Research Article

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Sublethal effects of spinosad on survival, growth and reproduction of *Helicoverpa armigera* (Lepidoptera: Noctuidae)

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Abstract

BACKGROUND: *Helicoverpa armigera* (Hübner) is one of the most important pests in many countries. Spinosad is used widely for the control of pests, but there is sparse information available regarding its sublethal effects on *H. armigera*. Here, the authors attempt to investigate the sublethal effects of spinosad on *H. armigera* in order to reveal the negative, non-lethal impact of insecticides on this pest.

RESULTS: The toxicity of spinosad against *H. armigera* was determined under laboratory conditions by oral exposure of late second-instar larvae to the compound. The 48 h LC_{50} and 72 h LC_{50} values of spinosad to this pest were found to be 0.41 mg kg^{-1} and 0.35 mg kg^{-1} respectively. Spinosad at sublethal concentrations significantly extended the developmental time of survivor larvae, and reduced larval wet weight. Post-exposure effects were indicated by decreased pupation ratio and pupal weight, by prolonged prepupal and pupal periods and by decreased emergence ratio, fecundity and longevity of adults.

CONCLUSION: These results suggest that the combination of lethal and sublethal effects of spinosad might affect pest population dynamics significantly by decreasing its survival and reproduction, and by delaying its development.

Keywords: spinosad; *Helicoverpa armigera*; sublethal effects; toxicity

1 INTRODUCTION

The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), is a polyphagous pest that damages numerous kinds of cultivated crop, including cotton.[1] The outbreak of this pest is partly due to its ability to develop resistance to commonly used insecticides.[2] Owing to heavy selection pressure over the past three decades, *H. armigera* has exhibited resistance to all conventional insecticide classes such as organochlorine, organophosphate, carbamate and pyrethroid insecticides.[3–6] Resistance of *H. armigera* to various kinds of insecticide has been increasingly enhanced in many areas.[7]

To control this important pest effectively, the development of alternative pesticides seems to be necessary. Spinosad is a selective biological insecticide and is classified as a reduced-risk compound by the US Environmental Protection Agency.[8] This insecticide is a naturally derived product from the fermentation of the actinomycete *Saccharopolyspora spinosa* Mertz & Yao, which comprises two macrocyclic lactones, spinosyn A and spinosyn D. It has been reported that spinosad has many unique advantages. For example: it works both by contact and by ingestion; it has strong insecticidal activity, particularly against Lepidoptera and Diptera; it shows low levels of mammalian toxicity and relatively low toxicity to non-target insects;[9,10] and its mode of action appears to be unique, with the primary site of attack being the nicotinic acetylcholine receptor,[11] and a secondary site of attack being γ-aminobutyric acid receptors.[12]

Better management of *H. armigera* could be achieved by implementing integrated pest management (IPM) programmes, and spinosad has been used in practical IPM as a biorational insecticide.[13] Previous studies have suggested spinosad as a promising insecticide that may be used in *H. armigera* control owing to its high and selective insecticidal activity against this pest. In addition, only a low level of spinosad resistance has been recorded in *H. armigera* in India, Australia and Pakistan,[14–16] and no cross-resistance between spinosad and commonly used insecticides such as fenvalerate, cyfluthrin (pyrethroid), phoxim (organophosphate), methomyl (carbamate) and abamectin (a naturally derived fermentation product of *Streptomyces avermitilis*) has been found.[17] Until now, most toxicological studies on spinosad against *H. armigera* have focused on toxicity assay against this pest.[16] Insects that survive toxicant exposure may still sustain significant injury, which may be manifested as reduced longevity, development, fertility or fecundity. Research on sublethal effects that aims at revealing the negative, non-lethal

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impacts of insecticides on pests will provide practical information for forming effective pest control strategies. Unfortunately, there is sparse information available regarding the sublethal effects of spinosad on *H. armigera*. In the present study, the authors attempted to investigate the sublethal effects of spinosad on this pest by recording and analysing various physiological and toxicological parameters.

