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Selective toxicity of some pesticides to *Pullus mediterraneus* Fabr. (Coleoptera: Coccinellidae), a predator of *Saissetia oleae* Bern. (Homoptera: Coccoideae)

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Abstract

1 To contribute to the development of IPM strategies in olive groves, the selectivity of several insecticides to *Pullus mediterraneus* Fabr. (Coleoptera: coccinellidae) was investigated in the laboratory. The study assessed the toxicity of seven chemical pesticides and one bacteriological insecticide to adult *P. mediterraneus*.

2 The LC_{50} was estimated by applying the pesticides on the dorsal side of adults. Lambda-cyhalothrin, methomyl and cypermethrin resulted in the highest toxicity followed, in decreasing order of toxicity, by malathion, fenthion and dimethoate. Parathion had the least effect on the coccinellid.

3 Consumption of *Bacillus thuringiensis* bacteria (applied as a commercial formulation), over a period of 10 consecutive days, resulted in low mortality of adult *P. mediterraneus*.

4 The results of these laboratory experiments indicate that the most widely used chemical pesticides were toxic to *P. mediterraneus*. Their use in olive groves must take account of the activity period of this predator. In contrast, *B. thuringiensis* seemed to be a suitable candidate to be included in pest management systems.

Keywords Coccinellid, olive groves, IPM programme, pesticides, *Pullus mediterraneus*, toxicity.

Introduction

The development of integrated control programmes in olive groves has gained increased attention in many parts of the world. However, many chemicals available for treatment of insect pests are also toxic to natural enemies. Use of the pesticides that are least harmful to beneficial organisms can potentially ameliorate this problem (Jepson, 1989; Croft, 1990; Bellows & Morse, 1993).

The coccinellid *Pullus mediterraneus* Fabr. (Coleoptera: Coccinellidae) is a common insect in the Mediterranean region, both in Europe and North Africa, and is abundant in crops such as conifers (*Pinus*), citrus, grenadier and olive groves (Gourreau, 1974). *Pullus mediterraneus* appears to be a particularly effective polyphagous predator of *Saissetia oleae* Bern. (Homoptera: Coccoidae), a pest of olive trees (Panis, 1977). Its presence in Morocco was first recorded by Smirnoff in 1956 (Smirnoff, 1956). It is widely distributed in the orchards of the Haouz region (south of Morocco) (Chemseddine, 1988) and has shown a good numeric response and a chronological coincidence with *S. oleae* (Ba M'hamed, 1993). Given its voracity and its biological characteristics (Ba M'hamed & Chemseddine, 1996, 2001), *P. mediterraneus* has potential as a control agent in the context of integrated pest management (IPM) programmes. The success of such programmes depends, in part, on the optimal use of selective pesticides that are less damaging to this natural enemy. However, no data on the susceptibility of *P. mediterraneus* to chemical or bacteriological insecticides are available. This study was undertaken to quantify the toxic effects of seven chemical pesticides and one bacteriological insecticide, which are widely used in olive crops in which *P. mediterraneus* is found.

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Materials and methods

Adult *P. mediterraneus* were collected from four olive orchards in different regions of the Haouz. In the laboratory, rearing was carried out at 27 ± 2 °C, 50–60% r.h. and LD 16 : 8 h in Plexiglas boxes (9 × 6 × 2 cm). The bottom of the box was covered with filter paper and the top was cut and covered by mesh to allow circulation of air. Excess eggs of *S. oleae*, collected directly from the olive groves, were offered as food. *Pullus mediterraneus* eggs were collected daily. After hatching, individual larvae were placed in a separate Petri dish (9 cm diameter) and larval development was observed twice daily (at 08.00 and at 17.00 hours), to produce a batch of physiologically identical adults of the same age (4–5 days old).

