Control of the Mupli Beetle, *Luprops tristis* (Coleoptera: Tenebrionidae), and Dormancy Phase-related Variation in Insecticide Susceptibility[1]

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ABSTRACT Massive home invasions by the Mupli beetle, *Luprops tristis* F. (Coleoptera: Tenebrionidae), with the onset of monsoon rains, aggregation in a prolonged state of dormancy at specific locations, and repeated selection of the same locations for years provides an excellent opportunity to control the beetles employing suitable insecticides. The weak physical stature of the surviving beetles during the last phase of dormancy lead to the proposition that there were differences in insecticide susceptibility during the early- and late-dormancy phases. Hence, efficacy of three pyrethroid insecticides were tested with filter-paper bioassays to determine LC_{99} and KC_{99} values in the early and late phases of dormancy and to analyse whether the efficacy of the compounds vary between the early and late phases. During the late-dormancy phase, these compounds caused immediate knockdown but not immediate mortality at the lowest application rates. However, because the beetles have to be controlled during the early phase of dormancy when home invasions occur, the best control strategy for this nuisance pest is to knockdown the beetles and physically remove them instead of direct mortality.

KEY WORDS Nuisance pest, rubber plantation, insecticide, dormancy, darkling beetles

Home invasions by large numbers of litter-dwelling Mupli beetles, *Luprops tristis* (F.) (Coleoptera: Tenebrionidae), in the range of 500,000 to over 4 million per residential building following summer showers, and their prolonged stay in a state of dormancy (Fig. 1), are a regular event in rubber plantation tracts in south India (Sabu et al. 2008), where about 90% of India's natural rubber is produced (Anonymous 2010, DSIR 2013). Litter stands of monoculture rubber plantations during pre-summer and summer periods are the feeding and breeding habitat for *L. tristis*. Rain-soaked litter stands induce annual migrations of Mupli beetles to tile-roofed and palm-frond thatched residential buildings in the vicinity of rubber plantations (Vinod & Sabu 2010). Additionally, *L. tristis* aggregates in a variety of other over-wintering quarters in the vicinity of rubber plantations, including palm fronds and husks in firewood piles, hay stacks in cattle sheds, hollow wooden blocks, piles of worn-out polyethylene rain guards, and crevices below boulders. Following home invasion, clusters of the beetles crawl inside living areas, fall into beds and food from ceilings, and when

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disturbed by picking them off the walls or when they are squeezed, release an odorous secretion that causes skin irritation and eye inflammation (Sabu et al. 2008, John et al. 2010). Subsequently, they congregate in attics and gaps between palm fronds in thatched sheds and remain dormant during the 8–9 mo wet monsoon period. The onset of the dry season leads to their arousal from dormancy and return to the freshly fallen leaf litter in rubber plantations, coinciding with the annual leaf shedding of rubber trees during the pre-summer period (Sabu et al. 2008, Sabu & Vinod 2009). Availability of nutrient-rich prematurely fallen leaves is the major factor contributing to the unusual abundance of *L. tristis* in rubber plantation belts.

Attempts to control the beetles with physical and mechanical means, such as installing window screens, caulking cracks in walls, smoking the aggregation sites with camphor and frankincence, manually collecting beetles from aggregation sites, and placement of light traps in makeshift thatched sheds in and around the plantations have not been successful due to the following reasons. Residential buildings in rubber plantation regions are either tile-roofed buildings with multiple windows and doors or palm frond-thatched sheds, and both types of structures have multiple gaps allowing the entry of beetles into the buildings. The abundance of the aggregated beetles, their swift movements, difficulty to reach the aggregation sites in the attics, and the release of odorous secretions make manual collection impossible.

Indoor application of insecticides remains the only control solution for *L. tristis* infestations, and no research exists on the efficacy of insecticides against this pest. Interactions with the rubber planters who have experimented with various insecticides indicated that low insecticide concentrations can knock down (but not kill) the beetles for 6–8 h, but timely physical removal of the beetles is difficult due to the odorous excretions. We propose to select concentrations of insecticides that will provide knockdown of the beetles for a 12-h period to allow removal of the knocked-down beetles from buildings before their recovery.

