BIOLOGICAL CONTROL OF INSECT PESTS OF SUGARCANE BY PARASITIC NEMATODES

ABSTRACT

THESIS
SUBMITTED FOR THE AWARD OF THE DEGREE OF
Doctor of Philosophy
IN
ZOOLOGY

BY
NAYER IQBAL

DEPARTMENT OF ZOOLOGY
ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA)
2003
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ABSTRACT

*Heterorhabditis* and *Steinernema*, the two rhabditid nematodes, have drawn attention of the workers around the globe for being potential biological control agents of insects. These soil dwelling nematodes, together with their bacterial symbionts, are obligate and lethal parasites of insects and are usually referred to as Entomopathogenic Nematodes (EPN). Nematodes’ non-specific development, which does not rely on specific host nutrients, allows them to infect a large number of insect species, as a result these nematodes have got a wide host range and can be used successfully against numerous pests. India being a country where sugarcane is a major cash crop, it was found necessary here to study the control the control status and level of pathogenicity of entomopathogenic nematodes in controlling the major pests of sugarcane. This study is an attempt to test the potential of the two entomopathogenic nematodes, *Steinernema* sp. and *Heterorhabditis* sp. against three lepidopteron pests; shoot borer (*Chilo infuscatellus* Snellen), Gurdaspur borer (*Acigona steniellus* Hampson), and root borer (*Emmalocera depressella* Swinhoe), and a coleopteran pest sugarcane beetle (*Holotrichia consanguinea* Blanch.) in the laboratory. The study further includes laboratory analysis of the effect of abiotic factors, namely, temperature, humidity, solar irradiations and soil type on the virulence and the control status of the entomopathogenic nematodes.

The larvae of these four insect species were exposed for infestation to the infective juveniles of both the entomopathogenic nematode species under
study. The present study was divided in two forms of experiments; the one named as 'infectivity assays' was conducted as a preliminary study to test the potential of both the entomopathogenic nematodes individually against the larvae of the four insect pest species one by one. The other experiment was termed 'efficacy studies' where the two concentrations of the infective juveniles (IJs) of entomopathogenic nematodes (100IJs/ml., Hb1&Sn1 and 1000IJs/ml., Hb2&Sn2) were utilized to be employed against 100 pest larvae kept in the petridishes at constant optimum condition set at 25°C, 85% RH in the sandy loam soil and kept in the dark. Paper bioassay was selected for preliminary studies in the infectivity assays and petridish bioassay for laboratory trials in the efficacy studies. The percent mortality of pest larvae on nematode applications was a tool to determine the control status of the entomopathogenic nematodes. The pathogenicity or persistence level inside the host was determined through percent infectivity. The efficacy studies were then also conducted under variable ecological factors to investigate the effect of changes in temperature, humidity, sunlight and soil texture on the control status of entomopathogenic nematodes. The ecological studies were planned under conditions taking one of the aforementioned factors under varied limits and the other factors taken to be constant within optimum range. The various temperature ranges selected were 5-10°C, 10-15°C, 15-20°C, 20-25°C, 25-30°C, 30-35°C, 35-40°C, 40-45°C, and 45-50°C. The various levels of relative humidity (RH) selected were 30%RH, 50%RH, 70%RH, and 90% RH with standard error of ± 5%. The various sunlight conditions selected were direct and diffused sunlight and dark. The various soil types selected were clay soil,
loam, sandy soil and coarse sand (Badarpur soil). Adopting the procedure identical to that employed for studying the efficacy of nematodes in controlling the larvae of the four insect pests under study, two different concentrations of \( \gamma \)-BHC (Lindane) were applied on the insect larvae, under constant ecological conditions, in order to compare the results obtained by using the nematodes as control agents with those obtained by using \( \gamma \)-BHC (Lindane). The results were statistically analyzed by using ‘analysis of variance’, ‘LSD test’, and ‘student’s t-distribution test’.

The infectivity assays indicated positive results showing that both the entomopathogenic nematodes *Heterorhabditis* sp. and *Steinernema* sp. under study were effective against the larvae of all the four pest species studied here. In the efficacy studies done at constant ecological factors, it was found that while the low concentrations of both these nematodes were less effective against all the four species of insect pests and showed low persistence level and low control status, their high concentrations showed significant effect on these four different insect pests and had high persistence level and high control status. *Steinernema* sp. was found to show significantly higher control status and persistence level than that showed by *Heterorhabditis* sp. when used against the larvae of shoot borer, *C. infuscatus*; Gurdaspur borer, *A. sterniiellus*; and root borer, *E. depressella*. In the case of sugarcane beetle, *H. consanguinea*, however, *Heterorhabditis* sp. was found to be comparatively more effective than *Steinernema* sp., and showed higher control status as well as higher persistence level in comparison to the other nematode species. Both *Heterorhabditis* sp. and *Steinernema* sp. caused higher percent
infectivity than percent mortality in the larvae of all the four species of insect pests of sugarcane studied here, thus indicating that the persistence level of both the entomopathogenic nematodes was higher than their control status. The control status of the two biocontrol agents, *Heterorhabditis* sp. and *Steinernema* sp. was found to be meaningful when compared to the control status of chemical pesticide γBHC, recommended by U.P.C.S.R., regarding control of the larvae of four insect pest species under study. (FIG- 1-, 2., 5)

In the efficacy studies at variable temperature ranges it was found that both low as well as high temperature ranges had adverse effect on the control status of *Heterorhabditis* sp. and *Steinernema* sp. Best results for *Heterorhabditis* sp. as control agent were obtained between 20-25°C; and, *Steinernema* sp. gave best results between 20-30°C. As compared to *Steinernema* sp., members of *Heterorhabditis* sp. were more susceptible to deviations from the optimum temperature range, both on the lower side of this range as well as on its higher side. As far the deviation from the optimum range of temperature in the case of *Steinernema* sp. it was found that the members of this nematode species were more tolerant to temperatures lower than the range of optimum temperature (20-30°C) than to the deviations on higher side of their optimum. The efficacy studies conducted here at various humidity levels shows that both these species of nematodes had best control status at 90 percent relative humidity. The control status of these two species of nematodes reduced with the reduction in humidity levels. Pathogenicity of these nematodes was directly related to the humidity levels, being favourably affected by high humidity levels and adversely affected by its low level. The
efficacy studies conducted here at various sunlight conditions revealed that both these nematodes acted poorly under conditions of bright sunlight. Both of these showed high control status under diffused sunlight and dark conditions with the results for them under these two conditions of light being almost similar. Efficacy studies conducted in different soil types revealed that loam was best suited for the activities of both the entomopathogenic nematodes under study. *Heterorhabditis* sp. showed highest control status in loam, followed by clay soil, sandy soil and lowest in coarse sand (Badarpur soil). *Steinernema* sp. showed highest control status in loam, followed by sandy soil, coarse sand (Badarpur) and lowest in clay soil.

It can therefore be concluded in the light of the present study and the studies done by various other scientists that entomopathogenic nematodes are potential biocontrol agents. Further it is evident in the laboratory studies that *Steinernema* sp. has got a good potential in controlling shoot borer, *C. infuscattellus*; Gurdaspur borer, *A. steniellus*; sugarcane beetle, *H. consanguinea*, and to a considerable extent root borer, *E. depressella*. Moreover, *Heterorhabditis* sp. has also been found in the laboratory studies to have a good potential in controlling three of the four insect pests under study namely shoot borer, *C. infuscattellus*; Gurdaspur borer, *A. steniellus*; sugarcane beetle, *H. consanguinea* with no significant control observed in the case of root borer, *E. depressella* through this nematode. However, further work is required, particularly in commercial growing conditions in the field, before the entomopathogenic nematodes studied here can be regarded as
suitable alternatives to gamma BHC (Lindane) for the control of the four insect pest species of concern.

In spite of low success rate in the field trials there is a need of laboratory studies to be conducted to test the potential of entomopathogenic nematodes against a wide variety of insect pests still to be tested. Current strategies for the control of insect pests in agriculture rely increasingly on the development of transgenic crops expressing insecticidal protein toxins, and there is a continuous search for novel insecticidal toxins (Constant & Bowen, 2000). Biotechnologists all over the world are working on various aspects of the pesticidal toxins isolated from entomopathogenic nematodes and their bacterial symbionts (Bowen & Ensign, 1998 and Morgan et al, 2001). The significance of our studies and its findings for further investigation is that the aforementioned entomopathogenic nematodes against the pest larvae studied here can be tested in the field and the consideration that toxins isolated can also be applied with a transgenic option in sugarcane plant.

The various biological and chemical pesticides and their different doses used are:

**Stock solutions with infective juveniles (IJ's) of *Steinernema* sp.**
1. Sn 1: 100 + 10 IJ's per milliliter of stock solution.
2. Sn 2: 1000 + 10 IJ's per milliliter of stock solution.

**Stock solutions with infective juveniles (IJ's) of *Heterorhabditis* sp.**
1. Hb 1: 100 + 10 IJ's per milliliter of stock solution.
2. Hb 2: 1000 + 10 IJ's per milliliter of stock solution.

**Stock solutions of Gamma BHC (Lindane).**
1. L1: 20% EC of 0.625 ml. yBHC per 187.5 ml. of water.
2. L2: 20% EC of 1.25 ml. yBHC per 187.5 ml. of water.

**Stock solution of control experiment.**
Co: normal saline (0.65%).
Fig. 1. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of shoot borer, *C. infuscatus* under laboratory conditions.

Fig. 2. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of Gurdaspur borer, *A. steniius* under laboratory conditions.
Fig. 3. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.

Fig. 4. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of root borer, *E. depressella* under laboratory conditions.
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ALIGARH MUSLIM UNIVERSITY
ALIGARH (INDIA) 2020
CERTIFICATE

This is to certify that the thesis entitled "BIOLOGICAL CONTROL OF INSECT PESTS OF SUGARCANE BY PARASITIC NEMATODES" submitted for the award of the Degree of Doctor of Philosophy in Zoology of Aligarh Muslim University, Aligarh embodies the original research work carried out by Mr. Nayer Iqbal under my guidance and supervision and has not been submitted for the award of any other degree or diploma of this or any other university to the best of my knowledge.

(Dr. Wajihullah)
Supervisor
“We ourselves feel that what we are doing is just a drop in the ocean. But the ocean would be less because of this missing drop.”

Mother Teresa
"They would walk a thousand miles just to get a glimpse of my face. They would writhe in pain at the slightest hint of any discomfort to me. And, on seeing me smile, the shine in their eyes could put the radiance of the sun to shame. The desire of bestowing me with all the possible pleasures of life has been a large part of their pain. Beyond doubt, they are a lighthouse of inspiration to me forever and a day."

Dedicated to MY PARENTS

because of whom I am what I am.
ACKNOWLEDGEMENTS

Anything that is neoteric, unusual, and unorthodox should be carried out delicately, with a proper direction and immaculate supervision. My supervisor Dr. Wajihullah, Section of Parasitology, Deptt. Of Zoology, AMU, with his proactive approach, tireless effort, savvy navigation, and keen interest fits the bill earnestly.

Thank you, Sir for your enthusiastic and energetic attitude towards the completion of this venture. You shall always inspire me, in all my endeavours.

I put on record my sense of requital and gratitude to Dr. S. B. Singh, Director, UPCSR, whose wholehearted help and cooperation inspired and guided me during the course of this work.

I also take opportunity to offer my deep sense of gratitude to Prof. Durdana Shamim Jairajpuri, Chairman, Deptt. of Zoology, AMU, for providing me the necessary facilities to carry out this work.

I also ventilate my profound sense of obligation to Prof. Shahid H. Khan, Section of Nematology, Deptt. of Zoology, AMU, for his unbridled support throughout the compilation of this work and otherwise I must add that without his help it would have not taken this shape.

I would also like to express my indebtedness to the staff of Sugarcane Research Institute, UPCSR, for their technical help together with creative and valuable inputs. I have nothing special to offer them but the small piece of work as a token of love and respect.
My heart goes out to all my friends who have helped me in this compilation in his/her own way. To them I owe at least million thanks and just this maxim – “A friend in need is a friend indeed”.

I must add that there is a space between man's imagination and man's attainment that may only be traversed by his longing. What I am and hope to be I owe to my dear parents and loving sisters.

Last, but above all, I thank the Almighty God for guiding me with His divine light whenever I went astray, and for blessing me with the strength.

Nayer Iqbal
The species contributing to the 'web of life' are interdependent and this characterizes the dynamics of all ecosystems, the terrestrial being no exception to it. Their association is either close or distant due to their similar, constant and simultaneous requirements. This association may be harmful or beneficial and so there is struggle for existence. Man hasexcelled over the rest of the creation through adapted capabilities in devising adequate means to combat all his foes. Insect pests are among the greatest foes of man. They cause heavy damage to vegetables, cereal crops, fruits, stored grains, cotton and sugarcane.

Considering that pest species is one that we, as humans, consider undesirable because its members compete with us for food and other crops, transmit pathogens, and feed on man and domesticated animals, an abundance of pest population may threaten human health, comfort and welfare. So our aim is to control pests, involving manipulation of population's abundance, which is often drastically reduced, in order to allow the hungry to be fed and prevent the ravages of disease. The aim of pest control is an economic one to reduce the pest population to a level below which no further reductions are profitable, i.e., below the economic injury level (EIL) for the pest.

The origin of pest control dates back to the initial development of agriculture approximately 10,000 years ago. Sumerians are known to have used sulphur compounds for pest control more than 4500 years ago. Chinese used insecticides derived from plants about 2500 years ago and developed biological control practices by AD 300. The development in cultural and chemical pest control practices continued thereafter. In Europe, renaissance and the 17th century saw the introduction of a variety of natural pesticides for bringing down the damage caused by the insects. The 18th century was the time of agricultural revolution marked by considerable increase in the
agricultural products. But as could be expected, it was followed by greatest pest drawn disasters also. However, it was in the 19th century that a series of breakthroughs took place which included the adoption of sound and varied agricultural practices like biological, cultural, and mechanical control methods along with the development of new chemical pesticides. These methods were evolved to bring down the damages to agricultural crops caused by insect pests. Pest control was revolutionized after the Second World War, driven by the need to control insect vectors of human diseases. This was followed by the outburst of organic insecticides like DDT that seemed to be truly ‘miracle insecticides’. It went on till the publication of Rachel Carson’s ‘Silent Spring’ in 1962 asserting that the chemical barrage, as crude a weapon as the cave man’s club, has been hurled against the fabric of life. These doubts drove the scientists all over the world to look into the problems with chemical pesticides. Studies which were conducted subsequently, established several demerits with these pesticides like widespread toxicity, target pest resurgence, secondary pest outbreak and, the most serious among them all, the evolved resistance, followed by even more serious multiple resistance. In spite of the aforesaid problems, however, pesticide production increased rapidly because of their highly appreciable results and lack of preferable alternatives. Opposition for the use of pesticides grew steadily, until the XVth International Congress of Entomology in 1976 firmly rejected the wide spread use of broad-spectrum and persistent pesticides in favour of an Integrated Pest Management (IPM) approach, which had a clear seat for alternative methods like biological control.

Biological control may be defined as the use of natural enemies to suppress the pest species. The term natural enemy refers principally to predators, pathogens, and parasites that can be used for reducing pest population. The concept lies in working with the forces of nature rather than against them. This is inherent in Integrated Pest Management (IPM) approach.
Until now, insects have undoubtedly been the main agents of biological control against insect pests, where parasitoids have been particularly useful. These parasitoids were used for permanent long-term pest control through the process of introduction of natural enemies from another geographical area, often the area from where the pest originated and is termed as *importation*. The other methods were *inoculation* and *augmentation*. Our increasing interest in the direction of control of insect pests, and a search for alternative methods has now focused our attention on the use of insect pathogens as agents of biological control. There are several species of insect pathogenic bacteria, viruses and fungi known to us today; but the attention has so far been paid mainly on the use of bacterium *Bacillus thuringiensis*, baculoviruses and a few fungi as an alternative method for the purpose of biological control. These days the **entomopathogenic nematodes** are also being seriously considered as agents of biological control. Fundamental to their role as insect control agents, however, is the mutualistic relationship existing between the entomopathogenic nematodes and the insect pathogenic bacteria which is mainly responsible for bringing down the population of insect pests. These were the first commercially available biological control products with the name EPN products introduced in the market for control of a wide variety of pests. These biological control agents and their products are used through the process of *inundation*, i.e. the release of large numbers, and due to this analogy with the use of chemicals they are referred to as biopesticides.

The entomopathogenic nematodes of the genera, *Steinernema* and *Heterorhabditis* (Nematoda: Rhabditida) are known to be effective biological control agents. Glaser first of all reported it in 1932. Later, Welch (1958) made important suggestions regarding the use of nematodes as biological control agents. Since then the potential of these nematodes has been tested against a wide variety of insect pests. Encouraging results in this field have attracted both small-scale industries and high value market towards the mass production of these nematodes.
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CHAPTER # 1

INTRODUCTION
Heterorhabditis and Steinernema, the two rhabditid nematodes, have drawn attention of the workers around the globe for being potential biological control agents of insects. These soil dwelling nematodes, together with their bacterial symbionts, are obligate and lethal parasites of insects and are usually referred to as Entomopathogenic Nematodes (EPN). Both these nematodes have a global distribution and almost similar mode of action. Members of these two genera of entomopathogenic nematodes can provide effective biological control of some important lepidopteran and coleopteran pests of commercial crops.

The infective stage in the life cycle of Entomopathogenic nematodes is the third stage dauer juvenile, also known as infective juvenile (IJ). The infective juveniles are free living and non-feeding, representing the stage that possesses attributes of both insect parasitoids or predators and microbial pathogens. Like parasitoids and predators, they have chemoreceptors and are motile; like pathogens they are highly virulent, killing their hosts quickly, and can be easily cultured in-vitro, have a high reproductive potential, and have a numerical and no functional response (Kaya & Gaugler, 1993).

Entomopathogenic nematodes are known to have a broad host range, covering several insect pests of various agrosystems. In spite of being virulent to so many insect species, these nematodes have been found to be non-virulent to vertebrates, plants and other non-target organisms, thus being suitable biocontrol agents favouring the strategies of Integrated Pest Management (IPM) programme.

The possibility of their easy application using standard spray equipments, compatibility with various chemical pesticides and a great scope
of genetic improvement make the Entomopathogenic nematodes all the more useful as effective biological control agents.

The insect parasitic nematodes belonging to *Steinernema* spp. and *Heterorhabditis* spp. have got an almost similar life cycle. The parasitic phase of their life cycle is initiated by the third stage infective juveniles (IJ3s). Before getting entry into the host, and, under suitable climatic conditions, these infective juveniles can survive in moist soil for extended periods of time, but do not feed, surviving on stored energy resources until a host is located (Grewal et al, 1994). Infective juveniles navigate through soil and orient to host chemical cues, such as carbon dioxide and cuticular components. The host-location strategy may involve either "sit-and-wait" ambushing or "seek-and-destroy" cruising behaviour. These strategies have proven highly successful; so that entomopathogenic nematodes can be found in the soil practically anywhere where the insect host is available. Nematodes attracted to insect hosts, enter through the natural body openings (i.e., mouth, anus or spiracles) and in the case of *Heterorhabditis* sp., also through soft intersegmental membranes (Bedding & Molyneux, 1982), to gain access to the haemocoel. Some mermithid and tylenchid nematodes found associated with insects penetrate the host’s cuticle with the aid of their oral stylet and glandular secretions. However, this is not the case for Steinernematid nematodes that rely on entry through the natural body openings (i.e., mouth, anus and spiracles). The high hydrostatic head present in these nematodes, plus the extremely narrow diameter of the anterior region, enable these nematodes to punch through the soft internal parts of the insect. While being an additional method of entry into the body of the host, the cuticular penetration in the case of some insects which are characterized by having fine diameter spiracles, tightly closed oral and anal apertures or, in the case of beetles, sieve plates covering their
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spiracles, cuticular penetration is the only method of entry available to the heterorhabditid nematodes.

