"Management of *Parthenium hysterophorus* through the Establishment of Mexican beetle, *Zygogramma bicolorata* Pallister and Evaluation of Host Specificity in and around Kota Region"

"मेक्सिकन बीटल जाइगोग्रेमा बाइकोलोरेटा पेलिस्टर के स्थापन द्वारा पारथेनियम हिस्टेरोफोरस का प्रबंधन तथा कोटा एवं समीपवर्ती क्षेत्रों में परपोषी विशिष्टता का मूल्यांकन |"

A Thesis
Submitted for the Award of Ph. D. degree of

UNIVERSITY OF KOTA
in the
Faculty of Science
By

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M. Sc. in Zoology

Under the Supervision of

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A FEW WORDS OF GRATITUDE

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Place: Kota **Neeta Sharma**

Date: 27.6.2017

(Certificate given by the Supervisor)

CERTIFICATE

I feel great pleasure in certifying that the thesis entitled "Management of *Parthenium hysterophorus* through the Establishment of Mexican beetle, *Zygogramma bicolorata* Pallister and Evaluation of Host Specificity in and around Kota Region" by Neeta Sharma under my guidance. She has completed the following requirements as per Ph. D. regulations of the University.

a) Course work as per the university rules.
b) Residential requirements of the university (200 days).
c) Regularly submitted annual progress report.
d) Presented her work in the departmental committee.
e) Published/accepted minimum of one research paper in a referred research journal,

I recommend the submission of thesis.

Date: 27.6.2017

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Candidate's Declaration

I, hereby, certify that the work, which is being presented in the thesis, entitled "Management of *Parthenium hysterophorus* through the Establishment of Mexican beetle, *Zygogramma bicolorata* Pallister and Evaluation of Host Specificity in and around Kota Region" in partial fulfilment of the requirement for the award of the Degree of Doctor of Philosophy, carried under the supervision of Dr. Surabhi Shrivastava and submitted to the University of Kota, Kota represents my idea in my own words and where others ideas or words have been included. I have adequately cited and referenced the original sources. The work presented in this thesis has not been submitted elsewhere for the award of any other degree or diploma from any Institutions. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will cause for disciplinary action by the University and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

Neeta Sharma

Date: 27.6.2017 (Name of the student)

This is to certify that the above statement made by Ms. Neeta Sharma (Enrolment No. 06/13069 and Registration No. F-6( )/Res/UOK/2013/17061-62) is correct to the best of my knowledge.

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9	**Participating certificate in International conference on Innovative Research in Science, Technology and Management, 2017.**	**ix.**
10	**Published paper "Impact of Temperature and Relative Humidity on Development of *Zygogramma bicolorata* " Published certificate in International Journal of Advanced Research in Science and Engineering, 2017.**	**x.**
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14	**Presentation certificate from Department of Entomology, Rajasthan College of Agriculture, Udaipur, 2014.**	**xiv.**

Ph. D. THESIS

Research Scholar: Neeta Sharma

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Title of the Thesis:

Management of *Parthenium hysterophorus* through Establishment of Mexican beetle, *Zygogramma bicolorata* Pallister and Evaluation of Host Specificity in and around Kota Region.

Research areas: Government College, Kota and Selected sites of Kota region for field work.

Objectives:

- **Objective-A: Bio-control of congress grass, *Parthenium hysterophorus* through *Zygogramma bicolorata* Pallister.**
- **Objective-B: Mass multiplication of *Zygogramma bicolorata*.**
- **Objective-C: Host specificity tests for *Zygogramma bicolorata* on some indigenous and economically important flora species, closely related to *Parthenium hysterophorus.***
- **Objective-D: Evaluation of Quality compost of *Parthenium hysterophorus*.**

CHAPTER-1
INTRODUCTION

INTRODUCTION:

Parthenium hysterophorus Linnaeus (Asteraceae) commonly called 'gajar ghas', 'carrot grass' (Tower and Rao, 1992), 'white head', 'star weed', 'false ragweed', 'chatak chandani', 'ramphool', 'bitter weed', is one of the aggressive, noxious (Adkins and Navie, 2006; Paudel, 2009; Dahiya and Jakhar, 2015) invasive weed that has made wide distribution, globally affecting the growth of native species (Holdman, 1981; Sushilkumar, 2009; Blackmere and Johnson, 2010; Dogra *et al*., 2011; Dutta, 2015; Khaket *et al*., 2015 and Shreshtha *et al.*, 2015).

It is a native of the area around the Gulf of Mexico and Central South America (Navie *et al*., 1996; Evans, 1997; Navie *et al*., 2004 and Qureshi *et al*., 2014).

Parthenium hysterophorus was first introduced in India due to contaminated PL-480 wheat imported from United States in the 1950s (Bennet *et al*., 1978; Srikanth and Pushpalatha, 1991; Seldon *et al*., 2005 and Lakshmi and Shrinivas, 2007) (Map- 1,2,3 and 4).

The invasion of species into the forest areas has altered the native species composition affecting the forest structure and diversity.

It is estimated that about 35 million hectares of land has been invaded by *Parthenium* in India (Seetharamiah *et al*., 1981; Chamberlain, 2004; Patel, 2011; Shabbir *et al*., 2012 and Quaik and Ibrahim, 2013) (Plate- 1).

Morphology of *Parthenium hysterophorus*:

Parthenium hysterophorus is a branched annual herb with a deep tap root and an erect stem that becomes woody with an age, growing up to 2 meter under favorable conditions. Under dry conditions, the plant may mature and set seeds at its height of only 10 cm. Normally height varies between 50-150 cm where stem is highly branched; leaves are pale green, deeply lobed and covered with fine soft hairs.

The plant starts flowering 4-8 weeks after germination and flowering continues for a long time. Small creamy white flowers occur on the tips of the numerous stem branches. Flower heads are small and numerous in open panicles. Achenes are black. Each flower contains five black seeds that are wedge shaped.

A large plant can produce up to 15,000 seeds on an average. *Parthenium* does not reproduce vegetatively; the only method of reproduction is dispersal by seeds. Buried seeds last longer than the seeds on soil surface. Seed germination depends on high moisture but is inhibited by shading and plant competition. Most germination occurs in spring to early summer. *Parthenium* is able to grow on a wide range of soil types ranging from sandy to heavy clays. The optimum temperature for seed germination is 20 - 28^0C. The whole plant cycle is completed within about five months (Kushwaha and Maurya, 2012).

P. hysterophorus, a drought resistant plant can grow in almost all soil types, but is particularly successful on vertisols (Towers, 1992 and Karim, 2012).

Firstly, it is a highly adaptable weed and can grow anywhere, invade all types of pasture lands and causes substantive losses in the yield of agriculture (Qureshi *et al*., 1980).

Secondly it creates health hazards to livelihood (Trounce and Gray, 2004).

Parthenium weed (Asteraceae) is a vigorous weed or branched herb that can grow to 2 m high (Navie *et al*., 1996).

The neotropical weed *Parthenium hysterophorus* (Asteraceae) is one of the top 10 notorious weeds in India (Joshi, 1991; Navie *et al*., 1996 and Annapurna and Singh, 2003).

Parthenium weed is known to be allelopathic (Belgeri *et al*., 2012) with root and shoot leachetes capable of reducing growth or germination of numerous crops.

Cannabis sativa, Cassia occidentalis and *Calotropis procera* are naturally growing plants which are being replaced by this weed (Khalid, 2000; Gazziero *et al*., 2006; Knox *et al*., 2010 and Kumari *et al*., 2010).

The successful spread of the weed, in part, may be attributed to these allelopathic properties (Kanchan and Jayachandra, 1980; Sharma and Bhutani, 1998; Shafique *et al*, 2005; Mersie, 2009 and Devi *et al*., 2013).

Parthenin, a glycoside, is said to produce depressant effect on nervous system (Khaket *et al*., 2012).

Several cattle disorders have also been reported. During scarcity of fodder, cattles, sheeps and goats are forced to eat *Parthenium* which can taint their meat and make diary milk unpalatable due to its irritating odor (Ahmed *et al*., 1988).

This weed causes many problems to human health, agriculture, livestock production and biodiversity (Narasimhan *et al*., 1984; Rao *et al*., 1985; Evans, 1997; Sushilkumar, 2005 and 2009 and Devarinti, 2015).

Parthenium has been considered as one of the greatest sources of dermatitis (Devarinti, 2015), fever, asthama, nasal-dermal and bronchial (Ahmed *et al.*, 1988; McFadyen, 1995; Riley *et al*., 2001; Dhileepan, 2003 and Dhileepan *et al*., 2000 and 2009) types of diseases resulting from parthenin.

A revolution for the control of *Parthenium* has been achieved through a beetle *Zygogramma bicolorata* Pallister which is an effective bio-control agent of *P. hysterophorus* (Jayanth and Visalakashy, 1994; Jayanth and Bali, 1995; McClay *et al*., 1995; Sushilkumar and Bhan, 1995; Jayanth *et al.*, 1996; Bhatia *et al.*, 2005; Sushilkumar, 2005; Javaid *et al* 2007; Dhileepan, 2009; Singh, 2009; Shrestha *et al*., 2010; Dhileepan and McFadyen, 2012; Roy and Shaik, 2013; Singh and Negeri, 2013; Bahadur *et al*., 2015 and Bezuneh, 2015).

The insect, a chrysomelid beetle, *Zygogramma bicolorata* Pallister, was introduced in South India in 1983 from Mexico (Annadurai, 1989; Krishnamurthy, 1998; Dhileepan, 2001 and 2003; Gupta *et al.*, 2004 and Singh, 2014).

Therefore, biological control trials were initiated by Indian Institute of Horticulture Research, Bangalore by importing the leaf feeding beetle

Zygogramma bicolorata Pallister (Coleoptera: Chrysomelidae) from Mexico (Dhileepan, 2001 and 2009).

In India, *Z. bicolorata* was released in Bangalore in 1984 and became abundant within 3 years after being introduced, resulting in a significant reduction in *Parthenium* plants in localized areas (Jayanth and Visalakshy, 1994 and Shreshtha, 2011).

The adult *Zygogramma* beetles feed and oviposit on *Parthenium* leaves while the newly hatched larvae feed voraciously on the terminal and auxiliary buds, leaves, stem parts etc. Five generations of *Zygogramma bicolorata* were completed in a year (Bhumannavar and Balasubramanian, 1998; Gupta and Sood, 2005; Pandey *et al*., 2013; Powar and Korat, 2013 and Sharma *et al.*, 2014).

After investigation on host specificity and biological studies (Jayanth and Bali, 1993) the beetles were released in various parts of the world suffering from *Parthenium* invasions including India (McFadyen, 1992 and 1998; Jayanth and Bali, 1995; Kuhlmann *et al.*, 1999; Shappard *et al*., 2005; Dubey *et al.*, 2010).

The practice of host specificity testing has benefited much from such basic studies but we can continue to tune testing methodology by applying the latest information and concepts (Withers, 1997; Driesche *et al*., 1999; Klinken, 1999; McFadyen and Heard, 1999; Sands *et al*., 1999; Tallamy, 1999; Futuymma, 2000; Withers *et al*., 2000 and 2009; Sushilkumar and Ray, 2010 and McConnachie, 2015).

During the present research work for testing of feeding and oviposition behavior of *Zygogramma bicolorata* (both adults and instars) a host specificity test was carried out in the experiments. Firstly choice tests were conducted in which beetle (both adults and instars) had a choice to feed and oviposit on a given host plant varieties with target plant (*Parthenium*) but secondly in non-choice tests, beetle (both adults and instars) had no choice due to absence of target plant (*Parthenium*) and only economically important plants were provided.

Thus among various control measures, the mexican beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) has been found as a biological control agent of *Parthenium* (McFadyen, 1992; Withers, 1999; Dhileepan, 2001; Kumar and Chaudhary, 2005; Carson *et al*., 2008; Visalakshy and Jayanth, 2008; Dhiman and Bhargava, 2010; Sushilkumar and Ray, 2011; Kulshreshtha and Kumar, 2013).

Vermitechnology is a bio-oxidation and stabilization of organic material involving the joint action of earthworms and microorganisms. Although microbes are responsible for biochemical degradation of organic matter, earthworms are the important drivers of the process, conditioning the substrate and altering biological activity (Rajpal *et al*., 2011).

One of the major benefits of green manures is their ability to suppress the weed (Naikwade *et al.*, 2012).

Green manuring enriches diversity and reduces the opportunities for weeds to become adapted to a particular cropping pattern. Some green manures also secrete specific chemicals into the soil that inhibit weed seed germination (Eastmen *et al*., 2001).

Earthworm species convert the waste into better end product and provide solution to the problem of organic waste degradation (Nagavallemma *et al*., 2006; Aalok *et al*, 2008; Manyuchi *et al*., 2013; Pirsaheb *et al*., 2013; Saravanan and Aruna, 2013; Abrahamson and Bertoni, 2014).

Earthworms have been long recognized by farmers as beneficial to soil (Singh, 2014) and as one of the major soil macro fauna which constitute an important group of secondary decomposers.

Earthworms are key biological agents in the degradation of organic wastes (Ndegwa *et al*., 2000 and Adhikari, 2012).

For a long time composting is applied as a biological process of organic wastes in many parts of the world and in recent decades using some species of red worms in compost process as vermicomposting makes many advantages for the process of

biological degradation of organic wastes and finally obtaining fertilizers (Rostami, 2011).

Organic wastes passing though the gut of the earthworm recycle the organic wastes which are excreted as casting or worm manure, an organic material rich in nutrients that look like fine- textured soil (Dickerson, 2001 and Yadav and Garg, 2011).

Eisenia foetida, is an organism that produces stable humus and nutrient available for the plants (Garg *et al*., 2005; Beetz, 2010; and Kushwaha and Maurya, 2012).

During the process to produce vermicompost, it is considered important to monitor the routine parameters, namely: temperature, moisture, pH and airing that predict stability, quality and the maturity of the vermicompost (Borah *et al*., 2007; Juarez *et al*., 2011; Lalitha *et al*., 2012; Manyuchi and Whingiri, 2014 and Sharma *et al*., 2014).

Integrated weed management is a weed population management system that uses all the suitable techniques in a compatible manner to reduce weed population and maintain them at levels below those causing economic injury (Maniyappa *et al*., 1980; McFadyen and Heard, 1988; Jayanth *et al.*, 1998; Singh *et al*., 2004; Javaid and Shabbir, 2006; Boyetchko *et al.*, 2009; Kumar *et al*., 2009; Palmer *et al*., 2009; Kishor *et al.*, 2010; Kumar, 2011; Upadhyay *et al*., 2011; Gnanavel, 2013; Masum *et al*., 2013; Winston *et al*., 2013; Jae *et al.*, 2014 and Knox *et al*., 2014).

Presence of *Parthenium* in different areas

Rocky land

Waste land

Agriculture land

Residential land

PLATE- 1

CHAPTER-2
REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

- The study of Bennet *et al* (1978) discussed in review about *Parthenium hysterophorus* history, ecology and biology in detail.

- Kanchan and Jayachandra (1980) studied about the allelopathic effects of *Parthenium hysterophorus.*

- Researchers Muniyappa *et al* (1980) had discussed about the comparative effectiveness and economics of mechanical and chemical methods to control of *Parthenium hysterophorus*.

- According to Qureshi *et al* (1980) a study on sub acute toxicity of *Parthenium hysterophorus* L. in goats was carried over successfully.

- Researcher Holdman (1981) had discussed about the *Parthenium* weed threatening in detail in their research paper.

- Seetharamiah *et al* (1981) study indicated that *Parthenium* plant was present in the atmosphere in significant amount as free pollen grains or in the form of clumps during the months of June to august.

- The investigations of Narsimhan *et al* (1984) indicate that fractionation of methanolic extracts of air dried aerial parts of *Parthenium* resulted in the isolation of a toxic constituent which was identified as *Parthenin,* the major sesquiteropene lactone from the weed. When *Parthenium* was given orally or by intravenous administration, radioactivity appeared.

- Rao *et al* (1985) studied about the allergy by *Parthenium* pollen.

- Jayanth (1987) discussed in his research paper about introduction and establishment of *Z. bicolorata* on *Parthenium* in Bangalore.

- Ahmed *et al* (1988) studied that a total of male, 1-year old buffaloes were given fresh chaffed *Parthenium* weed 10g/Kg body weight for 8 weeks (group1), an aqueous extract of *Parthenium* as stomach drench 10g/ Kg body weight for 8 weeks (group2) of sun-dried, powdered *Parthenium* 10g/Kg body weight for 20 weeks (group 3) with the normal diet of green fodder, paddy straw and concentrates. Buffaloes in group 2 had symptoms and lesions of acute toxicity including itching, alopecia and skin depigmentation. In week 3 erythematous eruptions occurred on the head around the ears, shoulders, neck and abdomen. In weeks 6 and 8 a total of 3 buffaloes died.

- McFadyen and Heard (1988) discussed in their studies about the host range tests in Queensland department of natural resources and CSIRO Entomology Australia.

- Nath (1988) observed that *Parthenium hysterophorus* competed with pasture species reducing pasture carrying capacity up to 90%.

- Annadurai (1989) studied the reproductive potential in terms of quantitative food utilization of *Z. bicolorata* and a gravimetric method was used to estimate quantitative food utilization. A possible (-or+) correlation was indicated between host preference in terms of chemical parameters of the host plant and changes in reproductive ability of the insect.

- Joshi (1991) in its research paper discussed about the bio-control of *Parthenium hysterophorus*.

- Srikanth and Pushpalatha (1991) showed that integrated strategies for wasteland using insects, pathogens, allelopathic plants and agroecosystem were beneficial for management of weed.

- The study of McFadyen (1992) had discussed about the biological control against *Parthenium* weed in Australia.

- Towers and Rao (1992) have investigated that *Parthenium,* a weedy species from the New World has invaded Asia, Africa and Australia. It now occupies much agricultural land in India and has been shown to be responsible for toxicity to livestock with allergy. Biological control with the use of insect herbivores such as *Z. bicolorata* has been implemented in Australia and in India.

- Jayanth and Bali (1993) had discussed about the effect of some commonly used weedicides on *Parthenium* beetle, *Z. bicolorata.*

- Jayanth and Bali (1993) while studying the life cycle of *Z. bicolorata* reported that this leaf feeding beetle extremely defoliated *Parthenium hysterophorus*.

- According to Jayanth and Visalakshy (1994) *Z. bicolorata* of Mexican origin, released in Bangalore in 1984, resulted in defoliation of *Parthenium hysterophorus* in 1988. The insect appears to have potential in reducing weed density in those parts of India having moderate weather conditions.

- Fifteen varieties of Sunflower *(Helianthus annuus* L.) were evaluated by Jayanth and Visalakshy (1994) under field conditions for their feeding attraction to the mexican beetle, *Zygogramma bicolorata*.

- Jayanth and Bali (1995) reported that full grown larvae of *Z. bicolorata* were neither capable of burrowing into dry soil for pupation nor they could pupate on the surface of the soil, although they survived up to 10 days.

- McClay *et al* (1995) in their research paper discussed about some phytophagous Arthropods associated with *Parthenium* in North America.

- The aim of a study was to estimate *Parthenium* and its effect on human health in research work done by McFadyen (1995) in Australia.

- The study of Sushilkumar and Bhan (1995) discussed control of *Parthenium* by insects in India.

- Easy method for mass rearing the *Parthenium* beetle, *Z. bicolorata* under laboratory conditions was done by Jayanth *et al* (1996).

- In the investigation of Navie *et al* (1996) biological study of *Parthenium hysterophorus* was carried over.

- The aim of Evans (1997) was to study the biological control of *Parthenium hysterophorus*.

- Withers (1997) estimated that if no-choice tests are conducted for extended period, food and oviposition site deprivation can induce broadening of host utilization in the field conditions.

- Adair *et al* (1998) discussed about the oviposition preference of *Diaprepes abbreviates* on the different physical substrates provided in laboratory conditions.

- Quantitative food utilization indices were measured by Bhummanavar and Balasubramanian (1998) in different larval instars and physiological ages of adult *Z. bicolorata* Pallister. Among the larvae and adult stages of the beetles, third instar larvae and egg laying females ingested maximum food. Various growth parameters like relative consumption rate (RCR), relative growth rate (RGR) and approximate digestion (AD) were higher in the first, third and fourth instars and egg laying females. Efficient of conversion of digested food (ECD) were maximum in first, third and fourth instar larvae and increasing trend was observed in second instar larvae.

- Jayanth *et al* (1998) found that freshly emerged adults of *Z. bicolorata* for biological control trials against the noxious weed *Parthenium* were seen to feed and lay eggs on sunflower leaves under laboratory conditions but no feeding behaviour was noticed on sunflower plant.

- A complete study related to *Z. bicolorata* preference had been done by Krishnamurthy (1998) on *Parthenium* and sunflower.

- McFadyen (1998) had discussed about the biological control of weeds in his investigations.

- Sharma and Bhutani (1998) had discussed about the amoebiciadal activity of the weed *Parthenium hysterophorus*.

- Drieshce *et al* (1999) discussed about the host specificity testing of Arthropod Biological control control agents for weeds.

- Heard (1999) discussed the means by which practitioners can incorporate these concepts into the design implementation and an interpretation of host specificity tests was discussed. Practical matters affected by these include: choice of area size and design; duration of tests; use of behavioral observations to examine the process instead of the end results and interpretation of the results of choice vs. no-choice tests, sequential versus parallel tests, and open field versus cage tests.

- According to Klinken (1999), host specificity testing can be divided into three steps: i) identification of aspects of life history that need to be host-specific if the insect is to be safe for release; ii) description of the fundamental host range of the organism; iii) if non-target species was included within the fundamental host-range, prediction of whether they will be attacked under field conditions and the frequency and severity of such attacks.

- Kuhlman *et al* (1999) had discussed about the host specificity assessment of European *Peristeness* parasitoids for classical biological control of native *lygus* species in North America.

- McFadyen and Heard (1999) discussed in their research paper that the decision to release a potential weed biological control agent into the field was based on judgment about risk of damage to non-target plants of economic or conservation valve. This risk should be balanced against the possible benefits of biological control of the target weed in Australia.

- Sands *et al* (1999) studied that before releasing exotic natural enemies for biological control of weeds, host range tests are almost universally required by authorities to ensure that agents are unlikely to have determined impacts on non-target plants. Current methods for evaluating the host range of agents for biological control of arthropod pests are discussed taking into account issues of insect taxonomy and behavior that influence testing procedures as well as some environmental and faunistic considerations that need to be considered in making decisions relating to safety or risk assessments of potential agents.

- Tallamy (1999) discussed about recent evidence from *Luperine* chrysomelid beetle and other pharmacophagous insects and suggested that evolutionary novel compounds can elicit feeding or oviposition responses when their polarity molecular configuration and stereochemistry at binding sites meet the criteria for depolarization of stimulatory input at peripheral neuroreceptors. Mechanisms for identifying plants with such compounds were also discussed.

- Withers (1999) studied that *Z. bicolorata* had a very narrow host range within the family Asteraceae and it is restricted to the sub family in which *Parthenium* occurs.

- The study of Dhileepan *et al* (2000) evaluated the impact of defoliation by *Z. bicolorata* on *Parthenium hysterophorus* from 1996 to 1998. *Z. bicolorata* caused 91-100% defoliation resulting in reduction in weed density by 32-93%, plant height by 18-65%, plant biomass by 55-89%, flower production by 75-100%, soil seed bank by 13-86% and seedling emergence in the following season by 73-90%. In all sites with continued outbreaks of Z. *bicolorata*, it

is expected that the existing soil seed bank would be minimized, resulting in reduced density by *Parthenium* in 6 to 7 years.

- Effect of defoliation by the introduced bio-control insect *Z. bicolorata* on the annual Asteraceae weed *Parthenium hysterophorus* were studied in green house and field cages. Feeding by *Z. bicolorata* caused damage to meristems, resulting in shorter height of the primary stem and changed branching pattern. Defoliation caused the plant height to be reduced by 13-56% and flower production by 25-45% and the reductions were more significant when defoliation was started at early stages of plant growth. Under water stress, defoliation caused the plant height to be reduced by 10-31%, flower production by 31-75%, leaf production by 23-51% and plant biomass by 2-9% (Dhileepan *et al* 2000).

- Futuyma (2000) reported that many clades of Herbivorous insects were remarkably conservative in the plants that they attack and in many groups, relative insects tend to feed on related plants. However, rapid evolution of host range had been documented in several species managers who contemplate introducing a host-specificity insect for biological control of a weed would like to predict whether or not the species to be introduced poses an appreciable risk that it might evolve rapidly in host range and adapt to non-target plants.

- Khalid (2000) confirmed that due to invasive capacity and allelopathic properties, *Parthenium* has the potential to disrupt natural ecosystem.

- The study of Ndegwa *et al* (2000) discussed in detail about the effect of stoking density and feeding rate vermicomposting of bio-solids.

- According to Withers *et al* (2000) *Parthenium* leaf feeding beetle *Z. bicolorata*, were tested in two different assays involving differently ranked plants. First, beetles differing in their time-dependent level of responsiveness were in two choice assays with plants in the sub tribe Ambrossinae of the Heliantheae. Second, group of beetles were tested in no-choice sequential assays alternating exposure between the highest and lower ranked plants. Then data support predictions that choice tests using insects in a non-deprived state and short duration sequential no-choice assays, will not adequately reveal the acceptability of lower ranked host plants.

