DISCUSSION
Germination

Of three different treatments (distilled water, 2 and 5 per cent sucrose solutions), 5 per cent sucrose solution was found superior over 2 per cent sucrose solution and distilled water for germination of *M. anisopliae*. These findings are in consonance with that of Dillon and Charnely (1990). They reported that spores of *M. anisopliae* failed to initiate spherical growth in distilled water. However, a prolonged period of soaking (up to 20 h) in distilled water accelerated spherical growth and germ - tube formation once a nutrient was provided. Hall et al. (1994) reported the influence of culture age on rate of conidial germination in Deuteromycetous entomogenous fungi. Unlike *M. anisopliae*, highest conidial germination was obtained on distilled water in different isolates of *B. bassiana* which decreased significantly with 2 and 5 per cent sucrose solution. However, in case of Bbr-1 conidial germination did not vary with the treatments. This shows that for conidia of *Beauveria* spp. no extra source of nutrients was required for initiation of germination. The reserve food present in the body of conidia may probably be adequate to initiate their germination. In the present study, some isolates (Ma-4, Bb-3 and Bbr-1) showed higher germination over others within the same treatment, indicating their high viability or ability to germinate and eventually high virulence against their target pest.

Liquid media

The media containing same source of carbon or nitrogen may not be equally good for growth and sporulation of a fungus. It is also not necessary that a
medium which is good for growth may also be good for sporulation or vice versa. Source of carbon and nitrogen in a liquid medium determines the growth and sporulation in *M. anisopliae* (Li and Holdom, 1995). In the present study, Richards' medium supported an excellent growth of *M. anisopliae* but did not produce good sporulation. Whereas in other media having same source of carbon that is sucrose had produced good growth and good sporulation. The molasses yeast broth having same source of carbohydrate but in crude form (Gur) produced good growth and highest sporulation in all the three entomopathogens (*M. anisopliae, B. bassiana, B. brongniartii*) under test. This may probably be due to yeast extract (0.5 %) as a source of nitrogen in the medium. This is in accordance with the findings of Rombach (1989) who reported that conidia production and hyphal growth of *B. bassiana* were the highest in the medium containing 2 % sucrose and 0.5 % yeast extract. Whereas high amount of sucrose (2.5 %) and yeast extract (2.5 %) enhanced the hypal growth only. Molasses yeast medium had also been found superior for excellent sporulation in *Trichoderma* sp. (Jeyarjan et al., 1994), an antagonist to plant pathogens. Sabouraud's dextrose was at par to molasses yeast in growth and sporulation for all the fungi. Li and Holdom (1995) found that soluble starch and soy peptone used as a source of carbon and nitrogen in a agar medium supported highest growth and sporulation in *M. anisopliae*, however we did not use this combination in any of the medium tested. Good growth and sporulation in *M. anisopliae* and *Beauveria* spp. with Sabouraud's liquid medium might be due to presence of peptone as a source of nitrogen.
Agar media

On agar media, trend of growth in test fungi measured in terms of colony diameter was different than it was in liquid media. Semi-synthetic media like potato dextrose agar (PDA) and tapioca potato dextrose (TDP) supported maximum growth for all the isolates of *M. anisopliae* over carbohydrate rich synthetic media like Richards’, Czapek’s and Sabouraud’s. In case of *B. bassiana* and *B. brongniartii*, higher growth was measured on all the agar media except the Richards’ medium. It is mainly because colony density or thickness are not measured on agar medium (Li and Holdom, 1995). Because of this reason, Richards’ medium despite supporting the highest growth in liquid medium exhibiting poor growth on agar medium. However, trend of sporulation on agar media was same as in liquid media. The highest sporulation was obtained either on Sabouraud’s agar (*M. anisopliae*) or on molasses yeast agar (*Beauveria* spp.). High spore production of *M. anisopliae* on Sabouraud’s agar medium is in conformity of the findings of Daoust and Roberts (1983).

