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CONTROL OF *LUPROPS TRISTIS* F. DURING ITS DORMANCY WITH INSECT GROWTH REGULATORS

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ABSTRACT

Hypersensitive and neurotoxic side effects of permethrin compounds and possibility of buildup of insecticide resistance against pyrethroid insecticides in darkling beetle *Luprops tristis* F. (Coleoptera: Tenebrionidae), a home-invading nuisance pest necessitates efficacy studies on other class of insecticidal compounds with more target specific action and less mammalian toxicity. The baseline dose- response bioassays conducted on *L. tristis* during their dormancy phase with three insect growth regulators (IGRs), fenoxycarb, diflubenzuron and 20-hydroxyecdysone (20E) revealed interesting results. Mortality (LC_{50} and LC_{90}), fecundity and egg hatchability were estimated by exposing dormant beetles to a range of concentrations. These data revealed that their LC_{50} values are within the permissible mammalian toxicity level. The capacity to reduce fecundity and hatchability of eggs (progeny production) together makes fenoxycarb a safer alternative to other pesticides in tackling *L. tristis*.

Key words: *Luprops tristis*, insect growth regulator, fenoxycarb, diflubenzuron, dormancy, 20-hydroxyecdysone (20E), bioassay, aggregated beetles, mortality, fecundity, egg hatchability

Massive home invasion of litter-dwelling Mupli beetle *Luprops tristis* (F.) (Coleoptera: Tenebrionidae), in the range of 500,000 to over 4 million/ residential building following summer showers, and their aggregation and prolonged stay in a state of dormancy are a regular event in rubber plantation belts in south India (Sabu et al., 2008). Litter stands of monoculture rubber plantations during non-rainy season are the feeding and breeding habitats for *L. tristis*. Rain-soaked litter stands during the monsoon period induce annual migrations of Mupli beetles to tile-roofed and palm-frond thatched residential buildings and other over-wintering quarters in the vicinity of rubber plantations (Vinod and Sabu, 2010). Following home invasion, clusters of these beetles crawl inside living areas, fall off from the ceilings into beds and food, and when 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 months in wet monsoon period (Sabu and Vinod, 2010).

Attempts to control the beetles with physical and mechanical means were all ineffective and failed for various reasons. Dormancy period of *L. tristis* is the most convenient time for their control as the home-invaded beetles in a specific locality, aggregate and enter into 8-9 months long dormancy in the attics of specific buildings and knocking down the home-invaded dormant beetles with permethrin-based compounds is the recommended methodology for *L. tristis* control (Aswathi et al., 2013). However, hypersensitive and neurotoxic side effects of permethrin compounds (Vijverberg and Bercken, 1990) and possibility of buildup of insecticide resistance against pyrethroid insecticides necessitates efficacy studies on *L. tristis* with other class of compounds having more target specific action and less mammalian toxicity. It leads to the present effort to analyze the utility of Insect growth regulators (IGRs). Insect growth regulators adversely interfere with the growth and development of insects (Dhadialla et al., 2005). Insect growth regulators are grouped under three categories: (i) Juvenile hormones (JHs) and their analogues, (ii) Ecdysone agonists and (iii) Chitin synthesis inhibitors (CSIs) based on their mode of action (Wing and Aller 1990). Juvenile hormone, fenoxycarb; Chitin synthesis inhibitor, diflubenzuron and Ecdysone agonist, 20- Hydroxyecdysone (20E) are effective against tenebrionid beetles especially against *Alphitobius diaperinus* Panzer which colonizes poultry and grain storage houses (Grenier and Grenier 1992; Singh and Johnson 2013). It is hypothesized that the

above three insect growth regulators (IGRs) which were effective against *A. diaperinus* with similar habits of aggregaion will be effective against *L. tristis.* Results will provide baseline data on the susceptibility of dormant *L. tristis* to the tested IGRs, which can be used as reference points for application of the compounds to the aggregated dormant beetles in residential areas.

MATERIALS AND METHODS

Aggregated dormant beetles were collected from a residential building near to 15 years old 5- ha rubber plantation (*Hevea brasiliensis* [Wild.ex ADR. De Jus] Muell. Arg. Of RRII 105 clone) from Kodenchery, Kozhikode, Kerala (11.4719^0N,75.96899^0E) by third week of August 2018. Collected beetles were maintained in laboratory by providing the cultural setup for dormancy (Sabu et al., 2008). Three insect growth regulators viz., a juvenile hormone analog (JHA), fenoxycarb (98%); a chitin synthesis inhibitor (CSI), diflubenzuron (99%); and the molting hormone, 20-hydroxyecdysone (20E) (98%) (Sigma Aldrich Laborchemikalien GmbH) were assayed. Stock solutions of 1000 ppm concentration of fenoxycarb, diflubenzuron and 20E were made by dissolving them in acetone. Serial dilutions of stock solutions were done to achieve five different concentrations-- of IGRs such as 0.01 ppm, 0.1 ppm, 1 ppm, 10 ppm and 100 ppm. Collected dormant beetles were exposed to selected concentration of three IGRs following the filter paper bioassay method (Tomberlin et al., 2002; Sheppard and Hinkle, 1987). Experimental setup containing Whatman No.1 filter paper (30 cm^2) placed in PVC vials (Tarsons; 5.5 × 4.5 cm; 50 ml capacity) for each concentration of the IGR was used. One ml of specific concentration of IGR was applied to the filter paper placed in individual vial with a micropipette for getting concentrations 3.33 mg/ cm^2, 0.33 mg/ cm^2, 0.03 mg/ cm^2, 0.003 mg/ cm^2 and 0.0003 mg/ cm^2. Filter paper wetted with acetone alone served as control.

