

Biorational Approach for *Rhyzopertha dominica* Control in Storage System for *Triticum aestivum*

PRACHI SINGH



CENTRE FOR RURAL DEVELOPMENT AND TECHNOLOGY

INDIAN INSTITUTE OF TECHNOLOGY DELHI

OCTOBER 2018

Biorational Approach for *Rhyzopertha dominica* Control in Storage System for *Triticum aestivum*

by

PRACHI SINGH

Centre For Rural Development And Technology

Submitted

In fulfillment of the requirements of the degree of Doctor of Philosophy

to the



INDIAN INSTITUTE OF TECHNOLOGY DELHI

OCTOBER 2018

CERTIFICATE

This is to certify that the thesis entitled, “**Biorational approach for *Rhyzopertha dominica* control in storage system for *Triticum aestivum***” being submitted by **Ms. Prachi Singh** to the **Indian Institute of Technology Delhi** for the award of ‘**Doctor of Philosophy**’ is a record of bonafide research work carried out by her. She has worked under our guidance and supervision, and has fulfilled the requirements for the submission of this thesis. To the best of our knowledge, the results contained in this thesis have not been submitted, in part or full, to any other university or institute for the award of any degree or diploma.

(Dr. S. N. Naik)

Professor

Centre for Rural Development and Technology
Indian Institute of Technology Delhi

(Dr. Santosh Satya)

Professor

Centre for Rural Development and Technology
Indian Institute of Technology Delhi

Acknowledgements

At the outset, I would like to thank whole heartedly my supervisors, Prof. Santosh Satya and Prof. S. N. Naik, Centre for Rural Development and Technology for paving the way towards successful completion of this research work. Their indepth knowledge and deep fervour for research has helped me a lot. My thesis is an outcome of their novel ideas, constructive suggestions and careful editing. It is my privilege to work under their able guidance.

I am also thankful to Prof. Satyawati Sharma (Former Head) and Prof. V.K. Vijay, Head, Centre for Rural Development and Technology for providing me with facilities and financial approval as and when required for my research work. I thank my SRC members – Prof. Rajendra Prasad, Prof. K.K. Pant and Prof. Anushree Malik for their valuable suggestions and comments during my progress presentations which have strengthened my research findings.

I would like to extend my heartfelt thanks to Director, Indian Grain Management Research Institute (IGMRI), Hapur (U.P.) for granting me permission to use their lab facilities. I am thankful to Mr. Sunil Sharma, Lab technician, IGMRI for the help during experimentation. I thank Dr. P. K. Patanjali and Dr Saurabh Dubey, Institute of Pesticide Formulation Technology (IPFT) for providing me training on development of bioformulations.

I am indebted to my Husband – Mr. Anil Malik for standing by my side and lending a sympathetic ear through all odds. Constant encouragement from my in laws' side is also appreciated. Being from an altogether different field of study, they boosted me to be determined to achieve my goals.

I profusely thank my parents and siblings - Ms. Chhaya Singh, Ms. Ritu Singh and Mr. Pratap Singh for their wise counsel and unconditional support. Chhaya didi, your perspective that the spirit of resilience reigns supreme, has pushed me through all ups and downs. You have always been there for me! Thanks a lot.

I am gratified to have such great friends from other departments who had never allowed pessimism to sink in. Special thanks to Dr. Swati Sidana, Dr. Mainisha, Dr. Rabia (Maths Deptt), Dr. Pankaj Parashar, Ms. Avinash Kaur (CBME), Ms. Namrata Pachori, Ms. Deachan, Mr. Aditya Gokhale and Dr. Sanjeet Kumar. Transcending the boundaries of cultural difference, I would like to extend my heartfelt gratitude to my friends- Dr. Linda from Nigeria, Dr. Jakob from Denmark and Dr. Martiros from Armenia who have amused me with their PhD tales and have boosted my morale to keep going.

Thanks a ton to Dr. S. Kumar, my senior who has pacified me in tough times. I gratefully acknowledge Ms. Vinod Kotwal for guiding me and keeping me in high spirits. Thanks for the numerous level discussions held at CCD along with chilled Caffè frappe and sumptuous pie. Ma'am, there is a lot to learn from you – a person with Type A Personality.

I also acknowledge and thank the cooperation extended by my labmates (Dr. Neeru Kadian, Mr. Ajay Kumar, Mr. Umesh Sharma, Mr. Mukesh Jain, Dr. Perminder Jit Kaur, Dr. Vikas Kardam, Ms. Arjoo, Ms. Monu), CRDT office staff and lab attendants (Mr. Gopi Mr. Satender and Mr. Lokender).

