EVALUATION OF THE EFFECT OF INSECT GROWTH REGULATORS ON THE NUISANCE PEST *LUPROPS TRISTIS* FEBRICIUS, 1801 (COLEOPTERA: TENEBRIONIDAE)

Thesis submitted to the

UNIVERSITY OF CALICUT

For the award of the Degree of

DOCTOR OF PHILOSOPHY IN ZOOLOGY

(Under the Faculty of Science)

BY

BINSHA P

Under the Guidance of

DR. SABU K. THOMAS

P.G. & RESEARCH DEPARTMENT OF ZOOLOGY,

ST. JOSEPH'S COLLEGE (AUTONOMOUS), DEVAGIRI, CALICUT, KERALA

MARCH 2020

P.G. & RESEARCH DEPARTMENT OF ZOOLOGY
ST. JOSEPH'S COLLEGE (AUTONOMOUS), DEVAGIRI
KOZHIKODE - 673 008
KERALA, INDIA
Ph: 9447349744
e-mail: sabukthomas@gmail.com

Dr. Sabu K. Thomas
Associate Professor of Zoology

04 March, 2020

CERTIFICATE

This is to certify that the thesis entitled **"EVALUATION OF THE EFFECT OF INSECT GROWTH REGULATORS ON THE NUISANCE PEST *LUPROPS TRISTIS* FABRICIUS, 1801 (COLEOPTERA: TENEBRIONIDAE)"** submitted to the University of Calicut for the award of the degree of Doctor of Philosophy in Zoology, is the record of the original work done by **Ms. BINSHA P. i**n the PG & Research Department of Zoology, St. Joseph's College (Autonomous), Devagiri, Kozhikode, under my supervision and guidance, and that it has not formed the basis for the award of any degree / diploma or other similar title to any candidate of any University.

Dr. Sabu K. Thomas
Supervising Teacher

P.G. & RESEARCH DEPARTMENT OF ZOOLOGY
ST. JOSEPH'S COLLEGE (AUTONOMOUS) DEVAGIRI
KOZHIKODE - 673 008
KERALA, INDIA
Ph: 9447349744
email: sabukthomas@gmail.com

Dr. Sabu K. Thomas
Associate Professor of Zoology

28 September, 2020

CERTIFICATE

Certified that the thesis entitled **"EVALUATION OF THE EFFECT OF INSECT GROWTH REGULATORS ON THE NUISANCE PEST *LUPROPS TRISTIS* FABRICIUS, 1801 (COLEOPTERA: TENEBRIONIDAE)"** submitted to the University of Calicut for the award of the degree of Doctor of Philosophy in Zoology by **Ms. BINSHA P.**, has made the corrections/ suggestions from the adjudicators have been incorporated.

Dr. Sabu K. Thomas
Supervising Teacher

DECLARATION

I do hereby declare that the work entitled "**EVALUATION OF THE EFFECT OF INSECT GROWTH REGULATORS ON THE NUISANCE PEST *LUPROPS TRISTIS* FABRICIUS, 1801 (COLEOPTERA: TENEBRIONIDAE)**" is an authentic record of the work carried out by me under the supervision and guidance of Dr. Sabu K. Thomas, Associate Professor, PG & Research Department of Zoology, St. Joseph's College (Autonomous), Devagiri, Kozhikode, and that no part of this has been published previously or submitted to the award of any other degree / diploma.

Place: Devagiri

Date: 04-03-2020

BINSHA P

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Dedicated to my Parents and Teachers

CONTENTS

CHAPTER 1

INTRODUCTION

1.1 Study organisms: *Luprops tristis* (Mupli beetles)

Luprops tristis, Fabricius, 1801 (Coleoptera: Tenebrionidae) is generally regarded as an inconspicuous, detritivorous litter dwelling darkling beetle found in rubber plantation belts in Kerala (Doyen *et al*.,1990). Their home invasion in huge aggregation following summer showers and their prolonged stay in dormancy state is a regular event in rubber plantation belts along the western slopes of southern region of Western Ghats. In central and south regions of Kerala, they are popularly known as "Mupli Vandu", in reference to the first reporting of their huge aggregations in rubber estates at Muplium in Central Kerala during the 1970s. Its familiar presence in firewood stocks, below coconut sheaths in thatched sheds and on wood works supporting earthen tile roofing has led to popular names Otteruma, Ola prani, Oadu vandu, Karichellu in North Kerala. Earlier it was wrongly considered as *Luprops curticollis* Fairmaire, 1896 (Narendran 1998) and re-verification of its taxonomic status revealed that *Luprops tristis* Fabricius, 1801 is the species found in rubber plantation belts of south Western Ghats (Sabu *et al*., 2007). Members of *Luprops* Hope, 1833 are inconspicuous, detritivorous and litter dwelling beetles found from tropical Africa through Asia and East Indies to Papua New Guinea (Doyen *et al*., 1990).

Luprops tristis beetles were not so common and not recorded from south and north region of Kerala during 1972 (Kaszab 1978) due to their absence in the extensive darkling beetle collections from the Western Ghats (Kaszab 1979). This leads to the conclusion that *Luprops tristis* became wide spread along with the spread of rubber plantation across Kerala during 1970 – 1980 periods after its first sighting in Muplium plantation belts.

Earlier presence of *L. tristis* recorded from Madurai and Andipatti in the eastern rain shadow belts of the Western Ghats (Fabricius 1801) and the presence of *Luprops* beetles also recorded in the poultry manure regularly brought to the rubber plantations from Madurai and Andipatti region. This leads to the conclusion that it is an invasive species that reached the Kerala rubber belts from Tamilnadu along with poultry dung (Sabu *et al*., 2007).

Litter stands of rubber tree (*Hevea brasiliancis*, (willd. Ex Adr.De Jus) Muell. Arg.1865) are the feeding and breeding habitat of *L. tristis* and tender rubber leaves as the most favored food resource. Perfect synchronization of the life cycle of the beetle with the leaf phenology of the rubber and availability of tender rubber leaves from the powdery mild dew disease mediated leaf fall to the mating females and young ones are the major reason for its high abundance in rubber plantation belts (Sabu and Vinod, 2009a, b). The entire population of *L. tristis* leaves the litter floor and invades nearby residential buildings, other over-wintering quarters in the premises of residential buildings and cervices below the boulders with the onset of summer rains. Clumps of many hundreds to thousands of beetles invading residential building crawl inside living rooms, fall off into beds and food items from ceilings. Subsequently they concentrate in undisturbed dark areas like attics and wall voids and remain dormant for 8-9 months (Figure 1). Their frustrating nocturnal movements and high abundance in the range of 0.5 to over 4 million per residential building make them a serious nuisance pest in the rubber plantation belts. They do not sting or bite, but when disturbed (such as picking them off the walls or they

are squashed or pressed against while sleeping), they release an odoriferous phenolic secretion that causes irritation to eyes and skin burning (Dona *et al*., 2010).

The long duration dormancy shown by *L. tristis* adults does not fall under either quiescence or diapause. Term quiescence used to denote the life situation with a shorter period without an induction phase and with active feeding throughout the stage (Mansingh 1971; Danks 1987), on the other hand diapause is the stage that preceded by a long preparatory phase, in which energy reserves are accumulated for a specific stage in life cycle. Entry of *L. tristis* beetles irrespective of the active feeding time available in pre-dormancy indicates that preparations for dormancy by accumulation of energy reserve prior to it are not mandatory in *L. tristis*. In the present case beetles are attracted towards food resources and water during pre-dormancy phase and entered into dormancy irrespective of the active feeding refractory phase duration without a preparatory phase or an induction period and only response to an environmental token stimulus, the short mid-summer rain fall that arbitrate wetness in the rubber plantation litter floor. These observations favored our categorization of dormancy in *L. tristis* as oligopause, which is an intermediate between quiescent and diapauses (Mansingh 1971; Danks 1987; Leather *et al*., 1995).

Wetness mediated by summer showers act as cue which induces the annual migration of *L. tristis* to the shelters and their entry into dormancy and postponement of reproduction (Vinod and Sabu, 2009). Post dormancy beetles returned to the rubber plantation litter floor in time with the annual rubber leaf shedding during the pre- summer period (Sabu *et al*., 2008). At the time of leaf sprouting and tender leaf fall, parental adults

peaks in their number and larvae and teneral adults peaks at the time of premature fall of green leaves and flowers. Teneral adults feed intensely till the onset of summer shower and migrate to shelters in the next season. With their detritivorous litter dwelling habitats, harmless nature, diurnal passivity in lower litter layers, and nocturnal surface activity make them inconspicuous facilitators of litter decomposition and nutrient cycling in monoculture rubber plantation belts. However, their massive seasonal invasion in and around residential buildings for a prolonged period make them most dreaded beetles in rubber growing regions (Sabu *et al*., 2007, 2008).

1.1.1 Control measures

Residential buildings in the rubber plantation belts with climatic condition coupled with heavy humidity and harsh tropical summers are either tile roofed buildings with multiple doors and windows and ventilated attics with wooden ceilings or palm frond thatched sheds. Gaps in the palm frond thatched sheds and between the tiles in tile roofed buildings act as an inlet for the entry of beetles into residential buildings. Hence, attempts to control the beetles with various physical and mechanical means such as installing window screens, cauling cracks in walls, smoking the aggregation site with camphor and frankincence (kunthirikkam in Malayalam) and placement of light traps in and around the plantation to makeshift thatched sheds were not successful. Very high abundance in aggregation, their swift movements, difficulty in reaching the aggregation sites in the attics and release of odorous phenolic defensive gland secretion that causes skin burns and eye inflammation makes manual collection of *L. tristis* impossible.

Insecticide based control measures for *Luprops tristis* were impossible in the rubber plantation fields mainly due to their litter dwelling habitat, short life duration in the fields and large area of rubber plantation. Pyrethroid insecticide based methodology have been developed recently for the control of home invaded *L. tristis*. Beetles in their aggregation could be knocked down for a shorter period with low concentration of insecticides. But, higher dosages of insecticides are required for their immediate mortality. However, following insecticide application to the aggregated beetles, beetles secreted their odoriferous defensive gland secretion with repelling odour and it cause eye irritation. So, entry to the aggregation sites for the removal the knockdown or mortile beetles practically difficult for 6– 8 hrs following insecticide application. In the absence of any other possible control methods, knocking down with permethrin based compounds, that makes the aggregated beetles immobile and enable their collective removal after a short waiting period in the residential areas and direct killing with its higher dosage and removal of the dead beetles after 13–15 days are the current recommended methodology for *L. tristis* control (Aswathi *et al*., 2013; Aswathi and Sabu, 2013). But, the hypersensitive and neurotoxicological side effects of permethrin compounds (Vijverberg and Bercken, 1990) and the possibility to develop insecticide resistance in test insect necessitates efficacy studies on *L. tristis* with other class of compounds having more target specific action and less mammalian toxicity. It leads to analysis of the utility of Insect Growth Regulators.

1.2. Insect Growth Regulators (IGRs)

Williams (1956) provided a boost to the research on hormonal control of growth regulation in insects and put forwarded that Juvenile hormone (JH) may be employed in insect control. His constant research on various physiological effects of juvenile hormone led him to come to an end that these hormonal compounds can be also used as specific control agents for insects to which pest species may not be able to develop resistance. Williams (1967) announced insect hormones as "third generation pesticides" and suggested that it would not only be environmentally benign but that the pest insects would also be unable to develop resistance. Since hormones regulate insect development and differentiation, their analogues are collectively called as Insect Growth Regulators (IGRs).

Insect Growth Regulators have been known to contribute effects on biological aspects of the treated insects, for example, embryonic and post embryonic development, reproduction (fecundity and fertility), behavior and mortality. Irregular morphogenesis, mainly of the integument is broadly irreversible and is a readily observed aspect of IGR action on insects (Grosscurt 1978; Mian and Mulla, 1982; Eisa *et al*., 1984). Many IGRs are influential even against the egg stage (Mian and Mulla, 1982; Mondal *et al*., 1999). External and internal morphogenetic effects also influence mating and other reproductive functions of the insects directly or indirectly (Oberlander *et al*., 1975; Edwards 1976; Parween 1996). Further- more, morphological abnormalities also result indirect mortality through the impairment of sensory functions, behavior, feeding and so on (Retnakaran *et al*., 1985). IGRs may disturb larval growth by affecting larval weight and length, increasing larval period and lead to larval mortality at different instars resulting in reduced

percentage of pupal eclosion. Direct topical treatment to pupae, or the effect of larval treatment, can cause pupal mortality, loss of pupal weight, prolonged pupal period and defeat adult emergence. Compared with conventional insecticides, IGRs do not exhibit a quick knock- down effect in insects (Fox 1990); fairly long- term exposure to micro doses of these compounds pause generation growth due to the effects mentioned earlier in both parent and progeny (Mian and Mulla ,1982; Parween 1996).

Since the target sites of common insecticides on insects and mammals are known to be similar, it is desirable to develop insecticides whose primary target site does not exist in mammals for selective toxicity. IGRs belong to this type of selective insecticides. On the basis of the mode of action, workers (Willis 1974; Stall 1975; Marx 1977; Bengston 1986; Reynold 1987; Wing and Aller, 1990) grouped Insect Growth Regulators into three categories: (i) Juvenile hormones (JHs) and their analogues also called as Juvenoids; (ii) Ecdysone agonists and (iii) Chitin Synthesis Inhibitors (CSIs) or moult inhibitors.

1.2.1 Juvenile Hormone Analogues (JHAs)

Juvenile hormones (JH) are responsible for the maintenance of the juvenile state of insects that is programming and governance of metamorphosis (Edwards and Menn, 1981). Juvenile hormones analogues can operate as agonists or antagonists or a combination of both with natural juvenile hormone (Kramer and Stall, 1981). JHs have two specific biochemical effects: one during the larval stage and other in the adult stage. During the larval stage JHs suppresses metamorphic change during moulting; and in adult it induces vitellogenin synthesis during ovarian development. JH analogues interfere with critical biochemical mechanisms such as the production and transportation of natural JHs from the

secretary site to the target site, decadence, excretion and feedback control (Retnakaran *et al*., 1985).

Biological effects of JH analogues are very complex and differ from one analogue to other and have considerable capability as pest control agents and their low vertebrate toxicity makes them specifically suitable for use in areas of public health, human food manufacture and livestock rearing (Edwards and Menn, 1981). JH analogues such as methoprene and fenoxycarb have been shown to be highly active againts a wide range of pests (Strong and Diekman, 1973) including the tenebrionid beetles *Tribolium castaneum*, *Tribolium confusum*, *Tenebrio molitor* and *Alphitobious diaperinus* (Loschiavo 1976; Thind and Edwards, 1986; Grenier and Grenier, 1992; Wijayaratne *et al*., 2011; Singh and Johnson, 2013). However, there is no published information on the activity of such compounds againts *Luprops tristis*. According to Edward and Abraham (1985) fenoxycarb was found to be more effective than methoprene in *A. diaperinus* and non- neurotoxic nature of fenoxycarb prompted to test the effect of fenoxycarb on different life stages of *L. tristis.*

1.2.2 Chitin Synthesis Inhibitor (CSI)

This class of compounds disturb the biosynthesis of chitin in insects (Post and Vincent, 1973; Post *et al*., 1974; Deul *et al*., 1978; Hajjar and Casida, 1978; Gijswijt *et al*., 1979) and thus inhibits moulting, or produces an inexact cuticle (Mulder and Gijswijt, 1973; Mulder *et al*., 1975; Ishaaya and Casida., 1974; Hammock and Quistad, 1981). These compounds are active suppressors of development for the entire life cycle of insect pests (Verloop and Ferrell, 1977; Grosscurt 1978). Insufficiency or excess in synthesis of

chitin during either morphogenetic cycle in an insect can produce deleterious and lethal effects. Therefore, chitin biosynthesis is an absolute target for the development of pesticides. Many compounds ranging from natural products like plumbagin (Kubo *et al*., 1983) to antibiotics such as polyoxins and nikkomycin (Hori *et al*., 1971), insecticides such as benzimidazoles (Kuvano *et al*., 1982) and fungicides such as Captan® (Becker *et al*., 1978) have been shown to inhibit chitin synthesis in insects.

Chitin Synthesis Inhibitors are primarily stomach poisons (Wellinga *et al*., 1973; Fox 1990) and effective against the early instar larvae of holometabolous insects (Hammann and Sirrenberg, 1980). The insects' vulnerability to these compounds is an important factor, as chitin synthesis in treated insects regain after a short exposure (Grosscurt 1978; Neumann and Guyer, 1987). Contact activities of these compounds against eggs and larvae have also been reported (Hammann and Sirrenberg, 1980; Wang *et al*., 1994). CSI compounds are well reported as larvicides of lepidopteran and coleopteran insects of the stores. However, low doses of these compounds produce deformities at the pupal and adult stages (Retnakaran *et al.*, 1985; Parween 1996).

Several compounds of a new class of insecticides benzoylphenylureas, which are able to hamper with chitin synthesis, have been evaluated for their insecticidal activity against wide range of insect pests (Magagula and Samways ,2000; Khaled 2009; Bakr *et al*., 2010; Khan and Qamar, 2011; Misrhe *et al*., 2013; Talch *et al*., 2015). The most widely used benzoylphenylurea, diflubenzuron, was found to induce the deterioration of newly synthesized chitin in the insects (Wellinga *et al*., 1973; Post *et al*., 1973, 1974 and Ishaaya and Casida, 1974). Diflubenzuron was found to be effective against many

tenebrionid beetle pests such as *Tribolium castaneum* (Herbst) (Elek and Longstaff, 1993; Merzendorfer *et al*., 2012), *Tribolium confusum*, (Mc Gregor and Kramer, 1977) and *Alphitobious diaperinus* (Singh and Johnson, 2013). Present study aims to evaluate the toxic effect of diflubenzuron on *Luprops tristis* life stages.

1.2.3 Ecdysone agonists

Ecdysone agonists are quite disparate from Juvenile hormone analogues and Chitin synthesis inhibitors in their nature (Aller and Ramsay, 1988). Zooecdysteroids have essential functions in the activation of genes for cuticle formation, spermatocyte growth and induction of diapauses (Koolman 1989). Broadly, ecdysone in insects acts as a trigger in several systems (Willis 1974) appropriate for coordinating the numerous events at the moult cycle (Locke 1964). Application of these compounds drive to premature cuticle synthesis, specifically around the head region where occlusion of the functional mouth parts can cause a feeding inhibition (Wing and Aller, 1990) regardless of the age or instar of the treated insect (Schneiderman 1972; Fox 1990). Compounds like RH-5849 and RH-5992 are dibenzoyl hydrazines, possessing unique characteristics of mimicking insect molting hormones (Wing 1988; Wing *et al*., 1988; Oberlander *et al*., 1997). Ecdysone agonists also have chemosterilant activity on female insects. They are effective at low doses, moderately persistent and safe for non- target organisms (Fox 1990; Heller *et al*.**,** 1992).

