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Short communication

Side-effects of botanical insecticides derived from Meliaceae on coccinellid predators of the date palm scale

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Abstract

Bioassays were conducted in Mauritania to determine the toxicity of botanical insecticides from the tree *Melia volkensii* Gürke (Meliaceae) to ladybird predators (Coleoptera: Coccinellidae) of the date palm scale, *Parlatoria planchardi* Targ. (Homoptera: Diaspididae). *M. volkensii* seed extract was formulated in neem oil or a mixture of neem and maize oil. Three preparations were tested on *Chilocorus bipustulatus* L. var. *iranensis*, an introduced species, and one on the indigenous *Pharoscymnus anchorago* F., a species already used in previous bioassays. Fourth instar larvae were exposed for 2 days to treated scale-infested date palm leaves. The botanical insecticides were toxic to *C. bipustulatus*. Median lethal application rates (LR_{50}s) were close to the recommended application rate of 1 l/ha. In contrast, *P. anchorago* showed no increased mortality at this rate. Hazard quotients (application rate divided by the LR_{50}) were generally less than 2, suggesting a low risk for both species. However, risk mitigation measures are recommended when using oil formulations because the threshold value for *C. bipustulatus*, the more susceptible of the two ladybird species, would be exceeded at higher dose rates or when conducting multiple applications. Sublethal effects included an extension of the larval stage and morphogenetic defects. These effects were again more pronounced in *C. bipustulatus* than in *P. anchorago.*

Keywords: Beneficial arthropods; *Chilocorus bipustulatus*; *Pharoscymnus anchorago*; Botanical insecticides; *Melia volkensii*; *Azadirachta indica*; Side-effects

1. Introduction

Botanical insecticides derived from the mahogany family (Meliaceae) have been advocated as biorational control agents, compatible with biological control and integrated pest management (Schmutterer, 1997; Castagnoli et al., 2002; Tedeschi et al., 2001). Apart from the Indian neem tree, *Azadirachta indica* A. Juss., several other meliacious plants contain compounds with insecticidal activity, including the East African "tree of knowledge", *Melia volkensii* Gürke (Rembold and Mwangi, 1995). Oil formulations of *M. volkensii* seed extract have been tested to control nymphs of the Desert Locust, *Schistocerca gregaria* (Forskål) (Orthoptera: Acrididae) (Nasseh et al., 1993; Diop and Wilps, 1997).

Several studies have examined side effects of meliacious insecticides on ladybird beetles (Coleoptera: Coccinellidae). Topical treatment of larvae (Banken and Stark 1997; da Silva and Martinez, 2004) and imagos (Roger et al., 1995; Smith and Krischik, 2000; Ulrichs et al., 2001) with neem preparations had no serious adverse effects. In contrast, larvae subjected to multiple exposure routes were highly susceptible to neem (Banken and Stark, 1998; Ahmad et al., 2003). Furthermore, pure neem oil was more toxic than emulsifiable concentrate (EC) formulation, despite lower concentration of the principal active ingredient azadirachtin A (Ahmad et al., 2003; Basedow et al., 2003).

Only one study has investigated side effects of *M. volkensii* seed extract (Peveling and Demba, 1997). This study has found a low risk to larval *Pharoscymnus anchorago* F., an indigenous predator of the date palm scale, *Parlatoria planchardi* Targ. (Homoptera: Diaspididae) in Mauritania. Our study follows up this finding by testing for side effects of different oil formulations of

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M. volkensii seed extract on *Chilocorus bipustulatus* L. var. *iranensis*. This ladybird was introduced in the 1960s and 1970s and has kept scale infestations of date groves at moderate levels (Tourneur and Hugues, 1975).

The study had three objectives:

1. to establish median lethal application rates (LR_{50}s) for 4th instar *C. bipustulatus*;
2. to compare the susceptibility of 4th instar *C. bipustulatus* and *P. anchorago*;
3. to study the effect of application mode (microapplicator vs. Potter tower) on the toxicity of botanicals to 4th instar *C. bipustulatus*.

