CHAPTER - V
**DISCUSSION**

5.1. **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*):

Result of this experiment revealed that all 10 isolates of *B. bassiana* could infect the all three test insects. However, their virulence varied greatly with LC$_{50}$s ranging from 0.30 x 10$^5$ to 116.63 x 10$^5$ conidia ml$^{-1}$ for *H. armigera* (Table-7), 20.17 x 10$^5$ to 9781.55 x 10$^5$ conidia ml$^{-1}$ for *S. litura* (Table-10) and 0.06 x 10$^5$ to 177.83 x 10$^5$ conidia ml$^{-1}$ for *S. obliqua* (Table-13). In general, the BB10 isolate from bark-eating caterpillar proved most virulent for all three, test species. *B. bassiana* pathogen is already known for its wide geographical distribution and host range (Hall and Papierok, 1982). However, intra-specific differences in pathogenic activity may exist between isolates (Fargues and Remaudiere, 1977). The results of present studies also clearly demonstrated difference in virulence of the test isolates. Similar results have been reported earlier in case of larvae of *H. armigera* (Prasad et al., 1990). In this case, the LC$_{50}$ values ranged from 2.17 x 10$^5$ to 5007 x 10$^5$ conidia ml$^{-1}$, showing a wide variation. It was also to note that these isolates were originally obtained from different hosts and different geographical regions. Prasad et al. (1989) used the same isolates against larvae of *S. litura* also and obtained similar results, with LC$_{50}$s ranging from 19.98 x 10$^5$ to 7571.40 x 10$^5$ conidia ml$^{-1}$. In both the cases, *B. bassiana* (BPT isolate) was found most virulent. In the present studies BB10 isolate was found the most virulent with minimum values of LC$_{50}$s for the test species. BB10 isolate
was, however, found seven times more virulent against *H. armigera* (*LC₅₀ = 0.30 \times 10^5*), and equally virulent against *S. litura* (*LC₅₀ = 20.17 \times 10^5*) as compared to BB (BPT) isolate used by the above workers. Similarly, variations were also observed in LT₅₀ values of different isolates reported in the above two species by the earlier workers. Results of the present investigations further confirm these observations as shown in Fig. 1. Results further revealed that BB10 isolate proved most virulent against *S. obliqua* with least LC₅₀ of 0.06 \times 10^5 conidia ml⁻¹ and causing cent per cent mortality (Table-12). These results are the first report on the screening and evaluation of different isolates of *B. bassiana* for this pest. However, Pandit and Samanta (1994) conducted some preliminary work and reported this pest susceptible to infection of *B. bassiana*.


It is generally assumed that the strain isolated from a particular host, remain highly virulent to that host (Ferron *et al.*, 1972; Fargues, 1976). In the present studies, it was true for BB2 isolate of *B. bassiana*, which was originally derived from *H. armigera* and found highly virulent to this species, causing mortality even up to 100 per cent (Table-6). Similar observations were also made by Gopalkrishnan and Narayanan (1990) and Sandhu *et al.* (1993) in case of *H. armigera*; by Maniania...
(1992) against *B. fusca*; by Rajak *et al.* (1993) in case of *E. machaeralis* and *H. puera*, and by Lecuona *et al.* (1996) in case of *D. saccharalis*. But at the same time, result of the present investigations also showed that isolates derived from hosts belonging to different group of insects, different species of insects and from different geographical regions, varied in their virulence. Among these isolates some of them (BB1, BB2, BB3, BB4, BB7 and BB10) were found highly virulent to the three test species (Table-6, 9 & 12 and Fig. 1). Among the isolates evaluated in the present studies, except BB2, all other potential isolates were originally derived from host other than the test species as listed in Table-1. Besides these, one isolate BB5, derived from Coleopterous host also proved virulent to Lepidopterous test species, *H. armigera* (Table-6). Diaz and Lecuona (1995) recorded similar observations in evaluation of 31 isolates of *B. bassiana* against *D. saccharalis*. In this case, mortality due to infection of different isolates varied from 30-90 per cent. But the isolate originally derived from different species (*Spilosoma virginica*) proved most virulent (96% mortality) in comparison to the isolates derived from the test species (*D. saccharalis*) resulting in 80-90 per cent mortality. Similar results were also reported by other workers (Poprawski *et al.*, 1985; Xu *et al.*, 1986; Houle *et al.*, 1987; Prasad *et al.*, 1989, 1990; Khan *et al.*, 1993; Leathers and Gupta, 1993; Martins and Lima, 1994; Diaz and Lecuona, 1995; Rosa *et al.*, 1997).

The above results thus suggest that neither the origin of host nor the phylogenetic relationship between potential hosts is a reliable indicator of the probable virulence of a specific fungal isolate to a specific host as also suggested by Diaz and Lecuona (1995).

It is thus concluded from the results of the above studies that different isolates of *B. bassiana* showed great variations in their virulence
to the three test species (Fig. 1). However, no distinct relationship could be established between different parameters discussed above and the virulence of different isolates of *B. bassiana*. The differential susceptibility of the test larvae to the fungal isolates used may be due to inherent variations in the susceptibility of host insect to a particular fungal pathogen, the biochemical interaction in the infection process, different requirements of optimum environmental factors for each isolate, the culture medium, etc. This emphasizes the importance of careful selection of fungal isolates for use as microbial control agents. In the light of the results obtained, BB1, BB2, BB3, BB4, BB7, BB8 and BB10 were the promising isolates of *B. bassiana* identified. However, BB10 the most virulent isolate was selected for detailed studies related with its development into mycoinsecticide for control of the major polyphagous pests, viz. *H. armigera*, *S. litura* and *S. obliqua*. Further studies were, therefore, conducted on bioassays related with pathogen-pest-host plant/host stage/temperature relationship, standardization of its cultural conditions and comparative evaluation, compatibility tests with pesticides and non-target insect species, host range and mass multiplication.

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

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

*Effect of different synthetic nutrient media:*

Result of this experiment show that the isolates of *B. bassiana* grew and sporulated on all kind of media tested (PDA, SDA, CDA, MEA, CMA and OMA) (Table-15, 16 & 17). These findings thus indicate that *B.*
B. bassiana can grow and sporulate on a wide variety of medium (both solid and liquid substrates). Many workers have already evaluated a variety of media for culture of this pathogen (Samsinakova et al., 1981; Smith and Grula, 1981; Galani, 1983; Filho et al., 1985; Lim et al., 1989; Hegedus et al., 1990; Paccola-Meirelles and Azevedo, 1990; Ibrahim and Low, 1993; Padin et al., 1996; Yamashita and Satomi, 1996).

