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TOXICOLOGICAL EVALUATION AND BIOLOGICAL POTENCY OF PETROLEUM ETHER EXTRACT OF TWO PLANTS AND THEIR ISOLATES TOWARDS THE TWO SPOTTED SPIDER MITE “TETRANYCHUS URTICAE” KOCH

by Nadia Z. Dimetry 1, S. A. A. Amer 1, S. El-Genaihi 2

(Accepted October 2002)

SUMMARY: Six compounds isolated from unsaponified fraction of crude petroleum ether extracts of Curcuma longa L. rhizomes and Nicandra physaloides L. herb were compared for their acaricidal activity with the crude petroleum ether extract of both plants against Tetranychus urticae Koch females. The biological potency of the crude petroleum ether extract of both plants as well as the compounds isolated were assayed against the adult females.

Petroleum ether extract of C. longa and the different compounds isolated from the unsaponifiable fractions were more toxic to the adult females than N. physaloides extract or its isolates. At LC 50 level, Cu I isolated from C. longa ranked the first between the different compounds isolated and the triterpenoids isolated from N. physaloides were the least effective.

Newly emerged adult mite females offered discs treated with median concentration (0.153 %) of crude petroleum ether extract of C. longa or its isolates and the median concentration (0.5 %) for N. physaloides crude extract and their isolates induced serious effect on the biotic potential of female adults. A high significant reductions in egg production and percentage mortality together with serious increase in percent sterility were recorded. However, no significant effect was recorded on eggs hatchability.

In studying the bioresidual effect of the crude extract of both plants under semi field conditions, both extracts proved to have a lasting effect of 7 days against the adult females.

INTRODUCTION

Chemical control of the two-spotted spider mite has become difficult on account of an increasing resistance of this pest to the chemical pesticides, beside the problems of toxic residues and pollution of the environment. From this point of view, it is evident that the necessity exists to develop more environmentally sound technologies for its control. Higher plants contained many different compounds, which play a crucial role in their defense mechanisms against pest attack, which are broadly speaking classified as secondary plant metabolites. Extensive review are available concerning the role of neem seed oil, seed extracts and commercial formulations against several pests attac-
king different plants (Schmutterer & Ascher, 1987; Kleeberg, 1997).

Many plant components have insecticidal activity against several insect species. Su et al., (1982) carried out extensive studies towards the isolation and identification of the naturally insect repellent compounds from *Curcuma longa* L. Higher concentrations of Eucalyptus, *Mentha* spp. and rhizomes of curcuma extracts significantly decrease the population build up of the mite *Tyrophagus putrescentiae* (Schrank) in wheat flower (Gulati & Mathur, 1995). Dimetry et al., (2000) evaluated the efficacy of different successive extracts of different plants using different organic solvents on both the adult and egg stages of *Tetranychus urticae* Koch and concluded that petroleum ether extracts of *C. longa* and *Nicandra physaloides* L. and the alcoholic extract of *Dodonaea viscosa* L. were the most efficient extracts against the two spotted spider mite.

El-Gengaihi et al., (2000) found that the unsaponifiable matter of *D. viscosa* leaves was the most toxic, while the saponifiable matter of *C. longa* was the most potent against *T. urticae*.

The objective of the present work is to study the toxicity and biological potency of *C. longa* and *N. physaloides* petroleum ether extracts and their different isolates against the two -spotted spider mite *T. urticae*.

**Material and methods**

Mites were obtained from a laboratory culture of the two- spotted spider mite *T. urticae* on lima bean (*Phaseolus vulgaris* L.) under 25 ± 3 °C, 65 ± 5 % relative humidity.

**Preparation of petroleum ether extracts**

250 g of each plant powder were extracted with petroleum ether (b.r 40–60 °C) in soxhlet apparatus. The solvent was removed by evaporation using rotary evaporator under reduced pressure and temperature did not exceed 30 °C. The residue obtained was kept in dessicator until used in biological investigation.

**Saponification of the petroleum ether extract**

Ten grams of the petroleum ether extract of each plant was saponified using the method adopted by Farag et al. (1986), to obtain the saponifiable (fatty acids) and unsaponifiable matter. The unsaponifiable matter of the two plants was chromatographically fractionated by preparative thin layer. Benzene-ethyl acetate mixture in a ratio of (86:14) was the developing system which isolate 3 compounds from *C. longa* having the Rf-values of 0.23, 0.53, 0.86.