2 MATERIALS AND METHODS

2.1 Insect

The field *H. armigera* strain was collected from Hebei Province, China, in 2002, and maintained in the laboratory under insecticide-free conditions. The larvae were reared on an artificial diet at 27 ± 1 °C with a 14 : 10 h light : dark photoperiod until the prepupal stage.[18] Adult males and females were collected and released into 40 × 40 cm cages for mating and egg laying. Adults were held under the same temperature and light conditions at an RH of 60% and supplied with a 10% honey solution.

2.2 Acute toxicity assays

Spinosad 480 g L^{-1} SC (Tracer; Dow AgroSciences) was added to artificial diet after appropriate dilution in water. Late second-instar larvae of uniform size were provided with artificial diet containing spinosad at 0–0.6 mg kg^{-1}. All experiments were conducted in a climate chamber at 27 ± 1 °C with a 14 : 10 h light : dark photoperiod. For each treatment, three replicates with 20 individuals per replication were set up. Mortality was recorded after 48 h and 72 h exposure. Insects that did not respond to stimulation with a fine-haired brush were judged to be dead. LC_{50} values for spinosad were estimated by probit analysis.

2.3 Sublethal effects of spinosad

Two insecticide treatments at sublethal concentrations [6% lethal concentration (LC_{06}) = 0.04 mg kg^{-1} and LC_{26} = 0.16 mg kg^{-1}] and one insecticide-free control were set up in this study. Three replicates were made for each treatment, with 60 individuals per replicate. Uniform late second-instar larvae were used. Fresh diet was provided every 2 days. The survival and growth of each individual were checked twice daily until adult emergence. The duration of each larval instar, the prepupal and pupal stages, the first-day wet weight of larvae at each instar, the pupal weight, and the ratio of pupation and adult emergence were recorded. For the fecundity study, surviving moths were held in 40 × 40 cm cage to mate for about 2 days after emergence at 27 ± 1 °C with a 14 : 10 h light : dark photoperiod at an RH of 60%, and supplied with a 10% honey solution. Then mating pairs were transferred to a smaller box covered with gauze and reared under the same conditions as those above. The numbers of eggs laid were counted daily until all females died. The longevity of adult male and female was recorded as well. Fifty eggs were taken randomly from each pair of adult moths, and the numbers of hatching eggs were recorded. Larvae and adults were regarded as alive if they had the ability to crawl when stimulated with a fine-haired brush, while pupae were regarded as having survived if they could moult successfully to moths.

2.4 Statistical analysis

The data were statistically analysed using one-way analysis of variance (ANOVA) followed by Fisher's LSD test ($P < 0.05$). Non-overlap of 95% confidence limits was the criterion for significance of difference in both treated and untreated larvae for each parameter. All percentage data were arcsine transformed before being subjected to analysis of variance.

3 RESULTS

3.1 Acute toxicity of spinosad to larvae

Larval survivorship was evaluated for a range of spinosad concentrations from 0.04 to 0.6 mg AI kg^{-1} diet after 48 h and 72 h of exposure (Table 1). In the control group, no dead insects were found. Increased mortalities of insects were observed as the concentration of spinosad increased, both after 48 h ($F = 102.1$; df = 14; $P < 0.0001$) and after 72 h of exposure ($F = 155.8$; df = 14; $P < 0.0001$). The 48 h LC_{50} values and 72 h LC_{50} values were 0.41 mg kg^{-1} and 0.35 mg kg^{-1} respectively. Based on LC_{50} values, spinosad showed excellent activity against *H. armigera* when compared with other insecticides.[4,19]

3.2 Sublethal effects on larvae

Spinosad at doses of 0.04 mg kg^{-1} and 0.16 mg kg^{-1} caused death of *H. armigera*. Mortality in both insecticide treatments was significantly higher than in the untreated control (third instar: $F = 54.1$; df = 8; $P < 0.0001$; fourth instar: $F = 226.5$; df = 8; $P < 0.0001$; late instar: $F = 3873.9$; df = 8; $P < 0.0001$). Mortality increased as the spinosad concentration in the diet increased (Fig. 1).

