REVIEW OF LITERATURE
Chapter II

REVIEW OF LITERATURE

The literature on rice hispa, its pathology as caused by fungal pathogens and development of mycoinsecticides is reviewed under the following heads:

2.1. Rice hispa and its distribution

2.2. Entomopathogenic fungi in pest control.

2.3. Entomomycoses in rice ecosystem

2.4. Biological parameters of *B. bassiana*

2.5. Pathogenicity of Entomopathogenic fungi

2.6. Mass production of *B. bassiana*

2.7. Development of mycoinsecticides formulation of *B. bassiana*

2.8. Field efficacy of entomopathogenic fungi against rice pests

2.9. Impact of *B. bassiana* to non-target organisms.
2.1 Rice hispa and its distribution

The rice hispa *Dicladispa armigera* (Olivier 1808) (Coleoptera: Chrysomelidae) occurs in South East Asia and Africa, and is one of the major pests of rice in many rice growing states of India (Deka and Hazarika 1996, Palaszek *et al.* 2002, Islam *et al.* 2004, Hazarika and Puzari 2005) more particularly in Assam, West Bengal, Andhra Pradesh and Tamil Nadu, and also in some parts of North Eastern, Eastern and Central the country. In recent years incidence of rice hispa is on increase, particularly in the wet season. Both grub and adult beetles feed on leaves by scraping the surface of leaf and leave white streaks as mark of feeding. Pest population build up is favored by rainy and cloudy days. In case of severe damage entire crop bears whitish look and leaves soon dry and turn yellow. Though rice is considered as the principal host of rice hispa, many species of Poaceae, such as *oryza rufipogon* Griff (Dutta and Hazarika 1993) and *Panicum dichotomiflorum* Michx and *Brachiaria ramosa* (L.) (Sharma and Verma 2011) serves as alternate hosts. It was first reported from Barisal in 1905 followed by June 1912 from Sylhet and in September1913 from Noakhali. Since then its occurrence either as a major or sporadic pest was reported. Besides wide-spread out break was common in many districts of Bangladesh, Assam, Bihar, West Bengal and Madhya Pradesh, Andhra Pradesh (Hazarika *et al* 1998, Pasalu 2011). In Northern India after the rains, the prevailing high humidity and intermittent bright sunshine (BSH) seem to have favored hispa development in epidemic proportions. The maximum temperature during day never exceeded beyond 20°C, while the minimum temperature ranged between 17-20°C until the end of
January, decline in the beetle population occurred synchronously with the rise in temperature and the lowering of the relative humidity. It is major pest in Bihar, West Bengal and Madhya Pradesh, Andhra Pradesh. It has a long record of sporadic outbreak in Bangladesh, India and Assam. The intensity of outbreak seems to increase following the large scale adoption of high yielding varieties and their association of varities and their associated production technologies. In India both rice crop, kharif and rabi are subject to sporadic out breaks of D. armigera and may be severally attacked (Karim 1986). High temperature and high humidity a conductive to the buildup of rice hispa proportion in kharif (Islam and Husan 1999) suggested the warm conditioned of South. Rice hispa the patented pest is endemic to Sivasagar, Lakhimpur, Nalbari, Barpetam Cachar and Karimganj district of Assam. It is abundant during Sali and Ahu season than in Boro (Hazarika and Puzari 1990). In the year 2010-11, rice hispa, caseworm, leaf folder has been reported from 44,531 hectares of Sali paddy cultivation which cause heavy losses of rice production. (AssamTribune 2010). Adult beetles appear in February and the population gradually increased until June when the larvae as well as adult caused heavy damage to young plants. The population declines after August (Hazarika and Puzari 1997). Adult beetles in small number can be controlled from rice crop fields up to September-October (Pathak 1975). Many factors have been reported to influence the incidence of rice hispa in rice field. Rao et al. (1971) reported that top dressing high level of nitrogenous fertilizers during period of pest abundance resulted in greater susceptibility of the crop to the pest. Adults beetls prefer to feed and oviposite on rice crops in the vegetative stage (plants from 25-60 days old), after this the leaf become silicified and less succulent perhaps
offering mechanical resistance to feeding (Rao et al. 1971). Basu and Banerjee (1957) showed that plants just after transplanting were more prone to damage than those of other stages. Date of planting influence the ultimate damage caused by the hispa. Rao, P.S. 1975 reported that the July planting suffered in considerable infestation where as August suffered severe damage with less chance of recovery. In North eastern India, rainfall was reported to have a negative effect on the activity of hispa (Ghose et al. 1960). Heavy rainfall in July followed by unusually low rainfall in August and September was characteristics of epidemics. Rice hispa is endemic to low laying swampy areas of Assam particularly in Jorhat and Sivasagar districs, in which it feeds and breeds on wild rice (Oryza ruffipogon) and some other grass during non-rice seasons and this pest undergo reproductive hibernation from October to November till early February, before the onset of monsoon and rise in temperature during late February –March the next year (Hazarika and Puzari 1997). It has 6-7 gernerations per year in Assam, Bangladesh, Hyderabad and WestBengal (Hazarika and Puzari 1990, Karim 1986, Khan and Murthy 1954, Sen and Chakravarty 1970). Sharma and Verma (2011) observed that in the Kangra valley of Himachal Pradesh adult beetles emergence is influenced by the onset of monsoon rains. During the early monsoon rains of May and June, there is a sudden emergence of winter diapausing adults and feed on the alternate hosts, particularly Andropogon gayanus Kunth, Sorghum halepense(L.) Pers., and C. dactylon.
2.1.1 Rice hispa damage and yield loss

