Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar

Thesis

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(Himanshu Patwal)
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August, 2019

CERTIFICATE-I

This is to certify that the thesis entitled "**Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar"** submitted in partial fulfilment of the requirements for the degree of **Master of science in Agriculture** with major in **Entomology** of the college of Post Graduate Studies, G. B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona fide* research carried out by **Mr. Himanshu Patwal, Id. No. 44427**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation and source of literature have been duly acknowledged.

Pantnagar,
August, 2019

(R. P. Maurya)
Chairman
Advisory Committee

CERTIFICATE-II

We, the undersigned, members of the Advisory Committee of **Mr. Himanshu Patwal, Id. No. 44427,** a candidate for the degree of **Master of Science in Agriculture** with major in **Entomology** agree that the thesis entitled **"Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar"** may be submitted in partial fulfilment of the requirements for the degree.

(R. P. Maurya)
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(N. K. Singh)
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(R. M. Srivastava)
Member

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Introduction

CHAPTER 1 INTRODUCTION

In India, estimated annual production losses due to pests are as high as US$ 42.66 million **(Sushil, 2016)**. Pesticide in the form of insecticides, fungicides and herbicides are commonly used for pest control in agriculture. In India, there is significant increase in consumption of pesticide. Although chemical pesticides are very effective for controlling of insect pests, but resistance development in pests against these chemicals and their detrimental effect on environment become a serious issue in current prospective **(Abrol and Shankar, 2014).** Due to lack of technical information about the chemical content and increased number of sprays every time may enhance the pressure on farmer's pocket, health of peoples and soil content of land. Hence, in present situation, development of an organic agriculture-based (organic based agriculture) system that is linked with the growth of land productivity along with a financial security and support to farmer is become mandatory to improve and sustain the agro-ecosystem. The government of India is also emphasizing on adaptation of organic farming in the different states of country. Sikkim, the first organic state of India is an excellent example for this new trend. The State government of Uttarakhand is also stepping forward towards the organic farming. Consequently, there is an urgent need of noble and eco-friendly approach for maintaining the balance between the human need for food and need for sustainability of our environment.

The problem of insect pests in crops may be overcome by utilization of biological control technology (utilization of natural enemies). By virtue of wide host range and unique mode of attack on host by natural enemies, it will develop a sound framework of Integrated Pest Management (IPM) to harness economic advantage to farmers for organic farming. Biological control is an integral part of IPM, which involves the use of predators, parasites and microorganisms for the control of pests. Among the biological control agents, coccinellid beetles (also known as Lady birds or lady beetles) are an important group of predator which are predaceous on different agricultural pests *viz.* aphids, scale insects, mealy bugs, mites, whiteflies, thrips, psyllids, plant hoppers, eggs and larvae of other insects **(Omkar and Pervez, 2002).** The ladybird beetles have been used as biological control agents for the ancient time and were thought to evolve in the lower Permian period *i.e.* about 280 million years ago **(Dixon, 2012).** The very first and most successful case of classical biological control was the introduction of the Australian ladybird beetle, *Rodolia cardinalis* into California for the suppression of cottony cushion scale, *Icerya purchesi* in 1888 **(Caltagirone and Doutt, 1989).** Majority of coccinellids (about 90%) are beneficial predators, others are phytophagous on cucurbitaceous and solanaceous vegetables or fungivorous.

The coccinellids belongs to the family coccinellidae of order coleoptera. The coccinellidae is a well known, abundant and diverse family and nearly 6000 species are known worldwide **(Vandenberg, 2002)**. **Sasaji (1967)** reported that a total of 4800 species of coccinellids belonging to 490 genera have been described from different parts of the world. In Indian subcontinent there are 400 coccinellid species (including six subspecies) reported **(Poorani, 2012). Sathe and Bhosale (2001)** described that more than 4500 species of coccinellids are predaceous in nature. Coccinellids are extremely diverse in their habitat and are found in various agro-ecosystems as well as in forest ecosystems. They live in all terrestrial ecosystems *viz*., tundra, forest, grassland agro-ecosystems and from the plains to mountains **(Skaife, 1979)**. However, the species will compete strongly for prey resource under limited food conditions. It is likely that the larger size predatory stages between and within species will dominate over the smaller ones **(Yasuda *et al.*, 2001).** These predatory beetles are also regarded as bio-indicators **(Iperti, 1999)** and provide general information about the ecosystem in which they occur **(Andersen, 1999)**.

Although coccinellidae fauna of Indian subcontinent is rich and diverse but it is poorly studied in comparison to other geographical regions of world. This is a major disadvantage with this predator in India for its successful utilization in biocontrol programme **(Kumar *et al***.**, 2017)**. Hence, species delimitation of coccinellidae predators is necessary for biodiversity, conservation and biological control program to understand their natural world. The host specificity of these predators and their eco-friendly nature encourages its use in insect pest management.

In India, attack of sucking pests has been increased in past few years in different agricultural crops. Main reasons of this outbreak are introduction of different GM crops and indiscriminate use of insecticides in crop ecosystems. This has led to development of insect-pests resistance against various insecticides. To overcome their harmful effects, biological control methods are need of the hour. Coccinellid beetles are potential predators as they devour the sucking pests and thus control their population. The feeding habits of their grubs and adults are similar. Generally, the bright coloured species of predatory beetles feed on aphids, while the darker coloured and smaller size species feed on scale insects, mealybugs, whiteflies and spider mites. On scarcity of preferred prey, ladybirds rely on alternate food sources such as honeydew, extrafloral nectar, pollen etc. **(Iperti, 1999).** Hence, these predators offer an environmentally safe alternative of pest management over chemical pesticides and therefore, the utilization of suitable strain of coccinellids for commercial biological control applications is mandatory. Exploration and utilization of suitable native species and strains of coccinellid predators associated with different host insects in different crop ecosystems will help in maintaining normal equilibrium position of the environment. Thus, study of biodiversity of coccinellid predators will help in development and standardization of their mass production techniques and delivery system against sucking pests of different crops.

Therefore, in this context, it becomes mandatory to explore the different species of these predatory beetles associated with different crops in the region. It is also important to determine natural variability and distribution of predatory beetles to elucidate the relationship with hosts and their crop ecosystem. To study the variability and inter relation between or within the species, molecular tools can play very important role. To determine the genetic variability among inter or intra species of beetles, molecular characterization using mitochondrial DNA can provide the suitable information. Study of their phylogenetic relationships through the use of DNA markers is necessarily important as cytochrome oxidase (CO) region of mitochondrial DNA provide information at molecular level **(Hebert *et al.*, 2003; Halim *et al.*, 2017)**.

The overall aspect of the present study was to enlighten the ecological and economical benefits of coccinellidae in farming system. This would help in selection of suitable lady bird beetle for a particular crop and host at particular time. As the same would lead to breeding and mass production program of coccinellid beetle, so that it could be made commercially available for farmers.

Keeping in view the above, an extensive study of coccinellid species at Pantnagar were carried out with the following objectives:

1. Diversity of different coccinellid species from different crop ecosystem of Pantnagar.
2. Study on genetic variability among the different species of predatory coccinellid beetles thriving in Pantnagar region.
3. Seasonal abundance of predatory coccinellid beetles in different cropping systems.

Review of Literature

CHAPTER 2 REVIEW OF LITERATURE

In this chapter work carried out on the following given headings have been reviewed:

2.1. Diversity and distribution of different coccinellid species in different crop ecosystem.

2.2. Study on genetic variability among the different species of predatory coccinellid beetles.

2.3. Seasonal abundance of coccinellid in different cropping systems.

2.4. Biological control of pest population by using predatory coccinellid beetles.

2.1 Diversity of different coccinellid specie in different crop ecosystem.

Ladybird beetles attracted the interest of researchers and workers from all over the world due to their attractive color pattern, worldwide distribution and great contribution towards the biological control of agricultural pests. Their diversity has been studied by different scientist in all over the world. In Indian subcontinent **Agarwala and Ghosh (1988)** reported 36 species of true aphidophagous coccinellids. Later 5200 species of coccinellid beetles were reported by **Poorani (2002).**

Rao and So (1967) exploared and reported twelve species Coccinellid from Hong Kong which included *Chilocorus rufitarsis* (Fabricius), *Coccinella repunda* (Linneous), *Coccinella arcuata* (Fabricius), *Brumoides suturalis* (Fabricius), *Coccinella axiridis* (Fabricius), *Calvia albolineata* (Hope), *Chilocorus circumadatus* (Gyllenhal), *Coelophora biplagiata* (Swartz), *Coelophora circumusta* (Mulsant) and *Coelophora pupillata* (Mulsant).

Rawat *et al.* (1969) reported nine species of predacious coccinellid beetles *i.e*. *Menochilus sexmaculatus* (F.), *Hyperaspis maindroni* (Sic.), *Rodolia fumida* (Mulsant). *Nephus regularis* (Sic.), *Scymnus* (Pullus) *coccivora Ram.* Ayyar, *Illeis cincta* (Fabricus), *Stethorus pauperculus* (Weise), *S. gilvifrons* (Mulsant) and *Oligota sp*. were recorded feeding in 1966-68 on aphid and mite pests in Jabalpur, Madhya Pradesh, India.

Shantibala and Singh (1991) reported 38 species of coccinellid beetles from Manipur, 8 from Nagaland, 35 species from Arunachal Pradesh, 16 species from Mizoram and 49 species from Meghalaya in the northeast region of India.

Iperti (1999) studied the biodiversity of predatory coccinellid beetle and their economic importance. 90% of approximately 4500 coccinellid beetles were considered beneficial because of their predacious nature. They were mainly predaceous on hompterous insects and mites. These coccinellid beetles reproduce in different habitats and showed a wide range of behavior. Although coccinellid beetles were polyphagous in nature, adult preferred certain type of food or essential prey. Some coccinellids showed migratory behavior during unfavorable condition but most of them withstand this hostile environment by entering into a temporary dormant stage *i.e*. hibernation, aestivation and aestivo-hibernation. In spite of the adoptive behavior showed by coccinellid beetle they were vulnerable for several limiting factors including population of natural enemy and anthropogenic influences.

Singh and Sanjeev (2000) studied the association of coccinellids predators with the mustard aphid, *Lipaphis erysimi* (Kalt), infesting mustard crops during the year 1992-93 and 1993-94 at B.H.U. research farm. Six farmers' fields were selected in villages around the university campus. This study revealed the presence of four coccinellids species *i.e. Coccinella Septempunctata* (Linnaeus), *C. transversalis* (Fabricius), *M. sexmaculatus*, *Cheilomenes sexmaculata* and *Brumoides suturalis* from mustard crop. Among these, *C. septempunctata* and *C. transversalis* were important aphidophagous coccinellid predators of the mustard aphid.

Kapur (2002) conducted an extensive survey on different coccinellid species in Bhutan and recorded five species of coccinellids namely *Oenopia sauzeti* (Mulsant), *Scymnus* (pullus) *posticalis Sicard*, *Oenopia luteopustulata* (Mulsant), *C. septempunctata* Linnaeus and *M. sexmaculata* (Fabricius) were recorded for the first time from Bhutan.

Zahoor *et al*. (2003) selected two types of habitats for study of the biodiversity of coccinellid beetles. Data was recorded from May to October 2002. A total of 2027 coccinellids beetles were recorded in crop ecosystem while 2945 coccinellid beetles were recorded from forest ecosystem. It revealed that the diversity of coccinellid beetles was more in forest ecosystem then the crop ecosystem.

Mayadunnga (2007) conducted a survey on predatory coccinellid beetles in Srilanka. A total of 2682 coccinellid adult were collected from different vegetable crops. 15 species were identified which were belonged to twelve genera of four tribes and three subfamilies. Three subfamilies were coccinellinae, chilocorinae and cymninae. Most coccinellid beetles were observed as feeding on aphids and plant hoppers.

An extensive survey on predatory and mycophagus coccinellid beetles in Dehradun district of Uttrakhand was conducted by **Joshi and Sharma (2008)** for a period of two years. A total of 4382 adult were collected from four different ecosystems having altitudes from 310m to 640m within the Dehradun. A total of 26 species were identified out of which 14 were recorded first time .These belonged to sub-family Coccinellinae and tribe Coccinellini: *Anegleis cardoni* (Weise), *Megalocaria dilatata* (Fabricius), *I. cincta* (Fabricius), *C. sexmaculata var undulata* (Fabricius), *Hippodamia variegata* (Goeze), *Hippodamia sp*, *Micraspis discolor* (Fabricius), *M. vincta* (Fabricius), *Harmonia dimidiata* (Fabricius), *Micraspis sp*., and *Psyllobora bisoctonata* (Mulsant). Two species were from sub-family Chilocorinae and tribe Chilocirini: *B. suturalis* (Fabricius) and *Chilocorus nigrita* (Fabricius). Only one species namely *R. sexnotata* (Mulsant) represented tribe Noviini of the subfamily Coccidulinae

Khan *et al.* (2009) an extensive survey on predatory coccinellid beetle fauna was conducted in Srinagar district of Kashmir valley for a period of 6 month during 2006-2007. A total of 12 ecosystems *viz*., willow, apple, pear, cherry, kale, cabbage, cauliflower, plum, blue pine, rose, mustard, and Euonymus were explored during the survey program and 5525 samples were collected. Out of the 15 identified species, 11 belonged to the subfamily Coccinellinae [*Harmonia eucharis* (Mulsant), *Callicaria superba* (Mulsant), *Adalia tetraspilota* (Hope), *Oenopia conglobata* (Linnaeus), *Propylea luteopustulata* (Mulsant), *H. dimidiata* (Fabricius), *Aiolocaria hexaspilota* (Hope), *C. septempunctata* Linnaeus, *Calvia punctata* (Mulsant), *H. variegata* (Goeze), and *M. sexmaculata* (Fabricius)] while four belonged to the subfamily Chilocorinae [*Chilocorus rubidus* (Hope), *Platynaspis saundersi* (Crotch), *Chilocorus infernalis* (Mulsant), and *Priscibrumus uropygialis* (Mulsant)].

Tara and Feroz (2009) studied the biodiversity of coccinellid beetles in Kargil region during the years 2007 and 2008. Three different areas were selected for purpose of the survey. Five species of coccinellid beetles recorded during the survey periods which were identified as *Halyzia* sp., *Adalia* sp., *Coelophora* sp., *H. variegata* (Goeze) and *C. septempunctata* (L).

A comparative study on diversity of predatory coccinellid beetles in two different ecosystems *i.e.* forest and crop ecosystem was conducted by **Hemchandra *et***

***al*. (2010)** in Guwahati. The data was obtained by trapping, hand picking and netting during March– September 2009 and 2010. A total of 4119 specimens of coccinellids beetles were collected out of which 3612 were aphidophagous predator representing 35 species. In forest ecosystem a total of 1756 specimens were collected out of which 1372 were the aphidophagous beetles while 2290 aphidophagous samples were collected out of a total 2363 coccinellids in the crop ecosystem. The study reveals that the diversity of aphidophagous coccinellids beetles in crop ecosystem is more than forest ecosystem.

Desai *et al.* (2011) studied the diversity of coccinella species in Nashik distict of Maharastra. 300 samples were collected from different localities of which 16 species were recognized. These 16 species were belonging to 13 genera of 6 tribes and 5 subfamilies. The subfamilies coccinellinae, sticholotidinae, cocidulinae and scymnina represented nine, two and one species respectively. The study reveled the good genetic diversity of lady bird beetle in different localities and they were predatory on pest such that aphid mealy bugs and white flies

An extensive study on diversity, bio-ecology and biosystematics of aphidophagous predators of eastern Himalayan and north east India was conducted by **Chakrabarti *et al.* (2012)**. Coccinellids have been surveyed from different agro and forest agro-ecosystem. The diversity of coccinellids shows that 78 species in 31 genera in 4 subfamilies prey upon 122 species of aphids in 7 subfamilies. The major genera were *Coelophora* (Mulsant), *Cryptogonus* (Mulsant), *Chilocorus* (Leach), Oenopia (Mulsant) and *Scymnus* (Kugelann). There were 31 genera represented by single species. *C. septempunctata* has very wide prey range with 25 aphid prey species, *C. sexmaculata* (Fabricius) (11 prey species). The other polyphagous species were *Oenopia kirbyi* (Mulsant) (15 prey species), *O. sauzeti* (Mulsant) (14 prey species and *H. eucharis* (Mulsant) (10 prey species). These predators are mostly associated with the aphid species of the subfamily Aphidinae. Only *M. dilatata* (Fabricius) was associated with hormaphidine aphids. Species diversity was higher in the agro-ecosystem than the forest ecosystem.

Poorani (2012) analyzed that the coccinellide family (excluding epilachninae) in Indian subcontinent comprehended 400 species under 79 genera, 22 tribes and five subfamilies.

Dutta *et al.* (2012) conducted a survey on coccinellid beetles in a vegetable garden of Ranchi women's college. The survey was conducted for three consecutive years (2005-08). A total of 3077 individuals were collected which were belonged to 10 species. They also reported that coccinellid beetles play an important role in suppression of insect pests in vegetables.

Majumder *et al.* (2013) studied the distribution, diversity and habitat of predatory coccinellid beetles in Agricultural and Forest ecosystem of Tripura. A total 1627 individuals of coccinellid beetles were collected. They were categorized under 24 species and 17 genera. Diversity and species richness of coccinellid beetles more in forest ecosystem than the agro-ecosystem.

Shanker *et al*. (2013) suggested that the abundance of adult *M. discolor* in rice at flowering phase does not correspond to prey abundance in the crop but rather reflects a preference to pollen feeding more than entomophagy.

Shah and Khan (2014) conducted a survey in major fruit and vegetable growing area of Kashmir valley during the year of 2011. Total fifteen and seventeen species of predaceous coccinellid were collected from vegetable and fruit ecosystems respectively. Values of diversity indices of fruit orchard were higher than the vegetables field. In fruit orchard most dominant species was *C. septempunctata* while in the vegetable field *A. teraspilota* was the predominant species.

Choudhary *et al.* (2014) observed the exploration of different predaceous coccinellid beetles in mango ecosystem of Jharkhand. A total of 2301 beetles were collected out of which 16 species were identified. Among these 7 species were recorded for the first time. Peak population of coccinellids in mango orchards was recorded in the month of February and March. These results showed the significance of Coccinellids as an important part of integrated pest management in mango ecosystems.

Ahirwar *et al*. (2015) reported three species of ladybird beetles in soybean field during the kharif season 2012. These species were *C. transversalis, M. sexmaculata* and *C. septumpunctata.* The peak activity of these beetles was observed during the 2^{nd} week of August and September. They were found predaceous on jassids and aphids.

Chowdhury *et al*. (2015) collected and identified ten genera of coccinellid beetles under four tribe of the family Coccinellidae from indo Bangladesh border. They are *C. septempunctata* (Linnaeus), *B. suturalis* (Fabricius), *C. montrouzieri* (Mulsant) *C. nigritus* (Fabricius), *C. transversalis* (Fabricius), *H. dimidita* (Fabricius), *I. cincta* (Fabricius), *M. crocea* (Fabricius), *P. sp. nr. Japonica* (Thunberg) and *S. nubilus* (Mulsant). Among all ten species *H. octomaculata* was dominated (22.9%) followed by *M. crocea* (18.5%), *B. suturalis* (12.36%) and *C. nigritus* (9.06%) respectively.

Harit (2015) observed diversity of coccinellid fauna at various ecosystems in Champhai district of Mizoram state, north east India. A total of 13 species of coccinellids which were belongs to 3 sub families. Sub family Coccinellinae is represented by *C. sexmaculata, C. sexempunctata, C. transversalis, O. kirbyi, O. sexareata, Oenopia* sp.and *Psyllobora* sp. Chilocorinae is represented by *C. scircumdatus* and *Chilocorous sp.* sub family Epilachninae is represented by for species of Epilachna.

During their study on the coccinellid communities **(Lami *et al*., 2016)** conducted a surveyed during and around the typical flowering periods of maize in order to check their diversity and association with pollen shedding**.** A total of 11 species were recorded. In central Italy *T. sedecimpunctata* (L.) and *C. quatuordecimpustulata* (L.) species were abundant while in Northern Italy *H. variegata* (Goeze), *C. septempunctata* L. and the exotic *H. axyridis* (Pallas) were more dominant. The potential exposure to maize pollen changes according to different areas, in Central Italy the ladybird population peaks occurred roughly one month earlier than the anthesis while in Northern Italy coccinellid population was high during the period of flowering in maize.

During the study of the insect predator diversity in irrigated rice flora and fauna of west Bengal **Mondal *et al*. (2016)** recorded *M. discolor* (Fab.) and *C. transversalis* (Fab.) as major predators. The coccinellid predators *M. discolor* (Fabricius) appeared at the time of flowering stages whereas *C. transversalis* (Fabricius) dominated the other species at time of reproductive stage of the crop.

Ankalgi and Murali (2016) reported the occurrence of 12 species of coccinellidae family which belongs to 4 different subfamily and 9 genera. Out of these subfamilies coccinellidae dominant with 6 species and followed by subfamily scymninae with 3 species while subfamily chilocorinae and epilachninae each with 1 species.

Ramya *et al.* (2016) studied the diversity of predatory coccinellids in the citrus orchard of Assam Agricultural University, Jorhat. Twelve species of coccinellid beetles *viz.*, *C. montrouzieri* (Mulsant), *C. transversalis* (Fabricius), *H. dimidiata* (Fabricius), *C. bowringii* (Crotch), *Cryptogonus* sp., *Coelophora saucia* (Mulsant), *C. bimaculatus* (Kapur), *H. conglobata* (Linnaeus), *I. confusa* (Timberlake), *Propylea* sp., *Platynaspis kapuri* (Chakraborty and Biswas) and *Scymnus* sp. were recorded feeding on various sucking insect pest. Among the beetles, *C. transversalis* and *H. dimidiata* were recorded in highest number (9.0 and 8.4 per tree respectively) with a relative abundance of 17.79 and 16.60 per cent respectively while *Cryptogonus* sp. was recorded lowest (0.9 per plant with relative abundance of 01.79 per cent).

An extensive survey was conducted by **Ahmed K. S. (2017)** on predatory coccinellid beetles of Sargodha region of Pakistan to access their richness and distribution. A total of 1470 coccinellid beetle were collected from different vegetation and 9 species were recognized which belonged to four subfamilies along with 10 trophic association .These beetles were categorized up to species level on the basis of external morphology, published description and male genitalia micrometry. Among these species five were belonged to subfamily coccinellinae, two belonged to chilocorinae, one beloged to epilachninae and one species represented by subfamily scymninae. *C. septempunctata* was dominant species among all the nine species.

Kumar *et al.* (2017) reported twenty one species of predatory coccinellid beetle during their survey from western plain zone of Uttar Pradesh .This survey was carried out during the month of January to December. Out of these 21 species 17 were identified *i.e. C. sexmaculata* (Fab.), *C. transversallis* (Fab.), *H. axyridis* (Pallas), *C. sexmaculata* ab *rufafasciata* (Fab.), *C. septempunctata, R. breviuscula* (Weise), *P. dissecta* (Muls.), *S. nubilus* (Mulsant), *C. nigrita* (Fab.), *A. cardoni* (Weise), *M. discolour* (Mulsant), *B. suturalis* (Fab.) *I. cincta* (Fab.), *P. horni* (Weise), *P. bisoctonotata* (Muls.), *H. variegata* (Goeze), *H. maindroni* (Sicard), and 4 were unidentified. Dominant species was *C. sexmaculata* (46%) followed by *C.septempuncta* (30.58%) and *C. transversalis* (8%).While least population was of *Harmonia axyridis* (1.56%) followed by *M. allardi* (1.99%) and *P. hornii* (2.61%).

Sharma *et al.* (2017) conducted a periodical survey in agro-climatic zone of Himanchal Pradesh for the study of diversity of coccinellids. A total of 65 coccinellid adults were found and most of them were predaceous on the sucking pest of agricultural crops and wild vegetation. Diversity of coccinellids was also varied from one agro-climatic zone to another. Agro-climatic zone II was most diversified zone with 35 species followed by zone I having 25 species. Zone III and IV were found with 16 and 13 species respectively. *C. septempunctata* (Linnaeus), *H. variegata* (Goeze) and *C. sexmaculata* (Fabricius) were most widely distributed coccinellid species among all species.

2.2 Study on genetic variability among the different species of predatory coccinellid beetles

Kobayashi (1998) worked on molecular phylogeny of 12 Asian species of epilachnine and determined a nucleotide sequence of a gene (cyt-oxydase-I) having 1000 bp from mitochondrial region. Molecular phylogenic tree was assembled for ten *Henosepilachana* species using two *Epilachna* species as outgroup and taxonomic issues were discussed on the basis of suggested phylogenetic tree.

According to **Herbert *et al.* (2003)** specific DNA sequence of all living organism can be used as identification tool which was known as DNA barcode. A specific mitochondrial gene cytochrome oxidase I (COI) can serve as the core of a global bio-identification system for animals. 200 species of lepidopteron were identified successfully on the basis of analysis of COI region.

Kergoat *et al.* (2004) Studied on molecular phylogeny of European seed beetle (Bruchidae) using partial sequencing of three mitochondrial genes i.e. 12S rRNA, cytochrome b, and cytochrome c oxidase subunit I. Adult beetles examined in this study were collected from larvae bred from seeds directly collected in the field. Phylogenetic relationship between different species was studied with the help of three analytical methods i.e. Parsimony, maximum likelihood, and Bayesian inference. Both genera, Bruchidius and Bruchus, formed monophyletic groups in all analyses. A significant level of specialization was highlighted among these seed beetles after critical analysis.

Kavar *et al.* (2006) studied on genetic variation of green stink bug *Nezara viridula* (Linnaeus) from 11 geographically separated locations (Slovenia, France, Greece, Italy, Madieria, Japan, Guadaloupe, Galapagos, California, Brazil, and Botswana) by the sequencing of four mitochondrial genes 16S and 28S Rdna, cytochrome b and cytochrome c oxidase subunit I gene fragments. After sequencing 11 distinct haplotypes were clustered into lineage A, B and C. Lineage B and C were characteristics for Japan and Botswana respectively while haplotypes of America and European were belonged to lineage A. Halotypes of specimen from Slovenia, France, Madieria, Brazil were highly similar to subgroup A1 and specimen from Guadaloupe, Galapagos, California and Greece were similar to subgroup A2.

Foottit *et al.* (2008) test the effectiveness of DNA barcode for identification of 300 different aphid species from more than 130 genera. A 658-bp fragment of mitochondrial DNA from the 5′ region of the mitochondrial cytochrome oxidase 1 (COI) gene was adopted as standard DNA barcode. Most of species were differentiated and sequence variation within species was low, averaging just 0.2%.

Magro *et al.* (2010) studied phylogenetic relationship among the species of coccinellidae family using five genes *i.e.* 18S and 28S rRNA nuclear genes and mitochondrial 12s ,16s rRNA cytochrome oxidase subunit I (COI) gene. Phylogenetic tree was constructed using different approaches including Bayesian inference with partitioning strategies. Recovered phylogenies showed that coccinellinae was monophyletic while scymninae, epilachninae, chilochorinae, coccidulinae were paraphyletic.

