STUDY ON COMBINED EFFECT OF BROMADIOLONE AND CHOLECALCIFEROL (VITAMIN D_3) AGAINST HOUSE RAT, *Rattus rattus* LINNAEUS

Thesis

Submitted to the Punjab Agricultural University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE
in
ZOOLOGY
(Minor Subject: Biochemistry)

By

Navjot Kaur
(L-2008-BS-204-M)

Department of Zoology
College of Basic Sciences and Humanities

LUDHIANA-141004

2010

CERTIFICATE I

This is to certify that the thesis entitled, **"STUDY ON COMBINED EFFECT OF BROMADIOLONE AND CHOLECALCIFEROL (VITAMIN D_3) AGAINST HOUSE RAT, *Rattus rattus* LINNAEUS"** submitted for the degree of **Master of Science,** in the subject of **Zoology** (Minor subject: **Biochemistry**) of Punjab Agricultural University, Ludhiana, is a bonafide research work carried out by **Ms. Navjot Kaur (L-2008-BS-204-M)** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

_____

Major Advisor
(Dr. (Mrs.) Devinder Kaur Kocher)
Zoologist (Rodents)
Department of Zoology
Punjab Agricultural University
Ludhiana – 141004

CERTIFICATE II

This is to certify that the thesis entitled, **"STUDY ON COMBINED EFFECT OF BROMADIOLONE AND CHOLECALCIFEROL (VITAMIN D_3) AGAINST HOUSE RAT, *Rattus rattus* LINNAEUS"** submitted by **Ms. Navjot Kaur (L-2008-BS-204-M)** to the Punjab Agricultural University, Ludhiana, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Zoology** (Minor subject: **Biochemistry)** has been approved by the Student's Advisory Committee along with Head of the Department after an oral examination on the same.

_____ _____

Head of the Department **Major Advisor**
(Dr. (Mrs.) R.K. Parshad) **(Dr. (Mrs.) Devinder Kaur Kocher)**

_____

Dean Post-Graduate Studies
(Dr. Gursharan Singh)

ACKNOWLEDGEMENT

At the very outset with folded hands, I bow my head with reverence and dedicatedly accord by recondite to the "Almighty" Lord for His blessing hand throughout my life.

Though the debt of learning can not be repaid, it is my sovereign privilege to express my gratitude and moral obligation to my esteemed Major Advisor, ***Dr. (Mrs.) Devinder Kaur Kocher****, Zoologist (Rodents), Department of Zoology for her enlightened, invaluable and inspiring guidance. I shall remain ever indebted for her care and affection during the course of investigation as well as in the preparation of this manuscript. Her multifaceted personality and commitment to work motivated and encouraged me to work, even harder and hence developed right attitude not only for my research work but also as a managed human being.*

I am extremely thankful to ***Dr. (Mrs.) R.K. Parshad****, Head, Department of Zoology for providing necessary inputs and research facilities for this study.*

I am greatly indebted to the members of my advisory committee viz. ***Dr. S.S. Hundal****, Professor (Dean PGS nominee), Department of Zoology,* ***Dr. (Mrs.) B.K. Babbar****, Assistant Zoologist (Rodents), Department of Zoology,* ***Dr. (Mrs.) Sucheta Sharma****, Associate Professor, Department of Biochemistry for their considerate behavior, valuable advice and suggestions.*

I thankfully acknowledge the financial assistance received from ICAR.

I express my sincere thanks to ***Ashu mam*** *and* ***Lakshmi mam*** *(computer centre) for their time to time help, valuable suggestions and guidance.*

It is rightly said that "Every effort is motivated by ambitious and all ambitions have an inspiration behind." I owe this pride place to my grandmother ***Sdn. Pritam Kaur*** *and my parents,* ***S. Avtar Singh*** *and* ***Sdn. Karamjeet Kaur****. I am forever indebted to my parents for their understanding, endless patience and encouragement when it was most required and for providing me the means to learn and understand. I cannot weigh my feelings with words for my dearest brothers* ***Navdeep*** *and* ***Amanjot*** *for their motivation, encouragement, ever lasting love and affection and moral support. Special love thoughts for my dearest friend* ***Gurpreet Sidhu****.*

I have been fortunate to come across my funny & good friends without whom life would be bleak. I am happy to acknowledge the shadow support and moral upliftment showered upon me by ***Manveen****,* ***Kulwinder****,* ***Ruby****,* ***Pichu****,* ***Mann****,* ***Simran*** *and my seniors* ***Sherry didi****,* ***Navi didi****,* ***Priya didi, Nishi didi****,* ***Jas didi.***

I express my special thanks to ***Pappu bhayia*** *and* ***Raju bhayia*** *for necessary help at animal house laboratory during my research work.*

Last but not the least, I duly acknowledge my sincere thanks to all those who love and care for me. Every name may not be mentioned but none is forgotten.

(Navjot kaur)

Title of the Thesis	**:**	STUDY ON COMBINED EFFECT OF BROMADIOLONE AND CHOLECALCIFEROL (VITAMIN D_3) AGAINST HOUSE RAT, *Rattus rattus* LINNAEUS
Name of the student and Admission No.	**:**	Navjot Kaur L-2008-BS-204-M
Major Subject	**:**	Zoology
Minor Subject	**:**	Biochemistry
Name and Designation of Major Advisor	**:**	Dr. (Mrs.) Devinder Kaur Kocher Zoologist (Rodents)
Degree to be Awarded	**:**	M.Sc.
Year of award of Degree	**:**	2010
Total Pages in Thesis	**:**	73
Name of University	**:**	Punjab Agricultural University, Ludhiana, Punjab, India

ABSTRACT

Commensal rodents, especially house rat (*Rattus rattus* Linnaeus) causes extensive losses by feeding and contaminating the food products and also plays a role in spreading several diseases of health importance. House rats were trapped from various commensal situations and were fed for 5 days in no-choice on standard baits of bromadiolone (0.005%) and cholecalciferol (0.075%) and their combinations having different concentrations mixed in WSO-mix bait. Male and female house rats showed 81.30% and 95.80% average acceptability of standard bait of bromadiolone over plain bait respectively. Hundred per cent mortality of male house rats was observed within 3-6 days, while all female rats died on 4th day of feeding of 0.005% bromadiolone. Both the sexes showed a significant increase in blood clotting time (sec) after 48 hours of feeding as compared to that of 0 hour. The acceptability of standard bait of cholecalciferol (0.075%) over plain bait was 40.10% and 35.30% in male and female house rats, respectively and 100% mortality was observed within 4-10 days in male and 7-14 days in female house rats. Feeding of standard bait of cholecalciferol resulted in stop feeding action in the form of significantly less consumption of this bait from 3rd day onward. Serum calcium level (mg/dL) was found to be significantly high in 0.075% cholecalciferol fed male and female rats after 48 hours of its feeding as compared to 0 hour. *R. rattus* when fed on four formulated baits having different concentrations of bromadiolone and cholecalciferol i.e. combination-I (0.0025% bromadiolone+0.05% cholecalciferol), combination-II (0.001% bromadiolone+0.05% cholecalciferol), combination-III (0.0025% bromadiolone+0.01% cholecalciferol) and combination-IV (0.001% bromadiolone+0.01% cholecalciferol). Out of these four tested formulated baits, combination-IV (having the lowest concentration of bromadiolone and cholecalciferol) showed efficient rodenticidal potential because of synergistic effect of bromadiolone and cholecalciferol in this combination. It was able to produce 100% mortality and showed a significant delay in blood clotting time (sec) and rise in serum calcium level and was cost effective also. *R. rattus* L trapped from commensal situations of Ludhiana area showed no resistance towards standard bait of bromadiolone (0.005%) as both male and female house rats (n=60) showed 100% mortality.

Keywords: Bromadiolone, cholecalciferol, resistance, synergistic effect, *R. rattus*

_____ _____

Signature of Major Advisor Signature of the Student

ਖੋਜ ਪੱਤਰ ਦਾ ਸਿਰਲੇਖ	:	ਘਰਾਂ ਦੇ ਚੂਹੇ (ਰੈਟਸ ਰੈਟਸ ਲਿਨੇਇਸ) ਉੱਤੇ ਬਰੋਮਾਡਾਇਲੋਨ ਅਤੇ ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ ਦੇ ਸਾਂਝੇ ਪ੍ਰਭਾਵ ਦਾ ਨਿਰੀਖਣ।
ਵਿਦਿਆਰਥੀ ਦਾ ਨਾਮ ਅਤੇ ਪ੍ਰਵੇਸ਼ ਨੰ.	:	ਨਵਜੋਤ ਕੌਰ **(L-2008-BS-204-M)**
ਪ੍ਰਮੁੱਖ ਵਿਸ਼ਾ	:	ਜ਼ੂਲੋਜੀ
ਸਹਿਯੋਗੀ ਵਿਸ਼ਾ	:	ਬਾਇਓ-ਕੈਮਿਸਟ੍ਰੀ
ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦਾ ਨਾਮ ਅਤੇ ਅਹੁਦਾ	:	ਡਾ. ਦੇਵਿੰਦਰ ਕੌਰ ਕੋਚਰ ਜੀਵ ਵਿਗਿਆਨੀ (ਚੂਹੇ)
ਡਿਗਰੀ	:	ਐਮ.ਐਸ.ਸੀ.
ਡਿਗਰੀ ਨਾਲ ਸਨਮਾਨਿਤ ਕਰਨ ਦਾ ਸਾਲ	:	2010
ਖੋਜ ਪੱਤਰ ਵਿੱਚ ਕੁੱਲ ਪੰਨੇ	:	73
ਯੂਨੀਵਰਸਿਟੀ ਦਾ ਨਾਮ	:	ਪੰਜਾਬ ਖੇਤੀਬਾੜੀ ਯੂਨੀਵਰਸਿਟੀ, ਲੁਧਿਆਣਾ

ਨਿਚੋੜ

ਕਮੈਨਸਲ ਚੂਹੇ, ਵਿਸ਼ੇਸ਼ ਰੂਪ ਤੇ ਘਰਾਂ ਦਾ ਚੂਹਾ (ਰੈਟਸ ਰੈਟਸ ਲਿਨੇਇਸ) ਖਾਧੇ ਜਾਣ ਵਾਲੇ ਪਦਾਰਥਾਂ ਨੂੰ ਆਪਣੇ ਮਲ-ਮੂਤਰ ਨਾਲ ਖਰਾਬ ਕਰਕੇ ਅਤੇ ਉਹਨਾਂ ਨੂੰ ਖਾ ਕੇ ਬਹੁਤ ਵਧੇਰੇ ਨੁਕਸਾਨ ਕਰਦਾ ਹੈ ਅਤੇ ਨਾਲ ਹੀ ਸਾਰਵਜਨਿਕ ਸਿਹਤ ਅਤੇ ਮਨੁੱਖੀ ਬਿਮਾਰੀਆਂ ਨਾਲ ਜੁੜੀਆਂ ਕਈ ਸਮੱਸਿਆਵਾਂ ਵਿੱਚ ਮਹੱਤਵਪੂਰਨ ਭੂਮਿਕਾ ਨਿਭਾਉਂਦਾ ਹੈ। ਘਰਾਂ ਦੇ ਚੂਹੇ ਨੂੰ ਵੱਖ-ਵੱਖ ਸਥਾਨਾਂ ਤੋਂ ਪਿੰਜਰਿਆਂ ਰਾਹੀਂ ਫੜਿਆ ਗਿਆ ਅਤੇ ਉਹਨਾਂ ਨੂੰ ਪ੍ਰਮਾਣਿਕ ਬਰੋਮਾਡਾਇਲੋਨ (0.005%), ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ/ਵਿਟਾਮਿਨ ਡੀ$_3$ (0.075%) ਅਤੇ ਉਹਨਾਂ ਦੇ ਵੱਖ-ਵੱਖ ਮਾਤਰਾਵਾਂ ਦੇ ਮੇਲ ਨੂੰ ਸਾਦੇ ਭੋਜਨ ਡਬਲੀਊ ਐਸ ਓ ਵਿੱਚ ਰਲਾ ਕੇ ਬਣਾਏ ਗਏ ਚੋਗ ਨੂੰ ਨੋ-ਚੁਆਇਸ ਵਿੱਚ ਪੰਜ ਦਿਨਾਂ ਵਾਸਤੇ ਪਾਇਆ ਗਿਆ। ਨਰ ਅਤੇ ਮਾਦਾ ਚੂਹਿਆਂ ਨੇ ਬਰੋਮਾਡਾਇਲੋਨ (0.005%) ਦੇ ਚੋਗ ਨੂੰ ਸਾਦੇ ਭੋਜਨ ਨਾਲੋਂ ਕ੍ਰਮਵਾਰ 81.30% ਅਤੇ 95.80% ਸਵੀਕਰਿਤੀ ਦਿਖਾਈ। ਸਾਰੇ ਨਰ ਚੂਹੇ 0.005% ਬਰੋਮਾਡਾਇਲੋਨ ਦਾ ਚੋਗ ਖਾ ਕੇ 3-6 ਦਿਨਾਂ ਵਿੱਚ ਮਰ ਗਏ, ਜਦਕਿ ਸਾਰੇ ਮਾਦਾ ਚੂਹਿਆਂ ਦੀ ਚੌਥੇ ਦਿਨ ਮੌਤ ਹੋ ਗਈ। ਦੋਵਾਂ ਲਿੰਗਾਂ ਵਿੱਚ ਬਰੋਮਾਡਾਇਲੋਨ ਦੇ ਚੋਗ ਨੂੰ ਖਾਣ ਤੋਂ 48 ਘੰਟੇ ਬਾਅਦ ਖੂਨ ਜੰਮਣ ਦੇ ਸਮੇਂ ਵਿੱਚ 0 ਘੰਟੇ ਦੀ ਤੁਲਨਾ ਨਾਲੋਂ ਮਹੱਤਵਪੂਰਨ ਵਾਧਾ ਦੇਖਿਆ ਗਿਆ। ਨਰ ਅਤੇ ਮਾਦਾ ਚੂਹਿਆਂ ਵਿੱਚ ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ (0.075%) ਚੋਗ ਦੀ ਸਵੀਕਰਿਤੀ ਸਾਦੇ ਭੋਜਨ ਨਾਲੋਂ ਕ੍ਰਮਵਾਰ 40.10% ਅਤੇ 35.30% ਦੇਖੀ ਗਈ। ਨਰ ਚੂਹੇ 4-10 ਦਿਨਾਂ ਵਿੱਚ ਅਤੇ ਮਾਦਾ ਚੂਹੇ 7-14 ਦਿਨਾਂ ਵਿੱਚ ਸੌ ਫ਼ੀਸਦੀ ਮਰ ਗਏ। ਚੂਹਿਆਂ ਨੇ ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ ਦੇ ਪ੍ਰਮਾਣਿਕ ਚੋਗ ਨੂੰ ਖਾਣ ਤੇ ਸਟਾਪ ਫੀਡ ਐਕਸ਼ਨ ਦਰਸਾਇਆ ਯਾਨੀਕਿ 3 ਦਿਨ ਚੋਗ ਖਾਨ ਤੋਂ ਬਾਅਦ ਮਹੱਤਵਪੂਰਨ ਤੌਰ ਤੇ ਇਸ ਚੋਗ ਨੂੰ ਖਾਣਾ ਘੱਟ ਕਰ ਦਿੱਤਾ। ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ ਦਾ 0.075% ਚੋਗ ਖਾਣ ਤੋਂ 48 ਘੰਟਿਆਂ ਬਾਅਦ ਚੂਹਿਆਂ ਦੇ ਖੂਨ ਵਿੱਚ ਕੈਲਸ਼ੀਅਮ ਦੀ ਮਾਤਰਾ (ਮਿਲੀਗ੍ਰਾਮ/ਡੈਸੀਲਿਟਰ) ਵਿੱਚ 0 ਘੰਟੇ ਦੇ ਮੁਕਾਬਲੇ ਮਹੱਤਵਪੂਰਨ ਤੌਰ ਤੇ ਵਾਧਾ ਦੇਖਿਆ ਗਿਆ। ਰੈਟਸ ਰੈਟਸ ਨੂੰ ਬਰੋਮਾਡਾਇਲੋਨ ਅਤੇ ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ ਦੀਆਂ ਵੱਖ-ਵੱਖ ਮਾਤਰਾਵਾਂ ਨੂੰ ਮਿਲਾ ਕੇ ਬਣਾਏ ਗਏ ਚਾਰ ਤਰ੍ਹਾਂ ਦੇ ਮੇਲ ਵਾਲੇ ਚੋਗ ਜਿਵੇਂ ਕਿ ਮੇਲ-I (0.0025% ਬਰੋਮਾਡਾਇਲੋਨ + 0.05% ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ), ਮੇਲ-II (0.001% ਬਰੋਮਾਡਾਇਲੋਨ + 0.05% ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ), ਮੇਲ-III (0.0025% ਬਰੋਮਾਡਾਇਲੋਨ + 0.01% ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ) ਅਤੇ ਮੇਲ-IV (0.001% ਬਰੋਮਾਡਾਇਲੋਨ + 0.01% ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ) ਨੂੰ ਘਰਾਂ ਦੇ ਚੂਹਿਆਂ ਨੂੰ ਖੁਆਇਆ ਗਿਆ। ਇਹਨਾਂ ਪਰਖੇ ਗਏ ਚਾਰਾਂ ਮੇਲ ਵਾਲੇ ਚੋਗ ਵਿੱਚੋਂ ਮੇਲ-IV ਚੋਗ (ਜਿਸ ਵਿੱਚ ਸਭ ਤੋਂ ਘੱਟ ਬਰੋਮਾਡਾਇਲੋਨ ਅਤੇ ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ ਦੀ ਮਾਤਰਾ ਸੀ) ਵਿੱਚ ਮਹੱਤਵਪੂਰਨ ਚੂਹਾਨਾਸ਼ਕ ਸ਼ਮਤਾ ਦੇਖੀ ਗਈ। ਇਸ ਚੋਗ ਨੂੰ ਖਾਣ ਮਗਰੋਂ, ਬਰੋਮਾਡਾਇਲੋਨ ਅਤੇ ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ ਦੇ ਸਾਂਝੇ ਪ੍ਰਭਾਵ ਕਾਰਨ ਸੌ ਫੀਸਦੀ ਚੂਹੇ ਮਰ ਗਏ, ਖੂਨ ਜੰਮਨ ਦੇ ਸਮੇਂ ਵਿੱਚ ਮਹੱਤਵਪੂਰਨ ਵਾਧਾ ਹੋਇਆ ਅਤੇ ਖੂਨ ਵਿੱਚ ਕੈਲਸ਼ੀਅਮ ਦੀ ਮਾਤਰਾ ਵਿੱਚ ਵੀ ਮਹੱਤਵਪੂਰਨ ਵਾਧਾ ਦੇਖਿਆ ਗਿਆ, ਨਾਲ ਹੀ ਮੇਲ-IV ਦਾ ਚੋਗ ਸਸਤਾ ਸਾਬਤ ਹੋਇਆ। ਲੁਧਿਆਣੇ ਵਿੱਚੋਂ ਫੜੇ ਗਏ ਰੈਟਸ-ਰੈਟਸ ਲਿਨੇਇਸ (ਗਿਣਤੀ=60) ਵਿੱਚ 0.005% ਬਰੋਮਾਡਾਇਲੋਨ ਵਾਲੇ ਚੋਗ ਲਈ ਕੋਈ ਬਚਾਅ ਨਹੀਂ ਦੇਖਿਆ ਗਿਆ ਕਿਉਂਕਿ ਇਹਨਾਂ ਚੂਹਿਆਂ ਦੇ ਦੋਨਾਂ ਲਿੰਗਾਂ ਵਿੱਚ ਸੌ ਫ਼ੀਸਦੀ ਨਸ਼ਵਰਤਾ ਦੇਖੀ ਗਈ।

ਸ਼ਬਦ ਕੁੰਜੀ: ਬਰੋਮਾਡਾਇਲੋਨ, ਕੋਲੇਕੈਲਸੀਫੀਰੋਲ, ਬਚਾਅ, ਸਾਂਝਾ ਪ੍ਰਭਾਵ, ਘਰਾਂ ਦਾ ਚੂਹਾ।

ਪ੍ਰਮੁੱਖ ਸਲਾਹਕਾਰ ਦੇ ਹਸਤਾਖਰ

ਵਿਦਿਆਰਥੀ ਦੇ ਹਸਤਾਖਰ

CONTENTS

CHAPTER –I

INTRODUCTION

Rodents are ubiquitous and colonise where food and shelter are available. Rodents have played havoc with man's economy as they damage each and every food item in fields, godowns, storage houses, poultry farms and residential premises (Jackson 1987, Prakash and Ghosh 1992). The house rat, *Rattus rattus* is a native of Indian sub-continent and is now found worldwide and nominated as among 100 of the world's worst invaders (Gillespie and Myers 2004). It is the most abundant, widely distributed and cosmopolitan commensal rodent (Parshad 1999a). House rat not only causes severe damage by consuming the stored food items, but also contaminate the food materials by urination and defecation, thus making it unfit for human consumption (Parshad *et al* 1994) and also destroys sacks, bags, boxes and other packaging materials and infrastructure under storage conditions (Ahmad and Parshad 1987). Due to its close proximity to human habitations, it is involved in spreading several diseases of public health importance especially bubonic plague (WHO 1974, Weber 1982, Pai *et al* 2005, Sullivan 2007) and act as reservoirs of organisms that cause debilitating diseases in humans and livestock (Mills 1999, Neelanarayanan and Kanakasabai 2000).

The economic magnitude and health problems associated with rodent pests emphasize the need to develop techniques for their management. Integration of other methods like mechanical and environmental control, do form the technology package, but to a very limited extent. The methods used for the management of rodent population such as trapping, habitat manipulation, use of repellents/attractants/pathogenic agents, that induce mortality or migration of rodents have never produced consistent results (Buckle and Muller 2000). Chemical control by rodenticides is the most widely used and efficient method of all the available methods for the control of rodent pests both under agricultural and commensal situation (Prakash and Mathur 1987, Chopra *et al* 1996). A number of highly toxic substances like strychnine, zinc phosphide, barium carbonate, red squill, bromethalin have been commonly used for the control of rodents (Fishel 2005). However, rodenticides which are common in use for rodent control have their own drawbacks like poison aversion, bait shyness, lack of specificity and genetic resistance (Buckle *et al* 1994, 2007, Gould and Holmes 2008). With the introduction of first and second generation anticoagulant rodenticides, the rodent control strategies have undergone a complete change (Buckle and Muller 2000) and anticoagulants *viz.* brodifacoum, bromadiolone, warfarin are becoming more popular for rodent control (Koehler and Kern 2005). But these anticoagulants are the frequent cause of non-target animal poisoning and also potentially dangerous to all mammals, birds and humans (Buckle *et al* 1994, Brar and Sandhu 2000, Koehler and Kern 2005). Therefore, the chemical control of commensal rodents in human residential areas and

animal dewellings requires the use of safe and less toxic rodenticides. Among various rodenticides, cholecalciferol (vitamin D_3) sub-acute rodenticide is being considered as more potent and relatively safe than anticoagulants. Use of cholecalciferol as a rodenticide in bait has been found to lower the risk of secondary poisoning, minimiz the toxicity of non- target species and to overcome anticoagulant resistance in rats and mice (Eason *et al* 2000). No reports are available regarding the human poisoning from the use of cholecalciferol (Fishel 2005) and genetic resistance after its ingestion (Saini and Parshad 1992). But cholecalciferol alone is also not suitable to control rat population, as it often shows the poor acceptance of bait. Another aspect of using vitamin D_3 as rodenticide is its higher cost, as its effective concentration is more expensive than that of effective concentration of most of the anticoagulants used (Gould and Holmes 2008).