Among the various insecticides widely used in the region for the control of *L. tristis*, efficacy of three pyrethriod compounds were tested 1) to determine their LC_{99} and KC_{99} values in the early-dormancy and late-dormancy phases; 2) to establish the lowest levels required for obtaining prolonged 99% knockdown followed by 99% mortality and; 3) to analyse whether the efficacy of the compounds vary between the early- and late-dormancy phases. Pyrethroids were selected because of their low acute human toxicity and because they are widely used to control pests in and around homes (Freeman et al. 2004, Pine et al. 2008).

Materials and Methods

Test insects. This study was conducted during April–December 2011, on the Devagiri College campus, which is located 6 km east of the Malabar Coast at Calicut (11°15′ N, 75°50′ E), Kerala State, India. Bioassays were done during the early phase of dormancy in April 2011 and during the late phase of dormancy in December 2011 employing teneral beetles and pupae collected from a 10-year-old, 4-ha. rubber plantation [*Hevea brasiliensis* (Wild. ex Adr. de Jus) Müll. Arg.] (Malpighiales: Euphorbiaceae) adjoining the campus. Two sets of beetle cultures were maintained in large circular clay vessels (13 × 35 cm) half-filled with rubber litter and soil. The first culture, with pre-dormancy beetles, was maintained until

the beginning of home invasions in April. The second culture with dormancy-induced beetles (Sabu et al. 2008), categorized as post-dormancy beetles, was maintained until the onset of rubber leaf-fall and return of *L. tristis* to rubber plantation litter during December. The beetles were fed tender rubber leaves.

Test compounds. The compounds used in the study were Tiktox (permethrin 1.5% EC, Shree Agro Industries, Mumbai, India), Tatafen (fenvalerate 20% EC, Rallis India, Ltd., Mumbai, India), and Ticomax (fenvalerate 20% EC, Ivorychem India, Pvt. Ltd., Bangalore, India). Recommended concentrations listed on the insecticide labels were 0.5 μg/cm^2 for Tiktox and 1.3 μg/cm^2 for Tatafen and Ticomax. Preliminary tests were conducted for each insecticide to determine the appropriate concentration ranges necessary to compute KC and LC values. Thereafter, seven dilution concentrations of each insecticide, 1×10^{-3} ml, 4×10^{-4} ml, 2×10^{-4} ml, 1×10^{-4} ml, 5×10^{-5} ml, 2.5×10^{-5} ml, 1.25×10^{-5} ml, were used for bioassay against both early- and late-dormancy *L. tristis*.

Experiment set up. Beetles were exposed to selected concentrations of insecticides using a filter-paper bioassay method (Sheppard & Hinkle 1987, Tomberlin et al. 2002). The experiment setup contained Whatman (Maidstone, Kent, U.K.) Grade No. 1 filter paper (30 cm^2) placed in PVC vials (Tarsons Products Pvt. Ltd., Kolkata, India) (5.5 × 4.5 cm; 50 ml capacity). One ml of the labelled concentration of solution was applied to filter paper in each vial, and the entire setup was left undisturbed for 1 h allowing the filter paper to air dry. Then, twenty beetles each were introduced into each vial, and the vials were covered with nylon mesh net. The experiment for each concentration was replicated six times for each insecticide. Filter papers treated with water served as the controls. Knockdown counts were recorded at 2, 4, 6, 8, and 12 h after insecticide exposure and mortality counts were made at 24-h intervals. Knockdown was defined as the inability of a beetle to walk or fly, and mortality was defined as the inability of a beetle to move its body or appendages when touched with an insect pin (Steelman 2008).

The time required for 99% mortality varied greatly among the three insecticides and between the two seasons. For reliable results, mortality was estimated after 80% mortality was reached by at least one test solution of each test insecticide (Yu 2008).

Data analysis. PROBIT analysis was used to determine regression equations for estimation of knockdown concentrations (KC) and lethal concentrations (LC) values and respective 95% confidence limits (Finney 1971, Robertson et al. 2007). All analyses were done with Minitab software for Windows (Minitab 2010).

