

DISCUSSION

Metarhizium anisopliae and *Beauveria bassiana* are wide spread entomopathogenic fungi on many insect species. Their high pathogenicity to termites was already recognized and their suitability in pest control was verified. However, all attempts to use the fungi as biological pest control agents failed for various reasons.

M.anisopliae and *B.bassiana* produce two types of spores, blastospores in the living host and conidia on the surface of the dead host. Blastospores are susceptible to environmental conditions and consequently less persistent than conidia. Artificial culturing of the fungi offer no particular difficulties; a utilizable source of carbon for germination, a nitrogen source for continuous hyphal growth, high humidity (80-100% R.H.), and temperatures between 20⁰C and 28⁰C, with an optimum at 25⁰C - 28⁰C, are necessary. The most common species, *M. anisopliae* and *B. bassiana* are already being successfully reared in mass cultures and formulated. In the present study the mass production of the fungi, their formulation and their molecular characterization are described. The mass production was necessary for field assay.

Isolates of *B. bassiana* and *M. anisopliae* showed high virulence towards the termites (*O. obesus*) as evidenced from the present laboratory and field evaluations. From the bioassays with spore concentrations of the isolates it was possible to determine accurately the LC₅₀ value for each isolate.

Previous studies have indicated that *B. bassiana* isolates induced >90% mortality by direct inoculation of *H. hampei* adult females at 1% spore concentration, whereas *M. anisopliae* isolates caused *H. hampei* mortality between 57% (Ma4) and 89% (MA3, Ma5) under similar conditions (Rosa *et al.* 2000). This difference may be related in

part to the origin of the various isolates; all *B. bassiana* materials were isolated from local populations of *H. hampei*, whereas the *M. anisopliae* materials originated from Lepidoptera and Homoptera from northern Mexico and the United States.

The LC₅₀ values of Bb25 and Ma4 towards the coffee berry borer were 4.1×10^6 and 4.2×10^6 spores per ml, respectively (Rosa *et al.* 2000). These figures represent half the LC₅₀ value of Bb25 to *P. nasuta* and a nearly identical value for the LC₅₀ of Ma4 to *P. nasuta*. It is therefore evident that these isolates are highly virulent to both the coffee berry borer and the parasitoid *P. nasuta*. In another study with *C. stephanoderis*, LC₅₀ values were found to be greater than that of *P. nasuta* which reflected that *C. stephanoderis* was substantially more resistant to fungal infection compared with *P. nasuta*. Niranjana (2002) also made an attempt to use *B. bassiana* in managing coffee berry borer.

Field evaluations on the impact of applications of bio-insecticides for the control of insects natural enemy populations are very limited in number. An evaluation of the impact of aerial applications of *Beauveria brongniartii* spores to forest habitat for control of *Melolontha melolontha* L. reported that only 1.1% of non target insects and spiders were infected by the pathogen of >10,000 individuals sampled.

In the present investigation it was clear that surface contamination had good effect on the incidence of fungal infection in the worker termite populations. Thus, it would be appropriate to apply the fungus in the field during the phase of termite infestation. With the field application of *B. bassiana* and *M. anisopliae* isolates the infestation ratio of the plants was significantly reduced.

In the present study, four isolates of *B.bassiana* and two isolates of *M.anisopliae* were tested against the worker termites (*O. obesus*) under laboratory condition. 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 1.334×10^4 , 2.818×10^4 and 5.012×10^4 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*.

In a bioassay test with the conidial suspension of five isolates of three entomopathogenic fungi for their infectivity to second instar larvae of *Spodoptera litura* , *B. bassiana* was found to be the most virulent (Prasad *et,al.*1989). The data on the dosage-mortality response of the larvae to the different fungal isolates following direct application of the conidial suspension indicated a good fit of the observed and expected responses based on a chi-square test. The regression coefficients in general were very low with all the fungal isolates. The comparison of LC_{50} however, revealed the differential susceptibility of the pest to the fungal isolates. There was a sharp increase in the values of LC_{50} in other isolates . When second, third and fourth instar larvae of the test insect were bioassayed for their susceptibility to *B.bassiana*, it was observed that the susceptibility to infection decreased with the age of the larvae.

