

**GEOGRAPHICAL VARIABILITY OF WOOLLY APPLE  
APHID, *Eriosoma lanigerum* (Hausmann) AND  
ITS MANAGEMENT IN APPLE CROP**

*Thesis*

by

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(H-2020-01-M)**

submitted to



**Dr. YASHWANT SINGH PARMAR UNIVERSITY  
OF HORTICULTURE AND FORESTRY  
SOLAN (NAUNI) HP - 173 230 INDIA**

in

partial fulfilment of the requirements for the degree

of

**MASTER OF SCIENCE  
(AGRICULTURE)  
ENTOMOLOGY**

**DEPARTMENT OF ENTOMOLOGY  
COLLEGE OF HORTICULTURE**

**2023**

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### **CERTIFICATE-I**

This is to certify that the thesis titled “**Geographical variability of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop**”, submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science (Agriculture) Entomology** in the discipline of **Plant protection** to Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173 230 India is a bonafide research work carried out by **Mr Abhishek Dhanta (H-2020-01-M)**, son of Shri Manoj Dhanta under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

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


**Place: Nauni, Solan (HP)**  
**Date:**


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**(Dr. Rakesh Kumar Daroch)**  
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## CERTIFICATE- II



This is to certify that the thesis titled, "Geographical variability of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop" submitted by Mr. Abhishek Dhanta (H-2020-01-M) son of Shri Manoj Dhanta to the Dr. Yashwant Singh Parmar University of Horticulture and Forestry, (Nauni) Solan (HP) – 173 230 India in partial fulfilment of the requirements for the degree of **Master of Science (Agriculture) Entomology** in the discipline of **Plant Protection** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an external Examiner.

  
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## ACKNOWLEDGEMENTS

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*First and foremost, I would like to thank “The Almighty God” for providing me all the strength and wisdom to complete this manuscript and helping me through all the ups and downs of my life.*

*I am extremely grateful to my grandparents **Mr. Devi Singh** and **Mrs. Krishna Devi** for their great affection, prayers and constant inspiration. Whenever I looked back they were always stand by me with their best wishes and encouragement in everything I do.*

*I express my profound sense of gratitude and indebtedness to my mentor and guide **Dr. Rakesh Kumar Daroch** for his valuable guidance, suggestions, cooperation, encouragement, constructive criticism, parental love, help and moral support throughout the period of my investigation. He has inspired me to become an independent researcher. It was great privilege and honour to work and study under his guidance.*

*I am immensely thankful to **Dr. Devinder Gupta**, Professor and Head, Department of Vegetable Science, member of my advisory committee **Dr. Pramod verma** for their valuable suggestions and needful help during the course of investigations.*

*It would never be possible for me to accomplish this work without the help and support rendered to me by my beloved seniors. I specially want to thank **Manish, Sakshi, Diksha, Neha, Nirvik, Ishan, Dhruv and Nikita** for their valuable advice and moral support.*

*My heartfelt thanks to, **Pratyush, Ronin, Ikshit, Abhishek, Pragun, Rohan, Aman, Priti, Rohan, Samrit, Rakesh, Vikas, Gunjali, Mansi, Shabnam, Isha, Akanksha, Kritika, Saksham, Shushant, Divyansh, Prateek, Rajan, Sanjneesh, Gaurav, Rishuraj, Lakshay and Rajneesh** for their timely help and suggestions.*

*I also owe sincere gratitude to office and field staff members of the Department for their generosity and help throughout my research work.*

*Lastly, thanks to all beloved and respected people around me who directly and indirectly helped me during my degree programme.*

*I solely claim the responsibility for all errors and omissions*

**Place: Solan (Nauni)**

**Dated:**

**(Abhishek Dhanta)**

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## ABBREVIATIONS USED

%	:	Per cent
&	:	And
/	:	Per
@	:	At the rate
+	:	Plus
=	:	Equal to
×	:	Multiplication
<i>viz.</i> ,	:	viedelicet (namely)
ANOVA	:	Analysis of Variance
CD	:	Critical Difference
cm	:	Centimetre
cm <sup>2</sup>	:	Square centimetre
cv.	:	Cultivar
df	:	Degree of freedom
DMSO	:	Dimethyl sulphoxide
ed.	:	Edition
eds.	:	Editors
<i>et al.</i>	:	Co-workers
etc.	:	Et cetera
F-cal	:	F-calculated
g	:	Gram
i.e.	:	That is
°C	:	Degree Celsius
RBD	:	Randomized Block Design
SE	:	Standard Error
μl	:	Microlitre
i.e.	:	Id Est. (that is)
CTAB	:	Cetyltrimethylammonium bromide
Fig.	:	Figure
NCBI	:	National Center for Biotechnology
bp	:	Base pair

kb	:	Kilo base pair
BLAST	:	Basic Local Alignment Search Tool
PCR	:	Polymerase chain reaction
mtDNA	:	Mitochondrial DNA
COI	:	Cytochrome-c-oxidase subunit- I (or COX1)

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## *Chapter-1*

# INTRODUCTION

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The cultivated apple (*Malus x domestica Borkh*) is one of the most important temperate fruits of the world. It is native to South Western Asia (Brown, 1975) and is fourth among the most widely produced fruits in the world after banana, orange and grape. China is the largest apple producing country in the world with 40.5 million tons (Anonymous, 2021). Other major apple producing countries are USA, European Union, Australia, Mexico, Egypt, India, Turkey and Japan. Apple is a chief commercial crop of the Himalayan region and most of India's apple is produced from Jammu & Kashmir, Himachal Pradesh, Uttaranchal, Arunachal Pradesh and Nagaland. The total area in India under apple cultivation in 2020 is 3,08,000 hectares with a production of 27,34,000 metric tonnes and the productivity is 8.8 metric tonnes per hectare. The area under apple cultivation in Himachal Pradesh during 2018-19 is 113 thousand hectares with a production of 368 thousand metric tons (Anonymous, 2019).

To overcome the problem of low productivity and long gestation periods of traditional varieties, a shift towards High Density Plantation (HDP) in apple orchards has been seen in Himachal Pradesh. Based on their performance, the major varieties recommended for commercial cultivation in India till date are- Oregon Spur, Red Chief, Vance Delicious, Ruby Red, Red Fuji, Jeromine, Red Velox, Red Cap Valtod, Scarlet Spur-II, Super Chief, Gale Gala, Redlum Gala and Auvil Early Fuji grafted on M9 and MM106 rootstocks. Several biotic and abiotic factors contribute and control the production and productivity of the crop. The main biotic factors affecting apple cultivation are pests and diseases in addition to insufficient pollination. Bhardwaj and Bhardwaj (2000) surveyed in 580 apple growing localities in the state and reported that woolly apple aphid, red spider mites, San Jose scale, blossom thrips, apple leaf roller and root borer infested 78-89 per cent orchards while stem borers, defoliating beetles, hairy caterpillars and bugs infested 5-9 per cent orchards.

Thereby, it is apparent that woolly apple aphid, *Eriosoma lanigerum* (Hausmann) (Homoptera: Aphididae), is a serious pest of apples in many production area till date. It feeds on the roots of apple trees, causing the formation of woody galls that reduce the capacity of the roots to transport water and nutrients (Brown *et al.*, 1991). During spring

(March and April) crawlers from subterranean populations migrate up the trunks and settle in leaf axils and pruning wounds where they form colonies (Pringle, 1998; Sood and Gupta, 2005). However, there is apparently no correlation between aboveground and underground populations (Brown and Schmitt, 1990), indicating that sampling aboveground populations yields no information on the abundance of *E. lanigerum* on the roots.

These aphids produce white, waxy filaments, making the colonies in which they live and are easily visible from far away. The feeding of *E. lanigerum* in the leaf axils destroys the buds. Favourite feeding sites are new growth shoots, branches, pruning and other wounds, leaf axils and the roots of apple trees (Mueller *et al.*, 1992). Their feeding activity causes galls to form on the woody tissue, which can lead to the destruction of young lateral shoots and buds. The galls, which are ideal feeding sites for aphid offspring, frequently give rise to the formation of densely packed colonies (Heunis and Pringle, 2003). The calyces of apples may also be infested by aphid colonies, resulting in contamination with honeydew and sooty mould (Shaw and Walker, 1996). Therefore, where heavy infestations are encountered, more crop losses can occur. However, this has never been quantified. Although, the colonies are attacked by a parasitic wasp, *Aphelinus mali* (Haldeman), which often appears too late in the season to prevent colony formation and bud damage. Therefore, corrective sprays are sometimes required essentially (Heunis and Pringle, 2003).

At present the only recommended insecticides for the management of woolly apple aphid are chlorpyrifos and malathion. Both are the contact insecticides with short residual period; especially the latter one but are highly toxic to biological control agents especially to *A. mali* (Heunis and Pringle, 2003). Being old pesticides, the CIBRC have placed them under the list of pesticides proposed to be banned. Introduction of new chemicals is therefore, necessary so that the woolly apple aphid can be controlled effectively without having much ill effects to consumers.

Biological control of *E. lanigerum* by *Aphelinus mali* is however, possible. De Bach (1964) reported *A. mali* as a successful biological control agent was introduced to over 50 countries with establishment occurring in at least 42 countries. Excellent control was noticed in northwestern United States, Australia, British Columbia, New Zealand, Chile and Uruguay while low to moderate control was recorded in Europe and parts of Asia. This is a solitary and host-specific parasitoid wasp from Aphelinidae and is cited as one of the most successful examples of classical biological control of insect pests (Mueller *et al.*, 1992; Goossens *et al.*, 2011). Although a complex of natural predators have been reported to

control the population of woolly apple aphid but the syrphids, coccinellids and hymenopterans have successfully been observed (Lessando *et al.*, 2012). At present, effective limitation of woolly aphid populations relies on a good synergy between chemical control treatments and biological suppression by beneficial insects, and use of entomopathogenic agents. Therefore, Biopesticides such as *Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecani* and azadirachtin are considered to be good treatments that enhance the activity of natural enemies such as *A. mali* and coccinellids to control the population of woolly apple aphid (Vineet and Gupta, 2019). Correct timing of insecticides application is also important, as colonies do not appear at the same time in different orchards and during different seasons (Heunis, 2001; Heunis and Pringle, 2003; Goossens *et al.*, 2011, Bangels *et al.*, 2021).

Knowledge of the patterns of genetic diversity of an insect pest population can help understand the biology, its resilience to changes in environmental stimulus greatly affecting its response to different management practices. However, information regarding the genetic diversity of *E. lanigerum* is lacking and to date no molecular studies have been conducted on this pest from Himachal Pradesh. Therefore, one of the objectives of this study was to know the value of genetic diversity among the populations of different areas of Himachal Pradesh.

Various molecular markers have been evaluated for characterizing the genetic structure of aphid populations. The most popular have been random amplification of polymorphic DNA (RAPD) (Black *et al.*, 1992), mitochondrial DNA analysis (Barrette *et al.*, 1994). However, amplified fragment length polymorphism (AFLP) analysis (Vos *et al.*, 1995) has not been used to evaluate genetic diversity of the Aphididae even though the technique has provided excellent results for analysis of the closely related phylloxerid *Daktulosphaira vitifoliae* Fitch (Hemiptera: Phylloxeridae) (Forneck *et al.*, 2000, 2001) as well as for other insect taxa (Reineke *et al.*, 1999; Salvato *et al.*, 2002). One of the aims of this study was therefore to evaluate genetic diversity in different populations of woolly apple aphid from potential apple growing areas of this state.

In the present study, entitled “**Geographical variability of Woolly Apple Aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop**” research was conducted with the following objectives:

1. To study the geographical variability of Woolly Apple Aphid collected from different locations in Himachal Pradesh.

2. To study the effect of different treatments for the management of Woolly Apple Aphid in apple crop.

## Chapter 2

# REVIEW OF LITERATURE

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All living things on this earth contain a unique code within them called DNA. The DNA is organized into genes. The DNA code is different even between individuals within the same species which is called as genetic diversity. This difference in diversity causes differences among individuals and is very important because it gives species a better chance of survival. Although the studies on genetic diversity is a tool for the breeders but additionally the knowledge is also useful in other disciplines of agriculture.

In order to understand this diversity, it is important to remember that insect families have been diversity from millions of years and producing huge number of species which have expanded their range in response to environmental or geographical changes or by the influence of biotic factors. Consequently, the genetic variation within species is a product of these processes operating over the time. To know the diversions may be of utmost importance to know the changes in the insect behavior, food and development and therefore, altering the response to management practices in agriculture. These studies were thus conducted to know the differences among the populations of different area having stressful environment for the production of commercial apple. The pertinent literature on genetic diversity and management of *Eriosoma lanigerum* (Hausmann) has been reviewed as under:

### 2.1 Genetic diversity of *Eriosoma lanigerum*

Bloomers in 1994 confirmed that the woolly apple aphid, *Eriosoma lanigerum* (Hausmann), cannot only harm American elm, mountain ash and hawthorn in its native North America but also can damage apple *Malus × domestica* and is regarded as an important pest in apple-growing areas worldwide. The damage induced by this pest includes the formation of galls on roots and buds in leaf axils, decreasing the uptake of nutrients and water and reducing tree vigor. The pest is difficult to control because it produces wax, which covers its cuticle rendering pesticides ineffective. *E. lanigerum* was introduced to Europe in 1801 and to Japan in 1885 on plantlets from its native North America. Currently its distribution covers more than 70 countries. This pest caused severe production problems in many countries including the United States, France, England, Holland, Japan, Israel, India, South Africa, Australia, New Zealand and China. To reveal the possible routes of the spread of the recently

established populations of *E. lanigerum* in China, 469 *E. lanigerum* adult females from 24 populations in 10 provinces of China were genotyped at eight microsatellite loci, and their genetic diversity and structure were analyzed.

Bradley *et al.* 2000 studied the Apple Genetics Population which mainly consisted of families derived from open-pollinated cultivars from around the world, was evaluated for resistance to apple scab, fire blight, and woolly apple aphid. Large scale patterns and clumping were present in the distributions of pest and diseases, but the experimental design of randomised incomplete blocks was found to adequately accommodate them. From the variance components, estimated using restricted maximum likelihood (REML), heritabilities and combining abilities were computed. In some cases where the data showed a skewed distribution, jackknife estimates of the standard errors for the heritabilities were derived. The combining abilities were ranked, and the confidence intervals for each rank determined by bootstrapping the residuals. The heritability for apple scab resistance was about 0.35, although it was much lower for some sub-populations due to high frequencies of resistant seedlings. Similarly, heritability for fire blight resistance was 0.10 for subline 94, while they were 0.29 for subline 92, and 0.70 for subline 93. The preliminary estimate for woolly apple aphid resistance in subline 93 was 0.52. Test-crosses confirmed the presence of major gene resistances to this pest in the open-pollinated seedlings derived from ‘David’, ‘Korichnoe Polosatoje’, Mildew Immune Seedling, and *M. floribunda* 821. Analysis of the infestation data on the Kazak accessions suggested a strong genetic component in the differences for the apple leaf curling midge, but not for the light brown apple moth.

Sandanayaka and Bus in 2005 concluded that the *E. lanigerum* populations and its parasitoids, *A. mali* from Xinjiang might have been secondarily introduced from Yunnan regions rather than directly from the “supercolony” in Northern China. However the possibility cannot be completely ruled out that the two Xinjiang *E. lanigerum* populations may have a unique genetic cluster when  $k=6$ . These characteristics of the genetic diversity and structure of *E. lanigerum* in China indicate that human activities have played a very important role in the spread of this pest. If the *E. lanigerum* populations in Xinjiang have been introduced from Yunnan regions by human activities, it indicates the geographical barriers do not appear to be a major factor modulating the genetic structure of *E. lanigerum* in China, which is similar with that in Chile.

