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LABORATORY AND FIELD EVALUATION OF DELTAMETHRIN AND BENDIOCARB TO CONTROL *CIMEX LECTULARIUS* (HETEROPTERA: CIMICIDAE)

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Abstract Pyrethroid, carbamate, and organophosphate insecticides are frequently used for bed bug control. A baseline susceptibility study for commonly used insecticides was carried out with a susceptible *C. lectularius* laboratory strain. LD_{50}-values against a susceptible strain in a contact bioassay format are 1.4 mg a.i./m², and 6.5 mg a.i./m² for deltamethrin, and bendiocarb, respectively. The studies are supplemented with biochemical investigations on enzyme systems commonly known to confer resistance in insect pests. Data from laboratory trials are compared to field trials with deltamethrin and the combination of deltamethrin with natural pyrethrins plus the synergist piperonyl butoxide as tank mix. This combination achieved control results against strong infestations and less susceptible *C. lectularius*.
Key Words Bed bug control, pyrethrins

INTRODUCTION

The temperate bed bug *Cimex lectularius* (Heteroptera, Cimicidae) was common in many parts of the world until the first half of the 20th century. With the development of effective insecticides, better sanitary conditions and pest awareness of the general public, bed bugs became rare over several decades. However, an apparent resurgence has been noted over the past 15 years (Boase, 2001, 2007; Gulmahamed, 2002; Doggett et al., 2004; Romero et al., 2007). Due to their ability to hide in narrow cracks and crevices and to survive without feeding over several weeks or months, depending on the development stage, bed bugs are hard to control, and often require a second or even third treatment until eradication.

Because of the increased bed bug problems a susceptibility baseline of commonly used insecticides against bed bugs should therefore be established to be compared with field strains with regard to possible resistance development.

MATERIAL AND METHODS

Laboratory Trials

The LD50/LD95 for the technical compounds deltamethrin and bendiocarb were assessed on filter paper disks with 10 cm dia (71 cm²) which were placed on aluminium foil and treated with 1.5 ml acetone + insecticide in different concentrations. The amount of insecticide was expressed in mg/m². A glass ring was placed 24 hr later on the treated filter paper and 10 adult bed bugs (males and females) were introduced. Mortality was assessed at 3, 6 and 24 hours. Dead bugs were transferred into new containers and observed for the 3 following days.

The products Deltamethrin 10 SC (Crackdown®, registered trademark of Bayer Group) and Bendiocarb 80 WP (Ficam® W, registered trademark of Bayer Group) were mixed with water and a serial dose range was prepared with the factor 3. The treatment of the filter paper disks and the trial design was done in the same way as described for the technical compounds. The LD 50 and LD 95 values, 24 hr post-exposure, were calculated with MS Excel-add-in XLfit (IDBS, UL, Vs. 3.0), dose response model 205.

The residual activity of bendiocarb and deltamethrin was assessed on plywood panels and glazed tiles. The surfaces were sprayed with 96 mg a.i./m² and 120 mg a.i./m² of bendiocarb, respectively. Deltamethrin was applied at a rate of 15 mg a.i./m². The efficacy of the dried surfaces was tested against *C. lectularius* at 1 DAT, 1 WAT, 2 WAT, 4 WAT, and 6 WAT. A susceptible *C. lectularius* laboratory strain (Bayer Environmental Science, Monheim, Germany) was used in these trials. The LT 50 and LT 95 values were calculated with MS Excel-add-in XLfit (IDBS, UL, Vs. 3.0), dose response model 205.

Biochemical Assays

The activity of enzymes such as carboxylesterases, glutathione S-transferases and microsomal monooxygenases, known to be involved in insecticide detoxification and resistance was also measured to the following methods.

Carboxylesterases. One adult *C. lectularius* was manually homogenized in 1.5 ml ice-cold 0.1M sodium phosphate buffer, pH 7.6 containing 0.1% (w/w) Triton X-100. The homogenate was centrifuged at 4°C, 10,000 g, for 5 minutes and the resulting supernatant was used as the enzyme source for the assay. The staining solution was prepared dissolving 15 mg of Fast Blue RR salt in 25 ml 0.2M sodium phosphate buffer, pH 6.0. The solution was filtered and 250 µl of 1-naphthylacetate (100 mM in acetone) was added. For bioassay purposes 25 µl of homogenate and 25 µl of 0.2M sodium phosphate buffer, pH 6.0, were added into the wells of a 96-well-microplate. Controls were prepared using buffer only instead of homogenate. After the addition of 200 µl staining solution to each well, the carboxylesterase activity was measured for 10 min at 450 nm and 23°C in a Thermomax kinetic microplate reader (Molecular devices). A standard curve of 1-naphthol was used to calculate enzyme activity. Protein concentrations were measured according to Bradford (1976).

