CHAPTER - II
REVIEW OF LITERATURE

The relationship that exists between entomogenous microorganisms on the one hand and the insect pests on the other was variable and provided interesting and promising problems with genuine significance of economic importance. One of the most important aspects of this natural relationship had been the biological control of insect pests affecting field crops and other plant products in storage. Among the microorganisms i.e. fungi, bacteria and viruses, fungi form major group as bioagents of insect pests. These fungi range from species that possess a high degree of parasitism to those which are little more than saprophytes. The highly parasitic species appear to be important in bringing a certain degree of natural control, which may under favourable and special situations amount to commercial control (Fawcett, 1944).


Among the entomogenous fungi, white muscardine fungus (*B. bassiana*) is the most widely prevalent species. It has worldwide distribution, parasitising many insect species and regarded as an important fungal agent for insect pest control (Ferron, 1978, 1981; Hall and Papierok, 1982; McCoy et
The history of *B. bassiana* induced diseases traces back with the report of diseased condition of silkworm, *Bombyx mori* L. The ancient Chinese manufacturers were affected by fungus attack upon the silkworm. The relationship between insect and fungi firmly established towards the beginning of 19th century in 1826 through the work of Kirby and Spence. Later, Bassi (1835) described the fungus of silkworm as *B. bassiana* which was commonly known as white muscardine. France and Italy suffered heavy losses of silkworm because of the disease caused by *B. bassiana*. During 1925 silk production in Italy, suffered losses of approximately 11 million pounds of cocoons annually (Bell, 1974). Later in United States, Shimer (1867) noticed white fungus on chinch bug (*Blissus leucopterus leucopterus* Say.), which was later identified as *B. globulifera* Speg. Forbes (1887) also observed the white fungus on chinch bug in Illinois and soon thereafter, the fungus was reported in Iowa, Kansas, Ohio and Minnesota. This entomopathogenic fungus was the most worked out. *B. globulifera* Speg recorded on this pest earlier by Shimer was later confirmed as a strain of *B. bassiana* by MacLeod (1954b).

At the end of 19th century and later on in the middle of 20th century, this microbial agent was evaluated in laboratory and in field trials against important crop pests with varying degree of success in various countries. Some of the early trials of historical importance were targeted against the major pests, i.e. *B. leucoperterus* and Colorado beetle, *Leptinotarsa decemlineata* Say (Lugger, 1888; Snow, 1890, 1895; Steinhaus, 1963; Roberts and Yendol, 1971). It had been studied more than a century and was soon identified as the most potential entomopathogen to be used as biocontrol agent. Research related with the use of commercial preparation of Boverin was started some 30-40 years ago in Russia against *L. decemlineata*. 
Later on this product was also used against codling moth, *Cydia* (*Carpocapsa*) *pomonella* L. (Sikura, 1974; Drozda and Lappa, 1974). Use of Boverin resulted in satisfactory control of this pest (Bajan *et al*., 1975a; Fargues, 1975).

The studies were also carried out, on the susceptibility to muscardine of several orchard pests (Lepidoptera) e.g. *Carpocapsa* sp., *C. (Laspeyresia) funebrana* Treits, *Zeuzera pyrina* L. (Sikura *et al*., 1974); on the physiological alterations in adults surviving infection (Sikura and Gritsaenko, 1973; Lappa and Goral, 1975) and on influence of climatic conditions on treatment efficiency (Lappa and Goral, 1975). *B. bassiana* infection was reported on a number of species of insect pests belonging to almost all the major orders throughout the world. But *B. bassiana* mycoses were common with certain taxonomic group, e.g. Coleoptera, Diptera, Hemiptera, Lepidoptera etc. (Roberts and Yendol, 1971; Cantwell, 1974; Burges, 1981). The major tested target pests include green house white fly, *Trialeurodes vaporariorum* Westwood on green house crops in Syria (Trefi, 1984); *L. decemlineata* on potatoes in United States (Campbell *et al*., 1985; Hajek *et al*., 1987; Anderson *et al*., 1988); *Scotinophora coarctata* F on rice in Philippines (Rombach *et al*., 1986a); *Nilaparvata lugens* Stal on rice in Korea (Rombach *et al*., 1986b; Aguda *et al*., 1987); pests of potato and apple (Tverdyukov *et al*., 1993); pests of cotton (Wright and Knauf, 1994); green house pests (Wright and Kennedy, 1996); sugarcane pests (Lecuona *et al*., 1996); stored grain pests (Bichoff and Reichmuth, 1997). These are some of the examples of pest control using *B. bassiana* as bioagent. That is why this entomopathogen is widely regarded as one of the most promising species known for potential development in to a practical insect biocontrol agent.

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 Veeresh, 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 \textit{B. bassiana} in IPM strategies for vegetable crops in our country.

\textit{B. bassiana} (Bals.) Vuill [Syn. \textit{B. tenella} (Sacc.) MacLeod; \textit{B. densa} (Link. per Pers.) Vuill; \textit{B. globullifera} (Speg) Pic \textit{B. effusa} (Beauv.) Vuill and] has been described in detail by Fassatiova et al. (1983). The genus \textit{Beauveria} has been thoroughly studied by MacLeod (1954b), who concluded that 14 species had been named which were characteristic of the genus but that only two were tenable. Sympodial development is the characteristic of the genus. Conidiogenous cells are with a swollen basal part terminating in a zigzag rachis. \textit{B. bassiana} species is parasitic to insect and has globose to sub-globose conidia (2.3 x 2.0-2.5 \textmu m). Conidiogenous structures form dense clusters which is the characteristic of the species (Samson, 1981).

Fungi have unique mode of infection. Unlike bacterial, viral or protozoans, entomogenous fungi need not be consumed by their hosts in order to be infective, instead, germinating fungal spores are able to grow directly through the insect’s cuticle. Infection, therefore, results from contact between a virulent infectious inoculum and a susceptible insect cuticle, germination, penetration of the germ tubes through the integument and finally spread of the pathogen through the haemocoel and the host tissues (Ferron, 1981). The exact site of infection depends on the fungus, the insect, the conditions, and the opportunity. The four main routes of infection are: through outer integument, digestive tract, tracheae and wounds. In case of \textit{B. bassiana}, infection through all the above routes has been observed histologically
(Madelin, 1963). But main and common route of infection is through the integument. The spore is germinated on the surface of cuticle. Penetration of hyphae through the cuticle involves both mechanical and enzymatic factors. St. Leger et al. (1986) has reported that there is involvement of cuticle degrading enzyme of entomopathogenic fungi during penetration of cuticle and the cuticular chain is degraded by chitinase and proteinase enzymes. Level of chitinolytic activity varied during the development of different entomopathogenic fungi. Undoubtedly, many enzymes are important determinants of virulence because they enable the pathogen to coexist with the changing metabolic processes associated with the host's diseased state (Hajek and St. Leger, 1994).

Once fungi invade the haemocoel, the host may be killed by some combination of mechanical damage produced by fungal growth, nutrient exhaustion, and toxicosis (Gillespie and Claydon, 1989). The relative importance of these mechanisms varies with the specific fungal isolate or host. B. bassiana is one of the fungi, which produce toxins. These include cyclic depsipeptides beauvericin, cyclotetradepsipeptides beauverolides and cyclodepsipeptide bassianolides (Roberts, 1981). The production of toxins by the fungi may be correlated with toxicosis and differential virulence of isolates against insects. After the death of the host, the fungus grows saprophytically through all tissues in competition with the intestinal bacterial flora. Oosporein, a red antibiotic pigment is also produced by B. bassiana which gives colour to the cadaver and curbs bacteria. The fungus grows outside the integument and produces conidophores when the atmosphere is humid (Ferron, 1977). The mode of action and process of disease development in entomogenous fungi are summarized below:
2.1. Evaluation and characterization of different isolates of *B. bassiana* on the basis of their pathogenic potential to the test insects (*Helicoverpa armigera*, *Spodoptera litura* and *Spilosoma obliqua*):

Different isolates of fungi may differ in their pathogenicity to an insect species indicating strain differences. According to Bell (1974), differences might be expected to result from one or more of these reasons: (i) The different fungus strains/isolates may have been collected from preferred or secondary hosts, (ii) The different culture media or storage methods may affect virulence, (iii) Optimum environmental factors for each strain are probably different, (iv) Length of time the fungus strain was maintained on an artificial medium.
There are reports showing that how virulence of an insect may be increased up to a certain level by repeated passage through a preferred host, or decreased through secondary host or by artificial culture methods (Steinhaus, 1963). Steinhaus (1960a) also found that longevity of spores was different for the three strains he tested. Similarly, the temperature requirements for different strains/isolates of fungi were found different (Illicheva, 1976; Sosa Gomez and Alves, 1985; Kleespies and Zimmermann, 1992; Fargues et al., 1992).