- In the investigation of Dhileepan (2001) six species of insects and a rust fungus were successfully established for biocontrol of the weed *Parthenium hysterophorus* in Queensland, Australia. Effectiveness of bio-control insects was evaluated at two selected sites in Queensland during 1996-1997. The feeding beetle *Zygogramma bicolorata* caused 96% defoliation on the weed *Parthenium hysterophorus* L. evaluated at two selected sites (Mount Panorama and Plain creek in Queensland.

- Dickerson (2001) discussed about the detailed technology of vermicomposting. Composting with earthworms was an excellent technique for recycling food waste in the apartment as well as composting yard wastes in the backyard. Red worms, in vermicomposting act in a similar fashion, breaking down food wastes and other organic residues into nutrient-rich compost. Nutrient in vermicompost are often much higher than traditional

garden compost. i.e. P% (0.35), K% (0.48), Ca% (2.27), Zn ppm (128.00) of garden compost was smaller than P% (0.47), K% (0.70), Ca% (4.40), Zn ppm (2.78) of tested vermicompost.

- Eastmen *et al* (2001) discussed in their research paper about the effect of vermicomposting in human pathogen reduction for USEPA bio-solids stabilization.

- The aim of Riley *et al* (2001) was to discuss about the new record rates found in selected North American leaf beetles (Coleoptera: Chrysomelidae) in their research paper.

- Annapurna and Singh (2003) discussed in their research paper about the variation of *Parthenium* in response to soil quality.

- According to Dhileepan (2003), *Zygogramma bicolorata* and *Epiblema strenuana* were found as bio-control agents of *Parthenium hysterophorus*. Effectiveness of *Z. bicolorata* and *E. strenuana* was dependent on weather conditions and as a result had only limited impacts on the weed in three out of four years.

- A full description, habitat, distribution, germination and reproduction of *Parthenium* weed and problem created by *Parthenium* weed have been provided by Chamberlain (2004).

- Gupta *et al* (2004) suggested that foreseen consequence regarding the unlimited use of exotic beetle, *Z. bicolorata* in the absence of the native natural enemies have started adopting this exotic beetle as a prey in the ecosystem.

- Navie *et al* (2004) discussed that germinability soil seed bank was determined at two sites in central Queensland on four separate occasions between Feb. 1995 and Oct. 1996. These sites were infested with *Parthenium* weed a serious invasive exotic weed. During this period, the seed bank varied between 3282 and 5094 seeds m^{-2} at the Clermont site, and between 20599 and 44639 seeds m^{-2} at the Moolayember Creek site.

- Field experiments were conducted by Singh *et al* (2004) to evaluate control of 90 to 100 cm tall ragweed *Parthenium* in a non cropped situation in Haryana state, India, during 2000 and 2001. Atrazine, 2, 4-D ethyl ester, atrazine plus 2, 4-D, metribuzin, etc chemicals were sprayed on ragweed *Parthenium* to control.

- A brief review on control of *Parthenium* was presented in the research paper of Trounce and Gray (2004).

- Vanit *et al* (2004) studied comparison of different materials as *Helicoverpa armigera* oviposition substrates.

- Bhatia *et al* (2005) studied that *Parthenium* is a serious problem in Jammu region with the weed spreading to all the site district, adverse effect on human being, livestock, agriculture and biodiversity. The current distribution and status of this weed in Jammu region, besides effectiveness of the bio-control agent in controlling *Parthenium* has been discussed.

- Dhiman and Bhargava (2005) reported that with the biology and population dynamics of *Zygogramma* bio-control agent on *Parthenium* in Saharanpur and adjacent area of western U.P. will

arm the economic entomologists to control this obnoxious weed biologically.

- The effect of various animal wastes on growth and reproduction of an epigenic earthworm *Eisenia foetida* was studied by Garg *et al* (2005) under identical laboratory conditions. Its biomass gain, mortality, sexual maturity, cocoon production periodically for 15 weeks were also studied. The earthworms grew rapidly in cow, sheep and goat wastes.

- In an investigation of Gupta and Sood (2005) the beetle resumption of feeding during late March to June was a sign of determination of the diapause. The total duration of the generation ranged from 20-70 days during April- November. Five generations were completed in a year. Defoliation was seen by beetle on *Parthenium*.

- Kumar and Chaudhary (2005) studied on the effect of weather parameters on the population dynamics of *Z. bicolorata* on *Parthenium hysterophorus* and calculated correlations and co-efficient of determination values of *Zygogramma bicolorata* with weather parameters.

- Mahna and Sharma (2005) studied the biology of the Mexican beetle, *Z.* b*icolorata* on *Parthenium hysterophorus* and its development, fecundity, longevity and sex ratio on *Parthenium*.

- Author Seldon *et al* (2005) discussed about the preparation of compost through earthworm in the bins. Here, small-scale vermicomposting system is meant to introduce the basic principles of vermicomposting. Systems can be as simple as a stack of plastic food-storage containers or as complex as an automated unit capable of processing hundreds of pounds of organic matter daily.

- Shafique *et al* (2005) discussed that aqueous extract bioassays were conducted to evaluate the allelopathic potential of most hazardous weeds.

- Shappard *et al* (2005) had discussed about scientific advances in the analysis of direct risks of weed biological control agent to non-target plants.

- Sushilkumar (2005) discussed in research paper about the need of intensified introduction of mexican beetle *Zygogramma bicolorata* for biological control of *Parthenium* in India.

- A brief review was given by Adkins and Navie (2006) on the *Parthenium hysterophorus*. Its biology, ecology, management were also tasked in this review.

- Gazziero *et al* (2006) had discussed about Ragweed *Parthenium* cross-resistance to acetolactate synthase inhibiting herbicides.

- According to author Javaid and Shabbir (2006) during field surveys of different *Parthenium* growing areas of province Punjab from 2003-2006, in search of a natural enemy of this weed of a mexican beetle *Z. bicolorata* were found feeding on leaves of this weed in different parts of Lahore and Chhanga manga forest. However, at present the population of this beetle is not large enough to control the *Parthenium* weed effectively.

- According to Nagavallema *et al* (2006) brief description about vermicompost quality, earthworm varieties, vermicompost use, protection, biodiversity in vermicompost were investigated.

- According to Borah *et al* (2007) vermicompost samples were tested for chemical constituents. Effect of temperature and density of cocoons on the production of vermicompost units installed in the laboratory as well as in the field provided an opportunity to analysis quality assessment of the vermicompost and introduction of a three pit system of vermicomposting.

- During field survey of different *Parthenium* growing areas in the province of Punjab from 2003-2005, Javaid *et al* (2007) found severe attack of a mealy bug species in an undisturbed area in Punjab University. The mealy bugs were found feeding on leaves, stems and flower heads of *Parthenium*. The infected plant first showed symptoms of dieback and ultimately dried to death. Five other weed species namely *Boerhavia diffusa* L., *Achyranthes aspera* L., *Malvestrum tricuspidatum* A. *Graysida spinosa* L. and *Xanthium stumarium* L. were also found to be attacked by the mealy bug.

- Distribution, botanical aspects, allergenic compositae and non compositae with sesquiterpene lactones, human and animal health issues originated in investigation of Lakshmi and Srinivas (2007).

- Acoording to Aalok *et al* (2008), an innovative discipline of vermicomposting biotechnology, the breeding and propagation of earthworm and the uses of its castings has become an important tool of waste recycling the world over. In FRI, the division of Ecology and Environment has started a project on vermitechnology using the earthworm species *Eisenia foetida*.

- Research program evaluated by Carson *et al* (2008) estimated the post-release phase of bio-control programme that use insect herbivores to control invasive plant species.

- Visalakshy and Jayanth (2008) studied that *Z. bicolorata*, a biological control agent of *P. hysterophorus* was feeding leaves of *Helianthus annuus* in India, raising concerns of its host range. Based on the age grading technique it was shown that the majority of *Z. bicolorata* on sunflower plants at any time were reproductively immature. This established that *Z. bicolorata* does not pose any risk to sunflower in India.

- Boyetchko *et al* (2009) studied the current biological weed control agents- *Sclerotinia minor* for dandelion weed in Turfgrass and on other bioherbicides, Pseudomonas fluorescent for the control of green foxtail in wheat crop.

- Accordng to Dhileepan (2009), a CLIMAX model based on the current distribution of *Z. bicolorata* in India suggests that the geographic range of this agent in India and Pakistan can extend to other *P. hysterophorus* infested areas in the region. The CLIMAX model also suggests that all the Bangladesh and Sri Lanka and parts of Nepal are climatically suitable for *Z. bicolorata*.

- Dhileepan *et al* (2009) studied the new initiative in the biological control of *Parthenium*. They studied the parameters on impact of the height, density and seed production potential of *Parthenium* in Central Queensland.

- Kumar *et al* (2009) studied the bio-control potential of *Cladospoprium* Sp. (MPCL-461), against a noxious weed *Parthenium hysterophorus* L. they investigated colony diameter and sporulation capability of *Cladosporium* sp. on different media and also evaluated host range for pathogen symptoms.

- According to Mersie (2009), environment assessment was done for release of the biological control agent, *Z. bicolorata* for invasive weed, *P. hysterophorus* in Ethiopia and was modeled in 2007 using the CIMEX program.

- Palmer *et al* (2009) discussed in their research paper that Some 43 new arthropod or pathogen agents were released in 19 projects. Effective biological control was achieved in several projects with the outstanding successes being the control of rubber vive, *Cryptostegia grandiflora* and *Brindal creeper*, *Asparagus asparagoides*. Scientific developments included greater emphasis on climate matching, plant and prioritization and agent evaluation.

- Paudel, (2009) studied *Parthenium hysterophorus* L., as noxious invasive weed and focused on different control measures and their effects in the management of the weed. The weed possesses different allelochemicals, specialy sesquiterpens and phenolics, Parthenin being the chief one. The weed adversely affects crop plants and sensitive grass species. Besides it causes hay fever, dermatitis, skin infections, bronchitis and several other health impacts.

- Singh *et al* (2009) discussed about the population dynamics of Chrysomelid beetle (*Zygogramma bicolorata*) and its role in management of *Parthenium*.

- Sushilkumar (2009) discussed about the biological control of *Parthenium* in India.

- Withers (2009) identified solitary endoparasitoid of the eggs of *Zygogramma bicolorata*, a bio-control agent of the weed *Parthenium.*

- Beetz (2010) studied for entrepreneurs interested in a commercial earthworm enterprise. Where information about using the worms, usually *Eisenia foetida*, to process waste into vermicompost was studied.

- Blackmere and Johnson (2010) studied about detailed aspects of the continued successful eradication of *Parthenium* weed from New South Wales (NSW). This simple fact highlights the remarkable success of the NSW *Parthenium* eradication campaign. It also highlights that containment of weeds to one area of a contiguous land mass and eradication from other areas is possible.

- Dhiman and Bhargava (2010) discussed in detail about the bio-control efficiency of *Z. bicolorata* after its release in district Saharanpur fields.

- Dubey *et al* (2010) reported an entomopathogen *B. bassiana* for the first time from the laboratory culture by *Z. bicolorata.*

- Kishor *et al* (2010) discussed about the potential use of *Parthenium hysterophorus* in agriculture.

- Knox *et al* (2010) discussed in their research paper about the allelopathic effect of selected weeds on Bio-chemistry activity of *Parthenium*.

- The results by Kumari *et al* (2010) had clearly revealed that *Hyptis suaveolens* and *Senna uniflora* were highly effective in the

management of *P. hysterophorus*. The results further showed that the physical dominance and the ability of the competitive species to deprive *Parthenium hysterophorus* of light are mainly responsible for the decline of *P. hysterophorus*.

- According to author Shreshtha *et al* (2010) observation showed that *Zygogramma bicolorata* had already been established in some parts of southern Nepal and an interesting phenomenon of 'fortuitous biological control' of *Parthenium* is operating.

- Sushilkumar and Ray (2010) investigated that *Zygogramma* is an effective biological control agent of *Parthenium* in India. Adult diapause in soil between December to May. As a result there is delay in its effectiveness on the plant which reaches to flowering and seed production by the time the beetle is able to build up its population after emerging from diapause Therefore, a study was conducted to explore possibilities of diapause aversion by temperature regulation. Results indicate that exposure of newly emerged adults to heat treatment of 35^0C to low temperature of 10^0C could reduce diapause in *Z. bicolorata*. The low temperature can also be used as a medium for the storage of the mass reared beetles for a long time without having negative effect on their longevity and fecundity.

- Dogra *et al* (2011) had discussed about the distribution, Biology and Ecology of *Parthenium hysterophorus* as an invasive species in the North-Western Indian Himalaya region.

- According to Juarez *et al* (2011) the bibliographic review underscores vermicomposting importance via the use of diverse

sorts of organic waste to reinforcement them into the environment. The parameters considered to produce vermicompost are: pH, temperature, moisture, total solid contents, nitrogen, carbon, C/N ratio and humic acids. They discussed about the vermicomposting as a process to stabilize the organic waste and sewage sludge as an application for soil.

- According to Kumar (2011) quality management and plant protection for enhanced competiveness in Agriculture export had been done.

- Patel (2011) considered that *P. hysterophorus* weed is considered to be a cause of allergic respiratory problems, contact dermatitis, mutagenicity in human and livestock. Crop production is drastically reduced owing to its allelopathy. Also aggressive dominance of his weed threatens biodiversity. Eradication of *Parthenium* by burning, chemical herbicides, eucalyptus oil and biological control by leaf feeding beetle *Zygogramma bicolorata*, stem-galling moth, stem-boring weevil and fungi have been carried out with variable degrees of success.

- In the study of Rajpal *et al* (2011) efficiency of vermicomposting in stabilizing sludge without pre-treatment was estimated. The results of the study showed significant reduction in initial C/N ratio from an initial value of 19 to 9 for all reactors with earthworms, total organic matter reduced by 50% and pH also reduced to neutral but total nitrogen was increased upto 95% and total phosphorus of 0.76 to 1.36%.

- According to the Rostami (2011) importance of vermicomposting, physiology, method of vermicomposting, the effect of embient conditions and wastes on vermicomposting etc had been discussed.

- Author Shreshtha (2011) had explained that the occurrence and distribution of beetle *Z. bicolorata* in Nepal had been poorly documented. They monitored and mapped the occurrence of this beetle in Kathmandu valley for two years from August 2009 to September 2011. The effect of bio-controlling process is likely to be limited by short period of defoliation activity of the beetle, prolific seed production by *Parthenium* round the year, and environmental pollution.

- Sushilkumar and Ray (2011) discussed about augmentative release of larvae or adults of *Zygogramma bicolorata* to three sites severely infested with *Parthenium* at Jabalpur, India for a period of three years. The *Parthenium* plants at augmentation sites were completely defoliated in 45 and 60 days by larvae and adults respectively.

- According to Upadhyay *et al* (2011), losses caused by different pests in agriculture produce are 45% and it may be more or less equal in the case of medicinal plants. Integrated weed management increases the factor productivity, income of the farmer, quality of produce and is eco-friendly in nature.

- According to Yadav and Garg (2011) recycling of organic wastes by employing *Eisenia fetida* had been done successfully.

- A brief review was given by Adhikari (2012) on the worms composting technology its important use and some salient results obtained in the globe so far. Update of vermicomposting research studies reported that vermicompost application suppressed 20%-40% infections of insects pests e.g. aphids (*Myzus persicate*),

mealy bugs (*Pseudococcus spp.*) and cabbage white caterpillars (*Peiris brassicae*) on pepper (*Capsicum annuum*).

- Belgeri *et al* (2012) discussed about the screening of *P. hysterophorus* seedlings for its allelopathic potential.

- Bhat and Limaya (2012) vermicompost was prepared vermicompost using *Eisenia foetida*. Compost and plain soil were kept as controls throughout the study. The physicochemical parameters like pH, organic carbon, nitrogen, available phosphorus, calcium, and magnesium and chloride contents were analyzed at regular intervals, over a period of 48 days. On the 48th day the pH of the vermicompost was found to be 7, with organic carbon-10.30%, nitrogen-0.85%, phosphorus-0.15%, calcium-1.96%, magneshium-0.80%, chloride-0.30 mg/ml. The efficiency of the prepared vermicompost was studied on the three flowering plant sp. *Mirabilis Jalapa, Calendula officinalis and Clitoria ternatea* over a period of 75 days by sowing the seeds of the plants in the pots. Significant differences were observed in the plants grown in the vermicompost as compared to the plants grown in soil and compost without the vermicompost.

- Current status of *Parthenium* biological control in Australia was studied and for its control, 9 species of insects and 2 rust fungi had been introduced in at two sites in Queensland (Dhileepan and McFadyen, 2012).

- Hodek (2012) reviewed adult diapauses in the Coleoptera as a kind of supplement to the classic compendia. A polyphonic character of diapauses is a prominent feature in *C. septempunctata* and *L. decemlineata,* but has been found also other Coleoptera and in

insects generally and often generates voltinism heterogeneity within populations.

- In an investigation, Karim (2012) had discussed about the impact of *P. hysterophorus* weed on human health, livestock production and environment in detail.

- Khaket *et al* (2012) had discussed about the biochemical characterization of consortium compost of toxic weed *Parthenium hysterophorus* and *Eichhornia crassipe* and found them rich in nutrients.

- The review of Kushwaha and Maurya (2012) discussed prominent biological utilities of *Parthenium hysterophorus* as it contains several important chemical constituents mainly histamine, saponine, glucosides and triterpene (sesquiterpene) etc.

- Lalitha *et al* (2012) discussed about the *Parthenium hysterophorus* importance to increase the agriculture productivity as an economical tool.

- In feeding preference study of Malkapure *et al* (2012), there were different treatment or hosts plants tested for feeding preference of *Z. bicolorata*, where *Parthenium* was found to be most preferred host of *Z. bicolorata* and other may be stated as non preferred hosts e.g. sunflower, *Chrysanthemum*, marigold, niger, gokhari leaves etc.

- Mishra and Pathak (2012) estimated the role of physical characters of the ovipositional substrates like cloth, leaves, cellophane tape,

glass and board for preference of egg laying and viability in *Z. bicolorata* and found maximum viable eggs on cloth.

- Naikwade *et al* (2012) discussed the management of waste by composting, vermicomposting and its use for improvement of growth, yield and quality of fodder maize in agriculture.

- Shabbir *et al* (2012) had discussed in detail about the spread of *P. hysterophorus* weed and its biological control agent in Punjab, Pakistan.

- Devi *et al* (2013) discussed about the effect of allelopathic plant extracts (i.e. *Parthenium hysterophorus* and *Chromolaena odorata*) on the seed germination and seedling vigor of rice (*Oryza sativa* L.) in laboratory conditions.

- According to Gnanavel (2013) the infestation of the weed causes yield losses up to 40% in several crops and reduces forage production up to 90%. The rapid spread of *Parthenium* in India would be a bigger risk to the expansion and sustainable production of many crops. Hence, an attempt was made to review the habitats and adaptability, morphology and biology, allelopathic properties of *P. hysterophorus* and its impact on agriculture and human and animal health and management of the weed.

- Field survey experiments were conducted by Kulshrestha and Kumar (2013) during 2008, 2009 and 2010 years in different areas of Agra region to study the impact of weather parameters on incidence of *Z. bicolorata* in gajar ghas. August and September months showed highly positive significance correlation with temperature and rainfall.

- Phosphorus and potassium contents in dried vermicompost had been compared to the raw vermicompost respectively by Manyuchi *et al* (2013).

- In the review of Masum *et al* (2013) light shaded on the biology of *Parthenium* and its possible mechanisms of dispersal and dissemination. They also discussed the possible ways to manage this weed.

- From the research findings of Nishanthan *et al* (2013) control of *Parthenium hysterophorus* and its effect on the yield of tomata were estimated.

- Pandey *et al* (2013) studies was designed for investigating the existence of slow and fast developing individuals and their effect on reproductive attributes of *Parthenium* beetles, *Zygogramma bicolorata*. Under constant rearing conditions, the emergence ratio of slow and fast developers was determined to be 0.47 and 0.53, respectively.

- Pirsaheb *et al* (2013) had detailed studied in detail on the domestic scale vermicomposting for solid waste management in their research.

- In the investigation of Powar and Korat (2013) an attempt was made to study the life cycle of *Z. bicolorata* a potential bio-control agent of *Parthenium* weed while working with different aspects of the insects.

- According to Quaik and Ibrahim (2013), chemical fertilizers are still in extensive use these days, people are more aware of the

effects brought by these chemicals. Vermicomposting has been getting attention due to its environmental friendly approach. This paper reviews the common terms used for vermicomposting derived liquids and its potential in agricultural use.

- The aim of the review of Roy and Shaik (2013) was to explore the toxicological reports of *Parthenium hysterophorus.* They summarized the active compound responsible for different pharmacological properties and the effective control measures that can be implemented as well as to unravel the beneficial prospects of this weed.

- According to Saravanan and Aruna (2013) attempts had been made to find out if supplementation with probiotics such as Lacto bacillus, essential microbes and *Saccharamyces cerevisiae,* could enhance the vermicomposting process and improve nutrient levels in compost. The result is discussed in the light of enhancement of N, P and K level by supplementation with probiotics.

- Laboratory experiments were conducted by Singh and Negerei (2013) for fixing optimum dose requirement of the bio-control agents *Listronotus setopsipennis* Hustache and *Zygogramma bicolorata* for the control of *Parthenium.* The beetle was most effective feeder on *Parthenium* plant and up to 99.33% defoliation was noticed in report.

- A detailed report of FHTET (Forest Health Technology Enterprise Team) had been presented by Winston *et al* (2013) about the biological control of weeds.

- Abrahamsson and Bertoni (2014) research paper discussed about the vermicomposting in detail.

- According to Jae *et al* (2014) studies were understood to assess the phytosociology of *Parthenium* with other weeds growing in its vicinity and its control by some potent bio-agents. Studies were also undertaken to assess the chlorophyll content and percentage nitrogen of *Parthenium*. Maximum significant inhibition in chlorophyll content and percentage nitrogen was observed in shoot leacheates of *Cassia occidentalis* and *Calotropis procera*, respectively.

- Knox *et al* (2014) discussed about the phytosociology of *P. hysterophorus* and its management through some potential bio-agents.

- According to Manyuchi and Whingiri (2014) vermicomposting periods of 30-50 days, substrate quantities of 2.5-5 kg and cow dung composition of 20-40% were employed in 80.59 m * 31 m wormbins where a loading rate of 150 worms/m^2 was used for earthworm's growth in vermicomposting length of 50 mm and mass of 1 kg. Earthworm's growth in vermicomposting was promoted by increase in vermicomposting period, substrate quantity and substrate composition.

- Qureshi *et al* (2014) had discussed in their research paper about the invasive flora *Parthenium hysterophorus* in Pakistan.

- The study was conducted by Sharma *et al* (2014) with the objective to find out the efficiency of the low cost vermicomposting unit as compared to conventional units involving higher cost of construction. The experiment was laid out in a five replicated randomized block design with 4 treatments using *Perionyx*

excavates in low cost vermicomposting unit of various dimension with conventional unit as control. Among the 4 treatments T^1, T^2 and T^3 were found to be for *Parthenium* in terms of quantity of vermicompost harvested.

- Sharma *et al* (2014) discussed about the management of an aggressive weed, *P. hysterophorus* through introduction of mexican beetle *Z. bicolorata*.

- Singh (2014) investigated that The Punjab agriculture department decided to introduce mexican beetles to control the growth of *Parthenium* weed. The department called in experts from the DWSR in Jabalpur, M.P, to demonstrate the technique to the farmers in the regions.

- According to Bahadur *et al* (2015) ecological weed management strategy is to integrate the options and tools, rather than on specific control practices which are available to make the crops and cropping system unfavorable for weed and to minimize the impact of any weed that survive. Maintaining appropriate crop rotation with legume and non-legume crops, and growing of cover crop helps to suppress weeds by smothering and allelopathic effects. Further development and testing of alternative weed management practices that can be utilized along with herbicide applications must be pursued in order to make the practice sustainable and successful.

- The aim of Bezunen (2015) is mainly to explore the chemical components and antimicrobial activity of *Parthenium*. In this review the effect of *Parthenium* on environment, human being and

agriculture and major controlling practices have also been summarized.

- According to Dahiya and Jakhar (2015) the wide adaptability of *Parthenium*, its photo and thermo insensitivity and drought tolerance capacity in addition to allelopathic potential makes it a strong competitor in all habitats. Looking at the harmful effect of the weed, there is an urgent need to control the population of this weed. However, it is a big challenge because of its high regeneration capacity, production of huge amount of seeds, high seed germinability and extreme adaptability to a wide range of ecosystems.

- Devarinti (2015) discussed about common allergenic behavior and weed management measures of *Parthenium* by pollen in their research paper.

- In Malda districts mango cultivation is prime agriculture activity. Now *Parthenium* population is rapidly increased in mango fields and the people working in mango fields were suffering from several diseases. So, from this research work an attempt has been made to protect people and environment from *Parthenium* (Dutta, 2015).

- Khaket *et al* (2015) studied about the toxic weed *P. hysterophorus* with its industrial, agricultural and medicinal applications.

- Author McConnachie (2015) had discussed about the host range and risk assessment of *Zygogramma bicolorata*, a defoliation bio-control agent released in South Africa for the biological control of *Parthenium hysterophorus* weed.

