Effects of bio-pesticides on *Eretmocerus warrae* (Hym., Aphelinidae), a parasitoid of *Bemisia tabaci* (Hom., Aleyrodidae)

P. Kumar[1], M. Whitten[2], G. Thoeming[3], C. Borgemeister[4] & H.-M. Poehling[5]

1 Department of Entomology and Agricultural Zoology, Rajendra Agriculture University, Pusa, Samastipur, India
2 Department of Integrative Biology, University of Queensland, Qld, Brisbane, Australia
3 Department of Ecological Plant Protection, University of Kassel, Witzenhausen, Germany
4 ICIPE, Nairobi, Kenya
5 Institute of Plant Protection and Plant Diseases, Leibniz Universität Hannover, Hannover, Germany

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Correspondence
H.-M. Poehling (corresponding author), Institute of Plant Diseases and Plant Protection, Leibniz Universität Hannover, Herrenhäuser Street 2, 30419 Hannover, Germany.
E-mail: poehling@ipp.uni-hannover.de

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Abstract

The sweet potato whitefly *Bemisia tabaci* (WF) can be controlled by two commercial neem products, NeemAzal-T/S® (1% azadirachtin) for foliar application, and NeemAzal-U (17% azadirachtin) for soil application, alongwith two biorational products of microbial origin, Abamectin (avermectin) and Success® (spinosad). Side effects of these products were tested in a laboratory bioassay against a native aphelinid, *Eretmocerus warrae* (EW). Eggs and early larval instars of the parasitoid, commonly found outside the host body, were highly susceptible to foliar spray of neem with only 8%, 18% and 55% emergences of adults from treated eggs. larval and pupal stages respectively at recommended dose-rates of 5 ml/l and 1%, 8% and 40% at twice recommend dose-rate (10 ml/l). Soil application with NeemAzal-U marginally affected EW. At highest tested dose-rate of 3.0 g/l, 46%, 64% and 81% emergence was recorded after treatement of plants harbouring WF parasitized by egg, larval and pupal stages of EW respectively. In contrast to neem application, Success® and Abamectin caused high mortality in development stages of the parasitoids. In particular, abamectin was highly toxic to the parasitoids with less than 1% emergence from either of the three development stages if treated with 1–2 ml/l.

Introduction

Whiteflies (Homoptera; Aleyrodidae) are key pests of vegetable, ornamental and agronomic crops throughout the world (van Lenteren and Noldus 1990; Gerling and Mayer 1996). The geminiviruses transmitting sweet potato whitefly (WF) *Bemisia tabaci* Gennadius (Hom., Aleyrodidae) is one of the major production constraints for successful tomato cultivation in the field and greenhouses in the humid tropics (Attathom et al. 1990; Kumar et al. 2005).

Chemical management is costly and, at best, provides only partial control for WF because of rapid development of resistance, a problem worldwide (Prabhaker et al. 1989; Dittrich et al. 1990; Cahill et al. 1995; Byrne et al. 2003). For these reasons, research was focused on biological control agents (for overviews of biocontrol options: Gerling 1990; Gerling and Mayer 1996; van Lenteren and Martin 1999; Gerling et al. 2001). Most successfully used worldwide against whiteflies in general are parasitoids dominated by two genera of aphelenid hymenopterans, *Encarsia* and *Eretmocerus*. Concerning WF different *Eretmocerus* species with widespread natural distribution in all warmer regions of the world such as *Eretmocerus eremicus* Rose and Zolnerowich and *Eretmocerus mundus* Mercet., are described as effective parasitoids (Avidov 1956; Rodryguez et al. 1994; Hoddle et al. 1998; Qiu et al. 2004). In our study area in Thailand, different *Eretmocerus* species such as

E. transvena Timberkale or *E. adrianae* Lopez-Avila are commonly found with apparent field parasitism rates up to 65% (Kirk et al. 2000). Recent exploration revealed the presence of additional species identified as *Eretmocerus nr. warrae* (EW). EW is an ecto-endoparasitoid. Females oviposit between the venter of the host nymph and the leaf surface. The first instar larva penetrates into the host which engulfs the young larva by a kind of cellular capsule protecting the young larvae from the host's immune response during a first dormant phase (Gerling 1990). Once the host reaches the pupal stage the capsule is dissolved and the larval parasitoid starts to digest the host tissue.

