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ORIGINAL PAPER

Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L. against adult *Tetranychus urticae* (Koch)

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Abstract The ethanol extracts obtained from both leaf and seed in the Thorn apple (*Datura stramonium* L.) (Solanaceae) were investigated for acaricidal, repellent and oviposition deterrent properties against adult two-spotted spider mites (*Tetranychus urticae* Koch) (Acari: Tetranychidae) under laboratory conditions. Leaf and seed extracts, which were applied in 167,250 and 145,750 mg/l concentrations, respectively (using a Petri leaf disc-spray tower method), caused 98 and 25% mortality among spider mite adults after 48 h. The simple logistic regression analysis showed that the independent variable, an increase in the dose of leaf extract was associated with a significant increase in the death rate of *T. urticae* females, but an increase in the dosage of seed extracts did not have a significant effect. Using probit analysis and estimating the parameters with a confidence limit of 95%, we determined the LC_{50} values of leaf extract to be 70,593 mg/l. According to Pearson's χ^2 test, mites showed the strongest run off to bean leaf surfaces sprayed with both leaf and seed extracts (in sub-lethal doses: <7,500 mg/l and <25,000 mg/l, respectively) and moved towards surfaces that had not been sprayed with extracts. Furthermore, repeated measures ANOVA indicated a significant difference between the number of eggs laid on unsprayed bean leaves compared to bean leaves that were sprayed with leaf and seed extracts at sub-lethal doses, 2,500 and 25,000 mg/l concentrations, respectively. These results suggest that *D. stramonium* extracts could be used to manage the two-spotted spider mite.

Keywords Acaricidal · Oviposition deterrent · Repellent · Spider mite · Thorn apple

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Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acarina: Tetranychidae), is a pest that is of critical importance to several kinds of cultivated crops, such as tomato, bean, eggplant, pepper, cucumber and potato (Jeppson et al. 1975; Helle and Sabelis 1985). Large populations of *T. urticae* can cause webbing, as well as spotty yellowing and curling in leaves and thus reduce the quality of yields. In order to manage the pest, different kinds of chemicals such as synthetic acaricides are used. Such strategies aim to prevent damage and forestall an epidemic by keeping the populations below a critical threshold. However, widespread use of these chemicals can cause serious ecological problems. These chemicals kill non-target organisms, including insects and mites that prey on the pest, and threaten human health. The major threats include residual chemicals on crops, as well as the evolution of resistance to these chemicals among spider mite populations. Researchers have noted the increased frequency of resistance to currently existing pesticides, even those with different chemical structures. This trend, as well as awareness of the hazardous effects of using these pesticides on human health and environmental ecosystems, has decreased the frequency with which these chemicals are used (Nauen et al. 2001; Kim et al. 2004).

These factors have driven the search for effective pesticides with low rates of toxicity to mammals and plants. Since 1990, synthetic pesticides have been used less frequently. Consumption rates dropped 61% by 2001, but the market share remains high. In contrast, the consumption of botanical and biological products had increased 69% by 2001 (FAO 2001). In recent years, the use of plant-derived chemical compounds has increased, reaching 7.6% of the world market (FAO 2001; Yu 2008). Prior to the discovery of synthetic pesticides, plant-derived chemicals were the main pesticide compounds used by farmers. Even today, farmers frequently use plant-based products such as the oil derived from the seed of the neem tree [*Azadirachta indica* A. Juss, (Meliaceae)]. Azadirachtin, one of the most active constituents in neem oil, acts as a feeding deterrent and limits the growth of insects and mites (Immaraju 1998; Martinez-Villar et al. 2005; Ismann 2006; Kumral and Susurluk 2007). Similarly, nicotine derived from *Nicotiana tobaccum* L. (Solanaceae) is used to slow the growth of pests with sucking and piercing mouth parts, such as spider mites, white flies and leaf hoppers (Sugavanam and Copping 1998; Ismann 2006). Georges et al. (2008) reported that *Datura innoxia* Mill. (Solanaceae) compounds possess insecticidal activity [e.g. mosquitocidal on *Ochlerotatus triseriatus* Say (Diptera: Culicidae); larvicidal on *Helicoverpa zea* Boddie, *Heliothis virescens* Fabricius (Lepidoptera: Noctuidae) and the white fly *Bemicia tabaci* Genn. (Homoptera: Aleyrodidae)]. In addition, whole-plant extracts of the perennial common herb *Datura stramonium* L. were defined as toxic for their insecticidal and antifeedant properties against *Dysdercus cingulatus* Fabricius (Hemiptera: Pyrrhocoridae), *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae), and *Pericallia ricini* Fabricius (Lepidoptera: Noctuidae) (Prakash and Rao 1997). Furthermore, the effectiveness of the compounds extracted from different parts of *Datura* spp. on spider mites were investigated under laboratory conditions (Mateeva et al. 2003; FangPing et al. 2006). For example, partial extracts of *Datura metel* L. (Solanaceae) had repellent effects on adults and oviposition in the Cassada red mite, *Oligonychus biharensis* Hirst (Acarina: Tetranychidae) (FangPing et al. 2006). *D. stramonium* extracts were tested for their acaricidal action on *T. urticae*. The compound was found to be 100% toxic to the organism during its active stages (Mateeva et al. 2003). However, this investigation studied only the lethal toxicity of the extracts. Repellency and oviposition deterrent tests were not performed against *T. urticae*. While some of the insecticidal, repellent, oviposition deterrent and antifeedant activities of the leaf and seed extracts of *D. stramonium* were studied, sub-lethal doses of the extracts were not investigated clearly.

