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The Efficacy of Spinosad on Different Strains of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)

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Abstract: The cotton leafworm, *Spodoptera littoralis* (Boisduval), was collected from commercial cotton production fields in Adana, Turkey, and tested for susceptibility to the insecticide spinosad. The susceptibility of the field strain was also compared to the susceptible strain (S) of *S. littoralis*. Lethal dose bioassays were performed with third instar larvae using the leaf dip method. The LC₅₀ values for field and susceptible strains were 43.691 and 10.037 ppm, respectively. When LC₅₀ values and 95% confidence intervals were compared with a susceptible laboratory reference strain, the field strain was approximately 4.4-fold less sensitive than the susceptible strain. The present study suggests that spinosad is potentially important in the control of *S. littoralis*.

Key Words: Spinosad, *Spodoptera littoralis*, Toxicity, Biological Activity

Spinosad'ın *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae)'in Farklı Populasyonları Üzerindeki Etkinliği

Özet: Pamuk Yaprakkurdu *Spodoptera littoralis* (Boisduval)'in bir popülasyonu Türkiye, Adana ili ticari pamuk tarlalarından toplanmış ve insektisit, spinosad'a karşı hassasiyeti test edilmiştir. Tarla popülasyonunun hassasiyeti *Spodoptera littoralis*'in hassas popülasyonu (S) ile karşılaştırılmıştır. Letal doz denemeleri yaprak disk yedirme metodunun 3. dönem larvalara karşı kullanılması ile gerçekleştirilmiştir. Tarla ve hassas popülasyonların LC₅₀ değerleri sırasıyla 43,691 ve 10,037 ppm olarak bulunmuştur. Tarla ırkının LC₅₀ değeri ve % 95 güven aralıkları hassas laboratuvar ırkı ile karşılaştırıldığında bu ırkın hassas ırka göre spinosad'a yaklaşık olarak 4,4 kat daha az duyarlı olduğu bulunmuştur. Bu çalışma, *S. littoralis*'le savaşımında spinosad'ın potansiyel bir insektisit olduğunu göstermektedir.

Anahtar Sözcükler: Spinosad, *Spodoptera littoralis*, Toksikite, Biyolojik Aktivite

Introduction

There are many insects causing reductions in cotton production in Turkey. The cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae), is responsible for the greatest part of this loss and it threatens several economically important crops such as cotton, corn, peanuts, vegetables and soybean in Turkey. Synthetic insecticides are often a part of management programs to control lepidopterous pests in Turkey (1). However, the pressure of insecticide selection causes a resistance problem in the control of lepidopterous, especially in Çukurova province in Turkey. The time until the development of resistance depends on a number of factors, including the frequency and nature of resistance genes, pest management strategies, and the relative fitness of the resistant strains relative to the wild type

(which is still sensitive to the insecticide in question). To prevent this cycle, there is a need for different insecticides having different modes of action. Spinosad is a naturally derived biorational insecticide with an environmentally favourable toxicity profile (2). It is an insecticide based on an aerobic fermentation product of the bacterium *Saccharopolyspora spinosa* on nutrient media, and was discovered during the 1980s (3). Spinosad (a mixture of spinosyns A (C₄₁H₆₅NO₁₀) and D (C₄₂H₆₇NO₁₀)) belongs to a new class of polyketide-macrolide insecticides (Figure 1). In many countries, spinosad is used in control of lepidopteran pests in cotton, tobacco and other crops (4). Spinosad has been used in some vegetables and cotton since 1998 in Turkey.

It has a novel mode of action, acting primarily at the nicotinic acetylcholine receptor in the nerve synapses (5).