As a result of present studies SDA medium was found to provide best results when compared with other media on the basis of different growth parameters (with mean colony diameter of 36.71 mm, conidial density of $2.093 \times 10^7$ conidia ml$^{-1}$ and dry mycelium weight of 0.2942g) (Fig. 2). However, BB3 isolate had significantly higher growth in CDA medium when compared individually for different media (Table-15). Lim et al. (1989) also found SDA to provide best growth with respect to colony diameter (36.63 mm at 12$^{th}$ day) and conidial production ($2.26 \times 10^8$ conidia ml$^{-1}$ at 21$^{st}$ day) for B. bassiana isolate from H. theobromae, a pest of cocoa. The other media observed to give better growth, were PDA and MEA as reported in our results also. These results were nearly similar as observed in the present studies. Knudsen et al. (1990) also found biomass production significantly superior in Sabouraud broth medium in case of isolate derived from cereal aphid. PDA medium was however, found to provide significantly higher conidial yield and growth diameter in different isolates as reported by Paccola-Meirelles and Azevedo (1990) and Padin et al. (1996). Wide variations in growth diameter (24.40 to 36.20 mm) and in spore production ($1.30 \times 10^7$ to $27.20 \times 10^7$ conidia ml$^{-1}$) at 11$^{th}$ day of growth were reported in different isolates by Paccola-Meirelles and Azevedo (1990). Similar variations in these growth parameters were also observed in results presented in Table-15 to 17. In another study, the potato glucose medium was found less favourable to fungal growth in comparison to corn residue, potato flakes and synthetic
Wolenweber medium (Galani, 1983). These authors recorded poor growth of different isolates of *B. bassiana* in corn meal agar as recorded also in the present studies.

The differences in growth parameters of *B. bassiana* in different media of cultivation may be due to differences in their nutritional compositions, i.e., carbohydrate and nitrogen sources, the basal salts and even the coagulating agents. The media evaluated in the present studies also differed in their composition (Table-2). The Sabouraud’s agar and broth, which proved the best medium, for growth of BB10 isolate contained 4% dextrose, 1% peptone and 2% agar and 2% dextrose and 1% peptone respectively. CDA, the next better medium contained 3% sucrose, 0.3% sodium nitrate and 0.21% basal salts. The important role of different nutrient, in growth and sporulation of *B. bassiana* has been reported in studies carried out by various workers (Samsinakova *et al.*, 1981; Motobayashi *et al.*, 1988; Rombach, 1989; Hegedus *et al.*, 1990; Yamashita, 1996).

The other important observations made from the present investigations were that the isolates differed significantly in the production of conidia and hyphal growth. It was also observed that each isolate had different responses to the media used. Figure 3 shows the response of different isolates on SDA medium with respect to different growth parameters. Wide variations were noticed in different isolates with respect to various growth parameters. In the ten isolates evaluated the growth diameter varied from 25.90 to 37.66 mm, conidial production from $6.09 \times 10^7$ to $24.81 \times 10^7$ conidia ml$^{-1}$ and dry mycelial weight from 0.2036 to 0.3208 g (Table-15 to 17). These results are in general agreement with observations made earlier in the case of *B. bassiana* and other important fungal pathogens including *M. anisopliae* and *P.*
farinosus (Machowicz-Stefaniak, 1988; Paccola-Meirelles and Azevedo, 1990; Kleespies and Zimmermann, 1992; Feng et al., 1994; Lecuona et al., 1996; Arcas et al., 1999).

It was also observed that not all of the most virulent isolates were good hyphal or conidial producers. For example, our observation showed that isolate BB2, which is next to BB10 in virulence (Fig. 1), has comparatively lower conidial and hyphal yield (Table-16 & 17). The conidial production was $6.39 \times 10^7$ and $23.95 \times 10^7$ conidia ml$^{-1}$ respectively in BB2 and BB10 isolates (Table-16), while dry mycelial weight was 0.2485 and 0.3208 g respectively in both the isolates (Table-17). In contrast, BB6 a less virulent isolate had better biomass production in comparison to more virulent isolates, BB1 and BB2, or isolate BB4 had better conidial production but found less pathogenic to the three test species. These observations are in confirmation with the findings of Lecuona et al. (1996).

In general, from the above results, no definite correlation between growth parameters of different isolates and their pathogenicity could be established. Variations were observed in each characteristic and also in virulence of different isolates. However, isolate BB10 was found most virulent as well as productive in respect to different growth parameters (Fig. 3). On the basis of these characteristics this isolate may therefore, be considered for the development into potential mycoinsecticide. Therefore, this isolate was selected for further studies

Effect of different initial pH values:

Results of this experiment revealed that the different isolates of B. bassiana were able to grow well in media with pH 5 to 7 (Table-18). Most of the isolates, however, registered higher hyphal growth between pH 6
and 6.5. Galani (1988) also reported highest biomass values at pH 6 for B. bassiana. Thereafter the yield tended to be higher towards basic media rather than acidic media. Shimazu and Sato (1996) in their investigations reported B. bassiana to grow well at high pH of more than 10. These differences in hyphal growth may be due to natural variability existing between different isolates in response to different factors of growth. Results of present studies also indicated that different isolates differed in biomass production at different pH values (Fig. 4). Vast differences in pH values required for growth of different isolates have also been reported by Kleespies and Zimmermann (1992) in case of M. anisopliae.

Range of pH between 5-7 was also found suitable for growth of other entomogenous fungi, i.e., V. lecanii (Easwaramoorthy and Jayaraj, 1977); M. anisopliae (Sundra Babu et al., 1986); Hirsutella spp., M. flavoviride and N. rileyi (Im et al., 1988) and Paecilomyces cicadae (Chen, 1991).

Effect of different temperature:

In the present studies, although temperatures from 15 to 35 °C supported growth of the ten isolates of B. bassiana, the optimum was between 20 and 30 °C (Table-19, Fig. 5). These findings further confirm the observations of Roberts and Campbell (1977); Kuberappa and Jayaramaiah (1987); Fargues et al. (1992); Khan et al. (1993); Fargues et al. (1997). Lim et al. (1989) however, reported the temperature range between 5 and 30°C for growth of an isolate of this fungus from cocoa mirid, Helopeltis theobromae.

