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Sub-lethal effects of the anticoagulant rodenticides bromadiolone and chlorophacinone on breeding performances of the barn owl (*Tyto alba*) in oil palm plantations

Subletálny vplyv antikoagulačných rodenticídov bromadiolonu a chlórofacinonu na hniezdnu úspešnosť plamienky driemavej (*Tyto alba*) na plantážach palmy olejovej

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Abstract: This study investigated the effects of the first generation anticoagulant rodenticide chlorophacinone and the second generation rodenticide bromadiolone on the population and breeding performances of barn owls at oil palm plantations. Three treatment plots were established: one baited with chlorophacinone, one with bromadiolone, and the third kept rodenticide-free. Four rat-baiting campaigns which coincided with barn owl breeding season were carried in the rodenticide-treated plots. The occupancy rate of nest boxes, clutch size, brood size and fledging rates of the barn owls in each plot were monitored weekly throughout the study. Freshly regurgitated pellets from barn owls were collected from all occupied nest boxes at weekly intervals during the development of nestlings in each breeding season. The results show that the occupancy rate of the nest boxes was significantly higher in the rodenticide-free area compared with both rodenticide-treated areas. Similarly, the breeding performances, such as clutch size, brood size and fledging success, were higher in the rodenticide-free area compared with the rodenticide-treated areas. Results of high performance liquid chromatography (HPLC) analysis showed that 20.56% (mean residue: 1.335 ± 0.073 µg/g) and 28.89% (mean residue: 0.777 ± 0.032 µg/g) of the collected regurgitated pellet samples from the rodenticide-treated areas contained bromadiolone and chlorophacinone residue, respectively. The mean brood size and fledging success of the barn owls showed a strong negative correlation with the mean concentration of rodenticide residues present in the regurgitated pellets and with the percentages of pellets detected having such residues (R2 ≥ 0.44, P < 0.05). Similarly, the mean clutch size of barn owls was negatively correlated with the mean concentration of rodenticide residues and with the percentages of pellets detected with these residues. However, the correlation was not significantly different (R2 ≥ 0.34, P > 0.05). In general, the higher the amount of residues detected, the lower the breeding performance parameters measured in this study.

Abstrakt: Štúdia skúma vplyv prvej generácie antikoagulačného rodenticídu chlórofacinonu a druhej generácie bromadiolonu na parametre hniezdenej úspešnosti plamienky driemavej na plantážach palmy olejovej. Založili sa tri pokusné plochy: jedna ošetrená chlórofacinonom, ďalšia s bromadiolonom a tretia kontrolná bez rodenticídu. Na chemicky ošetrených plochách sa počas hniezdnej sezóny rodenticíd vykladal štyri krát. Obsadenosť hniezdnych búdok, veľkosť znášky, počet vyliahnutých mláďat a podiel vyletených mláďat plamienky sa zisťovali v týždenných intervaloch. Z obsadených hniezd sa tiež v týždenných intervaloch zbierali vývržky. Z výsledkov vyplýva, že podiel obsadených búdok bol štatisticky významne vyšší na chemicky neošetrenej ploche než na plochách s vyloženou otrávenou návnadou. Rovnako aj parametre hniezdenia – veľkosť znášky, počet vyliahnutých mláďat a počet vyletených mláďat – dosahovali vyšších hodnôt na neošetrenej ploche. Z výsledkov z vysokoúčinnej kvapalinovej chromatografie (HPLC) vyplýva, že 20,56 % (priemerná koncentrácia rezíduí: 1,335 ± 0,073 µg/g) a 28,89 % (priemerná koncentrácia rezíduí: 0,777 ± 0,032 µg/g) z vývržkov zozbieraných v rodenticídom ošetrených plochách obsahovalo rezíduá bromadiolonu alebo chlórofacinonu. Priemerná veľkosť znášky a počet vyletených mláďat negatívne korelovali s priemernou koncentráciou rezíduí rodenticídov vo vývržkoch a s relatívnym podielom vývržkov obsahujúcich tieto rezíduá ($R^2 \geq 0.44$, $P < 0.05$). Negatívne koreloval s týmito charakteristikami aj priemerný počet vyliahnutých mláďat, avšak štatisticky nevýznamne ($R^2 \geq 0.34$, $P > 0.05$). Zovšeobecňujúc možno konštatovať, že čím vyššie bolo množstvo chemických rezíduí vo vývržkoch, tým nižšie boli parametre hniezdnej úspešnosti.