Results

Knockdown for early-dormancy phase. Knockdown values (KC_{50}, KC_{99}) after 2, 4, 6, 8, and 12 h of exposure for three insecticides against *L. tristis* during the early-dormancy phase are shown in Table 1. KC_{99} values decreased after 2 h of exposure for all three insecticides, and they increased after 6 h of exposure for Tiktox and 8 h of exposure for Tatafen and Ticomax (Table 1, Figure 2). The lowest KC_{99} values were recorded for Tiktox (0.28 μg/cm^2) after 4 h of exposure, and for Tatafen (0.25 μg/cm^2) and Ticomax (0.79 μg/cm^2) after 6 h of exposure. KC_{99} values after 12 h of exposure for Tiktox, Tatafen, and Ticomax were 0.65 μg/cm^2,

Table 1. Knockdown values (KC_{50}, KC_{99}) of three insecticides for *Luprops tristis* during the early and late phases of dormancy with increasing exposure time ($P < 0.05$).

Insecticide	Exposure time (days)	KC_{50} (95% CL) (μg/cm^2)	KC_{99} (95% CL) (μg/cm^2)	Slope (±SE)	Chi square
Early-dormancy phase					
Tiktox	2	0.00 (0.00–0.00)	0.57 (0.18–15.1)	1.63 (0.48)	1.12
	4	0.02 (0.01–0.02)	0.29 (0.18–0.55)	1.20 (0.20)	4.57
	6	0.02 (0.01–0.02)	0.38 (0.23–0.80)	1.78 (0.19)	4.42
	8	0.02 (0.01–0.02)	0.66 (0.36–1.7)	1.53 (0.17)	6.66
	12	0.02 (0.01–0.02)	0.66 (0.35–1.7)	1.50 (0.17)	4.73
Tatafen	2	0.04 (0.02–0.06)	0.55 (0.35–1.5)	2.10 (0.45)	1.00
	4	0.04 (0.02–0.06)	0.45 (0.29–1.3)	2.28 (0.52)	0.98
	6	0.05 (0.02–0.07)	0.25 (0.18–0.77)	3.13 (0.88)	0.41
	8	0.05 (0.02–0.07)	0.39 (0.26–1.0)	2.62 (0.59)	3.09
	12	0.04 (0.01–0.06)	0.62 (0.38–2.0)	1.89 (0.42)	1.18
Ticomax	2	0.01 (0.00–0.03)	7.6 (2.3–201)	0.79 (0.20)	2.35
	4	0.01 (0.00–0.04)	1.4 (0.7–9.9)	1.19 (0.30)	1.52
	6	0.00 (0.00–0.02)	0.79 (0.36–39.9)	1.01 (0.37)	0.63
	8	0.00 (0.00–0.01)	8.0 (1.7–146,246)	0.57 (0.22)	2.01
	12	0.00 (0.00–0.02)	56.4 (8.6–29,013)	0.56 (0.16)	0.57
Late-dormancy phase					
Tiktox	2	0.00 (0.00–0.00)	0.02 (0.01–0.07)	2.72 (0.67)	0.21
	4	0.00 (0.00–0.00)	0.03 (0.02–0.10)	2.29 (0.51)	1.10
	6	0.00 (0.00–0.01)	0.05 (0.03–0.14)	2.16 (0.38)	1.25
	8	0.01 (0.00–0.01)	0.06 (0.04–0.15)	2.34 (0.35)	2.35
	12	0.01 (0.01–0.02)	0.11 (0.07–0.23)	2.44 (0.30)	4.95
Tatafen	2	0.02 (0.00–0.04)	0.17 (0.10–1.5)	2.74 (0.94)	0.88
	4	0.02 (0.00–0.04)	0.28 (0.15–2.4)	2.03 (0.62)	0.62
	6	0.03 (0.01–0.04)	0.30 (0.17–1.5)	2.23 (0.59)	1.42
	8	0.04 (0.02–0.05)	0.55 (0.30–2.0)	1.99 (0.41)	0.43
	12	0.05 (0.03–0.07)	0.96 (0.51–3.4)	1.78 (0.33)	1.71
Ticomax	2	0.02 (0.00–0.03)	0.12 (0.07–21.8)	3.18 (1.41)	0.10
	4	0.02 (0.00–0.03)	0.12 (0.07–21.7)	3.81 (1.41)	0.10
	6	0.03 (0.01–0.04)	0.14 (0.09–0.92)	3.38 (1.15)	0.25
	8	0.03 (0.02–0.05)	0.20 (0.12–0.72)	3.11 (0.83)	1.41
	12	0.04 (0.03–0.06)	0.37 (0.23–1.0)	2.53 (0.50)	1.71

