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Laboratory Evaluation of Neem Formulations with and without Additives against the Two Spotted Spider Mite, *Tetranychus urticae* Koch

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ABSTRACT

Neem Azal T/S (a commercial formulation of neem seed kernel extract) was tested for its efficacy as a deterrent toxicant or growth inhibitor against the two spotted spider mite, *Tetranychus urticae* Koch. Addition of T/S Fort increased its effect on mite females and eggs. Neem Azal T/S with T/S Fort at the ratio of 1:2 showed the highest acaricidal activity compared with the other formulations tested. Newly emerged females sprayed with the median concentration (0.086%) of Neem Azal T/S showed a serious chronic effect on their biotic potential. A high significant reduction in the egg production with an increase in percent of females sterility and a significant decrease in longevity were recorded. However, egg hatchability was slightly reduced while durations of immature stages were significantly increased.

Key Words: Toxicological and Biological Activity, Neem Azal T/S, T/S Fort, *Tetranychus urticae*.

INTRODUCTION

Although synthetic organic pesticides appeared to provide a solution to problems of pest control, it has become apparent that the repeated application of pesticides can be an inadequate method of control. This is due to the fact that different problems have arisen such as potential resistance, environmental hazards, outbreak of secondary pests and general public disapproval. Such environmental problems increased interest on pesticides occurring naturally in plants. These botanical agents are considered as biodegradable to non toxic products and can be suitable for use in integrated pest management programs (Isman *et al.*, 2002).

Use of neem is especially prevalent in the developing countries, where neem is grown locally and cheaper for farmers to use as alternative to than synthetic chemicals (Schmutterer, 1995). Neem Azal T/S is a neem formulation which has been registered against aphids, leaf miners and whiteflies in ornamental and vegetable crops (Zuber, 2000). It has both contact and stomach activity against different pests. Its stability in the field is short not exceeding one week; for this reason addition of T/S Fort is essential to increase its activity and stability.

Aim of the present work is to elucidate the role of Neem Azal T/S as a botanical pesticide with or without the additive T/S Fort against the two spotted spider mite, *Tetranychus urticae* Koch.

MATERIALS AND METHODES

Mite culture

A stock culture of *T. urticae* was maintained on lima bean, *Phaseolus vulgaris* L. under the laboratory conditions (25±2 ºC and 65±5% RH).

Neem formulation

Neem Azal T/S is a commercial formulation of neem seed kernel extract containing 1% Azadirachtin. This product was produced by Trifolio Company as an emulsifiable concentrate. Its chemical structure is $C_{35}H_{44}O_{16}$.

Additive

The additive was T/S Fort with a concentration of 2ml/liter water. Both products were kindly obtained from Dr. Kleeberg in Germany.

Toxicity to the adult females

Adult females of *T. urticae* were confined on the lower surfaces of detached raspberry (*Morus alba* L.) leaf discs (3cm in dia.) with the upper surfaces placed on cotton wool saturated with water in Petri dishes. Mites were sprayed with different concentrations of Neem Azal T/S alone or with a mixture of Neem Azal T/S and T/S Fort in a ratio of 1:1 and 1:2. Triton X-100 (0.01%) was added to each extract for emulsification and then dilution was made with water to obtain the required concentration. Each test contained 5 concentrations and each concentration was replicated five times (20 females/replicate). A control experiment was conducted using 0.01% Triton X-100 added to water. Mortality was recorded 48 hours after application.

Toxicity to the egg stage

Ten females of *T. urticae* were transferred to the lower surfaces of raspberry leaf discs, left for 24 hours for oviposition then removed. The deposited eggs (24 hrs old) were sprayed with different concentrations of Neem Azal T/S alone or with the

additive (1:1) and (1:2). Each concentration was replicated 5 times (20 eggs/replicate). Six days after treatment, the number of unhatched eggs was counted.

Mortality counts of eggs and females were corrected according to Abbott's formula (1925) and submitted to probit analysis according to Finney (1971). The toxicity index was determined according to Sun (1950).