Several non- steroidal agonists of ecdysone appear to cause a variety of effects such as stomach and contact poisoning, paralysis and death, reducing oviposition and feeding

and also other molting and behavioural effects in coleopterans (Fox 1990; Darvas *et al*., 1992; Salgado 1992; Wing *et al*., 1992; Smagghe and Degheele, 1994; Aller and Ramsay, 1998) and also in tenebrionid beetle *Tenebrio molitor* (Tiabi *et al*., 2002; Smagghe *et al*., 2003). 20- Hydroxyecdysone (20E) formed by ecdysone 20- monooxygenase- catalyzed hydroxylation of ecdysone, is the major active hormone that controls embryonic development, moulting, metamorphosis and reproduction in insects (Dhadialla *et al*., 1998) and it was found to be effective against *Alphitobious diaperinus*, cosmopolitan pest present in poultry production facilities (Singh and Johnson, 2013). So, analyzing the effect of 20E in the life stages of *Luprops tristis* is pertinent as it may lead to development of a management strategy using ecdysone agonists**.**

A

B

Figure 1: Multi layered aggregation of *Luprops tristis* A) in the wall of a residential building ., B) in a tile roofed building .

CHAPTER 2

REVIEW OF LITERATURE

2.1. *Luprops tristis*

Luprops beetles in general considered as an inconspicuous litter-dwelling detritivore which extend from tropical Africa through Asia to Australia (Doyen *et al*., 1990). Taxonomic studies by Sabu *et al*., (2007) *Luprops* in Kerala regions taxonomically reverified as *Luprops tristis* Fabricius, 1801 and not as *L. curticollis* Fairmaire, 1896 and arrival of *Luprops tristis* as an invasive pest to Kerala.

Studies on the biology, dormancy and habits of *Luprops tristis* disclosed that litter floor of rubber plantations are the feeding and breeding habits of this beetles and the reproductive activities are limited to the phase after dormancy (Sabu *et al*., 2008). Strong preference of *L. tristis* toward wilted tender rubber leaves and perfect synchronism between the beetle life cycle and the leaf phenology of rubber tree contributed to their unrivalled abundance in rubber belts (Sabu and Vinod, 2009 a, b). Vinod *et al*., (2008) detailed out about the detection of a sexual dimorphic character in *L. tristis* and sternal notch methodology of sex determination. Morphology of its defensive gland was described by Abhitha *et al*., (2010) and Vinod and Sabu (2010) confirmed the rain fall-mediated wetness from summer showers act as the dormancy inducing cue in *L. tristis*. Aswathi *et al*., (2013) observed the knockdown status of *L. tristis* beetles exposed to low dosage of pyrethroid insecticides for sufficient period of time to enable their physical removal from aggregation sites. Variation in insecticide susceptibility of *L. tristis* in different stage of their dormancy was detailed (Aswathi and Sabu, 2013). Nirdev *et al*., (2014) extracted air borne volatiles from the natural aggregation site of *L. tristis* during

different states of dormancy and reported distinct seasonality in the distribution of volatile chemicals during dormancy.

2.2. Insect Growth Regulators

Insect Growth Regulators (IGRs) evolved rapidly following the discovery of juvenile hormone and its activity in Cecropia moth by Williams (1956). Williams (1967) coined the term "third generation pesticides" to describe hormone-based insect growth regulators. As their name indicates, this new class of chemicals regulate the growth and development of insects (Staal 1975), control insects either through regulation of metamorphosis or interference with reproduction (Riddiford and Truman, 1978), affecting either egg production, the development of brood or both (Vargo and Laurel,1994). An IGR, therefore, does not necessarily have to be toxic to its target, but may lead instead to several abnormalities that impair insect survival (Siddall, 1976). The subtle, delayed effects of IGRs are vastly different to quick direct kill chemicals they replaced (Mondal and Parween, 2000). IGRs are quite selective in their mode of action and potentially act only on target species (Tunaz and Yugun, 2003). A number of IGRs have been developed, and the selection of which chemical to use in a given case is dependent on both the target organism and the environment in which it will be applied. In the USA, an IGR is provisionally approved for use by the EPA (Environmental Protection Agency), based on the results of laboratory and field testing (US EPA 1991).

2.2.1. Juvenile Hormone analog: Fenoxycarb

The search to apply knowledge of Juvenile hormones to the development of effective insecticides has since been limited to the area of Juvenile hormone analogs (JHAs). JHAs act in the same manner as JHs but are much more chemically stable (Matolcsy *et al*., 1988). Manser *et al*., (1980) defined the JH mimetic effects of Fenoxycarb which are mainly morphogenetic: metamorphosis is disturbed or blocked at the end of the last larval instars. Lohri and Masner (1981) detailed the mortality caused by fenoxycarb treatment on green peach aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae) and the chrysanthemum aphid *Macrosiphoniella sunhorni* (Gillette). Fenoxycarb was efficient to control *Pandemis heparana* after fenoxycarb treatment on substratum, the growth of the different larval instars was affected and metamorphosis was disrupted with production of supernumerary instars, adultoid in appearance (Cavalloro and Piavaux, 1984) and inhibits the transformation into adults of *Archips podana* (Lepidoptera: Tortricidae) larvae even at much lower concentrations (0.001%) (Molnar *et al*., 1985). Besides the morphogentic effects on larva and pupa, there are other effects generally occur with this JH analog on embryogenesis, reproduction, pheromone production, caste differentiation or diapauses (Retnakaran *et al*., 1985). Fenoxycarb also shows a JH mimetic action on *Triatoma infestans* (Klug) (Hemiptera: Reduviidae), the principal Chagas disease vector in Argentina, the effective dose (ED 50) is 0.02 pg/insect, when it reaches 10.8 pg for malathion (Villar *et al*., 1986). In California, fenoxycarb disturbs the larval development of the citrus thrips, *Scirtothrips cirri* (Moulton) (Thysanoptera: Thripidae)

and slightly reduces the reproduction rate of adults, without inducing mortality (Grout and More, 1986).

In the laboratory as well as in fields in India, different formulations of fenoxycarb are effective to control dipterans such as *Culex quinquefasciatus, Aedes aegypti* and *Anopheles srephensi* (Tyagi *et al*., 1987). According to Ishaaya (1990) fenoxycarb inhibit the last stage of oogenesis and both larval and pupal development in treated insects.

Fenoxycarb possesses ovicidal properties against the fall armyworm *Spodoptera frugiperda* Smith (Gardner, 1991). When fenoxycarb was topically applied at 100 pg per newly moulted fourth instar larva of the migratory grasshopper *Melanoplus sanguinipes* (F.) (Orthoptera: Acrididae), larval duration was prolonged, nymphal and adult survival reduced and egg production by surviving females is completely inhibited (Capinera *et al*., 1991). Fenoxycarb at 0.50 and 1.00 ppm concentrations behaves as insecticides that severly hamper the normal growth, development and metamorphosis of *Coreya cephalonica* (Lepidoptera: Pyralidae) (Singh and Tiwari, 2014).

Fenoxycarb at very low doses (<20 mg/kg) suppressed the larval development in coleopteran pests (*Sitophilus oryzae* (L.), *S. granarius* **(**L.), *S. zeamais* Motschulsky. *Tribolium confusum* Duv, *T. castaneum* (Herbst), *Oryzaephilus surinaniensis* (L.), *Rhpzopertha dominica* (F.), *Cryptolestes pusillus* (Schonh.), *Trogoderma vuriabile* Ballum) and lepidopteran pestes (*Plodia interpunctella* (Hb.), *Sitotroga cerealella* (Olivier), *Ephestia cautella* (Walker)) in stored wheat (Kramer *et al*., 1981; Eisa and Ammar, 1992). In rice fenoxycarb at 5 and 10 mg/kg controls *S. cerealella, R. dominica, S. oryzae* and *T. castaneum* for 18 months. Whereas, malathion no longer acts after six

months (Cogburn, 1988). After treatment of insects or bark with fenoxycarb decreased the female fecundity and egg hatching success in scolytid species *Ips paraconfusus* Lanier (pest of *Pinus ponderosa* Laws) (Chen and Borden, 1989). Fenoxycarb was ovicidal for the Fuller rose beetle *Pantomorus cervinus* (Boheman) by dipping in 100 mg/liter solution, leading Coats (1990) to recommend the use of fenoxycarb as a dip for fruits, in quarantine treatment before export, to limit dispersion of the pest. Edwards *et al*., (1991) proved that treatment of wheat grain at 8.2 mg/kg concentration was effective in controlling populations of four stored wheat pests *O. surinamensis*, *R. dominica, S. granaries* and *T. castaneum* for the entire period of trial. Letellier *et al*., (1994) observed 45.5% adult mortality and 95% inhibition of progeny in *S. zeamais* after three weeks exposure to grain treated with fenoxycarb at 10 mg/kg.

There is no scientific data available on the fenoxycarb efficacy in *Luprops tristis*. Search for IGR efficacy in other darkling beetles reported as pest in agricultural fields and poultry farms showed that Juvenile hormone fenoxycarb treatment at any immature stage reduces adult emergence in yellow mealworm, *Tenebrio molitor* L., and confused flour beetle, *Tribolium confusum* (Pallos *et al*., 1971). Edwards and Abraham (1985) studied that fenoxycarb was more effective than methoprene in tenebrionid beetle *Alphitobius diaprinus*. Trials were undertaken in the US by Kramer *et al*., (1985) to study the insecticidal effectiveness of fenoxycarb at 10ppm againts *Tribolium confusum*, it was found that fenoxycarb controlled *T. confusum* population for two seasons. White (1987) examined the residual action of fenoxycarb on forth instar larvae of *T. castaneum* were kept in contact with and it was suggested that a surface treatment would control

populations in grain residues up to 2 cm deep. Smet *et al*., (1989) observed that in *Tribolium confusum*, the larval period increased with increased dose of fenoxycarb. Fenoxycarb significantly delayed the developmental times of insect larvae from the stage treated to adult emergence (Liu and Chen 2001). The mean number of days to pupation increased and pupation was delayed in fenoxycarb-treated seventh instar larvae of *Alphitobius diaperinus* in feeding, residual, and topical bioassays (Singh and Johnson, 2013).

2.2.2. Chitin Synthesis Inhibitor: Diflubenzuron

Diflubenzuron, better known by its trade name Dimilin ^{TM} was the most extensively studied benzoylphenylureas that is commercially available and finds wide application in forestry, some agricultural crop pests, fly and mosquito control (Maas *et al*., 1980). It was found to induce the degradation of newly synthesized chitin in the insects (Wellinga *et al*., 1973; Post *et al*., 1973, 1974; Ishaaya and Casida, 1974).

In the larvae of *Musca domestica* (Diptera: Muscidae) it was observed that diflubenzuron disturbs the synthesis of chitin by reducing the rate of production of chitin during cuticle deposition (Grosscurt, 1976). In Orthopterans, the presence of diflubenzuron was found to reduce the amount of chitin deposited in the peritrophic membrane and gives it loose and fibrous texture (Clark *et al*., 1977; Becker, 1978). In the spruce budworm, *Choristoneura fumiferana*, (Lepidoptera: Tortricidae) larvae in the fifth and sixth stadia were more susceptible to diflubenzuron than in the earlier stages (Granett and Retnakaran, 1977). Some species like the forest tent caterpillar, *Malacosoma disstria*, (Lepidoptera: Lasiocampidae) and the gypsy moth, *Lymantria dispar* (Lepidoptera: Erebidae) are very

sensitive to diflubenzuron (Retnakaran *et al*., 1985). It has been used to control cockroaches, locusts, grasshoppers and most leaf-feeding larvae (Weiland *et al*., 2002). Both topical treatment and contact with IGR-treated peach leaves caused significant mortality and inhibition of moulting of first instar *Chrysopa owlata* Say (Neuroptera: Chrysopidae)., reduced the emergence of *Macrocentrus ancylivorus* (Rohwer) (Hymenoptera: Braconidae) adults from treated larvae that had successfully pupated (Broadbent and Pree, 1984). Diflubenzuron was generally found to be safe for hymenopteran honey bee brood in fields treated at 35 to 400 g/ha and it was harmful to bumble bees at 300 g/ha (Tasei 2001).

Mc Gregor and Kramer (1977) examined the reduction in F1 progeny of stored coleopteran pests adult *Sitophilus granaries* (Coleoptera: Curculionidae), *S. oryza* (Coleoptera: Curculionidae), *Rhyzopertha dominica* (Coleoptera: Bostrichidae), *Tribolium confusum* (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) and *Lasioderma serricorne* (Coleoptera: Ptinidae) on diflubenzuron treatment on maize and wheat. Desmarchelier and Allen (1992) also suggested that difubenzuron can be used as a grain protectant to control *Sitophilus* species such as *S. oryzae* and *S. granarius*. Untreated adults of Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) the mechanical penetrability of the elytra decreases until about 10 days after adult emergence. At any time during this period, this change in penetrability can be blocked by administering diflubenzuron (Grosscurt, 1978). Eggs from white-fringed weevil *Naupactus leucoloma* (Coleoptera: Curculionidae) adults fed with Lucerne treated with diflubenzuron showed reduced hatch (Henzell *et al*., 1979).

Mian and Mulla (1982) found that diflubenzuron induced higher rates of oviposition in *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *R. dominica* (Coleoptera: Bostrichidae) compared with control samples; however, it also showed ovicidal activity against *R. dominica, 0. surinamensis* and *T. castaneum*, producing 100%, 98.4% and 80.3% egg mortality respectively.

Diflubenzuron topically applied to *Trogoderma granarium* (Coleoptera: Dermestidae) (0-24 h old adults) at rates of 5-20 µg IGR/insect reduced the fecundity and egg viability up to 90% (Saxena and Kumar, 1982). In a subsequent study by Rajendran and Shivaramaiah (1983), the effect of diflubenzuron on *T. granarium* larvae was examined. It was found that susceptibility of larvae to the compound varied with age. Schroeder (1996) stated that diflubenzuron significantly affected the reproductive potential of *Diaprepes abbreviates* (Coleoptera: Curculionidae) for more than a month after application to citrus foliage. Kemabonta and Odebiyi (2005) detailed that when *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) was placed on diflubenzuron-treated seeds, the number of eggs oviposited decreased significantly as the concentration of diflubenzuron increased. Emergence of adults from the eggs also decreased considerably.

Studies conducted on several stored product beetles belonging to the family tenerionidae revealed that diflubenzuron can be used to control their population. Soltani *et al*., (1984, 1987) detailed diflubenzuron, applied by dipping on newly emerged pupae of *Tenebrio molitor* disturbs the pupal-adult development and defective cuticle secretion. Reduced larval weight was reported in diflubenzuron treated *Tribolium confusum* (Ishaaya *et al*., 1984). The potency of diflubenzuron was much greater in inhibiting growth and

development of first instar larvae of *Tribolium castaneum* than of forth instar larvae, as expressed by death and retardation of larval development (Ishaaya and Ascher, 1977). Experiments carried out by Webley and Airey (1982) showed that diflubenzuron applied to woven polypropylene bags at 500 mg/m^2 was ineffective against *T. castaneum* confined to the treated surface. It was also observed that its fecundity reduced after exposure to wheat treated with diflubenzuron (Elek and Longstaff, 1993). In feeding and contact bioassay conducted in *Alphitobius diaperinus*, first instar larvae were found to more toxic to diflubenzuron than fifth instar larvae and treatment also reduced larval survivability in this species (Singh and Johnson, 2013).

2.2.3. Insect Moulting hormone: 20-Hydroxyecdysone

Insect moulting is the result of the expression of a cascade of genes that is sequentially both up and down-regulated by the moulting hormone, 20-hydroxyecdysone (20E), which is secreted as a pulse during each instar. Overdose of 20E itself, known classically as `hyper ecdysonism' causes various developmental abnormalities in insect species (Carlson *et al*., 2001). The efficacy studies of 20E to control insect pests mainly occurred in Lepidopteran insects and it was found that oligophagous or polyphagous species of insects which feed on host plants from families which are known to contain phytoecdysteroid-positive species, were able to tolerate low levels of 20-hydroxyecdysone in their diets, but exhibited developmental defects at high concentrations (Blackford and Dinan, 1997).

The use of ecdysteroid agonists generates the premature synthesis of the insect's cuticle especially close to the area of the head, causing feeding inhibition irrespectively of

the age or instar of the insect (Schneiderman 1972; Fox 1990; Wing and Aller, 1990). These compounds show chemo-sterilant activity of females, act both through stomach and contact, and can also penetrate the insect's cuticle (Heller *et al*., 1992). An agonist of 20E, RH-5849 not only induced premature molting in insects, but also reduced feeding activity and weight gain (Oberlander *et al*., 1995). The decreased body weight of RH-5849-treated larvae has been confirmed in *Plodia interpunctella* (Lepidoptera: Pyralidae) (Silhacek *et al*., 1990) and *Spodoptera littoralis* (Lepidoptera: Noctuidae) (Smagghe and Degheele, 1992) and this agonists also found effective against Japanese beetle *Popillia japonica* (Coleoptera: Scarabaeidae) grubs at concentrations as low as 1 ppm in soil, sub lethal effects on overwintered third instars included earlier formation of prepupae (Monthean and Potter, 1992). When newly molted sixth instar spruce budworm *Choristoneura fumiferana* (Lepidoptera: Tortricidae) larvae were each fed with 100 mg of RH-5992, another agonist of 20-hydroxyecdysone, the larvae went into a precocious moult (Retnakaran *et al*., 2003).

After topical application of RH-0345, an agonist of 20-hydroxyecdysone on female adult beetle of mealworm *Tenebrio molitor* (Coleoptera: Tenebrionidae), first oviposition was delayed, the number of eggs per female was reduced to 32%, the size of the deposited egg was reduced and egg viability was lost by 68% (Taibi *et al*., 2003). Decreased mean number of days to pupation and occurance of abnormal larval molts observed in 20E treated *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) larvae through topical and feeding application (Singh and Johnson, 2004). Reduction of insect food consumption resulting in fat body lipolysis during molting and pupation in insects was reported in response to the application of 20-hydroxyl ecdysone (Wang *et al*., 2010)

CHAPTER 3

METHODOLOGY

3.1. Laboratory culture of test insects: Adult *Luprops tristis* beetles in their dormancy were collected from their aggregation site in a residential building near a fifteen-year-old 5- hectares 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 2015, to ensure uniformity of age at each stage of experiment. Collected dormancy beetles were transferred to six large circular clay vessels (16 × 40 cm) capped with nylon mesh net. Dry coconut husk was provided as resting substratum in each clay vessel and placed in an environmental chamber (YORCO, India) at 55% relative humidity and temperature 30^oC (representing the average temperature and humidity in the natural aggregation site). Water was sprayed over the collected beetles kept in the clay vessels and also on the exterior of the vessel at alternate days with a mist sprayer to make the living conditions wet. This cultural set up was maintained till the end of their dormancy period in the first week of December 2015. By first week of January 2016, arousal of aggregated dormancy beetles and active movements in the cultural set ups (entry into post-dormancy phase) were noted. Subsequently, the beetles in post-dormancy phase were separated into 10 circular clay vessels (12 × 30 cm) half filled with rubber litter and soil, capped with nylon mesh net and placed in environmental chamber (YORCO, India) at relative humidity 70% and temperature 33^oC (representing the average temperature and humidity in the rubber plantation litter) (Sabu *et al*., 2014). Post-dormancy beetles were fed with wilted tender rubber leaves and cotton balls (2 cm^3) soaked in water placed above the mesh net topping as water source and they were moistened daily. Unfed leaves and excreta were removed on alternate days. Towards the end of post-dormancy period, egg laying and

hatching of eggs were noted in cultural setups. Neonate larvae were transferred with a moist fine hair brush into another set of 30 clay pots (5 × 5 cm) half filled with rubber plantation litter and soil, placed in the environmental chamber with relative humidity and temperature same as that of rubber plantation litter. Mouth of the clay vessels were capped with fine cotton cloth until the emergence of third instar larvae to prevent the escape of comparatively smaller size larvae and thereafter with nylon mesh net (figure 2A). All Larval instars were fed with finely sliced tender rubber plant leaves and circular pieces of moistened filter paper (~ 7cm^2) placed in a petri dish were kept in the clay vessels for provision of water, and moistened on alternative days. Newly eclosed teneral adult beetles were fed with wilted tender rubber leaves and this beetle culture was maintained till the beginning of home invasion of the beetles in the region with the onset of summer rains by last week of April, 2016. Wet litter conditions were stimulated in the cultural setups set ups by spraying water with a mist sprayer until the leaves and upper layers of soil were wet as in the plantation to induce dormancy in beetles. Rectangular wooden boxes (15 × 7 × 3 cm), half filled with hay and with a hinge door kept half open in the clay vessel, was provided as the dormancy shelter for the beetles. Spraying continued until the beetles moved into the dry shelter and the litter was kept moist for a week to ensure the aggregated beetles remain in dormancy (Sabu *et al*., 2005). Stock culture was maintained during the period of the study that lasted till December 2019.