- According to Paul (2015), the study was undertaken to convert vegetable waste into vermicompost. Waste of surroundings was dumped in compost pit, wooden craters then it was left for 30 days for decomposition. After that slurry of cow dung was also added into the organic bio-wastages. Then earthworms were inculcated to decompose it into manure. This process helped in recycling waste which also reduced the pressure on the surrounded dumping site.

- According to Shrestha *et al* (2015) the rapid expansion of the alien invasive *Parthenium hysterophorus* is a new agriculture and environmental problem for Nepal which is currently found in the Tarai, Siwalik and Hill region of Nepal. A CLIMAX modeling projection has shown these regions to be climatically suitable for the growth of *P. hysterophorus.* However, the fortuitorous arrival of the biological control agents *Z. bicolorata* and *Puccinia* had some impact upon the weeds growth and abundance.

- Sharma *et al* (2017) discussed in detail, about the emergence percentage and egg laying behaviour of *Zygogramma bicolorata* in favor of its mass multiplication.

- Vyas *et al* (2017) studied in their research paper about the impact of temperature and relative humudity on development of *Z. bicolorata* (a bio-control agent of *Parthenium*).

CHAPTER-3
MATERIALS AND METHOD

MATERIALS AND METHOD

1. Materials and method for objective-A:

1.1 Morphological study of *Zygogramma bicolorata* in natural conditions:

For morphological study of *Z. bicolorata* Pallister, its biology, ecology and life cycle were studied under the natural conditions in Kota region. For this purpose four sites (Govt. College Kota, Zoology Department Campus, around DCM, across the sides of Baran road and Railway Colony, Kota) were selected as investigation areas in Kota. 500 *Z. bicolorata* were released in each of the selected site.

Regular visits were made to all the selected sites throughout the year from July 2013 to June 2014. Visits were repeated in the succeeding years also (2014-15, 2015-16) for investigation. The sites were marked properly to avoid any error in the observations.

1.2 Rearing of adult *Z. bicolorata* in laboratory conditions:

For raising *Parthenium* plants in laboratory conditions, 50 gm seeds of *Parthenium* plants were collected and spread out in a series of 1 sq. m beds prepared in the selected areas at Govt. College, Kota and in some earthen pots also. Rearing was done under laboratory conditions at 27 ± 2^0C temperature and 75 ± 5% relative humidity.

1.2.1 Oviposition cages –

Adults of *Z. bicolorata* were released at the rate of 5 pairs in wide mouth clear plastic jars of 2 liter capacity, with brass wire-mesh windows on their lids for aeration. Bouquets of *Parthenium* twigs were kept in the clear plastic containers, for adult feeding and oviposition.

1.2.2 Larval rearing cages –

After oviposition by females the bouquets of *Parthenium* leaves with eggs were collected out every 2-3 days and fresh leaves were placed inside the oviposition cages and the leaves with eggs were kept safe for placement in larval rearing cages.

In the larval rearing cages, *Parthenium* plants in the pre-flowering stage were implanted in net covered plastic containers (15*10 cm) and fish net covered plastic tubs (40*40*25 cm). One long stick was also installed in the centre of tubs for lifting the surface of net.

Eggs were collected with the help of "0.5" point brush from the leaves taken out from oviposition cages and were placed in between the plants in plastic containers. Thus about 50 eggs were introduced into each cage container. The newly hatched larvae fed initially on the leaves on which eggs were laid or placed and later they moved on to the healthy plants. Fully grown larvae were collected with the help of "0.5" brush and released into pupation cages.

1.2.3 Pupation cages –

Pupation cages were similar to oviposition cages. Before releasing the larvae, enough water was added to moisten the full soil bed. A twig of *Parthenium* was provided inside for completion of feeding, if required. After completion of pupation period, the emerged adults were collected out and used for succeeding studies (month wise).

1.3 Diapause percentage of released *Z. bicolorata* in laboratory conditions:

For this experiment at the rate of 50 adults were released in each diapause cage (20*15 cm, six cages). Diapause cages were similar to pupation cages, with a bed of moist soil at the bottom. Known numbers

(50) of adults were released into each of these cages, along with *Parthenium* leaves and retained for five days. The number of adults that had entered the soil for diapause during period was determined by counting the ones left out alive and dead on the soil surface.

1.4 Defoliation of *Parthenium hysterophorus* by *Z. bicolorata* (in field conditions):

Experiments were set up to calculate defoliation of *Parthenium* plants by *Z. bicolorata*. Four different sites were selected (Site "A"- Akashwani, Site "B"- Borkheda, Site "C"- selected areas of Baran road and Site "D"- Govt. College, Kota were selected sites). After counting the number of *Parthenium* plants per meter square in the selected sites, 50 beetles were released in each site.

2. Materials and method for objective – B:

2.1 Mass multiplication of *Z. bicolorata* was done under laboratory conditions at 27 ± 2^{0}C temperature and 75 ± 5% relative humidity in insect rearing cages.

2.1.1 Study of the pupation of instars and adults emergence:

The experiments to notice the adult *Zygogramma bicolorata* emergence percentage were conducted in five net covered containers (15*10 cm). For feeding the instars, bouquets of *Parthenium* leaves were introduced in sufficient amount in the net covered containers. In each container ten 4th instars larvae were released and to make soil spongy for pupation purpose, sufficient amount of water was also been sprinkled in the soil at regular intervals.

2.1.2 Egg laying behavior of female on selected physical substrates:

For more accuracy, the experiments related to egg laying behavior of *Z. bicolorata* were conducted in replicate of five.

In each plastic jar of five replicates, a muslin cloth and a glass sheet were kept as physical substrates for egg laying. Along with physical substrates *Parthenium* bouquets were also kept. Simultaneously on one *Parthenium* bouquet upper surface of leaves was covered with cellophane tape so that lower surface remains available for egg laying. Similarly, in another bouquet of *Parthenium* lower surface of the leaves was covered with cellophane tape where upper surface was exposed for oviposition.

In these experiments five pairs of 5 days old male and female beetles, were released to know the preference of the physical substrates and *Parthenium* for egg laying for 48 hours.

3. Materials and method for objective – C:

3.1 Host specificity tests in field conditions:

Various indigenous and economically important plants of different seasons were selected in choice and non choice tests to observe the food and oviposition preference of the beetle, *Zygogramma bicolorata*.

Indigenous and economically important plants like *Oryza sativa, Vigna radiata, Vigna mungo, Abelmoschus esculentus, Zea mays, Sorghum bicolor, Glycine max, Thevetia peruviana, Tagetes erecta* and *Helianthus petiolaris* were grown in selected research fields (some selected areas of Borkheda).

The selected sites were marked properly to avoid any kind of error in the observations.

3.1.1 Choice tests with *Parthenium*:

The behavior of beetles whether in the presence of *Parthenium* they were feeding or ovipositing on other host plants, was also investigated.

For choice test, two separate fields were selected and in each field 10 plants of *Parthenium hysterophorus* were established with each indigenous and economically important plant species (10 plants in number) separately. Thus 10 plants of *Parthenium* were grown with 10 plants of known species, covered with nylon net, to avoid mexican beetle flying behavior.

Before covering with net, 10 pairs of beetle (*Zygogramma bicolorata*) were released for observation of feeding and oviposition behavior. In another field twenty 2nd instar larvae were placed near *Parthenium* plants and indigenous and economically important plant species to notice only feeding behavior on host plants.

3.1.2 Non-choice tests without *Parthenium*:

Host specificity tests for non- choice were also conducted in the same manner as mentioned in the choice test, without taking *Parthenium* plant.

During non-choice tests only indigenous economical plants were sown in the area where no *Parthenium* was implanted. Then 10 pairs of *Z. bicolorata* were released for observation of feeding and oviposition behavior of the beetle. In another field with indigenous plants without *Parthenium*, twenty 2^{nd} instar larvae were released to notice only feeding behavior of instars.

The choice and non-choice tests for host specificity were set up for 48 hours.

3.2 Host specificity tests in laboratory conditions (Feeding preference of *Z. bicolorata* on different hosts in laboratory conditions):

The present investigation on the feeding preference of larvae and adults of test insect *Z. bicolorata* on different hosts (11 selected species) were carried out under laboratory conditions.

Oryza sativa, Vigna radita, Vigna mungo, Abelmoschus esculentus, Zea mays, Sorghum bicolor, Glycine max, Thevetia peruviana, Tagetes erecta, Helianthus petiolaris and *Parthenium hysterophorus* as some host plants were taken for feeding preference of host insect.

The equipments and materials used were digital balance, plastic containers of 11*14 cm (for 1^{st} instars) and 15*10 cm (for 2^{nd}, 3^{rd}, 4^{th} instars) with a lid having plastic mesh windows.

1^{st}, 2^{nd}, 3^{rd}, 4^{th} instars and adults (*Z. bicolorata*) were transferred to plastic containers for recording the observations on feeding potential of *Z. bicolorata* beetles. 5 plastic containers were taken. In each container ten numbers of different stages of *Zygogramma* were transferred, offering fresh leaves (weight 1000 mg) of host plants (HP_1 - HP_{11}) to mexican beetle. Extra weights of leaves were cut by scissors (The twigs were

placed in water containing Petridish for keeping them fresh). The experiments were set up for 48 hours.

4. Materials and method for objective – D:

4.1 Vermicomposting with and without *Parthenium*:

4.1.1 Vermicompost preparation (Including *Parthenium*)-

Parthenium hysterophorus (Asteraceae) plants were collected from the sites of Baran road and Govt. College Kota for vermicomposting.

10 kg *Parthenium* plants (soft stem part) were mixed with 15 kg cow dung and were kept for initial degradation into the cemented tanks measuring 4.5*2.5*3 feet.

The cemented tanks were covered with gunny bags and water was sprinkled every day. The stirring of the material in the cemented tank was carried out every day to remove methane gas and the other gases from the tank. The initial degradation was carried out for 4 days. On fifth day approx 500 earthworms (*Eisenia foetida*) were released in each tank. Everyday water was sprinkled in the tank. After 45-50 days, watering was discontinued and the tank was observed for 5-6 days.

The completed decomposed biomass was sieved to separate earthworms from vermicompost. This was considered as vermicompost for further analysis and efficiency study. Samples were taken at regular intervals for chemical analysis.

4.1.2 Vermicompost preparation without *Parthenium*-

Vermicompost (Cow dung and earthworms) was prepared by the same method as above but here *Parthenium* was not used.

4.2 Experiments to investigate the effect of quality compost on the growth of some selected plant species:

Six earthen pots were taken and three types of seeds were sown in 2 pots each (*Helianthus petiolaris, Calendula officinalis* and *Tagetes erecta*). Then *Parthenium* based vermicompost was added in one pot and only cow dung vermicompost in another pot. The process was done for all the three species. All the experiments were done in triplicate.

CHAPTER-4
OBSERVATION

OBSERVATIONS:

1 Observations of objective- A:

1.1 Life cycle of *Zygogramma bicolorata* in natural conditions:

Egg-

In the present research study from 2013-15, it was noticed that *Z. bicolorata* laid eggs on lower surface of old and new leaves of *Parthenium,* but occasionally the eggs were also found on stem, flowers and upper surface of leaves (Plate- 2).

Female laid eggs singly or in a group of 2 to 8. The eggs were light yellow during winter and summer season but light orange during rainy season. It was also observed during research work that yellowish and orange eggs turned slightly reddish in color just before hatching.

The eggs were cylindrical 1.18 - 1.20 mm long. The number of eggs was very less during extreme hot and cold conditions (Plate- 3).

Instars-

During favorable conditions, 80.58-89.32% instars emerged out but during extreme cold and hot conditions, the percent was only 4-16%.

In the present research work newly emerged instars were light yellow in color and later changed into white creamy instars. *Zygogramma bicolorata* had four instar stages (Plate- 4, 5 and 6).

In case of first instar larvae, a faint line located centrally on dorsal side of larval body was visible while body segments were not easily distinguishable. All the body segments were distinguishable in second instars.

Third and fourth instar larvae could be easily differentiated from the earlier instars by their spiracles. Nine pairs of spiracles were seen on the thoracic and abdominal region of the third instars larvae.

A set of six, black, button like spots were clearly observed on each lateral side of head. These spots were situated just below the base of antennae. Instars had three pairs of thoracic legs. Larval body in these instars was more curved than the earlier instars (1st and 2nd instars).

Fourth instar larvae were found to be similar in appearance as the third instars, except in size. Larval body became more convex in this stage.

Instars fed voraciously on *Parthenium*. Observations indicate that instar stages existed for 9-13 days. During metamorphosis, instars stopped the feeding for some time and penetrated in the soil.

Pupa-

The fully developed instars (4th instar) made pupal chambers at about 1 cm to 6 cm level of soil under the ground for their metamorphosis. The instars, after entering the pupal chamber sealed the holes made by themselves. The metamorphosis was observed in pupal chambers only. Adults were emerged after 8-13 days of pupation, which came out by making holes in the soil (Plate- 2).

Adults-

Adults were elongated and oblong in shape. Newly emerged adult's body was slightly black and soft. After 5-7 days of emergence, dorsal part of adults became black with white patches which were also tough in structure (Graph- 1, 2 and Plate- 7, 8 and 9).

During observation it was noticed that newly emerged beetles didn't fly but after few days they started flight behavior from one place to another for feeding and mating.

Present research indicates that males were smaller in size than females, which helped in to be identified on the basis of gender of the beetle *Zygogramma*.

Longevity-

During the present research work it was found that the average longevity of adult males range 69 to 77 days which was less than female range (78 to 84 days).

Fecundity-

During observation it was found that after 4-5 days of emergence, males and females started copulation. In a day they mated many times. After coitus, females started eggs laying on the lower surface of *Parthenium* leaves (Plate-10).

Female laid eggs many times in a day. Single female laid 40-45 eggs in one day.

Diapause-

In the present research work it was observed that during unfavorable conditions like extreme hot and cold and humidity (months of May to June and January to February) beetles were diapaused for their survival. (Table- 1, 2, 3 and 4).

1.2 Rearing of adult *Z. bicolorata* in laboratory conditions:

In the present research work observations indicated that in wide mouth clear plastic jars of 2 liter capacity 412 eggs were collected within 2-3 days laid by 5 pairs of beetles. 50 eggs were introduced into each of the larval cages (15*10 cm). In all cages *Parthenium* plants in the pre-flowering stage were implanted.

It was recorded that newly hatched larvae (332-368 instars) fed initially on the leaves on which eggs were laid and later moved on to the healthy plants in the containers. Fully grown instars (4^{th} instars) were collected and released into pupation cages.

In the pupation cages a twig of *Parthenium* was provided inside for completion of feeding, if required.

It was observed during the present research work that after 12-13 days of pupation 235-267 adults emerged out (Plate- 11).

1.3 Diapause percentage of adult *Zygogramma bicolorata* in laboratory conditions:

Experiments were conducted to observe the diapause behavior of *Z. bicolorata* in laboratory conditions. In the observations it was studied that after releasing 50 beetles in each cage (total 6 cages), adult *Z. bicolorata* going in the diapause condition during January and February months ranged from 76 to 88%. It was also observed that the beetles diapaused in two ways. Some of the beetles penetrated the soil and diapaused inside while some beetles stopped the metabolic activities at the surface of soil and diapaused above the soil surface. *Z. bicolorata* was observed to remain in diapaused condition for about 65-78 days (Plate- 12).

Observations also showed that at the surface of the soil the dead adult beetles ranged from 8-24%. It may be due to completing their full age.

Observations indicate the inability of adult *Zygogramma bicolorata* to consume *Parthenium hysterophorus* during diapause period (during unfavorable conditions). These conditions of the beetles favor the growth of *Parthenium* which flourishes to a great extent during this period and becomes havoc for the environment in field conditions (Table- 5, Graph- 3 and 4).

1.4 *Parthenium hysterophorus* defoliation affected by *Z. bicolorata* at selected sites:

After carrying out the defoliation experiments observations were noticed on the basis of defoliation percentage of *Parthenium* at selected sites (A, B, C and D).

50 beetles (adult *Zygogramma bicolorata*) were released in each selected field (Site "A"- Akashwani, Site "B"- Borkheda, Site "C"- selected areas of Baran road, Site "D"- selected areas of Govt. College, Kota) and defoliation was observed after 20 days of releasing the beetles. Beetles fed voraciously on *Parthenium* in all the four selected sites. Maximum defoliation (83.69%) of *Parthenium* was noticed at selected sites "D", which showed as better consuming percentage by mexican beetle, *Zygogramma bicolorata* in the favorable months (September to December). In the present observation it was also found that mexican beetle voraciously fed firstly on soft new leaves of *Parthenium* and later on old leaves, followed by stem parts etc. Feeding on leaves, this beetle stopped photosynthesis activity of a plant which later caused stop in metabolic activities due to absence of food in the plant and at last caused death of *Parthenium* plants (Table- 6, Graph- 5 and Plate- 13).

Life cycle of *Zygogramma bicolorata*

Egg

1st Instar

Mating

2nd Instar

Female beetle

3rd Instar

Male beetle

4th Instar

Pupation

PLATE- 2

Eggs of *Zygogramma bicolorata*

Winter and summer season

Rainy season

PLATE-3

Different instar stages of *Zygogramma bicolorata*

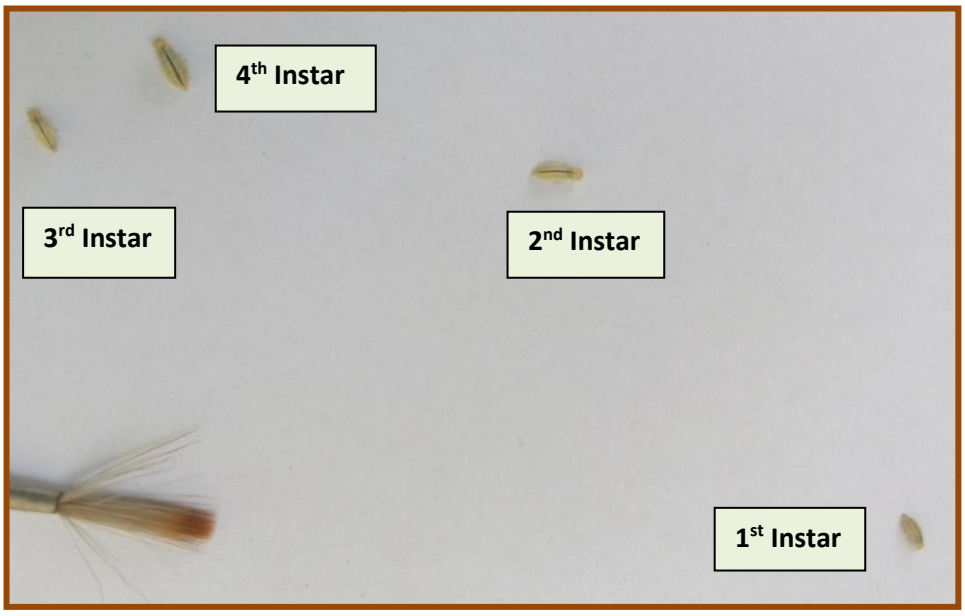


PLATE-4

Comparison between 2nd and 3rd instars of *Z. bicolorata*

PLATE-5

Comparison between 1st and 4th instars of *Z. bicolorata*

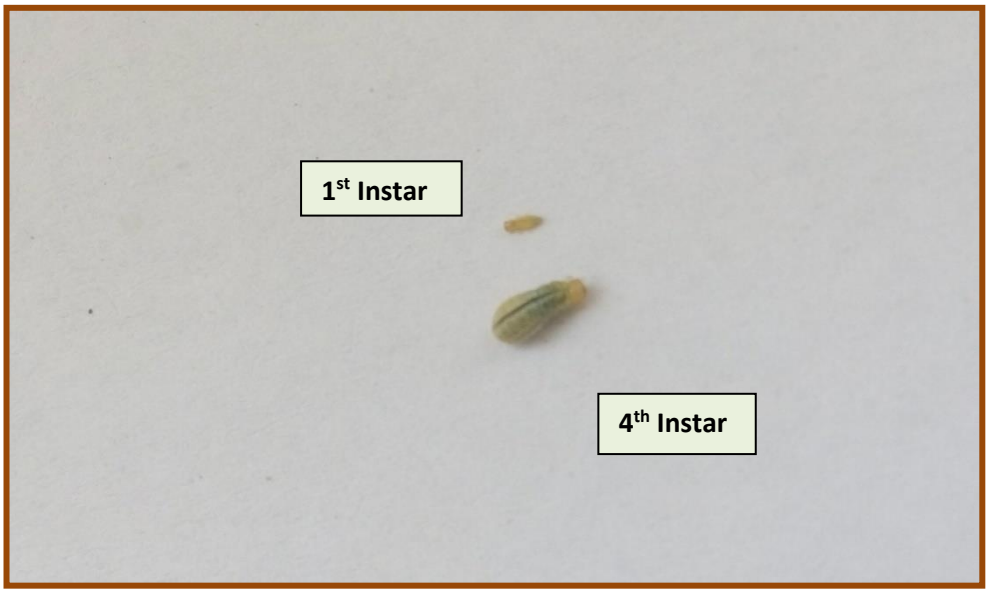


PLATE-6

Different views of *Zygogramma bicolorata*

PLATE-7

Z. bicolorata at old stage


PLATE-8

Excreta of *Z. bicolorata*


PLATE-9

Mating behavior of *Z. bicolorata*




PLATE-10

Rearing of *Z. bicolorata*






PLATE-11

Inactive diapaused and dead adults of *Z. bicolorata*

PLATE-12

Defoliation of *Parthenium* by *Z. bicolorata*

PLATE-13

2 Observation-B:

2.1.1 Mass multiplication of *Zygogramma bicolorata* during laboratory conditions:

2.1.2 Study of the pupation of instars and adult emergence:

In the observations regarding pupation and adult emergence percentage, the number of pupated instars (4^{th} instar) was 45 (90%) from number of originally released 50 larvae (100%). The number of total emerged adults (*Z. bicolorata*) was 71.11%, the percentage of females (46.66%) was higher than emerged males (24.44%). Hence in the observation it was seen that the number of female emergence was higher than male which was experimentally profitable to environment as greater number of females lay eggs in larger number. Thus mass multiplication of *Zygogramma bicolorata* resulted in high defoliation of *Parthenium* (Table- 7, Graph- 6 and 7 and Plate- 14).

2.1.3 Egg laying behavior of female on selected physical substrates:

Observations were carried out for the most proferred physical substrates for egg laying in the laboratory conditions. Four physical substrates like muslin cloth, upper surface of *Parthenium* leaf covered, lower surface of *Parthenium* leaf covered and glass plates were taken. The eggs were also laid hafazardly on the inner wall of plastic container. This number of eggs was also added with the number of eggs on different substrates to find out the egg laying capacity of female during its life time.

Among the physical substances, the highest egg deposition by the beetle was observed on muslin cloth surface (76.80 eggs/ female/ 48 hours) due to the roughness. Female beetles easily attached their eggs on rough surface of muslin cloth.

During observation second priority of egg laying of female *Z. bicolorata* was upper surface covered leaf of *Parthenium* (41.40 eggs/ female/ 48 hours) in which female laid eggs on lower surface of *Parthenium* leaves because generally adults of *Z. bicolorata* laid their eggs on the lower surface of *Parthenium* leaves for saving their eggs from predators and direct contact of sunlight. Due to direct contact with sunlight the eggs may dessicate. 3rd priority on lower surface covered leaves of *Parthenium* (19.80 eggs/ female/ 48 hours). Next 4th and 5th priority observed for egg laying were plastic jar surface (18.8 eggs/ female/ 48 hours) and on glass plates (5.40 eggs/ female/ 48 hours).The experiments for observation of egg laying behavior of female on selected physical substrates were replicated five times for getting more accurate values through ANOVA test (Table- 8, Graph- 8 and Plate- 15).

4th Instars ready for pupation and adult emergence of *Z. bicolorata*

Before going in pupation instar feeding sufficient amount of *Parthenium*

Adult emerged out after pupation

PLATE-14

Egg laying behavior of *Z .bicolorata* and boxes used during experiments

PLATE-15

3 Observations of objective- C:

3.1 Host specificity tests in field conditions:

Host specificity tests were conducted with 11 plant species belonging to five families. Observations were taken to choice (*Parthenium* with indigenous and economically important plant species) and non-choice (indigenous and economically important plants without *Parthenium*) tests which had been carried out during research work in field conditions.

But during observations in choice tests it was noticed that *Zygogramma bicolorata* showed host specificity on the basis of feeding and oviposion only towards *Parthenium hysterophorus* and no other plant was fed or oviposited by mexican beetle except that this leaf feeding beetle laid some eggs on *Helianthus petiolaris* but no feeding was noticed on this plant.

Other experiments were conducted for non-choice tests where mexican beetle *Z. bicolorata* in the absence of *Parthenium* plant did not feed on any other host plant but some eggs were laid on *Glycine max, Tagetes erecta* and *Helianthus petiolaris* leaves but no feeding was reported on these plants (Table- 9, Plate- 16 and 17).

3.2 Host specificity tests in laboratory conditions (Food preference by the instars and adult *Z. bicolorata* in laboratory conditions):

For testing food preference of *Zygogramma bicolorata* (adults and instars) verities of leaves were offered to the *Z. bicolorata* e.g. *Oryza sativa, Vigna radiata, Vigna mungo, Abelmoschus esculentus, Zea mays, Sorghum bicolor, Glycine max, Thevetia peruviana, Tagetes erecta, Helianthus petiolaris* and *Parthenium hysterophorus*. But in the observations report feeding behavior was reported only on *Parthenium hysterophorus* leaves by *Zygogramma bicolorata* (adults and instars) and no other host plants leaves were damaged by the beetle.

