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Effects of some botanical pesticides on *Tetranychus urticae* Koch (Acariformes, Tetranychidae) and its predator *Cydnodromus californicus* (McGregor) (Parasitiformes, Phytoseiidae) in laboratory trials

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The first three Authors have equally worked on the bioessays; the other two Authors have developed the chemical part of the work.

Abstract

Extracts of *Annona cherimola* Mill., *Melia azedarach* L., *Artemisia absinthium* L. and *Quassia* spp., were tested in laboratory trials on *Tetranychus urticae* Koch (Acariformes, Tetranychidae) and *Cydnodromus californicus* (McGregor) (Parasitiformes, Phytoseiidae). Water and acetone were the solvents used for the extraction. Results obtained showed that water and acetonic extracts of *Annona*, and acetonic extracts of *Melia* had a negative influence on both prey and predator. Acetonic extracts of *Quassia*, water extracts of *Melia,* and both extracts of *Artemisia* showed a toxic action on *T. urticae,* being harmless on *C. californicus.*

Some extracts also showed a repellent action on *T. urticae* (acetonic extracts of *Melia* and *Artemisia*) and on *C. californicus* (acetonic extracts of *Quassia* and water ones of *Melia*).

Introduction

There are no doubts that the continuous use of chemical products in agriculture in the last half century caused negative effects on human health, the appearance of a great number of resistant strains, a negative influence on useful arthropods, as well as a stimulating action towards some pests (Spear *et al.* 1975; Murphy 1986). Alternative pest control methods as integrated control or agricultural process method as organic farming is spreading more and more especially in the USA and in Europe. In Italy, for example, the diffusion of the latter has passed from 91.500 Ha in 1993 (MIRAAF, 1998) to 958.687 Ha in 1999 (FAO 1999). When necessary, pest control is carried out using botanical extracts that are quite often prepared in farms, using as solvent only water. The effectiveness of homemade products is unclear, as it is not possible to define their concentration, and water is often not the best solvent to extract biocide substances (Merz, 1987).

Recent research has been addressed towards finding substances that are effective against pests, selective towards vertebrates and useful arthropodofauna, with rapid environmental degradation. The world literature reports about 2000 botanical species with negative effects towards pests (Bezzi and Caden 1991). However only few have been studied for insect or mite control: extracts of seeds of *Azadirachta indica* A. Juss (Schmutterer and Ascher 1984, 1987; Stephens and Schmutterer 1982; Barnby *et al.* 1989; Stark *et al.* 1990; Mordue

and Blackwell 1993; van Randeng and Roitberg 1998). Little is known of other botanical pesticides (Jacobson *et al.* 1978; Klocke and Kubo 1982; Mansour *et al.* 1986; Merz 1987; Ewete *et al.* 2000).

Some of the botanical extracts control a wide range of phytophagous insects and mites. Their wide spectrum of activity, however, is mainly due to the mixture of different substances contained in the extracts more than to the activity of the dominant one (Tsolakis *et al.* 2002). The solvents used during the extraction play an important role too. Mansour and Ascher (1983, 1995) showed that extracts obtained from neem using lipophilous solvents were more active towards tetranychids than those obtained using hydrophilous solvents.

This paper report results from a cooperative project of Italian Institutions, to verify if some indigenous or exotic plants, could give, via extraction, materials, with effects on two spotted spider mite, *Tetranychus urticae* Koch, or on predaceous phytoseiid mites, e.g. *Cydnodromus californicus* (McGregor), that is often associated with *T. urticae* on herbaceous crops.

Materials and Methods

Plant extractions

Test plants for botanical extracts were *Annona cherimola* Mill., *Melia azedarach* L., *Artemisia absinthium* L. and *Quassia* spp. Seeds of *Annona* Cv Fino de Jete were collected in a commercial field at Sciacca (Agrigento) in November 2001. Fruit of *Melia* were collected in April 2002 in Palermo and kept in a warm and dry room to allow the pulp desiccate. Seeds of the above plants were milled to obtain a micron level powder. *Artemisia* and *Quassia* were purchased as micronised powder. Extracts were obtained by shaking 40 g micronised powder with 200 ml solvent (acetone or water) at room temperature for 48 hours; the filtered solutions were then dried in a Rotovapor (Buchi) under vacuum. Residues were weighed to determine the amount of dry extract and were analyzed in HPLC in order to check the presence of traces of the solvent. The extracts were dissolved in the minimum amount of solvent in order to obtain the maximum concentration (Table 1).