The three compounds were scraped from the pHc after visualized with 50 % sulphuric acid of a margin edge of the plate. The scraped spots were eluted with methanol, then the solvent was removed to obtain a residue. This residue was rechromatographed on TLC plate to check purity of the compound.

Three substances from curcuma were subjected to different spectral tools to elucidate its structure and named Cu I, Cu II and C III Mohamed et al., (2002).

Chromatographic fractionation was carried out with the unsaponifiable matter of *Nicandra physaloides* to obtain their major substances which have the following Rf-values, 0.39 (a compound with sterols nature), 0.56 (compound with triterpene nature) and 0.89 (compound having hydrocarbon structure), El-Gengaihi, & Ibrahim (2002)

**Toxicity of *C. longa* and *N. physaloides* petroleum ether extracts and their isolated compounds towards the adult stage of *T. urticae***

The toxicity of petroleum ether extracts of the two plants and their isolates towards *T. urticae* female was studied by spraying adults placed on raspberry leaf discs with different concentrations of each extract or their isolates, 0.01 % Triton X-100 was added to each extract for emulsification and then dilution was made with water to obtain the required concentrations. Each concentration was replicated 4 times (80 females/treatment). A parallel control experiment was done using Triton X-100 added to the water. Mortality of the females was recorded daily till 96 hours and corrected using Abbott’s formula (1925). The corrected mortality was analysed by the method
Biological efficacy of crude petroleum ether extracts of C. longa and N. physaloides and their isolates against T. urticae females.

Newly emerged T. urticae females were transferred singly to the lower surface of 3 cm diameter raspberry leaf discs previously treated with either crude petroleum ether extract of C. longa or its isolates (Cu I, Cu II or Cu III) at a median concentration of 0.153 % or with that of N. physaloides or its isolates (sterols, triterpenoid and hydrocarbon) at a median concentration of 0.5 %. Triton X-100 (0.01 %) was added to each extract for emulsification. The discs were placed with the upper surfaces in contact with moist cotton in Petri dishes. The effect of each compound on the different biological aspects of the mite was studied over a period of 10 days. The percentage of females sterility was calculated according to Toppozada et al., (1966) as follow:

$$\text{% Sterility} = 100 - \left( \frac{a \times b}{A \times B} \times 100 \right)$$

Where a= number of eggs/female in treatment, b= % hatch in treatment, A = No. of eggs/female in control, B= % hatch in control.

Another group of mites were exposed to raspberry leaf discs treated with water and 0.01 % Triton X-100 which served as control.

Each experiment consisted of 20 replicates. Statistical analysis was carried out using F-test.

Bioresidual effect of C. longa and N. physaloides petroleum ether extracts against T. urticae adults.

The bioresidual activity of petroleum ether extracts of C. longa and N. physaloides was evaluated by spraying Vicia faba L. plants cultivated in pots with the LC50 value of crude extracts of both plants and left in the open field.

Samples of treated leaves were taken after 1, 3, 5 and 7 days after spraying. The sprayed leaves were kept in Petri dishes coated with moistened cotton and the newly emerged female mites were transferred singly to the lower surface of the treated leaves. A similar number of control leaves were offered to the females.

Twenty replicates were carried out for each test. The percentage mortality of the adults and the number of eggs laid were recorded daily through 7 days.

RESULTS AND DISCUSSION

Toxicity of the crude petroleum ether extract of C. longa and N. physaloides and their isolates against the adult females of T. urticae.

The results obtained in Table (1) show that the different extracts tested (crude and isolates) show variable toxicities to the adult female mites. In case of C. longa plant, Cu I was found to be the most toxic compound tested. At the LC50 level, the relative potencies between the crude extract or isolates compared with Cu III as being the least toxic indicate that crude petroleum ether extract, Cu II and Cu I were 1.96, 2.04 and 3 folds more toxic than Cu III.

Different trends were followed up at LC90 level. Curcuma II ranked the first, it was obvious that the two spotted spider mite was highly susceptible to even low concentration (LC90 = 0.995 %). Petroleum ether extract was the least toxic (LC90 = 40.51 %).