Timm *et al.* (2005) conducted research and he found out that the woolly apple aphid *Eriosoma lanigerum* (Hausmann) is one of South Africa's most damaging apple pests. There

is a scarcity of information on its genetic diversity, and this study, which characterized the genetic structure of parthenogenetic *E. lanigerum* populations in South Africa's Western Cape Province, is the first of its kind. A total of 192 people from four different regions were gathered and tested for amplified fragment length polymorphism (AFLP). 250 fragments were scored for analysis using five selective AFLP primer pairs. The results showed that there was little genetic variation in *E. lanigerum* populations in the Western Cape ( $H = 0.0192$ ). Furthermore, populations collected from geographically distant regions were highly related, which can be explained in part by the fact that agricultural practices were responsible for the spread of populations from a common ancestor to geographically distant areas. The low level of variation discovered indicated that using host plant resistance to control *E. lanigerum* in the Western Cape is a viable option. This is the first report of AFLP being used to characterize an aphid species' genetic structure. The findings suggest that this marker could be useful for analyzing other aphid species.

Lavandero *et al.* (2008) conducted research and he found out that landscape genetics has been particularly useful in assessing the impact of landscape characteristics on genetic variability and in identifying gene flow impediments. The efficiency of these programmes would be increased by linking current practices of area-wide pest management information on pest population genetics and geographical barriers. The woolly apple aphid, *Eriosoma lanigerum* (Hausmann), a major pest of apple orchards worldwide, was collected on apple trees (*Malus × domestica* Borkh) from various locations along a 400-kilometer north-south transect in central Chile. Diversity and flow were evaluated to see if there was population structure. Inter Simple Sequence Repeat (ISSR) markers were used to analyze 215 people from these locations. Four ISSR primers resulted in 114 polymorphic loci. These tools could be useful. The percentage of molecular variation among locations was 18%. Because the STRUCTURE algorithm may be unsuitable for inferring the number of genetic clusters in a data set with an IBD relationship, the number of genetic clusters in the samples was also analyzed using a Bayesian clustering method implemented in software BAPS version 4.14. We deduced the presence of four genetic clusters in the study region. Individual clustering followed a pattern explained by geographical barriers. Using partial Mantel tests, we discovered barriers to gene flow other than distance, which were caused by a combination of major rivers and mountains. Although landscape genetics is rarely used in pest management, our findings suggest that these tools could be useful in the design of area-wide pest management programmes.

Christians in 2003 studied that woolly apple aphids (*Eriosoma lanigerum* L. (Hausmann)) have a significant effect on the apple industry in the Western Cape. Damage caused is two-fold, occurring aerially and terrestrially. In 1962, Northern Spy was identified as a woolly apple aphid resistant rootstock and has since then formed the basis for traditional rootstock breeding programmes. The Er1 gene in Northern Spy confers resistance. The main objectives of this study was to firstly identify molecular markers more closely linked to the woolly apple aphid resistance gene, Er1, than existing markers, by applying AFLP technology to selected seedlings, identified to be resistant by conventional phenotyping. Three families were derived from crosses of each of three resistant genotypes, namely Northern Spy, Rootstock 5 and Russian Seedling and a susceptible cultivar, Braeburn. For the three successive years of the study, each resistant genotype was allowed to cross-pollinate in isolation with the susceptible parent. The amplified fragment length polymorphism (AFLP) technique was used in an attempt to identify markers in the resistant and susceptible seedlings. No markers were identified using this technique. Known sequence characterised amplified regions (SCAR) and random amplified polymorphic DNA (RAPD) markers were used due to their suitability in marker-assisted selection for woolly apple aphid resistance. Varying results were obtained with these markers and no conclusive information was acquired with regard to the segregation of the Er gene in any of these rootstocks and crosses.

Bus *et al.* 2010 evaluated that apple is host to a wide range of pests and diseases, with several of these, such as apple scab, powdery mildew and woolly apple aphid, being major causes of damage in most areas around the world. Resistance breeding is an effective way of controlling pests and diseases, provided that the resistance is durable. As the gene pyramiding strategy for increasing durability requires a sufficient supply of resistance genes with different modes of action, the identification and mapping of new resistance genes is an ongoing process in breeding. The mapping of an apple scab, a powdery mildew and a woolly apple aphid gene from progeny of open-pollinated mildew immune selection. The scab resistance gene *Rvi16* was identified in progeny 93.051 G07-098 and mapped to linkage group 3 of apple. The mildew and woolly aphid genes were identified in accession 93.051 G02-054. The woolly aphid resistance gene *Er4* mapped to linkage group 7 to a region close to where previously the genes *Sd1* and *Sd2*, for resistance to the rosy apple leaf-curling aphid, had been mapped. The mildew resistance gene *Pl-m* mapped to the same region on linkage group 11 where *Pl2* had been mapped previously. Flanking markers useful for marker-assisted selection have been identified for each gene.

Wu *et al.* (2012) investigated the melon fly's genetic relationship. *Bactrocera cucurbitae* (Diptera: Tephritidae) from 23 different locations in nine different countries were identified using DNA sequences from the mitochondrial cytochrome oxidase I (COI) gene. Among the 38 haplotypes, 42 polymorphic sites were identified. The most common haplotype, HI, was found in 73 percent of the samples from all populations. Within populations, there was the greatest genetic diversity, and no isolation by distance was observed. The western regions (Nepal, Bangladesh, Thailand, Burma, and western China) had greater haplotype diversity than the eastern regions (China-east). China-Yunnan had the highest levels of genetic diversity in the country. From west to east, haplotype diversity decreased with longitude. These analyses suggested that *B. cucurbitae* spread from west to east within a limited geographic scale and was recently discovered.

Prabhakar *et al.* (2013) investigated the genetic structure of the pumpkin fruit fly population. *Bactrocera tau*, a cucurbit fruit fly pest, used mitochondrial cytochrome oxidase I (mtCOI) gene sequences in Himachal Pradesh (India). With the possible exception of population Solan, levels of differentiation (genetic distances and FST values) among samples from different locations are minimal, implying the local occurrence of a large and geographically undifferentiated population. Local *B. tau* is closely related to *B. tau* species A from Thailand, according to phylogenetic analysis of local *B. tau* samples in the context of the different sibling species that comprise the *B. tau* complex in its South-East Asia region of origin. They proposed that the sterile insect technique (SIT) may be a viable option due to the observed local genetic uniformity and predominance of a single species within the species complex.

San jose *et al.* (2013) sequenced one mitochondrial and two nuclear genes from 73 specimens from 19 species in order to build phylogenies and examine species relationships and limits within the genus *Bactrocera* and several species of the *B. dorsalis* complex, with a focus on the placement of *B. invadens*. Their findings suggested that the *B. dorsalis* complex was polyphyletic. *B. invadens* and several other species in the *B. dorsalis* complex (*B. dorsalis*, *Bactrocera papayae* Drew & Hancock, and *Bactrocera philippinensis* Drew & Hancock) were also paraphyletic with respect to each other and most likely represent a single genetically indistinguishable, phenotypically plastic pest species that has spread worldwide.

Asokan *et al.* (2013) studied life stage independent molecular identification using 28S rDNA. They assessed the utility of 28S rDNA for quick and accurate species identification of *Bactrocera spp.* eggs, larvae, pupae, and adults. The *Bactrocera spp.* phylogeny suggested

that 8 sau is phylogenetically distant from the other three species, *B. dorsalis*, *B. zonata*, and *B. correcta*, as supported by a 100 percent bootstrap value. Furthermore, in the 288 DNA sequences, *B. dorsalis*, *B. zonata*, and *B. correcta* had the highest sequence identity (98 percent), with very few variable sites. In these species studied, the 28S rDNA region had high reliability for species identification, which had never been reported before. This study unequivocally demonstrated the utility of 28S rDNA for the rapid and accurate identification of *B. zonata* Saunders, *B. tau* Walker, and *B. dorsalis* Hendel eggs, larvae, pupae, and adults.

Zhou *et al.* (2015) investigated that in China, the woolly apple aphid, *Eriosoma lanigerum* (Hausmann), was first detected as an invasive species during the 1910s to 1930s, restricted to Shandong, Liaoning and Yunnan provinces. In order to determine the possible spread routes of the recently established populations, the genetic diversity and genetic structure of 24 populations in 10 provinces were analysed using eight microsatellite loci. Analyses using structure software identified two genetic clusters overall. Three populations from Yunnan and Xinjiang consisted of individuals originating from a single cluster. Nineteen populations from eight northern provinces consisted only of individuals from another cluster, which formed a single large and panmictic population, resembling a distinct “supercolony” in Northern China. The possible routes of spread of the recently established populations of *E. lanigerum* in China revealed the populations in Northern China (including these from Henan, Hebei, Shanxi, Jiangsu and Gansu) may have been introduced from Shandong or Liaoning Provinces; the populations in Yunnan consisted of an early established population and a population introduced secondarily from Shandong or neighbouring areas, indicating that the population in Yunnan has at least two sources and the recently established populations of *E. lanigerum* in Xinjiang might not have been introduced from the “supercolony” in Northern China. Knowledge of these routes of spread is useful for avoiding further dissemination and/or additional introductions.

Asghar *et al.* (2015) reviewed on DNA Extraction from insects using different techniques and was compiled through a detailed review of many research articles published in various journals of international repute. Different molecular techniques were used to identify insects and research their roles. High quality DNA was required for each molecular technique, which was extracted from the insects using various techniques. The technique used for DNA extraction was chosen based on the specimen under study, the amount of time required for extraction, the cost of the reagents and equipment used for extraction, and, most importantly, the quality of the extracted DNA.

Zhou *et al.* 2016 reported that during the 1910s and 1930s, the woolly apple aphid, *Eriosoma lanigerum* (Hausmann), was discovered as an invasive species in Shandong, Liaoning, and Yunnan Provinces of China. However, since the 1990s, the pest were spread to many other parts of China. The genetic diversity and genetic structure of 24 populations from 10 provinces were analysed using eight microsatellite loci to determine the possible spread routes of recently established population. STRUCTURE software analysis revealed two genetic clusters in total. Three populations from Yunnan and Xinjiang were made up of individuals from a single cluster. Nineteen populations from eight Northern provinces were made up entirely of individuals from another cluster, which formed a single large and panmictic population, resembling a distinct "supercolony" in Northern China. He found out that the following are possible routes of spread for newly established populations of *E. lanigerum* in China: 1) the populations in Northern China (including Henan, Hebei, Shanxi, Shannxi, Jiangsu, and Gansu) may have been introduced from Shandong or Liaoning Provinces; 2) the populations in Yunnan consisted of an early-established population and a population introduced secondarily from Shandong or neighbouring areas, indicating that the population in Yunnan has at least two sources; and 3) the recently established populations of *E. lanigerum*. Knowing these routes of spread can help you avoid further spread and/or introductions.

Wang *et al.* 2017 reported that *Aphis gossypii*, one of the world's most important agricultural pests, can cause significant economic losses in major crop-producing areas. The investigated genetic diversity and population structure of *A. gossypii* collected from 33 different climatic zones in China, used 10 nuclear microsatellite loci (SSR) and two mitochondrial gene sequences (COI and Cytb). SSR and mtDNA data suggested that genetic diversity was low to moderate. According to a star-shaped network of mtDNA haplotypes, the maternal ancestor of China cotton aphids most likely originated in Xinjiang. The POPTREE, STRUCTURE, and PCoA (principal coordinate analysis) analyses revealed two genetic clusters: one in the east and one in the west. The results of isolation by distance (IBD) revealed a positive relationship between geographic distance and genetic distance in the vast eastern region but not in the western region. In most populations, neutrality testing and mismatch distribution analysis revealed strong evidence of a recent rapid expansion. There was no evidence of a genetic bottleneck in *A. gossypii* populations from China. The current research can be used to develop pest management strategies.

Dorji and Saengyot (2019) conducted study on population dynamics of woolly aphid, *Eriosoma lanigerum* (Hausmann) and its natural enemies in apple orchard in Thimphu valley, Bhutan. The study revealed that aphid densities were observed higher during the growing season (June-September) when tender shoots and plants resource are abundant. The study was conducted in two apple orchards, Hongtsho-2 and Kausumche-3 (coordinates: 27°28'20"N - 89°38'10"E, altitude from 2,248 to 2,648 meters, Temp. 5-30 °C). Data was collected randomly from five trees comprising sample unit of four terminal shoots (approx. 10 cm) each tree. During the apple growing season significantly an independent sample t-test revealed that abundance of WA infestation was higher in Hongtsho-2 than Kausumche-3 averaging  $1.40 \pm 0.30$  and  $1.27 \pm 0.38$  aphids per tree respectively ( $t = .94$ ,  $P = 4.3410$ ,  $P=0.0001$ ).

Biello *et al.* (2020) conducted a research and found that Woolly apple aphid is a major pest of apple trees (*Malus domestica*) and is critical to the economics of the apple industry in most parts of the world. A chromosome level genome assembly of WAA representing the first genome sequence from the aphid sub family Eriosomatinae using a 10X genomics linked reads and in vivo Hi-C data. The contig and scaffold N50 values are 158 kb and 71 Mb, respectively, and we predicted a total of 28,186 protein-coding genes. The assembly is highly complete, including 97% of conserved arthropod single-copy orthologues based on Benchmarking Universal Single Copy Orthologs (busco) analysis. Phylogenomic analysis of WAA and nine previously published aphid genomes, spanning four aphid tribes and three subfamilies, reveals that the tribe Eriosomatini (represented by WAA) is recovered as a sister group to Aphidini + Macrosiphini (subfamily Aphidinae). Identification of syntenic blocks of genes between WAA assembly and the genomes of other aphid species and was concluded that two WAA chromosomes (El5 and El6) map to the conserved Macrosiphini and Aphidini X chromosome. The high-quality WAA genome assembly and annotation gave a valuable resource for research in a broad range of areas such as comparative and population genomics, insect–plant interactions and pest resistance management.

## **2.2 Effect of different treatments for the management of Woolly Apple Aphid**

Management of woolly apple aphid (WAA), *Eriosoma lanigerum* (Hausmann), a major insect pest of apple, has a long history which includes the use of different insecticides and biological control agents. Recently some insecticide molecules with different modes of

action have also been developed to counteract the adverse effects of the use of old group of insecticides.

### 2.2.1 Spirotetramat

Alston *et al.* (2007) studied the efficacy of Movento 150SC® (spirotetramat), for suppression of woolly apple aphid (*Eriosoma lanigerum*) and green apple aphid (*Aphis pomi*) populations in ‘Delicious’, ‘Gala’, ‘Idared’, ‘Jonathan’, ‘Mutzu’, and ‘Prime Gold’ apple cultivars. Treatments were replicated four times and plots were arranged in a randomized complete block design. Movento 150SC was applied @ 8 oz and 12 oz/acre + 1% v/v horticultural mineral oil, singly during starting May and also after one month. The mean percentage of apple tree shoots infested with woolly apple aphid (WAA) was very low before treatment and increased post-treatment in the untreated and horticultural oil. Shoot infestation did not exceed 2.5% in any treatment throughout the trial period. Infestation levels were < 0.2% in all Movento treatments on preand post-treatment dates. Numbers of WAA in all of the Movento treatments remained low throughout the trial. Significant differences in densities among treatments occurred on 4, 6, and 8 WAT.