Preliminary experiments suggested that EW may be an effective biocontrol agent for WF in protected cultivation systems. Because of the mostly complex pest communities (WF, thrips, leafminers) occurring in tropical greenhouses releases of single specific natural enemies are often insufficient to keep the crop economically viable and integrative approaches by combining different natural enemies or with pesticides are more promising, however selective use of pesticides is a prerequisite then.

Bio-rational pesticides, derived from plants or microbes, represent potentially convenient ingredients matching requirements of integrated pest management (IPM) systems (Thompson and Hutchins 1999; Kumar et al. 2005; Kumar and Poehling 2006). In previous studies (Kumar et al. 2005; Kumar and Poehling 2007), we assessed the effects of these bio-rational pesticides on WF. However, any information on the possible side effects of these pesticides on the above mentioned candidate biocontrol agents are still missing. Therefore, this paper will elucidate the effect of neem, spinosad and abamectin on the growth and development of EW to optimise sustainable control of WF in protected cultivation in the humid tropics.

Materials and Methods

Plant sources

The experiments were conducted using 2-week-old potted (pot size-7.5 cm high and 6.5 cm Ø) tomato plants (*Lycopersicon esculentum* Mill (Solanaceae), cv. King Kong II) with two fully expanded leaves (plant– 16 ± 2 cm tall) in air conditioned rooms at 26 ± 3°C and 60–70% relative humidity (RH). The plants were grown in a locally available substrate composed of silt, sand and clay (39.2%, 29.9% and 30.9%, respectively) and organic mater of 27.9%. Pots were watered manually with tap water and grown without any additional fertilization.

Whitefly and parasitoid cultures

The initial WF culture, obtained from the Department of Agriculture (Virology section, *Chatuchak*, Bangkok), had been maintained without any pesticide exposure for the two previous years. Individuals for the experiments were drawn from mass reared cultures established on tomatoes grown inside air conditioned rooms. The WF cultures were kept in insect-proof Plexiglas cages (120 × 65 × 65 cm with top and side walls covered with 52 mesh nylon nets) at 26 ± 2°C and 60–70% RH. WF stages of same age, i.e. L1, L2 and adults, were obtained by allowing female WF to lay eggs for 24 h on caged tomato plants. Adults were then removed from the cages using an aspirator. Plants with eggs were further cultivated for synchronized development of WF. Plants with L1, L2, L3 or pupae were used for the experiments (see below); or kept until adult emergence in order to obtain adults of similar age.

The parasitoid, *Eretmocerus nr. warrae*, was initially collected in August 2002 from WF infested tomato plants in the greenhouse complex at the Asian Institute of Technology (AIT), Bangkok, Thailand and later mass reared on WF-infested tomato plants under laboratory conditions, using first and second larval instars of WF. Parasitoid adults of the same age were produced using similar procedure as described for WF above. The parasitoid species was identified by Dr Stefan Schmidt, Hymenoptera Section, Zoologische Staatssammlung Muenchen, Muenchhausenstr. 21, 81247 Munich, Germany. All experiments were conducted in air-conditioned laboratory rooms at the AIT campus with 6–18 plants (one plant was used as one replication) per experiment; and repeated twice in time. The experiments were conducted in acrylic cages (45 cm × 40 cm × 40 cm) with upper side and two perforated side holes (25 cm Ø) covered by 72-mesh net (Econet®; Ludvig, Swensson, Sweden) to allow sufficient ventilation.

Bio-pesticides (NeemAzal-U, NeemAzal®-TS, Success® and Abamectin)

NeemAzal-U, NeemAzal®-T/S, Success® and Abamectin were tested in different dilutions of the stock product in tap water (see table 1). NeemAzal-U and NeemAzal®-T/S solutions were prepared, based

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Table 1 Biorational pesticides tested against *Eretmocerus nr warrae*