In our experiment, seed and leaf extracts from the Thorn apple (*D. stramonium*) were evaluated in terms of their acaricidal, repellent and oviposition deterrent activity on *T. urticae*. We examined each extract at various dosages and at different time points.

Materials and methods

Spider mite culture

For this experiment, we used a *T. urticae* Koch population. This particular population had not come into contact with any chemicals from the outdoor ornamental plant, Mudanya (Northern Bursa, Turkey), used in this experiment. The susceptible population was reared on *Phaseolus vulgaris* L. (c.v. Magnum, MayAgro Seed Corp., Bursa, Turkey) that were grown in a climate-controlled room with a 16-h light (500 μmol/m^2/s PAR; at 27 ± 1°C and 60 ± 5% r.h.)/8 h dark (18 ± 1°C and 60 ± 5% r.h.) cycle supplemented with white lamps.

Preparation of plant materials

The plant (*D. stramonium*) samples used in the experiment were collected between the end of October and the beginning of November from the experimental land at the University of Uludağ (Bursa/Turkey). Samples were collected in the mornings from flowering plants. The sample plants were identified by a member of the Faculty of Natural Science, Department of Biology, Uludağ University, according to the Index of Botanical Plants of Turkey. Fresh seeds and leaves were dried under indoor conditions for 5 days. The dried seeds and leaves were powdered with a trade asset (Model Bosch).

Preparation of extracts

Plant materials (50 g each) were extracted with EtOH (96%) (600 ml) using a Soxhlet Extractor at 78–80°C. The extraction was completed in three cycles. The solvent was removed with a rotary evaporator at 55°C and 200 mm Hg. After the evaporation, a 16.73% (6.69 g/40 ml) leaf extract and a 14.56% (5.83 g/40 ml) seed extract were synthesised (167,250 mg/l leaf and 145,750 mg/l seed).

Acute toxicity bioassays

Acaricidal activity bioassays were tested by using the adult leaf disc method recommended for spider mites (Kumral and Kovanci 2007). Leaves sprayed with *D. stramonium* leaf and seed extracts were taken from mite-free bean plants and placed with the undersurface facing up on damp filter paper in 5.0 cm plastic Petri dishes. For each assay, 3 replicates and 4–6 concentrations were analysed under a

Potter precision spray tower at the 10-bar and 3 s settings (Burkard Manufacturing Co. Ltd. Rickmansworth U.K.). The responses of *T. urticae* females were observed for various concentrations of leaf extract (27285, 55750, 83625, 111500, 139375 and 167250 mg/l) and seed extract (24292, 97167, 121458 and 145750 mg/l). For each treatment, 2 ml of the leaf and seed extracts were applied to the undersurface of the leaf disc, to the bottom of the Petri dish, and to the inner side of the Petri lids. Petri dishes were then dried at 27 ± 1°C for 30 min (Potter 1952). EtOH (96%) was used as a control treatment. Young *T. urticae* females (precise age was not known, but 1–2 days old females were tested) were placed on sprayed Petri dishes using a paint brush, and the dishes were sealed with Parafilm to prevent the escape of the spider mites. Then, the Petri dishes were placed in a climate chamber that was maintained at 27 ± 1°C with 16 h of light. Live mites were counted at 24, 48 and 72 h. Mites were considered to be alive if they moved when probed with the hairbrush under a stereomicroscope. Each mite that was exposed to various doses of the extracts, leaf or seed, was examined and recorded as either dead or alive. The response variable for an individual mite was then either 1 if dead, or 0 if not. Then, the logistic regression analysis was performed (SAS Institute 2005). Moreover, mortality percentages were calculated using Abbott's formula, which accounts for the mortality rate in the control Petri dishes (Abbott 1925). The extracts with a significant effect were evaluated by probit analysis, and LC_{50} value was estimated (Finney 1971) with a program developed by Analystsoft (2008).