Eight insecticides were tested: (1) malathion, an organophosphate (Malathion 50, EC [emulsifiable concentrate], 500 g/L, S.A.O.A.S. Agadir, Morocco); (2) cypermethrin, a pyrethroid (Ripcord 5, EC, 50 g/L, Cyanomid Agro. S.I.P.P. Casablanca, Morocco); (3) parathion (Platte, CO, EC, 50%, Amaroc S.A. Casablanca, Morocco); (4) fenthion, an organophosphate (lebaycid 40, EC, 550 g/L, Bayer S.A. Casablanca, Morocco); (5) dimethoate, an organophosphate (Callidim.40, EC, 40%, Philea Casablanca, Morocco); (6) Lambda-cyhalothrin (Karate, EC, 50 g/L, MARBAR Chimie Casablanca, Morocco); (7) methomyl (Lannate, WP [wettable powder], 25%, E.I. DuPont de Nemours, Geneva, Switzerland); (8) *Bacillus thuringiensis* (*Bt*) (Bactrospeine PM 16000S, WP, 50%, Abbott, Ait Melloul, Morocco). The field dilution rates recommended by the Moroccan Ministry of Agriculture were 1.15 mL/L for malathion, 0.06 mL/L for cypermethrin, 0.8 g/L for parathion, 1.25 g/L for fenthion, 1.25 mL/L for dimethoate, 0.04 mL/L for Lambda-cyhalothrin, 0.8 g/L for methomyl, and 0.7 g/L for *Bt*. In each case the effect of the full field dilution rate and reduced rate concentrations of the insecticides were investigated.

A well established method was used to test contact toxicity on adult *P. mediterraneus* (Broumas, 1979; Kay, 1979; Broadley, 1983; Tondeur *et al.*, 1993; Roger *et al.*, 1994). A 1-μL distilled water solution containing the desired quantity of pesticide was applied to the pronotum (dorsal side) of each beetle using a Hamilton syringe (0.05 μL precision). Beetles in the control groups were treated with distilled water only. To reduce locomotor activity during the topical applications, the beetles were maintained at 4 ± 2 °C for 10–15 min prior to treatment. After treatment, the beetles were placed in a Petri dish containing excess *S. oleae* eggs as prey and returned to the rearing chambers for observation.

For each concentration of each product tested, the experimental design consisted of six replicates of 10 individuals. Four different concentrations were used to estimate the LC_{50} for each of the seven insecticides: fenthion (1, 0.25, 0.43 and 0.87 g/L), malathion (0.5, 0.14, 0.34 and 0.67 g/L), dimethoate (0.15, 0.2, 0.5 and 0.8 g/L), parathion (0.3, 0.55, 1.5 and 2.5 g/L), cypermethrin (0.02, 0.05, 0.08 and 0.1 mL/L), methomyl (0.1, 0.05, 0.08 and 0.02 g/L) and lambda-cyhalothrin (10, 5, 2 and 1 mL/L, 5 μL/L). Mortality was assessed 24, 48 and 72 h after application. The criterion for death was failure of the coccinellid beetle to move its legs when stimulated with a fine brush.

In order to establish the LC_{50} of *Bt*, the technique described by Giroux *et al.* (1994) was used. Eggs of *S. oleae*, taken from infested olive branches, were dipped for 2 s in the appropriate solution and dried in still air for 20 min. Cotton wool moistened with the test solution was also presented to the adults as a source of water. This procedure was repeated using water as a control. Four concentrations were tested (0.07, 0.04, 0.025 and 0.01 g/mL) and mortality was noted every 24 h for a period of 10 days, using the method described above.

The recorded mortality was corrected using Abbott's formula (Abbott, 1925). The LC_{50} for each pesticide was estimated using probit regressions (EPA probit analysis program). One-way analysis of variance (ANOVA), followed by a pair-wise multiple comparison using the Student-Newman–Keuls method, was carried out to identify significant differences between pesticides.