Biopesticides (Bp)	Active ingredients (a.i.)	Concentrations used: Bp g or ml (a.i. mg)/l	Manufacturers
NeemAzal-U	Azadirachtin 17%	0.75 g (127.5 mg) 1.5 g (255 mg) 2.25 g (382.5 mg) 3.0 g (510 mg)	Trifolio–M GmbH, Lahnau, Germany
NeemAzal®-T/S	Azadirachtin 1%	5 ml (50 mg) 10 ml (100 mg) 15 ml (150 mg)	Trifolio–M GmbH, Lahnau, Germany
Success®	Spinosad 12%	2 ml (240 mg) 4 ml (480 mg)	Dow Agrosciences, Indianapolis, IN, USA
Abamectin	Avermectin 1.8%	2 ml (36 mg) 4 ml (72 mg)	Exphoreflex, Industrial, Thailand; Imported by: Inter Crop Co., Ltd, Bangkok, Thailand

on recommended dose rates and according to previous studies (Kumar et al. 2005). NeemAzal-U is specifically developed for soil drenching, whereas NeemAzal®-TS is registered for spray applications. As Success® and Abamectin are not registered for controlling *B. tabaci* in Thailand, the concentrations of Success® and Abamectin used were based on our previously reported study (Kumar and Poehling 2007). Approximately 50 ml of the test solutions were used to drench the growing substrate (NeemAzal-U); or were uniformly sprayed (NeemAzal-TS, Abamectin, Spinosad) on the top and bottom surfaces of leaves until runoff, using a 1l capacity locally available handheld sprayer Apollo Sprayer (Apollo International Spray Co. Ltd, Bangkok, Thailand).

Experiment 1. Effect of soil and foliar application of neem on the different development stages and longevity of EW

Under laboratory conditions (see above) EW completed its life cycle in 12 (±2)days. Three developmental stages of EW were selected from the synchronized cultures: eggs just deposited with 8–9 days old WF L2; larval stages about 7 days old (WF 15–16 days); and pupal stages after 10 days when fully developed parasitoids were visible inside WF pupae (WF 18–19 days). After reaching these stages, host plants were treated with three concentrations each of Neem-Azal-U (0.75, 1.50 and 3.0 g/l) drench application and Neem-Azal-T/S (1.0, 5.0 and 10.0 ml/l) foliar spraying with tap-water as a control.

The treated plants were kept inside ventilated plexi-glass cylinders (30 cm high and 10 cm Ø, 100 μm mesh size). Approximately, after 13, 8 and 3 days (d), adult emergence started in the different age groups, and enclosed adults were counted. In addition, at the end of experiments, the numbers of emerging parasitoids were re-confirmed by counting the round opening holes on the WF pupal case; and a 'T' shaped opening in the case of WF adults. For longevity evaluation, a single pair of newly emerged (male and female) parasitoids was confined inside a 14 cm diameter petri dish lined with filter paper. The lid of the dish was perforated with 3 holes each 3 cm diameter. Two holes were covered with nylon tissue (pore size 64 μm) to serve as ventilation holes while the third served as entry and exit outlet and for feeding. This comprised a solution of honey and water (1 : 10 ratio). Dead parasitoids were removed and counted daily. Since only a few parasitoids emerged with the foliar neem applied at the egg stage, only six replications split over three times could be evaluated for adult EW longevity.

A parallel experiment with plants bearing unparasitized WF was run to compare the pesticidal effects on parasitized and non-parasitized WF. This involved 18 replications split over three time periods.

Experiment 2. Effect of spinosad and abamectin on different development stages and longevity of EW

An experimental procedure similar to the foliar neem treatments (see Experiment 1) was performed to evaluate the effect of spinosad (Success®) and abamectin at dose-rates of 2 and 4 ml/l of water, with tap-water treatments as controls. Longevity of the emerged EW for abamectin and spinosad were assessed as per the procedure discussed above. Since no parasitoids emerged from abamectin-treated plants at dose-rate of 4 ml/l, longevity measurement with this treatment was not possible. Moreover,

since fewer parasitoids emerged in this series of experiments, a total of six replications for each treatment were performed over three time periods.

Statistical analyses

Data of different life stages (time intervals of treatment) were analysed separately and not combined. Data with percentage adult emergences and longevity were subjected to Levene test to check for homogeneity of variance and normality. In the case of non-homogeneity, percent values were transformed using arcsine–square-root (arcsin$\sqrt{}$) transformation. Longevity in days was transformed by square-root ($\sqrt{}$) transformation before running an ANOVA (PROC GLM, SAS Institute, Cary, NC, 1999). ANOVA was performed separately for each life stage (time of treatment) to compare on the one hand the different Neem concentrations (and water treatment as control) used and the different treatments of abamectin and spinosad on the other hand. Where ANOVA yielded significant F-values, means were compared using Tukey's HSD procedure unless stated otherwise. A significance level of $\propto = 0.05$ was used in all analyses.