Analyses of the extracts were carried out with a Shimadzu liquid chromatography coupled with a DAD (Diode Array Detector) in gradient mode with a RPC18 column with a methanol/water (HPLC grade) mobile phase. This allowed us to estimate the number of compounds in each extract.

The identification of compounds is being carried out with a mass spectometer detector coupled with a HPLC instrument.

For successive trials, extracts were dissolved in water and 0.1 % w/w of Marseilles soap was added to acetonic extracts to facilitate solubility.

Test procedures for *T. urticae* and *C. californicus*

T. urticae was collected on weeds in a field near Palermo (Sicily, Italy) and kept in a laboratory on bean leaves at 24±1°C, 70±5 % R.H. and a 16:8 LD photoperiod .

C. californicus was collected on weeds in Chile and reared in plastic cages according to Swirski *et al.* (1967), on a mixture of pollen of *Carpobrotus edulis* L. and *Oxalis corniculata* L. as food.

Each experimental unit (EU) consisted of 3cm disk (diameter) from a citrus leaf placed upside, on wet cotton in a Petri dish. Each test was replicated five times and all tests were carried out at 24±1 °C, 70±5 % of R.H. and with a photoperiod of 16:8 LD.

Newly laid eggs (max 24 hours old) of *T. urticae* were obtained from 5 females placed on each EU for

Table 1. Dry extracts (in grams) obtained from the plants and concentration of solutions used in the tests.

Solvent		*Quassia*	*Melia*	*Artemisia*	*Annona*
Water	Weight	0.87 g	17.5 g	3.09 g	4.13 g
	Concentration (ppm)	3,500	70,000	10,300	30,600
Acetone	Weight	0.15 g	1.80 g	1.00 g	11.60 g
	Concentration (ppm)	1,192	15,117	6,620	60,830

24 hours for oviposition, removed afterwards and 10 eggs were left on each leaf.

For *C. californicus*, 5 eggs (max 24 h old) were put in each EU with pollen granules as food. Eggs were sprayed with 8 ml of solution per leaf disk using the Potter tower. Eggs or larvae observated daily until adult. Only distilled water was used in the control tests as no traces of the solvent were found in the dry extracts.

Mortality of eggs was calculated from the initial number of eggs; the mortality of the other stages was calculated from the number of individuals reaching each stage. The total negative effect was calculated on the number of individuals that did not reach adulthood (sum of dead and disappeared individuals).

Results were analyzed by Student *t* test and Analysis of Variance (ANOVA) followed by Tukey's test when the ANOVA was significant ($P < 0.05$) using "Statistica" computer program (StatSoft Inc., 1995).

RESULTS AND DISCUSSION

Chromatograms of samples extracted in water were similar to samples extracted in acetone suggesting the presence of similar classes of compounds extracted from each solvent The large number of peaks in the chromatograms of acetone extracts could be ascribed to essential oils or esters of fatty acids, being more soluble in acetone than in water.

Neither type of *Quassia* extract had a negative influence on the eggs of *T. urticae* (Fig. 1). Water extracts had also no negative influence on larvae while the acetonic extracts caused a very high mortality of them (86.5%). Water extracts of *Melia* were more toxic towards the larvae (52.3%) than towards the eggs (34%), while the acetone extracts caused a same mortality to both stages (66%). No significant differences were found between the results obtained with the two extracts (Fig. 1). A slight ovicidal effect was shown by both extracts of *Artemisia* (28% and 32% for water and acetonic extracts respectively) but not significantly different from the control. Acetone extracts showed mortality of larvae of *T. urticae* (59%). Water extracts of *Annona* showed significantly different mortality to the eggs (44%) and high mortality to the larvae (80.1%). Acetone extracts were very toxic to both stages (98% and 100% of mortality for eggs and larvae respectively). Eggs adversely affected usually dehydratated, often showing the embryo had already formed.

With *C. californicus*, both extracts of *Annona* were toxic to eggs and larvae while extracts of all the other compounds were harmless (Fig. 2). No significant differences were found between the results obtained with the two extracts of *Annona* for the same post embryonic stage even if water extracts showed a greater larvicidal effect (Fig. 2). Mortality caused by the different extracts to protonymphs and deutonymphs was very low for *T. urticae*. The acetone extracts of *Melia* was very toxic for the protonymphs of *C. californicus* (table 2 and 3). It should be said that seeds of *Melia* contain many terpenoids and limonoids which have been reported to be insecticide (Bohenenstengelet *et al*. 1999).