3.2. Test compounds: Three different types of Insect Growth Regulators namely, a juvenile hormone analog (JHA), fenoxycarb (98%); a chitin synthesis inhibitor (CSI), diflubenzuron (99%); and the moulting 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.

3.3. Experimental Design

3.3.1. Feeding Bioassay: Each life stage of the beetles was exposed to selected concentrations of the three IGRs in feeding bioassay with IGR treated tender rubber leaves as food (Tomberlin *et al*., 2002). Sliced tender rubber leaves were weighed into diet batches of 1gm, required number of diet batches were treated with 2 ml of each concentration (100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm) of three IGR solutions by using a micropipette. One-gram diet batch of sliced rubber leaves with acetone alone served as the control. The treated diet mixtures were separately kept in wire gauze for one hour to evaporate the acetone completely. PVC vials (Tarsons; 5.5 × 4.5 cm; 50 ml capacity) were labeled for each IGR and the specific concentration used. One-gram diet batch treated with labeled concentration of each IGR was used. Water was provided by using water-soaked filter paper for first and fifth instar larvae and moistened cotton balls for all adult stages. First and newly eclosed fifth instar larvae, pre- and post-dormancy adults were tested in feeding bioassay. Six replicates were kept for all concentrations of each IGR in experimental set ups and three replicates of control set ups were maintained. Unfed leaves and excreta were removed and new batches of leaves were added on alternate days. Larval stages were allowed to feed on treated leaves till the eclosion into next stage. Pre- and post-dormancy adults were fed on IGR treated leaves for 10 days and were

transferred to normal untreated diet for the remaining feeding period (Sing and Johnson, 2013). Treated larval and adult stages were observed in regular periods (Figure 2B).

3.3.2. Residual contact bioassay: Each life stage of the beetle was 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 concentrations 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. Filter paper wetted with acetone alone served as control. The entire set up was left undisturbed for a period of 1 hr and for evaporating acetone completely. *Luprops tristis* eggs, first instar larvae, newly eclosed fifth instar larvae, one day old pupae, pre-dormancy, dormancy and post-dormancy adults were tested in residual contact bioassay. 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. Larval and adult feeding stages were fed with untreated tender rubber leaves and water was provided by placing water-soaked filter paper for first and fifth instar larvae and moistened cotton balls for all adult stages. Unfed leaves and excreta were removed and fresh leaves were added on alternate days. Eggs were exposed to the IGR treated filter paper till hatching. Larval and pupal stages were exposed to the treated filter paper till their eclosion to next stage. Pre-dormancy, dormancy and post-dormancy adults were allowed to contact with the IGRs for ten days. Treated egg, larval and adults stages were observed at regular intervals (Figure 2C).

3.3.3. Efficacy of fenoxycarb, diflubenzuron and 20E on eggs of *Luprops tristis*: From the laboratory stock culture hundred number of post-dormancy *Luprops tristis* beetles was separated. They were sexed based on sternal notch method (Vinod r*et al*., 2008). Sexed beetles were separated into three mating pairs and transferred to each clay pots (5 × 5 cm) half filled with rubber litter and soil, covered with nylon mesh net and placed in environmental chamber (YORCO, India) with setting relative humidity and temperature similar to that of rubber plantation litter (relative humidity 70 % and temperature 33ºC). These post-dormancy beetles were fed with sliced tender rubber plant leaves and water was provided by placing moistened cotton balls. Unfed leaves and excreta were removed and new batches of leaves were provided on alternate days. Egg laying was noted at the end of the post-dormancy period. Ten eggs were transferred into each PVC vials, labeled for each IGR and its concentration used in contact bioassay and also to the control vials. Both control and experiment set ups were observed at three hours intervals to record mortality of eggs and hatched larvae were transferred to labeled clay vessels with rubber litter and soil and fed with wilted tender rubber plant leaves and water. Larval stages were observed till pupation to get percentage of larval survivability.

3.3.4. Efficacy of fenoxycarb, diflubenzuron and 20E on first instar larvae of *Luprops tristis*: Ten number of first instar larvae from the stock culture were transferred to each labelled PVC vial for each concentration of IGRs. Mouth of the both control and experiment vials were covered with fine cotton cloth. Larvae in both control and experiment set ups were observed at every three hours interval till they eclosed into second instar to record their mortality. Live larvae in both control and experiment setups were

eclosed into second instar and were transferred to labeled clay pots (5 × 5 cm) arranged with soil and litter, fed with untreated tender rubber leaves and water also provided. Observations were taken at six hours intervals to record larval and pupal survivability. Small body size (~ 2 mm), delicate body nature and short larval duration of first instar larvae makes it practically difficult to record larval weight of first instar larvae in both control and experimental set ups.

3.3.5. Efficacy of fenoxycarb, diflubenzuron and 20E on fifth instar larvae of *Luprops tristis*: Batches of ten fifth instar larvae were separated from the stock culture and transferred into individual PVC vials labeled for IGRs and its concentration used for feeding and contact bioassays and also to the control vials. Mouth of both control and experiment vials were covered with nylon mesh net. Observations were taken at six hours to record mortality, mean number of days to pupation and survivability of pupae that were eclosed from treated fifth instar larvae.

3.3.5.1. Larval weight gain: Larval weight was measured at five-day intervals for fifth instar larvae fed on fenoxycarb treated feed, and exposed to fenoxycarb treated filter papers. The weight of all fifth instars was nearly identical at the beginning of the bioassay. Weight gain was calculated as the difference between the final weight at the time of observation and the initial weight of each larva to eliminate the variation in the initial weight on analysis. The weight at first day was used as initial weight to determine weight gain analysis.

3.3.6. Efficacy of fenoxycarb, diflubenzuron and 20E on pupae of *Luprops tristis*: Batches of ten one day old pupae were transferred from stock culture into PVC vials loaded with IGR of specific concentrations and also to the control vials. Observations were taken at six hours intervals for recording pupal mortality. Teneral adults from immortile pupae were transferred into labeled clay vessels and maintained as stock culture. Observations were noted.

3.3.7. Efficacy of fenoxycarb, diflubenzuon and 20E on pre-dormancy adults of *Luprops tristis*: Pre-dormancy adult beetles in stock culture were sexed and five males and five females were transferred into each PVC vials labeled for IGRs and its concentrations used for feeding and contact bioassay and also to the labeled control vials for both. After ten days exposure to the IGRs, beetles in feeding and contact bioassays and control set ups were transferred to untreated labeled clay pots. Observations were taken at regular intervals. Towards the end of pre-dormancy period, immortile beetles in both experiment and control set ups were induced to enter into dormancy, by providing dormancy inducing cues and conditions as in stock culture. Observations were taken regularly. Towards the end of nine month dormancy period, mating pairs in both control and experiment were transferred to another set of labeled clay vessels (5 × 5 cm) half filled with rubber plantation litter and soil, placed in environmental chamber set with relative humidity and temperature similar to rubber plantation litter (relative humidity 70% and temperature 33^{o}C), fed with wilted tender rubber leaves and water also provided. Egg laying was noted, fecundity and percentage of egg hatchability were recorded.

3.3.8. Efficacy of fenoxycarb, diflubenzuron and 20E on dormancy adults of *Luprops tristis*: Dormancy adult beetles in stock culture were sexed and five mating pairs were transferred into PVC vials labeled for specific concentration of IGR to be tested in contact bioassay and also to the labeled control vials. Small pieces of dry coconut husk were provided as a resting substratum during dormancy. Placed in environmental chamber with temperature and humidity similar to natural aggregation site (55% relative humidity and temperature 30^{o}C). Water was sprayed with a mist sprayer into each vial to induce dormancy. After ten days of exposure to IGRs, beetles in the experiment and control setups were transferred to untreated labeled clay vessels with dry coconut husk. Beetles were observed regularly to record their mortality. Towards the end of their dormancy period each mating pair of live beetles was transferred into another set of labeled clay vessels (5 × 5 cm) half filled with rubber plantation litter and soil, and placed in an environmental chamber set with temperature and humidity similar to that in rubber plantation litter (relative humidity 70% and temperature 33^{o}C). Fed with wilted tender rubber plant leaves and water also provided with moistened cotton balls. At the end of their post-dormancy period egg laying was noted. Fecundity and percentage of egg hatchability were recorded.

3.3.9. Efficacy of fenoxycarb, diflubenzuron and 20E on post dormant adults of *Luprops tristis*: Post dormancy adult *Luprops tristis* beetles in stock culture were sexed and five mating pairs were transferred to each PVC vials labeled for IGRs and specific concentration of IGR to be tested in both feeding and contact bioassay and also to the labeled control vials. After ten days of exposure to IGRs, each mating pair of beetles in both experimental and control set ups was transferred to labeled untreated clay vessels

(5 × 5 cm) half filled with rubber litter and soil, placed in the environmental chamber (relative humidity 70% and temperature 33^{o}C). Observations were taken at regular intervals. At the end of their post-dormancy period, egg laying was noted and fecundity and egg hatchability were recorded.

3.4. Data analysis: Natural mortalities among the controls need to be corrected to obtain the actual mortality caused by IGR exposure so as to assess the toxicity of the IGRs in test insect. Corrected mortality of the beetle exposed to the test solutions of the IGRs were calculated with Abbot's Formula (Abbot 1925) as follows.

$$P = \frac{Po - P_c \times 100}{100 - P_c}$$

Where, P = Corrected mortality

P_o = Observed mortality

P_c = Control mortality, all expressed in percentages

The formula assigned to Abbot (1925) had in fact been used earlier by Tattersfield and Morris (1924).

For statistical examination of binary quantal response bioassays probit analysis is done. D.J Finney (1971) developed the probit analysis techniques for analyzing data from dose- quantal response experiments and detailed in Finney (1978) and Robertson *et al*., (2007). The intent is to estimate the dose level just required to produce a particular

response (mortality or an effect) within the given proportion of insects and to use the dose-response estimate to make comparisons.

Data obtained from bioassays, generally transferred into percent response (mortality or affected) at the corresponding concentration, are plotted against the concentrations and a sigmoid (S- shaped) curve is obtained. This sigmoid relationship can be linearized by transforming concentrations to logarithm of concentrations and percent responses to probits (probability units obtained from a normal probability curve). This is because toxicity is better fitted to the logarithm of concentrations; thus, in the analysis, the concentration or dose variable is transformed in to its logarithmic scale. Since the toxicity induction of a chemical in biological systems are concentration dependent, the toxicity of a chemical to an organism is generally denoted or expressed in terms of lethal concentration (LC) or lethal dose (LD). It is usually easy to estimate the median (50%) response level of the population so, the most commonly used values to denote toxicity are LC_{50}, concentrations of a chemical in external media lethal to 50% of the population of the tested organisms and LD_{50}, the dose per unit body weight lethal to 50% of the tested population of the organisms. Additionally, bioassays aimed at determining the higher response level (90%), LC_{90}/ LD_{90} value denote the concentration/dose of chemicals lethal to 90% of the population of tested organisms, is also used (Heong *et al*., 2011; Robertson *et al*., 2007). To determine dosage mortality regression equations and to estimate LC values and respective 95% confidence limits, probit analysis was done using the corrected mortality values. A faster and more accurate way for estimation of critical LC values from probits is using a Minitab software.

Significance levels of variation in percentage of pupation, mean number of days for pupation, larval and pupal survivability, fecundity and percentage of egg hatchability among the tested concentrations of the fenoxycarb, diflubenzuron and 20E and in different life stages of *Luprops tristis* were assessed with two-way ANOVA and pair wise differences among the IGRs with Tukeys- Kramer Post hoc testes (t-tests). Datas were transferred to percentage before analysis. Significance level of variation in mean larval weight gain in fenoxycarb treated fifth instar larvae were assessed by one-way ANOVA. Significance was determined at $P < 0.05$. All statistical analysis was performed by using Minitab 16 academic software for windows (Minitab, 2010) and with the support of Thomachen K.T., Associate Professor, St. Joseph's College, Devagiri, Calicut-8.

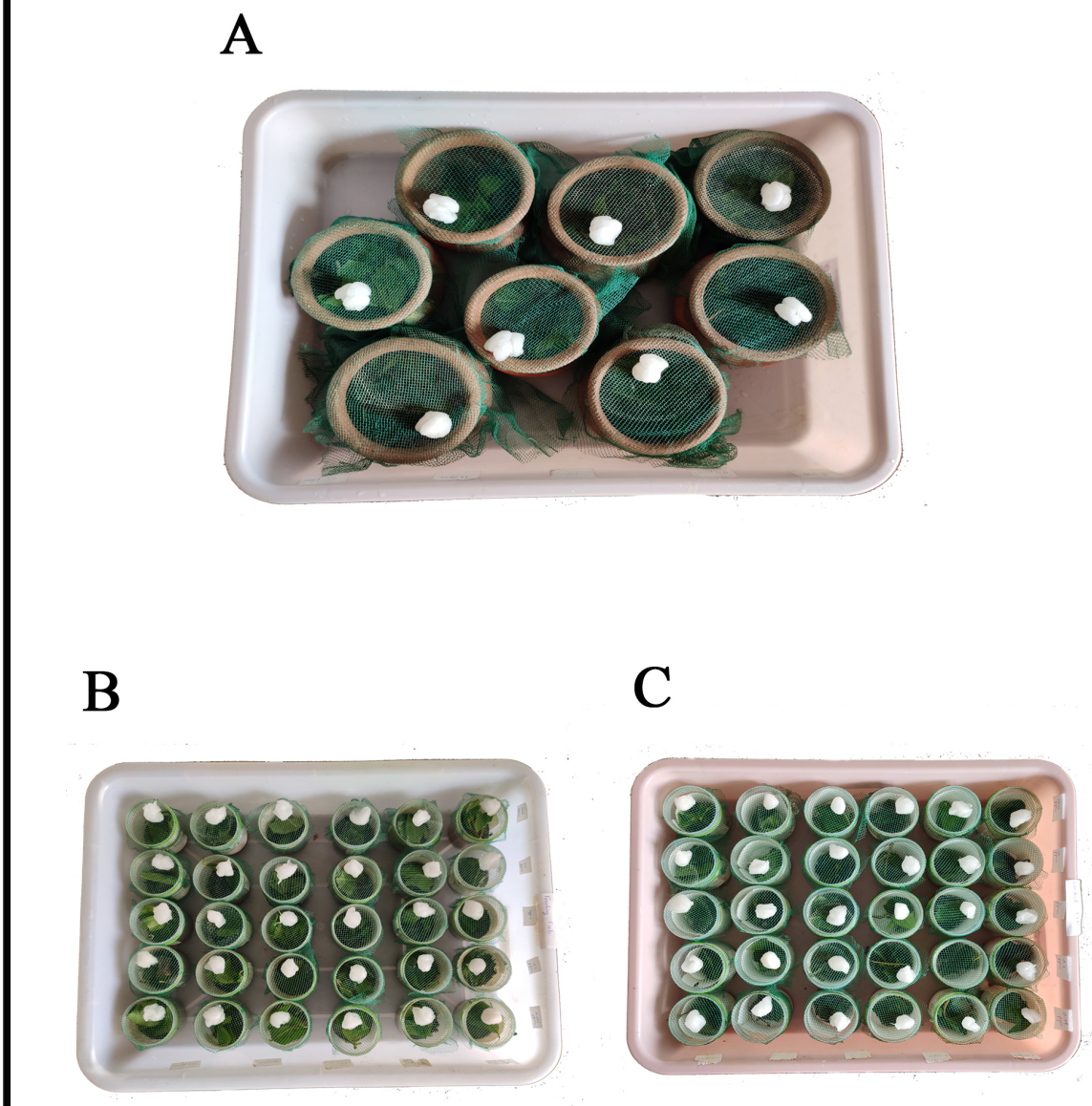

Figure 2: A) *Luprops tristis* laboratory stock cultural setup; experiment set up showing IGRs exposure to *L. tristis* following B) feeding bioassay method; C) residual contact bioassay method.

CHAPTER 4

RESULTS

Exposure of various life stages of *Luprops tristis* such as *eggs*, first instar larvae, newly eclosed fifth instar larvae, one day old pupae, pre-dormancy, dormancy and post-dormancy adult beetles to the three Insect Growth Regulators (IGR) namely fenoxycarb (Juvenile hormone analogue), diflubenzuron (Chitin synthesis inhibitor) and 20-hydroxyecdysone (20E, Insect moulting hormone) resulted in mortality and abnormalities in growth and development. Extend of effect varied depending on the IGR tested, concentration used and the method of IGR application.

4.1. Efficacy of fenoxycarb, diflubenzuron and 20E on eggs of *Luprops tristis*:

4.1.1. Mortality of eggs treated with fenoxycarb, diflubenzuron and 20E: Analysis of the efficacy of different concentration of fenoxycarb, diflubenzuron and 20Eon the eggs of *Luprops tristis* showed that the LC_{50} values and fiducial limits indicated greater toxicity on filter paper treated with diflubenzuron (LC_{50} = 10.95 ppm) than 20E (LC_{50}= 73.57 ppm). Fenoxycarb was the least effective IGR on eggs (LC_{50} = 6.5 $\times 10^{2}$ ppm) (Table 1).

4.1.2. Hatchability of eggs treated with fenoxycarb, diflubenzuron and 20E: Percentage hatchability rate of eggs exposed to different concentrations of three IGRs were different ($p < 0.05$). Eggs treated with diflubenzuron showed lower hatchability rate than eggs exposed to 20E, fenoxycarb and eggs in control ($df = 2$; $F = 92.45$; $p = 0.00$). Among the tested concentrations of IGRs, diflubenzuron at 100 ppm showed lowest mean egg hatchability (3.00 ± 0.63; N= 10) and 20E treatment at 0.01 ppm showed highest mean egg hatchability rate (8.50 ± 0.55). Percentage hatchability rate of eggs treated with fenoxycarb were not different from that of untreated control eggs ($p > 0.05$) (Tables 2, 3 & 4).

