

EFFECT OF NEEM AZAL T/S ON THE TWO-SPOTTED SPIDER MITE *Tetranychus urticae* KOCH (ACARI: TETRANYCHIDAE)

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ABSTRACT

The effects of Neem Azal-T/S, a commercial product, on various developmental stages of the two-spotted spider mite, *Tetranychus urticae* Koch were tested in the laboratory. Neem Azal T/S was tested for its effect as a deterrent, toxicant, developmental retarding and antiovipositional of the pest *T. urticae*. The product exhibited strong deterrent for *T. urticae* females, and the percentage of ODI was increased as the concentration increased to reach 94.17% at 0.4 % conc. The developmental period of both treated larval and protonymphal stages were significantly prolonged. Female longevity resulted from treated larvae, protonymphs and newly-emerged females was shortened and a remarkable depression in fecundity was noted at all tested concentrations. A decrease in the percentage of egg hatchability was recorded at 0.4 and 0.2 % concs., while an increase in female mortality was observed.

INTRODUCTION

The two-spotted spider mite, *Tetranychus urticae* Koch is one of the most serious pests on various crops and vegetables in Egypt. To control this pest, biological control techniques have been adopted, mainly in greenhouses using the predacious mite, *Phytoseiulus persimilis* Athias-Henriot (Oatman *et al.* 1968; Mori and Moriama 1970; Pralavorio 1973; Markkula and Tiittanen 1976; Stenseth 1980; Picket *et al.* 1987; Waite 1988; Ragusa and Ciulla 1989; Decou 1994).

The predator is sometimes, unable to control the infestation of the pest *T. urticae* in a short time and chemical control is usually necessary to protect the crop. Because of the possible hazardous effects of synthetic, broad - spectrum pesticides application on the environment and human health (Pimentel *et al.* 1992), the attention has been directed to use natural pesticides.

Azadirachtin, a triterpenoid of the neem tree, *Azadirachta indica* A. Juss (Meliaceae) can prove to be one of the most promising botanical substances for use in insect control (Arnason *et al.* 1985). It has deterrent, antiovipositional, antifeedent, growth inhibition, and fitness reducing properties on insects (Butterworth and Morgan, 1971; Ruscoe, 1972; Ladd *et al.*, 1978; Kraus *et al.*, 1981; Saxena, 1986 and Koul *et al.*, 1990). While there is an ample of information on the effect of neem extracts on pest insects, there are very few studies focusing on mite (Schauer and Schmutterer, 1981; Mansour *et al.*, 1987, 1993; Dimetry *et al.*, 1993 Momen *et al.*, 1997 and Villar *et al.*, 2005). The aim of the present work is to evaluate the efficacy of the neem formulation namely; Neem Azal-T/S on the two-spotted spider mite,

T. urticae in the laboratory. The work presented here included its effect as a deterrent, antiovipositional, toxicant, antifeedant, developmental retarding of the pest *T. urticae*.

MATERIALS AND METHODS

Maintenance of Mite Stock Cultures

The two-spotted spider mite, *T. urticae* was collected from leaves of cucumber plant in Kaliobia governorate and maintained on lima bean (*Phaseolus vulgaris* L.) in the laboratory at $28 \pm 2^\circ\text{C}$ and $60 \pm 5\%$ R. H.

Commercial Product

Neem Azal-T/S is a commercial formulation of neem seed kernel extract containing 1% Azadirachtin (1% azadirachtin A and 5% plant oils, emulsifiable concentrate EC as recorded by Kleeberg 1997 and Kliche-Spory *et al.* 1997). Three concentrations of the product were selected (0.4, 0.2 and 0.1 %) according to Dimetry *et al.* (1993), were prepared and tested for their biological effect on the eggs, immature stages and females of *T. urticae*.