Several strains and isolates of *B. bassiana* have been recognized from diverse sources including different species of host insects, collection from different geographical regions, single spore isolation and mutant strains derived from genetic manipulation. Comparative studies have been done on virulence of different strains/isolates of *B. bassiana* towards a number of insect species belonging to various orders and pests of agricultural and forestry importance, the world over, so as to select the best possible isolates for control of important pests. Some of the important research findings on this aspect are mentioned here. Among the lepidopterous pests, Xu et al. (1986) tested two strains of *B. bassiana* against *Ostrinia furnacalis* Gn in field experiments in China and found strain 147 more effective than strain 9, in bringing down the pest population. In laboratory studies in India, Prasad et al. (1989,1990) evaluated the virulence of three different isolates of *B. bassiana* against larvae of *S. litura* and *H. armigera*. The authors found *B. bassiana* (BPT) isolate originally isolated from Bapta, most virulent for both the pests having lowest LC50 values (19.98 x 10^5 and 2.17 x 10^5 spores ml^-1 of suspension respectively).

Maniania (1991) evaluated different isolates of *B. bassiana* against the egg and neonate larvae of *Chilo partellus* Swinhoe on the
basis of results of the laboratory trials. Maniania (1992) in further bioassay tests, evaluated several strains of hyphomycetes fungi against 2nd instar larvae of *C. partelus* and 5th and 6th instar larvae of *Busseola fusca* Fuller and found *B. bassiana* isolate ICIPE 4 and *M. anisopliae* isolate ICIPE 18 and ICIPE 30, being the most effective.

Easwaramoorthy and Santhalakshmi (1993) found two isolates of *B. bassiana* equally effective in causing mortality (100%) in 3rd instar larvae of sugarcane root borer, *Emmalocera depressella* Swinhoe at concentration of $10^7$ spore ml$^{-1}$.

Leathers and Gupta (1993) evaluated five different isolates of *B. bassiana* against tent caterpillar, *Malacosoma americanum* F and reported that all five isolates at concentration of $4 \times 10^7$ spores ml$^{-1}$ were found able to kill the larvae of this pest. But isolate ARSEF 1775 and NRRL 20699 were more rapid acting than other isolates, as revealed from data on daily mortality. Data on production of cuticle degrading enzymes of these isolates revealed that rapidity of insect death was not related to the enzyme production in highly virulent isolates.

Fuentes and Carballo (1995) selected five isolates of *B. bassiana* as virulent isolates against *Plutella xylostella* L on the basis of determination of LC$_{50}$ values, percentage mortality and sporulation capacity. However, LC$_{50}$ value was minimum ($2.2 \times 10^5$ conidia ml$^{-1}$) for the isolate 447, which was identified as the most effective for microbial control of this pest as revealed in laboratory and greenhouse studies in Costa Rica. Selman *et al.* (1997) also found a number of isolates of *B. bassiana* significantly pathogenic to the mature larvae of *P. xylostella* with two isolates causing higher mortality at 20 and 24 °C.
temperatures.

Martinez et al. (1995) noticed differential susceptibility of *Diatraea saccharalis* Fab to different isolates of *B. bassiana* and *M. anisopliae* as a result of comparative bioassays. The results also demonstrated that eggs of this pest were not susceptible to isolates used in this study. Diaz and Lecuona (1995) evaluated the pathogenicity of 21 isolates of *B. bassiana* collected from different hosts and localities in Argentina and Brazil against *D. saccharalis*. Results showed that isolates originally isolated from this host were virulent against it while isolates from other insect species showed no virulence towards the larvae of *D. saccharalis*. Mortality rates ranged from 50 to 90 per cent, with an LT$_{50}$ of 2.1 to 8.4 days for different isolates. Conidial production and isoenzyme patterns were also determined but did not show clear relationship with the virulence factor. Lecuona et al. (1996) evaluated 31 isolates against the above pest and reported differential susceptibility with different isolates irrespective of their hosts and place of origin. Arcas et al. (1999) noted vast differences in mortality of *D. saccharalis*. One strain gave rise to 82.5 per cent mortality, whereas only 21.3 per cent mortality was observed with the other strain.

Several strains of *B. bassiana*, *M. anisopliae* and *P. farinosus* were identified as potential biological control agents of stored grain pests, *Plodia interpunctella* Hub and *Ephestia kuehniella* Zeller on the basis of assessment of different virulence factors (Bischoff and Reichmuth, 1997).

Besides Lepidopterous pests, large number of insect species belonging to different orders, have also been evaluated for their susceptibility to the different isolates of *B. bassiana*. The important
insect pests tested are, *Artipes floridonus* (McCoy et al., 1985); sweet potato weevil, *Cylas formicarius F* (Castineiras et al., 1986; Burdeos and Villacarlos, 1989); *Neochetina eichorniae* Warner (Haag and Boucias, 1991); boll weevil, *Anthonomus grandis* Boheman and pecan weevil, *Curculio caryae* Horn (Harrison et al., 1993); banana weevil, *Cosmopolites sordidus* German (Kaaya et al., 1993); Colorado potato beetle, *L. decemlineata* (Miranpuri and Khachatourians, 1995); coffee berry borer, *Hypothenemus hampei* Ferrari and stem borer, *Monochamus leuconotus* Pascoe (Varela and Morales, 1996; Schoeman and Schoeman, 1997); pest of *Rhus chinensis* Mill, *Ophrida spectabilis* (Yang et al., 1997); and stored grain pests, *Sitophilus zeamais* Motsch, *S. oryzae* L. and *Rhyzopertha dominica* Fab. (Adane et al., 1996; Moino and Alves, 1997) from the order Coleoptera; rice hoppers, *N. lugens*, *Nephotettix* sp. and *Sogatella furcifera* Horvath (Aguda et al., 1984; Li, 1986); aphid, *Diuraphis noxia* Mordvilko (Feng and Johnson, 1990; Vandenberg, 1996); *Thrips tabaci* Lindermann (Gindin et al., 1995); *Lygus lineolaris* Palisot de Beauvois (Steinkraus and Tugwell, 1997); pentatomid bugs, *Plautia stali* Scott and rice stem bug, *Tibraca limbativentris* Stal (Tsuda et al., 1997; Martins et al., 1997) from the order Homoptera; *Odontoterms obesus* Ramb and *O. wallonensis* Wasmann (Khan, 1992; Khan et al., 1993) and *Captotermes formosanus* Shiraki and *Heterotermes tenuis* Hagen 1858 (Jones et al., 1996 and Almeida et al., 1997) from Isoptera; locusts and grasshoppers (Nowierski et al., 1996) from Orthoptera; *Delia antiqua* Meigen (Poprawski et al., 1985) and *Liriomyza trifolii* Burges and *L. sativae* Blanchard (Bordat et al., 1988) from Diptera; formicids *Solenopsis invicta* Burem and *S. saevissima* F. R. Smith (Alves et al., 1988) ant, *Atta sexdens piiventris* Santschi, 1919 (Silva and Fleig, 1988) from Hymenoptera.
Scanning of literature shows that, selection of an optimal strain is crucial to successful control of a pest, which depends on the virulence, growth characteristics and the adaptability of the candidate organism to a production medium, which is economically viable. Virulence of fungi to insects is the most important characteristics in selection of candidate pathogen for pest control and is assessed by bioassay under standardized conditions. It was therefore, thought necessary to first evaluate pathogenic potential of different isolates with a view to select the most aggressive isolate for its incorporation in IPM of the test species.

2.2. Evaluation and characterization of different isolates of *B. bassiana* on the basis of physiological and morphological parameters:

2.2.1. Effect of different synthetic nutrient media, pH and temperature on the growth of *B. bassiana*:

An important factor in the success of microbial control is the potential for mass production in the laboratory. Among the parameters that influence fungus development and sporulation, the composition of the culture medium, temperature, light, pH and humidity are important.

*Effect of different synthetic nutrient media:*

For maintenance of culture in the laboratory and for small-scale production for experimental purposes a proper synthetic media is required for optimum growth, sporulation and viability of *B. bassiana*.

Significant variation in growth parameters has been noticed with respect to different media in different strain/isolates of *B. bassiana* as
revealed from literature. Samsinakova (1966), Bajan and Kmitova (1973) and Kmitowa (1978) evaluated the effect of various media on growth and sporulation of *B. bassiana* and other entomogenous fungi.