Key words: secondary poisoning, rodenticides, barn owls, oil palm plantations, non-target organisms

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Introduction

Rats have been considered an agricultural problem in Malaysia for many years, particularly in relation to oil palm cultivation (Wood 1994, 2001, Turner & Gillbanks 2003, Chung 2012). Damage to oil palm by rats is inflicted throughout the growth of the palm, regardless of age, leading to losses of seedlings through attacks in nurseries, damage to both newly planted palms and mature plants, and damage to inflorescences and fruit, causing severe loss in oil yields (Khoo et al. 1991, Turner & Gillbank 2003). At commercial plantations, chemical control using anticoagulant rodenticides integrated with biological control using the barn owl Tyto alba has been used against rat populations at Malaysian oil palm plantations since the late 1980's (Wood & Chung 2003). However, application of anticoagulant rodenticides in combination with the natural propagation of barn owls has raised concerns of potential secondary poisoning of the latter. Owl populations were reported as declining in numbers when anticoagulant rodenticides were introduced at oil palm plantations against rat pests (Duckett 1984, Naim et al. 2011).

Monitoring programmes on exposure to anticoagulant rodenticides in the United States, Canada and Europe have shown evidence of extensive contamination of anticoagulant rodenticides in owls (e.g. Stone et al. 2003, Thomas et al. 2011, Albert & Wilson 2009, Walker et al. 2012, Sanchez-Burbudo et al. 2012). Contamination with anticoagulant rodenticides is considered to be one of the most detrimental threats to wildlife (Erickson & Urban 2004). Recently, there have been concerns about the effects of anticoagulant rodenticides on the breeding performance and subsequently on populations of barn owls. Anticoagulant rodenticides are suspected of affecting animal reproduction and may also have detrimental effects on the breeding performance of barn owls (Naim et al. 2011, Olsen et al. 1993). A non-invasive technique for detecting residues in pellets for the assessment of secondary risk of poisoning of barn owls has been developed and is useful for determining contamination by anticoagulant rodenticides (Gray et al. 1994, Eadsforth et al. 1996). However, no attempt has been made to study the relationship between anticoagulant residues in owls and their reproductive performance. Hence, the specific objective of this study is to evaluate the secondary risk of poisoning from anticoagulant rodenticide, with particular reference to bromadiolone as a second generation anticoagulant and chlorophacinone as a first generation rodenticide, on population and breeding performances of barn owls at an oil palm plantation.

Material and methods

Study sites and treatments

The study was conducted at mature oil palm plantations, at FELDA Jengka 24 (N 03°45′, E 102°25), FELDA PPPTR (N 03°53′, E 102°30) and FELDA Kota Gelanggi 5 (N 03°57′, E 102°35′), Jerantut, in the State of Pahang, Malaysia. All of the areas had a similar planting density of 136 palms per ha (Fig. 1). The age of the palms was from 20–22 years old and their height about 9 meters. Previous data (2008–2010) provided by the plantations showed the occupancy rate of nest boxes at the sites to be about 40–50% of the total number of nest boxes. The nest boxes, made of highly durable fiberglass (50 cm width × 15 cm length × 61 cm height), were installed at a height of 7 m above ground and were distributed approximately at a density of one box per 15 ha.

Forty-two nest boxes (14 nest boxes/area) were chosen for sampling. Each study site comprised approximately 210 ha. The distance between the sites was approximately 15 to 20 kilometers. Thus, the sites were

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Fig. 1. Collecting of pellets on the study site (a), nestling ready to fledge from nest box (b)
Obr. 1. Zber vývržkov na študijnej ploche (a), mláďa opúšťajúce hniezdnu búdku (b)

sufficiently far apart to prevent barn owls from hunting across the sites. Two types of anticoagulant rodenticides – chlorophacinone (0.005% a.i.) and bromadiolone (0.005% a.i.) – were applied at FELDA PPPTR and FELDA Kota Gelanggi 5, respectively. FELDA Jengka 24 was kept rodenticide-free as the control plot. All areas had been previously baited with chlorophacinone in a single application. The last application of chlorophacinone in the areas had been conducted by the estate management in February 2010, or a year before this study commenced.