Results

Experiment 1: Effect of soil and foliar application of neem on different development stages and longevity of EW

Foliar application of NeemAzal-T/S significantly affected development of WF, when treated in different developmental stages corresponding to the egg, larva and pupa stage of the EW; L2 ($F = 3181.76$; d.f. = 3, 68; $P < 0.0001$); late L3 ($F = 671.24$; d.f. = 3, 68; $P < 0.0001$); pupa ($F = 604.52$; d.f. = 3, 68; $P < 0.0001$) (table 2; without parasitoid). A similar dose-dependent response was observed at the different development stages of the EW, egg ($F = 474.80$; d.f. = 3, 68; $P < 0.0001$); larva ($F = 436.48$; d.f. = 3, 68; $P < 0.0001$); pupa ($F = 222.43$; d.f. = 3, 68; $P < 0.0001$) (table 2; with parasitoid). Similarly, NeemAzal-T/S resulted in significant effects on the longevity of both male and females of EW, only when treatments were made at either egg (male, $F = 24.61$; d.f. = 3, 23; $P < 0.0001$; female, $F = 32.22$; d.f. = 3, 23; $P < 0.0001$) or larval stage (male, $F = 18.89$; d.f. = 3, 23; $P < 0.0001$; female, $F = 12.80$; d.f. = 3, 23; $P < 0.0001$). No adverse effect on longevity of either sexes was recorded when the pupa stage was treated with foliar NeemAzal-T/S (male, $F = 1.28$; d.f. = 3, 23; $P > 0.2891$; female, $F = 0.28$; d.f. = 3, 23; $P > 0.8744$) (table 3). The soil application of NeemAzal–U significantly affected all three corresponding ages of unparasitized WF: L2 ($F = 8181.76$; d.f. = 3, 68; $P < 0.0001$); later L3 ($F = 671.24$; d.f. = 3, 68; $P < 0.0001$); and pupa ($F = 604.82$; d.f. = 3, 68; $P < 0.0001$) (table 4; without parasitoid). Similar to the foliar neem spray, the soil application significantly affected emergence of the EW in a dose-dependent manner: egg ($F = 474.80$; d.f. = 3, 68; $P < 0.0001$); larva ($F = 486.48$; d.f. = 3, 68; $P < 0.0001$); pupa ($F = 222.48$; d.f. = 3, 68; $P < 0.0001$) (table 4; with parasitoid). Longevity of the emerged male and female parasitoid was reduced only at highest tested dose-rates of 3.0 g/l: egg stage (male, $F = 19.06$; d.f. = 3, 71; $P < 0.0001$; female, $F = 14.09$; d.f. = 3, 71; $P < 0.0001$); larval stage (male, $F = 18.72$; d.f. = 3, 71; $P < 0.0001$; female, $F = 12.21$; d.f. = 3, 71; $P < 0.0001$); and pupal stage (male, $F = 0.94$; d.f. = 3, 71; $P > 0.4266$; female, $F = 0.84$; d.f. = 3, 71; $P > 0.4772$) (table 5).

Table 2 Mean (±SE) percentages of adult emergence of *Eretmocerus warrae* (EW) and *Bemisia tabaci* (WF) adults, treated with different dose-rates of NeemAzal®-TS by foliar application

NeemAzal®-T/S dose-rates	Adult emergences %*		
Eretmocerus warrae	Egg	Larva	Pupae
Water (=0 ml/l)	90.56 ± 0.95a	91.33 ± 1.42a	92.33 ± 1.10a
1 ml/l	37.89 ± 1.43b	47.11 ± 2.29b	90.56 ± 1.60a
5 ml/l	8.33 ± 1.63c	18.56 ± 1.07c	55.78 ± 1.76b
10 ml/l	1.00 ± 0.37d	8.33 ± 1.26d	40.67 ± 1.46c
Bemisia tabaci	(L2; 8–9 d†)	(late L3; 15–16 d)	(Pupa; 18–19 d)
Water (=0 ml/l)	92.22 ± 0.68a	92.67 ± 1.51a	91.44 ± 1.03a
1 ml/l	30.11 ± 0.50b	40.44 ± 1.69b	71.78 ± 1.37b
5 ml/l	8.67 ± 0.76c	13.22 ± 0.83c	38.78 ± 1.28c
10 ml/l	0.00 ± 0.00d	6.44 ± 1.03d	20.44 ± 0.70d

Within columns, mean (±SE) percentages of adult emergence followed by the same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

*Mean values ± standard error (SE).