Barnes *et al.* (1975), Campbell *et al.* (1978) and Smith and Grula (1981) studied the nutritional requirements for conidial germination and hyphal growth of this fungus. Samsinakova *et al.* (1981) determined the optimal requirement of carbon and nitrogen sources. On the basis of these observations they developed a simple liquid medium which enhanced the production of conidia. Samsinakova and Kalalova (1983) also found that the optimization of the culture medium resulted in a multiple increase in the virulence of all types of isolates. Galani (1983) evaluated the effect of five different natural and synthetic media on growth of fungi *B. bassiana*, *P. farinosus* and *V. lecanii* and found wide variations on growth of different species on different nutrient media.

Filho *et al.* (1985) in laboratory studies in Brazil, evaluated different culture media for characterization of five isolates of *B. bassiana* pathogenic to cotton weevil, *A. grandis*. The growth parameter studied were conidial production and germination. Chase *et al.* (1986) screened different culture media and found oat meal agar based medium as most superior, for isolation of *B. bassiana* and *M. anisopliae*, the most commonly used fungi for biological control of agricultural pests as revealed from the studies conducted in USA.

Pandit and Som (1988) screened different synthetic and natural media in terms of fungal mass for maintenance and mass multiplication of *B. bassiana* strain pathogenic to insect pests of jute and mesta.

Motobayashi *et al.* (1988) determined the optimal nutritional
composition of medium, the carbon and nitrogen sources and coagulating agent for optimum production of conidia of *B. bassiana* isolate from the pine sawyer, *Monochamus alternatus* Hope. Sorbitol (1.2%) was found most effective carbon source while (0.8% dry milled pupae) along with peptone (1%) was found most effective source of nitrogen.

Gafurova (1988) established the correlation between morphological-cultural properties and the level of accumulation of biomass in strains of *Aspergillus, Beauveria, Verticillium* and *Mucor* isolated from insect pests of fruit crops in laboratory studies in USSR.

Rombach *et al.* (1988) and Rombach (1989) evaluated several simple liquid media with different concentrations of carbon and nitrogen sources. Optimal nutritional requirements for maximum production of conidia and hyphal bodies were determined. Sucrose and yeast extract were found most productive carbon and nitrogen sources, respectively. Lim *et al.* (1989) also studied the effect of different agar based artificial medium on conidial production of *B. bassiana* and Sabouraud dextrose agar was found to give best sporulation. Knudsen *et al.* (1990) demonstrated that Sabouraud’s broth was significantly superior over potato dextrose broth for biomass production of *B. bassiana*, an isolate of cereal aphid.

Paccola Meirelles and Azevedo (1990) studied effect of different media on linear growth and sporulation of different isolates of this pathogen to show the natural variability among the isolates. Significant differences were observed with respect to growth of different isolates in different media. Hegedus *et al.* (1990) also studied the effect of different synthetic liquid media on submerged conidiation of *B.*
bassiana and found N-acetyl-D-glucosamine to be better than yeast extract-peptone-glucose and other media tested. Grajek and Sobeza (1990) investigated the influence of various natural and synthetic media along with the water potential, on growth and sporulation of B. bassiana and V. lecanii.

This fungus was successfully cultured on PDA medium for laboratory and small-scale field application by Puzari et al. (1994). Later Mazumder et al. (1995) also found potato dextrose agar and broth medium to give sufficient production of conidia for the same isolate. Padin et al. (1996) compared two synthetic media to see their effect on conidial production of B. bassiana pathogenic to stored grain pests R. dominica, S. oryzae and Tribolium castaneum Herbst. Potato-dextrose-agar (PDA) was found to give higher production of conidia in comparison to Sabouraud yeast agar (SDA+Y). Among the six agar media tested by Yamashita and Satomi (1996), yeast manitol (0.5% yeast extract + 2% manitol) medium gave the best conidial production of B. bassiana. Arcas (1999) evaluated glucose yeast extract medium (10+10 g/l) for evaluation of biomass and conidial production.

Besides the above reports related with the work on B. bassiana, wide variations have been noticed in growth of other entomogenous fungi (M. anisopliae, Paecilomyces spp., V. lecanii, Hirsutella sp.) and their isolates with respect to different media, as reported by Galani (1983), Alves (1986), Sundra Babu et al. (1986), Frigo and Lucio De Azevedo (1986), Jin and Chang (1987), Bastos Cruz et al. (1987), Im et al. (1988), Machowicz Stefaniak (1988), Kleespies and Zimmermann (1992). Isolates of M. anisopliae were characterized on the basis of conidial production on different media (Alves, 1986; Frigo and Lucio De Azevedo, 1986; Kleespies and Zimmermann, 1992).
Effect of different initial pH values:

It was reported that the soil pH could affect the behavior of pathogenic fungi. Pospelov (1940) recorded that *M. anisopliae* infected proportionally more individuals of *Cleonus punctiventris* Germer in acid than in other soils. While Pyatritzki (1940) reported that the mortality of immature stages of this weevil was high due to *M. anisopliae* where the soil were acidic whereas, mortality due to *Sorospora* sp. were high where the soil were more alkaline. It was also reported that mineral fertilizers might alter the pH of soil to favour either one or the other of these two fungi.

Bell and Nebeker (1969) studied the pH tolerance of ten aquatic insect species and found that the lethal values ranged from 4.65 pH for mayflies to 1.50 pH for caddisflies. If these species of insects were found in habitats where the pH values were lower than could be tolerated by fungus pathogens, then the insects could escape the infection because of particular environmental conditions.

Falcon (1971) reported that pH of material deposited on the surface of plants may deactivate pathogens and even the pH of plant tissues itself, or the substances produced by plants, may alter the feeding insect’s susceptibility to infection by a pathogen. Campbell *et al.* (1980) also evaluated growth and reproduction responses of *B. bassiana* to various pH concentrations under laboratory conditions.

Galani (1988) also reported that the initial pH values influenced biomass production obtained at the end of growth in a stirred liquid medium in case of *V. lecanii, P. farinosus, B. bassiana* and *Agerita webbi* Fawcett. The highest dried biomass values were obtained when fungi were cultivated in media with initial pH ranging from 6 to 8.5.
Variation in biomass production was also noticed in different species of fungi at a particular pH value.

Im et al. (1988) determined the growth and sporulation of *Hirsutella* spp., *M. flavoviridae* and *N. rileyi* with various pH values of the medium and found pH of 5.0 – 8.0 suitable for growth of these pathogenic fungi.

Lingg and Donaldson (1981) evaluated the effect of various pH regimes of soil on survival of *B. bassiana* conidia and found that pH has little effect on conidia survival at different moisture content. However, out of the two strains evaluated, one was found more sensitive to pH 5 than the other. Samsinakova et al. (1981) determined the optimum pH value for one of the *B. bassiana* strain pathogenic to *L. decemlineata*.


Chen (1991) determined the range of pH 4-12 for growth of *P. cicadae* but pH 5-6 was preferred for maximum growth of the fungus. Kleespies and Zimmermann (1992) from their studies, concluded that pH was also an important production parameter for different strains of *M. anisopliae*. Different strains required different pH values ranging from basic to acid media for growth and sporulation. Shimazu and Sato (1996) investigated the effect of pH on growth of *B. bassiana*. The fungus was found to be able to grow well at high pH values of more than 10.
Effect of different temperature:

Temperature is one of the important factors affecting development and sporulation of entomopathogenic fungi. Scanning of literature revealed that, with regard to physical environment, temperature and humidity in relation to fungus pathogens have received more attention and testing than any of other climatic conditions. Ferron (1967) has considered temperature the most important single factor of the physical environment for the success of fungi in the field. Hall and Bell (1960, 1961) found variation in temperature ranges and optimums for hyphal growth in different species of entomogenous fungi including *B. bassiana*. The optima ranged from 24 °C - 33 °C and the limits for survival from 1 °C - 36 °C. In general, the limits for growth, range between 5 °C and 35 °C and the optima falls between 20 °C and 30 °C (Roberts and Yendol, 1971). Other important studies conducted on influence of temperature on entomogenous fungi including *B. bassiana* were by Bajan and Kmitova (1973), Roberts and Campbell (1977) and Hall and Papierok (1982).

According to Schaerffenberg (1963), the influence of temperature on insect infections with *Beauveria* and *Metarhizium* depends upon the specific requirements of the fungus. The genus *Beauveria* normally develops within a temperature range from 0 °C - 40 °C, *M. anisopliae* inhabited below 10 °C with an optimum between 25 °C - 30 °C.