For the purposes of the study, baiting was conducted twice a year, in March and September, which coincided with the peak breeding season of the barn owls in the study areas. The first baiting campaign was carried out from 7–10 March 2011, the second from 20–24 September 2011 and the third baiting campaign was carried out from 26–29 March 2012. The fourth and final baiting campaign was conducted from 10–13 September 2012. Each campaign involved two rounds of baiting. Bait was placed at the base of every palm tree in the designated plots.

Breeding performance of barn owls and collection of regurgitated pellets

Nest boxes occupancy, clutch size, brood size and number of juveniles fledged were recorded at weekly intervals for all selected nest boxes throughout the study period. Nestlings (more than 8 week-old) were considered to have successfully fledged if they were no longer found in the nest box. Data was collected for four breeding seasons.

Fresh regurgitated pellets from barn owls were collected from all occupied nest boxes at weekly intervals during the development of nestlings in each breeding season (approximately 9 weeks). The regurgitated pellets from each nest box were counted, weighed, pooled and labelled before being sent to the laboratory. The pellets were stored at –20°C and thawed to ambient temperature prior to laboratory analysis. The residue content in the pellets was then quantified using high-performance liquid chromatography (HPLC) analysis.

Analysis of rodenticide residues

Anticoagulant rodenticide residues in regurgitated pellets of barn owls were used to determine the barn owls' level of exposure to the rodenticides. The residues were determined by HPLC coupled with fluorescence and UV detectors (Naim 2010). Whole samples were placed in a 100 mL polypropylene tube, and 40 mL of the extraction solvent, an acetone–chloroform mixture (1:1, v/v), were then added into the tube. Two grams of anhydrous sodium sulphate was added to enhance the extraction of analytes. The mixture was then homogenized by shaking the tube in a vortex for 3 minutes and leaving it to stand for approximately one hour. The mixture was then filtered using filter paper and re-extracted with the extraction solvent (20 mL). The combined filtrates were then evaporated to dryness using a rotavapor at 35 °C. The resulting residue was dissolved in 10 mL methyl tert-butyl ether. The extract was purified in an aminopropyl column, eluting with 10 mL of methyl tert-butyl ether–acetic acid glacial (90:10, v/v) after being conditioned with 2 mL methyl tert-butyl ether. The extract was again evaporated to dryness using a rotavapor at 40 °C, reconstituted with 1 mL of mobile phase and filtered using a Cronus Filter Yellow. The solution was then transferred into vials and placed in an auto-sampler for HPLC analysis.

Tab. 1. Occupancy rate (mean % ± S.E.) of barn owls nest boxes (n = 42).
Values in rows with different letters are significantly different (P < 0.05) by Tukey's test (in Tabs. 1–4)
Tab. 1. Obsadenosť búdok (n = 42) plamienkou driemavou (priemerné % ± S.E.).
Hodnoty v riadkoch s rozdielnymi písmenami sa líšia štatisticky významne (P < 0.05, Tukeyov test). 1 – hniezdna sezóna, 2 – bromadiolon, 3 – chlórfacinon, 4 – bez rodenticídu, 5 – priemer (v Tabuľkách 1 – 4)

breed. season[1]	bromadiolone[2]	chlorophacinone[3]	rodenticide-free[4]
1	50.00 ± 0.84 a	42.86 ± 1.46 a	40.48 ± 0.20 a
2	35.71 ± 1.00 a	57.14 ± 0.38 b	92.86 ± 0.13 c
3	34.52 ± 0.88 a	64.29 ± 1.19 b	100.00 ± 0.00 c
4	28.57 ± 0.92 a	42.86 ± 0.53 a	100.00 ± 0.00 b
mean[5]	37.20 ± 1.14 a	51.79 ± 1.34 bc	83.33 ± 3.60 c

Tab. 2. Clutch size (mean ± S. E.) of barn owls
Tab. 2. Veľkosť znášky (priemer ± S. E.) plamienky driemavej

breed. season[1]	bromadiolone[2]	chlorophacinone[3]	rodenticide-free[4]
1	4.67 ± 0.10 a	4.88 ± 0.24 a	4.04 ± 0.10 a
2	3.03 ± 0.22 a	3.07 ± 0.07 a	4.47 ± 0.18 a
3	3.08 ± 0.15 a	3.14 ± 0.37 a	4.21 ± 0.12 a
4	3.44 ± 0.08 a	3.67 ± 0.12 a	6.02 ± 0.31 b
mean[5]	3.56 ± 0.10 a	3.69 ± 0.10 a	4.69 ± 0.11 a