2. Materials and methods

2.1. Methodology

The bioassays were designed as extended laboratory studies (Candolfi et al., 2001) in which laboratory-reared 4th instar larvae were exposed to insecticide-treated, scale-infested date palm leaves.

2.2. Rearing of test organisms

Colonies were established from field-collected beetles. They were reared in Petri dishes (9 cm diameter) at indoor temperatures (25–30 °C). Dishes were filled with scale-infested date palm leaves (1–2 cm wide, 7–9 cm long) and kept in a shaded place. Relative humidity was <35% in the rearing room but higher within Petri dishes due to evaporation from the leaves. Fresh leaves were provided if the scales were depleted. Leaves with eggs were removed daily and kept in separate dishes. After hatching, larvae were transferred onto fresh leaves until moulting into the 4th instar. These larvae were used in bioassays or allowed to pupate to maintain the colony.

2.3. Botanical insecticides

Three ultra-low-volume (ULV) preparations were tested, each consisting of *M. volkensii* seed extract formulated in neem oil or a mixture of neem and maize oil (Table 1). The agents were originally prepared to control locust nymphs (Diop and Wilps, 1997; Rembold, 1997). One agent (MV-095) was formulated at the University of Nairobi, Kenya, and the other two (MV-127 & MV-128) at the Max-Planck-Institute for Biochemistry, Martinsried, Germany. Recommended application rates were 1 l/ha. Concentrations of *M. volkensii* seed extract ranged from 10–45 g/l (Table 1). In bioassays with *Aedes aegypti* L. (Diptera: Culicidae), the toxicity of the seed extract was equivalent to one tenth of azadirachtin (H. Rembold, pers. commun.). Concentrations of azadirachtin A & B and azadirachtinin were very low (<0.01% and <0.05%) (Table 1).

2.4. Insecticide treatment

Treatments were initially conducted using a microapplicator (Burkard, Rickmansworth, UK) (Peveling and Demba, 1997). Subsequent treatments were conducted with a Potter spray tower (Burkard, Rickmansworth, UK). Volume application rates were 500 nl/cm^2 for microapplicator and 126 nl/cm^2 for spray tower treatments, corresponding to 50.0 and 12.6 l/ha, respectively. Scale-infested leaf strips (cover >75%) measuring 1.3 × 4.5 cm were treated with the microapplicator by depositing six equidistant 0.5 µl droplets on either side (1 droplet per cm^2). Treated leaves were left for 6 h until the oil was evenly dispersed over the surface. Batches of 10 leaf strips (dimension as above) were treated with the Potter spray tower. Leaves were placed on small wire racks in Petri dishes and sprayed on either side in two successive runs. A volume of 0.5 ml was atomised at 69 kPa to yield an average spray deposit of 126 nl/cm^2 (0.14 mg/cm^2).

2.5. Bioassays

Bioassays were conducted at the *Centre de Lutte Antiacridienne* field station in Akjoujt, Mauritania.

2.5.1. Test conditions

One-day old 4th instar larvae were used. Treatment groups comprised 20 larvae. Polyethylene vials 1.4 cm in diameter and 5.5 cm in length served as test chambers. Each vial received one leaf strip and was stocked with one larva. Vials were closed with cotton mesh. After 2 days

Table 1
Characteristics of the botanicals tested

Code	Carrier oil (formulation)	Botanical			
		Melia volkensii extract (g/l)	*Azadirachta indica* oil active ingredients (mg/l)		
			Azadirachtin A	Azadirachtin B	Azadirachtinin
MV-095	Neem/maize oil (30/70%)	10 (methanolic extract)	71	85	495
MV-127	Neem oil	45 (methanolic extract)	—[a]	—[a]	99
MV-128	Neem oil[b]	25 (CO_2-extract)	58	53	58

[a]Not quantifiable due to overlapping peaks in HPLC chromatograms.
[b]Oil with depleted azadirachtin content.

(44 h), leaves were replaced with untreated leaves. Thereafter, fresh leaves were supplied daily (*C. bipustulatus*) or every other day (*P. anchorago*). Organic sunflower oil was used as dilutant and oil control.