Grain media

All the isolates sporulated moderate to profusely on barley, chickpea, cowpea, maize, pearlmillet, rice, sorghum and wheat whole grain substrates. In the present investigation, media requirement differed slightly for isolate to isolate. In general, pearl millet whole grain medium yielded the highest sporulation in case of Ma-3 and Ma-4 isolates, while chickpea and sorghum were better for other isolates (Ma-1 and Ma-2). Earlier, a culture medium containing 6 per cent of rice (Frigo and Lucio - de - Azévedo, 1986) and use of coarse grain rice rather than the whole grain rice (Quintela, 1994) were found superior for high conidia
production in *M. anisopliae*. Similarly, rice media used in plastic trays and then inoculating with mycelia had produced $10^{10}$ conidia g$^{-1}$ (Alves and Pereira, 1989). Daoust and Roberts (1983) obtained the highest yield of conidia on rice with water content of 200 per cent.

All the isolates of *B. bassiana* and *B. brongniartii* sporulated on eight whole grain media used under study. However, rice (7.56 x $10^7$ - $3.05 \times 10^8$ g$^{-1}$) and cowpea (2.54 x $10^7$ - $6.34 \times 10^8$ g$^{-1}$) grain substrate supported the highest sporulation. The highest spore production on rice grain substrate is in conformity to the findings of Frigo and Lucio - de - Azevedo (1986). Aregger (1992) reported the best sporulation of *B. brongniartii* on whole barley grains after 14 days of incubation.

In the present study, though adequate sporulation was achieved on barley grains, but it was less ($1.24 \times 10^8$ g$^{-1}$) as compared to cowpea grain ($6.34 \times 10^8$ g$^{-1}$). This may probably be due to difference in water content of the grains and the incubation period. Apart from this, differential response of media may be attributed to either differential behaviour of isolates, different techniques of culturing, length of incubation and moisture content of the medium.

**Temperature**

*M. anisopliae* could grow at the range of 18 to 40 °C, but 28 °C was optimal for growth and sporulation of all the four isolates. This was closely followed by 25 and 22 °C. The optimum temperature for growth of *M. anisopliae* was 27-28 °C (Burges, 1981); 20-30 °C (Glare and Jackson, 1992); 27.5 °C (Mietkiewski et al., 1994) and 28 °C (Sharma et al., 1998). Temperatures below 20 °C and above
35 °C were not suitable for sporulation in the present isolates. Similarly, Walstad et al. (1970) found that spores of *M. anisopliae* did not sporulate at temperatures below 10 °C or above 35 °C. According to them most rapid sporulation occurred at 30 °C, *in vitro*, Maximum growth of *M. anisopliae* occurred at 25 °C with upper temperature limits of 28 - 37 °C. (Fargues et al., 1992). However, selected strains of *M. anisopliae* have also been reported which could tolerate temperatures below 15 °C (Milner, 1989) and as low as 5 °C (Rath et al., 1989). In the present study, a temperature below 18 °C, was not tried hence it is difficult to infer that the isolates tested were not able to resist the lower temperatures. The growth at 40 °C was significantly more over the lowest temperature of 18 °C which indicates that the isolates tested in present case had a tendency to tolerate high temperatures. However, Rath et al. (1989) have reported an optimum growth temperature of 22 °C for one of the four Australian isolates of *M. anisopliae*, which could even grow at very low temperature of 5 °C. This might be due to either variation in strains or different cultural conditions. Sporulation in all the four isolates of *M. anisopliae* was found maximum at 28 °C and none at 18, 35 and 40 °C in the present investigation.

Similarly, *B. bassiana* isolates could grow at 18-40 °C, but 20-28 °C were significantly superior over 18, 35 and 40 °C. The growth at 20, 22, 25, and 28 °C was statistically at par with each other, supporting the findings of Walstad et al. (1970), Burges (1981), Fargues et al. (1992), Metkiewski et al. (1994) and Sharma et al. (1998). In the present study, sporulation in all the three isolates was maximum at 25 °C followed by 22, 28 and 20 °C in pathogenic isolates of Bb-3 and Bb-1. The absence of sporulation at 35 and 40 °C indicates that
Beauveria isolates can not resist to higher temperatures. This finding is in consonance with the results of Walstad et al. (1970). Kuberappa and Jayaramaiah (1987) have reported that 20-30 °C was the optimum for growth, sporulation and development of B. bassiana. For the growth of B. brongniartii 28 °C was the optimum, but maximum sporulation occurred at 22 °C. Ferron (1967) reported that 23 °C was the optimum temperature for vegetative growth as well as for infection of Melolontha melolontha with B. brongniartii. The mortality of Rhizotrogous majalis due to B. brongniartii has been reported to be significantly higher in soil at 21 °C than at 27 °C (Krueger et al., 1991). The optimum temperatures required for infection of M. melolontha and R. majalis are in accordance with the optimum temperatures of 22 °C required for sporulation of B. brongniartii in the present study.