The optimal temperature for the majority of the isolates studied, was 25 °C (Table-19, Fig. 5). Fargues et al. (1992) also reported 25 °C as optimal temperature for majority of isolates out of 31 evaluated. Fargues
et al. (1997) found relative growth rate for each isolate significantly affected by temperature. These authors further reported that the optimal temperatures for *B. bassiana* varied between 25 and 28 °C. However, several isolates exhibited optimal growth at temperatures as low as 20 °C or as high as 30 °C. Results of the present studies also show that growth was optimum for most of the isolates at 25 °C except for one isolate (BB6) at 20 °C and for BB3 and BB8 isolates at 30 °C. Junianto and Sri Sukamto (1995) also found optimum hyphal growth and sporulation to vary around 25 °C for the 5 isolates of this fungal pathogen (ranging from 25 to 27 °C).

Optimum temperature range of 20 to 30 °C has also been found suitable for sporulation and germination of *B. bassiana* by various research workers (Walstad et al., 1970; Kuberappa and Jayaramaiah, 1987; Fernandes et al., 1989; Lim et al., 1989; Khan et al., 1993; Itoh et al., 1994; Junianto and Sri Sukamto, 1995).

Significant variations reported in growth among isolates in response to different temperatures may be due to intra-specific variability of isolates. These differences were more pronounced among the isolates at temperature < 20 °C or > 30 °C as also observed by Fargues et al. (1992, 1997).

It is thus concluded from above results that the isolates of *B. bassiana* were capable of growth within wide temperature range with adaptation to different geoclimatic conditions. However, there is a wide variation in specificity between isolates. Significantly higher radial growth was observed in BB10, the most virulent isolate selected and evaluated against the test insects. The optimum temperature was 25 °C for growth of this isolate. BB3 and BB8, the other virulent isolates with 30 °C
as optimum temperature requirements may be considered for use in tropical and sub-tropical climates for control of test insects.

5.2.2. Study of morphological characters of *B. bassiana*:

Present studies regarding the morphological characters revealed that 10 different isolates did not differ much in shape and size of conidia as also reported earlier in 4 strains of *B. bassiana* by Li (1987). Results of his studies showed that different isolates had spores of similar size varying from 2.7-3.2 μm in diameter. In the present studies the size of conidia varied from 1.88-2.56 μm x 1.80-2.44 μm between 10 isolates studied. Varela and Morales (1996) however reported variations in the morphology of conidia among 6 isolates of *B. bassiana*. They recorded three size ranges and two shapes. Mesquita Palva *et al.* (1996) also studied the conidial morphogenesis of different strains of the fungus under SEM.

In general conidia of *B. bassiana* are globose to subglobose and varying in size from 2-3 μm x 2.0-2.5 μm as described by Samson (1981) and Fassatiova *et al.* (1983). Similar shape and size around this range were also observed in conidia of different isolates of *B. bassiana* in the present studies. BB10 isolate was observed to have globose to subglobose conidia with the average diameter size of 2.05 μm x 1.80 μm and basal stalk of 0.55μm as revealed by SEM studies (Plate- IV & Fig. 1 to 3). Lim *et al.* (1989) as a result of SEM observations reported, globose to broadly ellipsoid, blastic conidia, measuring 1.86 – 3.0 μm x 1.86 - 2.70 μm with stalk size of 0.5 – 1.0 μm, both on cadavers of *H. theobromae* and on agar media. Bidochka *et al.* (1993) conducted SEM studies of conidia for an isolate from *Lygus* sp. and recorded conidial size ranging from 2.33 – 2.25 μm x 1.83 – 2.08 μm while Ambethgar (1997) recorded spherical to oval
conidia with a diameter range of 2.26 – 4.54 µm under microscopic examination under 40X.

Rombach (1989) observed that submerged produced conidia and aerially produced (as on agar) conidia did not significantly differ in shape or size. He recorded size ranging between 1.78 – 2.8 µm x 1.71 – 2.5 µm for conidia produced in submerged culture. It was also observed that both types of conidia possess a pedicel and are produced by sympodial proliferation from conidiogenous cells born on conidiophore. Similar structures were observed in the present studies (Plate- IV, Fig. 1 to 3) and also reported by other workers (Samson, 1981; Fassatiova et al., 1983; Lim et al., 1989; Ambethgar, 1997).

It is concluded from the above observations that in general conidial shape varied from globose to subglobose or spherical or oval and size varied round 2-3 µm in diameter in different isolates of *B. bassiana* with the only exception of maximum diameter of 4.5 µm reported by Ambethgar (1997). It is also observed from the various size ranges recorded by different workers for different isolates that the size of conidia varied not only between the isolates but within the isolates also. It is thus further concluded that conidial size can not be used as adequate criteria for identification of isolates as also suggested by Ferron (1981).

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

Histopathological observations on infection of *B. bassiana* in larvae of *H. armigera*, *S. litura* and *S. obliqua* showed that accumulation and germination of conidia occurred on host cuticle, which was later penetrated, followed by hyphal proliferation, development and sporulation in hypodermis, haemocoel and gut lumen resulting in degeneration and
disintegration of viscera and tissues and again hyphal emergence to the exterior (Plate- V, VI & VII). Thus results of the present studies also confirm the general infection process of B. bassiana in insect host as determined histologically by various other research workers (Wasti and Hartmann, 1975; Atuahene and Doppelretter, 1982; Fasih and Srivastava, 1988; Su, 1989; Hazarika and Puzari, 1990; Bidochka et al., 1993; Kaaya et al., 1993; Sohafer et al., 1993; Ramlee et al., 1996).

Present studies revealed that penetration of epidermis by germination of conidia occurred within 24 to 48 hr post-inoculation in case of S. obliqua. Similar observations were also recorded in case of lepidopterous larvae of M. plana (Ramlee et al., 1996) and larvae of coleopterous weevil, C. formicarius (Su, 1989), while penetration of epidermis and fat body was noticed at 72 hr post-inoculation in case of silkworm, B. mori (Sohaf et al., 1993).

Further, observations revealed that the whole body cavity was filled with B. bassiana hyphae and spores and massive disintegration of internal tissues occurred. Emergence of hyphae to the exterior occurred at 96-120 hr post-inoculation in case of H. armigera and S. obliqua (Plate- VI & VII, Fig. 1-3) but within 120 to 144 hr post-inoculation in case of S. litura (Plate- V, Fig. 5 & 6). In case of M. plana (Ramlee et al., 1996) and C. formicarius (Su, 1989), also the process of growth and development of hyphae and spores inside the body cavity was completed within 96-120 hr post-inoculation while at 132 hr and after in case of B. mori (Sohaf et al., 1993).