Tab. 3. Brood size (mean ± S. E.) of barn owls
Tab. 3. Počet vyliahnutých mláďat (priemer ± S. E.) plamienky driemavej

breed. season[1]	bromadiolone[2]	chlorophacinone[3]	rodenticide-free[4]
1	3.83 ± 0.14 a	3.08 ± 0.14 a	3.83 ± 0.27 a
2	3.00 ± 0.35 a	3.00 ± 0.71 a	3.38 ± 0.11 a
3	2.88 ± 0.04 a	3.25 ± 0.46 a	4.11 ± 0.36 b
4	2.73 ± 0.02 a	4.17 ± 0.18 b	5.52 ± 0.26 c
mean[5]	3.11 ± 0.06 a	3.38 ± 0.07 a	4.21 ± 0.12 b

Tab. 4. Fledging success (mean ± S. E.) of barn owls
Tab. 4. Počet vyletených mláďat (priemer ± S. E.) plamienky driemavej

breed. season[1]	bromadiolone[2]	chlorophacinone[3]	rodenticide-free[4]
1	3.38 ± 0.19 a	3.25 ± 0.27 a	3.83 ± 0.06 a
2	1.75 ± 0.09 a	2.17 ± 0.21 a	3.25 ± 0.07 b
3	2.00 ± 0.12 a	2.42 ± 0.09 a	4.00 ± 0.09 b
4	1.67 ± 0.12 a	2.78 ± 0.12 b	4.70 ± 0.18 c
mean[5]	2.20 ± 0.10 a	2.65 ± 0.06 a	3.95 ± 0.07 b

The HPLC system (Water, USA) consisted of a controller (model 600) with a multi-solvent delivery system, plus an auto-sampler (model 717), an ultra violet (UV) detector (model 2996) and a fluorescence detector (model 2475). A reversed-phase Nucleosil & Nucleodur® C18 column (5 μm particle size, 4.6 mm (ID) x 250 mm (L)) was used for analysis (Macherey-Nagel, Strasbourg, France).The mobile phase for detection of chlorophacinone was acetonitrile–Ortho phosphoric acid, 0.5% (90:10 for v/v), while the mobile phase for detection of bromadiolone was Methanol–distilled water–Acetic acid glacial (75:20:5 for v/v). The injection volume was set at 20 μL, and the mobile phase flow rate was 1.0 ml/min. The UV detector was monitored from 240 to 340 nm, with quantification done at 285 nm for detection of chlorophacinone. The fluorescence detector was set at 310 nm excitation and 390 nm for detection of bromadiolone.

Quality assurance

Rodenticide residue concentrations were quantified by comparison with analysis of bromadiolone and chlorophacinone standards (Sigma-Aldrich, Germany). The response of both rodenticides was linear for 6 standard solutions at concentrations of 0.01, 0.1, 0.5, 1, 5 and 10 mg/kg. The linearity of calibration was assessed from linear regression of the response (area) versus concentration of rodenticides, resulting in a R^2 value of 0.999. The average retention time of bromadiolone and chlorophacinone was 4.5 and 6.0 minutes, respectively. To validate the methodology, samples of pellets were spiked with three fortification concentrations of 1.0, 5.0 and 10.0 mg/kg of each standard. Recovery rates were assessed from the spiked control samples of rodenticides in the regurgitated pellet samples with the fortification concentration. It was evident that both rodenticides showed good recoveries at low and high concentrations ranging from 85% to 89%, respectively. Detection limits for each rodenticide were assessed from calibration standards using statistical regression with three replications. The estimated limit of detection of the bromadiolone and the chlorophacinone were 0.005 mg/kg and 0.002 mg/kg, respectively. A blank containing methanol was injected between each sample to monitor for possible contamination of the carry over.