2.5.2. Series I: MV-127 vs. MV-095 (microapplicator treatment)

Formulations were applied at 0 (oil control), 6.4, 16, 40, 100, 250, 500 nl/cm^2 (MV-127) and 0, 1.5625, 3.125, 6.25, 12.5, 25, 50 nl/cm^2 (MV-095), respectively. Bioassays with *C. bipustulatus* were performed in triplicate (total number: $N = 840$). Fresh stock solutions and dilutions were prepared for each replicate. Only one test was conducted with *P. anchorago* and MV-127 ($N = 140$), using the same dose rates and preparations. Further testing was not necessary because no effects were observed. MV-095 was not tested because its toxicity to *P. anchorago* had been established in a previous study (Peveling and Demba, 1997).

2.5.3. Series II: MV-128 vs. MV-095 (microapplicator and Potter tower treatment)

The second test series was conducted with *C. bipustulatus* only ($N = 480$). MV-128 was applied at 0, 3.125, 6.25, 12.5, 25 and 50 nl/cm^2 and MV-095 at 0, 1.875, 3.75, 7.5, 15 and 30 nl/cm^2. The four bioassays (2 formulations × 2 treatment modes) were not replicated.

2.6. Endpoints and test validity

Survival was recorded daily until 7 days after adult eclosion. Developmental, morphological and behavioural features were also noted daily. Four development stages were distinguished, mobile larvae, immobile larvae (attached to the surface before pupation), pupae and imagos. The observation period ranged from two (oil control) to 4 weeks (botanicals). The duration of the 4th instar was used as a sublethal endpoint. Larvae that died at an age of $\leqslant 3$ days were not included in this analysis. Tests were considered as valid if mortality in the oil control was $\leqslant 20\%$ (Schaub et al., 2002).

2.7. Data analysis

Median lethal application rates (LR_{50}s) were calculated using probit analysis (PriProbit 1.63). Log-likelihood ratio tests were employed to test hypotheses of equality and parallelism in response lines (Robertson and Preisler, 1992). The following comparisons were made: MV-127 vs. MV-095, MV-095 microapplicator vs. MV-095 Potter tower, MV-128 microapplicator vs. MV-128 Potter tower and MV-095 vs. MV-128 (application modes pooled). Kruskal–Wallis analysis followed by Nemenyi multiple range test was used to detect differences in the duration of the 4th instar stage of *C. bipustulatus* among dose levels. Replicate data were pooled for this analysis. Data for *P. anchorago* were analysed using one-way analysis of variance and Newman–Keuls test. For series I, percentages of malformed imagos were calculated. No statistics were applied to these descriptive data.

3. Results

3.1. Lethal effects

Mortality in oil control treatments was generally $<15\%$. Thus all bioassays were valid.

Series I: The LR_{50}s of MV-127 and MV-095 for *C. bipustulatus* were close to the label rate (1 nl/cm^2 or l/ha; Table 2). The probit regression lines had equal slopes and intercepts ($\chi^2 = 1.2$, df = 2, $P > 0.05$), i.e., the two formulations had similar toxicity. The relative potency estimate of MV-127 relative to MV-095 was 0.93 (95%-CL 0.74–1.16). In contrast, *P. anchorago* was not susceptible to MV-127. Mortality rates were similar among all dose groups and the oil control (10–15%).

Series II: The mode of application had no effect on the toxicity of MV-128 and MV-095. In both cases, regression lines were similar for Potter tower and microapplicator treatments ($\chi^2_{\text{MV-128}} = 3.0$, $\chi^2_{\text{MV-095}} = 3.9$, df = 2, $P > 0.05$). Therefore, data for the two modes of treatment were pooled to derive single LR_{50}s (Table 2). The hypothesis of equal regression lines was rejected at $P < 0.001$ ($\chi^2 = 20.8$, df = 2), but the lines were confirmed to be parallel ($\chi^2 = 0.9$, df = 1, $P > 0.05$). MV-128 was about half as potent as MV-095 (relative potency estimate = 0.52, 95%-CL 0.39–0.70).

At high dose rates, most individuals died during the larval stage, whereas at low to medium rates, they died during the early adult stage, resulting in a distinct second mortality peak (Fig. 1). An elevated adult mortality at lower dose rates was observed with all formulations but was most pronounced with MV-095. Malformations were rarely found among beetles that died within a week of emergence.