The effect of extract residues on spider mite avoidance

For the repellency test, the leaf disc method was used. Both leaf and seed extracts of *D. stramonium* were applied at sub-lethal doses of 2416, 3625, 7250 mg/l and 9000, 13500, 27000 mg/l. These doses cause a <10% death rate among spider mites, as determined by our acute toxicity test. For each experiment, we performed 3 replicates in 5.0 cm Petri dishes. The leaves taken from mite-free bean plants were placed with the undersurface facing up on damp filter paper. Half of the Petri dishes were enclosed with Parafilm, which extended until the leaf's median vein. The area that was not enclosed with Parafilm was then sprayed under the Potter precision spray tower at 10 bar and 3 s settings (Burkard Manufacturing Co. Ltd. Rickmansworth U.K.). After spraying, the Parafilm was taken off, and the Petri dishes were dried for 30 min at 27 ± 1°C. EtOH was sprayed on the other half of the Petri dishes as a control treatment in all applications.

For each application, 20 susceptible *T. urticae* females were transferred with the camel hairbrush and placed on the middle vein of the leaves. With the lids closed, the Petri dishes were sealed with Parafilm. They were held at 27 ± 1°C, 60 ± 5% r.h. and a photoperiod of 8:16 (D:L) in a climate chamber. After 24, 48 and 72 h, we counted the number of mites and eggs. We also recorded which side of the leaf disc the eggs had been laid on and the percentage of *T. urticae* mortality.

Pearson's χ^2 test was performed on the total number of females attracted to the area sprayed with leaf or seed extracts as opposed to the space with no substance (Van den Boom et al. 2002). The null hypothesis was that for each replicate female exhibited a 50:50 distribution across the sprayed and non-sprayed surfaces. Simultaneously, egg-laying activity of female *T. urticae* adults, either on the extract-treated or non-treated areas, was evaluated. Average eggs per female was transformed to logarithmic values $[\log(x + 1)]$. Then, the values were tested by ANOVA. In addition, Levene's test for homogeneity of variance was performed. Post-hoc testing ($P < 0.05$) of the multiple comparisons was performed by either the LSD or Games–Howell test depending on whether Levene's test was insignificant or significant, respectively (SPSS 2004).

Egg-laying effect

Egg-laying was evaluated in female two-spotted spider mites exposed to *D. stramonium* leaf and seed extracts. For each application, 20 replicates were tested, and sub-lethal doses of the leaf and seed extracts (2,500 and 25,000 mg/l, respectively) were sprayed. Prior to the fecundity test, several resting deutonymphs were separated onto different bean leaves. One newly matured female (1 day old) and two male spider mites were used for each application. The methodology for extract application and transferring the spider mites to Petri dishes was the same as in the toxicity bioassay. The Petri dishes were placed in a climate chamber that was maintained at 27 ± 1°C with 60 ± 5% r.h and a photoperiod of 8:16 (D:L). Egg-laying processes were observed daily throughout the entire life of the female. For each day, the variance analysis (ANOVA) and Levene's test for homogeneity of variance were performed. Post-hoc testing ($P < 0.05$) of the multiple comparisons was performed by Games–Howell test due to Levene's test was significant. Also, an analysis of egg-laying activity was performed by Repeated Measure ANOVA considering days (SPSS 2004).

