



Sublethal effects of spinetoram on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)



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ARTICLE INFO

Article history:

Received 7 August 2015

Received in revised form 27 January 2016

Accepted 8 February 2016

Available online 10 February 2016

Keywords:

Tetranychus urticae

Spinetoram

Sublethal effects

Life table

ABSTRACT

The two-spotted spider mite *Tetranychus urticae* is a serious pest of many agricultural crops and ornamental plants. The sublethal effects of a new chemical, spinetoram, on *T. urticae* were investigated by treating adult females and eggs with LC₁₀ and LC₂₀ in the laboratory. The data were assessed based on age-stage, two-sex life table analysis. The results showed that *T. urticae* developmental time from egg to adult was reduced and that fecundity was increased by treatment with LC₁₀ and LC₂₀ of spinetoram. The LC₁₀ and LC₂₀ of spinetoram also increased the intrinsic and finite rate of increase and the net reproductive rate and reduced the mean generation time, egg duration, and larval duration whether eggs or adult females were treated. These laboratory results suggest that sublethal or lethal doses of spinetoram may cause outbreaks of *T. urticae*.

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1. Introduction

The two-spotted spider mite *Tetranychus urticae* Koch is a widespread and notorious agricultural and horticultural pest with an extensive host range [1,2]. *T. urticae* uses its mouthparts to penetrate host cells and ingest cell contents. *T. urticae* is able to develop resistance to pesticides very rapidly [3] not only because of its short life cycle and high reproductive potential [4,5], but also because of how pesticides are applied in the field.

In China, insect pests and spider mites are mainly controlled by the application of chemical pesticides. As a consequence, *T. urticae* populations in the field are frequently exposed to sublethal or lethal concentrations of pesticides. Spider mites are often exposed to sublethal or lethal concentrations when growers apply pesticides to control other pests that coexist with spider mites, and spider mites are also exposed to sublethal concentrations as chemical pesticides degrade. In some cases, sublethal effects of pesticides can be integrated into pest control. For example, sublethal or lethal concentrations may increase pest developmental time and reduce adult longevity and fecundity [6–9]. Sublethal or lethal doses of some pesticides, however, can cause an increase in the pest population [10,11]. Therefore, it is important to understand the sublethal effects and risks of pesticide application [12].

Spinetoram, a reduced-risk insecticide, belongs to a novel class of chemicals called spinosyns [13–15]. Spinetoram has both contact and ingestion activities [16]; it primarily activates the insect's nervous system and causes involuntary muscle contractions, paralysis, and ultimately death [17–19]. In addition to being highly effective against a broad spectrum of insect pests, including lepidopteran larvae, leaf miners, and thrips, spinetoram has a low impact on most beneficial insects [20]. In 2007, spinetoram was registered for use on many agricultural crops in the USA [21]. Since then, spinetoram has been found to be highly effective in China against rice pests [22], Noctuidae pests [23], stored-grain insects [24], and thrips [25]. Spinetoram has also been found to be more effective than spinosad [26–28].

Previous studies have suggested that spinetoram is especially effective for control of thrips [15], but in some crop fields and especially in fields of ornamental crops, thrips simultaneously occur with *T. urticae* [29]. It follows that *T. urticae* will inevitably contact the residual spinetoram when that pesticide is used to control thrips. In addition, when spinosad was sprayed on bean plants infested with *T. urticae*, an unexpected outbreak of the *T. urticae* population occurred [30]. This undesirable outcome motivated us to consider the effects of spinosad on *T. urticae*. However, spinetoram rather than spinosad is the first choice when a spinosyn pesticide is used on ornamentals. Therefore, the current study examined the sublethal effects of spinetoram on *T. urticae*.

The LC₁₀ and LC₂₀ are often selected to evaluate the sublethal effects of pesticides on insects or mites [31,32]. In the present study, we investigated the sublethal effects of spinetoram at LC₁₀ and LC₂₀ levels on *T. urticae*. The results will provide practical information for the control of *T. urticae*.

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2. Materials and methods

2.1. Mite and pesticide

The laboratory strain of *T. urticae* used in this study was originally collected from an apple orchard in Tai'an Shangdong Province, China, in June 2009. The population was reared on bean leaf discs (var. Shuangqing 12) on moist sponges in Petri dishes (12 cm diameter) under pesticide-free conditions in an incubator at 26 ± 1 °C, 80% RH, and a photoperiod of 16 h:8 h (L:D). Cotton strips were placed around each leaf disc to prevent the mites from escaping. These conditions were used for all experiments in this study unless noted otherwise.

The spinetoram formulation used was an emulsifiable concentrate (Dow Agrosciences China Ltd., China) containing 60 g/kg of active ingredient.

2.2. Bioassay

Bioassays were conducted with adult females and eggs of *T. urticae* using a leaf dip method [33]. Spinetoram was serially diluted with pure water, and six concentrations (including the control) were tested until a satisfactory range was identified, i.e., a range of concentrations that resulted in 10–90% mortality with spinetoram dilutions and 5% mortality with the pure water. Each bean leaf disc (2 cm diameter), which contained either 30 recently deposited eggs or 30 24-h-old adult females, was dipped into one of the six spinetoram solution for 5 s. The excess solution around the surface of the mites and leaf disc was then quickly removed using small pieces of filter paper. Each leaf disc was then placed on a sponge in a Petri dish (one disc per dish), and the dishes were placed in an incubator. One Petri dish with one disc was regarded as a replicate, and four replicate dishes were used for each concentration. Mortality of adult females was evaluated after 24 h; female mites that could not crawl and were non-functional when touched with a small fine brush were regarded as dead. Egg mortality was assessed daily starting with the eclosion of the first protonymph (about 6 days after treatment) and continuing for five successive days. When more than 90% of the eggs in the control had hatched, eggs that had not developed into larvae were regarded as dead. Egg mortality was determined by subtracting the number of protonymphs from the total number of eggs.