Table 2
Toxicity of three botanical insecticides to *C. bipustulatus*. LR_{50}-values are expressed as formulation volume per leaf surface area (nl/cm^2)

Formulation	No. tested	Slope (standard error)	LR_{50} (95%-CL) (nl/cm^2)
Series I			
MV-127	420	3.87 (0.53)	10.7 (8.8–12.6)
MV-095	420	3.30 (0.46)	9.9 (8.2–11.6)
Series II[a]			
MV-128	240	2.83 (0.52)	14.6 (11.0–18.3)
MV-095	240	3.50 (0.61)	7.7 (5.9–9.5)

Corresponding field rates (l/ha) are 1/10 of the nominal values given in the table.

[a]Data from Potter and microapplicator treatments pooled because of similar LR_{50}-values.

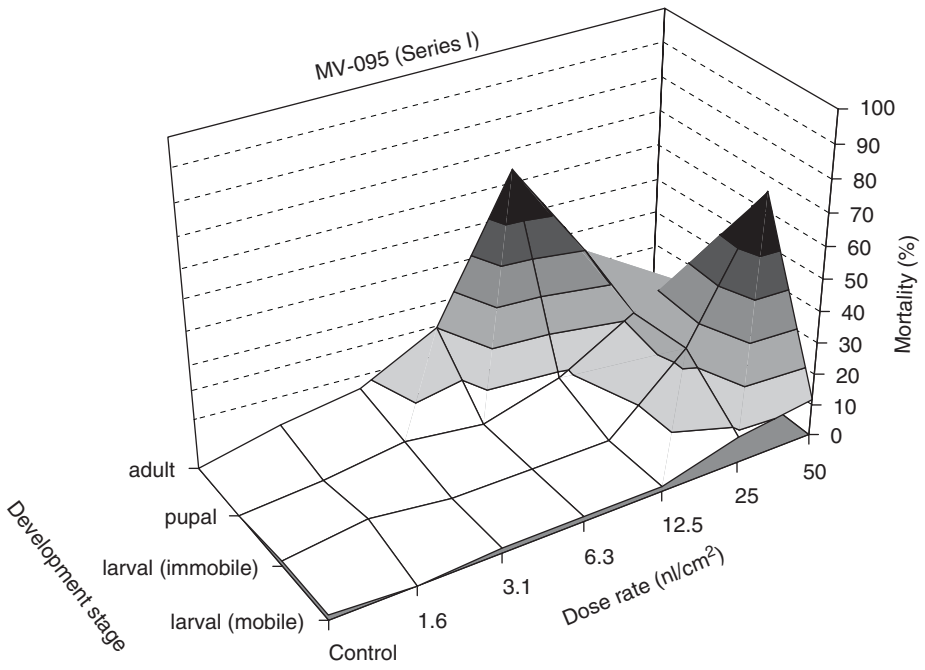

Fig. 1. Mortality by development stage of *C. bipustulatus* treated with MV-095 during the early 4th instar larval stage. Values are the means of three replicate bioassays.