Overcoming the poison resistance and poison shyness is critical to the success of rodent control operation, so there is a need to formulate such compounds which have efficient rodenticidal potential, low or no resistance, good susceptibility, safe against non-target species and cost effective. To achieve this success some workers have studied the effect on mortality of rodents by preparing attractive bait formulations having combination of anticoagulant rodenticides with hypercalcaemia causing agent i.e. cholecalciferol (Muktha Bai *et al* 1978, Zatsepin *et al* 2006). The combination of anticoagulant and cholecalciferol could overcome the inefficacy of anticoagulants caused by resistance or vitamin K containing food (Fuhrmann 1991, Lund 1991, Schnorbach 1992). Thus combination of ingredients leads to an obvious increase in efficacy against rodents, even under difficult conditions (Pospischil and Schnorbach 1994).

Cholecalciferol and anticoagulant though are acting on different locations but are involved in the same process of blood physiology. Therefore, the excess of calcium due to cholecalciferol action and unavailability of vitamin K due the presence of anticoagulant can explain the added effectiveness of cholecalciferol not only against the efficient killing of normal rats but also against the anticoagulant resistant rodents (Muktha Bai *et al* 1978, Redfern and Gill 1980, Zatsepin *et al* 2006). The combination of the multiple dose anticoagulant with vitamin D_3 in an attractive bait formulation is a good agent for modern rodent control even under difficult conditions caused by resistance problems or antidote containing foodstuffs (Pospischil and Schnorbach 1994). Therefore, usage of anticoagulant in combination with cholecalciferol may come out to be beneficial in the form of retaining the efficacy and controlling the anticoagulant resistant as well as non-resistant rats, thus leading to the formulation of a good, safe and cost effective agent for modern usage in rodent control.

Keeping in mind the properties and advantages of combination of anticoagulant with cholecalciferol, the present study was carried out with the following objectives:

1) To standardize the combination of bromadiolone anticoagulant and cholecalciferol (vitamin D_3) as a rodenticide against house rats (*R.rattus*).
2) To study the effect of combined/developed rodenticide on biochemical parameters of blood of house rats.

CHAPTER – II

REVIEW OF LITERATURE

About half of the world population is actively engaged in agriculture. Despite many advances in agricultural technology, millions of people suffer from hunger, malnutrition, and starvation. The reasons for this pathetic situation are several and complex. One important factor is food loss due to pests. Vertebrate pests, especially rodents, are responsible for much of this loss (Kurian 2000). In developing countries, rodent infestation poses a serious threat by not only reducing income, but also by causing widespread food shortage as well (Milan 1990, Parshad 1999a). Rodents are highly successful in many environments throughout the world as they constitute more than 40% of known mammalian species (Myers 2000). Rodents are broadly categorized into field and commensal rodents. The Punjab state is rich in rodent fauna as about 25 species and sub-species of rodents have been reported (Chopra *et al* 1996). Out of these, *Rattus rattus*, commonly called as the house rat is one of the most abundant, widely distributed and cosmopolitan commensal rodent. The genus *Rattus* has about 570 forms which is considered to be the greatest genus amongst other genera in animals according to Walker (1964). Out of 28 species of *Rattus* genus, *R. rattus* is the biggest one and atleast its 14 subspecies are recognized within the territories of India alone (Biswas and Tiwari 1969). Commensal rodents are universally distributed and occur in residential premises, godowns, stores and poultry farms. In this chapter, problems of house rats and its management by using anticoagulant and their combination with vitamin D_3 has been reviewed under the following points:

2.1 General Biology of house rat, *Rattus rattus* Linnaeus

2.2 Losses due to house rat

2.3 Control of house rat

- 2.3.1 Chemical control
- 2.3.1.1 Control by acute- rodenticides
- 2.3.1.2 Control by anticoagulants
- 2.3.1.3 Control by sub- acute rodenticides
- 2.3.1.4 Synergistic/combined effect of chemicals on rodenticidal potential

2.1 General Biology of house rat, *Rattus rattus* Linnaeus

The house rat *Rattus rattus* Linnaeus is a native of the Indian sub-continent and now spread throughout the world. It feeds and damages almost all edible things. It is the most abundant, widely distributed, highly adaptable and cosmopolitan commensal rodent species worldwide. It is most common in coastal areas as well as on large ships. For this reason,

these rats are often called ship rats. Some of the common names of this species include house rat, black rat and roof rat (Pye *et al* 1999, Grzimek 2003).

Taxonomically house rat belongs to (Gillespie and Myers 2004)

Kingdom – Animalia

Phylum – Chordata

Subphylum – Vertebrata

Class – Mammalian

Order – Rodentia

Suborder – Sciurognathi

Family – Muridae

Subfamily – Murinae

Genus – *Rattus*

Species – *rattus*

R. rattus is nocturnal in habitat and has definite feeding patterns, with keen sensory organs, highly developed adaptive qualities, high rate of breeding potential and litter size of 1-9 rats (Grzimek 2003, Gillespie and Myers 2004). Its sense of sight is poor, but its senses of smell, taste, touch and hearing are excellent (Jackson 1982). *R. rattus* can be readily recognized by its slender body, long tail and pointed snout. The colour of the fur on the dorsal side has various shades of grey and the underpart is paler depending primarily upon its habitat (Krishnakumari *et al* 1992). Wild rodents have been known to live over a year, whereas, captives have survived for over four years (Linzey 1998). House rat co-exist along with other species, *viz*. *Mus musculus, R. norvegicus and Bandicota indica* in various ecosystems. It lives in houses (both urban and rural), godowns, mills, ware houses, bakeries and various types of industrial establishments (Krishnakumari *et al* 1992). House rat infestation is easily recognized by the presence of scattered and kidney shaped fecal matter, peculiar odour and contours of gnawing left on the damaged structures like wood, paper, fabrics, grains and other material. Sometimes rat paths, foot and tail marks are detected in dusty and muddy floor. Its climbing and jumping capacities are high. Its home range does not exceed more than 10-15 meters when the food supply is adequate and nearer from its shelter (Krishnakumari 1968, Majumder 1968).

2.2 Losses due to House rat

Rodents, the vertebrate pests cause considerable loss to agricultural products or commodities and articles of economic value of man (Vyas *et al* 1985). Commensal rodents, especially *R.rattus* causes extensive losses by feeding and contaminating the products with their urine, droppings and hair and occasional damage to clothes, wooden articles, books,

electrical appliances and other infrastructure in residential premises (Prakash and Mathur 1987, Parshad 1999a). Rodent pests are known to cause immense losses to various production systems (Tripathi *et al* 1992). According to Dykstra (1996), the quantitative loss of food grains due to rodent pests at storage level in India is to the tune of 25 to 30%. Out of the 4.7% of spilled wheat grains collected from four mills, grain stores and rural houses, 0.6% samples of rice and 0.4% samples of green grains in grocery shops had signs of rodent contamination (Parshad *et al* 1994). Krishnakumari and Majumder (1965) have noted that generally the bagged grains are attacked and in vegetables godowns, potatoes, onions, cabbages, pumpkins, tomatoes were nibbled generally by *R. rattus.*

Rodents, mainly rats and mice inflict incalculable loss to poultry industry. They are always present in poultry farm premises in large numbers due to constant availability of favourable habitat, enough nutrient food, multiplication without any competition and predator free environment (Parshad 1999b). They cause direct damage to poultry structures, poultry feed, chicks and eggs and indirect damage by contaminating the poultry house environment and by spreading several dreaded disease to poultry birds and workers (Chopra and Dhindsa 1987, Parshad *et al* 1987, Chopra 1992, Gora *et al* 1995, Chopra *et al* 1996, Parshad 1999b, Hussain *et al* 2006). At times, these notorious mammals have been reported to even attack and kill young chicks (Malik and Ichhponani 1970, Gupta and Mangat 1977, Khatri and Veda 1984). Commensal rats play a significant role in public health, chiefly due to their role as carriers or reservoirs for micro-organisms associated with infections and diseases that can be transmitted to humans. These diseases include plague, salmonellosis (food poisoning), leptospirosis, murine typhus, rickettsial pox, rat-bite fever, haemorrhagic fever (Singleton *et al* 2003). Some are transmitted through contact with infected rodent urine or faeces, other through fleas and lice and still other through mosquito bites (Ruiz 2004).

2.3 Control of house rat

Rodents have become a global problem due to their high rate of reproduction and adaptive behavior. To manage their population, several methods are being used which can be grouped into lethal or reductional and non-lethal or preventive methods (Parshad 1999a, Zhang *et al* 1999). Lethal methods include mechanical methods such as physical killing and trapping and chemical methods include use of rodenticides and biological control methods using predators and parasites are common now a days. Non-lethal methods include environmental and cultural control, use of repellents, barriers, rodent proofing etc. In developing countries like India, as the storage facilities at the village level are always very primitive, there is an easy access for rodents. Often fairly small changes or improvement of such storage facilities would reduce damage considerably, but such improvement is rarely

implemented and maintained (Lund 1994). In India, inspite of extensive research on ecology, behavior and control of rodents, the damage problems due to these pests still persist in severe proportions due to poor adoption of rodent control measures by farmers at their fields (Prakash and Ghosh 1992).

Out of all these the easiest and effective method of rodent control is to kill them with poisonous chemicals/rodenticides. The use of rodenticides is still considered to be the most potent technology available for rodent pest management (Prakash and Ghosh 1992, Chopra *et al* 1996), but they generally provide effective control for short term. There is dire need to manage these pests effectively to minimize the economic losses caused by rodents (Chopra *et al* 2008). Rodenticides play a significant role in combating the rodents and selection of rodenticide for control operations is of prime importance (Soni and Prakash 1981). Control success depends upon the pest species infesting the area, rodent density, size of area (Ahmad and Parshad 1989a), method of bait application (Ahmad and Parshad 1989b, Malhi and Parshad 1995, Sheikher and Jain 1996, Mathur 1997), timings of treatments (Ahmad and Parshad 1991, Malhi and Parshad 1993) and the method of determining area efficacy (Mathur and Prakash 1984). The effect of rodenticidal treatments are generally short lived as the left over rats reproduce to rebuild their population to their original level in short span of time. The resiliency in rodent population is due to their high rate of productivity particularly under tropical and subtropical climates (Parshad *et al* 1989). Therefore, for developing effective methods of rodent control with long lasting effects, it is desirable to develop methods to check resiliency in rodent population. Presently, the main approach being followed to tackle rodent problems all over the world is to reduce their population by killing them with rodenticides (Jackson 1979, Buckle 1994, Parshad 1999a, Jain and Tripathi 2000), but these can result in environmental hazards due to direct and indirect poisoning of non-target organisms (Saunders and Cooper 1981).

2.3.1 Chemical control of house rat

Under commensal situations, the management of rodent population number constitutes a difficult and greatly unsolved contemporary problem. House rat has high reproductive potential, as well as immense adaptive capabilities, it is considered to be complex technology to tackle with its population. Different types of rodenticides which had been used and are being used are as follows.

2.3.1.1 Rodent control by acute rodenticides

In the past, highly toxic substances like arsenic, strychnine, cyanide and compound (1080) have been commonly used in managing rodents (Prakash and Ghosh 1992). Strychnine, a poisonous alkaloid was first registered for use in U.S. in 1947. It is an acute rodenticide with toxic hazards to non-target species and a number of accidental exposures to strychnine pesticides are reported anually to poison control centers (Fishel 2005). Previously, the inorganic rodenticides like zinc phosphide, barium carbonate and organic rodenticides were commonly in use for managing the rodent problems (Fishel 2005). Out of these, the most commonly used was zinc phosphide. It was first registerd in the U.S in 1947 and labelled for controlling rats, moles, gophers and mice in a wide variety. It is used in 80-90 per cent of rodent control operations particularly under agricultural situations (Parshad 1992). Zinc phosphide is an acute poison that may kill a target rodent as a result of single dose of feeding. Once ingested, zinc phosphide reacts with moisture in the gastrointestinal tract to liberate phosphine gas, which is a lethal agent (Buckle and Muller 2000). Later it was classified as a federally restricted use pesticide due to its hazards to non-target species and its acute oral and inhalation (Brar and Sandhu 2000).

2.3.1.1.1 Disadvantages of acute rodenticide

A number of reports are there indicating the problems of bait shyness, poison aversion and genetic resistance being emerged after the usage of commonly used acute rodenticide for the control of rodent pests (Buckle *et al* 1994, 2007, Gould and Holmes 2008). Since rodents usually share environment with humans and other mammals, the risk of accidental exposure is an integral part of the placement of poison for their control (Fishel 2005) and it also poses risks of poisoning the non-target animals (poultry, cattles, pets etc.) and human beings (Koehler and Kern 2005). So there is a continous need to develop new and more potent rodenticides with low risk of secondary poisoning and non-hazardous to non-target species. Also, these chemical methods have certain drawbacks such as concerns about the safety of their long term use, inconvenience of use, permanence of effect, limited acceptance and availability in the widely differing social, cultural, religious, service in the developing world (Buckle *et al* 1994, 2007, Gould and Holmes 2008).

2.3.1.2 Rodent control by anticoagulants

Anticoagulant inhibits the formation of prothrombin, a key protein in blood clotting process thus leading to capillary damage, internal bleeding, and eventually to death. Anticoagulants generally take at least four days to inhibit the formation of prothrombin, a key protein in the blood clotting process, from the onset of feeding until rodents begin to die (Anonymous 2007). Anticoagulant toxicants act by interfering with the normal synthesis of

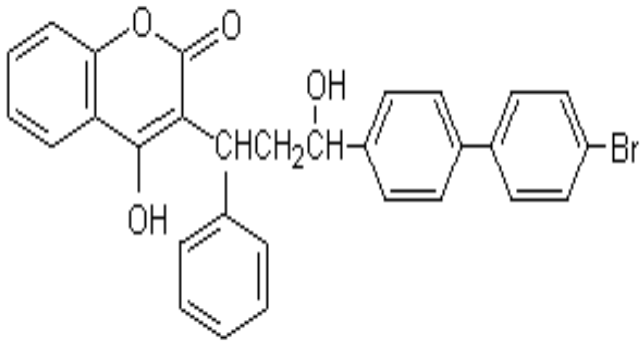
vitamin-k-dependent clotting factors in the liver of animal (Halder and Shadbolt 1975). This results in an increase in blood clotting time to the point where no clotting occurs. These days, anticoagulants like warfarin, bromadiolone, brodifacoum etc are being used more commonly for the control of rodents (Koehler and Kern 2005). Among the first generation anticoagulant rodenticides (multi-dose) 0.025% warfarin and 0.025% fumarin are generally used in cereal baits or as ready-to-use wax cakes. Racumin (coumatetralyl) to be used as 0.75% tracking powder or 0.0375% cereal bait in crop fields has been commercialized in India (Parshad 1999a).Treatment with 0.0375% Racumin bait for five days in a residential area resulted in 88.88% reduction in activity of rodents like *B. bengalensis*, *R. rattus* and *M. musculus* (Pathak 1995). Among the second generation anticoagulant rodenticides which are effective after ingestion of single dose by the pest species, poison baits – bromadiolone (0.005%), broadifacoum (0.005%) and flocoumafen (0.005%) have broad spectrum effect against major rodent pest species. These are also effective against rodents resistant to first generation anticoagulant rodenticides (Greaves 1994) and poison shy rodents to zinc phosphide (Saxena and Mathur 1996), thus are more effective for rodent control with single and multiple application of freshly prepared loose cereal bait or ready-to-use wax cake formulation (Parshad 1999a). Bromadiolone was found better than 2% zinc phosphide (Saxena *et al* 1991, Sheikher and Jain 1996). Mathur and Prakash (1984) conducted trials to evaluate brodifacoum (0.002%), chlorophacinone (0.0075%) and coumatetralyl (0.0375%) for control of rodents, predominantly *M. hurrianae* and got 90.5, 83.2 and 81.1 % control, respectively. The main advantage of second generation anticoagulant rodenticides is their potency, which makes them highly effective. In comparison, the first generation anticoagulants are more rapidly metabolized and excreted, and there is less risk to non-target species (Eason 1991).

2.3.1.2.1 Control of rodents by bromadiolone rodenticide

Bromadiolone is a potent second generation rodenticide (4-hydroxy coumarin derivative), often called a “super-warfarin” for its added potency and tendency to accumulate in the liver of the poisoned organism. When first introduced in 1980, to the UK market, it was effective against the population that had become resistant to first generation anticoagulant (Anonymous 2010). Bromadiolone sharply reduced the blood platelet count and also resulted in degeneration of liver and kidney in *M.musculus* fed on bromadiolone bait (Revathi and Yogananda 2006). Bromadiolone is a non-food use pesticide, therefore it is unlikely that there will be any exposure through food sources or residues in ground and surface water. Bromadiolone is absorbed rapidly and effectively by the dermal route. Oral administration of bromadiolone results in substantial retention of the chemical in the liver for an extended period of time (Rao 2005).

2.3.1.2.1.1 Chemistry of bromadiolone

Chemical name of bromadiolone is 3-[3- [4-(4-bromophenyl) phenyl]-3- hydroxy-1-phenylpropyl] - 2-hydroxychromen-4-one, its common name is bromadiolone (BSI, E-ISO, F-ISO); broprodifacoum (Republic of South Africa), its chemical formula is C_{30} H_{23} BrO_4 and chemical structure is:



Structure of bromadiolone

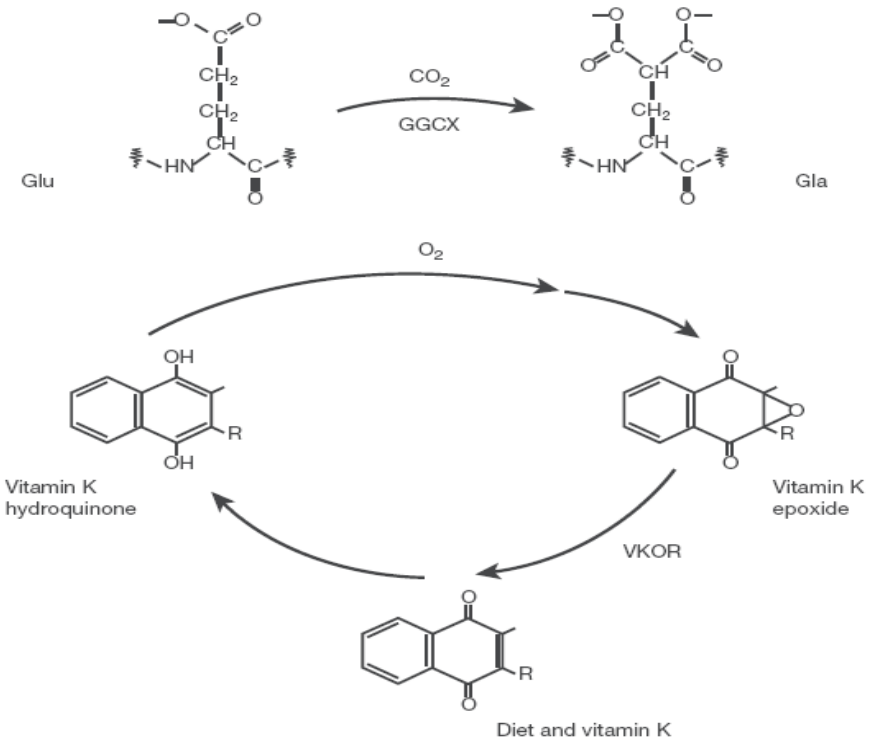
The technical material (97% pure) is an odourless, yellow-white powder. A mixture of the two diastereoisomers melts at 200 -210 ºC. It is soluble at 20ºC in 19 mg/litre water, 8.2 g/litre ethanol, 10 g/litre acetone, 25 g/litre ethyl acetate, 750 g/litre dimethyl formamide. It is stable under recommended application and storage conditions that is 200 ºC. Vapour pressure is negligible (0.002m Pa at 20ºC).

Bromadiolone is highly toxic to birds with an LC_{50} of 37ppm in northern Bob white. The result of the 96 hours blue gill sunfish and rainbow trout acute toxicity studies indicated that bromadiolone is moderately toxic to fish with an LC_{50} of 3ppm. Bromadiolone is considered moderately to highly toxic to fresh water invertebrates on an acute basis with an EC_{50} in the range of 0.1- 10 ppm (Rao 2005). Its toxicity, single dose is in rat 1.125 mg/kg bw, in mouse 1.75 mg/kg bw, in rabbit 1mg/kg bw, in dog >10 mg/kg bw, in cat >25 mg/kg bw. Bromadiolone is non-irritant to the skin and a slight irritant to the eye.

2.3.1.2.1.2 Mechanism of action of bromadiolone

Bromadiolone, like other anticoagulants act by interrupting the vitamin K cycle in liver microsomes which is necessary for the synthesis of prothrombin i.e. inactive protein (MacNicoll 1986, Anonymous 2007). In the functioning cycle (Fig. 1), the blood clotting factors II, VII, IX and X are produced as a result of post-translational γ- carboxilation of glutamyl residues. The active form of the vitamin K hydroquinone, is required as a co- factor in this process, during which is converted into the inactive vitamin K 2,3 epoxide. The epoxide is converted into vitamin K quinone by the enzyme epoxide reductase and then back to the hydroquinone by a third enzyme activity, vitamin K reductase. Anticoagulants inhibits

epoxide reductase enzymes and block the recycling of the active hydroquinone form of the vitamin which is necessary in the formation of prothrombin which further helps in clotting of blood. With this process of recycling blocked, only dietary vitamin K is available and this is insufficient to maintain clotting factor synthesis. For some time after the ingestion of an effective dose, sufficient factors are circulating in the blood to maintain clotting. These are eventually depleted, however, the mechanism fails and fatal haemorrhage results (Buckle 1994).



R= phenyl group

Fig. 1: Mechanism of inhibition of clotting cycle by anticoagulants

Ingestion of bromadiolone results in mortality within 9-10 days. This delayed action prevents rodents from associating the symptoms of toxicant with the anticoagulant which has caused it and, therefore, bait shyness is unknown. This mode of action brings with it a further important benefit. The supply of active form of vitamin can be preserved, and the ability of the blood to clot maintained, by the administration of excess amount of vitamin K, hence, this provides a specific antidote for use in cases of accidental poisoning.

2.3.1.2.1.3 Resistance towards anticoagulant rodenticides

The phenomenon of rodenticide resistance was first discovered among Norway rat (*R. norvegicus*) in Scottland in 1958 (Boyle 1960), but the initial outbreak was quickly followed by others occurring in the United Kingdom (Drummond 1966), United States

(Jackson and Kaukeinen 1972) and elsewhere. Meanwhile, the other important commensal rodent pest species, the house mouse (*M. musculus/domesticus*), were never very susceptible to early anticoagulant compounds such as warfarin, diphacinone and coumatetralyl and also developed resistant population in several countries (Ashton and Jackson 1984, Greaves 1994). The development of resistance in rodents to the anticoagulant rodenticides threatened the great strides towards improved efficacy and safety that the introduction of these compounds had made possible. The discovery of the second generation compounds (Hadler and Shadbolt 1975) redressed the balance for several years but, in a few localities, resistance to the first generation anticoagulant brought with it a measure of cross resistance to the second generation compounds and soon population of rats and mice began to appear with reduced susceptibility to these more potent compounds (Greaves *et al* 1982). However, resistance to the modern anticoagulants, such as brodifacoum, bromadiolone and difenacoum, has never become as widespread as that to the first generation compound and nowhere it is impossible to control rodents with at least one of these materials (Buckle 1994).

2.3.1.2.1.4 Advantages of anticoagulants

The main benefit of using anticoagulants for the control of rodents is the longer time taken for poison to induce death means that the rats do not associate death with eating the poison that is no poison shyness (Anonymous 1998). Bromadiolone and brodifacoum are effective as single dose poisons and this will mean economic saving in rat control (Singh and Saxena 1989).

