



# CHARACTERISATION OF THE TWO-SPOTTED SPIDER MITE (*TETRANYCHUS URTICAE* KOCH, *ACARI: TETRANYCHIDAE*) RESPONSE TO AQUEOUS EXTRACTS FROM SELECTED PLANT SPECIES

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**Abstract:** Characterisation of two-spotted spider mite (*Tetranychus urticae* Koch, *Acari: Tetranychidae*) response to aqueous extracts from selected plant species. Aqueous extracts from four plant species of known insecticidal activity on arthropods were tested against two-spotted spider mite females to describe their mode of action on the spider mite. The extracts effects on: (a) the mite establishment on host plant; (b) initiation of feeding (probing behaviour); (c) initiation of permanent feeding and (d) the mite mortality (a toxic effect) was measured using various experimental techniques. The highest toxic action was shown by NeemAzal-T/S (an extract from *Azadirachta indica;* production of Trifolio-M GmbH company) and repellent by water extract from *Allium sativum* and *Urtica dioica*. NeemAzal T/S also shown a strong activity as a feeding suppresant for *T. urticae*.

**Key words**: *Tetranychus urticae* Koch, white mustard, NeemAzal®-T/S; common garlic, common stinging nettle, feeding deterrent, feeding suppressant, antifeedants

# **INTRODUCTION**

In recent years there is increasing interest in using substances which interfere with the host plant location (repellents) and the feeding activity (fagosuppressants and fagodeterrents) of a pest on the treated plant offering yet another novel approach to arthropod pest control. Antifeedants are defined as substances which, when tasted, can induce cessation of feeding either temporary or permanently, depending on the potency (Munakata 1977). Several authors have tried to distinguish between "true antifeedants" and "feeding deterrents" (Higgins and Pedigo 1979). A perusal of the

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literature shows that such terms as feeding repellent, rejectant, inhibitory chemical and feeding deterrent are often used synonymously with antifeedant. Generally these chemicals retard the feeding activities of pests and reduce damage caused by them by rendering treated plants unattractive and unpalatable. The arthropod often remains near the treated foliage containing the antifeedant and may die from starvation. Antifeedant compounds for insect control have been dealt by Wright (1967), Chapman (1974), Bell and Carde (1984). Antifeedants in some plants constitute important defense barries against pest attack. They are generally pest-specific and therefore harmless to nontarget organisms. Interest in these compounds has intensified when the disadvantages of synthetic insecticides in crop protection have been reported by a number of scientists. Because of their biodegradable nature and relative safety to beneficial organisms or the environment, the research on the biological activity and chemistry of antifeedants has included a large number of plant species as their sources (Jacobson 1975; Norris 1986; Saxena 1987). Diterpenoids, saponins, isoflavan, alkaloids, phenolics have been identified as insect feeding deterrents. Presently they are especially recommended for ecological production of crops and small home gardens (Legutowska 2004).

The experiments showing a drastic reduction in feeding and unexpected effects on insect metamorphosis in brown plant-hopper [*Nilaparvata lugens* (Stal)] on rice plants treated with neem seed oil (crude oil, extracted from decorticated seeds) by Saxena et al. (1981) have triggered wide interest in using various formulations of neem seed kernel extracts in pest management (Saxena 1987; Schmutterer 1990). The commercial formulation of plant extract from the seed kernels of the neem tree – NeemAzal® -T/S was tested against 140 species of mites (*Acari*), *Thysanoptera*, *Homoptera*, *Heteroptera*, *Coleoptera*, *Lepidoptera* and *Hymenoptera*. The producer – Trifolio-M GmbH has obtained the first registration for NeemAzal in 1998 mainly for control of sucking arthropod pests as: spider mites, white flies and aphids on ornamental plants in greenhouses. Later high efficacy of the formulation was confirmed for young larval stages of a number of insects species with chewing type of mountparts as: *Lepidoptera*, *Coleoptera* and *Hymenoptera* (Kleeberg and Hummel 1999). The authors have also characterised the NeemAzal effects on tested insect pests as: feeding inhivition after hours; inactivity after days to 1–2 weeks and fertility reduction after weeks (next generation).

In addition to neem tree, a wide range of other tropical plant species as e.g.: chinaberry (*Melia* spp.); *Walburgia* spp., *Quillaja saponaria* Molina; *Gmelina arborea* L. were extensively studied for their insect repellent and antifeedant properties (e.g. Oparaeke 2005; Waligóra 2006). There are also reports that the Polish fauna comprise a number of species of insecticidal activities (Legutowska 2004).