4.1.3. Survivability of larvae hatched from eggs treated with fenoxycarb, diflubenzuron and 20E: Survivability of larvae hatched from IGR treated eggs was different for each IGR and the concentration exposed to eggs (df = 2; F = 70.88; p = 0.00). Post hoc comparison of means shows that the percentage larval survivability of untreated control (100%) was greater than that of all three IGRs treated (p > 0.05). Diflubenzuron at 100 ppm showed lowest mean larval survivability (0.50 ± 0.54), fenoxycarb at 100 ppm showed mean larval survivability of 3.67 ± 0.51and 20E at 100 ppm showed mean larval survivability of 2.67 ± 0.81 (Tables 2, 5 & 6).

4.2. Efficacy of fenoxycarb, diflubenzuron and 20E on first instar larvae of *Luprops tristis*:

4.2.1. Mortality of first instar larvae treated with fenoxycarb, diflubenzuron and 20E: In feeding bioassay, first instar larvae fed with fenoxycarb treated rubber leaves showed more toxicity (LC_{50} = 0.54 ppm) than 20E (LC_{50} = 0.90 ppm) or diflubenzuron (LC_{50} = 7.50 ppm). In contact bioassay also larvae exposed to fenoxycarb treated filter paper shows more toxicity (LC_{50} = 1.71 ppm) than 20E (LC_{50} = 2.40 ppm) and diflubenzuron (LC_{50} = 30.84 ppm) (Table 7).

4.2.2. Survivability of larvae treated with fenoxycarb, diflubenzuron and 20E: The percentage survivability of larvae treated with three different IGRs were different from each other and also from the untreated control both in feeding **(**df = 2; F = 114.75; p = 0.00**)** and residual contact bioassay (df = 2; F = 41.64; p = 0.00). There were no first instar larvae survived after treatment with fenoxycarb and 20E at 100 ppm in both feeding and residual contact bioassay. Diflubenzuron treatment at 100 ppm showed mean larval survivability of 2.00 ± 0.63 in feeding bioassay and 5.83 ± 0.75 in residual contact bioassay. At 10 ppm concentration both fenoxycarb and 20E suppressed 100%

larval development in feeding bioassay only. Both fenoxycarb (2.67 ± 0.81) and 20E (3.00 ± 0.00) showed low mean larval development in residual contact bioassay with 10 ppm treated filter paper. All the three IGRs tested showed significantly lower larval survivability than control. There was no difference in the survivability rate of larvae exposed to fenoxycarb and 20E in both feeding and contact bioassay ($p > 0.05$) (Table 8, 9 & 10). Fenoxycarb treated first instar larvae do not moult into second instar and died as abnormal first instar larvae (Figure 3). Diflubenzuron treatment caused abnormal larval segmentation and body abnormalities in first instar larvae (Figure 4). First instar larvae in treatment with 20E do not moult into second instar and died as larval-larval intermediate with occluded mouth parts (Figure 5).

4.2.3. Survivability of pupae eclosed from first instar larvae treated with fenoxycarb, diflubenzuron and 20E: Percentage survivability of pupae treated with fenoxycarb, diflubenzuron and 20E were different from each other in both feeding and contact bioassays and were lower to the untreated control in both ($p < 0.05$). There were no pupae that were eclosed from larvae treated with 100 ppm diflubenzuron in their first instar stage. In both feeding and residual contact bioassays, fenoxycarb showed lowest percentage of pupal survivability (Feeding = 55.83%, Contact = 44.72%) than 20E (Feeding = 56.33%, Contact =72.18%) and diflubenzuron (Feeding = 70.66%, Contact = 89.97%) (Tables 8, 11 & 12).

4.3. Efficacy of fenoxycarb, diflubenzuron and 20E on fifth instar larvae of *Luprops tristis*:

4.3.1. Mortality of fifth instar larvae treated with fenoxycarb, diflubenzuron and 20E: Fenoxycarb showed low LC_{50} value (LC_{50} = 0.02 ppm) than 20E (LC_{50} = 2.64 ppm) in feeding bioassay. Low LC_{50} value (0.08 ppm) were recorded for larvae

exposed to filter paper treated with fenoxycarb than 20E (LC_{50} = 5.65 ppm) in contact bioassay. Diflubenzuron showed higher LC_{50} values in both bioassays (LC_{50} Feeding = 15.35 ppm, LC_{50} contact = 459.65 ppm) (Table 13).

4.3.2. Mean number of days for pupation in fifth instar larvae treated with fenoxycarb, diflubenzuron and 20E: Mean number of days to pupation was different for fifth instar larvae treated with different concentrations of three IGRs in both feeding and contact bioassays ($p < 0.05$). Post hoc comparison of means showed difference in the mean number of days taken for pupation in fifth instar larvae in untreated control and treated with fenoxycarb and 20E ($p < 0.05$). No significance difference was recorded in the mean number of days taken for pupation following treatment of fifth instar larvae with diflubenzuron in comparison with untreated control larvae. Fifth instar larvae exposed to fenoxycarb at 1 ppm concentration took longer duration for pupation in feeding bioassay (22.85 ± 0.42 days) and in residual contact bioassay (19.99 ± 0.33) and larvae exposed to 100 ppm concentration of 20E showed lowest mean number of days to pupation in feeding bioassay (5.02 ± 0.64) and residual contact bioassay (5.46 ± 0.58) (Tables 14, 15 & 16) .

4.3.3. Mean larval weight gain of fifth instar larvae treated with fenoxycarb:

4.3.3.1. Mean larval weight gain in feeding bioassay: Mean larval weight gains of fifth instar fed with different concentrations of fenoxycarb treated diet were different after 5^{th} day ($p = 0.00$), 10th day ($p = 0.00$), 15^{th} day ($p = 0.00$) and 20^{th} day ($p = 0.00$). Mean larval weight was highest at 100 ppm after 5^{th} day (3.81 ± 0.37 mg) and 10^{th} day (7.12 ± 0.19 mg), and at 10 ppm after 15^{th} day (9.21 ± 0.16 mg) (Table 17). By 20^{th} day of larval life, larvae fed with a diet treated with 1 ppm attained the highest weight gain of 12.56 ± 0.09 mg. By fifth day, there was significant difference in larval weight gain

existed between untreated check larvae and larvae treated with all the concentration of fenoxycarb in feeding bioassay (df = 5; F = 2.17; p = 0.00). The untreated control larvae developed to pupae within 10 days, so the weight gains in this group were not reported thereafter. Larvae treated with 100 ppm and 10 ppm of fenoxycarb attained mortality after 10^{th} day and 15^{th} day of larval life respectively and larvae treated with 0.01 ppm fenoxycarb pupated after 15^{th} day (Tables 17 & 18).

4.3.3.2. Mean larval weight gain in residual contact bioassay: The mean larval weight gain of fifth instar exposed to different concentrations of fenoxycarb were different after 5^{th} day (p = 0.00), 10^{th} day (p = 0.00), 15^{th} day (p = 0.00) and 20^{th} day (p = 0.00) of fifth instar larval period (Table 18). The mean larval weight was highest with 100 ppm after 5^{th} day (3.26 ± 0.47 mg) and 10^{th} day (6.27 ± 0.15 mg), and highest with 10 ppm after 15^{th} day (8.43 ± 0.85 mg). By 20^{th} day of larval life, larvae exposed to filter paper treated with 1 ppm fenoxycarb attained the highest weight gain of 10.39 ± 0.06 mg. Difference existed between untreated check larvae and larvae treated with the different concentrations of fenoxycarb by fifth day in residual contact bioassay (df = 5; F = 2.92; p = 0.00). The untreated control larvae developed to pupae within 10 days, so weight gains in this group were not reported thereafter. Larvae treated with 100 ppm and 10 ppm of fenoxycarb attained mortality after 10^{th} day and 15^{th} day of larval life respectively and larvae treated with 0.01 ppm fenoxycarb pupated by 15^{th} day (Tables 19 & 20). There was no significant difference existed in mean larval weight gain of fifth instar larvae exposed to different concentrations of fenoxycarb through both feeding or contact bioassay.

Fifth instar larval treatment with fenoxycarb resulted the formation of abnormal fifth instar larvae with large body size and body weight (Figure 6), treatment with

diflubenzuron caused body abnormalities in fifth instar (Figure 7) and lethal larval-pupal intermediate was formed as a result of 20E treatment to the fifth instar (Figure 8).

4.3.4. Survivability of pupae eclosed from fifth instar larvae treated with fenoxycarb, diflubenzuron and 20E: Percentage survivability of pupae treated with three different IGRs were significantly different from each other in both feeding ($df = 2$; $F = 66.47$; $p = 0.00$) and residual contact bioassay ($df = 2$; $F = 71.47$; $p = 0.00$) and also significantly lower to the untreated control (in feeding = 58.00 ± 0.51; residual contact = 57.00 ± 0.54) in both bioassays ($p < 0.05$). There were no pupae that were eclosed from larvae treated with 100 pmm diflubenzuron in their first instar stage. In both feeding and residual contact bioassays, fenoxycarb showed lowest percentage of pupal survivability (Feeding = 13.67%, Contact = 14.72%) (Tables 21, 22 & 23).

4.4. Efficacy of fenoxycarb, diflubenzuron and 20E on pupae of *Luprops tristis*:

4.4.1. Mortality of pupae treated with fenoxycarb, diflubenzuron and 20E: LC_{50} values and fiducial limits indicated that towards the pupal stage of *Luprops tristis*, diflubenzuron was more toxic (LC_{50} = 11.25 ppm) than 20E (LC_{50} = 187.72 ppm). Fenoxycarb was found to be least toxic to the pupae ($LC_{50} = 3.06 \times 10^5$ ppm) (Table 24).

Pupal treatment with fenoxycarb resulted in the formation of lethal blocked pupae (Figure 11), damaged pupae were formed with pupal treatment with chitin synthesis inhibitor (Figure 12) and lethal pupal-adult intermediate was formed following the treatment of pupae with 20E.

4.5. Efficacy of fenoxycarb, diflubenzuron and 20E on pre-dormancy adults of *Luprops tristis*:

4.5.1. Mortality of pre-dormancy adults treated with fenoxycarb, diflubenzuron and 20E: 20E were found to be more toxic in both feeding (LC_{50} = 25.83 ppm) and contact bioassay (LC_{50} = 25.83ppm) on pre-dormancy *Luprops tristis* adults than diflubenzuron and fenoxycarb. Diflubenzuron showed LC_{50} value of 1.17×10^3 ppm in both feeding bioassay and contact bioassays. Fenoxycarb treatment showed LC_{50} value of 1.9×10^4 ppm in feeding bioassay and 9.5×10^5 ppm in contact bioassay (Table 25).

4.5.2. Fecundity of adults treated with fenoxycarb, diflubenzuron and 20E in their pre-dormancy phase: No difference was recorded in the fecundity among the beetles treated with fenoxycarb, diflubenzuron and 20E ($p > 0.05$), and untreated control beetles ($p > 0.05$) in both feeding bioassays with IGR treated tender rubber leaves and contact bioassays with IGR treated filter papers. Pre-dormancy beetles exposed to 20E at 100 ppm showed lower fecundity rate (Feeding = 26.83 ± 2.48, Contact = 29.33 ± 2.42) among the tested samples and control (Tables 26, 27 & 28).

4.5.3. Hatchability of eggs laid by adults treated with fenoxycarb, diflubenzuron and 20E in their pre-dormancy phase: Percentage hatchability of eggs laid by *Luprops tristis* exposed to various concentrations of the three IGRs during their pre-dormancy phase was different ($p < 0.05$) both in feeding and contact bioassay. In feeding bioassay, Post hoc comparison of means showed that percentage egg hatchability of pre-dormancy beetles treated with 20E (91.29%) were lower than that of fenoxycarb (94.81%), diflubenzuron (94.52%) and untreated control (96.17%) ($p < 0.05$). Percentage hatchability of eggs laid by beetles fed with fenoxycarb, diflubenzuron and untreated leaves were not different ($p > 0.05$).

In contact bioassay, percentage hatchability of eggs laid by beetles exposed to 20E (92.36%) treated filter paper in their pre-dormancy phase was lower compared to that of fenoxycarb (95.48%) and diflubenzuron (96.04%) ($p < 0.05$) and there was no difference from that of control ($p > 0.05$). In both feeding and contact bioassays, lower hatchability rate of laid eggs was observed in pre-dormancy beetles exposed to 100 ppm concentration of 20E (Tables 26, 29 & 30).

4.6. Efficacy of fenoxycarb, diflubenzuron and 20E on dormant adults of *Luprops tristis*:

4.6.1. Mortality of dormant adults treated with fenoxycarb, diflubenzuron and 20E: Mortality of all the three IGRs treated in dormancy phase of *Luprops tristis* showed higher LC_{50} values. Fenoxycarb was more toxic ($LC_{50} = 8.14 \times 10^3$) than 20E ($LC_{50} = 6.29 \times 10^5$) and diflubenzuron ($LC_{50} = 1.14 \times 10^{13}$) (Table 31).

4.6.2. Fecundity of adults treated with fenoxycarb, diflubenzuron and 20E in their dormancy phase: Fecundity of dormant beetles treated with fenoxycarb, diflubenzuron and 20E were lower than that of untreated control beetles (40.83 ± 2.63; $p < 0.05$). Dormant beetles exposed to filter paper treated with diflubenzuron at 100 ppm showed lowest fecundity among the tested samples (24.67 ± 1.03) and 20E treatment at 0.01 ppm showed highest fecundity of 41.50 ± 2.17. There was no difference in the fecundity rate among the beetles treated with fenoxycarb, difubenzuron and 20E in their dormancy ($p > 0.05$) (Tables 32, 33 & 34).

4.6.3. Hatchability of eggs laid by adults treated with fenoxycarb, diflubenzuron and 20E in their dormancy phase: Percentage hatchability of eggs laid by dormant beetles exposed to IGR in filter paper bioassay was different for fenoxycarb, diflubenzuron and 20E ($p < 0.05$). Post hoc comparison of means showed that there

was no significant difference in the hatchability rate of eggs laid by beetles treated in the untreated control with fenoxycarb treated and diflubenzuron treated beetles in the dormancy phase. Lowest hatchability rate of eggs was observed in beetles exposed to 100 ppm concentration of diflubenzuron in their dormancy period (19.67 ± 0.81) and highest hatchability rate was observed in dormancy beetles treated with 20E at 0.01 ppm concentration (Tables 32, 35 & 36).

4.7. Efficacy of fenoxycarb, diflubenzuron and 20Eon post-dormancy adults of *Luprops tristis*:

4.7.1. Mortality of post-dormancy adults treated with fenoxycarb, diflubenzuron and 20E: Among the three different IGRs tested, 20E was more toxic than fenoxycarb and diflubenzuron to the post-dormancy beetles with LC_{50} value 857.13 ppm in feeding bioassay and 3.08×10^5 in contact bioassay. Both fenoxycarb and diflubenzuron were less toxic to post dormancy phase with higher LC_{50} values in both feeding (LC_{50} fenoxycarb = 2.1×10^5 ppm; LC_{50} diflubenzuron = 4.6×10^{23} ppm) and residual contact (LC_{50} fenoxycarb = 3.1×10^7 ppm; LC_{50} diflubenzuron = 1.00×10^{11} ppm) bioassay (Table 37).

4.7.2. Fecundity of adults treated with fenoxycarb, diflubenzuron and 20E in their post-dormancy phase : Fecundity of *L. tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in their post-dormancy phase were different from each other and from the fecundity rate of untreated control beetles in both feeding bioassays with IGR treated rubber leaves and in contact bioassay with IGR treated filter paper (feeding, df = 2; F = 811.91; p = 0.00; residual contact, df = 2; F = 251.78; p = 0.00). In both feeding and contact bioassays, beetles exposed to fenoxycarb at 100 ppm showed lowest fecundity (Feeding = 8.67± 1.75 and Contact = 15.83 ± 1.83) and

diflubenzuron treatment at 0.01 ppm showed higher fecundity rate (Feeding = 38.33 ± 0.82 and Contact = 39.33 ± 1.21) (Tables 38, 39& 40).

4.7.3. Hatchability of eggs laid by adults treated with fenoxycarb, diflubenzuron and 20E in their post-dormancy phase: Percentage hatchability of eggs laid by *L. tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in their post-dormancy phase were different from each other and also from the untreated control in both feeding bioassay with IGR treated tender leaves of rubber plant and in contact bioassay with IGR treated filter paper (feeding, df = 2; F = 587.52; p = 0.00., residual contact, df = 2; F = 32.71; p = 0.00). Percentage hatchability of eggs of fenoxycarb treated beetles (51.68%) was lower to that of diflubenzuron (75.57%), 20E (88.24%) and untreated control (95.16 %) ($p < 0.05$) in feeding bioassays. In contact bioassay, percentage hatchability of eggs in untreated control (97.86%) was significantly higher than that of diflubenzuron (89.15 %), 20E (83.24 %) and fenoxycarb (79.51 %) (Tables 38, 41 & 42).

Table 1: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and 20E on egg stage of *Luprops tristis.*

IGR	LC_{50} (95% CL)	LC_{90} (95%CL)	SE	Chi squre
FXB	6.5×10^{2} (181.56 – 3.16)	5.5×10^{4} (226.41– 1.58)	0.07	7.44
DFB	10.95 (1.60 – 415.73)	791.19 (59.94 – 9.28)	0.05	18.57
20E	73.57 (39.32– 170.49)	7.18×10^{3} (1976.17– 500093.06)	0.07	3.43

Table 2: Number of hatched eggs of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E and survived larvae hatched from fenoxycarb, diflubenzuron and 20E treated eggs.

Concentration	IGR	Egg hatchability	Larval survivability
100 ppm	FXB	6.33 ± 0.51	3.67 ± 0.51
	DFB	3.00 ± 0.63	0.50 ± 0.54
	20E	4.33 ± 0.52	2.67 ± 0.81
10 ppm	FXB	7.83 ± 0.40	6.50 ± 0.54
	DFB	3.17 ± 0.75	1.16 ± 0.75
	20E	5.67 ± 0.51	4.33 ± 0.52
1 ppm	FXB	8.33 ± 0.51	8.71 ± 0.75
	DFB	6.17 ± 0.75	4.50 ± 0.83
	20E	7.33 ± 0.52	6.33 ± 0.52
0.1 ppm	FXB	8.33 ± 0.51	8.33 ± 0.51
	DFB	8.33 ± 0.51	7.00 ± 0.63
	20E	8.33 ± 0.81	6.83 ± 0.41
0.01 ppm	FXB	8.33 ± 0.51	8.33 ± 0.51
	DFB	8.33 ± 0.51	8.16 ± 0.75
	20E	8.50 ± 0.55	6.83 ± 0.41
	Control	8.50 ± 0.54	8.50 ± 0.54

Table 3: Two way ANOVA for percentage hatchability of eggs of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E.

Source	SS	df	MS	F	p-value
IGR	62.02	2	31.01	92.45	0.00
Concentration	210.37	4	52.59	156.80	0.00
IGR × concentration	51.42	8	6.42	19.16	0.00
Error	26.30	80	0.33	□□	□□

Table 4: Tukey's Post hoc comparison of hatchability (percentage) of eggs of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E.

