CHAPTER - I
INTRODUCTION

In the last four decades, widespread use of pesticides and fertilizers has helped growers to meet the demand for large quantity and variety of vegetables and relatively inexpensive and attractive vegetable products. India is second largest producer of vegetables in the world, next to China. Production of vegetables in India in 1997 was 72832 X 1000 MT against world production of 595565 X 1000 MT (FAO Year Book, 1997). Vegetables are grown in about 56.29 lakh hectares forming 3 per cent of the total cropped area in the country (Indian Horticulture data base, 1999, National Horticulture Board).

As a result of monoculturing and large-scale cultivation of high yielding hybrids and varieties, the population of pests have been found increasing and some times the minor pests also assume the status of major pests. Emergence of tomato fruit borer, Helicoverpa armigera Hub. and tobacco caterpillar, Spodoptera litura Fab. as a foremost pests of national importance are such examples. These are polyphagous pests causing damage to a variety of crops including important vegetables. The pest problems on vegetables can be more serious because of the favourable conditions, which are provided for pest multiplication as a result of present methods of cultivation (including intensive cultivation, multiple cropping systems, dense planting, application of fertilizers and pesticides etc.).

In India, crop losses due to all pests range from 10-30 per cent (Anonymous, 1998; Upadhyay et al., 1998) depending upon the type of crops and the environmental conditions. Complete crop failures may occur in case of serious attack and vegetables are no exception to this. Annual crop loss due to all pests in India was estimated at Rs. 6,000 crores in 1973-74 which
at today's price may exceed 20,000 crores or more. Insect-pests cause about 20 per cent of these losses (Lal, 1996).

In India, pest control for vegetable crops is still largely dependent on the use of synthetic chemicals and more than 70 per cent of pesticides used in the country, are insecticides. There has been a steep growth in pesticide usage in the country and the estimated demand for pesticide has risen to 75,000 MT in 1991. There after declining trend was noticed in pesticide consumption. In 1996-97 pesticide consumption was 56110 MT in our country (Singhal, 1999). India stands seventh in pesticide consumption in the world market. Among pesticide, insecticides occupy the largest share of 52.54 per cent of the total consumption as recorded for the year 1997-98. Vegetables alone account for nearly 9 per cent of the market share of pesticides (Singhal, 1999). Pesticides use had a positive and dramatic impact on agricultural production of crops including vegetables, against insect pests and diseases. But indiscriminate and overuse of pesticides have led to a number of pest problems (insect pest resistance, secondary pest outbreaks, pest resurgence etc.). Besides, pesticides cause adverse effects on non-target organisms and leave toxic residues, hazardous to human being, animals and environment. During this century there has been an almost 100 per cent increase in the number of insects and mites due to indiscriminate use of pesticides. In an assessment of out break of H. armigera and S. litura during 1987 and 1997 in Andhra Pradesh, alarming reduction in population of natural enemies of these pests was reported (Singh, 1999). Out of the larvae collected by the author, none yielded any parasitoid though as many as 77 have been recorded earlier. Only two entomofungal pathogens (Nomuraea rileyi Samson and Beauveria bassiana (Bals.) Vuill.) could withstand pesticide onslaught, were recorded on both these pests.
Similarly a number of major insect-pests of vegetable crops have developed resistance to recommended dosages of several pesticides, i.e. *H. armigera* on several crops; *S. litura* on groundnut and several other crops; diamond back moth, *Plutella xylostella* L. on crucifers, mustard aphid, *Lipaphis erysimi* Kalt.; leaf miner, *Liriomyza trifolii* Burges on tomato and several other hosts.

Many farmers apply pesticides in a routine manner and in large quantities when there is no need to do so. This is particularly true with the vegetables. Sometimes, farmers use pesticides just before harvesting the vegetables, even dipping them in pesticide solutions under the false impression that it will retain its freshness for longer time and be more attractive against its market value. Such indiscriminate use of pesticides can be considered as one of the most hazardous operations, which pose threat to our living environment. Miss Rachel Carson's book, ("Silent Spring", 1962) on the catastrophic consequences of use of pesticides created a furore and an active campaign was started against the use of synthetic organic pesticides at global level in the years to come. As a result, many persistent and toxic chemicals were banned and concept of integrated pest management (IPM) was developed as the practical approach towards solving the insect pest problems at global level. The Government of India has adopted IPM as a major thrust area and main plan of crop protection programme during the Eighth Plan including vegetables (India, 1995). During the Eighth Plan, an amount of 45 crores have been yearmarked for IPM which includes 15 crores for the establishment of biocontrol laboratories.