Patel and Patel (2014) studied abundance of the lady bird beetles, *Coccinella septempunctata* L. (Coleoptera:Coccinellideae) in relation to climatic factor in two consecutive cropping seasons of 2005-06 and 2006-07. The study revealed that, the cotton *C. septempunctata* was first recorded in the 27th SMW *i.e.* first week of July during both the year of study and remained up and remained active till 50th SMW (2nd week of December). The peak population was observed (9.76 / 5 plant) during 37th SMW i.e. 3rd week of September. The correlation studies between population of LBB and weather factors revealed that the *C. septempunctata* population had a significant positive correlation with maximum temperature (0.542) & minimum temperature (0.560). The multiple correlation coefficient value between the *C. septempunctata* population and group of variable clearly indicated that 79.50% change in *C. septempunctata* population were affected by maximum temperature, minimum temperature, morning relative humidity, evening relative humidity, sunshine hours, wind velocity, rainfall and rainy days respectively. The data also revealed that 20.50% variation caused by inexplicable reason or due to error beyond the control of experiment or due to factors not included in the investigation. Path coefficient analysis of *C. septempunctata* population and abiotic factors revealed that the minimum temperature had positive and high direct effect (1.6592) followed by morning relative humidity (0.1972), rainfall (0.1535), and sunshine hours (0.1519) and evening relative humidity (0.016) respectively.

Sayeda (2016) studied genetic diversity of two coccinellid beetle *Coccinella undecimpunctata* L. and *H. variegata* (Goeze). Study included partial sequencing of three mitochondrial gene *i.e.* 12S, 16S ribosomal gene and COI gene. Phylogenetic tree of sequences was constructed by using three analytical methods *i.e.* maximum parsimony, neighbor-joining and maximum-likelihood. The pair-wise genetic distances (D) among the studied populations ranged from 0.0013 to 0.0041 in case of *C. undecimpuntata* and from 0.0048 to 0.0128. From the study it was concluded that both coccinellid population having low genetic variability.

Rain *et al.* (2016) conducted an extensive survey on aphid predatory coccinellid beetles during October 2014 to march 2016. Total nine beetles were identified predatory on aphids. Among them, *M. discolor* was most abundant. Along with biological study, phylogenenetic analysis was also constructed with the help of mitochondrial COI gene. Out of nine identified coccinellid, five species showed more conserve region than the rest in alignment. The interspecific genetic distance range for nine beetles was very low (0.15-0.24). Among these nine species *H. axyridis* showed highest interspecific genetic diversity. Phylogenetic tree was constructed with the help of, maximum likelihood and neighbor-joining. Molecular analysis demonstrated that evolution of these species has been occurred from common ancestor.

Halim *et al.* (2017) presented first ever DNA barcode of eight coccinella species *i.e. C. nigritus, H. vigintioctopunctata, H. kaszabi, C. sexmaculata, M. discolor, H. reticulata, C. inaequalis* and *C. transversalis* from Malaysia. These all coccinellid species were collected from different crops of familes solanaceae, moraceae, malvaceae, convolvulaceae and rutaceae. The association between different coccinellid was anticipated by using phylogenetic analyses of Neighbor Joining (NJ), Maximum Parsimony (MP), and Bayesian Inference (BI) by taking *Cassidinae* sp. as an out-group.

2.3. Seasonal abundance of coccinellid in different cropping systems.

Kazimi and Ghani (1964) reported *C. sexmaculata* on *Hibiscus* sp., brinjal and *Cucurbita pepo* infested by mites at Rawalpindi. **Nagaraja and Hussainy (1967)** recorded six species of Chilocorus beetle predaceous on the San Jose scale and other scales in Bangalore *viz., Chilocorus Kuwanae* Silvestri, *C.nigritus* (Fabricius), *C. bijugus* Mulsant, *C. rubidus* Hope, *C. hauseri* Weise and *C. circumdatus* Schonherr.

Rawat and Modi (1968a) reported *R. fumida* Mulsant predaceous on *Perissopneumon tamarindus* (Green) from Madhya Pradesh. They **(1968b)** also reported that the coccinellid predators *H. maindroni* Sciard, *c.* Aiyar, *Nephus regularis* (Sicard) and *Scymnus* (Pull.), were found as natural enemies of *F. virgata* in Jabalpur, Madhya Pradesh.

Ahmed (1973) allied a new tribe Telsimini and Sticholotidini and new genus were erected for *Ghanius karachiensis* predator of *Cerococcus hibisci* Green in Pakistan

Nath and Sen (1976) examined the occurrence of aphidophagous coccinellid beetles on mustard crop in west Bengal. Four species of coccinellid beetles were found predatory on Mustard aphid (*L. erysimi*). Out of these, *C. septempunctata* was very efficient during early and late phase of the crop. During January when the aphid population was quite dense, grubs were more often seen then adult beetles. In the late phase *i.e.* during February-March when crop was mostly in pod stage, the adults were found, devouring the aphids.

Natarajan and Mathur (1981) recorded two predaceous coccinellid beetles *viz*., *Oenopia* sp. and *Coccinella billieti* (Mulsant) feeding on brown plant hoper *Nilaparvata lugens* (Stal) from Cuttack. **Samal and Misra (1982)** observed first time the grubs of *C. rependa* was feeding on nymphs of brown plant hopper, *N. lugens* (Stal.). **Vazirani (1983)** reported *Sticholotis chittagongi* sp. was feeding on aphids infesting *Ziziphus mauritiana* in Bangladesh.

Srikanth and Lakkundi (1990) observed that the activity of four coccinellid species *M. sexmaculatus, H. axyridis C. transversalis* and *H. convergens* on cowpea directly coincided with the peak aphid infestation. Predatory beetles were observed in field 1 to 3 weeks after appearance of aphids.

Sureja (1991) observed four different species of coccinellid beetles *viz., C. septempunctata, M. sexmaculatus, A. variegata and B. suturalis* of coccinellids were predaceous on different species of aphids, coccids, thrips, jassids etc. in various crops during April to November. During summer, the beetles remained inactive in adult stages. Predatory coccinellid beetle *Harmonia dimidiate* reported from north and north eastern region of India during the month of August to September **(Shantibala and Singh, 1991)**

C. septempunctata is one of the most common and widely studied ladybird beetles, reported from India as well as different parts of the world **(Singh and Singh, 1991; Shantibala and Singh, 1991; Omkar and Bind, 1993)**. It feeds upon *Aphis pomi*, *L. erysimi*, *Myzus persicae*, *Brevicoryne brassicae* and other aphids. It can be seen throughout the year.

Varma *et al.* (1993) observed that the two species of coccinellid beetles *viz., M. sexmaculata* (Fabricius) and *C. septempunctata* (Linnaeus) were active during the month of March to November and the population initiated (12.4/ 5 cm central shoot) in second week of January continued till March second week (20.4/ 5 cm central shoot) and reached its peak (83.0/ 5 cm central shoot) in last week of February.

Behera *et al.* (1999) recorded the feeding potential of *C. septempunctata* (Linnaeus) on the chrysanthemum aphid *Macrosiphoniella sanbormi* (Gillette).

Kulkarni and Patel (2001) observed that the population of *C. septempunctata* on Mustard field appeared during last week of January (0.67 per plant) and reached at peak level (2.90 per plant) during third week of February. There was a sharp declined in the population (1.22 per plant) in last week of February month.

Sunil Joshi *et al.* (2001) for the first time recorded *Sticholotis cribellata* as a potential predator of sugarcane scales, *Melanaspis glomerata* in Bangalore. **Inamulla Khan *et al*. (2003)** described 12 different species belonging to nine genera of three tribes and three subfamilies. Eight species belongs to Coccinellinae and 3 species belongs to Chilocorinae, one species belongs to Psyloborini in Pakistan.

Singh and Nath (2007) observed effect of time of sowing of *Brassica juncea* on coccinellid beetles of *Lipaphis erysimi*. This study revealed that, maximum population predatory coccinellid beetle was observed in last week of February while minimum during last week of December. Results also indicated that when population of coccinellids increased significantly, aphids were already in their migration phase.

Tank and Korat (2007) studied the relationship between *C. sexmaculata* on cotton crop and weather parameter. The study of correlation revealed that all the meteorological parameters showed negative association, except morning relative humidity. Significantly negative correlation was observed between the grubs of *C. sexmaculata* with minimum temperature (r = -0.493*), wind speed (r = -0.411 *) and morning (r = -0.433*) and evening (r = -0.385*) vapour pressure, whereas significant and negative correlation existed between the adult population with minimum temperature (r = -0.688*) and morning (r = -0.612*) and evening (r = -0.448*) vapour pressure. On cowpea Negative and significant correlation was recorded between *C. sexmaculata* with minimum temperature (r = -0.829 * for grubs and r = -0.923* for adults), evening relative humidity (r = 0.897* for grubs and r = - 0.965* for adults), wind speed (r = 0.874* for adults) and morning (r = -0.751 * for grubs and r = 0.889* for adults) and evening (r = -0.881 * for grubs and r = 0.939* for adults) vapour pressure. Similarly positive and significant association was noticed between sunshine hours and grub (r = 0.873*) as well as adult (r = 0.907*) population on cowpea crop.

Hugar *et al.* (2008) reported three aphidophagous coccinellid predator *viz.*, *C. sexmaculata*, *C. septempunctata*, *C. transversalis*, appeared during the last week of January to first week of March (0.20 to 1.55 Coccinellids per plant). Population of coccinellid beetle started in second week of March and attained two peaks (2.54 and 2.81/plant) at fourth and fifth week of March. Thereafter population of coccinellid beetles decreased and reached at low level (0.92/plant).

Mandal and Patnaik (2008) observed that *C. transversalis* and *C. sexmaculata* appeared during mid- November and attain their peak population during January and February (7.29 to 10.94 and 4.26 to 5.08 grubs and adults /10 plants respectively.

According to **Patel (2009)** in cowpea crop coccinellid beetles population had positive significant correlation with average temperature (r = 0.68*) and bright sun shine hours (r = 0.69*) whereas there was a negative significant correlation with morning and evening RH (r = -0.90** and 0.72**, respectively). Positive correlation was also established with maximum and minimum temperature, vapour pressure and wind speed.

Chanmamla (2009) reported twelve species of predatory coccinellid beetles from different agro-ecosystem of Tirupati. Among all the twelve species *C. transversalis* (38%) was most abundant species followed by *C. sexmaculata* (34%) and *Illeis cincta* (6%).

An extensive survey in farmer's fields of cauliflower was conducted by **Ali and Rana (2010)** at three districts (Mathura, Agra and Firozabad) of Uttar Pradesh in two subsequent year 2009-10 and 2010- 11. Aphid, *L. erysimi* (Kalt.) was found to attack on cauliflower field at every examined site. However, three species of ladybird *viz*., *C. septempunctata* (Linnaeus), *C. transversalis* (Fabricius) and *M. sexmaculatus* (Fabricius) were also recorded to be associated with aphid colonies. The populations of coccinellids have been synchronized with the infestation of aphids in the field. The maximum number of ladybeetles were obtained as 5.00, 3.00 and 3.50; 7.00, 3.50 and 4.00; 9.00, 4.00 and 3.00 in March 2010; and 5.00, 3.50 and 2.50; 5.00, 4.00 and 3.00; 8.00, 5.00 and 3.50 during March 2011, respectively. It was also noticed that *C. septempunctata* was found to be most abundant species at every experimental site than other lady beetle species.

Roy *et al*. (2010) recorded twenty species of predatory coccinellid beetles during their survey in tea plantation of North Bengal. The survey was carried out from 2004 to 2006. Among all the species *M. discolor* was most dominant (42.5%) and it was found predaceous on red spider mites and tea aphid. The preferred prey of the .*M. discolor* was tea aphid. Their study revealed that the Female beetle *of M. discolor* consumed significantly higher number of aphids then male beetle. . Among these species *M. discolor* was found most abundant followed by *C. septempunctata* and *C. transversa*lis in the conventionally managed tea plantation. It was also observed that the population of *M. discolor* reached its peak during the month of January and March.

Ghosh and Chakrborty (2012) studied the correlation between the population of *C. septempunctata* and different weather parameters. A significant positive correlation (p= 0.05) was showed by *C. septempunctata* with maximum and mean temperature and a significant negative correlation with maximum and gradient relative humidity and rainfall. In case of humidity gradient and rainy days the correlation was negative but non-significant.

Chakraborty *et al*. (2013) studied the relationship between weather parameter and feeding habits of two coccinellid species *i.e. B. suturalis* Fab, and *S. coccivora* Ayyar. Among the different species of coccinellid beetles these two species generally prefers mealy bugs as a host. Various life stages (grub, pupa and adult) of the predators were observed at weekly interval in cotton crop during two subsequent years i.e. 2009-

10 and 2010-11 and correlated with the weekly mean values of weather parameters. The values of correlation coefficient indicated that the maximum temperature influenced negatively with different stages of both the coccinellids beetles. Significantly negative correlation was found between maximum temperature and grubs as well as adults of both the coccinellids during 2010-11. Minimum temperature also influenced negatively on the population of both the coccinellids during 2010-11. Significantly negative association was revealed between minimum temperature and grubs and adults of *B. suturalis* during 2010-11.

Kumar and Kumar (2015) studied the effect of biotic and abiotic factors on abundance of predator in cowpea crop. *C. septempunctata* was most abundant predator in cowpea during kharif 2007. It was appeared in cowpea field at 21 DAS (1.73 per plant) during last week of august, 2007. Maximum population (2.60 per plant) of coccinellid beetles were recorded during third week of November.

Nelaballe and Meruva (2015) studied the feeding habits of three coccinellid beetles *i.e. C. montrouzieri, S. coccivora* and *I. cincta.* Among these three beetles *C. montrouzieri* and *S. coccivora* were used for the management of aphids, scale insects and mealy bug while *I. cincta* was observed feeding on conidia of powdery mildew.

An experiment was conducted by **Goswami *et al.* (2016)** in two consecutive years for the study of abundance of different coccinellid beetles in Mustard, Chickpea, Linseed and Lentil crops. In Linseed crop only two coccinellid species were found *viz., C. transversalis* and *M. discolor* while in Mustard crop there coccinellid species were recorded. Among all the species *C. septempunctata* was found most abundant species in Mustard crop. Among the pulses coccinellid predators namely, *M. sexmaculatus* and *C. septempunctata* were observed. In lentil *C. septempunctata* was the abundant species.

Rain *et al*. (2016) conducted the extensive survey of predatory coccinellids beetles (Coleoptera: Coccinellidae) in Jahangirnagar University campus, savar during October 2014 to March 2016. Total of nine ladybird beetles *viz., A. dipunctata, C. septempunctata, C. transversalis, C. munda, H. axyridis, H. convergens, M. discolor, S. nebulosus* and *Sticholoyis* sp. were identified as predator of aphid. Among all species *M. discolor* was found most abundant.

Sharma *et al*. (2017) reported that most of coccinellid beetles were active during last week of March or later in Himachal Pradesh. Abundance of *C. septempunctata* was more during first week of March in Cabbage crop.

Kumar *et al.* (2017) reported twenty one species of predatory coccinellid beetle during their survey from month of January to December .Out of these 21 species 17 were identified and 4 were unidentified. Dominant species was *C. sexmaculata* (46%) followed by *C. septempuncta* (30.58%) and *C. transversalis* (8%).While least population was of *H. axyridis* (1.56%) followed by *M. allardi* (1.99%) and *P. hornii* (2.61%).

During their study on relationship between population of ladybird and weather parameter **Jadhav *et al.* (2017)** found significant positive correlation and regression coefficients between lady bird beetle population on okra and maximum temperature (r= 0.568*), minimum temperature (r= 0.547*) and bright sunshine (r=0.629*). Whereas there was a negatively significant relationship with relative humidity (r= 0.617*) and evening relative humidity (r= 0.594*). The non-significant and negative correlation was observed between lady bird beetle population and rainfall and rainy days.

Mishra and Yousuf (2019) conducted a survey of coccinelid beetles in Dehradun district of Uttrakhand during the period of June 2016 to May 2018. Survey was conducted in forest ecosystem at different altitudes. Total fifteen coccinellid species were collected by handpicking and sweep net methods. These species were identified as *C. septempunctata* Linnaeus, *A. hexaspilota* (Hope), *H. dimidiata* (Fabricius), *H. vigintioctopunctata* (Fabricius), *A. cardoni* (Weise), *I. confusa* Timberlake, *B. suturalis* (Fabricius), *C. transversalis* Fabricius, *M. univittata* (Hope), *P. saundersi* (Crotch), *H. variegata* (Goeze), *M. sexmaculatus* (Fabricius), *M. allardi* (Mulsant), *O. sexareata* (Mulsant) and *P. dissecta* (Mulsant). Out of these species *I. confusa* was found mycophagous while *H. vigintioctopunctata* was phytophagous in nature. Rest of the other species were predaceous on mealy bugs and aphids. Among all the species *O. sexareata*, *I. confuse*, *A. hexaspilota* and *P. saundersi* were recorded first time in Uttrakhand.

2.4 Biological control of pest population by using coccinellid beetles.

Deng *et al*. (1987) conducted an experiment on the mass rearing and field release of two coccinellid beetles *i.e. S. grandis* and *C. biplagiata* [*Lemnia biplagiata*] in Guangxi, China. These beetles were collected from sugarcane fields, and stored in the laboratory. The survival rate after about 5 months storage during the winter was 62.96-82.9% for *S. grandis* and 45.7-100% for *L. biplagiata.* A total of 44660 beetles were released into sugarcane fields in 1982-85 after storage. The coccinellids reduced infestation of plants by the aphid *Ceratovacuna lanigera* by 96.6% and of the leaves by 90%.

Trouve *et al*. 1997 conducted an experiment for the control of damson-hop aphid Phorodon *humuli* (Schrank) in a dwarf-hop garden by using the predatory coccienllid beetle *H. axyridis* (Pallas) in northern France. Second and third instar larvae of *H. axryridis* were released at different stages. In the control, the number of aphids reached 191.3 ± 30.8 per leaf at the end of June. In the plots where 50 larvae per plant were released, the best control was obtained when larvae were released early and the aphid population was approximately 20 per leaf. The average number of aphids per leaf was not exceeded 54.5 ± 10.3 aphids at the end of June. While in insect treated condition aphid population was 80 aphids per leaf. It was also observed that the indigenous predatory coccinellid beetle *A. bipunctata* L. complemented the effect of *H. axyridis*.

Zaki *et al.* (1999) conducted an experiment to check the biological control potential of two predatory coccinellid beetles *C. carnea* Steph and *C. undecintpunctata* Reich. These beetles were released against *A. gosspii*, *B. tabaci*, and *B. brassicae* in open field as well as in green house condition. Double releases of *C. Carnea* (1:5 predator: aphids) achieved 100% reduction in *A. Gosspii* after 12 days. A single release of *C. undecitnpunctata* (1:50 predator: aphids) resulted in 99.97% reduction in the same aphid. Three releases of *C. undecimpunctata* adults in a green- house cultivated with soybean decreased *A. fabae* population from 207 to 7.6 aphids/ plant.

Ellis *et al.* (2001) studied a dynamic change in population of *Bemisia argentifolii* on caged poinsettia plant when coccinellid predator *Serangium parcesetosum* Sicard was used against their population. *B. argentifolii* were introduced to caged poinsettias plants at 1 or 10 adults per plant. *S. Parcesetosum* was introduced after 6 week of the introduction of *B. Argentifolii* at the rate of 0, 2 or 4 adults per plant. Population of the *B. Argentifolii* was decreased dramatically after two week of the *S. Parcesetosum* release. Population of the pest remains constant for the next 10 weeks. Larvae of predatory beetles were observed after 2 to 10 week of release of beetles. They observed that the population of pest was controlled due to the continuous feeding and prolonged survival of the predatory beetles. It was concluded that the *S. Parcesetosum* having potential for *B. argentifolii* control.

Legaspi *et al*. (2001) conducted a field cage experiment on *S. parcesetosum* adult as a predator of egg of citrus blackfly. This study was conducted in a commercial grapefruit orchard of Hargill, which was heavily infested with the citrus blackfly. Screen cages were placed around individual terminals consisting of 6 to 10 leaves. The predators were starved for approximately 12 hours and released at the rate of one beetle per cage into the treatment cages. The control cages received no predators. The predators were allowed to feed on the citrus blackfly eggs for 12 days then the leaf terminals were brought to the laboratory to record numbers of eggs that hatched. Egg hatch rather than predation was measured because in previous observations, *S. parcesetosum* punctured eggs without consuming them completely. The pre-release egg counts showed no significant differences between the control and treatment. In the predator treatment, numbers of eggs averaged 383.4 per cage, compared to 367 in the control. After predation, significantly fewer eggs hatched in the treatment cages, compared to the control. Even in the absence of predation, only 42.8% of citrus blackfly eggs hatched. Predation by *S. parcesetosum* for 12 days reduced egg hatch to 12.5%.

Kuroda and Miura (2003) conducted two experiments for the control of *A. gossypii* on cucumbers by using the predatory coccienllid beetle *H. axyridis.* During first experiment 1, 5 and 10 egg mass sheet of *H. axyridis* per plant in each greenhouse were released only one time. It was observed that the population of *A. gossypii* was lower in the green house with egg mass as compare to the green house without the egg mass. Within 5 days after release, the greenhouses receiving 5 egg mass sheets and 10 egg mass sheets per plant showed reduction of aphid densities to less than 1% of that without the sheets. During second experiment, five egg mass sheets along with 5 flightless adults per plant were released in each greenhouse three times at about one week intervals. The population of *A. gossypii* in greenhouses receiving flightless adults showed fluctuation to a lower level than in the greenhouses receiving egg mass sheets.

Materials and Methods

CHAPTER-3 MATERIALS AND METHOD

Systemic procedure and protocol were adopted to accomplish the objectives set forth under the present study entitled **"Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar"** The details of the materials used, experiments conducted and the methodology followed during experiments are presented below in this chapter.

Geographical Situation:

Pantnagar is situated in Kumaon region of Uttrakhand with 29 ^{0} N latitude, 79.30^{0} E longitudes and at altitude of 243.83 meters above the mean sea level. The general climate of this place is humid and subtropical characterized by maximum temperature during summer from 32^{0}C to 45^{0}C and minimum temperature during winter ranging from 0^{0}C to 9^{0}C. The relative humidity during the month of July and February remain 90% and reduces up to 55% during the month March and April. The average annual rainfall is around 1400 mm.

Experimental Location

The present survey carried out in different locations of Govind Ballabh Pant University of Agriculture and Technology. While the study on genetic variability of different coccinellid beetles was carried out in Biological Control Laboratory of department of Entomology. Details of experiments are given below.

3.1 Study on biodiversity of coccinellid beetles in different crop ecosystems of Pantnagar

3.1.1 Method field survey and collection

Field survey was undertaken at Govind Ballabh Pant University of Agriculture and Technology, Pantnagar from May 2018 to April 2019. Three different sites were selected for the survey of Coccineellid beetles *viz.*, Vegetable Research Centre (VRC), Crop Research Centre (CRC) and Model Floriculture Research Centre (MFRC). The collection of coccinellid beetles was carried out at different crop ecosystems *viz.*, field crops, vegetables and floriculture crop at weekly time interval. Different field crops (Wheat, Rice, Soybean, Maize, Pigeonpea Black gram, Chickpea, Mustard and Green gram), vegetable crops (Okra and Cowpea) and floricultural crops (Rose and Marigold) were observed during the period for occurrence of coccinellid beetles. Collection was done generally in morning hours from 7:00 AM to 9:30 AM, when insect activity is least and they can easily spot. Samples were collected from a crop by making 'W' shape pattern within the field (**Zehnder, 2014**). Each corner of 'W' was considered as a spot for the collection. At a spot one meter square area was selected and each plant of square was observed for predators. All the life stages of predatory coccinellid beetles (eggs, grub, pupa and adult) were collected and brought to the laboratory. Immature stages were reared up to the adult stage for the further study. Collection of adult coccinellid beetles were carried out continuously during the study period. The beetles were collected by hand picking method **(Hemchandra *et al.*, 2010).**

3.1.2 Identification of collected specimens

Collected specimens were killed using chloroform in killing bottles and they were left for drying. The collected specimens were studied and identified using available keys **(Omkar and Pervez, 1999; Poorani, 2002b; Omkar and Bind, 1996, 1995, 1993)**. The species which were not identified with the help of keys were sent to Entomology Division of IARI, New Delhi for identification by experts.

3.2 Study on genetic variability of coccinellid predators prevailing at Pantnagar region.

3.2.1 Material required

3.2.1.1 Microscope

A binocular microscope (4.5X zoom) was used to remove wings and elytra of coccinellid beetles. Beetles were dissected with the help of scissors, needle, and forceps after anesthetized by chloroform.

3.2.1.2 Autoclave

All the centrifuge tubes, micro-tips, spatula, mortar and pestle, PCR tubes autoclaved at 15 psi at 121.5 ºC for 15 minutes, so that to prevent all types of contamination.

3.2.1.3 Insect DNA isolation kit

Insect DNA was isolated with the help of Purification Kit manufactured by Himedia. Various chemicals like Lysis solution C, Lysis solution AL, RNAs, Protienase K, Elution buffer along with High Shradar spin column, Micro-centrifuge tube, liquid nitrogen, mortar and pestle, spatula, micropipette micro-tips centrifuge, vortex, and water bath were also required for isolation of genomic DNA of insects.

3.2.1.4 Micropipette

Micropipette and micro-tips having Capacity of 10 µl, 20 µl, 100 µl, 200 µl, 1000 µl were used for pipetting of various chemical during whole experiments.

3.2.1.5 Glass wares

Measuring cylinders with volume 5 ml, 20 ml, 100 ml, 250 ml and 1000 ml were used for preparation of wash solution and TAE buffer.

Conical flask (1000 ml) was used for the melting and preparation of agarose gel medium during the process of gel electrophoresis.

3.2.1.6 Spectrophotometer

Qualitative and quantitative investigation of genomic DNA was done with the help of UV spectrophotometer.

3.2.1.7 Deep freezer and refrigerator

Chemicals required during the molecular work were stored in Refrigerator (8ºC) and Deep freezer (-20ºC).

3.2. 1.8 Chemicals

Double distilled water was used for different processes like DNA isolation, spectrophotometery and buffer preparation to avoid the chances of contamination of isolated samples.

Ethanol (70%) was required to sterilize the instruments to avoid the chances of contamination.

3.2.1.9 Preparation of working primers

1) **Gene specific primer:** Eight mitochondrial gene specific primers of CO1 region (synthesised by Chromos biotech, Bangalore, India) were used for amplification of genomic DNA. In molecular methods, CO region of mitochondrial DNA is considered as an excellent marker because it is the fastest variable mitochondrial region (**Kumazawa *et al.*, 2004),** so it opened a wide range of new approaches to insects research, particularly with regard to molecular phylogenetic and taxonomic studies. Details of gene specific primers were given in table 3.1.