Investigations of Walstad *et al.* (1970) for the two pathogenic fungi, *B. bassiana* and *M. anisopliae*, revealed that these fungi required temperatures between 15 °C and 35 °C for spore germination, hyphal
development and spore production. The optimum temperature for this series of developmental stages in the fungi were between 25°-30 °C. Spores of both fungi were killed near 50 °C. B. bassiana, however, kills overwintering insects in environments where temperatures average well below the optimum. The optimum temperature for growth of strains of this species was found reduced from south to north in the USSR. This may account for the bark beetle mortality noted with a finish isolate at temperatures below 14 °C and averaging considerably less than 10 °C as reported by Roberts and Yendol (1971). They also observed that the temperatures, which support only moderate growth of the fungus, are adequate for disease initiation and development.

Goral (1973), Isaenko et al. (1974), Illicheva et al. (1976) also studied the influence of temperature on different strains/isolates of B. bassiana. Sanzhimitupova and Kalvish (1979) reported that there was no conidial formation at 35 °C in B. bassiana. Campbell et al. (1980) also evaluated the effect of temperature on growth and development of B. bassiana, a strain of pecan weevil, C. caryae in laboratory studies in USA. Ferron (1981) reported optimum growth temperature near 23 ° - 25 °C for B. bassiana. Hussey and Tinsley (1981) reported that B. bassiana could be cultured at any temperature between 8 °C and 30 °C with the optimum temperature being 24 °C. Wen (1983) found 20 °C as suitable temperature for infection of B. bassiana.

Kuberappa and Jayaramaiah (1987) determined 20° - 30 °C as range of temperature for optimum growth, sporulation and development of B. bassiana strain pathogenic to silkworm larvae.

Fernandes et al. (1989) evaluated the effect of different
temperatures on conidiogenesis of *B. bassiana* infecting cadavers and adults of *Ceratoma arcuata* Olivier. These studies revealed that conidiogenesis of this isolate were limited by higher temperature and did not occur at 30 °C.

Lim *et al.* (1989) determined 25 °C as optimum temperature for growth and sporulation of *B. bassiana* isolate from *Helopeltis theobromae* Miller, although growth was observed at temperature between 5°-30 °C.

Hywel Jones and Gillespie (1990) demonstrated that the intra-specific differences occurred in the germination response of *B. bassiana* and *M. anisopliae* at temperature varying between 25 ° -30 °C.

Fargues *et al.* (1992) studied the effect of temperature on 31 isolates of *B. bassiana, B. brongniartii, M. anisopliae, M. flavoviride, N. rileyi* and *Paecilomyces fumosoroseus* (Weize) Brown and Smith and established that maximum growth occurred at 25 °C for 26 isolates but upper temperature limit varied from 27 ° - 37 °C. Inter-specific and intra-specific differences were also noticed. It was concluded that the temperature ranges established according to *in vitro* experiments might be useful for selecting candidate species/isolate of fungi for microbial control suited in particular climatic condition. Khan *et al.* (1993) evaluated the effect of temperature on mycelial growth, sporulation and viability of *B. bassiana* and *M. anisopliae* and their effect on pathogenicity to *Odontotermes brunneus* Hagen. Considerable variation was noticed in various growth parameters at different temperatures.

Itoh *et al.* (1994) determined 30 °C and 27.5 °C as optimum
temperature for mycelial growth and conidiation respectively, for \textit{B. bassiana}, an isolate from \textit{Chilo suppressalis} Walker. Junianto and Sri Sukamto (1995) determined the temperature ranges for growth and sporulation of 5 different isolates of \textit{B. bassiana} pathogenic to \textit{H. hampei} Ferr. Intra-specific differences among the isolates were found to exist in response to different temperature for growth and sporulation.

\text{Kim et al.} (1996) evaluated four strains of \textit{B. bassiana} for growth at 25 °C. They found conidial production to differ in different strains at this temperature. Strain F101, however, showed greatest conidial production and proved the best as compared to other strains at 25 °C.

\text{Fargues et al.} (1997) determined the effect of temperature on growth of 65 isolates of \textit{B. bassiana} from different geoclimatic and host origins. In general, wide temperature range of 8 °- 35 °C was detected. Significant differences were found among the isolates for requirement of optimum temperature for the growth and sporulation.

\text{Nelson et al.} (1997) found 23 °C as optimum temperature for conidial production of New Zealand strain of \textit{B. bassiana} after 3 weeks, as a result of their laboratory studies.

Temperature has been reported as one of the important factors influencing the growth of other potential fungal pathogens (\textit{M. anisopliae}, \textit{Paecilomyces} spp., etc.), as evidenced by the work of Zimmermann (1982), Alves \textit{et al.} (1984), Sundara Babu \textit{et al.} (1986), Jin and Chang (1987), Machowicz-Stefaniak (1988) and Chen (1991).

\text{Sosa Gomez and Alves} (1985) and \text{Kleespies and Zimmermann} (1992) characterized isolates of \textit{M. anisopliae} on the basis of their
optimal temperature requirements.

Scanning of literature revealed that the isolates of entomogenous fungi not only differ in their virulence but may also differ in their physiology and morphology. Therefore, the present studies were aimed at analyzing the different isolates of *B. bassiana* on the basis of physiological and morphological parameters also. The ultimate aim of this study was to select the best isolate on the basis of growth parameters.

### 2.2.2. Study of morphological characters of *B. bassiana*:

Isolates of entomogenous fungus can differ significantly with respect to pathogenicity, sporulation etc. as evidenced by number of publications (Steinhaus, 1963; Roberts and Yendol, 1971; Burges, 1981; Maniania, 1992; Leathers and Gupta, 1993; Selman et al., 1997). But intra-specific variation with respect to morphological characters of conidia and hyphae has not been studied by many workers. Variations are reported among isolates of *B. bassiana* based on microscopic morphology of conidia (Varela and Morales, 1996). Morphogenesis of *B. bassiana* strains was observed with scanning electron microscopy by Mesquita et al. (1996) for intra-specific comparison of strains. Kleespies and Zimmermann (1992) showed clear differences in size, morphology and physiology of the three strains of *M. anisopliae* when grown on liquid media. Conidiogenesis studies are, however, stated to be inadequate to identify strains (Ferron, 1981). The present studies aimed at exploring the possibilities to differentiate between the isolates of *B. bassiana* on the basis of morphology of conidia, which will be an easier and quick method for the identification of isolates.

### 2.3. Studies on the histopathology of *H. armigera, S. litura* and
S. obliqua infected with BB10 isolate of B. bassiana:

Histopathological studies are important for understanding the mode of action, disease development and structural changes of the host body due to pathogenesis caused by fungus. Paillot (1930) and Lefebvre (1934) studied the penetration and development of the fungus B. bassiana in the tissues of the silk worm, B. mori and corn borer, Pyrausta nubilalis Hb.

Histopathological evidence with respect to progression of disease by B. bassiana in larvae of gypsy moth, Lymantria dispar L has been provided by Wasti and Hartmann (1975). Atuahene and Doppelreiter (1982) did histopathological studies in larvae of Lamprosema lateritalis Humps. Penetration of cuticle and disease development was clearly observed in histopathological studies of B. bassiana in larvae of Indarbela sp. (Fasih and Srivastava, 1988). The authors observed penetration of cuticle, multiplication of fungal hyphae and spores, disintegration of hypodermis, gut wall and other internal structures in infected larva as compared to the transverse section passing through the body of a healthy larva. Su (1989) in histopathological examination of B. bassiana in C. formicarius after varying inoculation periods, showed timely progression of disease in internal body tissues and proved the pathogenicity of the fungus to its host pest. Hazarika and Puzari (1990) reported the progression of disease due to B. bassiana in rice hispa, Dicladispa armigera Olivier. Similar observations were also recorded by Kaaya et al. (1993) in larvae of banana weevil, C. sordidus.

Bidochka et al. (1993) in their microscopic examination of infected cadavers of lygus bug, Lygus spp. L showed massive disintegration of internal muscle tissues due to parasitization of B.
Sohaf et al. (1993) studied the progression of disease in internal tissues of silk worm larvae infected with this pathogen. The hyphal growth was observed after 48 hr and hyphal emergence outside the integument was noticed after 132 hr.

Ramlee et al. (1996) conducted histopathological studies in 4\textsuperscript{th} instar larvae of psychid *Metisa plana* Walker to determine the mode of *B. bassiana* infection and studied in detail the periodical development of fungus in the internal tissues of the infected larva.

Histopathological studies have been done to understand the mode of infection and disease development in other entomopathogenic fungi also. Srivastava and Mathur (1970) observed disease development in larvae of *Chilo zonellus* Swinhoe infected with *B. densa*. Yadava et al. (1979) did histopathological studies in *Sesamia inferens* Walker infected with *B. brongniartii*. Penetration of hyphae through cuticle and its progressive development in the tissues of body was clearly observed. Wasti et al. (1980) also established the clear progression of disease in larvae of gypsy moth, *L. dispar* L., infected with *M. anisopliae* and *Paecilomyces fumosoroseus* (Weize) Brown and Smith by histological examination. Vicentini and Magalhaes (1996) did similar studies in 5\textsuperscript{th} instar nymphs of grasshopper, *Rhammatocerus schistocercoides* Rehn infected with the fungus, *Metarhizium flavoviridae* Gams and Rozsypal.

