BIOCONTROL POTENTIAL OF THE FUNGUS *BEAUVERIA BASSIANA* (Balsamo) AGAINST THE DESERT LOCUST, *SCHISTOCERCA GREGARIA* (Forskal)

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by

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DECLARATIONS

This thesis is my original work and has not been presented for a degree in any other university

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ABSTRACT

Pathological potential of the fungus *Beauveria bassiana Balsamo* on the desert locust *Schistocerca gregaria Forsk* was evaluated with an aim to develop a mycoinsecticide as a supplement to the persistent chemical insecticides used for desert locust control in order to minimize the pollution in agro-ecosystems.

Conidial suspension of *B. bassiana* when topically applied to locusts was found to be effective under 27.5 $\pm$ 2.50°C and 90% RH. Comparative stage susceptibility studies showed instars I and II as the most susceptible followed by III and IV. Adults showed the lowest mortality. Bioassays to establish the critical pathogenicity indices showed the lethal dose (LD_{50}) to be $5x10^7$ conidia/ml at 8 days post-innoculation. Mortality was found to be directly proportional to conidial concentration with the highest cumulative mortality at $5x10^{12}$ conidia/ml. However sub-lethal doses could be improved through serial *in vivo* passage of fungi through the host.

Dose-mortality relationship studies to determine optimum environmental requirements of *B. bassiana* when conducted under laboratory conditions showed that a temperature of 27.5 $\pm$ 2.5°C and 90.0% $\pm$ 10% RH produced maximum mortality while the optimum RH range was 90% $\pm$ 10%. Conidial activity was suppressed by prolonged periods of irradiation.

Conidial efficacy outside the optimum conditions was enhanced in oil formulations and addition of UV-protectants. Oil formulated conidia showed improved mortalities even at higher temperatures and low relative humidity compared to non-formulated conidial suspensions. UV-protectants improved conidial stability even after prolonged UV exposures. A combination of oil and molasses formulation proved to be the most stable at different environmental conditions.

Screening tests against beneficial insects to determine toxicity to bees and rats respectively showed *B. bassiana* was safe against both organisms. The biocontrol potential of *B. bassiana* to *S. gregaria* under field cage conditions showed mortality to be higher in formulated than non-formulated Pathogen. This indicated that *B. bassiana* has a potential for controlling *S. gregaria* in the field.

DEDICATION

To my beloved parents

JAMES OYUGI OGOT

AND

DORCAS AKEYO OYUGI

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CHAPTER 1

INTRODUCTION

Locusts are large to medium-sized insects belonging to the order Orthoptera, characterized by powerful chewing jaws and enlarged hind legs for jumping (Uvarov, 1966). They are further categorized in the suborder Caelifera which is divided into five superfamilies. Locusts belong to the superfamily Acridoidae which is the largest, having about 10,000 species, distinguished by short antennae. Within the family Acrididae only 10-12 species could be referred to as locusts while the rest are known as grasshoppers. Locusts are not differentiated from grasshoppers by any particular morphological feature and certain species commonly show closer morphological affinities with grasshoppers than with other locusts. For instance, there are several locust species in the subfamily Cyrtacanthacridinae which also contains a number of typical grasshoppers (Chapman, 1976).

An important feature of locusts is their ability to reversibly transform between two extreme phases, solitaria and gregaria, which differ in morphology, physiology and behaviour (Uvarov, 1966; Steedman, 1988). The most striking feature of gregarious locusts is their ability to change into dense groups, march in bands as wingless hoppers or swarm over long distances as adults when stimulated by favourable climatic conditions (Steedman, 1988). As solitaria,

individual locusts are relatively insignificant pests and only attain a major pest status when they aggregate.

Mankind has probably been plagued by locusts ever since he first began to grow crops some ten thousand years ago. The earliest records at Saqqara in lower Egypt consist of carvings of locusts in tombs of the sixth dynasty (2420-2270 BC). The eighth plague in the book of Exodus (1300 BC) emphasizes the fear which desert locust swarms created in ancient times (Chapman, 1976).

Africa and the adjoining regions continue to be liable to widespread and prolonged infestations by locust swarms. The main locust species responsible for plagues experienced in Africa and Asia are:

(a) Desert locust, *Schistocerca gregaria*;

(b) African migratory locust, *Locusta migratoria*;

(c) Red locust, *Nomadacris septemfasciata*;

(d) Tree locusts, *Anacridium* spp;

(e) Brown locust, *Locustana pardalina*;

(f) Sudan Plague Locust, *Ailopus simulatrix*; and

(g) Madagascar Locust, *Locusta migratoria capito.*

The desert locust is perhaps the most successful species because of its ability to respond to favourable weather systems, and its remarkable flight performance (Bullen, 1969). Its diffuse distribution makes it difficult to monitor its occurrence in recession areas. With an invasion area of about 29 million Km^2 at its disposal and a recession area of 14 million km^2, the desert locust affects 57

countries, covering over 27% of the total land surface of the world (Fig.1) (Steedmann, 1988). The desert locust is the most voracious pest known, devouring most vegetation in areas they swarm over resulting into enormous losses (Alomenu 1985; FAO, 1988). The amount of damage they cause stems from the fact that each locust in a swarm has the potential of consuming approximately its own weight (1.5-3.0g), of fresh vegetation daily (Chapman, 1976). Considering that there are often some 50 million individuals in every km^2 of a medium density swarm, they have the potential to eat 100 tonnes of food daily, which is enough to feed 500,000 people for a year (FAO, 1988).

Inspite of the long historical occurrence of plagues, locust control is still a difficult problem in the affected countries (Brader *et al.,* 1988). Early measures were defensive and aimed at minimizing crop damage once the locust or grasshoppers had invaded cultivated areas. During the last 50 years, the strategy has gradually switched towards attacking and containing the small populations to their breeding sites and not allowing them to reach proportions congenial to swarming. This strategy of control comprises mainly of intensive ground and aerial application of synthetic insecticides which do sometimes prevent outbreaks, but only provide short term solutions (Steedman, 1988; Duranton *et al.*, 1987; OTA, 1990). Synthetic insecticides are costly, especially when measured against the national budgets of the countries affected. Moreover apart from being hazardous to an ecologically conscious world (UNEP, 1987), most insects are now developing resistance to these insecticides (Alomenu,

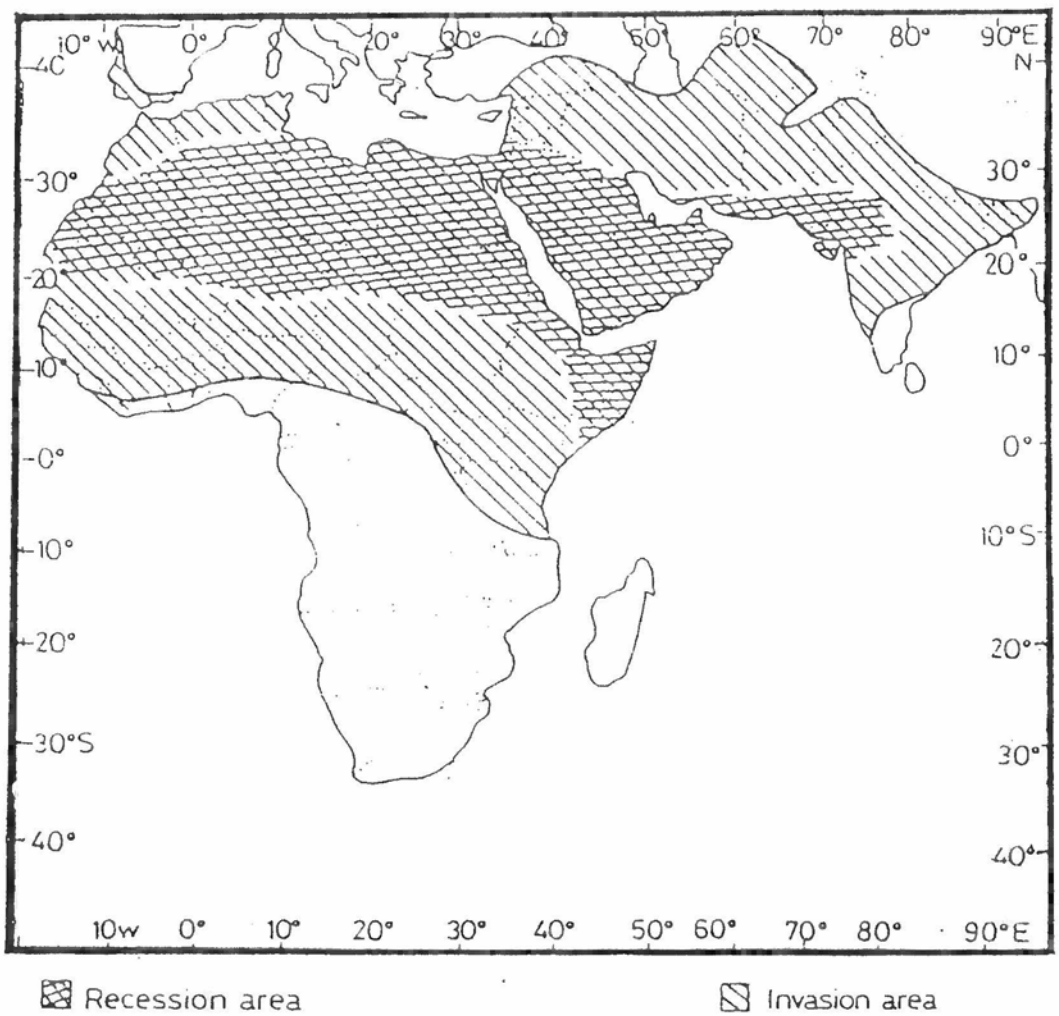

Fig. 1. Distribution of the Desert locust.

1985). This predicament has made the search for a sound alternative control strategy more urgent and of paramount importance.

Scanty research has been carried out into developing better and environmentally acceptable locust control methods. An additional handicap has been presented by the banning of the organochlorine insecticide, dieldrin. This insecticide, although for a long time had been the most effective against locusts, it exhibits high toxicity to non-target organisms and has a high bio-magnification potential. However, due to its persistence in the environment dieldrin needed to be applied only once on the locust breeding ground in barriers at long intervals. Locust hopper bands crossing dieldrin barriers became contaminated and died. Although dieldrin was banned, the consequences to locust control in Africa has not been sufficiently analyzed. Accordingly no alternative agent or methods had yet been developed to replace it.

The massive upsurge of all major species of locusts, and particularly that of the desert locust in 1985 could be attributed partly to civil wars in the affected countries, but it highlighted the inadequacy of the current use of the less persistent organophosphates such as malathion and fenitrothion (Bie, 1989; Brader *et al.*,1988). It meant that much larger areas had to be treated with multiple applications with all the accompanying negative impact on the environment. On the basis of this unsatisfactory situation in locust control, it became conceivable that alternative environmentally friendly and effective methods of control be developed.

An environmentally acceptable control strategy for locusts should be based on the principles of integrated pest management (IPM) which maximizes the natural mortality factors of the pest while minimizing the need and impact of chemical insecticides. There is evidence that a complex fauna of natural enemies including birds, arthropods and micro-organisms play an important role in the regulation of locust population in their natural habitats (Chapman, 1976; Greathead, 1989). The same biotic factors could be manipulated to provide a biological means of safely controlling locusts (Greathead, 1989; Panos, 1993). Among the natural factors affecting locusts and grasshoppers populations are entomopathogenic fungi.

Fungal diseases in insects are common and widespread and often wipe out insect populations during epizootics (MacLeod, 1963; Burges, 1981; McCoy, 1988). The Fungal pathogens which have the most potential in locust control are members of the Deuteromycetes which include the species *Beauvera bassiana* (Balsamo), *Metarhizium anisopliae* and *Metarhizium flavoviride*. There are several records of this fungi infecting grasshoppers (Dresner, 1949; Macleod, 1954; Humber and Soper, 1986; Li 1987; Moore and Erlandson, 1988) and it has been documented as the cause of an epizootic in the swarm of red locusts *N. septemfasciata* in South Africa (Schaefer, 1936). Some recent experiments have demonstrated pathogenicity of *B. bassiana* to grasshoppers under laboratory conditions (Marcandier and Khachatourians, 1987; Moore and Erlandson, 1988). Grasshoppers have also been shown to be highly susceptible to a strain of *B.*

bassiana isolated from a dead grasshopper in Montana (Johnson, *et al.,* 1988).

B. bassiana has advantages as potential myco-insecticides because:

(a) unlike most other insect pathogens which must be ingested in order to invade their host, entomopathogenic fungi usually invade via the external cuticle (Charnley, 1984);

(b) can be produced on simple culture media (Goettel and Roberts, 1991) as well as *en masse*;

(c) can be formulated as myco-insecticides suitable for spraying using con-ventional chemical spraying equipment (Mathews 1983; Bateman, 1989); and

(d) they have a proven safety record (Geottel, 1990).

These phenomena illustrate the promising potential of fungi and has resulted in considerable discussions on the attempt to exploit them as insect control agents. In dealing with a system involving two living organisms, the host and its pathogen, a full understanding of the factors affecting the ability of a fungus to enter and kill its host is paramount as a prelude to the development of a bio-insecticide. The factors that interfere in any of the critical steps could render the pathogen less efficient if not useless. They include abiotic factors such as solar radiation, temperature and humidity. It is only in the immediate past that work has been done on unravelling the complex interaction between the host integument and fungal pathogens (St Leger, *et al.*, 1986).

The major stimuli to the current advances in the field of fungal pathology has been the realization that infection from an initial application can occur independent of the high humidity (Prior *et al.*, 1989). Indications are that the micro-humidities at the surface of the host integument or on the foliage may be sufficient for spore germination and host penetration (Ferron, 1977). Fungal spores formulated in oil have been shown to kill locusts at humidities of 35% RH (Prior *et al.*, 1991). High humidity is however required for the production of spores and it is this constraint that limits subsequent spread of the fungal disease (Ferron, 1977; Hall, 1981; McCoy, 1981; Marcandier and Khachatourians, 1987). The aim therefore would be to maximize the kill from the initial application in the same way as a chemical insecticide. In this study, my objective was to isolate, characterize and formulate *B. bassiana* strain and determine its efficacy and suitability in the management of *S. gregaria*.

CHAPTER 2

LITERATURE REVIEW

2.1 Biology and behaviour of *S. gregaria*

The life cycle of *S. gregaria* comprises 5-6 phenological stages from egg through hopper to adult with the duration of each stage varying considerably depending on environmental conditions (Uvarov, 1977; Waloff, 1976). The female locusts dig into the soil 3-4cm deep by stretching their ovipositors and lay between 20-100 eggs covered in a frothy secretion forming an egg pod. There is a plug on one end of the egg pod to prevent dessication and also to provide a medium through which the young hoppers can easily reach the surface on hatching (Stower *et al.*, 1958; Duranton *et al.*, 1987). Incubation period varies according to soil temperature but normally takes 10-14 days before hatching into nymphs (Waloff, 1976). There are 5 nymphal instars with a developmental period lasting 20-45 days at 24°C. The final moult, also known as fledging, produces pink immature adults which mature 2-4 weeks later. Adult locusts last for 2-5 months. *S. gregaria* is bivoltine, but a third generation could develop late in the year (Chapman, 1976; Waloff, 1976).

