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Effects of azadirachtin on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae)

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Abstract. Effects of sublethal azadirachtin exposure to the biological performance of *Tetranychus urticae* Koch was studied under laboratory conditions. Bioassay was used to asses the effect of different concentrations of azadirachtin on longevity, fecundity, fertility, and offspring development. Azadirachtin (64 and 128 ppm) affected fecundity and mortality but had no effect on fertility and offspring development. A subsequent life-table study with 80 ppm of azadirachtin found that the compound caused a reduction of 50% in survival to adult stage. The peak of reproduction was reached at 5 days causing a decrease in the mean fecundity to almost eight times than of untreated females. The net reproductive rate (R_0), the intrinsic rate of increase (r_m), and the finite rate of increase (λ) of treated females were lower. Treatment showed a negative value of r_m, resulting in a declining population. These results suggest that azadirachtin could be incorporated in Integrated Pest Management (IPM) programmes of *T. urticae*.

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) has been recorded on more than 150 hosts of economic value (Jeppson et al. 1975). It has recently become a more serious problem due to continuous pesticide use (Young-Joon et al. 1993) which causes reduction in populations of their natural enemies. In addition, the rise of resistance among mite populations necessitates the use of acaricides that are compatible with Integrated Pest Management (IPM) programmes.

Therefore, research in recent years has been turning more towards selective biorational pesticides, because they are generally perceived to be safer than the synthetics (Amason et al. 1989). Among these biorationals, azadirachtin, isolated from Neem tree seeds, *Azadirachta indica* A. Juss (Meliaceae), has attracted the greatest attention in recent years (Schmutterer 1990; Mordue and Blackwell 1993; Sundaram 1996). Neem extract is a potent repellent, antifeedant, growth regulator and oviposition deterrent affecting more than 200

species of pests (Ascher 1993) including *T. urticae* (Sanguanpong and Schmutterer 1992).

There are two major types of toxicological studies of pesticides. The acute toxicological study looking at endpoint mortality and the chronic exposure study that monitors the effects of repeated exposures to pesticides over longer time periods (Stark and Banks 2003). Several authors have argued that the best approach for evaluation of pesticide toxicity is life-table analysis or demographic toxicology studies (Robertson and Worner 1990; Robertson and Preisler 1992; Stark and Wennergren 1995; Stark and Banks 2003), and the use of the intrinsic rate of increase (r_m) has been recommended (Allan and Daniels 1982), because it is based on both survivorship and fecundity.

Several studies using demographic techniques to quantify sublethal effects of pesticides on spider mites have been reported (Boykin and Campbell 1982; Ahmadi 1983; Jones and Parrella 1984; Ibrahim and Knowles 1986; Stark et al. 1997; Marcic 2003) but none have considered azadirachtin. Therefore, we evaluated the effects of this compound against adult *T. urticae*, using demographic toxicological analysis and speculate the possibility of incorporating azadirachtin in the management of the two-spotted spider mite.

Materials and methods

Mite and pesticide tested

The *T. urticae* population used was obtained from *Impatiens balsamina* plants and reared in laboratory on young pesticide-free green bean plants (*Phaseolus vulgaris*, var. Garrafal), and mantained in a climatic chamber at 24 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D) photoperiod. Bioassays were performed under the same conditions.

A commercial preparate of Neem extract was used [Align® 32 g (ai)/kg emulsified concentrate, Sipcam Inagra].

Rearing units

Each rearing unit consisted of eight green bean leaf discs of 20 mm diameter (3.14 cm^2), placed on wet filter paper inside a 90-mm diameter Petri dish. The dish top had two holes of 6 mm diameter each, in order to prevent an excess of humidity.