IGRs	MD	p-value
FXB/ DFB	2.03	0.00
FXB/ 20E	1.00	0.00
FXB/ Control	0.67	0.06
DFB/ 20E	1.03	0.00
DFB/ Control	2.70	0.00
20E/ Control	1.67	0.00

Table 5: Two way ANOVA for survivability of larvae of *Luprops tristis* (percentage) hatched from eggs treated with fenoxycarb, diflubenzuron and 20E.

Source	SS	df	MS	F	p-value
IGR	11686.77	2	5843.38	70.88	0.00
Concentration	32534.16	4	8133.54	98.65	0.00
IGR × concentration	8174.02	8	1021.75	12.39	0.00
Error	6595.26	80	82.44	□□	□□

Table 6: Tukey's Post hoc comparison of the effect of fenoxycarb, Diflubenzuron and 20E on survivability of larvae *of Luprops tristis* (percentage) hatched from treated eggs.

IGRs	MD	p-value
FXB/ DFB	27.17	0.00
FXB/ 20E	8.06	0.00
FXB/ Control	12.17	0.01
DFB/ 20E	19.11	0.00
DFB/ Control	39.35	0.00
20E/ Control	20.24	0.00

Table 7: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and 20E tested on first instar larvae of *Luprops tristis*.

Bioassay Method	IGR	LC_{50} (95% CL)	LC_{90} (95% CL)	SE	Chi squre
Feeding	FXB	0.54 (0.23– 1.25)	15.13 (5.27– 89.23)	0.06	5.45
	DFB	7.50 (0.00– 0.00)	113.04 (0.00– 0.00)	0.0	57.25
	20E	0.90 (0.24– 3.42)	37.97 (8.13–1093.60)	0.05	10.36
Contact	FXB	1.71 (0.40– 8.02)	47.09 (9.55– 2756.63)	0.06	14.14
	DFB	30.84 (4.60– 1646.31)	216.21(48.30–1.1×10^{9})	0.14	24.78
	20E	2.40 (1.03– 5.80)	50.78 (17.17– 349.80)	0.07	6.22

Table 8: Number of first instar larvae *of Luprops tristis* survived after IGR treatment and survived pupae enclosed from larvae treated with IGR during first instar larval stage.

Concentration	IGR	Larval Survivability		Pupal Survivability	
		Feeding	Contact	Feeding	Contact
100 ppm	FXB	0	0	-	-
	DFB	2 ± 0.63	5.83 ± 0.75	-	2.83 ± 0.40
	20E	0	0	-	-
10 ppm	FXB	0	2.67 ± 0.81	-	2.50 ± 0.55
	DFB	5.83 ± 0.40	7.83 ± 0.75	2.00 ± 0.00	5.50 ± 0.54
	20E	0	3.00 ± 0.00	-	2.16 ± 0.40
1 ppm	FXB	2.17 ± 0.75	3.00 ± 0.63	1.83 ± 0.41	3.00 ± 0.63
	DFB	8.33 ± 0.82	8.50 ± 1.22	6.00 ± 0.63	7.67 ± 0.51
	20E	3.00 ± 0.00	5.33 ± 0.51	1.5 ± 0.54	4.83 ± 0.75
0.1 ppm	FXB	6.00 ± 0.63	4.16 ± 0.41	5.67 ± 0.81	4.16 ± 0.41
	DFB	9.33 ± 0.82	9.33 ± 0.51	8.00 ± 0.89	9.00 ± 0.00
	20E	5.17 ± 0.40	8.33 ± 0.81	4.67 ± 0.51	8.16 ± 0.75
0.01 ppm	FXB	8.50 ± 2.95	6.33 ± 0.51	8.17 ± 0.98	6.33 ± 0.51
	DFB	9.67 ± 0.51	9.33 ± 0.51	9.33 ± 0.51	9.33 ± 0.51
	20E	8.33 ± 0.51	9.50 ± 0.54	7.16 ± 0.40	9.50 ± 0.50
Control		9.5 ± 0.54	9.50 ± 0.54	9.33 ± 0.51	9.50 ± 0.54

Table 9: Two way ANOVA for survivability of first instar larvae of *Luprops tristis* (percentage) treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

Bioassay Method	Source	SS	df	MS	F	p-value
Feeding	IGR	7077.62	2	3538.81	114.75	0.00
	Concentration	12031.07	4	30032.76	973.85	0.00
	IGR × concentration	9783.53	8	1222.94	39.65	0.00
	Error	2467.11	80	30.83	□□	□□
Contact	IGR	2597.34	2	1298.67	41.64	0.00
	Concentration	76227.40	4	19056.85	611.15	0.00
	IGR × concentration	18908.27	8	2363.53	75.79	0.00
	Error	2494.55	80	31.18	□□	□□

Table 10: Tukey's Post hoc comparison of effect of IGRs in survivability of first instar larvae *of Luprops tristis* (percentage) treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

IGRs	Feeding		Contact	
	MD	p-value	MD	p-value
FXB/ DFB	18.14	0.00	10.03	0.00
FXB/ 20E	1.26	0.81	2.36	0.36
FXB/ Control	2.40	0.00	20.83	0.00
DFB/ 20E	19.41	0.00	19.39	0.00
DFB/ Control	86.25	0.00	10.80	0.00
20E/ Control	105.67	0.00	23.19	0.00

Table 11: Two way ANOVA for survivability of pupae of *Luprops tristis* (percentage) eclosed from first instar larvae treated with fenoxycarb, diflubenzuron and 20E.

Bioassay Method	***Source***	SS	df	MS	F	p-value
Feeding	IGR	9724.80	2	4862.40	74.43	0.00
	Concentration	139422.49	4	34855.62	533.56	0.00
	IGR×concentration	13529.06	8	1691.13	25.88	0.00
	Error	5226.03	80	65.32	□□	□□
Contact	IGR	4769.68	2	2384.84	72.95	0.00
	Concentration	77594.47	4	19398.62	593.39	0.00
	IGR×concentration	16509.65	8	2063.70	63.12	0.00
	Error	2615.26	80	32.69	□□	□□

Table 12: Tukey's Post hoc comparison of effect of IGRs on the survivability of pupae (percentage) eclosed from first instar larvae of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

IGRs	**Feeding**		**Contact**	
	MD	p-value	MD	p-value
FXB/ DFB	14.83	0.00	9.97	0.00
FXB/ 20E	10.50	0.00	7.81	0.00
FXB/ Control	42.50	0.00	20.00	0.00
DFB/ 20E	25.33	0.00	17.78	0.00
DFB/ Control	27.66	0.00	10.02	0.00
20E/ Control	53.00	0.00	27.81	0.00

Table 13: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and 20E tested on fifth instar larvae of *Luprops tristis.*

Bioassay Method	IGR	LC_{50} (95% CL)	LC_{90} (95%CL)	SE	Chi squre
Feeding	FXB	0.02 (0.00– 0.38)	43.21 (1.82– 2.6×10^{4})	0.04	17.49
	DFB	15.35 (10.47–23.51)	387.57 (197.63–960.50)	0.07	1.57
	20E	2.64 (0.45–19.15)	47.13 (8.74– 11232.26)	0.07	20.75
Contact	FXB	0.08 (0.00–1.063)	42.82 (2.55– 2.1×10^{11})	0.04	18.97
	DFB	459.65 (157.49–2481.27)	1.60×10^{5} (18290– 6×10^{6})	0.06	2.24
	20E	5.65 (2.03– 17.78)	94.97 (27.00– 1553.37)	0.08	9.47

Table 14: Mean number of days taken for pupation by fifth instar larvae of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay

Concentration	IGR	Mean no.of days to pupation	
		Feeding	Contact
100 ppm	FXB	12.46 ± 0.42	12.52 ± 0.07
	DFB	9.08 ± 0.07	9.1 ± 0.07
	20E	5.02 ± 0.64	5.46 ±0.58
10 ppm	FXB	16.78 ± 0.41	15.21 ± 0.17
	DFB	9.19 ± 0.23	9.09 ± 0.11
	20E	6.24 ± 0.35	7.01 ± 0.11
1 ppm	FXB	22.85 ± 0.42	19.99 ± 0.33
	DFB	9.05 ± 0.35	9.09 ± 0.08
	20E	8.41 ± 0.50	8.89 ± 0.12
0.1 ppm	FXB	22.16 ± 0.43	18.14 ± 0.17
	DFB	9.04 ± 0.27	9.05 ± 0.08
	20E	8.51 ± 0.32	8.93 ± 0.13
0.01 ppm	FXB	16.73 ± 0.46	14.62 ± 0.42
	DFB	9.16 ± 0.09	9.05± 0.35
	20E	8.70 ± 0.29	9.22 ± 0.15
Control		9.28 ± 0.09	9.28 ± 0.09

Table 15: Two way ANOVA for mean number of days to pupation in fifth instar larvae of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

Bioassay Method	**Source**	SS	df	MS	F	p-value
Feeding	IGR	2020.46	2	1010.23	7.3E3	0.00
	Concentration	260.96	4	65.24	473.58	0.00
	IGR × concentration	256.79	8	32.09	233.00	0.00
	Error	11.02	80	138.00	□□	□□
Contact	IGR	1178.50	2	589.25	9.9E3	0.00
	Concentration	144.80	4	36.2	612.68	0.00
	IGR × concentration	129.31	8	16.16	273.56	0.00
	Error	4.72	0	0.05	□□	□□

Table 16: Tukey's Post hoc comparison for mean number of days to pupation in fenoxycarb, diflubenzuron and 20E treated fifth instar larvae in feeding and residual contact bioassay.

IGR	Feeding		Residual Contact	
	MD	p-value	MD	p-value
FXB/ DFB	9.07	0.00	7.02	0.00
FXB/ 20E	10.80	0.00	8.19	0.00
FXB/ Control	8.89	0.00	6.81	0.00
DFB/ 20E	1.72	0.00	1.17	0.00
DFB/ Control	0.17	0.71	0.20	0.25
20E/ Control	1.90	0.00	1.37	0.00

Table 17: Mean larval weight gain (mg) of fifth instar larvae of *Luprops tristis* treated with fenoxycarb in feeding bioassay.

Concentration (ppm)	Initial weight (mg)	Weight gain (mg)			
		5 day	10 day	15 day	20 day
100	18.86 ± 0.54	3.26±0.47	6.27 ± 0.15	-	-
10	18.86 ± 0.49	3.02 ± 0.44	5.89 ± 0.62	8.43 ± 0.85	-
1	18.76 ± 0.74	3.40 ± 0.78	5.19 ± 0.90	7.41 ± 0.06	10.39 ± 0.06
0.1	18.62 ± 0.68	2.43 ± 0.51	4.91 ± 0.74	6.76 ± 0.73	8.75 ± 0.58
0.01	18.58 ± 0.43	2.43 ± 0.79	4.49 ± 0.59	6.49 ± 0.81	-
Control	18.74 ± 0.96	2.23 ± 0.60	-	-	-

Table 18: One way ANOVA for mean larval weight gain in fifth instar larvae of *Luprops tristis* treated with fenoxycarb in feeding bioassay.

Days	SS	df	MS	f	p-value
5	10.87	5	2.17	6.85	0.00
10	31.37	4	7.84	27.88	0.00
15	27.87	3	9.29	27.84	0.00
20	15.70	1	15.70	40.25	0.00

Table 19: Mean larval weight gain (mg) of fifth instar larvae of *Luprops tristis* treated with fenoxycarb in residual contact bioassay.

concentration (ppm)	Initial weight (mg)	Weight Gain			
		5 day	10 day	15 day	20 day
100	18.78 ± 0.23	3.81 ± 0.37	7.12 ± 0.19	-	-
10	18.56 ± 0.28	3.61 ± 0.29	6.27 ± 0.16	9.21 ± 0.16	-
1	18.91 ± 0.47	3.19 ± 0.22	6.15 ± 0.17	8.60 ± 0.18	12.56 ± 0.09
0.1	18.63 ± 0.26	2.96 ± 0.28	5.09 ± 0.14	7.45 ± 0.14	9.86 ± 0.21
0.01	18.61 ± 0.38	2.84 ± 0.48	4.63 ± 0.25	6.02 ± 0.21	-
Control	18.47 ± 0.96	2.45 ± 0.38	-	-	-

Table 20: One way ANOVA for mean larval weight gain (mg) in fifth instar larvae of *Luprops tristis* treated with fenoxycarb in residual contact bioassay

Days	SS	df	MS	f	p-value
5	7.36	5	1.47	2.92	0.00
10	12.50	4	3.12	7.42	0.00
15	13.33	3	4.44	5.84	0.00
20	8.06	1	8.06	11.01	0.00

Table 21: Number of survived pupae eclosed from fifth instar larvae of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E

Concentration (ppm)	IGR	PupalSurvivability	
		Feeding	Contact
100	FXB	0	0
	DFB	5.00 ± 0.75	25.00 ± 0.75
	20E	0	0
10	FXB	4.00 ± 0.51	5.00 ± 0.40
	DFB	26.00 ± 0.81	34.00 ± 0.51
	20E	5.00 ± 0.51	5.00 ± 0.40
1	FXB	10.00 ± 0.82	6.00 ± 0.63
	DFB	42.00 ± 1.26	43.00 ± 0.98
	20E	14.00 ± 0.51	6.00 ± 0.63
0.1	FXB	12.00 ± 0.63	10.00 ± 0.52
	DFB	49.00 ± 1.47	51.00 ± 0.55
	20E	45.00 ± 0.54	10.00 ± 0.52
0.01	FXB	15.00 ± 0.84	17.00 ±1.67
	DFB	58.00 ± 0.51	51.00 ± 0.55
	20E	50.00 ± 1.03	17.00 ±1.67
Control		58.00 ± 0.51	57.00 ±0.54

Table 22: Two way ANOVA for survivability of pupae (percentage) that were eclosed from fifth instar larvae treated with fenoxycarb, diflubenzuron and 20E.

Bioassay Method	Source	SS	df	MS	F	p-value
Feeding	IGR	24024.56	2	12012.28	66.47	0.00
	Concentration	52146.33	4	13036.58	72.14	0.00
	IGR × concentration	6677.55	8	834.69	4.61	0.00
	Error	14456.75	80	180.70	□□	□□
Contact	IGR	40485.14	2	20242.57	71.47	0.00
	Concentration	17283.38	4	4320.84	15.25	0.00
	IGR × concentration	3379.85	8	422.48	1.49	0.17
	Error	22658.68	80	283.23	□□	□□

Table 23: Tukey's Post hoc comparison of effect of IGRs in survivability of pupae (percentage) eclosed from fifth instar larvae of *Luprops tristis* treated with fenoxycarb, diflubenzuron and 20E in feeding& residual contact bioassay.

IGR	Feeding		Contact	
	MD	p-value	MD	p-value
FXB/ DFB	39.12	0.00	51.87	0.00
FXB/ 20E	12.27	0.00	28.33	0.00
FXB/ Control	62.94	0.00	69.77	0.00
DFB/ 20E	26.84	0.00	23.54	0.00
DFB/ Control	23.81	0.00	17.89	0.09
20E/ Control	80.66	0.00	41.44	0.00

Table 24: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and20E tested on *Luprops tristis* pupae.

IGR	LC_{50} (95% CL)	LC_{90} (95%CL)	SE	Chi squre
FXB	306.00(7009– 7.87×10^{9})	1.52×10^{5}(1.2×10^{7}– 5.2×10^{18})	0.06	0.11
DFB	11.25(7.83– 16.64)	244.53(133.27– 545.76)	0.08	1.53
20E	187.72(0.00– 0.00)	13×10^{4}(0.00– 0.00)	0.08	28.11

Table 25: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and 20E tested on pre-dormancy *Luprops tristis* adults in feeding and residual contact bioassay.

Bioassay Method	IGR	LC_{50} (95% CL)	LC_{90} (95%CL)	SE	Chi squre
Feeding	FXB	1.92×10^{4} (0.00– 0.00)	1.77×10^{8} (0.00– 0.00)	0.06	11.00
	DFB	1.17×10^{3} (316 – 11101)	4.53×10^{5} (34674– 4.76×10^{7})	0.07	3.44
	20E	25.83 (13.86– 56.50)	6.17×10^{3} (1638.23– 42096)	0.05	1.94
Contact	FXB	9.5×10^{5}(12197– 1.59×10^{9})	1.13×10^{10} (64×10^{5}– 1×10^{19})	0.08	2.04
	DFB	1.17×10^{3} (316– 11101)	4.53×10^{5} (3×10^{4}– 4.76×10^{37})	0.07	3.44
	20E	758.04 (200.86– 6796.64)	9.76×10^{5} (61029– 1.24×10^{8})	0.05	1.07

Table 26: Fecundity and egg hatchability of pre-dormancy stage *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

Concentration (ppm)	IGR	Fecundity		Egg hatchability	
		Feeding	contact	Feeding	Contact
100	FXB	36.83 ±2.41	34.50 ± 4.13	34.67 ±2.81	32.50 ±4.67
	DFB	32.67 ±2.73	37.33 ± 3.72	30.67 ± 2.65	36.17 ±3.43
	20E	26.83 ± 2.48	29.33 ± 2.42	22.67 ± 2.33	25.33 ± 2.58
10	FXB	37.33 ± 3.72	37.83 ± 5.78	35.17 ± 3.92	36.17 ± 5.34
	DFB	38.00 ± 3.67	40.00 ± 3.46	35.67 ± 4.08	38.33 ±3.87
	20E	36.00 ± 1.79	39.00 ± 4.24	33.17 ± 1.83	36.50 ±4.08
1	FXB	37.17 ± 4.58	37.00 ± 5.66	35.67 ± 4.27	35.50 ±5.32
	DFB	37.67 ± 5.98	38.67 ± 3.07	35.50 ± 6.32	36.83 ± 2.79
	20E	39.17 ± 1.94	37.33 ±3.72	35.33 ± 0.82	46.67 ± 4.41
0.1	FXB	38.67 ± 3.07	38.51 ± 4.72	36.83 ± 3.00	37.00 ±3.84
	DFB	37.67 ± 4.80	37.67 ± 5.99	36.17 ± 4.17	36.33 ±5.31
	20E	38.50 ± 1.87	37.16 ± 4.58	36.67 ± 2.25	35.00 ± 4.50
0.01	FXB	39.00 ±4.24	40.00 ± 3.46	36.83 ± 3.87	38.16 ±3.43
	DFB	37.33 ± 3.27	37.83 ± 5.78	35.33 ± 2.66	36.17 ±5.78
	20E	38.50 ± 4.50	38.5 ± 4.72	36.33 ± 1.83	36.67 ±4.63
Control		36.17 ± 4.95	38.67 ± 5.00	34.83 ±5.30	36.33 ± 4.13

Table 27: Two way ANOVA for fecundity of pre-dormancy phase *Luprops tristis* that were treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

Bioassay Method	Source	SS	df	MS	F	p-value
Feeding	IGR	60.35	2	30.17	2.18	0.12
	Concentration	501.84	4	125.46	9.06	0.00
	IGR × concentration	280.08	8	35.01	2.53	0.01
	Error	11.07	80	13.84	□□	□□
Contact	IGR	63.62	2	31.81	1.55	0.21
	Concentration	324.37	4	81.09	3.96	0.00
	IGR × concentration	177.48	8	22.18	1.08	0.38
	Error	1637	80	20.46	□□	□□

Table 28: Tukey's Post hoc comparison for fecundity in fenoxycarb, diflubenzuron and 20E treated pre-dormancy phase *Luprops tristis* beetles in feeding and residual contact bioassay

IGR	**Feeding**		**Contact**	
	MD	p-value	MD	p-value
FXB/ DFB	1.13	0.64	0.73	0.92
FXB/ 20E	2.00	0.16	1.30	0.68
FXB/ Control	1.63	0.76	1.10	0.94
DFB/ 20E	0.86	0.80	2.03	0.31
DFB/ Control	0.50	0.99	0.36	0.99
20E/ Control	0.36	0.63	2.40	0.63

Table 29: Two way ANOVA for hatchability of eggs (percentage) laid by pre-dormancy phase *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and20E in feeding and residual contact bioassay.