Monooxygenases: For the monooxygenases assays a microsomal fraction was prepared. Adult bed bugs were manually homogenized in ice-cold 0.1M sodium phosphate buffer, pH 7.6, containing 1 mM EDTA, 1 mM DTT, 200 mM sucrose. The homogenate was then centrifuged at 4°C, and 5,000 g for 5 min. The pernatant was centrifuged again at 4°C, and 15,000 g for 15 min. The resulting pellet was discarded and the supernatant was centrifuged at 4°C, and 100,000 g for 60 min. After ultracentrifugation the upernatant was discarded and the pellet (microsomes) was re-suspended in 0.1 M phosphate buffer pH 7.6 and used for monoxygenase activity assays. The assay was done using 7-ethoxycoumarin as a substrate according to the method recently described by Rauch and Nauen (2003).

Glutathione S-transferases: For the glutathione S-transerase assays adult bed bugs were homogenized in 0.05M Tris-HCl-Puffer, pH 7.5 and afterwards centrifuged at 4°C, and 15,000 g for 15 min. The resulting supernatant was used as enzyme source for the assays. As substrate for activity measurement 1-chloro-2,4-dinitrobenzene was used. The methodology of measurement was recently described elsewhere (Nauen and Stumpf, 2002).

Field Trials

In 2007 two field trials were performed in the USA comparing results between deltamethrin 4.75% (Suspend® SC) alone treatments and deltamethrin plus 6% pyrethrins (Kicker® EC, registered trademark of Bayer Group) tank mix treatments. Kicker EC consists of pyrethrins synergized with 60% piperonyl butoxide. The ratio of deltamethrin and pyrethrins was 1 : 1.42 corresponding to an application rate of 22.7 mg deltamethrin /m² and 40.6 mg natural pyrethrin/m².

The trials were performed in cooperation with professional pest management firms in New York City (New York) and in Fairfield (Connecticut). The trials in New York City were performed in separate middle and upper income residences (condominiums and cooperative apartments), while the Connecticut trial was performed in a subsidized low income apartment building. In both trials standardized professional service was provided to units with confirmed infestations of bed bugs. This service can be briefly described as thorough inspections to all potential bed bug harbourage sites. Applications of insecticide solutions were made as spot and/or crack and crevice sprays into and around harbourages. Service included bed disassembling to provide access to all harbourage sites on frames, mattresses, springs and adjacent furnishings. Service was provided usually for multiple visits until no alive bugs are observed and/or the resident(s) no longer reported being bitten. In New York, one technician applied only deltamethrin as the chemical control agent in his assigned residences and another technician applied a tank-mix of deltamethrin and synergised pyrethrins to his assigned sites. In Connecticut, specific units were assigned as treatment replicates throughout the trial. In this trial the cooperator included 0.06% hydroprene (Gentrol EC, Wellmark International) in all his tank mixes. In both trials service technicians reported activity and results from visual counts of live bugs from defined harbourage/treatment sites from an initial count (before any treatment) and on each subsequent visit.

RESULTS

Laboratory Trials

The LD50 and LD95 for technical deltamethrin in acetone against *C. lectularius* were calculated as 1.4 mg a.i./m² and 19.2 mg a.i./m² on filter paper disks, and for Deltamethrin 10 SC <0.83 and 0.9 mg a.i./m², respectively. The LD50 and LD95 of the carbamate bendiocarb were 6.5 mg a.i./m² and 38.1 mg a.i./m² on filter paper for the technical compound and 12.1 and 53.8 mg a.i./m² for the formulation Bendiocarb 80 WP, respectively (Table 1).

Table 1. Establishment of baseline susceptibility data for insecticides used against *Cimex lectularius* (Laboratory reference strain – Monheim) Calculation of the LD 50 and LD 95 values after 24 hours in mg a.i./m²

Technical compounds	LD 50	LD 95
Deltamethrin	1.4	19.2
Bendiocarb	6.5	38.1
Formulated compounds		
Deltamethrin 10 SC	<0.83	0.9
Bendiocarb 80WP	12.1	53.8

(No mortality was found in the UTC and on the filter disks treated with acetone and water)

The residual action of Bendiocarb 80 WP and Deltamethrin 10 SC is presented in Table 2a and b. The LT50 for bendiocarb was reached after 44 minutes with the lower dose rate and after 46 minutes with the higher dose rate on plywood (Table 1a). The LT50 at 4WAT was reached after 78 minutes (96 mg a.i./m²) and 60 minutes (120 mg a.i./m²), respectively. The LT95 was reached between 1 to 2 hours from 1 DAT to 4 WAT and increased to 14 hours with the lower dose rate and 2 hours with the higher dose rate 6 WAT, respectively. With 15mg deltamethrin/m² 132 minutes were needed to reach the LT50 and 240 minutes for the LT95, respectively. 1 WAT to 4 WAT, a shorter time was required to reach the LT50 and LT95, respectively. As with bendiocarb an increase of the LT 50 to 2 hours and LT95 to 6 hours 6 WAT was also found with deltamethrin (Table 2a).

