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Toxicity of some insecticides to *Tetranychus urticae*, *Neoseiulus californicus* and *Tydeus californicus*

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Abstract. Three mite species are frequently found on vegetable crops in Italy: the pest *Tetranychus urticae* Koch (Acari: Tetranychidae), the predator *Neoseiulus californicus* (McGregor) (Acari: Phytoseiidae) and the unspecialised feeder *Tydeus californicus* (Banks) (Acari: Tydeidae). In laboratory trials, the direct and residual effects of six insecticides recommended for the control of aphids, whiteflies and thrips in vegetable crops, (Biopiren® plus (pyrethrins), Confidor® (imidacloprid), Oikos® (azadirachtin), Plenum® (pymetrozine), Naturalis® (*Beauveria bassiana*) and Rotena® (rotenone)), were evaluated for the three mite species. All the products affected the mites and their effect was often favourable towards *T. urticae* and unfavourable towards *N. californicus* and *T. californicus*. Rotenone was more toxic to eggs than females of *T. urticae*. It was highly toxic to *N. californicus* and caused the death of all treated females of *T. californicus*. Pyrethrins and imidacloprid increased *T. urticae* fecundity, but decreased fecundity of *N. californicus*. Imidacloprid decreased *T. californicus* fecundity more than pyrethrins. *Beauveria bassiana* was not toxic to *T. urticae* and *T. californicus*, but induced high mortality in the progeny of treated females of *N. californicus*. Azadirachtin and pymetrozine were the least toxic to *T. urticae* and *N. californicus*, but decreased production of larvae in *T. californicus*. Implications for integrated pest management on vegetables are discussed.

Key words: integrated pest management, pesticides, Phytoseiidae, side effects, Tetranychidae, Tydeidae

Introduction

The tetranychid *Tetranychus urticae* Koch, the phytoseiid *Neoseiulus californicus* (McGregor) and the tydeid *Tydeus californicus* (Banks) are frequently found simultaneously on annual crops in Italy

(Castagnoli and Liguori, 1990; Castagnoli et al., 1998; Castagnoli and Liguori, unpublished data). *Tetranychus urticae* is one of the most serious pests in a number of agricultural systems; its outbreaks are often a consequence of repeated and non-selective pesticide applications, which enhance pesticide resistance in the tetranychid and decimate its natural enemies (Helle and Sabelis, 1985). *Neoseiulus californicus* is a widespread predator known for its efficiency in the control of phytophagous mites, and for its ability to feed on alternative foods (Castagnoli and Falchini, 1993; Castagnoli and Liguori, 1994; Castagnoli et al., 1999). *Tydeus californicus* is considered a scavenger mite (Gerson, 1968) and it can also thrive by feeding on pollen (Liguori et al., 2002). Reports show that some tydeid species prey on tetranychid eggs and eriophyids (Laing and Knop, 1983) and also feed on pathogenic fungi (English-Loeb et al., 1999). A number of tydeids can act as alternative prey for phytoseiids (Knop and Hoy, 1983; Camporese and Duso, 1995) and their presence could contribute towards maintaining phytoseiid population levels when spider mite populations are low.

The use of selective pesticides, which do not affect interactions among tetranychids, phytoseiids and tydeids, is recommended in programmes to conserve natural enemies. The effects of pesticides on *T. urticae* are being widely studied and its resistance to new products is frequently monitored. In contrast, fewer phytoseiid mite species (e.g. *Phytoseiulus persimilis* Athias-Henriot and *Typhlodromus pyri* Scheuten) are studied in this context (e.g. Sterk et al., 1999) and information on endemic phytoseiids is scarce for many countries and for different crops. Since many factors (pesticide application history, cultural practices, environmental conditions, etc.) can affect mite susceptibility to pesticides, data on endemic species is quite important in integrated pest management (IPM) programmes. Toxicological data concerning tydeid mites are very scarce.