The symptom of rice hispa damage are characterized by the presence of parallel white streaks on the upper leaf surface and burning of field in patches. Adults scrape off the parenchymatous tissues from the upper surface of leaf. Larvae are the leaf miners residing inside mines. Eggs are deposited singly by partially inserting them into the tissues from the lower leaf epidermis (Hazarika et al. 2005). *D. armigera* attacks all four rice crops: *aus* (summer rice), transplanted *aman* (monsoon rice), deepwater rice, and *boro* (winter rice) in Bangladesh. In India, both rice crops, *kharif* and *rabi*, are subjected to sporadic outbreaks of *D. armigera* and may be severely attacked. This pest causes extensive damage to the vegetative stage of plant resulting 35-65% loss in yield throughout Assam (Rajek et al. 1986, Hazarika and Dutta 1991, Dutta and Hazarika 1992, Deka and Hazarika 1996). In India it causes yield loss of 28% (Nath and Dutta 1997) between 20-30% in Nepal (Dhaliwal et al. 1998) and 40-50% in deep water rice in Bangladesh (Islam 1989). However it may be as high as 100% in rice transplanted post flood in Assam (Hazarika and Puzari 2005). Annual yield loss estimates of 20% in Andhra Pradesh (Pasalu and Tewari 1989), up to 50% in Bihar (Agarwala 1955), 17-20% in West Bengal, India (Pasalu and Tewari 1989), 14-62% in Bangladesh (Islam, 1973), 40-50% in south China (Fey 1925), have been reported. Estimates suggest a wide variation in yield losses between 10 and 65% (average 20%) in affected areas (Islam, 1973, Pasalu and Tewari 1989). When the area (thousands of hectares) affected is considered, production losses amount to substantial losses in the quantity of food grains. However, a study with population density of 64
adults /m² indicated that yield losses of 4.5, 3.1, 2.2 and 2.1 kg per day resulted increased to 22.1, 19.6, 19.9 and 27.5% loss, when infested at the booting, tillering, flowering and milk stage, respectively (Gyawali et al. 1998). There has been a decline in its incidence in Andhra Pradesh since 1985, except sporadic incidences. Hispa population was found to be severe at KhawalaTrind and Dhanotu villages of Kangra district of Himachal Pradesh with yield loss of 34.3% (Kaushik et al. 2007). The damage caused by rice hispa reduced plant height, tiller number of grain per panicle and ultimately, grain yield. Affected deep water rice become vulnerable to rising flood water level (Karim 1986, Islam 1997, 1999). More recent studies by (Haque and Islam 2001) indicate that under high densities of rice hispa, the leaf growth of young plants (with greater than 20% leaf damage) is severally retarded. Estimates suggested a wide variation in yield loss between 10 and 60% with an average 20% is affected in ahu (Karim 1986, Islam 1999). Economic threshold level (ETL) of 10 percent leaf damage in the vegetative stage or 2-3 freshly damaged leaves per hill has been reported (Kaushik et al. 2007) and 2 adults or 2 damaged leaf per hill (Directorate of Agriculture, Assam 2004). The economic threshold level suggested for India is 4 adults/hill up the mid-tillering stage and 2 adults/hill after mid-tillering (Nath and Dutta 1997). The economic threshold level practiced in Bangladesh is 5 adults/hill or 5 grubs/tiller or 35% infested leaves (Anon. 1999).
2.1.2 Management of rice hispa

The concept of Integrated Pest Management (IPM) for rice hispa has been documented by several workers (Hazarika and Puzari 1997, Islam 1973, Karim 1986, Dutta and Hazarika 1992, Sehagal et al. 2001). It is now widely accepted as sound and long term pest management strategy. The IPM of rice hispa combines such components as plant resistance, cultural practices, biological control and chemical insecticide application (Sundara Babu et al. 1998). However management of rice hispa, using synthetic insecticides has been the most common method all over the world in the past several years. The insecticides suggested to control rice hispa are chloropyriphos 20 EC, Monocrotophos 36 SL, Quinolphos 25 EC Fenitrothion 100 EC Neem Seed Kernal extract (5%) (Chellaiah and Subramanian 1974, Hidaka et al. 2000, Verma and Gupta 2001, Hazarika and Puzari 1997). The use of chemical pesticides causing a serious problem of environmental pollution have promoted the scientist to seek alternatives to chemical control. Consequently a new series of bioinsecticides is gaining rapid prominence due to their high bio-efficiency against many crop pests including D.armigera (Sharma and Rahman 2010) and relatively safe to the environment as compared to the synthetic pesticides (Karim and Haque 1999). Field observations suggested that the highest plant is not a major limiting factor to rice hispa population growth. The winter relative humidity and also river flooding as mortality factor (CABI Report 2005). The pest infestation is highest during the hot and humid summer compared to the cooler (Sehagal et al. 2001). The highest mortality was obtained by using biopesticides Azacel (@0.03%) and Larvocel (@ 0.1% caused
86.36% reduction of hispa population after 5 and 10 days of treatment (Bhattacharjee and Ray 2010). WeiBing et al. (2008) reported that Beauveria bassiana and Metarhizium anisopliae @ 1.5 and 1.05 conidia /ha effectively controlled the Red spider mite population 85.8%-88.00% for M.anisopliae and 77.95-85.75% for B.bassiana with relative humidity 95 % and daily mean temperature 23.6°C for both. Hazarika and Puzari (1995) found that the egg, larva, pupa and adult were effectively controlled by B.bassiana both in laboratory and field conditions during summer, autumn and spring seasons. Baitha et al. (1993) reported different neem products viz., neem cake, NSKE, and neem oil were effective against D.armigera and reduced the leaf damage. Spraying of B.bassiana and neem seed oil effectively controlled the pest population of rice hispa in field (Hazarika and Puzari 1997). Chakraborti (1998, 2003) found neem products singly and in combined with chemical insecticides resulted significant reduction in pest population build up. Bora and Hazarika 2001 observed that neem oil proved to be effective antifeedent and antiovipositional compound against D.armigera. Jena and Dani 2011 reported that clothianidin and bifenthrion were the most effective insecticides in controlling the adults as well as grub population of rice hispa D.armigera.

2.2 Entomopathogenic fungi in pest control

The main drivers behind the push for mycoinsecticides are the need for specific agents as components of integrated pest management (IPM) programmes due to concerns over chemical residues on human health and the environment, the time-consuming and costly process of registration of new pesticides among other concerns.
Biological control agents as entomopathogens (beneficial fungi which infect insects) can be used as a component of IPM of *D. armigera*. It appears that 700 (Charnley and St-Leger 1991) to 750 species (Carruthers and Soper 1987) of entomopathogenic fungi are pathogenic to insects but only a dozen species have been exploited for insect control. Epizotic of fungal pathogens are common in nature (Carruthers and Soper 1987). All insect orders are more or less susceptible to fungal diseases (Hajek and St-Leger 1994). Several fungal species particularly members of hyphomycetes (Deuteromycotina and entomophthorals) (Zygomycotina), from about 100 genera are pathogenic to insects (Rombach *et al.* 1987). Many species under Hypomycetes have a wide host range and are pathogenic to different orders of insect (Zimmerman 1993). They are potentially the most versatile biological control agents, because they have wide host ranges, infect at different age and stages of their hosts and often cause natural epizootics. The best-known entomopathogen in this group is white muscardine fungus, *Beauveria bassiana* (Bals.) Vuill.(Deuteromycotina:Hyphomycetes) which has great potential in biological pest control programme (Pingel and Lewis 1996, Hazarika and Puzari 1989, 1990, 1991, 1995, 1997, Haraprasad *et al.* 2001).