The results on treated glazed tiles (Table 2b) gave faster mortality than those on plywood (Table 2a). The LT50 with Bendiocarb was 31 minutes with the lower dose rate and 29 minutes with the higher dose rate, and increased to 59 minutes and 55 minutes, respectively, at 6WAT. With Deltamethrin a slower onset of efficacy compared to 1 to 6 WAT was also found on glazed tiles (Table 2b). The LT50 and LT95 were between 46 minutes and 1 hour from 1 WAT to 6 WAT.

Biochemical Assays

The biochemical assays were carried out with adults of the susceptible laboratory strain of *C. lectularius* and revealed for well-known marker enzymes involved in metabolically driven insecticide resistance baseline activities of 13 ± 3.5 pmol/min µg protein, 131 ± 2.2 pmol/min µg protein and 4.2 ± 0.13 pmol/30min eq for carboxylesterases, glutathione S-transferases and 7-ethoxycoumarin O-deethylase monooxygenases), respectively. These enzyme activities represent baseline values for unfed bed bugs measured during this study, but can differ considerably with other strains depending on collection sites and resistance status. These values will be used as starting points for more detailed studies on field-collected resistant strains in the future.

Table 2a. LT 50 and LT 95 of Bendiocarb and Deltamethrin against *Cimex lectularius* (Laboratory reference strain – Monheim) on plywood

Product	Application rate	1DAT		1WAT		2WAT		4WAT		6WAT	
	mg a.i./m²	LT50/LT95		LT50/LT95		LT50/LT95		LT50/LT95		LT50/LT95	
		minutes		minutes		minutes		minutes		minutes	
Bendiocarb 80WP	96	44	75	57	61	60	65	78	120	207	838
Bendiocarb 80WP	120	46	47	58	63	70	119	60	65	119	129
Deltamethrin 10SC	15	132	240	67	137	57	85	59	64	125	367

(untreated control: no mortality)

Table 2b. LT 50 and LT 95 of Bendiocarb and Deltamethrin against *Cimex lectularius* (Laboratory reference strain – Monheim) on glazed tiles

Product	Application rate	1DAT		1WAT		2WAT		4WAT		6WAT	
	mg a.i./m²	LT50/LT95		LT50/LT95		LT50/LT95		LT50/LT95		LT50/LT95	
		minutes		minutes		minutes		minutes		minutes	
Bendiocarb 80WP	96	31	34	46	47	30	32	45	66	59	64
Bendiocarb 80WP	120	29	32	46	47	32	35	40	60	55	84
Deltamethrin 10SC	15	68	165	46	47	58	63	46	47	59	64

(untreated control: no mortality)

Field trials

In the first trial in New York City, the deltamethrin (alone) treatments reported between 50-75% reductions in visual counts from the initial inspection to the second treatment after 3 days. Bed bug counts declined by >95% in residences treated with the combination tank mix after one treatment. Three to six service visits were required using the deltamethrin-only treatment and overall insecticide application volumes (due to more required service) were higher than in the combination tank mix treatments. The cooperator reported that service visits in the deltamethrin/pyrethrin/piperonyl butoxide serviced units were reduced by 1-2 trips compared to the deltamethrin-only service (3 to 6 trips). Bed bugs were found moving and ‘dying’ after contact with the deltamethrin/pyrethrin/ piperonyl butoxide tank-mix spray. Residents reported no new bites after the second service visit.

In the second trial in Connecticut the cooperator reported an average 60% reduction from first visit from the deltamethrin (alone) treatments (Table 3). All deltamethrin-only units required 3 additional visits before elimination (defined as no new bites for over 2 weeks).

Table 3. Efficacy of Deltamethrin SC plus natural Pyrethrum/PBO in a tank mix versus Deltamethrin SC alone against *Cimex lectularius* in infested apartments (one example each)
Application rates: 0.06% Deltamethrin, 0.1% Pyrethrum, 1.0% Synergist

	a. Deltamethrin alone				b. Deltamethrin/Pyrethrum/PBO			
	initial	2 WAT	4 WAT	6 WAT	initial	2 WAT	4 WAT	6 WAT
	N° of bed bugs				N° of bed bugs			
Beds								
Mattress	>100	28	6	0	>100	3	0	0
Boxspring	>100	40	9	2	n.a.	-	-	-
Frame/headboard	26	20	0	0	>100	7	0	0
Other sites								
Baseboards	>100	19	3	1	62	4	1	0
Chairs/sofas	n.a.	-	-	-	>50	4	2	1
Nightstands/Dressers	>100	28	6	0				
Walls/Ceilings	56	39	0	0				
Applied liquid per apartment (liter)	7.6	3.8	1.9	1.9	9.5	1.9	0.9	0