Bioassay Method	**Source**	SS	df	MS	F	p-value
Feeding	IGR	228.57	2	114.28	13.72	0.00
	Concentration	227.70	4	56.92	6.83	0.00
	IGR × concentration	256.27	8	32.03	3.84	0.00
	Error	666.24	80	8.32	□□	□□
Contact	IGR	235.70	2	117.85	13.74	0.00
	Concentration	112.52	4	30.63	3.57	0.01
	IGR × concentration	209.12	8	26.14	3.04	0.00
	Error	685.93	80	8.57	□□	□□

Table 30: Tukey's Post hoc comparison for hatchability of eggs laid by (percentage) pre-dormancy phase *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

IGR	Feeding		Residual Contact	
	MD	p-value	MD	p-value
FXB/ DFB	0.28	0.98	0.55	0.88
FXB/ 20E	3.51	0	3.12	0
FXB/ Control	1.35	0.71	1.29	0.75
DFB/ 20E	3.22	0	3.67	0
DFB/ Control	1.29	0.58	1.84	0.49
20E/ Control	4.87	0	1.83	0.50

Table 31: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and 20E tested on dormant adults *Luprops tristis* in residual contact bioassay.

IGR	LC_{50} (95% CL)	LC_{90} (95%CL)	SE	Chi squre
FXB	8.14×10^{3} (127.66- 7.21×10^{36})	2.30×10^{7} (9462.91- 4.2×10^{21})	0.06	8.99
DFB	1.14×10^{13} (0.00- 0.00)	1.63×10^{25} (0.00- 0.00)	0.05	0.74
20E	6.29×10^{5} (0.00- 0.00)	1.12×10^{13} (0.00- 0.00)	0.04	10.36

Table 32: Fecundity and egg hatchability of dormant adult *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in residual contact bioassay.

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

Table 33: Two way ANOVA for fecundity of dormant adult *Luprops tristis* that were treated with fenoxycarb, diflubenzuron and 20E in residual contact bioassay.

Source	SS	df	MS	F	p-value
IGR	8.46	2	4.23	1.30	0.27
Concentration	1705.84	4	426.46	131.13	0.00
IGR × concentration	122.75	8	15.34	4.71	0.00
Error	260.16	80	3.25	□□	□□

Table 34: Tukey's Post hoc comparison for fecundity in fenoxycarb, diflubenzuron and 20E treated dormant *Luprops tristis* beetles in residual contact bioassay

IGR	MD	p-value
FXB/ DFB	0.63	0.52
FXB/ 20E	0.03	1.00
FXB/ Control	0.80	0.00
DFB/ 20E	0.66	0.48
DFB/ Control	5.46	0.00
20E/ Control	4.80	0.00

Table 35: Two way ANOVA for hatchability of eggs (percentage) laid by dormant adult *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in residual contact bioassay.

Source	SS	df	MS	F	p-value
IGR	1160.68	2	580.34	79.16	0.00
Concentration	610.29	4	152.57	20.81	0.00
IGR × concentration	416.11	8	52.01	7.09	0.00
Error	586.49	80	7.33	□□	□□

Table 36: Tukey's Post hoc comparison for hatchability of eggs laid by (percentage) dormant adult *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in residual contact bioassay.

IGR	MD	p-value
FXB/ DFB	3.24	0.00
FXB/ 20E	8.70	0.00
FXB/ Control	2.24	0.25
DFB/ 20E	5.45	0.00
DFB/ Control	1.21	0.84
20E/ Control	6.45	0.00

Table 37: Lethal concentrations (LC_{50}, LC_{90}) (ppm) of fenoxycarb, diflubenzuron and20E tested on post-dormancy *Luprops tristis* adults in feeding and residual contact bioassay.

Bioassay Method	**IGR**	**LC_{50} (95% CL)**	**LC_{90} (95%CL)**	**SE**	**Chi squre**
Feeding	FXB	2.1×10^{5} (5398– 3×10^{3})	2.01×10^{10} (1.47×10^{7}– 6.1×10^{18})	0.06	1.36
	DFB	4.6×10^{23} (0.00– 0.00)	4.6×10^{23} (0.00– 0.00)	0.08	2.28
	20E	83.14 (26.28– 6500)	2.19×10^{5}(20964– 1.5×10^{7})	0.07	0.73
Contact	FXB	3.1×10^{7}(0.00– 0.00)	6.1×10^{10} (0.00– 0.00)	0.08	1.89
	DFB	1×10^{11} (8×10^{5}– 1×10^{17})	1.8×10^{13} (3.5×10^{10}– 1×10^{23})	0.06	0.93
	20E	857.13 (701– 8.6×10^{5})	1.3×10^{10} (1. $\times10^{7}$– 5.1×10^{18})	0.06	3.30

Table 38: Fecundity and egg hatchability of post-dormancy phase *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

Concentration (ppm)	IGR	Fecundity		Egg hatchability	
		Feeding	contact	Feeding	Contact
100	FXB	8.67± 1.75	15.83 ± 1.83	3.00 ± 0.89	8.33 ± 1.21
	DFB	15.17 ±1.60	21.67 ± 1.75	8.67 ± 1.63	16.33 ± 2.16
	20E	16.17 ± 1.32	22.33 ± 1.50	12.33 ± 1.63	16.33 ± 1.21
10	FXB	10.07 ± 1.86	20.16 ± 1.47	4.33 ± 0.51	12.83 ± 1.94
	DFB	17.83 ± 1.16	26.50 ± 1.04	11.5 ± 1.04	22.17 ± 1.16
	20E	24.67 ± 1.03	25.83 ± 1.47	20.67 ± 1.37	19.17 ± 1.32
1	FXB	14.33 ± 2.33	25.5 ± 1.04	6.00 ± 0.63	21.66 ± 1.21
	DFB	27.17 ± 1.67	37.33 ± 1.63	21.5 ± 1.37	35.33 ± 1.37
	20E	34.50 ± 1.22	33.00 ± 1.57	30.83 ± 1.47	28.00 ± 1.54
0.1	FXB	18.00 ± 1.41	29.00 ± 1.67	10.67 ± 1.37	29.33 ± 3.55
	DFB	36.33 ± 1.63	38.33 ± 0.75	30.5 ± 1.38	37.33 ± 0.81
	20E	36. 67 ± 1.21	36.83 ± 1.16	35.00 ± 0.89	32.33 ± 1.36
0.01	FXB	25.17 ± 1.47	35.17 ± 1.47	20.67 ± 1.63	33.50 ± 2.07
	DFB	38.33 ± 0.82	39.33 ± 1.21	35.83 ± 0.75	37.83 ± 1.94
	20E	37.67 ± 1.03	39.17 ± 1.16	36.33 ± 1.03	37.67 ± 0.81
Control		38.17 ± 1.83	38.33 ± 1.21	36.33 ± 2.33	37.5 ± 0.83

Table 39: Two way ANOVA for fecundity of *Luprops tristis* that were treated with fenoxycarb, diflubenzuron and 20E in their post-dormancy phase in feeding and residual contact bioassay.

Bioassay Method	Source	SS	df	MS	F	p-value
Feeding	IGR	3555.48	2	1777.74	811.91	0.00
	Concentration	5212.06	4	1303.01	595.09	0.00
	IGR × concentration	511.40	8	63.92	29.15	0.00
	Error	175.16	80	2.19	□□	□□
Contact	IGR	991.40	2	495.70	251.78	0.00
	Concentration	4046.15	4	1011.53	513.79	0.00
	IGR × concentration	128.37	8	16.04	8.15	0.00
	Error	157.50	80	1.96	□□	□□

Table 40: Tukey's Post hoc comparison for fecundity in fenoxycarb, diflubenzuron and 20E treated post-dormancy *Luprops tristis* beetles in feeding and residual contact bioassay.

IGR	**Feeding**		**Contact**	
	MD	p-value	MD	p-value
FXB/ DFB	11.60	0.00	7.60	0.00
FXB/ 20E	14.56	0.00	6.30	0.00
FXB/ Control	22.80	0.00	13.20	0.00
DFB/ 20E	2.96	0.00	1.30	0.00
DFB/ Control	11.26	0.00	5.60	0.00
20E/ Control	8.23	0.00	6.90	0.00

Table 41: Two way ANOVA for hatchability of eggs (percentage) laid by post-dormancy phase *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

Bioassay Method	**Source**	SS	df	MS	F	p-value
Feeding	IGR	2460.58	2	10230.29	587.52	0.00
	Concentration	13524.95	4	3381.23	194.18	0.00
	IGR × concentration	2354.59	8	294.32	16.90	0.00
	Error	1393.00	80	17.41	□□	□□
Contact	IGR	1418.21	2	709.10	32.71	0.00
	Concentration	12177.31	4	3044.33	140.46	0.00
	IGR × concentration	2628.44	8	328.55	15.16	0.00
	Error	1733.4	80	21.67	□□	□□

Table 42: Tukey's Post hoc comparison for hatchability of eggs laid by (percentage) post-dormancy phase *Luprops tristis* beetles treated with fenoxycarb, diflubenzuron and 20E in feeding and residual contact bioassay.

IGR	**Feeding**		**Contact**	
	MD	p-value	MD	p-value
FXB/ DFB	23.70	0.00	9.64	0.00
FXB/ 20E	36.67	0.00	3.27	0.01
FXB/ Control	43.29	0.00	18.34	0.00
DFB/ 20E	12.67	0.00	5.91	0.00
DFB/ Control	19.58	0.00	8.70	0.00
20E/ Control	6.91	0.00	14.62	0.00

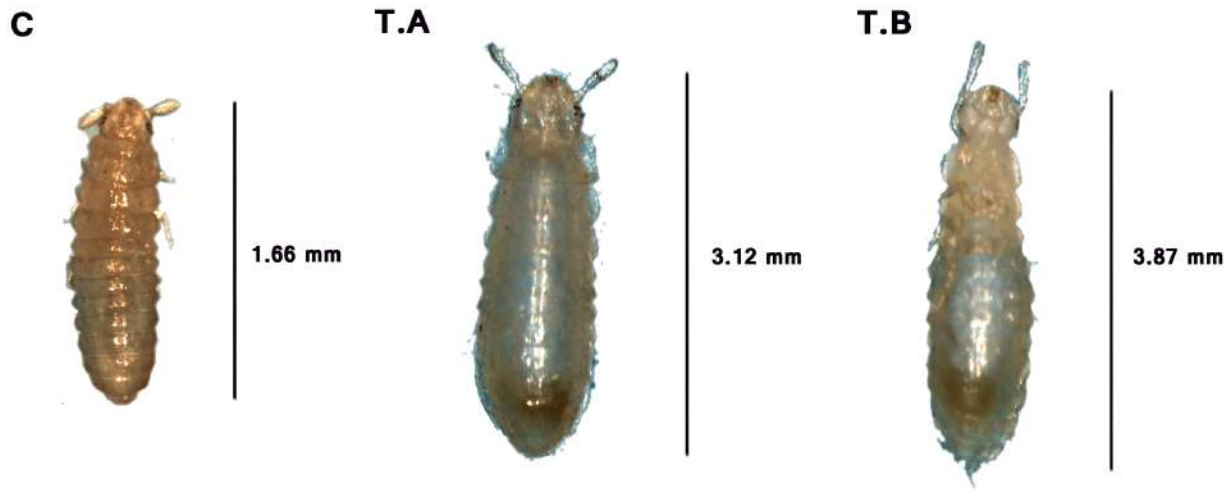

Figure 3. Relative size of first instar larvae of *L. tristis* after exposure to Juvenile hormone analogue Fenoxycarb at 1 ppm concentration in residual contact bioassay (T.A), in feeding bioassay (T.B) or acetone treated control (C) (C = Control; T = Treatment).

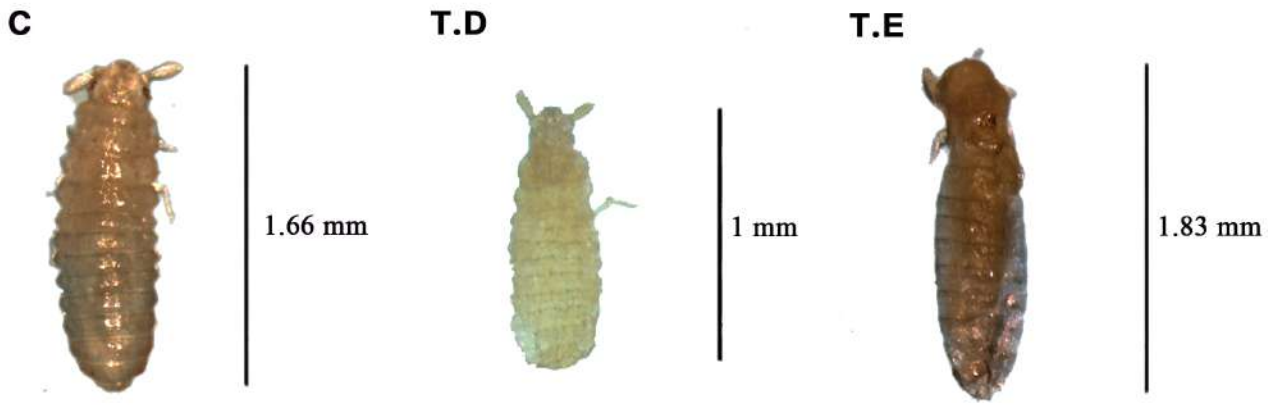

Figure 4. Lethal first instar larva of *L. tristis* with defective cuticle formed after Chitin synthesis inhibitor, Diflubenzuron treatment at 10 ppm in residual contact bioassay (T.D), in feeding bioassay (T.E) or acetone treated control (C) (C = Control; T = Treatment).

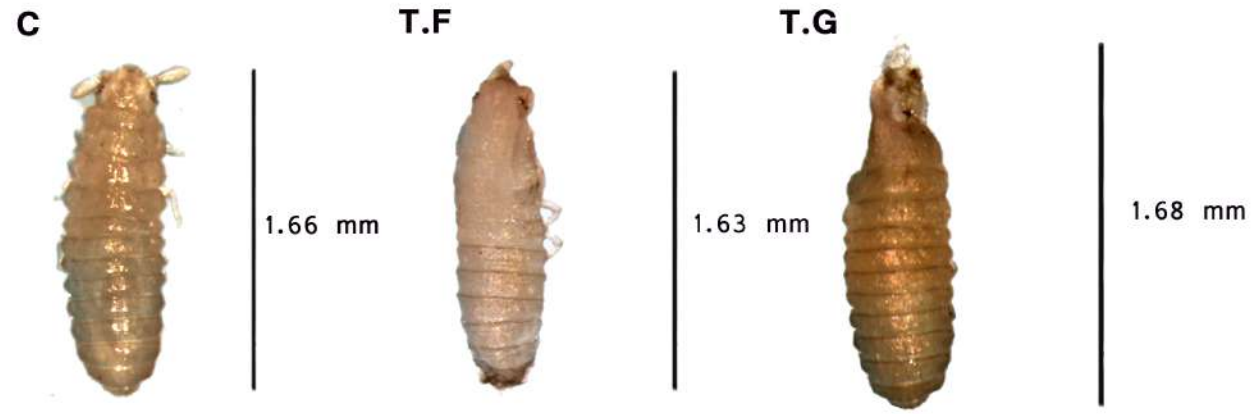

Figure 5. Lethal larval- larval intermediate with occluded mouthparts formed after first instar larva of *L. tristis* exposed to Moulting hormone, 20- Hydroxyecdysone at 10 ppm in residual contact bioassay (T.D) and feeding bioassay (T.E) or acetone treated control (T) (C = Control; T = Treatment).

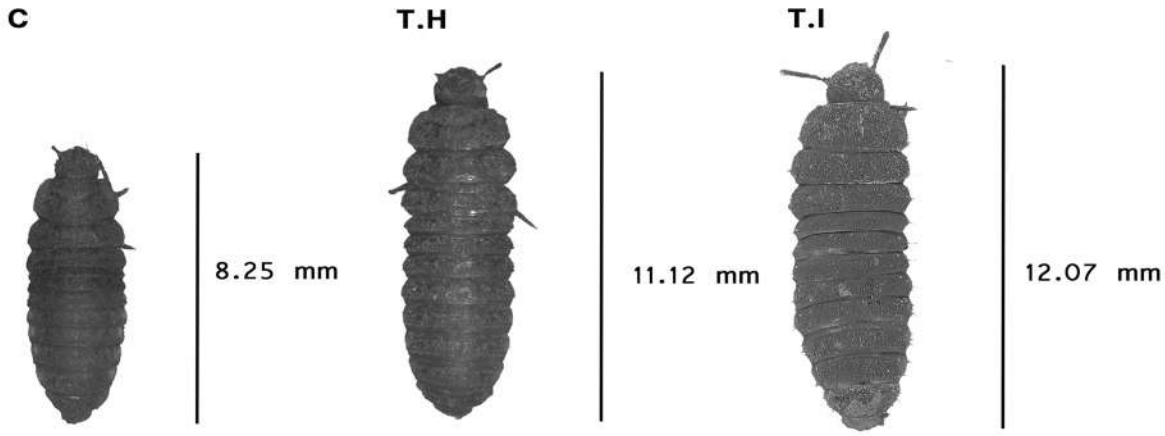

Figure 6. Relative size of fifth instar *L.tristis* after exposure to Juvenile hormone analogue, Fenoxycarb at 1 ppm concentration in residual contact bioassay (T.H), feeding bioassay (T.I) or acetone treated control (C) (C = Control; T = Treatment).