Results

After 24 h the mortality caused by the seven chemical pesticides tested was significantly higher than that of the control group ($F = 14.6$, d.f. = 29, $P \leq 0.05$). Parathion caused little mortality to *P. mediterraneus*, with a LC_{50} of 0.108 g (AI)/L, and the surviving beetles remained active throughout the observation period. Lambda-cyhalothrin, methomyl and cypermethrin resulted in the highest mortality followed by, in decreasing order of toxicity, malathion, fenthion and dimethoate (Table 1). All treatments were significantly different from each other, except for fenthion, dimethoate and parathion ($F = 122.9$, d.f. = 28, $P = 0.0001$). The estimates of LC_{50} established in the laboratory were much lower than the application rates recommended by the Moroccan Agricultural Ministry. For example, the mean rate of lambda-cyhalothrine recommended in crops was 77 times higher than the LC_{50}. For cypermethrin and methomyl, the ratios of the recommended concentration to the estimated LC_{50}s were 65 and 20 times higher, respectively.

The slopes of the mortality curve for different treatments varied significantly among themselves ($P \leq 0.0001$) except in the case of fenthion, dimethoate and parathion. Significant differences in toxicity were observed between the concentrations tested for each pesticide over the first 24 h following treatment (cypermethrin: $F = 24.8$; d.f. = 3; $P \leq 0.0001$; methomyl: $F = 38.1$; d.f. = 3; $P \leq 0.0001$; lambda-cyhalothrin: $F = 19.5$; d.f. = 4; $P = 0.0006$; fenthion: $F = 6.49$; d.f. = 3; $P = 0.0003$; malathion: $F = 10.5$; d.f. = 3; $P = .0002$; dimethoate: $F = 18.2$; d.f. = 3; $P \leq 0.0001$; parathion: $F = 4.49$; d.f. = 3; $P = 0.01$).

The pattern of mortality during the first 72 h after treatment differed, depending on the pesticide used. In some compounds, differences in mortality between insecticide concentrations were most evident in observations made at 24 h after application, and were higher than those obtained in control beetles (Figs 1 and 2). The toxic effect of other pesticides persisted beyond 24 h. The mortality rates recorded in coccinellids treated with lambda-cyhalothrin and methomyl were highest after 24 h and decreased to reach the level observed in the control after 48 h (lambda-cyhalothrin) or 72 h (methomyl) (Figs 1 and 2). Malathion, cypermethrin and methomyl were the most toxic products,

Table 1 Toxicity (after 24 h) of the eight pesticides applied on the dorsal side of adult *P. mediterraneus* ($n = 240$)

Pesticides	Slope ± SEM	LC_{50} g(AI)/L (95% CL)
Bactrospeine	a	a
Fenthion	1.51 ± 0.48	0.068 (0.044,0.23)
Parathion	1.52 ± 0.57	0.108 (0.061,2.08)
Dimethoate	1.93 ± 0.55	0.069 (0.048,0.164)
Malathion	1.82 ± 0.43	0.035 (0.026,0.015)
Cypermethrin	9.29 ± 2.88	0.0046 (0.0042, 0.005)
Methomyl	4.40 ± 1.42	0.01 (0.093,0.144)
Lambda-cyhalothrin	0.92 ± 0.31	0.0026 (0.001,0.06)

a, Slope, not significantly different from zero, LC_{50} and confidence limits cannot be computed because the mortality obtained was too low. AI: Active Ingredient; CL: Confidence Limit; SEM: Standard error of mean.