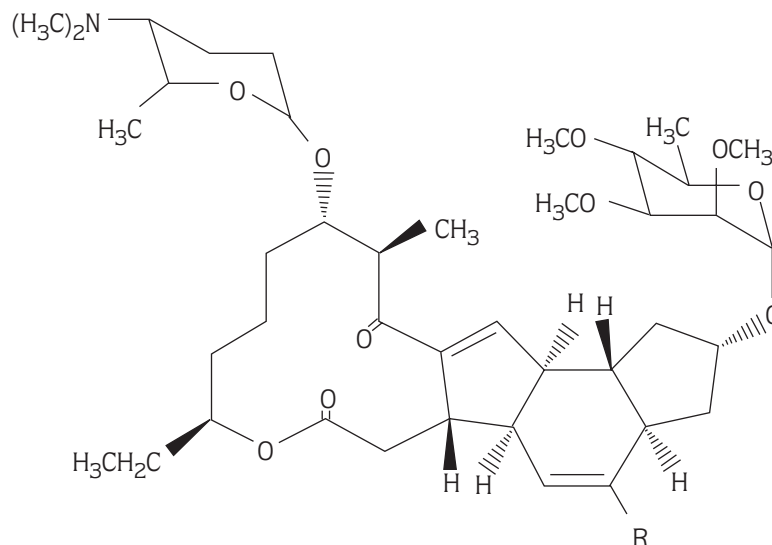


Figure 1. The structures of the spinosyn A and D (6)
 Spinosyn A, R=H-
 Spinosyn D, R=CH₃.

The mode of action is unique and not clearly understood yet. Continuous activation of motor neurones causes spasmodic paralyzes of muscles and the insect dies from exhaustion. There may be some effects on the GABA and other nervous system components (5-7). Due to this unique mode of action, spinosad is valued in resistance management programmes. Spinosad must be ingested by the insect; therefore it has little effect on sucking insects and non-target predatory insects. It has no systemic effects on plants, but will penetrate leaves. Thus, it is active against leafminers and has activity against flies and thrips. On crops, higher application rates are needed in the control of thrips and leafminers than for caterpillars (7). As part of a pest management programme on biorational control of cotton leafworm larvae, *Spodoptera littoralis* Bois. (Lepidoptera: Noctuidae), we have begun to assess the efficiency of spinosad and have determined the precise concentration-mortality relationship.

Materials and Methods

Insects

In this experiment, 2 different strains were used, an insecticide susceptible strain (S) and a field strain collected from Adana province in 2003. The S strain was obtained from Volcani Research Centre in Israel. *S.*

littoralis was reared on lettuce leaves under constant conditions (25 ± 3 °C and 60-70% relative humidity with a 16:8 (light:dark) light cycle).

Bioassay

Biological activity of the insecticide spinosad (Laser 480 g/l SC, Dow AgroSciences) was determined using a leaf dip bioassay. In the bioassays, third instar larvae of *S. littoralis* were used. Lettuce leaf discs were prepared using a cork borer with a diameter of 3.8 cm. For the *S. littoralis* (S) strain, spinosad doses of 0 (only water), 1.953, 3.906, 7.812, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 ppm, and for the *S. littoralis* (Adana) strain, doses of 0 (only water), 15.625, 31.25, 35, 40, 45, 50, 55, 62.5, 125, 250, 500 and 1000 ppm were used. All leaf discs were dipped into solution containing different concentrations of spinosad for 5 s and then they were air-dried for 1 h (8). Control leaf discs were dipped into double distilled water. Afterwards, the leaf discs were placed in a 16-well insect rearing plate and one third instar *S. littoralis* was added per well and allowed to feed on the treated leaf disc for 24 h. The larvae consumed the entire leaf disc within 24 h. Mortality percentages were measured after 24 h. The experiment was recorded 3 times using 16 larvae for each experiment.

Statistical Analysis

Data from the leaf dip assays were analysed using probit analysis models in the POLO-PC program (9). A significant difference between LC_{50} values was based on overlap of 95% confidence intervals. The percentage mortality data were corrected by using the Abbott formula (10). Data were analysed by analysis of variance (breakdown one way ANOVA) and followed by a least significant difference (LSD) test as post-hoc comparisons of the mortality means. Dose-response curves and the log of the dosage were plotted using percentage mortality rates in Microsoft Excel spreadsheets.