Table 3.1: List of gene specific primers used for amplification of DNA

S. No.	Primers Name	Sequence	Vol. for 100 pmol/µl	GC content	Tm [°C]
1	P1	F-AGCACCAATTTCTTCAATTTT	296	28.6%	44
		R-TGTAAATATGTGATGTGCTC	303	35%	
2	P2	F-GGTCAACAAATCATAAAGATATTGG	239	32%	50
		R-TAAACTTCAGGGTGACCAAAAAATCA	248	34.6%	
3	P3	F-AATTGGGGGATTTGGAAACT	370	40%	46.5
		R-CAGTTCCTGCCCCTATTTCA	993	50%	
4	P4	F-ATTGGGGGATTTGGAAACTG	950	45%	46.5
		R-CAGTTCCTGCCCCTATTTCA	552	50%	
5	P5	F-TGCAGGAACTGGATGAACTG	957	50%	46.5
		R-GGTTTATGCCAAATGGTCGT	1017	45%	
6	P6	F-CTCCCACCTGCCTTAACCTT	501	55%	47.75
		R-GGTTTATGCCAAATGGTCGT	910	45%	
7	P7	F-TGCCATATCTTTCTCATGTTCG	646	41%	44.5
		R-GATTTGCCCCACAAATTTCA	1203	40%	
8	P8	F-TGCCATATCTTTCTCATGTTCG	397	41%	46.5
		R-TGCCCCACAAATTTCAGAAC	1095	45%	

2) **TE buffer:** Used for preparation of stock primer solution from powdered formed primer.

3) Molecular biological grade water was used for preparation of working primer from stock solution.

3.2.1.10 Chemicals used for amplification of genomic DNA

1) DNA template: Genomic DNA molecule extracted from insect sample and produces multiple copies of DNA after amplification.
2) dNTPs mix: Mixture of dATP, dCTP, dGTP, dTTP. Where dATP and dGTP are purines and dCTP and dTTP are pyrimidines.

3) *Taq* DNA polymerase: This enzyme helps in copying of DNA molecules at higher temperature and derived from heat loving bacteria *Thermus aquaticus.*
4) Gene specific Primers.

3.2.1.11Thermal cycler

Amplification of genomic DNA with gene specific primer was performed in Thermal cycler Wee32 (Himedia).

3.2.1.12 Chemicals used in gel electrophoresis

1) 50X TAE: Helps in separation of DNA and RNA during the process of electrophoresis.
2) Ladder: 100 bp DNA ladder was used to determine the approx. size of different amplified sequences.
3) 6X Gel loading dye: Double dye made up of xylene cynanol (orange colour) and bromophenol blue (blue colour) was used to assess rate of movement of amplified DNA in agarose gel medium.
4) Ethidium bromide: Used as fluorescent tag (nucleic acid stain) during the process of gel electrophoresis.

3.2.1.13 Gel electrophoresis unit

Maxi-submarine gel unit (GEINI) was used for separation of amplified genomic DNA molecules on 2.5% agarose gel with TAE (50X) as running buffer.

3.2.1.14 Gel documentation system

Gel Imaging and documentation of nucleic acids molecules suspended in agerose gel was carried out with the help of Gel documentation system (Alpha InfoTech Alpha imager EC).

3.2.1.15 Miscellaneous

Gloves, plastic tray, tissue paper, eppendrop stand, PCR box, cello tape, marker, discard box, Heater, mini coolar.

3.2.2 Methods to study the genetic variability of coccinellid beetles

3.2.2.1 Selection of sample

Four samples of each coccinellids species were randomly selected from all the collected samples. Each samples were kept in seprate mortar pastel to avoid the chances of the contamination.

3.2.2.2 Preparation of sample

Coccinellid beetles are generally predatory in nature, to avoide cross contamination with prey DNA, these beetles were put under the starved condition for the 48 hours. Wings and elytra were removed with the help of Sterile Scapel Blade No. 11 under compound microscope and remaining parts of the body of insect were used for the DNA isolation.

3.2.2.3 Isolation of DNA

Procedure for isolation of genomic DNA from coccinellid beetles was according to the standardized protocole of HipurA^{TM} Insect DNA Purification Kit (Himedia). This isolation procedure used miniprep spin column (capped) comparies three steps *viz.*, binding of DNA molecules to the membrane of spin column, removal of other contaminants and elution of pure genomic DNA from membrane. HiElute miniprep spin column format allowed the rapid processing for DNA isolation of multiple samples. These spin columns had high binding capacity to the DNA molecules so that high quality DNA was obtained from insects samples. During the process of DNA isolation insect samples were grinded in the mortar and pestal with the help of liquid nitrogen. Liquid nitrogen was used for the removal of moisture content of insects body. Grinded samples were treated with lysis buffer along with RNAs and proteinase-K (to ensure good qality of DNA). The suspension was incubated at 55ºC for 2 hours and lysate (suspension) was loaded on HiShredder and centrifuged (13000 rpm) for two minutes. The precipitated fraction was washed with ethanol (96-100%) and mixed properly by vortexing. Then lysate was transferred to the spin columns (capped) and centrifuged (10000 rpm) for one minute. The precipitated fraction was discarded and 200 ml of Elution buffer was added to each spin columns. Again these spin columns were centrifuged at 10000 rpm for 2 minutes. Extracted DNA was transferred to the collection tubes for longer storage.

3.2.2.4 Qualitative and quantitative estimation of the genomic DNA

For the estimation of purity and concentration of genomic DNA, Spectrophotometer method (**Sambrook *et al.,* 2001)** is the most commonly used method. This method is based on Beer-Lambert's law, which states that the absorbance is directly proportional to both the concentration of the absorbent and thickness which allows the selection of wavelength that pass through the solution and amount of the solute in solution determined by measuring the absorbance of light at given wavelength.

Concentration and purity of DNA was evaluated with the help of Citizen UV Spectrophotometer. The absorbance was recorded at two different wavelengths (260 nm and 280 nm) against diluted DNA samples. Dilution of DNA samples was done with the help of double distilled water in the PCR-tubes. The dilution was prepared by mixing of 1 µl working DNA sample in 59 µl of double distilled water.

Purity of DNA was evaluated by finding the ratio of optical densities ($OD_{260/280}$). This ratio is an indicator of amount of impurities *i.e.* RNA and Proteins in the diluted DNA samples. The ratio of $OD_{260/280}$ above and below 1.8 in the samples indicates the contamination of RNA and DNA respectively.

The quantity of the DNA in the sample was correlated to absorbance by the given rule:

$$Amount\ \ of\ DNA\ (ng/\mu l\ ) = \frac{Dilution\ factor \times 50 \times OD_{260}}{1000}$$

3.2.2.5 Preparation of working DNA from stock solution

Working DNA samples were prepared form stock solution after calculating the concentration of DNA with Spectrophotometeric method. Working samples were prepared in such a way that each sample should have around 100 ng DNA per µl working sample. Working samples were stored in deep freezer at 20 ºC temperature for further use in amplification process.

3.2.2.6 Amplification of DNA by PCR

DNA amplification of eight gene specific primers was performed with the help of PCR thermo-cycler. The gene specific primers were synthesised by Chromous Biotech Bangalore India. Details of these primers were given in **Table 3.1** with their volume, nucleotide sequence, annealing temperature, and GC content. Annealing temperature was found with the help of temperature gradient in PCR thermal cycler.

3.2.2.7 Preparation of reaction mixture

Reaction mixture was prepared by two component *i.e.* master mix and working DNA template. Master mix (a mixture of *Taq*DNA polymerase, PCR Buffer, dNTP mix, Molecular biology grade water and Primers) was prepared to reduce the chances of pipetting errors. After that 13µl master mix was distributed in each PCR tube which had already pipetted with 2µl of DNA template.

At the end, PCR tubes containing reaction mixture were vortex and gently homogenised for 15 seconds.

Table 3.2: PCR ingredients used in preparation of reaction mixture

Ingredient	Working	Single reaction(15µl)
Diluted DNA sample	-	2 µl
Molecular biology grade water	-	9.1 µl
PCR Buffer	10X	1.5 µl
dNTP mix	10Mm	0.9 µl
*Taq*DNA polymerase	5U/µl	0.3 µl
Primer	10Mm	1.2 µl

The amplification of DNA was done with thermal-cycler (Wee 32 HIMEDIA), programmed as follows.

Table 3.3: Reaction cycle of PCR amplification

Cycles	Denaturation		Annealing		Extension	
	Temp.	Time	Temp.	Time	Temp.	Time
First cycle	94°C	5 min	-	-	-	-
40 cycles	94°C	45 sec	43-50°C	45 sec	72°C	2 min
Last cycle	-	-	-	-	72°C	5 min

3.2.2.8 Polymerase Chain Reaction

PCR (Polymerase Chain Reaction) technique was first invented by Mullis in 1983 **(Mullis *et al.*, 1986; 1987).** It was applied for the amplification of definite genomic fragment of DNA. PCR worked on three basic steps which constitute a single cycle.

i. **Denaturation**: The genomic DNA sequence denatured at 94-96ºC into two separate strands. The separation occurred due to rising of temperature.
ii. **Annealing**: Primers bind to their complementary sequences on either side of the target sequence at a particular annealing temperature.
iii. **Extension**: *Taq* DNA polymerase binds and extends a complementary DNA and new DNA strand made using original template by addition of nucleotides at 3'end of each primer.

3.2.2.9 Analysis of amplified products (Gel electrophoresis)

After amplification of genomic DNA, PCR product was analysed on 2.5% agarose gel medium by the process of gel electrophoresis.

Gel electrophoresis is a method of fractionation of amplified DNA molecules according to their size. It was achieved in agarose gel medium with the help of horizontal gel electrophoresis unit (Maxi-submarine gel unit GEINI). Gel medium of 2.5% was prepared by dissolving 6.25gm of agrose in 250ml TAE buffer. Buffer acts as a source of ions to set up an electric field during the process of gel electrophoresis. TAE buffer commonly prepared as a 50X stock solution. 5 ml of TAE dissolved in 245 ml of double distilled water and the mixture was boiled in a conical flask for ten to fifteen minutes. After that gel was poured in casting tray and left for solidification. Before the complete solidification 12 µl ethidium bromide added in the mixture with the help of micropipett. Amplified DNA and loading dye were loaded to the wells formed on agarose gel medium in a ratio of 7:3. DNA ladder (100bp) was also loaded as a standard marker. Electrophoresis process was performed at a constant voltage (70V) for two hours and fifty minutes with TAE buffer. Banding pattern was visualised with the help of UV transilluminator and stored in the Gel Documentation system.

3.2.2.10 Molecular marker data analysis

Scoring of gel was done manually and independently. Each image was scored thrice to minimise the chances of error. Band presence was indicated by one (1) and its absence by zero (0). All the unique bands were also incorporated in the analysis.

The polymorphism was found by the counting of monomorphic and polymorphic PCR bands. The percentage monomorphism and polymorphism was calculated individually for all the eight primers by using the given formulas.

$$Percentage\ monomorphism\ of\ individual\ primers = \frac{No.\ of\ monomorphic\ bands\ amplified\ by\ an\ individual\ primer}{Total\ no.\ of\ bands\ amplified\ by\ an\ individual\ primer}$$

$$Percentage\ polymorphism\ of\ individual\ primers = \frac{No.\ of\ polymorphic\ bands\ amplified\ by\ an\ individual\ primer}{Total\ no.\ of\ bands\ amplified\ by\ an\ individual\ primer}$$

$$Overall\ percentage\ monomorphis = \frac{No.\ of\ monomorphic\ bands\ amplified}{Total\ no.\ of\ bands\ amplified}$$

$$Overall\ percentage\ polymorphism = \frac{No.\ of\ polymorphic\ bands\ amplified}{Total\ no.\ of\ bands\ amplified}$$

Polymorphism information content (PIC) was according to **Rodan-Ruiz *et al*. (2001)** to detect ability of primer to establish polymorphism in the population depending on the number of alleles detected and on their distribution frequency.

PIC identifies the discriminatory capability of the primer. It depends upon the number of recognized alleles and their distribution frequencies; therefore it is equal to the gene diversity.

PIC value calculated as: $PIC = \Sigma PICi$

Where,

$PICi = (2pi(1 - pi))$

Pi=Frequency of i^{th} allele

PICi=Polymorphism content of i^{th} loci

PIC=polymorphism information content of primer

Marker index (MI) and Effective multiplex ratio (EMR) was calculated according to **Nagaraju *et al*. (2001)** and **Powell *et al.* (1996)** and these values are generally measured to know the usefulness of primers.

EMR was calculated by total number of polymorphic loci (per primer) multiplied by the proportion of polymorphic loci per total number of bands.

Higher the value of EMR, more efficient primer marker system will be.

$$EMR = \mathrm{np} \times \frac{np}{n}$$

EMR=Effective multiplex ratio

np =No. of polymorphic bands

n =Total no. of bands

Marker index (MI) is a statistical factor used to estimate the overall effectiveness of marker system. MI is the product of polymorphism information content value and effective multiplex ratio. The higher the MI, the better the primer is.

$$MI = PIC \times EMR$$

Resolution power (R_p) was assessed according to **Prevost and Wilkinson, (1999)** and **Gilbert *et al.* (1999)** to characterize the ability of primer combination to detect the difference between samples.

$$Rp = \Sigma BIi$$

Where,

$$BIi = 1 - (2 \times (|0.5 - pi|)$$

Bli=Band informativeness of ith loci

Pi=Frequency of the ith allele

Genetic distance based on Jaccard coefficient **(Jaccard,1908)** was calculated using NTSYSpc 2.11a software package **(Rohlf, 2002)** .The distance coefficient obtained was used to construct the dendrogram using UPGMA (the unweighted pair group method with arithmetic average) employing the SHAN clustering algorithm in same software package. Principal component analysis also used for clustering the population in bidimentional scatter plot **(Lopes *et al.*, 2017)** which was analysed by R software package **(Jombart, 2008).**

3.3. Seasonal abundance of coccinellid in different cropping systems of Pantnagar.

3.3.1 Method of sampling

Sampling of predatory coccinellid beetles (eggs, grub, pupa and adult) was conducted at a regular interval of 7 days. Sampling techniques that provide accurate assessment of coccinellid density are critical for evaluation. The beetles were collected by hand picking method **(Hemchandra *et al.*, 2010).** Samples from a particular crop were collected by making 'W' shape pattern within the field (**Zehnder, 2014**). Each corner of 'W' was considered as a spot for the collection. At a spot one meter square area was selected and each plant of square was observed for predators. All the life stages of predatory coccinellid beetles (eggs, grub, pupa and adult) were collected and brought to the laboratory. Immature stages were reared up to the adult stage for the further study. Collection of adult coccinellid beetles were carried out continuously during the study period.

3.3.2 Meteorological observations and Correlation studies.

The mean population was correlated with weather parameters including maximum and minimum temperature, rainfall, relative humidity morning and relative humidity evening for calculating value of Pearson's coefficient. Meteorological data was collected from Meteorological Observatory situated at Norman E. Borlaug Crop Research Centre Pantnagar. Analysis of Pearson's coefficient was done with the help of SPSS software.

Results and Discussion

CHAPTER-4 RESULTS AND DISCUSSION

The results of investigation on "**Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar"** are presented under following headings:

4.1 Diversity of different coccinellid species from different crop ecosystems of Pantnagar.

4.2 Study on genetic variability among the different species of predatory coccinellid beetles thriving in Pantnagar region.

4.3 Seasonal abundance of coccinellid in different cropping systems at Pantnagar.

4.1. Diversity of different coccinellid species in different crop ecosystems of Pantnagar.

A periodical survey was conducted from May, 2018 to April, 2019 in different crop ecosystems of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand. A total of fifteen species of coccinellid predators belonging to three subfamilies; Chilocorinae Mulsant (1846), Coccinellinea Latreille (1807) and Coccidulinae Mulsant (1846) were reported. Thirteen species were belonged to subfamily coccinellinae and rest of two subfamilies Chilocorinae and Coccidulinae had one species each **(Table 4.1)**.

Table 4.1: Different species of coccinellid predators reported from different crop ecosystems of Pantnagar.

Species reported	**Sub family**
Anegleis cardoni (Weise)	Coccinellinae
Cheilomenes sexmaculata (Fabricius)	
Coccinella septempunctata (Linnaeus)	
Coccinella transversalis (Fabricius)	
Harmonia dimidita (Fabricius)	
Illeis sp.1	
Propylea dissecta (Mulsant)	
Micraspis univittata (Hope)	
Micraspis allardi (Mulsant)	
Illeis sp. 2	
Micraspis discolor (Fabricius)	
Harmonia sedecimnotata (Fabricius)	
Hippodamia variegate (Goeze)	
Brumoides suturalis (Fabricius)	Chilocorinae
Rodolia fumida (Mulsant)	Coccidulinae

4.1.1 Diversity of different coccinellid species in rice crop at Pantnagar

A total of three coccinellid species viz., *C. transversalis* (Fab.), *C. septempunctata* (L) and *M. discolor* (Fab.) were reported from rice crop. These beetles were found predacious on *Nilaparvatha lugens* (Stal), *N. virescens* (Distant) and *Sogatella fursifera* (Horvath) during the month of August and September. These coccinellid species were also recorded by **Vinothkumar (2013)** during their study in rainfed rice ecosystem of Tamilnadu. In addition to that *M. discolor* (Fab.) and *C. transversalis* (Fab.) were also found by **Mondal *et al.* (2016)** in rice ecosystem of West Bangal **(Table 4.2)**.

4.1.2 Diversity of different coccinellid species in wheat crop at Pantnagar

Four species of coccinellid beetles were found in wheat crops *i.e. C. septempunctata* (L), *C. sexmaculata* (Fab.), *C. transversalis* (Fab.), and *P. dissecta* (Mul.). They were mainly predaceous on *Rhopalosiphum maidis* (Fitch) and *Macrosiphum miscanthi* (Takahashi) during the month of February and March. **Jat *et al.* (2009)** and **Megha *et al.* (2015)** also reported the presence of *C. sexmaculata* (L) and *C. transversalis* (Fab.) in wheat crop.

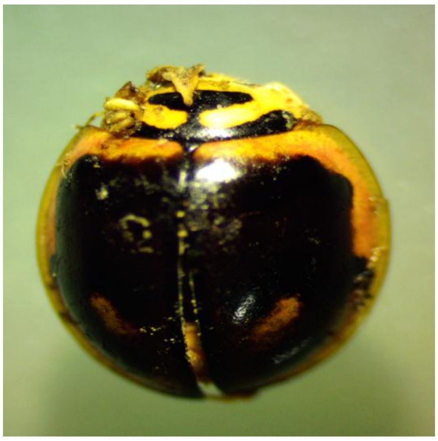
Table 4.2: Coccinellid species reported from particular crop along with their associated prey during 2018 and 2019.

Crops	Month	Coccinellid species observed	Prey insects
Wheat	November to April	*C. septempunctata* (L.), *C. sexmaculata* (Fab.), *C. transversalis* (Fab.) *P. dissecta* (Mul.)	*Rhopalosiphum maidis* (Fitch), *Macrosiphum miscanthi* (Takahashi)
Rice	June to October	*C. transversalis* (Fab), *C. septempunctata* (L.), *M. discolor* (Fab.).	*Nilaparvatha lugens* (Stal), *N. virescens* (Distant), *Sogatella fursifera* (Horvath)
Soybean	July to October	*C. septempunctata* (L.), *C. sexmaculata* (Fab.), *M. discolor* (Fab.), *P. dissecta* (Mul.), *H. dimidita* (Fab.), *M. univittata* (Hope), *A. cardoni* (Weise), *B. suturalis* (Fab.)	*Bemisia tabaci* (Gennadius), *Aphis gossypii* (Glover)

Coccinella septempunctata

Brumoides suturalis

Cheilomenes sexmaculata



Cheilomenes sexmaculata

Micraspis discolor

Micraspis univittata

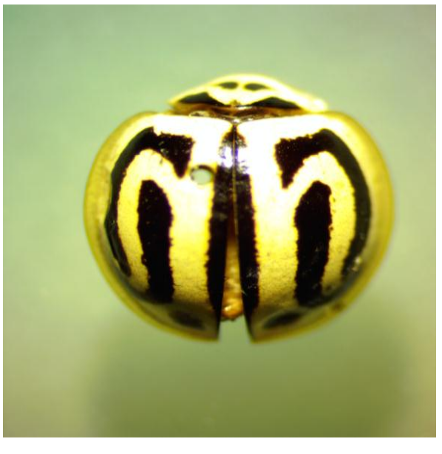
Plate 9: Coccinellid species reported from different crop ecosystems of Pantnagar during 2018-19

Illeis sp. 2

Illeis sp.1

Harmonia dimidita

Harmonia dimidita



Anegleis cardoni

Rodolia fumida

Plate 10: Coccinellid species reported from different crop ecosystems of Pantnagar during 2018-19

Maize	June to September	*C. septempunctata* (L.), *C. sexmaculata* (Fab.), *P. dissecta* (Mul.), *C. transversalis* (Fab.), *H. dimidita* (Fab.), *R. fumida* (Mul.)	*Rhopalosiphum maidis* (Fitch), Pollen grains.
Cowpea	August to October	*C. septempunctata* (L.), *C. sexmaculata* (Fab.), *M. discolor* (Fab.), *A. cardoni* (Weise), *B. suturalis* (Fab.), *H. variegate* (Goeze), *M. allardi* (Mul.).	*Aphis craccivora* (Koch). *Bemisia tabaci* (Gennadius)
Chickpea	November to April	*C. transversalis* (Fab.), *C. septempunctata* (L.), *C. sexmaculata* (Fab.)	*Aphis craccivora* (Koch)
Okra	July to October	*C. transversalis* (Fab.), *C. septempunctata* (L.)	*Aphis gossypii* (Glover) *Amrasca biguttula biguttula* (Ishida)
Coriander	November to March	*C. septempunctata* (L.)	*Hyadaphis coriandri* (Das.)
Mustard	October to April	*C. septempunctata* (L.), *C. sexmaculata* (Fab.), *C. transversalis* (Fab.), *M. allardi* (Mul.), *Illeis* sp.1 & 2	*Lipaphis erysimi* (Kaltenbach), *Myzus persicae* (Sulzer).
Rose	December to March	*C. septempunctata* (L.), *A. cardoni* (Weise).	*Macrosiphum rosae* (Linneaus)
Pigeon pea	June to February	*C. septempunctata* (L.), *H. dimidita* (Fab.)	*Aphis craccivora* (Koch)
Marigold	October to March	*C. septempunctata* (L.), *A. cardoni* (Weise), *B. suturalis* (Fab.)	*Lipaphis erysimi* (Kaltenbach)
Cauliflower	October to February	*H. sedecimnotata*(Fab.), *C. septempunctata* (L.)	*Lipaphis erysimi* (Kaltenbach)
Cabbage	October to February	*C. septempunctata*(L.), *C. sexmaculata* (F.). *H. variegate*(Goeze)	*Lipaphis erysimi* (Kaltenbach)

4.1.3 Diversity of different coccinellid species in maize crop at Pantnagar

Maize crop is rich in diversity of coccinellid species. Total five species *i.e. C. septempunctata* (L.), *C. sexmaculata* (Fab.), *P. dissecta* (Mul.), *C. transversalis* (Fab.), *H. dimidita* (Fab.) and *R. fumida* (Mul.) were found in maize field throughout the survey period. They were found predacious on maize aphid *i.e. R. maidis* (Fitch) during the month of August and September. In Maize crop three species *viz., P. dissecta* (Mul.), *H. dimidita* (Fab.), *Rodallia fumida* (Mul.) of coccinellid species, besides aphids, were also found feeding on the pollen grains. Similar findings were also observed by **Lami *et al.* (2016). Swaminathan *et al.* (2015)** observed a positive correlation between maize aphid and coccinellid population.

4.1.4 Diversity of different coccinellid species in soybean crop at Pantnagar

Coccinellid fauna found in soybean crop was more diverse than other crops because eight coccinellid species were collected and identified during the survey program. The identified species were *C. septempunctata* (L.), *C. sexmaculata* (Fab.), *M. discolor* (Fab.), *P. dissecta* (Mul.), *H. dimidita* (Fab.), *M. univittata* (Hope), *A. cardoni* (Weise), *B. suturalis* (Fab.). Two coccinellid species *i.e. A. cardoni* (Weise) and *B. suturalis* (Fab.) were found predacious on *Bemisia tabaci* (Gennadius) and rest of other species were found feeding on *Aphis gossypii* (Glover) during the month of August and September. Similar types of results were also recorded by **Ahirwar *et al.* (2015)** during their study. **Suyal *et al.* 2018** also reported the presence of *C. septempunctata* (L.) in soybean crop during the month of August.

4.1.5 Diversity of different coccinellid species in mustard crop at Pantnagar

Coccinellid species found in mustard crop were *C. septempunctata* (L), *C. sexmaculata* (Fab.), *C. transversalis* (Fab.), *M. allardi* (Mul.), *Illeis* sp.1 and *Illeis* sp.2. These all species were recorded feeding on *Lipaphis erysimi* (Kaltenbach) and *Myzus persicae* (Sulzer) during the month of January and February. These finding are in close confirmatory with the finding of **Pal and Bhatt (2018)** who studied feeding efficiency of six coccinellid species on mustard aphid**.** *Illies* sp. was also found feeding on mealy bug, scale insects, whitefly and causal agent (*Phyllactinia corylea*) of powdery mildew disease in mulberry crop (**Nelaballe and Meruva, (2015).**

Harmonia sedecimnotata

Micraspis allardi

Propylea dissecta

Propylea dissecta

Propylea dissecta

Hippodamia variegate

Plate 11: Coccinellid species reported from different crop ecosystems of Pantnagar during 2018-19

Hippodamia variegate

Coccinella transversalis

Cheilomenes sexmaculata in Maize crop

Propylea dissecta in Maize crop

Plate 12: Coccinellid species reported from different crop ecosystems of Pantnagar during 2018-19

4.1.6 Diversity of different coccinellid species in cowpea crop at Pantnagar

In cowpea total seven coccinellid species were found. The collected species were *C. septempunctata* (L), *C. sexmaculata* (Fab.), *M. discolor* (Fab.), *A. cardoni* (Weise), *B. suturalis* (Fab.), *H. variegate* (Goeze), and *M. allardi* (Mul). They were found predaceous on *Aphis craccivora* (Koch) and *Bemisia tabaci* (Gennadius) during the month of August and September. **Srikanth and Lakkundi (1990)** observed that the *C. sexmaculata* (Fab.) constitute major predator population during the Kharif and summer season. **Megha *et al.* (2015)** also reported eighteen species of predatory coccinellid beetles in cowpea crop which include most of above mentioned species**.**

4.1.7 Diversity of different coccinellid species in chickpea crop at Pantnagar

Three species of coccinellid beetles *C. transversalis* (Fab.), *C. septempunctata* (L.) *and C. sexmaculata* (Fab.) were found in chickpea crop. These species mainly found predaceous on *Aphis craccivora* (Koch) during the months of January and February**. Kumar *et al.* (2017)** also reported these three coccinellid beetles in chickpea crop from Western plain zone of Uttar Pradesh. **Megha *et al.* (2015)** also reported occurrence of *C. sexmaculatus* (Fab.) *and Illies* sp. from Dharwad region.