The above observations are important as they confirm the mode of infection and pathogenicity of B. bassiana in the host insects tested. These findings are also important from the point of view of virulence as the pathogenic process may take its own time and course, which may vary
with different isolates and in different host species. Pathogenicity further
depend upon the number of other factors i.e. production of cuticle
degrading and other enzymes, toxins etc. These factors play an important
role in infection process besides, the possible mechanism of pathogenicity
involving invasion of haemocoel tissues and/or organs leading to
malfuctioning of vital organs affecting physiological activities. In
addition, depletion of nutrients by the fungus during mycosis probably
resulted in death of the host insect.

5.4. 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:

Results of the bioassay test with regard to larval instar-dose-
mortality relationship in the test species, revealed that, in general, higher
percentage mortality were observed because of infection of *B. bassiana* in
early instar larvae as compared with the later instars. This shows that the
mortality rates decreased with advancement in age of the larvae (Table
22, 25 & 28). Similar results were reported by Gopalkrishnan and
Narayanan (1990) in *H. armigera*, recording maximum mortality (70%) in
2nd-instar larvae and significantly low mortality (56 to 58%) in 4th- and
5th -instar larvae using isolate derived from the same host. Whereas,
present studies showed 73 per cent mortality in 2nd -instar and 51.24 to
52.08 per cent in the 4th - and 5th - instar larvae of *H. armigera*. Similar
trend was also reported by Sandhu *et al.* (1993) in *H. armigera*. In case of
*S. litura*, maximum mortality of 48.15 per cent in 2nd - instar and 26.78 per
cent in 5th -instar were reported in the present studies. Jayanthi and
Padmavathamma (1996) recorded similar trend in instar-mortality
relationship in *S. litura*, supporting the author’s findings. Instar-dosage-
mortality relationship has been worked out for the first time in case of *S.*
obliqua. Mean mortality in this case varied from 64.16 per cent in 2\textsuperscript{nd} instar to 35.41 per cent in 5\textsuperscript{th} instar. Variations in the mortality percentage of different instars were also observed in *O. nubilalis* larvae (Feng et al., 1985), in teak defoliators, *H. puera* and *E. machaeralis* (Rajak et al., 1993).

Variations in the susceptibility of different larval instars of the three test insects were further confirmed by the results of probit analysis of dose-mortality and time-mortality relationship. It was found that the initial stages were more susceptible with lower LC\textsubscript{50} and LT\textsubscript{50} values for the pathogen with few exceptions. Present investigations showed that in case of *H. armigera*, the 4\textsuperscript{th} instar larva was most resistant with respect to LC\textsubscript{50} estimates (Table-20) and in case of *S. litura*, the 2\textsuperscript{nd} instar larva was more resistant than 3\textsuperscript{rd} instar with respect to LT\textsubscript{50} estimates (Table-24). Mohammad et al. (1977) also reported similar responses in *Helicoverpa zea* Boddie. Feng et al. (1985) also reported the 4\textsuperscript{th} instar larvae as most resistant in case of *O. nubilalis*, but no definite conclusion could be drawn about the cause of the susceptibility of the 5\textsuperscript{th} instar in the above case. They also viewed it to the chance of casting off inoculum through moulting before infection, because of shorter duration of initial instars as compared to later instars, particularly the last one.

General trend with regard to susceptibility of different larval instars based on LC\textsubscript{50} and LT\textsubscript{50} values of *B. bassiana* for the above three species, reported in present studies, were in agreement with the observations of Gardner and Noblet (1978) for *Helicoverpa virescens* F., *Spodoptera exidania* and *S. frugiperda* J. E. Smith; Samsinakova et al. (1981) and Feng et al. (1985) for *O. nubilalis*; Prasad et al. (1989) for *S. litura*; Prasad et al. (1990) and Sandhu et al. (1993) for *H. armigera*; Sivasankaran et al. (1990) for *Chilo infuscatusellus* Snellen; Rajak et al.
(1993) for *H. puera* and *E. machaeralis*; Jayanthi and Padmavathamamma (1996) for *S. litura*; Hafez et al. (1997) for *Phthorimaea opercullela* Zeller, all belonging to group lepidoptera.

LC$_{50}$ and LT$_{50}$ estimates for *B. bassiana* to *H. armigera* and *S. litura* worked out in the present studies differ with studies conducted by other workers. The LC$_{50}$ estimates of the fungus for *H. armigera* as reported in the present studies varied from $0.01 \times 10^5$ to $3.22 \times 10^5$ conidia ml$^{-1}$ for the 2$^{nd}$ to 5$^{th}$ -instar larvae, respectively (Table-20). Results of laboratory evaluation of Prasad et al. (1990) showed LC$_{50}$ for this species as $1.61 \times 10^5$, $6.45 \times 10^5$ and $11.72 \times 10^5$ conidia ml$^{-1}$ for the 2$^{nd}$-, 3$^{rd}$- and 4$^{th}$ -instar larvae. The LT$_{50}$s for the fungus reported as a result of present studies varied from 82.79 to 143.80 hr (Table-21) for the respective instars while LT$_{50}$s reported by the above workers were 101.16, 111.13 and 128.35 hr for the 2$^{nd}$-, 3$^{rd}$- and 4$^{th}$ -instar larvae at conidial concentrations of $1 \times 10^7$ conidia ml$^{-1}$. This shows that BB10 isolate was comparatively more virulent to this species. Sandhu et al. (1993) reported LC$_{50}$ of $0.9636 \times 10^4$ conidia ml$^{-1}$ and LT$_{50}$ of 98.80 hr at $1.2 \times 10^5$ conidia ml$^{-1}$, for the 3$^{rd}$ -instar larvae of *H. armigera*.

In case of *S. litura*, LC$_{50}$ estimates for *B. bassiana* varied from $7.20 \times 10^5$ to $8527.80 \times 10^5$ conidia ml$^{-1}$ for the respective instar of 2 to 5 (Table-23). Prasad et al. (1989) reported LC$_{50}$ estimates of $16.98 \times 10^5$, $58.23 \times 10^5$ and $235.60 \times 10^5$ conidia ml$^{-1}$ for the respective instars of 2 to 4 for this pest. LT$_{50}$ estimates for the fungus for this species was higher for all the instar (Table-24) as worked out in the present studies as compared to 112.87, 120.03 and 130.86 hr for the instars of 2-4 reported by the above workers earlier. In case of *S. obliqua*, different instars were evaluated for infection of *B. bassiana* for the first time in the present studies. The LC$_{50}$ estimates varied from $0.24 \times 10^5$ to $3269.56 \times 10^5$
conidia ml$^{-1}$ and LT$_{50}$ estimates varied from 79.30 to 188.09 h for the respective larval stages of 2$^{nd}$ to 5$^{th}$ (Table-26 & 27).