The wet weight of larvae treated with spinosad was obviously lower than in the control (Fig. 2). This effect was also dose related. Biggest weight differences were observed at the fifth instar ($F = 1188.4$; df = 8; $P < 0.0001$), followed by fourth-instar larvae ($F = 93$; df = 8; $P < 0.0001$). Relatively smaller differences were found at the third instar ($F = 380.1$; df = 8; $P < 0.0001$).

The time the larvae needed to complete each developmental stage differed significantly among the different treatments (third instar: $F = 405.9$; df = 8; $P < 0.0001$; fourth instar: $F = 462.6$; df = 8; $P < 0.0001$; fifth instar: $F = 281.7$; df = 8; $P < 0.0001$). Extended developmental time was observed when this pest was exposed to spinosad at the larval stage (Fig. 3).

Table 1. Acute toxicity of spinosad against the larvae of *Helicoverpa armigera*[a]

Spinosad concentration in diet (mg kg^{-1})	Mortality after treatment (%) 48 h	72 h
0.04	10.0 (±0.00)a	10.0 (±0.00)a
0.08	16.6 (±0.02)b	16.6 (±0.02)b
0.20	31.6 (±0.02)c	33.3 (±0.02)c
0.40	53.4 (±0.04)d	61.7 (±0.03)d
0.60	70.0 (±0.03)e	75.1 (±0.03)e

[a] Mortality was recorded 48 h and 72 h after the larvae were provided with artificial diet containing spinosad. Values are the means and standard deviations of three replicates. Means in the same column followed by different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test. Differences of mortality after different exposure times were analysed by the *t*-test. All percentage data were arcsine transformed before being subjected to analysis of variance.

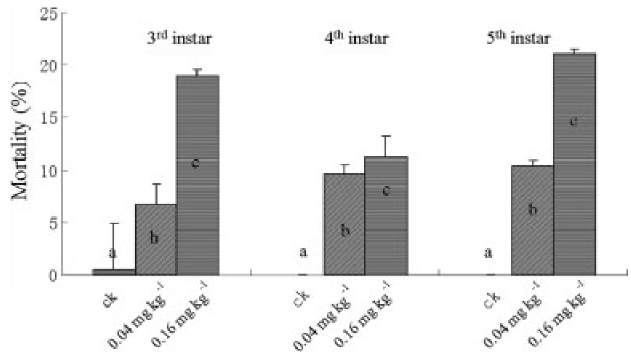

Figure 1. Mortality of *Helicoverpa armigera* occurring in different larval stages when larvae had been provided with diets containing spinosad at 0 (control, ck), 0.04 or 0.16 mg kg^{-1} beginning at late second instar. Values shown are the means and standard deviations of three separate experiments. Data marked with different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test. All percentage data were arcsine transformed before being subjected to analysis of variance.

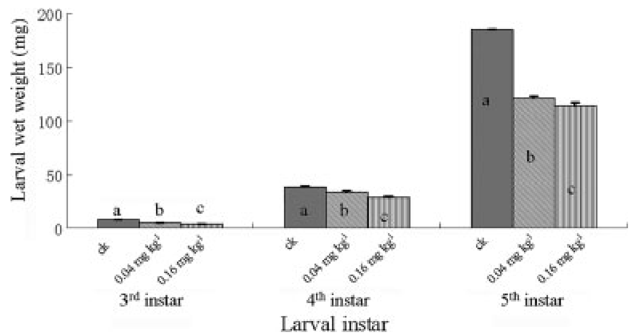

Figure 2. Larval wet weight of survivor *Helicoverpa armigera* exposed to different doses of spinosad at late second instar. Values are the means and standard deviations of three separate experiments. Data marked with different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test.

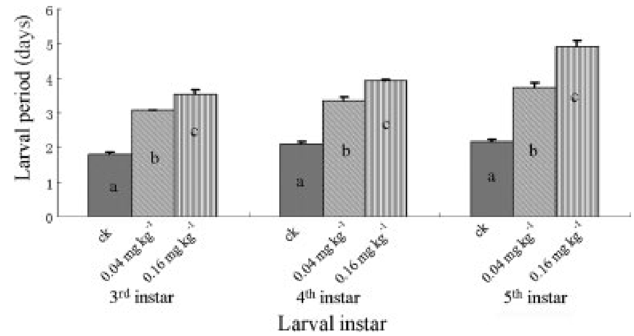

Figure 3. Developmental duration of each instar of survivor *Helicoverpa armigera* exposed to different doses of spinosad at late second instar. Values shown are the means and standard deviations of three separate experiments. Data marked with different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test.