Results

We investigated the acaricidal effect of leaf and seed extracts from *D. stramonium*. After 48 h, we observed visible acaricidal results (Figs. 1, 2). From the simple logistic regression analyses, *D. stramonium* leaf extract significantly

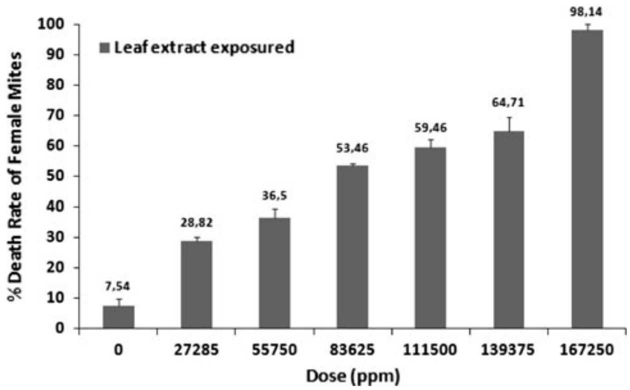

Fig. 1 Observed mortality of the two-spotted spider mite, *Tetranychus urticae* Koch., after a 48-h exposure to crude leaf extract from the Thorn apple, *Datura stamonium* L

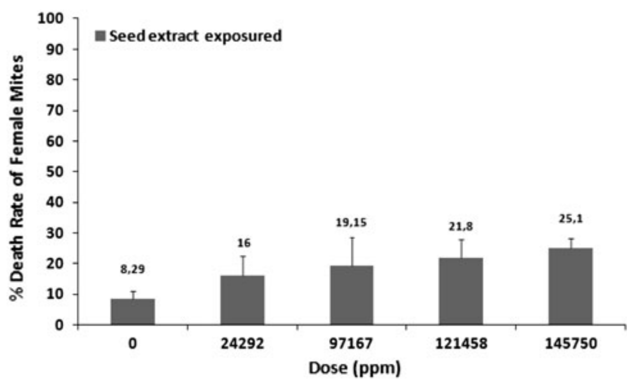

Fig. 2 Observed mortality of the two-spotted spider mite, *Tetranychus urticae* Koch., after a 48-h exposure to crude seed extract from the Thorn apple, *Datura stamonium* L

increased the death rate in the spider mite population compared to the control ($P < 0.01$), but the seed extract did not ($P > 0.05$) (Fig. 3a, b). Treatment with the control resulted in a death rate <10%. When two-spotted spider mites were exposed to leaf extract, the death rate ranged between 29 and 98% at 48 h (Fig. 1). This effect was dose-dependent. However, exposure to the seed extract resulted in a death rate ranging between 16 and 25% (Fig. 2). The results showed that the minimum effect of leaf extract was approximately equal to the maximum effect of seed extract. Thus, leaf extract from *D. stramonium* was much more effective than seed extract in controlling two-spotted spider mite infestations. Furthermore, according to probit analysis, the LC_{50} value of the leaf extracts was 70592.5 mg/l, with a 95% confidence interval (58810.3–83037.7 mg/l). The results shown above were obtained at 48 h because after 24 h the observed death rate of the spider mites decreased. We do not present the results at 72 h because the Abbott formula revealed that the death rate of the control bioassay replicates was high at this time point.

The repellency test results showed that both seed and leaf extracts from *D. stramonium* had a significant effect on the activity of the two-spotted spider mite when applied at sub-lethal doses. The results show spider mites run towards either the extract-exposed side or the non-treated side (Figs. 4, 5). Spider mite movement towards the non-treated side of the leaves and away from the leaf extract-exposed side is presented in Fig. 4. For each replicate in which sub-lethal doses (7250, 3625 and 2416 mg/l) were used, the results showed significant repellent activity ($X^2 = 62.5, 17, 74, 36, 36, 58.5, 78.5, 17, 36$; $P < 0.01$) according to Pearson's χ^2 test (Fig. 4).

Seed extract avoidance was significant for all replicates receiving doses of 27,000 mg/l ($X^2 = 16, 36, 25$; $P < 0.01$) (Fig. 5). While two of the replicates at the 13,500 mg/l dose displayed very high avoidance of seed extract ($X^2 = 25, 36$; $P < 0.01$), one showed no significant effect ($X^2 = 1$, $P > 0.05$). Also, a similar result with a previous dose was observed for leaf extract at the 9,000 mg/l dose ($X^2 = 36, 16$, $P < 0.01$; $X^2 = 1$, $P > 0.05$).