Mortality data for eggs and adult females were corrected using Abbott's formula [34], and the LC_{10} and LC_{20} values and their 95% fiducial limits and slope \pm SE were calculated from probit analysis using Polo Plus Version 1.0 software (LeOra Software, Berkeley, CA, USA). The regression equation was used to calculate the LC_{10} and LC_{20} values, which were selected for the subsequent experiments.

2.3. Exposure of *T. urticae* adult females to LC_{10} and LC_{20} of spinetoram

Adult females (24 h old) from the laboratory colony were transferred to fresh bean leaf discs (30 mites per 3.5-cm-diameter disc), each of which was placed on a sponge in a Petri dish. Once the mites began to feed (after about 30 min on the discs), the discs with mites were dipped into the pure water, the LC_{10} and LC_{20} of spinetoram for 5 s, respectively. After dried, they were transferred to the incubator as described earlier. Each of the three treatments was represented by 10 replicate discs. After 24 h, each surviving female mite was carefully transferred to a new, fresh bean leaf disc and was reared under the same conditions. Adult longevity and fecundity were recorded daily until death. During the peak of oviposition, about 100 eggs were collected as the F_1 generation for further observation. The development of the eggs of F_1 generation was observed daily. When the eggs had developed into larvae, the surviving larvae from each treatment were transferred onto a new, untreated leaf disc (one larva per disc) and incubated as before. The development of larvae was documented daily. When a nymph developed into the late second stationary phase, one male

mite from the stock colony was introduced for mating and then removed after 2 days. The discs were examined with a dissecting microscope, and when female adults oviposited, the number of eggs deposited was determined daily. After the number of eggs was recorded, a soft brush was used to transfer each female to a new leaf disc. This was repeated until the mites were dead. Dead or escaped females were excluded from the analysis. During the experiment, the leaf discs were kept moist and were changed when necessary.

2.4. Exposure of *T. urticae* eggs to LC_{10} and LC_{20} of spinetoram

Adult females were placed on bean leaf discs (about 30 females per 2-cm-diameter disc), which were placed on sponges in Petri dishes (3.5 cm diameter) in an incubator. After 24 h, the adults were removed, and eggs were moved until 30 remained per leaf disc. The discs with eggs were then dipped into the pure water, the LC_{10} and LC_{20} of spinetoram for 5 s, respectively. The discs were placed on sponges in Petri dishes and were incubated in the incubator. Each of the three treatments was represented by 10 replicate discs. The development time, survival, and reproduction of the treated mites and of the F_1 generation were observed daily as described in Section 2.3.

2.5. Data analysis

The raw data for the life history of *T. urticae* individuals were analyzed according to the age-stage, two-sex life table [35,36] using the computer program TWSEX-MSChart [37]. The sublethal effects of spinetoram on immature survival and development period, adult longevity, and fecundity were assessed with a paired bootstrap test ($100,000 \times$). The results were subjected to analyses of variance (ANOVAs) with SPSS version 17.0 for Windows (SPSS Inc., Chicago, IL, USA). Plots for survival, fecundity, life expectancy, and reproductive value were prepared with SigmaPlot 12.0 (Systat Software Inc., Point Richmond, CA, USA).

3. Results

3.1. Determination of LC_{10} and LC_{20} of spinetoram to *T. urticae* adult females and eggs

According to the results of the leaf dip bioassay with spinetoram, the LC_{50} , LC_{20} , and LC_{10} for adult females was 6.22, 2.09, and 1.18 mg/L, respectively, while the LC_{50} , LC_{20} , and LC_{10} for eggs was 4.29, 1.07, and 0.52 mg/L, respectively (Table 1).

3.2. Sublethal effects of spinetoram on *T. urticae* adult females

The sublethal effects of spinetoram on *T. urticae* adult females are summarized in Table 2. Compared to the control, the number of eggs laid per female was significantly increased after the adult females were treated with spinetoram; however, adult longevity was significantly reduced following treatment with LC_{10} and LC_{20} of spinetoram (Table 2).

The population parameters of the F_1 generation (the progeny of the adult females that had been exposed to the three treatments) are shown in Table 3. The intrinsic rate of increase (r), the finite rate of increase (λ), and the net reproductive rate (R_0) were significantly increased while

Table 1
Toxicity of spinetoram to the eggs and adult females of *T. urticae* in a leaf dip assay.

| Stage | Slope \pm SE | LC_{10} (mg/L) | LC_{20} (mg/L) | LC_{50} (mg/L) |
|--------------|-----------------|------------------|------------------|-------------------|
| Adult female | 1.78 \pm 0.18 | 1.18 (0.39–1.99) | 2.09 (0.98–3.15) | 6.22 (4.28–10.16) |
| Egg | 1.40 \pm 0.15 | 0.52 (0.29–0.78) | 1.07 (0.70–1.46) | 4.29 (3.43–5.36) |

Values in parentheses are 95% CL.

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