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Side effects of Neemark (*Azadirachta indica* A. Juss) and two new vegetable oils formulations on *Tetranychus urticae* Koch and its predator *Phytoseiulus persimilis* Athias-Henriot

Abstract - The effects of Neemark, commercial preparation obtained by neem (*Azadirachta indica*) seed kernels extract, on *Tetranychus urticae* Koch and *Phytoseiulus persimilis* Athias-Henriot were tested on bean leaf discs treated with different concentrations in laboratory experiments. Neemark can be considered moderately toxic for *T. urticae* but highly toxic for young stages and adults of *Ph. persimilis* at 3% and 5% concentrations. Also were investigated two new vegetable oils formulations 7094 and 1294 D/S on *T. urticae* in concentrations 0.5%, 1% and 2% caused high toxicity and promise for the near future an alternative solution in the control of tetranychids in greenhouse culture and field crops.

Riassunto - *Effetti collaterali del Neemark* (Azadirachta indica *A.Juss*) *e di due nuovi oli vegetali su* Tetranychus urticae *Koch e* Phytoseiulus persimilis *Athias-Henriot.*

É stata studiata l' efficacia del prodotto commerciale Neemark, estratto da semi di neem (*Azadirachta indica*) su *T. urticae* e su *Ph. persimilis* in laboratorio. Neemark puó essere considerato non tossico nei riguardi di *T. urticae* in quanto sono stati osservati solo effetti leggeri, mentre risulta molto tossico per *Ph. persimilis* (stadi giovanili e adulti) alle concentrazioni del 3% e 5%. Sono stati anche saggiati sempre in laboratorio due nuovi preparati di oli vegetali 7094 e 1294 D/S su *T. urticae* (stadi giovanili e adulti). Questi oli risultano molto tossici per il fitofago a tutte le concentrazioni saggiate (0,5%, 1%, 2%) e promettono una soluzione alternativa nel controllo dei Tetranichidi in serre e in pieno campo.

Key words: Neemark, vegetable oils formulations, *Tetranychus urticae, Phytoseiulus persimilis.*

INTRODUCTION

The use of phytosanitary compounds has been and continues to be the most fundamental means of protection for all crops, as it guarantees a good production in agriculture and improves the quality of the products.

The use of a.i. with a smaller impact on production, environment and consumer, is a fundamental condition for the control of pests (mites and insects).

Extracts from fruit, seed, leaves and bark of the exotic neem trees *Azadirachta indica* A. Juss, *A. integrifolia* and *Melia azaderach* L. of the Meliaceae family, are known by several authors (Butterworth & Morgan, 1971; Ruscoe, 1972; Ladd *et al.*, 1978; Kraus *et al.*, 1981) to contain substances with repellent, antifeedant and especially toxic effect on insects, mites and nematodes. In modern plant protection these formulated neem extracts as insecticides, acaricides and nematocides are used either in itself or in combination with chemicals compounds (Gill, 1972; Ketkar, 1976; Jacobson *et al.*, 1978; Warthen, 1979). As far as mites are concerned, so far a rather limited number of researchers have worked on the effect of neem on phytophagous mites *Tetranychus urticae* Koch, *Panonychus citri* (McGregor) and *Tetranychus cinnabarinus* (Bois.) (Schauer & Schmutterer, 1981; Mansour & Ascher, 1983; Dimetry *et al.*, 1993) and predacious mites *Typhlodromus fallacis* (Garmain), *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius gossipi* El-Badzy (Hamstead, 1970; Mansour *et al.*, 1987; Sopp *et al.*, 1990; Dimetry & Amer, 1992).

Apart from a commercial neem formulation, the authors have also applied preparations of certain mixtures of mono-, di- and tri-glycerides, free fatty acids and oily alcohols derived from vegetable oils, fish oils, animal fat and wax. The latter are formulations of the Greek industry of biological products "Bioryl" and are of pioneer use in the control of *T. urticae*, a serious pest in greenhouse culture and field crops.