We also assessed the impact on egg-laying activity in the above-mentioned repellency test during the 3 days of the experiment (Table 1). Seed extract at 27,000 mg/l showed a significant deterrent effect on egg-laying compared to the treatment with ethanol over the course of the 3 days ($F_{1, 5} = 24.1$, $P < 0.01$; $F_{1, 5} = 11.1$, $P = 0.03$; $F_{1, 5} = 41.4$, $P < 0.01$ for 24, 48 and 72 h, respectively). Although the average number of eggs laid by spider mites on leaves

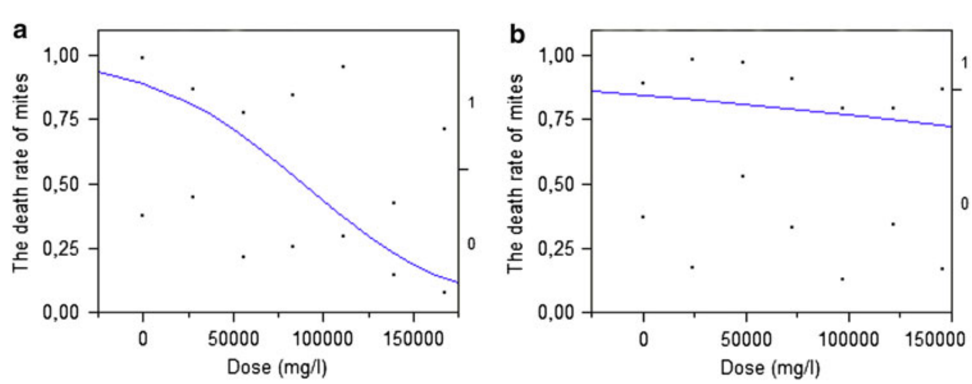

Fig. 3 The logistic fit of the death rate of *Tetranyhcus urticae* female by doses of leaf (**a**) and seed (**b**) extracts

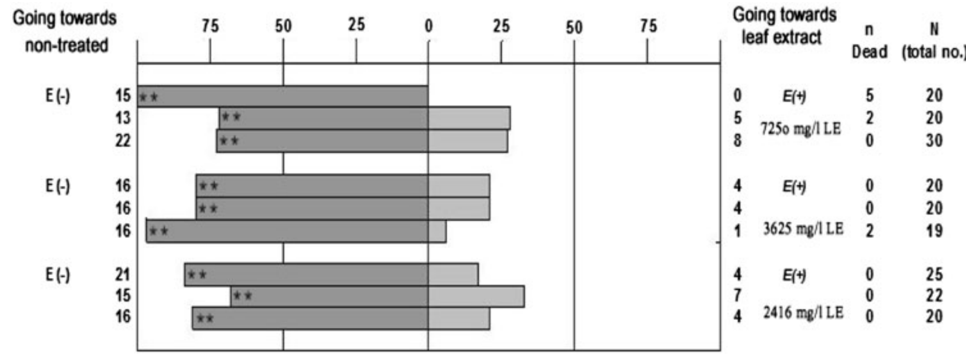

Fig. 4 Percentage avoidance of leaf extract in two-spotted spider mite, *T. urticae*, on both the non-treated or extract-treated side of the leaf after 24 h (** $P < 0.01$)

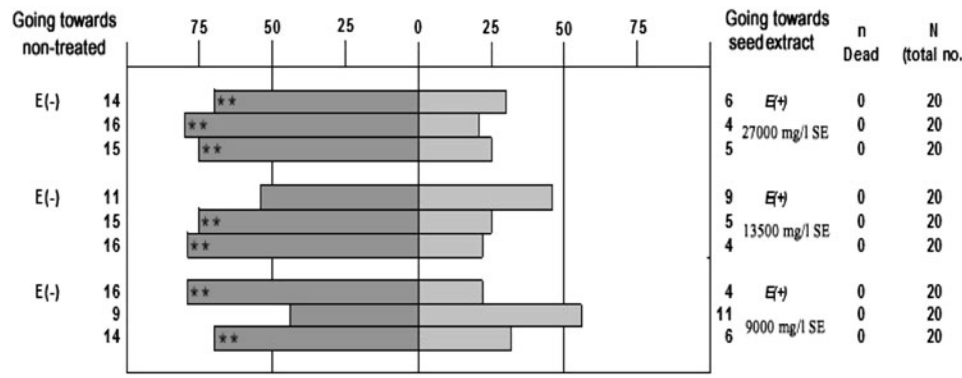

Fig. 5 Percentage avoidance of seed extract in two-spotted spider mite, *T. urticae*, on both the non-treated or seed extract-treated side of the leaf after 24 h (** $P < 0.01$)

Table 1 The observed changes in egg-laying activity of the two-spotted spider mite, *T. urticae*, at different doses of leaf and seed extracts in repellency tests after 24, 48, 72 h