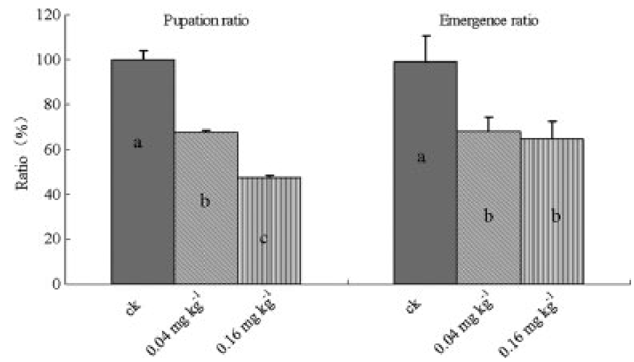

Figure 4. Post-exposure effects of spinosad on the pupation and adult emergence of *Helicoverpa armigera*. Values are the means and standard deviations of three separate experiments. Data marked with different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test after the percentage data had been transformed with arcsine square root.

3.3 Post-exposure effects on pupae

Post-exposure effects were observed in pupae if larvae were exposed to spinosad. Spinosad reduced the pupation ratio and pupal survival (Fig. 4). Pupation ratio was significantly different among the three groups ($F = 696.2$; df = 8; $P < 0.0001$), decreasing as the dose of applied spinosad increased. The adult emergence ratio of *H. armigera* was significantly decreased from 98.97% (control) to 68.26% (exposed to 0.04 mg kg^{-1} of spinosad) and to 64.74% (exposed to 0.16 mg kg^{-1} of spinosad). In addition, spinosad extended the prepupal and pupal periods of cotton bollworm. The development of prepupae with 0.16 mg kg^{-1} of spinosad in the diet was significantly delayed when compared with the control (Table 2). The pupal periods in both spinosad exposure treatments were obviously longer than the control and were dose dependent ($F = 119.6$; df = 8; $P < 0.0001$). Significant decreases in the pupal weight were also observed among the three treatments ($F = 260.6$; df = 8; $P < 0.0001$) (Table 2).

Table 2. Effects of spinosad on pupae of *Helicoverpa armigera*[a]

Spinosad concentration (mg kg^{-1})	Prepupal period (days)	Pupal weight (mg)	Pupal period (days)
0	2.79 (±0.07)a	282.9 (±2.95)a	9.90 (±0.14)a
0.04	5.10 (±0.05)b	252.5 (±1.06)b	12.70 (±0.51)b
0.16	5.17 (±0.07)b	245.9 (±1.09)c	13.94 (±0.21)c

[a] Values are the means and standard deviations from three separate experiments. Means in the same column followed by different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test.

3.4 Post-exposure effects on adults and eggs

Post-exposure effects of larval spinosad exposure were observed in the adult. The longevity of adults was reduced if the larvae were treated with spinosad. The mean life span of emerged adults was significantly different between the two insecticide treatments ($F = 10.39$; df = 8; $P = 0.011$). Female fecundity was reduced in the insecticide treatments as compared with the control (0.04 mg kg^{-1}: $F = 4.80$; df = 56; $P = 0.033$; 0.16 mg kg^{-1}: $F = 5.31$; df = 42; $P = 0.026$). However, there was no significant difference in the number of eggs laid per female between the two insecticide treatments ($F = 2.69$; df = 40; $P = 0.11$). Interestingly, the ratio of hatching eggs was greatly reduced (from 93% to around 35%) in larvae exposed to spinosad at both sublethal

Table 3. Effects of spinosad on adult longevity, fecundity and fertility of *Helicoverpa armigera*[a]

Spinosad concentration ($mg\ kg^{-1}$)	Adult longevity (days)	Number of eggs laid per female	Hatch ratio of eggs (%)
0	11.17 (±0.75)a	894 (±222.8)a	93.26 (±6.23)a
0.04	9.90 (±0.55)b	405 (±108.7)b	35.32 (±3.56)b
0.16	8.63 (±0.29)c	315 (±71.01)b	32.64 (±3.89)b

[a] Values are the means and standard deviations of three separate experiments. Means in the same column followed by different letters differ significantly ($P \leq 0.05$), based on the least significant difference (LSD) multiple comparison test after the percentage data had been transformed with arcsine square root.

doses (Table 3), but the dose effect was not significant ($F = 0.06$; df $= 40$; $P = 0.81$).