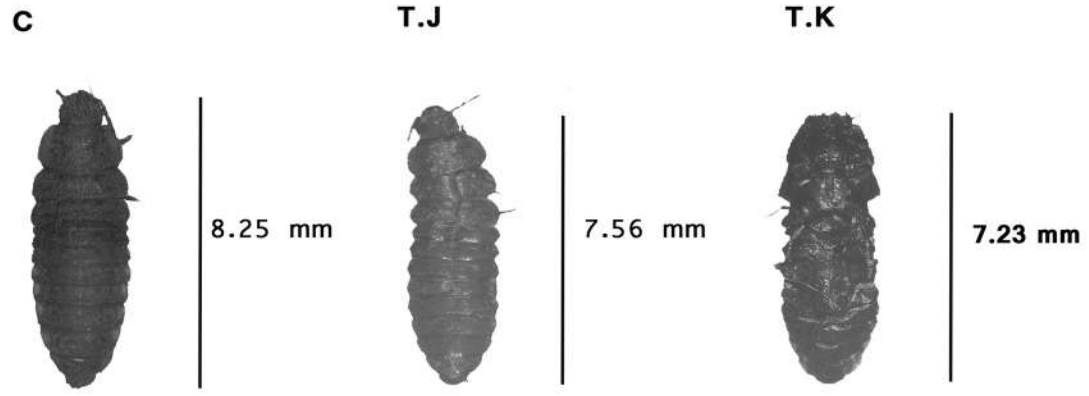

Figure 7. Lethal fifth instar larva of *L.tristis* formed after exposure to Chitin synthesis inhibitor, Diflubenzuron at 10 ppm concentration in residual contact bioassay (T.J), feeding bioassay (T.K) or acetone treated control (C) (C = Control; T = Treatment).

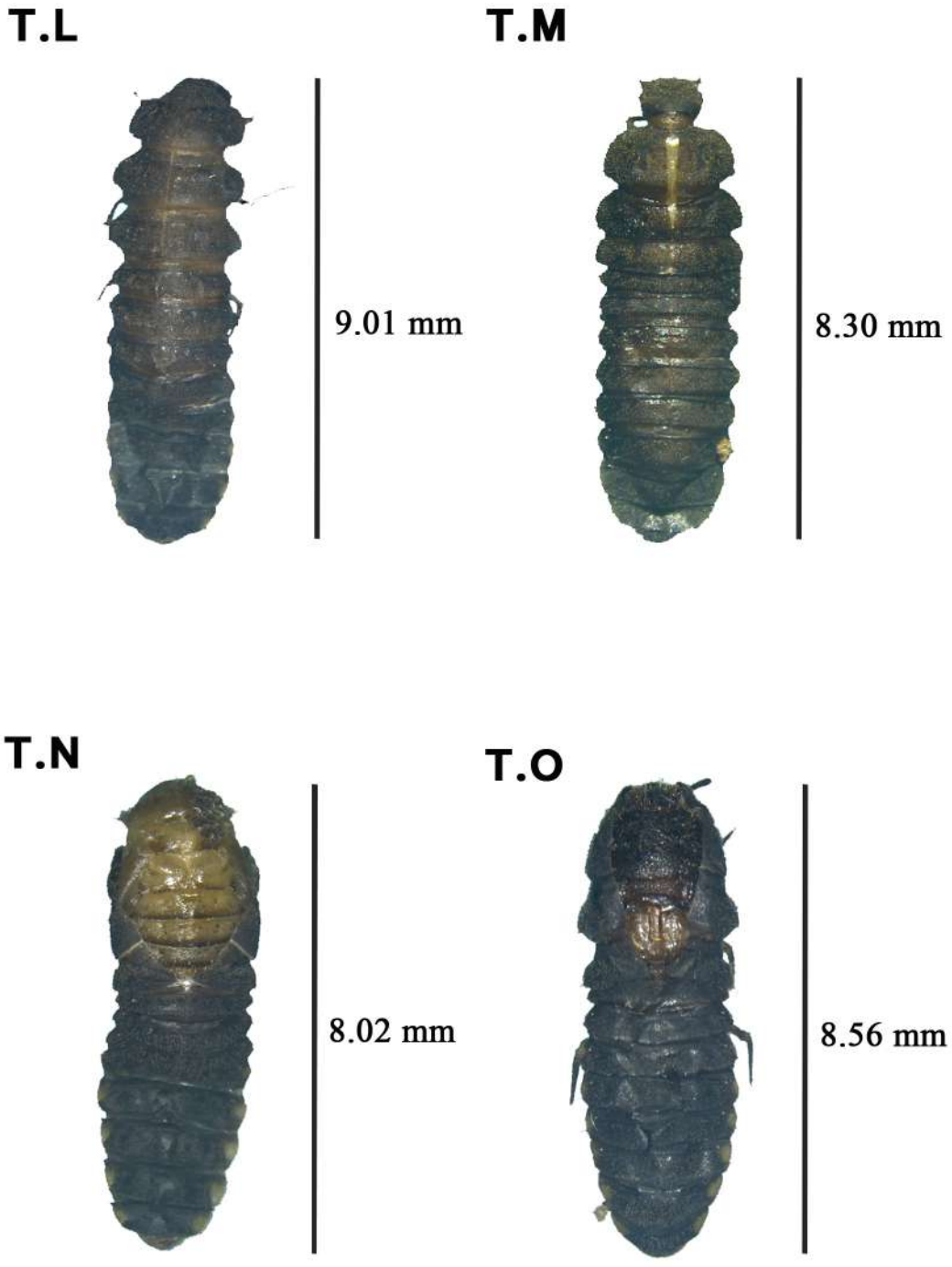

FIgure 8. Lethal larval- pupal intermediate formed after fith insttar larva of *L. tristis* exposed to Moulting hormone 20-Hydroxyecdysone at 10 ppm in residual contact bioassay (T.L); in feeding bioassay (T.M), and at 1 ppm in residual contact bioassay (T.N); in feeding bioassay (T.O) (T= Treatment).

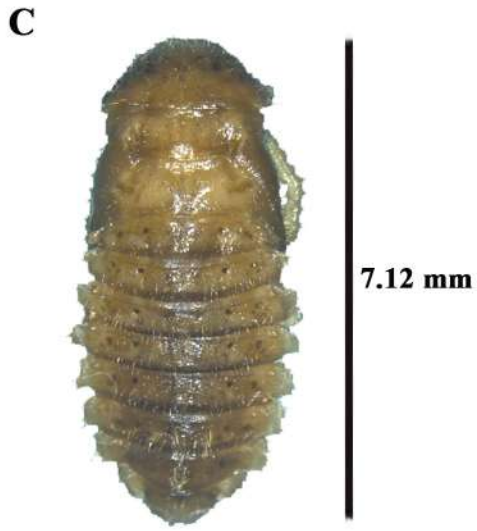

Figure 9. Acetone treated *L. tristis* pupa (C = Control)

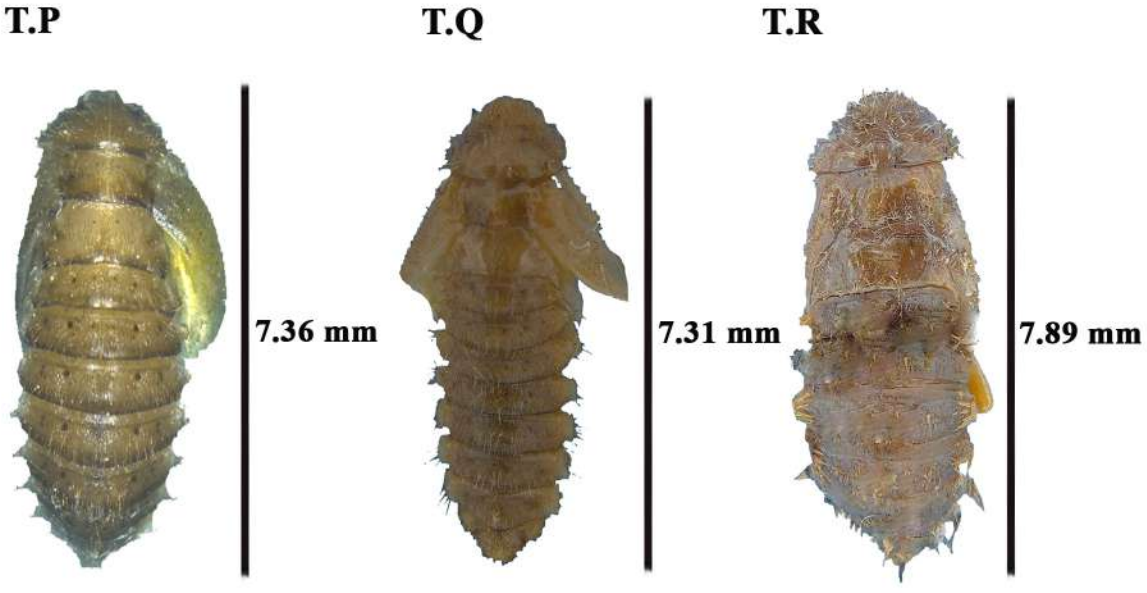

Figure 9. Lethal blocked *L.tristis* pupae after exposure to Juvenile hormone analogue Fenoxycarb at 100 ppm (T.P), 10 ppm (T.Q) and 1 ppm (T.R) (T = Treatment).

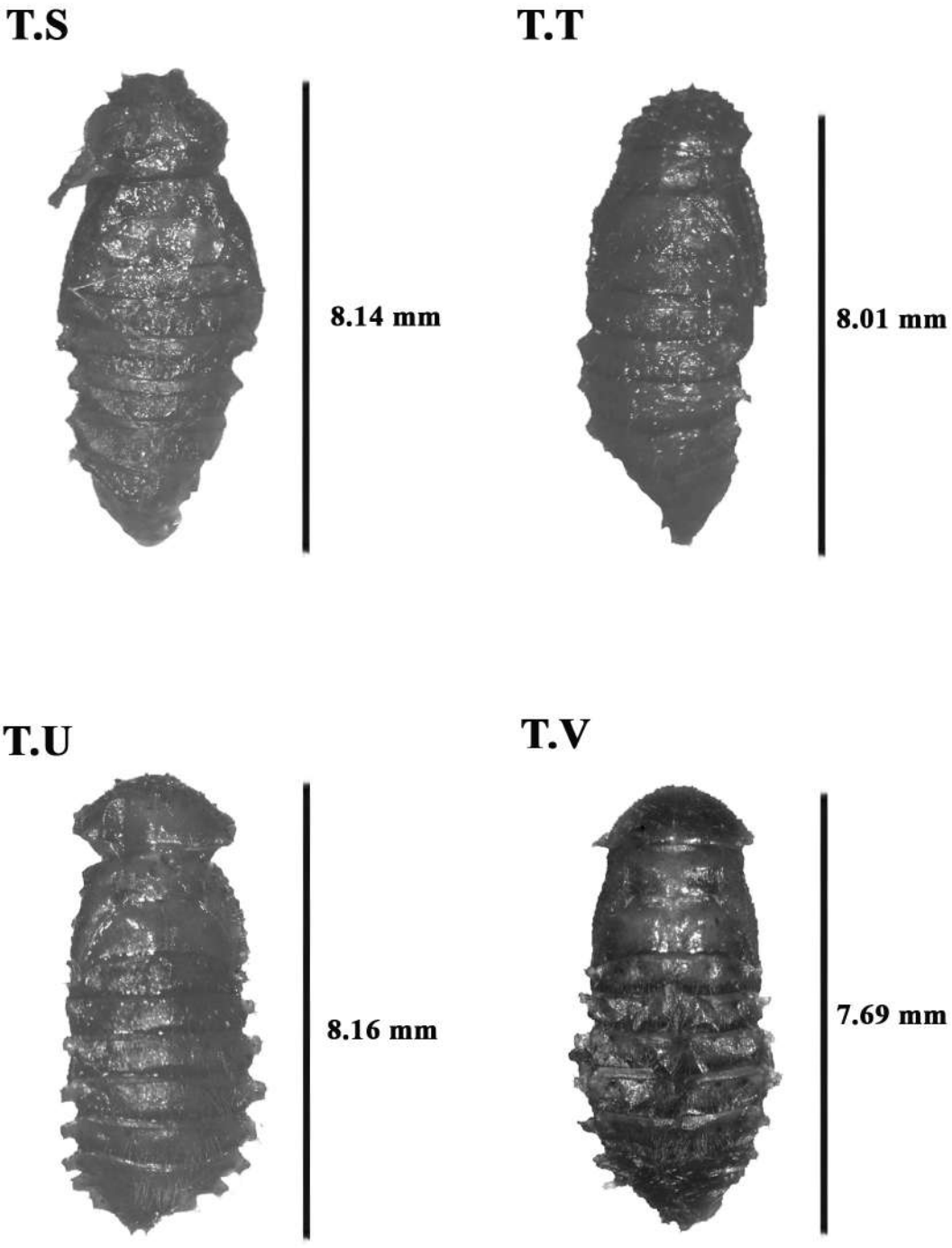

Figure 10. Damaged *L. tristis* pupae formed after exposure to Chitin synthesis inhibitor, Diflubenzuron at 100 ppm (T.S), 10 ppm (T.T) and 1 ppm (T.U & T.V) (T = Treatment).

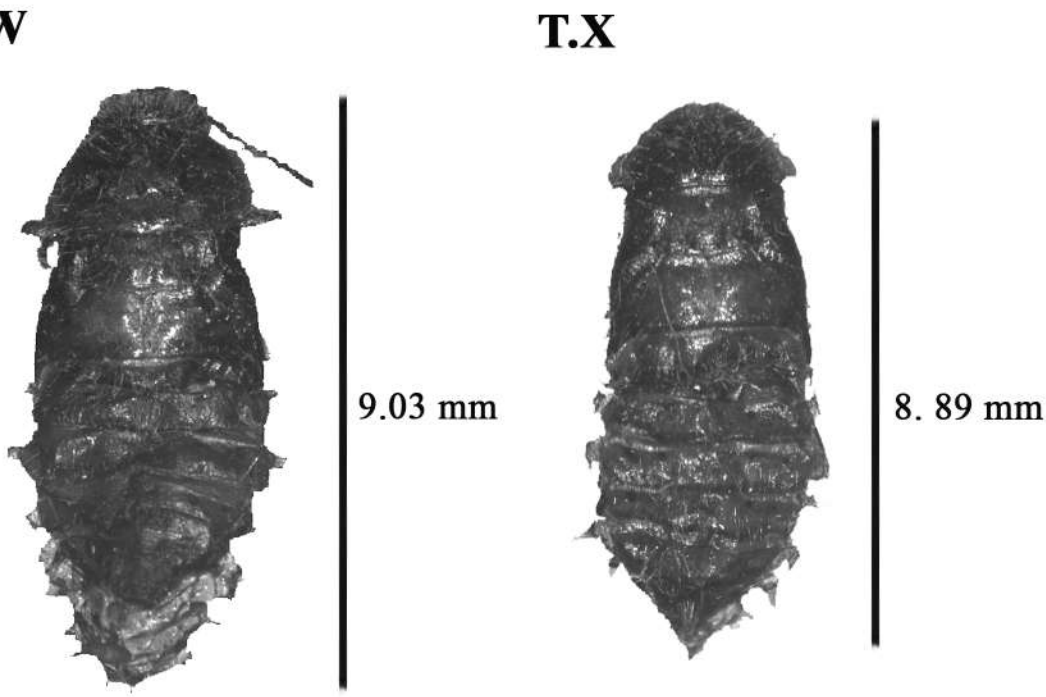

Figure 11. Pupal-adult intermediate formed after *L. tristis* pupa exposed to Moulting hormone 20-Hydroxyecdysone at 100 ppm (T.W) and at 10 ppm (T.X) (T = Treatment).

CHAPTER 5

DISCUSSION

5.1. Efficacy of fenoxycarb, diflubenzuron and 20E on eggs of *Luprops tristis*

Treatment of *Luprops tristis* eggs with insect growth regulators, fenoxycarb, diflubenzuron and 20-hydroxyecdysone (20E) lead to mortality of eggs (ovicidal effect) and incomplete hatching and low survivability of larvae (larvicidal effect) with respect to the insect growth regulator and its concentration exposed. All the three insect growth regulators, (fenoxycarb, diflubenzuron and 20E) showed both direct ovicidal effect and larvicidal effect on the larvae emerged from treated eggs. Direct ovicidal effect was higher than larvicidal effect (toxicity caused on emerged larvae from treated eggs) with all three IGRs tested.

Chitin synthesis inhibitor, diflubenzuron was more effective in exerting direct ovicidal effect on *L. tristis* eggs, with LC_{50} value (10.95 ppm) 59 times lower than LC_{50} value of fenoxycarb and 6.7 times lower than LC_{50} value of 20E. Considering the tested concentrations, diflubenzuron at 100 ppm showed higher ovicidal effect on *L. tristis* eggs (70%) than 20E (56.67%) and fenoxycarb (36.67%).

Based on cumulative effect (combining both the ovicidal and larvicidal effects) of each IGR on *L. tristis* eggs, difubenzuron was more effective than 20E and fenoxycarb as diflubenzuron treatment at 100 ppm lead to 95% cumulative mortality of treated eggs. Similar to this study, diflubenzuron showed very impressive ovicidal activity (80.3% mortality) against eggs of *Oryzaephilus surinamensis* Linnaeus, 1758 and *Rhyzopertha dominica* Fabricius, 1792 and incomplete hatching of treated eggs and low larval survivability in *Tribolium castaneum* Herbst (Mian and Mulla, 1982). As in the present study, ovicidal effect of juvenile hormone analogue fenoxycarb was weaker than other two

groups of IGRs in Colorado potato beetle *Leptinotarsa decemlineata* Say, 1824 (Koopmanschap *et al*., 1989).

In rubber plantation litter, eggs of *Luprops tristis* are present in the middle litter layers for a very short period of 3-4 days during February to March period (Sabu *et al*., 2008; Sabu and Vinod, 2009). Hence thought experimentally proved effective, field application of the most effective chitin synthesis inhibitor, diflubenzuron on eggs laid in the lower litter layers will be practically difficult.

5.2. Efficacy of fenoxycarb, diflubenzuron and 20E on first and fifth instar larvae of *Luprops tristis.*

5.2.1. Efficacy of fenoxycarb, diflubenzuron and 20E on first instar larvae of *Luprops tristis*.

In feeding bioassay with IGRs treated rubber plant leaves, LC_{50} value of fenoxycarb (0.54 ppm) was 13 times lower than LC_{50} of diflubenzuron and 1.6 times lower than LC_{50} of 20E. In residual contact bioassay, LC_{50} value of fenoxycarb (1.71 ppm) was 18 times lower than LC_{50} of diflubenzuron and 1.4 times lower than LC_{50} of 20E. Considering the lowest effective tested concentrations for instant mortality of first instar, fenoxycarb and 20E at 10 ppm concentration in feeding bioassay was effective and caused 100 % mortality in tested individuals. Diflubenzuron at higher concentration of 100 ppm, caused only 80% and 42% instant mortality in feeding and contact bioassay respectively. As concentration higher than 100 ppm is necessary to cause 100 % instant mortality in diflubenzuron treated first instar larvae application of lower concentrations of diflubenzuron on *L. tristis* first instar larvae is not effective for getting instant mortality.

The high cumulative toxic effect that considers toxicity in larval stages and in pupae those emerged from treated larvae, 20E and fenoxycarb at the lowest tested concentration at 1 ppm, was most effective and caused cumulative mortality of 85% and 81.67% respectively before entering into the adult stage. With only 20% cumulative mortality for diflubenzuron at 1 ppm, diflubenzuron is unsuitable for exerting the cumulative toxic effect at lowest concentrations. Though both fenoxycarb and 20E were effective in instant and cumulative toxicity, based on the lowest environmental persistence and low LD_{50} values reported on mammals for fenoxycarb, fenoxycarb is more suitable for application rather than 20E (Fischer and Hall, 1992; Sullivan 2000).