Treatment

Repellency and Toxicity Test Procedure

To study the deterrent effect of the product against the adult females of *T. urticae*, raspberry leaf discs (4 cm in dia.) were placed with the lower surface upwards in a Petri dish lined with moist cotton wool. One half of the lower surface of each disc was painted separately with the previously mentioned selected concentrations using fine brush, while the other half served as a check. Ten adult females were then placed on the mid-rib of each leaf disc, using a fine camel's hair brush. Ten replicates leaf discs were used per each concentration. After 2, 24 and 48 h. of treatment, the number of mites that moved to the treated and check halves, the number of eggs laid on each half and mortality of mites were recorded. The reduction in the total number of eggs / female was calculated according to Dimetry *et al.* (1993):

$$\% \text{reduction} = \frac{\text{Total no. of eggs laid on control} - \text{Total no of eggs laid on treated disc}}{\text{Total no. of eggs laid on control} + \text{Total no. of eggs laid on treated disc}} \times 100$$

Toxicity and Biological Effects

1. Effect of Neem Azal-T/S on-Eggs of *T. urticae*

Ten females of *T. urticae* for each concentration were transferred to the under surface of raspberry leaf discs and left to oviposit for 24 h. then removed. The accumulated eggs (0-24 h. old) were sprayed with the different concentrations using a glass atomizer. Each concentration was replicated five times (20 eggs / replicate). Hatchability was observed within one week from treatment. A similar number of untreated eggs was used as control.

2. Effect of Neem Azal-T/S on Larvae of *T. urticae*

Three groups of newly-emerged larvae, each contained twenty larvae, were sprayed separately with the same concentrations, after drying, they were transferred singly to clean raspberry leaf discs using a fine camel's-

hair brush. They allowed to develop and reach maturity. Life cycle, longevity and number of eggs laid within ten days were recorded. Each concentration was replicated five times. A similar number of larvae were sprayed with water and served as a check.

3. Effect of Neem Azal-T/S on Protonymph of *T. urticae*

Three groups of newly-emerged protonymphs (20 / each group) were sprayed by various concentrations, after drying they were transferred singly to clean raspberry leaf discs. They allowed to develop to reach maturity. Life cycle, longevity and number of eggs laid within ten days were recorded. Each concentration was replicated five times. A control was included.

4. Effect of Neem Azal-T/S on Adult Females of *T. urticae*

Three groups of newly-emerged females were sprayed separately with the selected concentrations. After drying, the females of each group were transferred singly on clean raspberry leaf discs. Each concentration was replicated five times (20 females / replicate). A similar number of females were sprayed with water and served as a check. Longevity, mortality of mites after 48 h. and the number of eggs laid over a period of 10 days were recorded.

RESULTS

1-Repellency, Mortality and Oviposition Deterrence

Neem Azal-T/S strongly deterred *T. urticae* adult females, showing > 70 % deterrence at 2-h. period for all tested concentrations. The higher concentration resulted in the higher percentage of deterrence. After 24 and 48 h. periods, the deterrence decreased gradually where an increased number of adults settled on the treated leaf part. Neem Azal-T/S caused considerable mortality of adult females which increased as the concentration was increased. Also, the treated half-leaf hindered the adult to lay eggs, hence, the ODI reached 94.70, 75.96 and 69.81 % at 0.4, 0.2 and 0.1 % conc., respectively (Table I)

Table I : Deterrent effect of Neem Azal – T/S on *T. urticae* females

Conc. %	% Mites distribution on treated leaf part after			% Mortality after 48 h	Average no of eggs / female after 48 h		ODI
	2 h	24 h	48 h		Treated	Control	
0.4	2.00	6.25	8.57	20.41	0.35	12.86	94.70
0.2	2.04	12.77	14.71	17.65	1.59	11.64	75.96
0.1	19.51	26.53	27.27	4.17	1.97	11.08	69.81

ODI : The oviposition deterrent indices

2- Influence of Neem Azal – T/S on Eggs of *T-urticae*

Data presented in Table II showed that the compound had no effect on eggs at 0.1 % conc. As the concentration increases the hatchability percent decreases, reaching 90 and 77.69 at 0.2 and 0.4 concs., respectively.

Table II: Effect of treated various stages with Neem-Azal T/S on hatchability (eggs), life cycle (larvae / protonymphs) and mortality of adult females of *T. urticae*.