Results

Third instar larvae of the pest displayed a concentration-dependent response to spinosad. According to LC_{50} values, a significant difference was observed between S (10.037 ppm) and Adana (43.691 ppm) strains 24 h after treatment, based on the overlap of 95% CI (Table). According to the present results, 100% mortality of the S and Adana strains was detected after using 62.5 and 55 ppm, respectively (for all doses on the S strain: $F = 30.742$; $df = 9, 20$; $P < 0.01$; on the Adana strain: $F = 44.188$; $df = 11, 24$; $P < 0.01$). Although approximately 15 ppm of spinosad caused 60% mortality on the S strain, only 15% mortality was observed at the same dosage on the Adana strain (Figure 2). Therefore, the S strain was highly susceptible to spinosad; however, the Adana strain was less susceptible (Table). The S strain of *S. littoralis* demonstrated $CI (95\%) = 4.743-15.914$, $slope = 1.759 \pm 0.229$ compared to the Adana strain ($CI (95\%) = 36.074- 46.506$, $slope = 14.495 \pm 3.259$).

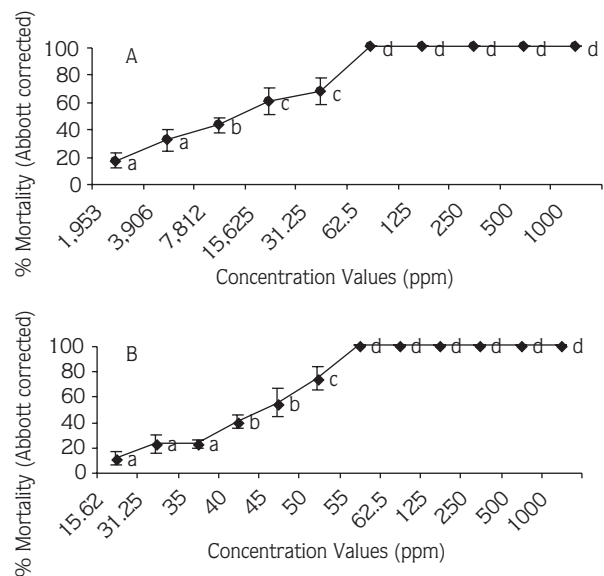


Figure 2. Percent mortality of *S. littoralis* (S) [A] and *S. littoralis* (Adana) [B] by spinosad at 10 and 12 different doses, respectively. Data present mean \pm SE. The means with the same letter are not statistically different according to the least significant differences (LSD) test ($P < 0.05$).

Although the susceptibility to spinosad decreased 4.4-fold, the Adana strain was a highly homogeneous population, because it had a very right angle log concentration-mortality slope value (Figure 3). The log concentration-mortality regression for S and Adana strains of *S. littoralis* is demonstrated in Figure 3. The slopes of regression lines and its locations varied according to the susceptibility and structure of the strains. The regression lines were located from left to right, parallel to the susceptibility of the strains. The

Table. Probit statistics for susceptible and Adana strains of *S. littoralis* against spinosad

| Strain | N ^a | Slope \pm SE | LC_{10} (ppm) (95% CI) ^b | LC_{50} (ppm) (95% CI) | LC_{90} (ppm) (95% CI) | RR ^c |
|-----------------|----------------|--------------------|------------------------------------------|-----------------------------|-----------------------------|-----------------|
| Susceptible (S) | 528 | 1.759 \pm 0.229 | 1.976* (1.390-4.309) | 10.037 * (4.743-15.914) | 50.983 (32.361- 104.905) | - |
| Adana | 624 | 14.495 \pm 3.259 | 35.643 (21.790-40.455) | 43.691 (36.074- 46.506) | 53.556 (50.505-63.215) | 4.4 |