The desert locust exhibits marked phase polymorphism and can change reversibly between a solitaria to gregaria phase. Individuals in the two phases differ in morphology, physiology and behaviour (Uvarov, 1966). This

transformation is triggered off by sufficient rainfall and abundant vegetation which must be maintained for at least 4 successive generations (Uvarov, 1966). Rapid build-up in locust population leads to development of plagues which poses the greatest danger to agriculture in the invasion areas (Skaf *et al.*, 1990).

2.2 Distribution of *S. gregaria*

The desert locust occurs in recession and invasion areas. The total invasion area includes the whole of Northern Africa, Southern and Western parts of the Iberian peninsula, West Africa north of the equatorial forests, Eastern Africa, the Arabian peninsula, the Middle east, north to eastern Turkey, the Southern republics of the former USSR, Iran, Afghanistan and the Indian sub-continent south to 10°N and east to the border of Burma (COPR, 1978; Skaf *et al.*, 1990). Desert locusts have also been recorded in the Atlantic Ocean islands, British Isles, the Mediterranean, Italy, Greece, Croatia and Serbia (Chapman, 1976; FAO, 1993).

During recession periods the desert locust is restricted to the arid and semi-arid central part of this area which covers 16 million km^2 extending from the Atlantic to North west India (COPR, 1978). Systematic cartographic studies of the distribution of breeding areas have established that the main ecological factor responsible for breeding patterns and seasonal occurence is rainfall (Donelly, 1947; Rao, 1960). Topography, vegetation and soils have also been shown to influence breeding (Brown, 1987).

2.3 Pest status of *S. gregaria*

In their gregarious swarming phase, locusts are probably the most spectacular and noticeable crop pest known to man (Bullen, 1969). The damage potential of locusts depend more on the stage of the crop at the time of attack (Chapman, 1976; Wright, 1986). The total grain loss due to desert locusts during the 1985-89 outbreak in the Sahelian countries of Mauritania, Senegal, Gambia, Burkina Faso, Mali, Niger, Chad, The Sudan and Ethiopia was estimated at 241,000 tons or 1.3% of their total food production with a direct monetary value of the loss being put at US$ 32.5 million (Odhiambo 1988).

2.4 Control of the desert locust

Early measures in desert locust control were traditional cultural practices which were defensive and aimed at minimizing crop damage once the locusts had invaded cultivated areas (Panos, 1993). These cultural practices involved;

(a) intercropping food crops with those less preferred by locusts,
(b) destroying food sources through burning,
(c) physical barriers to trap and bury hopper bands
(d) lighting fires
(e) scaring away adults (Chapman, 1976).

At best these methods, which were labour intensive, achieved some damage limitations at a local level. However, whenever natural conditions were

in the pest's favour, their population growth overwhelmed such efforts (Roffey, 1982; Panos, 1993).

Since the second world war, locust control in Africa in particular had depended entirely on the use of insecticides. Different types of compounds have been used (Brader et al, 1988) and include;

(a) organochlorines (e.g dieldrin, BHC and DDT);
(b) organophosphates (e.g fenitrothion, and malathion);
(c) carbamates (e.g carbaryl); and
(d) pyrethroids (e.g permethrin).

Regrettably, the heavy reliance on insecticides discouraged locust surveillance and research on alternative control methods. Thus when the locust plagues occurred in 1985, not only were the organizations charged with its control and monitoring unprepared, but they could also not use dieldrin due to the controversy on its environmental effects. The challenge then became the development of effective control strategies with reduced or no detrimental environmental effects.

2.5 Entomopathogenic fungi as biocontrol agents

The possible use of the entomopathogenic fungi to control crop pest populations was first considered at the end of the 19th century (Ferron, 1977). The hypothesis originated from studies on the silkworm diseases which at the time were severely inhibiting the development of sericulture in Europe (Bassi, 1836). Insect diseases were certainly known from previous generations but it

was Bassi (1836), who established that insect diseases were caused by infectious micro-organisms (Steinhaus, 1956). Among these, fungi causing muscardine diseases held an important place because of the spectacular symptoms they caused (Steinhaus, 1956).

During the second half of the nineteenth century, a number of researchers generated a lot of basic knowledge on entomopathogenic fungi demonstrating their potential as biocontrol agents (Robin, 1847; 1853; Fresenius, 1856; Tulasne, 1863, 1865; Brefeld 1870; Cohn, 1870; Leconte, 1874). A strategy for field application of pathogens which soon captured the interest of researchers was developed by Hagen (1879). Simultaneously, in the Ukraine the practical use of entomopathogenic fungi reached its highest development in efforts to control the wheat cockchafer, *Anisoplia austriacia* Hbst (Metschnikoff, 1879). A few years later Krassilstchik (1884) started industrial production of *Metarhizium anisopliae* for the control of *Cleonus punctiventris* Ger. In France, during the same period efforts were concentrated on the white muscardine of the European cochafer *Melolontha melolontha* L. caused by *Beauveria tenella* (Giard, 1892). While in the USA similar work was carried out to control the cinch bug *Blissus leucopterus* Say using *Beauveria globulifera* (Snow 1891, 1896).

Despite the reports on the potential of microbial agents in pest control, some researchers still questioned their effectiveness (Paillot, 1915 and Marchal, 1916). Even After the second world war when Steinhaus (1946, 1949) had extensively outlined the potential of using entomopathogenic fungi in

microbial pest control, the possibilities of using fungi continued to be under-estimated for a long time when compared to the use of bacteria and viruses. This could probably be explained by the increase in our knowledge of general microbiology, especially medical, which emphasised more on bacteriology and virology than mycology (Ferron, 1978). In the last 20 years several studies have shown the potential of entomopathogenic fungal control against insect pests (Burges, 1981; Cole and Kendrick, 1981; Davidson, 1981; Cantwell 1974; Roberts et al, 1981; Franz and Krieg, 1982). Approximately 750 species of entomopathogenic fungi, representing all major taxa, *B. bassiana* inclusive, are now known to infect insects (Fuxa 1987; McCoy *et al*, 1988).

2.5.1 *B. bassiana* as a microbial insecticide

B. bassiana attacks about 500 insect species of economic importance in the orders Lepidoptera, Coleoptera, Heteroptera and Diptera. In the USA high mortalities were recorded in test populations of the weevil *Curculio caryae* (Horn) after exposure of adults to inocula of *B. bassiana* and *M. anisopliae* Metcsch (Gottwald and Tedders, 1984). Similar results were observed in *Dendroctonus ponderosae* Hopk. when they were innoculated with *B. bassiana* conidia (Hunt *et al.*, 1984). In China, application of conidia to different planthopper and leafhopper populations also resulted in high infection rates 15 days after incubation (Li, 1987). *B. bassiana* has also been used in that country for the control of pine caterpillar *Dendrolimus punctata* (Walker) while in

France, field collected diapausing larvae of *O. nubilalis* showed high mortality after infection with *B. bassiana* (Marcandier and Riba, 1986). In Russia, *B. bassiana* has successfully been used in the control of Colorado potato beetle. Other isolates have been under investigation for control of other pests, including fire ants *Solenopsis* spp., black pine weevils *Otiorynchus sulcatus* (Fabricious) and the citrus root weevils *Diaprepes* spp (McCoy, 1990).

Within the same species, different activity spectra could be found (Rockwood, 1950; Latch, 1965; Paschke, 1965; Ferron, 1977). While assaying for the virulence of different strains of *B. bassiana* to the silk worm, *B. mori*, Shimuzu and Aizawa (1988) found that the lethal dose (LD_{50}) values of various isolates differed. The virulence and sporulation capacity of 50 isolates of *B. bassiana* collected world-wide were found to differ against the Colorado potato beetle, *Leptinotarsa decemlineata* (Soper and Ward, 1981). Studies with carabid beetle larvae infected by different strains of *M. anisopliae* indicated that there was a very strict adaptation of the strain to the original host (Ferron *et al.*, 1972).

Reports of *B. bassiana* infecting grasshoppers are available and include Dresner (1949) Macleod (1954), Humber and Soper (1986), Li (1987), Moore and Erlandson (1988). Schaefer (1936) documented that *B. bassiana* was the cause of epizootics in swarms of red locusts (*N. septemfasciata*) in South Africa. Some recent studies have also demonstrated its pathogenicity to

grasshoppers under laboratory conditions (Marcandier and Kachatourians, 1987; Moore and Erlandson, 1988).

2.6 Fungal pathogenesis

2.6.1 Adhesion of conidia to the cuticle

Contact between conidia and the insect cuticle is the prerequisite for the establishment of a mycosis (Charnley, 1989). The epicuticle is the site for the initial fungus-host interaction and in most cases conidia attach indiscriminately to host and non-host cuticle (Zacharuk, 1970a; Michel, 1981; Boucious and Pendland, 1989). The degree of adhesion depends on the fungal species under consideration. Most dry conidia attach passively to the host, but in *N. riley*, binding of conidia to the insect cuticle is very strong (Charnley, 1989). Spore adhesion has often been correlated with the aggressiveness or host specificity of a fungal species. For example Zeobold *et al.*, (1979) and Fargues (1981) were able to correlate host specificity of *Coelomomyces psorophorae* and *M. anisopliae* with the ability of conidia to attach to the cuticle of mosquitoes and scarabid larvae respectively. Similarly, hypovirulence of certain *M. anisopliae* strains pathogenic to mosquito larvae resulted from a defect in the attachment of the floating conidia to the larval siphon (Al-Aidroos and Roberts, 1978). However, adhesion is not always related to aggressiveness. It has been found, for instance that there is no difference in adhesion of conidia in

aggresssive and non-aggressive strains of *C. obscurus* to the pea aphid cuticle (Latge *et al.*, 1988). The same was observed with regard to the adhesion of *N. riley* to the cuticle of *Anticarsia gemmatalis* (Boucious *et al.*,1988).

2.6.2 Germination of conidia on the cuticle

Once the conidia has attached itself to the cuticle, it germinates to produce a germ tube which then penetrates the host cuticle (Al-Aidroos, 1978; Zeobold *et al.*, 1979; Hassan *et al.*, 1989). In addition to the germ tube, germinating conidia of most entomopathogenic species such as *M. anisopliae, B. bassiana, C. psorophorae* and *Neozygites fresesnii* produce an appressorial cell at the germ tube epicuticle interface. The appressorium is coated with mucilaginous materials responsible for the attachment of conidia to the epicuticular surface which is used to penetrate the host (Zacharuk, 1970a,; Brobyn *et al.*, 1977, Travland, 1979b; Michel, 1981; Zacharuk, 1981). It has been shown that conidial germination is dependent on macroclimatic factors, especially temperature and humidity (Vey *et al.*,1982).

Germination of conidia is also dependent on its nutritional environment. In the case of *B. bassiana,* the conidia germinate very poorly in the absence of nutrients (Boucious and Pendland, 1984; Woods and Grula, 1984). The quality and quantity of nutrients available has also been reported to influence the mode of germination of *B. bassiana* on *C. obscurus* (Sampdero *et al.*, 1984). Dillon and Charnley (1986) have further shown that although germination of *M.*

anisopliae is initiated by water, progress in the initial stages is dependent on a suitable exogenous nutrient.

2.6.3 Penetration of the conidia through the host integument

Penetration of the host exocuticle appears to involve both mechanical and enzymatic components of the developing germ tube (Charnley, 1984, 1989). The cuticle has a highly complex structure which is not well defined and so it is difficult to assign a role for specific enzymes in the penetration of this layer (St Leger, 1989). However, according to Zacharuk (1970) lipases and esterases might be implicated. Lipoprotein lipases are secreted by *M. anisopliae* and *B. bassiana* and aids penetration of the inner cuticle (St. Leger *et al.*, 1986).

2.6.4 Host death due to fungal pathogenesis

Death of an insect from an entomopathogenic fungus infection is attributed to toxicosis, starvation, invasion of the host tissues by the fungus, asphyxiation of the insect by fungal growth within the trachea and or the interference with the host physiology (Prasertphon and Tanada, 1968; Zacharuk, 1971). It is impossible to ascribe a cause of death to mycosis with any certainity since several factors may be involved (Charnley, 1986). Destruxins may also play a significant role in host death (Hall, 1981). A two-tier system for the involvement of destruxins in pathogenesis has been proposed (Samuels *et al.*,

1988). In this system, host death marks the end of the parasitic phase of fungal development. The mycelia then grow saprophytically producing antibiotics antagonistic to the intestinal bacterial flora. When the environmental conditions are favorable, the fungus grows outwards through the integument and develops conidiogenous structures (McCoy 1981; Pendland, 1982).

2.7 Environmental impact on fungal pathogenesis

Environmental conditions may have positive, indifferent or negative effect on the development, stability and efficacy of a pathogen (Zimmermann, 1986). Thus knowledge of the impact of environmental factors on entomopathogens is of great importance with respect to species and or strain selection, formulation and application techniques. The most important abiotic environmental factors are: temperature, humidity and sunlight (Ferron, 1977; Alves *et al.*, 1984; Soares and Pinnock, 1984).

2.7.1 Temperature

Different fungal species, even strains have different thermic preferences. The rates for germination, growth and sporulation of fungal pathogens have been reported to depend on temperature (Steinhaus, 1949; Muller-Kogler, 1965). Walstad *et al.* (1970) found that *B. bassiana* and *M. anisopliae* germinated at 15°C and 35°C with peak germination between 25°C and 30°C. Moore (1973) reported that the fungi, *B. bassiana, A. flavus* and *Fusarium*

solani (Mart) are infective to the Lepidoptera, *Trichoplusis ni* at 5°C to 30°C but more infective between 15°C and 20°C. Ferron (1978) also found that optimum values for most entomopathogenic fungi fall between 20°C and 30°C. For instance *B. brongniartii* exhibits peak development at 23°C, *Entomophthora obscura* (Hall and Dunn) and *Entomopthora exitialis* (Hall and Dunn) at 24°C, *B. bassiana* and *N.rileyi* between 25°C and 30°C, *M. anisopliae* at 27°C and *Entomopthora virulenta* (Hall and Dunn) at 30°C. However, Hall and Papierok (1982) reported that laboratory temperature requirements lie between 20°C and 25°C for *Beauveria, Peacilomyces and Nomurea*. The most suitable temperature for the application of *B. bassiana* was found to be 20°C at 80% relative humidity (Wen, 1983), while experiments on the effect of temperature on the control of *O. surinamensis* by *B. bassiana* showed that infection could occur down at 7°C (Searle and Doberski, 1984).

Temperature was also found to affect viability (Clerk and Madelin, 1965). They reported that viability of conidia of *B. bassiana, P. farinosus* and *M. anisopliae* decreased as storage temperature increased from 8°C to 25°C. According to Walstad *et al.*(1970), survival of *B. bassiana* and *M anisopliae* conidia were found to be affected by storage temperature. They found that at 8°C conidia remained viable for at least 12 months and at 21°C the two fungi survived well for 2½ months. The influence of temperature on the host insect is documented by Ferron (1978). Short periods between moults resulting from high temperatures may reduce the duration of instars to an extent that penetration

of the fungus through the integument is impeded (Ferron, 1978; Fargues, 1981).