0.61 μg/cm^2, and 56.4 μg/cm^2, respectively; and after 2 h of exposure they were 0.57 μg/cm^2, 0.55 μg/cm^2, and 7.6 μg/cm^2, respectively. Tatafen had the steepest regression line and Ticomax had the flattest regression line.

Knockdown for late-dormancy phase. Knockdown values (KC_{50}, KC_{99}) after 2, 4, 6, 8 and 12 h of exposure for three insecticides against *L. tristis* during the late-dormancy phase are shown in Table 1. KC_{99} values progressively increased with a rise in exposure time (Table 1; Figure 2). The lowest KC_{99} values were reached for Tiktox (0.02 μg/cm^2), Tatafen (0.16 μg/cm^2), and Ticomax (0.11 μg/cm^2) after 2 h of exposure. KC_{99} values after 12 h of exposure were

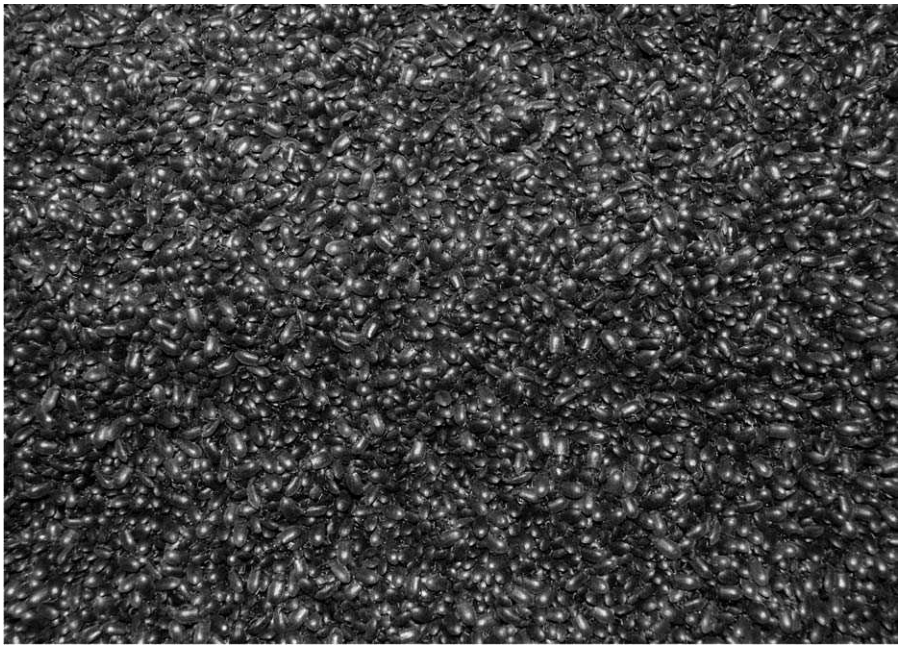

Fig. 1. Aggregated, dormant *L. tristis* beetles settled on the wall of a residential building.