During observation containers containing different stages of *Z. bicolorata* fed voraciously on *Parthenium* but maximum food was consumed by 3rd instars, which was followed by 4th instars and then feeding behavior was shown by 2nd instars while the adults consumed the least food (*Parthenium* leaves).

Observations indicated that *Z. bicolorata* show host specificity towards *Parthenium* plants only and specially its leaves (Table- 10 and Graph- 9).

Selected varieties for host specificity test

HP_1- *Oryza sativa*

HP_2- *Vigna radiata*

HP_3- *Vigna mungo*

HP_4- *Abelmoscus esculentus*

HP_5- *Zea mays*

HP_6- *Sorghum bicolor*

PLATE-16

HP_7- *Glycine max*

HP_8- *Thevetia peruviana*

HP_9- *Tagetes erecta*

HP_{10}- *Helianthus petiolaris*

HP_{11}- *Parthenium hysterophorus*

PLATE-17

4 Observations of objective- D:

4.1 Vermicomposting with and without *Parthenium*-

Observations were held on to find out quality of prepared N, P and K values in *Parthenium* based cowdung vermicompost as compared with only cow dung vermicompost.

During observations it was seen that *Parthenium* plant was completely decomposed by the earthworms (*Eisenia foetida*) and this earthworms species develops humus layer on upper surface of the soil. During quality testing of prepared vermicompost, it was indicated that the values of Nitrogen and phosphorus were maximum in prepared *Parthenium* based vermicompost in comparison to cow dung based vermicompost. Only the value of K was lower in *Parthenium* based vermicompost.

Observations indicated good values of N, P and K in *Parthenium* based vermicompost which was suitable for environment (Table- 11, Graph- 10 and Plate- 18 and 19).

4.2 Quality testing of prepared vermicompost (with and without *Parthenium*) on the growth of different plant species:

For testing of prepared vermicompost on different plant species, observations were conducted for the effect of *Parthenium* based vermicompost and cow dung vermicompost on three different plant species.

Growth was observed after providing *Parthenium* based vermicompost to three mentioned plants e.g. *Helianthus petiolaris, Calendula officinalis* and *Tagetes erecta* in comparison to providing cow dung vermicompost to the selected plant species (Table- 12, Graph- 11 and Plate- 20).

Eisenia foetida used for preparing vermicompost

PLATE-18

Vermicompost prepared in iron net covered tanks

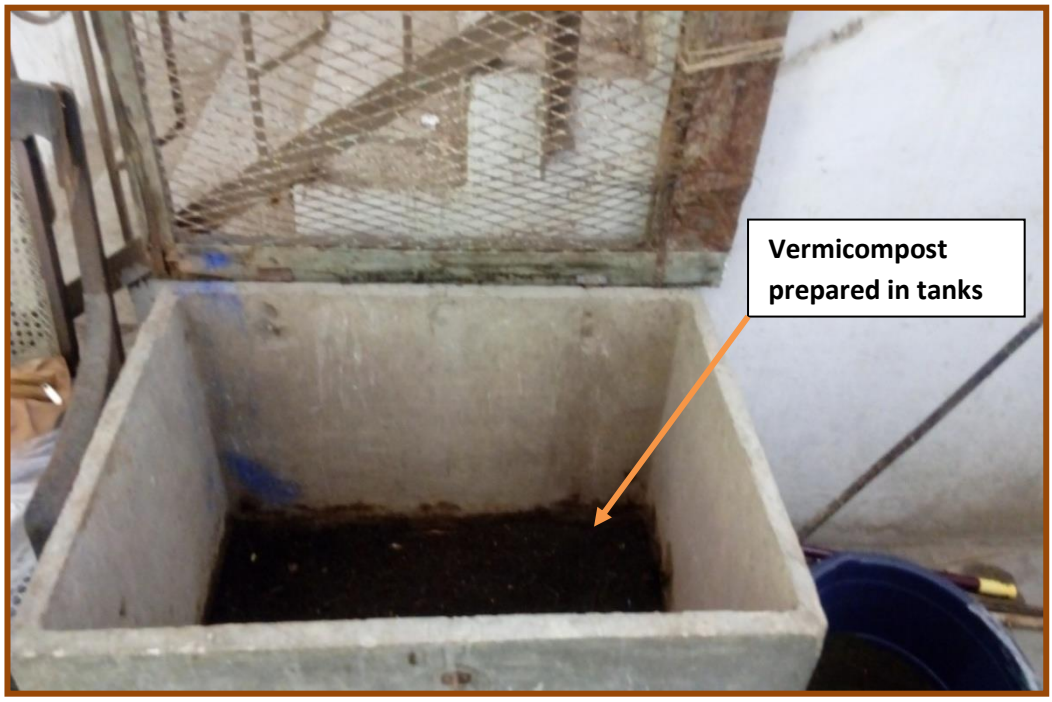


PLATE-19

Selected plants for quality testing of prepared *Parthenium* vermicompost


Helianthus petiolaris


Tagetes erecta

Calendula officinalis **PLATE-20**

OBSERVATION TABLES

Table-1

Life cycle of *Zygogramma bicolorata* in natural conditions during 2013

months	Duration in days						
	Hatching period	1st instars	2nd instars	3rd instars	4th instars	Pupation period	Total development period (Hatching period to adult emergence)
30. 12. 2012 to 2. 3. 2013	-	-	-	-	-	-	-
3. 3. 2013 to 29. 4. 2013	7	3	3	3	3	13	32
30. 4. 2013 to 2. 7. 2013	-	-	-	-	-	-	-
3. 7. 2013 to 31. 8. 2013	6	3	2	4	3	10	28
1. 9. 2013 to 30. 10. 2013	6	2	2	3	4	9	26
1. 11. 2013 to 28. 12. 2013	7	3	3	3	4	11	31

Table-2

Life cycle of *Zygogramma bicolorata* in natural conditions during 2014

months	Duration in days						
	Hatching period	1st instars	2nd instars	3rd instars	4th instars	Pupation period	Total development period (Hatching period to adult emergence)
29. 12. 2013 to 1. 3. 2014	-	-	-	-	-	-	-
2. 3. 2014 to 27. 4. 2014	6	3	3	3	3	12	30
28. 4. 2014 to 3. 7. 2014	-	-	-	-	-	-	-
4. 7. 2014 to 31. 8. 2014	6	3	2	3	3	10	27
1. 9. 2014 to 30. 10. 2014	5	2	2	2	3	8	22
1. 11. 2014 to 26. 12. 2014	7	3	3	3	4	11	31

Table-3

Life cycle of *Zygogramma bicolorata* in natural conditions during 2015

months	Duration in days						
	Hatching period	1st instars	2nd instars	3rd instars	4th instars	Pupation period	Total development period (Hatching period to adult emergence)
27. 12. 2014 to 5. 3. 2015	-	-	-	-	-	-	-
6. 3. 2015 to 26. 4. 2015	6	3	3	3	3	12	30
27. 4. 2015 to 1. 7. 2015	-	-	-	-	-	-	-
2. 7. 2015 to 31. 8. 2015	6	3	3	3	3	10	28
1. 9. 2015 to 30. 10. 2015	4	2	2	2	3	8	21
1. 11. 2015 to 31. 12. 2015	7	3	3	3	3	11	30

Table-4

Life cycle of *Zygogramma bicolorata* in natural conditions during 2013-15 as an average

months	Duration in days						
	Hatching period	1st instars	2nd instars	3rd instars	4th instars	Pupation period	Total development period (Hatching period to adult emergence)
Jan-Feb	-	-	-	-	-	-	-
Mar-Apr	6.33	3	3	3	3	12.33	30.66
May-Jun	-	-	-	-	-	-	-
Jul-Aug	6	3	2.33	3.33	3	10	27.66
Sep-Oct	5	2	2	2.33	3.33	8.33	23
Nov-Dec	7	3	3	3	3.66	11	30.66
Total mean	6.08	2.75	2.58	2.91	3.24	10.41	27.99
Standard deviation	0.83	0.5	0.5	0.41	0.31	1.68	3.61

Table-5

Diapause percentage of adult *Zygogramma bicolorata* in laboratory conditions

S.no	Dates of observation after 8 days of release	No. of adults released (b)	No. of diapaused adults penetrated in the soil (c)	No. of inactive adults at the surface of soil (d)	No. of dead adults at the surface of soil (e)	Total percentage of adult diapause (c+d)*100\b
1	2/1/14	50	18	26	6	88%
2	10/1/14	50	21	19	10	80%
3	18/1/14	50	27	11	12	76%
4	27/1/14	50	30	15	5	90%
5	3/2/14	50	28	18	4	92%
6	11/2/14	50	24	20	6	88%

Table- 6

Defoliation of *Parthenium hysterophorus* affected by *Zygogramma bicolorata* at selected sites (in the year 2014)

S.no	Selected sites	Duration of defoliation in *Parthenium* (in days)	No. of beetles (both sexes) released in a selected field	No. of plants (*Parthenium*) present in a selected sites	No. of remaining *Parthenium* plants (undefoliated)	Percent of defoliated *Parthenium* in selected sites
1	A	20	50	386	138	64.24%
2	B	20	50	320	134	58.12%
3	C	20	50	202	72	64.35%
4	D	20	50	233	38	83.69%
Total		80	200	1141	382	66.52%

Selected sites:

Site-A: Selected areas of Akashwani

Site-B: Selected areas of Borkheda

Site-C: Selected areas of Baran road

Site-D: A part of Govt. College Kota Campus

Table-7

Pupation and adult emergence percentage of *Zygogramma bicolorata* during laboratory conditions in the year 2015

S.no	Date	No. of released larvae	No. of pupated 4th instar	Adult emergence		
				Female	Male	Total
1	1/9/15	10	10	6	4	10
2	3/9/15	10	10	5	3	8
3	5/9/15	10	8	4	3	7
4	8/9/15	10	10	4	1	5
5	10/9/15	10	7	2	-	2
Total		50	45	21	11	32
%			90%	46.66%	24.44%	71.11%

Table-8

Egg laying behavior of 5 females of *Z. bicolorata* on different substrates

Physical substrates	1st investigation (no. of eggs laid after 48 hours)	2nd investigation (no. of eggs laid after next 48 hours)	3rd investigation (no. of eggs laid after next 48 hours)	4th investigation (no. of eggs laid after next 48 hours)	5th investigation (no. of eggs laid after next 48 hours)	Total no. of eggs laid in 10 days (240 hours)	Mean of eggs laid/ female/ 48 hours
Muslin cloth	78 eggs	85 eggs	52 eggs	97 eggs	72 eggs	384 eggs	76.80
Upper surface of *Parthenium* leaf covered	41 eggs	44 eggs	29 eggs	56 eggs	37 eggs	207 eggs	41.40
Lowered surface of *Parthenium* leaf covered	24 eggs	31 eggs	26 eggs	18 eggs	-	99 eggs	19.80
Glass plates	15 eggs	3 eggs	9 eggs	-	-	27 eggs	5.40
Eggs laid hafazardly on plastic jar surface	20 eggs	17 eggs	15 eggs	23 eggs	19 eggs	94 eggs	18.80

Table- 9

Host specificity on the basis of choice and non-choice situations provided to *Zygogramma bicolorata* showing feeding and oviposition

Name of Host plants (HP)	Common name	Family	Choice test (with *Parthenium*)				Non-choice test (without *Parthenium*)			
			Feeding		Oviposition		Feeding		Oviposition	
			A/I	A/I	A	A	A	I	A	A
			Host plants	*Parthenium*	Host plants	*Parthenium*	Host plants		Host plants	
HP1 *Orya sativa leaves*	Rice	Poaceae	0	+	0	+	0		0	
HP2 *Vigna radiata* leaves	Urad	Fabaceae	0	+	0	+	0		0	
HP3 *Vigna mungo* leaves	Mung	Fabaceae	0	+	0	+	0		0	
HP4 *Abelmoschus esculentus* leaves	Bhindi	Malvaceae	0	+	0	+	0		0	
HP5 *Zea mays leaves*	Maize	Poaceae	0	+	0	+	0		0	
HP6 *Sorghum bicolor* leaves	Jowar	Poaceae	0	+	0	+	0		0	
HP7 *Glycine max* leaves	Soyabean	Fabaceae	0	+	0	+	0		+	
HP8 *Thevetia peruviana* leaves	Kaner	Apocynaceae	0	+	0	+	0		0	
HP9 *Tagetes erecta* leaves	Genda	Asteraceae	0	+	0	+	0		+	
HP10 *Helianthus petiolaris* leaves	Surajmukhi (Small)	Asteraceae	0	+	+	+	0		+	
Probability			0	1	0.1	1	0		0.3	

HP- Host Plant

A- Adult

I- Instar

Table-10
Host specificity under choice tests

Mean weight of consumed food by the instars and adult *Z. bicolorata*

Host plants (HP)	Weight of fresh food offered (mg)	Weight of consumed food (mg/day/10 individual)				
		1st instars	2nd instars	3rd instars	4th instars	Adults
HP_1 *Orya sativa* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_2 *Vigna radiata* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_3 *Vigna mungo* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_4 *Abelmoschus esculentus* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_5 *Zea mays leaves*	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_6 *Sorghum bicolor* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_7 *Glycine max* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_8 *Thevetia peruviana* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_9 *Tagetes erecta* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_{10} *Helianthus petiolaris* leaves	1000 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg	0.00 mg
HP_{11} *Parthenium hysterophorus* leaves	1000 mg	928.068 mg	944.152 mg	991.196 mg	971.889 mg	870.07 mg

Table-11

Vermicomposting

Sample	%N	%P	%K
Parthenium **and cow dung vermicompost**	**1.397%**	**0.0952%**	**1.325%**
Cow dung vermicompost	**1.368%**	**0.0946%**	**1.482%**

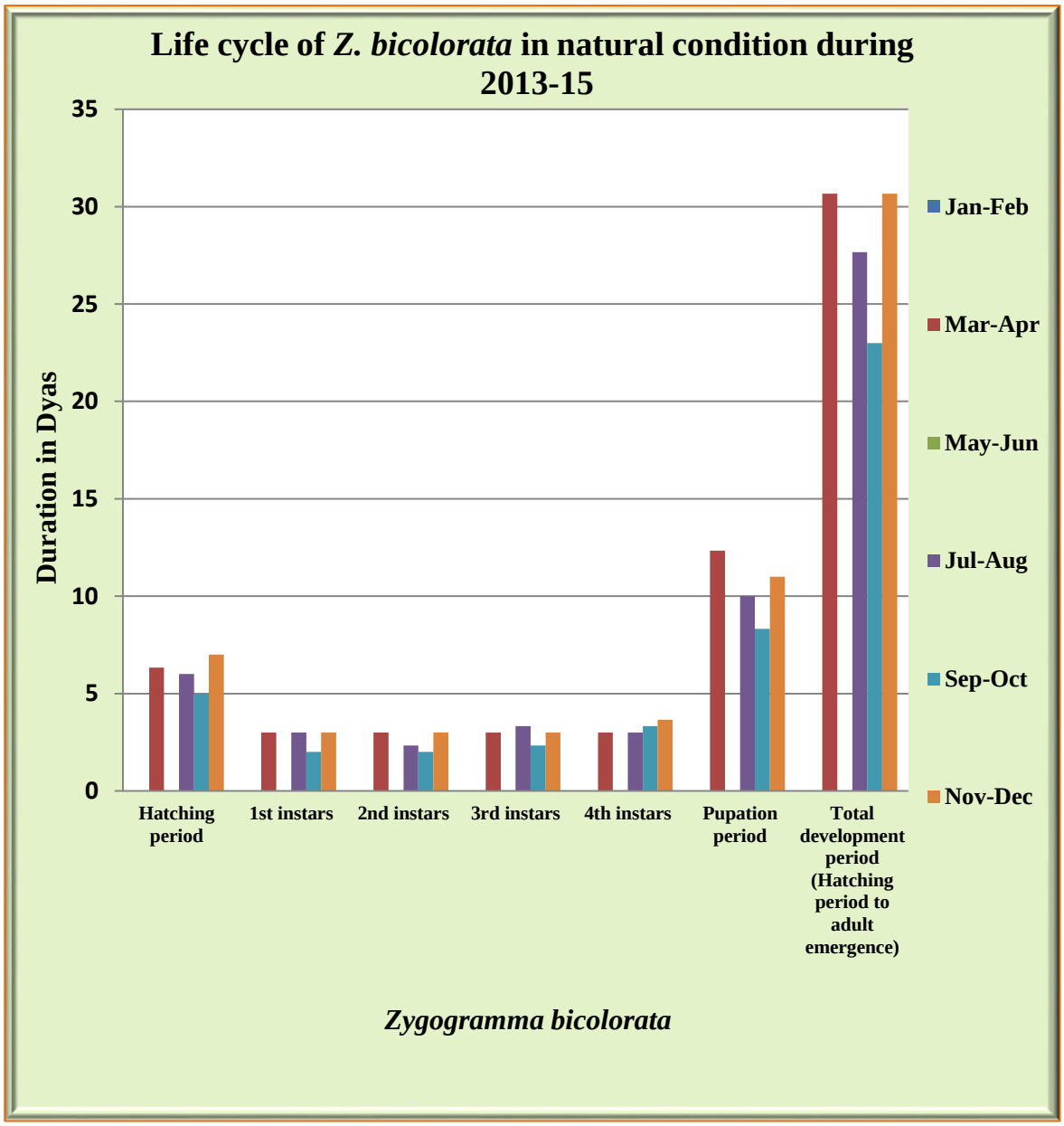
Table- 12

Height of three species of plants after application of *Parthenium* based vermicompost

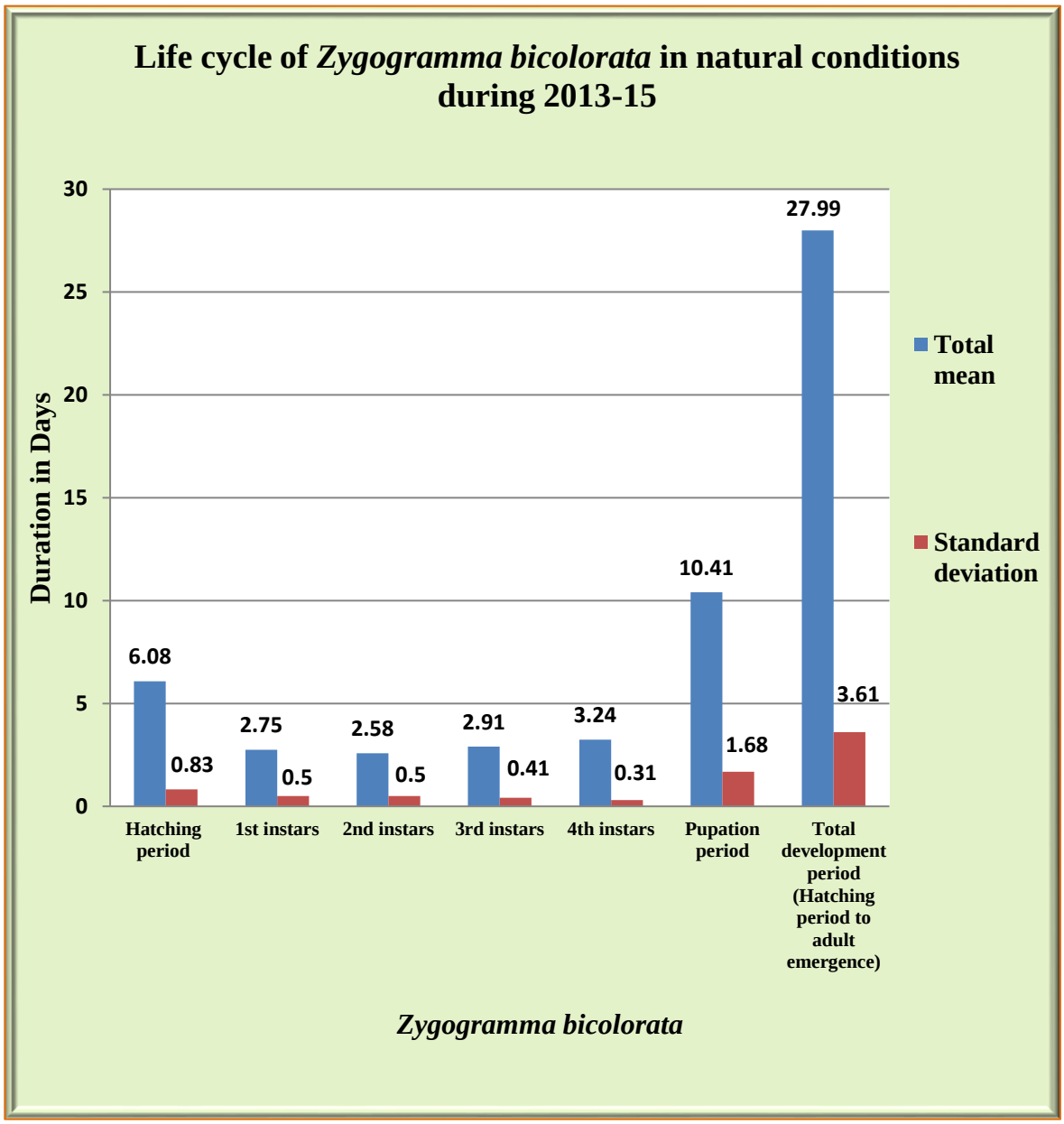
S.no	Plants	Height in inches after 75th day of plant growth	
		Treatment with *Parthenium* based vermicompost with cow dung	Treatment with cow dung vermicompost (standard)
1	***Helianthus petiolaris***	27.5 inches	25.33 inches
2	***Calendula officinalis***	33.56 inches	30.66 inches
3	***Tagetes erecta***	40.63 inches	38 inches

OBSERVATION GRAPHS

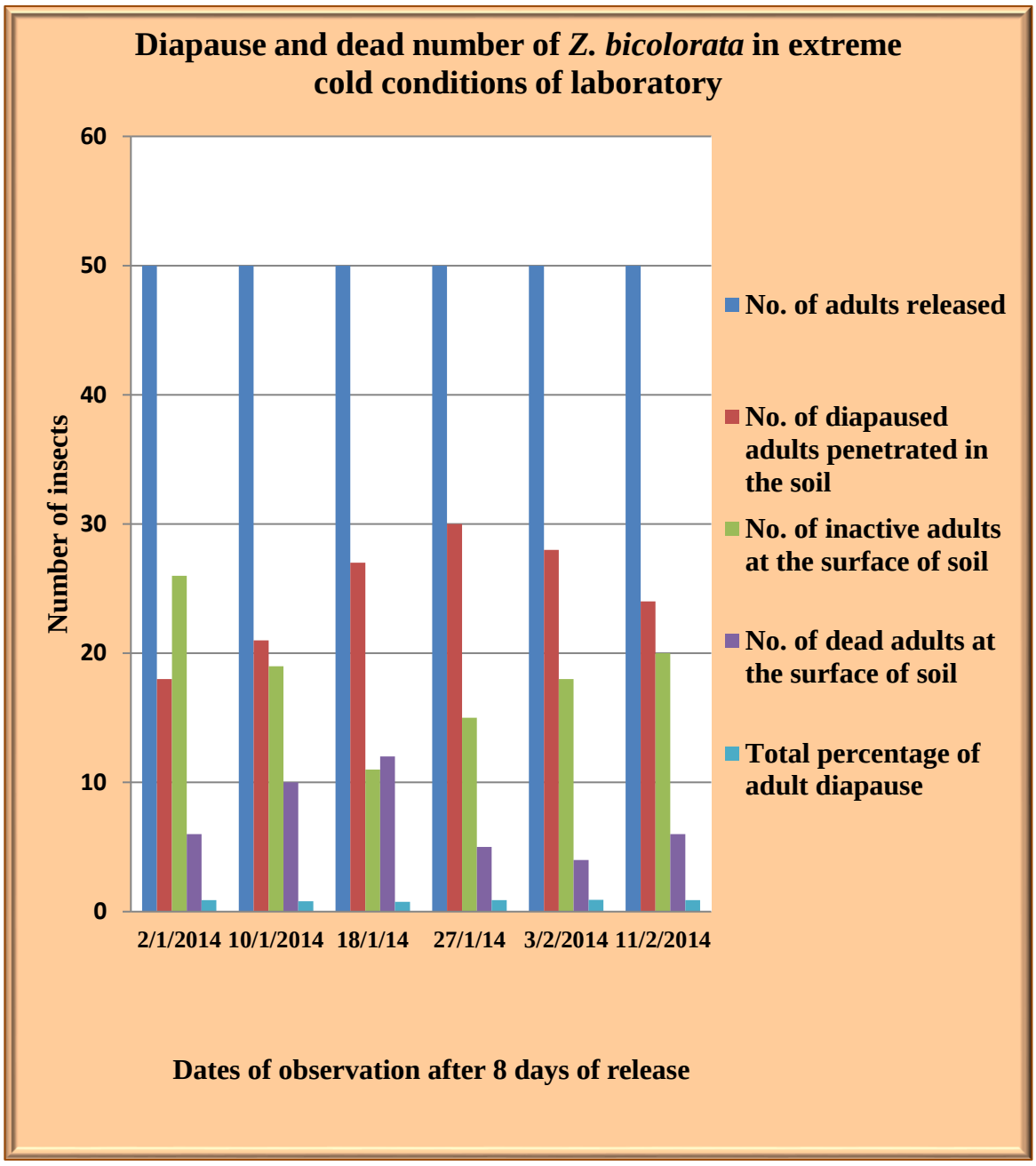
GRAPH-1


Life cycle of Z. bicolorata in natural condition during 2013-15
Duration in Dyas
35
30
25
20
15
10
5
0
Hatching period
1st instars
2nd instars
3rd instars
4th instars
Pupation period
Total development period (Hatching period to adult emergence)
Jan-Feb
Mar-Apr
May-Jun
Jul-Aug
Sep-Oct
Nov-Dec
Zygogramma bicolorata