After gaining entry into the body of insect the nematodes invade the haemocoelomic space of the host where they emit toxins which suppress the insect inducible immune system, and release the insect pathogenic bacteria which have a symbiotic existence in he intestine of nematode, thus killing the host insect. Bacteria multiply in the haemocoel and produce antibacterial compounds, which restrict the growth of other types of microorganisms, and as a consequence, create ideal conditions for reproduction of the nematode (Poinar, 1979). The nematode may appear as little more than a biological syringe for its bacterial partner, yet the relationship between these organisms is one of classic mutualism.

The nematode and bacterial toxins cause haemolysis and septicaemia, thus causing insect death. The nematodes feed upon the bacteria and degraded insect tissue and develop to first generation adults. Steinernematids are amphimictic in all generations. Large females and smaller males mate and produce progeny. This first generation is composed of larger adults than any succeeding generation (this is thought to be due to the abundant nutrition available). The infective juveniles of *Heterorhabditis* develop into hermaphroditic females. These females produce the next sexual generation. After mating, the female nematodes produce eggs which hatch within the uterus. The second generation of juveniles feed on the contents of uterus, then breaks out into the mother’s body cavity and feed within the parent’s cuticle. After having consumed the food material which was available within the parent’s body, the juveniles break out of the nematode cuticle and enter the insect haemolymph. These juveniles may develop into male or female second-generation adults. The adult nematodes mate and a third generation of juveniles
is then produced. These third generation juveniles continue to feed on the contents of insect’s haemocoel. If the amount of food available to them is adequate they develop into the adults which, in turn, will produce the next generation of juveniles. If however the third generation juveniles happen to face a depletion in the amount of food available to them in the insects haemocoel, a good number of these third generation juveniles are liable to transform themselves into third stage infective juveniles, to become the survival form of the life cycle as has been observed at least in an \textit{in vitro} study on \textit{Heterorhabditis} sp. by Strauch et al in 1994. The insect cuticle then ruptures and the third stage ensheathed infective juveniles escape into surrounding environment. Up to $5 \times 10^5$ infective stage juveniles per gram of larva are produced, which leave the dead body of the larva (henceforth referred to as ‘cadaver’ in this thesis) to locate new hosts. Symbiotic bacteria from the insect cadaver associate with the foregut of daughter nematodes prior to their release. (Poinar, 1990).

Nematodes’ non-specific development, which does not rely on specific host nutrients, allows them to infect a large number of insect species, as a result these nematodes have got a wide host range and can be used successfully against numerous pests. However the members of two insect orders, \textit{Lepidoptera} and \textit{Coleoptera} have so far been found to be most effected. Entomopathogenic nematodes have been tested against a wide variety of insect pests, in various geographical regions of this planet. In spite of appreciable success attained in this area of research, more efforts are needed for the welfare of increasingly complex human civilization. In view of the non specific development in the case of these nematodes and a long list of the agricultural pests among insects which are still to be screened for their susceptibility to the nematode attack, it can be emphasized here that as a major step towards the maximum utilisation of these nematodes as biopesticides, more work is
required in the direction of testing the so far unscreened pest species for finding out whether they are susceptible to entomopathogenic nematodes or not.

Importation, a classical means of biological control, is initiated by the introduction of an exotic species of entomopathogenic nematode for controlling the specified susceptible pest. The infective juveniles and their mutualistic bacteria infest the insect host and cause pest mortality. As mentioned supra the nematodes feed, develop and reproduce on the bacteria and decomposing host tissue, and a new generation of nematodes, complete with their charge of symbiotic bacteria, is then released to infect new hosts. This cycle is repeated and consequently the pest population does not increase above its economic threshold, thus providing long-term pest management. However importation of entomopathogenic nematode species can show the desired results of effective long-term control of pests only in those geographical areas, where climatic conditions favour the survival and successful pathogenicity of these nematodes, throughout the year.

Inoculation and inundation of the entomopathogenic nematodes are the two means of choice for biological control of insect pests in those areas where these nematodes are unable to survive or successfully perform throughout the year. Such areas require periodic release of nematodes at proper timings, insuring their presence and consequent pathogenicity. A better understanding of the variation in ecological factors- viz., temperature, humidity, sunlight and medium of persistence, i.e., soil and host body affecting survival and pathogenicity of entomopathogenic nematodes is needed. The wide insect host range of parasitic nematodes is due to their ability to survive environmental stress. The infective juveniles of entomopathogenic nematodes are tolerant to adverse environmental conditions. However, while their being equally tolerant to several other climatic factors, regarding their response to the factors of
radiation and dehydration, it has been found that members of *Steinernema* sp. are more tolerant to these stresses than the members of *Heterorhabditis* sp. (Gaugler, 1988). Although environmental stress has no major effect on nematode survival but their pathogenicity is greatly influenced by variations in climatic, edaphic and biotic factors (Ghally et al., 1994). The pathogenicity of entomopathogenic nematodes should be scrutinized regularly to examine the impact of varied ecological factors; and, therefore, the need for optimizing conditions to ensure nematode survival and its use as a selective control agent.

Many specific growing systems are still waiting for the entomopathogenic nematodes to be introduced for controlling a bulk of insect pests. Sugarcane is one such agro system. Sugarcane is grown in India for the production of white crystal sugar and is one of the major crops. It is cultivated under diverse agro-climatic conditions and is followed by one or two ratoon crops. This type of monoculture provides a sort of stable agro system for multiplication of insect pests. Though India tops the world in the total area under sugarcane cultivation, and sugar production, the average yield per unit area is low. The major factor destabilizing crop yield is high pest incidence. These pests cause heavy damage to the crop incurring heavy losses to the farmers. Insect pesticides are used to control these pests for their management below the economic injury level. Chemical control is not very successful, especially in the case of borers having concealed habit, and root feeders because of ratoon practices. In India Integrated Pest Management approach for sugarcane pests has been successfully launched with cultural, mechanical, chemical, and biological methods being used together in which biological control agents, which were used, did not include entomopathogenic nematodes. Biological control methods mainly involving indigenous insect parasitoids have shown notable success. In spite of high success in laboratory studies, the use of parasitoids has a major drawback because parasitoids are not being readily
available to the farmers. The importance of entomopathogenic nematodes as biological control agents of agricultural pests is being increasingly recognised the world over. In the case of some agricultural crops these nematodes are already being routinely used on commercial basis as biological control agents (Grewal and Georgis, 1997). However it is interesting to note that so far the efficacy of these entomopathogenic nematodes in the control of pests of sugarcane has not been tested. Thus there is a necessity of preliminary studies to be conducted for testing the potential of these nematodes against sugarcane pests.

India being a country where sugarcane is a major cash crop, it was found necessary here to study the control the control status and level of pathogenicity of entomopathogenic nematodes in controlling the major pests of sugarcane. This study is an attempt to test the potential of the two entomopathogenic nematodes, Steinernema sp. and Heterorhabditis sp. against three lepidopteron pests; shoot borer (Chilo infuscatellus Snellen), Gurdaspur borer (Acigona steniellus Hampson), and root borer (Emmalocera depressella Swinhoe); and a coleopteran pest sugarcane beetle (Holotrichia consanguinea Blanch.) in the laboratory. The larval forms of these pests are highly injurious to developing sugarcane plants and thereby incur heavy losses.

The present study further includes laboratory analysis of the effect of abiotic factors, namely, temperature, humidity, solar irradiations and soil type on the virulence and the control status of the entomopathogenic nematodes. The ecological studies were planned under conditions taking one of the aforementioned factors under varied limits and the other factors taken to be constant within optimum range.

As an extension of this study the control status of the entomopathogenic nematodes was compared with that of the chemical pesticide recommended by
U.P. Council of Sugarcane Research and currently being used by the farmers of the state of Uttar Pradesh.

Paper bioassay was selected for preliminary studies and petridish bioassay for laboratory trials. The percent mortality of pest larvae on nematode applications was a tool to determine the control status of the entomopathogenic nematodes. The pathogenicity or persistence level inside the host was determined through percent infectivity.
CHAPTER # 2

REVIEW OF LITERATURE
There is a complicated problem of gathering scientific information in the interior areas of a developing nation like India. Lack of established database, poor library facilities, and the, at times, bewildering logistics of simple communication systems create hindrance in collection of most relevant books, references and research articles & publications. So to study the not so popular subject of entomopathogenic nematodes we primarily referred to the books:

1. **Entomopathogenic Nematodes in Biological control** by Gaugler and Kaya, and,
2. **Nematodes and the Biological Control of Insect Pests** by Bedding, Akhurst and Kaya.

Further we collected many reviews communicated by Kaya (1985); Gaugler (1988); Woodring and Kaya (1988); Kaya (1990); Georgis and Hague (1991); Kaya and Gaugler (1993); Burnell and Stock (2000) and Liu, Poinar and Berry (2000). All these research articles were collected from National Medical Library (New Delhi) and ICAR Library (New Delhi). The information thus gathered led us to follow the basic principles and findings regarding entomopathogenic nematodes, and thus we established our objective of research. Further we collected the abstracts and reprints of various research papers being published in different journals. The studies relevant to our work has been compiled and presented as follows:

**Entomopathogenic nematodes**

**General Biology & Life Cycle:**

A lot of work has been done regarding the biology and life cycle pattern of the entomopathogenic nematodes (Kaya & Gaugler, 1993). The anatomy of the third stage infective juvenile has also been worked out (Poinar and Leutenegger, 1968). The parasitic life cycle of the entomopathogenic nematodes and its role in biological control has been deeply studied and made evident (Poinar, 1990).
The third stage dauer juvenile (DJ) occurs free in the soil and its role is to seek out and infest an insect larva (Poinar, 1979). Once a suitable host is found, the infective juveniles of *Steinernema* sp. gain entry through natural body openings (mouth, anus, or spiracles) or possibly wounds and penetrates into the haemocoel (Grewal et al. 1994, 1997). In addition to these modes of entry, the infective juveniles of *Heterorhabditis* sp. also gain entry by abrading the intersegmental membranes of the insect using a dorsal tooth. (Bedding and Molyneux, 1982). Once in the haemocoel of the insect, the infective juveniles release cells of a symbiotic bacterium that they carry in their intestines (Bird and Akhurst, 1983). The haemolymph of the insect is a rich medium for the propagation of bacterial cells, where they release toxins and exoenzymes to cause insect death through septicemia (Boemare et al. 1997). The insect dies rapidly, usually within 48-72 hrs. The nematodes resume development, molt to the J4 stage and reach adulthood within two (*Steinernema* sp.) or three (*Heterorhabditis* sp.) days when cultured in vivo in the larvae of the greater wax moth *Galleria mellonella* at 23°C (Wang & Bedding, 1996). The developmental cycle of the nematode continues over next two or three generations until the nutrients get exhausted, whereupon adult development is restrained and the infective juveniles accumulate in large numbers. These non-feeding infective juveniles leave the cadaver and enter into the soil in search of a new host, and can survive for an appreciable amount of time in the absence of a suitable host (Kaya & Gaugler, 1993).

In *Steinernema*, reproduction is amphimictic. Steinernematid infective juvenile mature to become either a male or a female and sex determination appears to be of the XX/XO type, typical of nematodes (Dix *et al*, 1994). In *Heterorhabditis*, by contrast, the infective juveniles mature to give first generation hermaphrodite females, but these females give rise to a second generation of amphimictic males and females and to self-fertile hermaphrodite females and infective juveniles (Dix *et al*, 1992; Strauch *et al*, 1994). In
nutritive conditions in liquid culture medium, the second generation *Heterorhabditis* infective juveniles recover and develop to hermaphrodites (Strauch *et al.*, 1994; Johnigk & Ehlers, 1999). Strauch *et al.* (1994) have also shown that when infective juveniles were starved for 24 hours in Ringer solution, 40% became hermaphrodite, 6.6% became amphimictic adults and 53% became infective juveniles. These data clearly show the importance of nutritional signals in *Heterorhabditis* sex determination. All the entomopathogenic nematodes have the same general history but species differ in host utilization (Dutky, 1956; Reed & Carne, 1967, Selvan & Blackshaw, 1990), searching behavior (Campbell & Gaugler, 1993, 1997; Grewal *et al.*, 1994) and reproductive strategies (Poinar, 1990). Intra specific competition affects progeny production in entomopathogenic nematodes (Kaya & Koppenhofer, 1996).

**Symbiotic Bacteria:**

Adamson (1986) suggested that the Rhabditid nematodes parasitising vertebrates or invertebrates have an ancestral relationship with the free-living bacterial feeding nematodes. Sudhaus and Schulte (1988) introduced the term ‘necromency’ for the rhabditid nematodes having an association with soil invertebrates. Sudhaus (1993) further advocated that the entomopathogenic nematodes have evolved from necromenic nematodes which develop a symbiotic association with an entomopathogenic bacterium. Thomas and Poinar (1979) established the account for specificity of association of *Xenorhabdus* bacterium with the nematode *Steinernema*. Grewal *et al.* (1997) further established the account for the mechanism of this specificity. Poinar and Thomas, along with Hess, (1977), also established the account of specificity of association of the bacterium *Photorhabdus* with its symbiotic nematode partner *Heterorhabditis*. Boemare *et al.* (1993, 1998) extended their work on the relation of the bacterial species and the nature of the pathogenicity.
The symbiotic bacteria *Xenorhabdus* sp. and *Photorhabdus* sp. being carried by the entomopathogenic nematodes *Steinernema* sp. and *Heterorhabditis* sp. are ultimately released in the haemolymph of insect where the insecticidal toxins released by the bacteria work and cause insect death through toxaemia or septicemia (Forst *et al.*, 1997; Boemare & Givaudan, 1998). These bacterial symbionts have not yet been isolated from the soil, leading to an assumption that they cannot exist in the soil in the absence of entomopathogenic nematodes (Morgan *et al.*, 1997).

**Taxonomic status:**

Entomopathogenic nematodes are categorized within two families. The family *Steinernematidae* (Chitwood & Chitwood) is digeneric, consisting of the genus *Steinernema* (Travassos) with 25 described species and a newly added genus *Neosteinernema* (Nguyen & Smart) with only one species. The family *Heterorhabditidae* (Poinar) is monogeneric, with the genus *Heterorhabditis* (Poinar) consisting of nine described species (Nguyen & Smart, 1996).

**Biodiversity:**

Entomopathogenic nematodes are worldwide in distribution. Steinernematids are globally distributed and have been recorded from all the continents except Antarctica (Hominick *et al.*, 1996). Heterorhabditids are also widely distributed and are found in America, Europe, Australia and Asia (Hominick *et al.*, 1996).

Several authors like Steiner (1994), Hominick *et al* (1995), Stock *et al* (1999) and Sturhan (1999) have advocated the preference and specificity of habitat types in some described *Steinernema* species. These habitat preferences may reflect not only the distribution of suitable insect hosts, but also physiological and behavioral needs along with ecological considerations that require specific niches (Kaya & Gaugler, 1993; Hominick *et al.*, 1996).
Behavior:

Of all the behavioral aspects, the most studied are the host search strategies and host penetration behavior. Host search is initiated by a proper selection of habitat. *Steinernema* sp. has been found to search for hosts at, or near, the soil surface (Moyle & Kaya, 1981). Contrary to this, the *Heterorhabditis* sp. looks deep into the soil for proper host (Choo & Kaya, 1991). The search strategies of entomopathogenic nematodes include two basic methods, either 'sit and wait' ambushing behavior or 'seek and destroy' cruising behavior (Lewis et al, 1992). Wallace (1963) and Lewis et al (1992) advocated the energy-conserving approach of ambushers for sedentary forms like *Steinernema corpocapsae*, waiting for mobile-surface-adapted hosts. Lewis et al, (1992) further proposed that actively motile forms like *Steinernema glaseri* adapt to cursing strategy by responding strongly to host chemical cues, thereby proving to be effective for parasitising sedentary subterranean hosts.

Gaugler and Kaya, (1990) in an overview of the infection process, proposed that entomopathogenic nematodes entering the host via mouth or anus must have been reaching the haemocoel by penetrating the gut wall, a behavior that is not well described. Nguyen and Smart further studied the case of *Steinernema scapterisci* where invasion occurs through the spiracles, and moving into the tracheal system to reach the haemocoel. They also observed that the movement reach a point where the tube diameter is only slightly greater than the nematode, and thus vigorous thrashing movement is followed, thereby rupturing the tube to reach haemocoel.

Host Range:

Entomopathogenic nematodes and their bacterial symbionts are lethal obligatory parasite of a wide range of insects, a fact well documented by Bedding et al (1993), Gaugler and Kaya (1990, 1993), Poinar (1990,1991) and
Smart (1995). They are so quick in killing the host they do not require highly
adapted host-parasite relations, a necessity evident in other insect nematode
infections (e.g. mermithids & allantonematids) (Kaya and Gaugler, 1993).
Entomopathogenic nematodes cover a lot of agrosystems, being effective
against a wide variety of insect pests. (Grewal & Georgis, 1997). The rapid
mortality permits the nematodes to exploit a wide range of hosts that cover
almost all insect orders (Poinar, 1979).

In the laboratory studies where the conditions are standardized to ensure
host contact, optimized environmental conditions and minimized ecological or
behavioral barriers, a wide range of insects from various orders and several
agrosystems can be successfully parasitized by steinernematid and
heterorhabditid nematodes (Gaugler, 1981, 1988). The studies conducted by
susceptibility of insects towards pathogenicity by entomopathogenic nematodes
in laboratory conditions but failure under environmental stress and ecological
imbalances that are a part of natural system.

Predictability of biological control, a key objective in the development
of sound pest-management strategies, can be achieved using nematodes. In
strictly controlled environmental conditions that exist in glasshouse industries,
entomopathogenic nematodes have replaced chemical pesticides because of an
unusual potential to exploit different insect pests as hosts (Richardson, 1990).
However, predictability is far more difficult to achieve in natural agrosystems
(Georgis and Gaugler, 1991). Klein (1990) and Begley (1990) have reviewed
the field efficacy of the entomopathogenic nematodes against various soil-
inhabiting pests and also against insects in habitats other than soil. Grewal and
Georgis (1997) have presented a brief overview of nematode performance in
selected markets in which they have been commercially successful, and are
routinely used. The table of this brief overview is presented below:
**Table: Commercialization of Steinernema and Heterorhabditis nematodes in different market segments.**

<table>
<thead>
<tr>
<th>Segment</th>
<th>Nematode species</th>
<th>Insects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artichoke</td>
<td><em>S. corpocapsae</em></td>
<td>Artichoke plume moth</td>
</tr>
<tr>
<td>Berries</td>
<td><em>S. corpocapsae</em></td>
<td>Block vine weevil, crown borers, cranberry girdler, strawberry root weevil</td>
</tr>
<tr>
<td></td>
<td><em>H. bacteriophora</em></td>
<td>Block vine weevil, white grubs</td>
</tr>
<tr>
<td></td>
<td><em>H. megidis</em></td>
<td>Black vine weevil</td>
</tr>
<tr>
<td>Citrus</td>
<td><em>S. riobravis</em></td>
<td>Sugarcane root-stalk borer weevil.</td>
</tr>
<tr>
<td>Greenhouses</td>
<td><em>S. corpocapsae</em></td>
<td>Black vine weevil</td>
</tr>
<tr>
<td></td>
<td><em>S. feltiae</em></td>
<td>Sciarid flies</td>
</tr>
<tr>
<td></td>
<td><em>H. megidis</em></td>
<td>Stem borers, black vine weevil</td>
</tr>
<tr>
<td>Mint</td>
<td><em>S. corpocapsae</em></td>
<td>Cutworms, mint flea beetle, mint root borer, root weevils</td>
</tr>
<tr>
<td>Mushrooms</td>
<td><em>S. feltiae</em></td>
<td>Sciarid flies</td>
</tr>
<tr>
<td>Sugar beet</td>
<td><em>S. carpocapsae</em></td>
<td>Sugar beet weevil</td>
</tr>
<tr>
<td>Pet/vet</td>
<td><em>S. carpocapse</em></td>
<td>Cat flea</td>
</tr>
<tr>
<td>Turf</td>
<td><em>S. carpocapsae</em></td>
<td>Bill bugs, bluegrass webworms, black cutworms, armyworms, European crane fly</td>
</tr>
<tr>
<td></td>
<td><em>S. riobravis</em></td>
<td>Mole crickets</td>
</tr>
<tr>
<td></td>
<td><em>S. scapterisci</em></td>
<td>Mole crickets</td>
</tr>
</tbody>
</table>


Since the last decade the list of agrosystems being saved and that of insect pests being controlled through the entomopathogenic nematodes, is getting augmented each day. The studies conducted on field efficacy of the entomopathogenic nematodes against a wide variety of insect pests in various agrosystems have led to a conclusion that these nematodes find a clear seat for being effective biological control agents against insects of cryptic and soil habitats. But the results are not equally promising for pests that do not dwell in soil (Begley, 1990; Georgis & Hague 1991; Klein, 1990).