Kumar *et al.* (2008a) conducted studies to assess the bioefficacy, persistence, and median lethal concentration of spirotetramat as a foliar application against mealy bug, *Phenacoccus solani* (Ferris) on cotton at Tamil Nadu Agricultural University revealed that the insecticide was highly effective in controlling mealy bug populations when applied at 75 gai/ha. Another study conducted by Kumar *et al.* (2008b) against the aphid, *Aphis gossyp*, at a similar dosage found that spirotetramat was highly effective in controlling the pest's population and was comparable to the standard check, imidacloprid 25 g ai /ha. The study also discovered that all insecticide treatments resulted in increased seed cotton yield under field conditions.

Nauen *et al.* (2008) found that treating apple seedlings infested with woolly apple aphid with spirotetramat (20-100 ug a.i, per leaf) resulted in a 70-90 per cent reduction in woolly apple aphid population after 28 days of application under laboratory conditions.

Brück *et al.* (2009) reported that in laboratory and greenhouse assays, as well as semi-field and field trials, the tetramic acid derivative spirotetramat (brand name Movento®) demonstrated outstanding performance against sucking insect pests. The product acts as a lipid biosynthesis inhibitor, affecting juvenile stages as well as adult fecundity.

Kumar *et al.* (2009) conducted field studies were to assess the bioefficacy of spirotetramat as a foliar application against cotton whitefly. *Bemisia tabaci* in Tamil Nadu, and it was discovered that spirotetramat (@) 75 g ai /ha) reduced whitefly population by up to 89.7% over control, with the chemical remaining active for up to 25 days

Udikeri *et al.* (2009) conducted bioefficacy studies against cotton sucking pests at the Agricultural Research Station in Dharwad, Karnataka, using spirotetramat 150 OD and a combination of thiamethoxam 141 SC+ lambda cyhalothrin 106 SC. The studies revealed that three sprays in 2006 and two sprays in 2007 reduced the population of thrips, leafhoppers, and aphids to levels below the ETL. Significantly higher seed cotton yields of 20.32 q/ha (2006) and 29.22 q/ha (2007) were obtained with the combiprodut (@ 300ml/ha) and spirotetramat (@500ml/ha), respectively, and were comparable to acetamiprid 20 SP, which was used as a standard check in the studies.

Wise *et al.* (2009) carried out a trial with the objective to testify the efficacy of certain insecticides including Movento 240 SC, for control of the late season WAA colonies. Application of insecticides to 21-year-old 'Red Delicious' apple plants was done with the help of airblast sprayer. Two tree plots were organised in RCB design with four replications. Rate of application of Movento 240 SC was fixed at 9 fluid ounces per acre. Survival rate of marked colonies were examined on 12 DAT and 34 DAT. Per-cent survival rate using Movento 240 SC at 12 DAT and 34 DAT was 3.3 and 6.7 respectively, when compared to control with 80% and 3.3 % survival rate at 12 DAT and 34 DAT, respectively.

Schoevaerts *et al.* (2011) carried out field trials aiming at 'multi-target approach' for controlling WAA infestation with spirotetramat (Movento 100 SC) in apple. Spirotetramat was applied at the registered dose rate of 75 g a.i/ha/m CH and water volume varied from 100-330 L/ha/m CH (300- 1000 L/ha standard orchard). In all trials a randomised block design was used with 3-4 replicates and units of at least six trees per plot. For *E. lanigerum* the best way to evaluate the efficacy is an assessment on the extension shoots at five weeks after application. Five weeks after one application, spirotetramat still controlled WAA (95% Abbott).

Baldessari *et al.* (2012) performed two field trials were performed to verify the field control activity of Movento® (a.i.spirotetramat) against the rosy apple aphid, *Dysaphis plantaginea* (Passerini) and woolly apple aphid *Eriosoma lanigerum* (Hausmann). The timing of spirotetramat 48 % SC application was post flowering (BBCH 69) with dose of 300 ml/ha.

One semi-field trial was also carried out to verify the action of spirotetramat on significant infestations of *E. lanigerum*. Movento guaranteed a high control of *E. lanigerum* and *D. plantaginea* infestation in field and semifield trials; the performances were comparable to that of the best references. The good selectivity observed on phytoseiids and the favourable ecotox profile make spirotetramat suitable for IPM programs.

Krystyna *et al.* (2012) in five field experiments conducted in Poland between 2009 and 2011, the effectiveness of spirotetramat (Movento 100 SC) at two doses, 1.8 l/ha and 2.25 l/ha, for the control of pear psylla (*Cacopsylla pyri*) on pear trees was investigated. Two treatments were administered in June, the first during the early stages of hatching and the second approximately two weeks later. In all experiments, a higher dose of insecticide effectively reduced the pest population by 75.3-91.4 percent after one week and by 83.7-97.6 percent after two weeks of single application, whereas a lower dose of the insecticide reduced the pest population by 58.4-91.3 percent and 56-92.6 percent. Spirotetramat was more effective than standard insecticides such as diflubenzuron and novaluron.

Steenwyk *et al.* (2012) conducted an experiment to evaluate the insecticidal properties of Spirotetramat and Diazinon for effective control of WAA. Trial was conducted on 'Gala' apples grafted on MM.111 rootstock. Seven treatments in RCB design were replicated four times. Movento 2SC was applied at the rate of 6 and 9 fl oz /acre. Treatments were given two times, in May and in September after harvest. WAA populations were assessed from June till mid-September at two weeks interval. WAA colonies were given an overall infestation rating, with 0 as no visible WAA colonies and 6 as colonies high density. Movento 2 SC and Diazinon 50 W controlled WAA to such an extent that there were insufficient colonies to do sampling for number of sampling dates.

Das and Islam (2014) conducted an experiment to evaluate the insecticidal properties of Spirotetramat and Diazinon for effective control of WAA. Trial was conducted on 'Gala' apples grafted on MM.111 rootstock. Seven treatments in RCB design were replicated four times. Movento 2SC was applied at the rate of 6 and 9 fl oz /acre. Treatments were given two times, in May and in September after harvest. WAA populations were assessed from June till mid-September at two weeks interval. WAA colonies were given an overall infestation rating, with 0 as no visible WAA colonies and 6 as colonies high density. Movento 2 SC and Diazinon 50 W controlled WAA to such an extent that there were insufficient colonies to do sampling for number of sampling dates.

Pasqualini and Scannavini (2015) during the spring conducted three field trials using spirotetramat, imidacloprid, thiamethoxam, and flonicamid to control *Myzus persicae* were set up in two nectarine farms with three varieties each in Italy. According to the findings, spirotetramat was the most persistent insecticide, while flonicamid was the most effective of the four test chemicals used.

Sunda *et al.* (2015) conducted an experiment to investigate the field efficacy of some newer insecticides against insect pests of brinjal and discovered that the combination of spirotetramat + imidacloprid (@ 75+ 75 g a.i./ha) was found to be the most effective for the control of jassids and white flies, accounting for more than 76.11 percent control of these pests, whereas the combination of spirotetramat + imidacloprid (@ 60+ 60 g a.i./ha) combination was found statistically at par with imidacloprid (@75 g a.i./ha).

Gong *et al.* (2016) carried out an experiment to study the effects of Spirotetramat on fecundity and carboxylesterase expression of *Aphis gossypii*, when exposed to a sub-lethal concentration (LC<sub>10</sub>) and to increasing (LC<sub>25</sub>, LC<sub>50</sub> and LC<sub>75</sub>). Seven serial concentrations (30–1920 mg/L for day 1 today 3 for bioassay; 3.75–120 mg/L for day 4 to day 5 for bioassay) of spirotetramat were used. Spirotetramat reduced aphid daily fecundity in all treatments, especially with upto 90 % reduction in case of exposure to LC<sub>75</sub>.

Jones and Mizuho (2016) conducted grapes mealy bug control trial was in Virginia, USA. *Pseudococcus maritimus* was studied using two chemicals, dinotefuran (Scorpion, 0.292 L/ha) and spirotetramat (Movento, 0.439 L/ha). Both chemicals significantly reduced mealybug counts, but spirotetramat was found high persistence and reduced mealy bug population.

Kumar and Gupta (2019) conducted an Evaluation of certain insecticides and biopesticides against the woolly apple aphid *Eriosoma lanigerum* (Hausmann) on apple and revealed that spirotetramat (0.015%) is the most effective resulting in a reduction of 68.48 and 61.04% in colony count and colony size, respectively followed by flonicamid (0.05%). Biopesticides with higher concentrations of *Beauveria bassiana* (2x10<sup>10</sup>cfu/l), fish oil 1.0 per cent, neem oil 2.0 per cent, and azadirachtin 0.02 per cent reduced colony count by 29.17, 31.32, 28.95, and 29.83 per cent, respectively. Neem oil was the safest, with 60 to 80 per cent parasitoid (*Aphelinus mali*) emergence, whereas chlorpyrifos (0.04%) was the most harmful, with 15 to 20 per cent parasitoid emergence. The parasitoid was fairly safe to spirotetramat and flonicamid. Pirimicarb, on the other hand, has a lesser efficacy when

treated during migration, but it has good control levels when applied early, at the onset of activity, or later, when applied as little fuzzy colonies emerge. Weather conditions, on the other hand, are a limiting factor in the usage of pirimicarb, because its vapour activity requires dry, warm weather at the time of application.

Smytheman and Beers (2020) carried out a study with objective to assess efficacy of certain insecticides against WAA in ‘Golden Delicious’, ‘Jonagold’, ‘Gala’, and ‘Fuji’ apple cultivars. Different insecticides used for the experiment were, spiropidon, spirotetramat, thiamethoxam, sulfoxaflor, isoclast (sulfoximines) and diazinon. Spirotetramat was applied at the rate of 6 fl oz /acre. By 13 DAT, better treatment separation was seen, with most treatments lower than the check.

### **2.2.2 Chlorpyrifos**

Molinari (1986), while working on WAA management, reported that serial spray application of mineral oil in winters followed by chlorpyrifos or oxydemeton methyl during the growing period, particularly after flowering (when predators are less abundant) is quite effective. Staubli and Chapuis (1989) reported reasonable aphid control using pirimicarb without adverse effects on the pest's natural enemy (*A. mali*), but the workers also noted that the chemical lacked persistence and was ineffective in killing hidden colonies of WAA.

Nicholas *et al.* (2003) carried out a trial to check the efficacy of different insecticides including chlorpyrifos in management of woolly apple aphid in apple cultivar ‘Granny Smith’ using soil root drenches. Treatments of chlorpyrifos consisted of 2.4 mg active ingredient plus 6 mL of the soil-wetting agent polypropylene glycol phenol in 1 L of water. Treatments were applied at petal fall by pouring into a 50–60 mm wide, 20–30 mm deep trench at the base of each tree.

Fahia *et al.* (2012) investigated the toxicity of six insecticides on *Aphelinus mali* Hald (Deltamethrin, Chlorpyrifos, Acetamiprid, Diflubenzuron, Lufenuron, and Fenoxycarb). The adult parasitoids were first tested for toxicity by exposing them to fresh-dry insecticide film applied on glass plates. The second test involved exposing adults to insecticide film applied to apple leaves. The third test was performed to determine the toxicity of insecticides on parasitoids found within the aphid mummy (pupa stage). The first test revealed that Chlorpyrifos was highly toxic to adult *A. mali*, resulting in 100 percent mortality. Diflubenzuron was completely safe, causing 27.16 percent mortality. The remaining four insecticides were classified as mildly to moderately hazardous. The second test revealed that

Chlorpyrifos was also highly toxic to adult *A. mali*, causing 100 percent mortality; the others (Deltamethrin, Acetamiprid, Diflubenzuron, Fenoxycarb, and Lufenuron) were less toxic, causing 94.81, 75.32, 18.18, 37.66, and 3.90 percent mortality, respectively. The tested insecticides showed low toxicity to *A. mali* pupa within-host mummies in the third test. Diflubenzuron, Lufenuron, and Fenoxycarb were all non-toxic, with adult emergence rates of 19.83, 23.96, and 29.75 percent, respectively. Deltamethrin, Chlorpyrifos, and Acetamiprid were mildly toxic, with adults emerging at 46.67, 48.67, and 52.67 percent, respectively.

Singh and Bhardwaj (2018) studied the field efficacy of new insecticides namely spirotetramat (Movento) @ 0.009, 0.012, 0.015%, flupyradifurone (Sivanto 200 SL) @ 0.02, 0.03, 0.04 %, thiamethoxam (Thompson 25 WG) @ 0.012, 0.025 % and flonicamid (Ulalal 50 WG) @ 0.20, 0.30, 0.40 % against woolly apple aphid. These were compared with chlorpyrifos (Dursban 20 EC) @ 0.04 %. The results revealed that all the treatments were superior, and 21 days after spray, chlorpyrifos (0.04%) and thiamethoxam 25 WG (0.025 %) were the best with no infestation; but these were statistically at par with flupyradifurone (0.03 and 0.04 %).

Singh and Sharma (2021) carried out an experiment to evaluate the efficacy of Phosmet and chlorpyrifos against the aerial form of the woolly apple aphid during October-November in apple orchards. Chlorpyrifos (0.05%) was found statistically superior (0.06 aphid colonies /twig) followed by phosmet @ 0.075%, 0.05% and 0.025% (0.36, 0.40 and 0.54 aphid colonies /twig, respectively).

### **2.2.3 Imidacloprid**

Nicholas *et al.* (2003) used insecticide soil-root drenches to control the woolly aphid, *Eriosoma lanigerum* (Hausmann) (Hemiptera: Pemphigidae), on mature apple trees. When administered as a root-soil drench, imidacloprid, a member of the chloronicotinyl family, is known to provide effective control of woolly aphids on trees up to the age of seven years. The results of a single application of chlorpyrifos, imidacloprid, pirimicarb, or vamidothion applied as a root drench across four growing seasons were compared in this study. To promote soil saturation and penetration, a soil wetting agent was added to each chemical. At the outset of the trial, imidacloprid offered excellent control of woolly aphids on trees that were 17 years old, and it continued to do so.

Singh and Gupta (2006) evaluated five different insecticides, including imidacloprid (0.04%), acetamiprid (0.01%), thiamethoxam (0.05%), acephate (0.12%), and profenofos

(0.10%), against the subterranean population of the woolly apple aphid, *E. lanigerum*, by drenching apple seedlings raised in earthen pots. Except for chlorpyrifos, which was only effective for 10 days, all of the test pesticides were comparable to phorate (1 g a.i./plant) and significantly superior to 0.08 percent chlorpyrifos and effective for 30 days against the root form of the aphid.

Das and Islam (2014) tested four insecticides (Fipronil, imidacloprid, buprofezin, and thiamethoxam + emamectin benzoate) against sucking pests (*Amrasca devastans* and *B. tabaci*) that infest brinjal throughout the winter months. Fipronil, imidacloprid, and buprofezin were shown to be effective in suppressing these two species, while thiamethoxam + emamectin benzoate was found to be ineffective.

Mandal *et al.* (2015) experimented to evaluate the efficacy of a few new groups of insecticides against two important sucking pests of cotton viz., cotton jassid (*A. biguttula biguttula*) and whitefly (*B. tabaci*) during the pre-Kharif season of 2011 and 2012. The insecticides viz., imidacloprid (18, 27 & 36 g a.i./ha), spiromesifen (100, 150 & 200 g a.i./ha) and buprofezin (150, 200 & 300 g a.i./ha) were applied twice at an interval of 15 days. From the experiment it was concluded that the most effective insecticide in controlling the jassid population was buprofezin (300 g a.i./ha) followed by imidacloprid (36 g a.i./ha) and the least effective was spiromesifen (100 g a.i./ha). The order of bio-efficacy based on per cent reduction of cotton jassid over the control plot was: buprofezin > imidacloprid > spiromesifen but in the case of whitefly, the most effective insecticide was spiromesifen (150 g a.i./ha) > buprofezin (300 g a.i./ha) > buprofezin (150 g a.i./ha). The order of bioefficacy based on per cent reduction of cotton whitefly over control was: spiromesifen > buprofezin > imidacloprid.