2.3.1.2.1.5 Disadvantages of anticoagulants

In 1970s and 1980s rats developed an increased resistance to anticoagulants in certain area worldwide (MacNicoll 1982, Greaves 1985, 1986). The effectiveness of rodent control was dramatically reduced when the resistant animals formed a large proportion of a population. Also vitamin K containing feed (like maize and chicken feed) caused severe problems in some places (Pospischil and Schnorbach 1994). Wide range of species are susceptible to bromadiolone, there are reports that hazards to wildlife do exist when using bromadiolone against field rodents even of secondary poisoning risk to carnivores (O'Connor and Eason 2000). Though these anticoagulant rodenticides are effective in controlling the rodent problems, but are also potentially dangerous to all mammals and birds and are frequent cause of poisoning in pets (Brar and Sandhu 2000). The Environmental Protection Agency (EPA) believes that there is a high risk of secondary poisoning to mammals that feed on anticoagulant poisoned rodents in rural and suburban areas (Rao 2005).

2.3.1.3 Control of rodents by sub-acute chemicals

Compounds like bromethalin, calciferol, flupropadine, alphachloralose, thallium sulphate are sometimes termed as sub-acute rodenticides. They have some of the characteristics of the acute rodenticides and some of that anticoagulants, but also differ from them in certain respects. Although rodents may take a lethal dose of these materials during first 24 hours like acute rodenticides, repeated feeding may occur and death is normally delayed for several days like anticoagulants. A further characteristic is that a period of anorexia may be apparent in animals that have taken both lethal and sub lethal doses (Prescott *et al* 1992). This is the 'stop feed' action commonly claimed as a benefit for these compounds, as if a lethal dose has been ingested by the target rodents prior to its onset but it is disadvantage if only a sub lethal dose is taken and may account for the occasional practical failure of these compounds (Buckle 1985). The distinction between acute and sub acute compounds is not clear-cut, however, because death may occasionally delayed beyond 24 hours with some acute rodenticides, particularly strychnine and thallium sulphate.

Vitamin D and its analogues (vitamin D_2/ calciferol and vitamin D_3/ cholecalciferol) are a group of sterol compounds with sub-acute rodenticidal action and their over doses had been and are being used to control commensal rats in several countries like U.S.A, England, New zealand, India and Japan (Lund 1974, Eason 1991, Saini and Parshad 1992, Tanikawa and Kusano 1993, Sheikher and Jain 1995, Anonymous 1998, Bennet 2002, Fishel 2005, Koehler and Kern 2005). Vitamin D_2, having a very close structural relationship, similar chemical properties and with same mechanism of action as vitamin D_3, has found to be very effective for the control of rodents (Buckle and Muller 2000). Calciferol was introduced to combat the anticoagulant resistant rodents such as house mice, roof rats, sewer rats and other rodents in England (Lund 1974, Renninson 1974). *R. rattus* was found to be more susceptible to calciferol (vitamin D_2), as 90% mortality was obtained with 500mg/kg bw and 100% mortality was found with 1000 mg/kg bw and there was no sign of bait aversion (Muktha Bai *et al* 1978). As vitamin D_2 is unstable in sunlight and remains effective in bait for 3 weeks only (Muktha Bai *et al* 1978), its another analogue compound, vitamin D_3 having 10 to 20 times more toxicity than vitamin D_2 was tested (Anonymous 1987).

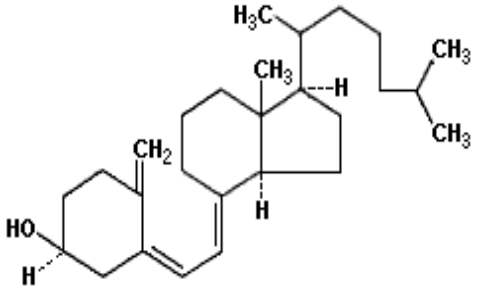
2.3.1.3.1 Use of vitamin D_3 as rodenticide

Vitamin D_3/cholecalciferol was initially registered as rodenticide (E.P.A. Reg No. 12455-39) and first used in USA in 1980's (Marshall 1984). Cholecalciferol (vitamin D_3) was developed in the 1980's as rodenticide (Marshall 1984, Tobin *et al* 1993). It was registered under the trade name of Quintox (0.075% cholecalciferol) in the USA, and in Europe it was added to baits (Racumin plus) to overcome anticoagulant resistance in rats and mice (Pospischil and Schnorbach 1994). In 1999 it was registered in New Zealand in a paste

bait containing 0.8% cholecalciferol (Feracol). The work carried out in early 1990's for evaluating the rodenticides potential of vitamin D_3 demonstrated the susceptibility of possums to cholecalciferol (Eason 1991, Eason *et al* 1996). Animal that ingests a lethal dose of cholecalciferol bait dies usually in 4-7 days (Eason *et al* 2000). Cholecalciferol baits are formulated to serve as chronic rodenticides, applied so that rats will have the opportunity to feed on the baits one or more times over the period of one to several days. After ingesting lethal amounts of vitamin D_3, house rats lose appetite and stop feeding which leads to clinical signs (Saini and Parshad 1992). Because the toxicant is slow acting, bait shyness is not reported to occur. It is claimed that rodents stop feeding once a lethal dose has been ingested. Even sex specific lethal dose observed by Kaur *et al* 2008 indicated LD_{50} of 30 mg/kg and 50 mg/kg in male and female house rats, respectively. In Norway rats, LD_{50} of vitamin D_3 is 43.6 mg/kg but there is some species variation in susceptibility among other mammals (Eason 1991, Eason 1993, Jolly *et al* 1995). In house mouse LD_{50} of vitamin D_3 is 42.5 mg/kg (Lund 1974). Possums and rabbits are sensitive to cholecalciferol as LD_{50} for possums is 16.8 mg/kg and for rabbits is 9 mg/kg (Jolly *et al* 1995, Eason *et al* 2000) but another report has indicated LD_{50} of 200 mg/kg for rabbits (Bennett 2002). Cholecalciferol has also proved effective in the US in controlling rock squirrels, gophers and gound squirrels (Beard *et al* 1988, Tobin *et al* 1993). Cats appear to be less susceptible to cholecalciferol than possums with some cats surviving dose up to 200 mg/kg while others died after doses of 50 mg/kg (Eason 1991), but dogs are reported to be more susceptible to cholecalciferol than cats (Dorman 1990). Craigmill (1988) found that a dose of 10 mg/kg had killed each of two dogs tested. Toxic doses reported for dogs ingesting bait products range from 0.5 - 20 mg/kg of cholecalciferol (Dorman 1990). But Marshall (1984) showed no sign of toxicosis in dogs eating rodent killed with bait containing 0.015% cholecalciferol and oral LD_{50} in dogs of technical cholecalciferol was found to be 88 mg/kg (Bennett 2002). The toxicity to birds is low e.g. the LD_{50} for mallard duck is 2000 mg/kg. The risk of secondary poisoning is also low (Marshall 1984). Natural dietary sources of cholecalciferol include liver, fish oils, egg yolk, and milk fat. Vitamin D_3 in toxic doses raises blood calcium levels (hypercalcaemia) and causes metastatic calcification of vital organs (Marshall 1984, Marsh and Turnberg 1986, Kaur *et al* 2008). Death usually results from heart failure. These effects are comparatively rapid when compared to anticoagulant rodenticides, animals normally take 3-7 days to die (Marshall 1984, Marsh and Turnberg 1986, Jolly *et al* 1995).

2.3.1.3.1.1 Chemistry of Vitamin D_3

Chemical name of vitamin D_3 is cholecalciferol: activated 7- dehydrocholesterol ($C_{27}H_{44}O$). It is solid resin, with molecular weight of 384.62 and melting point 84-85 ºC. It is soluble in most of the organic solvents, slightly to moderately soluble in vegetable oil and virtually insoluble in water (Deluca 1979) and its structure is:



Structure of Cholecalciferol

2.3.1.3.1.2 Mechanism of action and physiological effects of cholecalciferol toxicity

After a massive cholecalcifeol intake, excess of 25-hydroxycholecalciferol (calcifediol) is produced in the liver with the help of enzyme 25-hydroxylase. 1-25 dihydroxycholecalciferol (calcitriol, active form) is initially produced in the kidney by 1-α hydroxylase from 25-hydroxychaolecalciferol but once a set plasma concentrations of calcitriol is reached, it exerts a negative feedback on renal 1-α hydroxylase and more calcitriol is produced (Fig. 2). Because of the limited negative feedback on hepatic 25-hydroxylase, calcifediol concentrations continue to increase, and the plasma concentration of calcifediol become high enough to exert metabolic effects (Morrow 2001). Calcitriol is most metabolically active and binds to the vitamin D receptors 500 times greater than calcifediol and 100 times greater than cholecalciferol. Then active metabolities increase calcium and phosphorus concentration in plasma through three primary mechanism. First, they increase the amount of intestinal calcium-binding protein (calbindin) resulting in absorption of more calcium from intestine. Second, cholecalciferol metabolites stimulate calcium and phosphorus transfer from bones and third these metabolites decreases the calcium excretion by the kidneys. The net result is high concentration of calcium i.e. hypercalcaemia (Anonymous 1987, Scheftel *et al* 1991, Brar and Sandhu 2000, Hilbe *et al* 2000).

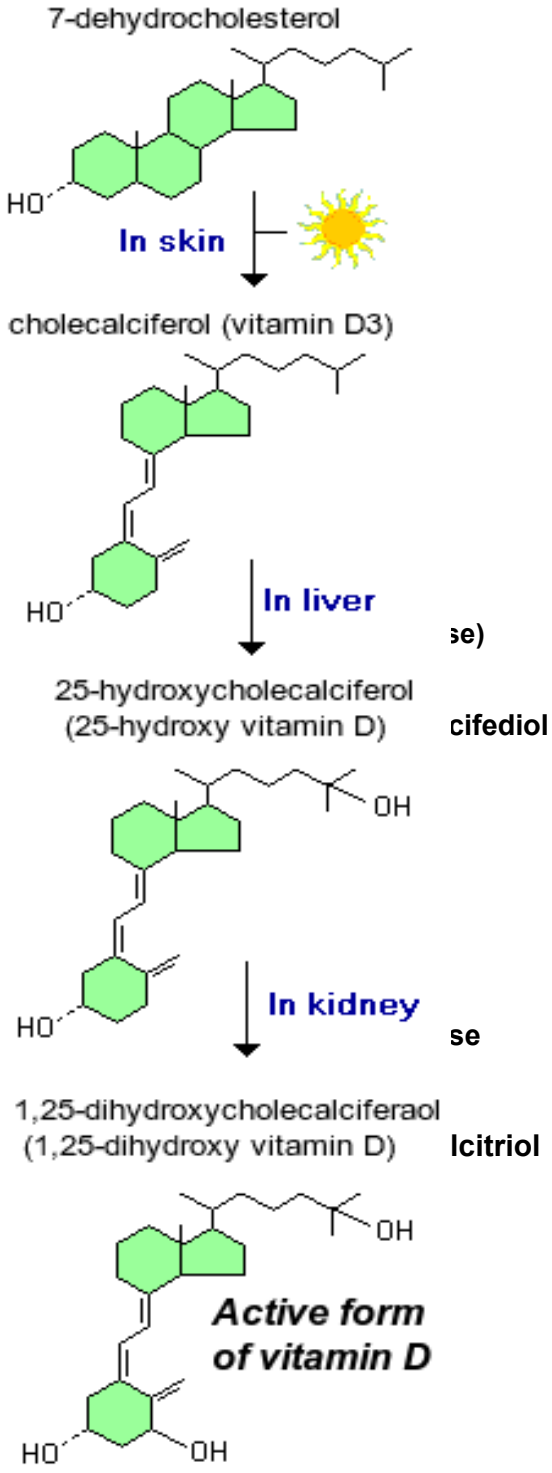


Fig. 2: Mechanism of action of cholecalciferol

Serum calcium level begins to increase about 24 hours after poisoning of cholecalciferol (Beasley *et al* 1997, Tischler *et al* 1999, Kocher *et al* 2008). Normal range of calcium in plasma/serum has been found to be 9-10.5 mg/dL or 2.2-2.6 m mol/L. At high calcium levels i.e. 12.0 mg/dL or 3 m mol/dL, symptoms are more common and severe calcium level like 15-16 mg/dL or 3.75-4 m mol/L may result in coma and cardiac arrest (Scheftel *et al* 1991). In case acute toxicosis, there is moderate rise in serum phosphorus and more severe rise in calcium concentration leading to higher level of calcium and phosphorus products (Morrow 2001, Kocher *et al* 2008). However in the control animal mean serum calcium concentrations ranged from 8.8-10 mg/dL (Tischler *et al* 1999).With an unregulated increase in plasma calcium and phosphorus, their product can rise above 60, which will cause soft tissue mineralization like kidneys, gastrointestinal tract, cardiac muscles, skeletal muscles, blood vessels and ligament and cause structural damage that leads to decreased functional capacity of these tissues and organs. The loss of function, contributes to the development of ongoing and end stage clinical signs as well as long term signs in animals that survived (Morrow 2001). The most common clinical signs associated with cholecalciferol intoxication appear within 12-36 hours after and include anorexia, dehydration, depression, muscle weakness, abdominal pain, constipation, vomiting and lethargy. As the toxicity increases additional clinical signs include hypertension, polyurea and elevated serum calcium, that may cause cardiac arythmias (Bennett 2002). Specific antidote for use in cases of accidental poisoning of vitamin D_3 is calcitonin.

2.3.1.3.1.3 Advantages of using vitamin D_3 as rodenticide

The formulations based on vitamin D_3 have good toxicological and ecological properties and the fact that no known cases of resistance make them recommendable for use against rodents in storage facilities (Vuksa *et al* 2007). There is considerable species variation in susceptibility to cholecalciferol amongst other mammals and birds. The risk of secondary poisoning is low and multiple exposures over several days would be required to cause toxicosis. Cholecalciferol is less toxic to birds than other rodenticides (Haydock and Eason 1997). However the lower toxicity to birds does not mean that they could not be poisoned, and the appropriate use of bait station would still be important. There have been few studies assessing the toxicity of cholecalciferol to invertebrates, but it appears to be low, based on one study which showd no deaths in weta (Ogilvie and Eason 1996). The risk of secondary poisoning to non-target species is also low (Marshall 1984, Eason *et al* 1996, Booth *et al* 2004). Calciferol (vitamin D), cholecalciferol (vitamin D_3) and ergocalciferol (vitamin D_2) are used as rodenticides, which are toxic to rodents for the same that they are beneficial to mammals as they are affecting calcium and phosphate homeostasis in the body. Vitamin D is essential in minute quantities. Upon single ingestion, solely calciferol based

baits are considered generally safer to birds than second generation anticoagulants or acute toxicants (Anonymous 1998). Vitamin D_3 is a safe rodenticide as it minimizes the risk of secondary poisoning and non-target species poisoning and is advantageous as it reduces the bait requirement (Saini and Parshad 1992). Therefore the use of vitamin D components viz. vitamin D_2 (calciferol/ergocalciferol) and vitamin D_3 (cholecalciferol) is a better option than other anticoagulants (Eason *et al* 2000).

2.3.1.3.1.4 Disadvantages of using vitamin D_3 as rodenticide

In family pets, accidental ingestion of vitamin D_3 is generally considered safe for cats but dangerous for dogs. Additional anticoagulant renders the bait more toxic to pets as well as humans (Anonymous 1998). Another aspect of using vitamin D_3 as rodenticides is its higher cost, as effective concentration of calciferol and cholecalciferol are more expensive that of effective concentration of most anticoagulants. Sometimes vitamin D_3 when mixed in > 0.05% concentration in bait causes bait shyness.

2.3.1.4 Synergistic/combined effect of chemicals on rodenticidal potential

In view of resistance reported to have developed towards second generation anticoagulants, the problem of bait shyness and neophobia when acute rodenticide are used and the expensive dose of vitamin D_3 like chemicals, it becomes imperative that methods be evolved to overcome these problems. Attempts to potentiate anticoagulant for effective rodent control is a new concept with very few studies. Experiments using two non-steroid anti-inflammatory drugs namely ibuprofen and phenylbutazone at 80 mg/kg and 50 mg/kg body weight respectively to potentiate the action of two second generation anticoagulants, brodifacoum and bromadiolone yielded positive results for control of *R. rattus.* The drugs reduced the lethal dose required for 100% mortality as well as days to death (Sridhara and Krishnamurthy 1992). There is an important feature of calciferols toxicology, that they are synergistic with anticoagulant toxicants, which means that mixture of anticoagulants and calciferols in same bait are more toxic than a sum of toxicities of anticoagulant and the calciferol in the bait. So that a massive hypercalcaemia effect can be achieved by substaintially lower calciferol content in bait, and vice versa. A more pronounced anticoagulant/hemorrhagic effects are observed when the calciferol was present. Synergism used in rodenticidal baits is association of an anticoagulant with a compound having vitamin D-activity i.e cholecalciferol. Typical formulas used are f.e warfarin 0.025-0.05% + cholecalciferol 0.01%. Sometimes, there are even fixed three component rodenticides, i.e anticoagulant + antibiotic + vitamin D_3 i.e. f.e difenacoum 0.005% + sulfaquinoxaline 0.02% + cholecalciferol 0.01%. Here antibiotic play a role in potentiating the rodenticidal effect (Anonymous 1998). Muktha Bai *et al* (1978) reported that when calciferol and warfarin

combined in bait resulted in increased percent mortality and reduced death time in *R. rattus* as compared to the baiting with calciferol (1000 mg/kg bait) alone or along with warfarin (50 mg/kg bait). Association of a second-generation anticoagulant with an antibiotic and/or vitamin D are considered to be effective even against most resistant strains of rodents, though some second generation anticoagulants (namely brodifacoum and difethialone), in bait concentration of 0.0025% - 0.005% are so toxic, that no known resistant strains of rodents against other derivatives are reliably exterminated by application of these most toxic anticoagulant (Anonymous 1998). Combination of anticoagulants with different types of vitamin D are generally described to increase the efficacy of action against rodents (Hadler 1973). Synergistic effect between warfarin and vitamin D_3 have also been apparently observed in oral acute toxicities to rats (Tanikawa and Kusano 1993). It was found in laboratory that, in particular, the combination of coumatetralyl and vitamin D_3 could overcome the inefficacy of anticoagulants caused by resistance or vitamin K containing food (Fuhrmann 1991, Lund 1991, Schnorbach 1992). The number of combinations of 0.02 to 0.04% coumatetralyl and 0.01 to 0.1% vitamin D_3 have been tested. In combination with vitamin D_3, the amount of coumatetralyl should not be less than 0.04%, but the percentage of vitamin D_3 can be diminshed to 0.025 or even 0.01% to avoid bait shyness (Prescott *et al* 1992). The described combination of the multiple dose anticoagulant (coumatetralyl) with the hypercalcaemia causing agent (vitamin D_3) in an attractive bait formulation is a good agent for modern rodent control even under food storage conditions. Different newer anticoagulant combinations viz, anticoagulant + calciferol (vitamin D_2), anticoagulant and Ratak (difenacoum) have been used for control of black rat, *R. rattus*. All anticoagulants tested, were found to be effective, because cent percent mortality in rats obtained with each compound (Arora *et al* 1982).

2.3.1.4.1 Advantages of using combined formulations

The combination of anticoagulant with different types of vitamin D are generally described to increase the efficacy of action against rodents and also to overcome the problem of increased resistance towards anticoagulants. The combination of these active ingredients leads to an obvious increase in efficacy against rodents, even under difficult conditions (Pospischil and Schnorbach 1994) and the cost of rodent control operation is also reduced.

CHAPTER - III

MATERIALS AND METHODS

The present study entitled "Study on combined effect of bromadiolone and cholecalciferol (vitamin D_3) against house rat, *Rattus rattus* Linnaeus", was conducted in Rodent Research Laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana.

3.1 MATERIALS

3.1.1 Collection of house rats

House rats were trapped live (both male and female) from poultry farms, grocery shops, store houses and godowns of Ludhiana city (Latitude 30°56'N, Longitude 75°52'E) with single and multicatch rat traps.

3.1.2 Maintenance of rats

The mature house rats, both male and female (having body weight >100 g) were maintained individually in laboratory cages. These rats were acclimatized for 15 days by providing them plain WSO-mix bait, prepared by mixing of cracked wheat, sugar powder and groundnut oil in the ratio of 96:2:2 and water *ad libitum* before starting any experiment. Food and water were replenished daily. Metallic trays were kept under each cage to collect the faeces and spilled food and were cleaned daily. Approval of Institutional Animal Ethics committee, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana was obtained for the usage of animals.

3.1.3 Chemicals used as rodenticide

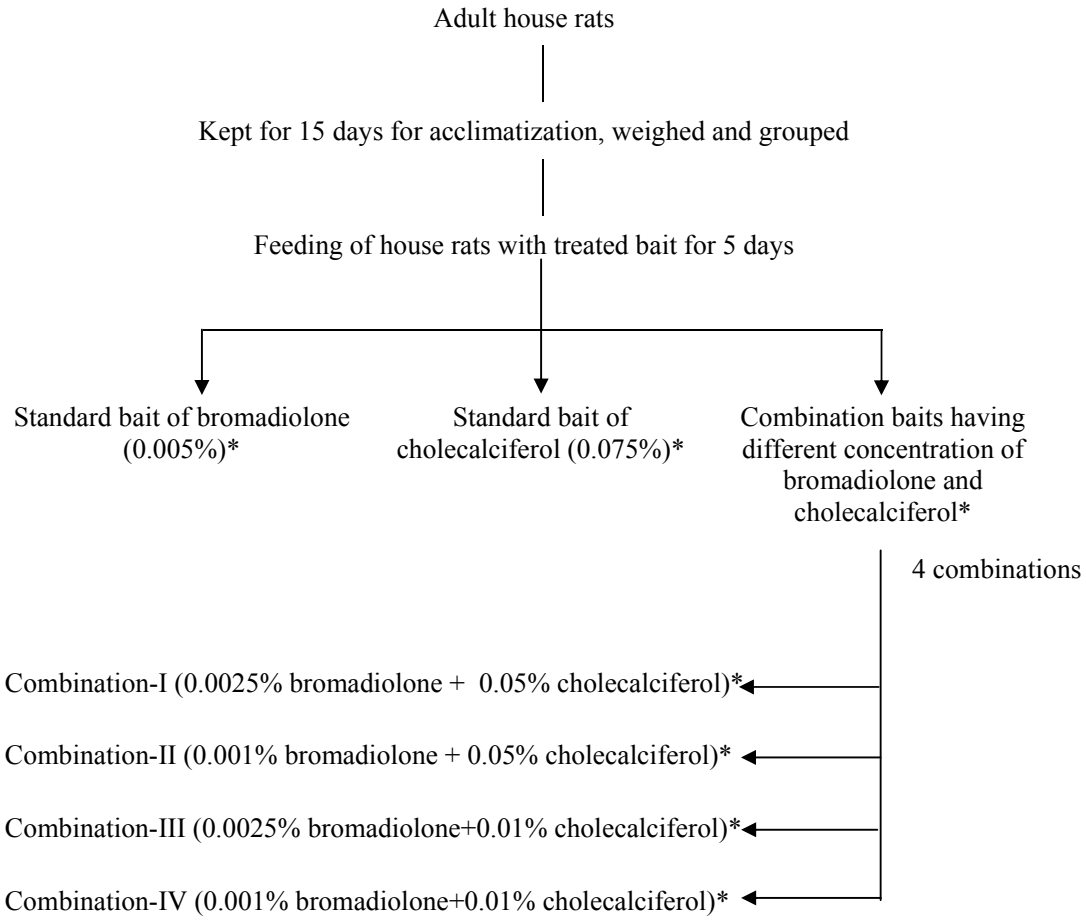
Two chemicals used as rodenticide during the present study were bromadiolone and cholecalciferol (vitamin D_3). Both these chemicals were purchased from standard sources i.e. bromadiolone from Pest Control (India) Pvt. Ltd., and cholecalciferol from MP Biochemicals, Inc.