^a Number of larvae

^b CI, confidence interval

^c RR= Resistance Ratio = LC_{50} Adana strain/ LC_{50} susceptible strain

* Values followed by an asterisk are significantly different from *S. littoralis* (Adana) strain's values based on the overlap of 95% CI

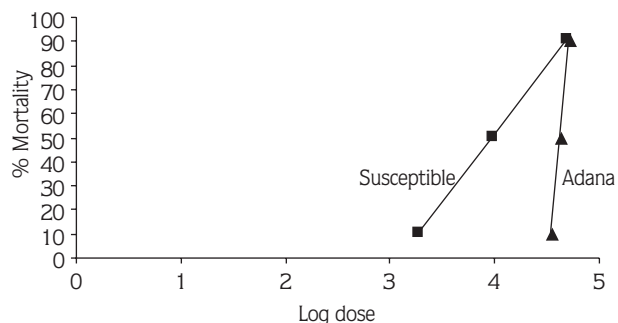


Figure 3. The log concentration-mortality regression for susceptible and Adana strains of *S. littoralis* tested with spinosad.

slope line of *S. littoralis* (S) was less than that of the other strain. The *S. littoralis* (Adana) strain's regression line was located on the right of the graph and showed a very right angle. Theoretically, with selective pressure from exposure to insecticides, a population will become heterozygous for resistant genotypes and, as the frequency of resistant genotypes increases, the slope of the regression line for will drop off and the line will shift to the right (11).

Discussion

The 24-h lethal concentration of the suspension, containing concentrate formulation of spinosad (LC₅₀) against *S. littoralis* third instar larvae was estimated. In this study, based on LC₅₀ values of spinosad against 2 strains, the field strain was susceptible. The field strain was approximately 4.4-fold more insensitive to spinosad than the S strain. The difference might be due to the differential susceptibility of strains and intensive insecticide selection pressure in that area. Similarly, a low level of spinosad resistance (5-fold) was recorded in *Heliothis armigera* (Lepidoptera: Noctuidae) (12). The selection of tobacco budworms each generation with topically applied technical spinosad produced a laboratory strain highly resistant to spinosad when exposed topically, by feeding on a treated diet or by injection (13). Substantial changes in susceptibility should be viewed seriously for timely management of the noctuid resistance development. When applied to both strains of *S. littoralis* the efficacy of spinosad was concentration dependent and resulted in 100% mortality at high concentrations.

The field and the laboratory strains of *Rhizopertha dominica* Fabr. (Coleoptera: Borstrychidae) were highly

susceptible to spinosad, and one of the field strains was less susceptible to spinosad than the laboratory strain (14). Flinn et al. (15) stated that spinosad was very effective in suppressing *R. dominica* and *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) populations in stored wheat. Toewss et al. (16) concluded that spinosad has excellent contact activity against adults of stored-product insects. Kristensen and Jespersen (17) reported that spinosad was relatively slow acting, but highly toxic to houseflies, *Musca* spp. (Diptera: Muscidae). Similarly Pineda et al. (18) recorded that spinosad and methoxyfenozide were potentially effective compounds for the control of *S. littoralis*. Stark et al. (19), on the other hand, found that spinosad was remarkably similar in toxicity to all 3 economically important fruit fly species, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae); the melon fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae); and the oriental fruit fly, *Bactrocera dorsalis* Hendel (Diptera: Tephritidae). Spinosad also effectively prevented breeding of *Culex* (Diptera: Culicidae) mosquitoes and chironomids (Diptera: Chironomidae) (2).

The results of the study showed that spinosad was very effective in the control of *S. littoralis*. Therefore, in order to maximise the negative effects of the chemicals on the environment and natural enemies in the management of pests, the natural insecticide could be integrated into IPM programmes. The present experiment showed the strong efficacy of the spinosad (i.e. 100% mortality) on *S. littoralis* when applied at high rates, resulting in complete control. Further research is needed to understand the reduced susceptibility to spinosad in the Adana strain.

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