The effectiveness of the different extracts against T. urticae females based on LC90 values were Cu II > Cu I > Cu III > crude petroleum ether extract. The cause of high mortality due to different isolates of C. longa could be due to contact toxicity. These findings are in agreement with the reports of Booth 1965 and Hamstead 1970 who obtained better control of the red spider mite T. urticae (Syn.) with a seaweed extract than with conventional fungicides and insecticides.

Also, Gulati & Mathur (1995) found that Curcuma rhizomes were very promising for possible use against the mite Tyrophagus putrescentiae.

Roth et al., (1998) found that fractionation of ethyl acetate extract from C. longa L. rhizomes yielded three curcuminoids which displayed topoisomerase I and II enzyme inhibition activity.
CHANDER et al., (1991) on the other hand, found that turmeric powder alone did not cause significant mortality among *Sitophilus oryzae* except at 2 or 4 % after a 3 month storage interval. They added that F₁ progeny was suppressed by more than 50% at a concentration of 1 % even after a 6 month storage interval.

In case of *N. physaloides* plant, the crude extract was found to be the most toxic one in comparison to its isolates (LC₅₀ value = 0.356 %) compared with 1.766 % for the triterpene compound isolated Table (2).

EL-GENGAIHI et al., (2000) reported that both hydrocarbons and sterols should occur together in similar proportions to produce acaricidal effect rather than each substance alone even at high concentrations.

Concerning the LC₉₀ level as in case of *C. longa* the crude petroleum ether extract was the least toxic while the hydrocarbon isolated was the most toxic.

The effectiveness of the different *N. physaloides* extracts against *T. urticae* based on LC₅₀ level are crude extracts > hydrocarbon > sterols and triterpenes. However, for LC₉₀ level are hydrocarbon > triterpenes > petroleum ether extract < sterols.

In conclusion petroleum ether extract of *C. longa* and the different compounds isolated from the unsaponifiable fraction were more toxic to the adult females than *N. physaloides* extract or its isolates. At LC₅₀ level, Cu I isolated from *C. longa* ranked the first between the different compounds isolated and the triterpenes isolated from *N. physaloides* was the least effective (Table 1 and 2).

### Table 1: Toxicological effect of different compounds isolated from petroleum ether extracts of *Curcuma longa* L. Rhizomes on *T. urticae* Koch.

<table>
<thead>
<tr>
<th>Extract</th>
<th>% LC₅₀</th>
<th>% LC₉₀</th>
<th>Slope</th>
<th>Toxicity index at:</th>
<th>No. of folds compared with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀</td>
<td>LC₉₀</td>
<td></td>
<td>Cu III</td>
<td>Pet. ether crude extract</td>
</tr>
<tr>
<td>Curcuma (pet. ether)</td>
<td>0.15</td>
<td>40.51</td>
<td>0.53</td>
<td>65.36</td>
<td>2.46</td>
</tr>
<tr>
<td>Cu I</td>
<td>0.10</td>
<td>3.66</td>
<td>0.82</td>
<td>100</td>
<td>27.19</td>
</tr>
<tr>
<td>Cu II</td>
<td>0.14</td>
<td>0.99</td>
<td>1.54</td>
<td>68.03</td>
<td>100</td>
</tr>
<tr>
<td>Cu III</td>
<td>0.30</td>
<td>4.04</td>
<td>1.07</td>
<td>33.33</td>
<td>24.63</td>
</tr>
</tbody>
</table>

### Table 2: Toxicological effect of different compounds isolated from petroleum ether extracts of *Nicandra physaloides* L. herb on *T. urticae* Koch.

<table>
<thead>
<tr>
<th>Extract</th>
<th>% LC₅₀</th>
<th>% LC₉₀</th>
<th>Slope</th>
<th>Toxicity index at:</th>
<th>No. of folds compared with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LC₅₀</td>
<td>LC₉₀</td>
<td></td>
<td>Triterpenoid</td>
<td>Steroid</td>
</tr>
<tr>
<td>Nicandra (pet. ether)</td>
<td>0.35</td>
<td>39.01</td>
<td>0.63</td>
<td>100</td>
<td>18.24</td>
</tr>
<tr>
<td>Triterpene</td>
<td>1.76</td>
<td>17.28</td>
<td>1.29</td>
<td>20.16</td>
<td>41.19</td>
</tr>
<tr>
<td>Hydrocarbon</td>
<td>0.71</td>
<td>7.11</td>
<td>1.28</td>
<td>50.14</td>
<td>100</td>
</tr>
<tr>
<td>Sterols</td>
<td>0.98</td>
<td>39.25</td>
<td>0.80</td>
<td>36.0</td>
<td>18.13</td>
</tr>
</tbody>
</table>

### Biological efficacy of crude petroleum ether extracts of *C. longa* and *N. physaloides* and their isolated compounds against *T. urticae* females.