**Hydrogen-ion concentration (pH)**

Effect of eight pH (4.5 - 8.0) on four isolates of M. anisopliae revealed that maximum growth and sporulation occurred at pH 6.0 - 6.5, closely followed by the growth at 7.0 pH and the least growth was obtained at pH 4.5 while none of the pH was found to be inhibitory for growth of any of the isolates. Data on pH of the filtrate recorded after 15 days of incubation revealed that the original pH were changed at all the levels. Change in pH of the filtrate was more pronounced in higher (7.0 - 8.0) original pH than the change in lower pH levels (4.5 - 5.5). This indicates that the pathogens can not grow in too acidic or too alkaline pH medium. Higher growth and sporulation were recorded at pH, close
to neutral (6.0 - 6.5) and this level remained unchanged with the result of growth of the pathogen. Likewise, pH level of 6.5 was found most favourable for growth and sporulation of B. bassiana and B. brongniartii. The requirement of pH did not vary among the isolates. Significantly less growth at 4.5 and 8.0 indicates that acidic and alkaline medium were not suitable for growth of the Beauveria isolates used under this study. The original pH of the medium changed after growth of the pathogen in all the isolates. The pH adjusted between 4.5 - 5.5 increased from 5.0 - 6.5 in the filtrate, whereas, original pH adjusted between 7.0 - 8.0 fell sharply in the filtrate. this further indicates that Beauveria isolate can be grown at pH near to neutral (6.0 - 6.5), as their was no change in original pH of 6.0 and 6.5. Similarly, trend of growth at various pH levels and shift in pH after growth were obtained by Galani (1987) in case of B. bassiana.

Relative humidity (RH)
In general, growth of Metarhizium isolates occurred over a range of 33 - 98 per cent, but 53 per cent RH was found optimum for growth of all the isolates. Like M. anisopliae, a RH level of 53 per cent was also optimum for the growth of Beauveria isolates.

Conidia of E₉ strains of M. anisopliae were most susceptible to RH values of 33 per cent, while 75 per cent RH was found detrimental to spores of other strains. Clerk and Madelin (1965) found that isolates of M. anisopliae to be most susceptible to RH values between 40 and 50 per cent. Merck and Fergus (1954) also investigated that viability of conidia decreased with increasing levels of RH. In present study, though no definite trend was observed, yet the highest growth
of all the entomopathogens at 53 per cent RH level is in consonance of the earlier studies on RH. Therefore, RH is not likely to affect the efficacy of entomopathogens, *Metarhizium* and *Beauveria* spp. to infect or kill the insects.

**Pathogenicity**

The pathogenicity of four isolates of *M. anisopliae* (Ma-1, Ma-2, Ma-3, Ma-4), three isolates of *B. bassiana* (Bb-1, Bb-2, Bb-3) and one of *B. brongniartii* was tested against *H. consanguinea* and *M. insanabilis*, using lab plate method (Gupta et al., 1998). Ma-4, Bb-3 and Bbr-1 isolates of *M. anisopliae*, *B. bassiana* and *B. brongniartii*, respectively were found most virulent and cause 100 per cent mortality with in one week time. Even the weakest isolate (Bb-2) took 13 days in causing mortality, showing the effectiveness of the test procedure. This shows that a considerable variations exists among the isolates. Earlier, such type of variations have been reported in *Metarhizium* (Samuel, 1989; Poprawski and Yule, 1991; Gupta et al., 1998) and *Beauveria* isolates (Ferron, 1981; Li, 1988; Poprawski and Yule, 1991, Khachatourians, 1992, Shimazu, 1994; Gupta et al., 1998).