Histopathological studies were therefore, thought important to establish the infectivity of *B. bassiana* in the test species. These investigations are also aimed at studying the histopathological events and changes occurring in the haemolymph and internal body structures after the fungus gain entry in to the body.
2.4. Studies on the susceptibility of different larval instars of *H. armigera*, *S. litura* and *S. obliqua* to BB10 isolate of *B. bassiana* at different inoculum levels:

Besides the major factors i.e. requirement of temperature, humidity, pH etc., certain special factors have also been reported to influence the infection of entomopathogenic fungi including *B. bassiana*. Different stages of host, age and inoculum level of pathogen are also some of the factors in host-pathogen relationship, influencing the incidence of disease and its further progress.

Gopalkrishnan and Narayanan (1990), Prasad *et al.* (1990) and Sandhu *et al.* (1993) determined the susceptibility of different larval instars of *H. armigera* on the basis of percentage mortality, LC$_{50}$ and LT$_{50}$ values. Susceptibility of different instars was also compared on different inoculum levels. Results of these studies showed that in general, 1$^{st}$ instar larvae of *H. armigera* were most susceptible followed by 2$^{nd}$ and 3$^{rd}$ instars, while 4$^{th}$ and 5$^{th}$ instars were significantly less susceptible as compared on the basis of percentage mortality. LC$_{50}$ and LT$_{50}$ values also varied. Young stages have less LC$_{50}$ and LT$_{50}$ values as compared to older larvae. Similar results were reported in larval instars of certain noctuids bioassayed for their susceptibility to *B. bassiana*, *N. rileyi* and *P. fumosoroseus* (Gardner and Noblet, 1978; Ignoffo *et al.*, 1978; Fargues and Rodriguez Rueda, 1980).

Carruthers *et al.* (1985) reported that *in vivo* incubation period of *B. bassiana* mycosis of the European corn borer, *Ostrinia nubilalis* Hub varied in response to inoculum level of initial exposure and the age of host larvae. Shorter incubation period was reported in case of initial stages with high dosages. However, studies made by Feng *et al.* (1985)
reported that larvae of 1\textsuperscript{st} and 5\textsuperscript{th} instar were most susceptible and 4\textsuperscript{th} instar is most resistant in case of \textit{O. nubilalis} as compared on the basis of LC\textsubscript{50} levels.

In several findings reported earlier also, inoculum level and age of the larvae were the factors found affecting the susceptibility of host insect. For example, in case of larvae of \textit{Malacosoma neustria} L. (Machowicz-Stefaniak, 1978); \textit{L. decemlineata} (Samsinakova \textit{et al.}, 1981; Fargues \textit{et al.}, 1991); \textit{B. mori} (Pitta \textit{et al.}, 1990); \textit{S. litura} (Prasad \textit{et al.}, 1989); \textit{Chilo infuscatellus} Snellen (Sivasankaran \textit{et al.}, 1990); \textit{Eutectona machaeralis} Walker and \textit{Hyblaea puera} Cramer (Agarwal \textit{et al.}, 1985; Rajak \textit{et al.}, 1993); \textit{S. litura} (Jayanthi and Padmavathamma, 1996); \textit{Phthorimaea operculella} Zeller (Hafez \textit{et al.}, 1997).

Walstead and Anderson (1971) found that the mortality was a function of the quantity of inoculum applied. Results of several workers on dosage-mortality relationship of \textit{B. bassiana} for different species of insect pests confirm this observation. Some of the recent findings on important pests are that of, sugarcane shoot borer, \textit{C. infuscatellus} (Easwaramoorthy and Santhalakshmi, 1987); lesser corn stalk borer (Gilreath and Funderburk, 1987); fig moth, \textit{Ephestia cautella} W. (Jassim \textit{et al.}, 1988); \textit{Chalcodermus bimaculatus} (Quintela \textit{et al.}, 1990); different species of aphids (Feng and Jhonson, 1990; Feng \textit{et al.}, 1990; Dorschner \textit{et al.}, 1991); chinch bug, \textit{B. leucopterus leucopterus} (Krueger \textit{et al.}, 1991); rice hispa, \textit{D. armigera} (Puzari \textit{et al.}, 1994); termite, \textit{Nasutitermes} sp. (Melagodi and Veiga, 1995); grass hopper, \textit{Melanoplus sanguinipes} Fab (Inglis \textit{et al.}, 1996); Russian wheat aphid, \textit{D. noxia} (Vandenberg, 1996); macadamia pest, \textit{Ecdytolopha torticornis} Meyrick (Gonzalez \textit{et al.}, 1996); \textit{S. zeamais} (Adane \textit{et al.}, 1996);
migratory grasshopper, *M. sanguinipes* Fab (Jeffs *et al.*, 1997).

On the basis of literature cited above it was thought pertinent to study the age-dose-mortality relationship with respect to infection of *B. bassiana* in the test species. The studies were mainly aimed at standardization of doses for different stages and to identify the most vulnerable stage of insect at which to strike control economically.

2.5. **Studies on the influence of different host plants on the susceptibility of *H. armigera, S. litura* and *S. obliqua* to BB10 isolate of *B. bassiana*:

Variations among host plants may also affect the relationship between herbivores and their natural enemies. Price *et al.* (1980) reviewed the roles host plants play in the relationship between herbivores and parasitoids. Development of fungal pathogens may also be indirectly affected by host food besides the host itself. For example, leaf cutter bees, *Megachile rotundata* reared on natural provisions were generally less susceptible to *Ascosphaera aggregata* as compared with bees reared on artificial provisions (Goettel *et al.*, 1993).

Most of the studies related with the trophic-level interactions between host plant-pest-pathogen, deals with the effect of different host plant species on development of fungal pathogens within herbivorous insects. Similar level of mortality was noticed among the larvae of *L. decemlineata* when reared on different levels of glycoalkaloids (Costa and Gaugler, 1989). In contrast, Hare and Andreadis (1983) found that the food plant species most suitable for *L. decemlineata* survival in the field produced the larvae that were least susceptible to *B. bassiana*. Larvae reared in the greenhouse also varied
in susceptibility to measured doses of *B. bassiana* conidia in the laboratory. Ramoska and Todd (1985) observed that adult chinch bug, *B. leucopterus leucopterus* inoculated with *B. bassiana* demonstrated higher mortality when fed wheat, barley or artificial diet compared with corn or sorghum. Results also revealed that only few cadavers of chinch bugs that had eaten corn or sorghum produced conidia, demonstrating an additional inhibitory effect of these foods, presumably resulting from fungistatic secondary plant chemicals. There are few reports of *in vitro* studies showing that the addition of glycoalkaloids or a diversity of plant extracts to media can inhibit growth of *B. bassiana* (Raghavaiah and Jayaramaiah, 1987; Costa and Gaugler, 1989).

Studies of Alves *et al.* (1990) and Macedo *et al.* (1990) indicated that *D. saccharalis* larvae differed in susceptibility to *B. bassiana* when reared on different host plants. Sporulation was found higher in *N. rileyi* killed larvae of *H. armigera* when larvae had eaten plants optimal for growth (Gopalkrishnan and Narayanan, 1989). Whereas time-mortality responses of *N. rileyi* to another pest, *Spodoptera littoralis* Boisd, did not differ for 3rd instar larvae reared on four readily utilized plant species as reported by Fargues and Maniania (1992). In another study, Hajek and St. Leger (1994) reared polyphagous larvae of *L. dispar* on leaves of five different host plants and found similar mortality level and disease incubation times for the fungus *Entomophaga maimaiga* Humber, Shimazu and Soper. However, the total duration from infection to host death was increased for the slow growing larvae on less preferred host *Acer rubrum* L.

Reports of the above studies show that disease development is linked with the plant nutritional quality ultimately affecting the pest in
various ways and which in turn affects parasitizing fungi. From the review of literature it is observed that very few studies have been done on the susceptibility of polyphagus insects to entomogenous fungi as influenced by different host plants. However, it is an important factor to be taken into consideration while using entomogenous fungi in IPM of insect pests infesting variety of important crops (as in case of *H. armigera, S. litura* and *S. obliqua* the test species selected for the present studies). It is with this point of view, it was thought desirable to study the influence of host plants on the susceptibility of test species to *B. bassiana*.

