PEST MANAGEMENT

Alternative Control of *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) on Tomato Plants Grown in Greenhouses

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ABSTRACT - *Tetranychus evansi* Baker & Pritchard is an important pest of solanaceous plants, including tomatoes. This mite is characterized by a high reproductive rate, which leads to high population growth in a short period of time causing important economic damage. Control of *T. evansi* is mainly through synthetic acaricides. In searching for environmentally friendly control measures, we evaluated the efficiency of alternative products to control *T. evansi* on tomato plants under greenhouse conditions. The products tested were lime sulphur and neem based products. We first estimated the lethal concentration (LC) and instantaneous rate of increase (r_i) of *T. evansi* exposed to different product concentrations in laboratory conditions, and later tested the efficacy of LC_{95} and the concentrations that restrained mite population growth ($r_i = 0$) in greenhouse conditions. The following treatments were repeated three times: NeemPro (81.0 and 71.6 mg a.i./l), Natuneem (31.1 and 20.4 mg ai/l), Organic Neem (39.1 and 30.4 mg a.i./l), lime sulphur (1.0 and 0.6%) and water (control). For all products, control provided by LC_{95} was higher than provided for lower concentrations ($r_i = 0$) one day after spraying. However, after five days, for both concentrations, the percentage of *T. evansi* population reduction was superior to 95% and increased over time. Only plants sprayed with Natuneem (31.1 mg a.i./l) showed symptoms of phytotoxicity. Lime sulphur and neem based products, applied in appropriate concentrations and formulations, bear out as a viable alternative to control *T. evansi* on tomato plants.

KEY WORDS: Acari, *Azadirachta indica*, lime sulphur, efficiency, phytotoxicity

The tomato red spider mite, *Tetranychus evansi* Baker & Pritchard, is an important pest of solanaceous plants, especially tomatoes (Flechtmann 1983, Bonato 1999, Ferragut & Escudero 2002). This mite is characterized by a high reproductive capacity, which leads to high population levels in a short time, causing important economic damage (Moraes & McMurtry 1986). Additionally, the mite produces large quantities of webs on the infested plants, hampering the action of natural enemies (Gerson 1985, Sabelis & Bakker 1992). The control of *T. evansi* in tomato is done mainly with application of synthetic pesticides. Despite its relative efficiency, chemical control has several negative impacts as the selection of resistant individuals due to the continuous use of certain active ingredients, the reduction or elimination of beneficial species, the high toxicity of products to applicators, and the presence of residues in food (Picanço *et al* 2007, Maniania *et al* 2008).

A viable alternative to the problems arising from excessive use of synthetic pesticides in tomato production is the use of methods that provide control with social and environmental safety. In the search for such methods, natural enemies are being evaluated as biological control agents of *T. evansi* (Wekesa *et al* 2005, Furtado *et al* 2007, Brito *et al* 2009). Another strategy that has been used where conventional pesticides are not allowed, such as in ecological and organic production systems, is the use of non synthetic products for pest control (Campanhola & Bettiol 2003, Venzon *et al* 2008a). Lime sulphur is among these products allowed in organic systems, primarily used as a fungicide (Smilanick & Sorenson 2001, Montag *et al* 2005), but is also employed as insecticide and acaricide (Guerra 1985, Penteado 2000, Afonso *et al* 2007). The toxic effect of lime sulphur on insects and mites is given by the reaction of its compounds when applied to the plant with water and carbon dioxide, resulting in hydrogen sulfide (Abbot 1945). Its acaricidal effect has been demonstrated for some mite species (Tuelher 2006, Venzon *et al* 2006, Andrade *et al* 2007, Beers *et al* 2009), but not yet for *T. evansi*.

Neem (*Azadirachta indica*) is another product that has been used as an alternative to control insect and mite pests.

Its major active ingredient (azadirachtin) may cause several negative effects on arthropods, such as feeding inhibition, repellency, decreased oviposition, reduced fertility and fecundity, changes in behavior and increased mortality (Schumutterer 1990, Dimetry *et al* 1993, Mordue & Nisbet 2000, Musabyimana *et al* 2001). Neem based products were shown to cause deleterious effects on several mites species as well (Mansour *et al* 1997, Gonçalves *et al* 2001, Makundi & Kashenge 2002, Venzon *et al* 2005, 2008b, Brito *et al* 2006a,b), but no studies have yet been carried out on their effect on *T. evansi*.