Grewal and Georgis, 1997, have affirmed the commercial use of *Heterorhabditis* sp. for controlling stem borers in many glass house crops. Schroeder in his studies during 1987, 1990 & 1994 has strongly recommended the use of Steinernematid nematodes for most effective control of citrus root weevil, *Diaprepes abbreviatus*, although Downing *et al*, 1991, reported through his studies that *Heterorhabditis* sp. is also effective but not equally good for the control of *D. abbreviatus*. Steinernema sp. was also found to be effective for controlling mint rot borer, *Fumibotrys fumalis*. Georgis and Gaugler (1991) noted that *Heterorhabditis* sp. is effective enough against white grubs to substitute chemical pesticides whereas *Steinernema* sp. show poor results against white grubs.

**Ecology:**

Steinernematids and Heterorhabditids differ in host-seeking behavior, tolerance to environmental parameters, behavior in the soil and pathogenicity to various insect species (Gaugler, 1988). It was also asserted by Gaugler (1988) that various environmental features and ecological considerations are limiting factors to the infectivity of entomopathogenic nematodes as well as to nematode dispersal and host-finding behavior.
Soil type: Blackshaw and Senthamizh selvan (1991) studied the effect of soil particle size on the activity of *Steinernema* sp. against *Galleria mellonella* larva. He demonstrated that sandy loam soil is best suited for nematode infectivity, and small particle-size causes restraint to persistence and infectivity of entomopathogenic nematodes. Choo & Kaya (1991), Georgis & Poinar (1983) and Molyneux & Bedding (1984) manifested complementary results through experimental evidences, thus advocating soil texture to be a limiting factor for the persistence of steinernematid and heterorhabditid nematodes. Kaya (1990) and Richardson & Grewal (1994) presented an overview of the relationship between insect parasitic rhabditid nematodes and soil ecology. They asserted that soil texture, soil moisture, soil temperature, soil pH and other soil factors influence the virulence of these rhabditid nematodes. McCoy, Shaprio and Duncan (Unpublished data) reported that the virulence of *Steinernema* sp. was greater than that of *Heterorhabditis* sp. in all soils. They also inferred from their studies that both the nematodes had higher virulence and persistence in marl soil compared with sandy soils. Walker (1984), in his studies on the efficacy of entomopathogenic nematodes against mole cricket found that small soil particle size, as in the case of clay soil, restricts nematode movement and hence has a negative impact on nematode virulence. Kung, Gaugler and Kaya (1990), in their studies on impact of soil type on persistence and virulence of entomopathogenic nematodes, have shown that sandy loam soil is the most desirable soil texture followed by sandy soil. They further demonstrated through their findings that clay soil is not suitable for efficacious use of these nematodes in biological control. Further, they suggested that the reason for better survival and virulence in case of sandy loam soil with larger pores than in case of soil with smaller pores, as clay, is that they might have to spend more energy to move in smaller pores. Raquel C. Ampos and Carmen G. Utierrez (unpublished data) in their studies on the effect of soil texture on *Steinernema* sp. virulence against *Spodoptera littoralis* (Lepidoptera) found
that nematode virulence was reduced in soil with higher clay contents. Hsiao and All (1996) concluded from their studies that the migration of infective juveniles tends to decrease as the proportions of silt and clay increase in soil. Their studies also showed that nematodes move more readily in sand and silty loam than in coarse sandy loam or sandy clay loam. Choo and Kaya (1991) reported that the *Heterorhabditis* sp. infection of final-instar larvae of *Galleria mellonella* was affected by soil texture, with the highest infection (71.1%) occurring in humus, followed by sand, loam and clay (52.2, 41.1 and 4.5% respectively). Barbercheck and Kaya (1991) found in their experiments that *Heterorhabditis* sp. was more motile than *Steinernema* sp. in organic and fine sandy loam soils, but less motile in clay soil. Barbercheck (1992) further reviewed the effect of soil physical factors on the virulence of entomopathogenic nematodes and came to the same conclusion. Kung, Gaugler and Kaya (1990) asserted that the survival of *Steinernema* sp. was highest in sandy loam followed by sand, clay loam and clay. The results were in accordance with the findings of Molyneux and Bedding (1984) showing less parasitism in soils of high clay content both for *Steinernema* sp. and *Heterorhabditis* sp. However, Fan and Hominick found no significant difference in virulence of entomopathogenic nematodes in infested soil or sterile sand. Duncan, McCoy & Terranova (1996) established through their experiments that persistence in soil of *Heterorhabditis* sp was lower than *Steinernema* sp. All these findings strongly recommend that sandy loam is the best soil type for virulence of entomopathogenic nematodes.

**Temperature:** Temperature limits the virulence of entomopathogenic nematodes by the influence on nematode activity, or bacterial symbiont, or both (Kaya, 1990). Kung, Gaugler and Kaya (1991) showed in their studies that survival and pathogenicity of *Steinernema* sp. were significantly restricted at lower and higher extremes. Griffin (1993) presented an overview on the response of entomopathogenic nematodes towards temperature variation. All
these researchers asserted that *Steinernema* sp. is significantly more tolerant to lower and higher ranges of temperature than *Heterorhabditis* sp. They also advocated the adverse effect of cold and hot conditions on the persistence and virulence of entomopathogenic nematodes. Miduturi *et al* (1994) in their laboratory studies obtained the optimal temperature, 20°C for *Heterorhabditis* and 20°C to 25°C for *Steinernema* sp. Any deviation from the optimum range showed negative impact on the virulence of these entomopathogenic nematodes. Danilov *et al* (1994) in their studies on the influence of temperature regarding virulence of entomopathogenic nematodes showed that *Steinernema* sp. appeared to be more adapted to survival at low temperatures as compared to *Heterorhabditis* sp. Mason and Hominick (1995) studied the effect of temperature on infection of heterorhabditids and found out that there was a drop in the level of infectivity at both the extremes of the temperature range tested. In this case the optimal infectivity was displayed at 25°C. Doucet *et al* (1996) communicated similar results with highest mortality of the pest recorded at 26°C. Steiner (1996) while studying the effect of low temperature on virulence of entomopathogenic nematodes inferred that they must be considered unsuitable control agents at low temperatures. Henneberry *et al* (1996) after their studies presented an opinion that higher range of temperature also adversely affect the virulence of entomopathogenic nematodes. The studies of Shamseldean *et al* (1996) support the preceding statement explaining that increasing temperature adversely effect nematode virulence, *Heterorhabditis* sp. being more affected than *Steinernema* sp. Hisiao and All (1996) advocated that *Steinernema* sp. show best results at 25°C which is most suited for their survival in soil whereas higher temperatures restrict their dispersal and have an antagonistic effect on their survival. Brown and Gaugler (1997) studied the influence of temperature on the emergence and survival of entomopathogenic nematodes. They found out from their experiments that low temperatures significantly reduced emergence of *Heterorhabditis* sp. Kiger and
Bornstein also presented their results showing adverse effect of low temperature on the infectivity of Steinernema sp. Lacey and Unruh (1998) studied the effect of temperature on entomopathogenic nematodes being used for control of codling moth. The results revealed adverse effect of low and high temperature ranges on the virulence of the entomopathogenic nematodes. No mortality was produced at 10°C. Whereas Steinernema sp. was more effective at 30°C and above as compared to Heterorhabditis sp. Shapiro et al. (1999) investigated the effect of temperature and host age n suppression of Diaprepes abbreviatus by entomopathogenic nematodes. They came out with interesting results where nematodes were found to be less virulent at 21°C than at 24°C or 27°C in case of older larvae and Heterorhabditis sp. was found to be more virulent at 24°C whereas Steinernema sp. at 21°C in case of younger larvae. These and many other studies strongly support the fact that temperature directly influences the persistence and infectivity of entomopathogenic nematodes. Gouge et al., (1999) established that the optimum temperature for the control of insect targets by an entomopathogenic nematode vary among target species. They also suggested that assuming existing nematode temperature optima and applying the same condition to untested insect species might not result in maximum biocontrol efficacy. Smith (1999) further suggested that it is better to apply entomopathogenic nematodes to a moist soil in the early morning or late evening when air temperatures are between 60 and 85°F.

Relative Humidity: Li et al, (1986) reported that relative humidity effect the survival rate, survival time and movement of entomopathogenic nematodes. Womersley (1990) suggested that entomopathogenic nematodes could survive slow desiccation at high relative humidities. Kung, Gaugler and Kaya (1991) studied the effect of relative humidity on survival and pathogenicity of steinernematids in the laboratory. They observed that survival and pathogenicity of the nematode decreased as relative humidity was decreased
from 100% to 25%. They also observed that at 100% RH, the nematode survived for 32 days, but as RH was lowered, survival time decreased. They further observed that survival for a few hours to a day or two was obtained at 25% RH and pathogenicity was reinitiated after rehydration and showed a trend similar to survival. Kung et al suggested that these differences in survival and pathogenicity might be attributed to the climatic origins or the soil habitats of the nematode. Brown and Gaugler (1995) presented their work on the effect of relative humidity upon infective juveniles of entomopathogenic nematodes considering emergence from the host cadaver and survival in the cadaver, at the annual meeting of the Society for Invertebrate Pathology. Brown and Gaugler (1997) later published the work asserting that the infective juveniles can survive adverse environmental conditions for limited periods in the host cadaver, but low relative humidities prevent emergence and survival of the infective juveniles. Kaya and Baur (1996) presented their work showing the adverse effect of low relative humidity on survival and infectivity of *Steinernema* sp. Lacey and Unruh (1998) suggested through their studies that more than 3 hrs. in high humidity was needed for entomopathogenic nematodes against codling moth larvae to attain 50% mortality. Results from a lot of studies have advocated that high relative humidity and high soil moisture content are essential for optimum activity of entomopathogenic nematodes. Shetlar (1999) thus recommended entomopathogenic nematode applications in the field before or even during the rainfall. Smith (1999) also suggested pre and post-application irrigation to moisten the soil and increase humidity for optimal use of nematodes as biocontrol agents.

**Sunlight:** Gaugler and Boush (1978) indicated that ultraviolet radiation and sunlight adversely effect the survival and pathogenicity of entomopathogenic nematodes. Ghally (1989) and he along his coworkers (1994) investigated the effect of gamma irradiation of different dosages (5-50 KR) on the infectivity of steinernematid nematodes. They established that the treatment of gamma
radiation inactivated the pathogen. Nematode motility and host infestation & mortality decreased at the higher dosages, and nematode development was inhibited at the highest dosage. They also indicated that no nematode reproduction took place after treatment at any dosage. Fujiie and Yokoyama (1998) studied the effect of ultraviolet light on the entomopathogenic nematodes. They found that the nematode mortality significantly increased after exposure to sunlight for 40mins. and their insecticidal activity decreased. They also found that sunlight decreased the density of viable symbiotic bacterium, *Xenorhabdus* sp., associated with the nematode in the body of infective juveniles.

**Nematode concentration:** Doucet *et al* (1996) studied the efficacy of *Heterorhabditis* sp. in relation to concentration of infective juveniles and found that increasing concentration of infective juveniles significantly increased the pathogenicity. Lacey and Unruh (1998) observed in their experiments that LC50 values were obtained at 5-6 IJs/cm². They also found that 50 IJs/cm² produced better results than 10 IJs/cm². Thus it was established that the concentration of infective juveniles also plays an important role in optimizing persistence and virulence of the entomopathogenic nematodes.

**Non-target organism:** Buck and Bathon (1993) studied the effect of field applications of entomopathogenic nematodes on the non-target fauna. Only a few case were recovered with appreciable parasitism of the members of Diptera, and only on one site a significant reduction of several non-target species or families was observed. On the contrary, four nematode plots indicated significant increase of non-target species or families. They thus concluded that the detrimental effects of a field application of entomopathogenic nematodes on non-target insect fauna must be considered negligible. Bathon (1996) further presented that entomopathogenic nematodes do not affect vertebrates under natural conditions. He further asserted that
mortality caused by the release of entomopathogenic nematodes among non-target arthropod populations can occur, but will only be temporary, will be spatially restricted and will affect only part of a population.

**Sugarcane Pests**

Sugarcane agro-ecosystem under tropical and subtropical conditions is diverse, owing to the variation in the climate, soil, pest complex and cultivation practices. Cultivating sugarcane in large contiguous areas around sugar factories and planting cane during spring and autumn in subtropical India, and almost throughout the year for jaggery production in tropical India, results in the availability of continuous and abundant food supply for the various species of insects attracting different growth phases of the crop. (Easwaramoorthy, 1993).

**Shoot Borer, C. infuscateullus:** The Crambid moth borer, *Chilo infuscateullus* Snell, (Crambiclae: Lepidoptera) is commonly known as shoot borer in the north Indian sugarcane belt and as early shoot borer in peninsular India. The shoot borer has a wide range of occurrence from Afghanistan through central Asia, India to Korea, Taiwan, Indonesia, Malaysia, as well as Philipes (Kapur, 1950; Bleszynski, 1669). The shoot borer is widely distributed in all sugarcane growing areas in India, infesting the crop during its early stages of growth (i.e., shoot stage prior to internode formation) in spring or *eksali* planted crop, during the period march through June. In *adsali* crop, borer damage occurs during September and October every year. (Avasthi & Tiwari).

It has been computed that the shoot borer destroys 26-65 percent mother shoots and 6.4, 27.1 and 75 percent primary, secondary and tertiary tillers respectively (Krishnamurthy Rao, 1954; Doss, 1956; Khan and Krishnamurthy Rao, 1956). Shoot borer has been found responsible for the elimination of 30-75 percent shoots in the early stages of crop growth in the different cane growing regions of the country (Rahman and Singh, 1942; Gupta and Avasthy, 1954;
Krishnamurthy, 1954). The study of Seshagiri and Krishnamurthy (1973) has revealed the economic threshold level of shoot borer to be 15 percent incidence. The shoot borer, *C. infuscatellus* is more active during hot period of the year both in tropical (Murthy, 1953; Sulaiman, 1954; Nagarja Rao and Chandy, 1957; Jagannatha Rao (1960); Varadharajan et al., 1972) and subtropical India (Khan and Singh, 1942; Agarwala and Huque, 1955; Gupta, 1959; Bains and Dev Roy, 1981). Moderate day temperatures coupled with high relative humidity is conducive for its multiplication (Kalra & Sharma, 1963; Kalyanaraman et al., 1963; Krishnamurthy Rao, 1966).

Larva is the infesting phase of the moth. Freshly hatched larva measure about 1.5 mm in length and have a black head and thorax. The body is dirty gray and the stripes though present are not prominent. The abdominal tubercles are present as black dots. Isaasc and Rao, 1941). Larvae after dispersal bore into the stalk at the bottom of the plant and kill the growing part in 7-8 days. The disease thus established is known as ‘dead heart disease’ (Usman et al., 1957). Pradhan and Bhatia (1956) presented in their studies that the lower and upper threshold limits for the developmental cycle of shot borer are 12°C and 40°C respectively. The optimum humidity level was found to be 90 percent relative humidity.

**Gurdaspur borer, *Acigona steniellus***: *Acigona steniellus* (Hampson) (Crombidae: Lepidoptera), is a serious pest of sugarcane in some pockets of north India (Maninder and Verma). In 1925, Dutta observed it as a major pest in Gurdaspur district of Punjab and named it as “Gurdaspur borer.” It has also been reported from Assam (Hampson, 1899), Punjab and Haryana (Dutta, 1925; Khan & Tandon, 1940; Kapoor, 1957), Uttar Pradesh (Gupta and Garg, 1943), Rajasthan (Panje, 196; Kalra and Kumar, 1965), Hmachal Pradesh (Pal, 1970) and Maharashtra (Patil et al., 1980). Other than in India, it is also reported from Pakistan (Hussain, 1923) and Vietnam (Joannis, 1930; Jepson,
1954). The infestation of this borer varies from 7-35 percent (Gupta and Garg, 1943; Gupta, 1954) in different varieties. Kalra (1963) reported that usually 15-20 percent of the crop is damaged and some times it may be even as high as 40-50 percent. Kapoor (1957) estimated a loss of 84.4, 59.4 and 19.5 percent in cane height and 100,64 and 38 percent in weight due to the damage caused by the first, second and third generations of this pest, respectively. Singh et al. (1957) estimated a loss of 5-15 percent in cane yield. Garg and Chaudhary (1979) observed that the pet flourishes well under moderate temperature and high humidity conditions. Commencement of monsoon early in the season, heavy rains and water logging are reported to favor its multiplication. Khan and Tandon (1940) observed that the borer infestation is more common in the 'barani' or rainfed crops. Kapoor (1957) made detailed studies on life cycle of Gurdaspur borer. He asserted that full grown larvae with four violet stripes present sub dorsally and laterally in pairs, hibernate by boring the lower portion of stem. Garg and Chaudhary (1979) asserted that after feeding about two-third of the inter-node in spiral manner, the larvae bore deeper and feed upwards by making a straight tunnel. Kapoor (1957) had shown from his studies that Gurdaspur borer is prevalent in the humid regions of Punjab and adjoining areas. Sunil Kumar (1975) presented that mean maximum temperature directly influences the migration of the larvae towards the root zone.

**Sugarcane Beetle (White Grubs): Holotrichia consanguinea Blanch:** The term white grub is generally used to denote the larvae of Scarabacid bettles (Imms, 1977). This species has since been reported from Uttar Pradesh, Rajasthan, Haryana, Punjab and Andhra Pradesh (Avasthy, 1967; krishnamurty Rao et al., 1978 and Sukhija et al., 1981). The yield loss due to white grubs has been reported to be as high as 80 percent (Veeresh, 1974 and Patil et al., 1981). Avasthy and Tiwari (unpublished data) estimated the loss to be 35.43 tonnes per ha and total loss in the country as 9.36 lakh tonnes. Generally, the damage caused by white grubs is in patches, but during epidemics the entire

Availability of adequate moisture and abundant roots for a long time in the sugarcane crop, tend to increase white grub build-up markedly (Karla & Kulshreshtha, 1961). Low temperature, below 10°C adversely affects the grubs, whereas their survival is good at 20°C (Veeresh, 1977). Gupta and Avasthy (1957) showed that the grubs migrate to a depth of 0.3±1.2 meters with the lowering of temperature at the end of October. It is observed that from October to January the grubs remain in soil at depths ranging from 40-70 cm. In February and March they move down when the atmospheric and soil temperatures start rising (Yadava et al., 1978). Kalra and Kulshreshtha (1961) observed that these beetles are active at night and hide in the soil during daytime. Another study by Gupta and Avasthy (1975) showed that these beetles are attracted to light in large numbers. David and Nandagopal (unpublished) asserted that a species of *Rhabditis* parasitises the eggs and first instar larvae of white grubs under Coimbatore conditions.

**Root Borer, *Emmalocera depressella* Swinhoe (Crambidae: Lepidoptera):** The species was first reported by Swinhoe in 1985. Hampson further described it as *Emmalocera depressella*. This is the only species of borer infesting the underground portion of canes and is therefore, generally referred to as the ‘root borer’. The root borer generally infests the young shoots, though it is known to infest grown up canes as well (Avasthy, 1967). Studies by Cheema (1953) reveal that borer infestation by the larvae of the first brood cause 52 percent canes to produce no tillers, 30 percent to produce only one tiller and 18 percent to produce, two tillers. Gupta et al. (1966) further revealed through his studies
that plants damaged by second brood form millable canes, showing a decrease of 66.2 percent in length and 73 percent in weight as compared to healthy canes. Furthermore canes damaged later in the season by larvae of third brood show a reduction of 14.3 percent in length and 17 percent in weight, whereas in the canes attacked by the further brood the decrease is 5.2 percent in length and 6.5 percent in weight.