### 2.2.1 The white muscardine fungus, *B. bassiana*

Historical records indicate that the diseases in honey bees were known from the time of Aristotle. However, Reaumur (1726) reported that first probable record of an insect pathogen, *Cordyceps* fungus, found on noctuid. Birth of insect pathology occurred in 19th century when the first infectious disease in silkworm *Bombyx mori* L. as caused by a fungus was reported by Agastino Bassi in (1835). This was later
identified as *B. bassiana* (Gillespie and Claydon 1989). Bassi in 1835 demonstrated the contagious and pathogenic nature of the fungus infecting silkworm, *Bombyx mori* (Anisworth 1956). Bassi not only elucidated the etiology of a contagious disease for the first time, but also implied that this infectious disease could be used to control insect (Ignoffo and Anderson 1979). The disease caused by *B. bassiana* is known as white muscardine. The name white muscardine has been derived from cookies produced in Italy, which are fully covered with sugar giving a whitish appearance. This was close in appearance to the white covering of the fungus recorded in silkworm resulting in naming this as white muscardine disease of silkworm (Weisser 1977). Italians also first called it *mal del segno* (the disease having a sign) or calcino (white powder like calcium). Therefore white mummified body of an insect is referred as white muscardine disease. However, Steinhus (1949) started that the term muscardine has been derived from the Italian word *muscardinio*, which means scented fruits, foliage or flowers.

Again coming back to the history of naming the white muscardine fungus *B. bassiana* that Balsamo (1835) described and named the fungus *Botrytis* (*Beauveria*) *bassiana*. The genus *Beauveria* was established in 1912 by Vuillemin and described *bassiana* as type species. Agastino Bassi’s (1835) discovery not only laid the foundation for microbial pest control, but also significantly influenced the work of Louis Pasteur, Robert Koch and other pioneers of microbiology (Ainsworth 1956, Porter 1973, Van Driesche and Bellows 1996). Bassi himself recognized the potential of using organisms such as *Beauveria bassiana* to control insect pests (Bassi 1836, cit.
in Van Driesche and Bellows 1996) and by the early 20th century, field trials had been conducted with *B. bassiana*, *B. brongniartii* (Sacc.) Petch and *Metarhizium anisopliae* (Metschn.) Sorokin. Today, over 100 years later, there are no known reports of significant adverse effects that can be attributed to the use of these organisms in biocontrol.


### 2.2.2 Mode of infection of *B. bassiana*

Unlike other microbial pathogens (bacteria and viruses) this fungus does not require ingestion for infection. The infective propagule of *B. bassiana* is a spore, usually conidium which germinates into a short germ tube giving out small swellings called appressoria. These structures secrete a complex of cuticle degrading enzymes
viz., chitinase, proteases and lipases, which are capable of hydrolyzing corresponding cuticular constituent’s viz., chitin, protein and lipids (Gabriel 1968, Samsinokova et al. 1981, McCoy et al. 1988, Bidochka and Khchatourians 1991, Charnley and St.Leger 1991, St.Leger et al. 1986). This facilitated the germ tube to invade hemocoel and fat bodies. The invading vegetative hyphae consume the contents of haemolymph for its growth and metamorphosis. On exhaustion of the haemolymph content, the host insect becomes moribund and the fungus sporulates after death of the host. Death of the insect is often due to a combination of the action of fungal toxins, physical obstruction of blood circulation, nutrient depletion and/or invasion of organs. After the host has died hyphae usually emerge from the cadaver and under suitable abiotic conditions, conidia are produced on the exterior of the host. These are then dispersed by wind or water (McCoy et al. 1988). Most of the toxins produced by microbial pathogens which have so far been identified are peptides, but they vary greatly in terms of structure, toxicity and specificity. The toxins produced by B. bassiana are known as beauvericin which is highly toxic to insects. The cuticle is the first barrier to infection by fungi. Hence, rapid and direct penetration of the cuticle is important for virulence (Pekrul and Grula 1979). The insect procuticle is primarily chitin fibrils embedded in a protein matrix and penetration appeared to involve both mechanical and enzymatic degradation. Penetration is a stage of infection where specificity may be determined since many pathogens are virulent after being injected into the haemolymph of an otherwise non-susceptible host. Virulent isolates, however, had 10-17 times more endochitinase activity and 15-28 times more exochitinase activity than a virulent isolates (El-Sayed et al. 1993). The development of mycosis involves a series of systematic events namely-
adhesion and germination of the spore on the insects’ cuticle, penetration into the haemocel and development of the fungus, which results in death of an insect. The cuticular penetration is both mechanical and enzymatic action. Development after invading the haemocel is by budding, which produces a network of vegetative hyphae (Pekrul and Grula 1979). The invading hyphae consume the contents of haemolymph for growth and development (Boucias and Latge 1988). According to McInnis and Domnas (1973), insect infection by fungal pathogens occurs through four successive steps: opportunity of contact between the host and fungal propagules, penetrations of cuticle or gut wall with subsequent invasion of host tissue and organs, and finally death of host by blockage of the gut, thachea, circulatory systems, histolysis, toxin production and or physiological starvation. After death of the host, saprophytic development of fungus is necessary for the completion of pathogenic cycle. Penetration of this fungus through the insect cuticle may also enhance the cuticular degrading enzymes such as proteinase, chitinase and lipase (St.Leger et al. 1986, Moraes et al. 2003, Fang et al. 2005).