2.7.2 Humidity

Relative humidity (RH) is a critical abiotic factor influencing the potential of fungal pathogenesis (Riba and Marcandier, 1984). Maximum germination of conidia has been reported to occur at 90-100% RH (Walstad *et al.*,1970; Ferron, 1977). For many entomophthorales, saturated or near saturated air is necessary for discharge of conidia and subsequent germination. Studies have however shown that infections of various host insects were possible in a wide range of ambient RH levels. Infection of elm bark beetle larvae (*Scolytus scolytus*) by *B. bassiana* and *M. anisopliae* was possible at RH as low as 51% (Doberski, 1981). Ferron (1977) demonstrated that while 92% RH was required for conidial germination of *B. bassiana*, this fungi would infect bean weevils *Acanthoscledes obtectus*, regardless of the ambient RH. *Entomopthora muscae* was found to infect house flies, *Musca domestica* at RH of 50% (Kramer 1980). However, it is important to distinguish between the atmospheric, macro-climatic RH and the micro-climatic RH existing around the insect cuticle (Roberts and Campbell, 1977).

Relative humidities higher than 90% are necessary to induce germ tube formation (Ferron, 1977). In certain Deuteromycetes, the presence of a film of free water is necessary to obtain maximum infection levels (Hall, 1981; McCoy, 1981). However, excessively high levels of humidity may be

unfavorable for the establishment of an entomophthoran fungi. Due to this reason it has been suggested that conidia of these genera should never be sprayed as a water suspension (Latge and Papierok, 1988).

2.7.3 Sunlight (Ultra-violet light)

The effect of visible and ultra-violet (UV) light on micro-organisms is multiple. Light radiation may stimulate, be indifferent or harmful to fungal activity. In germination, growth or sporulation process, light may be an indispensable abiotic factor (Leach, 1971; Roberts and Campbell, 1977). Alves *et. al.* (1984) tested 12 combinations of temperature and Photoperiods and found that a temperature of 28°C and a photoperiod of 16 hrs was most suitable for the fungal isolates. On the other hand, the longevity of conidia can be significantly reduced by exposure to light (Clerk and Madellin, 1965). In contrast to other pathogens, fungi seem to be more resistant to UV. Radiation experiments on the UV sensitive of entomopathogens such as granulosis virus (GV), nuclear Polyhedrosis virus (NPV), *Bacillus thuringiensis* and *B. bassiana* found that conidia of *B. bassiana* were more resistant to UV radiation of between 285-380nm which has the most deliterious effect (Kreig *et al.*, 1981). Detrimental effects of UV-A and UV-B which are components of the solar spectrum were also demonstrated in *M. anisopliae* by Zimmermann (1982). He observed that by exposing conidia to between 6-12 hr artificial solar radiation from UV lamps produced a significant decline in viability of the conidia and after 24 hr nearly

all spores were inactivated. These lamps, however, produce radiation intensity 10 times that of natural sunlight and would therefore be expected to be more deliterious than natural light (Zimmermann, 1982). He considered that in order to enhance the longevity of fungal propagules they should be protected against sunlight by UV-protectants.

2.8 Safety of *B. bassiana*

A review of the present knowledge of potential fungal control agents indicates that these organisms pose only a minimal risk to man (Siegel and Shadduck, 1990), domestic animals and wildlife (Saik *et al.*, 1990) and non-target invertebrates (Goettel *et al.*,1990). Nevertheless, the general consensus is that since fungi may pose inherent risks their use should be regulated. Most guidelines for registration of entomopathogenic fungi require laboratory testing for infectivity to non-target organisms including mammals (Hall *et al.*, 1982; Aizawa, 1990; Betz *et al.*,1990; Kandybin and Smirnov, 1990; Quinlan, 1990).

The potential hazards from microbial control agents to higher animals including man, centre on direct exposure to higher levels of inoculum. The hazards include toxicity and allergenicity and the danger could arise mainly due to improper handling. These are not new problems since the same types of hazards are encountered in the use of chemical pesticides (Prior, 1990).

Due to possible risks it is necessary that preliminary tests be conducted on susceptibility of non-target species to fungal pathogens. Most

requirements for registration and even limited field experimentation require laboratory testing to non-target invertebrates (Aizawa, 1990; Goettel, 1990). Such requirements usually include the honey-bee *Apis mellifera* as test organisms. Testing of entomopathogenic fungi against social insects such as the honey-bees may pose some problems. For instance, Vandenberg (1990) demonstrated that a strain of *B. bassiana* caused mycosis among honey-bees treated with very high doses of conidia, although the result was obscured by the fact that mortality was not significantly different from the control.

CHAPTER 3

MATERIALS AND METHODS

3.1 General procedures

3.1.1 Culturing of *S. gregaria*

S. gregaria were reared in the Insect mass rearing unit at ICIPE. The parental colony was developed from the stock collected from Tokar delta in the Red sea coast of Sudan. The nymphal instars placed 400 in each cage, were reared in wooden cages measuring 50cm x 50cm x 50cm (Fig. 2). Wooden cages were preferred since they maintained a stable temperature.

The cages were placed in a special room which was well aerated and fitted with a duct system that maintained a negative pressure and an airflow of 10-15 air flux per hour which prevented dampness in the room. Relative humidity was regulated at 40% using a (Model: Defensor) humidifier. A heater (Model: Phillips HD 3341/C) thermostatically controlled the temperature at 30°C ± 2°C during the day and 32°C ± 2°C at night. The rooms were sealed from any external source of lighting. Artificial lighting was provided by 60W bulbs. A 12:12h light and darkness photoperiod was maintained by a light-timer (Model: Orbis-Alpha QRD).

Locusts were fed on wheat (*Pennisetum variegatum*) seedlings. Fresh seedlings were cut each morning, cleaned in water and then fed to the

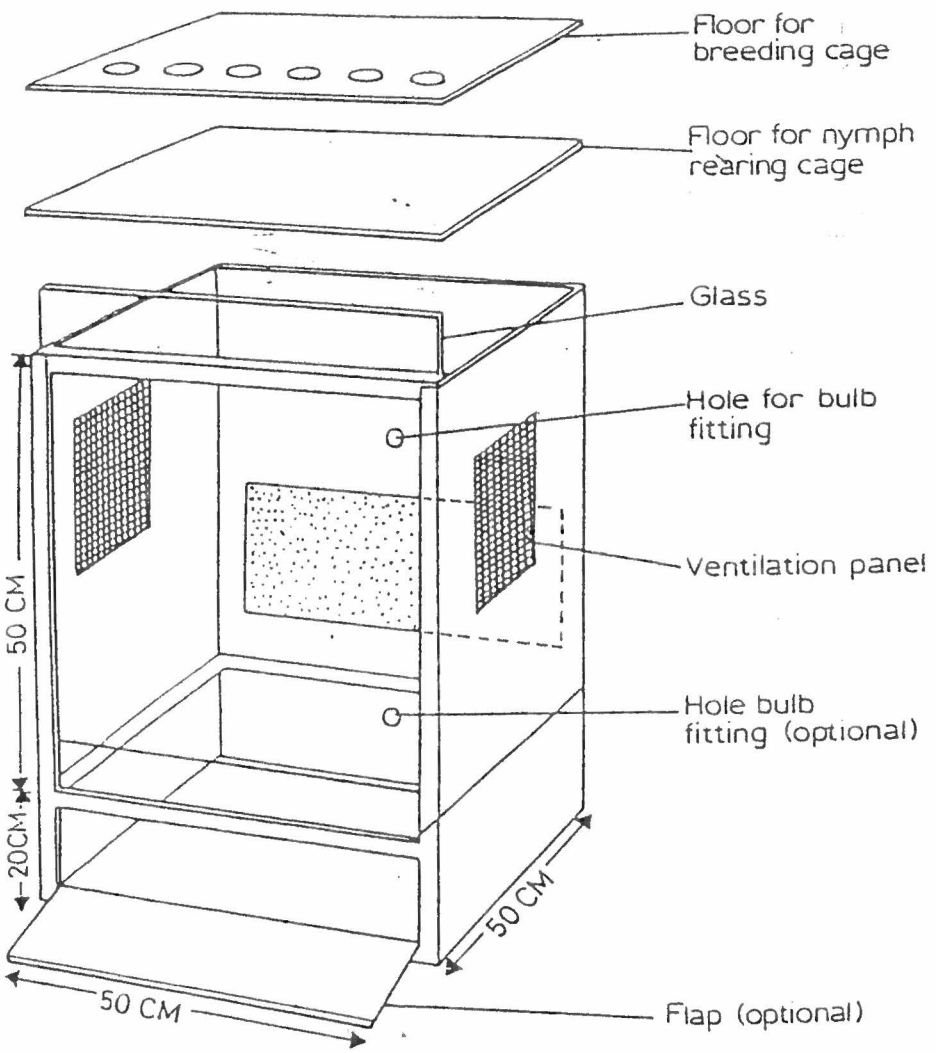

Fig. 2. Schematic representation of a locust rearing cage.

locusts in pots measuring 5.5cm in diameter and 7.5cm in height. In addition to wheat seedlings, wheat bran was also fed to the locusts. A high level of sanitation was maintained by daily cleaning of the cages using a hand held blower (Model: Black and Deckerr HC160) to remove feacal pellets and feed fragments from the cage. Dead and weak insects were removed and fixed in alcohol to avoid the spread of any disease. Rearing room floors were mopped with steam using a blower (Model: Karchier HDS 690) and disinfected with 0.5% Chlorochrysol weekly. At the second instar stage, insects were treated with 2.5% solutions of sulphonamide drug and Septrin for a period of 10 days to prevent infections by *Malamoeba locustae* and gregarines.

The cages were provided with compartments for egg laying. The sand used for this purpose was first washed and dried and then sterilized in a Memmert oven at 150°C for 2h. Before use the sand was cooled and moistened with distilled water at a ratio of 15:100 of water to sand and then filled into aluminium egg tubes measuring 3.5cm in diameter and 10cm height. These tubes were fixed into the holes in the false bottom of the cages. Tubes with egg pods were removed and covered with perforated polythene and incubated at 32°C. Freshly hatched nymphs were transferred into new cages. Records on fecundity, longevity, morphometrics and general health were kept as quality control.

3.1.2 Isolation and cultivation of *B. bassiana* strain

B. bassiana was isolated from dead grasshoppers collected from the field. Samples of the grasshoppers were washed with a 1% solution of sodium hypochlorite for three minutes, transferred onto a sieve and rinsed with a stream of distilled water. The specimens were put in a sterilized petri-dishes lined with moistened filter paper (Whatman's No.1) to induce high humidity. Fungal growth was initiated soon after the second day with subsequent mycelial growth after 6-8 days of incubation. After 14 days the conidia were picked from the cadaver surface using a fine sterile needle and streaked on media (Sabouraud Dextrose Agar-SDA) and incubated at 25°C

3.1.3 Identification of *B. bassiana* strains

The slide culture technique was used in preparing samples for characterization (Harris, 1986). Sterile water agar (10ml) was poured into 60mm plastic petri-dishes and allowed to solidify. A sterile $22mm^2$ cover glass was centered on the agar. In another 60-mm petri-dish 10 ml of SDA medium was poured and allowed to solidify, then cut with a sterile stainless steel surgical blade into $5mm^2$ blocks. Each block was placed on the cover glass and conidia from the host was subcultured on two opposite sides. The petri- dish was incubated at 25°C for 8 days. A semi-permanent mount was then prepared by staining the fungus growing on the coverslip with a drop of lactophenol cotton blue. The fungi preparations were identified under a microscope (Model: Ziess).

3.1.4 Preparation of *B. bassiana* inoculum

Fourteen day old sporulated cultures of *B. bassiana* were harvested by adding 10ml of distilled aqueous solution of 0.01% non-ionic surfactant (polyoxyethylene [20] sorbitan mono-oleate) commonly known as Tween-80 into petri-dishes. This was stirred using a sterilized glass rod. The suspension was poured through a 75 micron sieve to remove hyphal fragments before being agitated in a vortex for 3 min. The contents were then transferred into test tubes and centrifuged at 3,000 rpm for 10 min at 4°C using a Heraeus Sepatech 28RS Biofuge. The supernatant was discarded and the conidia resuspended in fresh surfactant. Conidial concentrations were obtained by diluting in a 1:100 ratio. The suspended conidia were counted using a Neubauer haemocytometer under a phase contrast microscope.

3.1.5 Bioassays with *B. bassiana* conidial supension

Conidial efficiency was estimated prior to each spraying by applying three drops of suspension on SDA media which was left to incubate for 24h at 25°C. Lactophenol Cotton Blue was then added onto the media and observed under a light microscope. Germinated conidia were recognized by the presence of germ tubes as opposed to ungerminated ones. Their numbers were estimated using a counter (Model: Assestant AC-12). Efficiency was calculated as percent conidia germinated over the total conidial count. Conidial suspension (100ml) was put into a 1 litre hand-held sprayer. Calibration of the sprayer was

done by relating flow rate to time of emission of 100 ml when the trigger was pressed. Conidia applications were done topically.

In all the bioassays, 3 replicates of 50 third instar nymphs of locusts were placed into wooden cages and sprayed with the desired conidial concentration. Controls were sprayed with 0.05% Tween 80 in distilled water. The treatments and controls were maintained at specific temperatures and humidities depending on the nature of investigation. Nymphs dying after the first two days were discarded, as death was due to natural causes. Subsequent mortality was recorded after every two days upto 14 days post-innoculation. Haemolymph from freshly dead locusts was collected on microscopic slides and stained with a drop of 1% lactophenol cotton blue to observe and confirm the presence of conidia and hyphae. Further verification of *B. bassiana* infection was done by placing each cadaver in a petri-dish with a moistened filter paper and incubating it for two days. Growth of the fungus from the intersegmental and anthrodal parts following incubation proved that infection was by the fungus being studied.

3.1.6 Mass production of *B. bassiana*

(a) Liquid phase

About 20g of sugar was dissolved in a litre of sterilized water. A similar amount of yeast extract was added to provide nitrogen. The constituted suspension (75ml) was transferred into ten 250ml conical flasks (Erlenmeyer).

Production of blastospores and mycelium was initiated by innoculating the yeast extract/sucrose broth with 1ml of $5x10^7$ conidia/ml suspended in sterilized aqueous suspension of 0.05% Tween 80. The flask was continuously stirred at 150 rpm for 3 days in an incubator/rotary shaker (Model: Lab-line) at 25°C± 2°C.

(b) Solid phase

The solid substrate was made by autoclaving 0.5g of white rice *Oryza sativum* at 121°C and 15 psi for 20 min. The autoclaved rice was transferred into sterile plastic bags (Gauge 150) measuring 15cm x 18cm and innoculated with blastospores produced from the liquid phase. Before sealing, sterile air was blown into the bags from a laminar flow hood. The bags were incubated at 30°C for 15 days until dense sporulation of *B. bassiana* was visible from the rice surface.

Extraction of conidia from the rice surface was done using 350ml of Tween 80 solution. To collect the conidial suspension, the bags were held upright and the lower tip cut off so that the suspension could trickle down into a beaker. The conidial suspension was then shaken for 5 mins using a mixer (Vortex K-550 GE) and sieved through a mesh of approximately 150μm into large airtight bottles for storage at 4°C.