The newly hatched larva measures 2mm in length and is pale yellow in color with a yellowish brown head and dark brown mouthparts. Spiracles are oval in outline with yellowish brown rim (Gupta, 1959). The larva has been observed to be active at high temperatures and moderate humidity levels and appears to be tolerant to rain to an extent of 45 cm. after which its population declines (Gupta, 1953). Peak population of borer is reached during July-August when the average maximum temperature is 35-37.8°C and mean relative humidity 50-75 percent (Gupta, 1959). Moreover, borer incidence and population are generally high in unirrigated fields and in sandy loam soils (Gupta & Avasthy, 1952).
CHAPTER # 3

MATERIAL & METHODS
3.1. Materials.

3.1.1. Entomopathogenic nematodes.

The two entomopathogenic nematode species, one belonging to the genus *Steinernema* and the other to the genus *Heterorhabditis* were reared through the white grub larvae and their infective juveniles were collected from the cadavers and formulated in normal saline (0.64%). One milliliter of sterilized sand moistened with normal saline (10%wt.:wt.) was placed at the bottom of a 15ml centrifuge tube. One hundred infective juveniles of that one of the two nematode species taken at a time whose specimens were immediately required for work were taken in a small drop of normal saline and were pipetted into the centrifuge tube. The tubes containing the members of a given nematode species were then incubated at 25°C for one hour. An aliquot of the stock solution which was prepared by dissolving 588gms. of sucrose in distilled water and making it to 1 liter, was taken and further dilution to 10% was added to each tube. These tubes were shaken and then kept on the vortex mixer for 10 seconds. These tubes were then centrifuged at 1700g for 10 minutes. Sugar solution containing nematodes was then isolated for preparing the stock solution of that nematode species. The stock solutions were prepared from the freshly collected infective juveniles of nematodes with concentrations as follows:

Stock solutions with infective juveniles (IJs) of *Steinernema* sp.

1. Sn 1 : 100 ± 10 IJs per milliliter of stock solution.
2. Sn 2 : 1000 ± 10 IJs per milliliter of stock solution.

Stock solutions with infective juveniles (IJs) of *Heterorhabditis* sp.

1. Hb 1 : 100 ± 10 IJs per milliliter of stock solution.
2. Hb 2 : 1000 ± 10 IJs per milliliter of stock solution.
Stock solution of control experiment.

Co : normal saline (0.65%).

These formulations were used within 24 hrs. of their preparation. The formulations were kept in a refrigerator to guard them from hot and humid conditions and to maintain cool and dry atmosphere.

3.1.2. Insect pests.

To assess the potential of entomopathogenic nematodes as effective biological control agents following insect pests were selected.

1. Shoot borer of sugarcane, *Chilo infuscattellus*.

The newly hatched larvae of all these insect pests were collected from the infected sugarcane plants in the field, 24 hrs. before the experiment and were kept on artificial diet.


3.2.1. Nematode bioassay.

The following two bioassays were conducted in the complete study presented over here.
1. **Paper bioassay:**

   The paper bioassay was undertaken here for doing preliminary studies to test the pathogenicity of entomopathogenic nematodes. Newly hatched larvae of the above-mentioned four species of insect pests were tested for their susceptibility to the entomopathogenic nematode attack. Taking one species at a time, twelve larvae of a given insect species were picked. Putting three of these twelve larvae per packet, four packets were prepared using Whatmann filter paper No. 1 to wrap the larvae in. Thus four packets per insect species were formed, two of which (taken in this study as the two replications of the experiment) were prepared by wrapping the insect larvae in those filter papers which had been freshly soaked in the suspension containing infective juveniles of *Heterorhabditis* sp. and two in the suspension containing infective juveniles of *Steinernema* sp. A portion of artificial diet was also wrapped along with the insect larvae in each packet. Putting each packet in a separate 50ml. beaker all packets were then incubated together in the dark at 25°C and 85% RH for 48hrs. At the end of this incubation period the cadavers and the surviving larvae were isolated from the packets for further examination.

2. **Petridish bioassay:**

   The petridish bioassay was undertaken here for doing laboratory trials to study the effect of entomopathogenic nematodes upon the mortality and infectivity of pest larvae. Each petridish was filled with loam soil which was obtained from the field and had been sterilized at 15lb/inch.\(^2\)in the autoclave prior to filling. The soil in the petridish was moistened with water and then allotting ten insect larvae per petridish, the larvae of given insect species were put over the soil in the dish. These dishes were used for
efficacy studies by treatment of various pesticidal formulations (both biopesticides as well as chemical pesticide) that were sprayed on to them, under constant and varied environmental conditions being provided in the laboratory. Each experiment and its part were performed with ten petridishes.

### 3.2.2. Infectivity assays.

In the present study, the newly hatched larvae of the four major pests of sugarcane, i.e., shoot borer, *Chilo infuscataellus*; Gurdaspur borer, *Acigona steniellus*; sugarcane beetle, *Holotrichia consanguinea* and root borer, *Emmalocera depressella* were used to assess the infectivity of the two entomopathogenic nematode species, *Steinernema* sp. and *Heterorhabditis* sp. The larvae of these four insect species were exposed for infestation to the infective juveniles of both the entomopathogenic nematode species under study by adopting the paper bioassay technique described above, for the purpose of establishing nematode infectivity and viability. In consonance with Georgis, R. (1992), and Kaya & Koppenhofer (1999), it was decided here to consider a nematode species to be a successful pathogenic agent if it brought about the mortality of the insect larvae within 72 hrs. of its application. This was taken as a criterion for selecting a given nematode species for its application in further experiments. After their exposure to nematode attack for 72 hrs. both the cadavers and the surviving insect larvae were dissected under binocular microscope and examined for the presence of infective juveniles of nematode, in order to establish the successful infestation. The experiment was run with two replicates. The format of the experiment is given below in a tabular form under the heading 'Experiment No.1':

---

**MATERIALS & METHODS**

---
### Experiment No. 1

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pest Larvae</th>
<th>Treatments</th>
<th>Heterorhabditis</th>
<th>Steinernema</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>Shoot borer, <em>C. infuscatellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
<td></td>
</tr>
<tr>
<td>1b</td>
<td>Gurdaspur borer, <em>A. steniellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
<td></td>
</tr>
<tr>
<td>1c</td>
<td>Sugarcane beetle, <em>H. consanguinea</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
<td></td>
</tr>
<tr>
<td>1d</td>
<td>Root borer, <em>E. depressella</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
<td></td>
</tr>
</tbody>
</table>
3.2.3. Efficacy studies.

Efficacy studies were conducted to determine the control status and persistence level of the two entomopathogenic nematode species, *Steinernema* sp. and *Heterorhabditis* sp. against the larvae of shoot borer, *C. infuscatus*; Gurdaspur borer, *A. steniellus*; sugarcane beetle, *H. consanguinea* and root borer, *E. depressella*. Efficacy studies were conducted under constant ecological factors as suggested by Gaugler, R.(1988). These studies were then also conducted under variable ecological factors to investigate the effect of changes in temperature, humidity, sunlight and soil texture on the control status and persistence level of entomopathogenic nematodes. For the purpose of study of efficacy of nematodes 10% concentration of the stock solutions of nematodes was used. Following the suggestions of Fisher regarding the randomized block design the experiments were performed here in five to ten replications depending on the number of treatments, in order to obtain more precise and accurate results.

Adopting the procedure identical to that employed for studying the efficacy of nematodes in controlling the larvae of the four insect pests under study, two different concentrations of γBHC (Lindane) were applied on the insect larvae, under constant ecological conditions, in order to compare the results obtained by using the nematodes as control agents with those obtained by using γBHC (Lindane). The studies are presented below.

3.2.3.1. Efficacy studies through nematode treatments.

3.2.3.1.1. Constant ecological factors.

Efficacy studies were conducted by adopting the petridish bioassay described above. Taking two concentrations of the infective juveniles of each
one of the two nematode species under study and the larvae of each one of the four insect species separately, an experiment was set up here by putting ten insect larvae of a species per petridish on ten petridishes and treating these 100 insect larvae to a given concentration of the infective juveniles of a particular nematode species. This experiment was performed in five replicates concurrent with a control experiment. After treatment the petridishes were placed in the incubator at $25^\circ$C and 85% RH for 72hrs. in the dark. The petridishes were then examined for counting the number of cadavers in each petridish to determine the percentage of mortality. Each of these was then dissected under the binocular for detecting the presence of infective juveniles to determine the percentage infectivity. The format of the experiment is given below in a tabular form under the heading 'Experiment No.2':

**Experiment No.2**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pest larvae (Ten in each petridish)</th>
<th>Treatments (On ten petridishes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2a</td>
<td>Shoot borer, <em>C. infuscataellus</em></td>
<td>Hb1</td>
</tr>
<tr>
<td>2b</td>
<td>Gurdaspur borer, <em>A. steniellus</em></td>
<td>Hb1</td>
</tr>
</tbody>
</table>

Contd....
Thus the two parameters determined here were percentage mortality and percentage infectivity with the level of infectivity, i.e., % infectivity signifying the persistence level and the level of mortality, i.e., % mortality, signifying the control status of the entomopathogenic nematodes.

3.2.3.1.2. Variable ecological factors.

The efficacy studies through petridish bioassay were also conducted under variable ecological factors to investigate the effect of changes in temperature, humidity, sunlight and soil texture.
3.2.3.1.2.1. Temperature variation.

Nematode bioassay was conducted to determine the persistence level, i.e., percent infectivity and control status, i.e., percent mortality of the pest larvae under various temperature ranges between 5-10°C, 10-15°C, 15-20°C, 20-25°C, 25-30°C, 30-35°C, 35-40°C, 40-45°C, and 45-50°C. The experiment was set at 85% RH and petridishes were incubated in the dark for 72hrs. at temperature ranges given above. The experiment was performed with five replicates. Manual adjustments were made at hourly intervals in order to keep the temperature inside the incubator within the desired temperature range. The format of the experiment is given below in a tabular form under the heading ‘Experiment No.3’:

**Experiment No.3**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pest larvae</th>
<th>Temperature ranges</th>
<th>5-10°C</th>
<th>10-15°C</th>
<th>15-20°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatments</td>
<td>Treatments</td>
<td>Treatments</td>
<td>Treatments</td>
</tr>
<tr>
<td>3a</td>
<td>Shoot borer, <em>C. infuscatellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
<td>Hb2</td>
</tr>
<tr>
<td>3b</td>
<td>Gurdaspur borer, <em>A. steniellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
<td>Hb2</td>
</tr>
</tbody>
</table>

Contd....
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<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>5-10°C Treatments</th>
<th>10-15°C Treatments</th>
<th>15-20°C Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugarcane beetle, <em>H. consanguinea</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Root borer, <em>E. depressella</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Shoot borer, <em>C. infuscatellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Gurdaspur borer, <em>A. steniellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Sugarcane beetle, <em>H. consanguinea</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Root borer, <em>E. depressella</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>20-25°C Treatments</th>
<th>25-30°C Treatments</th>
<th>30-35°C Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot borer, <em>C. infuscatellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Gurdaspur borer, <em>A. steniellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Sugarcane beetle, <em>H. consanguinea</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Root borer, <em>E. depressella</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>35-40°C Treatments</th>
<th>40-45°C Treatments</th>
<th>45-50°C Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoot borer, <em>C. infuscatellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
<tr>
<td>Gurdaspur borer, <em>A. steniellus</em></td>
<td>Hb2</td>
<td>Sn2</td>
<td>Co</td>
</tr>
</tbody>
</table>
### MATERIALS & METHODS

<table>
<thead>
<tr>
<th></th>
<th>35-40°C</th>
<th>40-45°C</th>
<th>45-50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatments</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3c Sugarcane beetle, <em>H. consanguinea</em></td>
<td>Hb2, Sn2, Co</td>
<td>Hb2, Sn2, Co</td>
<td>Hb2, Sn2, Co</td>
</tr>
<tr>
<td>3d Root borer, <em>E. depressella</em></td>
<td>Hb2, Sn2, Co</td>
<td>Hb2, Sn2, Co</td>
<td>Hb2, Sn2, Co</td>
</tr>
</tbody>
</table>

#### 3.2.3.1.2.2. Humidity variation.

Nematode bioassay was conducted to determine the persistence level, i.e., percent infectivity and control status, i.e., percent mortality of the pest larvae at various levels of relative humidity (RH) like 30%RH, 50%RH, 70%RH, and 90% RH with standard error of ±5%. The experiment was set at 25°C and incubated in the dark for 72hrs. The humidity of air is expressed in terms of relative humidity values. Relative humidity is the amount of moisture in air as the percentage of the amount that the air can hold at the existing temperature. The experiment was performed with five replicates. The format of the experiment is given below in a tabular form under the heading ‘Experiment No.4’:
3.2.3.1.2.3. Sunlight variation.

Nematode bioassay was conducted to determine the persistence level, i.e., percent infectivity, and control status, i.e., percent mortality, of the pest larvae under both direct sunlight and diffused sunlight to study the effect of various solar radiations on the entomopathogenic nematodes. The experiments were conducted in the open as specified below.

The experiment under direct sunlight was conducted on October 18th, 2001, which being a clear day, and the petridishes were exposed to sunlight for
the whole day, the maximum temperature of the day being 29.7\(^{\circ}\)C and the minimum temperature being 22.9\(^{\circ}\)C, the average relative humidity was 72.3\% and the day length was 11hrs.32mins. A large mirror was used to reflect the sunlight on the petridishes throughout the day in order to increase irradiation. At the end of the day these petridishes were allowed to incubate for 60 hrs. at 25\(^{\circ}\)C and 85\% RH.

The experiment under diffused sunlight was conducted on September 7\(^{\text{th}}\), 2001, which being a cloudy day for most of the period from sunrise till sunset, had diffused sunlight conditions almost throughout that day. The petridishes were exposed to diffused sunlight for the whole day, the maximum temperature of the day being 32.2\(^{\circ}\)C and the minimum temperature being 24.6\(^{\circ}\)C, the average relative humidity was 75.1\% and the day length was 11hrs.32mins. A white muslin cloth was placed at a height of one meter from the petridishes to mitigate the effect of intermittent direct sunlight. At the end of the day these petridishes were incubated for 60 hrs. at 25\(^{\circ}\)C and 85\% RH.

An experiment similar to those done under direct and diffused sunlight conditions was conducted under the laboratory conditions in the dark keeping the petridishes in incubator for 72 hrs. at 25oC and 85\% RH.

The experiments done under the direct sunlight, diffused sunlight, and dark conditions had 10 replicates each. Control experiments were also set up to run under all these three light conditions. Care was taken not to expose the insect larvae or the entomopathogenic nematodes to the sunlight before the start of the experiment. The format of the experiment is given below in a tabular form under the heading ‘Experiment No.5’:
### Experiment No.5

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pest larvae</th>
<th>Sunlight intensity levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Direct</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treatments</td>
</tr>
<tr>
<td>5a</td>
<td>Shoot borer, C. infuscetellus</td>
<td>Hb2</td>
</tr>
<tr>
<td>5b</td>
<td>Gurdaspur borer, A. steniellus</td>
<td>Hb2</td>
</tr>
<tr>
<td>5c</td>
<td>Sugarcane beetle, H. consanguinea</td>
<td>Hb2</td>
</tr>
<tr>
<td>5d</td>
<td>Root borer, E. depressella</td>
<td>Hb2</td>
</tr>
</tbody>
</table>

3.2.3.1.2.4. Soil texture variation.

Nematode bioassay was conducted to determine the persistence level, i.e., percent infectivity, and control status, i.e., percent mortality, of the pest larvae placed in various soil types like clay soil, loam, sandy soil and coarse sand (Badarpur soil). The experiment was set at 25°C and 85% RH in the dark for 72hrs. after the treatment was done. This experiment was
performed with eight replicates. The format of the experiment is given below in a tabular form under the heading ‘Experiment No.6’:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Pest larvae</th>
<th>Soil Types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Clay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Treatments</td>
</tr>
</tbody>
</table>

6a Shoot borer, *C. infuscataellus*  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2

6b Gurdaspur borer, *A. steniellus*  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2

6c Sugarcane beetle, *H. consanguinea*  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2

6d Root borer, *E. depressella*  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2  
Hb 2  
Sn 2  
Co 2

3.2.3.2. Efficacy studies through chemical treatments.

Efficacy studies through petridish bioassay were conducted to determine the control status, i.e., percent mortality of the insect larvae by the application of chemical pesticide, γBHC (Lindane), as recommended by the U.P. Council for Sugarcane Research, against the larvae of four insect pests studied here.
The following two concentrations of γBHC (Lindane) were used in the present study:

1. L₁: 20% EC of 0.625 ml. γBHC per 187.5 ml. of water.
2. L₂: 20% EC of 1.25 ml. γBHC per 187.5 ml. of water.

After application of pesticide the petridishes were incubated at 25°C and 85% RH in the dark for 72 hours. This experiment had ten replicates. The format of the experiment is given below in a tabular form under the heading 'Experiment No.7':

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Pest larvae</th>
<th>Treatment (γBHC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>Shoot borer, ( C. \text{infuscetellus} )</td>
<td>L₁, L₂, Co</td>
</tr>
<tr>
<td>7b</td>
<td>Gurdaspur borer, ( A. \text{steniellus} )</td>
<td>L₁, L₂, Co</td>
</tr>
<tr>
<td>7c</td>
<td>Sugarcane beetle, ( H. \text{consanguinea} )</td>
<td>L₁, L₂, Co</td>
</tr>
<tr>
<td>7d</td>
<td>Root borer, ( E. \text{depressella} )</td>
<td>L₁, L₂, Co</td>
</tr>
</tbody>
</table>
3.2.3.2. Comparative account of efficacy studies through nematode treatments and chemical treatments.

The results of both experiment no. 2 (Efficacy studies through nematode treatments at constant ecological factors) and experiment no. 7 (Efficacy studies through chemical treatments at constant ecological factors) are appended below in chapter 5. The results of experiment no. 2 were evaluated in the light of the results of experiment no. 7 to estimate the extent of biological control of the four species of insects used here, achieved through the agency of the two entomopathogenic nematode species under study in comparison to the chemical control of these insect species obtained by the use of γBHC (Lindane) as control agent. This was done by comparing the results of each one of the two concentrations of both *Steinernema* sp. and *Heterorhabditis* sp. with the two concentrations of γBHC (Lindane). Intraspecific and interspecific comparisons of the results of the two concentrations of *Steinernema* sp. and *Heterorhabditis* sp. were also done. Comparison was also made between the results obtained for the two concentrations of BHC (Lindane) with each other as well as with the results of each one of the two concentrations of each of the two nematode biocontrol agents under study.

3.2.4. Statistical Analysis.

The results were statistically analyzed by *analysis of variance* (ANOVA), the technique whereby the total variation present in a set of data is partitioned into several components. Associated with each of these components is a specific source of variation, so that in the analysis it is possible to ascertain the magnitude of the contributions of each of these sources to the total variation.
The model used for experiments was *randomized block design* (RBD), developed by R. A. Fisher in 1925. In this design the experimental units to which the treatments are applied are subdivided into homogeneous groups called blocks or replications, so that the number of experimental units in a block is equal to the number (or some multiple of the number) of the treatments being studied. The minimum number of replications required for obtaining precision in the experiment with varying number of treatments is as follows:

<table>
<thead>
<tr>
<th>Number of treatments</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of replications</td>
<td>13</td>
<td>7</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

For testing significant differences between individual pairs of means *least significant difference* test was applied. Fisher discussed it in the 1935 edition of his book, *The design of Experiments*. In all the cases where ANOVA leads to significant variance ratio, LSD test was applied. A difference between any two means that exceeds a least significant difference is considered significant at the level of significance used in computing the LSD.