Still very little is known about the effect of such compounds on phytophagous mites and Phytoseiidae predators in Greece. Aim of this study is therefore the evaluation of the toxic effect of the commercial formulation Neemark® (*Azadirachta indica*), Hellfarm product®, and the Bioryl® vegetable oils (codes 1294 D/S and 7094, which are waiting for registration) on *T. urticae* and its predator *Ph. persimilis.*

MATERIALS AND METHODS

T. urticae collected from cotton in the Kopais area, 100 km north of Athens, was reared on bean (*Phaseolus vulgaris* L.) in a greenhouse under controlled conditions, especially concerning temperature, at 28 ± 2°C. Populations of this mite were also

Table 1 - The formulations used.

Commercial name	Active ingredients
Neemark	*Azadirachta indica* – neem oil 80%
7094*	Mixture of vegetable oils 78% + mono- and di-glycerides of oleic acid 5% + glycerol 2% + volatile oils and emulsifiers 15%
1294 D/S*	Mixture of vegetable oils 78% + mono- and di-glycerides of oleic acid 5% + glycerol 2% + volatile oils** and emulsifiers 15%

* Waiting for registration

** The composition of volatile oils in this case is different

used to rear the predator *Ph. persimilis* in the insectary at 25 ± 2°C, 75 ± 5% R. H. and 16:8 hours photoperiod.

The formulations used are in Table 1.

The experiments were carried out using the Potter-tower for spraying; in each experimental unit a 2 ml solution was applied under 0.86 atm. Lids of plastic Petri dishes, diameter 9 cm, were used as experimental units. One of the plastic Petri lids was used as bottom and the other as cover over the materials put in it. In the center of this cover a 3 cm diameter opening was made to serve as an experimental unit (Fig. 1). Uniform pieces of filter paper were layered in the plastic dish and sprayed with distilled water. On top of this layer of filter paper a healthy bean leaf was put to serve as plant substrate for the mites. The leaf was covered with a thin piece of sponge of Wettex type with a hole in the middle corresponding to that in the cover. On one side the sponge stuck out over the leaf, reaching into the bottom of the dish in which was water. The opening was covered with a plexiglas type plastic cover carrying a 60μ mesh wire-netting at the upper side. The plastic cover was firmly attached to the bottom with elastic bands (Fig. 2). Then the technique of Papaioannou-Souliotis (1980) was applied; relevant modifications have been made to limit the losses that occurred in our laboratory when using other techniques (Tsolakis & Ragusa, 1993; Dimetry *et al.*, 1994). Ten individuals were used for each experimental unit to be drenched to assess the effects of the various formulations on the mobile forms (pronymphs, protonymphs, deuteronymphs and adults) of *T. urticae* and *Ph. persimilis*. When pronymphs were to be tested 10 eggs were applied previously and for testing of adults 8 females and

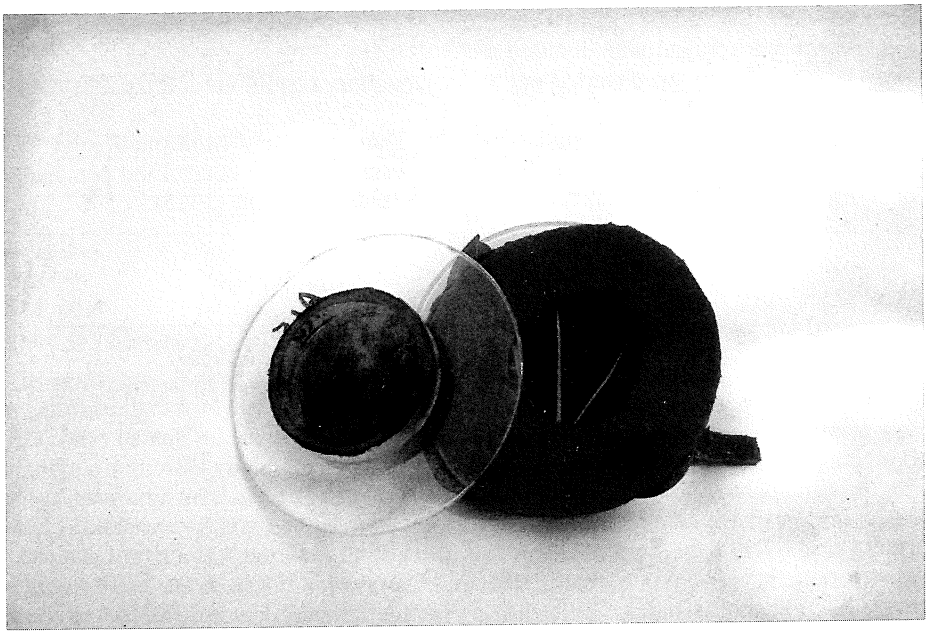

Fig. 1 - Plastic bottom and cover with hole of 3 cm diam.