3.2 Dose-mortality relationship of *B. bassiana* against of *S. gregaria*

3.2.1 Susceptibility of locust developmental stages to *B. bassiana* infection

Comparative bioassays were conducted to determine the susceptibility of the locust developmental stages to *B.bassiana*. Nymphs and adults of *S. gregaria* were tested for their susceptibility against *B. bassiana* conidia. Fifty individuals of each stage were placed in wooden cages and then sprayed with $5x10^7$, $5x10^8$, $5x10^9$, $5x10^{10}$ and $5x10^{12}$ conidia/ml. For every stage there were 3 replicates and a control which was sprayed with Tween 80 solution. Conditions in the cages were maintained as described earlier. Mortality and incidence of mycosis was recorded daily upto 14 days post-innoculation.

3.2.2 Lethal dose of *B. bassiana* to *S. gregaria*

Seven batches of concentrations of *B. bassiana* ($5x10^5$, $5x10^6$, $5x10^7$, $5x10^8$, $5x10^9$, $5x10^{10}$, $5x10^{12}$ conidia/ml) were tested against *S. gregaria*. Seven batches of 150 third instar nymphs (one batch with 3 groups of 50 each) were sprayed with the above concentrations. Controls were sprayed with Tween 80 in distilled water. Mycosis was monitored at temperatures from 25°C - 40°C and 60% RH. Likewise relative humidity ranges from 40% - 90% RH was fluctuated while keeping a constant temperature of 30°C. The experiment was replicated thrice. Analyses were conducted to obtain estimates of lethal dose (LD_{50}), lethal concentration (LC_{50}) and the lethal time (LT_{50}).

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3.2.3 Determination of the contact efficiency of conidial spray

Ten third instar nymphs were placed in wire mesh cage measuring 50cm x 50cm x 50cm and sprayed with conidial suspension from 0.5m, 1.0m and 2.5m. Lumogen is a yellow marker visible under UV light. The experiment was replicated 3 times. Sprayed locusts were then transferred into a dark room where the droplets could be counted when exposed to UV light and scaled using a droplet deposit gauge version 5 (Fig.3) (IITA, 1991).

3.3 Virulence improvement by serial *in vivo* passages

Three groups of 50 third instars of *S. gregaria* were sprayed with a sub-lethal dose ($5x10^5$ conidia/ml) then maintained at 30°C and 60% RH. Dead nymphs exhibiting mycosis in minimum time were bathed in 30 μg/ml sterile solution of chloromycetin for 5-10 min and placed on the surface of SDA in a petri dish. After 2 weeks of incubation at 25°C the sporulating fungus was subcultured on SDA and used for innoculating the next batch. Ten such serial passages were performed. Daily mortality upto 14 days post-innoculation of passaged and non-passaged cultures were compared together with those of controls which were sprayed with a solution of Tween 80 in distilled water

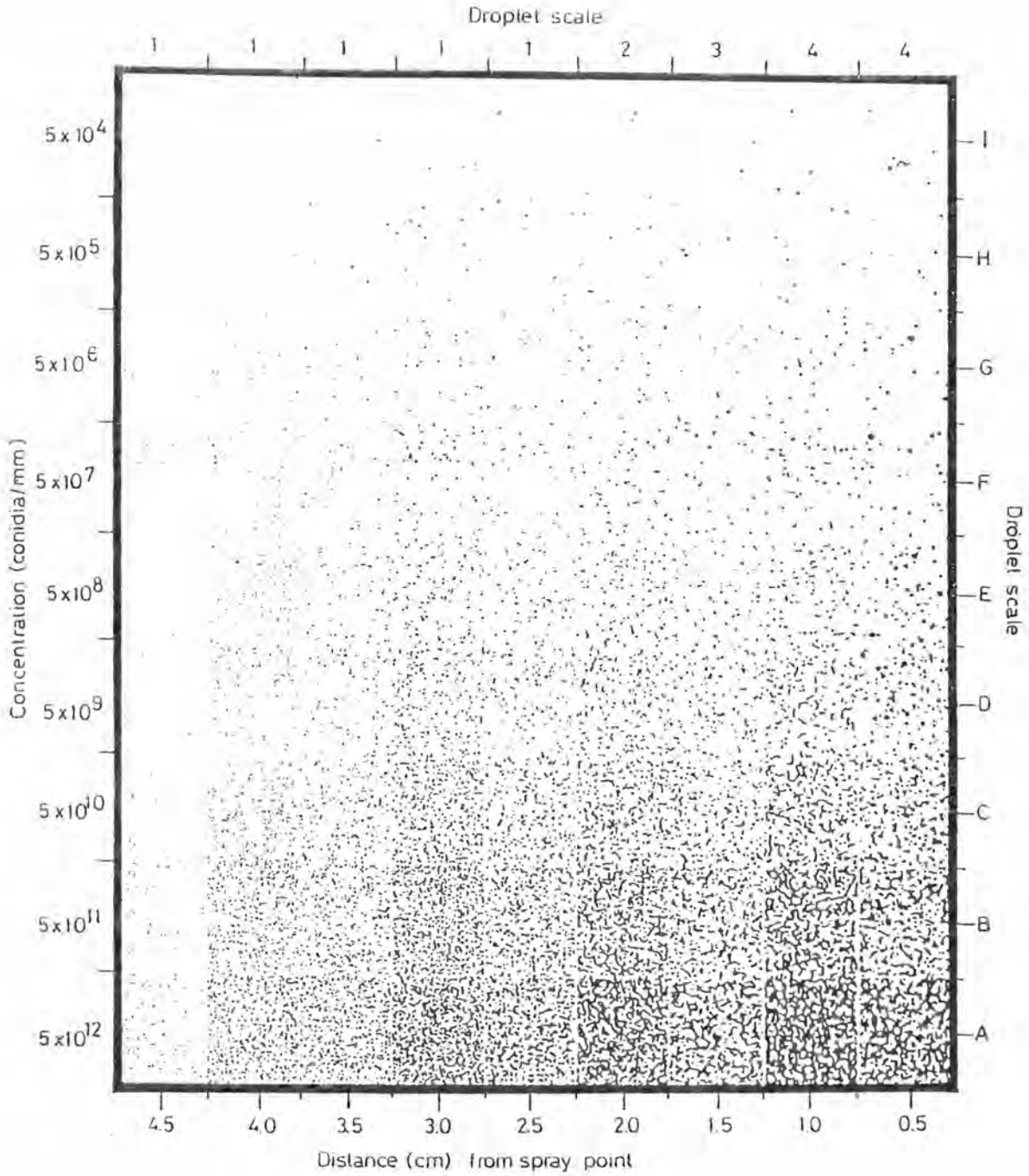

Fig. 3. Droplet deposit gauge: columns represent different concentrations and rows represent the distance from the spray point.

3.4 Influence of abiotic factors on the efficacy of *B. bassiana* against *S. gregaria*

3.4.1 Temperature

(a) Growth rate determination

In studying the involvement of temperature in pathogen efficacy, it was desirable that the conidial growth potential be independently assessed at different temperatures. One millimeter diameter of sporulating culture was cut with a sterilized cork borer and innoculated at the center of a petri-dish of fresh SDA media. These dishes were incubated in triplicates under 25°C, 30°C, 35°C, 40°C and 45°C at 60% RH. Non-innoculated agar controls were kept under similar conditions. Colony growth was measured daily in millimeters from the innoculation point outwards. After 14 days the rate of growth and colony size were compared.

(b) Pathogenicity

Pathogenicity was subsequently investigated by examining the influence of increasing temperatures on LD_{50} and LT_{50}. *B. bassiana* infectivity to *S. gregaria* was tested under 25°C, 30°C, 35°C, 40°C and 45°C at 60% RH. For each temperature regime a batch of 50 third instar nymphs replicated thrice were used. Each batch was sprayed separately with $5x10^7$, $5x10^8$, $5x10^9$, $5x10^{10}$, $5x10^{12}$ conidia/ml suspension. Mycosis and mortality were recorded at alternate days for 14 days.

3.4.2 Relative humidity (RH)

(a) Growth rate determination

The biophysical evaluation of the influence of relative humidity in determining growth rate was investigated. Sporulating cultures of *B. bassiana* were prepared as described in section 3.5.1 and incubated in triplicates under 40, 60, 80 and 100 %RH at 30°C. Daily recordings of colony growth were made upto 14 days post-innoculation.

(b) Pathogenicity

Four batches of 50 third instar nymphs replicated thrice were sprayed separately with $5x10^7$, $5x10^8$, $5x10^9$, $5x10^{10}$ and $5x10^{12}$ conidia/ml. Mortality at every humidity level from 40, 60, 80, and 100% RH was investigated. Temperature was constantly maintained at 30°C.

3.4.3 Ultraviolet light (UV)

(a) determination of conidial viability

The biophysical effect of UV irradiation on conidia viability was investigated before applying it to the host. Concentrations of fungal conidia were prepared from $5x10^7$ to $5x10^{12}$ conidia/ml and 10ml of each was placed in a glass petri dish (BS 160) and exposed to 360nm UV light from a laminar flow hood lamp for 1h, 12h, 24h and 48h, positioned 50 cm above the incident surface.

Samples were shaken during irradiation with UV light.

(b) Pathogenicity

Pathogenicity of UV irradiated conidia was determined using 50 third instars which were sprayed with conidia irradiated for varying periods of times from 1h, 12h, 24h, and 48h. Controls were sprayed with normal conidial suspension. Mortality was recorded daily upto 14 days.

3.5 Development of suitable formulations of *B. bassiana*

3.5.1 Oil formulation

(a) Effect of oil formulation on conidial viability

Viability of oil formulated conidia was tested by adding 10 ml of $5x10^7$ conidia/ml suspension to 1 ml of sterilized oils as follows;

(i) conidial suspension + 1% corn oil

(ii) conidial suspension + 1% coconut oil

(iii) conidial suspension + 1% mineral oil

(iv) conidial suspension + distilled water + Tween 80

These were thoroughly mixed to produce a homogenous emulsion. Three pipette drops were plated on a petri-dish (radius 8mm) of SDA and incubated at 25°C 30°C 35°C 40°C for 24h while maintaining 60% RH. Germination was compared with controls.

(b) Pathogenicity

A batch of 150 third instars of *S. gregaria* were sprayed with $5x10^7$ conidia/ml formulated with each of the oils mentioned in 3.6.1. The treated nymphs were kept in three cages of 50 each separately under 25°C, 30°C, 35°C and 40°C at 50% RH. Similarly, another batch of 50 third instar larvae were sprayed in triplicates with the same formulation and concentration and were each kept at 40, 60, 80, and 100% RH while maintaining the temperature at 30°C. Controls were sprayed with an aqueous conidial suspension. Mycosis and mortality were monitored daily for 14 days.

3.5.2 UV-protection of *B bassiana*

(a) Effect of UV on conidial viability

The efficacy of UV-protectants on conidial viability was investigated by mixing 10ml of $5x10^7$ conidia/ml suspension with the UV-protectants as follows:

(i) conidial suspension + 1% molasses.

(ii) conidial suspension + 1% orzan.

(iii) conidial suspension + 1% congo red.

(iv) conidial suspension + 1% cochneal

(v) conidial suspension + distilled water + Tween 80.

These conidial formulations were then exposed to 360nm of UV irradiation for different lengths of time; 1h, 12h, 24h, and 48h before they were then tested for viability. Both the formulated and control samples were plated in triplicates

by putting 3 drops on the SDA media and incubating at 30°C for 24h. Percent germination was calculated.

(b) Pathogenicity

A batch of 150 third instars were sprayed with the above formulations separately. Controls were sprayed with UV-irradiated aqueous conidial suspension. Mycosis and mortality were monitored upto 14 days.

3.5.3 Oil-molasses formulation of *B. bassiana*

(a) Effect on conidial viability

The effect of oil-molasses formulation on conidial viability was determined by mixing 10ml corn oil with a similar quantity of 1% molasses suspension. The mixture was added to 10ml of $5x10^7$ suspension of *B. bassiana*. Five such mixtures, replicated thrice were plated and incubated at the following temperatures; 25°C, 30°C, 35°C, 40°C and 45°C. Another set of 4 petri-dishes were also replicated 3 times and kept at 40, 60, 80, 100% RH. A third set of 4 petri- dishes treated as described above were exposed to UV light for 1h, 12h, 24h, and 48h before incubation.

(b) Pathogenicity

Corn oil and molasses mixed in similar ratios as mentioned in 3.5.3(a)

above were sprayed on the instars. The first set of experiments consisted of 5 batches of 50 locusts replicated thrice and incubated at 25°C, 30°C, 35°C, 40°C and 45°C. Other 4 batches with similar setting as above were kept at 40, 60, 80 and 100% RH. A third set of 4 petri-dishes were exposed to UV for 1h, 12h, 24h and 48h.

3.6 Screening of *B. bassiana* for safety against non-target organisms

3.6.1 Screening against Honey bees, *Apis mellifera*

Possible toxicity of the fungus towards the honey bee was investigated in which 100 adult bees were taken from a hive and placed 25 each in 4 cages measuring 20cm x 10cm x10cm. Three sets of 25 bees were fed with 10ml suspension of 5 x 10^{12} conidia/ml mixed with an equal amount of sucrose/water (1:1) solution. The fourth set of 25 bees were maintained as controls and fed with only sucrose/water solution.

Another batch of 100 bees were again divided into four sets of 25 bees kept in separate cages. Three sets were sprayed with a 5 x 10^{12} conidia/ml suspension and the control was sprayed with Tween 80. Cages were placed at 30°C and 70% RH. Daily observations of mycosis and mortality were made.

3.6.2 Screening against rats

Two cages, each containing 6 adult rats were used in the study. The first set was fed on marshed food impregnated with a 5x10^{12} conidia/ml in water daily

for 4 weeks. Controls were fed on marshed food and distilled water. Experimental conditions were 25°C and 50% RH. Test individuals were observed for any allergic reactions on the skins. Serological tests were perfomed on blood samples taken weekly.

3.7 Determination of the field cage efficacy of *B. bassiana* against *S. gregaria*

The field site was located at ICIPE Science Park in Kasarani, located 12 km from Nairobi city centre, Nairobi. It is a flat terrain with open grassland and scrubland. Nine blocks, each measuring 2.5 m x 2.0 m and separated from one another by a minimum barrier strip of 2m were demarcated on a 0.25 hectare and were planted with wheat. Cages of mosquito nets measuring 2.5m x 2.0m x 2.0m (Fig. 4) were used to cover the plots.

Cages had openings through which release, spraying and sampling of instars was done. In each cage 180 third instar nymphs were released. An initial pre-treatment random sampling was conducted after 7 days to determine the stable population within the cage after release. The conidial suspension for field-cage trials was produced through mass production mentioned earlier. Instars were sprayed with 5 $x10^{12}$ conidia/ml suspension using hand-held sprayers. Relative humidity range was between 43% - 63% while the average temperature was between 27°C - 32°C and a wind speed of 2m/sec.

Sampling of live instars was done using a $0.5m^2$ miniature quadrat thrown randomly 4 times on the wheat plot within the cage. The population was estimated by counting the instars within the quadrat. The average number of locusts in this area was multiplied by the total area of the plot and added to the number of locusts basking on the roof and walls of the cages. Dead locusts were collected daily for 20 days.