The aim of this work was to evaluate the effects of six pesticides generally used to control aphids and whiteflies in greenhouses and outdoor vegetable crops, on the survival and reproduction of *T. urticae*, *N. californicus* and *T. californicus*. We selected two organic insecticides (imidacloprid and pymetrozine), three insecticides containing botanical extracts (pyrethrins, azadirachtin, and rotenone), and one based on a fungus (*B. bassiana*). Imidacloprid and pymetrozine are currently used in conventional farms while the other products are mainly used in organic farms.

Materials and methods

Origin and rearing of mites

The *T. urticae* strain used originated from the greenhouse of the Istituto Sperimentale per la Zoologia Agraria of Florence (ISZA) and was reared on bindweed (*Convolvulus arvensis* L.) for several months. *Neoseiulus californicus* originated from strawberry fields near Florence and *T. californicus* originated from the ornamental shrub, *Weigela sp*., growing in the garden of the ISZA.

The three mite species were mass reared for at least five generations on strawberry leaves placed upside down on water-soaked foam in a Petri dish and surrounded by wet cotton-wool to prevent the mites from escaping and, at the same time, to provide water. *Neoseiulus californicus* was fed on the pollen of *Quercus* spp. and *T. urticae*, while *T. californicus* was fed exclusively on the pollen of *Quercus* spp.. Pollen was added to mite cultures twice a week. Mite cultures were maintained in a controlled climate chamber at 25 ± 1 °C, 75 ± 10% RH and a photoperiod of 16L:8D.

Pesticides

The following commercial pesticides were tested: Biopiren® plus (pyrethrins, 6.4 g of a.i./hl), Confidor® (imidacloprid, 13.3 g of a.i./hl), Oikos® (azadirachtin, 4.5 g of a.i./hl), Plenum® (pymetrozine, 20 g of a.i./hl), Naturalis® (*B. bassiana*, 8.6 g of a.i./hl), and Rotena® (rotenone, 18 ml of a.i./hl). Each pesticide was used at the concentration suggested for field applications. Distilled water was used as a control in all trials.

Toxicological tests

Eggs
The effect of different products was evaluated on 80 1-day-old eggs (20 × 4 replicates) of *T. urticae* and *N. californicus*. We used the leaf dip method (modified from Helle and Overmeer, 1985): a strawberry leaf, on which approximately 20 eggs were laid in the previous 24 h, was immersed in the test solution for 30 s. Eggs were examined daily and the number hatched recorded 5 days after the first egg hatched in the control. Egg toxicity was not tested on *T. californicus* since this species is viviparous (Liguori et al., 2002). Furthermore the handling of neonate

larvae was so difficult that observed mortality could not be considered reliable.

Females

The micro-immersion bioassay (modified from Dennehy et al., 1993) was used to test direct toxicity to females. Mites were drawn into a small pipette tip one at a time, after which the test solution was drawn up immersing mites for 30 s. Mites were then ejected from the pipette and dried on filter paper. Mites were then transferred using a fine brush to a treated (see above) strawberry leaf placed upside down on water-soaked foam in a Petri dish and surrounded by wet cotton-wool. Four replicates of about 20 females were used for each pesticide tested. Toxicity of the pesticides was evaluated 72 h after their application. Mites were considered dead if they were unable to react when gently prodded with a fine brush. Individuals that became entangled in the wet cotton-wool were excluded from the analysis. A number of surviving females (maximum 20 per product) were individually isolated on treated leaves to assess their fecundity for an 8-day period. The laid eggs were checked daily for hatching until the 5th day after the first egg hatched in the control. For tydeids the number of viable larvae produced was assessed for 8 days.

All trials on eggs and females were performed in controlled climate chambers at 21 ± 1 °C, 75 ± 10% of RH and a photoperiod of 16L:8D.

Data analysis

The data obtained were subjected to one-way ANOVA (after arc sine transformation for percentage data) and Duncan's test to separate the means (SPSS, 1994). The effect of each pesticide was expressed as:

$$E = 100\% - (100\% - \mathrm{M}) \times \mathrm{R}$$

where E is the coefficient of toxicity; M is the percentage of mortality calculated according to Abbott (1925); R is the ratio between the average number of hatched eggs produced by treated females and the average number of hatched eggs produced by females in the control group. For tydeids R was calculated as the ratio between the average number of larvae produced by treated females and the average number of larvae produced by females in the control group.