The present work aims to evaluate the potential of lime sulphur and commercial formulations of neem for *T. evansi* control. The toxicity of these products was first assessed in laboratory by estimating both the lethal concentration (LC) and the instantaneous rate of increase (r_i) of *T. evansi*. This is a direct measure of population growth in a given period of time and can be used to assess lethal and sub-lethal effects of toxic compounds at the population level (Stark *et al* 2003, Teodoro *et al* 2005). Subsequently, we evaluated in a greenhouse the efficiency of LC_{95} and of the concentration that restrains *T. evansi* population growth ($r_i = 0$), aiming to control the mite population on tomato plants. Additionally, phytotoxicity of the tested product concentrations was evaluated.

Material and Methods

The tomato red spider mite rearing was initiated with mites collected in the field in the municipality of Tocantins, Minas Gerais, Brazil. The rearing was established using tomato plants variety Santa Clara with 30 days of age as hosts kept in a greenhouse. Potted tomato plants were kept inside wooden framed cages (10 x 50 x 90 cm) covered with organza screens to isolate the rearing and to avoid contamination with other arthropods.

The alternative products evaluated were lime sulphur (31.5° Baume), prepared according to Penteado (2000) and three neem based commercial products: NeemPro [10 g/l of azadirachtin (AZA)], Natuneem (1.5 g/l of AZA) and Organic Neem (3.3 g/l of AZA).

Lethal toxicity. The concentration-response bioassays were conducted using 3-4 day old females of *T. evansi* at the beginning of their reproductive stage. Tomato leaf discs (3 cm diameter) were individually placed inside Petri dishes (3.5 cm diameter), and sprayed on a Potter tower (Potter 1952) (Burkard, Rickmansworth, UK). Product spraying was carried out under the pressure of 5 lb/pol^2 and the application of an equal volume of 2.5 ml per concentration, which corresponded to a deposit of 1.70 ± 0.07 mg/cm^2 on the treated area. The applied concentration agrees with recommendations of the IOBC/ WPRS (International Organization for Biological Control of Noxious Animals and Plants/West Paleartic Regional Section) (Overmeer & van Zon 1982). To fix the leaf disc on the Petri dish, a 10% water solution of carrageenan was prepared and the disc was placed before the solution solidification. The concentrations tested were chosen through initial bioassays and were between the lower (no mortality caused) and upper (100% mortality) limits of response. The tested concentration range for each product was: 0.15-1.80% of lime sulphur, 0.69-157 mg a.i./l of NeemPro, 0.32-57.7 mg a.i./l of Organic Neem, and 0.06-37.4 mg a.i./l of Natuneem.

Sprayed discs were exposed to the environment for 1h for drying out. Subsequently, eight females of *T. evansi* were placed on each disc. Five replicates were used for each product concentration. Treated discs were kept in a climatic chamber (25 ± 2°C, 60 ± 10% RH and 13h photophase). Mortality was assessed 24h after product application. A control treatment with distilled water was used to correct the data for natural mortality (Abott 1925). The concentration-mortality curves were estimated by probit analysis (Finney 1971).

Instantaneous rate of population increase (r_i). To evaluate the instantaneous rate of population increase of *T. evansi* (r_i) on different product concentrations, we used the same method as described for evaluating lethal toxicity, except for the evaluation time that was five days. The instantaneous rate of increase (r_i) was estimated using the following formula (Stark *et al* 1997): $r_i = ln (N_f / N_0) / \Delta t$;
where N_0 is the initial number of individuals in the population, and Nt is the number of individuals in the population at end of the time interval, *t*. The number of days (*t*) for the experiment runs was five. Positive values of r_i indicate a growing population, and $r_i = 0$ indicates a stable population, while a negative r_i value indicates population decline and heading towards extinction (Stark *et al* 1997).