Extract	Mean of eggs/female ± s.e			
	Dose (ppm)	24 h	48 h	72 h
Seed	Non-treated	15.0 ± 3.1a**	12.3 ± 3.2a*	16.3 ± 5.2a**
	27,000	1.3 ± 0.7b	1.7 ± 1.2b	0.3 ± 0.3b
	Non-treated	3.0 ± 2.1	3.3 ± 1.7	1.3 ± 1.3
	13,500	0.0 ± 0.0	1.0 ± 1.0	0.0 ± 0.0
	Non-treated	7.0 ± 2.5a*	7.7 ± 2.7	6.3 ± 5.4
	9,000	3.7 ± 3.7b	1.7 ± 1.7	0.0 ± 0.0
Leaf	Non-treated	39.3 ± 33.9	29.0 ± 27.5	13.3 ± 10.9
	7,250	21.3 ± 19.4	32.7 ± 31.7	17.3 ± 16.3
	Non-treated	4.3 ± 2.3	4.0 ± 2.1a*	2.7 ± 2.2
	3,625	0.3 ± 0.3	0.0 ± 0.0b	0.0 ± 0.0
	Non-treated	17.0 ± 8.6a*	12.7 ± 6.1	24.7 ± 16.1
	2,416	0.7 ± 0.7b	3.0 ± 2.1	0.3 ± 0.3

* Values not associated with the same letter(s) in a column are significantly different ($P < 0.05$)

** Significant at 0.01 level

exposed to *D. stramonium* decreasing doses of seed extract, except for 24 h of seed extract at the 9,000 mg/l dose, was not statistically significant compared to those on the control we feel, and the data show a clear trend towards a decrease due to the treatment ($F_{1,\ 5} = 3.1$, $P = 0.15$; $F_{1,\ 5} = 0.9$, $P = 0.39$; $F_{1,5} = 1.0$, $P = 0.37$ for 24, 48 and 72 h of seed extract at the 13,500 mg/l dose, respectively, $F_{1,\ 5} = 11.1$, $P = 0.03$; $F_{1,5} = 0.9$, $P = 0.39$; $F_{1,5} = 2.5$; $P = 0.19$ for 24, 48 and 72 h of seed extract at the 9,000 mg/l dose, respectively). In leaf extract treatments, a few significant deterrent effects were determined at 2,416 and 3,625 mg/l doses in different days ($F_{1,\ 5} = 14.7$, $P = 0.02$; $F_{1,\ 5} = 10.8$, $P = 0.03$), while no statistically significant result was observed at other doses during the 3 days ($F_{1,5} = 0.1$, $P = 0.80$; $F_{1,5} = 2.4$, $P = 0.19$; $F_{1,5} = 0.1$, $P = 0.98$; $F_{1,\ 5} = 2.1$, $P = 0.22$; $F_{1,\ 5} = 0.1$, $P = 0.95$; $F_{1,\ 5} = 2.3$, $P = 0.21$; $F_{1,5} = 2.9$, $P = 0.16$).

As two-spotted spider mite egg-laying activity appeared to be affected in the repellency test, we also investigated the deterrent effects of leaf and seed extracts from *D. stramonium* on the *T. urticae* oviposition process. We recorded the average number of eggs laid per female for 10 days. After 10 days, when the females were exposed to either leaf or seed extract, we observed a significant decrease in their oviposition compared with the females exposed to ethanol ($F_{1,\ 33} = 9.2$, $P < 0.01$; $F_{1,\ 33} = 9.2$, $P < 0.01$ for leaf and seed extracts, respectively). Additionally, we observed a significant difference between the ethanol-treated control bioassay replicates and leaf extract-treated bioassay replicates on the 2nd, 4th, 5th, 6th and 7th days ($F_{1,\ 33} = 6.6$, $P = 0.02$; $F_{1,\ 33} = 7.8$, $P < 0.01$; $F_{1,\ 33} = 9.0$, $P < 0.01$; $F_{1,\ 33} = 10.0$, $P < 0.01$; $F_{1,\ 33} = 6.7$, $P = 0.01$, respectively) (Fig. 6). For the seed extract, we observed the same trend on the 2nd, 4th, 5th, 6th and 7th