2.3 Entomomycoses in rice ecosystem

Rice provides a favourable environment for exploitation of fungi as mycoinsecticides. Fungal entomopathogens occurring on insect pests have been documented for diverse agro-ecosystems in many parts of the world both for the purpose of receiving potentially useful isolates and gaining an insight into the ecology of entomopathogenic fungi (Evans 1988, Samson and Rombach 1988). Similar records on entomopathogenic mycoses of rice insects have been made mostly from Japan.

2.3.1 Occurrence of B. bassiana on rice pests

The fungus B. bassiana causes devastating epizotics on a wide range of rice insects in different countries. Many a times spectacular examples of mycoses have been reported by several scientists (Ferron 1978, 1985, Brady 1981, Gillepse 1988, Ferron et al. 1991). Insect pests reported as being susceptible to this promising pathogen both under natural and experimental studies. In China, Korea, Philippines, Tiwan and India, B. bassiana has successfully been utilised for controlling several coleopteran, hemipteran and lepidopteran pests of rice both under natural and experimental studies. Ferron (1978), Fuxa (1987), Agarwal (1990) reported its effectiveness in controlling many pests. Similarly the fungus was reported from several Lepidopteran pests, such as, Scirpophaga incertulas Walker, (Padmanaban 1993, Danger 1998). Sesamia inferens Walker, (Yadava et al. 1979, Rao 1975) Cnaphalocrosis medinalis (Aguda and Rombach 1987, Rao 1975, Rao 1989, Ambethgar 1997, 2002, 2003); rice case worm and tiger moth Baruah et al. 2003; rice skippers, Parnara mathias (Nayak et al. 1978); Hazarika and Puzari (2004) reviewed occurrence and use of B.bassiana for management of rice pests. Among the coleopterans, Dicladispa armigera Olivier (Coleoptera: Chrysomelidae) (Puzari and Hazarika 1992, 1994, Hazarika and Puzari
1995, Puzari et al. 1994, Hazarika and Puzari 1997) had been successfully managed by use of this fungus. Several investigators reported the occurrence of *B. bassiana* in hemipteran pests like *Leptocorisa varicornis* Fabricious, *Niloparvata lugens* Stal. *Nephotettix nigropictus* Distant (Srinivas and Pasalu 1990, Ambethgar 1991, 1997, Loc and Chi 2005, Sivasundaram et al. 2008). Some investigators reported that *B. bassiana* has control some minor pests, which become a major one such as rice blue beetle, *Leptispa pygmaea* Baly (Karthikeyan et al. 2010); rice weevil *Sitophilus oryzae* (Sheeba et al. 2001). This fungus can be cost effectively mass-produced using locally available substrates. Therefore, this pathogen is considered as key component for experimentation of biological control of rice hispa pest and other pests of rice.

2.4 Biological parameters of *B. bassiana*

2.4.1 Effect of temperatures on biological parameters of *B. bassiana*

Temperature plays a vital role in growth and development of fungus. The growth of entomopathogenic fungus at different temperatures was studied by various authors. Walstad et al. (1970) reported that temperatures between 15 and 35°C were required for growth of mycelium, sporulation, and germination, the optimum being between 25 and 30°C. The thermal death point of spores was near 50°C for 10 minutes in water. According to Yendol and Hamlen (1973) germination of conidia of *B. bassiana* required water–saturated atmosphere and the optimal temperatures for growth was in the range of 25-30°C. Sanzhimitupova and Kalvish (1979) found that the minimum temperatures required for spore germination and mycellial growth of
B. bassiana was 5°C, while that for sporulation was 10°C. The fungus could be cultured at any temperature between 8 and 30°C, the optimum being 24°C (Hussey and Tinsley 1981, Butt et al. 1994, Sharma et al. 2010). According to Ferron (1981), the optimal growth temperature for growth of most of the strains is 27-28°C and requires a relative humidity of at least 92% to germinate. In field condition low level of infection of B. bassiana on adult of D. armigera may be attributed due to the high temperatures (35±2°C) that prevailed in rice field (Puzari and Hazarika 1994). Hart and Macleod (1955) found that the conidial germination of B. bassiana occurred between 10-28°C. According to Macleord et al. (1966), the medium development of most entomopathogenic fungi occurred at temperatures between 20-30°C. The highest sporulation of most isolates at 25°C temperature was reported by Parker et al. (2003). Shimazu (2004) revealed that B. bassiana could recover and grow at 25°C after exposure to higher temperature even if the fungus did not grow during high temperature exposures. Julio et al. (2004) also reported high radial growth and sporulation of Nomuraea releyi at temperature between 22-26°C. Sebastian (2006) reported radial growth was significantly affected by temperature, growth media and their interaction and optimum temperature for radial growth were between 24°C to 30°C but no growth was found at 36°C temperature. Dimbi Susan et al. (2007) who reported 28°C to be the best temperature for germination of B. bassiana and over 80% conidia germination at 20, 25 & 30°C with 24 hr incubation. The highest growth rate of B. bassiana at 25°C temperature with an exception for the two isolates which was exhibited highest growth rate at 20°C (Abdulhai et al. 2010).
2.5 Pathogenicity of entomopathogenic fungi

The pathogenicity of the fungal pathogen to specific host insects of a particular age should be established as stipulated by Koch’s postulates (Robert and Yendol 1971). Different investigators have established different experimental procedures for testing the pathogenicity of fungi with the host insect. The various technique includes exposing insect for a stipulated time on the fungal culture (Praserthphon, 1963), tropical application of conidial suspension (Robets and Yendol 1971) or dipping the insect in conidial suspension (Rombach et al. 1986b, Aguda et al. 1986). However, variation of infectivity was observed among different isolates/strains of this fungus (Deva Prasad 1989). Besides this pathogenicity depends upon the composition of nutrient media in which it is cultured and the insect immune system (Huxham 1989, Farugues et al. 1994, Baruah et al. 2003, Phukan 2003).