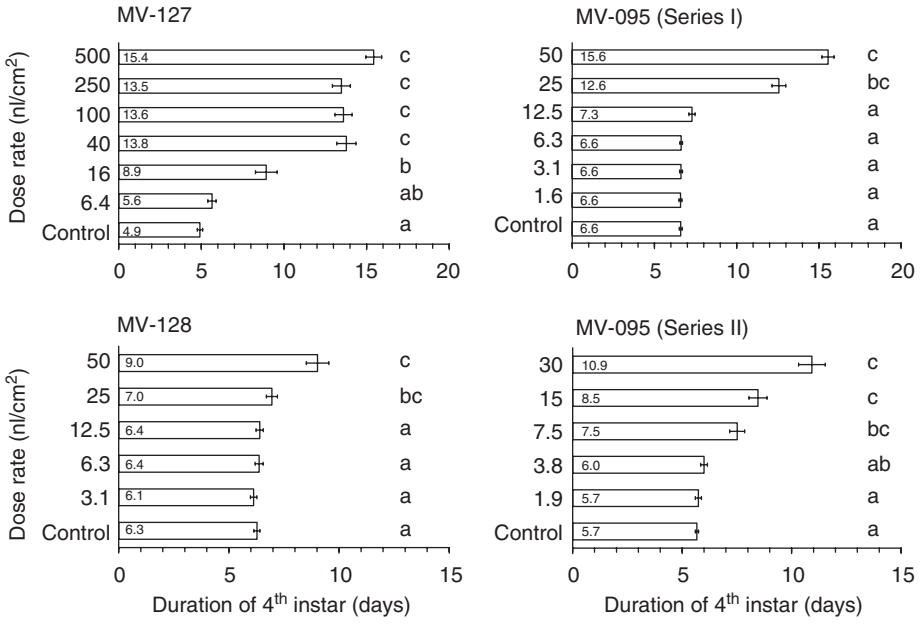

Fig. 2. Mean (±standard error) duration of the 4th instar stage of *C. bipustulatus* exposed to different dose rates of *M. volkensii* formulations. Corresponding field rates (l/ha) are 1/10 of the nominal values given in the figures. Means not sharing a letter are significantly different at $P<0.05$.

3.2. Sublethal effects

The duration of the 4th instar of *C. bipustulatus* in the oil controls ranged from 4.9±0.2 days (mean±SE; MV-127) to 6.6±0.1 days (MV-095, series I). Differences were due to seasonal changes in ambient temperatures. The 4th instar of *P. anchorago* in the MV-127 oil control lasted 4.0±0.2 days.

All formulations induced a dose-dependent extension of the larval stage (Fig. 2). The highest duration (15.6±0.4 days) was found in larvae exposed to MV-095 (series I) at 50 nl/cm^2, and the highest individual duration (23 days) in larvae exposed to MV-127 at 100 and 250 nl/cm^2. The lowest dose rates inducing significant effects ranged between 7.5 and 25 nl/cm^2 (Fig. 2). Corresponding 4th instar durations were 7.0±0.2 (MV-128), 7.5±0.3 (MV-

095, series II), 8.9±0.7 (MV-127) and 12.6±0.4 days (MV-095, series I). An extension of the larval stage was also noted in *P. anchorago* exposed to MV-127 ($F_{5,114} = 2.5$, $P = 0.035$), though not dose-dependent. The highest duration was 4.7±0.2 days at the second lowest and highest dose rate. These groups were significantly different from the oil control at $P < 0.05$.

In the MV-127 bioassay, morphogenetic defects were observed in 2.6% (16 nl/cm^2), 30.0% (40 nl/cm^2) and 50% (100 nl/cm^2) of the imagos of *C. bipustulatus*. The main defects were malformed elytra. In a few cases, legs were missing or malformed. No abnormalities were noted at lower dose rates. At higher rates, all test individuals died before reaching the adult stage. Percentages of malformed imagos in the MV-095 bioassay (series I) were 1.8% (6.25 nl/cm^2), 19.0% (12.5 nl/cm^2) and 53.6% (25 nl/cm^2). Again, no malformed beetles were found at lower dose rates. At the highest rate, the one larva that developed into the adult stage showed no malformations. No morphogenetic defects were recorded in *P. anchorago* (MV-127).

4. Discussion

4.1. Risk assessment

At the standard application rate, all botanicals were toxic to 4th instar *C. bipustulatus*. Hazard quotients (application rate divided by the LR_{50}) calculated according to the EPPO (2003) risk assessment scheme ranged between 0.7 (MV-128) and 1.3 (MV-095). Insecticides with a quotient of less than 2 are considered to pose a low risk to non-target arthropods. However, this threshold value would be exceeded at higher dose rates or when conducting multiple applications (EPPO, 2003). Following EPPO recommendations, higher tier semi-field or field tests would be necessary to conclude the risk assessment for *C. bipustulatus*.

Many seemingly unharmed beetles died within 1 week after adult eclosure (Fig. 2). LR_{50}s would have been overestimated, and hazard quotients underestimated, had the tests ended with successful adult eclosure. Therefore, and because exposure time was limited (one larval stage exposed for 2 days), our risk assessment must be considered as liberal.