The individual means together with the variance are reported in the tables after the application of *Student’s t distribution* test given by W. S. Gosset.

**NOTE**: (For statistically analyzed data in the tables presented at the following pages of Ch. 4: Observations :)

In a column of the table the data followed by same letters are not significantly different from each other. \((p \leq 0.05)\)
4.1. Infectivity assays.

The mortality and infectivity was determined for the larvae of four insect pests on treatment with two entomopathogenic nematodes through paper bioassay. Following observations were recorded in the investigation.

Observations from experiment no. 1:

Table 1(a); Effect of nematodes on larvae of shoot borer, *C. infuscatalellus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on insect larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality, m (n)</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>Occurs, 6(6)</td>
</tr>
<tr>
<td>Steinernema sp.</td>
<td>Occurs, 6(6)</td>
</tr>
<tr>
<td>Control</td>
<td>Not occurs, 0(6)</td>
</tr>
</tbody>
</table>

Table 1(b); Effect of nematodes on larvae of Gurdaspur borer, *A. steniellus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on insect larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality, m (n)</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>Occurs, 5(6)</td>
</tr>
<tr>
<td>Steinernema sp.</td>
<td>Occurs, 6(6)</td>
</tr>
<tr>
<td>Control</td>
<td>Not occurs, 0(6)</td>
</tr>
</tbody>
</table>

Table 1(c); Effect of nematodes on larvae of sugarcane beetle, *H. consanguinea*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on insect larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality, m (n)</td>
</tr>
<tr>
<td>Heterorhabditis sp.</td>
<td>Occurs, 6(6)</td>
</tr>
<tr>
<td>Steinernema sp.</td>
<td>Occurs, 6(6)</td>
</tr>
<tr>
<td>Control</td>
<td>Not occurs, 0(6)</td>
</tr>
</tbody>
</table>
Table 1(d); Effect of nematodes on larvae of root borer, *E. depressella*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Effect on insect larvae</th>
<th>Effect on insect larvae</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mortality, m (n)</td>
<td>Infectivity, i (n)</td>
</tr>
<tr>
<td><em>Heterorhabditis</em> sp.</td>
<td>Occurs, 0(6)</td>
<td>Occurs (low), 3(6)</td>
</tr>
<tr>
<td><em>Steinernema</em> sp.</td>
<td>Occurs, 4(6)</td>
<td>Occurs, 6(6)</td>
</tr>
<tr>
<td>Control</td>
<td>Not occurs, 0(6)</td>
<td>Not occurs, 0(6)</td>
</tr>
</tbody>
</table>

m: no. of larvae in which mortality occurred.
i: no. of larvae in which infectivity occurred.
n: total no. of larvae used in the experiment and investigated.

4.2. Efficacy studies.

4.2.1. Efficacy studies through nematode treatments.

In the observations recorded regarding efficacy studies under constant and variable ecological factors, the percent mortality determines control status and percent infectivity determines persistence level. These observations are as follows:

4.2.1.1. Constant ecological factors.

The constant ecological factors maintained during the course of experiment were:

Temperature = 25 °C; Relative Humidity (RH) = 85 %; Soil type = Loam; Light conditions = Dark; Experimental duration = 72 hrs.

The observations recorded regarding efficacy studies under the aforesaid conditions for the four insect pests of sugarcane being treated with two entomopathogenic nematodes are as follows:
Observations from experiment no. 2:

Table 2a. Effect of entomopathogenic nematodes regarding control of shoot borer, *C. infuscataellus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (stock solution)</th>
<th>% Mortality</th>
<th>% Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>33.40±4.18 d</td>
<td>71.80±6.94 d</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>70.40±5.74 c</td>
<td>92.80±2.70 b</td>
</tr>
<tr>
<td>Sn1</td>
<td>10% (100IJs/ml.)</td>
<td>73.80±5.16 b</td>
<td>86.80±4.16 c</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>98.00±3.63 a</td>
<td>99.80±0.57 a</td>
</tr>
<tr>
<td>Co</td>
<td>10% (0IJs/ml.)</td>
<td>05.40±0.68 e</td>
<td>00 e</td>
</tr>
</tbody>
</table>

C.V. = 3.51  
S.E. (±) = 1.25  
C.D. = 2.65

Table 2b. Effect of entomopathogenic nematodes regarding control of Gurdaspur borer, *A. steniellus*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (stock solution)</th>
<th>% Mortality</th>
<th>% Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>21.20±5.72 d</td>
<td>33.80±4.40 d</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>69.20±4.60 b</td>
<td>83.80±3.87 b</td>
</tr>
<tr>
<td>Sn1</td>
<td>10% (100IJs/ml.)</td>
<td>66.60±4.36 c</td>
<td>77.80±4.07 c</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>92.40±2.72 a</td>
<td>99.60±0.68 a</td>
</tr>
<tr>
<td>Co</td>
<td>10% (0IJs/ml.)</td>
<td>06.20±1.36 e</td>
<td>00 e</td>
</tr>
</tbody>
</table>

C.V. = 3.76  
S.E. (±) = 1.21  
C.D. = 2.57

C.V. = 2.93  
S.E. (±) = 1.63  
C.D. = 3.55
Table 2c. Effect of entomopathogenic nematodes regarding control of sugarcane beetle, *H. consanguinea*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (stock solution)</th>
<th>% Mortality</th>
<th>% Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>73.80±6.12c</td>
<td>84.80±3.45c</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>95.40±2.26a</td>
<td>98.80±1.62a</td>
</tr>
<tr>
<td>Snl</td>
<td>10% (100IJs/ml.)</td>
<td>68.80±4.69d</td>
<td>83.60±2.72c</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>82.80±3.34b</td>
<td>93.60±0.68b</td>
</tr>
<tr>
<td>Co</td>
<td>10% (0IJs/ml.)</td>
<td>02.20±0.56c</td>
<td>00d</td>
</tr>
</tbody>
</table>

\[\text{C.V.} = 2.79 \quad \text{C.V.} = 1.61\]
\[\text{S.E. (±)} = 1.14 \quad \text{S.E. (±)} = 0.92\]
\[\text{C.D.} = 2.42 \quad \text{C.D.} = 2.00\]

Table 2d. Effect of entomopathogenic nematodes regarding control of root borer, *E. depressella*.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration (stock solution)</th>
<th>% Mortality</th>
<th>% Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>04.40±1.89bc</td>
<td>01.60±1.42c</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>05.00±1.52bc</td>
<td>05.60±2.58c</td>
</tr>
<tr>
<td>Snl</td>
<td>10% (100IJs/ml.)</td>
<td>09.20±2.70b</td>
<td>16.60±5.02b</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>38.00±11.80a</td>
<td>62.60±10.73a</td>
</tr>
<tr>
<td>Co</td>
<td>10% (0IJs/ml.)</td>
<td>02.80±0.57c</td>
<td>00c</td>
</tr>
</tbody>
</table>

\[\text{C.V.} = 34.20 \quad \text{C.V.} = 20.55\]
\[\text{S.E. (±)} = 2.57 \quad \text{S.E. (±)} = 2.81\]
\[\text{C.D.} = 5.45 \quad \text{C.D.} = 6.12\]

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4.2.1.2. Variable ecological factors.

The observations recorded regarding efficacy studies under variable ecological factors are as follows:

4.2.1.2.1. Temperature variation.

Observations from experiment no. 3:

Table 3a. Effect of temperature variation on the control status of entomopathogenic nematodes in shoot borer, *C. infuscatus*.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>5-10°C</td>
<td>15.40±3.47 e</td>
</tr>
<tr>
<td>10-15°C</td>
<td>46.20±5.38 d</td>
</tr>
<tr>
<td>15-20°C</td>
<td>59.00±2.64 c</td>
</tr>
<tr>
<td>20-25°C</td>
<td>73.80±3.56 a</td>
</tr>
<tr>
<td>25-30°C</td>
<td>67.60±3.99 b</td>
</tr>
<tr>
<td>30-35°C</td>
<td>49.80±6.42 d</td>
</tr>
<tr>
<td>35-40°C</td>
<td>14.80±4.34 e</td>
</tr>
<tr>
<td>40-45°C</td>
<td>06.60±1.11 f</td>
</tr>
<tr>
<td>45-50°C</td>
<td>05.60±0.74 f</td>
</tr>
</tbody>
</table>

C.V. = 8.25  C.V. = 2.65  
S.E. (±) = 1.96  S.E. (±) = 1.39  
C.D. = 4.01  C.D. = 2.83
Table 3b. Effect of temperature variation on the control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>5-10°C</td>
<td>14.60±2.58 de</td>
</tr>
<tr>
<td>10-15°C</td>
<td>19.20±4.60 c</td>
</tr>
<tr>
<td>15-20°C</td>
<td>59.40±2.26 b</td>
</tr>
<tr>
<td>20-25°C</td>
<td>69.40±5.81 a</td>
</tr>
<tr>
<td>25-30°C</td>
<td>68.60±1.89 a</td>
</tr>
<tr>
<td>30-35°C</td>
<td>57.20±4.93 b</td>
</tr>
<tr>
<td>35-40°C</td>
<td>16.20±5.30 cde</td>
</tr>
<tr>
<td>40-45°C</td>
<td>12.40±1.67 e</td>
</tr>
<tr>
<td>45-50°C</td>
<td>07.80±1.36 f</td>
</tr>
</tbody>
</table>

\[ C.V. = 6.84 \quad \text{S.E. (±) = 1.56} \quad \text{C.D. = 3.19} \]
\[ C.V. = 3.25 \quad \text{S.E. (±) = 1.55} \quad \text{C.D. = 3.17} \]
Table 3c. Effect of temperature variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>5-10°C</td>
<td>13.00±2.64 h</td>
</tr>
<tr>
<td>10-15°C</td>
<td>44.60±3.72 f</td>
</tr>
<tr>
<td>15-20°C</td>
<td>82.20±4.53 d</td>
</tr>
<tr>
<td>20-25°C</td>
<td>97.00±1.76 a</td>
</tr>
<tr>
<td>25-30°C</td>
<td>93.00±3.83 b</td>
</tr>
<tr>
<td>30-35°C</td>
<td>87.80±1.04 c</td>
</tr>
<tr>
<td>35-40°C</td>
<td>62.20±9.28 e</td>
</tr>
<tr>
<td>40-45°C</td>
<td>17.00±3.17 g</td>
</tr>
<tr>
<td>45-50°C</td>
<td>07.60±0.68 i</td>
</tr>
</tbody>
</table>

C.V. = 5.37  C.V. = 3.27  
S.E. (±) = 1.91  S.E. (±) = 1.39  
C.D. = 3.89  C.D. = 2.84
Table 3d. Effect of temperature variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.

<table>
<thead>
<tr>
<th>Temperature Range</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>5-10°C</td>
<td>18.20±4.43 a</td>
</tr>
<tr>
<td>10-15°C</td>
<td>07.60±1.42 b</td>
</tr>
<tr>
<td>15-20°C</td>
<td>04.60±0.68 cde</td>
</tr>
<tr>
<td>20-25°C</td>
<td>05.40±1.11 c</td>
</tr>
<tr>
<td>25-30°C</td>
<td>05.20±0.57 c</td>
</tr>
<tr>
<td>30-35°C</td>
<td>04.40±1.11 cde</td>
</tr>
<tr>
<td>35-40°C</td>
<td>03.40±0.68 cd</td>
</tr>
<tr>
<td>40-45°C</td>
<td>03.20±0.57 cd</td>
</tr>
<tr>
<td>45-50°C</td>
<td>04.60±1.71 cd</td>
</tr>
</tbody>
</table>

C.V. = 21.05  S.E. (±) = 0.83  C.D. = 1.70  
C.V. = 11.43  S.E. (±) = 2.01  C.D. = 4.09
4.2.1.2.2. Humidity variation.

Observations from experiment no 4:

Table 4a. Effect of humidity variation on the control status of entomopathogenic nematodes in shoot borer, *C. infuscattellus*.

<table>
<thead>
<tr>
<th>Relative Humidity ( % )</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>30±5</td>
<td>12.80±3.10</td>
</tr>
<tr>
<td>50±5</td>
<td>25.60±3.58</td>
</tr>
<tr>
<td>70±5</td>
<td>50.20±3.87</td>
</tr>
<tr>
<td>90±5</td>
<td>73.20±3.22</td>
</tr>
</tbody>
</table>

\[ CV = 4.51 \]
\[ SE (±) = 1.13 \]
\[ CD = 2.47 \]

Table 4b. Effect of humidity variation on the control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*.

<table>
<thead>
<tr>
<th>Relative Humidity ( % )</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>30±5</td>
<td>11.6±1.67</td>
</tr>
<tr>
<td>50±5</td>
<td>18.8±0.57</td>
</tr>
<tr>
<td>70±5</td>
<td>47.8±5.08</td>
</tr>
<tr>
<td>90±5</td>
<td>70.8±3.45</td>
</tr>
</tbody>
</table>

\[ CV = 5.51 \]
\[ SE (±) = 1.29 \]
\[ CD = 2.83 \]
### Table 4c. Effect of humidity variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

<table>
<thead>
<tr>
<th>Relative Humidity (%)</th>
<th>% Mortality at different treatments</th>
<th>Hb2</th>
<th>Sn2</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>30±5</td>
<td>12.60±3.79 a</td>
<td>24.20±4.16 c</td>
<td>2.80</td>
<td></td>
</tr>
<tr>
<td>50±5</td>
<td>25.20±5.86 c</td>
<td>58.00±7.41 b</td>
<td>1.80</td>
<td></td>
</tr>
<tr>
<td>70±5</td>
<td>58.20±5.92 b</td>
<td>81.40±2.26 a</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>90±5</td>
<td>93.00±6.03 a</td>
<td>84.20±2.97 a</td>
<td>2.40</td>
<td></td>
</tr>
</tbody>
</table>

- C.V. = 9.51
- S.E. (±) = 2.84
- C.D. = 6.19

### Table 4d. Effect of humidity variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.

<table>
<thead>
<tr>
<th>Relative Humidity (%)</th>
<th>% Mortality at different treatments</th>
<th>Hb2</th>
<th>Sn2</th>
<th>Co</th>
</tr>
</thead>
<tbody>
<tr>
<td>30±5</td>
<td>12.80±5.66 a</td>
<td>07.80±2.04 d</td>
<td>7.60</td>
<td></td>
</tr>
<tr>
<td>50±5</td>
<td>10.80±2.97 a</td>
<td>14.00±3.17 c</td>
<td>6.80</td>
<td></td>
</tr>
<tr>
<td>70±5</td>
<td>02.60±1.11 b</td>
<td>36.40±2.26 b</td>
<td>1.60</td>
<td></td>
</tr>
<tr>
<td>90±5</td>
<td>06.40±1.42 b</td>
<td>43.40±3.99 a</td>
<td>2.80</td>
<td></td>
</tr>
</tbody>
</table>

- C.V. = 35.80
- S.E. (±) = 1.85
- C.D. = 4.02
4.2.1.2.3. Sunlight variation.

Observations from experiment no. 5:

Table 5a. Effect of sunlight variation on the control status of entomopathogenic nematodes in shoot borer, *C. infuscattellus*.

<table>
<thead>
<tr>
<th>Sunlight</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Direct</td>
<td>19.90±2.14 b</td>
</tr>
<tr>
<td>Diffused</td>
<td>72.00±4.73 a</td>
</tr>
<tr>
<td>Dark</td>
<td>70.90±3.83 a</td>
</tr>
</tbody>
</table>

C.V. = 9.65, S.E. (±) = 2.34, C.D. = 4.92

Table 5b. Effect of sunlight variation on the control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*.

<table>
<thead>
<tr>
<th>Sunlight</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Direct</td>
<td>18.70±1.94 b</td>
</tr>
<tr>
<td>Diffused</td>
<td>70.17±3.61 a</td>
</tr>
<tr>
<td>Dark</td>
<td>69.40±3.44 a</td>
</tr>
</tbody>
</table>

C.V. = 8.97, S.E. (±) = 2.12, C.D. = 4.46

C.V. = 3.98, S.E. (±) = 1.19, C.D. = 2.51
Table 5c. Effect of sunlight variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

<table>
<thead>
<tr>
<th>Sunlight</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Direct</td>
<td>22.20±2.31 b</td>
</tr>
<tr>
<td>Diffused</td>
<td>92.90±2.83 a</td>
</tr>
<tr>
<td>Dark</td>
<td>95.30±2.02 a</td>
</tr>
</tbody>
</table>

C.V. = 3.59
S.E. (±) = 1.13
C.D. = 2.36

Table 5d. Effect of sunlight variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.

<table>
<thead>
<tr>
<th>Sunlight</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Direct</td>
<td>03.30±0.48 b</td>
</tr>
<tr>
<td>Diffused</td>
<td>03.30±0.77 b</td>
</tr>
<tr>
<td>Dark</td>
<td>04.10±0.86 a</td>
</tr>
</tbody>
</table>

C.V. = 21.31
S.E. (±) = 0.34
C.D. = 0.71
4.2.1.2.4. Soil type variation.

Observations from experiment no. 6:

Table 6a. Effect of soil type on control status of entomopathogenic nematodes in shoot borer, *C. infuscatellus*.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Clay</td>
<td>63.38±2.28 b</td>
</tr>
<tr>
<td>Loam</td>
<td>71.63±4.89 a</td>
</tr>
<tr>
<td>Sand</td>
<td>56.38±3.95 c</td>
</tr>
<tr>
<td>Badarpur</td>
<td>31.25±4.52 c</td>
</tr>
</tbody>
</table>

C.V. = 8.25  S.E. (±) = 2.30  C.D. = 4.78

C.V. = 24.39  S.E. (±) = 2.00  C.D. = 4.16

Table 6b. Effect of soil type on control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Clay</td>
<td>65.63±6.51 b</td>
</tr>
<tr>
<td>Loam</td>
<td>71.38±7.04 a</td>
</tr>
<tr>
<td>Sand</td>
<td>53.75±7.04 c</td>
</tr>
<tr>
<td>Badarpur</td>
<td>32.13±6.48 c</td>
</tr>
</tbody>
</table>

C.V. = 5.74  S.E. (±) = 1.60  C.D. = 3.33

C.V. = 6.24  S.E. (±) = 1.86  C.D. = 3.87
### OBSERVATIONS

Table 6c. Effect of soil type on control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Clay</td>
<td>77.25±5.68  b</td>
</tr>
<tr>
<td>Loam</td>
<td>95.38±2.37  a</td>
</tr>
<tr>
<td>Sand</td>
<td>80.25±6.61  b</td>
</tr>
<tr>
<td>Badarpur</td>
<td>50.50±6.52  c</td>
</tr>
</tbody>
</table>

C.V. = 7.81  C.D. = 6.16
S.E. (±) = 2.96

Table 6d. Effect of soil type on control status of entomopathogenic nematodes in root borer, *E. depressella*.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>% Mortality at different treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hb2</td>
</tr>
<tr>
<td>Clay</td>
<td>03.50±0.78  b</td>
</tr>
<tr>
<td>Loam</td>
<td>05.00±0.90  a</td>
</tr>
<tr>
<td>Sand</td>
<td>05.25±1.07  a</td>
</tr>
<tr>
<td>Badarpur</td>
<td>05.00±1.55  a</td>
</tr>
</tbody>
</table>

C.V. = 23.33  C.D. = 1.14
S.E. (±) = 0.55

59
4.2.2. Efficacy studies through chemical treatments.

The observations recorded efficacy studies through the treatment of chemical pesticide, gamma benzene hexa chloride (γBHC) are as follows:

**Observations from experiment no. 7:**

**Table 7a. Effect of γBHC regarding control of shoot borer, *C. infuscattellus* under laboratory conditions.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. γBHC / 187.5ml. water)</td>
<td>87.50</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. γBHC / 187.5ml. water)</td>
<td>99.40</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>05.40</td>
</tr>
</tbody>
</table>

**Table 7b. Effect of γBHC regarding control of Gurdaspur borer, *A. steniellus* under laboratory conditions.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. γBHC / 187.5ml. water)</td>
<td>92.00</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. γBHC / 187.5ml. water)</td>
<td>99.80</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>06.20</td>
</tr>
</tbody>
</table>
Table 7c. Effect of γBHC regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. γBHC / 187.5ml. water)</td>
<td>99.40</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. γBHC / 187.5ml. water)</td>
<td>99.90</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>02.20</td>
</tr>
</tbody>
</table>

Table 7d. Effect of γBHC regarding control of root borer, *E. depressella* under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. γBHC / 187.5ml. water)</td>
<td>98.90</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. γBHC / 187.5ml. water)</td>
<td>100.00</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>02.80</td>
</tr>
</tbody>
</table>
4.2.3. Comparative account efficacy studies through nematode treatments and chemical treatments.