The simplicity of this procedure indicates its potential usefulness in the evaluation of entomogenous micro - organisms and the fungi attacking scarabs. The advantage include, large scale testing of diverse fungi isolated from insects and soil samples with in a short period of incubation without requiring any extra laboratory space. Laboratory methods for establishing pathogenicity of *B. bassiana* and *M. anisopliae* on *Culex tritataeniornynchus* and *Aedes aegypti* (Sandhu et. al., 1993 ) and *B. bassiana* on different stages of rice hispa on plant
host (Puzari et al., 1994; Hazarika and Puzari, 1995) have been tried but no information is available for testing large number of isolates of these pathogens against scarabs.

Compatibility

*M. anisopliae* was found more tolerant to all insecticides than to the fungicides. Blitox -50 (Copper oxychloride) and azadirachtin were very well tolerated even at 2000 ppm concentration. Whereas, kavach, ridomil MZ, thiram, dithane M -45, chlorpyriphos and monocrotophos were tolerated well at lower concentrations.

Compatibility of *M. anisopliae* with contact fungicides like blitox (copper oxychloride) and thiram (TMTD) are in agreement with the result of Duarte and Menendez (1989). In general, tolerance of the pathogen to a particular pesticide was dependent on its concentration. Increasing the concentration of the chemicals, caused significant reduction in fungal growth rate. Bavistin and endosulfan were found inhibitory to the fungal growth (*M. anisopliae*) by more than 50 per cent at lower concentrations, their contact with *Metarhizium* is likely to be detrimental and it will be important to avoid such contact in the field.

Toxicity of systemic fungicides like bavistin to *M. anisopliae* is in conformity to the earlier findings (Duarte and Menendez, 1989; Moorhouse et al., 1992; Li and Holdom, 1994). However, tolerance to ridomil MZ at lower concentrations as also reported by Vainio and Hokkanen (1990) could be due to the component of mancozeb in the formulation. Kavach (chlorothalonil) was well tolerated by *M. anisopliae* but according to Moorhouse et al. (1992), it inhibited the germination of *Metarhizium* conidia. Zimmermann (1975) also concluded that the germination of fungi was less sensitive to pesticides than growth. In contrast,
Hall (1981) found that germination of *Verticillium lecanii* was more sensitive. This partly supported the results of chlorothalonil in the present study. Difference in results may also be attributed to methods and doses of pesticides used. Tsai et al. (1992) reported that chlorpyrifos had the most inhibitory effect on *M. anisopliae* while in the present study, it was mildly tolerated at lower concentrations. This was in agreement with the findings of Li and Holdom (1994) who indicated that lorsban (chlorpyrifos) could be tolerated by *Metarhizium anisopliae* EF25 at lower concentrations. They have also reported differential response of a given pesticide to different isolates.

Like *M. anisopliae*, *B. bassiana* could grow well on Czapek's agar medium amended with blitox - 50, azadirachtin and kavach and was highly sensitive to ridomil MZ and bavistin. Dithane M-45, thiram, monocrotophos, chlorpyrifos, quinalphos and endosulfan were however, moderate in tolerance. The result of Vainio and Hokanen (1990) indicated that ridomil MZ did not affect the growth of *B. bassiana*. Results of blitox - 50 are in agreement with that of Olmert and Kenneth (1974) and Malo (1993) in respect of *B. bassiana*. Toxicity of benomyl to *B. bassiana* has earlier been reported (Aguda et al. 1988 and Olmert and Kenneth, 1974).

Blitox - 50 and azadirachtin were very well tolerated by the test pathogen even at the highest concentration of 2000 ppm. Toxicity of benomyl to *B. brongniartii* has been demonstrated by Zimmermann (1975). Thiram or mancozeb are generally used as seed treatment for the control of collar rot disease in groundnut, so the entomopathogens like *Metarhizium* and *Beauveria* could be used in conjunction with these chemicals. However, contact with bavistin (carbendazim) be avoided. Further study is needed to ascertain whether these
chemicals are toxic or compatible to entomopathogens in the field. Because addition of pesticides to media represents a severe test which, it could not normally occur in field conditions.

**Antagonism**

Soil micro-organisms interfere with the activity of pathogens directly by their growth inhibitory products, parasitism and indirectly by competitions for nutrients and oxygen (Sanford, 1926).