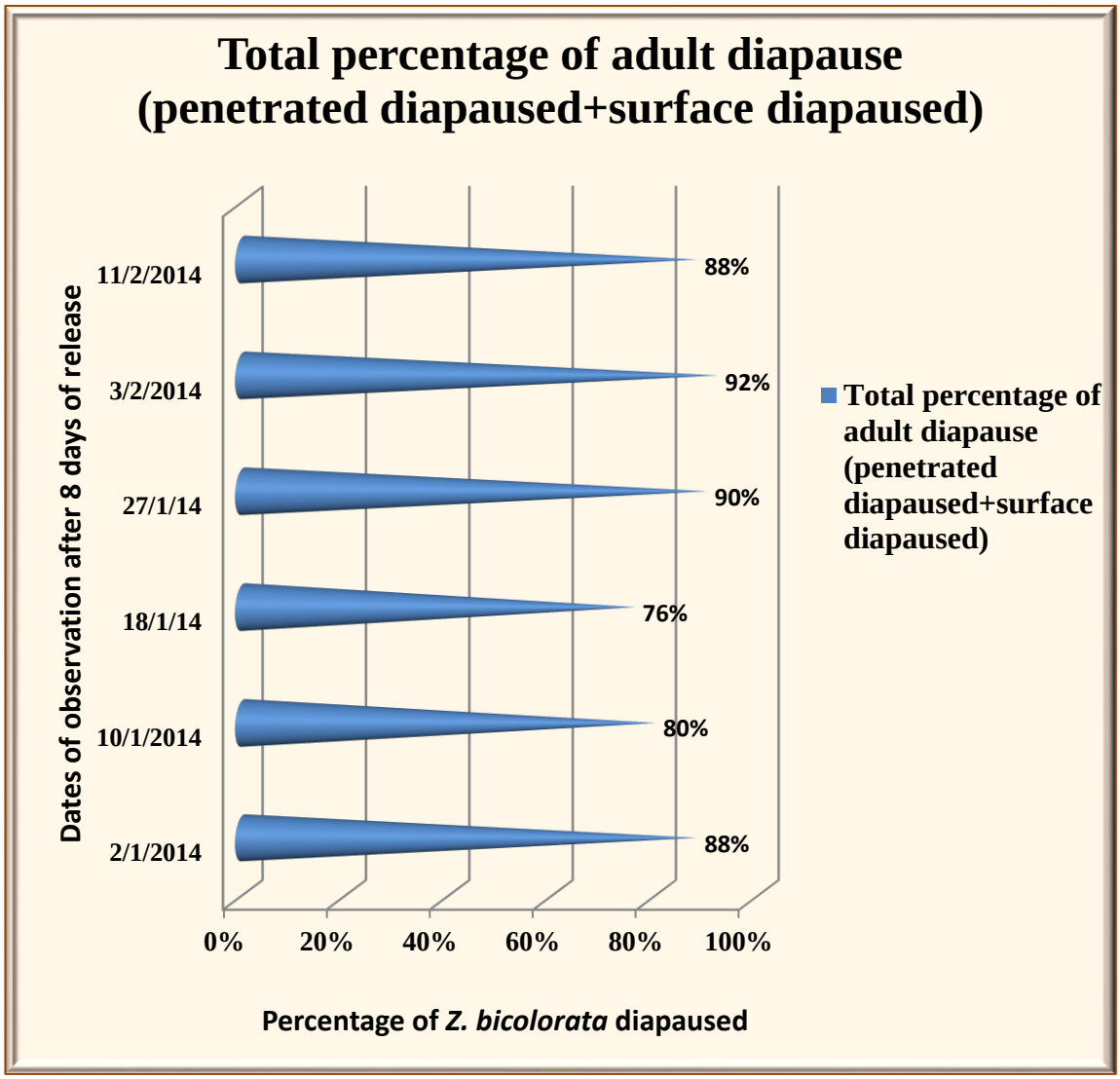
GRAPH- 2


Life cycle of Zygogramma bicolorata in natural conditions during 2013-15
Duration in Days
30
25
20
15
10
5
0
6.08
0.83
2.75
0.5
2.58
0.5
2.91
0.41
3.24
0.31
10.41
1.68
27.99
3.61
Hatching period
1st instars
2nd instars
3rd instars
4th instars
Pupation period
Total development period (Hatching period to adult emergence)
Total mean
Standard deviation
Zygogramma bicolorata

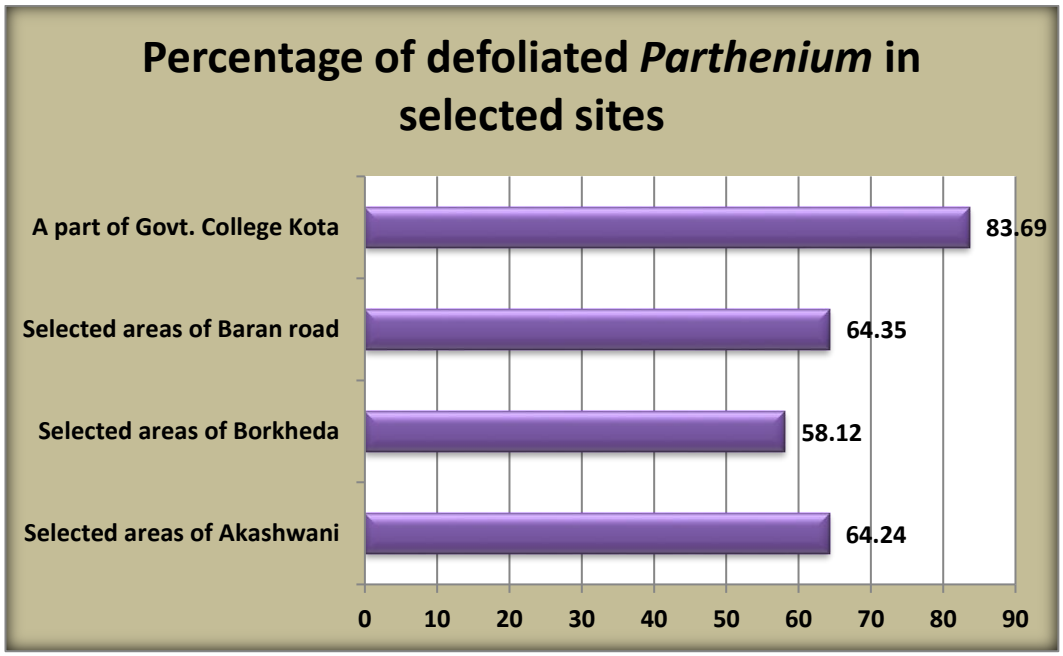
GRAPH- 3


Diapause and dead number of Z. bicolorata in extreme cold conditions of laboratory
Number of insects
60
50
40
30
20
10
0
2/1/2014
10/1/2014
18/1/14
27/1/14
3/2/2014
11/2/2014
Dates of observation after 8 days of release
No. of adults released
No. of diapaused adults penetrated in the soil
No. of inactive adults at the surface of soil
No. of dead adults at the surface of soil
Total percentage of adult diapause

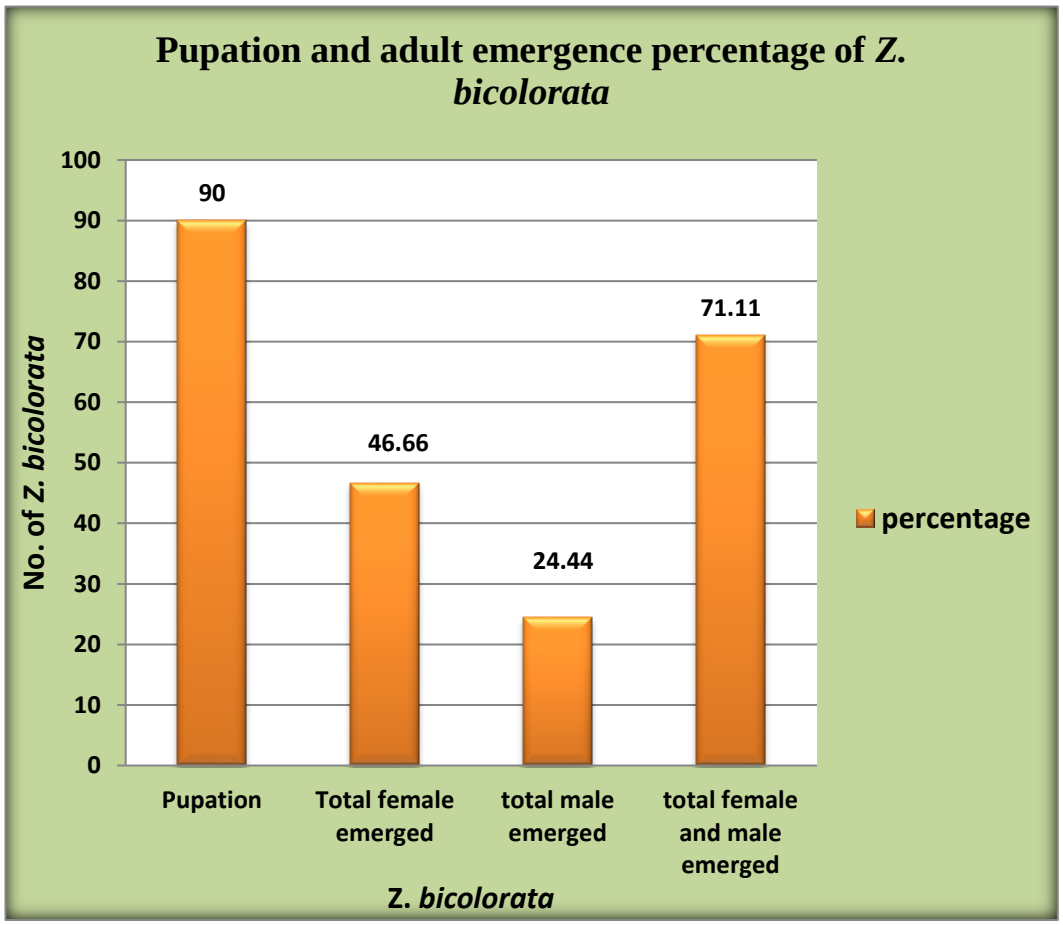
GRAPH- 4


Total percentage of adult diapause (penetrated diapaused+surface diapaused)
Dates of observation after 8 days of release
11/2/2014
3/2/2014
27/1/14
18/1/14
10/1/2014
2/1/2014
88%
92%
90%
76%
80%
88%
0%
20%
40%
60%
80%
100%
Percentage of Z. bicolorata diapaused
Total percentage of adult diapause (penetrated diapaused+surface diapaused)

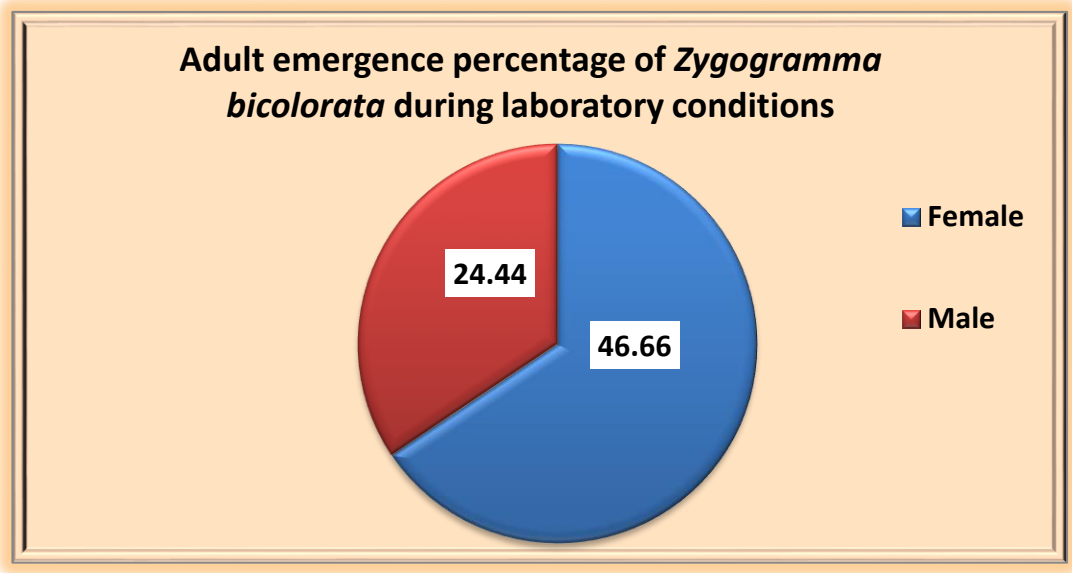
GRAPH- 5



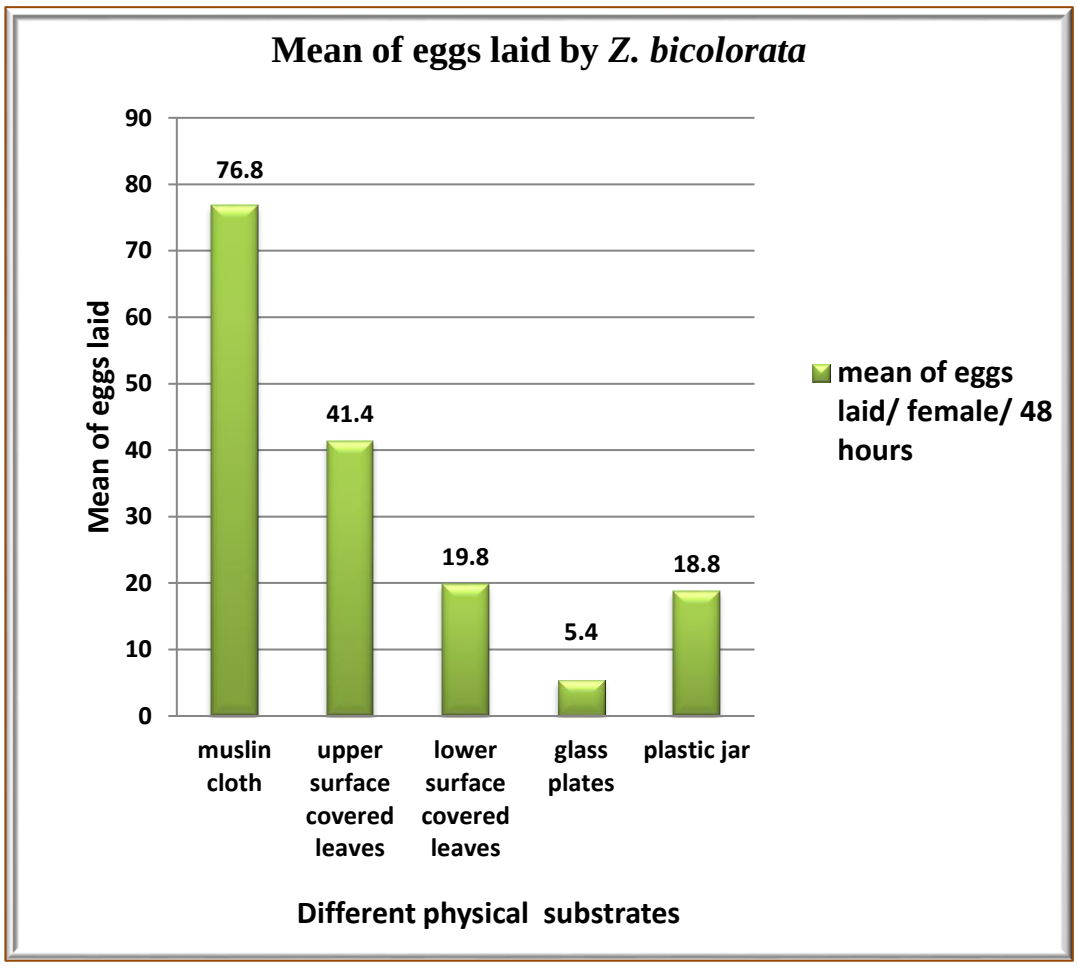
GRAPH-6



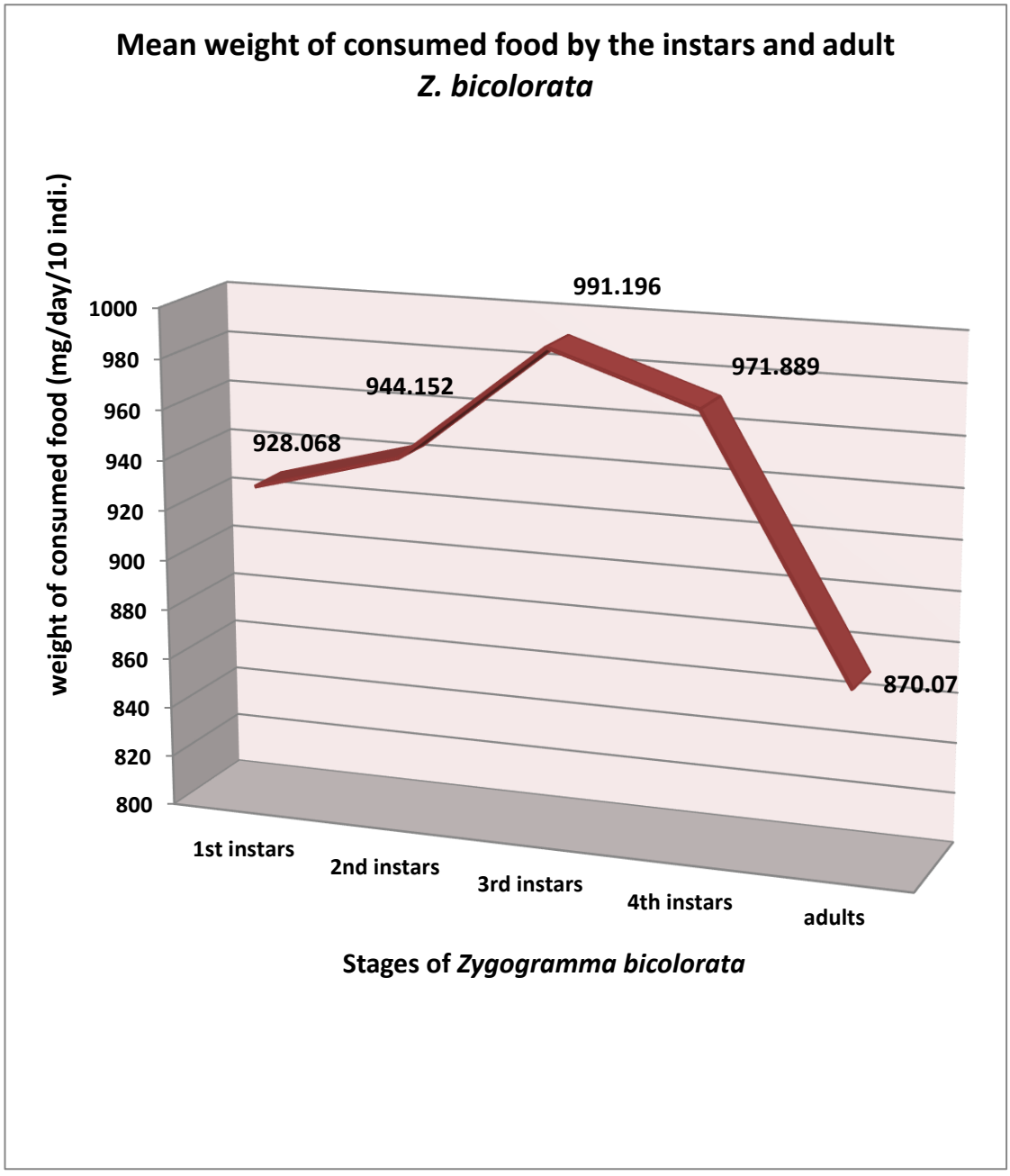
GRAPH- 7



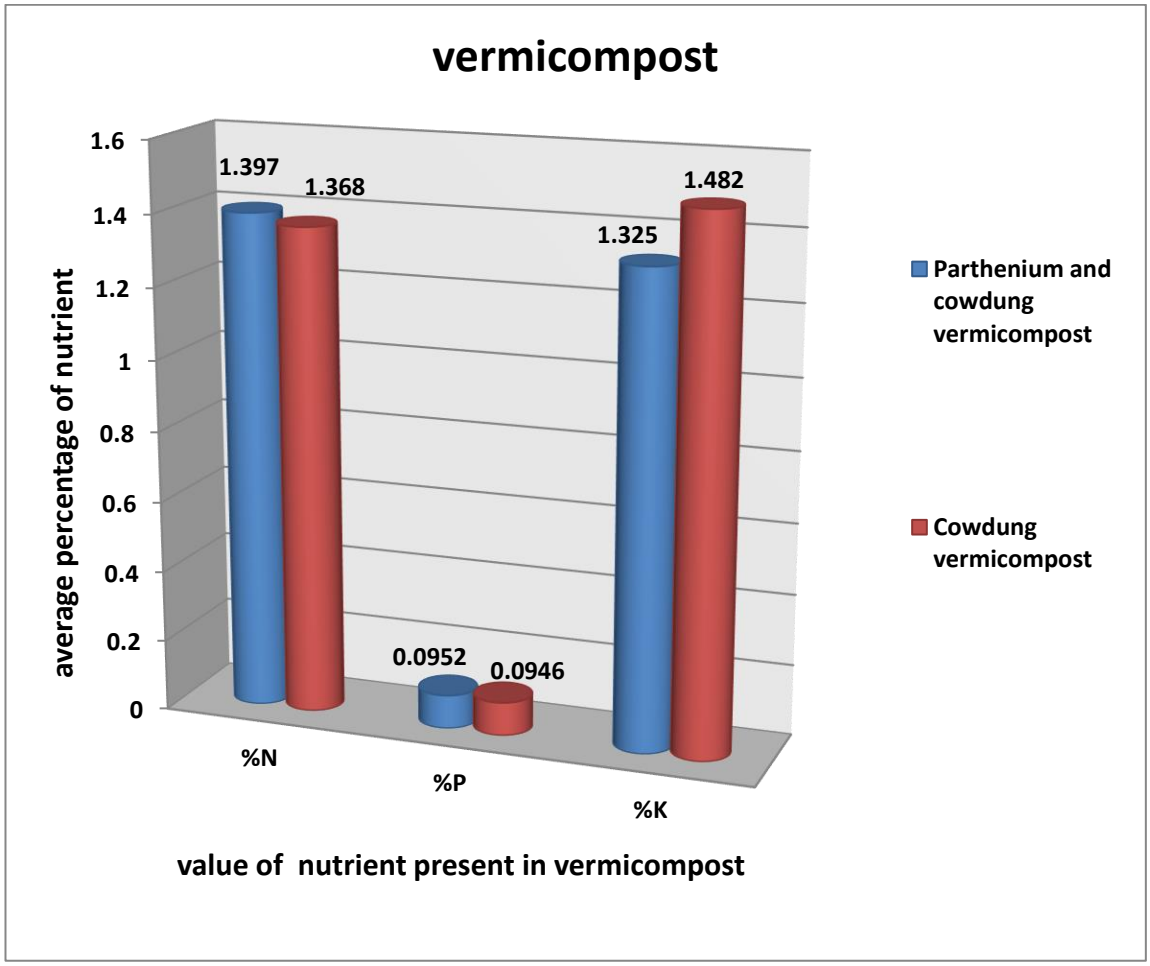
GRAPH- 8



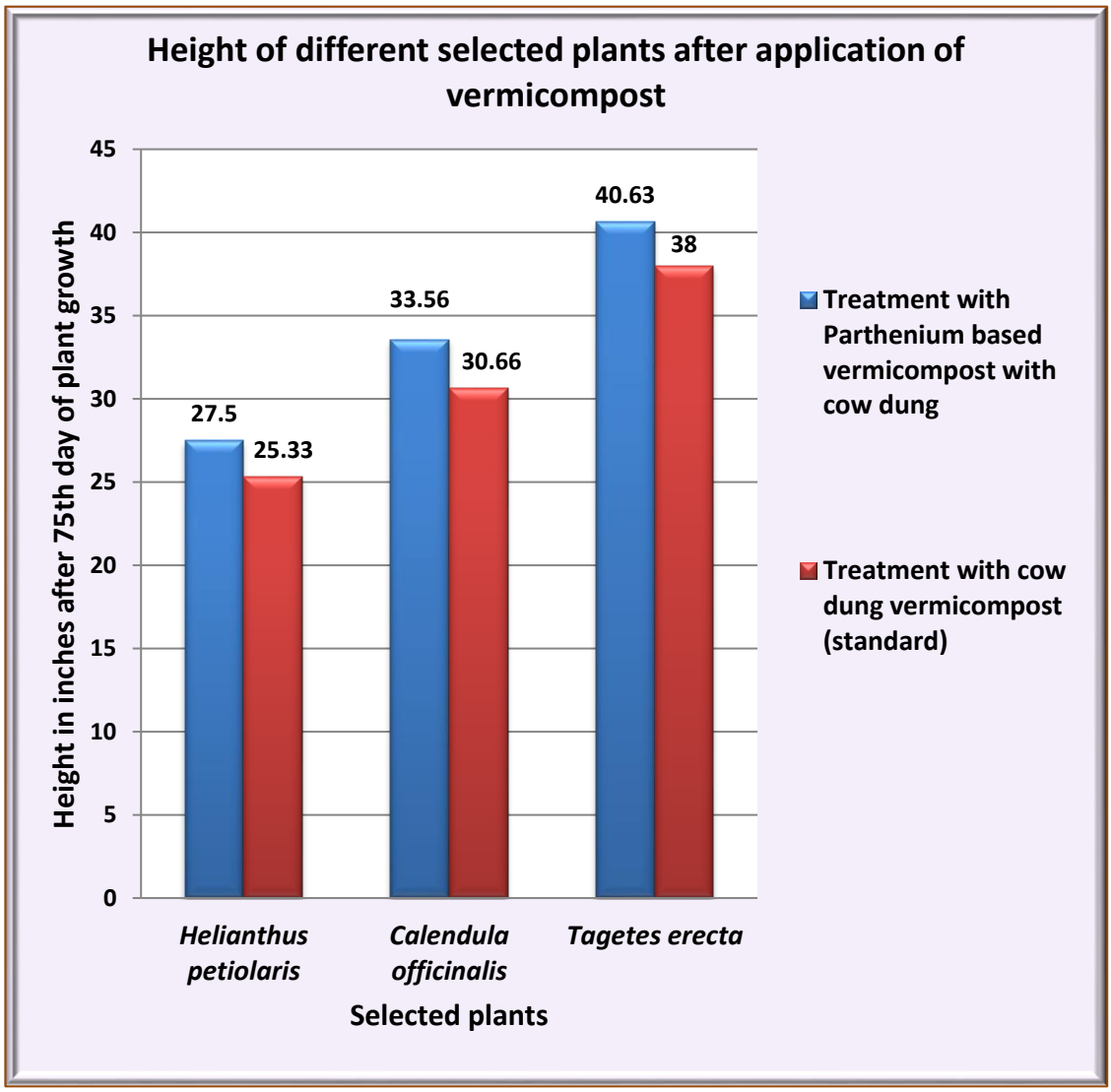
GRAPH-9


Mean weight of consumed food by the instars and adult
Z. bicolorata
weight of consumed food (mg/day/10 indi.)
1000
980
960
940
920
900
880
860
840
820
800
928.068
944.152
991.196
971.889
870.07
1st instars
2nd instars
3rd instars
4th instars
adults
Stages of Zygogramma bicolorata