The comparative analysis of the observations from efficacy studies through nematode treatments (Table 2a-2d) and efficacy studies through chemical treatments (Table 7a-7d) lead us to following findings.

Table 8a. Comparative account of biological and chemical agents regarding control of shoot borer, *C. infuscatus* under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>33.40</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>70.40</td>
</tr>
<tr>
<td>Sn1</td>
<td>10% (100IJs/ml.)</td>
<td>73.80</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>98.00</td>
</tr>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. (\gamma)BHC / 187.5ml. water)</td>
<td>87.50</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. (\gamma)BHC / 187.5ml. water)</td>
<td>99.40</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>05.40</td>
</tr>
</tbody>
</table>
Table 8b. Comparative account of biological and chemical agents regarding control of Gurdaspur borer, *A. steniellus* under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>21.20</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>69.20</td>
</tr>
<tr>
<td>Sn1</td>
<td>10% (100IJs/ml.)</td>
<td>66.60</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>92.40</td>
</tr>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. γBHC / 187.5ml. water)</td>
<td>92.00</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. γBHC / 187.5ml. water)</td>
<td>99.80</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>06.20</td>
</tr>
</tbody>
</table>
Table 8c. Comparative account of biological and chemical agents regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (1000 IJs/ml.)</td>
<td>73.80</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (10000 IJs/ml.)</td>
<td>95.40</td>
</tr>
<tr>
<td>Sn1</td>
<td>10% (1000 IJs/ml.)</td>
<td>68.80</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (10000 IJs/ml.)</td>
<td>82.80</td>
</tr>
<tr>
<td>L1</td>
<td>20% EC (0.625 ml. γBHC / 187.5 ml. water)</td>
<td>99.40</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25 ml. γBHC / 187.5 ml. water)</td>
<td>99.90</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>02.20</td>
</tr>
</tbody>
</table>
Table 8d. Comparative account of biological and chemical agents regarding control of root borer, *E. depressella* under laboratory conditions.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>% Mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb1</td>
<td>10% (100IJs/ml.)</td>
<td>04.40</td>
</tr>
<tr>
<td>Hb2</td>
<td>10% (1000IJs/ml.)</td>
<td>05.00</td>
</tr>
<tr>
<td>Sn1</td>
<td>10% (100IJs/ml.)</td>
<td>09.20</td>
</tr>
<tr>
<td>Sn2</td>
<td>10% (1000IJs/ml.)</td>
<td>38.00</td>
</tr>
<tr>
<td>L1</td>
<td>20% EC (0.625ml. γBHC / 187.5ml. water)</td>
<td>98.98</td>
</tr>
<tr>
<td>L2</td>
<td>20% EC (1.25ml. γBHC / 187.5ml. water)</td>
<td>100.00</td>
</tr>
<tr>
<td>Co</td>
<td>Normal saline</td>
<td>02.80</td>
</tr>
</tbody>
</table>
CHAPTER # 5

INFERENCE
Following inferences could be drawn from the experiments done here for testing the pathogenicity and control status of the two entomopathogenic nematodes, *Steinernema* sp. and *Heterorhabditis* sp. as biocontrol agents used against the larvae of four sugarcane pests, shoot borer, *C. infuscattellus*, Gurdaspur borer, *A. steniellus*; sugarcane beetle *H. Consanguinea*; and root borer, *E. depressella* in the laboratory.

**5.1. Infectivity assays.**

*Steinernema* sp. was shown in the infectivity assays to infect the larvae of all the four insect pests of concern, i.e., shoot borer, *C. infuscattellus*; Gurdaspur borer, *A. steniellus*; sugarcane beetle, *H. Consanguinea* and root borer, *E. depressella* with successful pathogenicity and successful infestation observed in all the four cases. However it was found that mortality was less in the case larvae of root borer, *E. depressella* (with four dead out of six treated larvae) than in the larvae of other four insects.

In the infectivity assays *Heterorhabditis* sp. was shown to infect the larvae of three of the four insect pests studied here, namely, shoot borer, *C. infuscattellus*; Gurdaspur borer, *A. steniellus*; and sugarcane beetle, *H. Consanguinea* with successful pathogenicity and successful infestation observed in all these three cases. *Heterorhabditis* sp. did not cause mortality in root borer, *E. depressella* and in the case of this insect infectivity was also found to be low, with only three insect larvae out of the six treated found to be infected on examination. Further in the case of root borer, *E. depressella* the insect larvae yielded fewer infective juveniles per larva than were found in the case of other three insects. Thus the laboratory paper bioassay involving root borer, *E. depressella* larvae and infective juveniles of *Heterorhabditis* sp. revealed failure in pathogenicity and mild infestation.
The infected larvae of all the four sugarcane pests studied here showed symptoms of pathogenicity like sluggish attitude of the larvae and change in body colour towards pale brown or dark brown. (Table 1a, 1b, 1c, and 1d.)

5.2. Efficacy studies through nematode treatments.

5.2.1. Constant ecological factors.

The percent mortality, i.e. control status, and percent infectivity, i.e. persistence level, were analyzed for shoot borer, *C. infuscacellus*. Highest mortality occurred with Sn2 (1000 IJs of *Steinernema* sp. / ml.) treatment \{98.00±3.63\%\} followed by Sn1 (100 IJs of *Steinernema* sp. / ml.) treatment \{73.80±5.16\%\}; Hb2 (1000 IJs of *Heterorhabditis* sp. / ml.) treatment \{70.40±5.74\%\}; Hb1 (100 IJs of *Heterorhabditis* sp. / ml.) treatment \{33.40±4.18\%\} [df = 04; F = 1717.67; C.D. = 2.65; S.E. = 1.25; P = 0.05]. Analysis of persistence level showed that infectivity was highest in case of Sn2 treatment \{99.80±0.57\%\}; followed by Hb2 treatment \{92.80±2.70\%\}; Sn1 treatment \{86.80±4.16\%\} and Hb1 treatment \{71.80±6.94\%\} [df = 03; F = 107.17; C.D. = 3.55; S.E. = 1.63; P = 0.05]. The percent mortality in the control experiment was as low as 5.40\% and zero percent infectivity was obvious in the control set up. (Table 2a)

Similar trends were observed for the data analyzed in case of Gurdaspur borer, *A. stenielliis*. Highest mortality occurred with Sn2 treatment \{92.40±2.72\%\} followed by Hb2 treatment \{69.20±4.60\%\}; Sn1 treatment \{66.60±4.36\%\}; Hb1 treatment \{21.20±5.72\%\} [df = 04; F = 1758.33; C.D. = 2.57; S.E. = 1.21; P = 0.05]. Concurrent to this, infectivity was also highest with Sn2 treatment \{99.60±0.68\%\}; followed by Hb2 treatment \{83.80±3.87\%\}; Sn1 treatment \{77.80±4.07\%\} and Hb1 treatment
The percent mortality in control experiment was low (6.20%) and zero percent infectivity was observed in the control experiment, as expected. (Table 2b)

Trends observed for the data analyzed in case of sugarcane beetle, *H. consanguinea* showed following results. Highest mortality occurred with Hb2 treatment (95.40±2.26%) followed by Sn2 treatment (82.80±3.34%); Hb1 treatment (73.80±6.12%); Sn1 treatment (68.80±4.69%) [df = 04; F = 2029.2; C.D. = 2.42; S.E. = 1.14; P = 0.05]. On the other hand infectivity was also highest with Hb2 treatment (98.80±1.62%); followed by Sn2 treatment (93.60±0.68%); Hb1 treatment (84.80±3.45%) and Sn1 treatment (83.60±2.72%). The results obtained regarding percent infectivity with Hb1 and Sn1 treatments were similar, with no significant difference. [df = 03; F = 125.09; C.D. = 2.00; S.E. = 0.92; P = 0.05]. The percent mortality in control experiment was very low (2.20%) and percent infectivity was again zero in the control experiment for obvious reasons. (Table 2c)

Results were not encouraging in case of root borer, *E. depressella*. Highest mortality occurred with Sn2 treatment (38.00±11.80%) followed by Sn1 treatment (09.20±2.70%); Hb2 treatment (05.00±1.52%); Hb1 treatment (04.40±1.89%). Results obtained from Sn1, Hb2, and Hb1 treatments were not significantly different, as were the results of Hb2 and Hb1 treatments compared to the control experiment, where mortality was 02.80% [df = 04; F = 66.26; C.D. = 5.45, S.E. = 2.57, P = 0.05]. Analysis of the persistence level showed that percent infectivity was highest with Sn2 treatment (62.60±10.73%); followed by Sn1 treatment (16.60±5.02%). The results with Hb1 and Hb2 treatments were insignificant as they were found not to be significantly different from zero percent infectivity in the control experiment [df = 03; F = 199.75; C.D. = 6.12; S.E. = 2.81; P = 0.05]. (Table 2d)
Fig. 2a. Effect of entomopathogenic nematodes regarding control of shoot borer, *C. infuscatellus*.

Fig. 2b. Effect of entomopathogenic nematodes regarding control of Gurdaspur borer, *A. steniellus*. 
Fig. 2c. Effect of entomopathogenic nematodes regarding control of sugarcane beetle, *H. consanguinea*.

![Graph showing the effect of entomopathogenic nematodes on sugarcane beetle mortality and infectivity.]

Fig. 2d. Effect of entomopathogenic nematodes regarding control of root borer, *E. depressella*.

![Graph showing the effect of entomopathogenic nematodes on root borer mortality and infectivity.]

66 d
The efficacy studies done here at constant ecological factors using low (100 IJs/ml.) and high (1000 IJs/ml.) nematode concentrations of *Heterorhabditis* sp. and *Steinernema* sp. against the larvae of four sugarcane pests presently under study, it was found that while the low concentrations of both these nematodes were less effective against all the four species of insect pests and showed low persistence level and low control status, their high concentrations showed significant effect on these four different insect pests and had high persistence level and high control status.

Among the two biocontrol agents studied here, *Steinernema* sp. was found to show significantly higher control status and persistence level (marked by % mortality and % infectivity respectively, in the treated pest larvae) than that showed by *Heterorhabditis* sp. when used against the larvae of shoot borer, *C. infuscetellus*; Gurdaspur borer, *A. steniellus*; and root borer, *E. depressella*. In the case of sugarcane beetle, *H. consanguinea*, however, *Heterorhabditis* sp. was found to be comparatively more effective than *Steinernema* sp., and showed higher control status as well as higher persistence level in comparison to the other nematode species.

Both *Heterorhabditis* sp. and *Steinernema* sp. caused higher percent infectivity than percent mortality in the larvae of all the four species of insect pests of sugarcane studied here, thus indicating that the persistence level of both the entomopathogenic nematodes was higher than their control status without any exception or deviation. (Fig. 2a, 2b, 2c, 2d)
5.2.2. Variable ecological factors.

5.2.2.1. Temperature variation.

The observed data regarding effect of temperature variation on the control status, i.e. percent mortality, of *Heterorhabditis* sp. (Hb2) treatment in shoot borer *C. infuscatus* revealed that the highest mortality occurred at 20-25°C {73.80±3.56%}, followed by 25-30°C {67.60±3.99%}; 15-20°C {59.00±2.64%}; 30-35°C {49.80±6.42%} & 10-15°C {46.20±5.38%} (showing insignificant difference); 5-10°C {15.40±3.47%} & 35-40°C {14.80±4.34%} (showing insignificant difference) and 40-45°C {06.60±1.11%} & 45-50°C {05.60±0.74%} (showing insignificant difference) [df = 08; F = 381.95; C.D. = 4.01; S.E. = 1.96; P = 0.05]. Similarly in case of *Steinernema* sp. (Sn2) treatment highest mortality occurred at 25-30°C {99.00±1.76%}; 20-25°C {98.20±2.39%}; and 30-35°C {98.00±2.92%} (showing insignificant difference) followed by 35-40°C {96.00±3.40%} (not significantly different from the results obtained at 20-25°C & 30-35°C); 15-20°C {90.80±2.22%}; 10-15°C {87.80±3.22%}; 40-45°C {79.00±5.05%}; 5-10°C {56.80±6.60%} and 45-50°C {39.00±4.57%} [df = 08; F = 468.70; C.D. = 2.83; S.E. = 1.39; P = 0.05]. (Table 3a)

The observed data regarding effect of temperature variation on the control status, i.e. percent mortality, of *Heterorhabditis* sp. (Hb2) treatment in Gurdaspur borer, *A. steniellus* revealed that the highest mortality occurred at 20-25°C {69.40±5.81%}, & 25-30°C {68.60±1.89%} (showing insignificant difference), followed by 15-20°C {57.20±4.93%}; & 30-35°C {57.20±4.93%} (showing insignificant difference); 10-15°C {19.20±4.60%}; 35-40°C {16.20±5.30%}; 5-10°C {14.60±2.58%} & 40-
45°C {12.40±1.67%} (showing insignificant difference) and 45-50°C {07.80±1.36%} [ df = 08; F = 580.81; C.D. = 3.19; S.E. = 1.56; P = 0.05]. Similarly in case of Steinernema sp. (Sn2) treatment highest mortality occurred at 25-30°C {92.60±2.39%}; & 20-25°C {99.40±2.44%} (showing insignificant difference) followed by 15-20°C {88.80±2.04%} & 30-35°C {87.00±3.17%} (not significantly different); 35-40°C {82.40±2.99%}; 10-15°C {77.00±4.48%}; 40-45°C {74.40±4.70%}; 5-10°C {54.20±5.92%} and 45-50°C {32.60±3.58%} [df = 08; F = 331.76; C.D. = 3.17; S.E. = 1.55; P = 0.05]. (Table 3b)

The observed data regarding effect of temperature variation on the control status, i.e. percent mortality, of Heterorhabditis sp. (Hb2) treatment in sugarcane beetle, H. consanguinea revealed that the highest mortality occurred at 20-25°C {97.00±1.76%}, followed by 25-30°C {93.00±3.83%}; 30-35°C {87.80±1.04%}; 15-20°C {82.20±4.53%}; 35-40°C {62.20±9.28%}; 10-15°C {44.60±3.72%}; 40-45°C {17.00±3.17%}; 5-10°C {13.00±2.64%}; and 45-50°C {7.60±0.68%} [df = 08; F = 732.45; C.D. = 3.89; S.E. = 1.91; P = 0.05]. Similarly in case of Steinernema sp. (Sn2) treatment highest mortality occurred at 25-30°C {83.40±2.02%}; & 20-25°C {82.00±3.05%} (showing insignificant difference) followed by 15-20°C {78.80±2.22%} & 30-35°C {78.20±3.22%} (not significantly different); 35-40°C {75.60±5.17%}; 40-45°C {68.60±0.68%}; 10-15°C {65.60±2.99%}; 5-10°C {47.00±3.17%} and 45-50°C {26.20±4.93%} [df = 08; F = 375.25; C.D. = 2.84; S.E. = 1.39; P = 0.05]. (Table 3c)

The observed data regarding effect of temperature variation on the control status, i.e. percent mortality, of Heterorhabditis sp. (Hb2) treatment in root borer E. depressella revealed that the highest mortality occurred at 5-10°C {18.20±4.43%}, followed by 10-15°C {07.60±1.42%}; 20-25°C
{05.40±1.11%}; 25-30°C {05.20±0.57%}; 45-50°C {04.60±1.71%}; & 15-20°C {04.60±0.68%} (showing insignificant difference); 35-40°C {03.40±0.68%} & 40-45°C {03.20±0.57%} (showing insignificant difference) [df = 08; F = 62.29; C.D. = 1.70; S.E. = 0.83; P = 0.05]. Similarly in case of Steinernema sp. (Sn2) treatment highest mortality occurred at 20-25°C {42.80±4.25%} followed by 25-30°C {38.60±5.17%}; 35-40°C {29.60±2.86%}; 15-20°C {28.80±2.04%}; 10-15°C {28.20±4.07%}; & 30-35°C {28.20±4.93%} (showing insignificant difference); 5-10°C {25.00±8.29%}; 40-45°C {24.40±3.99%}; and 45-50°C {04.00±2.33%} [df = 08; F = 57.97; C.D. = 4.09; S.E. = 2.01; P = 0.05]. (Table 3d)
Fig. 3a. Effect of temperature variation on the control status of entomopathogenic nematodes in shoot borer, C. infuscattellus.
Fig. 3b. Effect of temperature variation on the control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*. 
Fig. 3c. Effect of temperature variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*. 
Fig. 3d. Effect of temperature variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.
In the efficacy studies done here using high concentrations (1000 IJ/ml.) of the infective juveniles of *Heterorhabditis* sp. and *Steinernema* sp. against the larvae of the four insect pests of sugarcane presently under study, it was found that both low as well as high temperature ranges had adverse effect on the control status of the members of these two nematode species. Best results for *Heterorhabditis* sp. as control agent were obtained between 20-25°C; and, *Steinernema* sp. gave best results between 20-30°C. It can be easily inferred from the observations that, as compared to *Steinernema* sp., members of *Heterorhabditis* sp. were more susceptible to deviations from the optimum temperature range, both on the lower side of this range as well as on its higher side. As far the deviation from the optimum range of temperature in the case of *Steinernema* sp. it was found that the members of this nematode species were more tolerant to temperatures lower than the range of optimum temperature (20-30°C) than to the deviations on higher side of their optimum. (Fig. 3a, 3b, 3c, 3d.)
5.2.2.2. Humidity variation.

The observed data regarding affect of humidity variation on control status i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in shoot borer, *C. infuscatellus* revealed that highest mortality occurred at relative humidity of 90±5% {73.20±3.22%} followed by 70±5%RH {50.20±3.87%}; 50±5%RH {25.60±3.58%}; and 30±5%RH {12.80±3.10%} \( \text{df} = 03; F = 1121.82; \text{C.D.} = 2.47; \text{S.E.} = 1.13; P = 0.05 \). In case of *Steinernema* sp. (Sn2) treatment, highest mortality occurred at relative humidity of 90±5% {98.00±3.17} followed by 70±5%RH {85.60±2.86%}; 50±5%RH {64.00±6.46%} and 30±5%RH {41.20±5.38%} \( \text{df} = 03; F = 259.66; \text{C.D.} = 4.78; \text{S.E.} = 2.19; P = 0.05 \). (Table 4a)

The observed data regarding affect of humidity variation on control status i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in Gurdaaspur borer, *A. steniellus* revealed that highest mortality occurred at relative humidity of 90±5% {70.80±3.45%} followed by 70±5%RH {47.80±5.08%}; 50±5%RH {18.80±0.57%}; and 30±5%RH {11.60±1.67%} \( \text{df} = 03; F = 883.49; \text{C.D.} = 2.83; \text{S.E.} = 1.30; P = 0.05 \). In case of *Steinernema* sp. (Sn2) treatment, similar trends were observed with highest occurred at relative humidity of 90±5% {95.00±2.92%} followed by 70±5%RH {77.60±3.69%}; 50±5%RH {58.20±4.07%} and 30±5%RH {29.20±2.84%} \( \text{df} = 03; F = 594.43; \text{C.D.} = 3.57; \text{S.E.} = 1.64; P = 0.05 \). (Table 4b)

The observed data regarding affect of humidity variation on control status i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in sugarcane beetle, *H. consanguinea* revealed that highest mortality occurred at
relative humidity of 90±5% \{93.00±6.03\%\} followed by 70±5%RH \{58.20±5.92\%\}; 50±5%RH \{25.20±5.86\%\}; and 30±5%RH \{12.60±3.79\%\} \[df = 03; F = 321.90; C.D. = 6.19; S.E. = 2.84; P = 0.05\]. On the other hand *Steinernema* sp. (Sn2) treatment showed highest mortality at relative humidity of 90±5% \{84.20±2.97\%\} & 70±5%RH \{81.40±2.26\%\} (showing insignificant difference); followed by 50±5%RH \{58.00±7.41\%\} and 30±5%RH \{24.20±4.16\%\} \[df = 03; F = 282.89; C.D. = 5.09; S.E. = 2.34; P = 0.05\]. (Table 4c)

The observed data regarding affect of humidity variation on control status i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in root borer, *E. depressella* revealed that highest mortality occurred at relative humidity of 30±5% \{12.80±5.66\%\} & 50±5%RH \{10.80±2.97\%\} (showing insignificant difference); followed by 90±5%RH \{06.40±1.42\%\} & 70±5%RH \{02.60±1.11\%\} (showing insignificant difference) \[df = 03; F = 12.23; C.D. = 4.02; S.E. = 1.85; P = 0.05\]. On the other hand *Steinernema* sp. (Sn2) treatment showed highest mortality at relative humidity of 90±5% \{43.40±3.99\%\} followed by 70±5%RH \{36.40±2.26\%\}; 50±5%RH \{14.00±3.17\%\} and 30±5%RH \{07.80±2.04\%\} \[df = 03; F = 354.60; C.D. = 2.81; S.E. = 1.29; P = 0.05\]. (Table 4d)
Fig. 4a. Effect of humidity variation on the control status of entomopathogenic nematodes in shoot borer, *C. infuscatellus*.