n.a. = not assessed

In the combination tank-mix treated units the cooperator reported average reductions of 95-100% from the first service visit to the second. All units were defined as ‘bug-free’ by the third visit (Table 3). Residents in units treated with the deltamethrin/pyrethrin/piperonyl butoxide tank-mix reported no new bites after the second application. The cooperator reported that bugs move after direct application with tank mix and more dead bugs were seen in and around harbourage sites on subsequent inspections of the tank mix treated units, than in the deltamethrin alone treatments.

DISCUSSION

The LD50 and LD95 were assessed for the technical compounds deltamethrin and bendiocarb and for formulations on filter paper after 24 hours against the laboratory reference Monheim strain of *C. lectularius*. With the formulation Deltamethrin SC a lower LD95 was found with 0.9 mg a.i./m² than for the technical compound (19.2 mg a.i./m²). The reason for this difference is not known. The LD95 of 53.8 mg a.i./m² for the formulation Bendiocarb 80WP is in the range of the technical compound (38.1 mg a.i./m²). The LD95 for both formulations was within their label rates for bed bug control.

The onset of efficacy was faster on glazed tiles compared to plywood for both active ingredients. No differences between the two application rates of endiocarb were found on glazed tiles, the lower application rate was slower at 4 and 6 WAT on plywood.

One DAT, bendiocarb acted faster than deltamethrin on both surfaces. From 1WAT to 6 WAT the efficacy of both active ingredients was in the same range against the *C. lectularius* laboratory strain. Both formulations have proven to be effective for at least 6 weeks with their label rates on plywood and glazed tiles in the laboratory.

The effects of a tank mixture of Deltamethrin SC with formulated piperonyl butoxide synergized Pyrethrins were examined in a series of field trials in 2007, which have been described in the results. The efficacy of the treatment program was greatly enhanced by use of this tank-mix strategy, because initial knockdown of the bed bug population was increased and elimination of the infestation was achieved in a shorter time with fewer re-services. The number of treatments was half of that to be necessary without tank mix partner. Practitioners reported “seeing” more evidence of efficacy during treatment, which might be attributable to the flushing actions of the natural pyrethrins. Residents reported that biting episodes were stopped more quickly when applying the deltamethrin + piperonyl butoxide + pyrethrins tank mix. These results suggest that the synergist has increased the efficacy of the treatment against bed bugs.

Miller (2006) confirmed the results reported in the present paper with the combination of Deltamethrin SC and pyrethrum/ piperonyl butoxide EC as tank-mix in the laboratory against a susceptible laboratory strain and a pyrethroid resistant field strain of *C. lectularius*. Against the susceptible laboratory strain only small differences were found in the speed of control with the separate products and the combination. However, against the pyrethroid resistant field strain the combination provided faster and more effective control than Deltamethrin SC and pyrethrum/ piperonyl butoxide EC alone (Miller 2006), suggesting the possible involvement of monooxygenases as detoxification enzymes in partially resistant bed bug strains. Boase (2006) confirmed the performance of bendiocarb under field conditions in naturally infested rooms. Four sites (bedrooms in apartments), which were infested by *C. lectularius*, were treated with Bendiocarb 80 WP (application rate: 97 mg a.i./m²). At 1 DAT reduction of adults was 98% and of nymphs 94%, respectively. At 7 DAT and 30 DAT 98.5% and 100% reduction was found, respectively (Boase, 2006). Potter et al. (2006) conducted field trials with Deltamethrin 10 SC and deltamethrin dust and examined the effectiveness of deltamethrin, compared with selected other pyrethroids. Snell (2006) reported 100% mortality with 24 mg a.i. Deltamethrin SC after 48 hours on mattress ticking and 95% mortality after 48 hours on unpainted plywood directly after application in the laboratory. At 30 DAT 95% mortality was found after 48 hours exposure on plywood and 100% on mattress ticking. No evidence of repellency was found with Deltamethrin SC (Moore and Miller, 2006).

The monooxygenase levels which have been shown in the results for the susceptible laboratory *C.lectularius* reference strain - Monheim, can be much higher in field populations, so the suppression of monooxygenases with the synergist piperonyl butoxide could be useful in terms of reducing the level of pyrethroid detoxification in bed bugs. The results indicate that the rotation between deltamethrin and bendiocarb or the application of deltamethrin in a tank mix with pyrethrum and synergist are promising solutions against susceptible and less susceptible bed bug populations.

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