2.5.1 Bioassay of entomopathogenic fungi

For successful achievement for controlling of insect pest through entomopathogenic fungi in bio-control programme, bioassay is must. For quantifying the relative potency of any entomopathogenic species and strains, fungal inoculum load and time required to 50 % mortality of test populations is required (Burges and Thompson 1971). The relative potency/virulency is measured in terms of LC$_{50}$ by conducting bioassay tests with $B.bassiana$. Many such studies are available for example, LC$_{50}$ for $Plutella xylostella$ and $Trichopulsia ni$ were 0.025 and 0.27 per cent, respectively (Ignoffo et al. 1979). First instar larvae of $T. ni$ when exposed to a leaf disc
surface treated with viable conidia of this fungus the LC$_{50}$ is 139 conidia/mm$^2$ (Ignoffo et al. 1982). ED$_{50}$ of this fungus against Cnapholocrosis medinalis was determined as $7.4 \times 10^3$ conidia/ml (Aguda and Rombach 1987). Gillespie (1986) found that LC$_{50}$ value of different isolates of Metarhizium anisopliae Metsch. (Deuteromycotina: Hypomycetes) was $1.80 \times 10^6$ conidia/ml after four days of incubation at 25$^0$C. A formulation containing $50 \times 10^9$ conidia/g of B.bassiana produced 95 % mortality of larvae Ostrinia furnacalis with an LC$_{50}$ of $7.3 \times 10^5$ spores/ml (Zang et al. 1990). LC$_{50}$ value for this fungus against Plutella xylostella was $2.2 \times 10^4$ conidia/ml (Fuentes and Carballo 1995). LD$_{50}$ of B.bassiana against Sphenophorus levis was calculated as $8.8 \times 10^9$ conidia/500 ml (Badilla and Alves 1991). The fungus was highly pathogenic to pupa and adults of Anthonomus gandis with LD$_{50}$ at 6 and 7 days as $1.49 \times 10^6$ and $6.1 \times 10^7$ conidia/ml, respectively, for pupa and $2.88 \times 10^9$ conidia/ml for adults (Wright and Chandler, 1991). Ponce et al. (1992) also computed LC$_{50}$ and LT$_{50}$ value of B.bassiana as $2.1 \times 10^5$ conidia/ml and 16 days at $10^7$ conidia/ml, respectively, against cosmopolite’s sordidus. Puzari et al. (1994) observed that B. bassiana were highly pathogenic to Chrysomelid Dicladispa armigera with LC$_{50}$ of 90.16 conidia/ml. They observed that rate of infection of larvae; pupae and adults were comparatively lower under field condition than laboratory inoculation. More than 90 % adults were killed under laboratory and 34.38 % on field inoculation and when eggs were laid after inoculation, B.bassiana killed 77.78 per cent infection under laboratory condition. Wright et al. (1997) revealed that silverleaf whitefly, Bemisia argentifolii infected by B.bassiana showing white colour. The virulence of any pathogen can be enhanced by passing through the insect host (Taylor and Knowleden 1997). Padmaja (1998) studied
the virulency of *B. bassiana* at different developmental stages of *Henosepilachna vigintioctopunctata* and computed LT$_{50}$ values of the fungus against second instar larvae, which was 1.3 to 4.8 days in different isolates. The efficacy against adults of *Diabrotica virgifera* was evaluated in the lab and results revealed 50 percent mortality of the test insect was 5x10$^{13}$ conidia/ha (Molock and Chandler, 2000). Again Consolo *et al.* (2003) reported that against *D. speciosa* Germar (Coleoptera: Chrysomelidae) LC$_{50}$ of Isolate (F 12073) of this fungus was 3.48 × 10$^{7}$ conidia/ml.

Govindan *et al.* 2001 reported that LC$_{50}$ value of *B. bassina* against *Sitophilus oryzae* was 7.6 conidia /ml and their rate of mortality was 75.8 percent. Bioassay studies with native strains of *B. bassiana* on *Bemisia tabaci* showed no marked effect on the nymphs of whitefly even at 1x10$^{7}$ conidia/ml and percentage mortality could not reach above 47 percent (Herrera *et al.* 1999). Again LC$_{50}$ 1.20 × 10$^{4}$ to 1.55 × 10$^{6}$ conidia/ml against *Myzus persicae* was reported by Liu *et al.* 1999. Ihsan *et al.* (2004) while studying the laboratory bioassay of *B. bassiana* against broad mite; he found that the dose of *B. bassiana* @1x10$^{8}$ conidia/ml showed 80.88 % mortality within 3.349 days after treatment. The population of *Callosobruchus maculatus* reduced significantly in stored cowpea when apply *B. bassiana* (Vilas *et al.* 1996, Cherry *et al.* 2005). Loc *et al.* (2005) studied the efficacy of *B. bassiana* against ear head bug, *Leptocorisa acuta* under greenhouse condition. They found that 77.7 % mortality of bug was recorded in *B. bassiana* treated plot 10 days after treatment when applied at a concentration of 10$^{7}$ conidia /ml.
Candido et al. (2006) reported that the efficacy of *B. bassiana* against *B. tabaci* was significantly increased with time and mean mortality of nymph at 8 days after inoculation and ranged between 52.3 ± 7.3% for nymph on cotton and 91.8 ± 5.8% for nymphs reared on cucumber. Huynh Van Nghiep (1999) reported that *B. bassiana* and *M. anisopliae* effectively controlled the BPH population ranging from 60-80% and they could last rather longer from 7-12 days after spraying. Dhuyo et al. (2008) reported that the conidial concentration of two isolates No.272 and 373 of *B. bassiana* when applied @ 10^9 conidia/ml not only registered the highest mortality of yellow stem borer but also affected the hatchability percentage of yellow stem borer eggs under laboratory condition. Sivasundaram et al. (2008) reported that under *in vitro* condition among the different concentrations of *B. bassiana*, the conidial concentration @1 × 10^8 conidia/ml registered maximum mortality of 76.6 % with least LT_{50} value of 4.4 days. Lozano-Gutierrez (2008) registered 100% mortality of white grub, *Laniifera cyclades* in green house as well as field condition where the LT_{50} values were 5.1 days and 6.4 days in greenhouse and field condition respectively. Karanja et al. (2009) reported that *B. bassiana* @ 2.5 × 10^8 conidia /ml were successful to control the white coffee stem borer (*Monochamus leuconotus*) under laboratory condition when exposed the larvae to the *B. bassiana* treated filter paper for 24 hours at temperature 28°C.