The tested concentrations were based on the concentration-mortality curves obtained from lethal toxicity tests. The control consisted of water-sprayed leaf discs. Each product concentration was replicated five times. Mites were kept in a climatic chamber under the same conditions described in the experiments of lethal toxicity. Regression analyses were used to assess the effect of the product concentrations on the instantaneous rate of population increase of *T. evansi*.

Control of *T. evansi* in the greenhouse. Tomato plants of the variety Santa Clara with 30 days grown in plastic pots with soil (2 L) were used in the bioassay. They were infested with 100 adult females of *T. evansi* per plant, and adult mite population was counted eight days after infestation. Subsequently, the products were applied to the plants at concentrations corresponding to the LC_{95}, and at the concentration that stopped *T. evansi* population growth ($r_i = 0$). Thus, the following concentrations were tested per product: NeemPro (80.1 and 71.6 mg a.i./l), Natuneem (31.1 and 20.4 mg a.i. /l), Organic Neem (39.1 and 30.4 mg a.i./l), and lime sulphur (1.0 and 0.6%). Spraying was done with a hand sprayer Brudden® SS model with a capacity of five liters, the inlet diameter of 60 mm, equipped with an adjustable cone nozzle of the type and maximum working pressure of 14 kgf/cm^2.

The experimental design was completely randomized. Treatments were represented by the four products in two concentrations and the control (tomato plants sprayed with water). The nine treatments were repeated three times, and each replicate was represented by four potted plants placed inside a plastic tray (60 x 40 x 10 cm). After one, five, seven and 10 days from product application, mite population on plants was assessed by direct counting using a magnifying glass (10x). The mortality of mites was calculated using the formula proposed by Henderson & Tilton (1955).

Visual symptoms of phytotoxicity for each product concentration tested in the greenhouse were evaluated by two observers. The visual scale of phytotoxicity proposed by Vieira *et al* (2001) was used, with 0: plants with normal leaves and no signs of burns; 1: plants with slightly damaged leaves and/or with small burned areas; 2: plants with medium damaged leaves, yellow with burnt edges and tips; 3: plants with heavily damaged leaves, showing severe defoliation.

Data of mortality and phytotoxicity were *arcsen* $\sqrt{x} / 100$ transformed and subjected to repeated measure analysis of variance of the general linear model, using the indicator Wilks' Lambda (SAS Institute 1989) version 9.0. For the analysis of variance of mite mortality, the concentrations of the four products corresponding to LC_{95} were grouped as "high concentrations", and the product concentrations that stopped the population growth ($r_i = 0$) of *T. evansi* as the "low concentrations". Averages corresponding to phytotoxicity ranks were compared using the Tukey test at 5% of significance.

Results

Results for the concentration-mortality bioassays showed that LC_{95} for lime sulphur, NeemPro, Organic Neem and Natuneem were 1.03%, 80.97 mg a.i./l, 39.13 mg a.i./l and 31.14 mg a.i./l, respectively (Table 1).

The instantaneous rate of increase of *T. evansi* was null, indicating that the population is stable at concentrations of 0.62%, 71.6 mg a.i./l, 30.4 mg a.i./l and 20.4 mg a.i./l, for lime sulphur, NeemPro, Organic Neem and Natuneem, respectively (Fig 1).

In the greenhouse experiment, the analysis of variance for repeated measures showed no differences among products ($F_{3,16} = 3.00$, $P = 0.06$), but significant effects of concentration (low and high) ($F_{1,16} = 396.91$, $P < 0.0001$) and of the interaction of product and concentration on the reduction of *T. evansi* population ($F_{3,16} = 5.25$, $P = 0.01$). Significant effects of time (Wilks' Lambda = 0.0780, F = 55.12; DF = 3, 14, P < 0.0001), of the interaction of products and time (Wilks' Lambda = 0.2138, F = 3.36; DF = 9, 34.22, P = 0.004), of concentration and time (Wilks' Lambda = 0.2599, F = 13.28; DF = 3, 14, P = 0.0002), and of product, concentration and time (Wilks' Lambda = 0.1288, F = 5.02; DF = 9, 34.22, P = 0.0002) were found.