3.2 METHODS

The methodology followed has been presented schematically in flowchart-I.

3.2.1 Determination of rodenticidal potential of standard bait of bromadiolone (0.005%)

Already acclimatized adult and healthy house rats were selected, weighed and grouped in two sets, set I and set II (having 3 male and 3 female rats in each set). The rats in set I (treated set) were fed on the standard concentration of bromadiolone bait i.e. 0.005%. The rats in set-II (untreated set) were fed on plain WSO mix bait.



Flowchart-I: Schematic representation of the methodology followed.

* indicates that untreated set (house rats fed on plain WSO-mix bait) was also run simultaneously with each experiment.

3 male and 3 female house rats were used in each experiment (both for treated and untreated sets).

3.2.1.1 Preparation of standard bait of bromadiolone (0.005%)

This bait was prepared by mixing of 20 g of bromadiolone powder (0.25%pure) in 1 kg of plain WSO-mix bait. Bait was mixed properly with spoon or hands wearing gloves. Bait was put into transparent polythene bag and tied.

3.2.1.2 Feeding of house rats with standard bait of bromadiolone (0.005%)

House rats of treated set were fed on plain WSO-mix bait for five consecutive days. Then after this pre-treatment, rats were fed on standard bait of bromadiolone (0.005%) in no choice for five consecutive days. After treatment i.e. during post-treatment period survived rats were kept on feeding with plain WSO-bait for five consecutive days. Simultaneously rats of untreated set were also fed on plain WSO-mix bait.

3.2.1.3 Observations recorded

The following observations were recorded

3.2.1.3.1 Daily bait consumption

Rats of each set were provided with 20 g of plain WSO-mix bait during pre-treatment and post-treatment period and 20 g of 0.005% bromadiolone bait during treatment period daily. The left over bait on next day was weighed and again replenished with 20 g of bait. The daily bait consumption (g/100 g body weight) was calculated by the following formula:

$$\text{Daily bait consumption (g/100 g body weight)} = \frac{\text{Daily consumption of bait by rat (g)}}{\text{Weight of rat (g)}} \times 100$$

3.2.1.3.2 Percent acceptance/palatability of treated bait

Percent acceptance/palatability of treated bait over plain bait in no-choice feeding test was determined as per the formula given by Johnson and Prescott (1994) as given below:

$$\text{Acceptance of treated bait (\%)} = \frac{\text{Consumption of treated bait during treatment period}}{\text{Consumption of plain bait during pre-treatment period}} \times 100$$

3.2.1.3.3 Mortality rate

The mortality data was calculated by recording the death of 0.005% bromadiolone treated rats every day and percentage mortality was determined by following formula:

$$\text{Mortality (\%)} = \frac{\text{Number of rats died}}{\text{Total number of rats fed on treated bait}} \times 100$$

3.2.1.3.4 Determination of blood clotting time

Clotting time of 0.005% bromadiolone treated rats was recorded by Sabraze's capillary tube method. Tail region of mildly anaesthetized rat was punctured. First two drops of blood were discarded. Capillary tube (8 cm in length with diameter 0.8 to 1.2 cm) was held horizontally in the blood drop to rapidly allow the blood to run into non-heparinized capillary tube. Capillary tube was broken off about 1 cm length of tubing after every 30 seconds and recorded the coagulation time as the interval from the time the blood appeared on the skin of rat until a fibrin thread bridged the broken ends of the capillary tube. Blood clotting time was recorded at 0 hour (before treatment), after 48 hours and 72 hours of feeding with 0.005%, bromadiolone bait.

3.2.2 Determination of rodenticidal potential of standard bait of cholecalciferol/ vitamin D_3 (0.075%)

Already acclimatized adult and healthy house rats were selected, weighed and grouped in two sets, set I and set II (having 3 male and 3 female rats in each set). The rats in set I (treated set) were fed on the standard concentration of cholecalciferol i.e. 0.075%. The rats in set II (untreated set) were fed on plain WSO-mix bait.

3.2.2.1 Preparation of standard bait of vitamin D_3/cholecalciferol (0.075%)

This bait was prepared by mixing of 750 mg of cholecalciferol (99% pure) in 1 kg of plain WSO-mix bait. Bait was mixed properly with spoon or hands wearing gloves. Bait was put into transparent polythene bag and tied.

3.2.2.2 Feeding of house rats with standard bait of cholecalciferol (0.075%)

The same methodology was followed as mentioned in 3.2.1.2, except that during treatment period feeding of rats was done with standard bait of cholecalciferol (0.075%).

3.2.2.3 Observations recorded

The following observations were recorded:

3.2.2.3.1 Daily bait consumption

Daily bait consumption was recorded as already mentioned in 3.2.1.3.1

3.2.2.3.2 Percent acceptance/palatability of treated bait

Percent acceptance of treated bait over plain bait was already calculated as mentioned in 3.2.1.3.2

3.2.2.3.3 Stop feed action

The observation of daily consumption of treated bait by rats was used for determining another parameter known as stop feeding action i.e. the day when rats stop feeding of cholecalciferol bait during treatment period.

3.2.2.3.4 Mortality rate

The mortality data was recorded by the method as mentioned in 3.2.1.3.3

3.2.2.3.5 Serum calcium level

Blood was collected from the tail region of mildly anaesthetized treated house rat (fed on cholecalciferol and its various combinations with bromadiolone) and untreated rats. The collected blood was kept tilted for 24 hours at 4°C and centrifuged for 15 minutes at 1750 rpm to collect serum. Serum was collected with help of dropper by puncturing the blood clot with help of needle from the sides. Calcium level was measured spectrophotometerically by Cresolphthalein complex one method (Henry and Dryer 1963). Bayer Diagnostic autopak reagent kit was used for qualitative determination of calcium in serum of control and treated rats.

Reagents used

i. Reagent 1 (Cresolphthalein complex one) :

Dimethyl sulfoxide	:	1.4 mol/L
8-Hydroxy quinolin	:	17 m mol/L
Cresolphthalein complex one	:	0.06 m mol/L

ii. Reagent 2 (Buffer)

Potassium cyanide	:	7.6 m mol/L
Diethylamide	:	0.38 mol/L

iii. 0.9% normal saline solution

0.9 gm of NaCl dissolved in 100 ml of distilled water.

iv. Standard calcium (10 mg/dL)

Calcium	:	0.1 g/L

Preparation of working solution

Working solution was prepared fresh according to need by mixing equal volumes of reagent 1 and reagent 2 in clean beaker.

Procedure

In 1 ml of working solution, 10 µl of serum sample and 1 ml of 0.9% saline solution were added and incubated for 5 min at 30°C. After incubation the colour was measured at

575 nm by using the reagent blank (1 ml of working solution + 1 ml of 0.9% saline solution). Standard of calcium (0.2 – 1.2 µg) was run alongside and standard curve was plotted (Fig. 3). Blood serum was collected at 0 hour (before treatment), 48 hours and 72 hours after feeding of cholecalciferol treated bait and calcium level was determined.

3.2.3 Determination of rodenticidal potential of combination baits having bromadiolone and cholecalciferol in different concentrations

Already acclimatized adult and healthy house rats were selected, weighed and divided into 4 groups (having 3 male and 3 female rat each) and were exposed to four combinations of bromadiolone and cholecalciferol (vitamin D_3).

Group I : Combination-I (0.0025% bromadiolone and 0.05% cholecalciferol)

Group II : Combination-II (0.001% bromadiolone and 0.05% cholecalciferol)

Group III : Combination-III (0.0025% bromadiolone and 0.01% cholecalciferol)

Group IV : Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)

3.2.1.3.1 Preparation of combination baits

Different combinations, *viz.* combination I, II, III & IV were prepared as mentioned in table 3.

Table 1: Preparation of combination baits having bromadiolone and cholecalciferol at their different concentrations

Combinations	Prepared by mixing of		
	WSO-mix bait	Bromadiolone	Cholecalciferol
Combination-I (0.0025%bromadiolone+ 0.05% cholealciferol)	1 kg	10 g	500 mg
Combination-II (0.001%bromadiolone + 0.05% cholealciferol)	1 kg	4 g	500 mg
Combination-III (0.0025%bromadiolone+ 0.01% cholealciferol)	1 kg	10 g	100 mg
Combination-IV (0.001%bromadiolone + 0.01% cholealciferol)	1 kg	4 g	100 mg

Simultaneously, control set (having 3 male and 3 female rats) was also run separately with each concentration.

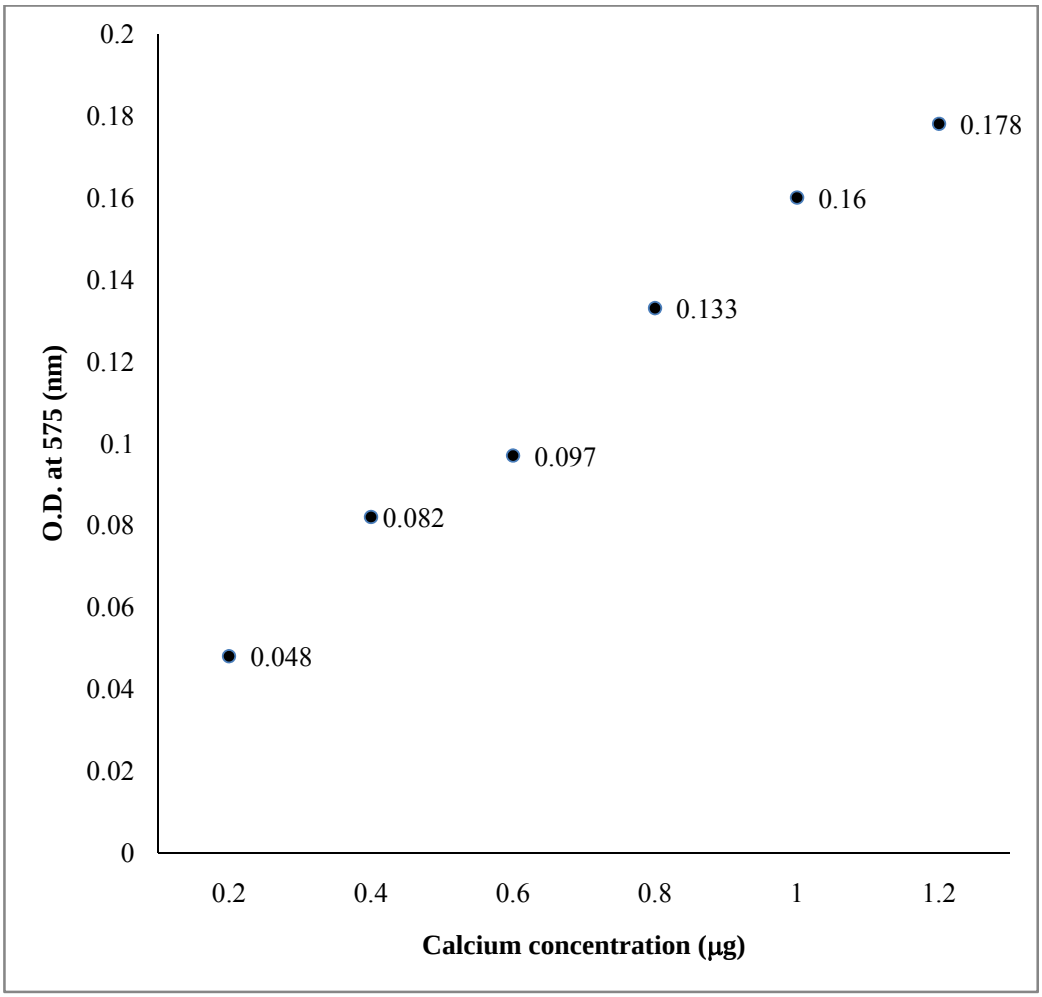


Fig. 3: Standard curve for calcium

3.2.1.3.2 Feeding of house rats with different combinations of bromadiolone and cholecalciferol

The same methodology was followed as mentioned in 3.2.1.1.2, except that different combinations of bromadiolone and cholecalciferol bait were used for feeding of rats during treatment.

3.2.1.3.3 Observation recorded

Daily bait consumption (as mentioned in 3.2.1.3.1), acceptance of treated bait over plain bait (as mentioned in 3.2.1.3.2), mortality rate (as mentioned in 3.2.1.3.3), blood clotting time (as mentioned in 3.2.1.3.4), stop feeding action (as mentioned in 3.2.2.3.3) and serum calcium level (as mentioned in 3.2.2.3.5) were recorded.

3.2.4 Testing of resistance of house rats towards standard bait of bromadiolone (0.005%)

Already acclimatized adult and healthy house rats were selected, weighed and grouped in 20 sets, 10 sets of male and 10 sets of female (having 3 rats in each set). These sets were fed on standard bait of bromadiolone i.e. 0.005%. Before treatment, house rats were fed on plain WSO-mix bait for five consecutive days. Then after pre-treatment, rats were fed on standard bait of bromadiolone (0.005%) in no-choice for five consecutive days. After treatment i:e during post-treatment, survived rats were kept on feeding with plain WSO-bait for five consecutive days (if same rats survived after feeding of bromadiolone bait, then their testing on the best combination bait formulated will be tested for the efficacy of that formulated bait against the bromadiolone resistant rats).

3.2.4.1 Observations recorded

3.2.4.1.1 Daily bait consumption

Daily bait consumption was recorded as already mentioned in 3.2.1.3.1

3.2.4.1.2 Mortality rate of house rat

The mortality data was recorded by the method as mentioned in 3.2.1.3.3

3.2.5 Statistical analysis

Significance of difference between two mean values were determined using student's t-test, significance of difference between two mean values within the same group were determined using 'paired t-test' and significance of differences among mean values of different treatments were determined using one way 'ANOVA' (Singh *et al* 2004).

CHAPTER-1V

RESULTS AND DISCUSSION

4.1 Rodenticidal potential of standard bait of bromadiolone (0.005%) against *R. rattus*

4.1.1 Acceptance and efficacy of standard bait of bromadiolone (0.005%)

Feeding of male house rats on standard bait of bromadiolone (0.005%) in no-choice for 5 days under laboratory conditions, resulted in 81.50 ± 0.97% acceptance of this bait over plain bait consumed during pre-treatment period (n=5 days). Similarly, female house rats when were fed on 0.005% bromadiolone bait in no-choice for 5 days, they showed 95.80 ± 3.19% acceptance of this bait over plain bait consumed during pre-treatment period. This acceptance in the form of average daily consumption (g/100g bw) of treated bait was non-significantly different from that of plain bait during pre-treatment period, showed good acceptability of standard bait of bromadiolone. Also this bait was found to be effective as it resulted in 100% mortality of both male and female house rats. In case of males, rats started dying on the 3rd day of treatment and all the rats died till 6th day after consuming the 0.005% bromadiolone bait and in females all the rats died on 4th day of treatment (Table 2). Anticoagulant rodenticidal potential of bromadiolone have chronic toxicity and rodents are capable of withstanding these rodenticides generally for 48 hours after ingestion and with increased intake of their dose, delayed mortality occurs (Revathi and Yogananda 2006). Standard bait of bromadiolone (0.005%) has been found to cause mortality of commensal rodents upto 100% by different workers with in a period of 2-15 days (Taley *et al* 1987, Dubey *et al* 1991, Renapurkar 1993, Kocher and Parshad 2003, Revathi and Yogananda 2006, Chaudhaury and Tripathi 2009) However, untreated groups of rats (both male and female) showed non-significant difference in the average daily consumption (g/100g bw) of the bait throughout the feeding trials (Table 2).

4.1.2 Blood clotting time of 0.005% bromadiolone fed house rats

Blood clotting time (sec) was recorded in both male and female house rats at 0 hour (before treatment), 48 hours and 72 hours after feeding of 0.005% bromadiolone bait. Results clearly indicated significant increase i.e. 118.3 ± 1.86 sec in the clotting of blood after 48 hours of feeding as compared to that of 0 hour in male house rats, where the clotting time was found to be 71.6 ± 1.00 sec and this delay in blood clotting time was further increased upto 142.5 ± 0.63 sec after 72 hours of feeding of treated bait. Similar results were obtained in female house rats, where significant increase in blood clotting time i.e. 100.0 ± 3.08 sec and 116.6 ± 1.90 sec was recorded at 48 hours and 72 hours, respectively after feeding of standard bromadiolone bait as compared to the blood clotting time, 68.3 ± 1.02 sec observed at 0 hour of treatment. Anticoagulant toxicants interfere with the normal synthesis of

Table 2: Acceptance and efficacy of standard bait of bromadiolone (0.005%) against *Rattus rattus* in no-choice feeding trial.

Sex	Set	Boyd weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Acceptance of treated bait over plain bait (%)	Mortality	
			Pre-treatment	During treatment	T value at 5% level		Percent	Range (days)
Male	Treated	168.30±1.59	5.12±0.50	4.07±0.24^{NS}	2.26	81.50±0.97	100	3 – 6
	Untreated	138.30±2.24	7.03±0.66	6.50±0.97^{NS}	1.37	-	Nil	Nil
Female	Treated	126.60±1.47	5.51±0.038	5.30±0.75^{NS}	0.97	95.80±3.19	100	4
	Untreated	125.00±0.73	6.90±0.48	5.98±0.50^{NS}	1.40	-	Nil	Nil

- All values are Mean±S.E.
- T value at 5% level of significance is 2.92
- NS represents non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within the same group of rats.

vitamin-K dependent clotting factors in the liver and also inhibits the formation of prothrombin, a key protein required for formation of blood clott, this results in an increase in blood clotting time leading to internal bleeding and eventually to death (Anonymous 2007, Revathi and Yogananda 2008) A non-significant difference in the blood clotting time of both male and female house rats of untreated groups was observed starting from 0 to 72 hours of feeding on plain bait (Table 3).

4.2 Rodenticidal potential of standard bait of cholecalciferol (0.075%) against *R.rattus*

4.2.1 Acceptance/palatability of standard bait of cholecalciferol (0.075%)

Feeding of standard bait of cholecalciferol (0.075%) by male house rats for 5 days resulted in significantly less average daily consumption (g/100g bw) i.e. 3.66 ± 0.88 as compared to that of plain bait during pre-treatment period (8.91 ± 0.83), which resulted in 40.10 ± 1.55 % acceptance of cholecalciferol treated bait over plain bait. Similarly, a significantly less average daily consumption (g/100g bw) of 0.075% cholecalciferol bait i.e. 2.70 ± 0.61 was found in female rats as compared to that of plain bait during pre-treatment period (8.06 ± 1.38). This resulted in 35.30 ± 0.64% acceptance of cholecalciferol treated bait over plain bait by females. Though the acceptance of 0.075% cholecalciferol by male and female house rats was significantly less but whatever amount of cholecalciferol was ingested by them was able to cause their 100% mortality. Delayed mortality of house rats was observed, as male and female rats started dying respectively on 4^{th} and 7^{th} day and all died till the 10^{th} and 14^{th} day after feeding of treated bait. Rodents generally take a lethal dose of cholecalciferol (sub-acute poisons) during first 24 hours like acute rodenticides and repeated feeding may occur causing delayed mortality of rats like anticoagulants (Buckle and Muller 2000). Different formulations of cholecalciferol i.e. cake, pellets and bait have also been used for effective killing of rodents and delayed effects have been reported (Kamath *et al* 1986, Eason *et al* 2002, Gould and Holmes 2008). However, untreated group of rats (both male and female) showed non-significant difference in the average daily consumption (g/100g bw) of the bait (Table 4).

4.2.2 Stop feed action of cholecalciferol

Male house rats when fed on standard bait of cholecalciferol (0.075%) showed non-significant difference in average daily consumption (g/100g bw) of treated bait as compared to that of plain bait during pre-treatment period for the first two days. However, the consumption of 0.075% cholecalciferol bait was significantly reduced on 3^{rd} day (1.60 ± 0.59 g/100g bw) as compared to average daily consumption of plain bait during pre-treatment i.e.

Table 3: Blood clotting time (sec) of *Rattus rattus* after feeding of standard of bromadiolone (0.005%) in no-choice feeding trial.

Sex	Set	Blood clotting time (sec)			T value at 5% level
		0 hour	48 hours	72 hours	
Male	Treated	71.6±1.00	118.3±1.86^{S}	142.5±0.63	7.58
	Untreated	70.0±0.84	80.0±1.58^{NS}	71.6±1.00	1.53
Female	Treated	68.3±1.02	100.0±3.08^{S}	116.6±1.90	2.97
	Untreated	70.0±0.84	65.0±0.87^{NS}	65.0±0.87	1.81

- All values are Mean±S.E.
- T value at 5% level of significance is 2.92
- NS represents the non-significant difference in blood clotting time at 48 hours with respect to 0 hour within the same group of house rats.
- S represents the significant difference in blood clotting time at 48 hours with respect to 0 hour within the same group of house rats.

Table 4: Acceptance and efficacy of standard bait of cholecalciferol (0.075%) against *Rattus rattus* in no-choice feeding trial.

Sex	Set	Body weight (g)	Average daily consumption (g /100g body weight), n=5 days			Acceptance of treated bait over plain bait (%)	Mortality	
			Pre-treatment	During treatment	T value at 5% level		Percent	Range (days)
Male	Treated	121.10±0.85	8.91±0.83	3.66±0.88^{S}	7.50	40.10±1.55	100	4 – 10
	Untreated	138.30±2.24	7.03±0.66	6.50±0.97^{NS}	1.37	-	Nil	Nil
Female	Treated	128.30±1.10	8.06±1.38	2.70±0.61^{S}	3.87	35.30±0.64	100	7 – 14
	Untreated	125.00±0.73	6.90±0.48	5.98±0.50^{NS}	1.40	-	Nil	Nil

- All values are Mean±S.E.
- T value at 5% level is 2.92
- NS represents the non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.
- S represents the significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.

8.91 ± 0.83 g/100g bw. This acceptance of bait was further reduced on 4th day and 5th day of treatment (Table 5, Fig. 4a). Similarly, female house rats showed non-significant difference in average daily consumption (g/100g bw) of treated bait for 1st and 2nd day as compared to that of plain bait and this consumption was significantly reduced on 3rd day (1.13 ± 1.02g/100g bw) onward as compared to average daily consumption of plain bait during pre-treatment i.e. 8.06 ± 1.38g/100g bw (Table 5, Fig. 4b). However, in untreated group of rats (both male and female) showed non-significant difference in the average daily consumption (g/100g bw) of the bait (Table 5). Such a data represented the stop feeding behavior of house rats against cholecalciferol bait i.e. after consuming lethal dose of cholecalciferol, rats stopped further feeding of its bait. Though rats stopped accepting any more bait, but 100% mortality (both in male and female) results indicated the ingestion of its lethal dose. As cholecalciferol toxicant is slow acting, no bait shyness has been reported to occur. But rodents show stop feeding behavior as after ingesting lethal amounts of vitamin D_3, house rats lose appetite and stop feeding, which leads to clinical signs (Saini and Parshad 1992). This stop feed action is commonly claimed as a benefit for such compounds like vitamin D_3, as it prevents further baiting of such expensive chemicals (Buckle *et al* 1985).

