SUMMARY

1. A review of literature pertaining to this investigation has been presented which deals mainly on termites and entomopathogenic fungi

2. Materials used in this investigation and experimental procedures followed have been discussed in detail.

3. *B. bassiana* strains 135,984, 2028, 1216 and *M. anisopliae* strains 140 and 892 were grown initially on four different culture media : yeast extract-glucose-agar (YGA) , potato-dextrose agar (PDA), potato-carrot-agar (PCA), and beef extract-agar (BA). Since maximum mycelial growth and sporulation took place in PDA and YGA media, these two media were selected for further experiments. Experimental results showed that 28-day-old cultures of *B. bassiana* isolate 2028 produced highest conidial concentration while *M. anisopliae* isolate 140 registered lowest conidial concentration. To determine the compatibility between the isolates, they were grown together in the same media. Excellent growth was observed between *B. bassiana* isolates 2028 and 984; 1216 and 984 and between *B. bassiana* isolate 135 and *M. anisopliae* isolate 140. In this study no antibiosis effect was observed between the isolates.

4. Four isolates of *B.bassiana* and two isolates of *M.anisopliae* were used for haemocytometer counts and transmission measurements from 28-day- old cultures for a period of 12 months.

5. Bioassays were performed with the isolates of *B.bassiana* and *M.anisopliae* to test against the worker termites (*Odontotermes obesus*) under laboratory conditions. All the isolates proved to be pathogenic to the termites at different degrees. *B.bassiana* isolate 2028 gave the lowest

lethal concentration (LC_{50}) of 1.334 x 10^4, 2.818 x 10^4 and 5.012 x 10^4 conidia / ml at bioassay - I, II and III respectively.. The Lethal time for 50% mortality (LT_{50}) was also recorded to be lowest (56.23 hrs, 50.12 hrs and 53.09 hrs respectively) when treated with isolate 2028 of *B.bassiana*.

6. The cumulative percent mortality among workers of *O.obesus* at different intervals was studied using two isolates (135 and 984) of *B.bassiana*. The isolate 135 required 5, 4, 4, 3 and 5 days exposure period to kill 50% of the termite population by 1 x 10^6 conidia / ml concentration from 10, 14, 20, 28 and 34 days old cultures respectively ; while isolate 984 required 5, 4.5, 4, 3 and 5 days exposure period to kill 50% of the termite population with the same conidial concentration and ages of culture.

7. Seven different substrates, such as, bajra, maize, wheat, wheat bran, groundnut, barley and rice bran were used for production of conidia. Different percents of water and sunflower oil were combined with the substrates to increase the conidial production. The highest yield of conidia varied between 6.55 x 10^8 and 1.15 x 10^9 conidia / ml of harvest from the bajra substrate on addition of 8% of oil and maintaining 70-80% moisture. The spore concentration of different isolates was tested for its bioefficiency against termite workers and the result showed that the highest mortality (88.8%) was achieved with *B.bassiana* isolate 2028. Molasses yeast broth was selected as a synthetic medium for mass scale production for all the isolates. Production was highest with *B.bassiana* isolate 2028 followed by isolate 1216. Their bioefficiency were determined against worker termites (*O.obesus*). The mortality was highest (89.6%) with *B.bassiana* isolate 2028.

8. Food preference tests on termites were conducted with different substrates; maize, bajra, Glyricidia powder, rice bran, filter paper and

wheat bran, following two different methods such as ' glass trough test ' and ' four arm glass chamber test '. The test results showed that bajra and Glyricidia powder were the most preferred substrates by the termites. Food deterrence tests were also conducted by the methods of ' glass trough test ' and 'four arm glass chamber test' . The isolates of *B.bassiana* and *M.anisopliae* mixed with substrates deterred the termites. The highest and the lowest deterrence were provided by the *B. bassiana* isolates 984 and 135 respectively.