4 DISCUSSION AND CONCLUSIONS

The data obtained suggest that spinosad is a highly toxic insecticide against *H. armigera*. The 48 h and 72 h LC_{50} values of spinosad to second-instar larvae of this pest were as low as 0.41 $mg\ kg^{-1}$ and 0.35 $mg\ kg^{-1}$ respectively. This suggestion is in keeping with that of Burkness *et al*.[20] and Smirle *et al*.,[21] who have reported high toxicity of spinosad to lepidopteran pests. Besides the toxic effect, the present study revealed numerous sublethal effects of spinosad on *H. armigera*.

Spinosad at sublethal doses can cause a decrease in survival, suppress weight gain of larvae and delay larval development. In the present study, significant differences were found in the survival rate in the various life stages among the different treatments and the control (Fig. 1). For larvae treated with spinosad at 0.04 $mg\ kg^{-1}$, the wet weight of third-, fourth- and fifth-instar larval survivors decreased by 32, 13 and 35% respectively, and the time needed to complete each instar increased by about 1.3 days. For the higher dose treatment (0.16 $mg\ kg^{-1}$), the wet weight of third, fourth and fifth instars decreased by 49, 26 and 39% respectively, and the duration for each instar increased by 1.8–2.6 days. Similar results were found in Asian lady beetle, which showed that spinosad decreased the survival of first instars, extended the time from first instar to adult and decreased weight gain.[22] However, some insect predators, such as *Orius insidiosus* (Say), did not show changes in developmental time parameters following exposure to spinosad.[23]

In addition to the direct interference of spinosad with the development of the *H. armigera* larvae, post-exposure effects of spinosad on this pest were also observed. Spinosad reduced pupal formation, as indicated by a decrease in prepupal and pupal periods, and by a decline in pupal weight and adult emergence ratio (Table 2 and Fig. 4). Similar results were obtained by Schneider *et al*.,[24] who reported that spinosad reduced pupal formation and adult emergence in *Hyposoter didymator*.

The post-exposure effects of spinosad also carry over to the adult stage. It was found that spinosad treatment in the larval stage shortened adult longevity and reduced reproductive capacity and egg hatchability (Table 3). Egg production was reduced by 54.3% and 64.7% in larvae fed with diet containing 0.04 $mg\ kg^{-1}$ and 0.16 $mg\ kg^{-1}$ of spinosad respectively. The fertility (proportionate egg hatch) was greatly reduced (from 93% to around 35%) when larvae were exposed to spinosad at both sublethal doses. Similarly, Davey *et al*.[25] observed that spinosad reduced hatchability of *Boophilus microplus* Can.[25] Contrary to the present results, Medina *et al*.[26] and Viñuela *et al*.[27] reported that spinosad did not reduce the productivity of *Chrysoperla carnea* (Stephans) adults. The discrepancies of some effects of spinosad on different insects as shown in different studies indicate that spinosad may exert influences in a species-specific manner, revealing the selective insecticidal trait of the compound.

In summary, the present results suggest that spinosad has both lethal and sublethal effects on *H. armigera*. Spinosad effects such as reduction in population size, pupation ratio and female fecundity may affect the population density of the next generation. Impacts such as delayed development of larvae, prepupae and pupae may change the occurrence date and occurring period of this pest. All the lethal and sublethal effects could have a negative influence on the dynamics of this pest. Therefore, the authors propose that not only lethal effects but also sublethal effects of spinosad should be taken into consideration when pest control strategies are made. The reasons why spinosad confers these effects are still to be investigated.

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