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LABORATORY EVALUATION OF NEEM FORMULATIONS 
WITH AND WITHOUT ADDITIVE AGAINST 
THE TWO SPOTTED SPIDER MITE, 
TETRANYCHUS URTICAE KOCH 

BY N. Z. DIMETRY, S.A.A. AMER, & S.A. SABER ¹ 

(Accepted October 2008) 

SUMMARY: Neem Azal T/S (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 induced a serious chronic effect on their biotic potential. A high significant reduction in the egg production with an increase in percent of female sterility and a significant decrease in longevity were recorded. However, egg hatchability was slightly reduced while durations of immature stages were significantly increased.

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 are arising such as potential resistance, environmental hazards, outbreak of secondary pests and general public disapproval. Such environmental problems have focused 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).

The use of neem is especially prevalent in the developing countries, where neem (Azadirachta indica) grown locally is cheaper for subsistence farmers to use than synthetic chemicals (SCHMUTTERER, 1995).

Neem Azal T/S is a neem formulation which has been registered against aphids, leaf miners and white flies in ornamental and vegetable crops (ZUBER, 2000). It had 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.

The 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.

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Material and methods

Mite culture: A stock culture of *T. urticae* was maintained on lima bean, *Phaseolus vulgaris* L. under laboratory conditions (25 ± 2°C 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 is T/S Fort with a concentration of 2ml/liter water. Both products were kindly obtained from Dr. Kleeborg 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 (3 cm in dia.), while the upper surfaces were placed on cotton 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 and 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 five times (20 eggs/replicate). A control experiment was conducted using 0.01% Triton X-100 added to water. Mortality was recorded 48 hours after application.

Biological efficacy: Newly emerged females of *T. urticae* were transferred to the raspberry leaf discs. The disc was placed on moist cotton 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 the 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 the adult females

The data obtained in Table 1 show that females of *T. urticae* were more sensitive to Neem Azal T/S + the additive T/S Fort than using 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 1 part : 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 Dimetry *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-Genaihi *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.
Table 1: Toxicity of different neem formulations to adult females of *T. urticae*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% LC₅₀</th>
<th>% LC₉₀</th>
<th>Slope</th>
<th>Toxicity index at:</th>
<th>N. folds compared with Neem Azal T/S at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀</td>
<td>LC₉₀</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neem Azal T/S</td>
<td>0.086</td>
<td>0.256</td>
<td>2.71</td>
<td>52.33</td>
<td>1.00</td>
</tr>
<tr>
<td>Neem Azal T/S: T/S Fort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>0.071</td>
<td>0.185</td>
<td>3.07</td>
<td>63.38</td>
<td>1.21</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.38</td>
</tr>
<tr>
<td>1:2</td>
<td>0.045</td>
<td>0.149</td>
<td>2.40</td>
<td>100</td>
<td>1.91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.72</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% LC₅₀</th>
<th>% LC₉₀</th>
<th>Slope</th>
<th>Toxicity index at:</th>
<th>N. folds compared with Neem Azal T/S at:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀</td>
<td>LC₉₀</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neem Azal T/S</td>
<td>0.473</td>
<td>12.282</td>
<td>0.90</td>
<td>13.95</td>
<td>1.00</td>
</tr>
<tr>
<td>Neem Azal T/S: T/S Fort</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1:1</td>
<td>0.138</td>
<td>0.789</td>
<td>1.69</td>
<td>47.83</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.57</td>
</tr>
<tr>
<td>1:2</td>
<td>0.066</td>
<td>0.328</td>
<td>1.83</td>
<td>100</td>
<td>7.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>37.45</td>
</tr>
</tbody>
</table>

Table 3: Different biological aspects of *T. urticae* females treated with LC₅₀ of Neem Azal T/S under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Preoviposition</th>
<th>Oviposition</th>
<th>Postoviposition</th>
<th>Incubation</th>
<th>Immature stages</th>
<th>Female longevity</th>
<th>Generation</th>
<th>No. of eggs /female ± S.E.</th>
<th>% Hatchability</th>
<th>% Sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neem Azal T/S</td>
<td>5.33 ± 0.16</td>
<td>11.80 ± 0.58</td>
<td>3.67 ± 0.21</td>
<td>6.47 ± 0.13</td>
<td>14.53 ± 0.17</td>
<td>20.73 ± 0.64</td>
<td>26.27 ± 0.21</td>
<td>38.93 ± 3.11</td>
<td>80.31</td>
<td>67.10</td>
</tr>
<tr>
<td>Control</td>
<td>2.40 ± 0.16</td>
<td>26.90 ± 0.48</td>
<td>3.30 ± 0.15</td>
<td>5.30 ± 0.15</td>
<td>12.50 ± 0.17</td>
<td>32.60 ± 0.53</td>
<td>20.20 ± 0.39</td>
<td>95.30 ± 2.35</td>
<td>99.69</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>12.39 **</td>
<td>18.56 **</td>
<td>1.27 **</td>
<td>5.68 **</td>
<td>8.33 **</td>
<td>12.98 **</td>
<td>15.01 **</td>
<td>13.16 **</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* t 0.05 = 2.069    t 0.01 = 2.807   ** = highly significant
Toxicity to the egg stage

The results obtained in Table 2 show that Neem Azal T/S+T/S Fort at both LC50 and LC90 levels in the ratio of 1:2 was the most toxic formulation to the egg stage in comparison with the other formulations tested.

At LC50 level, it was 7.17 times more toxic to the egg stage than using Neem Azal T/S alone. At LC90 level, it was 37.45 times more toxic than using Neem Azal T/S formulation alone.

The Table 2 shows that adding T/S Fort to the neem formulation increases 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 chorion of the eggs, which increases its potency and causes high mortalities than using the neem formulation alone. The present data are in accordance with the findings of Huiessaar 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 & Schmutterer (1981) have 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 and in other cases, larvae hatched but had no vitality and they did not feed and died. The authors considered starving to be one of the possible causes of their dying.

Biological efficiency of Neem Azal T/S against T. urticae females

The different biological aspects of T. urticae females treated with LC50 of Neem Azal T/S were greatly disturbed. The results obtained 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.

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

The present investigations are in agreement with those of Dimetry & El-Hawary (1995 & 1997) who found that different neem formulations had deterrent and antifeedant effects, which hindered larviposition 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) have shown significant reproductive inhibitory effects presumably by way of derailing the physiological mechanism of egg development and significant ovipositional deterrence.

Abdel-Aziz & 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, where showed high mortality and reduced the number of eggs laid by female.

Tabatadze & Lojadze (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 (Butterworth & Morgan, 1968; Zanno et al., 1975 and Jacobson, 1987). Azadiractin may affect the neuroendrine centers by inhibiting the release of prothoracic hormone and other neurohormones (Sieber & 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 remained elongated in comparison with control individuals. The prolonged time between moulting has been explained with the reduction of ecdyson in haemolymph (Rembold et al., 1987). These data explain that neem formulation influences the chemosensory behaviour of the mite or...
acts on the physiological processes as a growth regulator and this is in agreement with the data obtained by Islam (1984) and Schmutterer (1984).

REFERENCES


Sun (Y.P.), 1950. — Toxicity index and improved method of comparing the relative toxicity of insecticides. — J. Econ. Entomol. 43: 45-53.