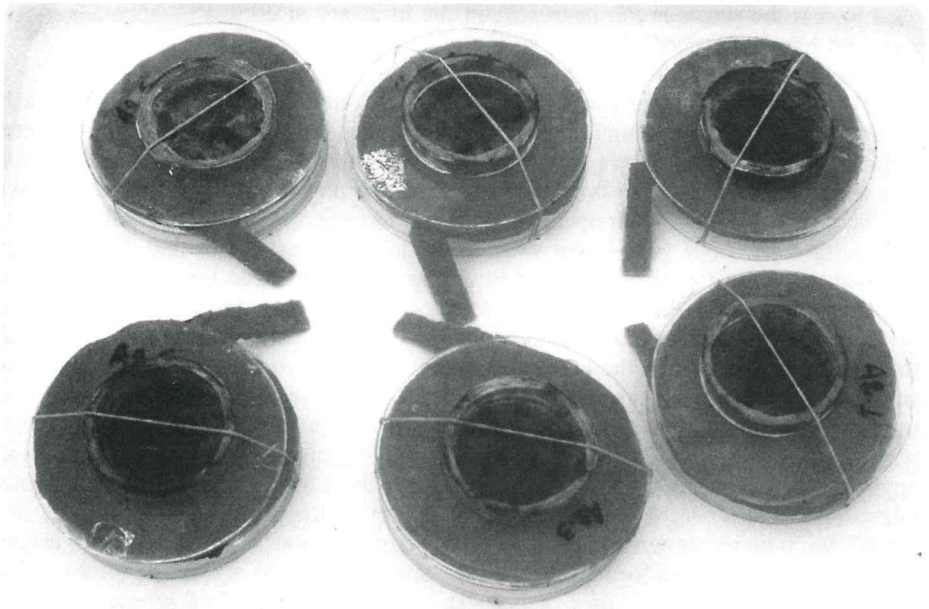

Fig. 2 - Petri dishes with 6 bean leaves used as experimental units.

2 males were used. Each formulation and dose was applied in five repetitions, while the control was sprayed with distilled water.

The experiments were carried out under laboratory conditions at 25 ± 2°C temperature and 75 ± 5% R.H.

The data were analyzed statistically by Duncan's New Multiple Range Test (Duncan, 1955). For all means statistically differing from the control the percentage of mortality was calculated according to the formula of Abbott (1925).

RESULTS AND DISCUSSION

Neemark

a) Effect on *T. urticae*: Evaluation of the data showed the formulation to be of low to moderate toxic effect on *T. urticae* according to the scale of Hassan (1985). At both concentrations, 3% and 5%, the formulation was moderately toxic to pronymphs and protonymphs (Table 2), especially 72 hours after treatment. No significant difference in percentage of mortality was observed between the two concentrations (59.6-70.6% and 69.3-76.3% respectively). At 5% concentration the formulation showed a moderate toxicity to deuteronymphs and adults 24 hours after treatment. At 3% concentration, on the other hand, it was harmless. The mortality of deuteronymphs and adults reached 68.7% and 77.4% respectively at 72 hours after treatment and the results does

Table 2 - Average of the surviving pronymphs, protonymphs, deuteronymphs and adults of Tetranychus urticae *(Means ± S.D.) and % mortality (%M) at 24, 48 and 72 hours after treatment with Neemark in two concentrations.*

Pronymphs of *Tetranychus urticae*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	13 (±2.9) b	45.4	13 (±2.9) b	45.4	9.6 (±2.9) a	59.6
Neemark 5%	8 (±3.1) a	66.4	7.4 (±3.8) a	68.9	7 (±3.1) a	70.6
Control	23.8 (±1.6) c		23.8 (±1.6) c		23.8 (±1.6) b	

Protonymphs of *Tetranychus urticae*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	13.6 (±5.6) a	40.3	12 (±6.2) a	47.4	5.4 (±1.9) a	76.3
Neemark 5%	12 (±6.1) a	47.4	9.8 (±5.4) a	57	7 (±2.9) a	69.3
Control	22.8 (±0.4) b		22.8 (±0.4) b		22.8 (±0.4) b	