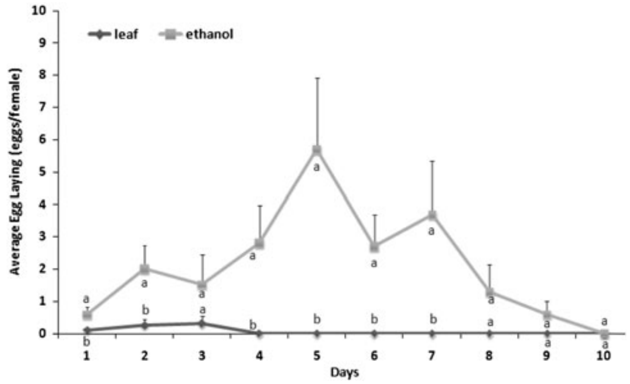

Fig. 6 Oviposition deterrent activity of *D. stramonium* leaf (2,500 mg/l) extract on the two-spotted spider mite, *T. urticae*, after 10 days. Means followed by a different letter differ significantly from the control ($\alpha = 0.05$)

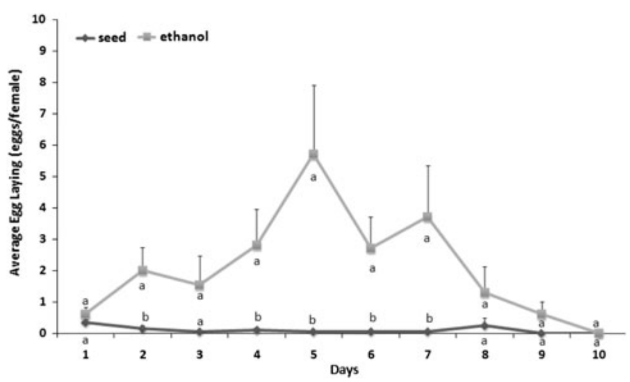

Fig. 7 Oviposition deterrent activity of *D. stramonium* seed (25,000 mg/l) extract on the two-spotted spider mite, *T. urticae*, after 10 days. Means followed by a letter differ significantly from the control ($\alpha = 0.05$)

days ($F_{1,\ 33} = 8.0$, $P < 0.01$; $F_{1,\ 33} = 7.2$, $P = 0.01$; $F_{1,\ 33} = 8.8$, $P < 0.01$; $F_{1,\ 33} = 9.6$, $P < 0.01$; $F_{1,\ 33} = 6.5$; $P = 0.02$) (Fig. 7). Thus, for both the leaf and seed extracts, egg-laying performance was markedly different between day 2 and 7 relative to ethanol performance. Besides, the egg-laying activity was at its overall maximum at day 5. Moreover, the experimental effects of time on the egg-laying activity of the females were considered. According to the repeated measured variance analysis, time had significant effects on egg-laying activity of female *T. urticae* ($F_{9,\ 25} = 5.4$; $P < 0.01$; $F_{9,\ 25} = 4.8$; $P = 0.001$ for seed and leaf, respectively).

Discussion

The current study demonstrated that leaf and seed extracts of *D. stramonium* had lethal effects on *T. urticae*. The death rate for females exposed to leaf extract was between 29 and 98% after 48 h. With the same conclusion when *D. stramonium* extracts were used for their acaricidal activity against *T. urticae* under laboratory conditions, the compound was toxic to all active stages of the spider mite (Mateeva et al. 2003). The larvacidal effects of *D. stramonium* against the rust-red flour beetle *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) were also observed (Pascual-Villalobos and Robledo 1997). In addition, the acaricidal effectiveness of *Datura* spp. extracts against the flat mite *Brevipalpus phoenicis* (Geijskes) and the coconut eriophyid mite *Aceria guerreronis* Keifer (Acari: Eriopyhidae) has been reported (Guirado et al. 2001; Thevan et al. 2005). In this study, the toxic effect of the seed extract, causing a death rate of females that varied from 16 to 25%, was lower than that of the leaf extract. These differences may be related to the presence of certain alkaloids, terpenoids and flavonoids that are believed to be responsible for many of this plant's insecticidal properties, though they are not harmful to humans (Rao and Nath 2005). Moreover, some studies showed that certain alkaloids are present in different amounts in different parts of *Datura* spp., thereby possibly explaining why leaf extracts were more effective than seed extracts in our studies (Philipov and Berkov 2002; Berkov et al. 2006; Anonymous 2008). For example, the alkaloid scopolamine is found at higher levels in the leaf extract compared to the seed extract (Berkov et al. 2006; Anonymous 2008). We hypothesised that the death rate of *T. urticae* may be related to the chemical contents of the leaf or seed extracts. However, this hypothesis is not supported by our experiments. Thus, in further research, chemical analyses in *Datura* extracts and simultaneous acute toxicity bioassays using these extracts on *T. urticae* populations should be conducted to clarify the relations between the mortality rates and chemical composition.