Sunda *et al.* (2015) experimented to investigate the field efficacy of some newer insecticides against pests of brinjal and it was revealed that a combination of spirotetramat + imidacloprid (@ 75+75 g a.i. / ha) was found to be most effective for the control of jassids and whiteflies. It accounted for more than 76.11 per cent control of these pests whereas, spirotetramat + imidacloprid (@ 60+ 60 g a.i. / ha) combination was found statistically at par with imidacloprid (@ 75 g a.i. / ha).

#### **2.2.4 *Metarhizium anisopliae***

Vu *et al.* (2008) screened twelve strains of entomopathogenic fungi for aphid control, including *Lecanicillium lecanii*, *Paecilomyces farinosus*, *Beauveria bassiana*, *Metarhizium anisopliae*, *Cordyceps scarabaeicola*, and *Nomuraea rileyi*. *L. lecanii* 41185 had the highest

virulent pathogenicity for both *Myzus persicae* and *Aphis gossypii* at 25 degrees C and 75 percent relative humidity (RH), and their control values were both nearly 100 percent 5 and 2 days after treatment, respectively. Furthermore, *L. lecanii* 41185 exhibited the highest virulence to *M. persicae* at a RH of 45 percent and a wide temperature range (20-30 degrees C). As the applied conidial concentration increased, the control value of *M. persicae* and the 50% lethal time (LT<sub>50</sub>) decreased significantly. The 50 percent lethal concentration (LC<sub>50</sub>) of this fungus's conidial suspension was determined to be  $6.55 \times 10^5$  conidia/ml. The control values of *M. persicae* obtained by using  $1 \times 10^7$  and  $1 \times 10^8$  conidia/ml were nearly identical and significantly higher than those obtained by using  $1 \times 10^6$  conidia/ml. The entomopathogenic fungi tested grew in a wide temperature range (15-30 degrees °C). *Lecanicillium* strains grew best at 25 degrees Celsius. *Lecanicillium* strains' aerial conidia germinated in a wide temperature range (15-30 degrees °C), with *L. lecanii* 41185 being the only strain with conidial germination at 35 degrees °C.

Araujo *et al.* (2009) investigated the effectiveness of the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* in controlling the aphid *Lipaphis erysimi* (Kalt.) (Hemiptera: Aphididae) in the kale *Brassica oleracea var acephala* D.C., as well as their compatibility with neem (Neemseto). Ten isolates of both fungi were tested, and the most pathogenic ones were *B. bassiana* CG001 and *M. anisopliae* CG30, with mortality rates of 90% and 4.4 days, and median lethal times of 64% and 3.8 days, respectively. Bioassays with neem at 0.5, 1.0, and 2.0 per cent concentrations were performed by dipping or spraying the aphids on the leaf discs. At a concentration of 2.0 per cent, neem spraying provided 90 per cent mortality. The use of *B. bassiana* isolate CG001 or *M. anisopliae* isolate CG30 with neem at 0.125, 0.25, and 0.5 percent showed that these isolates' spore viability or colony growth could be affected when exposed to neem concentrations greater than 0.25 percent. The isolates *B. bassiana* CG001 and *M. anisopliae* CG30 are the most virulent to *L. erysimi* in absolute values and could be used to manage this pest.

### **2.2.5 *Beauveria bassiana***

*Beauveria bassiana* is one of the most highly studied EPF which has received much developmental success as a microbial insecticide (Lacey, 2016). Commercially developed products as Real *Metarhizium* 69 (Meta 69 strain ICIP69), developed from a *M. anisopliae*, have been used in South Africa for management of such insect pests as thrips and whiteflies (Hatting *et al.* 2018). The use of indigenous strains of EPF, across

agricultural ecosystems, against crop-damaging insects is also considered as a preferred method, because the fungi concerned are already adapted to the local climatic conditions (Abaajeh and Nchu, 2015).

Stowke (2016) investigated the efficacy of entomopathogenic nematodes and fungi as biological control agents of the woolly apple aphid, *Eriosoma lanigerum* (hausmann) (Hemiptera: aphididae), in South African conditions and evaluated the use of two EPF, *Beauveria bassiana* and *Metarhizium anisopliae*, for WAA control in the laboratory. The commercial isolates *Beauveria bassiana* (Eco-Bb strain R444) and *Metarhizium anisopliae* (ICIPE 69) caused the highest rate of WAA mortality and were chosen for further study. When the effect of EPF concentration and exposure time of these two most virulent isolates was evaluated, it became clear that increased EPF concentration ( $1 \times 10^7$  to  $1 \times 10^{10}$  conidia ml<sup>-1</sup>) and exposure time increased WAA mortality. This led to the conclusion that a relatively long incubation period and a high EPF concentration are required to achieve full efficacy of these fungal isolates for controlling WAA.

#### **2.2.6 *Lecanicillium lecanii***

Jung *et al.* (2006) did a study called "Selection of *Lecanicillium* Strains for Aphid (*Myzus persicae*) Control," which found that *Lecanicillium* strains 4078 and 6543 were the best strains for aphid biocontrol at high temperatures and low relative humidity (RH) of 85 percent, respectively. After 3 days, strain 4078 had a 100% cumulative mortality, while strain 6543 had a 90% cumulative mortality at 85 percent RH after 5 days. At a wide temperature range, strain 4078 showed nearly 100% conidia germination and a high rate of mycelial development. The DNA sequences of the ITS sections of the fungi (accession no. EF026004 and EF026005, respectively) were used to identify the strains 4078 and 6543 as *Lecanicillium* species. Using steamed polished rice as the solid culture medium, an excellent generation of aerial conidia of strain 6543 was achieved.

Reddy and Sahotra (2018) conducted a study on the Multiplication of entomopathogenic fungus (*Lecanicillium lecanii*) on apple pomace and its toxicity against aphid (*Aphis craccivora*), and the results showed that AP medium at 2% recorded more mycelial growth and was comparable to AP medium at 3%, 4%, and 5%. *L. lecanii* spore yield was also significantly higher in AP at 5 percent and comparable to

AP 0.5 percent. *L. lecanii* at 1 109 spores ml<sup>-1</sup> had the highest mortality rate (43.33–93.33 percent mortality) against *A. craccivora*, followed by 1 108 spores ml<sup>-1</sup> (36.67–86.67 percent mortality).

### **2.3 Physical barriers for woolly apple aphid management**

Orpet *et al.* (2019) investigated the impact of employing sticky bands to prevent upward movement to the canopy and mulches and sand amendments to inhibit downward movement to trees on woolly apple aphid mobility between root and canopy populations. Bands were 10 cm high by 0.6 cm thick and placed 20 cm above ground on layers of cotton tape. During the observation period, aerial monitoring of woolly apple aphid colonies on the west side of trees up to a height of 2 m was done once a week. Crawlers that were travelling upward were caught on double-sided sticky tapes. The number of WAA colonies in the control and sticky band treatments was similar before the application of sticky bands. Colony counts remained low after that.

### **2.4 Effect on natural enemies**

Faiha (2012) investigated the toxicity of six insecticides on *Aphelinus mali* Hald (Deltamethrin, Chlorpyrifos, Acetamiprid, Diflubenzuron, Lufenuron, and Fenoxycarb). The adult parasitoids were first tested for toxicity by exposing them to fresh-dry insecticide film applied on glass plates. The second test involved exposing adults to insecticide film applied to apple leaves. The third test was performed to determine the toxicity of insecticides on parasitoids found within the aphid mummy (pupa stage). The first test revealed that Chlorpyrifos was highly toxic to adult *A. mali*, resulting in 100 percent mortality. Diflubenzuron was completely safe, causing 27.16 percent mortality. The remaining four insecticides were classified as mildly to moderately hazardous. The second test revealed that Chlorpyrifos was also highly toxic to adult *A. mali*, causing 100 percent mortality; the others (Deltamethrin, Acetamiprid, Diflubenzuron, Fenoxycarb, and Lufenuron) were less toxic, causing 94.81, 75.32, 18.18, 37.66, and 3.90 percent mortality, respectively. The tested insecticides showed low toxicity to *A. mali* pupa within-host mummies in the third test. Diflubenzuron, Lufenuron, and Fenoxycarb were all non-toxic, with adult emergence rates of 19.83, 23.96, and 29.75 percent, respectively. Deltamethrin, Chlorpyrifos, and Acetamiprid were mildly toxic, with adults emerging at 46.67, 48.67, and 52.67 percent, respectively.

## Chapter-3

# MATERIALS AND METHODS

Present studies entitled “**Geographical variability of Woolly Apple Aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop**” were carried in Fruit Entomology Laboratory of the Department of Entomology and for the management of woolly apple aphid, trials were conducted in the farms of Department of Fruit Science, Dr Yashwant Singh Parmar University of Horticulture and Forestry, Nauni, Solan. The trials to study the effect of band application were carried out in the year 2021 at farmer’s field (Baghi) located in Shimla district of Himachal Pradesh situated at an elevation of 8612 feets amsl.

### 3.1 Survey and collection of insect samples

Samples of woolly apple aphid were collected from different apple growing areas of Himachal Pradesh and stored in 99% ethanol for further molecular analysis. The detail of the areas visited for survey and sample collection is given in Table 3.1.

**Table 3.1 Details of apple growing areas surveyed for sampling**

District	Location	Elevation (amsl)
Shimla	Baghi	2350
	Dhangvi	1800
	Nehraghati	2300
Kullu	Seobhagh	1550
	Nagar	1800
	Banjar	1400
Mandi	Nihri	2065
	Churag	1830
	Pandar	2300
Kinnaur	Sharboo	3050
	Kalpa	2960
	Sangla	2643

## **3.2 Genetic variability**

The protocols used to study genetic diversity of *Eriosoma lanigerum* from different locations are discussed under the following headings:

3.2.1 DNA extraction

3.2.2 Preparation and running of standard Agarose gel

3.2.3 PCR amplification

3.2.4 Visualization of PCR product

3.2.5 DNA sequencing

3.2.6 Analysis of sequences

### **3.2.1 DNA extraction**

Total genomic DNA of woolly apple aphid was extracted from aphids (adult) using the HiPurA<sup>®</sup> Insect DNA Purification Kit. The DNA purification procedure using the miniprep spin column comprising of three steps viz., adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA.

#### **3.2.1.1 General Preparation**

1. The reagents were examined for any kind of precipitation and further warmed at 55-65°C until precipitate dissolved.
2. Wash Solution Concentrate (15ml) was diluted by adding 45 ml Ethanol (96-100 %).
3. Proteinase K powder was resuspended in Molecular Biology Grade Water to obtain a 20mg/ml stock solution by mixing 1.25 ml of Molecular Biology Grade Water in 25mg of Proteinase K provided in the kit. After reconstitution it was stored at -20°C. The Proteinase K solution was added directly to each sample preparation with the caution.

#### **3.2.1.2 Isolation of genomic DNA**

1. Ten nymphs of woolly apple aphid each from different locations (stored in 90% ethanol) were used for extraction of DNA.

2. The insects (not more than 50 mg) were ground using clean mortar and pestle in liquid nitrogen to a fine powder and the tissue powder was transferred to clean capped 2.0 ml microcentrifuge tube.
3. Lysis Solution 180  $\mu$ l (L) and Proteinase K solution 20  $\mu$ l (20mg/ml) were added to capped 2.0ml microcentrifuge tube containing the insect sample ground in liquid nitrogen and mixed thoroughly by vortexing and further incubated at 55°C till the insects were completely digested.
4. Lysis Solution 200  $\mu$ l (C1) was added to the sample. Vortex was done thoroughly for 15 seconds and then incubated at 70°C for 10 minutes.
5. The lysate was added onto HiShredder placed in a uncapped 2.0 ml collection tube and centrifuged for 2 minutes at 13,000 x g (=14,000 rpm).
6. The flow-through fraction from step 5 was transferred to a new 2.0 ml collection tube without disturbing the cell debris pellet.
7. Ethanol, 200  $\mu$ l (96-100 %) was added to the lysate and was mixed thoroughly by vortexing for 5-10 seconds.
8. The lysate obtained from step 7 was transferred onto HiElute Miniprep Spin Column (Capped) and it was centrifuged at 26,500 x g (=10,000 rpm) for 1 minute. The flow-through liquid was discarded and the column was placed in the same 2.0 ml collection tube.
9. Diluted wash solution 500  $\mu$ l was added to the column and centrifuged at  $\geq$ 6,500 xg (=10,000 rpm) for 1 minute. The flow-through liquid was discarded and the same collection tube with the column was re-used.
10. Another 500  $\mu$ l of diluted Wash Solution was added to the Column and it was centrifuged at 12,000-16,000 x g (=13,000-16,000 rpm) for 3 minutes to dry the storage.

### **3.2.1.3 Storage of purified DNA**

The genomic DNA was stored for further use at -20°C in a deep freezer.

### **3.2.2 Preparation and running of standard Agarose gel**

For gel preparation of 0.8% concentration, 1.6g of Agarose powder (SeaKem® LE Agarose) was mixed with 200ml of 1X TAE (Tris-acetate-EDTA) buffer Solution in 250ml volumetric flask and then the solution was heated until it was completely dissolved. For the

visualization of nucleic acids 2  $\mu$ l Ethidium Bromide was added to the solution after the solution get cooled to 50-55°C. The solution was poured into the casting tray with the comb for solidification. After the gel gets solidified the comb was removed immediately. During the electrophoresis the gel was kept in the plastic tray, the wells were toward the negative electrode and the gel was submerged under 1X TAE buffer. For the detection of DNA in the sample, 2  $\mu$ l of isolated DNA mixed with 2  $\mu$ l 6X loading dye (Bromophenol Blue) was placed into the well carefully. Alongside the DNA samples, a DNA ladder was also placed in well. Then the gel was run at 90 volts for 45 min to confirm the extraction. DNA was visualized by staining with ethidium bromide when ample migration has done (Corley, 2004)

### 3.2.3 DNA Amplification

The PCR amplification of DNA samples was carried out in a thermocycler (ProFlex™ Base) using 20  $\mu$ l reaction volume. The reaction mixture contained PCR pre-mix which is composed of Taq Buffer, dNTPs, MgCl<sub>2</sub>, Taq DNA Polymerase along with the pair of primers, distilled water and genomic DNA, making the reaction volume 20  $\mu$ l. The reagent and concentration along with their quantity are given in Table 3.2.

**Table 3.2 Reagent and concentration for 1X PCR reaction**

Reaction Mixture	Quantity ( $\mu$ l)
PCR pre-mix (Composition- Taq Buffer, MgCl <sub>2</sub> , dNTPs, Taq DNA polymerase)	10 $\mu$ l
Forward Primer (10 $\mu$ M) 20 pmol	1.0
Reverse Primer (10 $\mu$ M) 20 pmol	1.0
Water (SDW)	6.0
DNA	2.0
Total volume	20.0

#### 3.2.3.1 Primers

The PCR amplification of mitochondrial region (COX1) was done with the universal primer for arthropods LCO1490/HCO2198 as described in Table 3.3.