Results

Effects on Tetranychus urticae

Egg hatch of *T. urticae* was significantly reduced when rotenone and *B. bassiana* were used (Table 1). Mortality was more than three times higher with rotenone than with *B. bassiana*.

Four products (rotenone, *B. bassiana*, azadirachtin and pyrethrins) significantly reduced female survival, with mortality ranging from 5.1 to 13% (Table 1). Oviposition rate increased with all products, but only pyrethrins showed significantly higher values when compared with the control (Table 2). The mortality of eggs laid by treated females was significantly increased when azadirachtin and rotenone were used (Table 2). Combining the effect of the pesticides on oviposition rate and

Table 1. Toxicity of selected insecticides to *Tetranychus urticae* females and eggs

	Females		Eggs	
	Survival[a] (%)	Abbott mortality (%)	Hatching rate[a] (%)	Abbott mortality (%)
Control	97.47 a	–	98.75 a	–
Pyrethrins	92.50 b	5.10	93.75 a	5.06
Azadirachtin	92.50 b	5.10	97.50 a	1.27
Imidacloprid	98.73 a	−1.30	88.75 ab	10.13
Pymetrozine	97.50 a	−0.03	90.00 ab	8.86
Rotenone	84.81 b	12.99	7.50 c	92.41
B. bassiana	89.87 b	7.79	71.25 b	27.85

[a] Means followed by the same letter are not significantly different (Duncan's test, $p = 0.05$).

Table 2. Daily oviposition, mortality of eggs and coefficient of toxicity (*E*) of *Tetranychus urticae* females treated with selected insecticides

	n	Oviposition[a] (eggs/female/day)	Egg mortality[a] (%)	*E* (%)
Control	19	3.83 a	18.76 ab	–
Pyrethrins	20	5.82 b	11.49 a	−57.04
Azadirachtin	20	4.29 ab	25.66 cd	2.80
Imidacloprid	20	5.33 ab	21.69 bc	−35.89
Pymetrozine	20	4.43 ab	18.48 ab	−16.11
Rotenone	20	4.06 a	28.20 d	18.57
B. bassiana	19	4.61 ab	19.40 abc	−10.13

[a] Means followed by the same letter are not significantly different (Duncan's test, $p = 0.05$).

egg hatching, the R-value reached 1.66 when pyrethrins were used. The remaining products were characterised by R-values exceeding 1, with the exception of rotenone ($R = 0.94$). The coefficient of toxicity (E) was low for azadirachtin and rotenone (2.8 and 18.6, respectively) and reached negative values for the remaining products (E ranged from −10.1 to −57.0, Table 2). These values were largely affected by the increase in fecundity of the treated spider mites.

Effects on Neoseiulus californicus

With the exception of *B. bassiana*, which induced a mortality of 10.3%, the insecticides showed no significant effects on *N. californicus* eggs (Table 3).

Three products significantly reduced female survival (Table 3). The highest mortality was observed for pyrethrins, while lower values were recorded with rotenone and *B. bassiana* (Table 3). All products, with the exception of azadirachtin, reduced fecundity of *N. californicus* (Table 4). The daily oviposition rate was low for mites treated with imidacloprid, rotenone and *B. bassiana*. Pyrethrins and azadirachtin, but especially *B. bassiana*, showed a negative effect on egg survival (Table 4); R-values reached 0.03 for *B. bassiana* and imidacloprid. Pymetrozine and azadirachtin showed relatively low E-values compared to the other products whose values were above 92% (Table 4).

Effects on Tydeus californicus

Rotenone caused the death of all treated females and pyrethrins significantly reduced female survival. All other products had no significant

Table 3. Toxicity of selected insecticides to *Neoseiulus californicus* females and eggs

	Females		Eggs	
	Survival[a] (%)	Abbott mortality (%)	Survival[a] (%)	Abbott mortality (%)
Control	100 a	–	98.75 a	–
Pyrethrins	18.99 c	81.01	100 a	−1.27
Azadirachtin	96.05 a	3.95	98.75 a	0.00
Imidacloprid	98.73 a	1.27	100 a	−1.27
Pymetrozine	100 a	0.00	97.50 a	1.27
Rotenone	63.75 b	36.25	100 a	−1.27
B. bassiana	71.25 b	28.75	88.75 b	10.30

[a] Means followed by the same letter are not significantly different (Duncan test, $p = 0.05$).