Last but definitely not the least, I am thankful to Almighty for showering His blessings and enabling me to complete the research work.

Prachi Singh

Abstract

Wheat (*T.aestivum*) is an important dietary component serving as a good source of carbohydrates, B vitamins and some minerals. In order to preserve the quality, viability of grains and maintain the food supply, they are stored either at domestic level or large scale centralized system. During storage, the wheat grains are susceptible to insect-pest attack especially coleopteran species. Out of these species, *Rhyzopertha dominica* is the most dangerous insect-pest, an internal feeder which can damage both sound and cracked kernel. Infestation depreciates the grain mass and its nutritional as well as aesthetic value rendering it unfit for human consumption.

In the prevailing agricultural scenario, chemical pesticides are being used to mitigate the post-harvest losses. Aluminium phosphide is a chemical fumigant being widely used at domestic level. However, the excessive use of these insect control agents has led to pest resistance and pesticide residue in food commodity. These synthetic pesticides have deep negative impact on human health. Researchers worldwide, are exploring safer alternatives to chemical pesticides. In this background, present study focuses on following main aspects i) Effect of *R. dominica* infestation on quality parameters of wheat under storage ii) Development of suitable bioformulation against *R. dominica* and its testing under laboratory as well as simulated field conditions. In addition to this, a preliminary investigation on spatio-temporal movement of *R. dominica* to comprehend its biological characteristics useful for designing storage bins has been carried out.

Effect of *R. dominica* infestation on quality parameters of wheat under storage was studied by following standard protocols. At 50% level of *R. dominica* infestation, carbohydrate, protein and gluten content of infested wheat was found to decrease by 19.77%, 8.06% and 54.8%, respectively. 3-D profilometry using optical microscopy and SEM analysis have been performed to indicate zone of depth and cracks/ cavities within damaged layer of wheat grains, respectively. Spatio-temporal movement studies revealed that *R. dominica* adults prefer to colonize in the upper mass of the glass unit.

Bioefficacy screening of five essential oils (*M. arvensis*, *A. graveolens*, *C. longa*, *C. citratus* and *C. sinensis*) for their pesticidal potential against *R. dominica* was carried out by employing different bioassays (contact toxicity, fumigation and repellency). GC–MS analysis of selected essential oils was also performed to understand the chemical composition. The results from our study reveal that out of selected essential oils, *M. arvensis* essential oil showed the highest insecticidal potential as contact toxicant and fumigant against *R. dominica* population resulting in more than 85 % mortality. However, its effectiveness persists only for a few days.

However, literature document that essential oils' effectiveness reduces over time owing to their volatilization and decomposition. Therefore, spontaneous emulsification method was employed to develop *M. arvensis* EO based oil-in-water (O/W) nanoemulsion. Physico-chemical characterization of the developed nanoemulsion was performed. Particle size analysis reveals an average droplet size of 11.98 nm with unimodal distribution and rheological studies indicate viscosity of 140 cP. SEM and TEM analysis showed discrete and spherical morphology of the droplets. *Mentha arvensis* essential oil based nanoemulsion resulted in 85% mortality of *R. dominica* adults at 2 mg/ml dose after 72 h of exposure. In order to further

enhance the insecticidal property of developed nanoemulsion, synergistic combinations of other essential oils (*A. graveolens* and *C. longa*) and incorporation of aqueous filtrates of *Karnaja* (*P. pinnata*) de-oiled cake and *Neem* (*A. indica*) de-oiled cake in the developed bioformulation were also tried. However, these attempts failed to further improve the mortality percentage of model pest.

Finally, in order to achieve pronounced insecticidal property of developed nanoemulsion, 0.5% copperized *neem* oil was incorporated in 4.5% mentha oil and a novel integrated nanoemulsion was prepared. The integrated bioformulation resulted in ~ 92% mortality of *R. dominica* adults at 2 mg/ml dose after 72 h of exposure. SEM analysis revealed spherical morphology of nanoemulsion particles and ocular inspection showed that there was no colour change and phase separation. Thus, it was found to be stable. Small contact angle (32.93°) of formulation with galvanized steel surface proved its good wettability and surface adherence which are important aspects for field applications.