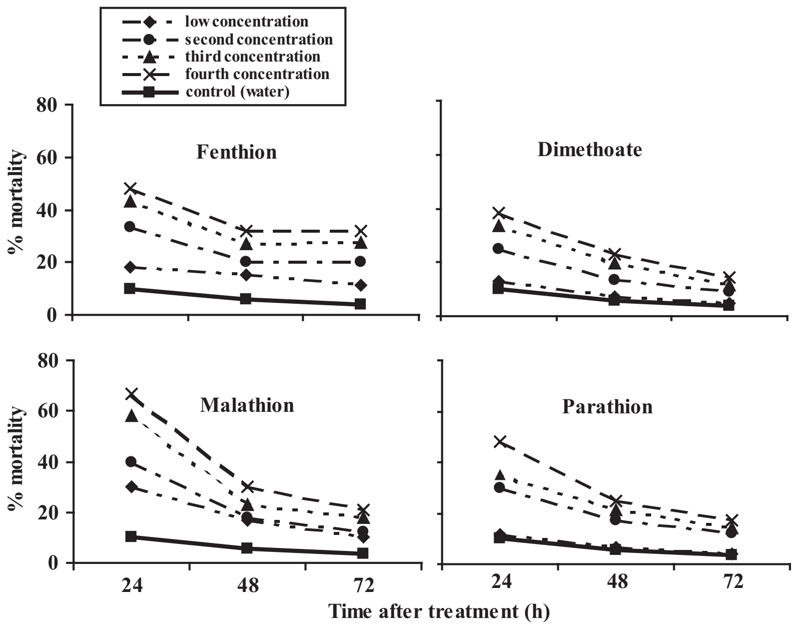

Figure 1 Mortality recorded for adult *P. mediterraneus* at 24, 48 and 72 h after treatment with the four concentrations of the chemical pesticides: fenthion (1, 0.25 , 0.43 and 0.87 g/L), malathion (0.5, 0.14, 0.34 and 0.67 g/L), dimethoate (0.15, 0.2, 0.5 and 0.8 g/L) and parathion (0.3, 0.55, 1.5 and 2.5 g/L).

causing rapid mortality with a ratio that exceeded 60% after 24 h. A dose–effect relationship was observed in all insecticides tested.

Mortality of adult *P. mediterraneus* after 10 days of feeding on eggs treated with any of the *Bt* concentrations tested did not exceed 13%. Percentage mortality did not differ significantly from that of the control at any concentration, except for those beetles treated with the recommended concentration (Fig. 3). The mortality following application of the field rate increased to 25% after 20 days.

Discussion

Ideal insecticides for use in IPM programmes will be toxic to the target pest but not to its natural enemies (Plapp & Bull, 1978; Coats *et al*., 1979; Rajakulendran & Plapp, 1982). Some studies have evaluated the susceptibility of coccinellids to synthetic insecticides. In standard toxicological studies, mortality counts are usually made at 24 h after the treatments are applied (Jepson, 1989), but it has been suggested that longer observation times (e.g. 72 h) should be used as standard procedure (Roger *et al*., 1994). *Pullus mediterraneus* was very susceptible to the seven insecticides tested in this study. All the chemical products used at recommended concentrations killed most adult coccinellids within a few hours of treatment. Lambda-cyhalothrin is an insecticide that is frequently used in olive groves and is recommended for control of *Bactrocera oleae* Gmel (Diptera: Tephritidae) and *Prays oleae* Bern (Lepidoptera: Yponomeutidae). However, in this study it resulted in a high and rapid mortality of adult *P. mediterraneus*. Its toxicity has previously been demonstrated in tests involving other natural enemies of olive tree pests, such as *Opius concolor* Siculus (Hymenoptera: Braconidae) (Jacas & Vinuela, 1997).