On some extracts, young stages did not remain on the leaf disk, suggesting those extracts were repellent. For the controls, no mites (phytophagous and predators) escaped from the leaf disk or remained on its margins, while 12% and 20% respectively of youngs of *T. urticae* disappeared on those leaf disks treated with acetone extracts of *Melia* and *Artemisia* (table 2). The same happened with *C. californicus* when acetone extracts of *Quassia* (48%) and water extracts of *Melia* (24%) were used (table 3). Therefore, specific and appropriate tests have to be carried out to confirm the repellent effect.

Another explanation for larvae found dead on the margin of the leaf disk, is that they may have preferred to remain there, perhaps because the concentration of the extracts was less high in the part of the leaf which was very close to water.

Repellency is reported for *Artemisia* towards *Cydia pomonella* (L.) (Suomi *et al.* 1986) and for powder of *Annona* seeds towards *Callosobruchus maculatus* (Fabr.) for at least 100 days (Pandey and Varma, 1978). A lasting repellent action was also shown by Neem oil for *T. urticae* (Dimetry *et al.* 1993; Mansour *et al.* 1993; Tsolakis *et al.* 2002).

Water extracts of *Quassia* were harmless to *T. urticae*, but all other extracts showed a high negative effect (from 60 to 100%) (Fig. 3). All the acetone extracts, except for *Annona*, were more effective than water extracts. Only the two extracts of *Annona* showed a strong negative effect (100%) on *C. californicus* whereas *Artemisia* and water extracts of *Quassia* were harmless. Acetonic extracts of *Quassia* and the two extracts of *Melia* gave a negative effect varying from 44 to 76% (Fig. 3).

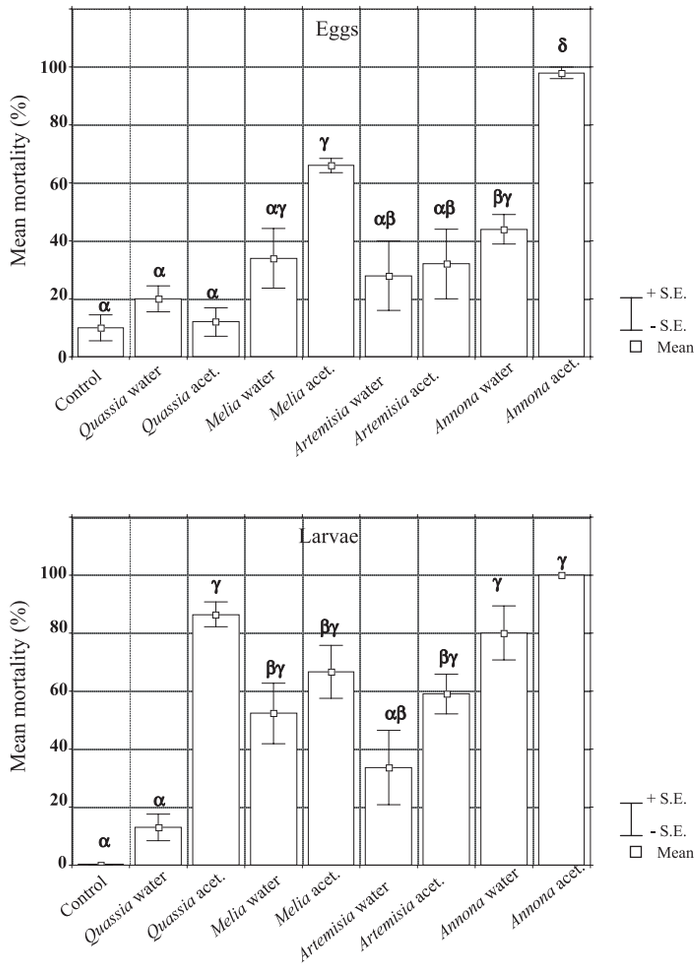

Figure 1. Toxicity of various extracts to the eggs and larvae of *T. urticae*. Different symbols denote significant differences ($P < 0.01$). Analysis of variance (ANOVA) was performed on the data. Means were separated using Tukey's studentized range (HSD) test.