In this paper we have compared the effects of extracts of three local plant species commonly grown in central Europe *versus* neem seed delivered commercial product-NeemAzal®-T/S of Trifolio-M GmbH. company on feeding behaviour and survival of two-spotted spider mite (*Tetranychus urticae* Koch). Special attention was given to characterise the mode of action of studied extracts on: (a) preliminary acceptance of treated leaf surface as a suitable host – repellent effect; (b) initiation of feeding (probing) – presence of feeding suppressants; (c) final acceptance of treated leaves for feeding – occurrence of feeding stimulants or deterrents and (d) on the spider mite survival during 6 days period.

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# MATERIALS AND METHODS

Our terminology used for the characterisation of *T. urticae* response to the water extracts in this paper is based on the Beck's (1965) revised Dethier's and associates original definitions (Dethier et al. 1960). The authors agreed that "arrestant" describes a stimulus that causes the insect to cease locomotion in close contact with the apparent source. "Repellents" elicit an orientation response away from the apparent source. However Beck (1965) distinguishes two steps in feeding behaviour of insects: initiation and maintenance of feeding as separate phenomena. The term "feeding incitant" describes a stimulus that evokes the biting or piercing reaction; conversely, a stimulus tending to prevent this response is designated as a "feeding suppressant". Stimuli tending to promote continuous feeding are termed "feeding stimulants"; and those preventing continuous feeding or hastening the termination of feeding are designated as "feeding deterrents". Dąbrowski and Rodriguez (1971) and Dąbrowski (1974), confirmed later by other scientists that the same behavioural responses to various plant properties are observed in the case of spider mites (*Tetranychidae*).

#### Preparation of water plant extracts

Tree plants species and one formulation based on neem seed extract (*Azadirachta indica* A. Juss) were included in the experiment: white mustard (*Sinapsis alba* L.); commen garlic (*Allium sativum* L.) and common stinging nettle (*Urtica dioica* L.). A formulation of NeemAzal®-T/S containing 1% of azadirachtin, produced by Trifolio-M GmbH company, was applied in 0.3% and 0.5% water solution as recommended by the manufacturer. Concentrations of plant extracts made from the other three species were based on previous experience and recommendations by other authors as: Górski (1986), Wasina (1987) and Legutowska (2004).

Three various extracts of common garlic were tested in the experiment. Fifty grams, 25 and 12.5g of garlic cloves were crashed by a kitchen hand squeezer and obtained extracts were mixed with 1l distilled water. After 15 minutes the mixture was filtered through filter paper. The quantity of 1.2g; 0.6g and 0.3g of powdered seeds of white mustard were mixed with 1l of distilled water; regularly stirred; left for 12 hours and finally filtered.

Three dosages: 40g, 20g and 10g of dry herb of common stinging nettle were mixed with 11 of distilled water, left for 24 hours and stirred from time to time and filtered through filter paper.

#### Measuring the repellent effect on T. urticae behaviour

Whole leaves of similar size of common bean (*Phaseolus vulgaris* L.) were dipped in the experimental solutions for 2–3s to assure uniform cover of leaf surface. The control untreated leaves were immersed in distilled water. All leaves were placed on wet cotton in Petri dishes. Two centimetres in diameter a vaseline ring was formed with help of syringe to restrict escaping the spider mites from treated area. Five *T. urticae* females were placed on each of five treated leaves used as a treatment. Counting of the mites staying inside the ring was done after 1, 2, 3 and 4 hours.

## Measuring the extracts effect on T. urticae feeding behaviour

Observations on the antifeedant effect of tested plant extracts were carried out on

the treated bean leaves as proposed by Dąbrowski (1974) and later successfully used for other spider mites species by other scientists (Skorupska 2003). To increase the female motivation for feeding, they were kept 12 hours without access to host plant and later transferred inside the treated leaf ring as described above. Observations on plant acceptance for feeding were carried out for 30 minutes. The hand drawings showing the mite behaviour inside the treated leaf ring were drawn and marked: (a) place of releasing a mite female inside the ring; (b) places of feeding probes and (c) places of initiation of a permanent feeding. The time period between the female release and consecutive stoppings for probing and finally for permanent feeding were noted on a separate sheet.

## Measuring the toxic effect on T. urticae female survival

Five young *T. urticae* females were transferred from the mass rearing colony with help of a fine brush onto treated bean leaves with experimental water extracts and untreated control leaves. The leaves were placed inside the Munger's rearing cages and kept under constant temperature of 24°C and 16D/8N hour photoperiod in the growing chamber. The number of living and dead mites was counted after 1, 3 and 6 days and expressed in the percentage of mortality.