†d = post-oviposition period (days).

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Table 3 Longevity (days) of emerged *E. warrae* adults, treated with different dose-rates of NeemAzal®-TS by foliar application

	Mean (±SE) longevity (days) of *Eretmocerus warrae* (adults developed from treated eggs, larvae and pupa)					
	Egg stage		Larval stage		Pupal stage	
NeemAzal®-T/S dose-rates	Female	Male	Female	Male	Female	male
Water (=0 ml/l)	14.50 ± 0.22a	11.33 ± 0.21a	14.28 ± 0.31a	11.28 ± 0.29a	14.33 ± 0.32a	11.67 ± 0.39a
1 ml/l	14.33 ± 0.21a	11.17 ± 0.17a	14.22 ± 0.26a	11.00 ± 0.27a	14.22 ± 0.22a	11.50 ± 0.36a
5 ml/l	12.00 ± 0.26b	9.33 ± 0.33b	13.06 ± 0.45a	9.61 ± 0.26a	14.11 ± 0.23a	11.11 ± 0.36a
10 ml/l	11.67 ± 0.33b	9.17 ± 0.17b	12.00 ± 0.36b	8.78 ± 0.24b	14.06 ± 0.10a	10.72 ± 0.35a

Within columns, mean (±SE) longevity (in days) of male and female adult *Eretmocerus warrae followed* by the same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Table 4 Mean (±SE) percentages of adult emergence of *Eretmocerus warrae* (EW) and *Bemisia tabaci* (WF) adults, treated with different dose-rates of NeemAzal-U by soil treatment

NeemAzal-U Dose-rates	Adult emergences %*		
Eretmocerus warrae	Egg	Larva	Pupae
Water (=0 ml/l)	88.33 ± 1.06a	87.56 ± 2.26a	89.56 ± 1.43a
0.75 g/l	73.89 ± 2.41b	86.44 ± 1.55a	88.33 ± 0.80a
1.50 g/l	52.78 ± 1.84c	71.00 ± 0.95b	87.33 ± 1.12a
3.0 g/l	46.22 ± 2.16c	64.11 ± 3.23b	81.00 ± 2.10b
Bemisia tabaci	(L2; 8–9 d†)	(late L3; 15–16 d)	(Pupa; 18–19 d)
Water (=0 ml/l)	86.56 ± 1.06a	86.78 ± 2.01a	91.44 ± 1.29a
0.75 g/l	68.67 ± 2.70b	66.56 ± 4.51b	69.22 ± 2.88b
1.50 g/l	49.33 ± 1.60c	49.67 ± 2.38c	56.89 ± 2.11c
3.0 g/l	36.44 ± 1.92d	29.56 ± 0.85d	28.78 ± 1.00d

Within columns, mean (±SE) percentages of adult emergence followed by the same letters are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).
*Mean values ± standard error (SE).
†d = post-oviposition period (days).

Table 5 Longevity (days) of the emerged EW adults, treated with different dose-rates of NeemAzal-U by soil treatments

	Mean (±SE) Longevity of *Eretmocerus warrae* adults (days) (adults developed from treated eggs, larvae and pupa)					
	Egg stage		Larval stage		Pupal stage	
NeemAzal-U Dose-rates	Female	Male	Female	Male	Female	Male
Water (=0 g/l)	14.11 ± 0.27a	11.17 ± 0.27a	14.28 ± 0.31a	11.28 ± 0.29a	14.06 ± 0.31a	11.56 ± 0.35a
0.75 g/l	14.22 ± 0.10a	11.06 ± 0.27a	13.00 ± 0.38a	10.87 ± 0.26a	14.22 ± 0.15a	11.78 ± 0.26a
1.50 g/l	13.56 ± 0.22a	10.94 ± 0.32a	12.89 ± 0.37a	9.98 ± 0.90a	14.06 ± 0.19a	11.56 ± 0.24a
3.0 g/l	12.00 ± 0.30b	9.00 ± 0.24b	11.98 ± 0.28b	9.17 ± 0.33b	13.72 ± 0.18a	11.33 ± 0.14a

Within columns, mean (±SE) longevity (in days) of male and female adult *Eretmocerus warrae* followed by the same letter are not significantly different ($P > 0.05$, ANOVA, Tukey's HSD test).