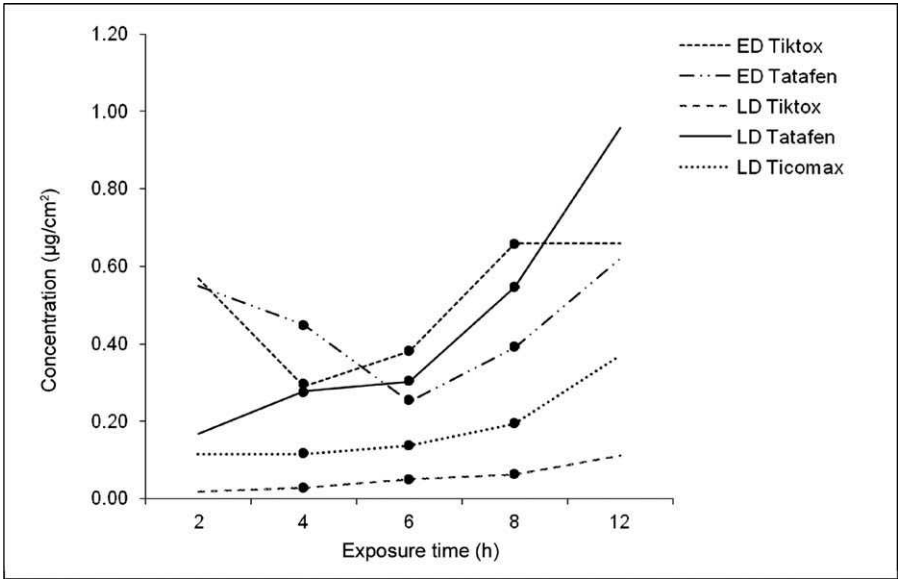

Fig. 2. Variation in KC_{99} concentration of three insecticides with increasing exposure time during the early- (ED) and late-dormancy (LD) phases of *L. tristis*.

Table 2. Lethal concentration values (LC_{50} & LC_{99}) of three insecticides for *Luprops tristis* during the early and late phases of dormancy with increasing exposure time ($P < 0.05$).

Insecticide	Exposure time (days)	LC_{50} (95% CL) (μg/cm^2)	LC_{99} (95% CL) (μg/cm^2)	Slope (±SE)	Chi square
		Early-dormancy phase			
Tiktox	10	0.04 (0.02–0.11)	19.7 (5.2–190)	0.89 (0.12)	2.83
	11	0.03 (0.01–0.05)	4.7 (1.7–25.7)	1.02 (0.13)	2.96
	12	0.01 (0.01–0.02)	1.6 (0.68–6.8)	1.13 (0.15)	5.67
	13	0.01 (0.00–0.01)	0.41 (0.14–8.2)	1.43 (0.17)	7.48
	14	0.01 (0.00–0.01)	0.18 (0.10–0.46)	1.63 (0.23)	4.01
	15	0.01 (0.00–0.01)	0.08 (0.05–0.18)	1.97 (0.32)	4.41
Tatafen	10	0.20 (0.13–0.28)	29.1 (11.5–135)	1.08 (0.14)	3.61
	11	0.12 (0.07–0.17)	9.9 (4.5–36.6)	1.21 (0.17)	3.90
	12	0.08 (0.04–0.11)	3.8 (1.96–11.8)	1.39 (0.20)	3.34
	13	0.07 (0.04–0.09)	0.87 (0.56–1.9)	2.12 (0.33)	1.29
	14	0.00 (0.00–0.00)	0.28 (0.21–0.55)	3.83 (0.79)	0.43
	15	0.00 (0.00–0.00)	0.28 (0.21–0.55)	3.83 (0.79)	0.43
Ticomax	10	0.49 (0.29–0.79)	826.7 (132–28,062)	0.71 (0.12)	0.07
	11	0.22 (0.13–0.33)	219.9 (49.2–35,475)	0.78 (0.12)	1.80
	12	0.14 (0.04–0.25)	21.5 (5.0–1588)	1.06 (0.15)	6.79
	13	0.22 (0.09–0.40)	2.2 (0.87–101)	2.36 (0.24)	20.60
	14	0.07 (0.00–0.14)	1.9 (0.68–485)	1.63 (0.25)	10.90
	15	0.50 (0.00–0.10)	1.3 (0.52–106)	1.66 (0.30)	7.78
		Late-dormancy phase			
Tiktox	5	0.04 (0.03–0.06)	3.2 (1.2–16.4)	1.23 (0.16)	6.69
	6	0.02 (0.02–0.03)	0.20 (0.12–0.39)	2.33 (0.28)	1.06
	7	0.02 (0.01–0.02)	0.10 (0.07–0.18)	2.86 (0.34)	5.50
	8	0.01 (0.00–0.01)	0.05 (0.03–0.31)	3.03 (0.41)	10.35
Tatafen	5	0.12 (0.07–0.17)	11.7 (4.4–70.8)	1.17 (0.18)	1.35
	6	0.05 (0.03–0.07)	0.90 (0.50–2.9)	1.92 (0.33)	1.90
	7	0.04 (0.02–0.06)	0.56 (0.31–2.0)	2.05 (0.42)	3.02
	8	0.03 (0.00–0.04)	0.11 (0.08–1.3)	3.75 (1.43)	0.08
Ticomax	5	0.36 (0.23–0.56)	62.9 (18.7–536)	1.04 (0.15)	2.21
	6	0.19 (0.13–0.28)	14.8 (5.9–69.6)	1.24 (0.17)	3.27
	7	0.12 (0.09–0.16)	1.61 (0.94–3.9)	2.08 (0.28)	0.94
	8	0.07 (0.05–0.09)	0.62 (0.38–1.5)	2.47 (0.40)	4.91