Effects on longevity, fecundity, fertility, and offspring development

One newly moulted adult female with two young males were transferred to a single leaf disc in a rearing unit. The whole rearing unit with spider mites was

then sprayed with azadirachtin using a Potter Precision Laboratory Spray Tower (Potter 1952) operating at 50 kPa with a 5.5 ml spray aliquot. This resulted in a homogeneous spray coverage of 5.0 ± 0.5 mg (mean ± SE) fluid per square centimeter. The treatment concentrations were selected after preliminary bioassays with a wide range of concentrations to determine the range needed to obtain low-hight fecundity reduction. After the proper range was obtained, six different concentrations were prepared within this range: 4, 8, 16, 32, 64 and 128 ppm. The azadirachtin was mixed with distilled water and controls were sprayed with distilled water alone. After spraying, the treated leaf discs were air-dried for 5 min and then covered. Each day female and males were transferred to another treated leaf disc of the same rearing unit. When all leaf discs of one rearing unit were used, the mites were transferred to another rearing unit treated at the same time as the previous ones. Egg laying, hatching, survivorship of females, and data about offspring development were recorded daily to determine both direct contact toxicity and residual activity of the compound. Twenty replicates per concentration and control were made.

Life-table bioassay

An adult female with two adult males was sprayed as above with 80 ppm of azadirachtin or distilled water, but adults were maintained in the same leaf disc throughout the whole bioassay. Eggs were counted daily and destroyed after counting (except 100 of each treatment, used to study the development from egg to adult). Also adult mortality data were recorded daily. Thirteen replicates were made.

To determine the effects of azadirachtin on offspring development of treated females, 100 eggs of each treatment were taken and placed individually on green bean leaf discs of new rearing units treated the same day and in the same way as above. Mortality and time of development of each stage were counted daily for each egg.

As above, both direct contact toxicity and residual activity of the compound were determined.

Statistical methods

Longevity, fecundity, fertility and offspring development were analysed by ANOVA with means separated by LSD ($p < 0.05$). If the assumption of normality or equality of variance was not met, a non-parametric Kruskal–Wallis test was used. To avoid the problem of variance heterogeneity, fecundity values were transformed using $\sqrt{x}$; in all cases untransformed means are presented. Analyses were performed using the SPSS programme (SPSS 1999).

After determining the preoviposition period, prereproductive survival and the emergence matrixes, the $r_m - 2.0$ programme (Taberner et al. 1993) was

used to establish the natural parameters (Table 1). Analysis of the data was carried out using Bootstrap technique to generate variances to allow analysis of life-table parameters, doing 1000 replicates as suggested by Meyer et al. (1986).

Results

Adult longevity, fecundity, fertility and offspring development

Longevity had decreased significantly at the 64 and 128 ppm concentrations (Table 2). Eggs laid per female had decreased significantly at 32, 64 and 128 ppm of azadirachtin, but no effect on fertility was observed. High percentages of egg hatching (> 80%) were scored in treated and untreated females. A high intrinsic variability in offspring development was recorded and, as a consequence, statistically significant differences were not scored among treatments.

Table 1. Definition and formulas for life-table parameters of *Tetranychus urticae*.

Symbol	Definition	Formula
l_x	Probability of an individual surviving to age x	
m_x	Reproductive expectation of a female at age x	
R_0	Net reproductive rate: number of daughters that replace an average female in course of a generation	$\Sigma\ l_x m_x$
T	Mean generation time: mean of the period over which progeny are produced	$\Sigma x l_x m_x$
r_m	Intrinsic rate of increase: number of progeny produced per unit of time	$\ln\ R_0/T$
λ	Finite rate of increase: number of times a population multiplies itself per unit time	e^{rm}
DT	Doubling time	$\ln\ 2/r_m$

Table 2. Influence of azadirachtin on longevity, fecundity, fertility and offspring development (mean ± SE) at different concentrations (ppm) on *Tetranychus urticae*, at 24 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D).