Data were analyzed by Statistical Analysis System (SAS, 1985). Percentage mortality was calculated and subjected to square root transformation according to Southwood (1966). The critical pathogenicity indices (LD_{50} and LT_{50}) were determined by Probit analysis. Means were compared using Duncan's multiple range test (DMRT). Differences from analysis of variance (ANOVA) were considered significant at $P < 0.01$.

CHAPTER 4

RESULTS

4.1 Pathogenicity of *B. bassiana* to *S. gregaria*

Pathological manifestations of *B. bassiana* infection on *S. gregaria* were observed on the second day post-inoculation. Infected nymphs first became sluggish and failed to feed normally. There was a decrease in nymphal response when touched. At death, after approximately 8 days, nymphs were found to be soft and usually distended, but hardened after 48 hours. Saprophytic growth of the mycelia was first seen from the intersegmental parts and subsequently, the fungus emerged from all parts of the integument (Fig. 5). As the disease progressed, mycelia appeared as a white covering over much of the integument and gradually mummified the cadavers.

4.2 Stage Susceptibility

All five nymphal and adult stages of *S. gregaria* showed varied susceptibility to *B. bassiana* infection. From the first and second nymphal stages, which were the most susceptible, a mean cumulative mortality of 76.10% ± 0.78 and 72.63% ± 2.60 respectively was recorded after 14 days when sprayed with $5x10^{12}$ conidia/ml (Table 1). In the third and fourth stages, mortalities were 66.87% ± 1.03 and 57.10% ± 1.57 respectively. The fifth nymphal instar only showed 51.60% ± 1.50 mortality. Only 46.43% ± 1.76 adults died in all

Fig. 4. Cages for field trials. The cages (arrowed), for field trials are in the foreground next to ICIPE office (background).

Table 1. Percent mortality of the different stages of *S. gregaria* infected with five concentrations of *B. bassiana* *Conidia*

	% Mean mortality per Concentration					
	10^{7}	10^{8}	10^{9}	10^{10}	10^{12}	C
Stage of insect						
I	70.70[a] ±3.50	71.50[a] ±1.06	71.80[a] ±1.27	73.71a ±1.27	76.10[a] ±0.78	6.90[b] ±1.10
II	67.13[a] ±2.13	76.37[a] ±2.35	71.70[a] ±1.70	71.90[a] ±0.05	72.63[b] ±2.60	8.07[b] ±0.06
III	60.90[b] ±0.50	60.83[b] ±1.44	72.35[b] ±0.07	63.57[b] ±1.37	66.87[c] ±1.03	6.07[b] ±0.12
IV	52.25[c] ±3.18	53.63[c] ±1.69	51.00[c] ±1.41	53.23[c] ±1.72	57.10[d] ±1.57	5.17[c] ±0.20
V	44.43[d] ±2.11	47.97[d] ±5.89	58.50[d] ±2.12	49.72[d] ±0.60	51.60[e] ±1.50	5.23[d] 0.50
A	39.43[e] ±1.01	43.17[d] ±1.24	43.47[e] ±0.15	47.67[d] ±2.75	46.43[f] ±1.76	5.12[c] ±0.50

. % mortality values are means ± standard error of the mean

. Values with the same letters in a column are not significantly different at ($P < 0.01$) according to Duncans Multiple Range Test (DMRT).

. C = Control

. A = Adult

Fig. 5. Mycelial growth of *B. bassiana* on infected *S.gregaria.*

concentrations (Fig. 6). Between 5.12 ± 0.05 - 8.07% ± 0.05 natural mortalities occurred in controls.

4.3 Lethal dose

Mortality of *S. gregaria* was found to be dependent on the conidial concentrations and the incubation period of the fungus respectively. Lethal dose as determined by probit analysis was $5x10^7$ conidia/ml (Table 2 and Fig. 7) after 8 days. All the nymphs treated with *B. bassiana* experienced mortality that was significantly greater ($P > 0.01$) than the control. However, there was no significant difference ($P < 0.01$) in the mortality among the nymphs inoculated with higher concentration ($5x10^4$ - $5x10^7$). In these groups mortality gradually increased upto the 50% kill after which variations were not significant ($P < 0.01$)). Highest mortality 70.33% ± 0.01 and 71.83 ± 0.80 in $5x10^6$ and $5x10^7$ respectively was obtained at $5x10^{12}$ conidia/ml. No apparent mortality was observed before the third day of infection other than natural deaths. Fungi infected nymphs developed characteristic symptoms of the disease after death.

4.4 Contact efficiency of *B. bassiana* on *S. gregaria*

The actual number of conidia coming in contact with the host was highest between 0.5m and 1.0m (Droplet Scale: 8A). The contact efficiency was reduced at 2.5m (Deposit Scale: 1A) (Fig. 3) (Table 3). Results show that as the distance increases conidia coming into contact with the host is in form of mist which

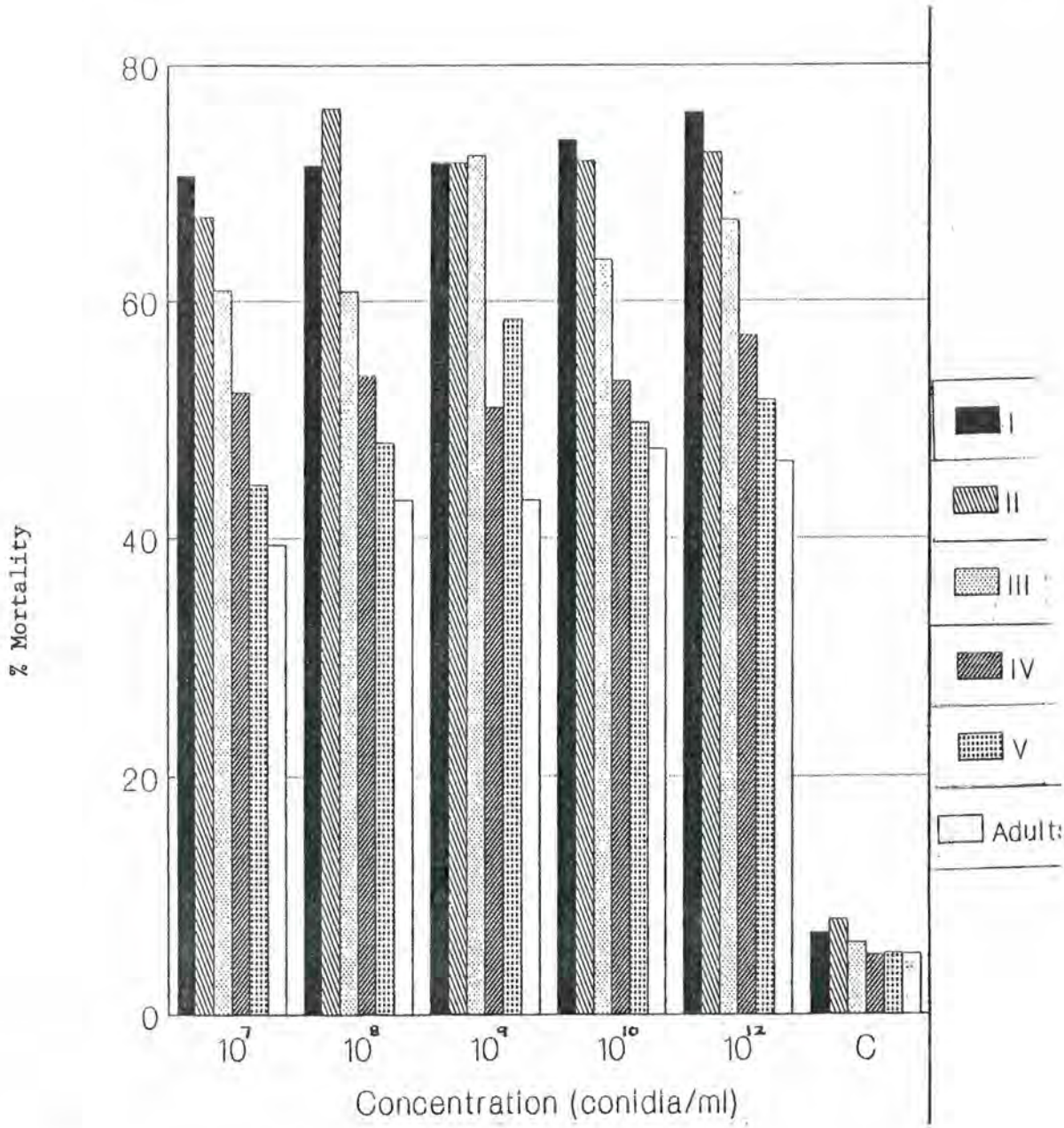

Fig. 6. Susceptibility of *S. gregaria* stages to different concentrations of *B. bassiana*.

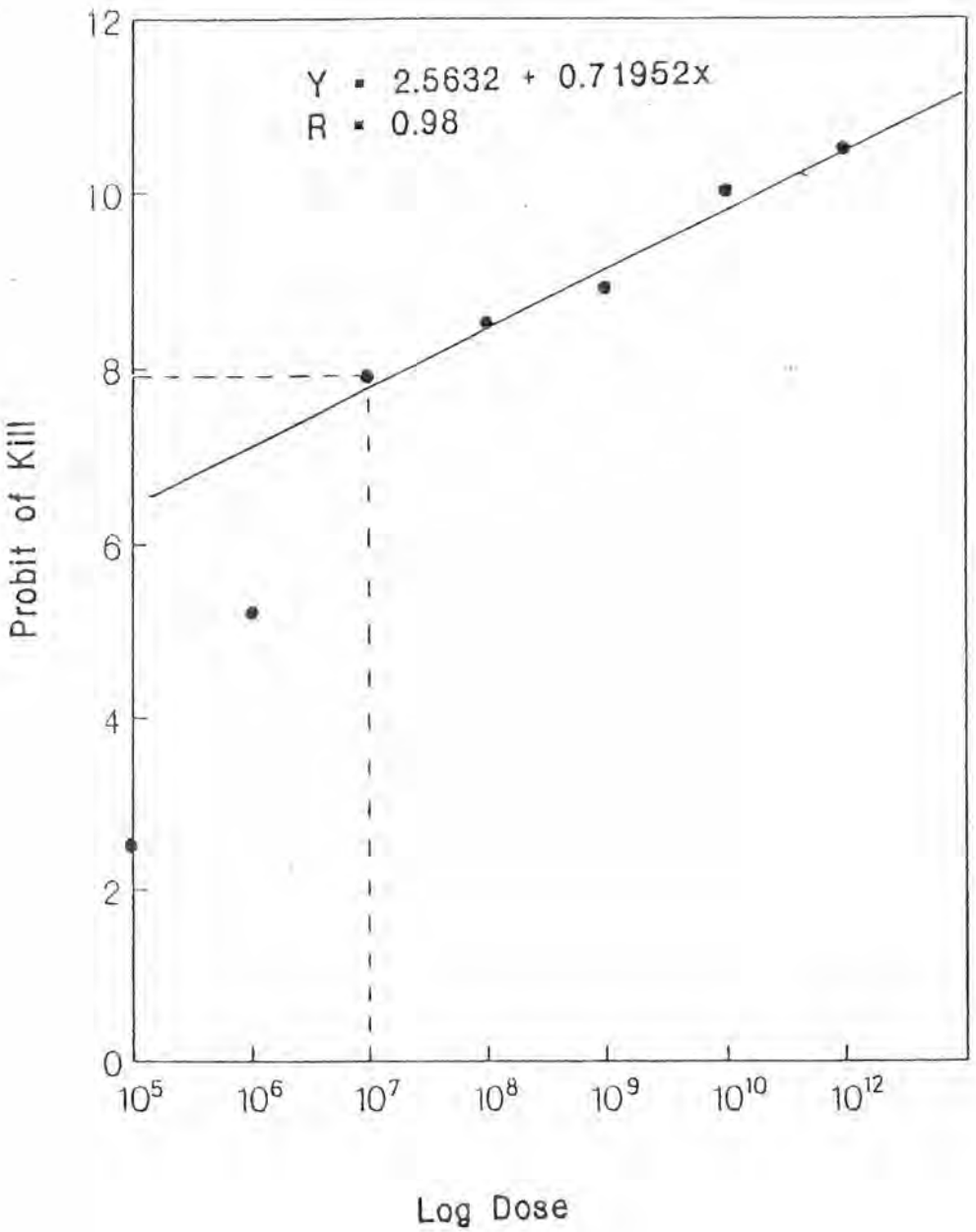

Fig. 7. **Mortality of III instars of *S. gregaria* at different *B. bassiana* concentrations.**

Table 2. Percent mortality of *S. gregaria* at seven concentrations of *B. bassiana* on days 2, 4, 6, 8, 10, 12 and 14 post innoculation.

	% Mortality per Days post-innoculation						
	2	4	6	8	10	12	14
Conidia							
$5x10^5$ (T_1)	3.33 ±0.20	5.15 ±0.32	7.03 ±2.56	12.33 ±0.28	28.60 ±2.72	45.60 ±0.17	47.83 ±2.46
$5x10^6$ (T_2)	4.33 ±4.33	5.16 ±0.28	15.33 ±0.28	28.33 ±7.63	43.90 ±0.01	45.00 ±0.01	45.00 ±0.17
$5x10^7$ (T_3)	3.33 ±0.00	4.00 ±0.28	13.50 ±1.73	50.40 ±0.17	57.50 ±0.00	59.06 ±0.00	62.13 ±0.23
$5x10^8$ (T_4)	5.00 ±0.86	6.50 0.86	13.83 2.88	56.83 ±0.17	59.66 ±0.57	63.16 ±0.28	66.50 ±0.00
$5x10^9$ (T_5)	4.00 ±0.00	9.20 ±3.27	28.50 ±3.04	57.96 ±0.05	60.16 ±0.32	64.00 ±0.57	67.83 ±0.76
$5x10^{10}$ (T_6)	4.16 ±0.28	5.86 ±0.80	18.93 ±0.92	53.00 ±1.00	59.00 ±0.40	65.73 ±0.57	70.33 ±0.00
$5x10^{12}$ (T_7)	3.33 ±0.42	4.00 ±0.00	4.20 ±0.34	55.50 ±0.28	61.66 ±29.44	66.50 ±0.05	71.83 ±0.80
C	4.30 ±0.55	5.86 ±0.00	5.93 ±0.57	6.50 ±0.28	7.60 ±1.00	9.20 ±0.28	8.66 ±0.80

. % mortality values are means ± standard error of the mean

. C = Control

Table 3. Mean number of *B. bassiana* droplets falling on *S.gregaria* at various spray distances.

Spray distance (m)	droplet scale	mean conidia/mm^2
0.5	8	$5x10^6$
1.0	4	$5x10^2$
2.0	4	$5x10^2$

moves to the locust surface, irrespective of the distance of the spray source.

4.5 Virulence improvement of *B. bassiana*

It was observed that the virulence of *B. bassiana* showed considerable improvement after successive *in vivo* passage of the sub-lethal dose ($5x10^5$) conidia/ml in *S. gregaria* (Table 4). An increase in virulence was evident from the fifth *in vivo* step when compared to the non-passaged conidia where 50% mortality was never attained. The gradual rise in pathogenicity occurred upto the 8th step when mortality of both passaged and non-passaged conidia stabilized but at different levels (51.32% ± 0.01 and 46.00% ± 0.02) respectively (Fig. 8). Controls which were sprayed with Tween 80 remained consistently low.