Surprisingly, 4th instar *P. anchorago* were far less susceptible than *C. bipustulatus*. The hazard quotient of MV-095, the most toxic agent, was only 0.09 (calculated from Peveling and Demba, 1997), which is about 1-14th of the one for *C. bipustulatus*. The difference in susceptibility was even more evident for MV-127, a formulation as toxic to *C. bipustulatus* as MV-095 (Table 2). Even the highest dose, which killed 100% of *C. bipustulatus* larvae, had no lethal effect on *P. anchorago*. The only discernable effect was a slightly extended larval stage (+0.7 days). According to EPPO criteria, both botanicals are therefore classified as posing a low risk to *P. anchorago*.

Several factors may explain the differential susceptibility of the two species. First, *C. bipustulatus* is larger than *P. anchorago*. The larvae are vigorous predators consuming up to 300 scales per day (Ely, 1996). No data are available for *P. anchorago*, but its predation rate is known to be much lower. It is therefore possible that *C. bipustulatus* larvae ingested, relative to their body size, more scales and hence more active ingredients than *P. anchorago*. Second, different feeding strategies (prey size and stage selection, feeding mechanics, proportion of scale covering in the diet) may lead to different insecticide exposure. Third, the two species may have a genuinely different susceptibility to the botanical formulations, e.g., a different capability to detoxify and eliminate active compounds.

The results show that extrapolation from one species to another may lead to erroneous risk classifications. It follows that separate risk assessments may be justified if predator guilds contain several species of economic importance belonging to the same family. Risk mitigation measures should then be designed to protect the most sensitive and economically important species, in the present study *C. bipustulatus*. Only one other study has examined the effect of the same meliacious botanical on different coccinellid species (Smith and Krischik, 2000). However, this study used adult beetles and is therefore not comparable. Besides, it found no adverse effects at all. Thus, our study provides the first evidence of differential susceptibly of coccinellids from the same predator guild to meliacious botanicals.

4.2. Formulation

Our results confirm findings that oil formulations are more harmful to ladybirds than EC formulations (Ahmad et al., 2003; Basedow et al., 2003). Vegetable oils have low volatility. Thus, larvae were exposed to relatively persistent oil films, resulting in enhanced insecticide uptake via the lipophilic cuticle.

4.3. Sublethal effects

Treated *C. bipustulatus* required a longer time to complete the larval stage. Even *P. anchorago* larvae were affected (MV-127). The formation of *dauer larvae* (Schlüter, 1995) is a common effect of meliacious insecticides (Banken and Stark, 1997; Ulrichs et al., 2001). For example, one 4th instar larva of *P. anchorago* treated with MV-095 survived for 45 days (Peveling and Demba, 1997).

The percentage of adult beetles with morphogenetic defects was highest at three (MV-095) and nine (MV-127) times the recommended dose rate. Thus, malformed imagos are unlikely to be encountered under field conditions. Similar to other studies with coccinellids (Banken and Stark, 1997; Kaethner, 1991; Schmutterer, 1997), malformed elytra and wings were the most frequent abnormalities.

4.4. Mode of application

The mode of application did not affect the outcome of the bioassays, despite a three-fold difference in volume application rate. Thus, a microapplicator or micropipette can be used if a spray tower is not available. Nevertheless, preference should generally be given to sprayers that are more representative of field applications.

4.5. Conclusions

It is a common attitude that laboratory bioassays overestimate insecticide risks (Schmutterer, 1997; Schaub et al., 2002). Yet, there is also evidence of the contrary. Banken and Stark (1998) found that neem insecticides classified as harmless to *Coccinella septempunctata* L. based on single exposure laboratory bioassays (Banken and Stark, 1997) were in fact harmful when tested under more realistic, multiple exposure conditions in microcosm experiments. Several authors proposed that botanical (neem) insecticides in biological or integrated pest management schemes must be used carefully to minimise side effects on ladybirds (Banken and Stark, 1998; Ahmad et al., 2003). The results from our study support this note of caution.

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