Shelf life studies depicted that menthol - the bioactive constituent of newly developed integrated nanoemulsion reduced by 4%, 17% and 31% at 10 °C, 30 °C and 40 °C, respectively over storage period of 02 months. Release kinetics study of the above emulsion from jute matrix was investigated under different temperature conditions. Retention of menthol (bioactive constituent) was found to be maximum at 10 °C (60%) followed by 30 °C (15%) and least at 40 °C (02%). The physico-chemical characterization of freshly prepared nanoemulsion and after 02 months of storage under different temperatures indicated the stability of the developed bioformulation. GC–MS analysis of nanoemulsion confirmed the entrapment of bio-active constituent – menthol in same concentration (65%) as present in pure essential oil.

The newly developed bioformulation exhibited ~ 92% mortality of *R. dominica* in contact toxicity bioassay. Further, in experiments under simulated field conditions, it proved to be successful in controlling the artificial infestation in wheat grains stored in small jute bags and in galvanized steel bins. The cytotoxic effect of developed formulations i.e. *M. arvensis* EO based nanoemulsion and integrated formulation was investigated using MTT assay on NIH3T3 fibroblast cell lines. The nanoformulation showed 73.7% and 50.7% cell viability at 3.0 mg/ml and 5.0 mg/ml concentration, respectively. The expected concentration to cause 95% insect mortality is slightly less than 3.0 mg/ml (2.77 mg/ml). Therefore, the integrated formulation at the selected dose applied in storage bins or bags are unlikely to cause any toxicity.

Overall, the results of the present study would provide a green alternative for domestic level grain storage system in rural sector. In fact these research findings form small steps in the journey of paradigm shift related to food safety.

सारांश

गेहूं (*T. aestivum*) एक महत्वपूर्ण आहार घटक है जो कार्बोहाइड्रेट, बी विटामिन और कुछ खनिजों का अच्छा स्रोत है। खाद्य आपूर्ति और गेहूं की गुणवत्ता को बनाए रखने के लिए, घरेलू स्तर या बड़े पैमाने (केंद्रीकृत प्रणाली) पर इसका भंडारण किया जाता है। सामान्य रूप से भंडारण के दौरान, गेहूं में *Rhyzopertha dominica* (Coleopteran प्रजाति) का संक्रमण हो जाता है। यह एक आंतरिक फीडर है जो साबुत व टूटे हुये दाने को नुकसान पहुंचाता है। कीट संक्रमण अनाज के भार और इसके पोषक तत्वों को कम करता है जो इसे मानव उपयोग के लिए अनुपयुक्त बनाता है।

वर्तमान कृषि परिदृश्य में, सुरक्षित अनाज भंडारण हेतु रासायनिक कीटनाशकों का उपयोग किया जा रहा है। एल्यूमीनियम फॉस्फाइड घरेलू स्तर पर व्यापक रूप से उपयोग किया जा रहा है। इन कीट नियंत्रण पदार्थों के अत्यधिक उपयोग से खाद्य वस्तुओं में कीटनाशक अवशेष रह जाते हैं तथा कीट प्रतिरोध विकसित हो जाता है। इन रासायनिक कीटनाशकों का मानव स्वास्थ्य पर गहरा नकारात्मक प्रभाव पड़ता है। इसीलिए दुनिया भर में शोधकर्ता, रासायनिक कीटनाशकों के लिए सुरक्षित जैव विकल्प तलाश रहे हैं। इस पृष्ठभूमि में, वर्तमान अध्ययन निम्नलिखित मुख्य पहलुओं पर केंद्रित है i) भंडारण के दौरान गेहूं के गुणवत्ता मानकों पर *R. dominica* संक्रमण का प्रभाव ii) *R. dominica* के नियंत्रण के लिए उपयुक्त जैव निरूपण (Bio-formulation) विकसित करना और प्रयोगशाला स्तर के साथ अनुरूपित क्षेत्रीय परिस्थिति के तहत इसका परीक्षण। इसके अतिरिक्त, भंडारण हेतु टंकी डिजाइन के लिए उपयोगी, कीट की जैविक विशेषताओं को समझने के लिए *R. dominica* के स्थानिक-अस्थायी गति (spatio-temporal movement) पर प्रारंभिक अन्वेषण किया गया है। भंडारित गेहूं के गुणवत्ता मानकों पर *R. dominica* का प्रभाव स्थापित प्रोटोकॉल द्वारा अध्ययन किया गया। 50% संक्रमण स्तर पर *R. dominica* संक्रमित गेहूं का कार्बोहाइड्रेट, प्रोटीन और ग्लूटेन में क्रमशः 19.77%, 8.06% और 54.8% की कमी आई है। 3-D profilometry ऑप्टिकल माइक्रोस्कोपी और SEM विश्लेषण का उपयोग गेहूं अनाज की क्षतिग्रस्त परत के भीतर गहराई और दरारों को इंगित करने के लिए किया गया है। स्थानिक-अस्थायी गति के अध्ययन से पता चला कि वयस्क *R. dominica* ग्लास इकाई के ऊपरी अनाज परत में एकत्रित होना पसंद करते हैं।