Of the eight soil fungi, *Penicillium* sp., *T. viride*, *R. bataticola* showed a strong antagonistic activity against all the three entomopathogenic fungi under study. However, *Penicillium* sp. was found more dangerous, reducing the growth of pathogenic fungi form 50 - 79 per cent. In general, the highest suppression of growth was recorded in *B. bassiana* and lowest in *M. anisopliae* which indicates that the latter has the ability to resist with the antagonist as compared to the former. This study suggests that the presence of these antagonists in soil may reduce the population of the entomopathogenic fungi. *P. urticae* has been reported to be the major cause in reduction of level of inoculum of *B. bassiana* (Lingg and Donaldson, 1981). *Penicillium*, *Trichoderma*, and *A. niger* have also been reported as potential inhibitors against plant pathogen (*Fusarium solani*) by Sharma and Sen (1991). Entomopathogens among themselves were also found to compete with each other in the present study and thus can affect the efficacy of potential bioagent.

Conversely, as a result of interaction between soil fungi and entomopathogens, growth of soil fungi was also suppressed by the entomopathogens, when grown
in dual culture. The growth of *T. viride*, *R. bataticola* and *Penicillium* sp. was not at all inhibited by any of the entomofungi. However, other soil fungi were markedly suppressed by *M. anisopliae* and *B. brongniartii*. However, *B. bassiana* did not suppress the growth of any of the test soil fungi, showing its poor ability to compete with saprophytic fungi. Some entomogenous fungi were reported to possess antibiotic properties against different common saprophytes (Walstad *et al.*, 1970).

Behaviour of known entomofungi among each other revealed that *M. anisopliae* suppressed the growth of *B. brongniartii* by 25 per cent and that of *B. bassiana* by 40 per cent. *B. bassiana* was also suppressed by *B. brongniartii*. It suggests that a considerable antagonism also exists among different entomopathogenic fungi. Although results of *in vitro* studies were not always related to degree of antagonism observed in the field but they reflect the antagonistic potential of the micro-organisms.

**Multiplication and formulation**

*M. anisopliae* (Ma-4) mass multiplied on molasses yeast broth had produced $8 \times 10^8$ conidia ml$^{-1}$ when cultured on 200 ml broth in 1000 ml capacity Erlenmeyer flasks after 25 days of inoculation. It was interesting to note that under liquid media study, the spore production on 25 ml of the same medium (molasses yeast broth) in 100 ml cap. flasks was obtained only $3.5 \times 10^7$ conidia ml$^{-1}$ as compared to $8.0 \times 10^8$ conidia ml$^{-1}$ in 1000 ml flask. Less production of conidia per ml in this study may probably be attributed to availability of less surface area
in 100 ml cap. flask for growth and sporulation of *M. anisopliae*. The high production of *M. anisopliae* was observed on YAA agar (Abali's, 1981) based on Ypss agar by Daoust and Roberts (1983). Therefore, the best conidial production in molasses yeast broth could be described due to the presence of yeast in the medium. In USSR, *Metarhizium* has been produced in two phase system (Goral and Lappa, 1973), the first phase involved submerged fermentation using corn extract and molasses with continuous aeration. The mycelial mass was transferred into open pans which yielded 5 to 7.5 x 10^{12} g^{-1} of dried preparation. This further confirms the usefulness of the molasses medium employed in the present study.

In case of *B. bassiana* and *B. brongniartii*, spore production (1 x 10^9 and 2 x 10^9 conidia ml^{-1}) was still higher on molasses yeast broth than it was in the *M. anisopliae*. Result of the present study are in consonance with the findings of Rombach *et al.* (1988, 1989), who investigated that a liquid medium containing sucrose and yeast extract produced maximum mycelial growth and many conidia of *B. bassiana*.