**Table 3.3 Primers along with their nucleotide sequences**

Name of the primer	Sequence (5' to 3')
MCOX-I (Forward)	5' GGTCAACAAATCATAAAGATATTG 3'
MCOX-I (Reverse)	5' TAAACTTCAGGGTGACCAAAAAATCA 3'

**3.2.3.2 PCR amplification**

The reaction mixture was added to PCR tubes and the thermal cycler was programmed for 35 cycles as given in Table 4 with one cycle of initial denaturation and step 2 to 4 were repeated 36 times and a final extension at 72°C for 30 minutes.

**Table 3.4 Temperature profile and number of cycles for PCR amplification**

Steps	Temperature (°C)	Time (minute)	Number of cycles
1. Initial denaturation	94	3.00	1
2. Denaturation	94	1.00	36
3. Annealing	50	1.00	
4. Elongation	72	1.00	
5. Final extension	72	30.00	1

**3.2.4 Visualization of amplified PCR product**

PCR product 2 µl from each tube was mixed with 2µl loading dye separately on a parafilm sheet and then loaded on 1.5 percent agarose gel (submerged in 1X TAE buffer and stained with 2 µl ethidium bromide for the detection of DNA) along with 2 µl DNA ladder (Genei 3kb DNA ladder) to determine the size of the amplified products. Then the gel was run at 90 volts for 60 minutes. Later the image analysis was done under UV light by using gel documentation system (BIO-RAD, Model- Universal Hood II) and Image Lab™ Software.

**3.2.5 DNA sequencing**

PCR product (10 µl) from each sample was sent to Eurofins Genomic India Pvt. Ltd., Bangalore, Karnataka for the sequencing. The Sanger dideoxy method was used for sequencing of *E. lanigeruma* samples.

### **3.2.6 Analysis of sequences**

The sequences were subjected to BLAST (Basic Local Alignment Search Tool) in search of the homology of the partial sequences and were compared with similar sequence from NCBI database in order to find their phylogenetic relationship. The phylogenetic tree was constructed based on the CO1 sequence of *E. lanigerum* with Neighbor Joining (NJ) method by using Mega X software (Saitou and Nei, 1987).

#### **3.2.6.1 Genetic divergence**

The genetic divergence was shown in terms of the number of base substitutions per site from between sequences. Analyses were conducted using the Maximum Composite Likelihood model (Tamura *et al.*, 2004) and evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

#### **3.2.6.2 Submission of sequences**

The aligned nucleotide sequence in FASTA format was submitted to NCBI with all the necessary details. NCBI was used for the submission of nucleotide sequences to NCBI (Wheeler *et al.*, 2001; Benson *et al.*, 2003), from where accession numbers for all the sequences were received.

## **3.3 Effects of different treatments for the management of woolly apple aphid in apple crop**

### **3.3.1 Management of aerial population of woolly apple aphid**

For the management of aerial form of woolly apple aphid, an apple orchard (10 years old) having aphid infestation was selected in the farms at Department of Fruit Science of UHF Nauni. One concentration each, of test insecticides/biopesticides namely, spirotetramat, spinosad, HMO, azadirachtin, *Lecanicillium lecanii*, *Metarhizium anisopliae*, *Beauveria bassiana* and chlorpyrifos was evaluated (Plate -1). The spray application was done in summer (May, 2022) with the high volume sprayer till the point of run off, and after each treatment, the pump was cleaned thoroughly with water to prevent any contamination of previous treatment. The trial was conducted in randomized block design having three replications with an infested tree as replicate. Four branches from all sides were selected and tagged for observations on colony count. The detail of the treatments used is given in Table 3.5.



**WAA infestation in aerial parts of apple tree**



**WAA infestation in fruiting areas**



**WAA infestation in water sprouts of apple tree**



**WAA infestation in roots of apple tree**

**Plate 1. Infestation of woolly apple aphid (WAA) in different parts of the apple plants**

**Table 3.5 Detail of different treatments used for management of woolly apple aphid.**

Treatment	Test product	Concentration (%)
T <sub>1</sub>	Spirotetramat	0.015
T <sub>2</sub>	Spinosad	0.04 %
T <sub>3</sub>	HMO	1.0 %
T <sub>4</sub>	<i>Metarhizium anisopliae</i>	2 x10 <sup>10</sup> cfu/lt
T <sub>5</sub>	<i>Beauveria bassiana</i>	2 x10 <sup>10</sup> cfu/lt
T <sub>6</sub>	Chlorpyrifos	0.05 %
T <sub>7</sub>	Azadirachtin (Neem formulation)	0.02 %
T <sub>8</sub>	<i>Lecanicillium lecanii</i>	2 x10 <sup>10</sup> cfu/lt
T <sub>9</sub>	Band application	10 cm width
T <sub>10</sub>	Control (untreated)	Water

**3.3.1.1 Colony count**

Pre-treatment aphid colony count on four marked branches/tree was taken before spray application in all the trees for experimentation. Thereafter, the colony count was recorded on the same branches at an interval of 3, 7, 14, 21 and 28 days of spray application. The reduction in colony count over pre-treatment count was calculated using following formula:

$$\text{Per cent reduction in colony count} = \frac{\text{Pre-treatment count} - \text{Post-treatment count}}{\text{Pre-treatment count}} \times 100$$

**3.3.1.2 Evaluation of physical barriers (sticky bands)**

This study was carried out in the farmer field at Baghi village of district Shimla (HP). The sticky monitoring band (Agri Pheroz Solutions, Hyderabad) of 10 cm width were installed 1ft above the ground on infested tree trunk. Average population of aphids trapped in sticky band

was calculated from all the replications and represented as aphids trapped per square cm of the band. The observations were recorded every month after replacing the old bands at the same site.

Four branches for calculating colony count and colony size per tree were selected from both control and the plants with sticky monitoring band. Monthly readings were taken from all the branches at the time of removal of sticky band so that a relation of the colony count and the colony size of aerial population of woolly apple aphid could be established between control and the plants with sticky monitoring traps.

### **3.4 Management of edaphic population of woolly apple aphid**

Orchard having aphid infestation was selected in the fields of Fruit Science Department of UHF Nauni. One concentration each of the test insecticides/biopesticides namely *Metarhizium anisopla*, *Beauveria bassiana*, chlorpyrifos and imidacloprid were used and compared with control where water was used (Table 3.6). Drenching was carried out in the first week of November. The trial was laid out in randomized block design having five replications with an infested tree as replicate.

#### **Sampling procedure:**

A standard soil sample was withdrawn from a depth of 5 cm using a soil auger with an internal diameter of 5 cm.

Samples were taken from the roots zone and soil with woolly apple aphid samples was placed in plastic packets and transported to the laboratory in ice boxice packs. The process of sampling was done at an interval of 0, 3,7,14 and 21 days of treatment.

The collected soil samples were rinsed through a sieve of 200  $\mu$  mesh size. The sieve was placed over a large glass beaker (1litre capacity) containing a solution of 600 g of sugar per litre of water, this sugar solution floated aphids to the surface and were taken to Petri dishes for count using stereoscopic microscope.

While taking count they were classified as alive or dead and the number of live aphids was noted.

Per cent decrease over pre count after drenching with various treatments was calculated from the observations on mortality of aphids.

**Table 3.6 Information regarding test insecticides and biopesticides used in present study**

Treatment	Test product	Concentration
T <sub>10</sub>	Chlorpyrifos	0.1 %
T <sub>11</sub>	Imidacloprid	0.035 %
T <sub>12</sub>	<i>Metarhizium anisopliae</i>	2 x10 <sup>10</sup> cfu/lit
T <sub>13</sub>	<i>Beauveria bassiana</i>	2 x10 <sup>10</sup> cfu/lit
T <sub>14</sub>	Control	Water

### 3.5 Effect of different treatments on natural enemies:

#### a) *Aphelinus mali*:

The effect of test insecticides/biopesticides was observed on Aphelinid endoparasitoid, *A. mali*. The parasitized aphids (lacking woolly strands and showing mummification) were collected from the experimental trees at an interval of 3, 7 and 14 days after spray and were brought to the laboratory where the parasitized aphids without exit holes were separated by observing under Stereoscopic Zoom Microscope using camel hair brush. Twenty aphids from each treatment collected after 3, 7 and 14 days of foliar application were kept in clean glass vials (15 ml Corning) for further observations in the laboratory. The data on emergence of parasitoid adults were recorded upto three months and thereafter the per cent emergence of parasitoid adults was calculated.

#### b) Coccinellids:

The adults of *Coccinella septempunctata* were collected from the field and were fed till three days with mustard aphids. The toxicity was evaluated using residue film method where test insecticide and biopesticides at the field concentrations were sprayed on the Petri plates (4.5x4.5 cm diameter) with the help of an atomizer, on the bottom, side walls and also on the inner side of

Petri plate (lid). The plates were allowed to dry, so that a film of the test product is obtained on the treated surface. Twenty adults were released thereafter and the mortality was recorded after 24, 48 and 72 hours of release.

### **3.6 Statistical Analysis**

The data recorded under field and laboratory conditions were subjected to statistical analysis. The data obtained from field trials were analysed using Randomized Block Design and data from laboratory studies was analysed by Completely Randomized Design. The transformation was applied to normalize the data as and when required (Gomez and Gomez, 1983).

## *Chapter-4*

# **RESULTS AND DISCUSSION**

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This chapter deals with the results and discussion of various experiments undertaken during research carried out on the topic “**Genetic variability of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop**”. Woolly apple aphid (WAA) infestation was observed in different districts of Himachal Pradesh and samples were brought to Fruit Entomology Laboratory, Department of Entomology to study the genetic variability among the populations under survey whereas, for the management of aerial and edaphic population of woolly apple aphid trials were conducted in the fields of Department of Fruit Science, Dr. Yashwant Singh Parmar University of Horticulture and Forestry. Trials on monitoring and mass trapping of test insect were conducted in farmer field at Baghi, Shimla district of Himachal Pradesh. The results of these studies have been presented and discussed here under following heads:

### **4.1 Survey and collection of insect samples**

Survey was conducted to know the woolly apple aphid incidence in the apple orchards in four districts of Himachal Pradesh viz., Kinnaur, Kullu, Mandi and Shimla. Each district was represented by three different locations where the plants were having symptoms of woolly apple aphid. The results of the survey of these locations have been presented in Table 4.1. The highest per cent infestation of WAA was observed at Seobagh (65.55) followed by the orchards at Sharbo with per cent infestation of 56.43. The infestation of WAA was found at Dhangvi (33.67 %). In the district wise comparison of WAA infestation, the highest per cent infestation was in district Kullu (49.30 %) followed by Kinnaur (47.30 %), Shimla (41.25 %) and least being in Mandi (37.21). There are not records of survey but the earlier studies shows that the maximum infestation of WAA in apple crop was observed during the months of June to August being the time of survey in all the locations. The extent of population is also correlated many times with the prevailing temperature and rainfall conditions in the area. The peak of population was observed during the scanty rainfall in Shimla hills during October to December (Thakur and Dogra, 1980). Low temperatures are conducive to aphid growth and high temperatures have a lethal effect. Maximum population was recorded during November-December at 4-8°C (Thakur, 1970), but below 2.8°C reproduction ceased. However, Lal and Singh, (1947)

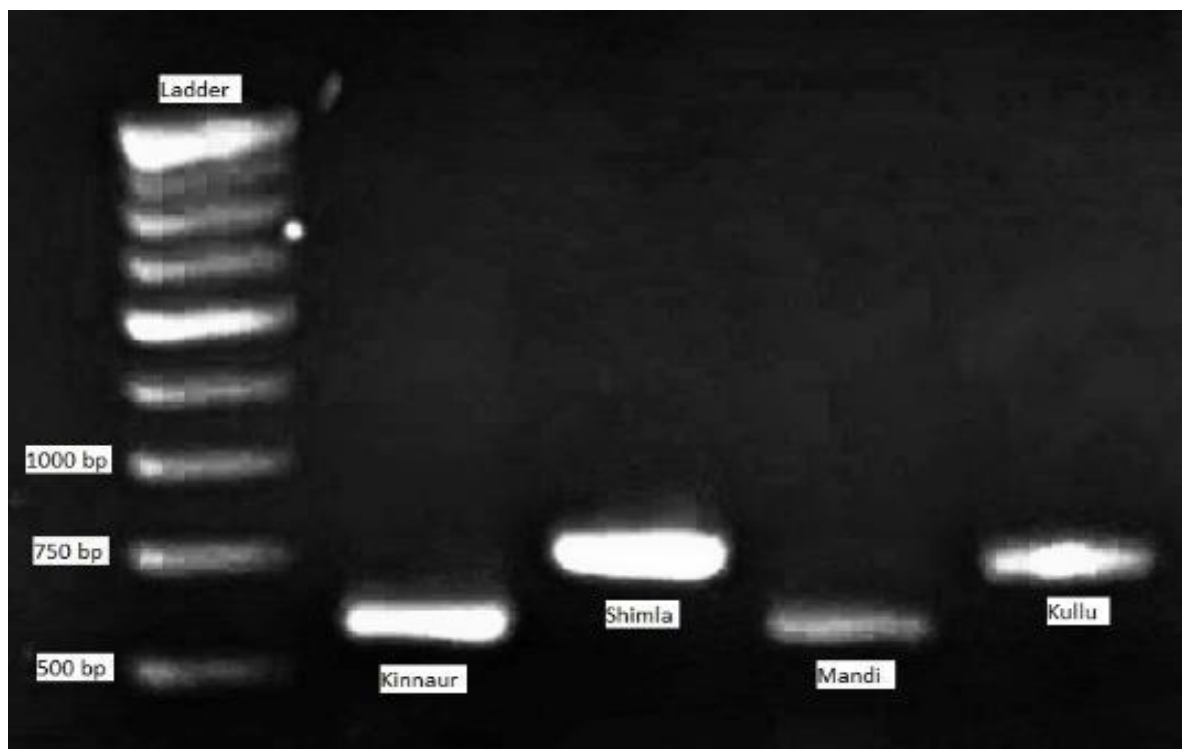
have correlated the high temperature upto 32 °C along with high humidity as responsible factors for highest population of WAA.

**Table 4.1 Per cent infestation of woolly apple aphid in different location under survey**

District	Location	Infestation (%)	Mean (%)	Elevation (m amsl)
Shimla	Baghi	47.56	41.25	2350
	Dhangvi	33.67		1800
	Nehraghati	42.42		2300
Kullu	Seobhagh	65.55	49.30	1550
	Nagar	35.67		1800
	Banjar	46.78		1400
Mandi	Nihri	34.78	37.21	2065
	Churag	36.43		1830
	Pandar	40.43		2300
Kinnaur	Sharboo	56.43	47.30	3050
	Kalpa	45.86		2960
	Sangla	39.60		2643

#### 4.2 Genetic variability

Samples from different locations under survey were collected and processed according to the procedures explained in Materials and Methods. The genomic DNA of *Eriosoma lanigerum* was extracted, amplified using the MCOX1 primers. The amplified PCR product of different DNA isolates was visualized in Gel Doc for its successful amplification (Fig. 1) and further these isolates were subjected for sequence analysis.



**Fig. 4.1 Visualization of PCR products on agarose gel electrophoresis (Ladder = 3Kb)**

#### 4.2.1 Analysis of nucleotide sequences

Comparative analysis of test sequence and reference sequence (NCBI database) were provided using BLAST tool (Basic Local Alignment Search Tool), the nucleotide sequence of *Eriosoma lanigerum* were aligned with the reference of NCBI (National Centre for Biotechnology Information) which revealed the taxonomic identifications viz., genus, species,

**Table 4.2 Gene Bank accession no. of MCOX1 sequence of the *Eriosoma lanigerum* collected from different location of Himachal Pradesh**

State	District	Place of Collection	Submission ID	Gen Bank accession no.
Himachal Pradesh	Shimla	Kotkhai	SUB11940735	OP236688
Himachal Pradesh	Kinnaur	Sharboo	SUB11912923	OP199033
Himachal Pradesh	Kullu	Seobagh	SUB11912923	OP199034
Himachal Pradesh	Mandi	Nihri	SUB11912923	OP199035

sub-species and family, to analyse the per cent similarities. The MCOXI sequences of *E. lanigerum* from all the 4 locations of the present study were submitted in NCBI GenBank vide accession numbers OP236688, OP199033, OP199034, OP199035 for Shimla (Kotkhai), Kinnaur (Sharbo), Kullu (Seobagh) and Mandi (Nihri) as given in Table 4.2.