Data analysis

The nest boxes occupancy rate, clutch size, brood size and fledging success were analysed using the Statistical Analysis System (SAS) for Windows version 9.2. Parametric variables were compared using one-way analysis of variance (ANOVA). Associations between breeding performances (nest boxes occupancy, clutch size, brood size and fledging success) with a concentration of residue detected in a regurgitated pellet and the numbers of pellets detected with anticoagulant rodenticides were analyzed using the Pearson correlation test. Values of $P < 0.05$ were considered to be statistically significant.

Results and discussion

Occupancy rate of nest boxes

Nest boxes in all areas were occupied by barn owls in all four breeding seasons (Tab. 1). The rodenticide-free area recorded the highest mean occupancy rate at 83.33%, with a 100% occupancy rate achieved in the 3rd and 4th breeding seasons. Furthermore, the results indicated that bromadiolone was the most detrimental to barn owls, with a mean occupancy rate of nest boxes at 37.20% compared with chlorophacinone (mean 51.79%). In the first breeding season, the occupancy rate was comparable in all areas, ranging between 40.48 to 50%. This result was predicted, since all of the areas had been previously baited with chlorophacinone. However, in the second breeding season, the occupancy rate of the nest boxes in the bromadiolone-treated area decreased to 35.71%. Similarly, in the third and fourth breeding seasons, the occupancy rate of the nest boxes in the bromadiolone-treated area showed a decreasing trend. The lowest occupancy rate was the 28.57% recorded in the fourth breeding season. In contrast, in the second breeding season the occupancy rate in the chlorophacinone-treated area increased to 57.14%. Similarly, in the third breeding season the occupancy rate of the nest boxes in the chlorophacinone-treated area increased to 64.29% but dropped slightly to 42.82% in the fourth breeding season.

Clutch size

The mean clutch size in the rodenticide-free area was consistently higher compared with those in both areas treated with rodenticides in all seasons except the first breeding season (Tab. 2). The highest mean clutch size was recorded in the untreated area in the fourth season, i.e. 6.02 ± 0.31 and was significantly higher ($P < 0.05$) compared with the rodenticides-treated areas. Similarly, Lenton (1984), who conducted his study in Peninsular Malaysia, found the mean clutch size of barn owls at a

Tab. 5. Number and percentage of pellets detected with residue of rodenticides. D = detected, ND = not detected
Tab. 5. Počet a podiel vývržkov obsahujúcich rezíduá rodenticídov. D = obsahujúci, ND = neobsahujúci. 1 – hniezdna sezóna, 2 – bromadiolon, 3 – chlórfacinon, 4 – bez rodentocídu, 5 – priemer

breed. season[1]	**bromadiolone**[2]			**chlorophacinone**[3]			**rodenticide-free**[4]		
	D	**ND**	**% D**	**D**	**ND**	**% D**	**D**	**ND**	**% D**
1	8	37	17.78	11	34	24.44	0.0	45	0.0
2	9	36	20.00	13	32	28.89	0.0	45	0.0
3	9	36	20.00	12	33	26.67	0.0	45	0.0
4	11	34	24.44	16	29	35.56	0.0	45	0.0
mean[5]	9.25	35.75	20.56	13.00	32.00	28.89	0.0	45.0	0.0

Tab. 6. Mean concentration of residues (ug/g) of rodenticides (mean ± S. E.) in collected pellets
Tab. 6. Priemerné hodnoty koncentrácie rezíduí (ug/g) rodenticídov (priemer ± S. E.) vo vývržkoch. 1 – hniezdna sezóna, 2 – bromadiolon, 3 – chlórfacinon, 4 – bez rodentocídu, 5 – priemer

breed. season[1]	**bromadiolone**[2]	**chlorophacinone**[3]	**rodenticide-free**[4]
1	0.769 ± 0.056	0.550 ± 0.028	0.00 ± 0.00
2	0.911 ± 0.073	0.680 ± 0.047	0.00 ± 0.00
3	1.970 ± 0.079	0.733 ± 0.059	0.00 ± 0.00
4	1.692 ± 0.042	1.144 ± 0.016	0.00 ± 0.00
mean[5]	1.335 ± 0.073	0.777 ± 0.032	0.00 ± 0.00

rodenticide-free mature oil palm plantation to be 6.6, and Naim et al. (2011) reported the mean clutch size of barn owls in Malaysia in an immature oil palm environment without baiting to be 5.43.