Vanmathi et al. (2011) carried out a laboratory test of *B. bassiana* at a concentration of (1×10^4 to 1×10^8 conidia /ml) against pulse beetle, *Callosobruchus maculatus* (F.). They found that *B. bassiana* caused maximum oviposition reduction and 100% adult mortality at higher concentration.
2.6 Mass production of *B. bassiana* both in liquid and solid state

The mass production of insect–pathogenic fungi is a necessary prerequisite for any large scale field application of this fungus. The technique for the cultivation of fungal spores has been carried out either by a surface culture with a solid substrate, or a submerged culture with a liquid medium.

2.6.1 Synthetic nutrient media for culturing the fungus

The nutritional requirement of entomopathogenic fungi vary with the fungal species or even the fungal strain. Deutomycocetes typically have low requirements and substantial growth of *B. bassiana* can be obtained in media containing only dextrose, a nitrate and a macro mineral solution. MacLeod 1954 reported that Sabouraud Maltose Agar (SMA) was considered the best for sporulation of *B. bassiana*. On agar media conidiogenesis starts after six days, while in liquid culture it takes 3-4 days (Samsinakova 1966). In stirred liquid cultures employed in mass production of this fungus, so-called blastospores developed which are thin walled larger (3-5x2-3mm) and less resistant than conidia (Mueller-Koegler and Samisinakova 1970). The production of entomopathogenic fungi had been successfully grown on many semisynthetic and synthetic media for its isolation, maintenance and mass culture, (Campbell *et al.* 1978, Smith and Grula 1981). The other media used for the culture of *B. bassiana* were zepek agar, cornmeal agar, casein hydrolysate medium, sabouraud dextrose agar (SDA), SAD with 0.2 % yeast and 0.5 g each of penicillin and steptomycin per litre of the medium (Wasti and Hartmann 1975). Barnes *et al.* (1975)
cultured *B. bassiana* in various commercial products, of which casitone, neopeptone and hydrolysate were effective as sources of peptone for the mycelial growth, while the first two products were conducive for sporulation. The media containing tryptophan and alanine were the most suitable for growth and sporulation of *B. bassiana* and the carbohydrate melezitose was best for growth, while sucrose, trehalose and D-glucose were the best for sporulation (Campbell *et al.* 1978). For harvesting dried Blastospores of *Beauveria bassiana* many authors utilized liquid medium (Ferron 1978). However Aquino *et al.* (1977), Filho *et al.* (1988) and Pandit and Som (1988) utilized rice grains and soyaben chunk respectively for mass culturing this fungus. Alvas and Periera (1989) obtained a yield of $2 \times 10^{11}$ Conidia/gm of powdery preparation of *B. bassiana* using rice as a basic growth substrate. liquid media such as rice guel, coconut water and potato broth and supplemented by different carbon and nitrogen sources and naturally available cheapest solid media such as rice husk, sawdust and rice bran for mass production of *B. bassiana* which help in management of waste for useful purpose and environmental pollution. Bidochka *et al.* 1987 observed the growth of *B. bassiana* was highest in four liquid media such as peptone, peptone-glucose, glucose and glucose-peptone-yeast extract. Chase *et al.* (1986) found that oatmeal agar was effective for the isolation of *B. bassiana* from an artificial potting medium. Pandit and Som (1988) recommended potato dextrose agar for the maintainance of *B. bassiana*. Feng *et al.* (1994) recommended potato dextrose agar medium with 0.2% yeast extract for isolation and mainantenance of *B. bassiana* cultures. Mazumder *et al.* (1995) reveald that rice husk in addition of 2 % dextrose showed higher sporulation of *B.bassiana*. Puzari *et al.* (1997) reported that rice husk: saw dust: rice bran + 2%
dextrose was successfully utilized yielding $39.33 \times 10^7$ conidia/ml water. They also found that among the liquids tested, potato broth on addition of 2% dextrose produced highest conidial population ($4.2 \times 10^7$ conidia/ml) with a considerable increase in its virulence (61.43% mortality). Sharma et al. (2002) observed that B. bassiana and M. anisopliae produced highest conidia in molasses yeast broth. Wadyaker et al. (2003) reported that potato broth supported maximum spore production of M. anisopliae. Francisco (2007) found that complete medium (CM) and potato dextrose agar medium provided highest germination of B. bassiana. Jackson et al. (2003) reported that liquid media supplemented with acid hydrolyzed casein or yeast extract supported the production of high concentrations of blastospores that were significantly more desiccation-tolerant when compared with blastospores produced in media supplemented with other nitrogen sources surviving up to 12-50% after drying. Sun and Liu (2006) reported that D-glucose, D-mannose, sucrose, trehalose, chitin, dextrin, gelatin, and lactic acid were suitable for fungal growth. These findings provided better understanding of the nutritional requirements of different fungi used as biocontrol agents that can improve the mass production process as a whole. Pham et al. (2009) observed that among the five different carbon sources, corn meal, while in case of nitrogen sources, peptone 2% enhanced the highest spore production to the tune of $16.5 \times 10^7$ spores/ml. Agar medium (Sergio et al. 2003) and Senthamizh Selvan et al. (2010) reported that PDA and SDA could produce maximum mycelial mass of B. bassiana. Sharma et al. (2002) reported that the peptone present in sabourauds liquid medium is a good source of nitrogen which results in good growth and sporulation of B. bassiana spp. Mass production of B. bassiana was carried out in solid state where
Aerial conidia are produced and in liquid state fermentation or submerged culture where blastospores are produced. Normally blastospores produced in deep agitated liquid, are more delicate and short lived than conidia. But can be equally effective or more virulent (Hall 1981, Barlett and Jaronski 1988, Jenkins and Goettel 1997). Blastospore can be formulated as an effective, durable, wettable powder by a sophisticated but commercially viable process.