Reasons for above-mentioned differences in susceptibilities of the same species may be attributed to the differences in virulence of isolates used by different workers and also inherent differences in geographical populations of species tested. Variations in the relative susceptibility of different larval instar of noctuids, were also noticed to the fungal pathogens other than *B. bassiana* (Fargues and Rodriguez Rueda, 1980; Ignoffo, 1981).

It was also revealed from the results that, dose-mortality response of different instars showed linear relationship. The three test species were found more susceptible to the infection of *B. bassiana* at higher concentrations than at lower concentrations. Walstead and Anderson (1971) also found that the mortality was a function of the quantity of inoculum applied. The present findings are also in confirmation with findings of Gopalkrishnan and Narayanan (1990); Prasad *et al.* (1990); Sandhu *et al.* (1993); Jayanthi and Padmavathamma (1996) in case of *H. armigera* and *S. litura*. This general pattern of susceptibility towards *B. bassiana* infection was noticed in number of species belonging to lepidoptera and other group (Carruthers *et al.*, 1985; Feng *et al.*, 1985; Gilreath and Funderburk, 1987; Sivasankaran *et al.*, 1990; Puzari *et al.*, 1994; Rosa *et al.*, 1997).

It was concluded from the above studies that both host age and exposure levels of fungus are responsible for influencing pathogenicity of *B. bassiana* among other factors (Fig. 6). These results also suggest that for control of the test insects, *B. bassiana* applications should be synchronized with the occurrence of early instars and dose giving desired mortality within safe period should be selected. The present studies also
established dose-mortality relationship for the first time for larvae of *S. obliqua* and further confirmed the pathogenicity of *B. bassiana* in this insect evaluated earlier by Pandit and Samanta (1994).

### 5.5. 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*:

Results of the present studies as shown in Fig. 7 revealed that larvae of *H. armigera*, *S. litura* and *S. obliqua* reared on different host plants in the laboratory, more or less varied in their susceptibility to *B. bassiana* infection as compared on the basis of percentage mortality and LT₅₀ values. *H. armigera* larvae were significantly more susceptible to *B. bassiana* infection when reared on *C. arietinum* and *P. sativum* as compared to when reared on *L. esculentum* and *C. cajan* (Table-29). Similarly, *S. litura* larvae were significantly more susceptible to this fungus when reared on *B. oleracea* varieties and *R. communis* and least susceptible when reared on *A. hypogea* (Table-30), while *S. obliqua* were more susceptible when reared on *B. oleracea* var. *capitata* and least susceptible when reared on *R. communis* (Table-31).

Hare and Andreadis (1983) recorded similar observations with *L. decemlineata* larvae showing variation in susceptibility to infection of *B. bassiana* when reared on different *Solanum* spp. and *L. esculentum*. Larvae of *L. decemlineata* were least susceptible to *B. bassiana* when reared on *L. esculentum* as observed in the present studies also in case of *H. armigera*. Ramoska and Todd (1985) also reported similar results in case of *B. leucopterus leucopterus*, adults and nymphs of which were found less susceptible to *B. bassiana* when reared on sorghum and maize as compared to other host plants, artificial diet and water. Observations of
Macedo et al. (1990) also confirmed the differential susceptibility in larvae of *D. saccharalis* when reared on different host plants. Larvae of this pest were more resistant to *B. bassiana* when reared on rice and maize than on cane and sorghum. Differential susceptibility was also reported by Alves et al. (1990) for *D. saccharalis* larvae reared on different types of diets in the laboratory.

In general, the various researchers were of the view that the fungistatic chemicals produced by different hosts may be responsible for differential susceptibility of insects to *B. bassiana* infection when reared on different host plants. This may be true with the present observations also. These studies also show that variations among host plant species may also affect the susceptibility of insect species to entomopathogenic fungi. This factor, therefore, may also be taken in to consideration while making decisions, where *B. bassiana* has to be used as bioagent for control of polyphagous pest species.

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

It is concluded from the results of this experiment that mortality in all the three species of larvae due to *B. bassiana* was found to depend on temperature (Table-32 to 34). However, 25°C was found as optimum temperature for all the three species for infectivity of *B. bassiana*. Lower LT$_{50}$ values and higher mortality percentage were reported in the larvae of all the three species at this temperature (Fig. 8).

Optimum temperature for *B. bassiana* determined in this study is similar to the findings of other workers in case of different insect species. Lower LT$_{50}$ values and disease incubation period were recorded at 25°
and 26 °C in lepidopterous larvae of European corn borer, *O. nubilalis* (Riba and Marcandier, 1984; Carruthers *et al.*, 1985). Similar results were obtained in case of larvae of *D. saccharalis* (Lecuona and Alves, 1988); *H. puera* (Rajak *et al.*, 1993), *H. armigera* (Sandhu *et al.*, 1993) and *P. xylostella* (Selman *et al.*, 1997).


It was further concluded that while making selection for a candidate strain for microbial control, one should take into consideration not only optimum temperature for the fungal isolate, but also the optimum temperature for disease development in the target insect in a particular environment.

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

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

*Effect of Fungicides*

Results pertaining to the effect of pesticides on radial growth of *B. bassiana* revealed that all the fungicides affected the growth significantly as compared to control. Reduction in mycelial growth varied from 38.58 to 100.00 per cent in different fungicides as assessed on the basis of colony diameter (Table-36). In all the cases where there was an effect, it was inhibitive and can be regarded as fungistatic. Only exception was the clearly fungicidal effect of carbendazim on this isolate of *B. bassiana*
(Fig. 9). Similar results were obtained by Machowicz-Stefaniak (1985), Kuberappa and Jayaramaiah (1988). Carbendazim was, however, found less inhibitory to *B. bassiana* isolate in our earlier studies (Masarrat Haseeb and Srivastava, 1996). These contradictions could be explained by the variations in sensitivity to a particular chemical among isolates of the same fungal species, as noted by Olmert and Kenneth (1974), Bajan *et al.* (1995).