Before statistical analysis, all data were transformed using the Bliss formula. Multifactorial analysis of variance in relation to time and a treatment was perfomed. The Tukey's test was used to estimate the significance of differences between average values at  $\alpha = 0.05$ .

## RESULTS

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## Measuring the repellent effect on T. urticae behaviour

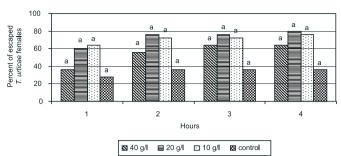
The plant repellent effect was indirectly measured by a number of *T. urticae* females staying inside the treated ring on bean leaves. The lowest repellent response of *T. urticae* mites was observed after the first hour of experiment. However, already during the first hour after transferring the mite females on treated leaves 60–64% of mites have escaped the treated leaf surface by the 20 g/l and 10 g/l nettle concentration (Fig. 1). On bean leaves treated by the 50 g/l garlic concentration, 50% of females have also escaped. Also the plant extracts of NeemAzal, garlic and mustard showed a high repellent effect already after 2 hour of observations (Fig. 1). The repellent effect demonstrated by higher than 80% of escaping females was demonstrated by: nettle at 20 g/l and 10 g/l concentration after 4 hours;.

NeemAzal concentration of 0.3% and 0.5% after 3 and 4 hours of exposure; garlic concentration of 500 g/l and 25 g/l after 3 and 4 hours (Fig. 1). The multifactorial statistical analysis has showed that both time period and treatment have significantly affected the repellent response of *T. urticae* females (p < 0.0001).

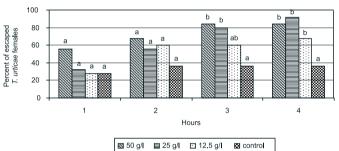
#### Measuring the extracts effect on T. urticae feeding behaviour

Generally, the first probing of treated bean leaves by experimental extracts took place significantly later than on untreated leaves. The first initial probing on untreated bean leaves was observed after 4–16 s. and permanent feeding after 2–3.5 minutes, with only 3–4 preliminary probing (Table 1; Fig. 2). Insignificantly larger numbers of probing by *T. urticae* females took place in following treatments: garlic extract at 12.5 g/l concentration (4–8 probes); mustard at 0.3 g/l; nettle at 10 g/l and 20 g/l con

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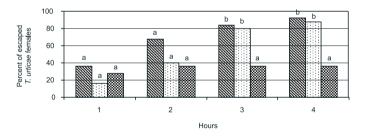






Allium sativum

NeemAzal



₿ 0.50%

5 ⊡ 0.30% ⊠ α Sinapsis alba

control

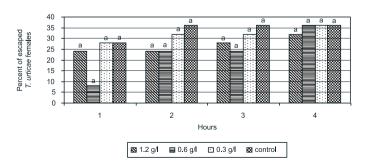


Fig. 1. Repelent action of tested water plant extracts as expressed by the percentage of escaping *T. urticae* females from the treated bean leaf surface into the vaseline sticky ring (barrier) after 1, 2, 3 and 4 hours



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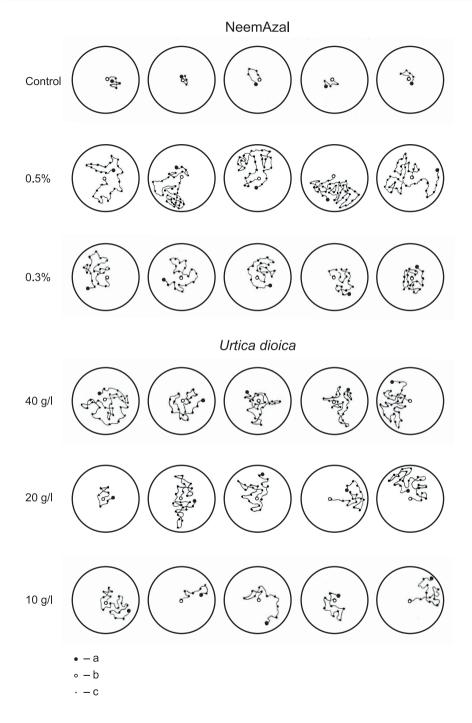


Fig 2. Behaviour of *T. urticae* females on bean leaf disks treated with various plant water extracts: a – the location of initial release of females into a leaf disk; b – the location of initial piercing and c – the location of permenent feeding