4.1.8 Diversity of different coccinellid species in pigeon pea crop at Pantnagar

Only two species of coccinellid beetles *C. septempunctata* (L.) and *H. dimidita* (Fab.) were recorded from pigeon pea crop from October to December. These species were found feeding on black aphid *i.e. A. craccivora* (Koch) during the months of August and September. **Chakravarty *et al.* (2016)** reported the presence of two coccinellid species *i.e. Coccinella septumpunctata* (L.) and *Menochiles sexmaculatus* (F.) from Pantnagar region of Uttrakhand.

4.1.9 Diversity of different coccinellid species in coriander crop at Pantnagar

Only one species of lady bird beetle *i.e. C. septempunctata* was found on coriander crop. It was found predaceous on *Hyadaphis coriandri* (Das.) during the months of January and February. Similar results were also observed by **Pareek *et al*. (2013).** Aphid population was highest during the third week of February and number of coccinellid beetles increases with the population of aphids. Similar results were also observed by **Swami *et al*. (2018)** and **Pareek *et al.* (2013).**

4.1.10 Diversity of different coccinellid species in okra crop at Pantnagar

Two species of coccinellid beetles *i.e. C. transversalis* (Fab.), *C. septempunctata* (L.) were recorded from okra crop and found feeding on *A. gossypii* (Glo.) and *Amrasca biguttula biguttula* (Ish). Maximum population of coccinellid beetles was recorded during third to fourth week of September when insect pest population was at its peak. These findings are in close conformity with **Bhatt *et al* (2018)** and **Bhatt and Karnatak (2018).**

4.1.11 Diversity of different coccinellid species in cabbage crop at Pantnagar

Coccinellid beetles found on cabbage crop were identified as *C. septempunctata* (L), *C. sexmaculata* (Fab.) and *H. variegate* (Goeze). These species were found predaceous on *L. erysimi* (Kaltenbach) during the month of January. C. *septempunctata* (L.) and *C. sexmaculata* (Fab.) were also reported by **Ali and Rana (2011)** in cabbage crop. **Thakur *et al.* (1989)** reported another species *H. variegate* (Goeze) preying upon cabbage aphid (*Brevicoryne brassicae*) in Kullu Valley, Himanchal Pradesh. **Megha *et al.* (2015)** also reported the occurrence of *H. variegate* in cabbage crop in Dharwad region.

4.1.12 Diversity of different coccinellid species in cauliflower crop at Pantnagar

Only two species coccinellid were collected and identified from cauliflower *viz., H. sedecimnotata* (Fab.) and *C. septempunctata* (L.) These species were found predaceous on *L. erysimi* (Kaltenbach) during the month of January. **Ali and Rana (2010)** reported three species of coccinellid beetles *viz., M. sexmaculatus* (Fabricius), *C. septempunctata* (L.) *and C. transversalis* (Fab.). **Gupta *et al.* (2017)** reported presence of *H. sedecimnotata* in Cauliflower and Brinjal crop from Nasik district of Maharastra.

4.1.13 Diversity of different coccinellid species in marigold crop at Pantnagar

Three species of coccinellid beetles were found in marigold crop *viz*., *C. septempunctata* (L.), *A. cardoni* (Weise) and *B. suturalis* (Fab.). **Ganai *et al.* (2017)** reported occurrence of *C. septempunctata* (L.) in marigold crop of Jammu district.

4.1.14 Diversity of different coccinellid species in rose crop at Pantnagar

Only two species of coccinellid beetles *i.e. C. septempunctata* (L.) *and A. cardoni* (Weise) were recorded in Rose crop. They were found predaceous on rose aphid *(M. rosae).* **Mahr *et al*. (2010)** also reported two predatory coccinellid beetles *i.e. C. septempunctata* (L.) and *A. bipunctata* (L.) on green house rose and chrysanthemum crop.

4.2 Study on genetic variability among different species of predatory coccinellid beetles thriving in Pantnagar region

The studies on genetic variability among different coccinellid beetles were conducted in Biological Control Laboratory of Department of Entomology at Govind Ballabh Pant University of Agriculture & Technology, Pantnagar. Polymerase Chain Reaction (PCR) reaction was conducted for 8 different primers and amplified primers were passed through agarose gel and image was viewed with the help of gel documentation system. Specific banding pattern revealed molecular profile of different species of coccienllid beetles.

4.2.1 Quantification of DNA extracted from different coccinellid species.

The values of optical density ($OD_{260/280)}$ of insects DNA was obtained with the help of spectrophotometer. OD value indicates the concentration, purity and yield of DNA and its value should be 1.8 for the preparation of working solution. Ratio of OD value above and below 1.8 indicates presence of contamination (RNA and Protein) in the solution. Values of OD presented in **Table 4.3.**

4.2.2Amplification of primers

During the study twenty eight samples of coccinellid beetles were used against eight gene specific primers. The amplicon pattern was recorded from gel image of all eight primers *viz.,*

1. Primer 1 (P1)
   F-AGCACCAATTTCTTCAATTTT
   R-TAAACTTCAGGGTGACCAAAAAATCA
2. Primer (P2)
   F-GGTCAACAAATCATAAAGATATTGG
   R-TAAACTTCAGGGTGACCAAAAAATCA
3. Primer (P3)
   F-AATTGGGGGATTTGGAAACT
   R-CAGTTCCTGCCCCTATTTCA
4. Primer (P4)
   F- ATTGGGGGATTTGGAAACTG
   R-CAGTTCCTGCCCCTATTTCA
5. Primer (P5)
   F-CAGTTCCTGCCCCTATTTCA
   R-GGTTTATGCCAAATGGTCGT
6. Primer (P6)
   F-CTCCCACCTGCCTTAACCTT
   R-GGTTTATGCCAAATGGTCGT

7. Primer (P7)
F-TGCCATATCTTTCTCATGTTCG
R-GATTTGCCCCACAAATTTCA

8. Primer (P8)
F-TGCCATATCTTTCTCATGTTCG
R-TGCCCCACAAATTTCAGAAC

Table 4.3: Quantitative analysis of DNA samples of different coccinellid beetles

S.N	Sample	OD at 260/280	Conc.in ng/μl	Working of PCR amplification (100ng/μl; DNA+TE)
1	CS1	1.68	158.2	63.21 +36.79
2	CS2	1.67	375.3	26.64 +73.36
3	CS3	1.84	258.2	38.72 +61.28
4	CS4	1.97	425.9	33.4+66.53
5	CT1	1.65	298.8	33.46 +66.54
6	CT2	1.83	325.9	30.67 +69.33
7	CT3	1.87	221.2	45.20 +54.8
8	CT4	1.69	382.8	26.12 +73.88
9	PQ1	1.72	122.2	81.83 +18.17
10	PQ2	1.93	398.8	25.07 +74.93
11	PQ3	1.80	218.4	45.78 +54.22
12	PQ4	1.66	126.2	79.23 +20.77
13	MD1	1.86	238.3	41.96 +58.04
14	MD2	1.84	231.2	43.25 +56.75
15	MD3	1.95	173.9	57.50 +42.5
16	MD4	2.0	525.9	19.01 +80.9
17	MS1	1.87	398.8	25.07 +74.93
18	MS2	1.65	241.9	41.33 +58.67
19	MS3	1.78	298.8	33.46 +66.54
20	MS4	1.87	283.8	35.23 +64.77
21	HD1	1.97	185.5	53.90 +46.10
22	HD2	1.83	281.6	35.51 +64.49
23	HD3	1.95	234.7	42.60 +57.40
24	HD4	1.75	176.4	56.68 +43.32
25	HY1	1.98	149.6	66.84 +33.16
26	HY2	1.85	371.9	26.88 +73.12
27	HY3	1.67	378.6	26.41 +73.59
28	HY4	1.78	221.2	45.20 +54.8

CS= *Coccinella septempunctata,* MS=*Cheilomenes sexmaculata,* CT= *Coccinella transversalis,* PQ= *Propylea dissecta,* HD= *Harmonia dimidita (Black),* HY= *Harmonia dimidita (Spotted),* MD=*Micraspis discolor*

Amplification of primers results into different banding pattern. The amplicon patterns were different for most of primers as they are having different nucleotide sequence. Most of the loci were polymorphic only few were monomorphic **(Table 4.4).**

The primer P1 showed amplifications with all coccinellid species. The total numbers of amplicons were 51. Maximum 18 amplicons were found in samples of *C. septumpuctata* and a minimum of 2 amplicons in samples of *M. disclor*. Range of amplicon was found from 100bp to 900bp. Total 8 amplified loci were polymorphic. The percent polymorphism was 100%. The PIC value, resolving power and marker index of the primer P1was found 0.27, 2.71 and 2.91, respectively.

The primer P2 showed amplifications with five Coccinellid species. The total numbers of amplicons were 14. Maximum 6 amplicons were found in samples of *H. dimidiate* (spotted) and a minimum of 0 amplicons in samples of *H. dimidiata* (Black) and *P. dissecta*. Range of amplicon was found from 180bp to 800bp. Total 6 amplified loci were polymorphic. The percent polymorphism was 100%. The PIC value, resolving power and marker index of the primer P2 was found 0.15, 1 and 0.90, respectively.

The primer P3 showed amplifications with four coccinellid species. The total numbers of amplicons were 10. Maximum 6 amplicons were found in samples of *M. disclor* and *M. sexmaculata* and a minimum of 0 amplicons in samples of *H. dimidiata* (Black) and *P. dissecta*. Range of amplicon was found from 150bp to 200bp. Total 2 amplified loci were polymorphic. The percent polymorphism was 100%. The PIC value, resolving power and marker index of the primer P3 was found 0.29, 0.71 and 0.58, respectively.

The primer P4 showed amplifications with six coccinellid species. The total number of amplicons was 21. Maximum 4 amplicons were found in samples of *M. sexmaculata, M. disclor, C. transversalis* and *P. dissecta*. Only single locus of 150bp was amplified. The percent polymorphism was 100%. The PIC value, resolving power and marker index of the primer P4 was found 0.37, 0.5 and 0.80, respectively.

The primer P5 showed amplifications with six coccinellid species. The total number of amplicons was 21. Maximum 5 amplicons were found in samples of *M. sexmaculata* and *C. transversalis.* Total 8 amplified loci were polymorphic in the range of 150bp to1000 bp. The percent polymorphism was 100%. The PIC value, resolving power and marker index of the primer P5 was found 0.14, 1.5 and 1.10, respectively.

Table 4.4: Analog polymorphisms of coccinellid samples with eight gene specific primers

S.No	Primer	No. of amplicon							No. of amplicon	Range of allele	No. of Loci	NPA	NMA	%PA	%MA	PIC	EMR	MI	RP
		CS	PQ	MD	HY	HD	MS	CT											
1	F-AGCACCAATTTCTTCAATTTT R-TGTAAATATGTGATGTGCTC	18	4	2	5	9	4	9	51	100-900	8	8	0	100	0	0.27	8	2.19	2.71
2	F-GGTCAACAAATCATAAAGATATTGG R-TAAACTTCAGGGTGACCAAAAAATCA	1	0	5	6	0	1	1	14	180-800	6	6	0	100	0	0.15	6	0.90	1
3	F-AATTGGGGGATTTGGAAACT R-CAGTTCCTGCCCCTATTTCA	0	1	4	0	0	4	1	10	150-200	2	2	0	100	0	0.29	2	0.58	0.71
4	F-ATTGGGGGATTTGGAAACTG R-CAGTTCCTGCCCCTATTTCA	3	4	4	2	0	4	4	21	150	1	1	0	100	0	0.38	1	0.38	0.5
5	R-CAGTTCCTGCCCCTATTTCA R-GGTTTATGCCAAATGGTCGT	4	2	0	2	3	5	5	21	150-1000	8	8	0	100	0	0.14	8	1.10	1.5
6	F-CTCCCACCTGCCTTAACCTT R-GGTTTATGCCAAATGGTCGT	8	4	4	4	2	1	4	27	220-1000	2	2	0	100	0	0.20	2	0.41	0.5
7	F-TGCCATATCTTTCTCATGTTCG R-GATTTGCCCCACAAATTTCA	4	7	6	2	7	4	4	34	180-450	4	3	1	75	25	0.10	2.25	0.22	0.43
8	F-TGCCATATCTTTCTCATGTTCG R-TGCCCCACAAATTTCAGAAC	4	6	4	4	4	4	4	30	180-500	2	1	1	50	50	0.07	0.5	0.03	0.14
	TOTAL								208		33	31	2	725	75	2.02	29.75	6.23	7.49
	AVERAGE								26		4.16	3.88	0.25	90.62	9.37	0.25	3.72	0.78	0.94

%MA=Percentage monomorphism, %PA=Percentage, NPL=Number of polymorphic loci, MI=Marker index, NML=Number of monomorphic loci,polymorphism, PIC=Polymorphism information content, Effective multiplex ratio, RP=Resolution power. CS= *Coccinella septempunctata*, MS=*Cheilomenes sexmaculata*, CT= *Coccinella transversalis*, PQ= *Propylea dissecta*, HD= *Harmonia dimidita* (Black), HY= *Harmonia dimidita* (Spotted), MD= *Micraspis discolor*

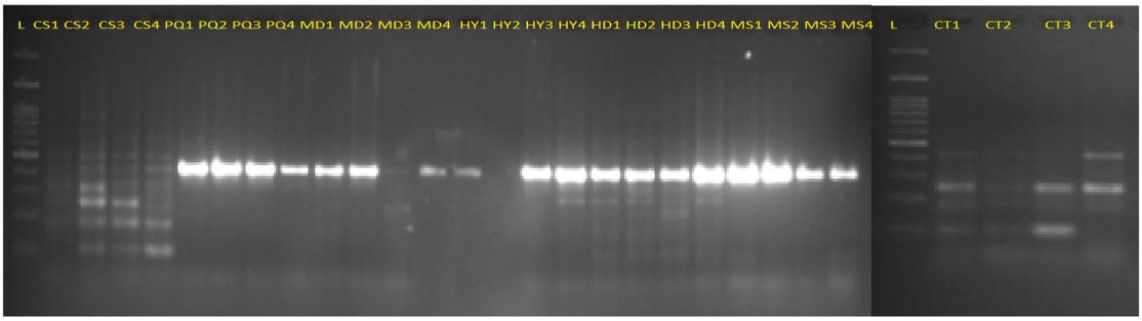


Plate 1: Amplification profile of different species of cocinellid with primer 1 (P1)

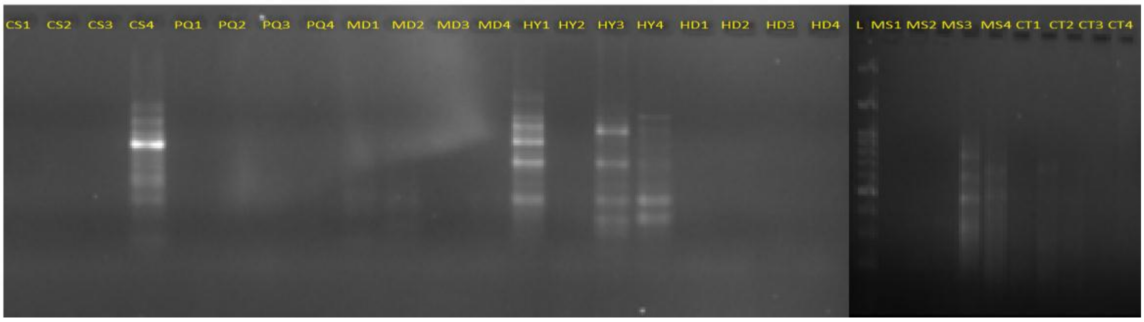


Plate 2: Amplification profile of different species of cocinellid with primer 2 (P2)

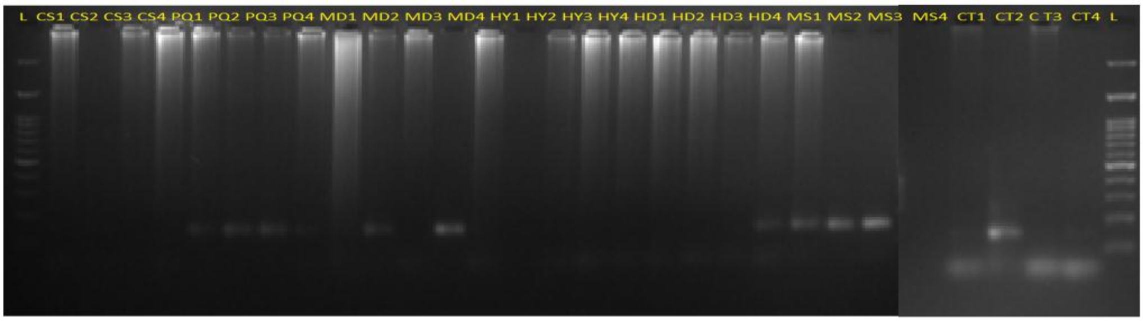


Plate 3: Amplification profile of different species of cocinellid with primer 3 (P3)

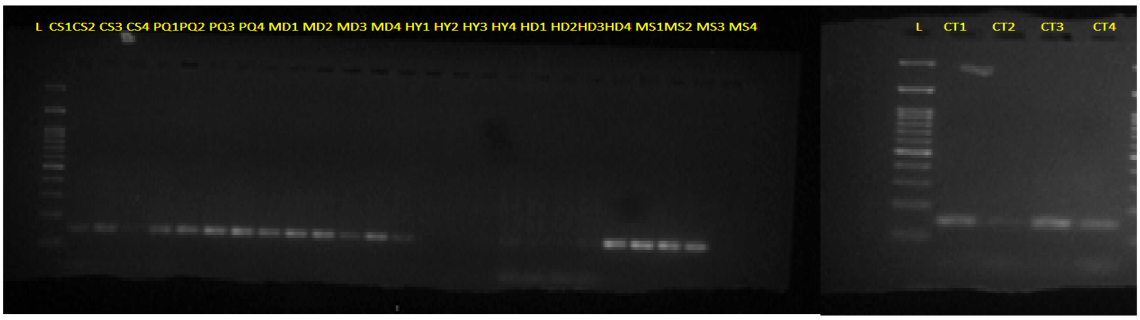


Plate 4: Amplification profile of different species of cocinellid with primer 4 (P4)

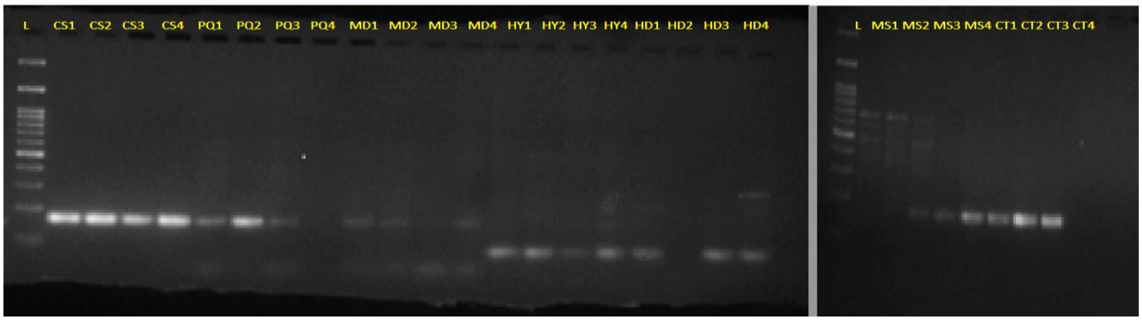


Plate 5: Amplification profile of different species of cocinellid with primer 5 (P5)

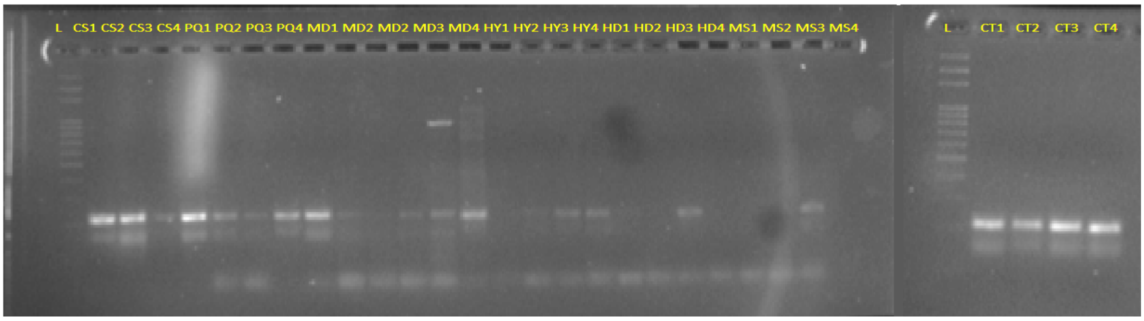


Plate 6: Amplification profile of different species of cocinellid with primer 6 (P6)

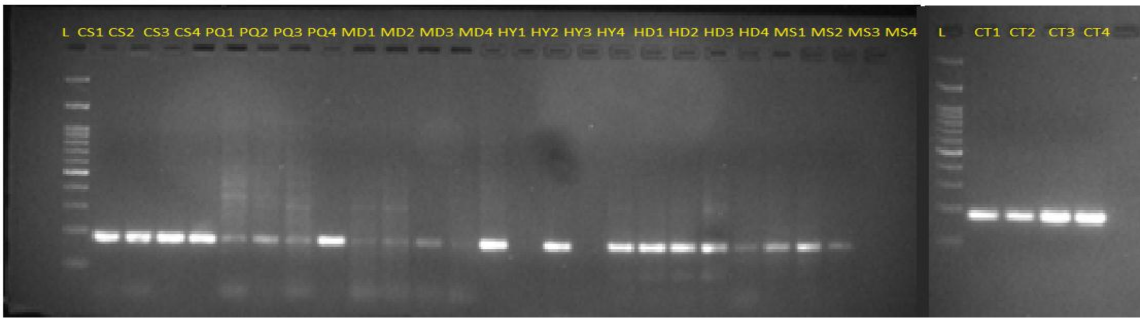


Plate 7: Amplification profile of different species of cocinellid with primer 7 (P7)

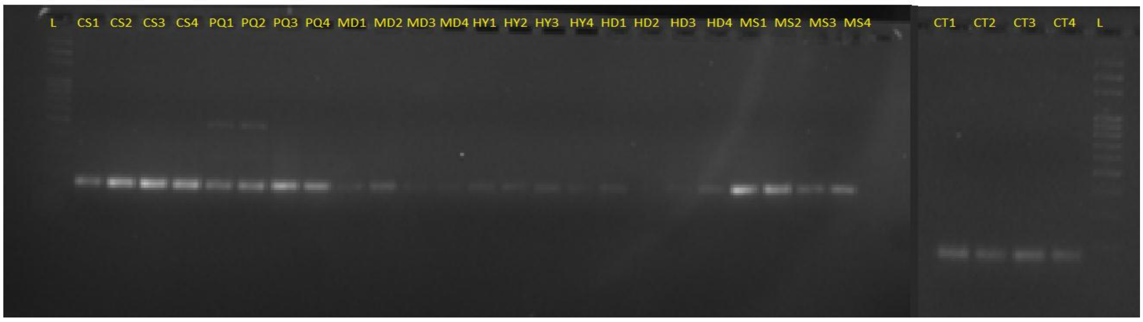


Plate 8: Amplification profile of different species of cocinellid with primer 8 (P8)

The primer P6 showed amplifications with all coccinellid species. The total number of amplicons was 27. Maximum 8 amplicons were found in samples of *C. septumpuctata.* Total 2 amplified loci were polymorphic in the range of 220bp to1000 bp. The percent polymorphism was 100%. The PIC value, resolving power and marker index of the primer P6 was found 0.20 0.5 and 0.41, respectively.

The primer P7 showed amplifications with all coccinellid. The total number of amplicons was 34. Maximum 7 amplicons were found in samples of *P. dissecta* and *H. dimidiata* (Black). Out of total 4 amplified loci, 3 loci were polymorphic. Range of amplicons was 180-450. The percent polymorphism was 75 %. The PIC value, resolving power and marker index of the primer P7 was found 0.10, 0.43 and 0.22, respectively.

The primer P8 showed amplifications with all coccinellid species. The total number of amplicons was 30. Maximum 6 amplicons were found in samples of *P. dissecta*. Out of total 2 amplified loci, 1 locus was polymorphic. Range of amplicons was 180-500. The percent polymorphism was 50%. The PIC value, resolving power and marker index of the primer P7 was found 0.07, 0.14 and 0.03, respectively.

4.1.2Amplification of primers

28 individuals of 6 different species and one varient of predatory coccinellidae from diverse crop ecosystem of Pantnagar were analysed with 8 primers of cytochrome oxidase gene. Which were able to generate scorable banding pattern **(Table 4.4).** The numbers of monomorphic and polymorphic loci were varied from primer to primer with respect to species wise. Out of 33 number of loci, 31 loci were polymorphic with 38.80% polymorphism while 2 loci were monomorphic with 25.00% monomorphism. The maximum 8 loci amplified by primer 1 and 5 and minimum 1 loci amplified by primer 4. The lowest percent polymorphism 50% detected in primer 8 while rest primer 1,2,3,4,5 and 6 showed maximum 100% percent polymorphism.

The value of average polymorphism information content (PIC) which depends upon the frequency of loci was found 0.25 for all 8 primers. The maximum value 0.80 of PIC was observed in primer 4 while minimum value 0.07 was observed in primer 8. The Effective Multiplex Ratio (EMR) value was ranged from 0.5 to 8.00 and it depends upon the number and types of loci. The Marker Index (MI) of primers in present study ranged from 0.03 to 2.19. The value of Marker Index, Resolving Power (RP) and Effective Multiplex Ratio was found maximum in case of primer 1. This primer also showed maximum number of amplicon at 8 different loci with 100% polymorphism. Due to these characteristics this primer is best primers among all the eight primers as it is able to distinguish the variation between different species of coccinellid beetles. Other primer like P2, P3, P4, P5 and P6 also having 100% polymorphism but they all are having lower value of MI and RP.

4.1.3 Analysis of gene cluster

28 individuals of coccinellidae generated a unine dendogram profile (**figure 2**) from 8 different primer of CO gene based on UPGMA, Jaccard Similarity Cofficent. Dendogram revealed that all coccinellidae species sharing quite similar pattern in CO gene with 35.00% of similarity cofficent, plotted in main cluster C which further subfragmented into subcluster C1 and C2. Cluster C1 containg only two species individuals *C. septempunctata* (CS) and *C. transversalis* (CT) with 50.8% genetic similarity into subfragmented cluster C1A and C1B. In subcluster C1A one individual of *C. septempunctata* (CS1) has 58.3% genetic similarity with rest all individuals of *C. transversalis* (CT) (1-4) in their CO gene while sub cluster C1B showed 71.0% similarity in rest three individuals of *C septempunctata* (CS) (2,3 and 4). *C. septempunctata* (CS1) having more similarity with *C. transversalis* (CT)(1-4) that mean its CO region has some variation which not physically appeared.