The mass multiplication of entomofungi done on the grain media indicated that *M. anisopliae* produced maximum yield of conidia (2 x 10^9 g^{-1}) on crushed maize grain medium. In the grain media study, though pearl millet grain produced the highest conidia but because of its sticky nature, the harvesting of conidia from this medium became little difficult. Therefore, the next best maize grain medium was selected for mass multiplication of *M. anisopliae*. Secondly, use of whole grain of maize in the grain media experiment may also be responsible for comparatively less conidial production. Therefore, use of crushed maize grain instead of whole grain in the mass multiplication might
have accelerated the conidia production. However, previous workers have reported that rice either used as a constituents of a medium (Moura Costa, 1974 and Frigo and Lucio-de-Azevedo, 1986) or as a whole grain (Daoust and Roberts, 1983 and Quintela, 1994) provided the highest yield of *M. anisopliae* conidia. This indicates that the suitability of a particular grain medium may vary with differential requirements of the strains or due to difference in production technology used. Nevertheless, rice grains, in present grain media study was comparable with that of maize grain medium, yet maize grain medium was preferred because of its low cost and economic feasibility over rice grains.

Grain media requirement varied among different species of fungal pathogens. For *Beauveria* spp. cowpea grain medium was proved best for their conidial production. *B. bassiana* and *B. brongniartii* mass cultured on cowpea grains filled in two kg. cap. polypropylene (PP) bags yielded $1.5 \times 10^9$ and $1.8 \times 10^9$ conidia g$^{-1}$ of dry grain weight, respectively in the present study. The conidial yield was much higher than that of the same grain media but used into 250 ml conical flasks instead of PP bags, in another experiment. In China, substrates like steamed wheat bran, rice powder compost or ground corn stalks were used to inoculate with fermented cultures of *B. bassiana* and found suitable for mass production of *B. bassiana* (Hussey and Tinsley, 1981), whereas rice media in plastic bags used by Alves and Pereira (1989) were found more suitable for higher mycelial and conidial production ($2 \times 10^{11}$ conidia g$^{-1}$) when mycelia developed in bags were transferred to plastic trays.

For *B. brongniartii*, most efforts have been directed towards blastospore production in deep tank fermentation even though, these spores were neither infectious nor persistent in the environment as that of true conidia (Ferron,
1974). In the present investigation, *B. brongniartii* was also cultured on cowpea whole grains, which produced $1.8 \times 10^9$ conidia g$^{-1}$ of dry weight. Aregger (1992) reported the high yield of $1 \times 10^8$ to $2 \times 10^9$ conidia g$^{-1}$ on whole grains of barley and documented that yield of conidia depend mainly on the addition of water and the length of incubation. In the present study, whole cowpea grains were preferred for mass multiplication of *B. brongniatii* as the whole grains of barley in our next experiment proved inferior to it in conidial production, yet, conidial production on whole grain barley was $1.2 \times 10^8$ g$^{-1}$. However, spore production varied amongst the strains (Aregger, 1992).

All varieties of *B. popilliae* are obligate pathogens that sporulate only in haemolymph of living scarabaeid (Steinkraus, 1959). Therefore, in the present study third instar grubs of *H. consanguinea* were used for mass multiplication of this bacterium. A spore suspension of $7.5 \times 10^8$ cells ml$^{-1}$ was obtained by macerating the infected grubs in sterilized water. The concentration of the spores in the suspension could be increased or decreased depending on the number of infected grubs used and the volume made up. As high as two billion cells in each infected grub has been reported (Fleming, 1968).

It is concluded that molasses yeast broth medium was found most suitable with regard to its low cost and easy preparation for mass multiplication of *M. anisopliae*, *B. bassiana* and *B. brongniartii*. Among the grains media, crushed maize grain for *M. anisopliae* and whole grains of cowpea for *Beauveria* spp. were the best for their mass production. However, further research is needed to pinpoint the various factors like moisture content, aeration, light, optimum amount of inoculum etc. which affects the conidial production.
Shelf life of formulation

Shelf-life is considered a pivotal factor that determines the commercial success of a biocontrol agent as well as its efficacy. Clark et al. (1968) considered that mycoinsecticide formulation could be successful if fungus conidia remained viable for 7 weeks. However, Couch and Ignoffo (1981) advocated a shelf life of 12-18 months. In the present investigation, conidial survival was studied in myco-insecticidal formulation only up to six months.