The high concentrations (LC_{95}) resulted in greater efficiency of control over time, regardless of the product. Moreover, the efficiency of high and of low product concentration on reducing *T. evansi* population increased with the duration of exposition (Fig 2). Despite the significant difference between "low" and "high concentration" of the products, especially in the beginning of the experiment, the products applied at "low concentration" causes more than 95% of population reduction after five days of application (Fig 2).

Regarding phytotoxicity, the analysis of variance with repeated measures over time showed significant effects of the products ($F_{3,16} = 46.26$, $P < 0.0001$), of concentrations ($F_{1,16} = 8.19$, $P = 0.01$) and of the interaction of product and concentration ($F_{3,16} = 3.66$, $P = 0.03$). No significant effect was found for the time (Wilks' Lambda = 0.8987, F = 0.53; DF = 3, 14, P = 0.67) and the interactions between product and time (Wilks' Lambda = 0.3993, F = 1.74; DF = 9, 34.22, P = 0.11), and for the concentration and time (Wilks' Lambda = 0.9612, F = 0.19; DF = 3, 14, P = 0.90), and the concentration, time and product (Wilks' Lambda = 0.5606, F = 1,02; DF = 9, 34.22, P = 0.44). Phytotoxicity symptoms were observed only on plants sprayed with Natuneem, especially at higher concentration (31.1 mg a.i./l) (Table 2).

Discussion

Control of *T. evansi* on tomato plants was achieved with the application of neem based products and of lime sulphur. For all products, the efficiency in reducing *T. evansi* population was above 95% five days after spraying, independently of using LC_{95} or lower concentrations at which the instantaneous rate equal to zero. The later concentration represented the LC_{78} for Organic Neem (30.4 mg a.i./l), LC_{94} for NeemPro (71.6 mg a.i./l), and LC_{71} for Natuneem (20.4 mg a.i/l).

It is important to consider the time needed for the tested products to reduce mite population. By evaluating the population effects of the products over time rather than only

Table 1 Toxicity of lime sulphur and neem based products to *Tetranychus evansi*.

Product	n	Slope ± SE[1]	LC_{50}[1] (50% fiducial limits)	LC_{95}[1] (95% fiducial limits)	X^1	P
Lime sulphur	140	0.17 ± 0.04	0.20 (0.15 -0.27)	1.03 (0.74- 1.57)	0.61	0.43
NeemPro	120	0.23 ± 0.48	16.03 (10.02-39.93)	80.97 (69.43-110.10)	0.68	0.17
Organic Neem	125	0.19 ± 0.31	15.11 (10.79-21.20)	39.13 (25.56-56.40)	0.57	0.36
Natuneem	260	0.38 ± 0.24	15.43 (11.41-28.42)	31.14 (26.72-45.27)	0.87	0.54

n = Number of mites tested.

[1]For lime sulphur, LC_{50} and LC_{95} are expressed as percentage of product (%); for neem based products LC_{50} and LC_{95} are expressed as mg a.i/l.