On the other hand cluster C2 formed a major cluster by including individuals of 5 different species of predatory coccinellids *P. dissecta* (PQ), *M. discolor* (MD), *C. sexmaculata* (MS), *H. dimidita* (Spotted) (HY) and *H. dimidita* (Black)(HD) respectively with 38.60% genetic similarity with in them. Cluster C2 further sub divided into C2A and C2B. The C2A represented mixed population relation in *P. dissecta* (PQ)(1-4), *C. sexmaculata* (MS)(1-4), *H. dimidita* (Black) (HD)(1-4) with two individuals of *M. discolor* (MD) (1 and 2) and one individula of *H. dimidita* (Spotted) (HY4) through subdivided into C2Aa and C2Ab. C2Aa showed 45.00% similarity in the all individuals of *P. dissecta* (PQ), *C. sexmaculata* (MS) and two individual of *M. discolor* (MD)(1 and 2) that mean they were closely related to each other by sharing smilar genetic pattern in CO gene. MS2 and MS3 had highest 83.00% similarty in their CO region. While C2Ab showed corelation between 4 individuals of *H. dimidita* (Black) (HD) (1-4) and one individual of *H. dimidita* (Spotted) (HY4) with 54.60% genetic smilarity. But rest *H. dimidita* (Spotted) (HY) (1,2 and 3) placed separately in C2B with two individual of *M. discolor* (MD) (3 and

4) with 42.8% genetic similarity. Population of different species in dendrogram represented that avilable primers were clearly distinguished the difference in CO gene species wise through correlating them to each other into cluster but intermixed separation like *M. discolor* (MD)(1 and 2) and HY4 indicated that there were some chnages in their mitochondrial DNA pattern that may be due to mutation in CO gene. Similar pattern of genetic variability showed by PCA plot **(figure 2)** in form of scatter plot where 28 individuals were sharing mixed plot area in bidimentional plot that representing even they all predatory coccinellidae but they have difference in their CO region according to species wise but with some similarity in the CO gene by overlapping at some area in PCA plot. The cumulative percentage of Eigen value generated was 13.7% in first dimension on other hand negative Eigen value had 16.8% in second dimension at reversed direction.

Among all of eight different primers of CO gene the primer 1 (F-AGCACCAATTTCTTCAATTTT, R-TGTAAATATGTGATGTGCTC) was act as suitable marker to distinguished the genetic variability present in CO region of coccinellid. Similar type of result was observed by **Sayed (2016)** during study the molecular diversity of Lady Beetles in Saudi Arabia where CO1 primer used against *C. undecimpuncata* and *H. variegata* to differentiate the variation between them. **Halim *et al.* (2017)** also found the CO region where being a source of identification between barcoding of eight ladybugs species from Peninsular Malaysia**. Kaur and Sharma (2017)** also use the CO region based barcoding for Pentatomidae species from North India, that mean CO region of mitochondrial gene were able to give suitable genetic variation because the Mitochondrial DNA have relevance to environmental adaption and migration events that may affect the genetic diversity (**Roderick and Navajas, 2003)** and the understanding about genetic variation in population within or between species which played a crucial role in substantially of biological control program and influence of genetic processes **(Kavar *et al.*, 2006).** Among the 6 species presently two species *C. septempunctata* (CS) and *C. transversalis* (CT) having very close evolutionary relationship (on the basis of UPGMA) however these two did not share many similarity yet they shared a clade in dendrogram. The *H. dimidita* (Black)(HD) and *H. dimidita* (Spotted) (HY) which are variant to each other (**poorani and booth, 2016)** but our present investigation resulted that the CO region of both having some variability which showing their evolutionary variable relationship (on the basis of UPGMA).

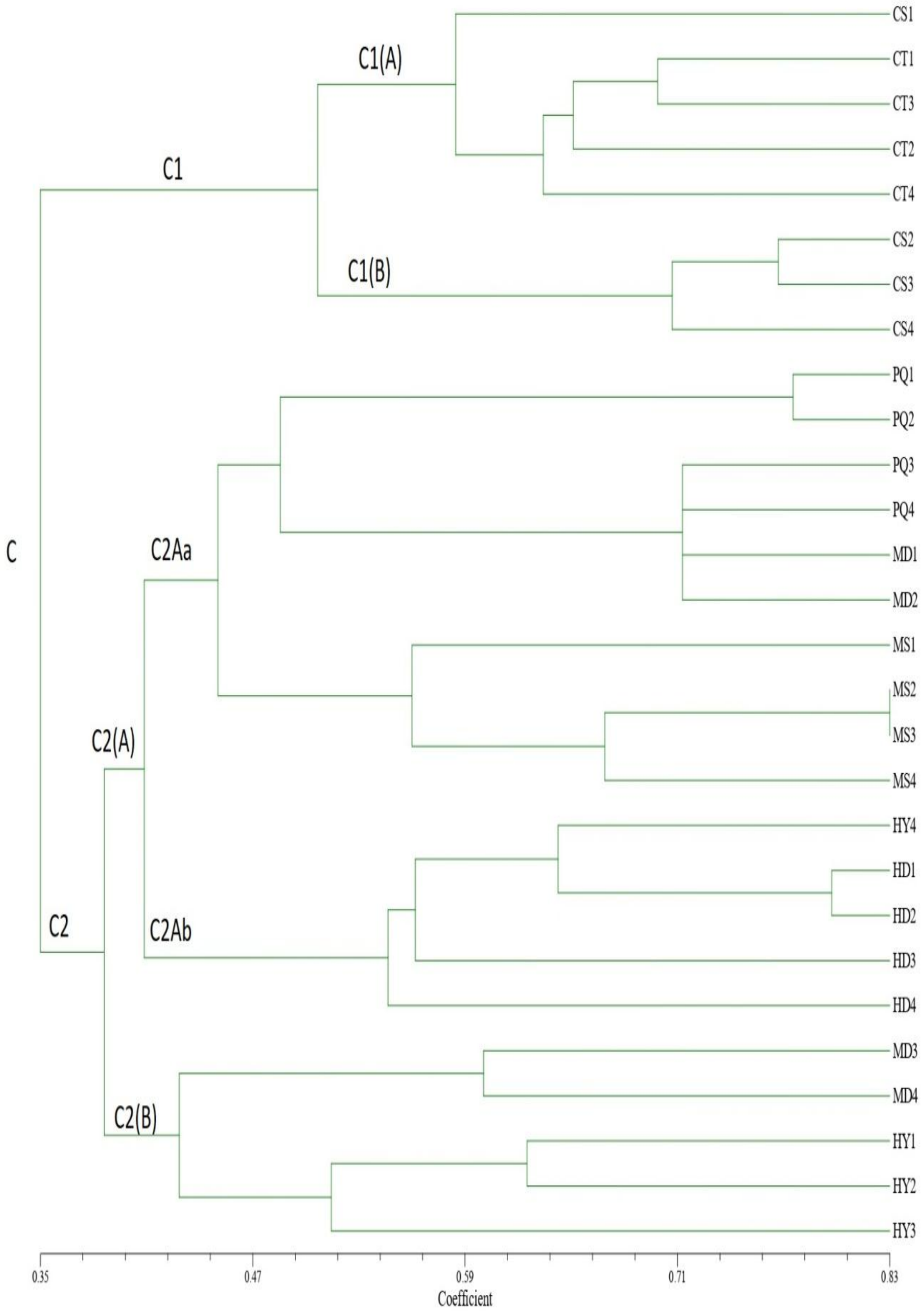


Figure 1: UPGMA dendrogram profile of different individual of coccinellids constructed using NTSYSpc 2.1a.

CS= *Coccinella septempunctata*, MS=*Cheilomenes sexmaculata*, CT= *Coccinella transversalis*, PQ= *Propylea dissecta*, HD= *Harmonia dimidita (Black), HY= Harmonia dimidita (Spotted)*, MD=*Micraspis discolor*

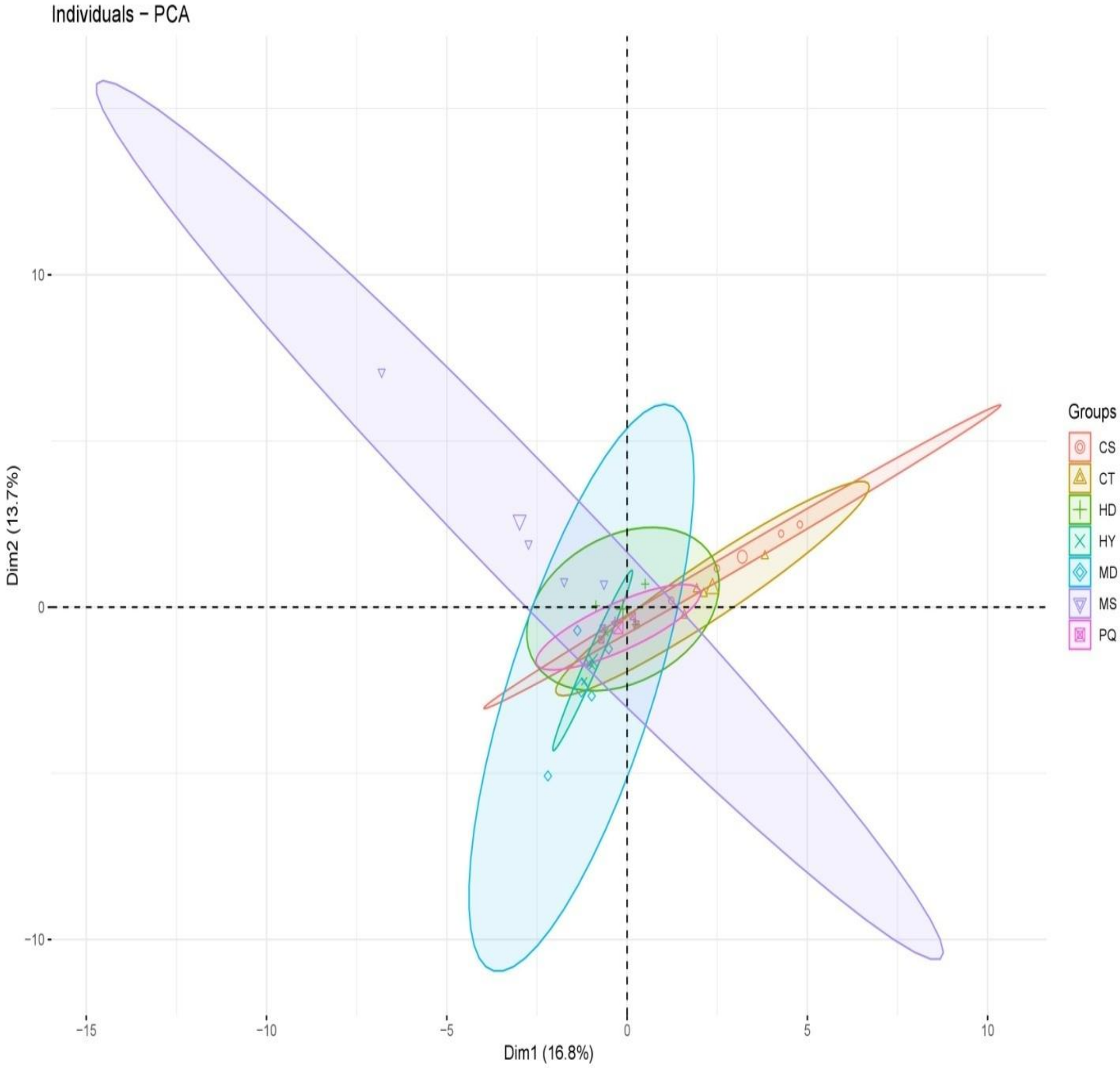


Figure 2: Bi-dimensional scatter plot for pair wise comparison analysis of different coccinellid beetles through R software.

CS= *Coccinella septempunctata*, MS=*Cheilomenes sexmaculata*, CT= *Coccinella transversalis*, PQ= *Propylea dissecta*, HD= *Harmonia dimidita (Black), HY= Harmonia dimidita (Spotted)*, MD=*Micraspis discolor*

4.3 Seasonal abundance of coccinellid beetles in different crop ecosystems of Pantnagar

The observations were taken on incidence of fifteen coccinellid beetles as number of insects per meter square area. The weekly observation on the occurrence of predatory coccinellid beetles was started from 18th standard week onward till 17th standard week. Recorded values are presented in **Table 4.6**. The values of correlation coefficient between weather parameters and cocinellid population is indicated in **Table 4.5**.

The weekly fluctuation of coccienllid beetles population is depicted in **Figure 3 to 7**. The seven spotted lady bird beetle (*C. septempunctata*) showed their presence throughout the year but their maximum population was recorded during the 13th standard week (SW). Similar type of findings was observed by **Soni *et al.* (2013)** who reported maximum population (17.6/m^2) of *C. septempunctata* during the first week of March. Simple correlation coefficient was find out with weather parameter and a highly significant negative correlation was found with maximum temperature (r=-0.653**) and rainfall (r=-0.468**). A highly significant positive correlation was found with minimum temperature (r=0.692**) and maximum relative humidity (r=0.451**) whereas, non significant positive correlation was found with minimum relative humidity (r=0.193).

C. sexmaculata also showed their dominance throughout the year and their maximum (18.3/ m^2) was recorded during the 42nd SW. **Karane *et al.* (2019)** reported the peak population of *C.sexmaculata* during the 47th and 48th standard weeks. Simple correlation coefficient with weather parameter revealed a highly significant negative correlation with maximum (r=0.620**) and minimum (r=-0.396**) temperature. A highly significant positive correlation was observed in case of maximum (r=0.669**) and minimum (r=0.506**) relative humidity whereas, a non significant negative correlation was found with rainfall (r=-0.192).

The population of *H. dimidiata* was observed for the first time on 31st standard week and remains active up to the 12th standard week. Peak population (13.3/ m^2) of *H. dimidiate* was observed during the 45th standard week. **Khan *et al*. (2009)** reported that the population of *H. dimidiate* in tropical region was highest during August-September. Analysis of correlation revealed that ladybird showed highly significant negative correlation with maximum (r=-0.487**) and minimum temperature (r=-0.416**). A highly significant positive correlation was recorded with maximum (r=0.548**) and minimum relative humidity (r=0.338*) whereas, non significant negative correlation was observed with rainfall (r=-0.202).

C. transversalis showed their activity throughout the year and their peak population (14.3/ m^2) was recorded during 1st standard week. This finding is close conformity with the finding of **Chakraborty and Korat (2014)** who also observed that the peak population of *C. transversalis* was from last week of December to first week of January. Lowest activity of these beetles was observed during 30th and 31st standard week. Analysis of correlation revealed that *C. transversalis* showed a highly significant negative correlation with maximum temperature (r=-0.455**), minimum temperature (r=-0.770**) and rainfall (r=-0.659**). A non significant positive correlation was found with maximum relative humidity (r=0.102) whereas, a non significant negative correlation was recorded with minimum relative humidity (r=-0.156).

P. dissecta was started exhibiting their activity during 31st standard week and remain active till 17th standard week with maximum population (16.7/ m^2) during 48th standard week. Simple correlation coefficient with different weather parameter showed a highly significant negative correlation with maximum temperature (r=-0.677**), minimum temperature (r=-0.666**) and rainfall (r=-0.330*). A highly significant positive correlation was found with maximum relative humidity (r=0.611**) and a significant positive correlation was recorded with minimum relative humidity (r=0.331*).

The initial incidence of *M. discolor* was recorded during 27 standard week and the population of these beetles gradually increased up to 45th standard week. These beetles remain inactive during the 13th to 27th standard week. The correlation study revealed that *M. discolor* showed highly significant positive correlation with maximum relative humidity (r=0.536**) and minimum relative humidity (r=0.395**). A non significant negative correlation was observed with minimum temperature (r=-0.252) and average rainfall (r=-0.004) whereas, a highly significant negative correlation was observed with maximum temperature(r=-0.425**).

The initial incidence of *M. allardi* was observed during 29th standard week and the highest population (13.3/ m^2) of these beetles was recorded during the 52nd standard week. Simple correlation coefficient with weather parameter revealed highly significant positive correlation with maximum (r=0.625**) and minimum relative humidity (r=0.385**). A highly significant negative correlation was observed with maximum (r=-0.651**) and minimum temperature (r=-0.545**) whereas, non significant negative correlation was observed with average rainfall (r=-0.137).

The population of *R. fumida* was appeared first time during 28^{th} standard week. Peak population (12.2/ m^2) of these beetles was observed during 43^{rd} standard week while minimum activity was recorded during 3^{rd} standard week. Analysis of correlation revealed that *R. fumida* showed highly significant positive correlation with maximum (r=0.397**) and minimum relative humidity (r=0.389**). A non significant positive correlation was found with minimum temperature (r=0.066) and average rainfall (r=0.028) whereas, a non significant negative correlation was found with maximum temperature (r=-0.169).

B. suturalis showed their presence throughout the year and their maximum population (7.8/ m^2) was recorded during the 42^{nd} standard week. Their minimum population was recorded during the 22^{nd} and 23^{rd} standard week. Analysis of correlation revealed a highly significant positive correlation with maximum (r=0.366**) and minimum relative humidity (r=0.416**). A non significant positive correlation was found with minimum temperature (r=0.170) and average rainfall (r=0.136) whereas, non significant negative correlation was observed with maximum temperature (r=-0.136).

A. cardoni started exhibiting their activity during 29^{th} standard week and persisted up to 20^{th} standard week. Maximum population (9.2/ m^2) was recorded during 36^{th} standard week. Study of correlation coefficient revealed a highly significant positive correlation with maximum (r=0.448**) and minimum (r=0.541**) relative humidity. A significant negative correlation was observed with maximum temperature (r=-0.276*) whereas, a non significant positive correlation was found with minimum temperature (r=0.229) and average rainfall (r=0.225).

The initial incidence of *M. univittata* was observed during 29^{th} standard week. Peak population (6.3/ m^2) of these beetles was recorded during 36^{th} standard week. Study of simple correlation coefficient revealed the highly significant positive correlation with minimum (r=0.554**) temperature, minimum relative humidity (r=0.500**), and rainfall (r=0.444**). A non significant positive correlation was found with maximum relative humidity (r=0.312*) whereas, a non significant positive correlation was found with maximum temperature (r=0.025).

H. variegate which is also known as variegated ladybug appeared first during 23^{rd} standard week. A gradual increase in the number of beetles was observed up to the 8^{th} standard week. Maximum population (14.4/ m^2) of these beetles was recorded during 42^{nd} standard week. Analysis of correlation coefficient with weather data revealed a highly significant positive correlation with maximum (r=0.521**) and minimum relative humidity (r=0.569**). A significant positive correlation was observed with the average rainfall (r=0.320*) whereas a non significant negative and positive correlation was found with maximum (r=-0.233) and minimum temperature(r=0.219), respectively.

Illeis sp1 started exhibiting their activity during 40th standard week and remain active till 15th standard week. Highest population (7.9/ m^2) was observed during 1st standard week. Simple correlation coefficient with different weather parameter showed a highly significant negative correlation with maximum temperature (r=-0.745**), minimum temperature (r=-0.867**) and rainfall (r=-0.362**). A highly significant positive correlation was found with maximum relative humidity (r=0.505**) and a non significant positive correlation was recorded with minimum relative humidity (r=0.165).

Illeis sp2 first appeared during 42nd standard week followed by a gradual increase and maximum population (7.1/ m^2) during 1st standard week and the population remained up to 13th standard week. Analysis of correlation coefficient with weather data showed a highly significant negative correlation with maximum temperature(r=-0.742**), minimum temperature (r=-0.841**) and rainfall (r=-0.327*). A highly significant positive correlation was found with maximum relative humidity (r=0.478**) and a non significant positive correlation was recorded with minimum relative humidity (r=0.150).

Population of *H. sedecimnotata* was observed for the first time on 34th standard week and remains active up to the 51st standard week. Peak population (6.9/ m^2) of *H. sedecimnotata* was observed during the 43rd standard week. Simple correlation coefficient with weather parameters revealed non-significant positive correlation with minimum temperature (r=0.013), maximum (r=0.235) and minimum relative humidity (r=0.209) whereas non significant negative correlation was found with maximum temperature (r=-0.061) and average rainfall (r=-0.111).

Table 4.5: Correlation of different species of coccinellid with weather parameters.

S.No	Coccinella species	Correlation with different weather parameters				
		Temp. (ºC) (Max)	Temp. (ºC) (Min)	RH (%) (Max)	RH (%) (Min)	Rainfall (mm)
1.	*Coccinella septempunctata*	-0.653**	0.692**	0.451**	0.193^{ns}	-0.468**
2.	*Cheilomenes sexmaculata*	-0.620**	-0.396**	0.669**	0.506**	-0.192^{ns}
3.	*Harmonia dimidiate*	-0.487**	-0.416**	0.548**	0.338*	-0.202^{ns}
4.	*Coccinella transversalis*	-0.455**	-0.770**	0.102^{ns}	-0.156^{ns}	-0.659**
5.	*Propylea dissecta*	-0.677**	-0.666**	0.611**	0.331*	-0.330*
6.	*Micraspis discolor*	-0.425**	-0.252^{ns}	0.536**	0.395**	-0.004^{ns}
7.	*Micraspis allardi*	-0.651**	-0.545**	0.625**	0.385**	-0.137^{ns}
8.	*R. fumida*	-0.169^{ns}	0.066^{ns}	0.397**	0.389**	0.028^{ns}
9.	*Brumoides suturalis*	-0.136^{ns}	0.170^{ns}	0.366**	0.416**	0.136^{ns}
10.	*Anegleis cardoni*	-0.276*	0.229^{ns}	0.448**	0.541**	0.225^{ns}
11.	*Micraspis univittata*	0.025^{ns}	0.554**	0.312*	0.500**	0.444**
12.	*Hippodamia variegate*	-0.233^{ns}	0.219^{ns}	0.521**	0.569**	0.320*
13.	*Illeis species1 (yellow)*	-0.745**	-0.867**	0.505**	0.165^{ns}	-0.362**
14.	*Illeis species2 (white).*	-0.742**	-0.841**	0.478**	0.150^{ns}	-0.327*
15.	*Harmonia sedecimnotata*	-0.061^{ns}	0.013^{ns}	0.235^{ns}	0.209^{ns}	-0.111^{ns}

Table 4.6: Seasonal occurrence of coccienllid beetles in different crops at Pantnagar.

S.W.	*C. septempunctata*	*C. sexmaculata*	*H. dimidiata*	*C. transversalis*	*P. dissecta*	*M. discolor*	*M. allardi*	*R.fumida*	*B. suturalis*	*A.cardoni*	*M. univittata*	*H. variegate*	*Illeis* sp1 *(y)*	*Illeis* sp2	*H.sedecimnotata*
01	9.4*	11.3	6.7	14.3	14.4	5.6	9.3	1.9	2.6	0.5	0.0	3.3	7.9	7.1	0.0
02	11.2	12.3	4.4	10.7	6.7	8.9	8.6	1.5	2.5	0.9	0.0	4.4	5.3	6.2	0.0
03	11.9	10.7	5.6	11.3	6.3	5.6	7.2	0.8	1.1	1.6	0.0	3.9	4.3	6.8	0.0
04	13.3	10.3	3.3	12.3	7.8	3.3	6.3	0.9	1.0	2.5	0.0	5.6	3.9	6.3	0.0
05	15.6	9.8	2.2	11.7	9.2	2.6	5.9	0.0	0.8	4.2	0.0	2.2	4.8	4.3	0.0
06	11.3	9.6	2.9	13.2	7.8	1.1	4.4	0.0	1.1	7.8	0.0	1.3	3.7	4.9	0.0
07	12.1	9.3	2.7	12.2	6.7	0.8	3.3	0.0	0.0	4.4	0.0	0.9	3.2	3.2	0.0
08	9.8	8.6	1.1	11.2	7.8	0.5	2.1	0.0	1.1	5.6	0.0	0.5	2.9	2.4	0.0
09	14.5	9.3	1.9	11.6	4.4	0.6	0.0	0.0	0.0	4.4	0.0	0.0	2.2	1.6	0.0
10	13.2	10.1	2.2	10.3	5.5	0.9	1.9	0.0	0.8	3.9	0.0	0.0	1.9	2.2	0.0
11	11.7	9.6	0.0	11.7	4.4	0.5	1.1	0.0	0.9	3.2	0.0	0.0	2.9	1.3	0.0
12	14.4	8.6	0.5	10.7	5.6	0.4	0.8	0.0	1.5	3.0	0.0	0.0	1.9	2.4	0.0
13	17.6	7.3	0.0	13.2	7.8	0.0	0.0	0.0	2.3	2.8	0.0	0.0	1.2	2.5	0.0
14	15.6	7	0.0	14.1	5.3	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.9	0.0	0.0
15	13.2	8.2	0.0	13.9	4.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.9	0.0	0.0
16	9.3	7.2	0.0	11.7	4.6	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
17	6.2	6.7	0.0	13	4.4	0.0	0.0	0.0	1.3	0.4	0.0	0.0	0.0	0.0	0.0
18	5.6	3.7	0.0	9.3	0.0	0.0	0.0	0.0	0.8	0.9	0.0	0.0	0.0	0.0	0.0
19	4.3	2.1	0.0	8.3	0.0	0.0	0.0	0.0	1.9	0.5	0.0	0.0	0.0	0.0	0.0
20	3.9	4	0.0	7	0.0	0.0	0.0	0.0	1.3	0.5	0.0	0.0	0.0	0.0	0.0
21	2.7	5.2	0.0	6.5	0.0	0.0	0.0	0.0	2.3	0.0	0.0	0.0	0.0	0.0	0.0
22	2.6	4.2	0.0	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23	1.9	3.3	0.0	6	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.5	0.0	0.0	0.0
24	1	2.1	0.0	4.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0
25	0	1.2	0.0	4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0
26	0	0	0.0	3.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
27	0.8	0	0.0	1.3	0.0	0.8	0.0	0.0	1.4	0.0	0.0	3.2	0.0	0.0	0.0
28	1.2	2.5	0.0	1.5	0.0	1.1	0.0	0.8	1.9	0.0	0.0	4.9	0.0	0.0	0.0