Benomyl, zineb, tricyclazole, thiophanate methyl, mancozeb, thiram, dodine continued to be strongly inhibitory for growth of *B. bassiana* as revealed from present studies also (Table-35 & 36). Present findings are in general agreement with the results reported by earlier workers (Galani, 1980; Tedders, 1981; Clark *et al.*, 1982; Loria *et al.*, 1983; Machowicz-Stefaniak, 1983, 1985; Sampson *et al.*, 1986; Vanninen and Hokkanen, 1988; Kuberappa and Jayaramaiah, 1988; Aguda *et al.*, 1988; Itoh *et al.*, 1994; and Feng and Chiang, 1995; Mietkiewski *et al.*, 1997. However, reduction in mycelial growth was comparatively low in sulfur and dinocap (38.58 and 45.08 % respectively). In the present studies, sulfur and dinocap was again found more or less compatible with *B. bassiana* as also reported by Olmert and Kenneth (1974), Masarrat Haseeb and Srivastava (1996) and Jaras *et al.* (1999). These results thus show that the pesticides, particularly most of the fungicides are detrimental to the growth of *B. bassiana* as also experienced by other researchers (Wright and Kennedy, 1996; Lee Sang Myeong, 1996).

**Effect of Insecticides:**

Studies regarding the effect of insecticides revealed that the organophosphate was more strongly inhibitory than the other insecticides. These insecticides resulted in 60.97 to 94.64 per cent reduction in average colony diameter (Fig. 10). Endosulfan, malathion and chlorpyriphos was
the most toxic to BB10 isolate as revealed in our findings. Hall and Papierok (1982) also found chlorpyriphos detrimental to another entomogenous fungus, *M. anisopliae*. Greatest reduction in colony diameter and mycelial growth was observed in *B. bassiana* at the recommended dosages of endosulfan for the control of cotton pests besides other organophosphates (Almeida et al., 1998). Anderson and Roberts (1983) found endosulfan greatly affecting germination of *B. bassiana*. Hall (1981) reported 100 per cent inhibition of germination and 66 per cent inhibition of mycelial growth in *Verticillium lecanii* Zimm when malathion was mixed with the media at recommended concentrations as also reported in case of *B. bassiana* in the present studies (94.20 %) (Table-37).

Insecticides next in order of toxicity were methyl parathion, monocrotophos, fenvalerate, kelthane and phosphamidon causing 69.90 to 87.5 per cent reduction in average radial growth (Fig. 10). Vyas et al. (1990) found quinalphos affecting the growth and sporulation of another species of *Beauveria* (*B. brongniartii*). In the present studies, this pesticide caused 60.05 per cent reduction in mycelial growth of *B. bassiana* at recommended concentration and 49.21 per cent reduction at lower concentration. It is concluded from these observations that among the organophosphate group of insecticides, quinalphos is comparatively safer and may be applied with *B. bassiana* with certain waiting period which may be determined by conducting the field trials.

Among the synthetic pyrethroids, fenvalerate was toxic at higher concentrations (0.015%) but resulted in comparatively low reduction in growth (52.76 %) at lowest concentration (0.005% a.i.) (Table-37). Anderson and Roberts (1983) reported 92.78 per cent reduction in spore germination of *B. bassiana* at 995 ppm of this insecticide. However,
pesticides effect on \textit{B. bassiana} spore germination may be quite different from those on mycelial growth. Anderson \textit{et al.} (1989) reported in their bioassays no significant antagonism when \textit{B. bassiana} and fenvalerate were mixed only briefly and applied against Colorado potato beetle. However, prolonged \textit{in vitro} exposure of \textit{B. bassiana} conidia to this insecticide formulation does appear to be detrimental as observed in the present studies. In this group of insecticides, cypermethrin was found comparatively safer with 43.78 per cent reduction in colony diameter. This insecticide was found comparatively less inhibitory to mycelial growth as also reported by earlier workers in case of insect pathogenic fungi, \textit{B. bassiana}, \textit{M. anisopliae}, \textit{Paecilomyces fumosoroseus} (Weize) Brown and Smith, and \textit{P. farinosus} (Vanninen and Hokkanen, 1988; Moorhouse \textit{et al.}, 1992). Results of present studies show 41 per cent reduction in mean growth of \textit{B. bassiana} (Table-37). The growth was further reduced at the lowest concentration, which may be safely combined with the pathogen for control of pests.

Cadatal and Gabriel (1970) and Gardner \textit{et al.} (1979) found that carbaryl inhibited \textit{in vitro} \textit{B. bassiana} mycelial growth. In further \textit{in vitro} studies, \textit{B. bassiana} germination (Anderson and Roberts, 1983) and colony growth (Olmert and Kenneth, 1974) were inhibited for carbaryl for only one \textit{B. bassiana} isolate out of 6 that were tested. Inhibitory effects were near minimum in case of carbaryl and \textit{B. bassiana} combination as noted in present studies. These results are significant from the point of view of IPM as carbaryl is one of the commonly used insecticides in horticultural crops including vegetables in our country.

As a result of present studies, dimethoate insecticide was found least inhibitory (32.37% reduction in average mycelial growth), while 19.30 per cent reduction was noticed at half the recommended
concentration. However, in reports of Vanninen and Hokkanen (1988), dimethoate was not found inhibitory at all. Mietkiewski and Gorski (1995) also recorded similar observations on effect of dimethoate on this fungus.

**Effect of pesticides of plant origin:**

Results of the present work demonstrate that, most of the neem-based products were compatible with *B. bassiana*. In this group of pesticides, average reduction in hyphal growth varied from 4.39 to 52.14 per cent with Neemark restricting the growth to minimum (Fig. 11). Neemark was also found to cause no harmful effect on mycelial growth of *B. brongniartii* and *M. anisopliae* as reported by Vyas *et al.* (1992). Aqueous neem seed extract was also found compatible with *B. bassiana* at the dose ranging from 0.5 to 5.0 per cent as it did not inhibit mycelial growth and spore viability (Arturo Rodriguez-Langunes *et al.*, 1997). Olson and Oetting (1999) reported reduction of growth of *B. bassiana* up to 50 per cent by use of azadirachtin as insect growth regulator as observed in their laboratory evaluation. In the present studies, Nimbecidine and Neemguard also caused reduction in growth (43.90 to 52.14 %). It appears that besides the active ingredient (azadirachtin), the formulation of particular product is also important for causing inhibition in growth of entomopathogenic fungi as reported in case of other pesticides (Anderson and Roberts, 1983).