GRAPH- 10


vermicompost
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
average percentage of nutrient
1.397
1.368
0.0952
0.0946
1.325
1.482
%N
%P
%K
value of nutrient present in vermicompost
Parthenium and cowdung vermicompost
Cowdung vermicompost

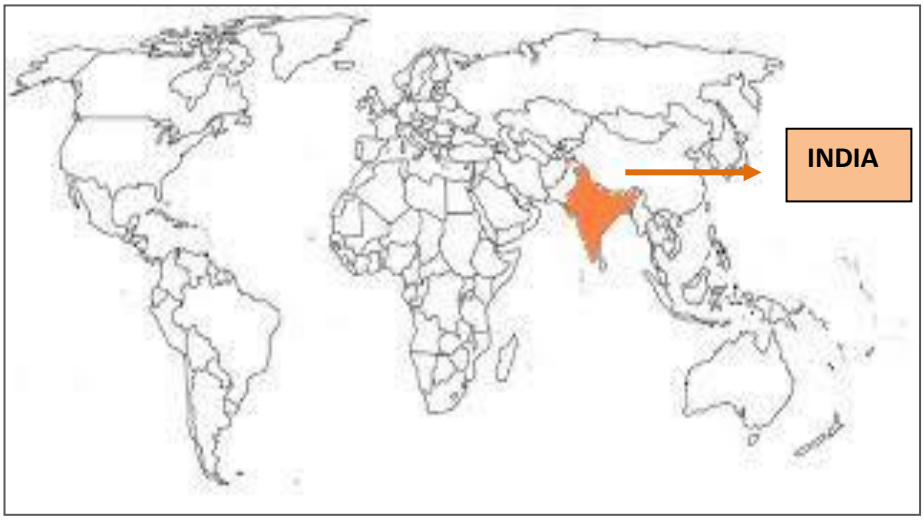
GRAPH- 11


Height of different selected plants after application of vermicompost
Height in inches after 75th day of plant growth
45
40
35
30
25
20
15
10
5
0
27.5
25.33
33.56
30.66
40.63
38
Helianthus petiolaris
Calendula officinalis
Tagetes erecta
Selected plants
Treatment with Parthenium based vermicompost with cow dung
Treatment with cow dung vermicompost (standard)

OBSERVATION MAPS

MAP- 1

WORLD MAP SHOWING LOCATION OF INDIA



MAP- 2

INDIA MAP SHOWING LOCATION OF RAJASTHAN

MAP- 3

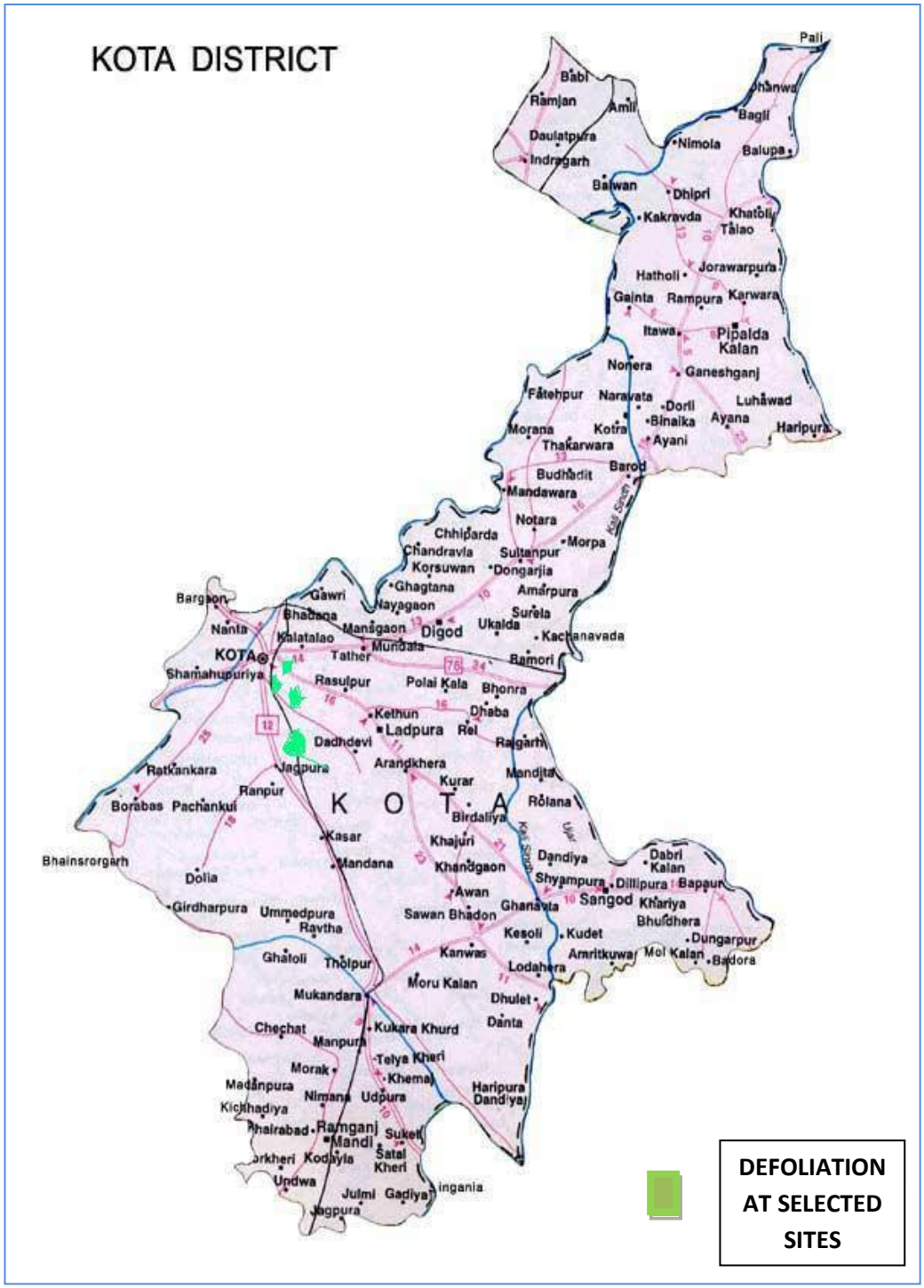
RAJASTHAN MAP SHOWING LOCATION OF KOTA

MAP- 4

KOTA MAP SHOWING LOCATION OF MORPHOLOGICAL STUDY SITES

MAP- 5

KOTA MAP SHOWING LOCATION OF *PARTHENIUM* DEFOLIATED SITES



CHAPTER-5
CALCULATION

During present investigation, observations from different treatments were recorded and calculated by using different formulae to obtain results. Following formulae were used during present investigation:

1) **Mean = Σ X ÷ N**

Here **Σ X** = Sum of Individual Observations of the Variables.

N = Total Number of observations.

2) **Standard deviation (SD)** = √ **Σ X ÷ N**

√ = Square root

Here **Σ X** = Sum of Individual Observations of the Variables.

N = Total Number of observations.

3) **Percentage (%) = x * 100/ n**

x = Number of Obtained Data
n = Total number of Data

4) **Classical Probability test (A) = f/N**

Probability of a simple event happening is the number of times the event can happen (**f**); divided by the number of possible events (**N**).

5) **Consumed food weight measurement = Weight of fresh food – weight of remaining food**

6) **Standard Error Mean = SD (σ) / √N**

Here **SD (σ) =** Standard deviation

√N = Square root of the number of Observations in the samples

7) **Analysis of Variance (ANOVA) =**
One way classification:

Source of Variation	Sum of Squares	Degree of Freedom	Mean Squares	F ratio
Between samples	**SSC**	**(c-1)**	**MSC = SSC/ c-1**	**MSC/ MSE**
Within samples	**SSE**	**(N-c)**	**MSE = SSE/ N-c**	
Total	**SST**	**n-1**		

Here **SSC** = Sum of the squares of variations amongst the sample
SSE = Samples sum of squares of variations within
(c-1) = Where c stands for number of samples
(N-c) = as the number of columns would be equal to the number of samples
MSC = Mean sum of squares of columns
MSE = Mean squares of the error

CHAPTER-6
RESULTS AND DISCUSSION

RESULTS AND DISCUSSION:

1 Results and discussion of objective-A:

1.1 Morphological study of *Zygogramma bicolorata* in natural conditions:

During the morphological study of mexican beetle, *Zygogramma bicolorata,* all the body parts and stages were thoroughly studied. It was noticed that egg size was 1.18-1.20 mm long, oblong in shape, slightly elongated, smooth and the surface was finely reticulated. They varied from light yellow (summer and winter season) to orange (spring season) in color.

Newly hatched larvae were yellowish in color and gradually turned creamy white with advancement in age. 1st and 2nd instars varied from 1.5-3 mm in length. First and second instar larvae were similar in appearance except in size. 3rd and 4th instars size varied from 5.5-7 mm in length. Fourth instar larvae were found to be similar in appearance as third instars, except in size. While larval body became more convex in 4th instars. Adults were elongated and oblong in shape. Dorsal surface was strongly convex and glabrous. There was no difference between the number of antennal segments of male and female beetles of *Z. bicolorata*. Female beetles were relatively larger (6.5 to 7 mm) in size than males (5.5 to 6 mm).

Females laid eggs either singly or in group ranging from 2 to 8 eggs on leaves, stem, buds and flowers of *Parthenium* plants. Per day per female laid 40-45 eggs with an average of 43 ± 1.26. The total number of eggs laid by a single female during its lifetime ranged from 1150 to 1600 eggs.

Results of 2013 to 2015 on the life cycle of *Zygogramma bicolorata* showed that the months of September to October were the best on account of favorable conditions to *Z. bicolorata*. In these months hatching period was 4-6 days, larval duration was 2 days for 1st instars,

2 days for 2nd instars, 2-3 days for 3rd instars and 3-4 days for 4th instars. Total pupation period was found to be 8-9 days. Adult beetles completed the development period in 21-26 days.

In the months of November to December life cycle of mexican beetle was found longer on the basis of days. Hatching period was 7 days, larval duration was 3 days for 1st instars, 3 days for 2nd instars, 3 days for 3rd instars and 3-4 days for 4th instars. Total pupation period was found to be 11 days and total development period was 30-31 days.

During last week of December months most of the adults started to hibernate and all pupae proceeded for diapause till favorable conditions achieved. The diapause period was found to occur for 65-78 days.

During the months of March to April, hatching period was 6-7 days, larval duration was extended to 3 days for 1st instars, 3 days for 2nd instars, 3 days for 3rd instars and 3 days for 4th instars. Total pupation period was 12-13 days. Adult beetles completed their development period in 30-32 days.

In the months of May- June (at high temperature) beetles didn't lay eggs or if laid then eggs were desiccated due to dry conditions.

In the months of July to August, life cycle of *Z. bicolorata* was found less longer on the basis of days. Hatching period was 6 days; larval duration was 3 days for 1st instars, 2-3 days for 2nd instars, 3-4 days for 3rd instars and 3 days for 4th instars. Total pupation period was 10 days. Adult beetles completed their development period in 27-28 days.

The over all hatching period, 1st instars, 2nd instars, 3rd instars, 4th instars and pupation period of *Z. bicolorata* varied from 6.08 (0.83), 2.75 (0.5), 2.58 (0.5), 2.98 (0.41), 3.24 (0.31), 10.41 (1.68) Mean (Standard deviation) with a total development period 27.99 (3.61) during 2013-2015. The average longevity of adult males and females was 73 ± 2.34 days (range 69 to 77 days) and 81.1 ± 1.85 days (range 78 to 84 days), respectively.

Dhiman and Bhargava (2005) mentioned that larval duration extends from 3-5 days for 1st instars, 3-3.5 days for 2nd instars, 2-3 days for 3rd

instars and 4-8 days for 4th instars, larvae and adults are voracious feeder for *Parthenium* weed, pupation period normally lasts for 10 to 12 days. Maximum population of adult *Zygogramma bicolorata* and larvae were recorded in July to Mid September. During winter in the last week of November most of the adults prepare to hibernate and pupae proceed for diapause, overwintering in adult and diapause in pupae last up to mid February taking a duration of about 2.5 to 3 months in district Saharanpur and adjacent area.

Approximately similar results were obtained on the basis of *Zygogramma* life cycle in the present research work also. Hatching period was 4-7 days, larval duration extended for 2-3 days for 1st instar, 2-3 days for 3rd instar, 3-4 days for 4th instars and total pupation period was 8-13 days respectively with toatl development period of 21-32 days.

Pandey *et al*. (2013) reported under laboratory conditions that a female could lay a maximum of 65 eggs per day during peak of production and on an average laid around 2500 eggs during its oviposition period.

In the present investigation also, the female laid 40-45 eggs per day and a single female laid 1150 to 1600 eggs in its life time.

Mahna and Sharma (2005) evaluated that the incubation, larval and pupal period of *Z. bicolorata* varied from 3 to 4 days, 9 to 11 days and 8 to 10 days, respectively with an average of 3.67 ± 0.48 days, 9.28 ± 0.63 days and 9.33 ± 0.64 days, total development period varied from 21 to 25 days with an average of 22.7 ± 1.04 days. The eggs laid by single female during its life time ranged from 1139 to 1602 with an average of 1370 ± 3.20.

In the present investigations it was estimated that incubation period, larval and pupal period of mexican beetle, *Zygogramma bicolorata* varied from 4-6 days, 9-13 days and 8-13 days respectively. During research work total number of eggs laid by a single female during its life

time was ranged from 1150-1600 eggs, which is very close to the results of Mahana and Sharma (2005).

According to Sushilkumar (2009) the developmental period of four grub stages is found to be 3.58, 2.22, 2.31 and 3.75 days in the laboratory conditions with a total of up to 11 to 20 days. He also reported that mature instars avoid feeding and penetrate in the soil for pupation. Present study showed in the results that months of September to October were the best on account of favorable conditions to the *Z. bicolorata*. In these months hatching period was 4-6 days, larval duration 2 days for 1^{st} instars, 2 days for 2^{nd} instars, 2-3 days for 3^{rd} instars and 3-4 days for 4^{th} instars. In the present research work it was reported that mature 4^{th} instars stop feeding *Parthenium* leaves and penetrate in the soil for pupation. Total pupation period was found to be 8-9 days Adult beetle completed the development period in 21-26 days.

1.2 Rearing of adult *Z. bicolorata* in laboratory conditions:

Results indicated that five pairs of beetles laid 412 eggs within 2-3 days. Hatching percent of eggs was recorded 80.58% to 89.32% at 27 ± 2^0C and 75 ± 5% relative humidity. Total laid eggs resulted in 332-368 instars. Then these emerged out into 235-267 adult *Zygogramma bicolorata*. This resulted in the successful development of mexican beetle in laboratory conditions and for further experimental work the number of adults were used during unfavorable conditions also.

Jayanth and Bali (1993) found that the hatchability of the eggs of *Z. bicolorata* ranged from 81.2 to 96.1 % at optimum temperature between 24 to 26^0C.

In the present research work hatching percent was also counted 80.58% to 89.32% at 27 ± 2^0C and 75 ± 5% relative humidity in laboratory conditions.

In the present research also for rearing mexican beetle, *Z. bicolorata*, *Parthenium* leaves were provided in each container under the laboratory conditions of 27 ± 2^0C and 75 ± 5% relative humidity. It was an improvement over previously available techniques for rearing the beetles, since it minimized handling and reduced labor involvement in rearing the insect.

1.3 Diapause percentage of released *Zygogramma bicolorata* in laboratory conditions:

During present research work, results estimated by calculating diapause percentage of total released *Z. bicolorata* adults (300 in numbers) showed that 36-60% of the adults had entered diapause (148 in numbers). The percentage of penetrated diapause was resulted by adding the number of inactive alive (109 in numbers) and substracting dead beetles (43 in numbers) from the number of released adults *Z. bicolorata.* It was observed that 109 adults remained inactive on the soil surface. They stopped metabolic activities till favorable conditions were achieved (March-April). Results showed that an adult was unable to consume such a sufficient quantity of food that it remained at one place for up to 1.5-2 months (during unfavorable conditions).

According to Jayanth and Bali (1993) *Zygogramma bicolorata* entered diapauses over an extended period of time between July and December in Bangalore. .

The present work indicated that January-February and May-June months were suitable for diapaused condition because in Kota region these months showed extreme cold and hot which were not better conditions for survival of *Z. bicolorata*. So beetles got diapaused in these seasons.

Hodek (2012) reported that adult diapause is the most common form of diapauses in coleopteran. It occurs in about 90% of beetle species, belonging mostly to the families of Coccinellidae, Chrysomelidae and Curculionidae, and partly also Carabidae (the so called carabid "autumn breeders" diapause as larvae). *Zygogramma bicolorata* Pallister (adults) can enter diapause from Aug to Dec with a peak in late November. They burrow into soil and are dormant at about 1-3 cm below the soil surface. The incidence of burrowing adults increases with soil moisture and is higher in salty soil (47%) than in sandy soil (24%).

In the present investigation it was estimated that mexican beetle *Zygogramma bicolorata* belonging to family Chrysomelidae also shows diapause behavior which favors with above researchers finding mentioned in their research paper.

Jayanth and Bali (1993) concluded that the beetles cannot tolerate the temperature above 40^0 C. They reported that beetles diapause only once during their life time.

On the basis of present investigation extreme cold and hot months, Jan-Feb and May-Jun months were declared diapaused month for *Z. bicolorata* and during their life time beetles were diapaused only once.

1.4 Defoliation of *Parthenium hysterophorus* by *Z. bicolorata*:

Results had shown that mexican beetle controlled the congress grass up to 64.24% at site "A", 58.12% at site "B", 64.35% at site "C" and 83.69% at site "D". (Site "A"- Akashwani, Site "B"- Borkheda, Site "C"- Selected areas of Baran road, and Site "D"- Govt. College, Kota were selected sites). Maximum defoliation was found at site "D" (83.69%)> followed by site "C" (64.35%)> site "A" (64.24%)> site "B" (58.12%).

Sushilkumar and Ray (2011) mentioned 100% defoliation of *Parthenium* stand by the 45 and 60 day by larval and adult augmentation of *Z. bicolorata*.

In the present investigation maximum defoliation percentage by *Zygogramma* counted on 20th day was 83.69% at selected site"D"- Govt. College, Kota. The results showed high defoliation capacity by leaf feeding beetle (*Z. bicolorata*).

Dhiman and Bhargava (2005) reported 90% damage to *Parthenium* stand by releasing 200 adults and 250 Larvae, in just 15 days under field conditions.

Jayanth (1987) reported in tropical countries like India, it is observed that after inoculative release of *Z. bicolorata* in an area, the insect colony takes about 3-4 years to get established followed by *Parthenium* suppression.

Javaid and Shabbir (2006) revealed that *Z. bicolorata* can be used as a potential biological agent to control *Parthenium* in Pakistan.

In the present research study it was also found that released *Z. bicolorata* voraciously damage higher percentage of *Parthenium hysterophorus* in a field and this biological control through *Z. bicolorata* suppressed *Parthenium* plants.

Shrestha *et al* (2011) reported that *Z. bicolorata*, a mexican beetle is the most widely distributed bio-control agent of the invasive weed *Parthenium*. A small population of the beetle was first encountered in a wasteland at Sundarighat of Kirtipur municipality in August 2010.

Approximately in the present study also 50 beetles (*Z. bicolorata*) were released in each selected sites and results showed positive defoliation of carrot grass, *Parthenium hysterophorus*.

Dhiman and Bhargava (2005) reported that *Z. bicolorata* is a potential bio-control agent of *Parthenium*. Field trials were made for the release of beetles; its population was established very rapidly. After 25 days of release, all stages of *Z. bicolorata* were observed at each site. Firstly beetle and their larvae ate only soft parts of leaf and buds, after that hard

and main stem was eaten. It was also observed when half plants were damaged; most of adult beetles migrated to nearby vicinity while larvae fed on the left over parts of the plants till these were fully damaged. After about a month of release, 50 to 90 % plants were damaged and after 35 to 85 days of release all the plants were found fully damaged at different sites.

In the present study it was estimated that after 20 days of released *Zygogramma* (50 beetles), 58.12% to 83.69% defoliation was resulted in the selected sites (Akashwani. Borkheda, Selected areas of Baran road and Selected sites of Govt. College, Kota).

Dhileepan *et al* (2000) observed the effect of *Z. bicolorata* on *Parthenium* during field as well as laboratory conditions and found that the beetle reduces the height, growth of flowers and leaves and weight of the *Parthenium* plant drastically.

Approximately similar results were found in present study where the mexican beetle controlled the congress grass up to 64.24% at site 'A', 58.12% at site 'B', 64.35% at site 'C', and 83.69% at site 'D', results showed the positive defoliation of *Parthenium* plant, which favored the growth of economically important crops in selected sites A, B, C, and D.

2 Results and discussion of objective-B:

2.1 Mass multiplication of *Zygogramma bicolorata* in laboratory conditions:

2.1.1 Study of the pupation of instars and adult emergence:

To study the pupation and adult emergence percentage (male and female) five plastic containers with net were taken and in each container ten 4th instars larvae were released**.** The results have shown that a total of 90% larvae pupated and 71.11% adults emerged. Out of total emerged adults, the percentage of females (46.66%) was far higher than emerged males (24.44%).

Sharma *et al* (2017) reported 71.11% adults of *Zygogramma biocolorata* emerged after pupation period.
In the present investigation also similar findings occurred.

2.1.2 Egg laying behavior of female on selected physical substrates:

In the present research work maximum mean eggs' output was obtained on muslin cloth (76.80)> followed by upper surface covered leaf of *Parthenium* (41.40)> lowered surface covered leaf of *Parthenium* (19.80)> plastic jar (18.8)> glass plates (5.40). Out of all the five substrates provided for eggs deposition, the highest egg output by the beetle was observed on muslin cloth surface due to the roughness of muslin cloth where the maximum eggs can stick properly.

To test the hypothesis of equality amongst several means ANOVA test was carried over. The results have shown that the calculated value F= 2.45 is less than the table value= 3.20 at 5% level of significance. The hypothesis was accepted.