Of the three entomopathogens (M. anisopliae, B. bassiana and B. brongniartii), M. anisopliae had the ability to survive up to 180 days at three storage temperatures viz. room temperature, refrigerator and deep freezer. Earlier workers have also reported that conidia remained highly virulent at lower temperatures. Daoust and Roberts (1983) and Daoust et al. (1983) working with the storage viability of unformulated conidia of M. anisopliae reported that as the temperature declined from 37 to 4°C, longevity of spore increased and remained virulent against Culex pipiens pipiens. On the other hand, Metarhizium conidia in dust formulations survived longer than unformulated conidia (Daoust et al., 1983). In the present study, survival period of Metarhizium conidia was longest at lower temperatures (4°C and -20°C) as compared to the room temperature (17.90 - 36.70 to 24.85 - 41.70 °C). These findings are in agreement with Daoust and Roberts (1983) and Abreu et al. (1987). Studies also suggest that prolonged storage of conidial formulations at lower temperatures might retained infectivity against the test insects as was found with M. anisopliae against Culex pipiens by Daoust and Robert (1983). Retention of conidial viability in deep freezer was still higher than in a refrigerator at 4°C which corroborates the findings of Abreu et al. (1987.)
Survival period of *B. bassiana* was shorter than *M. anisopliae* at all the three temperatures studied, however, it was higher at lower temperatures. Sandhu *et al.* (1993) found that conidia of *B. bassiana* survived longest at 0 to 20°C and at lower RH levels (0 - 53 %), but at higher temperature (30 - 40°C) conidia did not survive.

In the present study, role of different RH was not studied and only dry formulations were stored at different temperatures. Higher temperatures (30 - 40°C) coupled with different RH levels were found to be lethal for conidial viability of *B. bassiana*. Since in the present studies, formulations were stored under dry conditions, might have resisted to the higher temperatures prevailed during storage at room temperature (17.90 - 36.70 to 24.85 - 41.70 °C).

In *B. brongniartii*, survival period was almost at par with that of *M. anisopliae*, but the reduction in conidial viability was faster. Aregger (1992) also examined the effect of storage on *B. brongniartii* conidia at 2°C and found that the number of viable conidia decreased over a period of two year.

It is apparent from the studies that *M. anisopliae* conidia had the ability to survive for a prolonged period even at high temperatures, as compared to *Beauveria* spp. This suggest that *M. anisopliae* formulation can be stored for a considerable long period. It is therefore, imperative that storage conditions which increase the shelf life of a microbial insecticide should be identified and that routine determination of conidial viability in a laboratory should be performed before the formulation is applied in the field.
Bioefficacy against beetles

Bioassay studies with fungal entomopathogens against adults (beetles) of *H. consanguinea* and *M. insanabilis* had revealed their effectiveness under laboratory conditions. Among them, an isolate of *M. anisopliae* (Ma-4) showed the highest infectivity to the beetles with median lethal time of 6.02 and 4.84 days in respect of *H. consanguinea* and *M. insanabilis* when the adults were exposed to $7 \times 10^7$ conidia ml$^{-1}$. Lobo-lima (1990) reported over 90 per cent mortality in adults of sweet potato weevil (*Cylas puncticollis*), when they were exposed to $5 \times 10^7$ conidia ml$^{-1}$ suspension of *M. anisopliae*. This confirms the findings of present studies. According to Lacey et al. (1994) LT$_{50}$ values for adults of Japanese beetle (*Popillia japonica*) was 4.2 days on exposure to *M. anisopliae* at the rate of 10 mg/100 beetles. Similarly, *B. brongniartii* and *B. bassiana* also caused considerable adult mortality in the present study. The results of *B. bassiana* were slightly different as compared to the findings of Lobo-lima (1990) and Lacey et al. (1994), they found that *B. bassiana* was highly effective against adults of potato weevil and 8th Japanese beetle, respectively.

The LT$_{50}$ value for Japanese beetle was 3.1 days when exposed to 10 mg conidia of *B. bassiana* per hundred adults (Lacey et al., 1994). Low efficacy of *B. bassiana* in the present case may probably be attributed to the different strain or different incubation temperatures during the study.