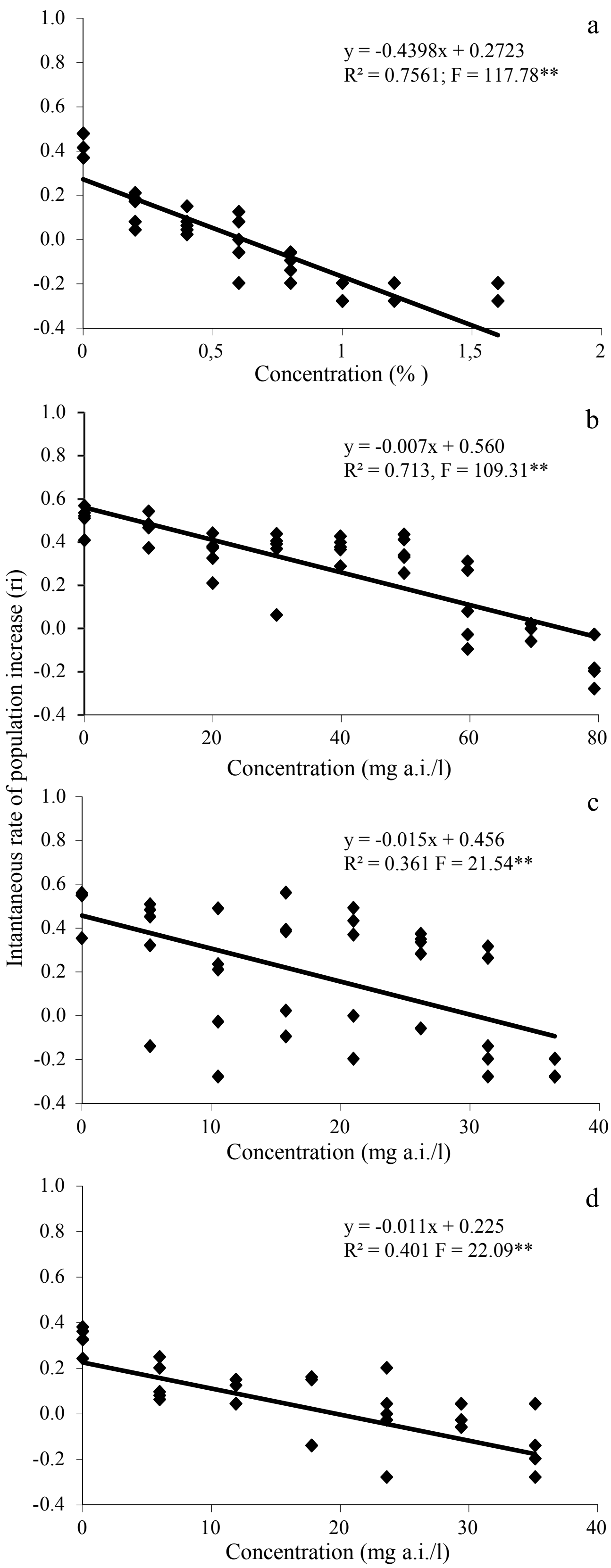

Fig 1 Instantaneous rate of increase (r_i) of *Tetranychus evansi* exposed to increasing concentrations of lime sulphur (a), NeemPro (b), Organic Neem (c), and Natuneem (d).

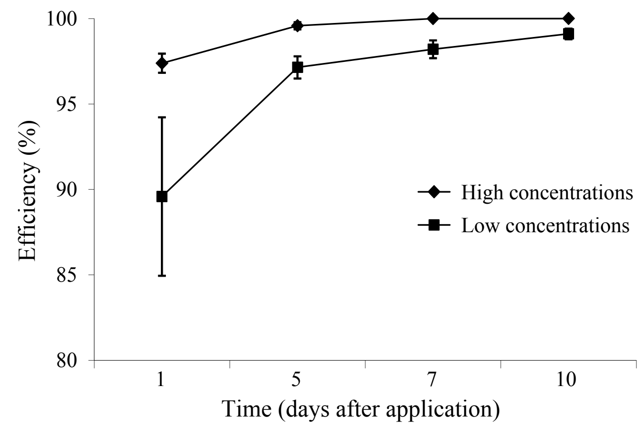

Fig 2 Efficiency (%) of alternative products for control of *Tetranychus evansi* in high (LC_{95}) and low concentrations (r_i = 0) over time. Showed significant difference between high and low concentrations of products ($F = 396.91$, $P < 0.0001$) and between the time after the application of products (Wilks' Lambda = 0.780, $F = 55.12$; DF = 3, 14, $P < 0.0001$), based on repeated measures analysis of variance.

mortality after a short period it is possible to select reduced concentration of products needed for a satisfactory control. The importance of such reduction is not only economic but mainly by the low impact on natural enemies. For neem based products, it has been demonstrated that selectivity towards natural enemies is related to formulation and concentration (Silva & Martinez 2004, Mourão *et al* 2004, Brito *et al* 2006b). For lime sulphur, recent studies showed the same tendency (Tuelher 2006, Soto 2009).