Experiment 2: Effect of spinosad and abamectin on the different development stages and longevity of the EW

Treatments of various development stages of WF with spinosad and abamectin significantly reduced emergences of WF: L2 ($F = 929.09$; d.f. = 4, 89; $P < 0.0001$); late L3 ($F = 780.73$; d.f. = 4, 89; $P < 0.0001$); and pupa ($F = 714.79$; d.f. = 4, 89; $P < 0.0001$). No emergences of WF were observed when larval and pupal stages of WF were treated with abamectin at dose-rates of 2 ml/l (table 6, without parasitoids).

All developmental stages of EW tested were found to be highly susceptible to these bio-rational pesticides; and, in general, only few parasitoids emerged

Table 6 Mean (±SE) percentages of adult emergence of *Eretmocerus warrae* and *Bemisia tabaci* adults, treated with different dose-rates of bio-pesticides (Spinosad and Abamectin)

Biorational pesticides Dose-rates	Adult emergence %*		
Eretmocerus warrae	Egg	Larva	Pupa
Water (=0 ml/l)	87.56 ± 0.57a	86.33 ± 2.05a	88.78 ± 1.14a
Spinosad 2 ml/l	3.33 ± 0.49b	8.22 ± 1.00b	14.67 ± 1.19b
Spinosad 4 ml/l	0.89 ± 0.33c	3.89 ± 0.68c	4.67 ± 0.97c
Abamectin 2 ml/l	1.44 ± 0.48c	0.83 ± 0.28d	1.06 ± 0.35d
Abamectin 4 ml/l	0.39 ± 0.12c	0.06 ± 0.06d	0.00 ± 0.00d
Bemisia tabaci	(L2; 8–9 d†)	(late L3; 15–16 d)	(Pupa; 18–19 d)
Water (=0 ml/l)	86.44 ±0.72a	86.44 ± 2.14a	88.67 ± 1.18a
Spinosad 2 ml/l	2.44 ± 0.66b	0.89 ± 0.49b	1.56 ± 0.80b
Spinosad 4 ml/l	0.11 ± 0.11bc	0.56 ± 0.27b	1.00 ± 0.46b
Abamectin 2 ml/l	1.00 ± 0.33c	0.78 ± 0.24b	1.11 ± 0.37b
Abamectin 4 ml/l	0.22 ± 0.15c	0.00 ± 0.00b	0.00 ± 0.00b

Within columns, mean (±SE) percentages of adult emergence followed by the same letters are not significantly different (P > 0.05, ANOVA, Tukey's HSD test).
*Mean values ± standard error (SE).
†d = post-oviposition period (days).

from the treated leaves. Emergence rates from differently treated stages such as egg stage ($F = 1074.40$; d.f. = 4, 89; $P < 0.0001$) larval stage ($F = 689.88$; d.f. = 4, 89; $P < 0.0001$) and pupal stage ($F = 679.86$; d.f. = 4, 89; $P < 0.0001$) were significantly different. Very few to nil parasitoids emerged with abamectin (table 6, with parasitoids).

Unlike neem, where little or no effect of treatments on longevity of emerged adults was observed, spinosad and abamectin seriously affected longevity of EW: egg stage (male; $F = 196.97$; d.f. = 3, 23; $P < 0.0001$; female, $F = 276.91$; d.f. = 3, 23; $P < 0.0001$); larval stage (male; $F = 124.26$; d.f. = 3, 23; $P < 0.0001$; female, $F = 271.26$; d.f. = 3, 23; $P < 0.0001$); and pupal stage (male; $F = 119.62$; d.f. = 3, 23 $P < 0.0001$; female, $F = 242.68$; d.f. = 3, 23; $P < 0.0001$) (table 7).

Discussion

There are some reports available dealing with side effects of neem products against natural enemies (e.g. Spollen and Isman 1996; Tedeschi et al. 2001; more specific reported studies pertaining to the results obtained are listed below) and biorational pesticides like spinosad and abamectin (Consoli et al. 2001; Takahashi et al. 2005). However, regarding WF control data on the impact of different application methods (foliar, soil) of neem and actinomycete biorationals on parasitoids like EW, as a prospective parasitoid species for biocontrol of WF, are missing.