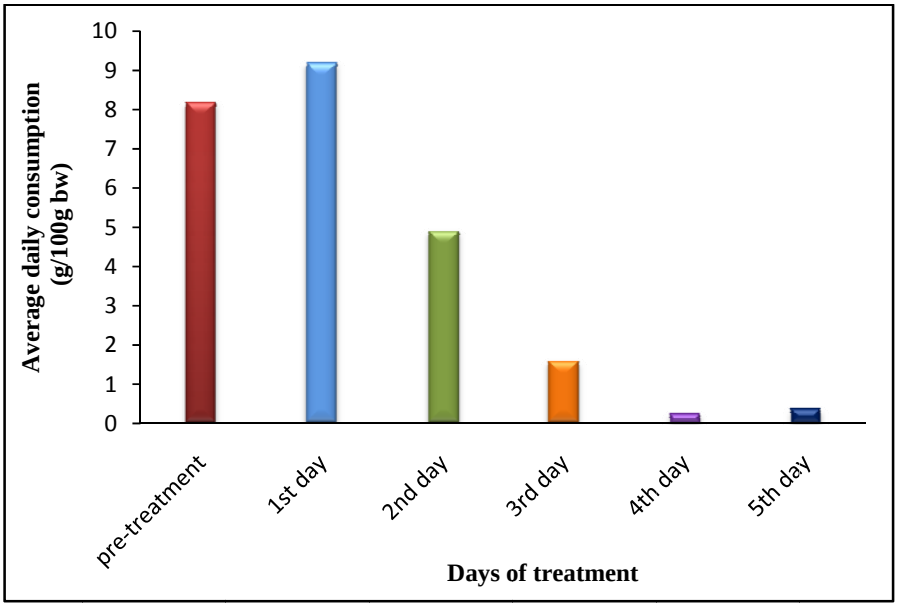
4.2.3 Serum calcium level of cholecalciferol (0.075% bait) fed house rats

Male house rats fed on standard bait of cholecalciferol in no-choice resulted in significantly higher value of calcium concentration in the serum i.e. 16.10 ± 0.36 and 15.73 ± 0.18 mg/dL, respectively after 48 hours and 72 hours of feeding as compared to that of 0 hour i.e. before treatment (7.50 ± 0.26 mg/dL). Similar type of results were also found in case of female house rats, where serum calcium level was found to be significantly high i.e. 13.09 ± 0.43 and 15.40 ± 0.27 mg/dL, respectively after 48 hours and 72 hours of feeding as compared to that of 0 hour serum calcium level (9.88 ± 0.50 mg/dL) as mentioned in Table 6. Serum calcium level (mg/dL) was found to be non-significantly different at 0 hour, 48 hours and 72 hours in both male and female rats of untreated sets. Various reports have shown that concentration of calcium in serum begins to increase about 24 hours after poisoning of cholecalciferol (Tischler *et al* 1999, Kocher *et al* 2008). This unregulated increase in serum calcium level (hypercalcaemia), cause metabolic calcification of vital organs like kidneys, gastrointestinal tract, cardiac muscles and lead to their severe functional disturbances and histopathological signs and death of animals (Jolly *et al* 1995, Morrow 2001, Kocher *et al* 2010).

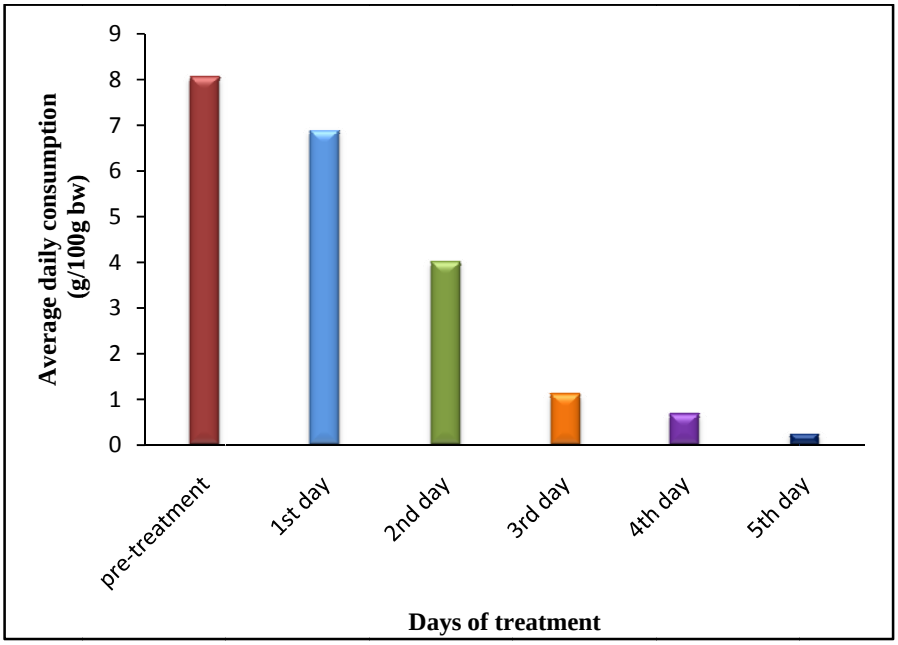
Table 5: Stop feeding action of standard bait of cholecalciferol (0.075%) against *Rattus rattus* in no-choice feeding trial.

Sex	Set	Body weight (g)	Average daily consumption (g /100g body weight)						CD at 5% level
			Pre-treatment (n=5 days)	During treatment					
				1st day	2nd day	3rd day	4th day	5th day	
Male	Treated	121.10±0.85	8.91±0.83	9.18±1.92^{NS}	4.99±0.44^{NS}	1.60±0.59^{S}	0.24±0.71^{S}	0.37±0.61^{S}	5.71
	Untreated	138.30±2.24	7.03±1.75	7.23±1.10	8.43±2.16	7.53±1.6	4.80±0.60	4.56±0.48	NS
Female	Treated	128.30±1.10	8.06±1.38	6.90±0.93^{NS}	4.08±0.28^{NS}	1.13±1.02^{S}	0.68±0.68^{S}	0.26±0.73^{S}	4.27
	Untreated	125.00±0.73	6.93±0.48	7.32±1.04	6.23±0.96	5.29±0.24	5.53±0.24	5.40±0.18	NS

- All values are Mean ± S.E.
- NS represents the non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.
- S represents the significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.



(a)



(b)

Fig. 4: Stop feed action of standard bait of cholecalciferol (0.075%) against *Rattus rattus* in no-choice feeding trial

(a) Male and (b) female house rats

Table 6: Calcium concentration (mg/dL) in the serum of *Rattus rattus* fed on standard bait of cholecalciferol (0.075%) in no-choice feeding trial.

Sex	Set	Blood calcium level(mg/dL)			T value at 5% Level
		0 hour	48 hours	72 hours	
Male	Treated	7.50±0.26	16.10±0.36^{S}	15.73±0.18	8.49
	Untreated	7.70±0.22	8.96±0.18^{NS}	7.24±0.26	2.56
Female	Treated	9.88±0.50	13.09±0.43^{S}	15.40±0.27	16.60
	Untreated	7.50±0.33	8.83±0.12^{NS}	7.43±0.16	2.44

- All values are Mean±S.E.
- T value at 5% level of significance is 2.92
- NS represents the non-significant difference in blood calcium level at 48 hours with respect to 0 hour within the same group of house rats.
- S represents the significant difference in blood calcium level at 48 hours with respect to 0 hour within the same group of house rats.

4.3 Rodenticidal potential of combination of bromadiolone and cholecalciferol at their different concentrations

Both male and female house rats were exposed to four different combinations of bromadiolone and cholecalciferol *viz.* combination-I (having 0.0025% bromadiolone and 0.05% cholecalciferol), combination-II (having 0.001% bromadiolone and 0.05% cholecalciferol), combination-III (having 0.0025% bromadiolone and 0.01% cholecalciferol), combination-IV (having 0.001% bromadiolone and 0.01% cholecalciferol). All these 4 combinations/formulated baits were having concentration of bromadiolone and cholecalciferol lesser than that in their respective standard baits. Results obtained after feeding of above mentioned 4 combinations to house rats for 5 days in no-choice are discussed below under the following parameters.

4.3.1 Acceptance and efficacy of formulated baits

When male house rats were fed on different combinations of bromadiolone and cholecalciferol (combination-I, combination-II, combination-III and combination-IV) in no-choice, rats in all the combinations showed significantly less average daily consumption of treated bait with respect to that of plain bait during pre-treatment period. The percent acceptance of treated bait over plain bait was found to be 47.90 ± 3.65, 52.20 ± 0.29, 59.20 ± 2.53, 35.10 ± 2.23, respectively after feeding of combinations I, II, III and IV. Hundred percent mortality was observed after feeding of all these four combinations and mortality ranged from 3-5 days during combination-I and 3-4 days in rest of combinations i.e. combinations II, III and IV (Table 7). Feeding of different combinations of bromadiolone and cholecalciferol in female house rats resulted in significantly less consumption of treated bait having combination I, II and IV respectively with an percent acceptance of 55.80 ± 1.73, 71.30 ± 0.72, and 47.90 ± 2.28 over plain bait. However, the combination-III was highly accepted i.e. 86.80 ± 1.88% over plain bait. Mortality was found to be 100% in all the feeding trials which ranged from 3-5 days during feeding of combinations I and IV and 3-4 days during combinations II and III (Table 8). Feeding of *R. rattus* on formulated bait having calciferol and warfarin resulted in complete kill at lower dosages of calciferol in the combination as compared to either calciferol or warfarin alone (Mukhta Bai *et al* 1978). Also 100% killing of rats and mice has been observed after feeding them on the bait having coumatetralyl (anticoagulant) with cholecalciferol in the formulation with optimal rodenticidal efficacy having their lower combination i.e. 0.04% coumatetralyl and 0.025% cholecalciferol (Pospischil and Schnorbach 1994).

Table 7: Acceptance and efficacy of different combinations of bromadiolone and cholecalciferol mixed in bait against male *Rattus rattus* in no-choice feeding trial.

Feeding Trial	Set	Body weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Acceptance of treated bait over plain bait (%)	Mortality	
			Pre-treatment	During treatment	T value at 5% level		Percent	Range (days)
Combination-I	Treated	141.66±2.91	11.81±0.70	5.61±1.20^{S}	4.49	47.90±3.65	100	3 – 5
	Untreated	118.33±1.32	10.36±0.32	10.10±0.24^{NS}	1.05	-	Nil	Nil
Combination -II	Treated	140.0±2.10	10.63±0.42	5.56±0.32^{S}	14.9	52.20±0.29	100	3 – 4
	Untreated	135.0±1.86	10.90±0.40	9.45±0.34^{NS}	2.04	-	Nil	Nil
Combination -III	Treated	156.6±0.13	5.04±0.13	3.03±0.61^{S}	4.80	59.20±2.53	100	3 – 4
	Untreated	125.0±0.73	9.04±0.28	7.25±0.40^{NS}	2.08	-	Nil	Nil
Combination -IV	Treated	146.6±2.03	9.75±0.55	3.30±0.68^{S}	5.91	35.10±2.23	100	3– 4
	Untreated	135.0±1.86	9.99±0.52	7.53±0.30^{NS}	1.64	-	Nil	Nil

- All values are Mean±S.E.
- Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- T value at 5% level is 2.92
- NS represents the non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.
- S represents the significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats

Table 8: Acceptance and efficacy of different combinations of bromadiolone and cholecalciferol mixed in bait against female *Rattus rattus* in no-choice feeding trial.

Feeding Trial	Set	Body weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Acceptance of treated bait over plain bait (%)	Mortality	
			Pre-treatment	During treatment	T value at 5% level		Percent	Range (days)
Combination-I	Treated	140.00±0.91	11.62±0.66	6.15±0.11^{S}	7.04	55.80±1.73	100	3 – 5
	Untreated	116.60±0.57	6.40±0.17	8.75±0.18^{NS}	2.28	-	Nil	Nil
Combination-II	Treated	121.0±1.09	12.20±0.68	8.63±1.06^{S}	5.61	71.30±0.72	100	3 – 4
	Untreated	148.3±0.77	9.56±0.68	9.64±0.59^{NS}	0.88	-	Nil	Nil
Combination-III	Treated	151.6±1.67	6.15±0.11	5.30±0.34^{NS}	1.93	86.80±1.88	100	3 – 4
	Untreated	141.6±1.58	5.24±0.67	5.88±0.49^{NS}	2.84	-	Nil	Nil
Combination-IV	Treated	140.3±1.21	9.05±0.95	4.04±0.63^{S}	4.43	47.90±2.28	100	3 – 5
	Untreated	135.0±1.05	8.86±0.60	9.05±0.50^{NS}	1.10	-	Nil	Nil

- All values are Mean±S.E.
- Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- T value at 5% level is 2.92
- NS represents the non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.
- S represents the significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.

4.3.2 Stop feed action of formulated baits by house rats

Due to the presence of cholecalciferol mixed in all these above said four formulations along with bromadiolone, house rats (both males and females) fed on these formulations showed the phenomenon of stop feeding (Tables 9 and 10, Figs. 5 and 6), similar to that observed during feeding of standard bait of cholecalciferol (Table 7, Fig 4). During the feeding of combination-I, all male house rats showed non-significant difference in consumption of treated bait for the first day only as compared to that in plain bait during pre-treatment. After that rats started consuming lesser and lesser amount of treated bait of all the formulations (Table 9, Fig 5). A direct co-relation between the concentration of rodenticides (bromadiolone and cholecalciferol) and daily consumption of treated bait indicated that combination-IV having lowest concentration (0.001% bromadiolone and 0.01% cholecalciferol) showed the minimum consumption of bait i.e. 0.85 ± 0.92 on the 3rd day (Table 9, Fig. 5d). From the 2nd day onward rat fed on all the 4 combinations showed higher rejection of treated bait as the its consumption found to be significantly low as compared to the plain bait (Table 9, Fig. 5). When female house rats were fed on combination-I, II and III, they showed non-significant difference in average daily consumption of these formulated baits on first and second day as compared to that of plain bait consumption during pre-treatment period and this consumption was significantly reduced on 3rd and 4th day of treatment (Table 10, Fig. 6). However, feeding of the combination-IV bait led to significantly less consumption of treated bait from 2nd day onward and minimum consumption of treated bait was found to be 0.66 ± 0.00 g/100g bw on the 5th day of treatment (Table 10, Fig. 6d). Similar results were also found in case of feeding of male house rats with combination-IV (Table 9, Fig. 5d). In both male and female house rats feeding of different concentrations of combined baits of bromadiolone and cholecalciferol resulted in 100% mortality even these rats well accepted the treated baits only for 1-2 days and after that a negligible amount of treated bait was consumed (Tables 9 and 10, Figs. 5 and 6). This was due to stop feed action indicating that house rats showed stop feed behavior after the ingestion of lethal amounts of vitamin D_3 (Buckle *et al* 1985, Saini and Parshad 1992). Literature also reveals the acceptance of lethal dose of cholecalciferol during first 24 hours of feeding with a success of 100% mortality (Buckle and Muller 2000). Cholecalciferol manifests symptoms slowly so that the rats ingests sufficient quantity of bait before becoming suspicious, which is an added advantage over other acute rodenticides. It scores over other chronic poisons in that it requires fewer baiting days while producing quicker response, thus saving labour, bait and expensive (Mukhta Bai *et al* 1978).

Table 9: Stop feed action different combinations of bromadiolone and cholecalciferol mixed in bait against male *Rattus rattus* in no-choice feeding trial.

Feeding Trial	Set	Body weight (g)	Average daily consumption of bait (g/100g body weight)						CD at 5% level
			Pre-treatment (n=5 days)	During treatment					
				1st day	2nd day	3rd day	4th day	5th day	
Combination-I	Treated	141.66±2.91	11.81±0.70	8.66±1.08^{NS}	4.52±1.57^{S}	4.83±2.0^{S}	Died	-	6.00
	Untreated	118.33±1.32	10.36±0.32	10.86±0.23	11.80±0.77	9.06±0.27	9.30±0.169	9.46±0.61	NS
Combination-II	Treated	140±2.10	10.63±0.42	7.70±0.70^{NS}	6.20±0.97^{S}	2.81±1.06^{S}	Died	-	3.40
	Untreated	135±1.86	10.90±0.40	13.30±0.25	10.63±0.23	9.13±0.58	7.26±1.27	6.96±1.03	NS
Combination-III	Treated	156.6±2.77	5.04±0.13	4.76±0.65^{NS}	3.16±0.68^{S}	1.23±0.80^{S}	Died	-	1.87
	Untreated	125.0±0.73	8.70±0.28	8.90±0.71	6.93±0.23	8.03±0.55	6.40±0.36	6.00±0.33	NS
Combination-IV	Treated	146.6±2.03	9.75±0.55	6.93±0.93^{NS}	1.46±0.68^{S}	0.85±0.92^{S}	Died	-	2.86
	Untreated	135.0±1.86	9.99±0.52	7.16±0.25	6.16±0.62	10.23±0.76	7.56±0.99	6.56±0.95	NS

- All values are Mean±S.E.
- Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- NS represents the non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.
- S represents the significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.

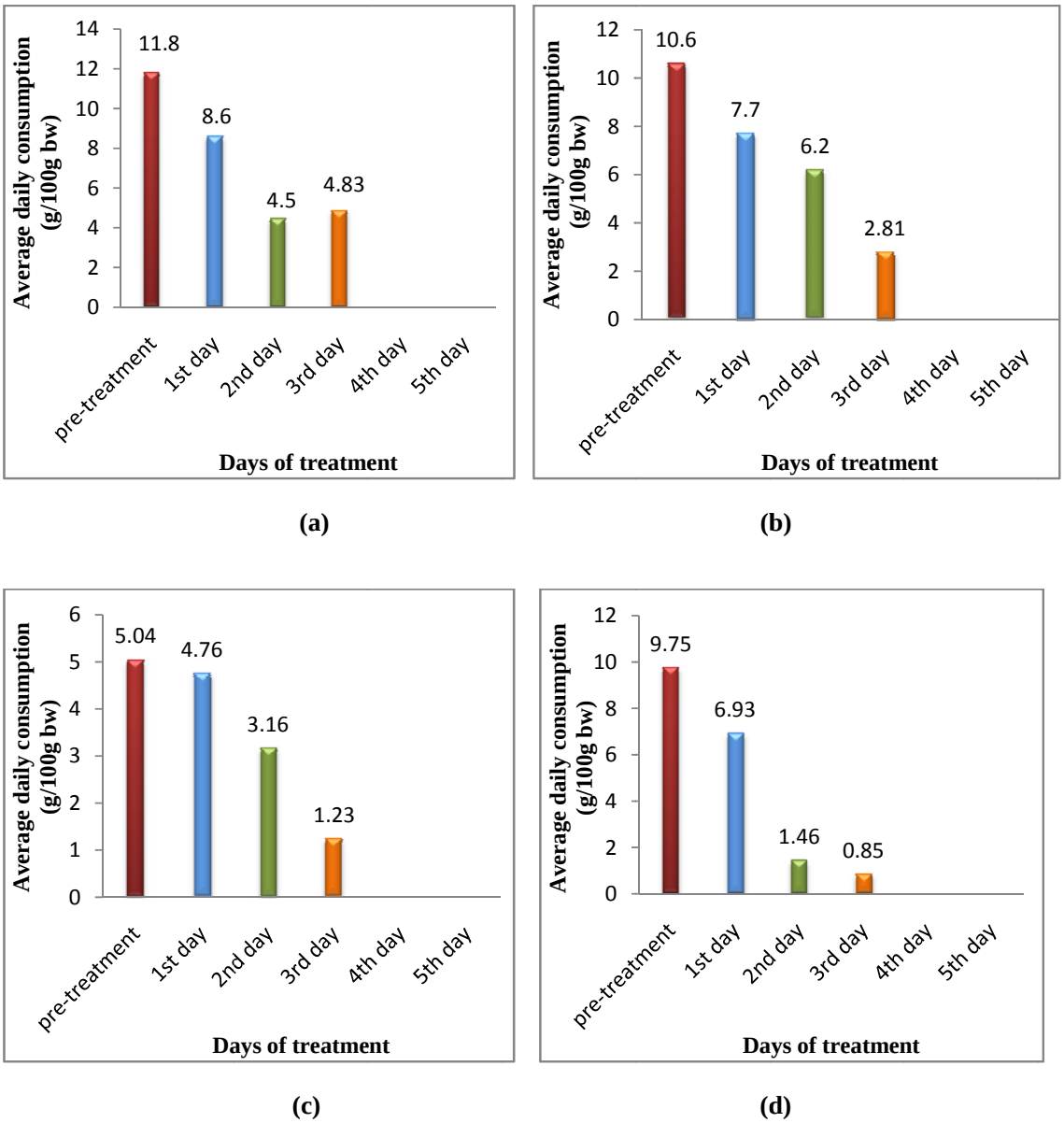


Fig. 5: Stop feed action of different combinations of bromadiolone and cholecalciferol mixed in bait against male *Rattus rattus* in no- choice feeding trial.

(a) Combination-I (0.0025% bromadiolone and 0.05% cholecalciferol)
(b) Combination-II (0.001% bromadiolone and 0.05% cholecalciferol)
(c) Combination-III (0.0025% bromadiolone and 0.01% cholecalciferol)
(d) Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol).

Table 10: Stop feed action of different combinations of bromadiolone and cholecalciferol mixed in bait against female *Rattus rattus* in no-choice feeding trial.

Feeding Trial	Set	Body weight (g)	Average daily consumption of bait (g/100g body weight)						CD at 5% level
			Pre-treatment (n=5 days)	During treatment					
				1st day	2nd day	3rd day	4th day	5th day	
Combination-I	Treated	140.00±0.91	11.60±0.66	10.53±0.32^{NS}	8.90±0.59^{NS}	2.45±0.74^{S}	0.75±0.76^{S}	Died	2.89
	Untreated	116.60±0.57	6.40±0.43	7.53±0.30	10.06±0.20	7.43±0.32	9.59±0.56	8.93±0.42	NS
Combination-II	Treated	121±0.85	12.20±0.68	12.73±0.62^{NS}	11.36±0.68^{NS}	3.80±0.90^{S}	1.13±0.00^{S}	Died	4.14
	Untreated	148.3±0.77	9.56±2.11	11.10±1.00	9.53±1.45	9.90±0.46	10.36±0.58	7.40±0.26	NS
Combination-III	Treated	151.6±1.67	6.15±0.11	7.06±0.65^{NS}	7.80±0.76^{NS}	1.16±0.90^{S}	Died	-	2.60
	Untreated	141.6±1.58	5.24±0.67	5.73±0.41	5.26±0.55	5.63±1.05	6.43±0.43	6.33±0.24	NS
Combination-IV	Treated	140.3±1.21	9.05±0.95	7.40±0.25^{NS}	4.60±0.89^{S}	2.36±0.53^{S}	1.28±0.54^{S}	0.66±0.00^{S}	3.29
	Untreated	135.0±1.05	8.86±0.60	8.33±1.02	9.33±0.79	8.16±1.30	11.76±0.20	7.76±0.80	NS

- All values are Mean±S.E.
- Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- NS represents the non-significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.
- S represents the significant difference in consumption of bait during treatment period with respect to the pre-treatment period within same group of house rats.