![Graph showing % mortality at different relative humidity levels for Hb2, Sn2, and Co nematodes](image)

Fig. 4b. Effect of humidity variation on the control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*.

![Graph showing % mortality at different relative humidity levels for Hb2, Sn2, and Co nematodes](image)
Fig. 4c. Effect of humidity variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

Fig. 4d. Effect of humidity variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.
The efficacy studies conducted here at various humidity levels, in which high concentrations (1000 IJs/ml.) of infective juveniles of *Steinernema* sp. and *Heterorhabditis* sp. were used against the larvae of the four insect pests of sugarcane, revealed that both these species of nematodes had best control status at 90 percent relative humidity. The control status of these two species of nematodes reduced with the reduction in humidity levels. Pathogenicity of these nematodes was directly related to the humidity levels, being favorably affected by high humidity levels and adversely affected by its low level. The two entomopathogenic nematodes exhibit similar response, and in a similar fashion, in being susceptible to low humidity levels. (Fig. 4a, 4b, 4c, 4d)
5.2.2.3. Sunlight variation.

The observed data regarding affect of sunlight variation on the control status, i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in shoot borer, *C. infuscataellus* revealed that highest mortality occurred in the diffused sunlight \(72.00\pm4.73\%\) & in the dark \(70.90\pm3.83\%\) (showing no significant difference); and low mortality rate in the presence of direct sunlight \(19.90\pm2.14\%\) \([df = 02; F = 323.35; C. D. = 4.92; S. E. = 2.34; P = 0.05]\). In case of *Steinernema* sp. (Sn2) treatment proportionate results were obtained with higher mortality occurring in the dark conditions \(98.20\pm0.81\%\) & diffused sunlight \(96.50\pm1.27\\%\) (showing insignificant difference); and low mortality in the presence of direct sunlight \(19.60\pm2.36\%\) \([df = 02; F = 4872.43; C. D. = 1.91; S. E. = 0.91; P = 0.05]\). (Table 5a)

The observed data regarding affect of sunlight variation on the control status, i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in Gurdaspur borer, *A. steniellus* revealed that highest mortality occurred in the diffused sunlight \(70.70\pm3.61\%\) & in the dark \(69.40\pm3.44\%\) (showing no significant difference); and low mortality rate in the presence of direct sunlight \(18.70\pm1.94\%\) \([df = 02; F = 390.44; C. D. = 4.46; S. E. = 2.12; P = 0.05]\). In case of *Steinernema* sp. (Sn2) treatment proportionate results were obtained with higher mortality occurring in the dark conditions \(92.60\pm2.27\%\) & diffused sunlight \(90.10\pm2.27\\%\) (showing insignificant difference); and low mortality in the presence of direct sunlight \(18.40\pm1.62\%\) \([df = 02; F = 2495.45; C. D. = 2.51; S. E. = 1.19; P = 0.05]\). (Table 5b)

The observed data regarding affect of sunlight variation on the control status, i.e., percent mortality of *Heterorhabditis* sp. (Hb2) treatment in sugarcane beetle, *H. consanguinea* revealed that highest mortality occurred in
the dark conditions {95.30±2.02%} & in the diffused sunlight
{92.90±2.83%} (showing no significant difference); and low mortality rate in
the presence of direct sunlight {22.20±2.31%} \([df = 02; \text{ F} = 2727.91; \text{ C. D.} = 2.36; \text{ S. E.} = 1.12; \text{ P} = 0.05]\). In case of Steinernema sp. (Sn2) treatment
proportionate results were obtained with higher mortality occurring in the dark
conditions {82.20±2.26%} & diffused sunlight {80.30±3.84%} (showing
insignificant difference); and low mortality in the presence of direct sunlight
{24.70±2.94%} \([df = 02; \text{ F} = 687.64; \text{ C. D.} = 3.70; \text{ S. E.} = 1.76; \text{ P} = 0.05]\).
(Table 5c)

The observed data regarding affect of sunlight variation on the control
status, i.e., percent mortality of Heterorhabditis sp. (Hb2) treatment in root
borer, E. depressella revealed that there was low mortality rate in the diffused
sunlight {03.30±0.77%} & in the direct sunlight {03.30±0.48%} (similar
results); and a little more in dark conditions {04.10±0.86%} \([df = 02; \text{ F} = 3.69; \text{ C. D.} = 0.71; \text{ S. E.} = 0.34; \text{ P} = 0.05]\). In case of Steinernema sp. (Sn2)
treatment higher mortality rate was observed occurring in the dark conditions
{39.40±6.34%} & diffused sunlight {37.50±5.81%} as compared to direct
sunlight {18.40±5.25%} where it was low \([df = 02; \text{ F} = 80.28; \text{ C. D.} = 5.71;
S. E. = 2.72; \text{ P} = 0.05]\). (Table 5d)
Fig. 5a. Effect of sunlight variation on the control status of entomopathogenic nematodes in shoot borer, *C. infuscataellus*.

![Figure 5a](image)

Fig. 5b. Effect of sunlight variation on the control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniellus*.

![Figure 5b](image)
Fig. 5c. Effect of sunlight variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

![Bar chart showing effect of sunlight variation on the control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.](image)

Fig. 5d. Effect of sunlight variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.

![Bar chart showing effect of sunlight variation on the control status of entomopathogenic nematodes in root borer, *E. depressella*.](image)
The efficacy studies conducted here at various sunlight conditions, in which high concentrations (1000 IJs/ml.) of infective juveniles of *Steinernema* sp. and *Heterorhabditis* sp. were used against the larvae of the four insect pests of sugarcane, shoot borer, *Chilo infuscattellus*; Gurdaspur borer, *Acigona steniellus*; sugarcane beetle, *Holotrichia consanguinea* and root borer, *Emmalocera depressella* revealed that both these nematodes acted poorly under conditions of bright sunlight. Both of these showed high control status under diffused sunlight and dark conditions with the results for them under these two conditions of light being almost similar.
5.2.2.4. Soil type variation.

The observed data regarding affect of soil type variation on the control status, i.e., %mortality of *Heterorhabditis* sp. (Hb2) treatment in shoot borer, *C. infuscataellus* revealed that highest mortality occurred in loam {71.63±4.89%} followed by that in clay soil {63.38±2.28%}; sandy soil {56.38±3.95%}; and coarse sand (Badarpur soil) {31.25±4.52%} \[df = 03; F = 115.22; C.D. = 4.78; S.E. = 2.29; P = 0.05\]. In case of *Steinernema* sp. (Sn2) treatment, highest mortality occurred in loam {98.13±1.45%} followed by that in sandy soil {84.75±5.18%}; coarse sand (Badarpur soil) {64.25±5.96%}; and clay {21.25±2.13%} \[df = 03; F = 565.39; C.D. = 4.16; S.E. = 2.00; P = 0.05\]. (Table 6a)

The observed data regarding affect of soil type variation on the control status, i.e., %mortality of *Heterorhabditis* sp. (Hb2) treatment in Gurdaspur borer, *A. steniellus* revealed that highest mortality occurred in loam {71.38±7.04%} followed by that in clay soil {65.63±6.51%}; sandy soil {53.75±7.04%}; and coarse sand (Badarpur soil) {32.13±6.48%} \[df = 03; F = 235.51; C.D. = 3.33; S.E. = 1.60; P = 0.05\]. In case of *Steinernema* sp. (Sn2) treatment, highest mortality occurred in loam {90.88±4.90%} followed by that in sandy soil {74.25±4.38%}; coarse sand (Badarpur soil) {56.75±4.76%}; and clay soil {16.50±3.17%} \[df = 03; F = 589.78; C.D. = 3.87; S.E. = 1.86; P = 0.05\]. (Table 6b)

The observed data regarding affect of soil type variation on the control status, i.e., %mortality of *Heterorhabditis* sp. (Hb2) treatment in sugarcane beetle, *H. consanguinea* revealed that highest mortality occurred in loam {95.38±2.37%} followed by that in sandy soil {80.25±6.61%} & clay soil {77.25±5.68%} (showing insignificant difference); and coarse sand (Badarpur
soil) \(\{50.50\pm6.52\%\}\) \([df = 03; F = 79.43; C.D. = 6.16; S.E. = 2.96; P = 0.05]\). In case of *Steinernema* sp. (Sn2) treatment, highest mortality occurred in loam \(\{83.13\pm4.21\%\}\) followed by that in sandy soil \(\{70.13\pm4.35\%\}\); coarse sand (Badarpur soil) \(\{41.50\pm2.65\%\}\); and clay soil \(\{19.13\pm3.37\%\}\) \([df = 03; F = 1510.90; C.D. = 2.18; S.E. = 1.05; P = 0.05]\). (Table 6c)

The observed data regarding affect of soil type variation on the control status, i.e., %mortality of *Heterorhabditis* sp. (Hb2) treatment in root borer, *E. depressella* revealed that the rate of mortality was low in loam \(\{05.00\pm0.90\%\}\), sandy soil \(\{05.25\%\pm1.07\}\), & coarse sand (Badarpur soil) \(\{05.00\pm1.55\%\}\) (with no significant difference); followed by that in clay soil \(\{03.50\pm0.78\%\}\) where it was lowest \([df = 03; F = 4.28; C.D. = 1.14; S.E. = 0.55; P = 0.05]\). In case of *Steinernema* sp. (Sn2) treatment, highest mortality occurred in loam \(\{38.87\pm4.81\%\}\) followed by that in sandy soil \(\{29.00\pm4.48\%\}\); coarse sand (Badarpur soil) \(\{15.75\pm3.71\%\}\); and clay soil \(\{08.38\pm1.09\%\}\) \([df = 03; F = 87.90; C.D. = 4.27; S.E. = 2.05; P = 0.05]\). (Table 6d)
Fig. 6a. Effect of soil type on control status of entomopathogenic nematodes in shoot borer, *C. infuscattellus*.

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<th>Co</th>
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</tr>
<tr>
<td>Badarpur</td>
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<td>5.5</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Fig. 6b. Effect of soil type on control status of entomopathogenic nematodes in Gurdaspur borer, *A. steniiellus*.

<table>
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</tr>
</tbody>
</table>
Fig. 6c. Effect of soil type on control status of entomopathogenic nematodes in sugarcane beetle, *H. consanguinea*.

![Graph showing % mortality by soil type for *H. consanguinea*]

Fig. 6d. Effect of soil type on control status of entomopathogenic nematodes in root borer, *E. depressella*.

![Graph showing % mortality by soil type for *E. depressella*]
Efficacy studies regarding the control status of infective juveniles of 
Steinernema sp. and Heterorhabditis sp. used here against the larvae of the four 
insect pests of sugarcane, shoot borer, Chilo infuscatellus; Gurdaspur borer, 
Acigona steniellus; sugarcane beetle, Holotrichia consanguinea and root borer, 
Emmalocera depressella placed in different soil types showed significant 
variation. Loam was found to be best suited for the activities of both the 
entomopathogenic nematodes under study. The data trend obtained for 
Heterorhabditis sp. (Hb2) reveals that for this nematode highest control status 
against all the four insect pests was achieved when they were allowed to 
operate in loam, followed by clay soil, sandy soil and coarse sand (Badarpur 
soil), with the lowest control status being achieved in the last mentioned soil 
type. The data trend obtained for the control status of Steinernema sp. (Sn2) 
indicated that highest mortality and hence highest control status against all the 
four insect pests was achieved when they were operating in loam, followed by 
sandy soil, coarse sand (Badarpur) and clay soil, with lowest control status 
being achieved when they were allowed to perform in clay soil. (Fig. 6a, 6b, 
6c, 6d)
5.3. Efficacy studies through chemical treatments.

The efficacy studies conducted by spraying γBHC on the larvae of the four concerned insect pests studied here showed almost complete rate of mortality of the insect larvae with both the concentration gradients L₁ (20% EC of 0.625ml. γBHC / 187.5ml. water), and L₂ (20% EC of 1.25ml. γBHC / 187.5ml. water), the exception being H. consanguinea (sugarcane beetle), in whose case lower γBHC concentration, L₁, was found to be less effective. In case of C. infuscatterellus (shoot borer), A. steniellus (Gurdaspur borer), and E. depressella (root borer) both the doses were found to be equally good, with promising results. It is apparent from these results that high dosage of γBHC in case of H. consanguinea (sugarcane beetle) and both low and high concentrations of this chemical when used against C. infuscatterellus (shoot borer), A. steniellus (Gurdaspur borer), and E. depressella (root borer) can give satisfactory control of these four species of insects even in the field conditions. (Table and Figs. 7a, 7b, 7c, 7d)
Table 7a. Effect of γBHC regarding control of shoot borer, *C. infuscatalles* under laboratory conditions.

Table 7b. Effect of γBHC regarding control of Gurdaspur borer, *A. steniellus* under laboratory conditions.
Table 7c. Effect of $\gamma$BHC regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.

Table 7d. Effect of $\gamma$BHC regarding control of root borer, *E. depressella* under laboratory conditions.
5.4. Comparative account of biological and chemical control agents.

The control status of the two biocontrol agents, *Heterorhabditis* sp. and *Steinernema* sp. was found to be meaningful when compared to the control status of chemical pesticide γBHC, recommended by U.P.C.S.R., regarding control of the larvae of four insect pest species under study. The present data indicate that the infective juveniles of *Steinernema* sp. brought about significant control of shoot borer, *C. infuscattellus*; and Gurdaspur borer, *A. steniellus*, with the results obtained by using these nematodes being significantly good and in accordance with the results obtained by the use of chemical against these insect pests. Furthermore, the data also indicates that *Heterorhabditis* sp. was found to give significant control of sugarcane beetle *H. consanguinea*; the results again being significantly good and in accordance with the results of chemical control. Even further the data indicates that the entomopathogenic nematodes are not much significant regarding control of root borer, *E. depressella; Steinernema* sp. being little effective and *Heterorhabditis* sp. having an insignificant effect; the results showing negative deviation to the results of chemical control. However, the role of these nematodes in the control cannot be ignored even in this case. (Table and Figs. 8a, 8b, 8c, 8d)
Fig. 8a. Comparative account of biological and chemical agents regarding control of shoot borer, *C. infuscatus* under laboratory conditions.

Fig. 8b. Comparative account of biological and chemical agents regarding control of Gurdaspur borer, *A. steniellus* under laboratory conditions.
Fig. 8c. Comparative account of biological and chemical agents regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.

![Graph showing comparative account of biological and chemical agents regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.]

Fig. 8d. Comparative account of biological and chemical agents regarding control of root borer, *E. depressella* under laboratory conditions.

![Graph showing comparative account of biological and chemical agents regarding control of root borer, *E. depressella* under laboratory conditions.]

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CHAPTER # 6

DISCUSSION
Human civilization all over the globe is struggling to preserve the originality of the Mother Nature. This contention with the forces against the nature seems to be converging on a single point agenda disfavouring the use of synthetic chemicals which are a major source diluting the vigour of our ambiance. But then there is an intraspecific struggle among the living creatures. Man, to sustain the supremacy among these living creatures is bound to defend and offend the negatives of nature. To conserve the positives of nature together with fighting the negatives create a complication in the battle against the odds. Here arises a need to use the forces of nature against the enemies in our surroundings without participation of any alien force. This undoubtedly will serve the purpose. Biological control is one of the finest strategies favouring the aforesaid idea.

The study presented over here is an attempt to analyse the role of entomopathogenic nematodes in the biological control of insect pests. For this purpose *Heterorhabditis* sp. and *Steinernema* sp., the two major genera of the entomopathogenic nematodes were employed against four major sugarcane pests, namely, shoot borer, *Chilo infuscatellus*; Gurdaspur borer, *Acigona steniellus*; sugarcane beetle, *Holotrichia consanguinea* and root borer, *Emmalocera depressella*. Laboratory studies were conducted to study the potential of these entomopathogenic nematodes against the four major pests of sugarcane under study that had not been previously tested. The effect of various ecological factors on the pathogenicity of the nematodes was also investigated. Due to unavailability of the commercial forms of these nematodes, the attempt was restricted to laboratory studies. Gauglar in 1981 and 1988 standardized the ecological conditions for optimising the virulence caused by the entomopathogenic nematodes. Such standard optimised conditions were set for the laboratory experiments done in the present study.
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In the laboratory conditions, the infectivity assays and the efficacy studies show successful pathogenicity in the four sugarcane pests studied here eliciting good potential of *Steinernema* sp. and *Heterorhabditis* sp. as biological control agents. These results strongly favour the findings of Bedding *et al* (1993), Gaugler & Kaya (1990, 1993), Grewal & Georgis (1997), Poinar (1990, 1991), and Smart (1995) asserting that the entomopathogenic nematodes are effective against a wide variety of insect pests. Rapid mortality within 72 hrs. was evident in the study done over here as was also found in the respective studies done by Georgis, R. (1992); Kaya & Kopenhoffer (1999); and Poinar (1979). The results were promising for shoot borer, *C. infuscatus* and Gurdaspur borer, *A. stenellus* that do not dwell in soil and thus contradicting the assertions of Begley (1990), Georgis & Hague (1991), and Klein (1990) that entomopathogenic nematodes are not very effective against those insect pests which do not dwell in soil.

In the present study increased level of pathogenicity was noted at high concentrations of infective juveniles of entomopathogenic nematodes compared favourably with the findings of Doucet *et al* (1996), Lacey & Unruh (1998) who also observed similar trends in their experiments with higher concentrations of infective juveniles of entomopathogenic nematodes being applied against pests and showing good better results than the application of lower concentrations of infective juveniles of entomopathogenic nematodes.

In the laboratory conditions both *Steinernema* sp. and *Heterorhabditis* sp. were found to be effective when used against shoot borer, *C. infuscatus* and Gurdaspur borer, *A. stenellus*. Grewal and Georgis in 1997 also found that *Heterorhabditis* sp. is useful in controlling stem borers in many glass house crops and recommended the use of this nematode species for field applications. The study presented over here signifies the better performance of *Steinernema*
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sp. as compared to the performance of *Heterorhabditis* sp. regarding control of *C. infuscatellus* and *A. steniellus*. These results are in accordance with the findings of Schroeder (1987, 1990, and 1994) and Dowing *et al.* (1991) where *Steinernema* sp. was showing better results when compared to that of *Heterorhabditis* sp. for the control of *Diaaprepes abbreviatus*. Cryptic habitat being an unusual residence for *C. infuscatellus* and *A. steniellus*, who dwell in the stem in natural conditions, it was found that the larvae of these pest species confined themselves to the surface of the soil placed in petridishes during the laboratory experiments. This might be the reason for *Steinernema* sp. being more effective as they search for the host at soil surface (Moyle and Kaya, 1981) where as *Heterorhabditis* sp. look for host deep into the soil (Choo and Kaya, 1989) and therefore less effective in this case. But it was shown in the findings of Bedding and Molyneux, 1982, that *Heterorhabditis* sp. has an additional mode of entry giving them an advantage over *Steinernema* sp. and thus, in spite of having lower control status than *Steinernema* sp., *Heterorhabditis* sp. gave significant results in controlling the larvae of both these insect pests.