Pesticide residues on vegetables were major health issues facing the vegetable industry in the 1990s. Residues of DDT and BHC has been found in vegetables including cabbage, cauliflower, cowpea, spinach, okra, brinjal
etc. (Lal, 1996). This is why strategy for insect control has to be different in vegetable crops as compared from other crops because of nature of utilization of this commodity. In recent years, vegetable growers have seen an increasing number of pesticides removed from the market with no replacement. There is substantial reduction in pesticide consumption, which declined from 75033 MT (1990-91) to 56110 MT (1996-97) (Singhal, 1999). Further reduction in pesticide consumption is anticipated in the coming years (Upadhyay et al., 1998). However, today, public and government concern about food and environmental safety are changing pesticide and fertilizer usage in vegetable production.

The concern for environmental safety and health risks have stimulated efforts to develop biological control agents as alternatives or supplements to these chemicals and as a key components of IPM of crop pests. As a result, there is growing interest in the development of biopesticides of which microbial pesticides are an important component. They are more environment friendly alternatives to chemical pesticides. The best known examples of biopesticides being used for control of pests of cotton, maize, tomatoes etc., the world over, are Bacillus thuringiensis (Berliner), B. bassiana and Metarhizium anisopliae (Metch.) Sorok.etc. Although at present, this group of biopesticides represent only a small fraction of world pesticide market. The biopesticide share of the market was estimated to be around 380 million in 1995, representing only 1.3 per cent of the total world pesticide market (Menn, 1997). Since the majority of biopesticides are currently marketed for insect control, biopesticides represent approximately 4.5 per cent of the world insecticide sale. However, the growth rate for biopesticides over the next 10 years has been forecast at 10-15 per cent per annum in contrast to 2 per cent for chemical pesticides (Menn and Hall, 1998).
Recently, entomopathogenic fungi (mycoinsecticides) are gaining increased attention as eco-friendly insect control agents. Although, over 750 species were reported to infect insects, only few have received, serious consideration as potential commercial candidates (Menn and Hall, 1998). Technological advances in production, formulation and shelf life have contributed substantially to the viability of mycoinsecticides as practical insect management agents that can compete economically with chemical insecticides in certain situations, such as fruit, vegetable and specialty crops.

The important insect pathogenic fungi belonging to the genera *Aspergillus*, *Beauveria*, *Entomophthora*, *Hirsutella*, *Isaria*, *Metarhizium*, *Paecilomyces*, *Spicaria*, *Verticillium* provided effective pest suppression (Roberts and Yendol, 1971; Burges, 1981; McCoy *et al*., 1988; Ferron *et al*., 1991). Some of these fungi (*B. bassiana*, *B. brongniartii* (Saccardo) Petch, *M. anisopliae*, *M. flavoviridae* Gams and Rozsypal, *P. farinosus* Brown and Smith, *V. lecani* Zimm., etc.) have been produced on mass scale and developed in to commercial formulations. These are being used successfully for control of some of the serious pests of agriculture and forestry in countries like USA, USSR, Canada, Japan, China and Brazil etc. (Steinhaus, 1963; Burges and Hussey, 1971; Ferron, 1978; Burges, 1981; Gillespie and Claydon, 1989; Feng *et al*., 1994; Wraight and Carruthers, 1998). In India, comparatively less work has been carried out. Different species of fungi have been reported on a large number of insect pests (Narsimhan, 1970; Ramakrishnan and Kumar, 1977). But very few studies have been carried out for practical utilization of these fungi in insect pest control (Easwaramoorthy and Jayaraj, 1977; Rajak *et al*., 1993; Puzari *et al*., 1994, 1998). Fungi have advantage over other pathogens in the sense that unlike bacterial, viral or protozoans, entomopathogenic fungi need not to be consumed by the hosts to be infective. Instead germinating fungal spores are able to grow directly
through the insect cuticle. Some of these fungi can be grown on cheap artificial media and this affords a good means of employing them for biological control.