Deuteronymphs of *Tetranychus urticae*

Treatments	24h	48h	72h			
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	17.8 (±2.1) b	22.6	17.8 (±2.1) b	22.6	16.6 (±2.3) b	27.8
Neemark 5%	10 (±3.8) a	56.5	9.8 (±3.7) a	57.4	7.2 (±4.2) a	68.7
Control	23 (± 0) c		23 (±0) c		23 (±0) c	

Adults of *Tetranychus urticae*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	20.4 (±5.8) b		18.6 (±4.7) b		16.2 (±4.9) b	29.6
Neemark 5%	9.4 (±3.9) a	59.1	7 (±3.6) a	69.6	5.2 (±3) a	77.4
Control	23 (±2) b		23 (± 2) b		23 (±2) c	

not differ significantly between these two biological stages nor does it increase significantly in time.

b) Effect on *Ph. persimilis*: The application of Neemark in 3% and 5% concentrations proved to be rather harmful to all biological stages of *Ph. persimilis*, with no statistical differences (Table 3).

The highly toxic effect of Neemark on all mobile forms (pronymphs, protonymphs, deuteronymphs and adults) is shown in rather high mortality measured 72 hours after treatment: 81.7-91.3% in pronymphs, 89.1-94.5% in protonymphs, 98.3-99.1% in deuteronymphs and 77.4-92.2% in adults.

Table 3 - Average of the surviving pronymphs, protonymphs, deuteronymphs and adults of Phytoseiulus persimilis *(Means ± S.D.) and % mortality (%M) at 24, 48 and 72 hours after treatment with Neemark in two concentrations.*

Pronymphs of *Phytoseiulus persimilis*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	5.8 (±4.9) a	74.8	2.6 (±1.5) a	88.7	2 (±1.7) a	91.3
Neemark 5%	5 (±2.3) a	78.2	2.4 (±1.3) a	89.6	1.6 (±0.9) a	81.7
Control	23 (±0.7) b		23 (±0.7) b		23 (±0.7) b	

Protonymphs of *Phytoseiulus persimilis*

Treatments	24h 48h 72h					
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	3.6 (±3.6) a	83.8	2.6 (± 3) a	88.2	2.4 (±3.2) a	89.1
Neemark 5%	1.8 (±0.8) a	91.9	1.4 (±0.5) a	93.6	1.2 (±0.8) a	94.5
Control	22.2 (±0.8) b		22 (±0.7) b		22 (±0.7) b	

Deuteronymphs of *Phytoseiulus persimilis*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	0.8 (±0.8) a	96.5	0.6 (±0.5) a	95.6	0.4 (±0.5) a	98.3
Neemark 5%	1.2 (±1.1) a	94.8	0.4 (±0.5) a	98.3	0.2 (±0.4) a	99.1
Control	23 (± 0.7) b		23 (±0.7) b		23 (±0.7) b	

Adults of *Phytoseiulus persimilis*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
Neemark 3%	2.4 (±1.1) a	89.6	1.8 (±1.1) a	92.2	1.8 (±1.1) a	92.2
Neemark 5%	2.4 (±2.2) a	89.6	2.2 (±2.4) a	90.4	1 (±1) a	77.4
Control	23 (±0.7) b		23 (± 0.7) b		23 (±0.7) b	

The toxic effect of Neemark seems in the case of *T. urticae* to be related to the concentration in which it is applied, while its acaricide activity classifies as low to moderate. Similar data have been obtained also by Mansour *et al.* (1983, 1987, 1993, 1997) and Dimetry *et al.* (1993, 1994), who using in laboratory conditions derivatives of neem oil in pure form as well as in commercial compounds, showed a difference in toxic effect on various species, but also between different concentrations of the same preparation. Thus, in contrast to RD9-Repelin and Neemgard, the preparations Margosan-O, Azactin and Neemix showed no toxic effect on *T. cinnabarinus*, while Margosan-O and Neem Azal-S were rather toxic to females of *T. urticae.*

Comparable observations have been also made on *Ph. persimilis*, *Typhlodromus athiasae* Porath & Swirski, *Neoseiulus barkeri* Hughes and *Typhlodromus richteri* Karg (Mansour *et al.*, 1983, 1987, 1993; Dimetry *et al.*, 1994). Neemark proved to be very toxic, in all concentrations tested, to *Ph. persimilis* and so RD9-Repelin to *T. athiasae*, but pure extracts of neem seed kernel were less toxic to *T. athiasae* than to *T. cinnabarinus*. Neem Azal-S and Margosan-O, tested on *N. barkeri*, caused a decrease in oviposition and voracity. Margosan-O was classified as harmless to *T. richteri*, while Neem Azal-S was harmful.