Roughness of the substrate was also reported by Gupta *et al* (2004) while working with *Andrallus spinidens* and *F. Cantheconidea furcellata*

in the laboratory in which gravid females after a pre-oviposition period of 1-3 days, moved up and down over the object on which the eggs were to be deposited, bent the abdomen and rubbed the object with its tip before egg deposition. The eggs hatched in 5.7 to 8.0 days, with a mean incubation period of 6.46 ± 0.51 days on glass surface, cotton or muslin cloth. They also recorded oviposition by reduvid bug *Sycanus pyrrhomelas* Walker incapacity condition, who laid most of the eggs on the underside of a muslin cloth tied upon the mouth of a glass jar, with occasional laying of eggs on glass surface. Ovipositional preference of *Diaprepes abbreviates L*. on immature citrus leaves, mature citrus leaves and doubled strips of various substances as potential oviposition sites was observed by Adair *et al* (1998). They recorded that both the laboratory and in caged outdoor experiments more egg masses were deposited between freezer paper strips than between mature leaves. Substrates were preferred in the following order: freezer paper> waxed paper> transparency film. Vanit *et al* (2004) observed preferred oviposition substrate for rearing of *Helicoverpa armigera* under laboratory conditions using pigeon pea leaves, muslin cloth, tissue paper and cotton wool as oviposition substrates as free-choice and no- choice tests in descending order which was found to be cotton wool> pigeon pea leaves> tissue paper> muslin cloth. The preference index of cotton wool was 65% higher than the least preferred substrates, muslin cloth.

Mishra and Pathak (2012) found that there was a significant variation in the total egg output in females of *Zygogramma bicolorata*. Maximum mean egg output was obtained on muslin cloth (52.00)> followed by upper surface covered leaf (25.00)> lower surface covered leaf (12.20)> non adhesive portion (7.60)> on adhesive portion of cellophane tape (6.20)> glass (0.60) and bits of card board (0.40) on all the seven substances provided for egg deposition.

Sharma *et al* (2017) reported maximum mean egg output obtained on muslin cloth (76.80)> followed by upper surface covered leaf of *Parthenium* (41.40)> lowered surface covered leaf of *Parthenium*

(19.80)> plastic jar (18.8)> glass plates (5.40). Out of all the five substrates provided for egg deposition, the highest egg output by the beetle was observed on muslin cloth surface due to the roughness of muslin cloth. Some eggs were also laid haphazardly on the inner surface of plastic container or near mouth of the container (To be added in total out put of eggs).

3 Results and discussion of objective-C:

3.1 Host specificity tests in field conditions:

Host specific tests related to choice (*Parthenium* with indigenous and economically important plants) and non- choice (indigenous and economically important plants without *Parthenium*) performance had been carried out during field research work.

3.1.1 Choice tests (*Parthenium hysterophorus* with indigenous and economically important plants):

The results revealed that on released twenty instars and 10 pairs of beetles in each of the selected sites for field experiments; probability test (P) for feeding on indigenous and economically important plants was "P=0" in the presence of *Parthenium hysterophorus* and for oviposition the adult females oviposited only on *Parthenium hysterophorus*, except that some oviposition was recorded on *Helianthus petiolaris* (P=0.1) in the presence of *Parthenium* but no feedings were seen on any other economically important plants except *Parthenium*.

3.1.2 Non- choice tests (indigenous and economically important plants without *Parthenium hysterophorus*:

Twenty instars and 10 pairs of beetles were released in each of the selected sites; probability test (P) for feeding on indigenous and economically important plants was "P=0". Further results have shown that little oviposition was reported on *Glycine max*, *Tagetes erecta* and *Helianthus petiolaris* leaves (P=0.3) but no harm was seen on these plants (no feeding behavior).

Some economically important plants like *Oryza sativa, Vigna radita, Vigna mungo, Abelmoschus esculentus, Zea mays, Sorghum bicolor, Glycine max, Thevetia peruviana, Tagetes erecta* and *Helianthus petiolaris* were selected for oviposition of *Z. bicolorata*. Little oviposition was reported on *Glycine max*, *Tagetes erecta* and *Helianthus petiolaris* leaves

but on no other plants. Feeding behavior was not shown for any species of plants. The results showed that *Z. bicolorata* prefer only *Parthenium* as food and didn't feed on any other plants.

Choice tests:

Feeding on economically important plants (P = 0), and on *P. hysterophorus* (P = 1)

Oviposition on economically important plants (P = 0.1), and on *P. hysterophorus* (P = 1)

Non-choice tests:

Feeding on economically important plants (P = 0)

Oviposition on economically important plants (P = 0.3)

According to Heard (1999), insects that have recently fed or oviposited have a higher threshold for host acceptance and are less likely to respond to plants In some circumstances choice tests may become stringent, when a resource of the preferred host is finite and its quantity drops below that of lower ranked hosts present, as a results of host marking or damage.

Jayanth *et al.* (1998) however say that feeding does not occur on sunflower when it is present in choice tests with *P. hysterophorus*. Choice tests are therefore useful for confirming that the target weed is the most preferred host plant, but do not ascertain the relative acceptability of other hosts.

Approximately similar results were obtained in the present research work in response to choice tests with *Parthenium*. No feeding behavior was noticed on host plant *Helianthus petiolaris* (Sunflower) in the presence of *Parthenium*. Only little oviposition was found on *Helianthus petiolaris* (Probability= 0.1) in presence of *Parthenium*.

McFadyen and Heard (1988) reported that given a choice, adults of *Z. bicolorata* would neither feed nor lay eggs on any plant except *Parthenium* and ragweed. However, when given no choice, after 1 to 2 days adults will feed on most plants in the *Helianthaceae* family and on

some, there was feeding and considerable damage. Few eggs were laid on these plants and larvae did not feed or survive on them.

But in the present research work no damage was found on the plants other than *Parthenium*. Further results have shown that little oviposition was reported on some plants (*Glycine max, Tagetes erecta* and *Helianthus petiolaris*) leaves (Probability= 0.3) but no feeding was seen on these plants.

Towers and Rao (1992) mentioned that host-specificity studies with *Zygogramma* taking 40 plant species representing 27 families, under quarantine conditions were carried out in India. They were satisfied with that the beetle feeds and reproduces only on *Parthenium*. A Field cage study showed that it breeds under Bangalore climate conditions and subsequently was released in Karnataka. In July 1991, when researches were carried in Bangalore, they found large populations of this beetle, feeding on *Parthenium*, throughout the city in its environment.

Hence, in the present study after examining 10 plant varieties in non-choice tests for host-specificity with *Z. bicolorata*, a little oviposition resembles were seen on *Glysine max, Tagetes erecta* and *Helianthus petiolaris* leaves but no feeding behavior was observed. Feeding behavior was seen on only *Parthenium* plant in choice tests. So it was not harmful to release *Z. bicolorata* in field conditions for defoliation of carrot grass.

3.2 Food preference by the instars and adult *Z. bicolor*ata in laboratory conditions:

During present investigations, it was found that HP_{11} (Here HP means host plant) – *Parthenium* was the most preferred host food of *Zygogramma bicolorata* in comparison to other plants ($HP_1 – HP_{10}$). The maximum food (*Parthenium*) was consumed by 3rd instars (991.196 mg/ day/ 10individual) followed by 4th instars (971.889 mg/ day/ 10indi.), 2nd instars (944.152 mg /day/ 10indi.), 1st instars (928.068 mg/ day/ 10indi.) and adults (870.07 mg/ day/ 10indi.) respectively. HP_1- *Oryza*

sativa (0.00 mg/ day/ 10indi.), HP_2- *Vigna radita* (0.00 mg/ day/ 10indi.), HP_3- *Vigna mungo* (0.00 mg/day/10indi.), HP_4- *Abelmoschus esculentus* (0.00 mg/ day/ 10indi.), HP_5- *Zea mays* (0.00 mg/ day/ 10indi.), HP_6- *Sorghum bicolor* (0.00mg/day/10indi.), HP_7- *Glycine max* (0.00 mg/ day/ 10indi.), HP_8- *Thevetia peruviana* (0.00 mg/ day/ 10indi.), HP_9- *Tagetes erecta* (0.00 mg/ day/ 10indi.) and HP_{10}- *Helianthus petiolaris* (0.00 mg/ day/ 10indi.) were the non preferred plants for *Zygogramma bicolorata*.

Bhumannaver and Balasubramanian (1998) reported the consumption and food utilization of 1st instar 2.67 ± 0.48 mg/ day/ individual, 2nd instar 5.08 ± 0.28 mg/ day/ indi, 3rd instar 11.01 ± 5.81 mg / day/ indi., 4th instar 8.10 ± 0.38 mg /day/ indi., and adult 0.37 ± 0.00 mg/ day/ indi., respectively, in their study.

However, the similar results were found in the present research work where maximum food was consumed by 3rd instars, followed by 4th instars, 2nd instars, 1st instars and adult (*Z. bicolorata*).

Malkapure *et al* (2012) mentioned that, there were different host plants tested for feeding preference of *Z. bicolorata* and other plants may be stated as non-preferred hosts. The maximum food was consumed by 3rd instar (111.93 mg/ day/ individual) followed by 4th (72.84 mg/ day/ indi.), 2nd instar (56.63 mg/day/indi.), 1st instar (28.11 mg/ day/ indi.) and adult beetles (7.80 mg /day/ indi.), respectively.

From above discussions the present study also showed maximum food consumed by 3rd instars (991.196 mg/ day/ 10 indi.) which was important instar for consuming *Parthenium* and defoliation of this invasive weed, followed by 4th instars (971.889 mg/ day/ 10 indi.), 2nd instars (944.152 mg/ day/ 10 indi.), 1st instars (928.068 mg/ day/ 10 indi.) and adult beetles (870.07 mg/ day/ 10 indi.) respectively.

4 Results and discussion of objective-D

4.1 Vermicomposting with and without *Parthenium*:

During the experiments on vermicomposting of *Parthenium*, the results have shown that *Parthenium* was converted into soluble and available ingredients that provided nutrients such as available N (nitrogen), P (phosphorus) and K (potassium) which can easily be absorbed by plants.

The values of N, P, K contents in the vermicompost made from *Parthenium* were found to be 1.397% (N), 0.0952% (P) and 1.325% (K) which were approximately similar to the contents of cow dung vermicompost (1.368% (N), 0.0946% (P) and 1.482% (K). No allelopathic effects were reported through the vermicomposting of *Parthenium.*

A difference of + 0.029 (N), + 0.0006 (P) and - 0.157 (K) was found in the *Parthenium* vermicompost results, which shows a good quality of *Parthenium* vermicompost as compared to cow dung vermicompost and it can be degraded easily without toxifying the environment. Neither it affected the life cycle of *Eisenia foetida.*

Paul (2015) reported that vermicomposting have better options for converting organic solid wastes into nutrients having rich organic bio-fertilizers for improving the productivity of crops. The study also revealed that the earthworm's species *Eisenia foetida* feed on any type of organic wastes and can convert it into organic compost. The vermicompost being easily and cheaply manufactured can be used as a source of additional income.

In the present study feeding behavior of *Eisenia foetida* showed better results of *Parthenium* vermicompost over cow dung vermicompost. Hence *Eisenia foetida* is able to convert any waste into vermicompost with high nutritive values.

Manyuchi and Whingiri (2014) in a study carried out at Harare Institute of Technology for a period of up to 50 days between April and May 2014,

used *Eisenia foetida* as earthworm and the substrates comprised of food waste and cow dung of varying compositions. The completion of vermicomposting period was 30-50 days.
Approximately the similar period of 45 to 60 days was found in the present study for completion of vermicomposting.

Nagavallema *et al* (2006) mentioned that when *Parthenium* mixed with cow dung as feed materials was used, there was an increase in population and size of earthworms during incubation for 90 days. Nutrient value was also higher in *Parthenium* vermicompost than cow dung vermicompost. In the present study also, the similar results were obtained where the nutrient values of N and P in *Parthenium* vermicompost were slightly higher {1.397% (N), 0.0952% (P)} than cow dung vermicompost. While the values of 'K' was 1.325 which was slightly lower.

Javaid *et al* (2007) also reported that *Parthenium* can be managed by using it as green manure. It is able to extract nutrients even from nutrient deficient soils. It has very high level of nitrogen (3%), phosphorus (0.2%), potassium (4.5%) and other macro and micro nutrients.
In the present study results show high level of N, P, K (1.397%, 0.0952% and 1.325%) respectively in *Parthenium* vermicompost in comparison to cow dung vermicompost (N (1.368%), P (0.0946%), and K (1.482%). Except the value of "K" which was somewhat less.

Adhikary (2012) reported that worm castings contain better available nutrient for plants than found in average potting soil mixes. Chemical analysis of the castings was conducted and it was found that it contains 5 times the available nitrogen, 7 times available potash and 1.5 times more calcium than that found in 15 cm layer of good top soil.
On the basis of present study *Parthenium* vermicompost and cow dung varmicompost contain high values of N, P and K in comparison to top soil.

Sharma *et al* (2014) found that daily temperature recorded in the vermicompost tanks was initially higher and gradually decreased with the decomposition process.

Approximately similar results were obtained in the present study in which initial temperature was higher and gradually decreased with decomposition.

4.2 Quality testing of prepared vermicompost (with and without *Parthenium*) on different plant varieties:

In the comparative study of *Parthenium* based vermicompost (*Parthenium* + Cow dung + Earthworm), the results have shown that a little increment was measured on the basis of height of the stem on 75th day (after germination) in *Helianthus petiolaris* (27.5 inches), *Calendula officinalis* (33.56 inches) and *Tagetes erecta* (40.63 inches) after application of *Parthenium* vermicompost in the soil of mentioned plant varieties. Besides it, cow dung as a standard vermicompost was also applied for the same species of plants, the height was measured on 75th day after germination in *Helianthus petiolaris* (25.33 inches), *Calendula officinalis* (30.66 inches) and *Tagetes erecta* (38 inches). The comparative results showed better measurement of height on providing *Parthenium* vermicompost in the soil of mentioned plants (*Helianthus petiolaris, Calendula officinalis and Tagetes erecta*).

Increment of 8.56% in height of *Helianthus petiolaris*, 9.45% in *Calendula officinalis* and 6.92% in the height of stem of the *Tagetes erecta* was found on providing *Parthenium* vermicompost.

The results thus show that *Parthenium* based vermicompost provides better nutrients to growing plants in comparison to cow dung vermicompost alone.

Bhat and Limaye (2012) mentioned that height of plants; breadth and length of leaves were found superior in all the three plants (*Mirabilis jalapa*, *Calendula officinalis* and *Clitoria ternatia*) when treated with vermicompost as compared to controls. Thus the prepared vermicompost

is better in all respects like high microbial load, high water holding capacity and moisture content and comparatively good for germination, chlorophyll contents, flowering capacity and overall growth of plants as compared to plain soil and composted material individually. Approximately similar results were obtained in present study where *Parthenium* vermicompost increased the height of some selected plant species *Helianthus petiolaris* (27.5 inches), *Calendula officinalis* (33.56 inches) and *Tagetes erecta* (40.63 inches) as compared to cow dung vermicompost (*Helianthus petiolaris* (25.33 inches), *Calendula officinalis* (30.66 inches) and *Tagetes erecta* (38 inches). All the results found through the present investigation draw a conclusion that use of *Parthenium* weed for making its vermicompost creates no harm to the environment and user; favor the growth of plants and is loaded with better quantity of nutrients in comparision to top soil and control specimen of vermicompost.

CHAPTER-7
CONCLUSION

Conclusion:

In the light of the results of studies carried out so far and in the interest of the science of biological control of weeds, it is high time that we should go by available facts and not by perceived threat perceptions in making a judgment on the pest potential of the insect *Zygogramma bicolorata* which has already brought about large scale defoliation of noxious weed *Parthenium hysterophorus* and has encouraged the growth of economically important indigenous flora varieties formerly suppressed by it.

Parthenium hysterophorus is one of the most serious alien weed to have invaded India during the 50s in last century. Due to the allelochemicals (Parthenin) produced by this plant, in the absence of natural enemies, it grows in pure stands suppressing local vegetation. *P. hysterophorus* has now spread throughout India and creating health hazards, agriculture and livestock losses. However, the threats to natural diversity due to uncontrolled weed growth, can lead to extinction of native economically important varieties of flora.

We proposed a comprehensive program to evaluate the release of bio-control agent insect herbivore (*Zygogramma bicolorata*) to control invasive weed *Parthenium hysterophorus*.

The release was done in follow up by well replicated sampling and experimental protocols that evaluate the degree of success or failure.

These follow- up studies should include landscape scale monitoring across relevant habitat gradients of mass multiplication of the bio- control agent; the impact of the bio- control agent on the target plant species in choice and non choice tests; the response of native species and communities etc. in the reduction of invasive weed *Parthenium.*

Zygogramma bicolorata, of mexican origin, has caused large scale defoliation of *Parthenium* in Kota and surrounding areas through the present investigation.

Defoliation by the beetle was also found to reduce flower production by the weed, besides encouraging the growth of other vegetation which were formerly suppressed by *Parthenium*.

Field observations over the past three years have shown that the adults of *Z. bicolorata* feed only on *Parthenium* plants in selected areas.

Host specificity testing is central to the prediction of non – target attack, one approach is to view it as a direct estimate of field host specificity and relative attack. The second approach can be translated into a methodology for host specificity testing which produces generalizable result with which to make accurate predictions of non- target attack in the field. Fundamental host range, which represents the absolute limits of the insect's host specificity, is described first for aspects of the insect's life history that need to be host specific.

If non- target species are included, prediction can be made as to whether non-target species within the fundamental host range will indeed be attacked in the field. It acknowledges that fundamental host range can be described for any aspect of an insect's life history where the insect interacts with plants.

Disposal of *Parthenium* is a serious problem. Our trials have demonstrated the vermicomposting as an alternate technology for the recycling of *Parthenium*, using an epigenic earthworm *Eisenia foetida* under laboratory conditions. Meanwhile the rejuvenation of degraded soils by protecting topsoil and sustainability of productive soils is a major concern at the international level.

Provision of a sustainable environment in the soil by amending with good quality organic soil additives enhances the water holding capacity and nutrient supplying capacity of soil and also the development of resistance in plants to pests and diseases. By reducing the time of humification process and by evolving the methods to minimize the loss of nutrients during the course of decomposition, the fantasy becomes fact.

Earthworms can serve as tools to facilitate these functions. They serve as 'farmer's friend' to produce upper humus layer of the soil, to fulfill the nutritional needs of crops.

The utilization of vermicompost results in several benefits to farmers, industries, environment and over all national economy.

Parthenium offers a big challenge to all attempts of control because of its high seed germinability and production of huge seed banks. So there is no single, full proof method of controlling *Parthenium*. Every method suffers from some limitations.

In this situation, here are some suggestions for proper management of *Parthenium*:

- There is an urgent need for an integrated approach by trying out more than one caption to root out this dangerous weed from the face of the earth.
- Integrated approaches like handpicked methods, use of *Parthenium* as vermicomposting, biological control methods etc are needed step by step to eradicate this toxic weed completely from district map and finally from over state and country.
- Public awareness has to be developed and participatory approach to control the invasive weed should be adopted.

Biological control strategies have been proved futile individually to curb proliferation of *P. hysterophorus*. So, integrated approaches are warranted to restrict the invasion of this weed. To address this problem, public awareness and participatory approach to control the weeds should be adopted by every person.

There is a need to encourage the research, which can be achieved through joint efforts of researchers, farmers, government and non government agencies. At present vermicomposting and biogas are some of the recently discovered implications of *P. hysterophorus*.

CHAPTER-8
SUMMARY

SUMMARY OF THE Ph. D. THESIS

Entitled "Management of *Parthenium hysterophorus* through Establishment of Mexican beetle, *Zygogramma bicolorata* Pallister and Evaluation of Host Specificity in and around Kota Region."

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Presently Co-ordinator of Wild Life Sciences, University of Kota, Rajasthan.

Title of the Thesis:

Management of *Parthenium hysterophorus* through Establishment of Mexican beetle, *Zygogramma bicolorata* Pallister and Evaluation of Host Specificity in and around Kota Region.

Research areas: Government College, Kota and Selected sites of Kota region for field work.

Objectives:

- **Objective-A: Bio-control of congress grass, *Parthenium hysterophorus* through *Zygogramma bicolorata* Pallister.**
- **Objective-B: Mass multiplication of *Zygogramma bicolorata*.**
- **Objective-C: Host specificity tests for *Zygogramma bicolorata* on some indigenous and economically important flora species, closely related to *Parthenium hysterophorus.***
- **Objective-D: Evaluation of Quality compost of *Parthenium hysterophorus*.**

Introduction:

Objective- A:

Parthenium hysterophorus Linnaeus (Asteraceae) is an alien and invasive weed which is cited as the seventh most devastating and hazardous weed. The weed is known by different names like “congress grass”, “star weed”, “carrot grass”, “white top”, “chatak chandani” etc. *Parthenium hysterophorus* L. is a native of southern parts of North America, Central America, the West Indies and the central parts of South America (Navie *et al*., 2004; Dhileepan and McFadyen, 2012). *P. hysterophorus* was first introduced in India due to contaminated PL-480 wheat imported from United States in the 1950s (Jayanth *et al*., 1998; Shreshtha *et al*., 2015). *Parthenium hysterophorus* weed has been considered as one of the worst weed because of its invasiveness, potential for spread and economic and environmental impacts (Dhileepan *et al.,* 2002; Chamberlain, 2004; Sushilkumar, 2005; Patel, 2011; Shabbir *et al.,* 2012; Sharma *et al*., 2014; Vyas *et al*., 2017).

This noxious weed is known for its adverse effect on environment, biodiversity, agriculture and health of animals and human being. This is because of the toxic chemicals produced by *Parthenium* plant (Narasimhan *et al*. 1984; Mersie, 2009; Bezuneh, 2015). Various methods for example, physical, chemical, bio herbicides and integrated, are being practiced to manage this weed around the globe but instead of all, there are several disadvantages of using the chemical herbicides, such as the environment hazards, soil fertility decrease etc. So, the mexican beetle *Zygogramma bicolorata* from Mexico was introduced into India in 1983 (Gupta *et al*., 2004; Sushilkumar and Ray, 2010). *Zygogramma bicolorata* (Coleoptera: Chrysomelidae) is a bio-control agent of *Parthenium* which is specific to *Parthenium* only (Jayanth, 1987). In India in some localities, *Zygogramma* has been credited for up to 90% in *P. hysterophorus* population decline (Nath, 1988). During the present research, the adults of *Zygogramma* were found to feed and oviposit on *Parthenium* leaves while the newly hatched larvae fed voraciously on the terminal and auxiliary buds moving on the peripheral leaves causing mass defoliation followed by feeding on the seeds.

Objective- B:

A revolution for the control of *Parthenium* has been achieved through a beetle *Zygogramma bicolorata* Pallister which is an effective bio-control agent of *P. hysterophorus* (Jayanth, 1987 and 1991; Dhileepan *et al*., 2000 and Withers *et a*.,*l* 2000 and 2009). However, in India bio-control of congress grass through *Z. bicolorata* has not been reported as a mass campaign. Hence, through the present research work mass multiplication in field and laboratory conditions of bio-control agent *Z. bicolorata* had been done successfully.

Objective- C:

The practice of host specificity testing has benefited much from such basic studies but we can continue to tune testing methodology by applying the latest information and concepts (Withers, 1997). The host specificity of selected species of *Zygogramma* for the management of *Parthenium hysterophorus* was also observed. Thus among various control measures, the mexican beetle, *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae) has been found as a biological control agent of *Parthenium* (McFadyen, 1992; Dhileepan *et al*., 2000; Dhileepan, 2001).

Objective- D:

An alternative management of *Parthenium* had also been done through 'vermicomposting'. A large mass of *Parthenium* was collected from different areas of Kota region for making its vermicompost.

Integrated weed management is a weed population management system that uses all the suitable techniques in a compatible manner to reduce weed population and maintain them at levels below those causing economic injury (McFadyen, 1992; Upadhyay *et al*., 2011; Gnanavel, 2013; Masum *et al*., 2013).

Materials and Method: (Details of materials and methods will be discussed in thesis)

Materials and method for objective-A:

Parthenium seeds had been sown in a series of 1 sq.m beds for rearing *Zygogramma bicolorata*. Day to day observations were noted regarding successful breeding and feeding of test insects. A complete life cycle of beetles had been studied during the research work.

Defoliation of *Parthenium* plants through *Z. bicolorata* had also been studied under the field conditions in four selected sites (Site "A"- Akashwani, Site "B"- Borkheda, Site "C"- Selected areas of Baran road and Site "D"- Selected areas of Govt. College, Kota) of Kota Rajasthan.

Materials and method for objective-B:

In the laboratory conditions, experiments for mass multiplication of the beetle, *Z. bicolorata* had been carried over throughout the research work.

Materials and method for objective-C:

Various indigenous and economically important plants of different seasons had been taken in choice and non choice tests to select the food and oviposition preference of mexican beetle *Z. bicolorata*.

Materials and method for objective-D:

Through the investigations, quality compost manure (from *Parthenium*) had been prepared. For this purpose handpicked *Parthenium* plants had been collected, placed in sockets, covered with some cow dung and soil and vermicompost was prepared by introducing earthworms (*Eisenia foetida*).

The prepared quality compost manures had been checked for its nutrients and for the growth of different economically important plants e.g. *Helianthus petiolaris*, *Calendula officinalis* and *Tagetes erecta* for comparative study. It was found that

the plants grow faster when treated with *Parthenium* based vermicompost as compared to control vermicompost (only cow dung vermicompost).