In the present investigation, dosage-mortality responses of *O. obesus* to the isolates of *M.anisopliae* and *B.bassiana* in different bioassays (I, II, III) showed the observed and expected responses which were achieved from chi square tests. The values ranged from 0.212 - 1.160 in bioassay - I, while in bioassay - II and III the chi square values were found between 0.160 - 2.451 and 0.1185 - 3.0966 respectively.

The lethal concentrations, LC₅₀ and LC₉₀ of 12 fungal isolates were determined by Burdeos and Villacarlos (1989). All the isolates of *M.anisopliae* were highly pathogenic to adult sweet potato weevils, while those of *B.bassiana* were moderately pathogenic. They observed that the spore germination of *M.anisopliae* was 96% and *B.bassiana* was 95%.

Marked differences were observed in the virulence of the various isolates. The isolate that required the lowest concentration of spores to cause 50% mortality of the weevil population was considered the most virulent. Thus, the isolates arranged in decreasing order of virulence were Ma1 for *M.anisopliae* (8.42×10^5) and Bb1 for *B.bassiana* (1.54×10^7). Although Bb1 was relatively virulent, it was not comparable to any of the isolates of *M.anisopliae*. Unlike the latter, the two isolates of *B.bassiana* differed considerably in their pathogenicity to the sweet potato weevil. This is probably a common characteristic of the group as shown also by other workers. For instance, Fargues (1972) in testing seven strains of *B.bassiana* against the Colorado potato beetle, *Leptinotarsa decemlineata*, also found great variation in pathogenicity so that the most virulent strain caused 100% mortality in 5 days, while the least virulent caused only 10% mortality after 26 days. *B. bassiana* had been reported to infect adult sweet potato weevils but it appeared to have contributed very little to the mortality of the weevils in the field. Barson (1977) reported that varying conditions of temperatures and humidity determine the success or failure of a pathogenic fungus. In their study, the adult sweet potato weevils inoculated with *M.anisopliae* and *B. bassiana* were incubated at 27⁰C-30⁰C and 70-90% RH. The upper temperature ranges were within the optimum range of 27⁰C-28⁰C reported for *M.anisopliae* and 20⁰C-30⁰C for *B.bassiana*.

The experiment of Burdeos and Villacarlos (1989) indicated that virulence was a function of fungal species. However, spore concentration was also found to be an important factor in the expression of the virulence of the best isolates that were previously selected. For instance, at 1×10^5 spores / ml, mortality of the treated weevils was significantly greater than that of the uninoculated control in Ma1 and Bb1 treatments. However, a concentration of 1×10^8 spores / ml of all species significantly reduced weevil population under laboratory conditions.

The calculated LC_{50} values for the three entomopathogens were probably an overestimate because the assay did not consider the actual number of spores which came in contact with the weevil. In a related study using *B. bassiana*, Fargues (1972) estimated 5×10^4 to 1×10^5 spores / ml were required to cause 85 - 90% mortality among fourth - instar larvae of the Colorado potato beetle, *L. decemlineata*. Barson (1977) on the other hand, obtained 100% mortality of *Scolytus scolytus* larvae after 7 days exposure to more than 1×10^6 spores/ml of *B. bassiana*. The highly sclerotized cuticle in the adult weevils used in the present work probably formed a barrier against fungal infection by *B. bassiana*, so that higher concentration was required to cause infection compared with the above works.

At 1×10^8 spores/ml, the highest daily mortality of the insects exposed to Ma1 (41%) and Bb1 (47%) occurred on the third and fourth day after exposure, respectively. In all the treatments, percent daily mortality gradually declined until the seventh day when almost all the insects had died. In the present study, the *B. bassiana* isolate 135 required 5,4,4,3 and 5 days exposure to kill 50% of the termite (*O. obesus*) population while isolate 984 required 5,4,5,4,3 and 5 days exposure to kill 50% of the population of *O. obesus* and 1×10^6 conidia / ml concentration taking 10,14,20,28 and 34 day-old cultures.