R. dominica के विरुद्ध कीटनाशक क्षमता के लिए पांच सुगन्धित तेलों (*M. arvensis*, *A. graveolens*, *C. longa*, *C. citratus* और *C. sinensis*) की जैव सुरक्षा परीक्षण विभिन्न bioassay (contact toxicity, fumigation और repellency) द्वारा किया गया है। रासायनिक संरचना को समझने के लिए चयनित सुगन्धित तेलों का GC-MS विश्लेषण भी किया गया है। हमारे अध्ययन के परिणाम बताते हैं कि चयनित सुगन्धित तेलों में से *M. arvensis* तेल उच्चतम दक्षता वाला कीटनाशक है। इसके द्वारा प्रयोगशाला परीक्षण में *R. dominica* का 85% मृत्युदर प्राप्त हुआ। लेकिन इसकी प्रभावशीलता केवल कुछ दिनों तक ही कायम रहती है, ऐसा साहित्य में भी वर्णित है। इसलिए, *M. arvensis* तेल आधारित आयिल-इन-वॉटर (o/w) नैनोइमल्सन स्वचालित इमल्सीफिकेशन विधि द्वारा बनाया गया। विकसित नैनोइमल्सन का भौतिक-रासायनिक विश्लेषण किया गया। इसका कण आकार 11.98 nm पाया गया तथा इसके प्रवाही गुण अध्ययन में इसकी श्यानता 140 cP पायी गयी। SEM और TEM विश्लेषण ने बूंदों का गोलीय आकार दर्शाया।

2 mg/ml सांद्रता वाले *M. arvensis* तेल आधारित नैनोइमल्सन से 72 घंटों के बाद *R. dominica* वयस्कों की 85% मृत्यु दर प्राप्त हुई। इस नैनोइमल्सन की कीटनाशक क्षमता को आगे बढ़ाने के लिए अन्य सुगन्धित तेलों (*A. graveolens* और *C. longa*) और करंज (*P. pinnata*) व नीम (*A. indica*) खल के जलीय खनक को विकसित नैनोइमल्सन में मिलाया गया। हालांकि, ये प्रयास कीट के मृत्यु दर वृद्धि में असफल रहे।

अंततः विकसित नैनोइमल्सन की वर्धित कीटनाशक क्षमता प्राप्त करने के लिए, 0.5% कॉपरआइज्ड नीम का तेल 4.5% मेन्था तेल में मिलाया गया और एक नवीन एकीकृत नैनोइमल्सन तैयार किया गया। 2 mg/ml सांद्रता वाले एकीकृत बायोफॉर्म्यूलेशन ने 72 घंटों के बाद *R. dominica* वयस्कों की ~ 92% मृत्यु दर प्राप्त की। SEM विश्लेषण ने बूंदों का गोलीय आकार दर्शाया। इस बायोफॉर्म्यूलेशन का नियमित निरीक्षण किया गया, इसमें कोई रंग परिवर्तन या चरण पृथक्करण नहीं पाया गया। गैल्वनाइज्ड स्टील सतह के साथ फॉर्म्यूलेशन के छोटे संपर्क कोण (32.93 °) इसकी उत्तम फैलाव और सतह पर चिपकना प्रमाणित करते हैं जो बड़े स्तर पर इसके अनुप्रयोगों के लिए महत्वपूर्ण पहलू हैं।