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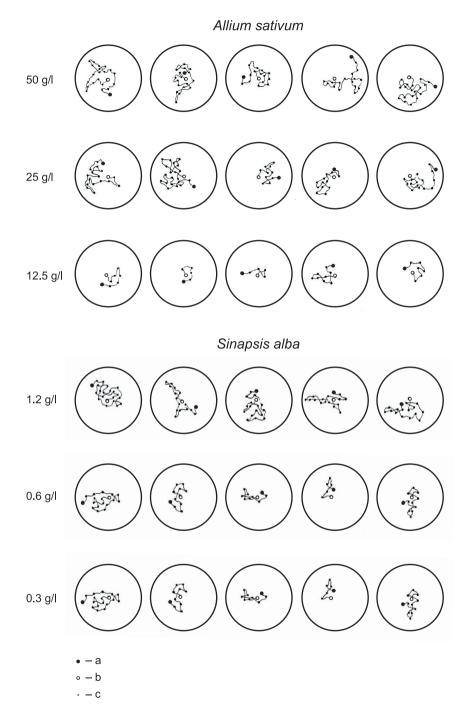


Fig 2. Behaviour of *T. urticae* females on bean leaf disks treated with various plant water extracts: a – the location of initial release of females into a leaf disk; b – the location of initial piercing and c – the location of permenent feeding centration (Fig. 2). On bean leaves covered by NeemAzal in 0.5% concentration, the first probing was observed after 2.5–4 minutes and after 1.5–2.5 minutes in the garlic at 50 g/l concentration and mustard at 1.2 g/l. The longest trail of wandering mites before initiation of probing was noted for the 0.5% NeemAzal; the 40 g/l and 20 g/l nettle extract and the 50 g/l and 25 g/l garlic treatments (Fig. 2).

Insignificant differences in the period between release of *T. urticae* females and initiation of permanent feeding on untreated and treated leaves were observed only for the garlic extract at 12.5 g/l treatment, with only 4–8 probings (Table 1). In the following combinations this period varied between 14 and more than 20 minutes: NeemAzal at 0.5%; garlic at 50 g/l and nettle at 40 g/l, indicating their strong antifeed-ant action to *T. urticae* (Table 1).

Treatment	Time period to:		No. of	
	first preliminary piercing	permanent feeding	piercings (probes)	Comments
Untreated control	4–6s	2–3.5 min	3–4	
NeemAzal 0.5%	2.5–4 min	15–20 min	25–35	after 7–15 min restless movements – espesially legs, clining chelicerae
NeemAzal 0.3%	55 s–1 min 40 s	11–18 min	15–19	as above
Allium sativum 50g/l	1.5 –2 min	16–18 min	15–20	cleaning chelicerae after 8 min
Allium sativum 25 g/l	30 s–2 min	9–13 min	10-20	
Allium sativum 12.5 g/l	8–30 s	2–7 min	4-8	
Sinapsis alba 1.2 g/l	1.5–2.5 min	7–12 min	15–19	
Sinapsis alba 0.6 g/l	30 s–3 min	6–13 min	15–19	
Sinapsis alba 0.3 g/l	30 s–1.5 min	10–16 min	5–16	
Urtica dioica 40g/l	30 s–1.5 min	14–28 min	16–28	after 6 min restless movements, clining chelicerae
Urtica dioica 20 g/l	15–55 s	9–14 min	6–21	after 4 min clining chelicerae
Urtica dioica 10 g/l	20–30 s	7–9 min	6–15	as above

 Table 1.
 Comparison of feeding behaviour of the *T. urticae* females on treated bean leaves with various extracts

# Measuring the toxic effect on *T. urticae* female survival

The high and significant mortality of *T. urticae* females were observed on leaves treated by two extracts made of NeemAzal and common stinging nettle in comparison to other extracts and untreated control (Fig. 3). The NeemAzal formulation even at the 0.3% concentration caused 50% mortality and 65% of the mite mortality at the

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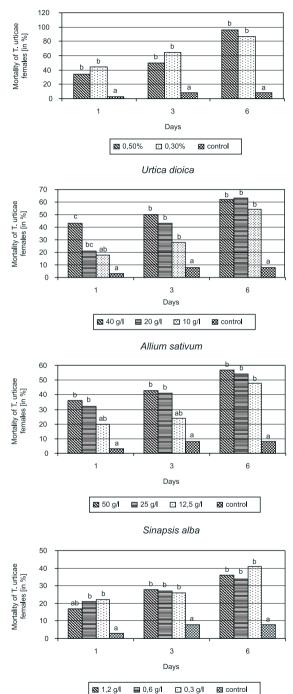