Conc. %	Egg stage		Larval stage		Protonymphal stage		Adult stage	
	Initial no	% Hatchability	Life cycle (Mean ± SE)	% Reaching maturity	Life cycle (Mean ± SE)	% Reaching maturity	Initial no	% Mortality after 48 h
0.4	100	77.69	-----	2.78	6.17 ± 0.17 ^a	31.25	100	47.83
0.2	100	90.00	5.58±0.19 ^a	57.89	4.09 ± 0.34 ^b	55.17	100	38.46
0.1	100	100	5.50±0.19 ^a	63.16	3.50 ± 0.22 ^{bc}	66.67	100	32.00
Control	100	100	4.75±0.13 ^b	100	3.33 ± 0.19 ^c	100	100	0.00
			F= 7.188**		F= 31.802**			

** Highly significant ; Different letters in each column denote a significant difference (level: P ≤ 0.01)

3-Influence of Neem Azal – T/S on Larvae of *T-urticae*

Data presented in Table II showed that Neem Azal-T/S application had a toxic effect and the mortality reached up to 97.22 % at 0.4 % conc. A significant influence on the development of larvae in 0.2 and 0.1% concs., as it prolonged the period to reach adulthood compared to control. The number of larvae reached adulthood was 2.78, 57.89 and 63.16 % at 0.4, 0.2 and 0.1 % concs., and the toxicity was increased as the concentration was increased. When the treated larvae reached adulthood there was a significant reduction in the total number of eggs laid per female during 10 days and the reduction gradually increased as the concentration was increased, also, the female longevity was significantly shorter (Table III).

Table III: Effect of treated various stages of *T. urticae* with Neem Azal T/ S on its longevity and fecundity of adult females.

Factors	Treated larvae (Mean ± SE)			Treated protonymphs (Mean ± SE)			Treated newly emerged females (Mean ± SE)			Control
	0.4	0.2	0.1	0.4	0.2	0.1	0.4	0.2	0.1	
Longevity	—	17.00±0.41 ^b	20.67±1.36 ^b	4.20 ± 0.73 ^b	15.50±0.93 ^b	18.00±1.86 ^b	3.77 ± 0.23 ^b	8.78 ± 0.94 ^c	17.00±2.19 ^b	37.80±2.99 ^a
No. of eggs/ 10 days	—	59.75 ± 2.70 ^b	71.33±5.08 ^b	4.00 ± 0.82 ^d	18.33±1.41 ^c	28.17±3.21 ^c	11.47±0.92 ^c	13.50±2.23 ^c	24.33±1.65 ^c	80.45±1.78 ^a

Longevity: F at 0.4 = 82.776**; at 0.2 = 43.891** at 0.1 = 18.604**

Eggs/10 days : F at 0.4 = 69.968**; at 0.2 = 23.289** at 0.1 = 102.791**

** : Highly significant ; Different letters in transverse row denote significant difference at similar concentrations in each treatment (level: P ≤ 0.01)

4-Influence of Neem Azal – T/S on Protonymphs of *T.urticae*

As shown in Table II, the developmental period of treated protonymphs was significantly prolonged in comparison to that of the control specially at the higher concentrations (0.2 & 0.4 %). There was a considerable low percentage number of protonymphs reached adult females i.e. 66.67, 55.17 and 31.25 % at 0.1, 0.2 & 0.4 % concs. The longevity of females resulted from the treated protonymphs were significantly shortened with respect to that of untreated ones at the aforementioned concentrations,

as the concentration increased the longevity decreased. Also, there was a significant reduction in the total number of eggs laid during 10 days at all concentrations used (Table III).

5- Influence of Neem Azal – T/S on Newly- Emerged Adult Females

As shown in Table III Neem Azal-T/S had a biological activity towards females, hence the longevity of treated females was shortened significantly compared with that of untreated ones ($P \leq 0.01$). Moreover, the total number of eggs laid was decreased significantly at the used concentrations (11.47, 13.50 & 24.33 eggs / adult female for 0.4, 0.2 and 0.1 %, respectively) compared with those of control (80.45 eggs / female). It was noticed from the presented data that adult females have been greatly affected more than protonymphs which in turn affected more than larvae.

DISCUSSION

The present study suggested the vital role of the commercial formulation of neem seed kernel extract, Neem Azal - T/S against *T. urticae* in its egg, immature and adult stages. The obtained results showed clearly that neem formulation has a pronounced deterrent effect on *T. urticae*. The deterrent effect of the extract resulted in low feeding activity of the females on the treated halves and low rates of oviposition. These results explain the significant decrease in the egg production rates of the females offered discs treated with the formulation and the ODI reached 94.70 & 75.96 % at 0.4 & 0.2 % conc. respectively. Our results are in agreement with those of Jacobson (1978), Mansour and Ascher (1983), Dimetry and Schmidt (1992), Dimetry *et al.* (1993), Mansour *et al.* (1997), Momen *et al.* (1997) and Tsolakis *et al.* (2002), who stated that 1.0 % hexane extract of neem kernels, neem seed kernels, prepared from various solvents, Margosan-O and Neem Azal-S, Neem gard, Neem-Azal-F and Neem oil strongly repelled *Panonychus citri* (McGregor), *Tetranychus cinnabarinus* (Boisd), the bean aphid, *Aphis fabae* Scop and *T. urticae* respectively.