Methomyl and cypermethrin were amongst the most toxic pesticides tested in this study, corroborating the results obtained by Broadley (1983), who observed a high mortality after application of the two products to *Coccinella repanda* and *Harmonia octomaculata*. Similar observations

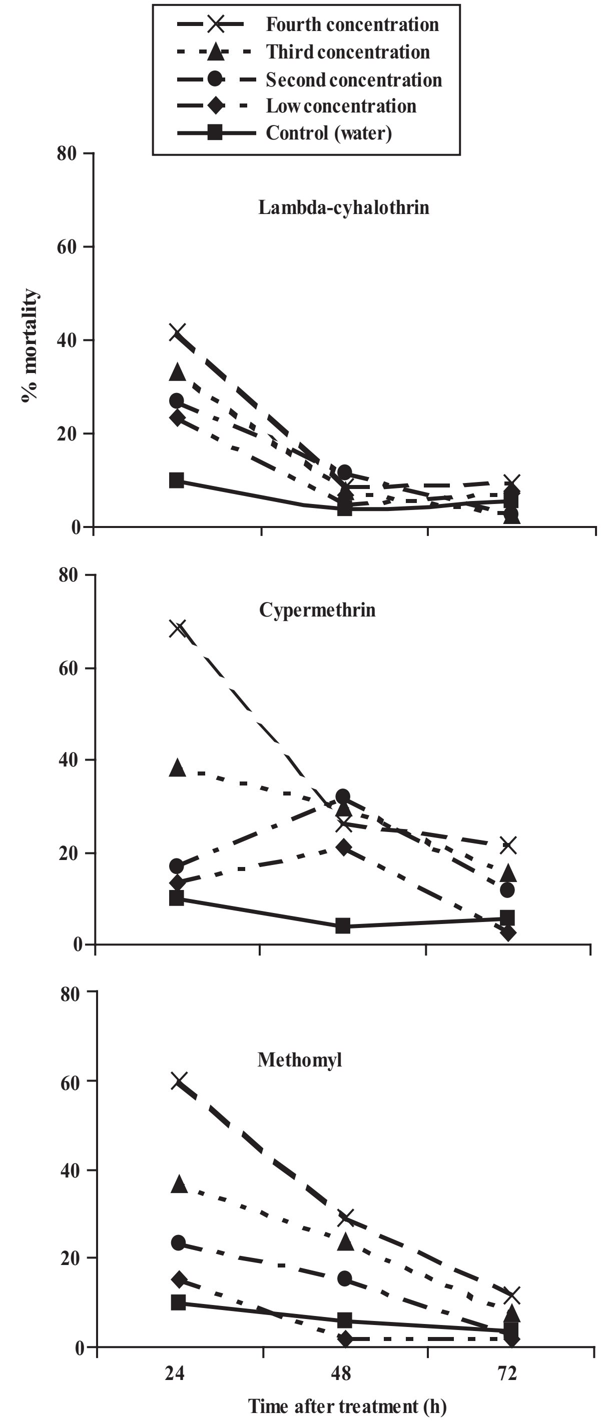

Figure 2 Mortality recorded for adult *P. mediterraneus* at 24, 48 and 72 h after treatment with the four concentrations of the chemical pesticides: lambda-cyhalothrin (100, 50, 20, 10 and 5 μL/L), cypermethrin (0.02, 0.05, 0.08 and 0.1 mL/L) and methomyl (0.1, 0.05, 0.08 and 0.02 g/L).

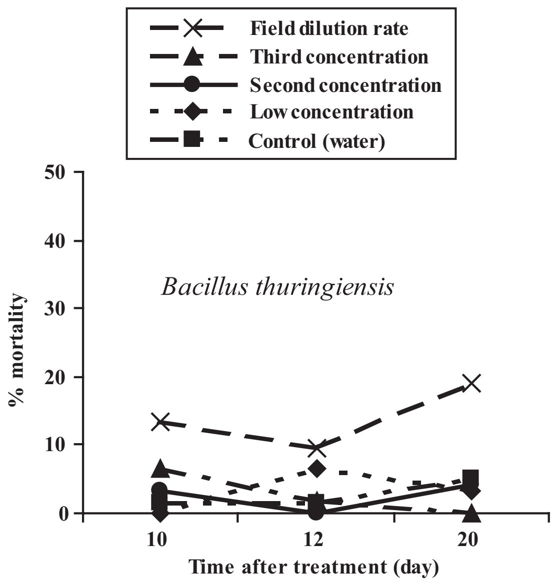

Figure 3 Mortality recorded after adult *P. mediterraneus* were fed for at 10, 12 and 20 days on *S. oleae* eggs treated with the four concentrations of the bacteriological insecticide *Bt* (0.07, 0.04, 0.025 and 0.01 g/mL).