Table 4. Daily oviposition, mortality of eggs and coefficient of toxicity (*E*) of *Neoseiulus californicus* females treated with selected insecticides

	n	Oviposition[a] (eggs/female/day)	Egg mortality[a] (%)	*E* (%)
Control	18	1.85 a	9.59 a	–
Pyrethrins	11	0.94 c	30.12 bc	92.53
Azadirachtin	20	1.76 a	31.32 c	30.88
Imidacloprid	20	0.06 d	10.00 a	96.66
Pymetrozine	19	1.48 b	9.78 a	20.33
Rotenone	12	0.17 d	25.00 ab	95.24
B. bassiana	18	0.10 d	60.00 d	98.23

[a] Means followed by the same letter are not significantly different (Duncan test, $p = 0.05$).

Table 5. Toxicity of selected insecticides to *Tydeus californicus* females and effects on production of progeny

	Survival (%)[a]	Abbott mortality (%)	Fecundity[a] (larvae/female/day)	E^b (%)
Control	93.02 a	–	1.88 a (n = 19)	–
Pyrethrins	81.11 b	12.81	1.66 a (n = 19)	22.60
Azadirachtin	87.91 ab	5.45	1.14 b (n = 20)	42.35
Imidacloprid	90.91 a	2.27	0.93 b (n = 20)	51.79
Pymetrozine	86.67 ab	6.83	0.79 b (n = 18)	61.01
Rotenone	0.00 c	100	–	100
B. bassiana	88.37 ab	5.00	1.78 a (n = 20)	10.07

[a] Means followed by the same letter are not significantly different (Duncan test, $p = 0.05$).
[b] E = Coefficient of toxicity.

effect (Table 5). Azadirachtin, imidacloprid and pymetrozine significantly reduced production of larvae by treated females; pyrethrins and *B. bassiana* had no effect on this parameter. The coefficient of toxicity was highest with rotenone, lowest with *B. bassiana* and pyrethrins, and was intermediate with the remaining products (Table 5).

Discussion

All the tested products affected the survival and/or fecundity of the mite species used in this study. A number of the products were favourable towards the mite pest *T. urticae* and unfavourable towards the predatory mite, *N. californicus* and the unspecialised mite *T. californicus*. Only rotenone could be considered helpful in the control of *T. urticae* for the slight effect it had on females and its significant toxicity on eggs. However, rotenone was toxic to *N. californicus* and *T. californicus*. Similar results have been obtained with other rotenone-based formulations on *T. urticae* and *N. californicus* (Castagnoli et al., 2000). Insecticide treatments based on rotenone (and pyrethrins) did not allow *Panonychus ulmi* (Koch) to be controlled by its predator *Neoseiulus fallacis* (Garman) in an experimental apple orchard (Rashid et al., 2001).