Azadirachtin concentration (ppm)	Longevity[A] (days)	Eggs/female[A]	% eclosions[B]	% offspring[B]
Control (0 ppm)	$9.93^{a} \pm 0.86$	$81.27^{a} \pm 8.81$	$97.14^{a} \pm 1.03$	$88.22^{a} \pm 1.87$
4	$11.47^{a} \pm 1.15$	$81.53^{ab} \pm 11.45$	$97.11^{a} \pm 0.84$	$89.90^{a} \pm 1.67$
8	$10.12^{a} \pm 0.99$	$71.12^{ab} \pm 8.61$	$94.56^{a} \pm 1.62$	$90.37^{a} \pm 1.59$
16	$9.24^{ab} \pm 0.94$	$59.59^{ab} \pm 9.26$	$89.54^{a} \pm 2.71$	$86.24^{a} \pm 1.75$
32	$9.33^{ab} \pm 0.89$	$53.07^{bc} \pm 9.60$	$95.7^{a} \pm 1.25$	$86.17^{a} \pm 1.90$
64	$7.25^{bc} \pm 0.87$	$37.69^{c} \pm 7.84$	$96.23^{a} \pm 1.35$	$73.25^{a} \pm 5.01$
128	$4.88^{c} \pm 0.50$	$8.06^{d} \pm 3.07$	$81.68^{a} \pm 6.43$	$56.86^{a} \pm 12.59$

Within the columns, the data followed by the same letter do not differ significantly ($\alpha = 0.05$).
[A] Means separated by ANOVA and LSD.
[B] Means separated by non-parametric test Kruskal–Wallis.

Effect on life-table parameters

Examination of the l_x (probability of an individual surviving to age x) and in (reproductive expectation of a female at age x) in untreated mites revealed low mortality in the immature stages (20% of eggs and 10% of larval and nymphal stages) with a 70% chance of reaching adulthood. In contrast, treated females showed higher mortality in the immature stages (25% of eggs and 25% of larval and nymphal stages) with a 50% chance of reaching adulthood (Figure 1). The untreated mites spent half of their lives in the adult stage and did not reach their peak of reproduction ($m_x = 10.5$) until the adults were 9 days old, but the probability of reaching this age was only 5%. The treated mites spent less than half of their lives in the adult stage, and reached their peak of reproduction 5 days after reaching adulthood ($m_x = 1.5$). The probability of reaching this age was only 5.6%. No delay in time reaching reproductive maturity was observed in treated mites.

The effects of mortality on the population growth can be demostrated by simply comparing the net reproductive rate (R_0) (Table 3). Due to mortality effects, the average treated female only produced 0.79 females, almost 20 fold less than untreated mites (14.4).

For untreated mites, the mean generation time (T) was 13.97 days, and the population increased daily by 1.22 times the previous day's total number (finite

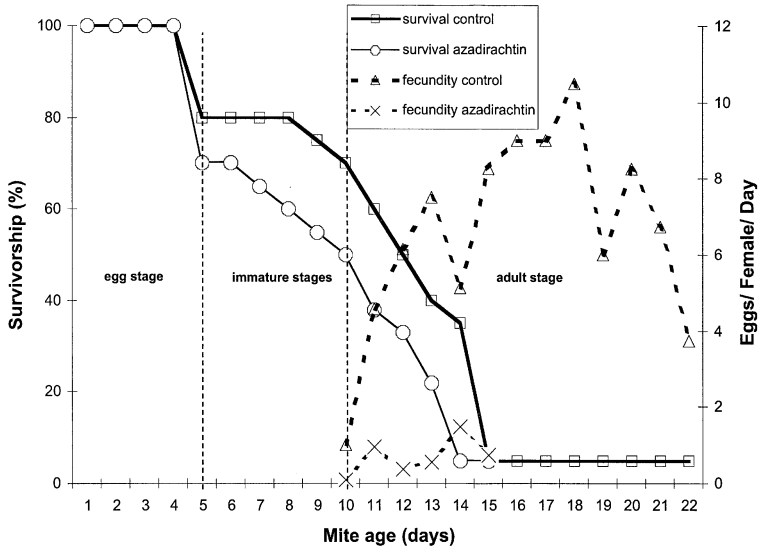

Figure 1. Survivorship (l_x) and fecundity (m_x) curves for *Tetranychus urticae* reared on green bean leaf discs treated with distilled water (control) or 80 ppm of azadirachtin, at 24 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D).

