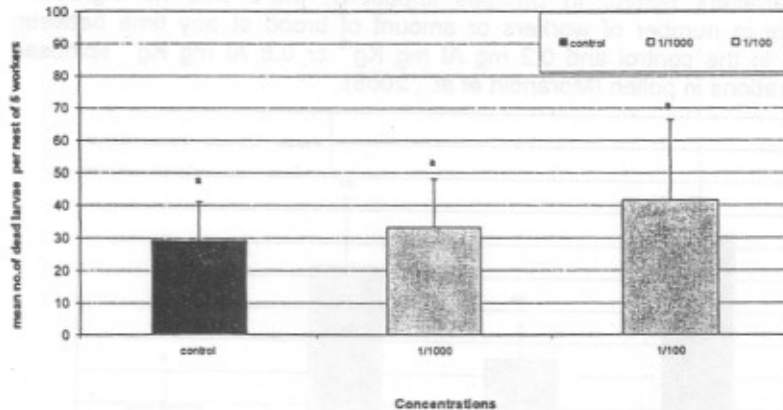


Fig. 9: Effect of exposed contacts to wet concentrations of spinosad for 24 hours on larval growth 7 weeks after oral sugar solution drinking. The scored numbers of dead larvae based on four replicates. Means  $\pm$  SEM followed by a different letter (a-b) are significantly different (Tukey-HSD post hoc with  $p=0.01$ ).

### Conclusion

Wet contact of spinosad to bumble bees is very risky. In 1/100 of (MFRC), the adult workers died after one week post treatment. However, the expose to ten fold of this concentration with dry contact did not affect so high in survival workers within 8 weeks post treatment and no negative effect on brood production. We estimated that MFRC under field conditions is not bad to bumble bees with avoiding direct contact of bees to wet solution. Stark *et al.* (1995) have pointed out the need for caution when making assumptions on pesticide impact on beneficial organisms based solely on laboratory-generated toxicity data. Laboratory assessment of direct and oral contact, although useful, is only one measure of potential impact, and mortality under field conditions may differ greatly depending on management practices (Scott-Dupree *et al.*, 2009). However, we need not only bees are survive with using pesticides, but also need to keep effective foraging.

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## التأثير الحاد والمزمن لمبيد سبينوساد على اليمبل بيس (بميس تيريسترس) تحت الظروف المعملية

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في هذا البحث تم دراسة السمية الحادة لأحد المبيدات الحيوية وهو سبينوساد على شغالات اليمبل بيس خلال ٩٦ ساعة بعد المعاملة باستخدام ثلاث طرق من المعاملة: طريقة خلط المبيد بمحلول التغذية السكري، تعريض الشغالات لسطح مبتل بمحلول المبيد، تعريض الشغالات لسطح جاف معامل بالمبيد تحت الظروف المعملية. بعد ٢٤ ساعة من المعاملة تسبب التركيز المختبر من المبيد ( قيمة أقصى تركيز موصى\*بأستخدامة) في موت ١٠٠ % من الشغالات في المعاملة بطريقة شرب المحلول، والنسبة تراوحت من ١٢ إلى ١٠٠ % في معاملة الأسطح. تم اختبار عدة تركيزات من المبيد على الشغالات من تركيزات ١/١٠٠٠٠ إلى ١/١ (من قيمة أقصى تركيز موصى به) لتحديد قيمة التركيز السام النصفى وكذلك تم تحديد قيم الزمن السام النصفى. سجلت أقصى قيمة للتركيز السام النصفى عند ٦ ساعات بعد المعاملة في طريقة شرب المحلول وكانت هذه القيمة ١٠٤٦.١٥ جزء في المليون (٢.٦٢ من قيمة أقصى تركيز موصى به). بينما كان أقل قيمة في طريقة شرب المحلول السكري عند ٩٦ ساعة وهي تساوى ٥.٠٣ جزء في المليون (٠.٠١ من قيمة أقصى تركيز موصى به). كان أطول وقت لازم لقتل ٥٠ % من الشغالات عند تركيز ٠.٠١ في طريقة معاملة السطح الجاف تساوى ٧٣ يوم. وكانت هذه القيمة عند تركيز ١/١ من أقصى تركيز موصى به هي ٠.٢٥ يوم. هذه النتائج السابقة أدت إلى دراسة تأثير التركيزات تحت المميتة وهي ١/١٠٠٠٠، ١/١٠٠٠، ١/١٠٠ (من قيمة أقصى تركيز موصى به) على الشغالات. أدى التركيز ١/١٠٠ في معاملة شرب المحلول إلى زيادة في موت الشغالات خلال ٨ أسابيع بعد المعاملة بالإضافة إلى نقص في حيوية الشغالات، عدد الذكور، تأخير في خروج الذكور، نقص في عدد اليرقات المميتة. بينما عند تركيزات ١/١٠٠٠٠، ١/١٠٠٠ لم تكن هناك أى فروق بين الكنترول وهذه المعاملات في الملاحظات السابقة. في طريقة معاملة السطح الجاف لم يكن هناك تأثير معنوي إلى تركيز ١/١٠٠. بينما في معاملة السطح الرطب كان هناك نقص معنوي لهذا التركيز. هذه النتائج تقترح أن مبيد سبينوساد له تأثير سام جدا على نحل اليمبل بيس (بميس تيريسترس) خاصة عند ملامسة النحل لمحلول المبيد. ويكون المبيد أقل خطورة عند جفافه.

كلمات افتتاحية: بميس تيريسترس - سبينوساد - تأثير مميت - تأثير تحت مميت-مبيدات

## قام بتحكيم البحث

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