In the present investigation, the varying effectiveness of the isolates of entomopathogens is reflected in the appearance of mycelial growth and the sporulation of the fungus on the body surface of the dead insect. Like many other entomopathogenic fungi, *M. anisopliae* and *B. bassiana* kill their host insect through the action of the hyphae which germinates from spores outside the body, penetrate the exocuticle, invade and ramify inside the body to subsequently destroy the internal tissues. Infected insects became restless, weakened and finally ceased feeding. Death due to mycosis occurred three days after inoculation. Dead termites became hard and their appendages turned brittle. About 24-48 hrs after death, whitish mycelia began to appear on the intersegmental regions and joints of the appendages of the dead insect. Sporulation on the body surface of the termite occurred on the fourth and fifth days due to infection by *M. anisopliae* and *B. bassiana*, respectively. By this time, the dead insects were almost covered with mycelial growth.

The present investigation proved that *B. bassiana* isolates 984, 2028, 1216 and 135 caused mycosis on *O. obesus* workers which was confirmed by direct microscopical examination and by culturing of the infected workers in water-agar plates. *M. anisopliae* isolates 892 and 140 also showed a similar response. The present observations agree with those of Burdeos and Villacarlos (1989)

The initial stages of insect infection by entomopathogenic fungi include the penetration of the host cuticle (Hajek and Leger, 1994). Cuticle solubilisation and subsequent hyphal penetration occurred by the action of extracellular enzymes and acid metabolites (Bidochka and Khachatourians 1987 and 1990). Following cuticle penetration, the fungus proliferates within the body of its host.

Burdeos and Villacarlos (1989) working with *Scolytus scolytu* larvae and *Cylas formicarius* adult weevils found that *B. bassiana*

required a higher spore concentration and a shorter post-treatment period to kill 50 percent of the worker population. Workers of *O. obesus* infected with *B. bassiana* and incubated at 27-30°C and 70-90% RH; experienced fast growth of the white muscardine fungus, thus causing high mortalities in the termites. They reported that the entomopathogenic fungi normally require about 100% RH and 20-30°C temperature for germination, growth and sporulation. The present study confirms the pathogenicity of *B. bassiana* and *M. anisopliae* against *O. obesus* workers.

The susceptibility of *Megalurothrips sjostedti* to *M. anisopliae* were evaluated on cowpea varieties by Ekesi *et al.* (2000). The result of their study showed that the susceptibility of *M. sjostedti* to *M. anisopliae* were dependent on the cowpea varieties. Mortality of the legume flower thrips was higher on the moderately resistant variety at all concentrations of inoculum and at all temperatures compared to the tolerant and susceptible varieties. Lethal time and lethal concentration values on the moderately resistant variety were shorter and lower, respectively, compared to the susceptible and tolerant varieties. These results suggest a faster kill of thrips on the moderately resistant variety with a low concentration of inoculum compared to the tolerant and susceptible varieties. Thrips feeding on moderately resistant cowpea varieties have longer developmental periods, lower body weights and lower reproduction potential (Ekesi *et al.*, 1998). These effects could increase the susceptibility of thrips to the fungal pathogen due to the physiological and metabolic stress imposed on the insect by feeding upon a suboptimal host (Butt and Brownbridge, 1997).

Temperatures of 15°C and 20°C caused a significant decrease in development of fungal infections but did not significantly affect the percentage mortality caused by any concentration of the fungus on the

susceptible and moderately resistant varieties. But in the present investigation it was observed that *M. anisopliae* and *B. bassiana* were more virulent when kept at 28 + 2°C and incubated for 28 days. The mortality percent of termites was also increased. Most authors agree that incubation period of most fungal diseases in insects is temperature-dependent (Ferron, 1978; Carruthers and Soper, 1987).

For mass multiplication of *B. bassiana* and *M. anisopliae* isolates, in the present investigation, molasses yeast broth was selected. *B. bassiana* isolates 2028, 1216, 135 and 984 produced 2.42×10^7 , 2.26×10^7 , 2.15×10^7 and 2×10^7 conidia/ml respectively when cultured on 200 litre broth in large scale production tanks. *M. anisopliae* isolates 892 and 140 produced 2.39×10^7 and 2.22×10^7 conidia/ml respectively on similar capacity tanks. Sharma *et al.*, (1999) also mass multiplied *M. anisopliae* and *B. bassiana* in molasses yeast broth (200ml) in 1 litre capacity Erlenmeyer flasks. *M. anisopliae* produced 8×10^8 conidia/ml while *B. bassiana* produced 1×10^9 conidia/ml which are relatively higher than the present findings. The results of the present study are also in consonance with the findings of Rombach *et al.*, (1987) who investigated that a liquid medium containing sucrose and yeast extract produced maximum mycelial growth and many conidia of *B. bassiana*.