भण्डारण सेवा अवधि अध्ययन ने दर्शाया कि दो महीने की अवधि के बाद 10 °C, 30 °C और 40 °C पर बायोफॉर्म्यूलेशन की मेन्थॉल मात्रा 4%, 17% और 31% कम हो गयी। इस बायोफॉर्म्यूलेशन का समय बद्ध प्रभावशीलता गतिज (release kinetics) अध्ययन विभिन्न तापमान पर जूट मैट्रिक्स में किया गया। 10 °C, 30 °C और 40 °C पर 60%, 15% और 02% मेन्थॉल (बायोएक्टिव घटक) जूट मैट्रिक्स में शेष पाया गया। ताजा तैयार नैनोइमल्सन के भौतिक-रासायनिक विश्लेषण और विभिन्न तापमान पर 02 महीने के भंडारण के बाद इस बायोफॉर्म्यूलेशन की स्थिरता का प्रमाण मिलता है। शुद्ध सुगन्धित तेल और नैनोइमल्सन के GC-MS विश्लेषण ने मेन्थॉल की समान मात्रा (65%) की पुष्टि की।

नवीन एकीकृत नैनोइमल्सन से संपर्क विषाक्तता bioassay में *R. dominica* का ~ 92% मृत्यु दर प्राप्त हुआ। अनुरूपित क्षेत्र की स्थितियों (छोटे स्केल) के आधारित प्रयोगों में भी इस बायोफॉर्म्यूलेशन ने उच्चतम कीट नियंत्रण दर्शाया। बायोफॉर्म्यूलेशन का कोशिका विषाक्तता प्रभाव MTT assay द्वारा NIH3T3 फाइब्रोब्लास्ट कोशिका पर किया गया। नैनोफॉर्म्यूलेशन ने क्रमशः 3.0 mg/ml और 5.0 mg/ml सांद्रता पर 73.7% और 50.7% कोशिका viability दिखायी। 95% कीट मृत्यु दर के लिए चयनित सांद्रता (2.77 mg/ml) अपेक्षित सांद्रता (3.0 mg/ml) से कम है। अतः चयनित सांद्रता का भंडारण टंकी या बैग में प्रयोग से किसी भी प्रकार की विषाक्तता होने की संभावना नहीं है।

कुल मिलाकर, वर्तमान शोध कार्य ग्रामीण क्षेत्र में घरेलू स्तर पर अनाज भंडारण के लिए जैव विकल्प प्रदान करता है। वास्तव में ये शोध परिणाम खाद्य सुरक्षा से संबंधित 'चिंतन मनन परिवर्तन' की यात्रा में एक छोटा सा कदम है।

Contents

	Page No.
Certificate	i
Acknowledgements	ii-iii
Abstract	iv-v
List of Figures	x-xi
List of Tables	xii
Chapter 1	Introduction
	1-12
1.1	Wheat production scenario in India
	1
1.2	India's wheat growing zones
	3
1.3	Wheat species grown in India
	3
1.4	Diversity of food products prepared from wheat
	4
1.5	Grain storage system in India
	5
1.5.1	Domestic level system
	5
1.5.2	Large scale centralized system
	6
1.6	Interventions to reduce storage losses due to infestation in centralized system and household level
	7
1.6.1	Large scale centralized grain storage system
	8
1.6.2	Small scale decentralized grain storage system
	10
1.7	Motivation
	11
1.8	Objectives
	11
1.9	Methodology and Scope
	12
Chapter 2	Literature Review
	13-30
2.1	Storage Pests of Wheat
	13
2.1.1	Life cycle of <i>R. dominica</i>
	14
2.1.2	Spatio – temporal studies on storage pests
	16
2.2	Effect of <i>R. dominica</i> infestation on nutritional quality of wheat
	19
2.2.1	Carbohydrate content
	20
2.2.2	Protein content
	20
2.2.3	Starch digestibility
	21
2.2.4	Protein digestibility
	21
2.2.5	Uric acid content and organoleptic characteristics
	22
2.3	Essential Oils for controlling storage pests
	22
2.3.1	Mentha essential oil against wheat storage pests
	25
2.3.2	Synergistic effect with Mentha essential oil
	27
2.4	Essential oil based formulations and their applications
	28
2.4.1	Integrated bioformulations
	29
Chapter 3	Materials and Methods
	31-43
3.1	Rearing of <i>R. dominica</i>
	31
3.2	Quality parameter assessment of <i>R. dominica</i> infested and uninfested wheat grains
	31
3.2.1	Sample preparation and analysis
	31