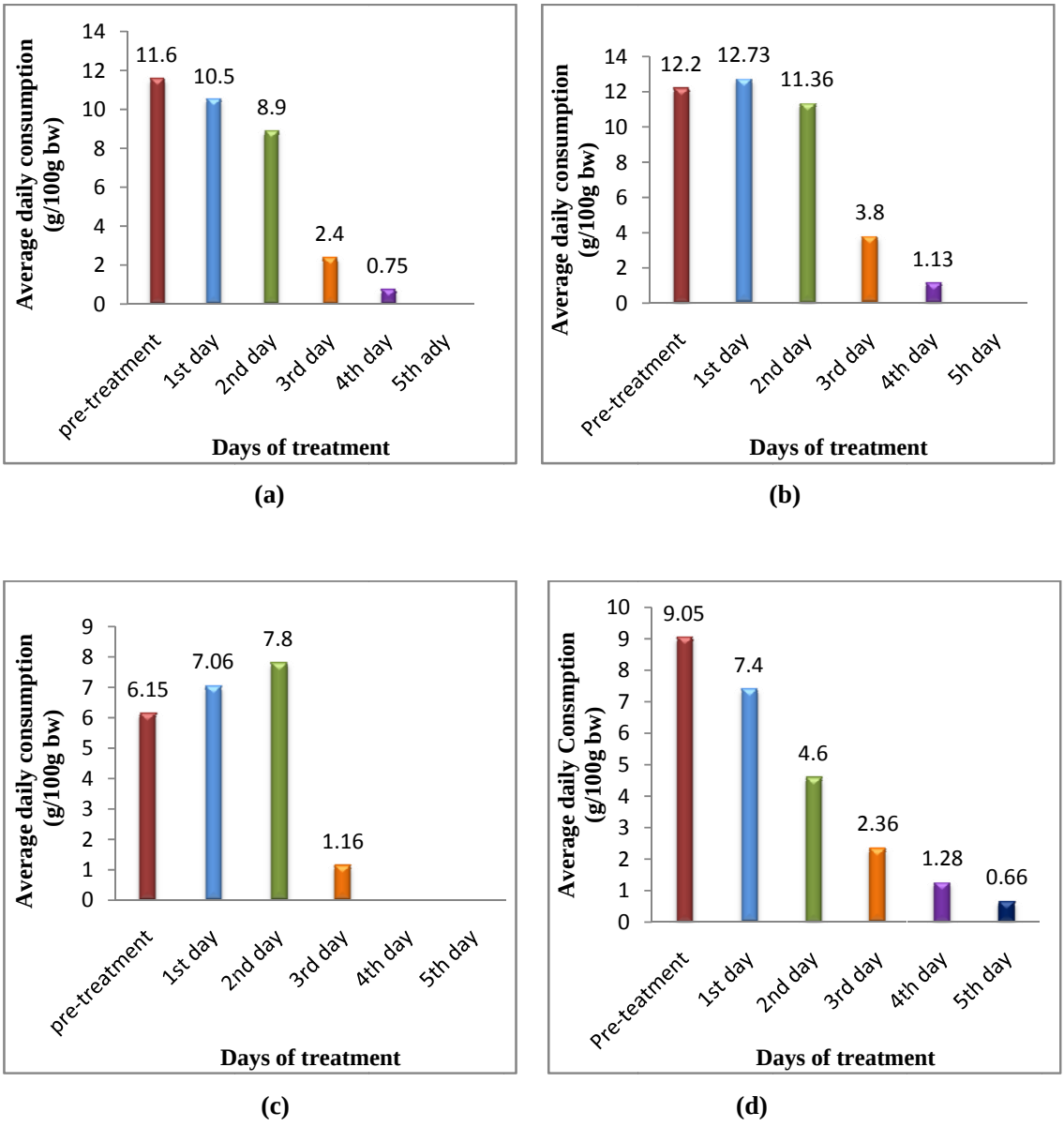


Fig. 6: Stop feed action of different combinations of bromadiolone and cholecalciferol mixed in bait against female *Rattus rattus* in no- choice feeding trial.

(a) Combination-I (0.0025% bromadiolone and 0.05% cholecalciferol)
(b) Combination-II (0.001% bromadiolone and 0.05% cholecalciferol)
(c) Combination-III (0.0025% bromadiolone and 0.01% cholecalciferol)
(d) Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol).

4.3.3 Blood clotting time of house rats fed on formulated baits

As all the four bait formulations have bromadiolone mixed in different concentrations along with cholecalciferol, the action of bromadiolone (anticoagulant) resulted in delaying of blood clotting time of house rats (both male and female) fed on these combinations (Tables 11 and 12), similar to that observed during feeding of standard bait of bromadiolone (Table 3). Blood clotting time of male house rats was found to be significantly enhanced after 48 hours of ingestion of all the four combination baits and further significant increase was also observed after 72 hours of feeding. Maximum blood clotting time (sec) was observed to be 195.0 ± 0.00 after 72 hours of feeding of combination-IV having lowest concentration i.e. 0.001% bromadiolone and 0.01% cholecalciferol (Table 11). Similar results were also found in case of female house rats, as feeding of combination-I, II, III and IV resulted in significant delay in blood clotting time from 48 hours onward with respect of 0 hour i.e. before treatment. After 72 hours of feeding of combination-I (0.0025% bromadiolone and 0.05% cholecalciferol), blood clotting time (sec) was found to be maximum i.e. 180.00 ± 0.00 which was comparable to blood clotting time (157.5 ± 1.80 sec) of rats fed on the lowest concentration (combination-IV) at 72 hours of feeding (Table 12). Even combination-IV having lowest concentration of bromadiolone mixed with lowest concentration of cholecalciferol was able to produce effective results in the form of significant delay in blood clotting time of both male and female house rats (Tables 11 and 12). This delay in blood clotting time was due to interruption caused by bromadiolone anticoagulant in the mechanism of blood clotting (Anonymous 2007, Revathi and Yogananda 2008).

4.3.4 Serum calcium level of house rats fed on formulated baits

Presence of vitamin D_3 in the formulated baits resulted in the enhancement of serum calcium level of house rats fed on these baits and results have been mentioned in Tables 13 and 14. Serum collected from male house rats fed on different combination (I, II, III and IV) after 48 hours of feeding showed significantly higher values of calcium level as compared to that of 0 hour of their feeding. Serum calcium level (mg/dL) was further enhanced at 72 hours and its maximum value was found to be 16.45 ± 0.08 after feeding of combination-III (0.0025% bromadiolone and 0.01% cholecalciferol) and this serum calcium level (mg/dL) was comparable to the values 14.80 ± 0.00, 14.35 ± 0.33 and 14.10 ± 0.00, respectively after feeding of combination-I, II and IV at 72 hours (Table 13). Female house rats also showed similar trend of significant increase in serum calcium concentration (mg/dL) after feeding of different combinations *viz*. combination-I, II, III and IV of formulated bait at 48 hours of their feeding as compared to that of 0 hour (Table 14). Even the lowest concentration (0.001% bromadiolone and 0.01% cholecalciferol) was able to significantly increase the serum calcium level (mg/dL) which was found to be 14.15 ± 0.09 at 72 hours as compared to

Table 11: Blood clotting time (sec) of male *Rattus rattus* after feeding on different combinations of bromadiolone and cholecalciferol mixed bait in no-choice feeding trial.

Feeding trial	Set	Blood clotting time (sec)			T value at 5% level
		0 hour	48 hours	72 hours	
Combination-I	Treated	70.0±0.85	90.0±1.13^{S}	150.0±0.00	9.87
	Untreated	75.0±1.42	65.0±0.88^{NS}	65.0±0.88	1.53
Combination-II	Treated	65.0±0.87	150.0±1.00^{S}	140.0±0.84	12.00
	Untreated	65.1±0.85	70.0±1.69^{NS}	65.0±0.87	1.14
Combination-III	Treated	65.0±0.87	120.0±2.24^{S}	165.0±1.17	3.76
	Untreated	66.6±0.76	70.0±0.84^{NS}	65.0±0.87	1.81
Combination-IV	Treated	75.0±1.41	130.0±0.62^{S}	195.0±0.00	7.87
	Untreated	71.6±1.00	60.0±0.00^{NS}	71.6±1.27	2.72

- All values are Mean±S.E.
- Combination-I (0.0025% bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- T value at 5% level of significance is 2.92
- NS represents the non-significant difference in blood clotting time at 48 hours with respect to 0 hour within the same group of house rats.
- S represents the significant difference in blood clotting time at 48 hours with respect to 0 hour within the same group of house rats.

Table 12: Blood clotting time (sec) of female *Rattus rattus* after feeding on different combinations of bromadiolone and cholecalciferol mixed bait in no-choice feeding trials.

Feeding trial	Set	Blood clotting time (sec)			T value at 5% level
		0 hour	48 hours	72 hours	
Combination-I	Treated	70.0±1.70	126.6±1.51^{S}	180.0±0.00	24.00
	Untreated	70.0±1.70	80.0±1.50^{NS}	75.0±1.40	1.81
Combination-II	Treated	70.0±0.84	135.0±1.05^{S}	165.0±1.17	18.40
	Untreated	60.0±0.00	65.0±0.87^{NS}	75.0±1.41	1.81
Combination-III	Treated	65.2±0.87	135.0±1.05^{S}	120.0±0.00	19.80
	Untreated	65.0±0.86	65.0±0.87^{NS}	65.0±0.87	0.81
Combination-IV	Treated	65.0±0.87	130.0±1.24^{S}	157.5±1.80	18.40
	Untreated	75.0±1.42	65.0±0.87^{NS}	70.0±0.84	0.81

- All values are Mean±S.E.
- Combination-I (0.0025% bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), C-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- T value at 5% level of significance is 2.92
- NS represents the non-significant difference in blood clotting time at 48 hours with respect to 0 hour within the same group of house rats.
- S represents the significant difference in blood clotting time at 48 hours with respect to 0 hour within the same group of house rats.

Table 13: Calcium concentration (mg/dL) of male *Rattus rattus* after feeding on different combinations of bromadiolone and cholecalciferol mixed bait in no-choice feeding trial

Feeding trial	Set	Blood calcium level(mg/dL)			T value at 5% level
		0 hour	48 hours	72 hours	
Combination-I	Treated	10.43±0.40	13.83±0.32^{S}	14.80±0.00	9.69
	Untreated	9.80±0.32	9.06±0.43^{NS}	7.23±0.26	1.20
Combination-II	Treated	8.88±0.40	12.59±0.24^{S}	14.35±0.33	7.39
	Untreated	9.40±0.18	8.90±0.26^{NS}	7.50±0.26	1.31
Combination-III	Treated	9.78±0.10	13.00±0.12^{S}	16.45±0.08	22.10
	Untreated	10.86±0.21	9.16±0.22^{NS}	9.53±0.10	2.09
Combination-IV	Treated	8.73±0.47	12.40±0.12^{S}	14.10±0.00	4.27
	Untreated	8.80±0.30	9.43±0.35^{NS}	7.33±0.19	1.28

- All values are Mean±S.E.
- Combination-I(0.0025% bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- T value at 5% level of significance is 2.92
- NS represents the non-significant difference in serum calcium level at 48 hours with respect to 0 hour within the same group of house rats.
- S represents the significant difference in serum calcium level at 48 hours with respect to 0 hour within the same group of house rats.

Table 14: Calcium concentration (mg/dL) of female *Rattus rattus* after feeding on different combinations of bromadiolone and cholecalciferol mixed bait in no-choice feeding trial

Feeding trial	Set	Blood calcium level(mg/dL)			T value at 5% level
		0 hour	48 hours	72 hours	
Combination-I	Treated	7.86±0.51	13.70±0.62^{S}	16.10±0.00	3.36
	Untreated	7.93±0.17	8.86±0.28^{NS}	7.43±0.16	1.94
Combination-II	Treated	7.91±0.20	14.08±0.09^{S}	15.35±0.19	29.20
	Untreated	6.60±0.17	8.46±0.26^{NS}	7.06±0.25	2.12
Combination-III	Treated	8.13±0.15	14.10±0.56^{S}	13.80±0.00	5.26
	Untreated	8.70±.33	10.13±0.35^{NS}	9.80±0.43	1.81
Combination-IV	Treated	8.16±0.45	11.10±1.09^{S}	14.15±0.09	3.87
	Untreated	8.50±0.35	8.70±0.39^{NS}	7.36±0.20	0.92

- All values are Mean±S.E.
- T value at 5% level of significance is 2.92
- Combination-I (0.0025% bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- NS represents the non-significant difference in serum calcium level at 48 hours with respect to 0 hour within the same group of house rats.
- S represents the significant difference in serum calcium level at 48 hours with respect to 0 hour within the same group of house rats.

8.16 ± 0.45 at 0 hour (Table 14). This rise in calcium level in serum of male and female house rats was due to mobilization of calcium from bone matrix into blood plasma starting from 24 hours of poisoning and resulting in death from hypercalcaemia (Tischler *et al* 1999, Kocher *et al* 2008).

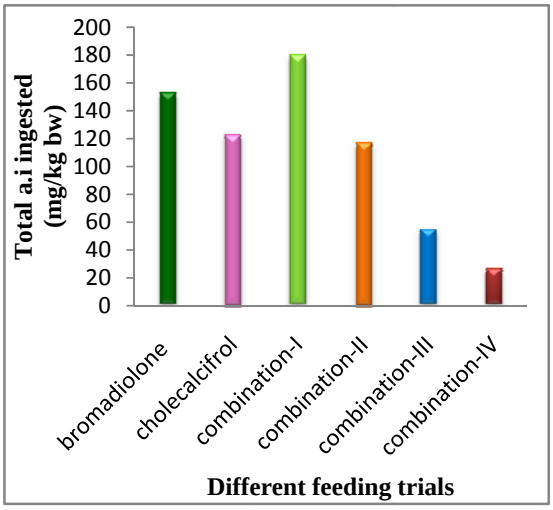
4.3.5 Comparative analysis of rodenticidal potential of standard baits of bromadiolone and cholecalciferol and their combinations

When standard bait of bromadiolone (0.005%) alone was fed to male house rats, total active ingredient of bromadiolone ingested was found to be 153.00 ± 4.49 mg/kg bw, which led to 100% mortality within an average of 4.33 ± 0.59 days, due to excessive flow of blood as a result of delay in 46.66 ± 1.82 sec in clotting time (as compared to normal clotting time). On the other hand the mortality of male rats was delayed as average time for 100% mortality was 7.33 ± 0.92 days after feeding of 122.30 ± 3.80 mg/kg bw of active ingredient of standard bait of cholecalciferol and rise in serum calcium level was observed to be 9.37 ± 0.72 mg/dL in the treated rats as compared to that of normal serum calcium level of male house rats (Table 15, Fig. 7). When male house rats were fed on baits having different combinations of bromadiolone and cholecalciferol (with lesser concentration than their standard one), total active ingredients of rodenticides ingested (bromadiolone + cholecalciferol) was found to be non- significantly different during the feeding of combination-I and combination-II as compared to that of standard bromadiolone and cholecalciferol feeding trials. However, a significantly less active ingredient i.e. 55.00 ± 2.67 and 26.90 ± 2.34 mg/kg bw, respectively was ingested by male rats during feeding of combination-III (0.0025% bromadiolone and 0.01% cholecalciferol) and combination-IV (0.001% bromadiolone and 0.01% cholecalciferol) as compared to that of the concentration in their standard doses (Table 15, Fig. 7a). However, talking in terms of ingestion of active ingredient of bromadiolone and cholecalciferol separately during feeding of these four combinations, results indicated a non-significant difference in feeding of bromadiolone and cholecalciferol during feeding of combination-I (0.0025% bromadiolone and 0.05% cholecalciferol) and significantly lesser consumption of active ingredient of bromadiolone and cholecalciferol during the rest of feeding trials i.e. combination-II, III and IV. Feeding of four different combinations of bromadiolone and cholecalciferol resulted in 100% mortality in all the experimental trials and there was non-significant difference in the average time taken for 100% mortality of rats (Table 15, Fig. 7b). Delay in blood clotting time was statistically similar after feeding of standard bait of bromadiolone (0.005%) and the four combination i.e. I, II, III and IV (Table 15, Fig. 7c). Rise in serum calcium level (mg/dL) was found to be 3.40 ± 0.38, 3.95 ± 0.55, 3.21 ± 0.16 and 3.66±0.93 respectively after feeding

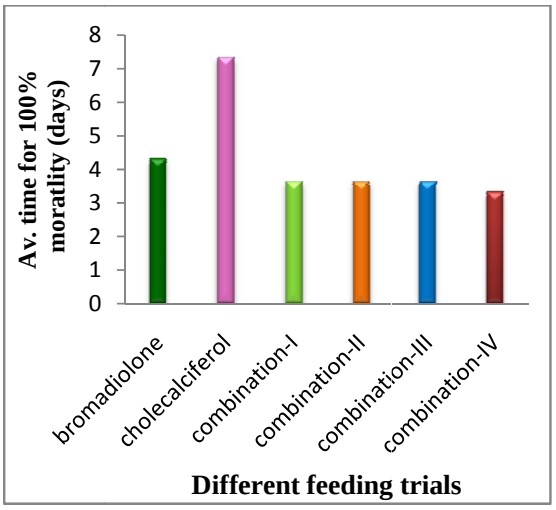
Table 15: Comparison of rodenticidal potential of standard bait of bromadiolone and cholecalciferol and their different combinations against male *Rattus rattus* in no-choice feeding trial.

Feeding trial	Body weight (g)	Active ingredient of rodenticide ingested (mg/kg bw) (n= 5 days)			Average time for 100% mortality (days)	Delay in blood clotting time(sec)*	Rise in serum calcium level (mg/dL)*
		Total Rodenticide (mg/kg bw)	Bromadiolone (mg/kg bw)	Vitamin D_3 (mg/kg bw)			
Standard bromadiolone	168.3±1.59	153.00±4.49	153.00±4.49	-	4.33±0.59	46.66±1.82	-
Standard cholecalciferol	121.2±0.85	122.30±3.80	-	122.30±3.80	7.33±0.92	-	9.37±0.72
Combination-I	141.6±2.91	180.31±5.06	90.15±3.58	90.15±3.58	3.66±0.49	16.662.08	3.40 ± 0.38^{b}
Combination-II	140±2.10	117.0±1.50	33.42 ± 0.80^{a}	83.57 ± 1.26^{b}	3.66±0.24	85.00±1.53	3.95 ± 0.55^{b}
Combination-III	156.6±2.77	55.00 ± 2.67^{S}	45.83 ± 2.44^{a}	9.16 ± 1.09^{b}	3.66±0.24	55.00±4.15	3.21 ± 0.16^{b}
Combination-IV	146.6±2.03	26.90 ± 2.34^{S}	17.93 ± 1.91^{a}	8.96 ± 1.35^{b}	3.33±0.25	55.00±1.90	3.66 ± 0.93^{b}
CD at 5% level	-	90.11	67.34	38.30	NS	NS	3.13

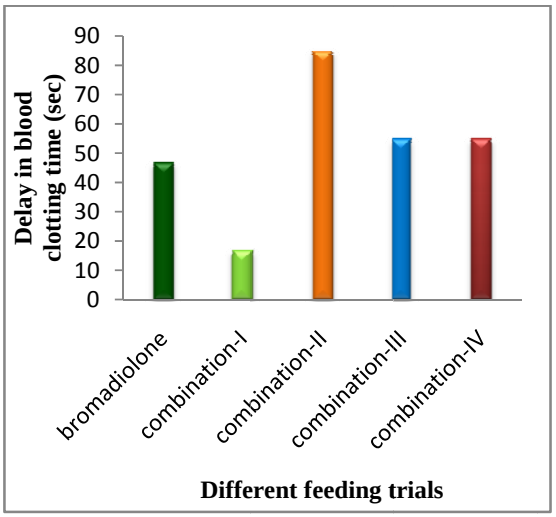
- * refers difference of the values between 48 hours and 0 hour of feeding
- Standard bromdiolone (0.005%), standard cholecalciferol (0.075%), Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- NS represents the non-significant difference among the different feeding trials
- S represents the significant difference among the different feeding trials
- a refers the significant difference of the values at 5% level among different combination with respect to standard bromadiolone feeding
- b refers the significant difference of the values at 5% level among different combination with respect to standard cholecalciferol feeding



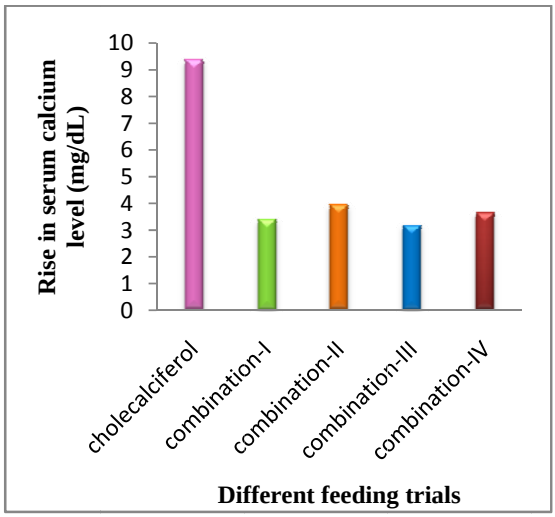
(a)



(b)



(c)



(d)

Fig. 7: Comparison of rodenticidal potential of standard baits of bromadiolone and cholecalciferol and their different combinations against male *Rattus rattus* in no-choice feeding trial.

(a) Total active ingredient ingested (mg/kg bw)

(b) Average time for 100% mortality (days)

(c) Delay in blood clotting time (sec)

(d) Rise in serum calcium level (mg/dL)

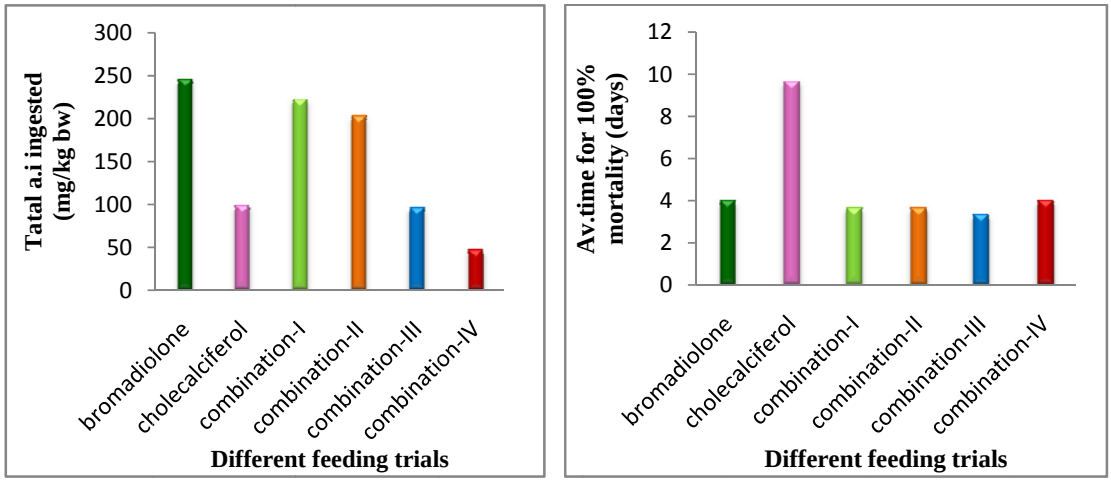
of combination-I, II, III and IV as compared to the serum calcium level in blood of normal house rats (before treatment), but these values of rise in serum calcium level was significantly less as compared to that observed in the male house rats fed on standard bait of cholecalciferol i.e. 9.37±0.72 mg/dL (Table 15, Fig. 7d).