Pyrethrins and, to a lesser extent, imidacloprid increased fecundity of *T. urticae* and thus the potential performance of this species. They had major impact on *N. californicus* by reducing fecundity. Previous report on the effect of pyrethrum-based products on *T. urticae* fecundity are contrasting: Castagnoli et al. (2000) observed an increase in fecundity, whereas Tsolakis et al. (1997) observed a decrease. The negative effects of pyrethrins on *N. californicus* are consistent with those observed on another phytoseiid, *Amblyseius andersoni* (Chant), in laboratory trials (Castagnoli et al., 2002). When tested in the field on the latter species, pyrethrins confirmed the negative effects in the short term, but were followed by re-colonisation 7 days after a single treatment (Castagnoli et al., 2002). Imidacloprid appeared to be non-toxic for phytophagous mites at field rates (Elbert et al., 1991). The increase in egg production, observed in *T. urticae* females in our experiment (39%), is slightly higher than that (10–26%) reported by James and Price (2002). The same authors observed that exposure to imidacloprid by ingestion caused a similar increase in egg production, although this was delayed by some days, and accompanied by increased female longevity. Ornamental plants treated with soil formulations of imidacloprid suffered greater damage by *T. urticae* than untreated plants (Sclar et al., 1998). These authors showed that imidacloprid was highly toxic for the predacious bug *Orius tristicolor* (White) and suggested that this factor could influence spider mite outbreaks. This broad-spectrum systemic insecticide has a contrasting reputation regarding its harmlessness to predatory mites. It was seen to be non-toxic for *Typhlodromus dossei* Schicha and *T. doreenae* Schicha (James and Vogele, 2001), and increased the fecundity of *Euseius victoriensis* (Womersley) (James, 1997). In contrast, *Galendromus occidentalis* (Nesbitt) and *N. fallacis* (Garman), the most important predators on hops in Washington, were completely eliminated at the recommended field rate (James and Coyle, 2001).

Products based on *B. bassiana* have been reported as being capable of controlling many arthropod pests, including *T. urticae* (Wright and

Kennedy, 1996). Alves et al. (2002) observed mortality ranging from 43 to 78% of some isolates for *B. bassiana* on this spider mite. However, different isolates of the fungus exhibited a certain variability in pathogenicity and virulence toward different arthropods (Almeida et al., 1997; Moino et al., 1998). In our test, *B. bassiana* had a slight, positive effect on *T. urticae* fecundity, but reduced female and egg survival. In contrast, *B. bassiana* was the most toxic to *N. californicus*, reducing fecundity and survival. Laboratory applications of *B. bassiana,* as well as its use in greenhouses, proved to be harmless to the thrips predator *Neoseiulus cucumeris* (Dosse) (Jacobson et al., 2001). In contrast, *P. persimilis* was highly susceptible to the pathogen under laboratory conditions, whereas lower infection rates were observed in greenhouse trials (Ludwig and Oetting, 2001).

Azadirachtin and pymetrozine were not toxic or slightly favourable to *T. urticae*, and scarcely detrimental to the survival and reproduction of the predatory *N. californicus*. Pymetrozine reduced oviposition of *N. californicus* more than azadirachtin, whereas the latter more markedly reduced the viability of the eggs laid by treated females. Different azadirachtin-based formulations are commercially available; the solvent used for the extraction of the active ingredient and the different ratio between azadirachtin and Neem and other vegetable oils can influence their effect on mites (Mansour et al., 1987, 1993). *Neoseiulus californicus* was susceptible to azadirachtin-based products, especially when they were mixed with vegetable oils and soybean lecithin; the same formulation increased egg production in *T. urticae* (Castagnoli et al., 2000). In other laboratory trials, residues of Neem oil were moderately toxic to *T. urticae* 24 h after application (but its effects decreased later) but were not toxic to *P. persimilis* (Cote et al., 2002). Pymetrozine was harmless to a number of predatory mites, e.g. *G. occidentalis, N. fallacis* and *A. andersoni* (James, 2002) and *T. pyri* (Sechser et al., 2002). Since this pesticide has been only recently introduced to Italy, there is a lack of data on its effects on native phytoseiid populations.

Tydeus californicus appeared to be less susceptible than *N. californicus* to the tested products, with the exception of rotenone, which was lethal to the females. For the other products, female mortality was less than 13%, and the coefficient of toxicity ranged from 10 to 61%. An explanation for the lower residual effects of pesticides on tydeids could be that in this species the time lapse between adult emergence and the birth of progeny is longer than for *N. californicus* and larval development occurs inside the mother (Liguori et al., 2002).

Caution is required in predicting field performance from laboratory bioassays; these data stress the importance of evaluating the effect of new products on local species/strains of phytophagous and predatory mites before their use in IPM programmes. In recent years, the use of botanical insecticides has increased. This study shows that some of them are harmful to predatory mites, but have a negligible impact (or even a favourable effect) on spider mites. The same occurs for imidacloprid. Since it is well known that strain features can influence these results, additional experiments are planned to investigate this topic further.

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