3.2.2	Surface imaging and 3-D profiling of grains of <i>R. dominica</i> infested grains	32
3.3	Spatio-temporal movement of model test insect	32
3.4	Selection of essential oils and their characterization by Gas Chromatography- Mass Spectrometry (GC-MS)	34
3.5	Bioassays Experiments	34
3.5.1	Contact toxicity bioassay	34
3.5.2	Fumigation bioassay	35
3.5.3	Repellency bioassay	35
3.6	Development of oil-in-water (O/W) nanoemulsion	36
3.6.1	Mentha essential oil based nanoemulsion	37
3.6.2	Physico-chemical characterization of <i>Mentha arvensis</i> essential oil based nanoemulsion	37
3.6.2.1	Particle size measurement	37
3.6.2.2	Viscosity measurement	38
3.6.2.3	Microscopic analysis	38
3.7	Enhancement of insecticidal effectiveness of developed nanoemulsion	39
3.8	<i>Mentha arvensis</i> essential oil based integrated copperized neem oil nanoemulsion	40
3.9	Physicochemical characterization of newly developed bioformulation	40
3.10	Surface tension and contact angle study	41
3.11	Cytotoxicity studies and storage stability	41
3.11.1	Cytotoxicity test	41
3.11.2	Shelf life study of nanoemulsion	42
3.11.3	Release kinetics of nanoemulsion	42
3.12	Bioefficacy evaluation of newly developed integrated nanoemulsion under simulated field conditions	42
3.12.1	Jute bags	42
3.12.2	Galvanized stainless steel bins	43
3.13	Statistical Analysis	43

Results and Discussion

Chapter 4	Effect of <i>R. dominica</i> infestation on quality parameters of wheat under storage	44-53
4.1	Effect of <i>R. dominica</i> infestation on physico-chemical parameters of wheat	44
4.1.1	Carbohydrate content	44
4.1.2	Protein content	45
4.1.3	Gluten content	46
4.1.4	Free fatty acidity	47
4.1.5	Alcoholic acidity	48
4.1.6	Moisture content	48
4.2	Microscopic analysis of <i>R. dominica</i> infested wheat samples	48
4.2.1	Optical Microscopy	49
4.2.2	Scanning Electron Microscopy (SEM)	50

4.3	Preliminary studies on spatio-temporal movement of <i>R. dominica</i>	51
Chapter 5	Bioefficacy screening of selected essential oils for their pesticidal potential against <i>R. dominica</i>	54-71
5.1	Characterization of selected essential oils	54
5.2	Bioassays for screening bioefficacy of selected essential oils	61
5.2.1	Contact toxicity	61
5.2.2	Fumigant toxicity	66
5.2.3	Repellency	69
Chapter 6	Development of <i>Mentha arvensis</i> essential oil based nanoemulsions and their bioefficacy evaluation against <i>R. dominica</i>	72-85
6.1	<i>Mentha arvensis</i> essential oil based nanoemulsion	72
6.1.1	Electron microscopic analysis of <i>Mentha arvensis</i> EO based nanoemulsion (F4)	75
6.1.2	Insecticidal activity of <i>Mentha arvensis</i> EO based nanoemulsion (F4)	76
6.2	Synergistic combination of <i>Mentha arvensis</i> EO with <i>Anethum graveolens</i> and <i>Curcuma longa</i> EO for nanoemulsion development	77
6.2.1	Insecticidal activity of blended essential oils based nanoemulsions	77
6.3	Incorporation of aqueous filtrate of <i>karanja</i> de-oiled cake (KDOC) and <i>neem</i> de-oiled cake (NDOC) in <i>Mentha arvensis</i> EO based nanoemulsion	78
6.3.1	Insecticidal activity of <i>Mentha arvensis</i> essential oil based nanoemulsion incorporated with varying concentration of aqueous filtrates of <i>neem</i> de-oiled cake (NDOC) and <i>karanja</i> de-oiled cake (KDOC)	80
Chapter 7	Development of a novel integrated bioformulation and its testing under laboratory as well as simulated field conditions	86-102
7.1	<i>Mentha arvensis</i> essential oil based integrated copperized <i>neem</i> oil nanoemulsion	86
7.2	Insecticidal activity of <i>Mentha arvensis</i> essential oil based integrated copperized <i>neem</i> oil nanoemulsion	87
7.3	Physico-chemical characterization of <i>Mentha arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	90
7.4	Cytotoxicity evaluation of <i>Mentha arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	92
7.5	Gas Chromatography - Mass Spectrometry (GC - MS) analysis of <i>Mentha arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	94
7.6	Scanning Electron Microscopy (SEM) Analysis	95