were also made after treatment of *Coccinella septumpunctata* with both insecticides (Brown *et al.*, 1983; Shires, 1985) and for *Rhizobius lophanta* when treated with methomyl (Bellows & Morse, 1993). However, cypermethrin was found to be only slightly toxic to *Coleomegilla maculata* (Coats *et al.*, 1979; Lecrone & Smilowitz, 1980), causing a temporary inactivation (apparent mortality) in adults, thereby reducing the insect's predation efficiency for 48 h (Roger *et al.*, 1991).

In this study malathion also showed a high toxicity to *P. mediterraneus* and was ranked fourth among the seven insecticides tested. This product was reported to be highly toxic to adult *Hippodania quinquesignata* (Bartlett, 1963) and Croft & Brown (1975) ranked it among the most toxic insecticides when they reviewed the responses of various coccinellids to pesticides. In laboratory studies, Mok Yun & Ruppel (1964) observed a high toxicity of malathion to *C. maculata* with 97% of individuals dead at 72 h after treatment. In the present study malathion was found to be more toxic than parathion toward adult *P. mediterraneus* and a similar comparative toxicity has been established in many other coccinellids (Pradhan *et al.*, 1959; Jotwani *et al.*, 1960; Bartlett, 1963; Hamilton & Kieckhefer, 1969; Bellows & Morse, 1993).

Dimethoate showed moderate activity when applied to adult *P. mediterraneus*, as has been reported for the coccinellid *Sterhorus paperculus* (Jotwani *et al.*, 1960); however, several authors have classed this product among the more toxic insecticides to many other species of coccinellids (Bartlett, 1963; Kehat & Swirski, 1964; Atallah & Newson, 1966; Gargav, 1968; Bellows & Morse, 1993). Moreover, this product has a high risk of phytotoxicity toward olive fruits. Fenthion, the product more frequently used in olive groves in the Haouz region, is known to be very toxic to certain pests, such as *B. oleae* and *P. oleae* (Broumas, 1979), and to some parasites such as the trichogrammes, parasites of *P. oleae* (Kot & Plewka, 1970; Broumas, 1979). Under laboratory conditions in this study it showed less toxicity toward adult *P. mediterraneus*.

Bt is a gram-positive bacterium, very effective against *P. oleae* (Yamvrias, 1985; Pastre, 1990). The side-effects of treatment by *Bt* on parasitoids of the olive moth have been evaluated (Herrnstadt *et al*., 1986, 1987; Bellows & Morse, 1993; Varlez *et al*., 1993). In the laboratory, over a period of 10 consecutive days, this product showed no lethal effects on adults of *P. mediterraneus*. The highest mortality was obtained only 20 days after ingestion of *Bt*, and followed the manufacturer's recommended concentration.

We conclude that the insecticides tested here have an important effect on the mortality of adult *P. mediterraneus* when applied at field-recommended concentrations and, in some cases, at lower concentrations. This study contributes to the establishment of a specific IPM programme for olive groves of the South Moroccan region. The high toxicity of the most frequently applied pesticides in this region, suggests that they must be used carefully, and must avoid periods of high activity of this predator. Moreover, it is known that coccinellids show a lower susceptibility than other groups of entomofauna to insecticides, because of their generally high level of the insecticide detoxifying enzyme activities (Shono *et al*., 1979; Yu, 1987). Thus, comparative field evaluation of selected insecticides within this class may be necessary to ascertain their effects on other natural enemy species, and to evaluate their relative usefulness for IPM programmes.

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