29	0.6	4	0.0	1.9	0.0	2.5	2.3	0.9	0.0	0.5	0.8	5.9	0.0	0.0	0.0
30	0.4	5.6	0.0	0	0.0	3.4	3.9	1.1	2.2	1.6	1.6	6.2	0.0	0.0	0.0
31	0.6	7.4	2.2	0	2.2	3.9	2.5	2.8	1.8	2.3	2.3	7.8	0.0	0.0	0.0
32	2.4	8.2	1.1	1.7	4.4	4.9	4.9	3.7	2.7	3.9	3.9	8.9	0.0	0.0	0.0
33	2.8	9.4	2.2	1.3	1.1	3.2	3.8	4.2	0.9	4.8	4.0	8.2	0.0	0.0	0.0
34	3.2	10.2	3.3	1.7	3.3	3.5	2.8	3.8	3.7	5.9	4.6	9.6	0.0	0.0	0.8
35	4.2	11	3.3	2.7	7.8	3.9	4.6	4.4	4.8	8.5	5.1	10.4	0.0	0.0	1.3
36	4.4	12.2	3.3	3.3	2.2	4.2	5.6	5.6	5.2	9.2	6.3	12.2	0.0	0.0	1.9
37	5.6	11.6	6.7	2.3	5.6	2.1	2.2	7.8	4.5	6.2	4.6	11.1	0.0	0.0	2.2
38	7	14.2	4.4	3	6.7	3.6	4.3	8.6	5.6	7.1	4.1	13.3	0.0	0.0	2.9
39	13.6	15.6	6.7	4.7	9.2	7.1	5.5	8.1	5.9	6.2	3.3	14.4	0.0	0.0	2.0
40	8.6	16.2	7.8	5.7	8.9	7.8	7.8	9.1	6.9	4.2	2.3	10.6	0.3	0.0	3.2
41	4.4	17.5	5.6	5	7.9	6.7	6.7	10.0	6.6	3.7	2.2	11.1	0.9	0.0	4.6
42	7.4	18.3	8.9	6.3	11.3	8.6	5.2	11.6	7.8	2.1	3.1	14.4	0.3	0.5	5.1
43	10.3	16.4	10.0	9	10.8	10.0	5.6	12.2	5.3	2.3	2.3	11.1	1.3	0.9	6.9
44	8.2	15.5	10.6	10.3	13.3	11.1	7.8	8.2	3.5	2.0	2.1	8.5	1.9	1.3	4.6
45	10.6	15.3	13.3	8.6	12.2	13.7	8.2	7.9	3.1	1.9	0.8	10.0	2.3	1.9	3.5
46	9.2	15.2	9.3	6.2	11.9	9.5	9.2	6.2	2.9	1.5	0.5	11.1	3.9	2.2	2.4
47	11.5	14.3	11.1	7.6	14.6	7.3	11.1	5.4	2.3	1.2	1.2	9.6	4.1	2.6	1.9
48	10.2	13.3	8.9	8	16.7	6.6	11.6	4.5	0.8	0.5	0.0	8.6	4.5	2.9	0.9
49	8.6	13	6.7	10.7	13.3	4.7	12.1	4.1	0.5	0.0	0.0	6.2	5.2	3.4	0.3
50	12.6	14.6	10.0	9	12.2	3.3	11.6	4.4	1.5	0.0	0.0	5.6	5.6	4.9	0.0
51	11.4	13.7	6.7	13.7	15.6	4.4	12.5	3.3	2.6	0.0	0.0	5.3	6.7	5.3	0.6
52	12.2	12.9	5.6	14	16.2	5.9	13.3	2.1	3.5	0.0	0.0	4.6	7.5	6.0	0.0

SW= Standard week

*values are per meter square of area

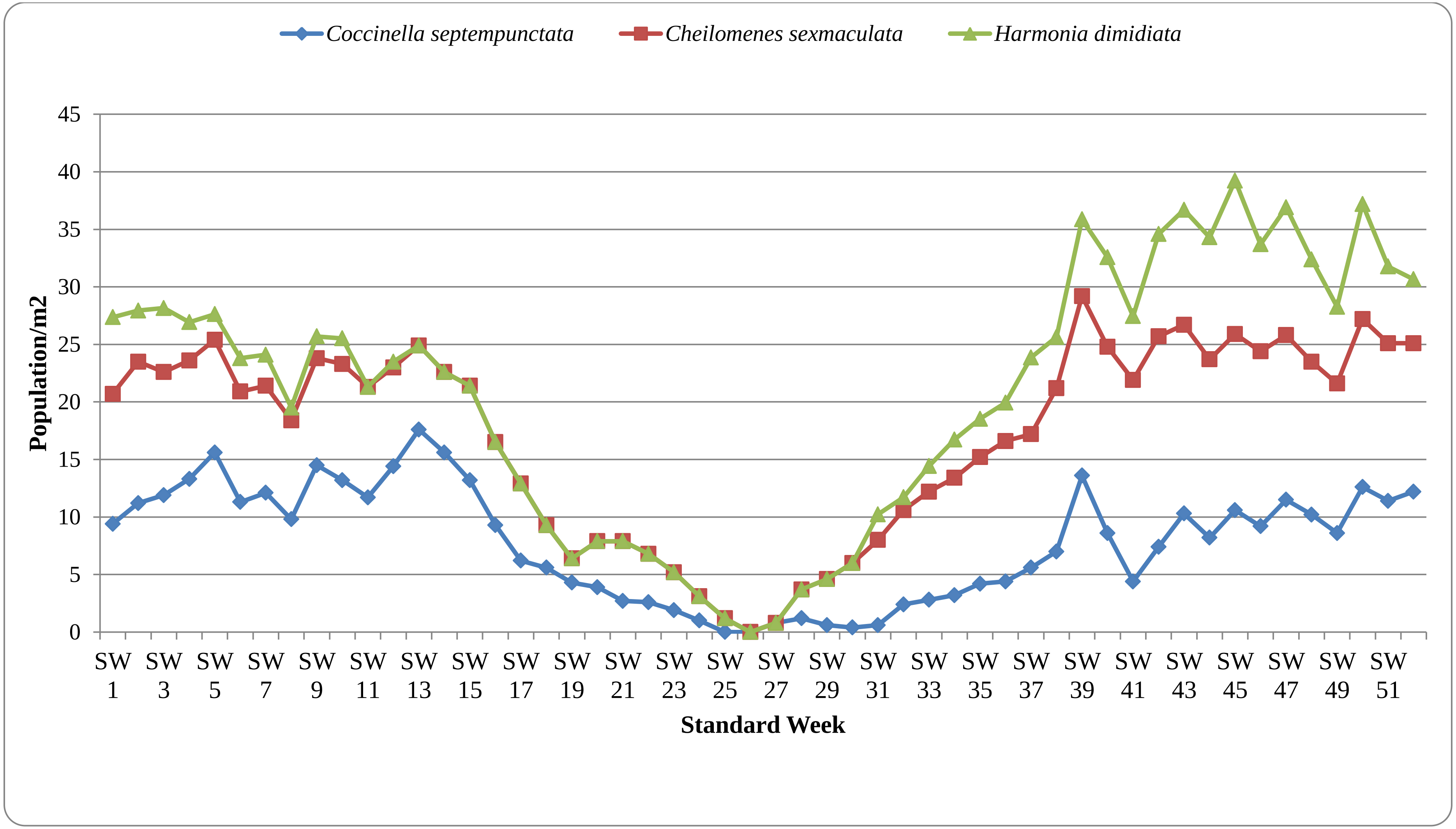


Fig 3: Population dynamics of *C. septempunctata, C. sexmaculata* and *H. dimidiata* during 2018-19

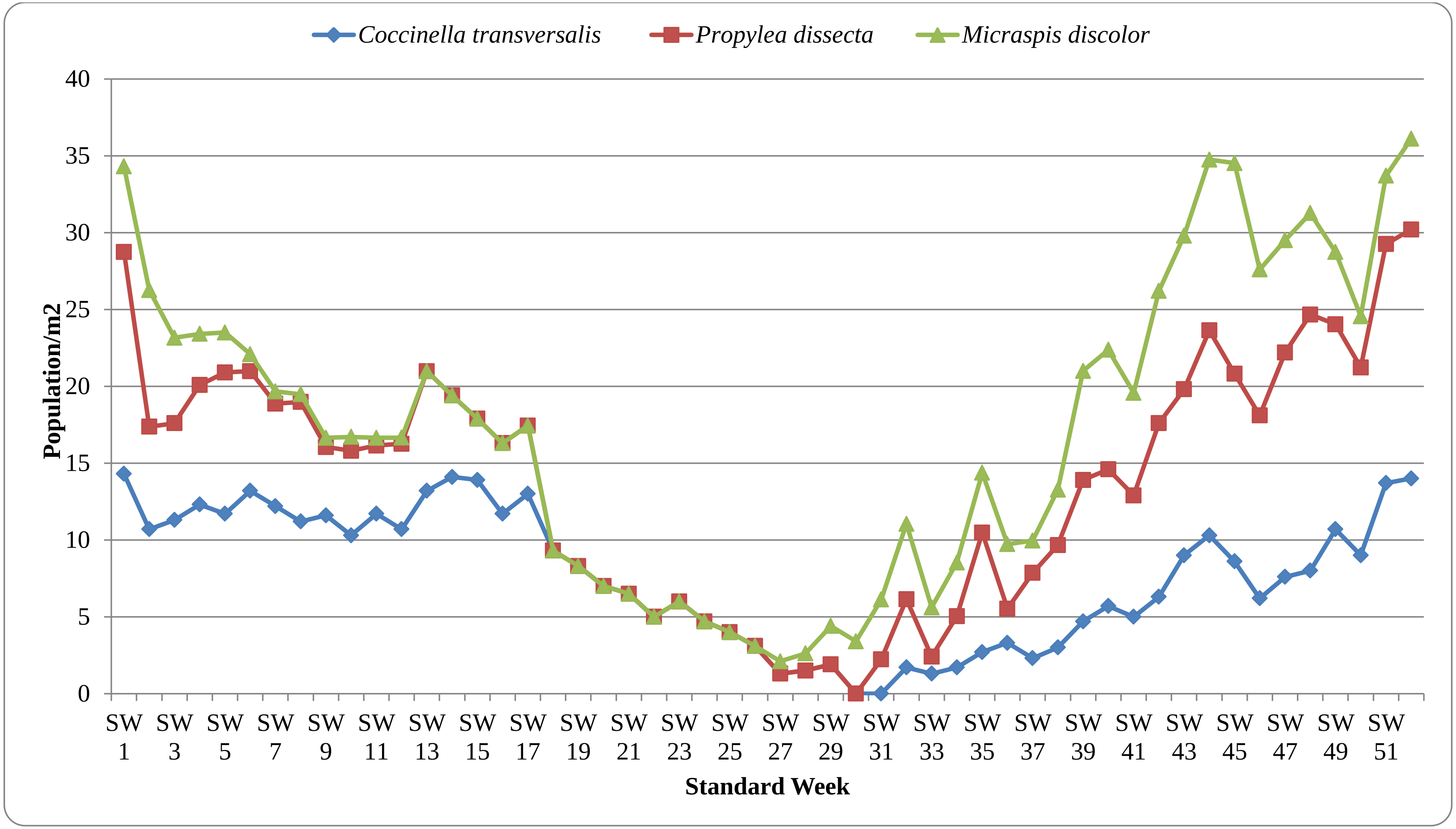


Fig 4: Population dynamics of *C. transversalis*, *P. dissecta* and *M. discolor* during 2018-19

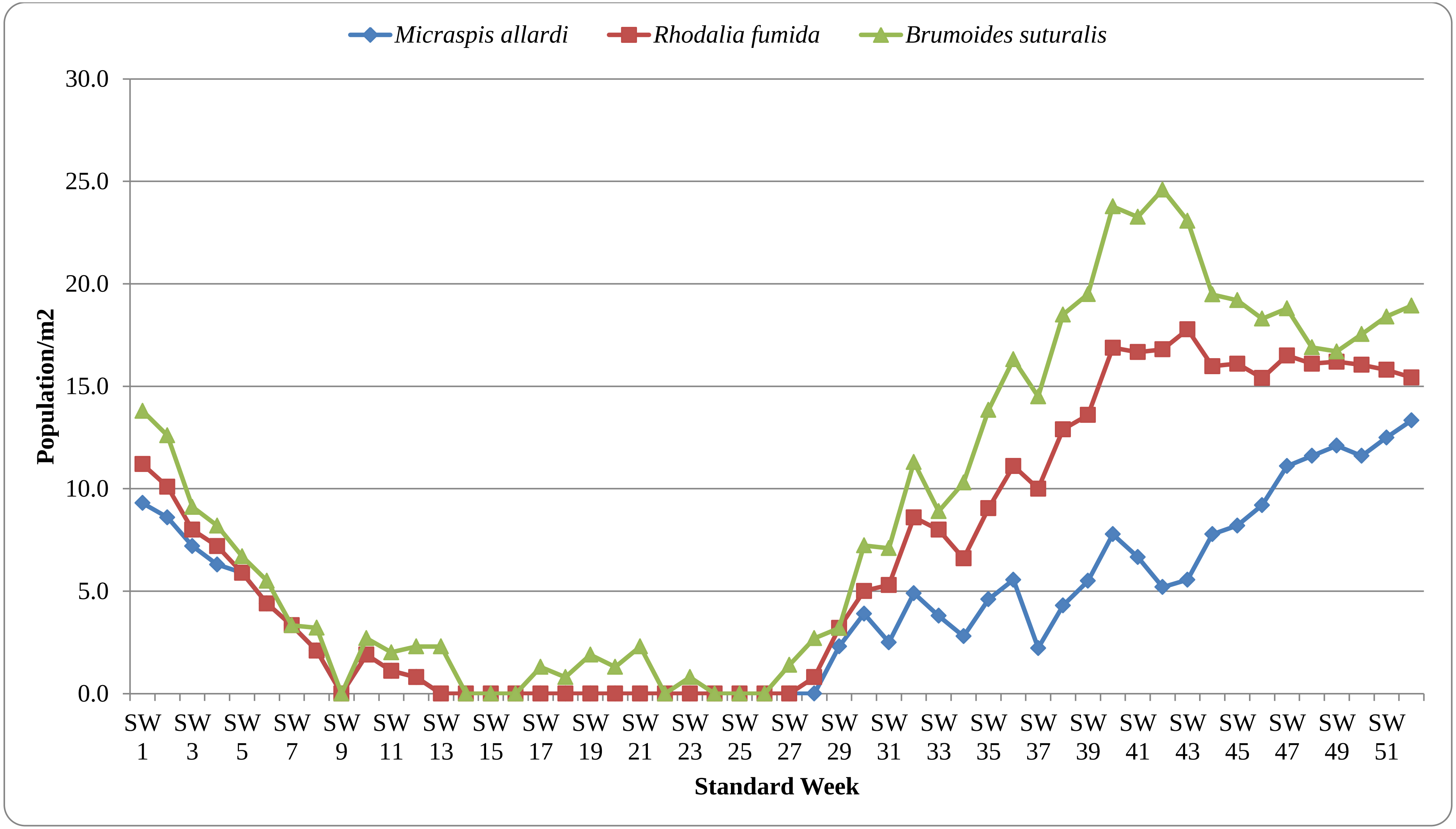


Fig 5: Population dynamics of *M.allardi, R. fumida* and *B. suturalis* during 2018-19

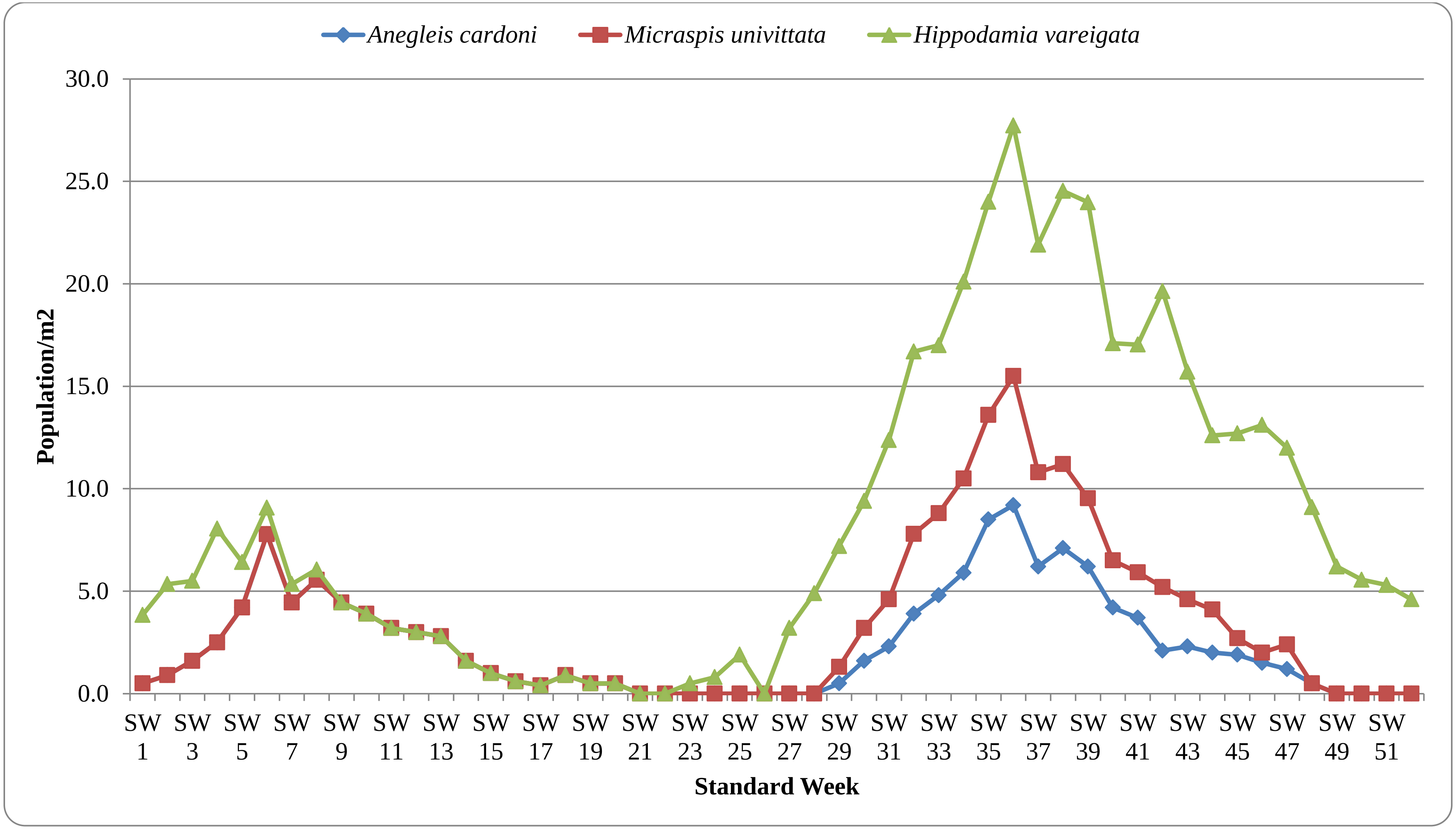


Fig 6: Population dynamics of *A. cardoni, M. univittata* and *H. variegate* during 2018-19

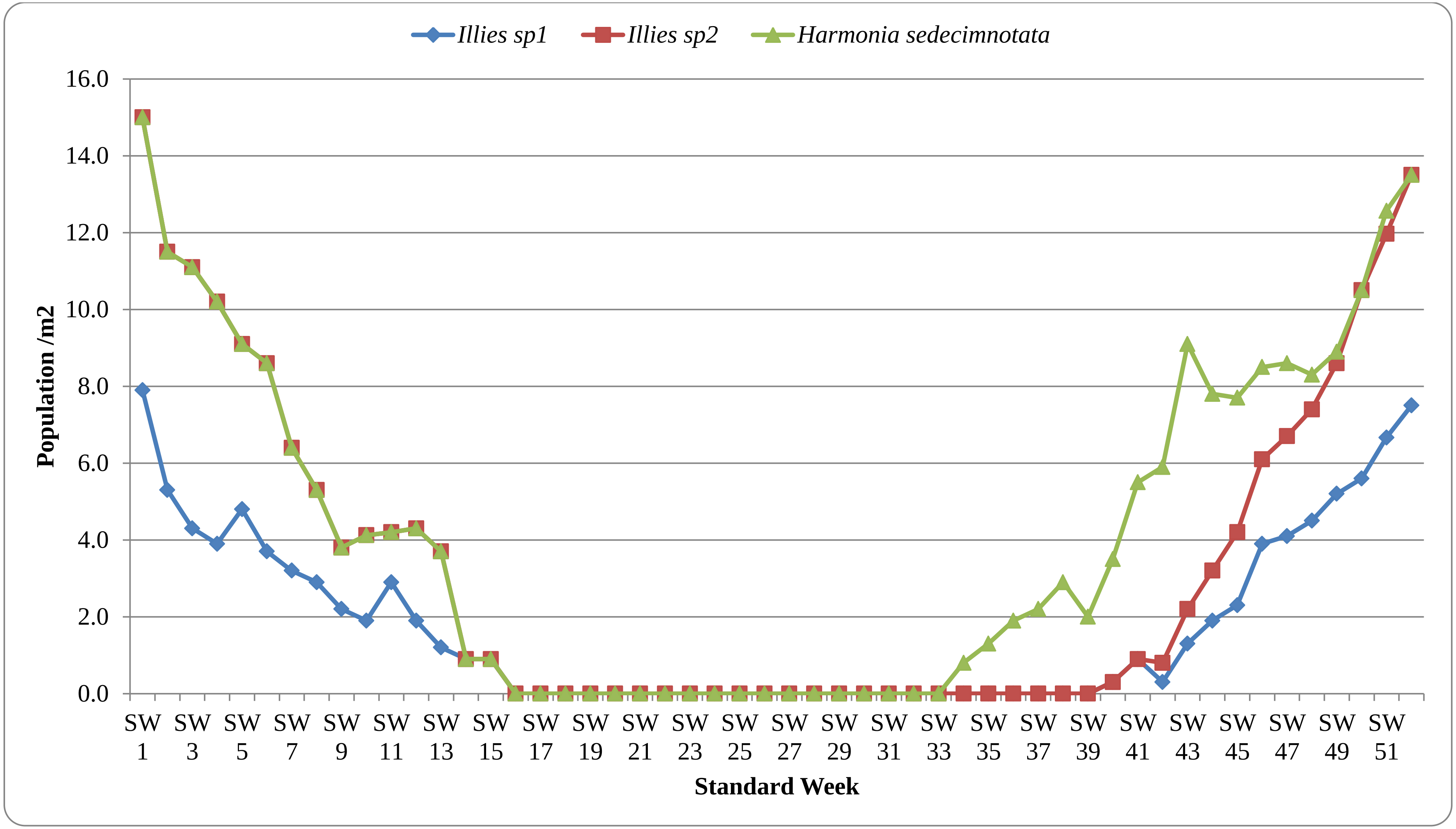


Fig 7: Population dynamics of *Illeis* sp1, *Illeis* sp2 and *H. sedecimnotata* during 2018-19

Summary and Conclusion

CHAPTER-5 SUMMARY AND CONCLUSION

The present study entitled **"Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar"** was carried out in three different locations of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, during 2018-19. While, the study on genetic variability of different coccinellid beetles was carried out in Biological Control Laboratory of Department of Entomology. An extensive survey of different coccinellid species had been carried out for a year to check their host range, seasonal incidence, and their fluctuation with the weather parameters. Genetic variability among different coccinellid beetles was carried out using eight gene specific primers. The silent findings of this study are summarized below.

5.1 Diversity of different coccinellid species in different crop ecosystems of Pantnagar.

Study on diversity of different predatory coccinellid beetles was carried out in three locations of Pantnagar *viz.,* N.E.B.Crop Research Centre, Horticulture Research Centre and Model Floriculture Research Centre. The predatory beetles were surveyed periodically from May 2018 to April 2019. A total of 15 species of predatory coccinellid beetles were observed from different crops of the region. Out of these recorded coccinellids, 13 species *viz., A. cardoni* (Weise), *C. sexmaculata* (Fab.), *C. septempunctata* (L.), *C. transversalis* (Fab.), *H. dimidita* (Fab.), *Illeis* sp1, *P. dissecta* (Mul.), *M. univittata* (Hope), *M. allardi* (Mul.), *Illeis* sp 2, *M. discolor* (Fab.), *H. sedecimnotata* (Fab.) and *H. variegate* (Goeze) were identified under subfamily Coccinellinae, while, rest of two species *i.e. B. suturalis* (Fab.) and *R. fumida* (Mul.) were belonged to subfamilies Chilocorinae and Coccidulinae, respectively. Different crops *viz.,* wheat, rice, soybean, maize, pigeonpea, chickpea, mustard, okra, cowpea, coriander, cauliflower, cabbage, rose and marigold were surveyed to study the predatory beetle. Among all the crops, maximum eight species of coccinellids were recorded in soybean crop followed by cowpea (7 sps.), maize (6 sps.), mustard (6 sps.), and wheat (4 sps.). Three coccinellid species were recorded from rice, chickpea, marigold and cabbage, while, 2 species were recorded in cauliflower, rose, and okra crops. Only one species of coccinellid was recorded in Coriander crop. Among all the species *C. septempunctata*, *C. sexmaculata*, and *C. transversalis* were found in all the cropping season of Pantnagar. Thirteen species of coccinellid were found predacious on the different aphid species, while, *A. cardoni* (W.) *and B. suturalis* (Fab.) were found predaceous on whiteflies. In Maize crop three species *viz., P. dissecta* (Mul.), *H. dimidita* (Fab.), *R. fumida* (Mul.) coccinellid species, besides aphids, were also found feeding on the pollen grains.

This investigation revealed that all the coccinellid species were predaceous on the different homopterous insect pests such as aphids, plant hoppers, scales and white flies in different crop ecosystems of Pantnagar. Hence, these predatory beetles could be utilized in an effective IPM module for the management of sucking pests in different crops.

5.2 Study on genetic variability among the different species of predatory coccinellid beetles thriving in Pantnagar region.

Gene specific markers were utilized to determine the genetic variability among the different species of coccinellid beetles collected from Pantnagar region. 28 samples of different coccinellid beetles were run against eight gene specific markers and all primers amplified scorable bands with the insect genomic DNA.

This investigation enlightened the genetic variability and phylogenetic relationship between different species of coccinellid in Pantnagar region. The study revealed that the 8 gene specific primers of CO region generated 208 number of different loci with 0.25 polymorphism information content (PIC), 0.78 marker index (MI), 0.94 resolving power (RP). Primer P1 found efficient primer to differentiate the variability among the samples because it had highest RP value 2.71 and MI value 2.19. All the samples of different species clustered with 0.35-0.83 similarity coefficient during cluster analysis using UPGMA method. The pair wise comparison analysis (PCA) showed an overlapped cluster of different coccinellid samples as they were having similar origin and evolutionary relationship.