7.7	Shelf life study of <i>Mentha arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	96
7.8	Release kinetics of <i>Mentha arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	97
7.9	Bioefficacy evaluation of <i>Mentha arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion under lab and simulated field conditions (small scale)	98
Chapter 8	Summary and Conclusion	103-109
	References	110-132
	Appendix I, II and III	133-139
	Biodata	

List of Figures

	Page No.
Figure 1.1. Major wheat producing states of India and their contribution in national wheat production	2
Figure 1.2. Wheat production and its cultivation area in India	2
Figure 1.3. Relationship between temperature and moisture content conducive for various insect –pest reproduction and growth of microorganisms in stored cereals	8
Figure 2.1. Life cycle of <i>R. dominica</i>	15
Figure 3.1. (a) Glass model fabricated for studying spatio-temporal dynamics of <i>R. dominica</i> (b) Top view of the mesh separating the units (c) Mesh size measurement (d) Grain filled units	33
Figure 3.2. Olfactometer for studying the repellent effect of essential oils	35
Figure 3.3. Mechanism of nanoemulsion preparation	36
Figure 3.4. Composition of developed nanoemulsion	37
Figure 3.5. (a) Surface tension and (b) contact angle measurement of integrated formulation on galvanized steel surfaces	41
Figure 4.1. 3-D Profilometry by optical microscopy analysis of wheat kernels of samples subjected to (a) 25% (b) 50% (c) 75% level of <i>R. dominica</i> infestation	49-50
Figure 4.2. SEM analysis of wheat kernels of samples subjected to (a) 25% (b) 50% (c) 75% level of <i>R. dominica</i> infestation	50
Figure 4.3. Schematic representation of spatio-temporal dynamics of <i>R. dominica</i> in glass model	52
Figure 4.4. Pictorial view of spatio-temporal dynamics of <i>R. dominica</i> in glass model	52
Figure 5.1. Contact toxicity exhibited by <i>M. arvensis</i> essential oil against <i>R. dominica</i> adults	61
Figure 5.2. Contact toxicity exhibited by <i>A. graveolens</i> essential oil against <i>R. dominica</i> adults	62
Figure 5.3. Contact toxicity exhibited by <i>C. longa</i> essential oil against <i>R. dominica</i> adults	62
Figure 5.4. Contact toxicity exhibited by <i>C. citratus</i> essential oil against <i>R. dominica</i> adults	62
Figure 5.5. Contact toxicity exhibited by <i>C. sinensis</i> essential oil against <i>R. dominica</i> adults	63
Figure 5.6. Fumigant toxicity exhibited by <i>M. arvensis</i> essential oil against <i>R. dominica</i> adults	66
Figure 5.7. Fumigant toxicity exhibited by <i>A. graveolens</i> essential oil against <i>R. dominica</i> adults	66
Figure 5.8. Fumigant toxicity exhibited by <i>C. longa</i> essential oil against <i>R. dominica</i> adults	67
Figure 5.9. Fumigant toxicity exhibited by <i>C. citratus</i> essential oil against <i>R. dominica</i> adults	67
Figure 5.10. Fumigant toxicity exhibited by <i>C. sinensis</i> essential oil against <i>R. dominica</i> adults	67
Figure 6.1. <i>M. arvensis</i> essential oil based formulations with varying surfactant concentration	73
Figure 6.2. Effect of surfactant concentration on particle size of <i>M. arvensis</i> essential oil based formulations	74