*B. bassiana* the white muscardine fungus is widely regarded as one of the most promising species known for potential development into practical insect biocontrol agent. It is a well known entomopathogen of Coleoptera, Hemiptera and Lepidoptera having wide host range and distribution (Bell, 1974; Burges, 1981; Houle *et al.*, 1987; Maniania, 1992; Stimac *et al.*, 1993; Leathers and Gupta, 1993; Feng *et al.*, 1994).

This fungus was recorded long back in silkworm (Bassi, 1835). Although mycoses caused by the fungus, *Beauveria* spp. and *Metarhizium* spp. have been studied for about a century, it is principally during the last 25 years or so that special attention has been focused to develop them in new methods of biological control of insects. For many years, they have been given secondary importance because of failures of some early attempts. In 1950s, East European countries started investigations with *B. bassiana*, as part of general strategy to control the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say. In the past few years, this entomopathogenic fungus has been extensively studied and used for control of many important pests of various crops around the world. The tested target pests included the CPB, *L. decemlineata* on potatoes in USA and USSR (Campbell *et al.*, 1985; Hajek *et al.*, 1987; Anderson *et al.*, 1988), pests of rice in Philippines (Rombach *et al.*, 1986a), *Nilapervata lugens* (Stal.) on rice in Korea (Rombach *et al.*, 1986b; Aguda *et al.*, 1987), green house white fly, *Trialeurodes vaporariorum* Westwood on greenhouse crops in Syria (Trefi, 1984) and the sugarcane borer, *Diatraea saccharalis* F. (Lecuona and Alves, 1988; Diaz and Lecuona, 1995; Lecuona *et al.*, 1996) are the few examples.
In India, *B. bassiana* has been recorded on large number of insect pests of various crops (Rao, 1975; Ramakrishnan and Kumar, 1977; Nayak and Srivastava, 1979; Jayaramaiah and Veeresha, 1983; Agarwal and Rajak, 1985; Prasad *et al.*, 1989, 1990; Easwaramoorthy and Santhalakshmi, 1993; Khan *et al.*, 1993; Masarrat Haseeb and Srivastava, 1996; Puzari *et al.*, 1998). But only scattered information is available on various aspects of this entomopathogen. So far, no systematic and detailed work has been done to find out the scope of utilizing *B. bassiana* in IPM strategies for vegetable crops in our country.

*B. bassiana* is known to have wide host range as mentioned above. But strains can differ in their specificity, virulence, production patterns and other characteristics (Prasad *et al.*, 1989, 1990; Maniania, 1992; Leathers and Gupta, 1993; Fuentes and Carballo, 1995; Lecuona *et al.*, 1996; Selman *et al.*, 1997; Arcas *et al.*, 1999). Several strains of *B. bassiana* have been identified on the basis of assessment of different virulence factors. The target insect as well may vary in their susceptibility, depending not only on age and instar, but also population density and geographical origin. It is, therefore, important to search for the best host pathogen combination (Keller, 1992).

After selection of strain, the next step is the evaluation of the selected strain for age-specific-dose-mortality relationship. These studies have been conducted for a number of important insect pests i.e. *H. armigera* (Prasad *et al.*, 1990; Sandhu *et al.*, 1993), *S. litura* (Prasad *et al.*, 1989; Jayanthi and Padmavathamma, 1996), *Ostrinia nubilalis* Hub. (Feng *et al.*, 1985), *L. decemlineata* (Fargues *et al.*, 1991) *Phthorimaea operculella* Zeller (Hafez *et al.*, 1997) and a number of other pests. It is also important to select isolates that grow rapidly at ambient temperatures occurring after spore application (Gillespie and Claydon, 1989).
Pathogenesis in *B. bassiana* involves both mechanical and biochemical means (Hajek and St. Leger, 1994). Production of enzymes helps in penetration of cuticle of insects (St. Leger *et al.*, 1986). Production of toxic elements (toxins) is considered to be an important aspect of pathogenicity of *B. bassiana*. Beauvericin and other toxins have been identified in this fungus (Roberts, 1981). Degeneration of tissues due to fungal growth and nutrient exhaustion are other reasons for diseased condition of host insect, as evidenced by histopathological studies (Wasti and Hartmann, 1975; Cheung and Grula, 1982).