Neem Azal-T/S can influence the hatchability of eggs at the higher concentration, this remark coincides with the data obtained by Tsolakis *et al.* (2002) who found that neem oil has a strong negative effect on eggs and larvae of *T. urticae* when citrus leaves were used as substrate, causing 100 % and was only 40 % mortality on bean leaves. Similar results were obtained by Hussein *et al* (2005) who reported the toxic effect of various extracts of the wild plant, *Capparis aegyptica* L. on eggs of *T. urticae* and there was a direct relation between the percentage mortality and concentration of the various extracts. Recently, Villar *et al.* (2005) demonstrated that Azadirachtin (64 and 128 ppm) decreased fecundity and increased mortality of *T. urticae* but had no effect on fertility and offspring development. In contrast, Momen *et al.* (1997) demonstrated that hatchability of *T.urticae* eggs was 100 % at all concentrations of Neem Azal-F.

It is worthy be mentioned also, that the formulation prolonged the developmental period of both larvae and nymphs of *T. urticae* as it became, 5.58 and 5.50 days at 0.2 and 0.1 % conc. respectively, compared to 4.75 days in control larvae and 6.17, 4.09 and 3.50 days compared to 3.33 days in

control protonymphs. Whereas 0.4% conc. was lethal and hindered the larvae to reach maturity. Moreover, the formulation affected negatively their longevity and fecundity when they reached maturity specially on the newly-emerged females. The depression in total number of eggs with high concentrations could be attributed to feeding inhibition and irritant effects of the formulation, causing depression on reproduction activity. This is related to the strong effect of the compound which penetrates directly the reproductive system of the female. These findings coincide with those of Tsolakis *et al.* (2002) who stated that there was a remarkable reduction of the oviposition rate in females contaminated with neem oil titolated in azadirachtin at 10,000 ppm. Similar results were also, reported by von Elling *et al.* (2002) who mentioned that neem treatment of early larval instars of the green house whitefly *Trialeurodes vaporariorum* Westwood significantly prolonged the time until adult emergence and reduced the number of eggs laid on exposing females to fresh neem residues. We can conclude that protonymphs and adult females were more sensitive to Neem Azal- T/S than larvae and eggs and this is may be due to the reproductive organs in larvae still unformed. More research is needed to throw more light on the effect of the formulation on the predacious mites associated with *T. urticae* in the field or glasshouses to fully evaluate its effect on benefit mites on application of the IPM programme.

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تأثير المركب المستخلص من نبات النيم (Neem Azal T/S) على العنكبوت

الأحمر *Tetranychus urticae*

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المركز القومي للبحوث - قسم افات ووقاية النبات - الدقى - القايره - مصر

تمت دراسة تأثير المركب المستخلص من نبات النيم على المراحل المختلفة للعنكبوت الأحمر وهي أطوار البيض واليرقات والحوريات الأولى والإناث الحديثة وذلك تحت الظروف المعملية من حيث تأثيره الطارد والسام والمثبط للنمو والمخفض لعدد البيض. ولقد أظهر المركب تأثيرا طاردا شديدا لإناث العنكبوت الأحمر مع زيادة في المعامل الطارد لوضع البيض (ODI) عند التركيزات الأعلى للمركب كما أظهر زيادة لفترة نمو كل من طورى اليرقة والحورية الأولى بالإضافة إلى نقص ملحوظ في عمر الإناث الناتجة من معاملة كل من اليرقات والحوريات والإناث الحديثة بالمركب بتركيزاته الثلاثة (٠,٤ - ٠,٢ - ٠,١ %) كما أنه أدى إلى نقص شديد فى خصوبة تلك الإناث. كما لوحظ نقص فى نسبة فقس البيض المعامل بالتركيزين ٠,٤ و ٠,٢ % وزيادة فى نسبة موت الإناث المعاملة بالتركيزات الأعلى.