2.6. Studies on the effect of different temperature on the susceptibility of *H. armigera, S. litura* and *S. obliqua* to BB10 isolate of *B. bassiana*:

Temperature is considered to be the most important factor of the physical environment for the success of fungi in the field. However, effect of temperature is not always clear, since it affects germination of spores, penetration of the insect by hyphae and the general pathogenic development that follow. Temperature dependent response of growth and development of *B. bassiana* has been demonstrated by many workers. These studies dealt with *in vitro* development of fungus. Carruthers et al. (1985) considered *in vivo* temperature-dependent development as one of the several stimulus-response of key importance in evaluating disease dynamics under field conditions and one of the first data requirements necessary for the construction of quantitative epizootiological models.

There are number of reports showing temperature ranges and optimum temperature-requirements for fungus, *B. bassiana* in relation
to its pathogenicity to host as reported earlier. Muller-Kogler (1942) reported that at 5 °C, *B. bassiana* could not infect larvae of *Bupalus piniarius* L. Whereas, it succeeded, though slowly, at 8° C. In case of larvae of *L. decemlineata*, *B. bassiana* declined in infectivity below 6° C and *B. densa* below 10 °C and *M. anisopliae* below 15 °C (Schaerffernberg, 1957b). Similarly temperature ranges were found to differ for different entomopathogenic fungi and in different isolates of the same species including *B. bassiana* (Steinhaus, 1963; Mohammad *et al.*, 1977; Barson, 1977; Fargues and Rodriguez Rueda, 1980).

Barson (1977) in his studies with *B. bassiana*, found maximum mortality of *S. scolytus* larvae, at 25 °C which decreased at 30 °C. Doberski (1981b) reported higher levels of mortality when experimental temperatures were increased from 2° to 20 °C. The most significant aspect of these results was that, given sufficient time, larvae of *S. scolytus* are killed at temperatures down to 2 °C, if inoculated with low temperature, isolates of *B. bassiana*. These findings also show the possibilities of using this fungus to control pests in temperate regions where temperature goes down to such extent.

Riba and Marcandier (1984) observed that at low temperature of 15 °C, *B. bassiana* was able to infect larvae of *O. nubilalis* but the LT$_{50}$ was much increased (11.3 days) while at 25 °C, LT$_{50}$ was 6.6 days only. This shows that temperature played an important role in causing early infection and mortality, necessary to get an effective control of the pest. Similarly, in laboratory tests, *B. bassiana* showed high rate of infectivity with lower LT$_{50}$ values at 30 °C in case of pest *Corythucha ciliata* Say in Italy (Marletto and Arzone, 1985). Carruthers *et al.*
(1985) reported that the in vivo incubation period of B. bassiana mycosis of O. nubilalis varied in response to temperature of incubation.

According to report of Houle et al. (1987), temperature seems to have variable effect on host-parasite relationship of Scolytus multistriatus Marsham and its pathogenic fungus B. bassiana. Results revealed significant differences between and within the 3 temperature regimes with respect to mortality caused by the different isolates of this pathogen. Higher percentage mortality was caused by B. bassiana to the curculionid, Hylobius abietis L. at higher temperature of above 20 °C i.e. at 23 °C as compared to 13 °C and 33 °C as reported by Wegensteiner and Fohrer (1988). Similar results were also reported by Diehl-Fleig et al. (1988) in case of infection of B. bassiana and M. anisopliae against sauva ant A. sexdens where medium lethal time was shorter under high temperature (23-28 °C). Nearly similar results were obtained in case of infection of this pathogen in larvae of D. saccharalis (Lecuona and Alves, 1988).

Fernandes et al. (1989) noticed higher infectivity of B. bassiana at 30 °C in case of Ceratoma arcuata Olivier in Brazil. Krueger et al. (1991) also reported high mortality of chinch bug, B. leucopterus leucopterus in soil was at 30 °C as compared at 20 °C. B. bassiana was reported to cause high mortality at temperature above 20 °C in Argentine stem weevil, Listronotus bonariensis (Goh et al., 1991); in Rhizotrogus majalis Razoumowsky (Krueger et al., 1991); in Ips typographus L. (Wegensteiner, 1992); in teak defoliator, H. puera and teak skeletonizer, E. machaeralis (Rajak et al., 1993); in H. armigera (Sandhu et al., 1993); in black tipped sawfly, Acantholyda posticalis posticalis Matsumura (Kim Hyeong Jun et al., 1996); in diamond back
moth, *P. xylostella* (Selman *et al.*, 1997).

Huwenjin *et al.* (1996) reported that pathogenicity of *B. bassiana* to nymphs and adults of coreid, *Riptortus linearis* Fab decreased with increase in temperature at 25 °C and above. In contrast Yasuda *et al.* (1997) found that, *B. bassiana* infected adults of the sweet potato weevil, *C. formicarius* irrespective of temperature between 15 °C and 31 °C.

It is revealed from the scanning of literature that temperature is an important determinant in host-pathogen relationship with respect to infection of entomopathogenic fungi. Present studies were therefore, thought to be undertaken to optimize the temperature of incubation for the maximum infectivity of the pathogen.

### 2.7. Studies on the compatibility of BB10 isolate of *B. bassiana* with pesticides and bioagents:

#### 2.7.1. Effect of pesticides on the radial growth of *B. bassiana*:

Scanning of literature regarding the effect of chemicals on insect pathogenic fungi revealed that the entomogenous fungi are more frequently adversely affected by pesticides, particularly fungicides. However, some accounts of beneficial combinations have also been reported. For effective use of fungal pathogens in IPM system, a moderately accurate estimate of compatibility of chemical pesticides and pathogen is necessary.

Comprehensive reviews by Benz (1971), Roberts and Campbell (1977), Osbarne and Boucias (1985), Rivera and Bustillo (1996) reveal that fungi are adversely affected by many pesticides in commercial use
for pest and disease control.

Several pesticides (fungicides, insecticides, nematicides etc.) were found to be harmful to various entomogenous fungi including, *B. bassiana*, *Entomophthora* spp., *M. anisopliae*, *N. rileyi*, *Paecilomyces* spp., *V. lecanii*, etc. (Hall and Dunn, 1959; Cadatal and Gabriel, 1970; Wilding, 1972; Ignoffo *et al.*, 1975; Soper *et al.*, 1974; Easwaramoorthy and Jayaraj, 1977; Kai *et al.*, 1990; Moorhouse *et al.*, 1992; Vyas *et al.*, 1990, 1992; Narahara *et al.*, 1992).

Various published reports indicate that the growth and germination of *B. bassiana* was affected by different fungicides (Olmert and Kenneth, 1974; Tedders, 1981; Galani, 1980; Clark *et al.*, 1982; Loria *et al.*, 1983; Machowicz-Stefaniak, 1983; Saito, 1984; Osborne and Boucias, 1985; Machowicz-Stefaniak, 1985; Sampson *et al.*, 1986; Kuberappa and Jayaramaiah, 1988; Coremans - Pelseneer and Tillemans, 1988; Vanninen and Hokkanen, 1988; Aguda *et al.*, 1988; Paccola-Meirelles and Azevego, 1990; Calderon *et al.*, 1991; Hasan *et al.*, 1994; Feng and Chiang, 1995; Wright and Kennedy, 1996; Rivera and Bustillo, 1996; Masarrat Haseeb and Srivastava, 1996; Lee Sang Myeong *et al.*, 1996).

Benomyl, zineb, mancozeb were among the most inhibitory fungicides against *B. bassiana* while fungicides compatible with *B. bassiana* in *in vitro* tests included sulphur, dinocap, copperoxychloride, daconil etc. (Jaques and Morris, 1981).

The insecticides were also found to affect the growth and development of *B. bassiana*. The insecticides affected the pathogen to the lesser or greater extent and some causing even synergistic effect (Ten, 1961; Urs *et al.*, 1967; Olmert and Kenneth, 1974; Galani, 1980;
Clark et al., 1982; Anderson and Roberts, 1983; Aguda et al., 1984; Foschi and Grassi, 1985; Coremans- Pelseneer and Tillemans, 1988; Vanninen and Hokkanen, 1988; Aguda et al., 1988; Vilas Boas and Alves, 1988; Su, 1988; Anderson et al., 1989; Dirlbek et al., 1989; Vyas et al., 1990; Calderon et al., 1991; Kurogi et al., 1993; Bajan et al., 1995; Rivera and Bustillo, 1996; Wright and Kennedy, 1996; Lee Sang Myeong et al., 1996; Quintela and McCoy, 1997).