In the present study, entomopathogenic fungi were also mass multiplied on different grains. Crushed grains of Bajra (*Pennisetum typhoides*) yielded spore dust of 4.43×10^7 , 4.6×10^7 , 4.97×10^7 and 4.33×10^8 conidia/gm by *B. bassiana* isolates 984, 2028, 135 and 1216 respectively. The spore production by *M. anisopliae* isolates 892 and 140 in crushed Bajra were 4.54×10^7 and 1.94×10^8 conidia/gm dry weight. The mass multiplication of entomopathogenic fungi by Sharma *et al.* (1999) on various grains indicated that *M. anisopliae* produced maximum

yield of conidia ($2 \times 10^9 \text{ g}^{-1}$) on crushed maize grain medium. In their study, though pearl millet grain produced higher conidia but, due to its sticky nature, the harvesting of conidia from this medium became a 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 conidial production.

Considering the above results, in the present investigation, maize grains were also used for mass multiplication and spore production. Suitability of a particular grain for preparation of medium may vary with differential requirements of the strains or due to difference in production technology used. Nevertheless, crushed Bajra grains in the present study was comparable with that of other grains, yet Bajra grain was preferred because of its low cost, high yield and economic feasibility over other grains.

Grain media requirement varied among different species of fungal pathogens. For *Beauveria* spp, cowpea grain medium proved best for their conidial production (Sharma *et al.*, 1999). *B. bassiana* and *B. brongniartii* mass cultured on cowpea grains filled in two kg capacity polypropylene bags yielded 1.5×10^9 and 1.8×10^9 conidia g^{-1} of dry grain weight, respectively. In their investigation, *B. brongniartii* was also cultured on cowpea whole grains, which produced 1.8×10^9 conidia g^{-1} of dry weight. Aregger (1992) reported high yield of 1×10^8 to 2×10^9 conidia g^{-1} on white grains of barley and documented that the yield of conidia depended mainly on the addition of water and the length of incubation. In the present study, bajra grains were preferred for mass multiplication of *B. bassiana* as the grains of barley in our experiment

provided very low conidial production, however, spore production varied among the isolates .

Esenther and Beal (1978) conducted a field trial in Southern Mississippi to determine if placing decayed wood bait blocks impregnated with merix could suppress termite (*Reticulitermes sp.*) populations when placed around the perimeter of an area. Bait blocks treated with 10mg merix / block were buried at 1.5 m spacing at the perimeter of six 7.5m-square plots, 3 of which also had treatments along the perimeter of an outer 30m square. Non-insecticidal bait blocks were used by them to monitor termite activity within the treatment lines. They observed that during a 3.5 yr period termite activity on the treated plots was suppressed.

Baits treated with diiodomethyl para-tolyl sulfone at 600ppm (wt/wt) were introduced into selected trap stations of 3 colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki in Hallandale, between November 1987 and November 1988 (Su and Scheffrahn ,1991). They observed that termites neither avoided foraging at sites containing treatment nor was feeding significantly reduced on the treated versus untreated baits. One year after bait application, foraging populations of 3 colonies that received A-9248 baits were reduced 65-98%. Their results demonstrated that a toxicant bait can be used to suppress foraging populations of subterranean termite colonies and hence reduced their damage potential. Su and Scheffrahn (1991) surveyed the foraging populations of colonies of the eastern subterranean termite, *Reticulitermes flavipes*, in residential and undeveloped environments of Southern Florida. A triple mark-recapture program using the dye marker Nile Blue A indicated foraging population of *R. flavipes* contain 0.2-5.0 million termites per colony, and the foraging territories encompass an area of up to 2,361 m² and a linear

foraging distance of 71m. Habitat type was not correlated with foraging population size.

Subterranean termites (Isoptera: Rhinotermitidae) were found to be ubiquitous in both wooded and desert regions of North America which were important contributors to nutrient cycling, energy flow, and ecosystem productivity (Grace, 1994). With the use of microbiological pesticides or microbial pest control agents (MPCAs), termites might suffer unintended exposure to these pathogenic agents.