From the above can be concluded, that the different effects of neem seed extracts (extracts or commercial compounds) on phytophagous mites and on their predators should be seriously considered, in particular when these neems products are meant to be used in programs of biological or integrated control of mites, either in greenhouses or in the field.

Vegetable oils 7094 and 1294 D/S

Effect on *T. urticae:* In the two higher concentrations applied, both oils proved very harmful to protonymphs and deuteronymphs (tested together) of *T. urticae* 48

Table 4 - Average of the surviving adults and juveniles stages of Tetranychus urticae *(Means ± S.D.) and % mortality (%M) at 24, 48 and 72 hours after the treatment with the two vegetable oils.*

Adults of *Tetranychus urticae*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
0,5% 7094	9 (± 0.8) e	45.1	3.6 (± 1.1) d	76	2 (± 1.6) b	86.6
1% 7094	4.2 (± 1.3) c	74.4	1 (± 1) b	93.3	0.4 (± 0.5) a	97.3
2% 7094	0 (± 0) a	100	0 (± 0) a	100	0 (± 0) a	100
0,5%1294 D/S	6 (± 0.8) d	63.4	2.2 (± 1.5) c	85.3	2.6 (± 1.1) b	82.6
1% 1294 D/S	2.8 (± 0.8) b	84.4	0.8 (± 0.8) b	94.6	0.6 (± 0.5) a	96
2% 1294 D/S	0 (± 0) a	100	0 (± 0) a	100	0 (± 0) a	100
Control	16.4 (± 1.2) f		15 (± 1) e		15 (± 1) c	

Juveniles stages of *Tetranychus urticae*

Treatments	24h		48h		72h	
	Means (±S.D.)	%M	Means (±S.D.)	%M	Means (±S.D.)	%M
0,5% 7094	7.8 (± 0.8) c	56.6	6.8 (± 1.3) b	57.5	4.4 (± 1.1) b	72.5
1% 7094	0 (± 0) a	100	0 (± 0) a	100	0 (± 0) a	100
2% 7094	0 (± 0) a	100	0 (± 0) a	100	0 (± 0) a	100
0,5%1294 D/S	9.8 (± 0.8) d	45.5	7.2 (± 0.8) b	55	5.6 (± 1.1) c	65
1% 1294 D/S	1.8 (± 0.8) b	90	0 (± 0) a	100	0 (± 0) a	100
2% 1294 D/S	0 (± 0) a	100	0 (± 0) a	100	0 (± 0) a	100
Control	18 (± 1.2) e		16 (± 1) c		16 (± 1) d	

hours after treatment and only the lower concentration (0.5%) showed slight toxicity (24 hours after treatment) and moderate (72 hours).

The data concerning laboratory testing of the two Bioryl® oils 7094 and 1294 D/S revealed that out of the three concentrations applied, the higher (2%) and intermediate (1%) proved to be very toxic to *T. urticae*, with no statistically significant difference between them (Table 4). At 72 hours after treatment the mortality of *T. urticae* adults was 97.3% - 100%. It should be also worth mentioned, that the effect of the lower concentration (0.5%) can be classified as harmful, according to the scale of Hassan (1985).

It can be concluded that Neemark (neem seed extract) showed to be moderately toxic to *T. urticae* and very toxic to *Ph. persimilis*. Because the various industrial neem formulations that are marketed, with azadirachtin, have a different toxic effect on phytophagous and predator mites, the degree of toxicity or non-toxicity of each separate formulation has to be known before planning its application in the field or greenhouses.

7094 and 1294 D/S, derived from a mixture of vegetable oils, showed a high toxic effect on *T. urticae* and promise for the near future an alternative solution in the control of spider mite of greenhouse crops.

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