From the effect of extract residues on spider mite avoidance experiments, we found that *T. urticae* females preferred non-treated areas over treated areas. Guarrera (1999) reported that one of the plants most frequently used for antiparasitics and repellents was *D. stramonium*. Our results are similar to those of FangPing et al. (2006), who suggested that leaf and seed extracts of *D. metel* have a repellent effect on Cassava red mites. Additionally, the whole-plant extracts of *D. stramonium* were found to have repellent and antifeedant properties against some insect pests such as the rice moth, *Corcyra cephalonica* St. (Lepidoptera: Pyralidae), *T. castaneum, D. cingulatus, S. litura* and *P. ricini* (Devaraj and Srilatha 1993; Pascual-Villalobos and Robledo 1997; Prakash and Rao 1997). Moreover, Van den Boom et al. (2002) concluded that the plant species vary in their degree of acceptance by the *T. urticae* population. The authors indicated minimal *T. urticae* acceptance for *D. stramonium* in comparison to Fabaceae and other Solanaceae species, namely tobacco and eggplant. Moreover, the researchers reported that some Solanaceae species, e.g. the sweet pepper and the thorn apple, are better directly defended than the others, probably due to the alkaloids in their leaves. Similarly, Cobanoglu et al. (2009) reported that *T. urticae* selectivity among plants in the same family, Solanaceae. *D. stramonium* presented the smallest population of *T. urticae,* probably related to the defensive feature of this plant. Moreover, in this study, crude leaf and seed extracts from *D. stramonium* had negative impacts on egg laying in the two-spotted spider mite. As determined in the repellency bioassay, the leaf and especially the seed extracts also deterred the egg-laying activity of *T. urticae*. Although the average number of eggs laid by spider mites on leaves exposed to *D. stramonium* leaf extract was not statistically significant at the sub-lethal doses of leaf extract compared to those on the control, we feel the data show a clear trend towards a decrease due to the treatment. In addition, this effect was not significant in each of the counts of all doses. Thus, the egg-laying activity may not have been measured accurately with this method because we did not know the exact ages of the females in our test. Sabelis (1985) reported that increasing the feeding rate in response to increasing temperature causes a subsequent increase in oviposition. In addition, the oviposition rate decreases with age. Moreover, Bancroft and Margolies (1999) revealed that the female offspring bias of *T. urticae* decreases with age and conspecific density.

We, therefore, performed a new oviposition experiment using females of the same age. After 10 days, we observed a clear difference between the control and the extract-treated adults in the egg-laying activity. Both leaf and seed extracts from *D. stramomium* markedly decreased oviposition in the two-spotted spider mite, *T. urticae*. Similarly, FangPing et al. (2006) demonstrated that partial extracts of *D. metel* had repellent effects on oviposition in Cassada red mite. In addition, Yogita et al. (2001) reported that extracts of *D. metel* significantly reduced the oviposition of *T. castaneum*. Liyanage et al. (2009) revealed that *D. metel* adversely affected to biological traits of *Earias vittella* (Fabricius) (Lepidoptera: Noctuidae), such as larval period, pupation, adult emergence and fecundity, because of larval feeding on food treated with extract fractions for 48 h. Similar to our results, the negative impact of some other plant extracts, such as *A. indica*, *Capsicum annuum* L. (Solanaceae), *Melodinus suaveolens* (Hance) (Apocynaceae), *Pterospermum heterophyllum* Hance. (Sterculiaceae) and *Haworthia angustifolia* Haw. (Asphodelaceae), was demonstrated on the oviposition activity of mites (Antonious et al. 1997; Metspalu and Mitt 2001; Martinez-Villar et al. 2005; FangPing et al. 2006).

With the increase of resistance in *T. urticae* populations to synthetic pesticides and threat of these chemicals to non-target organisms and human health, plants extracts may be valuable materials for natural pesticides (Prakash and Rao 1997; Nauen et al. 2001; Kim et al. 2004). To sum up, our results highlight the properties of crude leaf and seed extracts from *D. stramonium* that effectively deal with the two-spotted spider mite management. Therefore, these extracts from *Datura* spp. could be an important source for conducting new toxicological and repellent activity tests on spider mite species in further investigations.

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