Figure 6.3.	Effect of surfactant concentration on viscosity of <i>M. arvensis</i> essential oil based formulations	75
Figure 6.4.	SEM and TEM Micrograph of 5% <i>M. arvensis</i> based nanoemulsion (F4)	76
Figure 6.5.	Contact toxicity of 5% <i>M. arvensis</i> based nanoemulsion (F4) exhibited against <i>R. dominica</i> adults	76
Figure 6.6.	Contact toxicity of nanoemulsion 'D' against <i>R. dominica</i> adults	78
Figure 6.7.	Contact toxicity of nanoemulsion 'T' against <i>R. dominica</i> adults	78
Figure 6.8.	<i>M. arvensis</i> essential oil based nanoemulsion incorporated with varying concentration of aqueous filtrates of <i>neem</i> de-oiled cake and <i>karanja</i> de-oiled cake	79
Figure 6.9.	<i>M. arvensis</i> essential oil based nanoemulsion incorporated with varying concentration of aqueous filtrates of <i>neem</i> de-oiled cake (a) 1 % NDOC (b) 5 % NDOC (c) 10 % NDOC	80
Figure 6.10.	Contact toxicity of (a) KDOC aqueous filtrate (b) NDOC aqueous filtrate (c) combination of NDOC and KDOC aqueous filtrate (1:1) against <i>R. dominica</i> adults	81
Figure 6.11.	Contact toxicity of <i>M. arvensis</i> essential oil based nanoemulsion incorporated with (a) 1 % (b) 5 % (c) 10 % (d) 30 % (e) 50 % (f) 65 % of NDOC and KDOC aqueous filtrate against <i>R. dominica</i> adults	83
Figure 6.12.	Contact toxicity of <i>M. arvensis</i> essential oil based nanoemulsion incorporated with (a) 1% (b) 5% and (c) 10% of NDOC aqueous filtrate against <i>R. dominica</i> adults	84
Figure 7.1.	Contact toxicity of <i>M. arvensis</i> essential oil based integrated copperized neem oil (having 0.2 % of copper naphthenate) nanoemulsion	87
Figure 7.2.	Contact toxicity of <i>M. arvensis</i> essential oil based integrated copperized neem oil (having 0.3 % of copper naphthenate) nanoemulsion	87
Figure 7.3.	Contact toxicity of <i>M. arvensis</i> essential oil based integrated copperized neem oil (having 0.4 % of copper naphthenate) nanoemulsion	88
Figure 7.4.	Physical appearance of integrated formulation after (a) 1 week (b) 4 week (c) 8 week incubation at different temperatures (10 °C, 30 °C and 40 °C)	90
Figure 7.5.	Cytotoxic effects of <i>M. arvensis</i> essential oil based nanoemulsion and copperized <i>neem</i> oil integrated formulation on NIH3T3 fibroblast cell lines	93
Figure 7.6.	SEM micrographs of <i>M. arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	96
Figure 7.7.	Release kinetics of <i>M. arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	98
Figure 7.8.	Testing of integrated formulation under small scale simulated field conditions (a) jute bags (b) galvanized steel bins	100
Figure 7.9.	Pictorial view of newly designed galvanized steel storage bin	101

List of Tables

	Page No.
Table 1.1. Wheat species grown in India and their approximate area	3
Table 1.2. Chemical pesticides used in large scale grain storage system	9
Table 3.1. Analytical methods employed for quality parameters determination in wheat grains	32
Table 3.2. Synergistic combinations of <i>Mentha arvensis</i> essential oil with aqueous filtrates of de - oiled cakes (<i>karanja</i> and <i>neem</i>)	40
Table 4.1. Effect of <i>R. dominica</i> infestation on physico-chemical parameters of wheat (PBW-373)	46
Table 5.1. Chemical profile of <i>M. arvensis</i> essential oil	55
Table 5.2. Chemical profile of <i>C. longa</i> essential oil	56
Table 5.3. Chemical profile of <i>C. citartus</i> essential oil	57
Table 5.4. Chemical profile of <i>C. sinensis</i> essential oil	58
Table 5.5. Chemical profile of <i>A. graveolens</i> essential oil	59
Table 5.6. Repellency of selected essential oils to <i>R. dominica</i> adults in olfactometer	70
Table 6.1. LC ₉₅ (mg/ml) values of 5% <i>M. arvensis</i> based nanoemulsion (F4) against <i>R. dominica</i> adults in contact toxicity assay	77
Table 7.1. LC ₉₅ (mg/ml) values of <i>M. arvensis</i> essential oil based integrated copperized <i>neem</i> oil having 0.3 % and 0.4 % of copper naphthenate nanoemulsions against <i>R. dominica</i> adults in contact toxicity assay	89
Table 7.2. Analysis of variance for mortality exhibited by <i>M. arvensis</i> EO based integrated copperized <i>neem</i> oil (having 0.3 % of copper naphthenate) nanoemulsion	89
Table 7.3. Response surface model evaluation for <i>M. arvensis</i> EO based integrated copperized <i>neem</i> oil (having 0.3 % of copper naphthenate) nanoemulsion	89
Table 7.4. Physico-chemical characterization of <i>M. arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion over range of temperature	90
Table 7.5. Static contact angles and surface tension of integrated formulation on galvanized steel surface	92
Table 7.6. Chemical profile of <i>M. arvensis</i> EO based integrated copperized <i>neem</i> oil nanoemulsion	95