Besides the toxicity of pesticides, there are other factors responsible for varying degrees of reduction caused by different insecticides to entomopathogens (Moorhouse et al., 1992). Anderson and Roberts (1983) reported formulation of insecticides as one of the factors responsible for variation in toxicity of different insecticides. Insecticides in formulation of wettable powder were found less toxic to the fungal growth than the EC formulation. Some of the insecticides i.e. endosulfan, piperonyl, butoxide EC and permethrin EC caused significant reduction in growth while carbaryl WP was similar to control. In addition intra-specific variation in pesticide sensitivity of V. lecanii and M. anisopliae has also been observed.

Plant based pesticides are currently being used for control of insect pests as an alternative to chemical pesticides. There are numerous evidences available on compatibility and incompatibility of muscardine fungi with pesticides as stated above but little work has been done with natural pesticides. Antifungal properties of plant extracts/ plant products on plant pathogenic and zoopathogenic fungi have been reported by Odikodze (1960), Appleton (1975), Tansey and Appleton (1975), Pushpa (1978), Krishna Prasad et al. (1979), Misra and Dixit (1979), Rajput (1982), Aguda et al. (1986), Raghavaiah and Jayaramaiah (1987, 1990) Costa and Gaugler (1989) and Vega et al.
Raghavaiah and Jayaramaiah (1987) found growth and germination of *B. bassiana* affected by essential oils of medicinal plants i.e. lemongrass (*Citronella citratus* (D.C) Stapf), palmorosa grass (*C. maritinii* var. *motia* (Roxb.) Stapf.), citronella (*C. nardus* (L.) Rendle), *Eucalyptus globulifera* and *E. citriadora* Hook.

Most of the neem products were, however, found compatible with *B. bassiana* either not affecting or affecting to a lesser extent, the growth and germination of this pathogen including, RD-9 Replin (Vyas *et al.*, 1992), and aqueous neem seed extract (Arturo *et al.*, 1997).

Evaluation of seed kernel extracts of *Azadirachta indica* Juss, *Pongamia pinnata* (L) Pierre and whole extract of three other plants and vegetable oils from sunflower, safflower, groundnut, rape seed, sesame, coconut and cotton seed was done against *N. rileyi*. None of these oils were found detrimental (Devi and Prasad, 1996).

Rivera and Bustillo (1997) in their recent review have discussed in detail, the effect of agrochemicals on *B. bassiana* and *M. anisopliae*. This review included studies on the effect of plant extracts, their nature and mode of action.

### 2.7.2. Effect of *B. bassiana* on some insect predators:

The interaction of entomogenous fungi with other biological agents acting on pest species is a major concern for successful use of this pathogen in IPM system. Interference and competition with other pathogens and with parasites and predacious arthropods could reduce net effectiveness, perhaps, making introduction of a pathogen impractical (Jaques and Morris, 1981).
There are evidences indicating that some entomopathogenic fungi may influence populations of parasitic and predacious species, but in general, much less than do chemical insecticides. But the work on this important aspect is poorly documented. Many predators have been found parasitized by entomogenous fungi (Bell, 1974; Cartwright et al., 1984; Iperti, 1986; Joques and Morris, 1981; Magalhaes et al., 1988; James and Lighthart, 1994; Todorova et al., 1996; Jayanthi and Padmavathamma, 1996).

Cartwright et al. (1984) reported infection of \( B. \) \( bassiana \) in over-wintering beetles of \( Coccinella septempunctata \) L. Castineiras and Calderon (1985) evaluated the susceptibility of an ant predator \( Pheidole megacephala \) Fab to this fungus as to find out the possibility of using the bioagents in IPM of \( C. \) \( formicarius \). Iperti (1986) also reported over-wintering coccinellids were often found infected and killed by a fungus, \( B. \) \( bassiana \) as reported in a review on biology of predacious coccinellids in France.

Pavlyushin and Krasavina (1986) in the pathogenicity tests, found three predators of aphids i.e. \( Chrysopa carnea \) (\( Chrysoperla carnea \) Steph.), \( C. \) \( sinica \) and \( Cycloneda limbifer \) Casey, susceptible to the infection of \( B. \) \( bassiana \) and other fungi. Donegan and Lighthart (1989) also studied the effect of stress factors on susceptibility of neuropteran predator, \( C. \) \( carnea \) to \( B. \) \( bassiana \).

Beetle, \( Rhizophagus grandis \) Gyll predator of scolytid, \( Dendroctonus micans \) Kug was also found susceptible to the infection of \( B. \) \( bassiana \) (Anonymous, 1988). Magalhaes et al. (1988) tested the pathogenicity of \( B. \) \( bassiana \) to the coccinellid predators, \( Coleomegilla maculata \) De Geer and \( Eriopis connexa \) Germer and found them
susceptible to the infection of this fungus. Hemptinne (1988) also reported mortality of overwintering coccinellid predators, *Propylea quatuordecimpunctata* L and *C. septempunctata* due to infection of *B. bassiana*.

James and Lighthart (1994) evaluated the susceptibility of convergent lady beetle, *Hippodamia convergens* Guerin-Menville to four commonly used fungi for pest control, i.e., *B. bassiana*, *M. anisopliae*, *N. rileyi* and *P. fumosoroseus* (Weize) Brown and Smith. Except *N. rileyi* the beetle was found susceptible to other three fungal species. Steerberg et al. (1995) recorded infection of entomogenous fungi *B. bassiana*, *P. farinosus*, *V. lecanii* as well as *Zoophthora radicans* (Brefeld) Batko and *Z. philonthi* in overwintering carabidae and staphylinidae beetles from agricultural fields in Denmark. The incidence of *B. bassiana* was even recorded to the extent of epidemic level in staphylinid beetles of *Anotylus rugosus* and *Gyrohypnus angustus*. *B. bassiana* was the predominant fungus isolated from these predators.

Todorova et al. (1996) as a result of their studies, found the development of coccinellid predator, *Coleomegilla maculata lengi* Timberlake affected, when the larvae were fed food inoculated with fungus, *B. bassiana*.

There are reports of parasitoids i.e. *Apenteles flavipes* Cam and a parasitoid of coffee berry borer, *Cephalonomia stephanoderis* Betrem also being affected by the infection of fungi *M. anisopliae* and *B. bassiana* (Folegatti et al., 1990; Reyes Aristizabal, 1995).

Recently, in India, the negative impact of *B. bassiana* was also reported on predators, *C. septempunctata* L., *Menochilus sexmaculatus*
Fab and *C. carnea* Steph (Jayanthi and Padmavathamma, 1996).

With a view to incorporate BB10 isolate of *B. bassiana* in IPM of test species, it was thought necessary to study its compatibility with pesticides and some predatory beetles of common occurrence in agriculture fields in our country.

### 2.8. Studies on the host range of BB10 isolate of *B. bassiana*:

*B. bassiana* has wide host range and has been recorded from temperate and tropical regions. Occurrence of *B. bassiana* and its potential and commercial use for a number of host insect has been reported in various reviews published by Steinhaus (1963), Pramer (1965), Roberts and Yendol (1971), Bell (1974), Ramakrishnan and Kumar (1977), Burges (1981), Gillespie and Claydon (1989), Xu (1991) and Feng *et al.* (1994). These workers reported *B. bassiana* pathogenic to the insect species belonging to different taxonomic group. However, *B. bassiana* mycoses has been reported to be more common with certain taxonomic group, e.g. Lepidoptera (moth and butterflies, particularly larvae); Hemiptera (particularly aphids, white flies, scale insects, leaf hoppers and plant hoppers); Coleoptera (beetles and weevils of a wide variety); Diptera (mosquitoes and flies); Orthoptera (locusts and grasshoppers); Hymenoptera (bees and ants); Isoptera (termites).