4.6 Influence of temperature on the efficacy of *B. bassiana*

4.6.1 Growth potential

The development of *B. bassiana* cultures was observed at varying temperatures and a relative humidity of 60%. Growth was initially slow at all temperature regimes upto the fourth day (Table 5). A rapid increase was recorded after the fifth day at 25°C, 30°C and 35°C and the maximum growth (8mm) radius was attained in the petri-dish after day 7. At higher temperatures, development was slow even after the fifth day and did not reach the maximum, suggesting that conidial growth was affected by the heat.

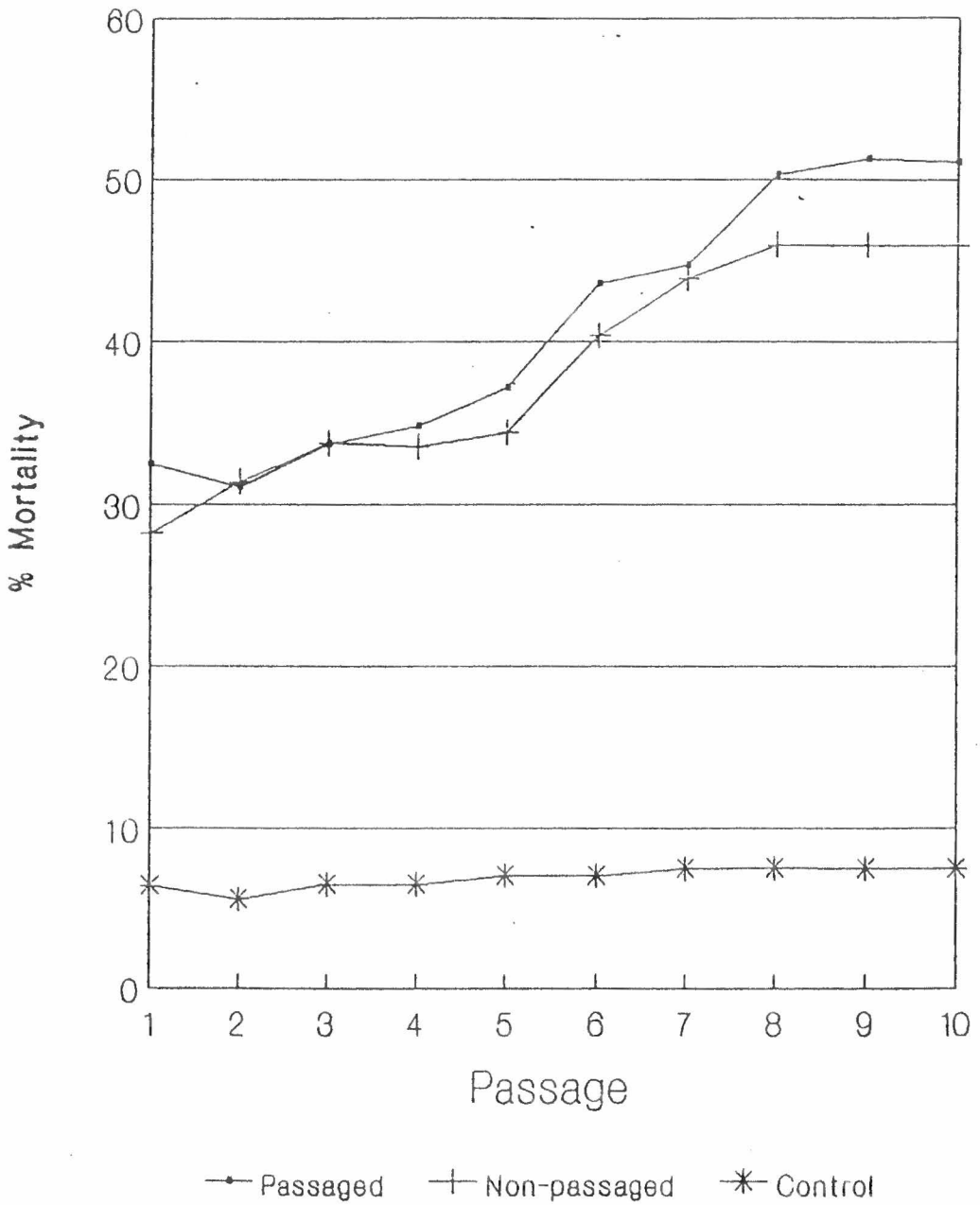

Fig. 8. **Mortality of *S. gregaria* treated with passaged and non-passaged *B. bassiana*.**

Table 4. Percent mean mortality of *S. gregaria* infected with passaged and non-passaged *B. bassiana* conidial suspension.

	% mortality		
Step	Passaged	non-passaged	control
1	32.56^{de} ±0.17	28.25^{e} ±0.04	6.40^{f} ±0.10
2	31.07^{e} ±0.02	31.49^{de} ±0.22	5.60^{f} ±0.04
3	33.75^{cde} ±0.13	33.80^{cde} ±0.22	6.50^{f} ±0.38
4	34.80^{c} ±2.01	34.58^{c} ±0.24	6.50^{f} ±0.04
5	37.15^{c} ±0.69	34.44^{c} ±0.12	7.05^{f} ±0.12
6	43.65^{b} ±0.87	40.40^{b} ±0.17	7.50f ±0.38
7	44.80^{b} ±0.07	43.95^{b} ±0.03	7.55^{f} ±0.10
8	50.25^{b} ±0.06	43.95^{b} ±0.12	7.50^{f} ±0.12
9	51.04^{a} ±0.04	46.00^{b} ±0.02	7.50^{f} ±0.12
10	51.32^{a} ±0.01	46.00^{b} ±0.02	7.50^{f} ±0.23

. % Mortality values are means ± standard error.

. Number with the same letters in a column are not significantly different at ($P < 0.01$) according to DMRT.

Table 5. Mean growth CM of *B. bassiana* under five temperature regimes

	Mean growth (cm) per Temperature (°C)				
	25	30	35	40	45
Day					
1	0.50 ±0.00	0.50 ±0.00	0.60 ±0.00	0.50 ±0.00	0.50 ±0.00
2	0.50 ±0.00	0.53 ±0.02	0.70 ±0.00	0.50 ±0.00	0.50 ±0.00
3	0.56 ±0.02	0.56 ±0.06	0.80 ±0.00	0.53 ±0.02	0.53 ±0.00
4	0.93 ±0.15	0.60 ±0.00	0.93 ±0.02	0.53 ±0.02	0.50 ±0.02
5	3.36 ±0.15	0.60 ±0.00	1.33 ±0.13	0.53 ±0.02	0.53 ±0.02
6	6.30 ±0.12	0.60 ±0.02	5.33 ±0.13	0.53 ±0.02	0.53 ±0.02
7	8.10 ±0.08	0.80 ±0.00	6.33 ±0.13	0.53 ±0.01	0.53 ±0.04
8	8.16 ±0.13	3.33 ±0.13	7.33 ±0.13	0.53 ±0.13	0.60 ±0.01
9	8.30 ±0.12	4.16 ±0.13	7.33 ±0.13	.0.82 ±0.13	0.60 ±0.00
10	8.30 ±0.32	7.33 ±0.13	7.00 ±0.13	1.33 ±0.01	0.60 ±0.04
11	8.23 ±0.00	7.33 ±0.13	7.33 ±0.13	2.00 ±0.13	0.60 ±0.00
12	8.50 ±0.00	7.33 ±0.27	7.33 ±0.33	2.00 ±0.00	0.60 ±0.05
13	8.50 ±0.00	7.66 ±0.27	7.33 ±0.33	2.50 ±0.00	0.73 ±0.05
14	8.50 ±0.00	7.66 ±0.27	7.33 ±0.33	3.00 ±2.00	0.73 ±0.05

4.6.2 Pathogenicity

Higher nymphal mortalities occured between 25 - 30°C but decreased at 35°C and above. No significant mortality was recorded at 45°C (Table 6). Percent mortality were high between 25°C - 35°C and decreased significantly ($P > 0.01$) even when concentrations were increased. Although mortalities were recorded at temperature levels above 35°C, nymphs did not show the characteristic symptoms of the white muscardine disease. This showed that conidia retained some ability to infect, but lost the vigour to germinate.

4.7 Effect of relative humidity on efficacy of *B. bassiana*

4.7.1 Growth potential

The growth rate was found to be directly proportional to the levels of humidity. Maximum growth was recorded in 5 days at 100% RH, but a delay in growth potential occured as humidity was lowered (Table 7). Growth below 50% RH was very slow upto the ninth day and only increased slightly from the tenth day onwards.

4.7.2 Pathogenicity

Mortality caused by *B. bassiana* ocurred at all levels of relative humidity although it was highest between 80 - 100% and dropped rapidly as humidity was lowered (Table 8). The external development of fungal mycelia occurred in all cases irrespective of the humidity although high RH was found to accelerate

Table 6. Percent mean mortality of *S. gregaria* to *B. bassiana* infection at five temperatures

Conc	% Mortality per Temperature (°C)				
	25	30	35	40	45
10^{7}	50.00^{b} ±0.00	52.33^{b} ±2.51	52.66^{b} ±2.51	43.66^{c} ±0.57	42.66^{c} ±0.57
10^{8}	53.33^{ab} ±2.88	47.33^{c} ±2.52	49.66^{bc} ±5.50	45.00^{c} ±8.60	40.00^{c} ±0.00
10^{9}	59.90^{a} ±1.73	56.66^{ab} ±0.60	52.44^{b} ±1.00	44.33^{c} ±0.50	40.35^{c} ±0.50
10^{10}	59.98^{a} ±0.72	58.33^{a} ±2.05	58.33^{a} ±2.00	48.50^{bc} ±0.80	41.00^{c} ±0.50
10^{12}	60.00^{a} ±1.40	61.00^{a} ±1.00	60.00^{a} ±0.57	47.90^{c} ±0.60	40.33^{c} ±1.55
C	6.00^{d} ±0.00	7.00^{d} ±1.00	7.50^{d} ±0.57	8.00^{d} ±0.57	6.66^{d} ±1.55

. Numbers with the same letters in a row are not significantly different at ($P < 0.01$) according to DMRT

. % mortality are means ± standard error of the mean

. C = Control

Table 7. Mean growth potential radius of *B. bassiana* under four relative humidities.

Mean growth (cm) per Relative humidity (%)				
	40	60	80	100
Day				
1	0.50 ±0.00	0.50 ±0.00	0.51 ±0.13	0.50 ±0.00
2	0.51 ±0.01	0.60 ±0.01	0.52 ±0.16	1.50 ±0.01
3	0.50 ±0.12	0.81 ±0.03	0.86 ±0.24	4.30 ±0.14
4	0.51 ±0.00	0.90 ±0.02	4.06 ±0.10	8.16 ±0.14
5	0.70 ±0.00	2.06 ±0.13	6.00 ±0.23	8.16 ±0.00
6	0.93 ±0.01	2.03 ±0.15	6.50 ±0.00	8.50 ±0.14
7	0.93 ±0.12	3.03 ±0.13	6.50 ±0.02	8.50 ±0.00
8	0.93 ±0.33	7.03 ±0.12	7.06 ±0.14	8.50 ±0.00
9	1.00 ±0.00	8.06 ±0.13	8.00 ±0.02	8.76 ±0.00
10	1.06 ±0.04	8.20 ±0.04	8.33 ±0.01	8.76 ±0.03
11	1.56 ±0.04	8.13 ±0.02	8.33 ±0.02	8.76 ±0.03
12	1.50 ±0.10	8.13 ±0.12	8.54 ±0.02	8.76 ±0.24
13	2.06 ±0.02	8.15 ±0.04	8.41 ±0.02	8.76 ±0.14
14	2.33 ±0.41	8.15 ±0.13	8.41 ±0.12	8.76 ±0.14

Table 8. Percent mean mortality of *S. gregaria* infected with *B. bassiana* at four relative humidities

Conc	% Mean mortality per Relative humidity (%)			
	40	60	80	100
10^7	40.33^{c} ±0.57	49.66^{b} ±2.51	61.33^{a} ±1.15	62.00^{b} ±0.00
10^8	41.66^{c} ±2.88	49.00^{b} ±1.78	61.90^{ab} ±2.08	63.66^{a} ±1.52
10^9	45.00^{bc} ±0.00	50.00^{b} ±1.73	62.66^{a} ±2.51	65.00^{a} ±0.00
10^{10}	45.70^{bc} ±0.00	49.78^{b} ±1.15	65.00^{a} ±1.73	67.00^{a} ±1.73
10^{12}	56.00^{b} ±2.08	50.06^{b} ±0.21	63.66^{a} ±1.50	72.00^{a} ±2.00
C	7.00^{d} ±1.50	8.00^{d} ±0.50	8.00^{d} ±0.55	8.00^{d} ±2.56

. Numbers with the same letters in a row are not significantly different at ($P < 0.01$) according to DMRT

. % mortality values are means ± standard error

. C = Control

fungal sporulation and subsequent nymphal mortality. LT_{50} varied with the dosages and the level of humidity.

4.8 Effect of ultra-violet light on efficacy of *B. bassiana*

4.8.1 Conidial viability

A decrease in viability of UV-irradiated conidial suspension was observed with increasing time of exposure. Growth reduction occured during the first hour followed by a gradual reduction upto 24 hours. After 48 hours the germination rate of conidia decreased to 40% (Table 9). In the controls, 95% viability was maintained.

4.8.2 Pathogenicity

The resulting nymphal mortality from *B. bassiana* pre-exposed to UV light showed a negative correlation to exposure time. As the UV exposure to conidial suspension was increased, the percent mortality of the locusts was consequently decreased. 50% mortality was achieved in exposures below 12h as compared to non exposed conidia (Table 10). The low mortalities at increased UV exposure could be attributed to loss in conidial viability from the exposure.

4.9 Development of suitable formulation of *B. bassiana* against *S. gregaria*

4.9.1 Oil formulation

Corn oil formulation had the least effect on the germination potential compared to coconut and mineral oils. However, the germination rate

Table 9. Mean growth potential radius of *B. bassiana* after exposure to four UV irradiation periods.

	Mean growth (cm) per Exposure Time			
	1	12	24	48
Day				
1	0.50 ±0.00	0.53 ±0.02	0.50 ±0.00	0.50 ±0.00
2	0.50 ±0.00	0.56 ±0.02	0.50 ±0.00	0.63 ±0.07
3	0.50 ±0.04	0.78 ±0.01	0.52 ±0.00	0.63 ±0.07
4	0.60 ±0.13	1.46 ±0.04	0.50 ±0.02	0.63 ±0.19
5	6.15 ±0.13	2.10 ±0.04	1.89 ±0.02	1.46 ±0.19
6	7.06 ±0.13	3.46 ±0.16	4.20 ±0.21	1.46 ±0.19
7	7.16 ±0.13	4.78 ±0.10	5.23 ±0.09	1.46 ±0.07
8	7.83 ±0.02	5.03 ±0.10	5.23 ±0.02	1.78 ±0.30
9	7.83 ±0.02	5.03 ±0.01	5.60 ±1.12	1.78 ±0.07
10	8.50 ±0.02	7.36 ±0.05	6.36 ±0.02	2.26 ±0.03
11	8.56 ±0.02	8.23 ±0.12	6.68 ±0.04	2.26 ±0.01
12	8.06 ±0.02	8.23 ±0.19	7.00 ±0.00	2.60 ±0.12
13	8.70 ±0.02	8.23 ±0.02	7.26 ±0.02	2.65 ±0.12
14	8.70 ±0.08	8.23 ±0.40	7.00 ±0.02	2.60 ±0.04

Table 10. Percent mean mortality of *S. gregaria* infected with four UV irradiated *B. bassiana*.