#### 4.2.2 Variation in composition of nucleotides in MCOX1 of different isolates

The present MCOX1 sequences of *E. lanigerum* of different isolates from different districts of Himachal Pradesh the mitochondrial gene of insect showed AT rich nucleotide in sequenced regions a (Table 4.3). The average GC and AT content of the entire specimen was 24.72 % and 75.28 %, respectively. In Kinnaur, maximum AT content (81.43 %) was recorded and minimum AT content (73.83 %) was found from the test insect of Kullu district of Himachal Pradesh. The highest GC content (26.17 %) was recorded from Kullu and lowest GC content (18.57 %) was recorded from Kinnaur district of Himachal Pradesh. Overall, the average nucleotide compositions for *E. lanigerum* were found to be thymine (37.44 %), cytosine (11.23 %), adenine (37.83 %), and guanine (13.50 %), respectively.

**Table 4.3 Average nucleotide composition of MCOX1 gene sequence among all *Eriosoma lanigerum* specimens collected from different locations of Himachal Pradesh**

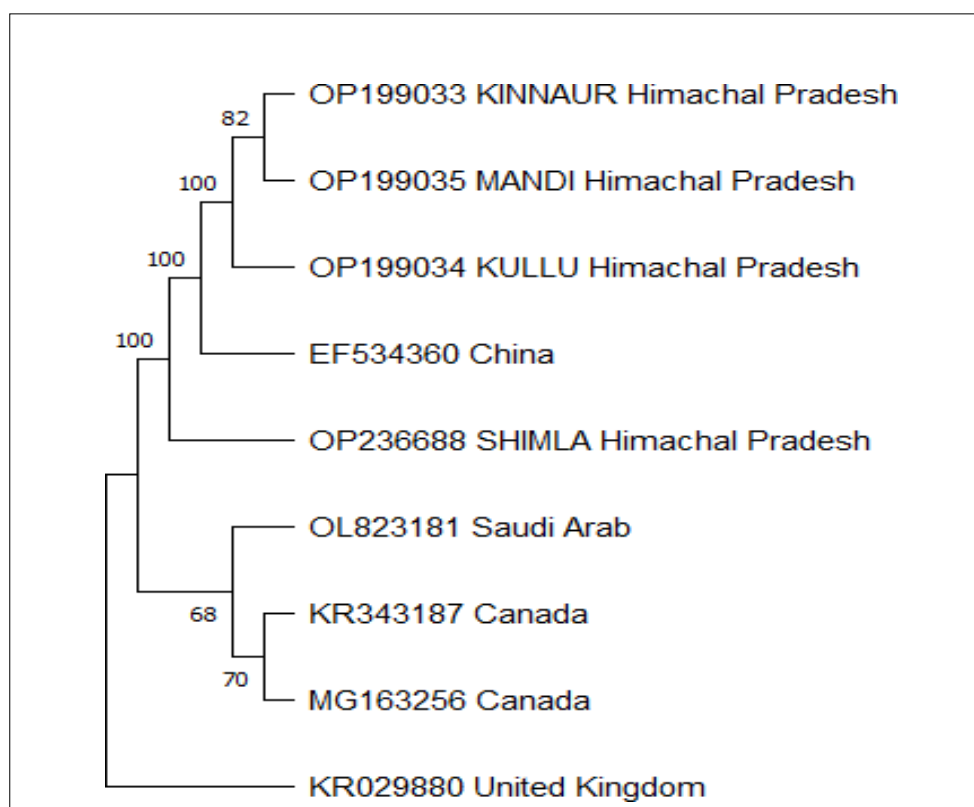
DNA Isolate	Thymine (T)	Cytosine (C)	Adenine(A)	Guanine(G)	AT	GC
Kullu Isolate	37.97	11.95	35.85	14.22	73.83	26.17
Mandi Isolate	36.52	9.87	39.03	14.58	75.55	24.45
Shimla Isolate	37.69	12.81	38.44	11.06	76.13	23.87
Kinnaur Isolate	40.00	8.57	41.43	10	81.43	18.57
Average	37.44	11.23	37.83	13.50	75.28	24.72

The highest thymine (T) percentage with the value of (40.00 %) was recorded from Kinnaur and minimum (36.52 %) from Mandi isolate. Specimen from Shimla had highest (12.81 %) percentage of cytosine and lowest (8.57 %) cytosine percentage was recorded from Kinnaur. The highest adenine percentage (41.43 %) was recorded from Kinnaur isolate and

minimum value (35.85%) from Kullu district. In case of guanine the highest percentage of 14.58 % was from Mandi isolate and lowest (10 %) from Kinnaur isolate.

### 4.2.3 Phylogenetic analysis

Genomic sequence data of WAA was used to work out their evolutionary evidences and a phylogenetic tree was assembled using Neighbour-joining (NJ) using Mega X programme (Saitou and Nei, 1987). The analysis of phylogenetic tree revealed that the population of China, Canada, Saudi Arab and UK formed three distinct clades in cluster analysis. All the woolly apple aphid collected from Himachal Pradesh matched the population of China. Population from Canada and Saudi Arabia were clubbed to form a separate clade whereas, the population of United Kingdom formed the most distant clade.



**Fig. 4.2 Phylogenetic tree of isolates of *Eriosoma lanigerum* that were collected from different districts of Himachal Pradesh**

The perusal of data in Table 4.4 obtained from pair wise analysis of genetic diversion of *E. lanigerum* population and collected from different districts of Himachal Pradesh revealed that the divergence ranged from 0.0679 – 3.3982. This suggests that there is no much variation in the population *E. lanigerum* within different locations. The population from

Kinnaur showed a genetic diversion of 3.3982, 3.3298 and 1.7769 when compared with Kullu, Mandi and Shimla, respectively. The genetic distance of *E. lanigerum* population collected from Shimla was 0.04548 when compared with Kullu and 0.04727 when compared with aphid population of Mandi. However, Mandi isolate had the least genetic diversion of 0.0679 when compared with aphid population of Kullu.

**Table 4.4 Genetic divergence of MCOX1 gene sequences among *Eriosoma lanigerum* specimens collected from different locations of Himachal Pradesh.**

Isolates	Kullu isolate	Mandi isolate	Shimla isolate	Kinnaur isolate
Kullu isolate				
Mandi isolate	0.0679			
Shimla isolate	0.4548	0.4727		
Kinnaur isolate	3.3982	3.3298	1.7769	

NOTE - Each entry is the probability of substitution (r) from one base (row) to another base (column). Substitution pattern and rates were estimated under the Tamura-Nei (1993) model. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in italics. Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 100, the nucleotide frequencies are A = 37.83%, T/U = 37.44%, C = 11.23%, and G = 13.50%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was -1519.254. This analysis involved 4 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 699 positions in the final dataset. Evolutionary analyses were conducted in MEGA11.

### **4.3 Effects of different treatments for the management of woolly apple aphid in apple crop**

#### **4.3.1 Management of aerial population of woolly apple aphid**

For the management of aerial form of woolly apple aphid, eight different treatments viz., spirotetramat, spinosad, HMO, azadirachtin, *Lecanicillium lecanii*, *Metarhizium anisopliae*, *Beauveria bassiana* and chlorpyrifos were evaluated. The application of different treatments was done during the summer (May, 2022). The effect was measured by

taking into consideration the parameters of colony count of aerial population and number of aphids in soil samples.

#### 4.3.1.1 Observation on colony count

The perusal of data in Table 4.5 and 4.6 revealed the effect of colony count and per cent reduction in woolly apple aphid population (Plate -2, 3, 4). The data taken one day after the treatment revealed that there was decrease in the aphid colony count from 11.58 to 7.17 in case of chlorpyrifos (0.05 %) which is the maximum quick reduction of population with 38.96 per cent in colony count. No other treatments resulted in a decline in aphid population i.e 0 per cent reduction in colony count.

After, the third day of treatment, chlorpyrifos (0.05 %) showed the maximum reduction of 60.57 per cent with decline in aphid colonies from 11.58 to 4.57. It was followed by spirotetramat 0.015 per cent that shows reduction from 11.42 to 6.85 colonies per 10cm shoot which means, a 40.07 per cent reduction in colony count of woolly apple aphid. Whereas, among mineral oil and biopesticide, 11.68 per cent, 15.73 per cent, 14.48 per cent, 17.17 per cent, 16.86 per cent reduction in colony count was observed in HMO 1 per cent, *Metarhizium anisopliae* ( $2 \times 10^{10}$  cfu/l), *Beauveria bassiana* ( $2 \times 10^{10}$  cfu/l), azadirachtin (0.02%) and *Lecanicillium lecanii* ( $2 \times 10^{10}$  cfu/l), respectively.

After seven days of treatment, there was huge decline in live aphid colonies which were treated with chlorpyrifos (0.05 %), followed by spirotetramat (0.015 %). The decline in chlorpyrifos, was from 11.58 to 2.27 followed by spirotetramat from 11.42 to 5.28 which resulted in 80.44 (%) and 53.79 (%) reduction, respectively.

In case of biopesticide, relatively low decline was observed in aphid colony count in comparison to treatments of chlorpyrifos (0.05 %) and spirotetramat (0.015 %). HMO resulted in reduction of live aphid colonies from 11.25 to 9.27, *Metarhizium anisopliae* ( $2 \times 10^{10}$  cfu/l) from 10.67 to 8.46, *Beauveria bassiana* ( $2 \times 10^{10}$  cfu/l) 13.42 to 10.45, azadirachtin (0.02 %) 11.58 to 11.01 and *Lecanicillium lecanii* ( $2 \times 10^{10}$  cfu/l) 10.75 to 8.11. Spinosad (0.04 %) being insecticide of organic origin resulted in 28.74 (%) reduction in colony count but was better than the microbial applications.

After 14 days of treatment, a drastic decrease was observed in aphid population of plants treated with spirotetramat (0.015 %). The decline was from 11.422 to 2.24 resulting in 80.41 (%) reduction in colony count which was at par with chlorpyrifos (83.7 %).

HMO was found to be least effective resulting in only 21.63 (%) reduction in the colony count. *Metarhizium anisopliae* ( $2 \times 10^{10}$  cfu/lit), *Beauveria bassiana* ( $2 \times 10^{10}$  cfu/lit), azadirachtin (0.02%), *Lecanicillium lecanii* ( $2 \times 10^{10}$  cfu/lit) resulted in per cent reduction of 29.50, 26.68, 29.32 and 31.11, respectively. The trend for spinosad (0.04 %) remained same and resulted in decrease of colony count from 12.00 to 7.89 resulting in only 34.22 (%) colony count reduction. The effect of spinosad (0.04 %) was meager as compared to chlorpyrifos (0.05 %).

After 21 days of treatment, chlorpyrifos (0.05 %) was most effective resulting in decrease in colony count from 11.58 to 0.92 and per cent reduction of 92.08. This was followed by spirotetramat (0.015 %) with a per cent reduction of 83.14 per cent and decrease in colony count from 11.42 to 1.93. HMO was found to be least effective with per cent reduction of 25.9 per cent and decrease in colony count from 11.25 to 8.34.

The treatment mean for all insecticide and biopesticide were calculated and data in Table 12 revealed that chlorpyrifos (0.05 %) was most effective with overall population reduction of 71.15 per cent and with quick knock down effect to woolly apple aphid. Spirotetramat (0.015 %) had a delayed effect in controlling woolly apple aphid colonies but remained effective upto 21 days of treatment. The findings are similar to that of Singh and Bhardwaj (2018) who studied the field efficacy of new insecticides namely spirotetramat (Movento) @ 0.009, 0.012, 0.015 per cent and compared with chlorpyrifos (Dursban 20 EC) @ 0.04 %. The findings of the authors revealed that both the treatments were superior, and 21 days after spray, chlorpyrifos was the best with nil infestation. The bioefficacy of chlorpyrifos, spirotetramat, *Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii* and azadirachtin has also been studied by Kumar and Gupta, (2019). The results of their studies have shown the superior effect of chlorpyrifos and spirotetramat with 68.37 and 61.04 per cent reduction in colony whereas, the biopesticides remained at par with each other. The application of *Beauveria bassiana* ( $2 \times 10^{10}$  cfu/lit), and azadirachtin (0.02 %) resulted in 29.17 and 29.83 per cent reduction of woolly apple aphid that is similar to our findings with reduction in population of 19.53 and 21.27 per cent.

**Table 4.5 Bioefficacy of insecticides and biopesticides against aerial population of woolly apple aphid, *Eriosoma lanigerum* (based on colony count)**

Treatment	Concentration (%)	Average number of live aphid colony after different days of treatment					
		Pre-treatment count	1 DAT	3 DAT	7 DAT	14 DAT	21 DAT
Spirotetramat	0.015	11.42(3.52)	11.42(3.52)	6.85(2.76)	5.28(2.47)	2.24((1.77)	1.93(1.70)
Spinosad	0.04	12.00(3.60)	12.00(3.60)	9.44(3.21)	8.55(3.07)	7.89(2.97)	7.64(2.93)
HMO	1.00	11.25(3.49)	11.25 (3.49)	9.94(3.28)	9.27(3.18)	8.82(3.13)	8.34(3.05)
<i>Metarhizium anisopliae</i>	2×10 <sup>10</sup> cfu/lit	10.67(3.41)	10.67(3.39)	8.99(3.13)	8.46(3.05)	7.52(2.91)	7.23(2.86)
<i>Beauveria bassiana</i>	2×10 <sup>10</sup> cfu/lit	13.42(3.78)	13.42(3.78)	11.48(3.51)	10.45(3.37)	9.84(3.28)	8.80(3.12)
Chlorpyriphos	0.05	11.58(3.54)	7.17(2.82)	4.57(2.29)	2.27(1.77)	0.73(1.24)	0.92(1.37)
Azadirachtin	0.02	14.58(3.94)	14.58(3.93)	12.08(3.60)	11.01(3.45)	10.31(3.36)	9.42(3.22)
<i>Lecanicillium lecanii</i>	2×10 <sup>10</sup> cfu/lit	10.75(3.42)	10.75(3.40)	8.94(3.06)	8.11(2.99)	7.51(2.91)	6.97(2.82)
Control	Water	11.20 (3.48)	11.20 (3.48)	11.56 (3.54)	12.30 (3.70)	16.70 (4.56)	18.8 (4.96)
Mean		11.95 (3.59)	11.46 (3.53)	9.31 (3.19)	8.41 (3.05)	7.94 (2.99)	7.78 (2.96)
C.D <sub>(0.05)</sub>		0.025	0.06	0.14	0.17	0.21	0.1

Figures in parentheses are square root transformed values

DAT: Days after treatment

**Table 4.6 Bioefficiency of insecticides and biopesticides against aerial population of woolly apple aphid, *Eriosoma lanigerum* (based on per cent reduction)**