Cornelius and Grace (1994) performed bioassays to test the responses of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, to dichloromethane extracts of whole *Iridomyrmex glaber* (Mayr) workers. They observed that termite workers were strongly repelled by filter papers treated with *I. glaber* extracts. In a choice test, termite workers fed significantly less on filter papers treated with *I. glaber* extract compared with solvent treated controls within a 24h period. Bioassays also were performed by them to evaluate the effect of different concentrations of ant extracts on termite tunneling behavior. The rate at which termites penetrated the treated sand was concentration dependent. They also observed that in 10d, termites failed to penetrate any of the sand barriers treated with 500 ant equivalents per gram of sand. Their results suggested that ant extracts may also be considered as a potential source of natural products for termite control.

A baiting procedure that incorporated a matrix containing a chitin synthesis inhibitor, hexaflunmaron, was evaluated by Su (1994) against field colonies of the eastern subterranean termite, *Reticulitermes flavipes*, and Formosan subterranean termite, *Coptotermes formosanus* shiraki. Wooden stakes were first

driven into the soil to detect the presence of termites. Bait tubes were placed in the soil where termites were detected. A self-recruiting procedure, in which termites collected from wooden stakes were forced to tunnel through the matrix in bait tubes, significantly increased bait intake by termites.

In the present investigation the suppression of termite population in the field was based on different experimental procedures. Field colonies of *O.obesus* and *O.distans* were identified, wooden stake survey and monitoring station application methods were followed. This was done to estimate the foraging territory and the population of termites before the introduction of baits in field to suppress their population. In this investigation the fungal bait matrix used was muscardine fungi which was in contrast to bait matrix of acetone solution of hexaflumuron used by Su (1994). However, the bait application with muscardine fungi in the present study resulted in the reduction of termite population in the field which was in accordance with the findings of Su(1994) where hexaflumuron bait application in field suppressed the population of subterranean termites. The only difference was that the present study was based on biocontrol agents while investigation of Su (1994) was based on the application of chemical.

Kenne *et.al.*(2000) studied the hunting behavior of *Myrmicaria opaciventris* in order to evaluate if it can be used as biological control agent against the termites that damage sugarcane plantations. Hunting workers foraged in groups and recruited nest mates at short-range when they encountered large termite soldiers or groups of small termite workers. Differences in prey capture concerned may be considered as (1) means of detection; (2) termite body part seized; (3) percentages of prey abandoned and (4) use of venom. Large termites were stretched by several workers whose adherence to the substrate is facilitated by well-developed aralias and claws on the legs while others spread venom on the body and carved it up. An adaptation to termite capture was noted

with a distribution of tasks between the workers which subdued prey, and those which transported it. In the former case, the workers easily eliminated termite soldiers, successively attacked several termite workers and even captured new individuals while holding the first ones captured between their mandibles before retrieving them all at once. The remaining individuals were retrieved by the transporting workers. Given this particularly effective predatory strategy, they concluded that, under certain conditions, *M.opaciventris* can be used as a biological control agent against termites.

Verkerk *et.al* (1999) has described an outline of the most important novel techniques which would be feasibly developed for use in an agricultural context (e.g. chemical and microbial baiting, transmissible coatings). These methods have been developed primarily for control of 'lower' termites in buildings in industrialized countries. They are not directly transferable to agriculture in the tropics where 'higher' termites cause the vast majority of damage to crops and pest management decisions are made largely by resource-poor, small-holder farmers. The three most serious obstacles to the development of bio rational termite management in tropical agriculture probably relate to cost, availability of active ingredients or pathogens and the lack of research on station or trap design appropriate for 'pest' termite species. Based on their own experiences with tropical pest management and baiting technologies, they considered the characteristics required for the development of baiting systems, transmissible repellants for use in agriculture in developing countries. This includes the adaptation of these systems to allow the transference of biological control agents, such as entomopathogenic fungi and nematodes. They argued that it is important that such developments pose a negligible hazard to applicators or farmers, and cause no significant harm to non-target organisms, including beneficial fauna such as non-pest termites, earthworms and natural enemies important in the regulation of crop pests.