2.6.2. Natural substrates for mass culturing the fungus

For mass culturing of fungus apart from synthetic media, natural substrates like grains, tuber and other plant origin substances have also been tried. In any biocontrol programme, production of adequate quantities of good inoculum is an essential component. Further, a simple reliable production system following basic multiplication procedure of submerged liquid fermentation, which is less time consuming and hydrophilic, is essential (Rombach 1989). Likewise, solid-state fermentation is also equally important to maximize production of aerial conidia (Rousson et al. 1983). However, the most viable mass production technology include making use of a diphasic strategy in which the fungal inoculum is produced in liquid culture, which can also be further utilized as the solid state fermenter for conidia production (Burges and Hussssey 1981). Roberts and Yendol (1971) stated that the basic culture substrates for mass producing spores of Beauveria were wheat, corn and potato products. Latch and Fallon (1976) suggested cereal grains for the mass production of several entomopathogenic fungi. Potato dextrose liquid broth medium and Richard’s medium were best medium for mycelia growth (Manisegarane and
Bean broth, rice broth and potato broth are good liquid media for *B. bassiana* spore production and these spores have more than 96 per cent germinating capacity (Batia-Filho *et al*. 1985). There are other natural substrates which have been utilized for mass production are soyabean chunk (Pandit and Som 1988), carrot medium (Prasad 1989) and rice grain (Rao 1989), rice husk, sawdust and rice bran (Mazumder *et al*. 1994), sugarcane wastes (Somasekhar *et al*. 1998), silkworm pupal powder (Chavan *et al*. 1998). Furthermore, other natural products such as steamed rice (Feng *et al*. 1994, 2004), carrot, jack fruit seeds, lady’s finger, coconut water, rice cooked water, rice wash water and wheat wash water (Sahayaraj and RajaNamasivayam 2008) were also utilized for production of *B. bassiana*.

Dangar *et al*. (1991) observed that abundance of glucose and minerals in coconut water contributed growth and production of *M. anisopliae* spores.

### 2.7 Development of mycoinsecticides based on *B. bassiana*

For successful utilization of any mycoinsecticoides, development of suitable formulation is pre-requisite. Commercial mycoinsecticides must be formulated to target insects within their habitats with shelf life and environmental persistence after application (Soper and Ward 1981). There are specific requirements for successful commercial production and use of entomopathogenic fungi (Samsinakova *et al*. 1981, Roberts and Hajek 1992, Sandhu *et al*. 1993). According to them, firstly a fungal isolate for mass production must be selected with rapid growth, abundant sporulation and sufficiently high pathogenicity to the target pests. Secondly, production cost must
be minimal by developing a cheaper and simpler medium, which should be in large quantities. Thirdly, microbial products must be formulated to control different target pests with distinct biological aspects. Finally formulated products must be suitable for long-term storage under natural conditions without significantly losing their viability and infectivity.

Shelf life is considered a key factor that determines the commercial success of biocontrol agent as well as field efficacy (Rombach et al. 1986 b, Ramarethinam et al. 2001). Besides due to short shelf life commercialization of mycoinsecticides is a serious problem which gives erratic results in different agro climatic conditions (Lane et al. 1991b). Loss of virulence for any biocontrol agent in transportation and storage (Tanada 1963, Roberts and Yendol 1971) of its use, results in a wasted efforts. Formulation has been reported to greatly enhance the efficacy of entomopathogens (Navon and Ascher 2000). A number of facilities for the production of entomopathogenic fungi have now been established in many countries.

Talc based formulation containing beneficial microbes was found to be effective and cheaper for pests and diseases in different crops (Saravanakumar et al. 2007, Rajendran et al. 2007, Kavino et al. 2007, Radjacommarra et al. 2002). Sivasundaram et al. (2008) evaluated the efficacy of talc-based powder formulation of *B. bassiana* against rice pest *Cnaphalocrocis medinalis* under green house condition. They found that application of talc based formulation of *B. bassiana* treated plot showed lowest damage (5.6%) compared to control (25.8%). Several authors worked on talc based powder formulation of *B. bassiana* and found good results (Padmaja and
Kaur 2001, Padmanaban 1993, Aguda et al. 1987). A wettable formulation of *B. bassiana* conidia based on isolate ARSEE 252 has been developed by Abbott Laboratories, USA used against the Colorado Potato Beetle during the 1980s (Campbell et al. 1985). Conidia of *B. bassiana* have also been formulated as granules for application against the European corn borer (Bing and Lewis 1991).

### 2.8 Field efficacy of certain entomopathogenic fungi against rice pests

Field evaluation of entomopathogenic fungi against rice pest had been started late 1980’s. The most important versatile fungus *B. bassiana* worked effectively under field condition. Rombach et al. (1986 a) tested five entomopathogenic hyphomycetes, including *B. bassiana* for the control of rice BPH, *Nilaparvata lugens* in the Philippines. Suspension of conidia were applied at the rate of 4-5 × 10^12 conidia/ha and preparation of dry mycelium at the rate of 1.5 - 2 kg/ha. They found that *B. bassiana* caused 63 to 93 per cent mortality after three weeks of application. Rombach et al. (1986 b) while working on three entomopathogenic fungi *B. bassiana*, *M. anisopliae*, and *P. lilacinus* under field condition against black bug, *Scotinophora lurida* in the Philippines, they found that single conidia and dry mycelium application significantly reduced the bug population over a two-month period. Rombach et al. (1987) observed the occurrence of *Metarhizium album* on rice leaf and plant hoppers in rice. Aguda et al. (1987) evaluated dry mycelium of *B. bassiana*, *M. anisopliae*, and *M. flavoviridae* at the rate 200 and 2000g/ha, of 4-7.5×10^12 conidia/ha, against *N. lugens* and black bug *S. lurida* F on rice in Korea and found Significant control of pest population 7 and 22 days after application. The effectiveness of *B. bassiana* in
controlling *N. lugens* and *N. viresceens* was studied by Li (1986) and found that it caused 91-96% mortality after 10 days of treatment. In China, while conducting field efficacy with 0.5% dust and 0.5% formulations of conidia of *B. bassiana* caused 58-84 per cent mortality of rice green leaf hopper, *Nephotettix bipunctata* and 72 per cent mortality of *N. lugens* (Xu, 1988). In India Rao (1989) evaluated unformulated conidial suspension of four entomopathogenic hyphomycetes *B. bassiana*, *Metarhizium flavovirds*, *Metarhizium anisopliae* and *Paecilomyces lilacinus* for the control of a range of rice pests. Among these only *B. bassiana* isolates (5×10^12^ conidia/ha) were found to be effective against the leaf folder larvae. Udayaprabhakar (1994) evaluated dust and wettable powder formulations of mycoinsecticides of entomopathogenic fungus, *Pandora delphacis* for their efficacy against rice brown planthopper in selected sites in Tamil Nadu and found that mycoinsecticides (70% WP) gave significant control of *N. lugens* with 66.51 and 64.11 per cent mortality in two sites. Hazarika and Puzari (1995) reported that *B. bassiana* effectively controlled the eggs, larvae, pupae stages of rice hispa during summer, autumn and spring seasons. In field test *B. bassiana* infection on egg was 16.95% to 45.15% depending upon the seasons, being more than that during other stages. Low (1.67%) to very high (40.63%) level of infection of adults was also observed in summer, autumn and spring seasons. Ambthgar (2003) demonstrated that under field conditions oil in water formulation @ 2 × 10^11^ conidia/l and water dispersable powder effectively controlled the leaf folder population in rice especially during *Rabi* season. The combination of *Pseudomonas fluorescens* strains and *B. bassiana* isolate effectively reduced the incidence of leaf folder insect and sheath
blight disease on rice plants and showed the possibility of controlling both pest and disease using a single dose.