Vegetable crops are susceptible to many plant pathogens and disease out-break often elicit heavy usage of fungicides. Under these circumstances, there is potential for negative interaction between these disease-control agents and beneficial fungi applied for insect control. It is, therefore, necessary to conduct study on the compatibility of chosen strain with other agrochemicals of common use. Results of many studies on this aspect have indicated that the pesticides have deleterious effects on *B. bassiana* but not all classes of chemicals are antagonistic. Benomyl, zineb, mancozeb were the most inhibitory fungicides against this species while sulphur, dinocap and daconil were compatible as reported by Jaques and Morris (1981), Bajan *et al.* (1995), Rivera and Bustillo (1996). However, studies conducted by Wraight and Carruthers (1998) at the University of Maine indicated no significant reduction in *B. bassiana* activity against CPB when kocide, mancozeb and bravo were applied 24-72 hr after mycotrol. These results suggest that *B. bassiana* will be compatible with many fungicides under field conditions, if treatments can be applied asynchronously. Like-wise, insecticides detrimental to this entomopathogenic fungus can not tankmixed i.e. endosulfan, can be applied separately. Combined application of *B. bassiana* with sub-lethal doses of insecticides and other pesticides have also
proved synergistic (Bajan et al., 1995; Rivera and Bustillo, 1996; Wright and Kennedy, 1996; Lee Sang Myeong et al., 1996; Quintela and McCoy, 1997; Wraight and Carruthers, 1998).

Once suitable isolate has been selected with respect to virulence and growth, consideration must be given to production - an excellent pathogen is of little commercial use if it can not be produced readily. B. bassiana has been produced on mass-scale by solid state fermentation or in submerged culture or combination of both (Burges, 1981). Mass production of B. bassiana using surface culture has been in progress for many years in the USSR and China. Various natural substrates such as potatoes, sugarbeet, grain etc. have been used for conidial production. Boverin, Boverol, Boverosil were produced for commercial use (Ferron, 1981; Aregger, 1992). Greater production efficiencies have been achieved with this entomogenous fungus (Feng et al., 1994; Wraight and Carruthers, 1998). Fully automated, commercial scale production of aerial conidia of this pathogen in the west has been most aggressively pursued by private industries. As a result, products like Mycotrol, Botani Gard, Naturalis-L have been developed. Naturalis-L is more recent addition and this strain has been found very effective in field evaluation of important pests (Wright and Knauf, 1994; Wright and Kannedy, 1996). Recently Wraight and Carruthers (1998) have extensively reviewed the progress in research with respect to development of formulation, product stabilization and application of fungi including B. bassiana.

The advances made in development of B. bassiana as mycoinsecticide has brought it on to the doors of commercial success. The work on genetic and physiological engineering has been initiated and have already shown promise to increase speed of kill (St. Leger et al., 1996). Studies on selection
of strain and compatibility of this entomopathogen with low doses of pesticides and other methods of control indicate considerable potential for integrated pest control (Rivera and Bustillo, 1996). Aspects like selection and development of strains for high virulence, higher growth potential and resistance to pesticides, need further investigation. Besides, search for cheaper substrate with growth potential for mass multiplication is also required from economic point of view for use in a developing country like India.

These collective studies advance our knowledge of fungal disease in general and *B. bassiana* in particular, and the findings will also be useful in further developing our ability to use this fungus in insect pest control.

Keeping in view the above facts, the present studies on entomogenous fungus, *B. bassiana* was undertaken for the control of pests of vegetable crops. For detailed studies three major pests of vegetable crops such as gram pod borer, *H. armigera*; tobacco caterpillar, *S. litura* and Bihar hairy caterpillar, *S. obliqua* were selected. These are polyphagous pests of national importance. Being polyphagous in nature, they cause economic damage to a number of crops. *H. armigera* and *S. litura* have gained more importance as they have developed resistance to recommended dosages of many pesticides (Lal, 1996; Singh, 1999).