	% Mean mortality per UV-irradiation			
	1	12	24	48
Conidia/ml				
10^7	52.66^b ±0.50	50.00^b ±0.33	46.66^d ±0.60	33.33^d ±0.80
10^8	55.00^a ±0.40	53.33^b ±1.20	48.00^d ±1.14	46.66^d ±2.88
10^9	50.00^b ±0.21	51.66^b ±0.90	41.00^c ±0.60	38.00^d ±0.03
10^{10}	56.00^a ±0.60	52.66^b ±1.0	45.00^c ±2.00	44.56^c ±0.08
10^{12}	54.33^a ±1.50	52.00^b ±0.33	45.00^c ±0.40	37.33^d ±2.06
C	6.50^e ±0.01	7.00^e ±0.21	8.00^e ±0.57	8.00^e ±0.52

. % Mortality values are means ± standard error

. Numbers with the same letters are not significantly different ($P < 0.01$) according to DMRT

. C = Control

was dependent on temperature and was considerably diminished at 40°C and above, especially for coconut and mineral oil (Table 11). When relative humidity was varied, viability was similarly reduced at very low RH, particularly for coconut and mineral oil (Table 12)

4.9.2 Pathogenicity

The oil-based formulations produced higher levels of percent mortality than non formulated conidia over a wider range of temperatures (Table 13). In non-formulated conidia, mortality at temperatures above 35°C were significantly low as compared to corn oil formulation. Similarly at low relative humidity, non-formulated suspensions gave lower mortalities than formulated (Table 14). In both environmental parameters controls were significantly lower than the treated locusts ($P < 0.01$).

4.10 Efficacy of *B. bassiana* formulated with UV-protectants

4.10.1 Conidial viability

UV exposures had deliterious effect on the viability of non-formulated conidia after 1 hour of exposure and by 48 hours, 60% loss in viability occured (Table 15). Molasses-formulated conidial suspensions showed consistent protection even after 48 hours of exposure.

4.10.2 Pathogenicity

Mortalities of locusts with all the UV-protectants were significantly higher than the control, even after 48h of exposure. However, prolonged

Table 11. Viability of oil-based *B. bassiana* under varying temperatures

Conidial viability at given Temperature (°C) levels					
	25	30	35	40	45
Oils					
Corn	92.96 ±0.09	88.86 ±0.09	87.40 ±0.02	60.30 ±0.01	40.60 ±0.01
Coconut	56.30 ±0.01	54.50 ±0.02	52.60 ±0.02	30.50 ±0.02	25.30 ±0.01
Mineral oil	45.50 ±0.01	40.70 ±0.04	36.50 ±0.08	30.50 ±0.02	25.00 ±0.01
Control	98.20 ±0.01	98.00 ±0.03	98.00 ±0.00	60.50 ±0.00	60.00 ±0.02

. C = Non formulated conidia

Table 12. Viability of oil formulated *B. bassiana* under four humidities

	% Viability at given RH (%) levels			
	40	60	80	100
Oils				
Corn	64.40 ±0.04	80.55 ±0.00	95.05 ±0.00	96.00 ±0.00
Coconut	66.50 ±0.04	68.55 ±0.02	72.55 ±0.01	80.55 ±0.01
Mineral oil	55.55 ±0.16	60.00 ±0.12	66.50 ±0.11	70.50 ±0.14
Control	94.00 ±0.02	96.00 ±0.02	96.00 ±0.00	97.00 ±0.00

Table 13. Effect of oil-based *B. bassiana* formulation on mortality of *S. gregaria* at five temperatures

		% Mean mortality per Concentration (Conidia/ml)					
		10^7	10^8	10^9	10^{10}	10^{12}	C
Temp (oC)							
25	F	53.33 ±0.55	56.66 ±2.51	58.00 ±0.00	57.00 ±1.54	60.33 ±1.00	8.00 ±0.10
	NF	50.00 ±0.55	50.66 ±0.83	52.46 ±2.00	55.00 ±0.33	54.00 ±0.52	6.87 ±0.23
30	F	59.00 ±1.52	66.00 ±4.01	66.33 ±2.08	67.66 ±1.57	70.00 ±0.50	8.00 ±0.33
	NF	50.00 ±0.00	52.00 ±2.70	54.00 ±2.00	57.33 ±2.00	58.00 ±1.67	7.10 ±0.20
35	F	54.00 ±0.30	57.00 ±1.80	57.33 ±0.52	58.45 ±2.04	59.00 ±0.10	6.00 ±1.50
	NF	49.00 ±2.54	50.10 ±1.57	60.00 ±0.30	52.80 ±0.40	53.00 ±0.57	7.10 ±1.00
40	F	52.00 ±1.53	52.80 ±0.52	53.00 ±0.02	53.03 ±0.01	53.00 ±1.00	8.00 ±2.00
	NF	42.00 ±1.50	43.40 ±0.02	43.80 ±0.20	45.55 ±0.22	45.90 ±1.00	7.50 ±1.20
45	F	48.00 ±0.02	49.50 ±1.00	50.00 ±1.22	50.80 ±1.20	50.08 ±0.12	8.00 ±1.00
	NF	41.20 ±1.00	41.50 ±1.06	41.70 ±0.56	42.70 ±0.20	43.00 ±0.57	6.00 ±0.66

. % Mortality values are means ± standard error

. F = Formulated conidia

. NF = Non formulated

. C = Control

Table 14. Effect of oil-based *B. bassiana* formulation on mortality of *S. gregaria* at four relative humidities.

	% Mean mortality per Concentration (Conidia/ml)					
	10^{7}	10^{8}	10^{9}	10^{10}	10^{12}	C
RH%						
F	40.33 ±0.50	41.66 ±0.86	42.46 ±0.50	47.00 ±0.22	54.00 ±1.02	6.87 ±0.23
40 NF	40.33 ±0.00	38.66 ±2.80	35.00 ±1.03	37.66 ±2.08	38.33 ±2.88	7.00 ±1.00
F	49.00 ±2.28	50.00 ±0.04	56.33 ±1.60	57.00 ±2.57	60.5 ±0.57	7.10 ±0.00
60 NF	49.80 ±1.67	49.88 ±1.70	50.00 ±1.00	50.33 ±2.52	52.00 ±0.67	7.10 ±0.20
F	56.00 ±0.40	55.00 ±0.80	55.00 ±2.52	64.45 ±1.04	69.00 ±0.00	6.60 ±0.50
80 NF	55.00 ±0.24	52.02 ±0.57	60.08 ±2.57	60.80 ±0.40	63.00 ±2.51	7.10 ±0.02
F	64.00 ±2.73	60.00 ±0.52	65.00 ±2.00	67.93 ±0.01	72.80 ±0.01	7.00 ±0.01
100 NF	62.00 ±0.50	62.50 ±0.12	62.80 ±0.24	65.00 ±1.02	65.00 ±1.22	6.50 ±1.10

. % Mortality values are means ± standard error

. F = Formulated conidia

. NF = Non formulated

. C = Control

Table 15. Effect of UV-protectants on the viability of *B. bassiana* conidia

	% Viability per Exposure time (hr)			
	1	12	24	48
UV-protectant				
Molasses	85.00 ±0.12	70.80 ±0.03	70.22 ±0.01	70.00 ±0.33
Orzan	95.50 ±0.21	84.00 ±0.33	71.26 ±0.44	65.00 ±0.13
Congo red	92.00 ±0.03	80.00 ±0.34	75.55 ±0.13	60.50 ±0.14
Cochneal	94.00 ±0.24	79.00 ±0.22	75.00 ±0.12	62.00 ±0.14
NF	60.00 ±0.00	55.00 ±0.01	49.0 ±0.00	40.00 ±0.00
C	96.00 ±0.00	96.55 ±0.01	96.0 ±0.01	96.00 ±0.02

. NF = Non-formulated

. C = Control

exposures produced varying LT_{50} for the different formulations. For instance the LT50 of congo red is 11 and 9 days in both molasses and cochneal (Table 16).

4.11 Effect of oil-molasses based formulations

4.11.1 Conidial viability

Minimal reduction in germination of oil-molasses based formulation at varying temperatures was observed (Table 17). Viability was higher in the formulated suspension at 40°C but it was low at 45°C in both groups. When exposed to varying relative humidity, viability remained stable (Table 18). At various temporal exposures conidial viability remained stable upto 24h, but declined after 48h (Table 19).

4.11.2 Pathogenicity

Mortality of *S. gregaria* infected with *B. bassiana* formulated with oil and molasses showed significantly improved ($P < 0.01$) mortality at higher temperatures as compared to controls (Table 20). Nymphal response in terms of LT_{50} indicated a progressive prolongation with the increasing temperature as compared to controls. When kept under varying relative humidities, mortality levels were also observed to be higher than in cases where conidia was unformulated (Table 21). Similarly at various UV exposures these nymphs showed marked increase in LT_{50} as compared to that of the controls. After 48h of exposures the formulated conidia showed prolonged potency. Non-formulated conidial suspension did not perform well after UV exposure (Table 22).

Table 16. Percent mean mortality in *S. gregaria* at four UV exposures times infected with molasses formulated and non-formulated *B. bassiana*.

	% Mean mortality per Concentration (Conidia/ml)					
	10^7	10^8	10^9	10^{10}	10^{12}	C
ET (hr)						
F	54.33 ±0.37	58.66 ±2.88	55.00 ±1.73	57.66 ±2.08	65.33 ±2.88	7.00 ±0.86
1 NF	50.33 ±0.57	53.33 ±0.86	52.46 ±2.50	54.00 ±0.33	60.00 ±0.52	6.87 ±0.40
F	51.00 ±4.52	52.00 ±1.64	56.33 ±0.73	58.00 ±0.57	62.5 ±0.57	7.00 ±0.33
12 NF	49.80 ±0.62	50.88 ±1.03	51.00 ±2.00	53.33 ±0.50	52.00 ±0.68	7.00 ±0.23
F	57.00 ±0.46	55.00 ±0.88	55.50 ±2.22	56.45 ±1.24	59.00 ±0.00	6.50 ±0.52
24 NF	50.00 ±0.44	53.02 ±2.50	53.08 ±0.35	53.60 ±0.52	54.00 ±0.57	8.10 ±2.51
F	54.00 ±1.73	50.00 ±2.52	55.00 ±2.52	57.93 ±0.10	57.80 ±0.11	8.00 ±0.02
48 NF	32.00 ±0.57	42.50 ±0.20	42.80 ±0.20	45.00 ±0.22	45.00 ±1.22	6.00 ±0.10

. % mortality values are means ± standard error

. ET = Exposure time

. F = Formulated conidia

. NF = Non-formulated

Table 17. Effect of oil-molasses formulation on viability *B. bassiana* conidia at five temperatures

Temp (°C)	% conidial viability	
	Formulated	non-formulated
25	98.55 ±0.12	95.00 ±0.42
30	98.00 ±0.04	92.50 ±0.14
35	97.00 ±0.16	85.20 ±0.20
40	60.55 ±0.04	71.50 ±0.14
45	60.05 ±0.02	52.00 ±0.00

Table 18. Effect of oil-molasses based formulation on viability of *B. bassiana* conidia at four relative humidities

RH (%)	% viability	
	Formulated	Non-formulated
40	65.50 ±0.42	80.00 ±0.12
60	96.50 ±0.02	95.00 ±0.14
80	98.50 ±0.24	98.00 ±0.14
100	98.50 ±0.04	100.50 ±0.24

Table 19. Effect of oil-molasses formulation on *B. bassiana* conidial viability at varying UV exposures.

ET (hr)	% viability	
	formulated	non-formulated
1	60.00 ±0.14	96.20 ±0.20
12	55.55 ±0.03	96.04 ±0.12
24	49.00 ±0.01	95.02 ±0.32
48	40.04 ±0.12	95.00 ±0.44

Table 20. Percent mortality of *S. gregaria* infected with oil-molasses formulated *B. bassiana* at five temperatures

	% Mean mortality per Concentration (Conidia/ml)					
	10^7	10^8	10^9	10^{10}	10^{12}	C
Temp (oC)						
F	53.33 ±0.57	56.66 ±2.51	58.00 ±0.00	57.66 ±1.54	60.33 ±1.00	8.00 ±0.10
25 NF	50.00 ±0.55	50.66 ±0.83	52.46 ±2.00	55.00 ±0.33	54.00 ±0.52	6.87 ±0.23
F	59.00 ±1.52	66.00 ±4.01	66.33 ±2.08	67.66 ±1.57	70.00 ±0.50	8.00 ±0.33
30 NF	50.00 ±0.00	52.00 ±2.73	54.00 ±2.00	57.33 ±2.00	58.00 ±1.67	7.10 ±0.20
F	54.00 ±0.30	57.00 ±0.88	57.33 ±0.52	58.45 ±2.24	59.00 ±0.10	6.60 ±2.50
35 NF	49.00 ±2.52	50.02 ±1.50	50.08 ±0.30	52.00 ±0.57	53.00 ±0.00	7.10 ±2.51
F	52.00 ±1.52	52.00 ±0.52	53.00 ±0.51	53.93 ±0.61	53.80 ±2.01	8.00 ±2.01
40 NF	42.00 ±0.50	43.50 ±0.20	43.80 ±0.24	45.50 ±0.22	45.90 ±1.24	7.50 ±0.12
F	48.00 ±0.02	49.50 ±1.00	50.00 ±1.22	50.00 ±1.20	50.08 ±0.12	8.00 ±1.00
45 NF	41.20 ±1.00	41.50 ±1.06	41.70 ±0.56	42.80 ±0.20	43.00 ±0.57	6.00 ±0.66

. % Mortality values are means ± standard error

. F = Formulated conidia

. NF = Non formulated

. C = Control

Table 21. Percent mortality of *S. gregaria* infected with oil-molasses formulated *B. bassiana* at four relative humidities.

RH (%)	% Mean mortality per concentration (Conidia/ml)					
	10^7	10^8	10^9	10^{10}	10^{12}	C
F	56.33 ±0.07	58.66 ±1.00	59.00 ±1.00	57.66 ±2.18	58.33 ±0.88	8.00 ±0.80
40 NF	45.33 ±0.55	47.66 ±0.86	47.46 ±2.50	49.00 ±0.23	50.00 ±1.52	7.80 ±0.23
F	50.00 ±0.28	50.40 ±0.64	52.33 ±1.73	57.33 ±0.57	58.5 ±0.52	8.00 ±0.23
60 NF	49.80 ±0.00	49.88 ±1.03	50.00 ±4.20	50.33 ±1.50	52.00 ±1.67	7.10 ±1.23
F	58.00 ±2.36	59.00 ±2.08	60.00 ±4.52	68.45 ±0.24	70.00 ±0.02	8.60 ±0.20
80 NF	54.00 ±0.02	56.02 ±0.40	61.08 ±0.04	62.80 ±0.57	64.00 ±0.57	8.10 ±2.51
F	63.00 ±1.73	65.00 ±1.52	67.00 ±2.51	70.93 ±0.00	72.80 ±0.01	8.00 ±0.01
100 NF	62.00 ±1.52	62.50 ±0.15	62.80 ±0.20	64.00 ±1.20	64.00 ±1.20	7.50 ±0.57

. % mortality values are means ± standard error
. F = Formulated conidia
. NF = Non formulated
. C = Control

Table 22. Percent mortality of *S. gregaria* infected with oil-molasses formulated *B. bassiana* at five UV exposure time.