There is a long list of insect species recorded as host of this pathogen by various workers. Most of the work includes individual host records of pests, some related with particular group of insects and others with pest complex of particular crops. The important work related with the studies on host range of *B. bassiana* included record of, white muscardine disease of over 80 different insect species (Pramer,
1965), rice pests as host of this pathogen (Rao, 1975; Nayak and Srivastava, 1979 and Ambethgar, 1997); lepidopterous larvae injurious to crops in Argentine Republic as hosts of this pathogen (Fresa, 1981), white grubs as host of this pathogen (Jayaramaiah and Veeresh, 1983), hopper species (Aguda et al., 1984), lepidopterous noctuid hosts of *B. bassiana* (Maniania and Fargues, 1984; Napiorkowska Kowalik, 1986), various pests as hosts of *B. bassiana* in Cuba (Diaz Sanchez and Grillo Ravelo, 1986), four species of trunk boring insects (Fan et al., 1986), three pests of coconut (Gallego and Gallego, 1988), entomophagous arthropods as host of this fungus (Magalhaes et al., 1988), two species of coleoptera (Sankaran et al., 1989), pests of medicinal and agricultural importance as host of isolates of *B. bassiana* (Messias, 1989), isolates from various host pests including Argentine weevil (Barker et al., 1991), four species of insect pests (Keller, 1991), grass hoppers as hosts (Muralirangan and Sanjayan, 1992), various forest pests (Sandhu et al., 1993), greenhouse pests (white fly and thrips), pests of potato and apple (Tverdyukov et al., 1993), reed stem borers as host of *B. bassiana* in China (Li, 1993), cotton pests (Wright and Knauf, 1994), pests of oil palm (Siti Ramlah, 1994), pests of stored grains (Moino and Alves, 1995, 1997), cereal aphids (Feng et al., 1990; Dromph et al., 1996), pests of mango in India (Masarrat Haseeb and Srivastava, 1996), *B. bassiana* as pathogen of crop pests in Taiwan (Hou, 1997), *B. bassiana* isolates from various host pests and their pathogenicity to pests of *R. chinensis* (Yang Shiz Hang et al., 1997), besides several other insect species recorded as hosts of *B. bassiana* from time to time by various research workers.

A bioagent with wide host range is considered economically more acceptable in IPM of crops with a variety of host insects or when
applied in inter-cropping or multi-cropping systems. Such bioagents can exert control simultaneously over a variety of insect pests of the same crop or different crops. It is also revealed from the literature cited above that different strain or isolate may differ in their host range. It was therefore, thought desirable to study the host range of BB10 isolate of *B. bassiana*.

### 2.9. Screening of different substrates for mass multiplication of BB10 isolate of *B. bassiana*:

Successful use of entomopathogenic fungi as microbial control agents of insects and their development into mycoinsecticides depends largely on their mass production. Samsinakova *et al.* (1981) stated that the principal conditions to be considered in the production of fungal pathogens for commercial use are: (i) Selection of a strain capable of producing large quantity of sufficiently virulent spores, (ii) Selection of medium enhancing an optimal production of conidia, (iii) Easy production at low costs, (iv) Well designed formulation procedures and conditions of storage.


In general, Deuteromycetes fungi including *B. bassiana* produce infectious aerial conidia on solid media and infectious blastospores in liquid culture, formed by hyphal budding. Ferron (1978), Aregger *et al.* (1989) found blastospores more susceptible to environmental
conditions and consequently less persistent than conidia. However, production of blastospores in submerged culture fermentation is regarded as simplest and more productive (Kral and Neubauer, 1953; Samsinakova, 1966; Ferron, 1974; Campbell et al., 1978; Smith and Grula, 1981; Samsinakova et al., 1981; Fogal et al., 1986; Rombach et al., 1988; Rombach, 1989; Lane et al., 1991; Miao et al., 1993; Feng et al., 1994). However, more persistent conidia produced on solid medium are more suitable to control the pest. A wide variety of solid substrates have been evaluated for mass culture of *B. bassiana* by various researchers.

McCoy and Carver (1941) described the mass production of *B. bassiana* on cereals. Various natural substrates such as potatoes, sugarbeet, soybean chunks, grains etc. have been used for conidial production of entomogenous fungi (Kral and Neubauer, 1956; Telenga, 1958; Aquino et al., 1977; Kononova, 1978). On the basis of these results, "Boverin" was introduced for production in the USSR.

Mass production of *B. bassiana* using surface culture has been in progress for many years in the USSR and Peoples Republic of China (Ferron, 1981). On the basis of results of these findings formulated products of conidia were commercially made available, eg. Boverin (UDSSR), Boverol and Boverosil (CSFR) (Ferron, 1981; Aregger, 1992). Ferron (1981) has extensively reviewed the work on mass production of *B. bassiana* and *M. anisopliae* in Russia, Brazil and other countries during the period of 1960-1980. In Peoples Republic of China, conidia are produced on wheat bran, rice powder, compost or ground corn stalks in flat trays, glass crocks or shallow out door pits (Hussey and Tinsley, 1981).
Goettel (1984) has described a technique for producing several fungi including *B. bassiana*, on bran in autoclavable alkathene bags. Barlett and Jaronski (1988) produced *B. bassiana* on solid substrate such as heat sterilized grains mixed with wheat bran to fermentation in flat trays. Filho *et al.* (1988) and Pandit and Som (1988) also utilized solid substrates of grains and soybean chunks for production of *B. bassiana*. Alves and Pereira (1989) developed a technique for production of *B. bassiana* and *M. anisopliae* utilizing rice as substrate in plastic bags and plastic trays. An isolate from banana root borer, was cultured on natural media of rice and soaked beans (Filho *et al.*, 1989). Grajek and Sobezak (1990) presented data for *B. bassiana* and *V. lecanii* on conidial production in natural media i.e. wheat bran, sugarbeet and wood shavings.

Aregger (1992) evaluated grains of barley for the conidial production of *B. brongniartii*. The highest yield varied between $1 \times 10^8$ and $2 \times 10^9$ conidia/g. Gupta *et al.* (1994) evaluated rice as solid substrate for the mass production of this pathogen in polypropylene bags and enamel trays, with maximum conidial yield of $10^8$/g grain after 25 days of incubation.

Ibrahim and Low (1993) evaluated loose solid media i.e. grated coconut flesh, mature papaya fruit, tapioca root, sweet potato tubers and rice grains for conidial production of *B. bassiana*. As a result, paddy, sweet potato and tapioca were found to give significantly better yield of conidia.

Mazumder *et al.* (1995) screened some solid substrate which were locally available industrial wastes i.e. press mud, bagacillo, sand, sawdust and rice husk for mass production of *B. bassiana*. Highest
inoculum density was reported in rice husk supplemented with dextrose. More recently, Calderon et al. (1995) produced *B. bassiana* by solid state fermentation using sugarcane bagasse with type B cane molasses and torula yeast in a glass reactor as substrate.

Vilas Boas et al. (1996) evaluated some low cost products commonly found in North East Brazil as an alternative to rice for mass multiplication of *B. bassiana* and *M. anisopliae*. Media used were cowpea, *Phaseolus vulgaris*, *P. lunatus* and *Sorghum*, which were cooked and sterilized. Rice still proved superior for *B. bassiana* while *P. vulgaris* L and *P. lunatus* L proved better for *M. anisopliae*.

Borges et al. (1997) evaluated 10-70 per cent distillery must for mass culture of *B. bassiana* and found 40 per cent distillery must to give the best results. In another experiment he evaluated 40 per cent distillery must supplemented with coffee husk and soybean flour and found this combination to give better sporulation than standard medium of molasses (20/lt.) and torula yeast (10/lt.).

Nelson et al. (1997) evaluated the effect of solid medium (rice, wheat and barley) with additives, glucose and yeast extract, on conidial production of *B. bassiana* and *M. anisopliae*, strains from New Zealand. Rice still proved the better medium in comparison to wheat and barley. Narvaez et al. (1997) compared the conidial production of different isolates of *B. bassiana* and *M. anisopliae* on substrate of rice and on coffee berry borer cadavers. Recently, Puzari et al. (1998) developed a culture medium comprising substrate of rice husk, saw dust and rice bran (72:25:100) for mass culture of a isolate of *B. bassiana* from *D. armigera*. They observed that spore production depends on the isolate and interaction with media of its cultivation.
There are number of reports on use of solid media including grains and industrial by products for production of other valuable and closely related fungi. *M. anisopliae* another important entomopathogenic fungus has been produced on mass scale on different substrates i.e. on rice in autoclavable polyethylene bags in Russia and Brazil (Ferron, 1981); on rice and bean culture (Bastos et al., 1985); on rice and potato-dextrose agar (Sosa Gomez and Alves, 1985); on rice media (Frigo and Lucio De Azevedo, 1986; Alves and Pereira, 1989; Gupta et al., 1994; Almeida et al., 1997); on rice bran or rice bran-husk mixtures (Dorta et al., 1990); on coconut water (Dangar et al., 1991). Another fungus *V. lecanii* has also been produced on mass scale on different grains i.e. sorghum, maize, pearl millet etc. (Easwaramoorthy and Jayaraj, 1977); on bran (Goettel, 1984); on millet grain solid (Xia et al., 1995).

From the review of literature, it is understand that various fungi and their isolates differ in their production parameters with respect to different media. It was therefore, thought necessary to test different cheaper and easily available substrates (including grains and various wastes) for culture of BB10 isolate of *B. bassiana* (the best isolate selected on the basis of its virulence and conidial productivity). Studies were aimed at finding out simple, easily available and economically viable substrates for mass multiplication of this isolate of *B. bassiana*. 