Table 3. Life-table parameters of *Tetranychus urticae* reared on green bean leaf discs and treated with 80 ppm of azadirachtin at 24 ± 1 °C, 65 ± 5% RH, and 16:8 (L:D).

Parameter	Control	Azadirachtin
Net reproductive rate (R_0)	14.40	0.79
Mean generation time (T)	13.97	12.45
Intrinsic rate of increase (r_m)[a]	0.197 ± 0.004	−0.033 ± 0.035
Confidence interval 95%	0.188–0.207	−0.114–0.048
Finite rate of increase (λ)	1.22	0.97
Mean doubling time (DT)[b]	3.51	−21.02
% Eggs surviving to adults	70	50

[a] Mean ± SE.
[b] Negative value indicates half-time (HT).

rate of increase, λ). Every 3.51 days (doubling time, DT) the population doubled. Generation time of the treated mites did not differ much from generation time of the untreated ones. The population decreased daily by 0.97 times. Every 21.02 days the population would decrease by half.

Untreated mites had significantly higher r_m values (0.197 ± 0.004) than the treated ones (−0.033 ± 0.035), with no overlap of their confidence intervals at 95% (Table 3).

Discussion

Azadirachtin affected adult longevity and fecundity of *T. urticae* at the highest tested concentrations but had no effect on fertility or offspring development. That concentrations applied to females were enough to affect egg production but not to harm the embryo suggests the lack of transovarial transmission of the compound at a significative rate. Similar effects on longevity and fecundity were recorded by Dimetry et al. (1993) and Sundaram and Sloane (1995), who treated *T. urticae* adult females with several azadirachtin formulations which caused increased mortality and decreased fecundity. However, Sundaram and Sloane (1995) also obtained a reduction in the percentage egg hatch and in the survival of emerged mites, which may be due to the effect of the formulation type or to different sensitivity of the mites.

The r_m value integrates age at first reproduction, survivorship, brood size and frequency, and longevity. However, it is also worth examining how chronic exposure affects the individual components of r_m. In our experimental conditions, the probability that individuals can reach the peak of reproduction and the physiological maximum of reproduction (l_x and m_x values in that maximum, respectively) was lowered by azadirachtin. This caused a reduction in the number of daughters that replace an average female in course of a generation

(R_0) to a value lower than 1.0, imphying that the population decreases. In fact, the population would be reduced to half in 21 days of treatment (T).

Longevity, fecundity, fertility and offspring development provide a reference of short-term effects of the treatment. But this information cannot predict the behaviour of the treated population after weeks with the same treatment. Comparison of r_m values provides insight beyond that available from independent analyses of several life-history parameters (Petitt et al. 1994). In our bioassay, a negative value of r_m was recorded for treated females, resulting in a declining population. These results are in agreement with Makundi and Kashenge (2002), who observed that repeated application on tomatoes of several neem formulations led to significant reduction of the population size of *T. urticae*.

In conclusion, our work shows that azadirachtin reduces longevity and fecundity of *T. urticae* adult females. However, to forecast the fate of a population over several generations, it is necessary to consider other important aspects, especially development of resistance. Obviously, a reiterated use of the same compound increase the probability of resistance develpment in *T. urticae*. More studies are needed to investigate whether sulethal concentrations of azadirachtin in successive treatments could be interesting in resistance management programmes. The effects of azadirachtin on *T. urticae* suggest that the combination of toxic and sublethal effects could lead to the incorporation of this compound in IPM programmes against this important pest.

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