The biological efficacy of crude petroleum ether extracts of both *C. longa* and *N. physaloides* and their isolates against newly emerged female mites are shown in Table (3 and 4). Both crude extracts and their isolates are significantly potent against the adult stage. The pre-oviposition period was significantly elongated in case of female mites offered discs treated with any one of the crude extracts or with the majority of the compounds isolated. Tables (3 & 4) show significant reductions in the total number of eggs laid during the ten days period for all treatments in comparison with the control.
Most of the compounds isolated from *N. physaloides* (the hydrocarbon & triterpenes) as well as the crude extract and Cu III isolated from *C. longa* are highly potent against *T. urticae* females as they exhibited high sterility effect on *T. urticae* females (more than 96 % sterility compared with 77.1 % & 64.15 % sterility due to compounds Cu I & Cu II, respectively).

Also, the different extracts or compounds tested had toxic effect on adult stage. The majority of the compounds isolated from the 2 plants caused more than 70 % mortalities to the adult females. However, both crude petroleum ether extracts (*C. longa* & *N. physaloides*) gave the least percentage mortalities among adults.

The incubation period of the resulting eggs from adults offered discs treated with different isolates of *C. longa* was unsignificantly affected in comparison with the control (Table 3). However, eggs obtained from adults offered crude petroleum ether extract of *C. longa* and *N. physaloides* and the different isolates from *N. physaloides* are significantly affected where the incubation period was elongated in comparison with the control (Table 4).

<table>
<thead>
<tr>
<th>Extract</th>
<th>Pre-oviposition (days)</th>
<th>Incubation period (days)</th>
<th>No. eggs/ Female/ 10 days</th>
<th>% M of mites after 10 days</th>
<th>% Hatchability</th>
<th>% Sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcuma (pet. ether)</td>
<td>2.3 **</td>
<td>4.7 **</td>
<td>5.0 **</td>
<td>20</td>
<td>82.0</td>
<td>92.61</td>
</tr>
<tr>
<td>Cu I</td>
<td>1.8</td>
<td>3.3</td>
<td>13.0 **</td>
<td>90</td>
<td>97.69</td>
<td>77.11</td>
</tr>
<tr>
<td>Cu II</td>
<td>1.6</td>
<td>3.5</td>
<td>20.1 **</td>
<td>70</td>
<td>99.0</td>
<td>64.14</td>
</tr>
<tr>
<td>Cu III</td>
<td>2.9 **</td>
<td>3.6</td>
<td>2.3 **</td>
<td>90</td>
<td>95.65</td>
<td>96.03</td>
</tr>
<tr>
<td>Control</td>
<td>1.2</td>
<td>3.2</td>
<td>55.5</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>0.635</td>
<td>0.649</td>
<td>5.646</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L.S.D. 0.01</td>
<td>0.841</td>
<td>0.859</td>
<td>7.474</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M: mortality ** Highly significant