At tested lower concentrations of fenoxycarb, first instar larvae (75.69%) did not moult into second instar and died as abnormal first instar larvae itself and those survived underwent abnormal pupation leading to low pupal survivability. Survived pupae eclosed as normal teneral adults. Similar trend was observed in a dose response study in second instar larvae of *Corcyra cephalonica* (Stainton, 1866) with fenoxycarb inhibiting the moulting process to the next instar, when the larvae were treated with higher concentrations and the lower concentration inhibiting moulting process to a lesser extent (Singh and Tiwari, 2014).

Treatment of first instar larvae with 20E caused immature moulting into second instar larvae which finally died as abnormal larval-larval intermediate with occluded mouth parts. Chitin synthesis inhibitor, diflubenzuron treatment caused abnormal larval segmentation and body abnormalities that leaded to mortality. Similar abnormalities were found when diflubenzuron treatment at a concentration of 250 ppm caused complete

mortality of first instar larvae in *Chilocorus bipustulatus* (Linnaeus 1758) and in *Leptinotarsa decemlineata* Say, 1824 (Colorado potato beetle) where, the normal wrinkles in the larval skin disappeared and larvae became balloon shaped and died (Peleg 1983, Grosscurt 1978).

5.2.2. Efficacy of fenoxycarb, diflubenzuron and 20E on fifth instar larvae of *Luprops tristis*

Compared to diflubenzuron and 20E, lowest LC_{50} value obtained for fenoxycarb in both feeding (0.02 ppm) and residual contact bioassay (0.0 ppm) make it as the most effective IGR against the fifth instar larval stage of *Luprops tristis*. Comparison of mortality of fifth instar larvae of *L. tristis* in feeding bioassay with 100 ppm concentrations of fenoxycarb, diflubenzuron and 20E, established that fenoxycarb and 20E treatment at 100 ppm were most effective and caused 100% mortality of tested individuals. Compared to fenoxycarb and 20E, diflubenzuron was least effective with 80 % mortality in feeding bioassay at 100 ppm concentration. In residual contact bioassay, fenoxycarb was most effective providing 100% mortality at 100 ppm concentration followed by 20E showing mortality rate of 93.33%. Diflubenzuron caused 41.67% at 100 ppm concentration indicating it as most ineffective.

Cumulative toxicity comprising mortality of treated fifth instar larval stage and the emergence of pupae from surviving larvae after IGR treatment, fenoxycarb treatment at 10 ppm concentration caused highest cumulative toxicity of 93.33% in feeding bioassay whereas 20E caused 86.67% of cumulative mortality. Diflubenzuron was least effective with the lowest cumulative toxicity of 56.67% at 10 ppm treatment.

Pupation was inhibited or delayed in fenoxycarb treated fifth instar *L. tristis*, which continued their feeding and gained weight with respect to the concentration exposed. Fifth instar larvae treated with high concentrations of fenoxycarb (100 ppm, 10 ppm) have prolonged larval period (11-18 days). During this prolonged larval period gain in larval weight occurred and larvae thus formed were dead before pupation or after entering into the pupal stage. Fifth instar larvae treated with lower tested concentrations (1 ppm, 0.1 ppm and 0.01 ppm) of fenoxycarb also displayed weight gain and prolonged developmental period (14–24 days) and died as abnormal fifth instar larvae or abnormal pupae. Similarly, larval period was prolonged with higher dose of fenoxycarb in *Tribolium confusum* Jacquelin du Val, 1986 (Smet *et al*., 1989). Fenoxycarb targeted at the seventh instar larval stage of *Alphitobious diaperius* Panzer, 1797 inhibited pupation, which caused prolonged larval periods and production of 'supernumerary' or 'gaint' larvae (Edward and Abraham, 1985; Sing and Johnson, 2013). In both feeding and residual contact bioassays, fenoxycarb treated fifth instars of *L. tristis* taken longer period to reach pupation than in the diflubenzuron or 20E treatments.

Edward *et al*., (1991) recommended fenoxycarb as a long-term protectant of stored wheat againts *Tribolium castaneum, Oryzaephilus surinamensis* (Linnaeus, 1758), *Rhyzopertha dominica* (Fabricius, 1792) and *Sitophilus granaries* (Linnaeus, 1758). Juvenile hormone treatment of the stored product pests markedly prolonged the larval feeding stage and weight and thus accelerated the damages to the stored products (Ishaaya and Yablouski, (1976). However, prolongation of larval stage of *L. tristis*, will turn out to be beneficial in the control of *L. tristis* as prolongation of larval maturity by 14– 24 days

prior to the onset of rainfall (March- April period) will lead to death of larvae in the field itself as only the emerged adults can escape from the wet litter conditions by flying towards residential buildings and shelters**.**

In feeding and residual contact bioassays at tested concentrations, it took shorter duration for (all the tested concentrations of 20E) 20E treated fifth instars of *L. tristis* to reach pupation (and formation of lethal larval-pupal intermediate or formation of abnormal pupae) than in the fenoxycarb or diflubenzuron treatments. Similar developmentally premature, lethal molting was observed in Japanese beetle *Popillia japonica* Newman, 1841 when treated with 20E agonist RH 5849 (Monthean and Potter, 1992).

Though significant variations in number of days taken for pupation (prolonged pupation) were not observed in treatment with various concentration of diflubenzuron in feeding and contact bioassay, abnormal pupation (pupae with morphological abnormalities) occurred in fifth instar treated with diflubenzuron. Similar abnormal pupation was reported in fourth instars of the 28- spotted potato ladybird beetle (*Henosepilachna vigintioctopunctata* Fabricius, 1775) topically treated with diflubenzuron (Rao *et al*., 1992). In contrast to the present result, fenoxycarb failed to exert toxic actions in *Poecilus cupreus* (Linnaeus, 1758) (Abdelgader and Heimbach, 1992).

All the five larval stages of *L. tristis* are nocturnally active on the surface and diurnally passive in the lower litter layers in the rubber plantation fields. Arrival of larval stages in rubber plantation litter coincides with the new leaf formation in rubber trees following annual leaf shedding and larval population in rubber plantations peak in synchronization with powdery mildew mediated leaf fall and the larval stages feed on

fallen wilted tender rubber leaves on the surface litter floor for nearly three months during February-April period (Sabu and Vinod, 2009). Hence, synchronized field application of the effective IGR with annual leaf fall of rubber tree will turn out to be an effective strategy for controlling *L. tristis* population in the larval stage and before they enter into the nuisance causing adult stage.

5.3. Efficacy of fenoxycarb, diflubenzuron and 20E on pupae of *Luprops tristis*

Difubenzuron was more toxic to pupae of *Luprops tristis* than fenoxycarb and 20E with LC_{50} (11.25 ppm) value 27 times lower than LC_{50} value of fenoxycarb and 16 times lower than LC_{50} value of 20E. Diflubenzuron treatment at 100 ppm concentration was more effective and caused 83.33 % pupal mortality. 20E treatment at 100 ppm caused 56.67 % pupal mortality. At tested higher concentration of 100 ppm, fenoxycarb caused only 25 % of pupal mortality and was proved to be least effective on the pupae of *L. tristis.* Diflubenzuron treated pupae were unable to eclose to normal adults and died as abnormal blocked pupae. Similarly pupal treatment with diflubenzuron resulted in the formation of abnormal blocked pupae in *Tenebrio molitor* Linnaeus, 1758 (Soltani *et al*., 1984).

Exposure to 20E resulted in the precocious incomplete lethal pupal eclosion into immature adults in *L. tristis* and died as pupal-adult intermediates. Likewise three ecdysone agonists, RH-5849, RH-5992 and RH-0345 reduced the pupal duration and caused the lethal pupal- adult intermediate formation with a dose- response relationship in Mediterranean flour moth *Ephestia kuehniella* Zeller, 1879 and suggested the usage of these analogues for sound integrated pest management strategy of Mediterranean flour moth (Hami *et al*., 2005).

Pupal stages of *L. tristis* were less succeptible to fenoxycarb with higher LC_{50} value (3.06×10^2 ppm). But, in *A. diaperinus* pupae, topical treatment with fenoxycarb showed low LC_{50} value (0.07 ppm) (Singh and Johnson, 2013).Higher LC_{50} value obtained for the pupae in the present study (3.06×10^2 ppm) indicated the difference in the method of application of IGRs will lead to the difference in the toxicity effect exerted.

Pupal stages of *L. tristis* are appeared in the middle litter layer of natural rubber plantation about two months (February- March) with a pupal duration of 2-3 days (Sabu *et al*., 2008; Sabu and Vinod, 2009). Even though chitin synthesis inhibitor, diflubenzuron were experimentally proved as most effective against pupal stage of *L.tristis* leding to the formation of abnormal blocked pupae, field application of diflubenzuron on the pupae present in the middle litter layers will be practically difficult.

5.4. Efficacy of fenoxycarb, diflubenzuron and 20E on adults of *Luprops tristis*

5.4.1. Efficacy of fenoxycarb, diflubenzuron and 20E on pre-dormancy adults of *Luprops tristis.*

With pre-dormancy beetles, 20E was more effective (LC_{50} = 25.83 ppm) and caused 70% instant mortality at 100 ppm in feeding bioassay. Both fenoxycarb and diflubenzuron showed comparatively lower and similar mortality rate of 46.67% at 100 ppm in feeding bioassay.

20E treatment caused the highest reduction in fecundity of 30% and reduction in egg hatchability rate of 34% in treated pre-dormancy beetles at 100 ppm concentration. Comparatively lower fecundity and egg hatchability reduction were observed with diflubenzuron (15% reduction in fecundity and 9% reduction in egg hatchability) and

fenoxycarb (5% reduction in fecundity, no reduction in egg hatchability rate) treatment at 100 ppm concentration.

While considering the cumulative effect on the fecundity and hatchability rate of eggs laid by IGR treated beetles, pre-dormancy beetles treated with 20E at 100 ppm showed highest cumulative effect and recorded reduced progeny production (fecundity) of 38% in feeding bioassay and 30% in residual contact bioassay. Progeny reduction was observed in adult beetle of mealworm *T. molitor*, with the number of eggs per female was reduced to 32%, the size of the deposited egg was reduced and egg viability was lost by 68% (Taibi *et al*., 2003).There were no significant variation in progeny production for pre-dormancy beetles treated with fenoxycarb and diflubenzuron indicating that these two compounds are ineffective on pre-dormancy adults. The cumulative effect on the fecundity and hatchability rate of eggs, higher mortality rate and reduced fecundity of treated beetles and the hatchability rate of eggs in the feeding and contact bioassays showed that 20E is more effective on pre-dormancy adults of *Luprops tristis.*

5.4.2. Efficacy of fenoxycarb, diflubenzuron and 20E on dormant adults of *Luprops tristis*

In dormant beetles, comparatively lower LC_{50} value (8.14×10^3 ppm) of fenoxycarb showed that the beetles are more susceptible to fenoxycarb than diflubenzuron and 20E. Fenoxycarb treatment at 100 ppm concentration was more effective causing 33.33% of mortality in treated beetles followed by 20E (18.13%). Diflubenzuron caused lowest mortality rate of 5% at 100 ppm concentration in contact bioassay.

At 100 ppm concentration, diflubenzuron treatment caused 33% reduction in fecundity and 46.13% reduction in egg hatchability in treated dormancy beetles. Reduction

in fecundity (30.1%) and reduction in egg hatchability (33.8%) were observed in fenoxycarb treated dormancy beetles. Comparatively lower fecundity reduction (28%) and egg hatchability reduction (25.12%) were observed in 20E treated dormancy beetles.

Considering the cumulative effect on progeny reduction, diflubenzuron treatment at 100 ppm showed highest reduction in progeny production (47%) even though low LC_{50} value obtained for fenoxycarb .at 100 ppm concentration 20E reduced next generation by 25.12% and fenoxycarb reduced next generation by 33.80%. Similar reduction in fecundity and egg sterilizing effect of diflubenzuron was observed in Nitidulid beetle *Carpophilus hemipterus* (Linnaeus, 1758) (Ascher *et al*., 1986).

Reduced fecundity of treated beetles and the reduced hatchability rate of eggs in the feeding and contact bioassays and the cumulative effect on the fecundity and hatchability rate of eggs laid by diflubenzuron treated dormant beetles showed that diflubenzuron is effective on dormant adults of *Luprops tristis.* Higher instant mortality rate of fenoxycarb treated dormant beetles showed that fenoxycarb is effective to induce mortality on dormant adults of *L. tristis.* So both the compounds are effective though both are unable to cause 90-100% reduction.

5.4.3. Efficacy of fenoxycarb, diflubenzuron and 20E on post-dormancy adults of *Luprops tristis*

LC_{50} values obtained for all the IGRs, fenoxycarb, diflubenzuron and 20E, were above the tested concentration range in post-dormancy adult *Luprops tristis*. Comparatively lower LC_{50} value obtained for 20E (LC_{50} feeding = 83.14 ppm and LC_{50} contact = 857.13 ppm) makes it as the most effective IGR to induce mortality in post-dormancy beetles. 20E treatment at the highest tested concentration of 100 ppm was more

effective and caused 63.33% mortality in feeding bioassay and 16.66% in residual contact bioassay. Fenoxycarb and diflubenzuron at 100 ppm in feeding bioassay caused mortality of 33.67% and 10% and in residual contact bioassay caused mortality of 6.66% and 5% respectively.

Highest reduction in fecundity rate of 77.30% and 92% and reduction in egg hatchability were observed in fenoxycarb (100 ppm) treated post-dormancy beetles in feeding bioassay. 60.27% reduction in fecundity and 75% reduction in egg hatchability were observed in diflubenzuron (100 ppm) treated post-dormancy beetles. Comparatively lower reduction in fecundity (56%) and reduction in hatchability rate of eggs (66%) were obtained 20E (100 ppm) treated post-dormancy beetles in feeding bioassay.

Based on cumulative effect on post dormancy beetles, fenoxycarb at 100 ppm reduced the progeny production up to 90% in feeding bioassay. Diflubenzuron treatment reduced progeny production by about 60.27% and 20E reduced about 57.65% at 100 ppm treatment in feeding bioassay. Reduction in progeny production was resulted in *Trogoderma granarium* Everts, 1898 and *Tribolium castaneum* (Herbst, 1797) following treatment with juvenile hormone analogues hydroprene and methoprene respectively (Saxena and Kumar, 1991; Williams and Amos, 1974) and production of non-viable eggs were noticed in mature females of *Lasioderma serricorne* (Fabricius, 1792) exposed to juvenile hormone analogue, methroprene (Marzke *et al*., 1977).

Luprops tristis adults were more susceptible to fenoxycarb, diflubenzuron and 20E in their active and feeding phase of pre- and post-dormancy than during the inactive, non-feeding, aggregated intermittent phase of dormancy. During pre-dormancy and post-

dormancy stages beetles were more susceptible to fenoxycarb, diflubenzuron and 20E in feeding bioassay with IGR treated rubber plant leaves than in residual contact bioassay with IGR treated filter paper. Higher reduction in progeny production in post-dormancy phase suggested the probability that either reproductive organs in *L. tristis* do not mature until their post dormancy phase or the beetles are undergoing reproductive diapause (Danks 1987; Mansingh 1971).

Luprops tristis adults in their pre-dormancy (Feburary- April) and post-dormancy (December- March) stages inhabited rubber plantation litter with a duration of 34.31 ± 4.72 days in pre-dormancy and 31.46 ± 3.35 days in post-dormancy with nocturnal surface activity in the upper and diurnal passivity under lower litter layers and beetles in both these stages feed on wilted rubber leaves on the surface litter floor (Sabu and Vinod, 2009). As the beetles passively aggregate in a specific location for a longer period (278.69 ± 3.34 days; April- December) dormancy period of *L. tristis* is the most convenient time for their control. But, higher LC_{50} values obtained in the present study for fenoxycarb, diflubenzuron and 20E in dormant beetles showed that higher concentration of IGRs needed for inducing mortality in dormant beetles than during pre and post-dormancy phases. Hence, field application of the IGRs (fenoxycarb, diflubenzuron and 20E) especially in the post dormancy phase of *L. trists and* inducing mortality in the parent generation and reducing progeny production becomes the effective measure to control *L. tristis* with IGRs.

CHAPTER 6

CONCLUSIONS

Tested IGRs, Fenoxycarb, Diflubenzuron, and 20E showed residual contact toxicity towards eggs, first and fifth instar larvae, pupae and pre-dormancy, dormancy and post-dormancy adult stages of *Luprops tristis* and feeding toxicity towards first and fifth instar larvae, pre and post-dormancy adults of *L. tristis*. The susceptibility of *L. tristis* towards the tested IGRs varied with respect to the phase and mode of application. Results from the present study provide baseline data on the susceptibility of *L. tristis* to the tested IGRs that can be used as reference points for field application of the compounds.

1) Chitin synthesis inhibitor diflubenzuron was more toxic to the egg stage of *L. tristis* while considering the direct ovicidal and cumulative toxic effect on treated eggs. But application of diflubenzuron on the eggs laid by *L. tristis* in the lower litter layer of rubber plantation will be practically difficult.

2) Juvenile hormone analogue, fenoxycarb was more toxic to first and fifth instar larvae of *L. tristis* causing direct mortality and cumulative toxicity. But, application of effective IGR to the first instar larvae inhabited in the middle litter layers is practically difficult. Pupation was delayed in fenoxycarb treated fifth instar larva by 14–24 days and prolongation of larval maturity prior to the onset of rainfall in rainy belts will lead to death of larvae in the field itself. Synchronized field application of fenoxycarb with the arrival of fifty instar larval stage and the premature leaf fall in rubber plantations will turn out to be an effective strategy for controlling the population build-up of *L. tristis* beetles in rubber plantation litter.

3) Diflubenzuron was more toxic to pupae of *L. tristis*. Field application of diflubenzuron on the passive pupae present in the middle litter layers will be practically difficult.

4) *Luprops tristis* adults were more susceptible to tested IGRs during pre and post-dormancy phase than in the inactive dormancy phase. Among the three IGRs tested, 20E was more toxic to adults in their pre and post-dormancy phase. Post-dormancy phase application of 20E is recommended due to the lower concentration required during the phase. Since 20E provided higher instant mortality of post dormancy beetles with lower concentration, 20E becomes a better IGR to control *L. tristis* than fenoxycarb that lead to higher reduction in progeny production in the next generation but less instant mortality compared to 20E.

5) Fenoxycarb was more toxic to *L. tristis* during the dormancy phase. Aggregation of *L. tristis* in specific locations followed by dormancy during the rainy seasons makes dormancy as the most convenient time for application of IGRs and control of *L. tristis*. However, the requirement of higher concentration of fenoxycarb to induce mortality in the aggregated dormant beetles present inside residential buildings makes it less attractive choice.

6) Application of 20E in the post-dormancy phase will induce mortality and will lead to reduction in progeny production. Hence, application of 20 E that lead to higher instant mortality of post dormancy beetles during the termination phase of dormancy and beginning of the post-dormancy phase becomes the recommended strategy for effective control of *L. tristis.*

CHAPTER 7

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20E = 20-Hydroxyecdysone

CSI = Chitin synthesis inhibitor

DFB = Diflubenzuron

FXB = Fenoxycarb

IGR = Insect growth regulator

JH = Juvenile hormone