Comparative results obtained after feeding of female house rats with standard bromadiolone bait, standard cholecalciferol bait and their four different combinations have been compiled in Table 16 and Fig. 8. Combination-I and II resulted in non-significant difference in the total active ingredient of ingested rodenticide. However, active ingredient ingested during the feeding of combination-III and IV (0.0025% bromadiolone + 0.01% cholecalciferol and 0.001% bromadiolone + 0.01% cholecalciferol) was found to be significantly less as compared to that of standard bait of bromadiolone and cholecalciferol and the minimum active ingredient ingested was 46.88 ± 1.35 mg/kg bw during feeding of combination-IV having the least concentration of bromadiolone and cholecalciferol mixed in bait i.e. 0.001% bromadiolone and 0.01% cholecalciferol (Table 16, Fig. 8a). When active ingredient of bromadiolone and cholecalciferol ingested separately during feeding of different combination was analysed, it showed a significant reduction in their ingestion during the various feeding trials (having bromadiolone and cholecalciferol in combinations) as compared to that of their standard one except the combination-I which showed a non-significant difference in the ingestion of vitamin D_3. Hundred per cent mortality was attained with a non-significant difference in the average time of mortality (days) during different combinations as compared to that of standard bromadiolone and cholecalciferol baits (Table 16, Fig. 8b). Even the delay in the blood clotting time was statistically similar in all the feeding trials irrespective of the concentration of the bromadiolone and cholecalciferol mixed in bait (Table 16, Fig. 8c). A non-significant difference in the rise in serum calcium level (mg/dL) was observed during the feeding of standard baits of bromadiolone, cholecalciferol and their different combinations (Table 16, Fig. 8d). Results from comparative data (Table 15, Fig. 7) clearly indicated efficient rodenticidal potential of the combination-IV (0.001% bromadiolone and 0.01% cholecalciferol) having the lowest concentration of bromadiolone and cholecalciferol as 100% mortality of male house rats was achieved earlier than standard bromadiolone (0.005%), standard cholecalciferol (0.075%), combination-I (0.0025% bromadiolone and 0.05% cholecalciferol), combination-II (0.001% bromadiolone and 0.05% cholecalciferol), and combination-III (0.0025% bromadiolone and 0.01% cholecalciferol). In females, the least concentration of bromadiolone and cholecalciferol mixed in bait (0.001% bromadiolone and 0.01% cholecalciferol) resulted in lowest ingestion of active ingredient of total rodenticide as compared to standard bromadiolone, cholecalciferol and other

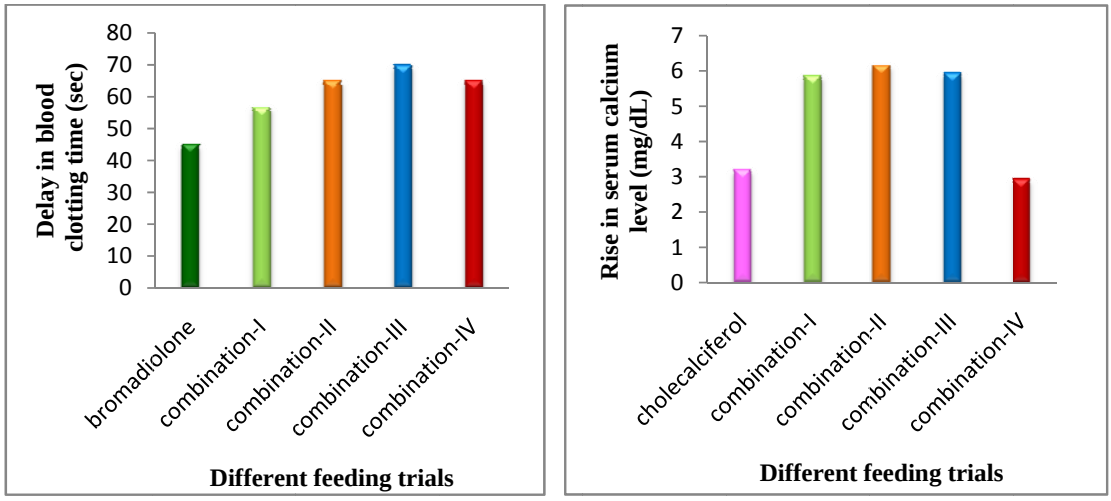
Table 16: Comparison of rodenticidal potential of standard bait of bromadiolone and cholecalciferol and their different combinations against female *Rattus rattus* in no-choice feeding trial.

Feeding trial	Body weight (g)	Active ingredient of rodenticide ingested (mg/kg bw) (n= 5 days)			Average time for 100% mortality (days)	Delay in blood clotting time(sec)*	Rise in serum calcium level (mg/dL)*
		Total Rodenticide (mg/kg bw)	Bromadiolone (mg/kg bw)	Vitamin D_3 (mg/kg bw)			
Standard bromadiolone	126.6±1.47	245.90±3.69	245.90±3.69	-	4.00±0.00	45.00±3.39	-
Standard cholecalciferol	128.3±1.10	98.02±3.28	-	98.02±3.28	9.66±0.80	-	3.21±0.21
Combination-I	140.0±0.91	222.43±2.45^{S}	111.21±1.81^{a}	111.21±1.81	3.66±0.49	56.66±0.62	5.86±1.53
Combination-II	121.0±0.85	203.23±3.16^{S}	58.06±1.68^{a}	145.16±2.66^{b}	3.66±0.24	65.00±0.87	6.17±0.16
Combination-III	151.6±1.67	96.20±1.46^{S}	80.16±1.34^{a}	16.03±0.60^{b}	3.33±0.25	70.00±0.84	5.96±0.95
Combination-IV	140.3±1.21	46.88±1.35^{S}	30.88±1.06^{a}	15.44±0.75^{b}	4.00±0.40	65.00±0.87	2.94±0.92
CD at 5% level	-	81.40	63.24	38.08	NS	NS	NS

- * refers difference of the values between 48 hours and 0 hour of feeding
- Standard bromdiolone (0.005%), standard cholecalciferol (0.075%), Combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), Combination-II (0.001% bromadiolone and 0.05% cholecalciferol), Combination-III (0.0025% bromadiolone and 0.001% cholecalciferol), Combination-IV (0.001% bromadiolone and 0.01% cholecalciferol)
- NS represents the non-significant difference among the different feeding trials
- S represents the significant difference among the different feeding trials
- a refers the significant difference of the values at 5% level among different combination with respect to standard bromadiolone feeding
- b refers the significant difference of the values at 5% level among different combination with respect to standard cholecalciferol feeding



(a) **(b)**



(c) **(d)**

Fig. 8: Comparison of rodenticidal potential of standard bait of bromadiolone and cholecalciferol and their different combinations against female *Rattus rattus* in no-choice feeding trials

(a) Total active ingredient ingested (mg/kg bw)

(b) Average time for 100% mortality (days)

(c) Delay in blood clotting time (sec)

(d) Rise in serum calcium level (mg/dL)

combinations and was able to cause 100% mortality with an average of 4 days of the feeding of combination-IV (Table 16, Fig. 8).

The combination (combination-IV) having lowest concentration i.e. 0.001% bromadiolone and 0.01% cholecalciferol in both male and female house rats was having the efficient rodenticidal potential because of the combined effect of bromadiolone (anticoagulant) and cholecalciferol (sub-acute poison), as synergism increases the rodenticidal activity of the formulated baits as compared to their standard baits and such combined preparations can also be widely used for the eradication of rats resistant to anticoagulants (Zatsepin *et al* 2006). Literature also indicated that calciferol or warfarin baits given individually to *R. rattus* produced partial mortality, however in combination produced a complete killing. Excess of calcium due to cholecalciferol and the unavailability of vitamin K due to the action of bromadiolone might explain the added effectiveness of cholecalciferol, indicating that cholecalciferol and bromadiolone act on different loci in the same process of blood physiology (Mukhta Bai *et al* 1978).

There is an important feature of calciferols (vitamin D_2 and vitamin D_3) toxicology that they are synergistic with anticoagulant toxicants, it means that mixture of anticoagulant and calciferols in same bait are more toxic than sum of toxicities of anticoagulant and the calciferol in the bait. Thus, a massive hypercalcaemia effect can be achieved by a substantially lower calciferol content in bait and vice-versa, a more pronounced anticoagulant/hemorrhagic effects are observed if the calciferol is present. This synergism is mostly used in calciferol low concentration baits, because effective concentration of calciferols is more expensive, than effective concentration of the most anticoagulants. Such type of synergistic calciferol based baits having less concentration of anticoagulant are considered generally safer to birds and other non-target species than anticoagulants or acute toxicants (Anonymous 1998).

4.3.6 Comparative costs of different formulated baits

When standard baits of bromadiolone and cholecalciferol were prepared, the cost of rodenticide for preparation of their 1 kg bait was Rs 24/- and Rs 1239.95/-, respectively. Cost of rodenticide used for preparation of 1 kg of combination-I, II, III and IV bait was Rs 838.63, Rs 831.43, Rs 177.32 and Rs 170.12, respectively (Table 17). Bromadiolone bait was though cheaper as it cost only Rs 24/kg bait, but it is very toxic and has been found to have primary and secondary toxic effects in non-target species (Brar and Sandhu 2000, Rao 2005).

Table 17: Benefit in terms of Rs. during the formulation of rodenticidal baits having different concentrations of bromadiolone and cholecalciferol.

Rodenticidal bait	Quantity of rodenticide		Cost of rodenticide (Rs/kg of WSO bait)		
	Bromadiolone (g/kg of WSO bait)	Cholecalciferol (mg/kg of WSO bait)	Bromadiolone	Cholecaliferol	Total cost
Standard bromadiolone (0.005%)	20	-	24.00	-	24.00
Standard cholecalciferol (0.075%)	-	750	-	1239.95	1239.95
Combination-I (0.0025%bromadiolone+ 0.05% cholealciferol)	10	500	12.00	826.63	838.63
Combination-II (0.001%bromadiolone + 0.05% cholealciferol)	4	500	4.80	826.63	831.43
Combination-III (0.0025%bromadiolone+ 0.01% cholealciferol)	10	100	12.00	165.32	177.32
Combination-IV (0.001%bromadiolone + 0.01% cholealciferol)	4	100	4.80	165.32	170.12

Out of all the other feeding trials of combination baits, the combination-IV (0.001% bromadiolone and 0.01% cholecalciferol) was found to be cost effective i.e. Rs 170.12/kg (Table 17), as less amount of the costly chemical i.e. cholecalciferol is needed to prepare it, while the efficiency and rodenticidal potential equivalent or more than that of standard baits of bromadiolone and cholecalciferol and other combination baits is achieved (Tables 15 and 16) while spending less money i.e. Rs 170.12/kg of bait. As the concentration of bromadiolone in combination-IV is reduced, its lethal effects to other non-target species will also be reduced. Such type of combination could benefit in terms of reduction of active ingredients whilst retaining the efficiency and minimizing the primary and secondary hazards (Anonymous 1998).

4.4 Study of resistance towards standard bait of bromadiolone (0.005%) by house rats

The feeding of 0.005% bromadiolone for 5 days in no-choice, showed 100% mortality of the male rats as all the rats (n=30) in these 10 feeding trials were found to be dead within 2-9 days (Table 18). Similarly when 30 female house rats (grouped into 10 sets having 3 rats in each) were fed on standard bait of bromadiolone (0.005%) in no-choice, showed 100% mortality of female house rats within 2-9 days (Table 19). As no male and female house rat was observed to survived after feeding of standard bromadiolone bait, indicating 100% susceptibility and no resistance towards standard bromadiolone bait (0.005%) by *R. rattus*. There are certain reports available in the literature showing resistance towards anticoagulant (both first and second generation) rodenticides (Jackson and Ashton 1986, MacNicoll 1987, Buckle *et al* 1994, Greaves 1994, Misenheimer *et al* 1994, Lasseur *et al* 2007, Yi *et al* 2009) which are specific against a particular rodent species like resistance towards standard bait of bromadiolone (0.005%) by *R. norvegicus* (Buckle *et al* 2007, Markussen *et al* 2007), *M. musculus* L (Rowe *et al* 1981), and *M. musculus domesticus* (MacNicoll 1987, Misenheimer *et al* 1994). But on the other hand literature also reveals efficient susceptibility of bromadiolone bait by different rodent species for e.g. *M. musculus* showed 100% mortality in single dose of bromadiolone (Revathi and Yogananda 2006) and *R. rattus rufescens* and *R. norvegicus* also showed 100% killing of these rats (Renapurkar 1993). These reports from different species also support the fact of no-resistance towards the bromadiolone anticoagulant rodenticide as also observed in our studies, revealing that *R. rattus* L found in the commensal situations of Ludhiana area showed no-resistance towards standard bait of bromadiolone (0.005%).

Table 18: Rodenticidal potential and resistance towards standard bait of bromadiolone (0.005%) by male *Rattus rattus* in no- choice feeding trial.

Set	Body weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Total active ingredient ingested (mg/kg bw) (n= 5 days)	Mortality		Survived or Resistant rats (%)
		Pre treatment	During treatment	Post treatment		Percent	Range (days)	
I	132.0±1.38	8.35±1.25	3.10±0.680	1.45±0.721	168.13±2.17	100	6 – 8	0
II	121.3±1.68	7.08±0.268	3.4±0.44	0.446±0.52	126.62±1.45	100	5 – 8	0
III	151.6±1.88	5.13±0.704	3.4±0.778	1.00±1.21	125.31±3.91	100	6 – 9	0
IV	124.6±0.42	5.28±0.342	5.7±0.050	2.5±0.126	219.90±0.87	100	4 – 8	0
V	106.0±0.48	6.7±0.265	6.6±0.138	1.91±0.00	215.66±2.31	100	4 – 7	0
VI	103.0±0.00	7.1±0.816	6.6±0.488	2.9±0.00	274.66±2.34	100	4 – 7	0
VII	120.0±1.84	8.7±0.105	7.4±0.829	0.675±0.00	337.63±7.10	100	4 – 6	0
VIII	143.3±2.01	8.3±0.145	6.2±0.362	3.87±0.00	225.97±3.30	100	2 – 8	0
IX	138.6±1.28	6.5±0.318	5.5±0.554	1.04±0.00	293.20±2.18	100	2 – 7	0
X	116.3±0.26	9.0±0.209	7.63±0.416	0.00±0.00	303.00±6.34	100	2 – 6	0

- All values are Mean±S.E

Table 19: Rodenticidal potential and resistance towards standard bait of bromadiolone (0.005%) by female *Rattus rattus* in no- choice feeding trial.

Set	Body weight (g)	Average daily consumption of bait (g /100g body weight), n= 5 days			Total active ingredient ingested (mg/kg bw) (n= 5 days)	Mortality		Survived or Resistant rats (%)
		Pre treatment	During treatment	Post treatment		Percent	Range (days)	
I	160.6±1.79	5.4±0.40	3.3±0.309	0.503±1.00	159.95±4.51	100	5 – 8	0
II	165.6±0.55	4.6±0.496	2.1±0.054	0.80±0.899	173.69±2.99	100	4 – 8	0
III	134.0±0.55	6.35±0.499	2.5±0.579	1.61±0.883	172.35±5.39	100	5 – 8	0
IV	126.3±1.47	5.9±0.634	4.3±0.124	2.58±0.428	286.30±3.59	100	4 – 8	0
V	138.0±2.51	4.7±0.87	4.3±0.328	2.77±0.280	333.33±1.00	100	5 – 9	0
VI	111.3±0.22	5.5±0.244	5.4±0.331	1.67±1.29	330.33±3.37	100	4 – 6	0
VII	133.0±1.95	7.6±0.751	6.7±1.00	1.83±0.048	367.66±5.86	100	4 – 9	0
VIII	111.0±0.61	7.2±0.179	6.5±0.556	4.15±0.00	224.30±3.54	100	2 – 9	0
IX	128.0±1.49	7.6±0.213	5.8±0.310	1.08±1.22	251.76±6.26	100	7 – 9	0
X	135.3±2.05	8.4±0.325	6.7±0.495	1.64±0.963	337.33±5.87	100	2 – 7	0

- All values are Mean±S.E

CHAPTER-V

SUMMARY

The present study entitled "Study on combined effect of bromadiolone and cholecalciferol (Vitamin D_3) against house rat, *Rattus rattus* Linnaeus" was carried out in the Rodent Research Laboratory, Department of Zoology, Punjab Agricultural University, Ludhiana. House rats were trapped from poultry farms, grocery shops, godowns, store houses etc. of Ludhiana city and mature male and female house rats were selected and acclimatized under laboratory conditions before starting the experiments. Standard baits of bromadiolone (0.005%) and cholecalciferol (0.075%) and their combinations having different concentrations of bromadiolone and cholecalciferol were prepared in WSO-mix bait and were fed to house rats (3 males and 3 female) for 5 days in no-choice feeding trials. Both male and female house rats showed good per cent acceptability of standard bait of bromadiolone (0.005%) over plain bait i.e. 81.50±0.97 and 95.80±3.19, respectively leading to 100% mortality within 3-6 days in males house rats and all female rats died on 4^{th} day of feeding. Ingestion of bromadiolone (anticoagulant) resulted in significant increase in blood clotting time (sec) 118.3±1.86 and 100.0±3.08, respectively in male and female house rats observed at 48 hours as compared to their values at 0 hour i.e. 71.60±1.00 and 68.3±1.02 sec.

Standard bait of cholecalciferol (0.075%) was less accepted than that of standard bait of bromadiolone and its per cent acceptability over plain bait was 40.10±1.55 and 35.30±0.64, respectively in male and female house rats. After feeding of standard bait of cholecalciferol complete kill of rats was observed within 4-10 days in males and 7-14 days in female rats. House rats showed stop feeding behavior, as the consumption of cholecalciferol treated bait was found to be significantly low from 3rd day onward as compared to the consumption of plain bait during pre-treatment period. Ingestion of cholecalciferol caused hypercalcaemia i.e. increase in the serum calcium level as the values of calcium concentration in serum (mg/dL) was found to be significantly high i.e. 16.10±0.36 and 13.09±0.43 respectively in male and female rats at 48 hours of feeding as compared to 0 hour i.e. 7.50±0.26 and 9.88±0.50 (mg/dL).

Out of the four combinations of formulated baits (having concentration of bromadiolone and cholecalciferol less than in their standard baits), i.e. combination-I (0.0025%bromadiolone and 0.05% cholecalciferol), combination-II (0.001% bromadiolone and 0.05% cholecalciferol), combination-III (0.0025% bromadiolone and 0.01% cholecalciferol) and combination-IV (0.001% bromadiolone and 0.01% cholecalciferol), the acceptance and ingestion of combination-IV having the lowest concentration of bromadiolone and cholecalciferol was found to be the lowest one, but even with this low

acceptance 100% mortality of both male and female house rats was achieved within the same range of days (3-5 days) observed during the feeding of other three combinations (combination-I, II and III). Male and female house rats consumed a good amount of all the combined baits only for the first day of feeding, second day onward rats showed stop feeding behavior. Delay in blood clotting time (sec) due to action of bromadiolone anticoagulant and rise in serum calcium level (mg/dL) due to presence of hypercalcaemia causing agent cholecalciferol in case of both male and female house rats fed on different combinations clearly indicated the synergistic effect of these two chemicals mixed in baits. The results obtained during the present study showed the comparable rodenticidal potential of the combination-IV having the lowest concentration of bromadiolone and cholecalciferol in comparison to the standard baits of bromadiolone and cholecalciferol and other three combinations (combination-I, II, and III). This combination/formulated bait was also cost effective, as the total cost for preparation of combination-IV was calculated to be Rs 170.12/kg which was lesser than the cost of standard bait of cholecalciferol and its other three combinations.

Twenty experimental sets of male and female house rats (having 3 rats in each) were conducted for evaluating the phenomenon of resistance by house rats towards bromadiolone standard bait (0.005%). Feeding of 0.005% bromadiolone for 5 days in no-choice showed 100 per cent mortality of both male and female house rats (n= 60) in these feeding trials and rats were found to be dead within 2-9 days. No resistance was observed in *R. rattus* L. trapped from commensal situations of Ludhiana area towards standard bait of bromadiolone (0.005%) as complete killing of male and female house rats was recorded.

The results of the present study thus can be concluded by the following points:

1. Combination having lowest concentration of bromadiolone and cholecalciferol (0.001% bromadiolone and 0.01% cholecalciferol) during the present study showed efficient rodenticidal potential against both male and female house rats, *R. rattus* L.
2. Male and female house rats when fed on standard bait of bromadiolone (0.005%) showed no resistance towards this anticoagulant.

REFERENCES

Ahmad N and Parshad V R (1987) Rodent control in sugarcane. *Sugarcane* : 3-6.

Ahmad N and Parshad V R (1989a) Effect of size of baiting area on rodent control success with bromadiolone in wheat crop. *Indian Journal of Plant Protection* **17** : 1-4.

Ahmad N and Parshad V R (1989b) Evaluation of rodenticidal baiting in small cane fields. *Sugarcane* 1989 : 2-4.

Ahmad N and Parshad V R (1991) Evaluation of rodenticidal baits in fields of sugarcane (*Sachharum officinarum*). *Indian J Agric Sci* **61**(4) : 281-84.

Anonymous (1987) *Vitamin Tolerance of Animals*. Subcommittee on Vitamin Tolerance. Committee on Animal Nutrition Board on Agriculture. National Research Council pp. 11-12. National Academy Press, Washington.

Anonymous (1998) Rodenticides. http://en.wikipedia.org/wiki/rodenticide.

Anonymous (2007) Impact Assessment for Proposed Rodenticide Mitigation (DP 332577) http://www.regulation.gov/fdmspublic/component/main.

Anonymous (2010) Bromadiolone. http://en.wikipedia.org/wiki/Bromadiolone.

Arora K K, Lal S S and Rani S (1982) Studies on Newer Anticoagulant Rodenticides for the Control of Black Rat, *Rattus rattus* Lin. *J Pestology* **6**(1) : 5-7.

Ashton A and Jackson W B (1984) Anticoagulant resistance in the House mouse in North America. In : Dubock A C (ed) *Proceedings of a Conference on the Organization and Practice of Vertebrate Pest Control*. Elvetham Hall, UK, 30 August -3 September, 1982, PP 181-88.

Beard M L, Montaman C E, Maupin G O, Barnes A M, Craven R B and Marshall E F (1988) Integrated Vector Control Rodenticide may reduce the spread of human plague. *J Environ Health* **51**(2) : 69-75.

Beasley V R, Dorman D C, Fikes J D, Diana S G and Woshner V (1997) Cholecalciferol based rodenticides and other vitamin D-containing products. In : A Systems Affected Approach to Veterinary Toxicology pp 445-450. University of Illinois Press, Urbana, Illinois, USA.

Bennett S M (2002) Cholecalciferol. www.thepiedpiper.co.uk/th15(h).htm.

Biswas B and Tiwari K K (1969) Taxonomy and distribution of Indian rodents In: Harris K L (ed.) *Proc Indian Rodent Symp Calcutta* pp 9-45.

Booth L H, Fisher P, Hepplewaite V and Eason C T (2004) Risk of Feracol baits to non-target invertebrate, native skinks, Weta. *Science for Conservation* **239** : 18p.

Boyle C M (1960) Case of apparent resistance of *Rattus norvegicus* to anticoagulant poisons. *Nature* **188** : 517.

Brar R S and Sandhu H S (2000) *Text Book of Veterinary Toxicology* Pp 181-200. Kalyani Publishers, Ludhiana, India.

Buckle A P (1985) Fields trials of a new sub-acute rodenticide flupropadine, against wild Norway rats (*Rattus norvegicus*). *Journal of Hygiene*, Cambridge **95** : 505-12

Buckle A P (1994) Rodent control methods : Chemicals. In : Buckle A P and Smith R H (ed) *Rodent Pests and Their Control.* pp 127-60. Cab International, UK.

Buckle A P and Muller F (2000) Rodenticides. In: Muller F (ed) Agrochemicals : *Composition, Production, Toxicology and Application*. Pp 667-686. Wiley-VCH, Weinheim.

Buckle A P, Endepols S and Prescott C V (2007) The relationship between resistance factors and treatment efficacy when bromadiolone was used against anticoagulant resistant Norway rats (*Rattus norvegicus* Berk) in wales. *Intl J Pest Mgmt* **53**(4) : 291-97.

Buckle A P, Prescott C V and Ward K J (1994) Resistance to the first and second generation anticoagulant rodenticides - A new perspective. *Proc 16th Vetebr Pest Conf* pp 138-42.

Buckle A P, Yong Y C and Abdul Rahman H (1985) Damage by rats to rice in southern-east asia with special reference to an integrated management scheme proposed for peninsular Malaysia *Acta Zool. Fennica*, 173 : 139-144.