0.11 μg/cm^2, 0.95 μg/cm^2, and 0.37 μg/cm^2, respectively for Tiktox, Tatafen, and Ticomax. Regression lines were steep for all three insecticides.

Mortality for early-dormancy phase. LC_{50} and LC_{99} values of the three insecticides during the early phase of dormancy and varying exposure periods are shown in Table 2. The lowest LC_{99} values were recorded after 15 d of exposure for Tiktox (0.08 μg/cm^2), Tatafen (0.28 μg/cm^2), and Ticomax (1.3 μg/cm^2). KC_{99} values for Tiktox (0.65 μg/cm^2) and Tatafen (0.61 μg/cm^2) that maintained knockdown for 12 h resulted in 99% mortality after 12–13 d of exposure for these insecticides. However, KC_{99} values for Ticomax (56.4 μg/cm^2) that maintained knockdown for 12 h were far beyond the recommended labelled rates, and hence the LC_{99} values for this insecticide were not considered. The shortest time to

reach 99% mortality was 13 d of exposure for Tiktox (0.41 μg/cm^2) and Tatafen (0.87 μg/cm^2) and 15 d of exposure for Ticomax (1.3 μg/cm^2). All KC_{99} concentrations of Tiktox and Tatafen during the early-dormancy phase provided 99% mortality after 13–14 d of exposure. All except the lowest KC_{99} concentration of Ticomax resulted in 99% mortality after 12–15 d of exposure (Table 1).

Mortality for late-dormancy phase. LC_{50} and LC_{99} values of the three insecticides during the late phase of dormancy and varying exposure periods are provided in Table 2. The lowest LC_{99} concentration was recorded after 8 d of exposure for Tiktox (0.05 μg/cm^2), Tatafen (0.11 μg/cm^2), and Ticomax (0.62 μg/cm^2). LC_{99} values that maintained knockdown for 12 h for Tiktox (0.11 μg/cm^2) and Tatafen (0.95 μg/cm^2) resulted in 99% mortality after 6 d of exposure and for Ticomax (0.37 μg/cm^2) after 8 d of exposure. The shortest time to reach 99% mortality was after 6 d of exposure for Tiktox (0.20 μg/cm^2) and Tatafen (0.90 μg/cm^2), and after 8 d of exposure for Ticomax (0.62 μg/cm^2).

KC_{99} concentrations after 6, 8, and 12 h of exposure for Tiktox resulted in 99% mortality by 8 d of exposure, and KC_{99} concentrations after 2 and 4 h of exposure did not lead to 99% mortality. All KC_{99} concentrations of Tatafen produced 99% mortality after 8 d of exposure. None of the effective KC_{99} concentrations for Ticomax provided 99% mortality (Table 1).

Slopes of regression lines were dependent on the period of insecticide exposure during the early- and late-dormancy phases. The regression lines were initially flat but became steeper with an increase in exposure time for all three insecticides.