Bioefficacy against grubs

Bioefficacy of three entomopathogens viz., *M. anisopliae*, *B. brongniartii* and *B. bassiana* was tested against three larval stages of *H. consanguinea* and *M.*
insanabilis using three different doses of each pathogen with two inoculation methods i.e. insect inoculation and soil inoculation. In general, it was noted that time required for hundred per cent mortality as well as median lethal time were prolonged progressively with the advancement of larval stages, irrespective of method of inoculation and test insect species. The concentration of inoculum and time required for grub mortality were inversely correlated.

Early mortality of *M. insanabilis* at a particular dose of inoculum of the pathogen could be due to high susceptibility of this species as compared to *H. consanguinea*. Similar observation have been reported by Moorhouse *et al.* (1993) that $5 \times 10^8$ conidia ml$^{-1}$ dose of *M. anisopliae* caused 60 and 43 per cent mortality in *Sikkimia japonica* and *Yiburnum plecatum*, respectively.

In the present investigation, it was observed that *M. anisopliae* showed high virulence against the test insects than *B. brongniartii* and *B. bassiana*. The findings are in consonance of the results derived by Garcia *et al.* (1990) in case of *Lissorhoptrus brevirostris*. The mortality due to *B. brongniartii* was almost comparable with that of *M. anisopliae* in all the instars and it was much higher than *B. bassiana*. The poor efficacy of *B. bassiana* over the former us in accordance with the findings of Shimzu (1994). The effectiveness of *B. brongniartii* has earlier been reported against *H. consanguinea*, *H. serrata* and *M. nathani* (Vyas *et al.*, 1990 and against *Rhizotrogous majalis* (Krueger *et al.*, 1991).

In insect inoculation method, median lethal time ($LT_{50}$) required was comparatively less than it was required by soil- inoculation method for both, *H. consanguinea* and *M. insanabilis*. Early mortality in insect inoculation method may probably be due to direct contact of the pathogen with the cuticle of the
insects and similar observation were also recorded by Poprawski and Yule (1991) using *M. anisopliae* against *Phyllophaga* sp. Enhanced mortality with the increase in dose of inoculum is in accordance with the findings of Vimla Devi (1994) in case of *Nomurea rileyi* tested against *Spodoptera litura* on castor. Over 90 per cent mortality was achieved when high doses of *M. anisopliae* and *B. bassiana* were used against red-head cockchafer (Rath, 1989).

Different kinds of fungal formulations like mycelial formulations, conidial formulations have been used by different workers, however, Aguda et al. (1987) while evaluating different doses of dry mycelium and conidial suspensions of *B. bassiana* reported that dry mycelium preparations were as effective as that of conidial applications. Thus, present studies with conidial preparations of different entomopathogens are comparable with that of mycelial formulations used by other workers.

It is apparent from the foregoing discussion that all the three fungal pathogens under test are potential candidates for microbial control of the whitegrubs. However, further investigations are needed for their formulations which could be effective under field conditions.

Efficacy of *B. popilliae* dust formulation mixed in soil was tested against three larval stages of *H. consanguinea* and *M. insanabilis*. Milky disease symptoms started developing in second and third instars of both the insects after twenty days of grub release in artificially infested soil. But maximum infection was achieved after 30 days of inoculation. In the first instars, however, disease symptoms did not develop until 15 days of inoculation. This shows that either the bacterium (Bp) required more incubation period to develop in the body of the insect or the first instar larvae were not able to ingest the required amount of
inoculum. A high infectivity of 54-61 per cent at higher doses of inoculum
\((2.5\times10^7 \text{ g}^{-1})\) as compared to 19-33 per cent at lower dose \((2.5\times10^6 \text{ g}^{-1})\)
indicate that an optimum level of inoculum was required for effective control of
white grubs. Besides, there were several other factors such as density of larvae
in the field (Fleming, 1968); pH (Beard, 1945); organic manures (Rao and
Veeresh, 1988) and nutritional state of the larvae (Milner, 1981) which could
affect the potency and development of milky disease bacterium. Yadava (1992)
reported that \textit{B. popilliae} applied as drilling in soil caused up to 46 per cent
infection in \textit{H. consanguinea}. Therefore, more information is needed on all of the
factors influencing milky disease development and to use \textit{B. popilliae}
formulations to their fullest potential.