The use of demographic studies together with lethal concentration is recommended to evaluate the toxicity of products (Walthall & Stark 1997, Teodoro *et al* 2005, Stark *et al* 2007). For neem based products, this approach is especially important due to their special mode of action and delayed effects on arthropods (Scmutterer 1990). Besides direct mortality (Momen *et al* 1997, Castiglioni *et al* 2002), neem causes reduction in fertility (Dimetry *et al* 1993), longevity and fecundity of mites (Martínez-Villar *et al* 2005). Thus, subletal effects should be considered when selecting neem concentrations for mite control. In our study, direct mortality after one day of application was responsible for higher reduction of *T. evansi* at the concentration corresponding to LC_{95}. When lower concentrations were used, averaged mortality at first day was inferior to when LC_{95} were applied. However, after the product had enough time to act, i.e. after five days, population reduction was above 95% using either lower or higher concentration. Moreover, the efficiency in reducing *T. evansi* increased over time.

The two concentration of lime sulphur tested in this study were effective against *T. evansi* (0.6 and 1.0%). In most crops were lime sulphur is applied, the commonly used concentrations ranged from 2% to 4% (29º to 32º Baume) (Penteado 2000, Silva *et al* 2009). The use of high concentrations of lime sulphur might be unnecessary also for controlling other mite species, as we showed for *T. evansi*. It is important to consider the use of reduced concentration of lime sulphur in order to decrease negative effects on natural

Table 2 Toxicity of lime sulphur and neem based products to tomato plants in greenhouse.

Treatments	Phytotoxicity ranks[1]			
	1 DAA	5 DAA	7 DAA	10 DAA
NeemPro (0.08 mg a.i./l)	0.22 ± 0.39 c	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
NeemPro (0.07 mg a.i./l)	0.11 ± 0.19 c	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
Natuneem (1.4 mg a.i./l)	2.00 ± 0.00 a	2.00 ± 0.00 a	2.00 ± 0.00 a	3.00 ± 0.00 a
Natuneem (0.87 mg a.i./l)	1.17 ± 0.17 b	1.45 ± 0.51 a	1.23 ± 0.10 a	1.89 ± 0.19 b
Organic Neem (0.37 mg a.i./l)	0.17 ± 0.00 c	0.11 ± 0.20 b	0.11 ± 0.10 b	0.00 ± 0.00 c
Organic Neem (0.28 mg a.i./l)	0.06 ± 0.09 c	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
Lime sulphur (1.0%)	0.06 ± 0.09 c	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c
Lime sulphur (0.6%)	0.06 ± 0.09 c	0.00 ± 0.00 b	0.00 ± 0.00 b	0.00 ± 0.00 c

DAA = Days after application

[1]Rank 0: plants with normal leaves and no signs of burns; Rank 1: plants with slightly damaged leaves and/or with small burned areas; Rank 2: plants with medium damaged leaves, yellow with burnt edges and tips; Rank 3: plants with heavily damaged leaves, showing severe defoliation.

Averages followed by the same letter in columns do not differ by Tukey test at 5% significance.

enemies (Daniel *et al* 2001, Beers *et al* 2009). According to Tuelher (2006), lime sulphur caused more negative effects on the instantaneous rate of increase of the predatory mite *Iphiseiodes zuluagai* Denmark & Muma than on its prey, the coffee red mite *Oligonychus ilicis* (McGregor). The effects of lime sulphur on natural enemies of *T. evansi* need further investigation.

Symptoms of toxicity to tomato plants were observed only for Natuneem, especially at high concentration. It is not clear why only this product showed phytotoxicity, as the other two neem based products did not. Probably other compounds used in the formulation might be responsible for plant toxicity. Abbasi *et al* (2004) reported the absence of neem oil toxicity to tomato plants. For other crops, neem toxicity has been variable (Xuan *et al* 2004, Carneiro *et al* 2007, Pinheiro *et al* 2009).

The application of lime sulphur and neem based products, in appropriate concentrations and formulations, represents a viable alternative to control *T. evansi* on tomato plants. It is important to consider the time required for the acaricide action for each of the products, especially the neem based products, in order to achieve successful control using reduced concentrations.

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