Besides *B. bassiana*, other entomopathogenic fungi were also found affected by various neem products and other plant extracts. Studies conducted by Aguda *et al.* (1986) showed that even lower concentration of 5 per cent neem seed oil had a significant inhibitory effect on growth and sporulation of *M. anisopliae*. Similar observations were also reported in the present studies in case of *B. bassiana*. Whereas, neem seed kernel
extracts did not had any detrimental effect on *N. rileyi* (Devi and Prasad, 1996).

Most of these results are based on combination of biocides in agar media. Results may, however, be different if they are used with sublethal concentrations and certain waiting period. It is concluded from the above discussion that *B. bassiana* may be used effectively in IPM of insect pest control by careful selection of neem based pesticides and their formulations. The neem-based pesticides have also been reported safer for other important natural enemies of pests, like predatory coccinellids, braconids, spiders and some other important egg parasites and predators (Schmutterer, 1995). Natural pesticides were also reported to have synergistic effects with other pathogens (*Bacillus thuringiensis* Berliner and nuclear polyhedrosis virus) in control of *S. litura* (Prasad et al., 1989; Joshi et al., 1990).

In general it is concluded from the above results and discussion that the fungicide as a group affected the growth of BB10 isolate of *B. bassiana* the most, followed by insecticides and pesticides of plant origin. In most of the cases pesticides had inhibitive effect and may be regarded as fungistatic. In general, all the pesticides affected the average hyphal growth significantly at different concentrations tested, as compared to control (Table-36, 38, & 40). The results also show increasing trend in percentage reduction of mycelial growth of *B. bassiana* with increasing concentration in SDA with almost all the pesticides tested. Tedders (1981) and Moorhouse et al. (1992) reported similar trend.

However, the above results gave some idea about compatibility of the tested pesticides to BB10 isolate of *B. bassiana* on the basis of mycelial growth. There are other parameters i.e. sporulation and viability which are important for virulence of a pathogen. There are conflicting
reports about sensitivity of mycelial growth and germination to pesticides. Zimmermann (1975) and Moorhouse et al. (1992) however, reported mycelial growth as more sensitive than germination of entomogenous fungi to different pesticides.

The results of this study suggest that it will be possible to use this pathogen along with selected pesticides in IPM programme of the test insects. Microbial control agents serve as useful tool for IPM, as combination of pesticides with alternative mortality agents such as B. bassiana and other fungi serve to reduce selection pressure for resistance by lowering the dose and perhaps the number of pesticide application as reported by Foschi and Grassi (1985), Joshi et al. (1990), Quintela and McCoy (1997, 1998). Such combinations also introduce multiple mortality factors, so that individual with genes for insecticide resistance may still fall prey to B. bassiana (Anderson and Roberts, 1983).

5.7.2. Effect of B. bassiana on some insect predators:

Data presented in Table-41 indicated that the predatory species of insects are also affected by BB10 isolate of fungus B. bassiana to varying degrees. However, M. sexmaculatus and Coccinella spp. were comparatively found more susceptible. Low susceptibility of M. sexmaculatus to B. bassiana was also reported recently by Jayanthi and Padmavathamma (1996). The pathogenic effect of this fungus has already been reported on a variety of predacious beetles (Anonymous, 1988; Magalhaes et al., 1988; James and Lighthart, 1994; Steerberg et al., 1995, Todorova et al., 1996). Besides, Chrysopa spp. another important predator of common occurrence was found susceptible to infection of this fungus (Castineiras and Calderon, 1985; Pavlyushin and Krasavina, 1986; Donegan and Lighthart, 1989; Pavlyushin, 1996; Jayanthi and Padmavathamma, 1996).
Experimental results further indicated that *Coccinella* spp. were found more susceptible showing shorter incubation period for disease and resulting in higher mortality in comparison to other predators evaluated (Table-41). The effect may be drastic at higher concentration as shown in Table-42, Fig. 12. But the LC$_{50}$ and LT$_{50}$ values for BB10 isolate for the different stages of this predator was higher (Table-43 & 44) as compared to *H. armigera* and *S. obliqua* (Table-7, 8, 10, 11, 13 & 14). BB10 isolate was however comparatively less virulent to these predators. Results also indicated that pupae of this predator were found comparatively more susceptible with higher per cent mortality and lower LC$_{50}$ and LT$_{50}$ values of the BB10 isolate (Fig. 12, Table-43 & 44). These differences may also occur in the field conditions because pupae being stationary stage of insect are more liable to get infected while larvae and adults are movable and hence, have better chances of escaping inoculum. However, *B. bassiana* appears compatible with *B. suturalis* and *Episyrphus* sp. as indicated by results in the present studies (Table-41).

These results are however based on laboratory trials and it is not necessary to get the same results in the field also. Overall actual effect of the entomogenous fungus on the predator population may vary greatly in the field where sub-optimal growing conditions, many different antagonists and adverse weather conditions prevail. Besides dosage of pathogen, method and time of its application, predator-pathogen behavior, are also important factors affecting the mortality in the field. But some reduction in predator population may be anticipated as evident from reports in case of *C. septempunctata, P. quatuordecipunctata, M. sexmaculatus* and *C. carnea* (Cartwright *et al.*, 1984; Hemptinne, 1988; Jayanthi and Padmavathamma, 1996).
On the basis of these results it is suggested that BB10 isolate of *B. bassiana* may be used in IPM of test species in a compatible manner, without causing much harm to the common insect predators, with appropriate management of dosage and time of its application.

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

Studies on the host spectrum reveals that the 22 insect species belonging to major taxonomic groups including major pests of agricultural and forestry importance (Table-45) and beneficial insects (coccinellid and syrphid predators) (Table-41), were found susceptible to *B. bassiana*, suggesting its wide host range. Bell (1974) also provided the list of host insects belonging to different taxonomic group and class of insects. A number of research workers have mentioned about the host spectrum of *B. bassiana* in various reviews published from time to time (Pramer, 1965; Bell, 1974; Rao; 1975; Ramakrishnan and Kumar, 1977; Burges, 1981; Gillespie and Claydon, 1989; Xu, 1991; Feng, 1994; Hou, 1997).