Table 2. Effect of different plant extracts on post embryonic stages of *T. urticae.*

Tests	*Initial number of eggs*	*Dried eggs*	*Dead individuals*			*Mortality (%)*	*Escaped individuals*			*%*	*Maturing adults*		
			Larvae	*Proto*	*Deuto*	*Mean ± S.E.**	*Larvae*	*Proto*	*Deuto*	*Mean ± S.E.**	—	—	*%*
Quassia wat.	50	10	5	0	1	32 ± 3.61 ab	0	0	0	0 ± 0.00 a	19	15	68
Quassia acet.	50	6	38	2	2	96 ± 2.34 c	1	0	1	4 ± 2.34 a	0	0	0
Melia wat.	50	17	17	0	0	68 ± 4.39 d	0	0	0	0 ± 0.00a	7	9	32
Melia acet.	50	33	11	0	0	88 ± 2.89 c	6	0	0	12 ± 2.89ab	0	0	0
Artemisia wat.	50	14	12	4	0	60 ± 5.48 bd	0	0	0	0 ± 0.00a	8	12	40
Artemisia acet.	50	16	20	2	1	78 ± 3.61 c	9	1	0	20 ± 3.98b	1	0	2
Annona wat.	50	22	22	3	0	94 ± 2.34 c	2	0	0	4 ± 2.34a	1	0	2
Annona acet.	50	49	1	0	0	100 ± 0.00 c	0	0	0	0 ± 0.00a	0	0	0
Control	50	5	0	0	0	10 ± 3.16 a	0	0	0	0 ± 0.00a	20	25	90

* Different letters denote significant differences ($P<0.01$). Analysis of variance (ANOVA) was performed on the data. Means were separated using Tukey's studentized range (HSD) test.

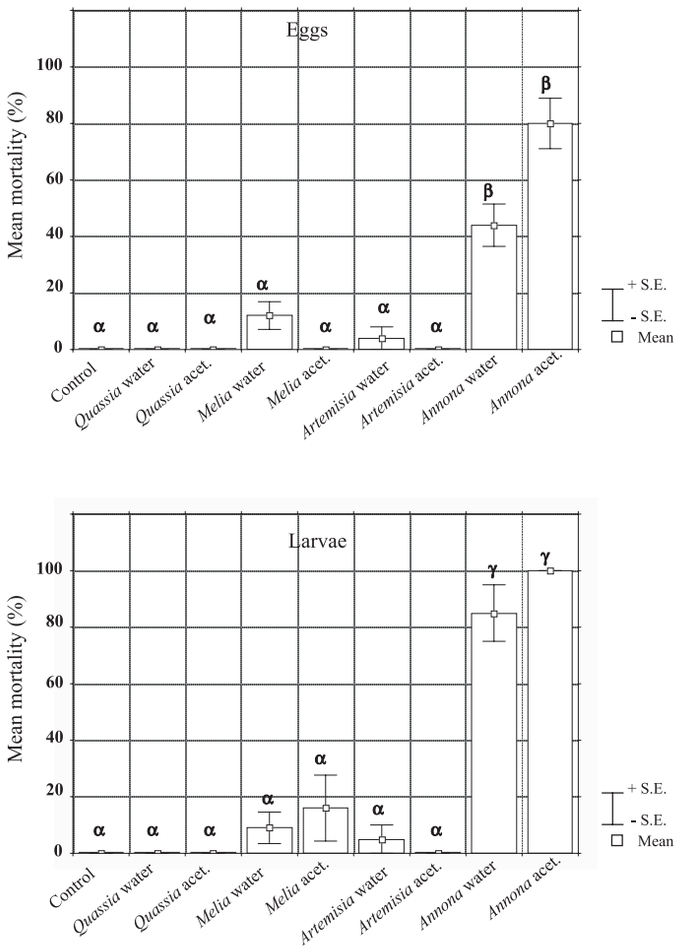

Figure 2. Toxicity of various extracts to the eggs and larvae of *C. californicus*. Different symbols denote significant differences (P<0.01). Analysis of variance (ANOVA) was performed on the data. Means were separated using Tukey's studentized range (HSD) test.