Results:

1. Results of objective-A:

1.1 Morphological studies of *Zygogramma bicolorata* in natural conditions:

During the morphological studies of mexican beetle, *Zygogramma bicolorata* all the body parts and stages were thoroughly studied under the natural conditions. It was noticed that egg size was 1.18 – 1.20 mm long, 1st and 2nd instars varied from 1.5 – 3 mm long and 3rd and 4th instars size varied from 5.5 – 7 mm long. Female beetles were relatively larger (6.5 to 7 mm) in size than males (5.5 to 6 mm). Females laid 1 or 2 to 8 eggs at a time on *Parthenium* leaves. 40–45 eggs were laid per day per female. The total number of eggs laid by a single female during its life time ranged from 1150 to 1600 eggs. On the basis of 2013-15 observations, the results have shown that the hatching period was 4-7 days, larval duration extended for 2-3 days for 1st instars, 2-3 days for 2nd instars, 2-4 days for 3rd instars, 3-4 days for 4th instars and total pupation period was 8-13 days respectively with a total development period of 21 – 32 days. Metamorphosis also occurred in it. The average longevity of adult males and females was found 69 to 77 days and 78 to 84 days respectively. September to October was most favorable duration for the development of mexican beetle (21 – 26 days). In winter season beetles showed diapause behavior (65 – 78 days).

1.2 Rearing of adult *Zygogramma bicolorata* in laboratory conditions:

The results clearly showed that five pairs of beetles laid 412 eggs within 2-3 days. Hatching percent of eggs was recorded 80.58% to 89.32% at 27 ± 2^0C and 75 ± 5% relative humidity. Total laid eggs resulted into 332-368 instars. Then these instars emerged out into 235-267 adult *Z. bicolorata.*

1.3 Diapause percentage of released *Zygogramma bicolorata* in laboratory conditions:

The experiments were set up for diapause percentage also. From 300 adult *Z. bicolorata* only 148 adults were found to undergo diapause and 109 adults were not reported to go underground for diapause but stopped their metabolic activities at the surface of soil for 1.5 – 2 months. 43 of the adults died. Hence the percentage of underground diapaused was 49.33%, surface diapaused was 36.33% out of 300 adults released.

1.4 Defoliation of *Parthenium hysterophorus* by *Z. bicolorata*:

Results had shown that mexican beetle controlled the congress grass up to 64.24% at site "A", 58.12% at site "B", 64.35% at site "C" and 83.69% at site "D". (Site "A"- Akashwani, Site "B"- Borkheda, Site "C"- Selected areas of Baran road, and Site "D"- Govt. College, Kota were selected sites). The detail will be highlighted in thesis.

2. Results of objective-B:

2.1 Mass multiplication of *Zygogramma bicolorata* in laboratory conditions:

2.1.1 Study of the pupation of instars and adults emergence:

The results have shown that a total of 90% larvae pupated and 71.11% adults emerged. Out of total emerged adults, the percentage of females (46.66%) was higher than males (24.44%).

2.1.2 Egg laying behavior of female on selected physical substrates:

Maximum mean eggs output was obtained on muslin cloth (76.80)> followed by upper surface covered leaf of *Parthenium* (41.40)> lowered surface covered leaf of *Parthenium* (19.80)> plastic jar (18.8)> glass plates (5.40). Due to the roughness of muslin cloth maximum eggs were laid on it.

To test the hypothesis of equality amongst several means ANOVA test was carried over. The results have shown that the calculated value F= 2.45 is less than the table value= 3.20 at 5% level of significance. The hypothesis was accepted.

3. Results of objective-C:

3.1 Host specificity tests in field conditions:

Some economically important plants like *Oryza sativa, Vigna radita, Vigna mungo, Abelmoschus esculentus, Zea mays, Sorghum bicolor, Glycine max, Thevetia peruviana, Tagetes erecta,* and *Helianthus petiolaris* were selected for oviposition of *Z. bicolorata*. Little oviposition was reported on *Glycine max, Tagetes erecta* and *Helianthus petiolaris* leaves but no feeding behavior was shown on these plants. The results showed that *Z. bicolorata* preferred only *Parthenium* as food and didn't feed on any other plants.

3.1.1 Choice tests (when *Parthenium* is also grown with selected economically important plants):

Feeding on economically important plants (P = 0) and on *P. hysterophorus* (P = 1)

Oviposition on economically important plants (P = 0.1) and on *P. hysterophorus* (P = 1)

3.1.2 Non-choice tests (when only economically important plants are grown without *Parthenium* plants):

Feeding on economically important plants (P = 0)
Oviposition on economically important plants (P = 0.3)

3.2 Host specificity tests in laboratory conditions (Food preference by the instars and adult *Z. bicolor*ata in laboratory conditions):

It was found that HP_{11} (Here HP means host plant) – *Parthenium* was the most preferred host of *Z. bicolorata* than others (HP_1–HP_{10}). The maximum food (*Parthenium*) was consumed by 3^{rd} instars (991.196mg/day/10individual) followed by 4^{th} instars (971.889mg/day/10indi.), 2^{nd} instars (944.152mg/day/10indi.), 1^{st} instars (928.068mg/day/10indi.) and adults (870.07mg/day/10indi.).

4. Results of objective-D:

4.1 Vermicomposting with and without *Parthenium*:

The values of N, P, K contents in the vermicompost made from *Parthenium* were found to be 1.397% (N), 0.0952% (P) and 1.325% (K) which was approximately similar to the contents of cow dung vermicompost which were 1.368% (N), 0.0946% (P) and 1.482% (K). No allelopathic effects were reported through the vermicomposting of *Parthenium*.

4.2 Quality testing of prepared vermicompost (with and without *Parthenium*) on the growth of different plant species:

The comparative results showed better growth of the plants provided with *Parthenium* vermicompost {*Helianthus petiolaris* (27.5 inches),

Calendula officinalis (33.56 inches) and *Tagetes erecta* (40.63 inches)} in comparison to plants treated with only cow dung vermicompost {*Helianthus petiolaris* (25.33 inches), *Calendula officinalis* (30.66 inches) and *Tagetes erecta* (38 inches)}.

Conclusion:

Thus through the present research work it has been estimated that a noxious weed *Parthenium* can be best managed through multiplication of an exotic beetle, *Zygogramma bicolorata* during field as well as laboratory conditions. However Indian climate is suitable only during the months between July last to November for multiplication of *Z. bicolorata* but its population can be maintained at sufficient number during laboratory conditions as well, throughout the year.

An another interesting control measure of *Parthenium* is to prepare its quality vermicompost by derooting sufficient number of plants and dumping in sockets along with known earthworm species and obtaining high quality compost which does not show any allelopathic effect due to its biodegradation. Only there is need to develop awareness among people against proper handling of this weed.

CHAPTER-9
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Management of an Aggressive Weed, Parthenium Hysterophorus Through Introduction of Mexican Beetle, Zygogramma Bicolorata.

Neeta Sharma, Sapana Vyas & Surabhi Shrivastava,
Department of Zoology, Govt. P.G. College, Kota.

Parthenium hysterophorus is an invasive weed colonizing disturbed areas, roadsides, railway tracks, crops fields etc. Its seeds are highly viable, can sprout any where and create allelopathic impacts and health hazard risks. The present study is focused on the biocontrol of congress grass, Parthenium hysterophorus through mexican beetle, Zygogramma bicolorata which has been introduced for the first time in Kota region. The investigations show that the growth rate of Parthenium was minimized through leaf feeding behaviour of various stages of Z. bicolorata. All the stages (1^{st}, 2^{nd}, 3^{rd}, and 4^{th} instars) were found to feed on Parthenium plants at their maximum in comparison to the adults. The selected Parthenium invaded areas were completely devoured by the beetles. Only about 10% of Parthenium spores sprouted which showed about 90% reduction of its invasion.

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A STUDY OF ADULT EMERGENCE PERCENTAGE AND EGG LAYING BEHAVIOR OF *Zygogrammabicolorata* IN FAVOR OF ITS MASS MULTIPLICATION

Neeta Sharma[1], Sapna Vyas[2], Surabhi Shrivastava[3]

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ABSTRACT

The present research work had been carried out to recognize mass multiplication activites of mexican beetle Zygogramma bicolorata To observe the adult emergence percentage of Z. bicolorata in the laboratory conditions experiment were carried out and the result showed that the percentage of emerged females (33.33%) was higher than the emerged males (15%).In the other experiment the egg laying behavior of a female Zyogramma was noticed on different substrates which was significantly higher on muslin cloth (55.52%) due to sticky and roughness of the substrate in comparison to upper surface covered leaf of Parthenium (22.39%), > lower surface covered leaf of Parthenium (13.49%)> and glass (8.58%).

Keywords: Mass multiplication, Parthenium hysterophorus, Zygogramma bicolorata

I INTRODUCTION

The beetle *Zygogamma bicolorata* Pallister is an effective bio control agent of *P. hysterophorus.* The insect was imported from Mexico in 1983. Biological control efforts were initiated in India with the introduction of *Zygogamma bicolorata* Pallister in 1983 (Jayanth and Nagarkatti, 1987) and field releases were initiated in India in 1984. Since then the beetles have brought about considerable reduction in *Parthenium* flower production and enabling local vegetation that had been suppressed by the weed to grow again. *Parthenium* has been considered as one of the greatest source of dermatitis, asthma, nasal-dermal and nasal-bronchial types of diseases resulting fom Parthenin (Nabum Yadi and Mandal, 2008). Parthenin, a glucoside is said to produce depressant effect on nervous system (Chandra and Vartak, 1970). The adult *Zygogramma* beetles feed and oviposit on *Parthenium* leaves while the newly hatched larvae feed voraciously on the terminal and auxially buds, leaves, stem parts etc. It is estimated that about 35 million hectare of land has been invaded by *Parthenium* in India (Sushilkumar and Varshney, 2007). Among various control measures, the mexican beetle, *Zygogramma bicolorata* Pallister

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IMPACT OF TEMPERATURE AND RELATIVE HUMIDITY ON DEVELOPMENT OF ZYGOGRAMMA BICOLORATA .

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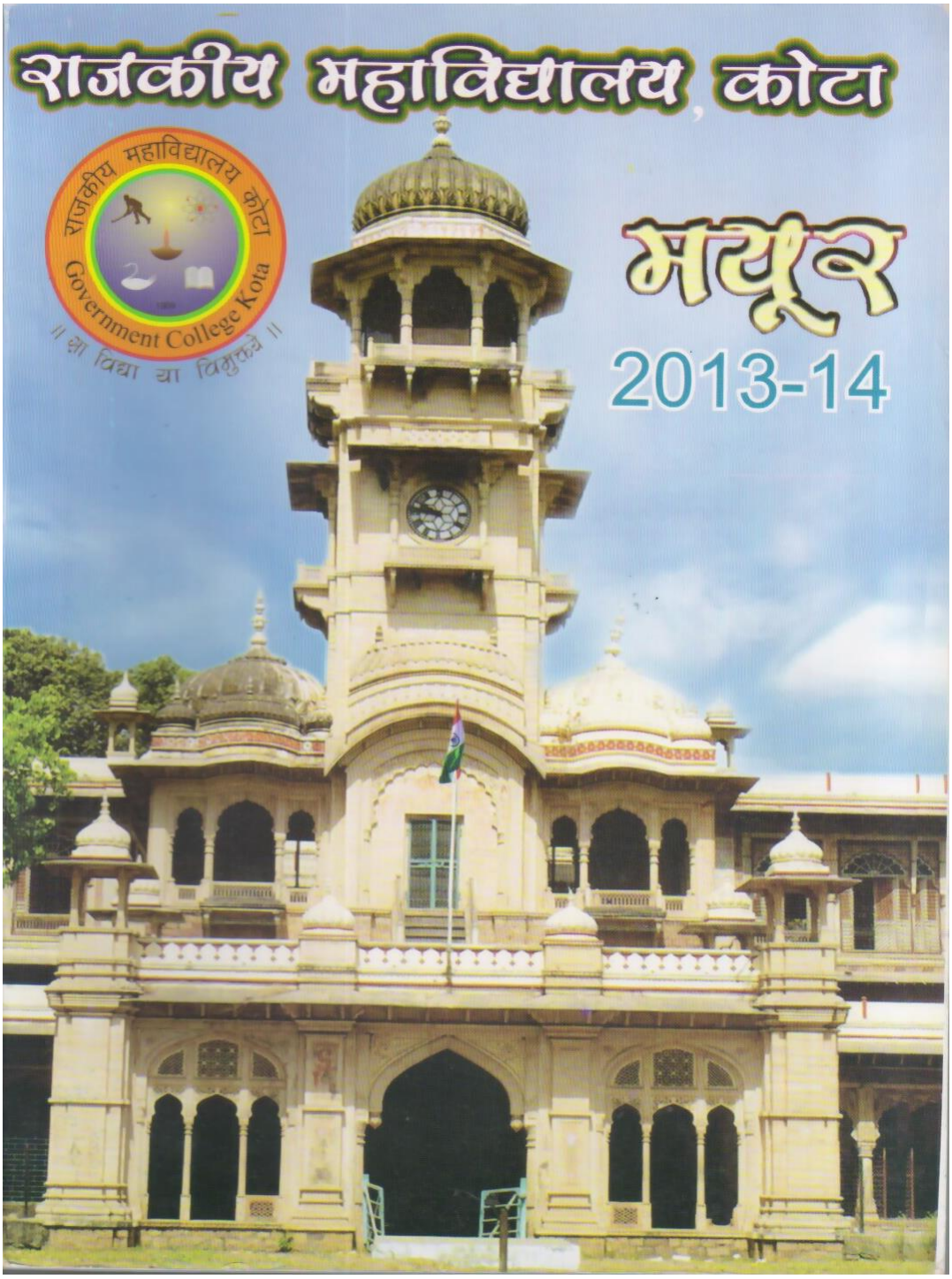
ABSTRACT

Parthenium hysterophorus, commonly known as carrot weed, native of Central America, is one of the first seven most dangerous weeds of the world. It shows strong allelopathic potential and replacement of native species along with health hazards to humans and animals. To control this noxious weed instead of using chemical weedicides, a cost effective and environmentally safe method through the release of a biological control agent (insect), Zygogramma bicolorata has been practiced. During the present investigation the effect of temperature and relative humidity was studied on the development of Mexican beetle (Zygogramma bicolorata). The developmental stages of Mexican beetle were studied under controlled conditions of temperature and relative humidity (20ºC, 65%; 24 ºC, 70%; 28ºC, 75%, 32 ºC,80%) reared on weed Parthenium hysterophorus. The aim of the study was to analyze the effect of temperature and relative humidity on Z. bicolorata so that mass multiplication can be done efficiently. Studies revealed that maximum development, fecundity and survival were recorded at 28 ºC and 75% RH. Four larval instars were seen and first stage instars were most fortified and defenseless while 3rd and 4th instars were least fortified. Lower temperature (20 ºC) and low humidity (65%) do not favor the mass multiplication and beetle showed diapause behavior, while maximum fecundity and survival was at 28 ºC, 75% RH. This study also established that percentage larval instars life span was almost same at all the temperatures and relative humidity but the duration of pupation was 12-16 days at 20 ºC, 65% RH and 32 ºC and 80% RH, while it was only 9-12 days at 28 ºC, 75% RH.

Key Words; Zygogramma Bicolorata , Mexican Beetle, Parthenium Hysterophorus.

I. INTRODUCTION

Ever since *Parthenium hysterophorus L.* (Asteraceae) introduced in India has become one of the most dangerous weed of wasteland, forestland, along roadside along railway tracks, agriculture land and over grazed pasture lands. It is a fast maturing annual short lived perennial herb with a deep tap root. It may eventually reach a height of 2m. leaves are pale green, branched and covered with soft fine hairs. The weed has strong allelopathic potential (Kanchan and Jayachandra 1980; Singh et al 2002) Persistent soil seed production (Pandey and Dubey 1988) and phenotypic plasticity in growth form (Annapurna and Singh 2003). Invasion of this weed has replaced native species and inturn native diversity has been affected (Grice 2006, Timisina et al 2011) and increased health hazard to animal and human (McFadyen 1995). Parthenium may cause various diseases such as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॥ सा विद्या या विमुक्तये ॥
मयूर
2013-14

Parthenium : A Threat to our Living beings and Environment

Neeta Sharma, Sapna Vyas and Dr. Surabhi Shrivastava

Weeds are represented as a major contributing factor of crop yield loss. The prolific seed production of most weeds also increases the weed seed bank which contributes to problems in subsequent time, lowering in crop grades, raising cost associated with harvesting and seed cleaning.

Parthenium weed commonly called as "gajar ghas" is a new, notorious and potentially major weed in India. It was accidentally introduced into India around 1956. P.hysterophorus is commonly known as ragweed, carrot grass, star weed, white top or municipal weed etc.

Parthenium does not reproduce vegetatively, the only method of reproduction and dispersal is by seeds. It can produce large amount of seeds (up to 100,000 per plant). The seeds can be easily spread by vehicles, machinery, animals, wind and water, although being a drought resistant plant it can grow in almost all soil types.

It is an invasive plant species that colonizes disturbed areas, cultivated lands, roadside vegetation and human settlement areas. This weed not only infests wasteland, but also invades cultivated fields and poses a threat to crops such as cereals, vegetables, oil seeds etc. This weed is also a serious threat to human and animal health by causing respiratory problems, allergy or severe dermatitis etc. Different herbicides and vermi composting methods are commonly used to get rid of this weed but herbicides create many problems in polluting of our environment. Hence to bio control *P. hysterophorus*, a leaf feeding exotic beetle has been introduced firstly in Kota region through present research work. In recent studies the *Parthenium* seed bank has covered many regions of Kota.

The exotic beetle has also been introduced in a part of Govt. College Kota, Zoology campus and it has been controlled to a great extent. The main objective of this article is to introduce the bio control agent and raise the general awareness for control of the weed *Parthenium* focusing on its status and possible hazards to human, cattle and vegetation. It also focuses on different control measures and their

अमेरिका से आए कीड़े ने कंट्रोल की घातक गाजर घास

अंतरराष्ट्रीय जैव विविधता दिवस पीजी कॉलेज में दो साल पहले जूलॉजी डिपार्टमेंट की ओर से मंगवाए गए थे कीड़े, 40 फीट एरिया में सफल रहा प्रयोग

प्रवीण जैन | कोटा

जंगल से लेकर शहरी इलाकों तक जैव विविधता के लिए घातक मानी जाने वाली गाजर घास की रोकथाम हो सकती है। कोटा में इन घासों को रोकने का प्रयोग भी सफल हुआ है। गाजर घास मानव स्वास्थ्य के लिए भी हानिकारक है। प्रयोग सफल होने की पुष्टि पीजी गवर्नमेंट कॉलेज की एसोसिएट प्रोफेसर डॉ. सुरभि श्रीवास्तव ने की है। उन्होंने बताया कि इस घास पर नियंत्रण करने के लिए 2012 में अमेरिका से करीब 1000 कीड़े (मैक्सिकन बीटल) मंगवाए गए थे।

गाजर घास वनस्पतियों के अलावा इंसान और जानवरों के लिए भी घातक है। डॉ. सुरभि के मुताबिक कीड़ों के लिए गाजर घास का करीब 40 फीट एरिया चिह्नित किया गया था। घास के खात्मे के लिए कीड़ों को करीब दो से ढाई फीट जमीन के नीचे रखा गया। धीरे-धीरे दो साल में कीड़ों ने यहां घास का खात्मा कर दिया। गाजर घास खत्म होने के बाद इसके आसपास के पौधों में अच्छी ग्रोथ होने लगी। दरअसल, यह घास अपने आसपास की वनस्पतियों के पोषक तत्वों को खींच लेती है। जिससे पेड़-पौधों का विकास रुक जाता है। कॉलेज के जिस एरिया में इन कीड़ों का प्रयोग नहीं हुआ, वहां खूब गाजर घास है। जिससे उनके अगल-बगल के पौधे विकसित नहीं हो रहे हैं।

गर्वमेंट कॉलेज में जहां मैक्सिकन कीड़े का प्रयोग हुआ, वहां से गाजरघास गायब हो गई। जहां नहीं प्रयोग हुआ, वहां अभी भी खूब फल-फूल रही है गाजर घास।

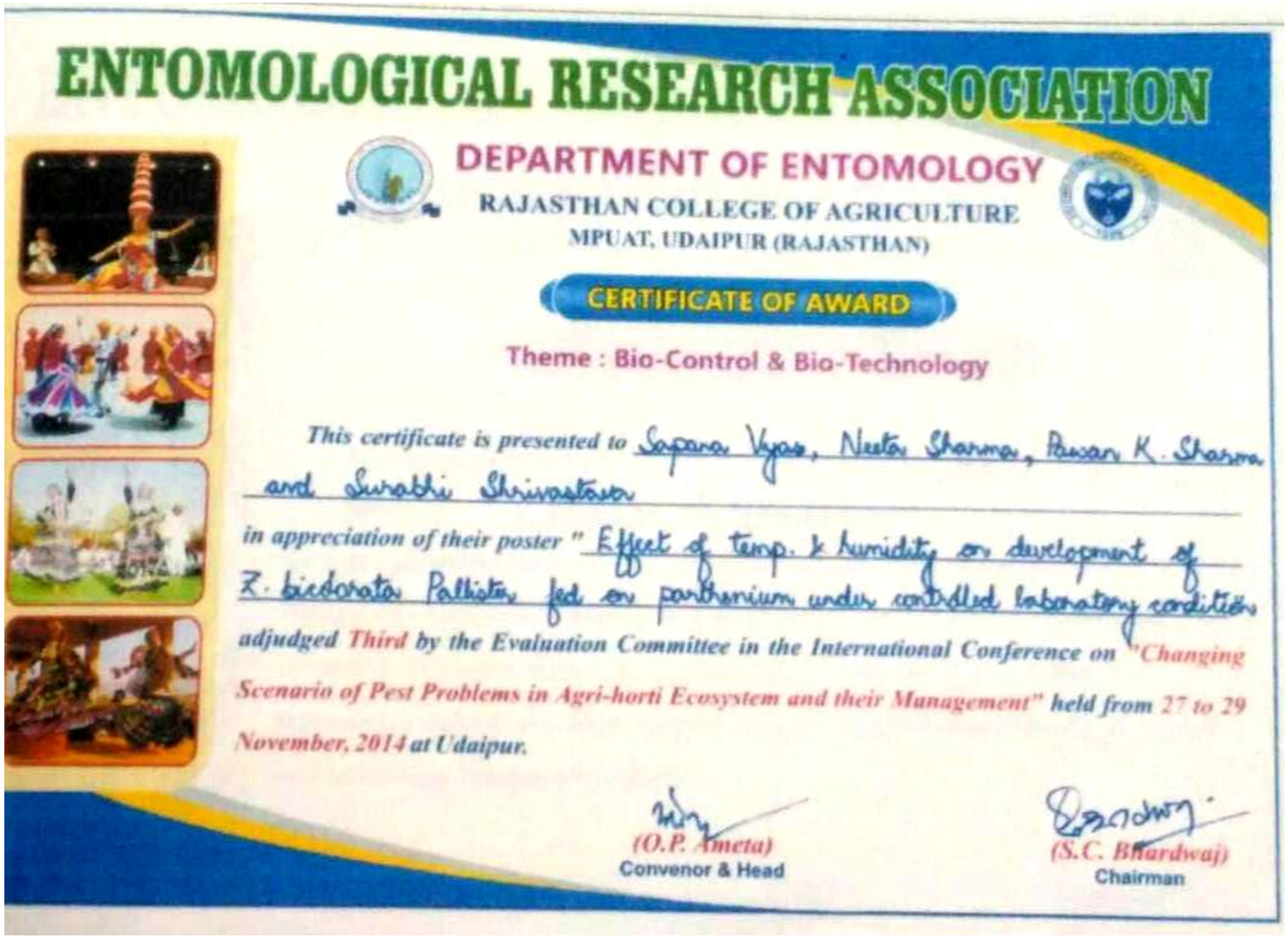
अमेरिका से आया था बीज

डॉ. श्रीवास्तव ने बताया कि 1950 में अमेरिका से गेहूं के साथ इस घास के बीज भारत आ गए थे। धीरे-धीरे ये खेतों समेत जंगलों और रिहायशी इलाकों में फैल गए। जो अब घातक साबित हो रहे हैं। रिसर्चर सपना व्यास और नीता शर्मा इस पर शोध कर रही हैं।

इंसान व जानवर दोनों के लिए खतरनाक

गाजर घास और इनके बीजों से सांस की तकलीफ, अस्थमा, दमा, एलर्जी जैसी बीमारियां होती हैं। जंगल में वनस्पतियों को तो नुकसान पहुंचाती ही है, साथ ही वन्यजीवों में गंभीर बीमारियां फैलाती है।

» **जैव विविधता पर कार्यक्रम आज:** जैव विविधता दिवस पर गुरुवार सुबह 10 बजे सूचना केन्द्र में वन विभाग और स्वयंसेवी संस्थाओं की ओर से कार्यक्रम होगा। कार्यक्रम समन्वयक डॉ. एलके दाधीच ने बताया कि मुख्य अतिथि सीसीएफ एमएल मीणा होंगे। उपवन संरक्षक विक्रम केसरी प्रधान ने बताया कि इस दौरान विजुअल प्रजेंटेशन के माध्यम से जैव विविधता के संबंध में एक्सपर्ट जानकारियां देंगे।



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CERTIFICATE OF AWARD

Theme : Bio-Control & Bio-Technology

This certificate is presented to Sapana Vyas, Neeta Sharma, Pawan K. Sharma and Surabhi Shrivastava

in appreciation of their poster " Effect of temp. & humidity on development of Z. bicdorata Pallisto, fed on parthenium under controlled laboratory conditions *" adjudged Third by the Evaluation Committee in the International Conference on "Changing Scenario of Pest Problems in Agri-horti Ecosystem and their Management" held from 27 to 29 November, 2014 at Udaipur.*

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