Out of list of host insects mentioned in Table-45, *B. bassiana* has already been reported pathogenic to *H. armigera* (Agarwal and Rajak, 1985; Prasad et al., 1990; Sandhu et al., 1993), *S. litura* (Rangaswami et al., 1968; Prasad et al., 1989; Jayanthi and Padmavathamamma, 1996), *S. obliqua* (Pandit and Samanta, 1994), *E. machaeralis* and *H. puera* (Agarwal et al., 1985; Rajak et al., 1993), *H. robusta* (Misra, 1993), *Plusia* sp. (Urs et al., 1965), *D. mangiferae* (Srivastava and Fasih, 1988), the major pests of various crops in India. Pests like, *P. xylostella*, *E. zinckenella*, different aphid species have also been found susceptible to *B. bassiana* as reported by researchers of various other countries of the world (Kato et al., 1989; Feng and Jhonson, 1990; Itoh et al., 1994; Vandenberg, 1996; Selman et al., 1997).
However, some of the coccinellid predators were also found susceptible to this isolate of *B. bassiana* as revealed from the results of the present studies (Table 41). Susceptibility of insect predators to this pathogen was also reported by other workers (Bell, 1974; Magalhaes *et al.*, 1988; Jayanthi and Padmavathamma, 1996).

On the basis of these results, the fungus was found highly pathogenic to some of the important insect pests i.e. *E. machaeralis*, *H. armigera*, *H. robusta*, *L. orbonalis*, *O. euadrusalis*, *P. xylostella*, *S. obliqua*, resulting in 70-100 per cent mortality (Fig. 13). It is thus concluded that this isolate may also be considered for use in IPM of these important pests of horticulture and forestry.

As a result of the present studies some of the important insect species have been tested for their susceptibility to *B. bassiana* for the first time in our country. These are *A. foevicollis*, *C. crocale*, *E. merione*, *Earias* sp., *L. orbonalis*, *P. ricini*, *P. cupreata*, *P. fulgurita* and thus, these pests further add to the list of susceptible hosts of *B. bassiana*.

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

Many fungi grow profusely on damp solid media and produce conidia aerially. Surface culture on solid or semisolid media is usually best suited for these fungi including the species *B. bassiana* (Goettel and Roberts, 1992).

As a result of present studies rice, sorghum and maize provided significantly better growth and sporulation of the BB10 isolate (Table-46). These media provided loose solid substrate, a condition reported by Muller-Kogler (1967) as necessary for good growth and sporulation to occur.
Substrate of cooked rice or other grains has been employed in the standard technology for mass production of *B. bassiana* and *M. anisopliae* in many countries. Trays or autoclavable plastic bags or glass jars or bottles were used for the purpose (Alves, 1986; Filho *et al*., 1988; Alves and Pereira, 1989; Filho *et al*., 1989; Quintela, 1994; Feng *et al*., 1994). The yield ranging from $4.38 \times 10^9$ to $2 \times 10^{11}$ conidia g$^{-1}$ of rice substrate are obtainable with selected isolates (Alves and Pereira, 1989; Nelson *et al*., 1997; Narvaez *et al*., 1997). However, in present studies, comparatively low yield of $4.28 \times 10^8$ conidia gm$^{-1}$ of rice in conical flask was obtained in BB10 isolate and which did not differ significantly with yield in maize ($4.26 \times 10^8$ conidia gm$^{-1}$) and sorghum ($4.36 \times 10^8$ conidia gm$^{-1}$). Rice also proved as better substrate for conidial production in comparison to seeds of cow-pea and sorghum as reported by Vilas Boas *et al*., (1996) and grains of wheat and barley as reported by Nelson *et al*., (1997). Results of present studies show wheat, barley, oat and pearl millet as inferior to rice.

Results of present studies also indicated that rice husk alone or supplemented with carbon and nitrogen sources gave better yield in comparison to other byproducts/wastes evaluated for mass multiplication of BB10 isolate of *B. bassiana*. Rice husk supplemented with 1 per cent glucose and 1 per cent yeast extract resulted in best yield ($42.04 \times 10^6$ conidia gm$^{-1}$) followed by rice husk alone ($33.07 \times 10^6$ conidia gm$^{-1}$) and wheat husk supplemented with 1 per cent glucose and 1 per cent yeast extract ($13.50 \times 10^6$ conidia gm$^{-1}$). Mazumder *et al*., (1995) also obtained the best yield in rice husk supplemented with 2% glucose ($5.8 \pm 0.15 \times 10^7$ conidia ml$^{-1}$) for the isolate from *D. armigera*. Later on Puzari *et al*., (1997) developed a culture medium for the same isolate using rice husk,
saw dust and rice bran at a ratio of 75 : 25 : 100, respectively with yield of 39.33 x 10^7 conidia ml^-1 after 24 days of incubation. The yield in this medium was nearly many fold higher than the yield obtained separately in the rice husk, and saw dust media evaluated earlier (Mazumder et al., 1995). Lee Sang Myeong et al. (1996) obtained better growth of *B. bassiana* on rice bran, barley and corn extract than on SMAY medium. They also reported better growth of *B. bassiana* and *M. anisopliae* in saw dust + rice bran medium than in an organic fertilizer + rice bran medium. Besides, various other by products/ wastes i.e. rice powder, wood shavings, sugarcane bagasse, distillery must, ground cornstalk, etc. have been evaluated with varying degree of success for conidial production of *B. bassiana* as reported by various workers (Hussey and Tinsley, 1981; Grajek and Sobezak, 1990; Ibrahim and Low, 1993; Feng et al., 1994; Calderon et al., 1995; Borges et al., 1997; Wraight and Carruthers, 1998; Arcas et al., 1999).

Progression of growth and higher conidial yield in rice husk and wheat husk media supplemented with glucose and yeast extract, can be attributed to the fact that growth of *B. bassiana* can occur up to certain extent by utilizing the endogenous carbon and nitrogen resources. However, addition of these energy sources exogenously enhanced fungal growth as also reported by Smith and Grula (1981). Further, in the present studies absence of growth or negligible growth in saw dust and rice bran may be due to insufficient amount of carbon and nitrogen sources as compared to the whole grains which are rich in essential nutrients. In general, growth and sporulation are fungal species and strain dependent as is well documented (Aregger, 1992; Kleespies and Zimmermann, 1992; Lee Sang Myeong, 1996; Lecuona et al., 1996; Narvaez, et al., 1997; Arcas et al., 1999).
It is concluded from the results of the above studies that there are possibilities of utilization of cheaper natural media as well as the wastes/by-products for cultivation of *B. bassiana*. Cultivation of entomogenous fungi on by-products and wastes will further help in better management of these materials for useful purposes and also in reduction of environmental pollution.