Table 3: Biological effects of different compounds isolated from petroleum ether extracts of *Curcuma longa* rhizomes on *T. urticae* adults.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Pre-oviposition (days)</th>
<th>Incubation period (days)</th>
<th>No. eggs/ female/ 10 days</th>
<th>% M of mites after 10 days</th>
<th>% Hatchability</th>
<th>% Sterility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nicandra (pet. Ether)</td>
<td>3.5 **</td>
<td>5.7 **</td>
<td>3.8 **</td>
<td>10</td>
<td>78.95</td>
<td>94.59</td>
</tr>
<tr>
<td>Triterpene</td>
<td>2.6 **</td>
<td>4.5 **</td>
<td>1.7 **</td>
<td>90</td>
<td>100</td>
<td>96.94</td>
</tr>
<tr>
<td>Hydrocarbon</td>
<td>2.1 *</td>
<td>4.6 **</td>
<td>1.2 **</td>
<td>100</td>
<td>100</td>
<td>97.84</td>
</tr>
<tr>
<td>Sterols</td>
<td>2.2 **</td>
<td>4.4 **</td>
<td>15 **</td>
<td>40</td>
<td>97.90</td>
<td>73.54</td>
</tr>
<tr>
<td>Control</td>
<td>1.2</td>
<td>3.2</td>
<td>55.5</td>
<td>0</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>L.S.D. 0.05</td>
<td>0.734</td>
<td>0.539</td>
<td>4.276</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L.S.D. 0.01</td>
<td>0.973</td>
<td>0.714</td>
<td>5.663</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

M: mortality * Significant ** Highly significant

Table 4: Biological effects of different compounds isolated from petroleum ether extracts of *Nicandra physaloides* L. herb on *T. urticae* adults.

The percentage of egg hatchability for all isolates was not affected or slightly decreased in comparison with the control eggs. However, eggs obtained from mites offered crude petroleum ether extracts of both plants had a decreased percentage of hatchability (82 & 78.95 %) for *C. longa* and *N. physaloides*, respectively, compared with 100 % for control eggs.

From the foregoing results, it could be concluded that newly emerged adult mite females offered discs treated with either crude petroleum ether extract of *C. longa* or any one of its isolates at a conc. of 0.153 % or *N. physaloides* crude extract or its isolates at conc. of 0.5 % induced serious effect on the biotic potential of *T. urticae* adults. High significant reductions in egg production and percentage mortality...
together with serious increase in percent sterility were recorded. This may be due to direct influence of the compound on the female ovaries or to the contact effect of any of the substances used on the cuticle of the pest, which may in turn disturbed pheromone production (DIMETRY et al., 1990).

Again N. physaloides crude extract and its isolates seemed to be a little bit more potent biologically than C. longa extract or its isolates inspite of the fact that last one and its isolates are more toxic against the adults.

These results agree with those obtained by GULATI & MATHUR (1995) who found that rhizomes of Curcuma were efficient even at a conc. of 0.1 % in reducing the mean egg laying to 7.66 per female mite T. putrescentiae in wheat flour.

Similar results were obtained by CHANDER et al., (1991) on the rice weevil Sitophilus oryzae. They indicated that 4 ml/kg dosage of mustard oil combining 1-20 g of turmeric powder/kg gave the best protection of rice by completely suppressing progeny. Roth et al., (1998) stated that curcumin III was the most active curcuminoid in inhibiting topoisomerases at 25 ug. REDA et al., (1989) and SCHAUER & SCHMUTTERER (1981) pointed out that the deterrent effect of crude petroleum ether extract of Abrus precatorius L. seeds and fresh extract of Ajuga remota against T. urticae females resulted from lower frequency or no oviposition occur following contact with treated leaf discs and this would indicate rejection in response to a contact stimulus.

RESIDUAL EFFECT OF CRUDE PETROLEUM ETHER EXTRACTS OF C. LONGA L. AND N. PHYSALOIDES L. UNDER SEMI FIELD CONDITIONS.

The results obtained in Fig. (1) show that the initial toxicity effect of petroleum ether extracts of either N. physaloides or C. longa was quite obvious. The activity, however, gradually decreased with time elapsed after treatment. The female mite offered treated discs after one day gave the highest percentage mortality (47.62 & 30% for N. physaloides & C. longa extracts, respectively) compared with 9.53 & 10 % only for those adults offered treated leaves after 7 days from treatment.

For all tested periods and for both plants (N. physaloides and C. longa), the number of eggs laid per female was drastically reduced in comparison with the control. Again, the females were more sensitive to petroleum ether extract of N. physaloides than C. longa extract.

Thus, it could be concluded that both crude petroleum ether extracts of N. physaloides & C. longa had a lasting effect of 7 days against T. urticae adults. The present findings are in agreement with the data obtained by DIMETRY et al., (1995) who found that the activity of neem Azal-S and Margosan-O (neem seed extract formulations) against Liriomyza trifolii decreased with the time elapsed after treatment.

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