### 2.9 impact of *B. bassiana* to non-target organisms

The success of microbial agent in biolcontrol programme depends not only on their higher efficacy against target pest, but also low virulence against non target insects. In addition, most guidelines for the registration of biopesticides require laboratory testing for infectivity to non target organism including mammals (Hall *et al.* 1982, Aizawa 1990, Goettel *et al.* 1990, Strasser *et al.* 2000). Fungi wide host range is frequently more specific under field conditions, especially during epizootics (Goettel *et al.* 1990). Some entomofungal species are highly specific and pose minimal threat to non- target invertebrates (Kandybin and Smirnov 1990, Goettel 1990, Roy and Pell 2000). However, the success of fungal entomopathogens as biological control agents depends not only on high efficacy against insect pests, but also on safety to non target organisms. Moreover, *B. bassiana* and *M. anisopliae* are known to have broad host ranges and use of such biological control agents to control many pests. In this section, safety of *B. bassiana* to certain non target insects like parasitoids, predators of rice ecosystem is reviewed.

### 2.9.1 Effect of *B. bassiana* on Natural enemies

Many times the white muscardine disease has been recorded on coccinellide populations (Iperti 1964). Different isolate of *B. bassiana* were reported pathogenic to larva, pupae and adults of predatory beetle, *coccinella septempunctata* under laboratory test (Haseeb and Murad 1997). Fargues (1981) recorded mycoses caused by
muscardines in certain braconid and ichneumonid parasitoids. Prasad (1989) reported that isolates of *B. bassiana* had no effect on the parasitization, adult emergence, adult longevity and life cycle period of two egg parasitoids, *Trichogramma chilonis* Ishi and *Chelonus blackburni* Cameron in two generations tested, and both the parasitoids exposed to treated Corcyra eggs did not show sings of mycosis. Rao (1989) investigated the effect of *B. bassiana* on certain natural enemies of rice insects, and found that direct conidial application of the pathogens at a dose of $10^8$ conidia/ml had no adverse effect on the hymenopteran parasitoids *Trichogramma Japonicum* Ashmead, *Platigaster Oryzae* Cameron and *Tetastichus schoenobii* Ferriere. Thungrabeab and Tongma (2007) reported that *B. bassiana* at concentration of $1 \times 10^8$ conidia /ml did not show any adverse effect on natural predators, *Coccinella sepempunctata*, *Crysoperla carnea Dicyphus tamaninii* and other soil benificial insects. Their results revealed that different genera or species of fungi had different pathogenicity and virulence. Entomopathogenic funguses are quite specific and they infect only certain type of host. *Bassiana*, *B. brongniartii*, *Hirsutella* spp. *M. anisopliae* and *V.lecanii* did not affect the mortalities of three collembolan species, *Folsomia fimetaria L*, *Proisotoma minuta* (Tullberg) (Collembola: Isotomidae) and *Hypogastrura assimilis* (Krausbauer) (Collembola: Hypogastruidae) (Broza et al. 2001, Dromph and Vestergaard 2002). James and Lighthart (1994) reported that *M. anisopliae*, *B. bassiana* and *P. fumosoroseus* have the potential to infect *Hippodamia convergens* Guerin Meneville (Col., Coccinellidae), whereas *Nomuraea releyi* (Farlow) did not show any deleterious effect. When *B. bassiana* sprayed on *Serangium parcesetosum* Sicard (Col., Coccinellidae) a predator of whitefly, it had significantly
lower survival than with *P. fumosoroseus* (Poprawski 1988). Magalhaes et al. (1988) noted that *B. bassiana* caused mycosis in 60% of adult coccinellid predator, *Coleomegilla maculata lengi* Timberlake (Col., Coccinellidae) and in 35% of adult *Eriopis connexa* (Col., Coccinellidae), when conidia were applied directly to the insect. Satish et al. (1999), have evaluated that *B. bassiana* was safer to *Crytorhinus lividipenis*, *Lycosa pseudoannulata* and coccinellid predators. Rosa et al. (2000) evaluated that *B. bassiana* isolate Bb25 and *M. anisopliae* isolate Ma4 caused the lowest infection in coffey berry borer parasite, *Prorops nasuta* with LC$_{50}$ values of $8.31 \times 10^6$ and $4.08 \times 10^6$ spores/ml. Neither pathogen significantly affects the predatory and parasitic capacity of *P. nasuta*. Ambethgar (2003) reported that water dispersable powder formulation, oil in water emulsifiable formulations and unformulated *B. bassiana* were found to be safe to natural enemies like *Trichogramma chilonis*, *Coccinella arcuata* and *Lycosa pseudoannulata* both in laboratory and field conditions and these formulations is also safe to non target species like honey bees and albino rats. Chatterjee and Senapati (2010) evaluated some microbial insecticides such as *Baccilus thuriengiensis* (Halt-B.t.k.-55000S.U./mg and Biolep- B.t.k.- 32000S.U./mg), Vertimec (Avermectin-1.8% w/v) and *B. bassiana* ($1 \times 10^7$ conidia /ml) against coccinellid predators, *Menochilus, Micraspis, Harmonia sp.*, and spiders (*Lycosa, Oxeopes and Argeope* sp) under field condition. They found among these biopesticides *B. bassiana* exerted its effect after a certain lag period and reduced the incidence of coccinelide and spider population on 14 days after spraying. The biopesticides avermectin showed very toxic effect to spider population within three days after spraying.