Effect on biology

Newly emerged females of *T. urticae* were transferred to the raspberry leaf discs. The disc was placed on moist cotton wool in a Petri dish. The females were sprayed with LC_{50} of Neem Azal T/S after adding Triton X-100 (0.01%) to the extract and left to dry. Alive females were singly transferred to leaf discs (3 cm in dia.). The effect of neem formulation on different biological aspects of the mites was studied.

Another group of females was transferred to raspberry leaf discs sprayed with water and 0.01% Triton X-100 which served as a control. Fifteen replicates were used for both neem treatment and the control.

The percentage of females sterility was calculated according to Toppozada *et al.* (1966). Statistical analysis was carried out using (t) test.

All experiments were performed under laboratory conditions of 25±2°C and 65±5% RH.

RESULTS AND DISCUSSION

Toxicity to *T.urticae* adult females

Data obtained in Table (1) show that *T.urticae* females were more sensitive to Neem Azal T/S + the additive T/S Fort than to Neem Azal T/S alone. For LC_{50} values of the tested formulations, toxicity was in a descending order as follows:

- Neem Azal T/S 1 part: T/S Fort 2 parts > Neem Azal T/S 1part: T/S Fort 1 part > Neem Azal T/S alone. At LC_{90} level, the same trend was followed up as at LC_{50} level.
- Neem Azal T/S + T/S Fort at the ratio of 1:2 was considered the standard formulation in calculating the toxicity index at both LC_{50} and LC_{90} levels, respectively. Table (1) shows that *T. urticae* females were highly susceptible to the low concentration of the mixture (LC_{50}=0.045%).

This is in agreement with the data obtained by Dimetery *et al.* (2003)who found that the two spotted spider mite was highly susceptible to the low concentration of curcuma II (LC_{90}= 0.995%). El-Gengaihi *et al.* (2000) found that both hydrocarbons and sterols should occur together in similar proportions to produce an acaricidal effect rather than each substance alone even at high concentrations.

Toxicity to egg stage

Results obtained in Table (2) show that Neem Azal T/S+T/S Fort at both LC_{50} and LC_{90} levels in the ratio of 1:2 was the most toxic mixture to the egg stage in comparison with the other combinations tested . At LC_{50} level, it was 7.17 times more toxic to the egg stage than using Neem Azal T/S alone. At LC_{90} level, it was 37.45 times more toxic than using Neem Azal T/S formulation alone.

Results presented in Table (2) also show that adding T/S Fort to the neem formulation increased its potency against *T.urticae* eggs. This may be due to the fact that T/S Fort helps in the penetration of Neem Azal T/S formulation through the chorions of the eggs, which increased its potency and caused

Table (1): Toxicity of different neem formulations to *T. urticae* adult females.

Treatment	% LC_{50}	% LC_{90}	Slope	Toxicity index at		N. folds compared with Neem Azal T/S at	
				LC_{50}	LC_{90}	LC_{50}	LC_{90}
Neem Azal T/S	0.086	0.256	2.71	52.33	58.20	1.00	1.00
Neem Azal T/S: T/S Fort 1:1	0.071	0.185	3.07	63.38	80.54	1.21	1.38
Neem Azal T/S: T/S Fort 1:2	0.045	0.149	2.40	100	100	1.91	1.72

Table (2): Toxicity of different neem formulations to *T. urticae* eggs.

Treatment	% LC_{50}	% LC_{90}	Slope	Toxicity index at		N. folds compared with Neem Azal T/S at	
				LC_{50}	LC_{90}	LC_{50}	LC_{90}
Neem Azal T/S	0.473	12.282	0.90	13.95	2.671	1.00	1.00
Neem Azal T/S: T/S Fort 1:1	0.138	0.789	1.69	47.83	41.57	3.43	15.57
Neem Azal T/S: T/S Fort 1:2	0.066	0.328	1.83	100	100	7.17	37.45

Table (3): Biological aspects of *T. urticae* females treated with LC_{50} of Neem Azal T/S under laboratory conditions.