Dormant adult beetles in stock culture were sexed based on sternal notch methodology (Vinod et al., 2008) and five mating pairs were transferred into each labeled vial. Six replicates were kept for each concentration of each IGR in experimental set up and three replicates were maintained for control in each set up. Beetles were observed at 24 hrs interval to record their mortality to fix the acute toxicity of each IGR tested. Towards the end of their dormancy period each mating pair of live beetles was transferred into post dormancy cultural set ups (Sabu et al., 2008). At the end of their post-dormancy period, egg lying was noted. Fecundity and % of egg hatchability were recorded. PROBIT analysis was used to determine lethal concentrations (LC) values and respective 95% confidence limits (Finney 1971, Robertson et al., 2007). All significance levels of variation in fecundity and % egg hatchability among the tested concentrations were subjected to two-way ANOVA and pair wise differences with Tukeys- Kramer Post hoc testes (t-tests). Significance was determined at $p < 0.05$. Analyses were done with Minitab software for Windows (Minitab 2010).

RESULTS AND DISCUSSION

Lowest LC_{50} and LC_{90} values were recorded for fenoxycarb compared to 20E and diflubenzuron. LC_{90} concentrations obtained for fenoxycarb, diflubenzuron and 20E were $2.30x10^7$ ppm, $1.63x10^{25}$ ppm and $1.12x10^{13}$ ppm and LC_{50} concentrations were $8.1x10^3$ ppm, $1.14x10^{13}$ ppm and $6.29x10^5$ ppm, respectively. For all the compounds, LC_{90} values were higher than mammalian toxicity levels. LC_{50} values of 20E and diflubenzuron, were higher than mammalian toxicity levels (LD_{50} of 20E for mammals > 6000 ppm; LD_{50} of diflubenzuron for mammals > 4640 ppm, (Fischer and Hall 1992, Dinan and Lafont 2006) and LC_{50} value of fenoxycarb was lower than mammalian toxicity level (LD_{50} for mammals >10000 ppm) (Sullivan 2000). Hence, though with the lowest LC_{50} value compared to other two compounds, lower mammalian toxicity level of fenoxycarb makes it as the safer IGR than 20E and diflubenzuron for direct killing of dormant *L. tristis* aggregated inside residential buildings.

Fecundity of dormant beetles treated with five tested concentrations of fenoxycarb, diflubenzuron and 20E were lower than that of untreated control beetles ($p < 0.05$). Fecundity of beetles treated with fenoxycarb, diflubenzuron and 20E did not vary for all treatments ($p > 0.05$) except for 100 ppm concentration treatment with diflubenzuron. Diflubenzuron at 100 ppm concentration recorded the lowest fecundity (24.67 ± 1.03) which was 40% lower than the fecundity recorded with untreated control and 13.4% and 15.5 % lower than the fecundity with fenoxycarb and 20E respectively. Hatchability of eggs laid by dormant beetles treated with fenoxycarb, diflubenzuron and 20E were different at 100 ppm and 10 ppm concentrations ($p< 0.05$) and not different for other concentrations. Lowest hatchability rate of eggs (19.67± 0.81) was observed in beetles exposed to 100 ppm concentration of diflubenzuron. When cumulative effect on progeny reduction (by combining

Table 1. Mortality, fecundity and egg hatchability of *L. tristis* as influenced by the IGRs

IGR	% mortality				
	100 ppm	10 ppm	1 ppm	0.1 ppm	0.01ppm
FXB	65	56.66	55	53.33	50
DFB	53.33	51.22	50	50	46.67
20E	60	55	55	50	50
Control	42				
			Fecundity		
FXB a	28.50± 1.87a	35.33± 1.86a	38.17± 1.47a	38.50± 2.16a	39.5± 1.38a
DFB a	24.67± 1.03	36.83± 1.47a	38.50± 1.37a	38.67± 1.03a	38.17± 2.93a
20E a	29.16± 1.47a	35.50± 1.97a	36.50± 1.64a	37.5± 1.05a	41.50± 2.17a
Control	40.83± 2.36				
			Egg hatchability		
FXB a	24.17±1.94	30.67± 1.03	33.83± 1.94	34.33± 2.33	34.17± 0.75
DFB b	19.67± 0.81	33.50± 1.04	35.67± 1.75a	36.67± 1.21a	35.83± 2.64
20E	27.33± 0.82	34.33± 2.25	34.83± 0.98a	36.00± 0.89a	40.5± 2.95
Control ab	36.5± 2.07				

Means in same column with similar letter (s) not significantly different (p> 0.05); values transformed to % for analysis, but actual values given here; FXB-fenoxycarb; DFB-Diflubenzuron; ZOE-Zohydroxy ecdyscre

both reduction in fecundity and hatchability rate of eggs laid) in the next life cycle stage, the post dormant phase alone is considered, diflubenzuron at 100 ppm with 47% reduction in progeny production was more effective than the 100 ppm concentrations of fenoxycarb and 20E with 33.80% and 25.12% reduction in progeny production.

Comparing the effects of three tested IGRs on dormant beetles, none of the three compounds was effective enough to control *L. tristis* by causing higher mortality of adults or significant reduction in progeny production with the permissible limits.
As fenoxycarb can bring down the population number of *L. tristis* substantially by causing 50% mortality of dormant beetles and later by causing 33.5% reduction in the progeny production in the surviving dormant beetles (during post dormancy phase after their return to the litter field after 5 months from application), collectively, it leads to a gradual 83.5% fall in the population of *L. tristis*, fenoxycarb is better than the other two tested IGRs and is a safer alternative to pesticides in controlling home invaded *L. tristis* and repeated applications in successive years can bring down the *L. tristis* populations**.**

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