Treatment	Concentration (%)	Percent reduction in colony count over pre count					
		1DAT	3DAT	7DAT	14DAT	21DAT	MEAN
Spirotetramat	0.015	0(0)	40.07(39.28)	53.79(47.18)	80.41(63.73)	83.14(65.76)	51.48(45.85)
Spinosad	0.04	0(0)	21.37(27.54)	28.74(32.42)	34.22(35.81)	36.34(37.07)	24.13(29.43)
HMO	1	0(0)	11.68(19.99)	17.58(24.79)	21.63(27.72)	25.90(30.60)	15.36(23.08)
<i>Metarhizium anisopliae</i>	2×10 <sup>10</sup> cfu/lt	0(0)	15.73(23.37)	20.71(27.07)	29.50(32.90)	32.20(34.58)	19.63(26.3)
<i>Beauveria bassiana</i>	2×10 <sup>10</sup> cfu/lt	0(0)	14.48(22.37)	22.09(28.04)	26.68(31.10)	34.41(35.92)	19.53(26.23)
Chlorpyrifos	0.05	38.96(38.6)	60.57(51.11)	80.44(63.76)	83.70(66.19)	92.08(73.65)	71.15(57.52)
Azadirachtin (Neem formulation)	0.02	0(0)	17.17(24.48)	24.48(29.65)	29.32(32.78)	35.38(36.50)	21.27(27.46)
<i>Lecanicillium lecanii</i>	2×10 <sup>10</sup> cfu/lt	0(0)	16.86(24.25)	24.59(29.73)	30.11(33.28)	35.14(36.36)	21.34(27.52)
Control*		-	-	-	-	-	-
Mean		4.32(14.57)	17.90(24.92)	34.05(35.68)	41.94(39.81)	46.80(43.18)	

C.D.(0.05)

Treatment 1.32

Days 1.02

T×D 2.94

\* There was increase in the insect population

\*\* Figures in parentheses are square root transformed values



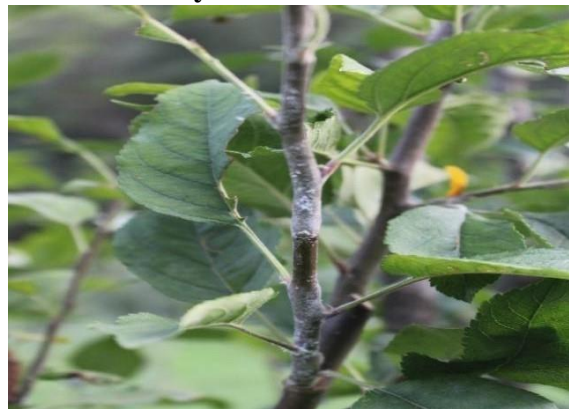
**Woolly apple aphid population, 0 days after treatment**



**Woolly apple aphid population, 3 days after treatment**



**Woolly apple aphid population, 7 days after treatment**



**Woolly apple aphid population, 14 days after treatment**



**Woolly apple aphid population, 21 days after treatment**

**Plate 2. Effect of chlorpyrifos on woolly apple aphid colonies**



**Woolly apple aphid population,  
0 days after treatment**



**Woolly apple aphid population,  
3 days after treatment**



**Woolly apple aphid population,  
7 days after treatment**



**Woolly apple aphid population,  
14 days after treatment**



**Woolly apple aphid population,  
21 days after treatment**

**Plate 3. Effect of spirotetramat on woolly apple aphid colonies**



**Woolly apple aphid population,  
0 days after treatment**



**Woolly apple aphid population,  
3 days after treatment**



**Woolly apple aphid population,  
7 days after treatment**



**Woolly apple aphid population,  
14 days after treatment**



**Woolly apple aphid population,  
21 days after treatment**

**Plate 4. Effect of azadirachitin on woolly apple aphid colonies**

### 4.3 Band application

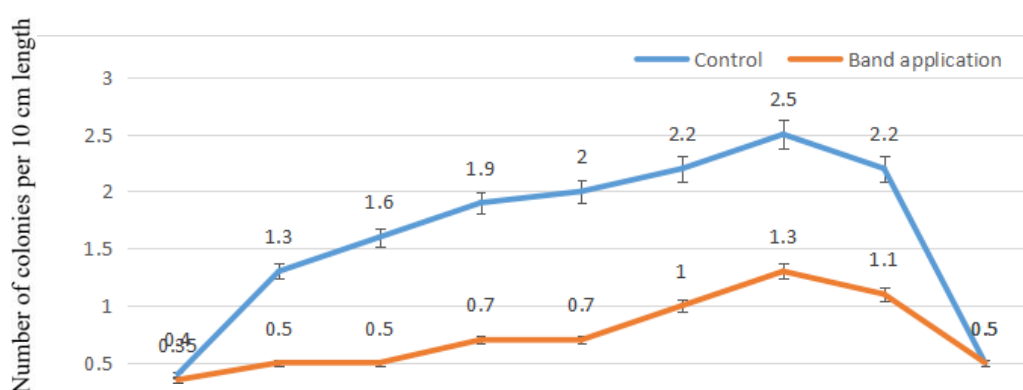
#### 4.3.1 Effect of band application on the colonies of Woolly apple aphid

The studies on the movement of woolly apple aphid from aerial parts to roots were conducted in the farmer's field at Baghi district Shimla in the year 2021. The moving nymphs were trapped using market available sticky bands (Plate -5) that are smeared with rat glue. The glue used in these traps was water proof and thus remained effective for months after its application on the band. The bands were applied in the month of April 2021 on apple tree trunk at a height of 1 to 2 feet above the ground. The crevices if any, on the bark of the tree were filled with mud so as to force the movement of WAAs over the band. The data in Table 4.7 shows the aphid trapped on the sticky band. It is apparent from the table that minimum number of aphids (14.50) were trapped in the month of December and therefore, minimum number of colonies on the tree (0.35) as compared to the control with 0.4 colonies per 10 cm shoots (Fig. 3). The trap catch continued to be increased during May onwards with maximum population (89.70 aphids) trapped in 1 cm<sup>2</sup> area of band in the month of November. Correspondingly, the number of colonies per 10 cm shoot was maximum in the month of October (2.5 in control and 1.3 in treatment). The reason for the movement of aphid nymphs have already been reported by many scientists in the past (Lal and Singh, 1947; Gautam and Verma, 1983; Bhardwaj *et al.*, 1995). In these studies, only first instar nymphs reported to show the movement from both the zone *viz.*, roots to

**Table 4.7. Number of aphids trapped in sticky bands monitoring band**

Month	Number of aphid per sq cm
April	14.50 ± 22.30
May	29.30 ± 22.30
June	32.80 ± 22.30
July	31.70 ± 22.30
August	33.56 ± 22.30
September	42.35 ± 22.30
October	61.70 ± 22.30
November	89.70 ± 22.30
December	13.20 ± 22.30

shoots and vice versa. The mean aphid movement per fortnight was 59.34 aphids. The ambient temperature 13.2 to 26 °C and the rainfall > 72 mm were assigned reasons for the movement of aphids from both zones (Bhardwaj *et al.*, 1995). There is increment change in the population of WAA in our studies also. The aphid colony number was found to be increased May onward to October with the increase in the temperature. After the month of October, 2021 the population was observed to be at decline reached minimum (0.5 colonies per 10 cm shoot) in December. These aphids have been observed to show both way movements through out of the year and reasons are uncertain.



**Fig. 4.3 Effect of band application on the colony number of woolly apple aphid.**

#### **4.4 Bioefficacy of test insecticides and biopesticides against edaphic population of woolly apple aphid**

Out of the two insecticides tested for the control of woolly apple aphid it was observed that in imidacloprid (0.035%) the reduction in aphid number was from 15.50 to 2.25 after third day of the treatment resulting in 85.39% reduction in the aphid number over the pre count whereas, in case of chlorpyrifos the decline in aphid number was from 12.50 to 3.50 resulting in 72.00 % of reduction in the aphid population. In biopesticides there was only a small reduction in the aphid number over pre count *Metarhizium anisopliae* had a decrease from 14.25 to 13.25 and *Beauveria bassiana* had a reduction of 13.50 to 11.50 resulting in 7.16 % and 14.36% reduction, respectively.



**Process of installation of sticky bands on apple tree trunk**



**Sticky band after installation**



**Monitoring bands in farmers field at Baghi**



**Woolly apple aphids trapped in sticky bands**

**Plate 5. Application of sticky bands for the monitoring of woolly apple aphid population in apple**

**Table. 4.8 Average number of live aphids after different days of treatment**

Treatment	Concentration (%)	Average number of live aphids after different days of treatment				
		Pre count	3 DAT	7 DAT	14 DAT	21 DAT
Chlorpyriphos	0.10	12.50(3.66)	3.50 (2.10)	3.00 (1.97)	4.23 (2.23)	4.25 (2.26)
Imidacloprid	0.035	15.50(4.06)	2.25 (1.79)	1.50 (1.53)	1.00 (1.39)	0.75 (1.31)
<i>Metarhizum anisopliae</i>	4×10 <sup>10</sup> cfu/l	14.25(3.89)	13.25(3.76)	13.00(3.73)	11.25(3.48)	10.50 (3.37)
<i>Beauveria bassiana</i>	4×10 <sup>10</sup> cfu/l	13.50(3.78)	11.50(3.51)	11.00 (3.42)	10.25 (3.33)	10.00( 3.30)
Control	Water	13.75(3.82)	14.80(3.99)	15.95(4.18)	15.80(4.17)	16.25(4.28)
Mean		13.83 (3.84)	10.05 (3.31)	10.15 (3.31)	9.84 (3.25)	9.875 (3.25)
CD <sub>(0.05)</sub>		0.43	0.35	0.39	0.26	0.18

\*Figures in parentheses are square root transformed values

DAT: Days after treatment

**Table. 4.9 : Bioefficacy of insecticides and biopesticides against edaphic population of woolly apple aphid, *Eriosoma lanigerum* (based on per cent reduction over pre-count)**

Treatment	Concentration (%)	Per cent reduction over control				
		Day-3	Day-7	Day-14	Day-21	Mean
Chlorpyrifos	0.10	72.00 (8.71)	76.86 (9.05)	65.24 (8.19)	66.64 (8.23)	70.19 (8.48)
Imidacloprid	0.035	85.39 (9.60)	90.20 (9.85)	93.72 (9.91)	95.20 (9.97)	91.13 (9.87)
<i>Metarhizum anisopliae</i>	4×10 <sup>10</sup> cfu/l	7.16 (4.54)	8.01 (4.68)	20.94 (5.93)	26.71 (6.59)	15.70 (5.23)
<i>Beauveria bassiana</i>	4×10 <sup>10</sup> cfu/l	14.36 (5.02)	20.05 (5.83)	23.62 (6.37)	24.61 (6.64)	20.66 (5.86)
Control**		-	-	-	-	-
Mean		35.74 (5.57)	29.56 (5.88)	40.70 (6.08)	42.63 (6.29)	

\*Figures in parentheses are square root transformed values

\*\* There was increase in the insect population

DAT: Days after treatment

CD<sub>(0.05)</sub>

Treatment 0.34

Days 0.42

T×D 1.02

After seventh day of the treatment the rapid decline in the aphid population was seen to be continued in case of insecticidal treatments. The aphid population reduced from 15.50 to 1.50 in imidacloprid and from 12.50 to 3.00 in chlorpyrifos treatment, resulting in 90.20 and 76.86% reduction respectively. The decline in aphid population in case of *Metarhizium anisopliae* and *Beauveria bassiana* was minimum and resulted in 8.01 per cent and 20.05 per cent reduction over the pre count.

Forteenth day observations of the treatment revealed that the aphid number increased when compared with seventh day of treatment in case of chlorpyrifos and resulted in 65.24% reduction over pre count. In imidachloprid treatment from day seven to fourteen day the aphid number reduced from 1.5 to 1.00 and hence 93.72 percent reduction over pre count was observed. The same was revealed in the work of Singh and Gupta (2006) where five different insecticides, including imidacloprid (0.04%), acetamiprid (0.01%), thiamethoxam (0.05%), acephate (0.12%), and profenofos (0.10%), were evaluated against the subterranean population of the woolly apple aphid, *E. lanigerum*, by drenching apple seedlings raised in earthen pots. Except for chlorpyrifos, which was only effective for 10 days, all of the test pesticides were comparable to phorate (1 g a.i./plant) and significantly superior to chlorpyrifos with reduction of 0.08 percent and remained effective for 30 days against the root form of the aphid.

In *Metarhizium anisopliae* and *Beauveria bassiana* 20.94 % and 23.62% reduction over pre count was calculated where aphid population decreased from 14.25 to 11.25 in case of *Metarhizium anisopliae* and from 13.50 to 10.25 in case of *Beauveria bassiana*.

After twenty one days of the treatment the aphid population of chlorpyrifos was at par with the aphid population on day fourteen and hence no change in per cent decrease in aphid population was observed. There was still a decrease in aphid population from 1.00 to 0.75 when the data for day fourteen and twenty one was analysed for imidacloprid treatment, this data revealed the maximum reduction of 95.20 per cent over pre count was in imidachloprid. Biopesticides, *Metarhizium anisopliae* and *Beauveria bassiana* resulted in 26.71 % and 24.61 % reduction in the colony count.

Imidacloprid proved to be the best treatment for the control of edaphic population of woolly apple aphid with the mean per cent decrease of 91.13, which was followed by chlorpyrifos with the mean reduction of 70.19 whereas, *Metarhizium anisopliae* and

*Beauveria bassiana* resulted in 15.70 and 20.66 per cent reduction of woolly apple aphid population.

#### 4.5 Toxicity of test insecticides and biopesticides to natural enemy

##### 4.5.1 Effect on *A. mali*

**Table 4.10: Toxicity of insecticides and biopesticides on aphelinid parasitoid, *Aphelinus mali* parasitizing woolly apple aphid**

Treatment	Concentration (%)	Percent emergence of parasitoid ( <i>Aphelinus mali</i> ) from parasitized aphids		
		3 DAT	7 DAT	14 DAT
Spirotetramat	0.015	60.56 (7.48)	56.14 (7.56)	64.96 (8.12)
Spinosad	0.04	52.80 (7.33)	48.15 (7.01)	64.44 (8.09)
HMO	1.00	38.51 (6.28)	43.30 (6.65)	39.44 (6.35)
<i>Metarhizium anisopilae</i>	2×10 <sup>10</sup> cfu/lt	48.15 (7.00)	43.87 (6.70)	52.80 (7.33)
<i>Beauveria bassiana</i>	2×10 <sup>10</sup> cfu/lt	54.31 (7.43)	44.42 (6.74)	61.49 (7.90)
Chlorpyrifos	0.05	10.83 (3.43)	14.89 (3.97)	15.82 (4.08)
Azadirachtin	0.02	60.24 (7.82)	56.84 (7.60)	65.66 (8.16)
<i>Lecanicillium lecanii</i>	2×10 <sup>10</sup> cfu/lt	55.34 (7.50)	47.20 (6.94)	62.74 (7.98)
Control	Water	90.00(71.57)	95.00(77.08)	90.00(71.57)
CD <sub>(0.05)</sub>		0.33	0.44	0.51

\*Figures in parentheses are square root transformed values

DAT: Days after treatment

The perusal of data in Table 4.10 revealed that after 3, 7 and 14 days of foliar application parasitized aphids were collected from experimental apple trees and brought to laboratory for calculating per cent emergence of adult parasitoids (Plate-6). The data revealed that particular trend in adult parasitoids emergence was recorded where the lower emergence of parasitoids was recorded from the sample collected after 7 days of treatment. Higher emergence of *A. mali* from parasitized aphids was recorded from the sample collected after 14 days of treatment, while moderate emergence of *A. mali* was recorded from samples collected after 3 days of treatment. While in case of chlorpyrifos the per cent emergence of adult



**Woolly apple aphid colonies on apple tree**



**Mummified aphids bodies due to *Aphelinus mali***



**Mummified aphids bodies showing the exit hole**



**Adult of *A. mali*. from mummified aphids**

**Plate 6. Toxicity of different insecticides and biopesticidal treatments on *Aphelinus mali***

parasitoids increased as the days after the treatment increases. The collections of parasitized aphids were done from field in the month of October and were put under observation till month of March. After last week of November no adult emergence was recorded which again started in month of February. These were due to over-wintering of parasitoids during winter months and resume its activity with warming of seasons (Gupta *et al.*, 2007).