Effects of neem on different development stages of the EW

Moreover, our results showed that the susceptibility of both WF and EW for the tested neem treatments depends on the development stages treated, the methods of neem application and dose-rate. We already described the different susceptibility of WF stages to the foliar application of neem. Eggs and hatching immatures were found most susceptible followed by other immature stages and pupa (Kumar

Table 7 Longevity (days) of the emerged EW adults, treated with different dose-rates of bio-rational pesticides (Spinosad and Abamectin)

Biorational pesticides dose-rates	Mean (±SE) Longevity of *Eretmocerus warrae* adults (days) (adults developed from treated eggs, larvae and pupa)					
	Egg stage		Larval stage		Pupal stage	
	Female	Male	Female	Male	Female	Male
Water	14.33 ± 0.21a	11.33 ± 0.33a	14.17 ± 0.17a	11.50 ± 0.67a	14.17 ± 0.31a	11.50 ± 0.67a
Spinosad 2 ml/l	6.33 ± 0.33b	3.83 ± 0.31b	5.67 ± 0.42b	3.63 ± 0.31b	5.17 ± 0.48b	3.17 ± 0.31b
Spinosad 4 ml/l	4.33 ± 0.21c	2.33 ± 0.21c	3.17 ± 0.17c	2.90 ± 0.22c	3.00 ± 0.00c	2.00 ± 0.26c
Abamectin 2 ml/l	3.33 ± 0.21d	2.17 ± 0.17c	2.83 ± 0.17c	1.50 ± 0.22c	2.33 ± 0.21c	1.33 ± 0.21c

Within columns, mean (±SE) longevity (in days) of male and female adult *Eretmocerus warrae* followed by the same letter is not significantly different (P > 0.05, ANOVA, Tukey's HSD test).

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et al. 2005). Similarly, it is obvious from our experiments though not directly compared that the egg stage and hatching larvae of EW are most susceptible to neem ingredients followed by larva and pupa stage independent of the application method (foliar or soil). For example, using the recommended dose-rates of NeemAzal-T/S for spray application (5 ml/l, 50 mg a.i./l) emergence rates of 8%, 18% and 55% at egg, larval and pupal stages were observed, respectively. Similarly, after soil treatment with the highest dose rate emergences of 46%, 64% and 81% (egg. larval, pupal stages, respectively) were recorded. Soil application resulted in relatively low effects on emergences of EW and WF treated in different developmental stages whereas with foliar treatments high mortality rates were obtained. Due to the mode of action of neem ingredients, especially the growth regulating properties, young insect stages in general are most sensitive to a.i. of neem (Schmutterer 1990; Banken and Stark 1997). In accordance, we recorded strong effects on egg and larvae stages whereas the pupal stage seems to be of low sensitivity. Moreover, the very low survival of treated eggs, especially after foliar treatment, could be a result of the specific parasitization behaviour of EW. The eggs were deposited outside the host-body, which makes them and the newly hatched larva most susceptible to contact toxicity of neem after such foliar treatments. The larval stage of EW inside the host body is also vulnerable to neem, which could be due to the ingestion of active ingredient by growing EW immatures with the host tissue. In addition, the presence of a chew hole at host body could facilitate the entry of neem, and, thereby increasing the chances of direct contact with neem ingredients after spray treatments. Once the EW reached the 'closed' pupal stage, the susceptibility decreases as it was protected inside the cocoon, and, risk of direct contamination with applied neem either through food or contact is drastically reduced.

Overall, the foliar application of NeemAzal-T/S showed very strong effects to both WF and EW. The soil application of NeemAzal-U however seems to by relatively safe for the parasitoids. Due to that low detrimental effect on EW, soil treatment with neem should be a convenient option for integrated WF control. However the moderate detrimental effect of soil neem application against whiteflies necessitates a comprehensive study integrating both control factors before giving practical advices. It has to be shown that the limited neem efficacy of soil treatments to WF could be compensated by the parasitoid effect.