This study provided first hand information about variation in CO gene of different predatory coccinellid species of Pantnagar region of Uttarakhand. In present study, mitochondrial region were able to differentiate the closely morphological similar species and it was found that the variants of *Harmonia dimidita* (HD) also had difference in their CO region and *Coccinella septempunctata* (CS) and *Coccinella transversalis* (CT) were more closer to each other than rest four species *viz., Propylea dissecta* (PQ), *Micraspis discolor* (MD), *Cheilomenes sexmaculata* (MS) and *H. dimidita* (variant HD and HY). The generated molecular data sets on diversity of coccinellid beeltes could be utilized as reference point for futurist for more depth studies related to specific gene responsible for active component synthesis and pigmentation. Overall present study elucidated the phylogenetic relation of different predatory cociinellids with crop ecosystems. This study would be helpful to select a suitable species of coccinellid for development of IPM module for a crop.

5.3 Seasonal abundance of predatory coccinellid in different cropping systems.

Study of the seasonal incidence of coccinellid beetles was carried out from 1st to 52nd SW during 2018-19. Three species *C. septempunctata*, *C. sexmaculata*, and *C. transversalis* were found active throughout the whole study period. The population of *C. transversalis* (14.3/ m^2), *Illeis* sp1 (7.9/ m^2) and *Illeis* sp2 (7.1/ m^2) was recorded maximum during 1st SW while, *C. septempunctata* was recorded maximum (17.6/m^2) during the 13th SW. Peak population of *A. cardoni* (9.2/ m^2) and *M. Univittata* (6.3/ m^2) was recorded maximum during 36th SW while, *C. Sexmaculata* (18.3/ m^2), *H. variegate* (14.4/ m^2) and *B. Suturalis* (7.8/ m^2) recorded maximum during the 42nd SW. peak population of *H. Sedecimnotata* (6.9/ m^2) and *R. fumida* (12.2/ m^2) found maximum during 43rd SW while, of *H. dimidita* (13.3/ m^2) and *M. discolour* (13.7/ m^2) during 45th SW, *P. dissecta* (16.7/ m^2) during 48th SW and *M. Allardi* (13.3/ m^2) during 52nd SW. Activity of coccinellid species were not recorded during hot and humid season *i.e.* from June to August. Study of correlation coefficient between weather parameters and coccinellid population revealed that most of coccinellid species exhibited a highly significant negative correlation with maximum temperature. A highly significant positive correlation was recorded with maximum and minimum relative humidity. Majority of coccinellid species showed a non significant correlation with the average rainfall.

Present study on seasonal abundance of different coccinellid beetles in various agro-ecosystems revealed the dynamics of these predators in relation to abiotic factors. Correlation study for *C. septempunctata* (-0.653**), *C. sexmaculata* (-0.620**) and *C. transversalis* (-0.455**) showed highly significant negative correlation with maximum temperature which means that the population of these beetles decreases with the increasing temperature. Correlation study of *C. septempunctata* (0.451**) and *C. sexmaculata* (0.669**) with maximum relative humidity exhibited significant positive correlation which means that as the relative humidity increases the population of these beetles also increases. This knowledge could be helpful for their mass rearing and utilization in biological control programs.

Literature Cited

LITERATURE CITED

Abrol, D. P. and Shankar, U. 2014. Pesticides, food safety and integrated pest management. In *Integrated pest management*. Springer, Dordrecht, 167-199. pp.

Agarwala, B.K. and Ghosh, A.L. 1988. Prey reports of aphidophagous coccinellidae in India. A Review and bibliography. *Journal of Tropical Pest Management*. **34**: 110-114.

Ahirwar, R.; Devi, P. and Gupta, R. 2015. Seasonal incidence of major insect-pests and their biocontrol agents of soybean crop (*Glycine max* L. Merrill). *Scientific Research and Essay*. **10**(12): 402-406.

Ahmad, K. F.; Baig, M. I. and Mustafa, S. G. 1999. Spatial distribution and phenology of adult *Chilocorus infernalis* (Mulsant) (Coccinellidae: Coleoptera) on apple trees in Kashmir-Pakistan. *Sarhad Journal of Agricultural Sciences*. **15**: 343-346.

Ahmed, K. S.; Majeed, M. Z.; Rafi, M. A.; Sellami, F. and Afzal, M. 2017. Biodiversity and Species Distribution of Coccinellids (Coccinellidae: Coleoptera) in District Sargodha (Punjab), Pakistan. *Pakistan Journal of Zoology*. **49**(5):1750-1759.

Ahmed, R. 1973. A new tribe of the family Coccinellidae (Coleoptera). *Bulletin of Entmological Research*. **62**: 449 – 452.

Ali, A. and Rana, K. S. 2010. Studies on population dynamics of ladybird beetles on cauliflower in some districts of Uttar Pradesh, India. *World Journal of Applied Sciences and Research*. **2**: 16-20.

Ali, A. and Rana, K. S. 2011. Collection of indigenous species of coccinellids from cabbage and their population dynamics in some districts of Uttar Pradesh. *Indian Journal of Biogical Studies and Research*. **1**:2.

Andersen, A.N. 1999. My bioindicator or yours? Making the selection. *Journal Insect Conservation*. **3**: 1–4.

Andrews, R. A. 1920. Insect control Rep. Proc. 3rd Ent. Meet. **1**:32.

Ankalgi, S. and Murali, J. 2016. Diversity and distribution of Coccinellidae (Coleoptera) in Ankalga village (Gulbarga district) Karnataka, India. *International Journal of Basic and Applied Sciences*. **5**(1): 1-5.

Audisio, P. and Canepari, C. 2009. Fauna Europaea–Coccinellidae. http://www. faunaeur. org/full_results.php.id=11062.

Ayyar, T. V. R. 1925. An undescribed Coccinellid beetle of economic importance. *Journal of the Bombay Natural History Society*. **30**: 491- 492.

Behera, M. K.; Behera, R. and Patro. B. 1999 Biology and feeding potential of *Coccinella septempunctata* Linnaeus (Coleoptera : Coccinelidae) on the Chrysanthemum aphid, Macrosiphoniella sanborni (Gillette). *Journal of Biological Control*. **13**:39-43.

Bhatt, B. and Karnatak, A. K. 2018. Population dynamics of sucking pests and their predators on okra agroecosystem. *Journal of Entomology and Zoology Studies*. **6**(2): 1289-1291.

Bhatt, B.; Joshi, S. and Karnatak, A. K. 2018. Biodiversity of insect pests and their predators on okra agroecosystem. *Journal of Pharmacognosy and Phytochemistry*. **7**(4): 84-86.

Bhattacharya, B. and Dutta, S.K. 1998. Black citrus aphid, *Toxoptera aurantii* Boyer (Aphididae: Homoptera) in Assam. *Insect Environment***. 3**(4): 109–109.

Booth, R. G.; Cross, A. E.; Fowler, S. V. and Shaw, R. H. 1995. The biology and taxonomy of *Hyperaspis pantherina* (Coleoptera: Coccinellidae) and the classical biological control of its prey .*Orthezia insignis* (Homoptera: Ortheziidae). *Bulletin of Entomological Research*. **85**: 307–314.

Brown, J. M.; Pellmyr, O.; Thompson, J. N. and Harrison, R. G. 1994. Phylogeny of Greya (Lepidoptera: Prodoxidae), based on nucleotide sequence variation in mitochondrial cytochrome oxidase I and II: congruence with morphological data. *Molecular Biology and Evolution*. **11**(1): 128-141.

Caltagirone, L. E. and Doutt, R. L. 1989. The history of the vedalia beetle importation to California and its impact on the development of biological control. *Annual Review of Entomology*. **34**(1): 1-16.

Chakrabarti, S.; Sarkar, S. and Debnath, M. 2012. Diversity, bioecology and biosystematics of aphidophagous predators of eastern Himalayas and Northeast India. In: Biversitat und Naturausstattung im Himalaya IV (eds. Hartmann and Weipert), Erfurt. pp. 129-147.

Chakravarty, S.; Agnihotri, M. and Kumar, L. 2016. Diversity of Insect fauna associated with pigeonpea and their succession in relation to crop phenology at Pantnagar, Uttrakhand. *Journal of Experimental Zoology of India*. **19:** 1327-1332

Chakraborty, D. and Korat, D. 2014. Population dynamics of coccinella transversalis fabricious in relation to weather parameters. *Population*. **8**(1-2): 141-147.

Chakraborty, D.; Korat, D.M. and Saneera, E.K. 2013. Correlation between coccinellids feeding on mealybugs and weather parameters. *Insect Environment*. **19**(1): 60-63.

Chanmamla, G. 2009. Taxonomic studies on predaceous coccinellidae, Order: coleptera. Thesis, M.sc. Acharya N.G. Ranga Agricultural University, Hyderabad. 79p.

Choudhary, J.S.; Naaz, N.; Mukherjee, D.; Prabhakar, C.S.; Maurya, S.; DAS, B. and Kumar, S. 2014. Biodiversity and seasonality of predaceous coccinellids (coleopteran: coccinellidae) in mango agro-ecosystem of Jharkhand. *The Ecoscan*. **8**(1-2): 53-57.

Chowdhury, S.; Sontakke, P.P.; Boopathi, T.; Bhattacharjee, J.; Bhattacharjee, D. and Malsawmzuali. 2015. Taxonomic studies on predatory coccinellid beetles and their species composition in rice ecosystem of indo Bangladesh border. *The Bioscan*. **10**(1): 229- 242.

Coeur d'acier, A.; Cocuzza, G.; Jousselin, E.; Cavalieri, V. and Barbagallo, S. 2008. Molecular phylogeny and systematic in the genus *Brachycaudus* (Homoptera: Aphididae): insights from a combined analysis of nuclear and mitochondrial genes. *Zoologica scripta*. **37**(2): 175-193.

Dempster, J and I. Mclean, 1999. Insets population: In theory and in Practice. Kluwer Academic Publishers, Boston, Massachsetts.506p.

Desai, A. E.; Bhamre, P. R., and Deore, S. R. 2011. First record of predatory coccinellid beetles (Coleoptera: Coccinellidae) from the Nasik district, (Maharashtra), India. *International Science Journal*. **2**(3): 7-16.

Dixon, A. F. G. 2012. Aphid ecology an optimization approach. Springer Science and Business Media**.**287p.

Dutta. S.; Prasad, A.; Lal, N.; Smita, A.; Dutta, A.; Naaz, M.; Kumari, S.; Farooqui, S. and Soren, S. 2012. The pool of coccinellids (coleoptera: coccinellidae) in a mixed vegetable garden of ranchi women's college. *The Bioscan*. **1**: 21-26.

FICCI .2015. Ushering in the 2nd Green Revolution: Role of Crop Protection Chemicals. Federation of Indian Chambers of Commerce and Industry, New Delhi.

Foottit, R. G.; Maw, H. E. L.; Von Dohlen, C. D. and Hebert, P. D. N. 2008. Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources*. **8**(6): 1189-1201.

Ganai, S. A.; Ahmad, H.; Sharma, D.; Sharma, S.; Khaliq, N. and Norboo, T. 2017. Diversity of arthropod fauna associated with marigold (*Tagetes erecta* L.) in Jammu. *Journal of Entomology and Zoology Studies*. **5**(5): 1940-1943.

Ghosh, S.K. and Chakraborty, K. 2012. Incidence and abundance of important predatory beetles with special reference to *Coccinella septempunctata* in Sub Himalayan region of north-east india. *International Journal of Plant, Animal and Environmental Sciences***.** **2**(3):157-162.

Gilbert, J. E.; Lewis, R. V.; Wilkinson, M. J. and Caligari, P. D. S. 1999. Developing an appropriate strategy to assess genetic variability in plant germplasm collections. *Theoretical and Applied genetics*. **98**(6-7):1125-1131.

Glenner, H.; Hansen, A. J.; Sørensen, M. V.; Ronquist, F.; Huelsenbeck, J. P. and Willerslev, E. 2004. Bayesian inference of the metazoan phylogeny: a combined molecular and morphological approach. *Current Biology*. **14**(18):1644-1649.

Gordon, R. D. 1987. A catalogue of the Crotch collection of Coccinellidae (Coleoptera). *Occasional Papers on Systematic Entomology*. **3**: 1-46.

Goswami, T.N.; Anil, Chandran, N. 2016. Lady bird beetles in major rabi oil seeds and pulse crops at Sabour, Bihar. *International Journal of Science, Environment and Technology*. **5**(4): 2382–2386.

Gupta, S.; Desai, A. and Ushir, S. 2017. Population Dynamics and Bio-Control of Insect Pest through Traps and Pheromone Lures. *International Journal of Agriculture Innovations and Research*. **5**(4): 2319-1473.

Halim, M.; Aman-Zuki, A.; Mohammed, M. A. and Yaakop, S. 2017. DNA barcoding and relationships of eight ladybugs species (Coleoptera: Coccinellidae) that infesting several crops from Peninsular Malaysia. *Journal of Asia-Pacific Entomology*. **20**(3): 814-820.

Harbhajan, K., and Kaur, S. N. 2017. DNA Barcoding of Six Species of Family Rhopalidae (Insecta: Hemiptera: Heteroptera) from India. *International Journal of Life Sciences*. **5**(4): 517-526.

Harit, D.N. 2015. Exploration of Coccinellid (Coleoptera : Coccinellidae) fauna of different Ecosystems in Champhai District of Mizoram state, North East India. *Research Journal of Agriculture and Forestry Science.* **3**(5): 21-24.

Hebert, P. D.; Cywinska, A.; Ball, S. L. and Dewaard, J. R. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*. **270**(1512): 313-321.

Hemchandra, O.; Kalita, J. and Singh, T. K. 2010. Biodiversity of aphidophagous coccinellids and their role as bioindicators in agro-forest ecosystem. *The Bioscan*. **1**: 115-122.

Hesler, L. S. and Kieckhefer, R. W. 2008. An Annotated and updated Species List of the Coccinellidae (Coleoptera) of South Dakota. *The Coleopterists Bulletin*. **62**(3): 443-454.

Hodek, I. 1973. Biology of Coccinellidae, Dr. W.J. Junk Publishers: The Hague (Netherlands). 259p.

Hodek, I. and Honek, A. 1996. Ecology of Coccinellidae. Dord. Kh. Acad. Publ.464 pp.

Hugar, P.G.; Anandhi, P.; Varma, S. and Sarvanan, L. 2008. Seasonal incidence of important insect pests of mustard and their natural enemies in relation to weather parameters in Allahabad region. *Journal of Entomological Research*. **32**(2): 109-112.

Iperti, G and Paoletti, M.G. 1999. Biodiversity of Predaceous coccinellidae in relation to bioindication and economic importance. Special issue: Invertebrate biodiversity as bioindicators of Sustainable landscapes. *Agriculture-Ecosystems-and-Environment*. **74**: 323–42.

Inamullah, Khan.; Sadrud-Din Khalil, S. K. and Rafi, M. A. 2003. Survey of predatory Coccinellids (Coleoptera : Coccinellidae)in District Chitral Pakistan. *Pakistan Entomology*. **25(**2):113-118.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. *Bulletin de la Societe Vaudoise des Science Naturelles*. **44**: 223-270.

Jadhav, Y.T.; Bhede, B.V. and Shinde, D.S. 2017. Influence of Meteorological Factors on Population Build-Up of Aphids and Natural Enemies on Summer Okra. *International Journal of Current Microbiology and Applied Sciences***. 6**(3): 2527-2537.

Jat, H., Swaminathan, R. and Upadhyay, B. 2009 Bio-ecology of aphidophagous coccinellids in maize-sorghum based cropping system. *Indian Journal of Entomology*. **71**(2):170-185.

Jinbo, U.; Toshihide, K. A. T. O. and Motomi, I. T. O. 2011. Current progress in DNA barcoding and future implications for entomology. *Entomological Science*. **14**(2): 107-124.

Jombart, T. 2008. Adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics**. 24**(11): 1403-1405.

Joshi, P. C. and Sharma, P. K. 2008. First records of coccinellid beetles (Coccinellidae) from the Haridwar, (Uttarakhand), India. *Tropical Natural History*. **8**(2): 157-167.

Joshi, S.; Poorani, J. and Singh, S. P. 2001. Bioecology of *Sticholotis cribellata* Sicard (Coleoptera : Coccinellidae), a potential predator of *Melanaspis glomerata* (Green) (Homoptera : Diaspididae) from Bangalore, *Journal of Biological Control*. **15**(1): 21-26.

Jurado-Rivera, J. A.; Vogler, A. P.; Reid, C. A.; Petitpierre, E. and Gómez-Zurita, J. 2008. DNA barcoding insect–host plant associations. *Proceedings of the Royal Society B: Biological Sciences*. **276**(1657): 639-648.

Kapur, A.P. 2002. On a collection of lady-bird beetles (Coccinelidae, Coleoptera) from Bhutan. *Oriental Insects*. **7(3)**: 457-460.

Karane, P.; Sharanabasappa, S. B.; Adivappar, N., and Satish, K. M. 2019. Population dynamics of coccinellid, *Cheilomenes sexmaculata* (Fab.) on cowpea aphid. *Journal of Entomology and Zoology Studies*. **7**(2): 1236-1238.

Kaur, H. and Sharma, K. 2017. COI-based DNA barcoding of some species of Pentatomidae from North India (Hemiptera: Heteroptera). *Mitochondrial DNA Part A*. **28**(5): 756-761.

Kavar, T.; Pavlovcic, P.; Susnik, S.; Meglic, V. and Virant-Doberlet, M. 2006. Genetic differentiation of geographically separated populations of the southern green stink bug *Nezara viridula* (Hemiptera: Pentatomidae). *Bulletin of entomological research*. **96**(2): 117-128.

Kazimi, S. K. and Ghani, M. A. 1964. A preliminary annotated list of predators of mites in West Pakistan Tech. Bull. *Common Institute of Biological Control*. **4**: 67-75.

Kergoat, G. J.; and Delobel, A. and Silvain, J. F. 2004. Phylogeny and host-specificity of European seed beetles (Coleoptera, Bruchidae), new insights from molecular and ecological data. *Molecular Phylogenetics and Evolution*. **32**(3): 855-865.

Khan M.R. and Khan M.R. 2002. Prey preference and prey switching behaviour of *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). *International Journal of Agriculture and Biology*. **4**: 110–112.

Khan, A. A.; Zaki, F. A.; Khan, Z. H.; and Mir, R. A. 2009. Biodiversity of predacious ladybird beetles (Coleoptera: Coccinellidae) in Kashmir. *Journal of Biological Control*. **23**(1): 43-47.

Kim, M. J.; Wan, X. and Kim, I. 2012. Complete mitochondrial genome of the seven-spotted lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae). *Mitochondrial DNA*. **23**(3): 179-181.

Kobayashi, N.; Tamura, K.; Aotsuka, T. and Katakura, H. 1998. Molecular phylogeny of twelve Asian species of epilachnine ladybird beetles (Coleoptera, Coccinellidae) with notes on the direction of host shifts. *Zoological science*. **15**(1): 147-152.

Kulkarni, A.V. and Patel, I.S. 2001. Seasonal abundance of mustard aphid (*Lipaphis erysimi*) and associated bioagents in Indian mustard (*Brassica juncea*) crop. *Indian Journal of Agricultural Sciences*. **71**(10): 681-682.

Kumar, A and Kumar, A. 2015. Effect of biotic and abiotic factors on incidence of pest and predators in cowpea [*Vigna uniguiculata* (L.) Walp.]. *Agriculture Research Communication Centre*. **31**(1): 121-125.

Kumar, A.; Singh, R.; Prasad, C. S.; Tiwari, G. N. and Kumar, S. 2017. New records of predatory coccinellids beetles (Coccinellidae: Coleoptera) in Western plain zone of Uttar Pradesh. *Journal of Entomology and Zoology Studies*. **5**(3): 1140-1147.

Kumazawa, Y.; Azuma, Y. and Nishida, M. 2004. Tempo of mitochondrial gene evolution: Can mitochondrial DNA be used to date old divergences. *Endocytobiosis Cell Research*. **15**(1):136-142.

Lami, F.; Masetti, A.; Neri, U.; Lener, M.; Staiano, G.; Arpaia, S. and Burgio, G. 2016. Diversity of Coccinellidae in ecological compensation areas of Italy and overlap with maize pollen shedding period. *Bulletin of Insectology*. **69**(1): 49-57.

Lopes, H. M.; Bastos, C. S.; Boiteux, L. S.; Foresti, J. and Suinaga, F. A. 2017. A RAPD-PCR-based genetic diversity analysis of *Helicoverpa armigera* and *H. zea* populations in Brazil. *Genetics and Molecular Research*. **16**(3):1-10.

Magro, A.; Lecompte, E.; Magné, F.; Hemptinne, J. L. and Crouau-Roy, B. 2010. Phylogeny of ladybirds (Coleoptera: Coccinellidae): are the subfamilies monophyletic? *Molecular Phylogenetics and Evolution*. **54**(3): 833-848.

Mahr, S. R.; Cloyd, R. A.; Mahr, D. L. and Sadof, C. S. 2010. Biological control of insects and other pests of greenhouse crops. North Central Regional. University of Wisconsin– Extension, Cooperative Extension. *North central regional publication* .**581**: 108.

Majka, C. G. and McCorquodale, D. B. 2010. Ladybird beetles (Coleoptera: Coccinellidae) of the Atlantic Maritime Ecozone. *In*: Assessment of Species Diversity in the Atlantic Maritime Ecozone. Edited by D.F. McAlpine and I.M. Smith. NRC Research Press, Ottawa, Canada. pp. 439–452.

Mandal, S.M.A. and Patnaik, N.C. 2008. Interspecific abundance and seasonal incidence of aphids and aphidophagous predators associated with cabbage. *Journal of Biological Control***.** **22**(1): 195-198.

Mayadunnage, S.; Wijayagunasekara, H.N.P.; Hemachandra, K.S. and Nugaliyadde, L. 2007. Predatory coccinellids (coleoptera: cocinellidae) of vegetable insect pest: A survey in mid country of Sri Lanka. *Tropical Agricultural Research*. **19**: 6977.

Megha, R. R.; Vastrad, A. S.; Kamanna, B. C. and Kulkarni, N. S. 2015. Species complex of coccinellids in different crops at Dharwad region. *Journal of Experimental Zoology (India)*. **18**(2): 931-935.

Mishra, A.K. and Yousuf, M. 2019. Notes on coccinellid beetles (Coleoptera: Coccinellidae) from forest ecosystem of Uttarakhand, India. *Journal of Biological Control.* **33**(1):1-6.

Mondal, A.; Raut, A.M. and Satpathi, C.R. 2016. Analysis of insect predator diversity in irrigated rice ecosystem of West Bengal, India, December 22, 2016. Ecology, Environment and Conservation. pp. S89-S94.

Mullis, K.; Faloona, F.; Scharf, S.; Saiki, R. K.; Horn, G. T. and Erlich, H. 1986. Specific enzymatic amplification of DNA in vitro: the polymerase chain reaction. In *Cold Spring Harbor symposia on quantitative biology*. **51**:263-273.

Nagaraja, H. and Hussainy, S. U. 1967. A study of six species of *Chilocorus* (Coleoptera : Coccinellidae) predaceous on san Jose and other scale insects. *Oriental Insects*. **1**: 249 -256.

Nagaraju, J.; Reddy, K. D.; Nagaraja, G. M. and Sethuraman, B. N. 2001. Comparison of multilocus RFLPs and PCR-based marker systems for genetic analysis of the silkworm, Bombyx mori. *Heredity*. **86**(5): 588.

Natarajan, K. and Mathur, K. C. 1981. New records of predaceous beetles on Brown plant hopper in India. *Current Sciences*. **50**(5): 239.

Nath, D. K. and Sen, B. 1976. Some observations on aphidophagous coccinellid beetles in mustard cultivation. *Science and Culture*. **42**(5): 288-299.

Nelaballe, V. K.; and Meruva, B. P. 2015. Coccinellidae Beetles in Pest and Disease Management. *International Journal of Chemical, Environmental and Biological Science*. **3**(5): 2320-4087.

Omkar, G. and Pervez, A. 2002. Intraguild predation by ladybeetles: An ultimate survival strategy or an aid for advanced aphid biocontrol. Prof. SB Singh Comm. Vol. Zool. Soc. India.pp 77-90.

Omkar. and Bind, R. B. 1993. Records of aphid natural enemies complex of Uttar Pradesh. II. The coccinellids. *Journal of Advance Zoology*. **14**: 96-99.

Omkar. and Bind, R. B. 1995. Records of aphid natural enemies complex of Uttar Pradesh. IV. The coccinellids. *Journal of Advance Zoology*. **16**: 67-71.

Omkar. and Bind, R. B. 1996. Records of aphid natural enemies complex of Uttar Pradesh. V. The coccinellids. *Journal of Advance Zoology*. **17**: 44-48.

Omkar. and Pervez, A. 1999. New record of coccinellids from Uttar Pradesh. I. *Journal of Advanced Zoology*. **20**(2): 106-112.

Pal, S. and Bhatt, J. 2018. Feeding Efficiency of Six Beetle Species against Mustard Aphid, *Lipaphis erysimi* (Kalt.) in Uttarakhand, India. *International Journal of Current Microbiology and Applied Sciences*. **7**(4): 1970-1976.

Pareek, R. K.; Sharma, A. and Kumawat, K. C. 2013. Seasonal abundance of insect pests and their major natural enemies in coriander, *Coriandrum sativum* Linn. *Indian Journal Applied Entomology*. **27**(1): 21-26.

Patel, S.K. 2009. Population dynamics and management of insect pest complex of vegetable cowpea, *Vigna unguiculata* (Lin.) in middle Gujarat condition. Thesis, M.Sc(Ag). AAU, Anand.114p.

Patel, Y and Patel, P. 2014. Relative abundance of Coccinellid in cotton ecosystem in relation to environmental factors. *International Journal of Current Microbiology and Allied Science*. **3**(3): 1067-1073.

Poorani, J. 2002. An annotated checklist of the Coccinellidae (Coleoptera)(excluding Epilachninae) of the Indian subregion. *Oriental Insects*. **36**(1):307-383.

Poorani, J. 2012. Annotated Checklist of the Coccinellidae (Coleoptera) of the Indian Subregion [www. angelfire. com/bug2/j_poorani/checklist. pdf].

Poorani, J., and Booth, R. G. 2016. *Harmonia manillana* (Mulsant), a new addition to Indian Coccinellidae, with changes in synonymy. *Biodiversity Data Journal*. **4:**1-6.

Powell, W.; Morgante, M.; Andre, C.; Hanafey, M.; Vogel, J.; Tingey, S. and Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. *Molecular breeding*. **2**(3): 225-238.

Prevost, A. and Wilkinson, M. J. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. *Theoretical and applied Genetics*. **98**(1): 107-112.

Puttarudraiah, M.; Channabasavanna, G. P. and Krishnamurty, B. 1952. Discovery of *Cryptolaemus montrouzieri* Mulsant (Coccinellidae: Coleoptera Insecta) in Bangalore, South India. *Nature.* **169**:377-378.

Rain, F. F.; Aslam, A. F. M.; Ringki, H. S.; Sultana, N.; Akter, N. and Howlader, A. J. 2016. Coccinellid Predators of Aphid and Their Phylogenetic Analysis Using COI Gene Sequences. *International Journal of Applied Sciences and Biotechnology*. **4**(3): 408-416.