Treatment	Durations (days) ± S.E.							No. of eggs /female ±S.E.	% Hatchability	% Sterility
	Preoviposition	Oviposition	Postoviposition	Incubation	Immature stages	Female longevity	Generation			
Neem Azal T/S	5.33±0.16	11.80±0.58	3.67±0.21	6.47±0.13	14.53±0.17	20.73±0.64	26.33±0.21	38.93±3.11	80.31	67.10
Control	2.40±0.16	26.90±0.48	3.30±0.15	5.30±0.15	12.50±0.17	32.60±0.53	20.20±039	95.30±2.35	99.69	
t value	12.39**	18.56**	1.27	5.68**	8.33**	12.98**	15.01**	13.16**		

$t_{0.05} = 2.069$ $t_{0.01} = 2.807$ ** = highly significant

high mortalities than using the neem formulation alone. The present data are in accordance with the findings of Hiiesaar *et al.* (2000) who found that the most susceptible stage to Azadirachtin and neem formulation (Neem Azal T/S) turned out to be the eggs of *T. urticae*. However, some of the eggs had completed their embryonic development, but their larvae died during hatching. Schauer and Schmutterer (1981) also found a strong toxic action on the eggs of the two spotted spider mite treated with an extract of neem. In some cases, no larvae hatched at all while in other cases, larvae hatched but had no vitality and did not feed then died. The authors considered starving to be one of the possible causes of their dying.

Effect of Neem Azal T/S against Biology of *T. urticae* females

The different biological aspects of *T. urticae* females treated with LC_{50} of Neem Azal T/S were greatly disturbed. Results in Table (3) show that Neem Azal T/S had a distinguished retardant effect; egg laying was significantly reduced and percent of females sterility increased.

Females longevity was also shortened in comparison with the control. The preovipositional period was highly significantly elongated, while the ovipositional period was significantly decreased. This may be attributed to the antifeedant or deterrent effect of the neem formulation. In this respect, Saxena *et al.* (1981) and Saxena and Khan (1985) reported that neem derivatives had diverse behavioural and physiological effects on insects ranging from repellency to feeding deterrency, growth disruption, sterilizing effect and oviposition inhibitor.

The present results are in agreement with those of Dimetry and El-Hawary (1995&1997) who found that different neem formulations had deterrent and antifeedant effects, which hindered larvipositon of adults of *Aphis craccivora* and decreased the reproductive period and longevity. Also, Kumar *et al.* (2001) reported that the antiovipositional activities of Nimbecidine and Neem Azal T/S containing 10 and 50 ppm azadirachtin concentrations respectively against the brown leaf hopper *(Nilaparvata lugens)* showed significant reproductive inhibitory effects presumably by way of derailing the physiological mechanism of egg development and significant ovipositional deterrency.

Abdel-Aziz and Kelany (2001) studied the effect of Neem Azal T/S (1% Azadirachtin) and Neem Azal T (5% Azadirachtin) on some biological aspects of the two spotted spider mite, *T. urticae*. They found that Neem Azal T/S was highly effective showing high mortality and reduced the number of eggs laid by female.

Tabatadze and Loiadze (2002) tested the efficacy of Neem Azal T/S against different coccids and confirmed its efficacy under laboratory conditions.

The insect repellent and antifeedant action of neem has been attributed to the triterpenoid azadirachtin and other related compounds (Jacobson, 1987). Azadiractin may affect the neuroendrine centers by inhibiting the release of prothoracic hormone and other neurohormones (Sieber and Rembold, 1983). Yolk incorporation was often abnormal and may account for the failure in egg development.

Neem Azal T/S hindered the development of the immature stages of *T. urticae* and thus the larvae, proto and deutonymphs durations were prolonged in comparison with control individuals. The prolonged time between moultings has been explained with the reduction of ecdyson in haemolymph (Rembold *et al.*, 1987).

These data explain the fact that neem formulation influenced the chemosensory behaviour of the mite or acted on the physiological processes as a growth regulator and this is in agreement with the data obtained by Schmutterer (1984).

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