Among insecticides, spirotetramat @ 0.015 (%) was the more safe to *A. mali* and resulted in 60.56 (%), 56.14 (%), 64.96 (%) emergence from parasitized aphids collected after 3, 7 and 14 days, respectively. The second best treatment was Spinosad @ 0.04 (%) which resulted in 52.80 (%), 48.15 (%) and 64.44 (%) emergence after 3, 7 and 14 days respectively. Chlorpyrifos resulted in least emergence of *A. mali* from parasitoids aphid *i.e.* 10.83 (%), 14.89 (%) and 15.82 (%) after 3, 7 and 14 days respectively. Among biopesticide, azadirachtin was observed to be the most safe for *A. mali* with 60.24 (%), 56.84 (%) and 65.6 (%) emergence respectively. Biopesticide were safer as compared with chlorpyrifos. *Beauveria bassiana* ( $4 \times 10^{10}$  cfu/l) resulted in 54.31 (%), 44.42 (%) and 61.49 (%) per cent emergence of *A. mali* after 3,7 and 14 days of treatment and which was at par with the emergence of *Metarhizum anisopliae* ( $2 \times 10^{10}$  cfu/l), *Lecanicillium lecanii* ( $2 \times 10^{10}$  cfu/l).

The results of this study are in line with Gupta *et al.* (2013) who recorded 12.50 (%) adult emergence in case of chlorpyrifos (0.04 %), which again corroborate the findings of present study where the adult emergence of parasitoids recorded was 10.83 (%), 14.89 (%) and 15.82 (%) from the parasitoids aphid collected after 3, 7 and 14 days of chlorpyrifos spray respectively.

#### **4.5.1 Effect on *C. septempunctata***

The data presented in Table 4.11 revealed the effect of different insecticidal and biopesticidal treatments on the *C. septempunctata* (Plate-7). Among various insecticide and biopesticide tested, it was reported that HMO @ 1(%) was most safe biopesticide for the *C. septempunctata* which resulted in 0 (%) mortality even after the interval of 72 hours treatment. Whereas, mortality rate of 87.23 (%), 100 (%) after 24, and 48 hours of treatment was observed in the case of chlorpyrifos @ 0.05 (%).

**Table 4.11 Toxicity of insecticides and biopesticides on *Coccinella septempunctata***

Treatment	Concentration (%)	Percent mortality of <i>C. septempunctata</i> adults		
		24 HAT	48 HAT	72HAT
Spirotetramat	0.01	23.86 (4.97)	28.24 (5.39)	31.92 (5.73)
Spinosad	0.04	20.60 (4.58)	27.86 (5.35)	33.10 (5.83)
HMO	1.00	00.00 (1.00)	00.00 (1.00)	0.00 (1.00)
<i>Metarhizium anisopliae</i>	2×10 <sup>10</sup> cfu/lt	12.90 (3.72)	21.66 (4.75)	23.66 (4.96)
<i>Beauveria bassiana</i>	2×10 <sup>10</sup> cfu/lt	15.30 (4.03)	21.71 (4.75)	23.50 (4.91)
Chlorpyrifos	0.05	87.23(9.39)	100.00 (10.05)	100.00(10.05)
Azadirachtin	0.02	18.73 (4.44)	26.56 ( 5.25)	28.36 (5.41)
<i>Lecanicillium lecanii</i>	2×10 <sup>10</sup> cfu/lt	12.53 (3.67)	19.83 (4.56)	22.12 (4.79)
Control		00.00 (1.00)	00.00 (1.00)	00 (1.00)
CD <sub>(0.05)</sub>		0.57	0.60	0.27

\*Figures in parentheses are square root transformed values

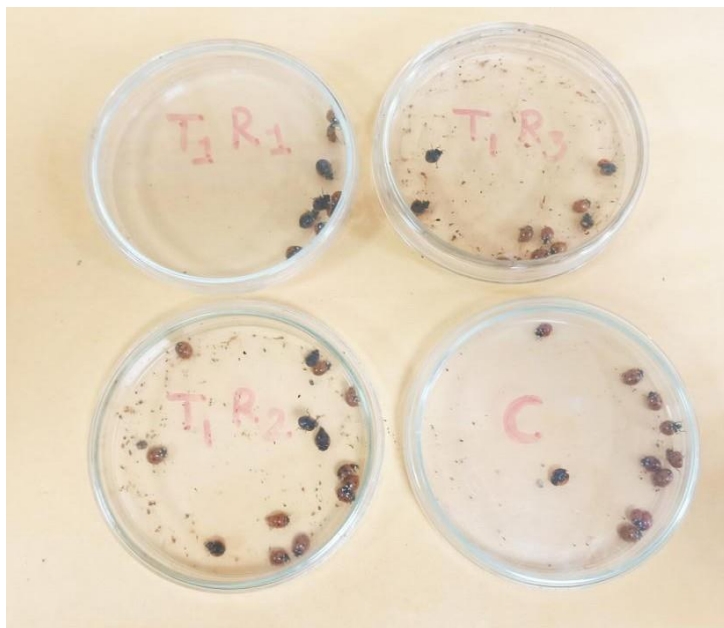
HAT: Hours after treatment

It was observed that mortality rate of spirotetramat for coccinellid predator, *C. septempunctata* was 23.86 (%), 28.24 (%) and 31.92 (%) for 24, 48 and 72 hours, respectively which was at par with the mortality rate of Spinosad that resulted in 20.60 (%), 27.86 (%) and 33.10 (%) in interval of 24, 48 and 72 hours, respectively.

Biopesticide, *Metarhizium anisopliae* resulted in 12.90 (%), 21.66 (%) and 23.66 (%) mortality after 24, 48 and 72 hours of treatment which were statistically at par with the mortality rate of *Beauveria bassiana* and *Lecanicillium lecanii* which reported with 15.30 (%), 21.7 (%), 24.57(%) and 12.54 (%), 19.83 (%), 22.12 (%) mortality, respectively.



*Coccinella septempunctata* adults feeding on woolly apple aphids



Laboratory study on toxicity of spirotetramat to the adults of *C. septempunctata*

Plate 7. Toxicity of different insecticides and biopesticidal treatments on *C. septempunctata*

## Chapter-5

# SUMMARY AND CONCLUSIONS

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The present study entitled Geographical variability of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop was divided into two parts i.e. checking the genetic variability of woolly apple aphids in different locations of Himachal Pradesh and testing the efficacy of insecticide molecules and biopesticides against woolly apple aphid. The first part of the study was carried out by collecting insect samples from Kullu, Kinnaur, Shimla and Mandi and brought to lab to check the genetic variability under laboratory conditions in Dr. Y S Parmar University of Horticulture and Forestry Nauli, Solan. The management of aerial and edaphic population of woolly apple aphid was conducted in the field of Fruit Science Department whereas band application was carried out in the farmer field in Shimla (Baghi) district of Himachal Pradesh. The efficacy of insecticides was tested against woolly apple aphid and the effect of tested products on the natural enemies of woolly apple aphid was observed. The results obtained are summarized as below:

- Maximum infestation was found in Kullu (49.30%) and Kinnaur (47.3%) district of Himachal Pradesh whereas, Shimla and Mandi district had a infestation of 41.25% and 37.21 % respectively.
- Kinnaur isolate showed a little genetic divergence when compared with Kullu, Shimla and Mandi.
- Three different clades were formed in phylogenetic tree and isolates of *Eriosoma lanigerum* collected from different locations of Himachal Pradesh were similar to China isolate as per the NCBI database.
- Chlorpyrifos (0.05%) was found to be the best treatment in reducing the aerial population of woolly apple aphid with reduction of 92.08 % after 21 days of treatment. This was followed by spirotetramat (0.015 %) which resulted in 83.14% reduction in aerial population. HMO was found to be least effective resulting in only 21.60 (%) reduction in the colony count. *Metarhizium anisopliae* ( $2 \times 10^{10}$  cfu/l), *Beauveria bassiana* ( $2 \times 10^{10}$  cfu/l), azadirachtin (0.02%), *Lecanicillium lecanii* ( $2 \times 10^{10}$  cfu/l) resulted in per cent reduction of 29.5, 26.68, 29.32 and 31.11 respectively.

- Imidacloprid proved to be the best treatment for the control of edaphic population of woolly apple aphid with the mean per cent decrease of 91.19, which was followed by chlorpyrifos with the mean reduction of 70.19. *Metarhizium anisoplae* and *Beauveria bassiana* resulted in 25.70 and 20.66 per reduction of woolly apple aphid population.
- Maximum first instar nymphs/per cm<sup>2</sup> were trapped in the month of November (89.70) followed by October (61.70).
- Per cent emergence of parasitoid (*Aphelinus mali*) was found least in case of chlorpyrifos (0.05%). Among biopesticide, azadirachtin was observed to be the safest for *A. mali* with 65.66 per cent after 14 days. Biopesticide were comparatively safe. *Beauveria bassiana* ( $2 \times 10^{10}$  cfu/l) resulted in 61.49 (%) per cent emergence of *A. mali* after 14 days of treatment and which was at par with the emergence of *Lecanicillium lecanii* ( $2 \times 10^{10}$  cfus/l), 62.74 per cent. The *Metarhizium anisopliae* resulted in 52.80 per cent emergence.
- Chlorpyrifos resulted in 100 % mortality of *C. septempunctata* adults while HMO has zero mortality rate. Biopesticide *Metarhizium anisopliae* resulted in 12.90(%), 21.66(%) and 23.66 (%) mortality after 24,48 and 72 hours of treatment which were statistically at par with the mortality rate of *Beauveria bassiana* and *Lecanicillium lecanii* which reported with 15.30(%), 21.7(%) 24.57(%) and 12.54 (%), 19.83 (%),22.12(%) mortality respectively.

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## APPENDIX

**Table 4.6. ANNOVA for Bioefficiency of insecticides and biopesticides against aerial population of woolly apple aphid, *Eriosoma lanigerum* (based on per cent reduction)**

Source of variation	DF	SS	MSS	F-calculated	Significance
Replication	2	2.09			
Factor A	3	2,986.41			
Factor B	7	16,032.75	995.469	456.564	0
Factor A X B	21	663.591	2,290.39	1,050.47	0
Error	62	135.182	31.6	14.493	0
Total	95	19,820.02	2.18		

**Table 4.5. ANNOVA for bioefficiency of insecticides and biopesticides against aerial population of woolly apple aphid, *Eriosoma lanigerum* (based on colony count)**

Source of variation	DF	MS			
		3DAT	7DAT	14 DAT	21 DAT
Replication	2				
Treatment	7	14.174	23.71	31.411	32.246
Error	14	1.424	1.186	0.899	0.865
Total	23				

**Table 4.10. ANNOVA for toxicity of insecticides and biopesticides on aphelinid parasitoid, *Aphelinus mali* parasitizing woolly apple aphid**

Source of variation	DF	MS		
		3DAT	7DAT	14 DAT
Replication	2			
Treatment	7	812.103	508.652	928.294
Error	14	6.997	8.512	12.631
Total	23			

**Table 4.11. ANNOVA for toxicity of insecticides and biopesticides on *Coccinella septempunctata***

Source of variation	DF	MS		
		After 24 hours	After 48 hours	After 72 hours
Replication	2			
Treatment	7	16.246	18.001	18.092
Error	14	0.106	0.117	0.023
Total	23			

**Table 4.8. ANNOVA for Average number of live aphids after different days of treatment**

Source of variation	DF	MS				
		Initial count	3DAT	7DAT	14 DAT	21 DAT
Replication	2					
Treatment	7	4.825	136.075	168.5	148.825	165.325
Error	14	4.292	3.642	5.4	3.258	4.325
Total	23					

**Table 4.9. Bioefficacy of insecticides and biopesticides against edaphic population of woolly apple aphid, *Eriosoma lanigerum* (based on per cent reduction over pre-count)**

Source of variation	DF	SS	MSS	F-calculated	Significance
Replication	2	3.915			
Factor A	1	98.554	98.554	51.246	0
Factor B	3	29,146.09	9,715.36	5,051.81	0
Factor A X B	3	21.22	7.073	3.678	0.03835
Error	14	26.924	1.923		
Total	23	29,296.71			

**Department of Entomology**  
**Dr. Yashwant Singh Parmar University of Horticulture and Forestry**  
**Nauni, Solan, (HP) -173230**

**Title of the Thesis** : “Geographical variability of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop”  
**Name of the student** : Abhishek Dhanta  
**Admission Number** : H-2020-01-M  
**Major Discipline** : Entomology  
**Minor Disciplines** : Fruit Science  
**Date of Thesis Submission** :  
**Total pages of the Thesis** : 61+ii  
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**ABSTRACT**

The present investigation entitled “Geographical variability of woolly apple aphid, *Eriosoma lanigerum* (Hausmann) and its management in apple crop” was conducted during the year 2021-22 in the fields of Fruit Science Department and Laboratory of Entomology, Dr. Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. In total four samples of *Eriosoma lanigerum* were collected from different regions of Himachal Pradesh. Samples formed the clade with the population of China and a little genetic divergence was seen in Kinnaur population when compared with Kullu, Shimla and Mandi. Bioefficacy studies proved that Chlorpyrifos (0.05%) was the best treatment in reducing the aerial population of woolly apple aphid with reduction of 92.08 % after 21 days of treatment. This was followed by spirotetramat (0.015 %) which resulted in 83.14 % reduction in aerial population. Among biopesticides *Metarhizium anisopliae* ( $2 \times 10^{10}$ cfus/Lt), *Beauveria bassiana* ( $2 \times 10^{10}$ cfus/Lt), *Lecanicillium lecanii* ( $2 \times 10^{10}$ cfus/Lt), Azadirachtin (0.02%) and HMO (1%) showed per cent reduction of 19.63, 19.53, 21.27 and 21.34 respectively. For edaphic population it was found that Imidachlorpid (0.035%) was most effective with reduction of 95.20% followed by chlorpyrifos (0.1%) which resulted in 66.64 % reduction. Biopesticides *Metarhizium anisopliae* ( $4 \times 10^{10}$ cfus/Lt) and *Beauveria bassiana* ( $2 \times 10^{10}$ cfus/Lt) resulted in 15.70% and 20.66% reduction. When the safety of test chemicals for natural enemies *Eriosoma lanigerum* was tested, per cent emergence of parasitoid (*Aphelinus mali*) was found least in case of chlorpyrifos (0.05%). Rest all other treatments had a moderate effect on the per cent emergence of *A. mali*. Chlorpyrifos resulted in 100 per cent mortality of *C. septempunctata* adults while HMO has zero per cent mortality rate. All the treatments resulted in 20-30 per cent mortality of *C. septempunctata*.

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**Name:** Abhishek Dhanta  
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**Signature of Major Advisor**  
Dr. Rakesh Kumar Daroch  
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**Scholarship/ Stipend/ Fellowship, any other financial assistance received during the study period** : University Stipend

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**(Abhishek Dhanta)**