Chaudhary V and Tripathi R S (2009) Field Efficacy of Second Generation Avticoagulant Rodenticides in Managing Rodents Pests in Poultry Farms. *Pestology* **33** (10) : 41-45.

Chopra G (1992) Production losses due to rodents and control strategies in poultry farms. In : Prakash I and Ghosh P K (eds.). *Rodents in Indian Agriculture,* pp. 309-330, Scientific Publishers, Jodhpur, India.

Chopra G and Dhindsa J S (1987) Rodent pest management in poultry farms. *Poultry Advisor* **20**(3) : 49-60.

Chopra G, Kandhwal S, Kumar P and Rai D (2008) Opinion survey of rodent manace and control in commercial poultry facilities. *J Pestology* **32** : 61-65.

Chopra G, Kaur P and Guraya S S (1996) Predominant rodent species in India. In : R Chand and Co. (ed) *Rodents : Ecology, Biology and Control*. pp. 3-19, New Delhi.

Craigmill A (1988) Hazards of new rodenticides to pets. Veterinary Toxicology. *Environ Toxicol Newsl* **8**(2) : 5.

Deluca H F (1979) Monographs on endocrinology : Vitamin D metabolism and functions pp 9-10. Springer - Verlag Berlin, Heidelberg, New York.

Dorman D C (1990) Toxicology of selected pesticides, drugs and chemicals. anticoagulants, cholealciferol and bromethalin based rodenticides. *Vet Clin North Am* (Small Anim Pract) **20**(2) : 339-352.

Drummond D C (1966) Rats resistant to Warfarin. *New Scientist* **30** : 771-72.

Dubey O P, Awasthi A K and Agarwal R K (1991) the bioefficacy of bromadiolone and flocoumafen against rodents. *Pestology* **15** : 39-42.

Dykstra W W (1996) The economic importance of commensal rodents. *Proceedings of the Seminar on Rodents and Rodent Ectoparasites,* Geneva. pp 9-14.

Eason C T (1993) The acute toxicity of cholecalciferol to the European rabbit, *oryctolagus cuniculus*. *Wildlife Res* **20** : 173-176.

Eason C T (1991) Cholecalciferol as an alternative to sodium monofluoroacetate (1080) for poisoning possums. *Proc 44th NZ Weed and Pest Control Conf* : 35-37.

Eason C T, Wickstorm M, Henderson R, Milne L and Arthur D (2000) Non-target and secondary poisoning risks associated with cholecalciferol. *NZ Plant Prot* **53** : 299-304.

Eason C T, Wright Meikle L and Elder P (1996) The persistance and secondary poisoning risks of sodium monofluoroacetate (1080), biodifacoum, and cholecalciferol in possums in Timm RM, Crabb A C (eds.) : *Proc 17th Vertebr Pest Conf.* pp 54-58. Davis, California, USA.

Eason C, Baigent D, Wilson L, Hix S, Macmorran D, Ross J, Miller A and Ogilvie S (2002) Toxicity of cholecalciferol to rats in a multi-species bait. *Sciences of conservatory* **12**: 1-9.

Fishel F M (2005) Pesticide toxicity profile : Miscellaneous rodenticides. *http://edis.ifas.ufl.edu* PI-78.

Fuhrmann R (1991) Labor-und Freilandstudien zur Wirksamkeit eines Kombinationsrodentizids mit den wirkstoffen coumatetralyl-unempfind-liche Wanderratten, *Rattus norvegicus.* Diplomarbeit, Fachhochschule fiir Technik, Sigmaringen pp 149.

Gillespie H and Myers P (2004) *Rattus rattus* (online) Animal Diversity Web at http://animaldiversity.ummz.umich/edu/site/accounts/information/*Rattus.*

Gora S, Sabhlok V P and Pasahan S C (1995) The population structure, damage and control measures of rodent in the poultry farms. *Rodent Newsl* **19**(4) : 5.

Gould E M and Holmes S J (2008) The effect of dextromethorphan in preventing cholecalciferol-induced poison shyness and sickness-induced anorexia in the laboratory Norway rat. *Pest Mgmt Sci* **64**(2) : 197-202.

Greaves J H (1985) The present status of resistance to anticoagulants. *Acta Zool Fenn* **173** : 159-62.

Greaves J H (1986) Managing Resistance to Rodenticides in Pesticide Resitance, Strategies and Tactices for Management (Committee on Strategies for Management of Pesticide Resistant Pest Population, ed.) National Academy Press, Washington, DC, pp 236-44.

Greaves J H (1994) Resistance to anticoagulant rodenticides. In : Buckle A P and Smith R H (eds). *Rodent Pests and Their Control.* pp 197-217. Wallingford : CAB International.

Greaves J H, Shepherd D S and Gill J E (1982) An investigation of difenacoum resistance in Norway rat population in Hampshire. *Annals of Applied Biology* **100** : 581-87.

Grzimek B (2003) Order Rodentia. In : Schlager N, Olendorf D and McDate (eds) *Grzimek's Animal Life Encyclopedia : Mammals.* Pp 126-28 Vol. **16**, 2nd Edition. Farmington Hills, MI : Gale group.

Gupta A K and Mangat I S (1977) Rats and mice poultry's enemy No. 1. *Poultry Guide* **14** (7) : 21-23.

Halder M R and Shadbolt R S (1975) Novel 4-hydroxy-coumarin anticoagulants active against resistant rats. *Nature* **253** : 277-82.

Halder R M (1973) Rodenticides Mittel. Ward Blenkinsop & Co. Ltd., Wembley, Middlesek. UK, DE2310636, filed March 2, 1973, published September 20, 1973.

Haydock N and Eason C T (1997) Vertebrate pest control manual - Toxins and poisons. Department of Conservation, New Zealand.

Henry R J and Dryer R L (1963) Standard methods of clinical chemistry pp 205. Acord Press, New York.

Hilbe M, Sydler T, Fischer L and Naegli H (2000) Metastatic calcification in a dog attributable to ingestion of a Tacalcital ointment. *Vet Pathol* **37** : 490-92.

Hussain I, Ahmed S B and Hassin D (2006) Importance of rodents in commercial poultry farming and their control measures. *Poultry Planner* **2** : 16-20.

Jackson W B (1979) Use of burrows for evaluating rodenticide efficacy in urban areas. *Special Tech Publ.* Pp 5-10. American Society for Testing Materials, Philadelphia.

Jackson W B (1982) Norway rat and allies. In : Chapman J A and Feldhamer G A (ed.) *Wild Mammals of North America.* Pp 1077-88. The Johns Hopkins University Press, Baltimore, USA.

Jackson W B (1987) Current management strategies for commensal rodents. In : Genoway H H (ed) *Current Mammology.* Pp 495-512. Plenum Press, New York and London.

Jackson W B and Ashton A D (1986) Case Histories of Anticoagulant Resistance. Pp 355. National Academy Press, Washington, DC.

Jackson W B and Kaukeinen D E (1972) Resistance of wild Norway rats in North Carolina to Warfarin rodenticide. *Science New York* **176** : 1343-44.

Jain A P and Tripathi R S (2000) An integrated approach to rodent pest management. In : Upadhya R K, Mukerji K G and Duby O P (ed). *IPM System in Agriculture.* Vol. **7**, Pp 275-307. Aditya Books Pvt Ltd., New Delhi, India.

Johnson R A and Prescott C V (1994) The laboratory evaluation of rodenticides. In: Buckle A P and Smith R H (ed) *Rodent Pests and their Control.* pp 161-79. Cab International, U K.

Jolly S E, Henderson R J, Frampton C and Eason C T (1995) Cholecalciferol toxicity and its enhancement by calcium carbonate in the common brushtail possum. *Wildlife Res* **22** : 579-83.

Kamath V R, Hemkar R P and Renapurkar D M (1986) Laboratory evaluation of rodenticidal activity of cholecalciferol against Indian species of commensal rats. *Pestology* **10** (1): 22-25.

Kaur G, Kocher D K, Sandhu H S and Brar R S (2008) Sex specific value of oral LD_{50} of cholecalciferol (Vitamin D_3) against house rat (*Rattus rattus*). *Toxicol Int* **15**(2) : pp 143-44.

Khatri A K and Veda O P (1984) Note on the incidence of house rat, *Rattus rattus* (Gray) in poultry farms in Jabalpur. *Poultry Advisor* **17**(3) : 47.

Kocher D K and Parshad V R (2003) Acceptance and Efficacy of baits of three rodenticides against *Rattus rattus alexandrinus*. *Pestology* **27** : 18-20.

Kocher D K, Kaur G and Sandhu H S (2008) Toxic levels of calcium and phosphorous in the serum of cholecalciferol (vitamin D_3) fed house rats (*Rattus rattus*) *Toxicology* **15** (2): 133-136.

Kocher D K, Kaur G, Banga H S and Brar R S (2010) Histopathological changes in vital organs of house rats given lethal dose of cholecalciferol (vitamin D_3). *Indian J Anim Res* 44 (3) : 193-96.

Koehler P G and Kern W H (2005) Rat and mouse control. UF/IFAS EDIS Document ENY-224 http://edis.ifas.uff.edu/DHO44.

Krishnakumari M K (1968) Studies on rodenticides and rodent repellents with special reference to the control of black rat (*Rattus rattus* L.). Ph.D. thesis, Mysore University, India, 1-230.

Krishnakumari M K and Majumder S K (1965) A new approach to rat control. *Symp Post Harvest Handling of Foodgrains, Banglore, India.* 10-14th July.

Krishnakumari M K, Yashoda L Urs and Muktha Bai K (1992) The house rat, *Rattus rattus* Linn. In : Prakash I and Ghosh P K (ed) *Rodents in Indian Agriculture.* Pp 63-94. Jodhpur Scientific Publishers.

Kurian P (2000) Crop losses to rodent pests in Kerala : A pre-harvest survey in select crop fields and survey on grain storage losses. *Discussion Paper No. 17.* Kerala Research Programme on Local Level Development Centre for Development Studies, Thiruvananthapuram.

Lasseur R, Grandemange A, Longin S C, Berny P and Benoit E (2007) Comparison of the inhibition effect of different anticoagulants on vitamin K epoxide reductase activity from warfarin –susceptible and resistant rat. *Pesticide Biochemistry and Physiology* **88** (2) : 203-208.

Linzey D W (1998) *The Mammals of Virginia.* Blacksburg Virginia: The McDonald & Woodward Publishing Company, Inc.

Lund M (1974) Calciferol as a rodenticide. *Intl Pest Control* **16** : 10-11.

Lund M (1991) Laboratory test with RACUMIN COMBI on bromadiolone - resistant brown rats. Danish Pest Infestation Laboratory, Report No. 63-1991 pp 4.

Lund M (1994) Commensal rodents. In : Buckle A P and Smith R H (ed). *Rodent Pests and Their Control.* pp 23-43. Cab International UK.

MacNicoll A D (1982) Rodent resistance. A review of studies on Warfarin resistance in rats and mice. MAFF/ADAS Agricultural Science Service Research and Development Report "Pesticide Science 1981". Reference Book **252**(81) : 38-43. HMSO, London.

MacNicoll A D (1986) Resistance to 4-hydroxycoumarin anticoagulants in rodents. In : *Pesticide Resistance*: Strategies and Trials for Management: National Academics Press, Washington D C, pp 87-99.

MacNicoll A D (1987) The influence of anticoagulant resistance on effective rodent control in the UK. *EEPO conference on rodents*, Rome.

Majumder S K (1968) Role of rodent proofing and sanitation on rodent population. *Proc Intl Symp Bionomics and Control of Rodents* (Ed Perti S L, Wal Y C and Srivastava C P). Kanpur, India : 86.

Malhi C S and Parshad V R (1993) Ridding heaven of rats : pest control in hen house. *FAO Review* 'Ceres' Italy **25**(6) : 10-13

Malhi C S and Parshad V R (1995) Comparative efficacy of three rodenticides with different baiting methods in wheat and rice crops. *Int Pest Control* **37**(2) : 55-57.

Malik N S and Ichhponani J S (1970) Control of diseases, parasites, rats and mice. *Poultry Guide,* **1**(11) : 40-43.

Markussen M D, Heiberg A C, Alsbo C, Nielsen P S and Kauppinen S (2007) Involvement of hepatic xenobiotic related genes in bromadiolone resistance in wild Norway rats, *Rattus norvegicus* (Berk). *Pesticide Biochemistry and physiology* **88** (3) : 284-295.

Marsh R and Turnberg A (1986) Characteristics of cholecalciferol. Rodent Control : Other options. *Pest Control Technology* **14** : 43-45.

Marshall E F (1984) Cholecalciferol : a unique toxicant for rodent control. *Proc 11th Vertebr Pest Conf* pp 95-98, Davis, California, USA.

Mathur R P (1997) Effectiveness of various rodent control measures in cereal crops and plantations in India. *Belg J Zool* **127**(1) : 137-44.

Mathur R P and Prakash I (1984) Field trials of bioadifacoum, chlorophacinone and coumatetralyl against desert rodents in India. *Trop Pest Mgmt* **30**(1) : 59-61.

Milan P P (1990) Evaluation of control methods for rats in Phillippines coconut plantations. *Current Mammalogy* **2**: 89.

Mills J N (1999) The role of rodents in emerging human disease : examples from hantaviruses and arenaviruses. In: Singleton G R, Hinds L A, Leirs H and Zang Z B (ed) *Ecologically based management of rodent pests*. Pp 134-60. Australian Center for International Agriculture Research, Canberra.

Misenheimer T M, Lund M, Baker A E M and Suttie J W (1994) Biochemical basis of warfarin and bromadiolone resistance in the house mouse, *Mus musculus domesticus*. *Biochemical Pharmacology* **47** (4) : 673-678.

Morrow Carla D V M (2001) Cholecalciferol poisoning. *Vet Med* **12** : 905-11.

Mukhta Bai K, Krishnakumari M K and Majumder S K (1978) Toxicity of Calciferol, Warfarin and their Combination to *Rattus norvegicus* (albino) and *R.rattus*. *Pestic Sci* **9** : 44-50.

Myers P (2000) Rodentia. Animal Diversity Web. Museum of Zoology, University of Michigan.*http://animaldiversity.ummz.umich.edu/site/accounts/information/Rodentia.html.*

Neelanarayanan P and Kanakasabai (2000) Rodent pest management-a traditional approach. *Pestology* **24** : 12 -19.

O'Connor C E and Eason C T (2000) Rodent baits and delivery systems for island protection. *Science for Conservation* **150** : 5-25.

Ogilvie S C and Eason C T (1996) Laboratory, pen and field trials of cholecalciferol for rat control. Landcare Research Contract Report LC 9596/124 (unpublished).

Pai H H, Hong Y J and Wang C H (2005) A community-based surveillance on determinants of rodent infestation. *Toxicol Rev* **24** (4) : 129-69.

Parshad V R (1992) State of the art technology for preventing major crop and grain losses due to rodents - scenario for 21st century. *Rodent Newsl* **16** : 6-11.

Parshad V R (1999a) Rodent Control in India. *Integ Pest Mgmt Rev* **4**: 97-126.

Parshad V R (1999b) *Rodent Management in Poultry Farms.* All India Coordinated Research Project on Rodent Control, Central Arid zone Research Institute, Jodhpur. *Tech Bull* No. 8, 11 p.

Parshad V R, Ahmad N and Chopra G (1987) Deterioration of poultry house environment by rodents and their control. *Ind Biodet* **21**(2) : 29-46.

Parshad V R, Kaur P and Guraya S S (1989) Reproductive cycles of mammals, Rodentia. In : Saidapur S K (ed) *Reproductive cycles of Indian Vertebrates*. Allied, New Delhi.

Parshad V R, Saini M S, Ahmad N and Malhi C S (1994) Food contamination by commensal rodents in Ludhiana : A Preliminary report. *Pestology* **18** : 30-32.

Pathak V R (1995) Efficacy of Racumin against different species of rats. *J Pestology* **19**(10) : 31-35.

Pospischil R and Schnorbach H J (1994) Racumin Plus, A New Promising Rodenticide against rats and mice. *Proc 16th Vertebrate Pest Conference* pp 180-87 University of Nebraska, Lincoln.

Prakash I and Ghosh P K (1992) *Rodents in Indian Agriculture.* Pp 707. Jodhpur Scientific Publishers.

Prakash I and Mathur R P (1987) *Management of Rodent Pests.* Pp 1-133, ICAR Publications.

Prescott C V, Elamin M and Smith R H (1992) Calciferols and bait shyness in the laboratory rat. *Proc 15th Vertbr Pest Conf,* pp 218-23 California, 1992.

Pye T, Swain R and Seppelt R D (1999) Distribution and habitat use of the feral black rat (*Rattus rattus*) Subantartic Macquarie Island. *J Zool* **247** : 429-38.

Rao K S (2005) Encyclopedia of Toxicology (second edition). Wexler P (ed) pp 338-340 National lib of medicine Bethesda MD, USA.

Redfern R and Gill J E (1980) Laboratory evaluation of bromadiolone as a rodenticide for use against warfarin-resistant and non-resistant rats and mice. *J Hyg Cambridge* **84** : 263-68.

Renapurkar D M (1993) Susceptibility of commensal rats to bromadiolone in Bombay. *Pestology* **17** : 39-43.

Renninson B D (1974) Field trials of calciferol against Warfarin resistant inestations of the Norway rat (*Rattus norvegicus* Berk). *J Hyg* **73** : 361-67.

Revathi K and Yogananda M (2006) Effect of bromadiolone on haematology, liver and kidney in *Mus musculus. J Env Biol* **27**(1) : 135-40.

Rowe F P, Plant C J and Bradfield A (1981) Trials of the anticoagulant rodenticides bromadiolone and difenacoum against the house mouse (*Mus musculus* L). *J Hyg Camb* **87** : 171-177.

Ruiz A (2004) Rodents in disasters. Article for Vaccination Program. Pan American Health Org., USA, pp : 18.

Saini M S and Parshad V R (1992) Control of *Rattus rattus* with cholecalciferol : Laboratory manual of freshly prepared and ready to use bait formulations. *Intl Biodet Biodr* **30** : 87-96.

Saunders G and Cooper K (1981) Pesticide contamination of birds in association with a mouse plague. *Emu* **82**: 227-29.

Saxena Y and Mathur A (1996) Efficacy of bromadiolone and difethiolone (anticoagulants) for the control of poison shy *M. musculus* albino. Biological Abstracts, **103** : Entry No. 109162, 1996 (Abstrs).

Saxena Y, Sahai K and Sahni K (1991) A comparative study of rodenticides on rats in fields. *J Ecotoxicology and Environ Monitor* **1 :** 65-66.

Scheftel J, Setzer S, Walser M, Pertile T, Hegstad R L, Felice L J and Murphy M J (1991) Elevated 25-hydroxy and normal 1, 25-dihydroxy-cholecalciferol serum concentrations in a successfully treated case of vitamin D_3 toxicosis in a dog. *Vet Hum Toxicol* **33**(4) : 345-48.

Schnorbach H J (1992) Ein neues Rodentizid : Ratten and Hausmause Wirksam Bekampfen. *Der Praktische Schadlingsbekampfer* **44**(11) : 262-66.

Sheikher C and Jain S D (1995) Rodenticidal efficacy of cholecalciferol. *Proc Indian Nat Vet Sci Acad Part B-Biol Sci* **61**(1) : 31-37.

Sheikher C and Jain S D (1996) Mode of application and performance of rodenticides in vegetable crops. *Int J Agr Sci* **66**(7) : 437-40.

Singh R and Saxena Y (1989) The susceptibility of house rat to some anticoagulant rodenticides. *Pesticide Bombay* **23**(3) : 27-28, 30.

Singh S, Bansal M L, Singh T P and Kumar R (2004) *Statistical Methods for Research workers.* Kalyani Publishers, New Delhi.

Singleton R G, Smythe L, Smith G, Spratt D M, Aplin K and Smith A L (2003) Rodent diseases in sSouth East Asia and Australia. *Biol Linn Sol* **84**: 565-83.

Soni B K and Prakash I (1981) Laboratory evaluatiuon of WBA 8119 (Brodifacoum) against *Gerbillus gleadowl* Murray. *Pestology* **5**(9) : 23-24.

Sridhara S and Krishnamurthy T R (1992) Potential of Anticoagulant Toxicity to *Rattus rattus*. *Proc 15th Vertebre Pest Conf* pp 212-17. University of Nebraska-Lincoln.

Sullivan L (2007) *Commensal rodents.* http://cals.arizona.edu/yavapai.

Taley Y M, Sharnagat B K, Patil K J, Sarode S V (1987) Evaluation of Bromadiolone against field and house rats. *Pestology* **11** (9) : 21-27.

Tanikawa T and Kusano T (1993) Acute toxicity of a mixture of warfarin and vitamin D_3 to laboratory rats. *Japanese J Sanitary Zoology* **44**(3) : 197-201.

Tischler A S, Powers J F, Pignatello M, Tsokas P, Downing J C and McClain R M (1999) Vitamin D_3-Induced Proliferative Lesions in the Rat Adrenal Medulla. *Toxicol Sci* **51** : 9-18.

Tobin M E, Matschke C H, Sugihara R T, McCann C R, Koehler A E and Andrews K T (1993) Laboratory efficacy of cholecalciferol against field rodents USDA Animal and Plant Health Inspection Services DWRS Research Report No. 11-55-002 pp. 15.

Tripathi R S, Jain A P, Kashyap N, Rana B D and Prakash I (1992) North Western Desert. In : Prakash I and Ghosh P K (ed) **.** *Rodents in Indian Agriculture,* vol 1Pp 357-96. Scientific Publishers, Jodhpur.

Vuksa M, Jokic G and Edovic S (2007) I P M strategy for rodent control in storage facilities. *Biotechnology in Animal Husbandary* **23**(5/6) : 375-82.

Vyas H J, Vyas H N and Singh B M (1985) Evaluation of Biomadiolone - A single dose anticoagulant against field and domestic rats. *Indian J Ent* **47**(1) : 45-48.

Walker (1964) *Mammals of the World.* Vol **2** : 665-973 The Johns Hopkins Press, Baltimore, USA.

Weber W J (1982) *Diseases Transmitted by Rats and Mice*. Pp 182. Thomson pub. Fresno, California, USA.

WHO (1974) Ecology and control of rodents of Public Health Importance. *WHO Technical Report*, Services No. **553**: 1-2.

Yi J, Duan J, Lin L, Zhang S, Liang L and Yin W (2009) Primary study on the resistance of *Rattus rattus sladeni* to anticoagulant rodenticide in Guangzhou. Chinese *J of Vector Biology and Control* **20** (4) : 317-318.

Zatsepin V G, Kadirov A F and Klement-eva S A (2006) Resistance of rats to first generation anticoagulants and ways of overcoming it using synergists. *Russ Agri Sci* **9** : 39-42.

Zhang Z B, Hinds L, Singleton G and Wang Z W (1999) Rodent biology and management. *ACIAR-Technical Reports Series* **45** : 146.

VITA

Name of the student	:	Navjot Kaur
Father's name	:	S. Avtar Singh
Mother's name	:	Sdn. Karamjeet Kaur
Nationality	:	Indian
Date of birth	:	25-11-1986
Permanent home address	:	VPO – Bhadalwad Teh/Distt – Barnala, 148101, Punjab

EDUCATIONAL QUALIFICATION

Bachelor degree	:	B.Sc.
University and year of award	:	Panjab University, Chandigarh, 2008
% marks	:	70.40
Master's degree	:	M.Sc. (Zoology)
University and year of award	:	Punjab Agricultural University, Ludhiana, 2010
OCPA	:	7.32/10.00
Title of Master's Thesis	:	STUDY ON COMBINED EFFECT OF BROMADIOLONE AND CHOLECALCIFEROL (VITAMIN D_3) AGAINST HOUSE RAT, *Rattus rattus* LINNAEUS