The results also indicated that there was no difference (P > 0.05) in the clutch size between the two rodenticide-treated sites in all seasons. In the bromadiolone-treated area the highest clutch size recorded was 4.67 ± 0.10, while the lowest was 3.03 ± 0.22. The clutch size in the chlorophacinone-treated area was comparable: the highest clutch size was 4.88 ± 0.24 and the lowest was 3.07 ± 0.07. This result was in agreement with Naim et al. (2011), who reported that the mean clutch size of barn owls in an immature oil palm environment under rodenticides application ranged from 3.95 to 4.83.

Brood size

The results show that brood size in the rodenticide-free area registered an increasing trend, with a total brood size mean of 4.21 ± 0.12. The highest brood size in the rodenticide-free area was 5.52 ± 0.26, and the lowest was 3.38 ± 0.11 in the first and fourth breeding seasons, respectively (Tab. 3). Lenton (1984) reported a mean brood size of 4.6 at oil palm plantations. In contrast, the bromadiolone-treated plot showed a total brood size mean of 3.11 ± 0.06. The highest mean brood size in the bromadiolone-treated area was 3.83 ± 0.14 chicks and was recorded in the first breeding season, while the lowest was in the fourth breeding season (2.73 ± 0.02). The chlorophacinone-treated area, meanwhile, showed a fluctuating trend, with a total mean brood size of 3.13 ± 0.07. The highest corresponding brood size mean was 3.25 ± 0.46, found in the third breeding season, and the lowest brood size mean was 3.00 ± 0.71, detected in the second breeding season. There was significant difference (P< 0.05) in the brood size among the treated areas in the third and fourth breeding seasons. In general, application of rodenticide in the field resulted in a lower brood size among barn owls.

Fledging success and productivity

The barn owls in the rodenticide-free plot consistently showed significantly higher (P < 0.05) mean fledging success compared with the rodenticides-treated plots in all breeding seasons except for the first breeding season, in which the number of fledglings from all of the study areas was comparable (Tab. 4). The mean fledging success in rodenticide-free plot was 3.95 ± 0.07 (78.04%) and significantly higher (P < 0.05) compared with the bromadiolone- and chlorophacinone-treated plots, which had values of 2.20 ± 0.10 (26.57%) and 2.65 ± 0.06 (39.84%), respectively. This finding is in agreement with Naim et al. (2011), who reported fledging success at 77.9% in a rodenticide-free area and as low as 10 to 36% in rodenticide-treated areas. Similarly, Lenton (1984) recorded the fledging success of barn owls in a rodenticide-free area to be 81.2%. In this study, no significant difference (P > 0.05) in fledging success was detected between the two rodenticide-treated areas except in the fourth breeding season. It declined drastically in the second breeding season (1.75 ± 0.09) and again in the fourth breeding season (1.67 ± 0.12), but