Discussion

Bioassays revealed variation in efficacy among the three insecticides and between the early- and late-dormancy phases of *L. tristis*. For the recommended application rates, no compound provided significant mortality before 6–7 d. However, 99% knockdown was possible for 2–12 h with lower concentrations of all the insecticides, except for Ticomax during the early-dormancy phase.

Knockdown. At the lowest concentrations, Tiktox was the most effective insecticide for providing quick and prolonged knockdown extending beyond 12 h during both the early- and late-dormancy phases. Although Tatafen was equally effective as Tiktox in the early-dormancy phase, this material required nine times higher levels than Tiktox during the late-dormancy phase. Thus, Tiktox was more effective in the broader perspective. However, the intense odour of Tiktox makes it a less preferred insecticide for indoor use. Either Tiktox or Tatafen could be used for control during the early-dormancy phase when most home invasion occur. Ticomax was the least effective insecticide for controlling *L. tristis*, and it required concentrations above the recommended rate to obtain prolonged knockdown.

Higher susceptibility of *L. tristi*s to all compounds during the late-dormancy phase (Tiktox, 30X susceptibility; Tatafen, 3X; and Ticomax, 2X after 2 h exposure) makes insecticide application at this time a better option than attempting to control during the early-dormancy phase. This higher susceptibility is attributed to the reduced vigour of the starved beetles following 8–9 mo of dormancy (Sabu et al. 2008). However, higher KC_{99} values after 8–12 h exposure in the late-dormancy phase indicates unpredictability in the response to Tatafen.

The reduced vigour of dormant beetles contributed to the homogeneous response of *L. tristis* towards low insecticide concentrations during the late-dormancy phase compared to the mixed response towards higher insecticide concentrations during early-dormancy phase. During the early-dormancy phase, the knockdown values declined in the early hours of exposure, which indicates that *L. tristis* is initially resistant to these insecticides. This is in contrast to the weaker late-dormancy beetles that were knocked down easily without resistance. Low resistance of late-dormancy beetles to insecticidal action is evidence of the weak physical stature of the late-dormancy phase beetle population.

Mortality. Tiktox provided early mortality using recommended concentration levels. Prolonged knockdown followed by 99% mortality was observed with the lowest concentration of Tiktox for both early- and late-dormancy phase beetles. Although these insecticides caused immediate knockdown (99% within 2–12 h), mortality progressed more gradually depending on the insecticide, its concentration, and the dormancy phase of the beetle. The period of exposure required for obtaining 99% mortality varied greatly between the two seasons for all of the insecticides, with the weaker, late-dormancy beetles attaining 99% mortality seven days earlier than the early-dormancy beetles. As explained earlier, higher susceptibility of late-dormancy phase *L. tristis* is attributed to the reduction in vigour following the food deprived nine month- long dormancy.

Home invasions into residential buildings during the early-dormancy phase are a great nuisance to residents (Sabu et al. 2008), and the affected people often demand quick removal of the beetles. The present study showed that LC_{99} values for quick mortality earlier than 12 days during the early-dormancy phase was in excess of the recommended limits of the insecticides, making them undesirable for indoor application. Higher concentration for quick mortality is unwarranted as the beetles could be knocked down immediately with much lower concentrations and mortality could be achieved after 12–13 d of exposure. Hence, the best method for immediate control of *L. tristis* following home invasion is to knockdown the beetles with the required concentrations. Then the beetles should be physically removed and destroyed. In places where immediate removal of *L. tristis* is not necessary, the focus of control should be on reducing the amount of insecticide used and restricting insecticide application to the late-dormancy phase when the beetles are weaker and 40–50% of the aggregated beetles are unable to survive the food-deprived dormancy (Sabu et al. 2008). Therefore 99% mortality could be attained within seven days compared to the 14 days required during the early-dormancy phase.

We conclude that (1) Tiktox is superior to the other compounds in providing mortality and knockdown during both dormancy phases of *L. tristis*. (2) At recommended application rates, no insecticide provided significant mortality before 6–7 d. (3) Removing beetles physically after knocking them down with insecticides at the KC_{99} rate is the best method for controlling home invasions while minimizing insecticide use. (4) Lower levels of insecticides are needed to control beetles during the late-dormancy phase.

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