The present study also reveals that higher control status of *Heterorhabditis* sp. than control status of *Steinernema* sp. was found in the case of controlling the larvae of sugarcane beetle, *H. consanguinea* under laboratory conditions. These findings strongly support the findings of Georgis and Gaugler (1991), recommending the use of *Heterorhabditis* sp. in controlling white grubs, being preferred, over the use of *Steinernema* sp. for the same.

The results of controlling the larvae of root borer, *E. depressella* by the application of infective juveniles of *Heterorhabditis* sp. and *Steinernema* sp., were not promising. *Heterorhabditis* sp. was not showing any significant control where as *Steinernema* sp. was showing less pathogenicity and thereby
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lower control status. The possible reasons behind this low success rate of entomopathogenic nematodes in this case are, incompetence in gaining entry into the larvae through any of the methods explained by Grewal et al (1994, 1997), strong immune system against bacterial symbionts carried by these nematodes, or improper host utilization as being discussed by Reed and Carne (1967) and Selvan and Blackshaw (1990). However, earlier studies show *Steinernema* sp. to be effective in controlling the larvae of mint root borer.

This difference in nematode virulence as shown in the aforementioned cases is attributed to their searching behaviour (Campbell and Gaugler, 1993, 1997; Grewal et al, 1994) and difference in host utilization (Dutky, 1956; Reed and Carne, 1967; Selvan and Blackshaw, 1990). However, in the laboratory studies and specially petridish bioassay as being done in the present study, both the nematodes and the target pests are in close proximity so that the need to seek the target by the nematodes is eliminated. Therefore it may be speculated that host-utilizing strategy including rate of penetration, mode of entry into the haemocoel, insect immune system and the rate of development and virulence of the symbiotic bacteria, played an important role in the nematode insect interaction.

The present study also implies that the rate of infection was found to be greater than the rate of mortality in the pest larvae being treated by the entomopathogenic nematodes. This shows that the difference of vigour and immunity status among the individuals of the same species play an important role in the pathogenicity of the entomopathogenic nematodes in the larvae of insect pests. However, the symptoms of pathogenicity, including sluggish attitude of the infected larvae after application of entomopathogenic nematodes, also participate in the prime objective of the efforts of saving the vegetation from pest attack. After infestation of infective juveniles of the
entomopathogenic nematodes in the pest larvae, the larvae are no more capable of causing damage to the plant. Thus it can be said that in spite of the intraspecific differences in vigour and immunity among the larvae of a pest species causing differences in the level of mortality and level of infectivity, the pathogenicity of entomopathogenic nematodes in all the cases serve the purpose of controlling the pest species.

The laboratory experiments conducted regarding efficacy studies at variable temperature ranges showed that low and high temperature ranges limit the virulence of the entomopathogenic nematodes, *Steinernema* sp. and *Heterorhabditis* sp. as was also found in the studies done by Kaya (1990), Griffin (1993), Miduturi *et al* (1994), Mason & Hominick (1995), and Lacey & Unruh (1998). It was also observed in the present study that *Steinernema* sp. gave best results between 20-30°C. Shaprio *et al* (1999) suggested 21°C as optimal temperature for virulence of *Steinernema* sp. in young insect larvae. Hsiao *et al* (1996) found best results for this nematode at 25°C. Midutari *et al* (1994) obtained optimal temperature for *Steinernema* sp. to be 25°C whereas Lacey & Unruh (1998) found it more effective at temperatures of 30°C and above. As far as *Heterorhabditis* sp. is concerned, it was observed in the present study that this nematode gave best results at 20-25°C. Shaprio *et al* (1999) suggested 24°C as optimal temperature for the virulence of *Heterorhabditis* sp. in young insect larvae. Optimal infectivity for this nematode species was displayed at 25°C in the studies done by Mason & Hominick (1995), at 26°C in the studies done by Doucet *et al* (1996), and at 20°C by the laboratory studies conducted by Miduturi *et al* (1994). It is thus evident that there is a difference in observations of various scientists regarding the optimum temperature established by them for the control of insect targets by any of the two entomopathogenic nematodes studied here as was also asserted by Gouge *et al* (1999), who further suggested that assuming existing
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nematode temperature optima and applying same condition to untested insect species may not result in maximum biocontrol efficacy. Thus it can be concluded that the optimal temperature, for virulence of entomopathogenic nematode, differs which can be attributed to the nematode species being employed, the pest species being targeted for control, and the climatic conditions of the geographical area under consideration. So it can be said that inspite of adhering to a particular temperature for being optimum for nematode virulence, it will be better to consider a range of temperature at which the nematode species significantly serve the purpose of biological control of the pest species in any practical suggestion for field applications.

The present study revealing the antagonistic effect of high temperature ranges towards the virulence of both the entomopathogenic nematodes supports the findings of Henneberry et al (1996) for both Steinernema sp. and Heterorhabditis sp., and the findings of Hsiao & All (1996) for Steinernema sp. advocating adverse effect of high temperatures on the virulence of these nematodes. The present study also reveals the antagonistic effect of low temperature ranges towards the virulence of Heterorhabditis sp. and Steinernema sp. This result supports the findings of Steiner (1996), and Brown & Gaugler (1997) for both these nematodes and the findings of Kiger & Bornstein (1997) for Steinernema sp. advocating adverse effect of low temperatures on virulence of these entomopathogenic nematodes.

In consonance with the findings of Griffin (1993), it was also found in the present study that the virulence of Heterorhabditis sp. is more affected by low and high temperature ranges than the virulence of Steinernema sp. Shamseldean et al (1996) and Lacey & Unruh (1998) found Steinernema sp. to be more effective regarding the control status than Heterorhabditis sp. at high
ranges of temperatures. Danilov et al (1994) found *Steinernema* sp. being more adapted to low temperature ranges than *Heterorhabditis* sp.

The present study shows that the high humidity level favours the pathogenicity of the entomopathogenic nematodes, *Steinernema* sp. and *Heterorhabditis* sp. and lowering of the humidity level has adverse effect on the pathogenicity of these nematodes. The results are in accordance with the findings of Kaya, Gaugler & Kaya (1991) which stated that there is an adverse impact of low humidity levels on the survival and pathogenicity of these entomopathogenic nematodes. Similar findings were stated by Brown & Gaugler (1995) showing adverse effect of low relative humidity on emergence, survival and pathogenicity of entomopathogenic nematodes as was also shown in the studies of Kaya and Baur (1996). The reason behind this lowering of pathogenicity as an effect of lowering of humidity is the adverse impact of low humidity levels on survival rate, survival time and motility of entomopathogenic nematodes necessary for host finding and this was clearly shown in the findings of Li et al in 1986. The present study shows that high humidity levels of 85 % RH are conducive for optimum virulence caused by entomopathogenic nematodes in the target species. This was also previously observed by Lacey & Unruh (1998) and thereby suggesting that a minimum of three hours in high relative humidity was necessary for attaining 50% mortality in the pest larvae being treated by entomopathogenic nematodes. But Kung et al (1990) further suggested that there is a difference in pathogenicity levels of individual species of entomopathogenic nematodes according to their climatic origins and native habitat.

The present study further reveals that pathogenicity of the entomopathogenic nematodes, *Steinernema* sp. and *Heterorhabditis* sp., is highly affected by exposure to direct bright sunlight. The experiments were
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performed on specified dates to provide optimum conditions with temperature, humidity, and soil type favouring the pathogenicity of these nematode species. The results are in full agreement with the findings of Gaugler & Boush (1978) who asserted the adverse effect of sunlight on the pathogenicity of entomopathogenic nematodes. Ghally in 1989 and along with his co-workers in 1994 provided evidences that exposure to gamma irradiations (an integral part of sunlight) cause inactivation of nematodes and inability to reproduce, along with decrease in nematode motility and host infestation. The reasons behind low pathogenicity of entomopathogenic nematodes on exposure to sunlight as was evident in our studies can be attributed to these factors. Fugiiee and Yokoyama in 1998 found similar results on exposing the entomopathogenic nematodes to ultra violet (UV) irradiations (an integral part of sunlight) as was found by Ghally in 1989 by exposing the same to gamma irradiations. They further attributed the lowering of pathogenicity level of entomopathogenic nematodes (when exposed to UV irradiations) to the decrease in the density of entomopathogenic symbiotic bacteria associated with the infective juveniles of these nematodes.

Sandy loam was the soil type selected for efficacy studies done here under constant and optimum ecological conditions. This selection was based on the recommendations of Blackshaw & Senthamizhselvan (1991); McCoy, Shapiro, & Duncan (unpublished data) and Kung, Gaugler & Kaya (1990). It was further observed in the present study that clay soil is not favourable for pathogenicity of *Steinernema* sp. as was also found in the respective studies by Walker (1984); Molyneux & Bedding (1984); Kung, Gaugler & Kaya (1990); Raquel & Carmen; Gaugler & Kaya (1990); Chu & Kaya (1991); Barbercheck & Kaya (1991); Barbercheck (1992) and Hsiao & All (1996). Further many of the scientists quoted above discussed in support of their findings that high clay content in the soil restrict nematode motility due to small particle size and thus
decrease the pathogenicity and virulence of entomopathogenic nematodes in the target pest species found in the soil. Blackshaw & Senthamizh selvan (1991) and Walker (1984) inferred from their findings that small pore size in addition to small particle size is responsible for causing restraint in the persistence and infectivity of pathogenic nematodes. Kung, Gaugler & Kaya (1990) pointed out that nematodes might have to expend more energy in order to move through small pores and in between fine particles and thus showing inefficacy when used for target pests in clay soil. In the present study it was also observed that *Heterorhabditis* sp. was found to show significant control in the clay soil, the control being lower than in the loam but being higher than in sand or coarse sand. This finding contradicts the findings of other scientists as mentioned supra. The possible reason behind such behaviour of heterorhabditid nematode is that the clay soil holds more water than sandy soil or coarse sand and as has been previously viewed by Kaya (1990) and Richardson & Grewal (1994) that apart from soil texture other factors like soil moisture, soil temperature, soil pH, etc. also limit the virulence of entomopathogenic nematodes. The soil used in the present experiments was sterilized before placing it in the petridishes to avoid any infection other than the nematodes applied for the test. However, Fan & Hominick (1991) found no significant difference in the virulence of these nematodes under sterilized and normal soil.
CHAPTER # 7

EXECUTIVE SUMMARY & CONCLUSION
Heterorhabditis and Steinernema, the two rhabditid nematodes, have drawn attention of the workers around the globe for being potential biological control agents of insects. These soil dwelling nematodes, together with their bacterial symbionts, are obligate and lethal parasites of insects and are usually referred to as Entomopathogenic Nematodes (EPN). Nematodes’ non-specific development, which does not rely on specific host nutrients, allows them to infect a large number of insect species, as a result these nematodes have got a wide host range and can be used successfully against numerous pests. India being a country where sugarcane is a major cash crop, it was found necessary here to study the control the control status and level of pathogenicity of entomopathogenic nematodes in controlling the major pests of sugarcane. This study is an attempt to test the potential of the two entomopathogenic nematodes, Steinernema sp. and Heterorhabditis sp. against three lepidopteron pests; shoot borer (Chilo infuscatellus Snellen), Gurdaspur borer (Acigona steniellus Hampson), and root borer (Emmalocera depressella Swinhoe); and a coleopteran pest sugarcane beetle (Holotrichia consanguinea Blanch.) in the laboratory. The study further includes laboratory analysis of the effect of abiotic factors, namely, temperature, humidity, solar irradiations and soil type on the virulence and the control status of the entomopathogenic nematodes.

The larvae of these four insect species were exposed for infestation to the infective juveniles of both the entomopathogenic nematode species under study. The present study was divided in to two forms of experiments; the one named as ‘infectivity assays’ was conducted as a preliminary study to test the potential of both the entomopathogenic nematodes individually against the larvae of the four insect pest species one by one. The other experiment was termed ‘efficacy studies’ where the two concentrations of the infective juveniles (IJs) of entomopathogenic nematodes (100IJs/ml. & 1000IJs/ml.) were utilized to be employed against 100 pest larvae kept in the petridishes at
constant optimum condition set at 25°C, 85% RH in the sandy loam soil and kept in the dark. Paper bioassay was selected for preliminary studies in the infectivity assays and petridish bioassay for laboratory trials in the efficacy studies. The percent mortality of pest larvae on nematode applications was a tool to determine the control status of the entomopathogenic nematodes. The pathogenicity or persistence level inside the host was determined through percent infectivity. The efficacy studies were then also conducted under variable ecological factors to investigate the effect of changes in temperature, humidity, sunlight and soil texture on the control status of entomopathogenic nematodes. The ecological studies were planned under conditions taking one of the aforementioned factors under varied limits and the other factors taken to be constant within optimum range. The various temperature ranges selected were 5-10°C, 10-15°C, 15-20°C, 20-25°C, 25-30°C, 30-35°C, 35-40°C, 40-45°C, and 45-50°C. The various levels of relative humidity (RH) selected were 30%RH, 50%RH, 70%RH, and 90% RH with standard error of ±5%. The various sunlight conditions selected were direct and diffused sunlight and dark. The various soil types selected were clay soil, loam, sandy soil and coarse sand (Badarpur soil). Adopting the procedure identical to that employed for studying the efficacy of nematodes in controlling the larvae of the four insect pests under study, two different concentrations of γBHC (Lindane) were applied on the insect larvae, under constant ecological conditions, in order to compare the results obtained by using the nematodes as control agents with those obtained by using γBHC (Lindane). The results were statistically analyzed by using ‘analysis of variance’, ‘LSD test’, and ‘student’s t-distribution test’.

The infectivity assays indicated positive results showing that both the entomopathogenic nematodes *Heterorhabdus* sp. and *Steinernema* sp. under study were effective against the larvae of all the four pest species studied here. In the efficacy studies done at constant ecological factors, it was found that
while the low concentrations of both these nematodes were less effective against all the four species of insect pests and showed low persistence level and low control status, their high concentrations showed significant effect on these four different insect pests and had high persistence level and high control status. *Steinernema* sp. was found to show significantly higher control status and persistence level than that showed by *Heterorhabditis* sp. when used against the larvae of shoot borer, *C. infuscetellus*; Gurdaspur borer, *A. steniellus*; and root borer, *E. depressella*. In the case of sugarcane beetle, *H. consanguinea*, however, *Heterorhabditis* sp. was found to be comparatively more effective than *Steinernema* sp., and showed higher control status as well as higher persistence level in comparison to the other nematode species. Both *Heterorhabditis* sp. and *Steinernema* sp. caused higher percent infectivity than percent mortality in the larvae of all the four species of insect pests of sugarcane studied here, thus indicating that the persistence level of both the entomopathogenic nematodes was higher than their control status. The control status of the two biocontrol agents, *Heterorhabditis* sp. and *Steinernema* sp. was found to be meaningful when compared to the control status of chemical pesticide γBHC, recommended by U.P.C.S.R., regarding control of the larvae of four insect pest species under study. (Fig. 9a, 9b, 9c, 9d)

In the efficacy studies at variable temperature ranges it was found that both low as well as high temperature ranges had adverse effect on the control status of *Heterorhabditis* sp. and *Steinernema* sp. Best results for *Heterorhabditis* sp. as control agent were obtained between 20-25°C; and, *Steinernema* sp. gave best results between 20-30°C. As compared to *Steinernema* sp., members of *Heterorhabditis* sp. were more susceptible to deviations from the optimum temperature range, both on the lower side of this range as well as on its higher side. As far the deviation from the optimum range of temperature in the case of *Steinernema* sp. it was found that the members of
this nematode species were more tolerant to temperatures lower than the range of optimum temperature (20-30°C) than to the deviations on higher side of their optimum. The efficacy studies conducted here at various humidity levels shows that both these species of nematodes had best control status at 90 percent relative humidity. The control status of these two species of nematodes reduced with the reduction in humidity levels. Pathogenicity of these nematodes was directly related to the humidity levels, being favourably affected by high humidity levels and adversely affected by its low level. The efficacy studies conducted here at various sunlight conditions revealed that both these nematodes acted poorly under conditions of bright sunlight. Both of these showed high control status under diffused sunlight and dark conditions with the results for them under these two conditions of light being almost similar. Efficacy studies conducted in different soil types revealed that loam was best suited for the activities of both the entomopathogenic nematodes under study. *Heterorhabditis* sp. showed highest control status in loam, followed by clay soil, sandy soil and lowest in coarse sand (Badarpur soil). *Steinernema* sp. showed highest control status in loam, followed by sandy soil, coarse sand (Badarpur) and lowest in clay soil.

It can therefore be concluded in the light of the present study and the studies done by various other scientists that entomopathogenic nematodes are potential biocontrol agents. Further it is evident in the laboratory studies that *Steinernema* sp. has got a good potential in controlling shoot borer, *C. infuscatellus*; Gurdaspur borer, *A. steniellus*; sugarcane beetle, *H. consanguinea*; and to a considerable extent root borer, *E. depressella*. Moreover, *Heterorhabditis* sp. has also been found in the laboratory studies to have a good potential in controlling three of the four insect pests under study namely shoot borer, *C. infuscatellus*; Gurdaspur borer, *A. steniellus*; sugarcane beetle, *H. consanguinea* with no significant control observed in the case of root
borer, *E. depressella* through this nematode. However, further work is required, particularly in commercial growing conditions in the field, before the entomopathogenic nematodes studied here can be regarded as suitable alternatives to gamma BHC (Lindane) for the control of the four insect pest species of concern.

In spite of low success rate in the field trials there is a need of laboratory studies to be conducted to test the potential of entomopathogenic nematodes against a wide variety of insect pests still to be tested. Current strategies for the control of insect pests in agriculture rely increasingly on the development of transgenic crops expressing insecticidal protein toxins, and there is a continuous search for novel insecticidal toxins (Constant & Bowen, 2000). Biotechnologists all over the world are working on various aspects of the pesticidal toxins isolated from entomopathogenic nematodes and their bacterial symbionts (Bowen & Ensign, 1998 and Morgan *et al*, 2001). The significance of our studies and its findings for further investigation is that the aforementioned entomopathogenic nematodes against the pest larvae studied here can be tested in the field and the consideration that toxins isolated can also be applied with a transgenic option in sugarcane plant.

The various biological and chemical pesticides and their different doses used are:

Stock solutions with infective juveniles (IJ$s$) of *Steinernema* sp.
1. Sn 1: 100 ± 10 IJs per milliliter of stock solution.
2. Sn 2: 1000 ± 10 IJs per milliliter of stock solution.

Stock solutions with infective juveniles (IJ$s$) of *Heterorhabditis* sp.
1. Hb 1: 100 ± 10 IJs per milliliter of stock solution.
2. Hb 2: 1000 ± 10 IJs per milliliter of stock solution.

Stock solutions of Gamma BHC (Lindane).
1. L 1: 20% EC of 0.625 ml. yBHC per 187.5 ml. of water.
2. L 2: 20% EC of 1.25 ml. yBHC per 187.5 ml. of water.

Stock solution of control experiment.
Co: normal saline (0.65%).
Fig. 9a. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of shoot borer, *C. infuscatus* under laboratory conditions.

![Bar graph showing % mortality for different treatments.](image)

Fig. 9b. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of Gurdaspur borer, *A. steniellus* under laboratory conditions.

![Bar graph showing % mortality for different treatments.](image)
EXECUTIVE SUMMARY & CONCLUSION

Fig. 9c. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of sugarcane beetle, *H. consanguinea* under laboratory conditions.

Fig. 9d. Effect of *Steinernema* sp., *Heterorhabditis* sp., and Lindane regarding control of root borer, *E. depressella* under laboratory conditions.
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