9. A study on the effect of soil factors on the pathogenicity of *B. bassiana* and *M. anisopliae* isolates showed that there was no significant difference in the mortality of the termites (*O. obesus*) on acid and alkaline soil treated with the isolates. Under higher (60 and 90%) and lower (15 and 30%) moisture content, 57.67 - 86.33% and 10-29.67 % mortality was observed respectively.

10. Different types of formulations such as dust, wettable powder and liquid solutions of the entomopathogenic fungi were prepared and their efficacy against the termites were determined. Among dust formulations, clay formulation gave the highest percentage of mortality (75.3%). Among the wettable powder formulations, Dedenol recorded highest mortality (80%). Whereas in liquid formulations, Tween 80 showed highest percentage of mortality (90.7%). Certain adjuvants and UV protectants were also applied in the formulations to enhance their efficacy. With the addition of 0.2% sunflower oil in the formulations and their application, mortality of termites increased up to 92.7 percentage. A field trial on tea bushes was conducted to evaluate the efficacy of the liquid formulation alone and in different combinations with chlorpyriphos against live wood termites. Chlorpyriphos alone could reduce the termite infestations on the tea bushes within 3 weeks while the liquid formulation could do the same within 6-7 weeks time.

11. For the suppression of subterranean termites population in field, bait application was undertaken which showed that the population of *O. obesus* in colony-I reduced from 742495 to 102528 after 9 months, that of colony-II from 1266894 to 71880 and that of colony-III from 674025 to 73782 after 7 months of baiting.

12. The formulated mycoinsecticide was also tested for its efficacy against the termites colonies in the plantation area of *Dalbergia sisso, Albizia lebbeck, Michelia champaca* and *Lagerstroemia speciosa*. After the application of the formulated mycoinsecticide mortality of the plants due to termite infestation was not observed.

13. The storage life for the formulated strains of *B. bassiana* and *M. anisopliae* was measured by comparing the number of conidia sporulated after 6, 12 and 18 months of storage at $4^{o}C$. With the increase of storage period, the conidial concentration decreased gradually from 1.8×10^{8} to 1.9×10^{5} conidia / ml within 18 months of storage of the formulated isolate 2028 of *B.bassiana*. Isolate 140 of *M.anisopliae* showed the highest reduction of conidial concentration within 18 months of storage which is about 1.5×10^{8} to 1.1×10^{4} conidia / ml. Pathogenicity of fresh and stored formulated isolates of *B. bassiana* and *M. anisopliae* against *O. obesus* showed that percentage mortality was higher with the fresh formulated strains compared to stored ones.

14. The effectiveness of antigen preparation from the mycelia of *M.anisopliae* (isolate 892) and *B. bassiana* (isolate 2028) in raising

antibodies was checked following agar gel diffusion technique. Strong precipitin bands were observed when PAbs of *M.anisophiae* (892) and *B. bassiana* (2028) were reacted against its own antigens. However, weak or no precipitin bands could be observed in cross reactions between PAbs and antigens of other isolates.

15. Optimization of antigen and antibody concentrations were done using DAC-ELISA formats. Cross reactivity of PAbs of *M.anisopliae* (892) and *B.bassiana* (2028) was tested against mycelial antigens prepared from isolates of entomopathogenic fungi as well as other soil fungi . Maximum absorbance values were observed in homologous reactions.

16. Immunofluorescence tests were performed with the mycelia and spores of *M. anisopliae* (892) and *B. bassiana* (2028). Bright fluorescence was evident on young hyphae and conidia of both the isolates.

17. *M. anisopliae* (892) and *B. bassiana* (2028) from soil were also detected using dot-blot technique.

18. Molecular probing of different antigens with PAb raised against mycelial antigen of *M. anisopliae* isolate 892 was performed through Western blotting technique. Homologous antigen of *M. anisopliae* (892) exhibited 6 bands with molecular weights between 55-16 KDa. Amended soil antigen of *M. anisopliae* (892) was also probed with PAb of *M. anisopliae* (892) where four bands similar to homologous antigen were found.

Based on our present study it is apparent that the entomopathogenic fungi *M. anisopliae* and *B. bassiana* can form effective biological agents for the control of termites.