*H. armigera* is widely distributed in the tropics, subtropics and warmer temperate regions of the world. It has been recorded feeding on about 181 plant species belonging to 45 families, 40 dicots and 5 monocots (Manjunath et al., 1989). It is in the recent past, this pest has assumed the status of serious pest in India. It is the most serious pest on tomato accounting for a yield loss ranging from 20-50 per cent (Mohan et al., 1996). Its main host is gram but it attacks castor, cotton, citrus, hemp, cowpea, groundnut, pulses,
okra, safflower, tobacco, peas etc. There can be 5-8 generations in a year depending upon environmental conditions and availability of hosts. Mainly chemical pesticides are used for controlling this pest. However, insecticides have failed to provide satisfactory control of *H. armigera* possibly due to rapid development of resistance to insecticides. Application of microbial control agents i.e. *B. thuringiensis* and nuclear polyhedrosis virus have proved very effective against this pest in field conditions (Jayaraj, 1986). *B. bassiana* has proved highly pathogenic in laboratory studies (Prasad et al., 1990; Sandhu et al., 1993).

*S. litura* popularly known as tobacco caterpillar is widely distributed in the old world tropics. It is a major pest of tobacco and tomato but it attacks banana, citrus, cauliflower, cabbage, colocasia, cowpea, gram, castor, cotton, hemp, maize, millets, mulberry, okra, peas, rice, sorghum, yam etc. In recent years, this pest has emerged as a dominant polyphagous pest (Rao and Dhingra, 1996). Chemical insecticide has been the main method of control in this case also. Nuclear polyhedrosis virus and *B.t.* are also found to give effective control of this pest on cotton, tobacco, black gram and cole crops (Jayaraj, 1986). Entomogenous fungal species of *Aspergillus, Beauveria, Metarhizium* and *Nomuraea* have been reported on this pest on several crops in India. Recently *B. bassiana* has also been tested against this pest and found very effective as revealed by laboratory studies (Prasad et al., 1989).

*S. obliqua* is widely distributed insect species in North India. It has attained serious proportions in tarai tract of Nainital and adjoining areas of Uttar Pradesh. In the recent past, this pest popularly known as Kamla destroyed crops worth crores of rupees in more than 100,000 hectares in Badaun district of U. P. (Hindustan Times, Oct. 25, 1983). Vevai (1969) studied the distribution and incidence of a hairy caterpillar on cabbage. It has
wide host range feeding on variety of food plants. Deshmukh et al. (1977) studied the host range of this pest. Varying degree of preference was observed on 75 plant species as compared to *Glycine max* Merr., besides recording 36 additional plant species as host of this insect. This species is major pest of pulses soybean, cabbage, cauliflower and other vegetables. The insect remains active throughout the year and migrates from crop to crop and weed to weed. Duration of lifecycle may vary, depending upon the climatic conditions. There may exist 6-8 generations in a year. The microbial control agents recorded on this insect are entomogenous fungus *Entomophthora* sp. and viruses (Ramakrishnan and Kumar, 1977). Recently Pandit and Samanta (1994) evaluated *B. bassiana* against this pest and reported 74-78 per cent mortality in the treated larvae.

However, no detailed studies have been conducted so far to explore the potential of *B. bassiana* in IPM of insect pests in vegetable crops. Therefore, studies were conducted on various aspects of this entomopathogen to explore the possibilities of using it in IPM of some of the important insect pests of vegetable crops. The present work was undertaken with the following objectives:

1. To evaluate and characterize different isolates of *B. bassiana* on the basis of their pathogenic potential to the test insects (*Helicoverpa armigera, Spodoptera litura* and *Spilosoma obliqua*).

2. To evaluate and characterize different isolates of *B. bassiana* on the basis of physiological and morphological parameters:
   
   (i) Effect of different synthetic nutrient media, pH and temperature on the growth of *B. bassiana*.

   (ii) Study of morphological characters of *B. bassiana*. 
3. To study the histopathology of *H. armigera*, *S. litura* and *S. obliqua* infected with *B. bassiana*.

4. To determine susceptibility of different larval instars of *H. armigera*, *S. litura* and *S. obliqua* to *B. bassiana* at different inoculum levels.

5. To study the influence of different host plants on the susceptibility of *H. armigera*, *S. litura* and *S. obliqua* to *B. bassiana*.

6. To determine the effect of different temperature on the susceptibility of *H. armigera*, *S. litura* and *S. obliqua* to *B. bassiana*.

7. To study the compatibility of *B. bassiana* with pesticides and bioagents:

   (i) Effect of pesticides on radial growth of *B. bassiana*.

   (ii) Effect of *B. bassiana* on some insect predators.

8. To study the host range of *B. bassiana*.

9. To screen different substrates for mass multiplication of *B. bassiana*. 