Fig. 3. Toxicity of various plant water extracts as estimated by the mortality of *T. urticae* females (in %) inside the Munger rearing cages after 1, 3 and 5 days

NeemAzal

0.5% concentration after 3 days of experiment. After 6 days, mortality increased to 87% and 96%, respectively for the two concentrations. Similar level of mortality in the case of *T. urticae* was caused by 40 g/l nettle extract after 1 and 3 days. The mortality has increased to 62–63% on leaves treated respectively by 20 g/l and 40 g/l nettle concentration (Fig. 3). Insignificant differences between various concentrations of garlic and white mustard extracts were observed after 3 and 6 days after treatments. The garlic extracts caused only 48–57% mite mortality and only between 34–41% by mustard extracts.

# DISCUSSION

The presented above experimental data confirmed that plant extracts from various species evoke various negative responses from two-spotted spider mite females affecting their feeding behaviour and survival. One of the first reports on the effect of neem extract on two-spotted spider mites behaviour and biology was published by Schauer and Schmutterer already in 1980 (after Schmutter 1990). The female fecundity and survival of both adults and juvenile stages was significantly reduced and a repellent effect was observed. The strong antifeedant and repellent effect of neem product was also obvious in our tests confirming that arthropod species of sucking mouthparts are more sensitive than insects with chewing type of mouthparts to neem chemical compounds (Saxena 1987). This may also be true for acarids with chewing mouthparts. Czajkowska (2002) found that neem extracts, commonly applied as antifeedants and deterrents to many orders and species of insects were found to be less effective in the stored product mite control than extracts from *Artemisia dracunculus* L. and *Piper retrofractum* (Vahl). Her studies included two species: *Tyrophagus putrescentiae* Schrank and *Rhizoglyphus echinopus* (F&R.).

Because intensive use of synthetic insecticides have caused outbreaks of spider mites on many crops and development of the mite resistance to acaricides (especially on treated ornamental crops), studies on the effect of extracts of various plant species on spider mites (*Tetranychidae*) have been intensified in 1980-ties and continue until the present. Deterrent and toxic effect of seed extracts from *Abrus precatorius* was confirmed by Amer et al. (1989). Essential oil extracted from *Tanacetum vulgare* and *Artemisia absinthium* strongly increased mortality of *Tetranychus urticae* (Chiasson et al. 2001). Extracts from: *Matricaria recutita, Achillea millefolium, Taraxacum officinale, Salvia officinalis* have strongly reduced the *T. urticae* fecundity, longevity and intensity of feeding (Tomczyk and Szymańska 1995; Kawka and Tomczyk 2002). Water extracts from needle surface of *Taxus baccata* stronly affected fecundity and oviposition period of two-spotted spider mite females (Furmanowa et al. 2001).

All presented above data indicate that extracts of various plant species, not only of tropical origin, affect various phenomena of spider mite behaviour and survival. Identification of chemical compounds responsible for repellent, antifeedant and toxic effects on spider mites and other arthropod groups may provide additional tools in pro-ecological method of integrated pest control.

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## POLISH SUMMARY

# CHARAKTERYSTYKA REAKCJI PRZĘDZIORKA CHMIELOWCA (*TETRANYCHUS URTICAE* KOCH) W STOSUNKU DO WODNYCH EXTRAKTÓW WYBRANYCH GATUNKÓW ROŚLIN

Badano wpływ wyciągów wodnych wybranych gatunków roślin, zalecanych w ochronie roślin przed szkodliwymi gatunkami owadów, na przędziorka chmielowca w celu scharakteryzowania ich sposobu działania. Wykorzystano różne metody doświadczalne w celu określenia: (a) zasiedlania traktowanych liści fasoli przez przędziorki; (b) zachowania związanego ze wstępnym żerowaniem (próbami); (c) okresu rozpoczęcia permanentnego żerowania i (d) śmiertelności testowanych roztoczy (efekt toksyczny). Najsilniejsze toksyczne działanie stwierdzono dla roztworów komercyjnego preparatu NeemAzal® – T/S firmy Trifolio-M GmbH (ekstrakt z nasion miotli indyjskiej, *Azadirachta indica*) a działanie repelentne wodnych ekstraktów czosnku, *Allium sativum* i pokrzywy, *Urtica dioica*. NeemAzal T/S również wykazał silne działanie jako czynnik zapobiegający żerowaniu ("feeding suppressant").