For the molecular characterization of the entomopathogenic fungi *M. anisopliae* and *B. bassiana* different experimental techniques have been adopted in the present investigation. Antigens were prepared from mycelia and conidia of all the isolates of *M. anisopliae* and *B. bassiana* as well as other soil fungi. Purified antigens prepared from the isolates of *M. anisopliae* and *B. bassiana* were resolved in SDS-PAGE and finally polyclonal antibodies (PAb) were generated against *M. anisopliae* (892) and *B. bassiana* (2028). These PABs were tested against homologous antigens as well as antigens prepared from other isolates of *M. anisopliae*, *B. bassiana* and also other soil fungi using agar gel double diffusion tests. The cross-reactions were evident in several immunoenzymatic assays such as Direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA), Dot immunobinding assay and Western blottings.

In the present investigation, cross reactivity of PABs raised against *M. anisopliae* and *B. bassiana* were tested with other soil fungi, such as *Fusarium graminearum*, *Sphaerostilbe repens*, *Fomes lamaoensis*, *Sclerotium rolfsii*, *Sclerotiana sclerotiorum*, *Trichoderma harzianum* and *Trichoderma viride*. Results revealed that among all the above fungi tested, PAb of *M. anisopliae* reacted to some extent with isolates of *B. bassiana* and *S. repens* while PAb of *B. bassiana* reacted with isolates of *M. anisopliae* and *S. repens*. Positive reactions in ELISA with higher absorbance were always obtained from the homologous fungal antigens of *M. anisopliae* (892) and *B. bassiana* (2028). Absorbance values were lower for other fungi. Mohan (1988) successfully raised antiserum against pooled mycelial antigens of five *Phytophthora fragariae* races. In indirect ELISA, it detected homologous soluble mycelial antigens. PAb of *Phytophthora fragariae* reacted strongly with antigens from several *Phytophthora* species. The sensitivity of detection was high and concentrations as low as 2ng proteins / ml were detectable.

The present experiment showed that ELISA positive material can be detected at early infestation stages of the entomopathogens. Therefore, the ELISA test is sufficiently sensitive to find important applications in detecting *B. bassiana* and *M. anisopliae* on termites. It could overcome the difficulty observed in visually detecting slight infection of termites in their colony. The ELISA test might prove useful in monitoring field samples. The sensitivity achieved in the present study by no means reflects the limit of detection possible in ELISA. The ELISA test described should prove valuable in screening field collected samples of entomopathogens for identification and ensure their infection potential.

Molecular probing of amended soil antigen was also performed with PAb raised against mycelial antigen of *M. anisopliae*(892). Soil amended with spores and mycelia of *M. anisopliae* (892) was probed with PAb of *M. anisopliae*(892) where bands were revealed with same molecular weights and patterns as the bands present in homologous mycelial antigen of *M. anisopliae*(892). This kind of test is very helpful in detection and identification of the entomopathogen(s) from the soil. This test also helped us to investigate the soil persistence of the entomopathogen(s) generally after the field application of the formulated mycoinsecticide. This kind of assay may be used to fix up spray schedule of the mycoinsecticide and monitor their persistence.

The experiments presented by Pendland and Boucias (1990) showed that the polyclonal antibodies generated against *Spodoptera exigua* hemocytes and hemolymph and those produced against cell wall surfaces of an entomopathogenic fungus (*Nomurace rileyi*) not only reacted to their own antigens but also showed activity against heterologous antigens. The cross-reactions were evident in their assays using both fluorescent microscopy and Western blotting techniques.

In the present study, using indirect immunofluorescence technique , cellular location of cross reactive antigen in mycelia and spores of *M.anisopliae* and *B.bassiana* were determined. This is an excellent technique for detection and identification of the entomopathogens with their PABs after their isolation from their hosts in different natural habitats. Detection of fungi from soil and plant tissues using immunological techniques have been elucidated by Chakraborty and Chakraborty (2002).

The present investigation on evaluation of *M. anisopliae* and *B. bassiana* as microbial insecticides to control termites provided positive results. *In vitro* and field study support the conclusion that the entomopathogenic fungi - *M. anisopliae* and *B. bassiana* may form effective biological agents for the management of termites in agro forest eco-systems.