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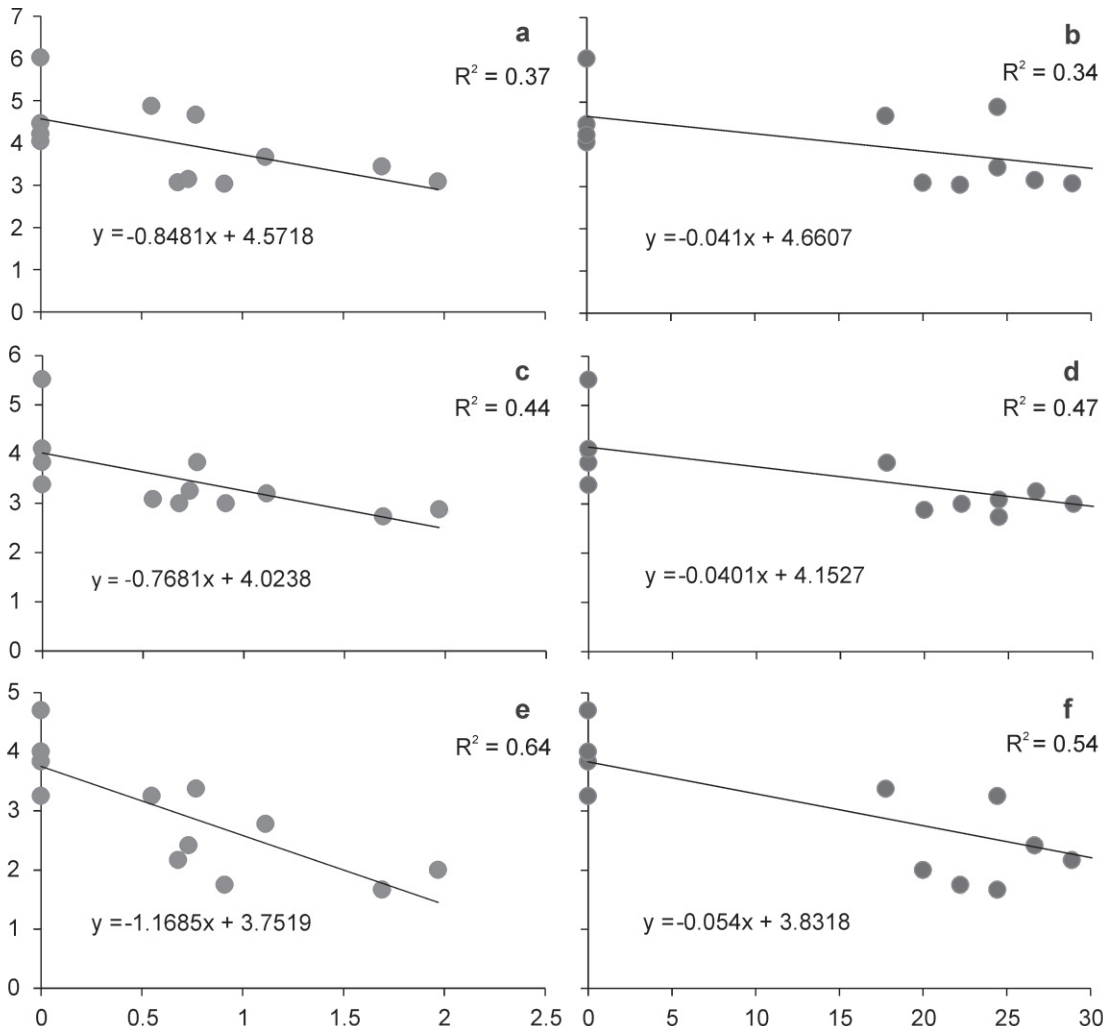

Fig. 2. Relationship between (a) mean concentration of residues (ug/g) and mean clutch size, (b) percent of pellets detected with residues and clutch size, (c) mean concentration of residues (ug/g) and mean brood size, (d) percent of pellets detected with residues and brood size, (e) mean concentration of residues (ug/g) and fledging success, and (f) percent of pellets detected with residues and fledging success

Obr. 2. Vzťah medzi (a) priemernou koncentráciou rezídzí (ug/g) a priemernou veľkosťou znášky, (b) podielom vývržkov s rezíduami (ug/g) a priemernou veľkosťou znášky, (c) priemernou koncentráciou rezídzí (ug/g) a priemerným počtom vyliahnutých mláďat, (d) podielom vývržkov s rezíduami (ug/g) a priemerným počtom vyliahnutých mláďat, (e) priemernou koncentráciou rezídzí (ug/g) a priemerným počtom vyletených mláďat a medzi (f) podielom vývržkov s rezíduami (ug/g) a priemerným počtom vyletených mláďat

the differences in fledging success in the second breeding season were not significant ($P > 0.05$).

Barn owls exposed to rodenticides have difficulty maintaining a suitable population. The fledging success in the rodenticide-treated areas, particularly the area treated with bromadiolone (just 1.67 in the final season of the study), suggests that the birds are at risk of not maintaining a suitable population. The lower fledging success in the rodenticide-treated areas in the fourth breeding season, particularly the bromadiolone area, was due to the death of several nestlings during their development stage. Henny (1969) calculated that a breeding success of at least 1.9–2.2 fledlgings per breeding pair is the minimum reproductive rate required to maintain a suitable barn owl population. Based on this calculation, barn owls in bromadiolone-treated plots have a reproductive rate too low to maintain a suitable population.

Rodenticide residues in pellets collected from barn owls

Residual analysis of rodenticides in regurgitated pellets has been acknowledged by many researchers as the most useful non-invasive technique to study the risk of secondary poisoning of owls through the consumption of rodenticide-ingested prey (Newton et al. 1990, Gray et al. 1994, Eadsforth et al. 1996, Coueurdassier et al. 2012). The residues of rodenticides, i.e. bromadiolone and chlorophacinone, in regurgitated pellets were evaluated to in order to assess the risks of secondary poisoning of barn owls and the subsequent impact on the reproductive performances of barn owls.