Table 3. Effect of different plant extracts on post embryonic stages of *C. californicus.*

Tests	*Initial number of eggs*	*Dried eggs*	*Dead individuals*			*Mortality (%)*	*Escaped individuals*			*%*	*Maturing adults*		
			Larvae	*Proto*	*Deuto*	*Mean ± S.E.**	*Larvae*	*Proto*	*Deuto*	*Mean ± S.E.**	__	__	*%*
Quassia wat.	25	0	0	1	2	12 ± 3.3 a	0	2	2	16 ± 4.1 a	10	8	72
Quassia acet.	25	0	0	3	1	16 ± 4.7 a	3	7	2	48 ± 3.3 bc	4	5	36
Melia wat.	25	3	2	0	0	20 ± 3.8 a	2	3	1	24 ± 5.1 ac	6	8	56
Melia acet.	25	0	4	15	0	76 ± 6.6 b	0	0	0	0 a	3	3	24
Artemisia wat.	25	1	1	0	0	8 ± 4.2 a	0	3	0	12 ± 4.2 a	8	12	80
Artemisia acet.	25	0	0	1	0	4 ± 3.0 a	1	0	0	4 ± 3.0 a	8	15	92
Annona wat.	25	11	12	0	0	92 ± 3.3 b	2	0	0	8 ± 3.3 a	0	0	0
Annona acet.	25	20	5	0	0	100 ± 0.0 b	0	0	0	0 a	0	0	0
Control	25	0	0	0	0	0 a	0	1	0	4 ± 3.3 a	16	8	96

* Different letters denote significant differences (P<0.01). Analysis of variance (ANOVA) was performed on the data. Means were separated using Tukey's studentized range (HSD) test.

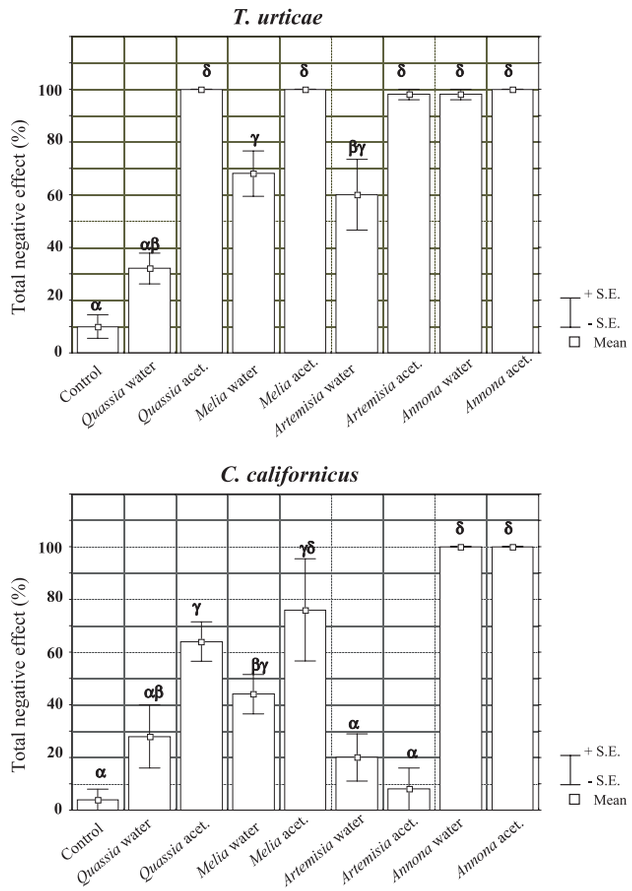

Figure 3. Total negative effect (mortality + repellence) of various extracts to post embryonic developmental stages of the two mite species. Different symbols denote significant differences (P<0.01). Analysis of variance (ANOVA) was performed on the data. Means were separated using Tukey's studentized range (HSD) test.

Considerations

It can be stated that:

- Water and acetone extracts of *Annona* and acetone extracts of *Melia* showed the same toxic effects to *T. urticae* and *C. californicus*.
- Acetone extracts of *Quassia*, water extracts of *Melia* and both extracts of *Artemisia*, caused a high toxic effect to *T. urticae*, but were harmless to *C. californicus*.
- Some extracts showed a repellent effect: acetone extracts of *Melia* and *Artemisia* towards *T. urticae*, and acetone extracts of *Quassia* and water extracts of *Melia* towards *C. californicus*.

These preliminary tests on harmful and useful mites ascertained that some of extracts had negative effects only on *T. urticae*, while others were active on both the prey and the predator. It is not known what components of the extracts were really active against mites, but further studies are underways to identify the different components, their effects, and to obtain products with a higher efficacy to the pest than its predator enemies.

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Mesalgoides anahoffmannae (Pérez & Ramírez, 1996), *Piona* sp.
Amblyomma imitator Kohls, 1958, *Leptus* sp., *Aceria* sp., and *Oppia* sp.

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