Ramya, H. R. and Rojeet, T. 2016. Predatory coccinellids of insect pests of Assam Lemon (*Citrus limon* L. Burmf) in Jorhat district of Assam. *Journal of Biological Control*. **30**(2): 121-123.

Rao, V.P. and So, P.Y. 1967. Notes on coccinellidae of Hong Kong. Tech. Bull. No. 9. Commonwealth Institute of Biological Control, p 9-24.

Rawat, R. R. and Modi, N. N. 1968b. A record of natural enemies of *Ferrisia virgata* ckll. In Madhya Pradesh (India). *Mysore Journal of Agricultural Sciences*. **2**: 51-53.

Rawat, R.R. and Modi, B.N. 1969. Record of some predaceous beetle on coccids, aphid, and mite pest from Madhya Pradesh. *Indian Journal of Agriculture Science*. **39**(11): 1057-1060.

Roderick, G.K. and Navajas, M. 2003. Genes in new environments: genetics and evolution in biological control. Nat Rev Genet **4**:889–899.

Rohlf, F. J. 2002. NTSYS-pc: numerical taxonomy and multivariate analysis system, version 2.11a. Applied Biostatistics, New York.

Roldan-Ruiz, I.; Van Euwijk, F. A.; Gilliland, T. J.; Dubreuil, P.; Dillmann, C.; Lallemand, J. and Baril, C. P. 2001. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. *Theoretical and Applied Genetics*. **103**(8): 1138-1150.

Roy, H. and Migeon, A. 2010. Ladybeetles (Coccinellidae). Chapter 8.4. *BioRisk*. **4**: 293.

Roy, S.; Mukhopadhyay, A.; Das, S and Gurusubramanian, G. 2010. Bioefficacy of coccinellid predators on major tea pests. *Journal of Biopesticides*. **3**(1): 033-036.

Samal, P. and Misra, B. C. 1982. Coccinella repanda thumb. a predatory Coccinellid beetle of rice brown plant hopper, *Nilaparvata lugens* (Stal.) from CRRI, Cuttack, *Oryza*, **19** : 212.

Sambrook, J.; Russell, D. W. and Russell, D. W. 2001. Molecular cloning: a laboratory manual . Cold spring harbor laboratory press, New York, pp. 999.

Sasaji, H. 1967a. A revision of the Formosan Coccinellidae (I). The subfamily Sticholotinae, with and establishment of a new tribe (Coleoptera). *Etizenia*. **25**: 1-28.

Sathe, T. V. and Bhosale, Y. A. 2001. Insect pest predators, Daya Publishing House, Delhi. pp.1-195.

Sayed, S. M. 2016. Molecular Diversity of the Lady Beetles, *Coccinella undecimpunctata* L. and *Hippodamia variegata* (Goeze)(Coleoptera: Coccinellidae) in Saudi Arabia. *Egyptian Journal of Biological Pest Control*. **26** (2): 351.

Shah, M. A. and Khan, A. A. 2014. Assessment of coccinellid biodiversity under pesticide pressure in Horticulture ecosystems. *Indian Journal of Entomology*. **76** (2): 107-116.

Shanker, C.; Mohan, M.; Sampathkumar, M.; Lydia, Ch. and Katti, G. 2013. Functional significance of *Micraspis discolor* (F.) (Coccinellidae: Coleoptera) in rice ecosystem. *Journal of Applied Entomology*. **137**: 601–609.

Shantibala, K. and Singh, T.K. 1991. Studies on the vertical distribution of aphidophagous coccinellids (Coleoptera: Coccinellidae) of Manipur and Nagaland. *Journal of Aphidology*. **5**: 39-43.

Sharma, P. K. and Joshi, P. C. 2010. New records of coccinellid Beetles (Coccinellidae: Coleoptera) from District Dehradun (Uttarakhand), India. *New York Science Journal*. **3**: 112-120.

Sharma, P. L.; Verma, S. C.; Chandel, R. S.; Chandel, R. P. S. and Thakur, P. 2017. An inventory of the predatory Coccinellidae of Himachal Pradesh, India. *Journal of Entomology and Zoology Studies*. **5**(6): 2503-2507.

Singh, K. and Singh, N.N. 1991. Biology and devouring propensity of lady bird beetle, *Coccinella septempunctata* Linnaeus on rapeseed-mustard aphid, *Lipaphis erysimi* Kaltenbach. *African Journal of Agricultural Research***. 9**(1): 61-64.

Singh, M.K. and Nath, P. 2007. Effect of dates of sowing of *Brassica juncea* on Coccinellid beetle of *Lipaphis erysimi* Kalt. *Annual of Plant Protection Sciences*. **15**(1): 8-11.

Singh, N.N. and Sanjeev, R. 2000. Relative abundance of different coccinellids in mustard ecosystem. *Indian Journal of Entomology*. **62**(4): 422-426.

Skaife, S.H. 1979. African Insect Life. Struik Publishers (Pty) Ltd., Conelis Struik House, Cape Town 8001. pp. 186–90.

Soni, R.; Deol, G. S. and Singh, S. 2013. Seasonal dynamics of wheat aphid complex and predator *Coccinella septempunctata* in relation to abiotic and biotic factors. *Journal of environmental biology*. **34**(4): 689.

Srikanth, J. and Lakkundi, N. H. 1990. Seasonal population fluctuations of cowpea aphid *Aphis craccivora* Koch and its predatory coccinellids. *International Journal of Tropical Insect Science*. **11**(1): 21-26.

Sundar Pal and Jitendra Bhatt. 2018. Feeding Efficiency of Six Beetle Species against Mustard Aphid, *Lipaphis erysimi* (Kalt.) in Uttarakhand, India. *International Journal of Current Microbiology Applied Science.* **7**(4): 1970-1976.

Sunil, J.; Poorani, J. and Singh, S. P. 2001. Bioecology of *Sticholotis cribellata* Sicard (Coleoptera : Coccinellidae), a potential predator of *Melanaspis glomerata* (Green) (Homoptera : Diaspididae) from Bangalore. *Journal of Biological Control*. **15**(1):21-26.

Sureja, B.V. 1991. Bioecology and utilization of predatory coccinellids for the management of aphid. Thesis, Ph.D. RAU, Udaipur.132 p.

Sushil, S. N. 2016. Emerging Issues of Plant Protection in India. Natural Resource Management: Ecological Perspectives. International Conference, SKUAST, Jammu.pp 529.

Suyal, P., Gaur, N., and Devrani, A. 2018. Seasonal incidence of insect pests and their natural enemies on soybean crop. *Journal of Entomology and Zoology Studies*. **6**(4): 1237-1240.

Swami, D.; Jat, B. L. and Dotasara, S. K. 2018. Population dynamics of insect pests of coriander and their correlation with biotic and abiotic factors. *Journal of Entomology and Zoology Studies*. **6**(4):460-464.

Swaminathan, R.; Meena, A. and Meena, B. M. 2015. Diversity and predation potential of major aphidophagous predators in maize. *Applied Ecology and Environmental Research*. **13**(4):1069-1084.

Tank, B.D.; Korat, D.M. and Borad, P.K. 2007. Determination of dominant species of predatory Coccinellid in Anand region of Gujarat. *Karnataka Journal of Agricultural Science*. **20**(3):637-638.

Tara, J. S., and Feroz, M. 2009. Lady Bird Beetles (Coleoptera: Coccinellidae) From Kargil. *The Bioscan*. **4**(4): 683-688.

Thakur, J. N.; Rawat, U. S.; Pawar, A. D. and Sidhu, S. S. 1989. Natural enemy complex of the cabbage aphid *Brevicoryne brassicae* L.(Homoptera: Aphididae) in Kullu Valley, Himachal Pradesh. *Journal of Biological Control*. **3**(1):69.

Vandenberg, N. J. 2002. The New World genus *Cycloneda* Crotch (Coleoptera: Coccinellidae: Coccinellini): Historical review, new diagnosis, new generic and specific synonyms, and an improved key to North American species. *Proceedings of the Entomological Society of Washington*. **104** (1): 221-236.

Varma, G.C.; Vyas, R. S. and Brar, K. S. 1993. Biology of *Menochilus sexmaculatus* (Fab.) PAU. *Journal Research*. **30**(1-2):27-31.

Vazirani, T. G. 1983. Sticholotis chittagongi sp.n. (Coleoptera : Coccinellidae), a predator on aphids on Ziziphus in Bangladesh. *Bulletin of Entomological Research* **73**:301-303.

Vinothkumar, B. 2013. Diversity of coccinellid predators in upland rainfed rice ecosystem. *Journal of Biological Control*. **27**(3):184-189.

Vrijenhoek, R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*. **3**(5): 294.

Yasuda, H.; Kikuchi, T.; Kindlmann, P. and Sato, S. 2001. Relationships between attack and escape rates, cannibalism, and intraguild predation in larvae of two predatory ladybirds. *Journal of Insect Behavior*. **14**(3):373-384.

Zahoor, M.K.; Suhail, A.; Iqbal, J.; Zulfaqar, Z. and Anwar, M. 2003. Biodiversity of predaceous coccinellids and their role as bioindicator in an agro-ecosystem. *International journal of Agriculture and Biology*. **5**(4):555-559.

Zehnder, G. 2014. Overview of Monitoring and Identification Techniques for Insect Pests.

Zibaee, A.; Hoda, H. and Fazeli-Dinan, M. 2011. A TAG-lipase activity in the salivary secretions of a predaceous bug, *Andrallus spinidens* Fabricius (Hemiptera: Pentatomidae). *Trends in Entomology*. **7**:27-32.

Zaki, F. N.; El-Shaarawy; M. F. and Farag, N. A. 1999. Release of two predators and two parasitoids to control aphids and whiteflies. *Anzeiger für Schädlingskunde= Journal of Pest Science*. **72**(1):19-20.

Ferran, A.; Niknam, H.; Kabiri, F.; Picart, J. L.; De Herce, C.; Brun, J. and Lapchin, L. 1996. The use of Harmonia axyridis larvae (Coleoptera: Coccinellidae) against *Macrosiphum rosae* (Hemiptera: Sternorrhyncha: Aphididae) on rose bushes. *European Journal of Entomology*. **93**:59-68.

Trouve, C.; Ledee, S.; Ferran, A. and Brun, J. 1997. Biological control of the damson-hop aphid, *Phorodon humuli* (Hom.: Aphididae), using the ladybeetle *Harmonia axyridis* (Col.: Coccinellidae). *Entomophaga*. **42**(1-2):57-62.

Kuroda, T. and Miura, K. 2003. Comparison of the effectiveness of two methods for releasing *Harmonia axyridis* (Pallas)(Coleoptera: Coccinellidae) against *Aphis gossypii* Glover (Homoptera: Aphididae) on cucumbers in a greenhouse. *Applied Entomology and Zoology*. **38**(2):271-274.

Deng, G. R.; Yang, H. H. and Jin, M. X. 1987. Augmentation of coccinellid beetles for controlling sugarcane woolly aphid. *Chinese Journal of Biological Control*. **3**(4):166-168.

Ellis, D.; McAvoy, R.; Ayyash, L. A.; Flanagan, M. and Ciomperlik, M. 2001. Evaluation of *Serangium parcesetosum* (Coleoptera: Coccinellidae) for biological control of silverleaf whitefly, *Bemisia argentifolii* (Homoptera: Aleyrodidae), on poinsettia. *Florida Entomologist*. **84**(2): 215.

Legaspi, J.; Ciomperlik, M.; and Legaspi Jr, B. 2001. Field cage evaluation of *Serangium parcesetosum* as a predator of blackfly eggs. *Southwestern Entomologist*. **26**(2):171-172.

Appendices

APPENDIX

Weekly meteorological data of April 2018 to April 2019

WEEKLY WEATHER DATA								
STATION NAME								
PANTNAGAR			LATTITUDE 29.0222° N	LONGITUDE 79.4908° E	ALTITUDE 243.84m AMSL			
Month	Date	Year	SMW	Temperature (°C)		Relative Humidity		Rainfall (mm)
				Max	Min	7:12 am	14:12 pm	
April- May	30-06	2018	18	35.1	22.0	69.7	41.0	2.8
May	07-13	2018	19	37.0	21.1	66.7	32.1	0.0
May	14-20	2018	20	38.9	20.3	63.7	23.3	-2.8
May	21-27	2018	21	40.9	19.5	60.7	14.4	-5.6
May- June	28-03	2018	22	42.8	18.6	57.7	5.6	-8.4
June	04-10	2018	23	44.7	17.8	54.7	-3.3	-11.2
June	11-17	2018	24	46.6	16.9	51.7	-12.1	-14.0
June	18-24	2018	25	48.6	16.1	48.7	-21.0	-16.8
June-July	25-01	2018	26	50.5	15.2	45.7	-29.9	-19.6
July	02-08	2018	27	32.6	25.4	90.0	70.0	180.8
July	09-15	2018	28	32.5	26.0	88.0	78.4	173.2
July	16-22	2018	29	33.3	26.9	82.1	73.1	79.6
July	23-29	2018	30	31.2	25.7	91.0	80.0	169.0
July-Aug.	30-05	2018	31	29.7	24.1	93.6	84.0	218.1
Aug	06-12	2018	32	30.9	24.9	90.4	79.7	126.4
Aug	13-19	2018	33	31.9	26.1	89.0	71.7	73.4
Aug	20-26	2018	34	30.9	25.5	95.1	83.0	160.8
Aug-Sep	27-02	2018	35	30.7	25.5	92.4	81.0	86.8
Sep	03-09	2018	36	32.3	25.3	90.4	76.3	76.6
Sep	10-16	2018	37	31.9	24.3	93.0	77.6	15.6
Sep	17-23	2018	38	32.1	22.6	90.1	73.4	49.2
Sep	24-30	2018	39	30.4	22.0	91.3	69.7	80.6
Oct	01-07	2018	40	32.6	18.5	84.3	60.1	0.0
Oct	08-14	2018	41	30.9	17.1	82.7	61.4	2.6
Oct	15-21	2018	42	30.7	14.3	86.9	59.1	0.0
Oct	22-28	2018	43	29.6	12.0	90.4	50.7	0.0
Oct- Nov	29-04	2018	44	29.9	13.7	87.7	54.3	4.2
Nov	05-11	2018	45	27.5	11.7	93.9	54.0	0.0
Nov	12-18	2018	46	26.5	11.8	93.3	63.1	0.0
Nov	19-25	2018	47	26.3	10.5	93.4	54.3	0.0
Nov-Dec	26-02	2018	48	26.4	10.7	94.6	60.6	0.0
Dec	03-09	2018	49	23.8	7.8	92.6	61.0	0.0
Dec	10-16	2018	50	22.6	6.6	94.6	60.4	0.8
Dec	17-23	2018	51	22.5	5.0	96.6	50.7	0.0
Dec	24-30	2018	52	20.3	2.7	97.1	51.9	0.0
Dec- Jan	31-06	2019	01	21.3	6.1	91.0	60.3	0.0
Jan	07-13	2019	02	21.6	5.7	94.4	57.0	0.0
Jan	14-20	2019	03	21.7	5.7	93.0	53.0	0.0
Jan	21-27	2019	04	20.5	8.6	88.4	49.9	14.2
Jan-Feb	28-03	2019	05	20.9	7.0	92.9	63.1	0.0
Feb	04-10	2019	06	21.1	9.1	94.9	65.9	15.0
Feb	11-17	2019	07	22.7	10.8	93.6	63.6	12.0
Feb	18-24	2019	08	24.3	11.2	91.9	63.7	3.2
Feb-Mar	25-03	2019	09	21.5	9.0	92.1	68.7	6.8
Mar	04-10	2019	10	26.0	8.9	88.3	50.0	0.0
Mar	11-17	2019	11	28.0	11.2	89.1	46.9	2.6
Mar	18-28	2019	12	30.7	11.7	85.4	40.6	0.0
Mar	25-31	2019	13	32.5	15.5	84.9	49.9	0.0
April	01-07	2019	14	33.3	18.0	70.4	43.3	0.0
April	08-14	2019	15	35.7	17.3	74.0	31.7	3.2
April	15-21	2019	16	33.2	17.8	72.4	37.1	11.2
April	22-28	2019	17	38.3	20.0	63.0	27.6	0.0

VITA

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ABSTRACT

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Sem. and year of admission	:	1st Sem., 2016-17	**Degree**	:	M.Sc. (Ag)
			Department	:	Entomology
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Thesis title	:	**"Study on the diversity of predatory Coccinellid species prevailing in different crop ecosystems of Pantnagar"**			
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The present investigation was carried out during 2018-19 at three different locations of Pantnagar and laboratory studies were conducted in Biological Control Laboratory, Department of Entomology, Govind Ballabh Pant University of Agriculture & Technology, Pantnagar. For the study of diversity of coccinellid beetles, 14 crops from three different ecosystems *i.e.* field crop, vegetables crop and floriculture crop were observed. Results of the diversity of different coccinellid species from different crop ecosystems revealed the presence of fifteen species of predatory coccinellid species in Pantnagar region. Among all the species, thirteen species were recorded under the subfamily Coccinellinae while, rest of two species were belonged to subfamily Chilocorinae and Coccidulinae each. Maximum eight number of coccinellid species *viz.*, *C. septempunctata* (L.), *C. sexmaculata* (Fab.), *M. discolor* (Fab.), *P. dissecta* (Mul.), *H. dimidita* (Fab.), *M. univittata* (Hope), *A. cardoni* (Weise) and *B. suturalis* (Fab.) were found in soybean crop while, minimum 01 species *C. septempunctata* (L.) was observed in coriander crop. Out of fifteen species, 13 species of coccinellids were found predaceous on different aphid species, whereas, two species *i.e. A. cardoni* (W.) and *B. suturalis* (Fab.) were found predaceous on whiteflies. Besides aphids, *P. dissecta* (Mul.), *H. dimidita* (Fab.) and *R. fumida* (Mul.) were also found feeding on pollen grains of maize.

Study of genetic variability of coccinellid beetles provided first hand information about variation in CO gene of different predatory coccinellid species from Pantnagar region of Uttarakhand. CO region of *C. transversalis* (CT) and *C. septempunctata* (CS) showed more similarity as they share a single cluster on dendogram profile while, The other species of coccinellid beetles *i.e*. *Propylea dissecta* (PQ), *Micraspis discolor* (MD), *Cheilomenes sexmaculata* (MS) and *H. dimidita* (variant HD and HY) form a separate cluster. Variants of *H. dimidita* (HD) also had difference in their CO region. This study showed that the mitochondrial region were able to differentiate the closely morphological similar species from each other.

Study on seasonal abundance of coccinellid species in different crops revealed that three species *C. septempunctata*, *C. sexmaculata* and *C. transversalis* were found active throughout the year. The population of *C. transversalis* (14.3/ m^2), *Illeis* sp1 (7.9/ m^2) and *Illeis* sp2 (7.1/ m^2) was recorded maximum during 1st SW while, *C. septempunctata* was recorded maximum (17.6/m^2) during the 13th SW. Peak population of *A. cardoni* (9.2/ m^2) and *M. Univittata* (6.3/ m^2) was recorded maximum during 36th SW while, *C. Sexmaculata* (18.3/ m^2), *H. variegate* (14.4/ m^2) and *B. Suturalis* (7.8/ m^2) recorded maximum during the 42nd SW. Peak population of *H. Sedecimnotata* (6.9/ m^2) and *R. fumida* (12.2/ m^2) found maximum during 43rd SW while, of *H. dimidita* (13.3/ m^2) and *M. discolor* (13.7/ m^2) during 45th SW, *P. dissecta* (16.7/ m^2) during 48th SW and *M. Allardi* (13.3/ m^2) during 52nd SW. A positive correlation with average relative humidity and negative correlation with average temperature, rainfall were found to be associated with the seasonal occurrence of these predatory coccinellid beetles. Present study showed that these predatory coccinellid species were well acclimatized with all the weather conditions at Pantnagar and three species *C. septempunctata*, *C. sexmaculata* and *C. transversalis* could be utilized in biological control programs throughout the year.

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शोध शीर्षक : "पंतनगर की विभिन्न फसल पारिस्थिकी में व्याप्त परभक्षी कॉक्सीनेलिड प्रजातियों की विविधता पर अध्ययन"

सलाहकार : डा0 आर. पी. मौर्य

प्रस्तुत शोध पंतनगर के तीन विभिन्न स्थानों एवं प्रयोगशाला अध्ययन, गोविंद बल्लभ पंत कृषि एवं प्रोधोगिक विश्वविधालय के कीट विज्ञान विभाग के जैव नियंत्रण प्रयोगशाला में वर्ष 2018–19 में संपादित किया गया । कॉक्सीनेलिड बीटल की विविधता के अध्ययन के लिए तीन विभिन्न पारिस्थितिकी तंत्र जैसे सब्जियों, फूलों एवं फसलों से 14 फसलों को देखा गया । पंतनगर के विविध फसल पारिस्थितिकी प्राणियों में परभक्षी कॉक्सीनेलिड प्रजातियों की विविधता पर किये गए परिणाम बताते हैं कि पंतनगर क्षेत्र में 15 परभक्षी कॉक्सीनेलिड की प्रजातियॉ पायी गयी । विभिन्न प्रजातियों के मध्य में 13 प्रजातियॉ उपकुत कॉक्सीनेलिनी के अंतर्गत एवं अन्य दो प्रजातियॉ कायलोकोरिनी एवं काक्सीडयूलिनी के अंतर्गत पायी गयी । सर्वाधिक 8 कॉक्सीनेलिड प्रजातियॉ जैसे कि *सी. सेंप्टमपंकटाटा* (एल.), *सी. सेक्समैक्यूलाटा* (फैब.), *एम. डिस्कलर* (फैब.), *पी. डाइसेक्टा* (मूल.), *एच. डाइमीडियाटा* (फैब.), *एम. यूनीविटाटा* (होप), *ए. कार्डोनी* (विस) और *डी. सुट्रालिस* (फैब.) सोयाबिन की फसल में, जबकि न्यूनतम एक *सी. सेंप्टमपंकटाटा* (एल.) धनिये की फसल में पायी गई । विभिन्न 15 प्रजातियों के बीच में कॉक्सीनेलिड्स की 13 प्रजातियॉ विभिन्न माहू की प्रजातियों पर परभक्षी पायी गई । जबकि 2 प्रजातियॉ *ए. कार्डोनी* एवं *बी. सुट्रालिस* सफेद मक्खी पर परभक्षी पायी गई । माहू के अलावा *पी. डाईसेक्टा, एच. डाइमीडियाटा* एवं *आर. फयूमिडा* मक्के के परागकणों पर भी भक्षण करते पाये गये ।

कॉक्सीनेलिड बीटल्स की आनुवांशिक विविधता के अध्ययन में उत्तराखंड के पंतनगर क्षेत्र में परभक्षी कॉक्सीनेलिड प्रजातियों में सीओ जीन की विविधता की प्रत्यक्ष जानकारी उपलब्ध करायी गयी । सीओ क्षेत्र में *सी. ट्रांसवर्सेलिस* (सी.टी) एवं *सी. सेप्टमपंकटाटा* (सी.एस) समानता प्रदर्शित करते हैं क्योंकि डेंडोग्राम प्रोफाइल अध्ययन में ये प्रजातियों एक समूह में आते हैं । जबकि *प्रोपाइलिया डायसेक्टा* (पी.क्यू), *माइक्रास्पिस डिसकलर* (एम.डी), *काइलोमीन्स सेक्समैक्यूलाटा* (एम.एस) एवं *एच. डाइमीडियाटा* (एच.डी एवं एच.वाइ. प्रकार) अलग समूह बनाते हैं । *एच. डाइमीडियाटा* के प्रकारों के सीओ क्षेत्र में भी विभिन्नता पायी गयी । इस अध्ययन ने बताया कि माइटोकांड्रियल क्षेत्र निकट रूप से आकारिकी में समान प्रजातियों के तुलनात्मक अध्ययन के लिए सक्षम था ।

विभिन्न फसलों के कॉक्सीनेलिड प्रजातियों की मौसमी प्रचुरता पर अध्ययन के परिणाम बताते हैं कि *सी. सेंप्टमपंकटाटा, सी. सेक्समैक्यूलाटा* एवं *सी. ट्रांसवर्सेलिस* पूरे वर्ष में सक्रिय पाये गये । *सी. ट्रांसवर्सेलिस* (14.3/मी.^{2}), *इल्लिस* एसपी1 (7.9/मी.^{2}) एवं *इल्लिस* एसपी2 (7.1/मी.^{2}) की जनसंख्या प्रथम मानक सप्ताह के दौरान अधिकतम देखी गयी जबकि *सी. सेंप्टमपंकटाटा* की जनसंख्या तेरहवें मानक सप्ताह के दौरान अधिकतम (19.6/मी.^{2}) पायी गयी थी । *ए. कार्डोनी* (9.2/मी^{2}) एवं *एम. यूनीविटाटा* (6.3/मी.^{2}) की अधिकतम जनसंख्या छत्तीसवें मानक सप्ताह में देखी गयी जबकि *सी. सेक्समैक्यूलाटा* (18.3/मी.^{2}), *एच. वैरिगेट* (14.4/मी^{2}) एवं *बी. सुट्रालिस* की अधिकतम जनसंख्या बियालिसवें मानक सप्ताह के दौरान देखी गयी थी । *एच. सेडिसिमनोटाटा* (6.9/मी^{2}) एवं *आर. फयूमिडा* (12.2/मी.^{2}) की अधिकतम जनसंख्या तैतालिसवें मानक सप्ताह में देखी गयी जबकि *एच. डाइमीडियाटा* (13.3/मी.^{2}) एवं *एम. डिस्कलर* (13.7/मी^{2}) की अधिकतम जनसंख्या पैंतालिसवें मानक सप्ताह के दौरान, *पी. डाइसेक्टा* (16.7/मी^{2}) अडतासिवें मानक सप्ताह के दौरान एवं *एम. आलार्डी* (13.3/मी.^{2}) की अधिकतम जनसंख्या बावनवे मानक सप्ताह के दौरान देखी गयी थी । परभक्षी कॉक्सीनेलिड बीटल की प्रजातियों के मौसमी उपस्थिति औसत आपेक्ष आद्रता के साथ सकारात्मक एवं औसत तापमान और वर्षा के साथ नकारात्मक संबंध दर्शाता है । वर्तमान परिक्षण के परिणाम बताते हैं कि परभक्षी कॉक्सीनेलिड बीटल प्रजातियॉ समस्त मौसमी परिस्थितियों के साथ अनुकूलित पायी गयी एवं तीन प्रजातियॉ *सी. सेंप्टमपंकटाटा, सी. सेक्समैक्यूलाटा* एवं *सी. ट्रांसवर्सेलिस* का उपयोग जैविक नियंत्रण कार्यक्रमों में किया जा सकता है।

(आर.पी.मौर्य)
सलाहकार

(हिमांशु पटवाल)
लेखक