The analysis of pellets regurgitated by barn owls in all breeding seasons shows that bromadiolone and chlorophacinone were successfully detected in the pellets samples collected from the rodenticide-treated sites (Tab. 5). Due to the large number of samples, only 45 samples (5 samples per week during 9 weeks of nestling development) were analysed in each treatment for each breeding season. Of the 180 pellets collected from the four breeding seasons in the bromadiolone-treated plot, 20.56% of regurgitated samples contained bromadiolone, with a mean residue of 1.335 ± 0.073. The highest residue was detected in the third breeding season, with a mean residue of 1.970 ± 0.079 µg/g. In the chlorophacinone-treated plot, 28.89% (n=180) of pellets contained residue, ranging from 0.550 to 1.144(g/g of net weight (mean: 0.777 ± 0.032 µg/g). No residue was detected in any of the samples (n=180) collected from the rodenticide-free plot (Tab. 6).

Pearson correlation analysis showed the mean clutch size of barn owls was negatively correlated to the mean concentration of rodenticide residues present in the regurgitated pellets (Fig. 2a) and to the percentages of pellets detected with residues (Fig. 2b). However, the correlations were not significantly different (P = 0.051). Similarly, the correlation between the mean cutch size and percentages of pellets detected with residue were not significantly different (P = 0.103). The results indicate that application of rodenticides in the field has no effects on the clutch size of barn owls. Lenton (1984) stated that if barn owls consume poisoned rats, the rodenticides will be transferred to eggs but will not disrupt egg production. This would indicate that the rodenticides are being metabolized and the rodenticide load presumably removed in the form of one batch of addled eggs. Barn owls are indeterminate egg layers and would thus keep laying until a clutch of rodenticide-free eggs is produced (Lenton 1984).

There was a negative correlation between brood size and the mean concentration of rodenticide residue present in regurgitated pellets (Fig. 2c) and with the percentage number of pellets detected with residue (Fig. 2d). Both parameters were significantly correlated to the brood size of the barn owls (P = 0.038 and P = 0.049). The high numbers of addled eggs in rodenticide-treated areas, particularly the bromadiolone plots, reduced the hatchability of eggs and consequently the brood size. The result clearly shows that exposure to an anticoagulant rodenticide as an environmental pollutant can lead to decreased hatchability of eggs and reduced brood size. There was a strong significant negative correlation between mean fledging success and mean concentration of rodenticide residues present in regurgitated pellets (Fig. 2e, P = 0.018) and with the percentage of pellets detected in the residue (Fig. 2f, P = 0.001). This observation indicates that some of the nestlings in rodenticide-treated areas failed to fledge and died during development. The nestlings in the bromadiolone- and chlorophacinone-treated areas faced the risk of secodary poisoning by feeding on rats that had ingested poisoned bait being brought to the nest.

Conclusions

The rate of nest boxes occupancy and breeding performance of the barn owls in the plots treated with both the first and second generation anticoagulant rodenticides were lower compared with areas not treated with rodenticide. The ingestion of poisoned rats by the barn owls poses a risk of secondary poisoning and produces a detrimental effect on the breeding performances of the birds. The lower occupancy rate and reduced breeding performances of the barn owls in both of the treated plots were due to the sub-lethal effects on the parents and the nestlings, substantiated by the detection of residue in the regurgitated pellets in both rodenticide-treated plots.

The findings of this study could help improve the implementation of Integrated Pest Management (IPM) in rat control at oil palm plantations which have the presence of barn owls, such as in the type of rodenticide used and the timing of its application. In general, the risk of exposure to bromadiolone in barn owls is higher than chlorophacinone, suggesting a greater potential for secondary poisoning from the former than the latter. Thus, chlorophacinone is recommended over bromadiolone. Single application of a rodenticide during the non-breeding season (April–June) of *T. alba* could reduce the damage to fruit bunches of oil palm while also reducing the risk of secondary poisoning of barn owls. Application of rodenticides should not be carried out during the breeding season in order to prevent mortality of nestlings and affect the breeding performance of barn owls.

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