Effect of spinosad and abamectin on EW

In our studies, spinosad and abamectin had strong toxic effects on EW after spray treatments on leaves bearing parasitized larvae of WF at different ages (egg, larva and pupa). Concerning the used dose rates, abamectin showed stronger effects than spinosad for any given development stage of EW in all experiments. Very few EW emerged from all tested development stages treated even with low dose-rates of abamectin, indicating a strong detrimental effect on the parasitoid. Similarly to previous experiments, very high mortalities of WF were also observed for all tested development stages (Kumar and Poehling 2007). The growing EW inside host body turned dark brown to black within 24–48 h post-treatments. Thus, a very fast mode of action of abamectin was obvious.

Spinosad showed slightly minor effects on WF and EW. However, only 3%, 8% and 14% and 0.89%, 3.89% and 4.67% emergences of EW (egg, larvae and pupal stage of EW) were observed at 2 and 4 ml/l dose-rates of spinosad, respectively, pointing out strong side effects of spinosad on the parasitoid. It also affected the longevity of both sexes of adult EW, e.g. from 14 days for females and 11 days for males treated with water compared to 1–2 days in case of spinosad treatments. The obviously much stronger detrimental effects for all life stages of WF and EW by the two actinomycetes products compared to the neem treatments could be mainly a result of the different modes of action. Preparations of actinomycetes like spinosad and abamectin affect the nervous system of insects (effect on nicotinic acetylcholine receptor and stimulation of neurotransmitter Gamma-aminobutyric acid production, respectively). Thus, after feeding contaminated plant parts an irreversible paralysis of the insect in all developmental stages occurs, resulting in rapid mortality (Gillham 2005). In contrast, neem ingredients are affecting the metamorphosis of insects (inhibition of the release of prothoracicotropic hormones, allatotropins and allatoinhibins), but moreover, manipulating the feeding behaviour, reproduction, growth, fitness and mobility as well as resulting in repellent effects (Schmutterer 1990; Banken and Stark 1997). Thus, neem shows varying and slow effects on insect development, affecting mostly the young stages and resulting only sometimes in the death of the insect.

Jones et al. (2005) found spinosad to be highly toxic for *Encarsia formosa* even 28 days after application, which corroborates our assumption of strong side effects of spinosad on parasitoids. Moreover,

Penagos et al. (2005) reported on 100% and 70% reduction of the reproduction of *Chelonus insularies* Cresson (Hymenoptera: Braconidae), the parasitoid of *Spodoptera frugiperda* J. E. Smith (Lepidoptera: Noctuidae), after application of spinosad at dosage rates of 200 and 20 ppm, respectively, to eggs of the parasitoid. Newman et al. (2004) found that Spinosad (Success®) at the field rate (96 g a.i./ha) caused 100% corrected mortality of the leafroller parasitoid *Dolichogenidea tasmanica* (Hym.: Braconidae).

For abamectin, side effects on some natural enemies were reported in several studies: Williams et al. (2003) described it as toxic for the egg parasitoid *Anaphes iole* (Hymenoptera: Mymaridae). Prijono et al. (2004) reported moderate toxicity and advised to use abamectin cautiously against the three parasitoids *Hemiptarsenus varicornis* Gerault (Hymenoptera: Eulophidae), *Opius* sp. (Hymenoptera: Braconidae), and *Gronotoma micromorpha* Perkins (Hymenoptera: Eucoilidae) commonly found as parasitoids of *L. huidobrensis* in Indonesia. In a recent study, larval and pupal mortality and sublethal effects of abamectin (dose rate 9.2 mg a.i./l) on two common Australian leafminer parasitoids, *Hemiptarsenus varicornis* Girault (Hymenoptera: Eulophidae) and *Diglyphus isaea* Walker (Hymenoptera: Eulophidae) were tested and, significant effects on mortality to larvae and pupae of both parasitoid species were recorded (Bjorksten and Robinson 2005).

In conclusion, both actinomycetes pesticide showed strong effects on WF, but, were also highly toxic to the growth and development of the EW. In contrast, neem applications were obviously a safer option regarding growth and development of the parasitoid. Due to the specific behaviour of the studied parasitoid with a relative long development phase outside the host (3–4 days) and uptake of host tissue by chewing on the (contaminated) hostbody, any kind of foliar application makes it highly vulnerable. Thus, for a sustainable control strategy relying of biocontrol with parasitoid treatments as a first option, soil treatments with Neem could be the most gentle way of additional insecticide applications if necessary to support the natural enemy. Only in case of strong pest pressure foliar treatments with neem or finally biorationals like spinosad and abamectin should be considered for IPM of WF.

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