ET (hr)		% Mean mortality per concentration (Conidia/ml)					
		10^7	10^8	10^9	10^{10}	10^{12}	C
1	F	57.33 ±1.37	60.66 ±0.88	60.00 ±0.73	61.66 ±0.08	65.33 ±0.88	8.00 ±0.82
	NF	50.33 ±0.07	53.33 ±0.80	52.46 ±2.00	54.00 ±0.30	58.00 ±0.50	6.87 ±0.42
12	F	54.00 ±0.52	55.00 ±0.64	56.30 ±0.70	58.00 ±0.07	62.5 ±0.27	7.00 ±0.30
	NF	48.80 ±0.60	51.88 ±1.00	51.40 ±2.00	52.33 ±0.20	52.80 ±0.608	8.00 ±0.82
24	F	60.00 ±0.46	56.00 ±3.88	56.50 ±0.22	57.45 ±1.24	59.12 ±1.30	6.50 ±0.52
	NF	50.00 ±0.44	50.02 ±2.50	51.60 ±0.35	52.00 ±0.52	53.66 ±0.57	8.20 ±2.51
48	F	56.00 ±0.70	52.00 ±0.52	55.00 ±2.52	52.93 ±0.10	58.00 ±0.11	8.00 ±0.02
	NF	33.00 ±2.50	44.50 ±0.20	44.80 ±1.20	45.00 ±2.02	46.00 ±2.02	7.50 ±2.00

* ET = Exposure time
* F = Formulated conidia
* NF = Non-formulated conidia
* C = Control

4.12 Life table analysis of age specific mortality of *S. gregaria* infected by *B. bassiana* at varying environmental factors

The potential of *B. bassiana* against *S. gregaria* and the subsequent k-values within a given temperature regime was found to decrease in the progressive stages from the first instar to the adult (Table 23). The total K-factor showed a decreasing trend with the increase in temperature. However, at 30°C and 35°C there was inconsistency. It decreased considerably at 40°C and 45°C. This was expected since the high temperature lowered the potential of the pathogen. The relative humidity ranging from 40% to 100% at 30°C showed decreasing k-values from L_1 to adults. However, the total K-factor showed higher values with increasing humidity (Table 24). From 80% RH upto 100% RH the K-values remained consistent. The UV tolerance of the pathogen from 1h to 48h was observed to decrease in terms of K-values with increasing exposure time and by 48h the value had declined considerably (Table 25).

4.13 Survivorship curves of *S.gregaria* infected with *B. bassiana*

Nymphs and adults both survived below 35°C, whereas temperatures higher than this resulted into more nymph survival than adults (Fig. 9a). Below 50% RH survivorship was high, while increasing humidity produced increased mortality giving low survival values (Fig.9b). The UV exposures above 12h

Table 23. Life table analysis of the effects of temperature on pathogenicity of *B. bassiana* against *S. gregaria*

Temp (°C)	Stage	% Killed (qx)	no. survived (nx)	k value	
25	L1	69.79	55.00	0.43	
	L2	76.70	51.20	0.46	
	L3	62.50	58.20	0.41	
	L4	54.16	66.00	0.36	
	L5	55.20	71.50	0.32	
	A	46.52	82.50	0.26	$\leq$ 2.24
30	L1	66.60	54.00	0.74	
	L2	67.70	52.50	0.46	
	L3	56.25	67.00	0.35	
	L4	52.08	73.92	0.31	
	L5	48.95	86.50	0.24	
	A	38.19	92.80	0.21	$\leq$ 2.31
35	L1	57.29	64.10	0.37	
	L2	52.08	71.88	0.29	
	L3	58.33	62.59	0.38	
	L4	50.00	75.00	0.30	
	L5	45.83	81.20	0.27	
	A	38.19	93.00	0.21	$\leq$ 1.82
40	L1	46.87	79.70	0.21	
	L2	43.75	87.38	0.33	
	L3	55.20	82.50	0.38	
	L4	46.87	81.00	0.30	
	L5	31.25	103.00	0.27	
	A	41.66	88.00	0.21	$\leq$ 1.70
45	L1	41.66	88.00	0.23	
	L2	41.66	88.34	0.23	
	L3	36.11	96.00	0.10	
	L4	39.58	91.42	0.27	
	L5	32.29	102.00	0.17	
	A	29.75	107.00	0.16	$\leq$ 1.16
				K-value	9.73

. k = kill factor ($\log_{Nx}$ - $\log_{nx}$; where Nx = initial no. of locusts used in stage and nx = no. surviving)
. Nx = 150
. L1 - L5 = first to fifth instars
. A = Adult

Table 24. Life table analysis on the pathogenicity of *B. Bassiana* against to *S. gregaria* at varying relative humidities

RH (%)	Stage	% killed (qx)	no. survived (nx)	k value	
40	L1	52.05	72.00	0.32	
	L2	52.08	72.00	0.32	
	L3	70.00	45.00	0.52	
	L4	50.00	75.00	0.30	
	L5	41.66	89.00	0.23	
	A	39.58	92.50	0.21	$\leq$1.90
60	L1	68.75	47.00	0.50	
	L2	62.00	57.20	0.42	
	L3	56.25	66.00	0.36	
	L4	57.29	65.92	0.36	
	L5	46.87	80.00	0.27	
	A	43.75	60.00	0.24	$\leq$2.15
80	L1	72.90	42.00	0.55	
	L2	69.79	47.00	0.50	
	L3	68.75	47.00	0.50	
	L4	64.58	54.00	0.44	
	L5	52.00	72.00	0.30	
	A	48.83	76.00	0.29	$\leq$2.58
100	L1	75.00	33.00	0.66	
	L2	72.91	42.00	0.55	
	L3	67.70	45.50	0.52	
	L4	96.00	06.00	1.40	
	L5	57.29	65.50	0.36	
	A	46.86	81.00	0.27	$\leq$2.76
				K-value	9.93

. k = kill factor($\log_{Nx}$-$\log_{nx}$;where N_x = initial no. of locusts used in each stage and nx = no. surviving)
. N_x = 150
. L1 - L5 = first to fifth instars; A = Adult
. Initial no. of instars used in the bioassay = 150

Table. 25 Life table analysis of the effects of UV on *B bassiana* pathogenicity to *S. gregaria*.

ET	Stage	% Killed (qx)	no. survived (nx)	k value	
1h	L1	66.60	50.10	0.48	
	L2	63.19	55.22	0.43	
	L3	63.19	55.55	0.43	
	L4	59.37	60.94	0.39	
	L5	46.52	80.30	0.27	
	A	41.66	89.00	0.28	$\leq$ 2.28
12h	L1	62.50	56.25	0.42	
	L2	64.58	52.00	0.46	
	L3	60.41	59.39	0.38	
	L4	58.33	62.51	0.26	
	L5	45.83	82.50	0.26	
	A	37.50	93.75	0.26	$\leq$ 2.04
24	L1	61.45	57.80	0.41	
	L2	60.41	59.85	0.40	
	L3	59.02	62.00	0.38	
	L4	67.00	61.42	0.39	
	L5	38.70	91.95	0.22	
	A	33.33	78.00	0.28	$\leq$ 1.70
48h	L1	41.66	87.57	0.23	
	L2	43.70	59.85	0.25	
	L3	42.18	62.00	0.24	
	L4	36.11	61.42	0.19	
	L5	38.70	91.95	0.20	
	A	29.16	78.00	0.17	$\leq$ 1.28
					K- value 8.2

. ET = Exposure time
. k = kill factor ($k = \log_{Nx} - \log_{nx}$ where Nx = initial no. of locusts used in each stage = 150)
. L1 - L5 = first to fifth instars 1-5; A = Adult

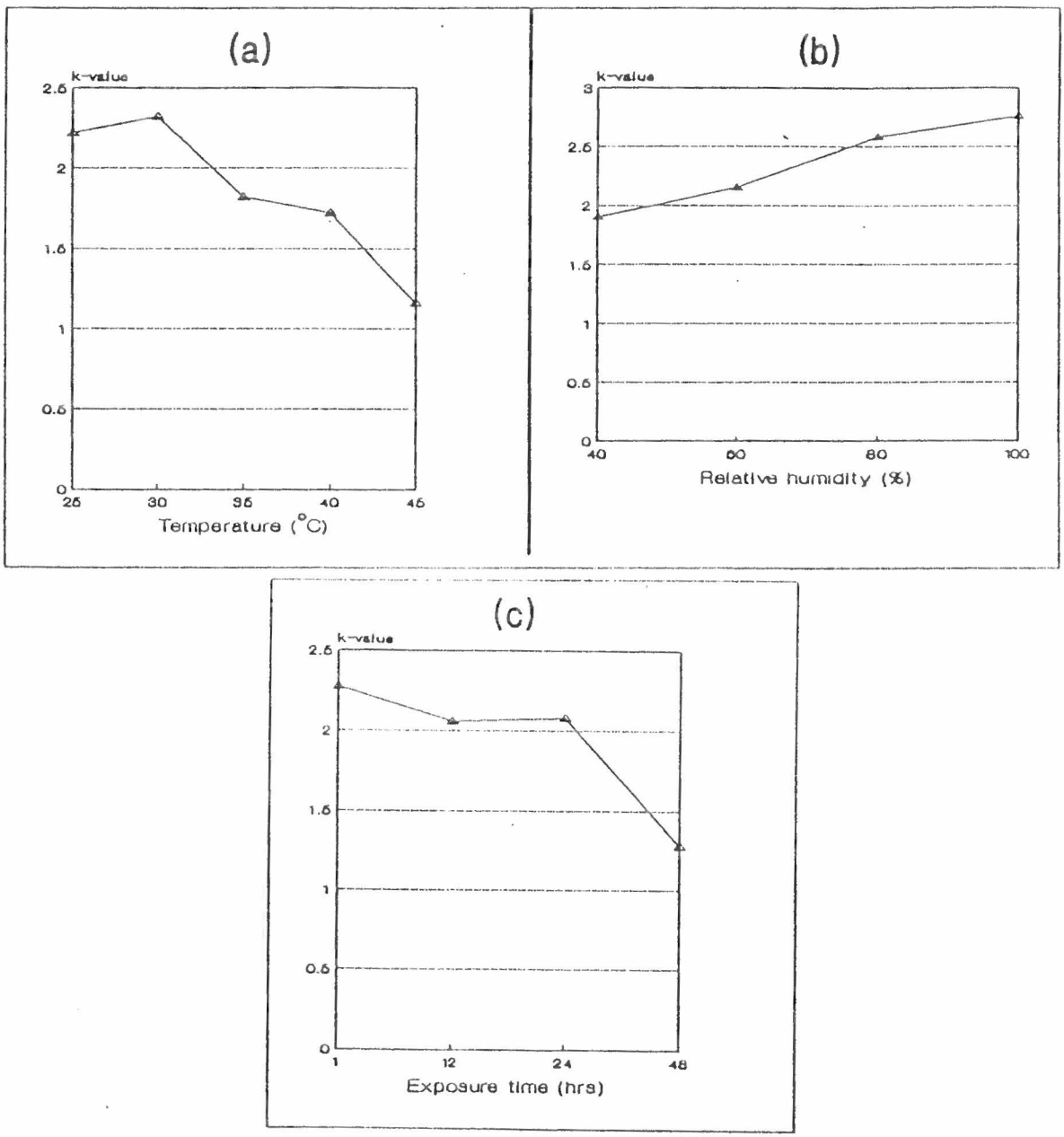

Fig. 9. Survivorship curve of *S. gregaria* to *B.bassiana* infection under different abiotic factors.

proved deliterious to the pathogen and consequently resulted into increased survival of the locusts. At all levels of exposure, survival was observed to increase with the development stage (Fig. 9c).

4.14 Safety tests of of B. bassiana against non-target organisms

4.14.1 Screening against Honey bees

Mortality was observed in both the *B. bassiana* treated and control bees. There was however, no difference in the mortality of bees exposed to *B. bassiana* and the controls which were sprayed with Tween 80 and water (Table 26). It was also observed that there was no reduction in active behaviour and food intake in either the treated bees or controls. This indicated that death could have resulted from natural factors.

4.14.2 Screening against Rats

Activities of the rats fed on *B. bassiana* conidia were normal, although food consumption was found to be higher in treated rats than in controls (Table 27). No skin lesion was observed in either the treated rats or the control. Serological tests detected no conidia in the blood.

4.15 Field-cage trials of *B. bassiana* against *S. gregaria*

Mortality of *S. gregaria* due to *B. bassiana* infection was recorded in field cages (Table 28). It was found that mortality increased slowly with increasing time and the LT_{50} was attained after 10 days in the formulated conidial

Table 26. Percent mean mortality of honey bees sprayed with *B. bassiana* suspension

Day	% Mortality	
	B. bassiana	Control
0	2.00 ±0.00	3.00 ±0.11
2	4.20 ±0.01	4.00 ±0.12
4	10.00 ±0.14	8.50 ±0.13
6	10.00 ±0.21	12.50 ±0.24
8	10.00 ±0.12	12.00 ±0.31
10	10.50 ±0.12	13.50 ±0.14
12	10.50 ±0.04	13.50 ±0.06
14	10.50 ±0.05	13.40 ±0.26

Table 27. Mean food consumption in rats treated with *B. bassiana*

Day	Weight of food left (g)	
	Treated	Control
0	14.52 ±0.17	78.30 ±0.18
2	52.75 ±0.10	73.20 ±0.11
4	53.40 ±0.02	61.00 ±0.00
6	91.60 ±0.40	102.50 ±0.24
8	102.80 ±0.40	111.35 ±0.24
10	80.75 ±0.13	57.30 ±0.25
12	93.50 ±0.22	35.40 ±0.56
14	45.90 ±0.00	33.30 ±0.56

Table 28. Percent mean mortalities of field-cage trials of *S. gregaria* infected with *B. bassiana*.

Day	Control	Aqueous	Formulated
4	4.90[c] ±0.50	9.20[c] ±0.70	13.10[c] ±0.50
8	6.83[c] ±1.40	33.30[c] ±2.33	27.93[c] ±5.40
12	7.43[c] ±3.92	45.63[b] ±1.30	50.83[b] ±5.03
16	10.40[c] ±1.70	58.86[b] ±4.02	77.06[ab] ±5.10
20	12.70[c] ±1.56	71.00[ab] ±2.85	85.46[a] ±0.92

* Means with the same letter are not significantly different at (P< 0.01) according to DMRT

suspension and delayed to 15 days in the non-formulated suspension (Fig 10). Mortality in the control remained consistently low throughout the entire period. Results indicate that even after 21 days mortality could still be expected.

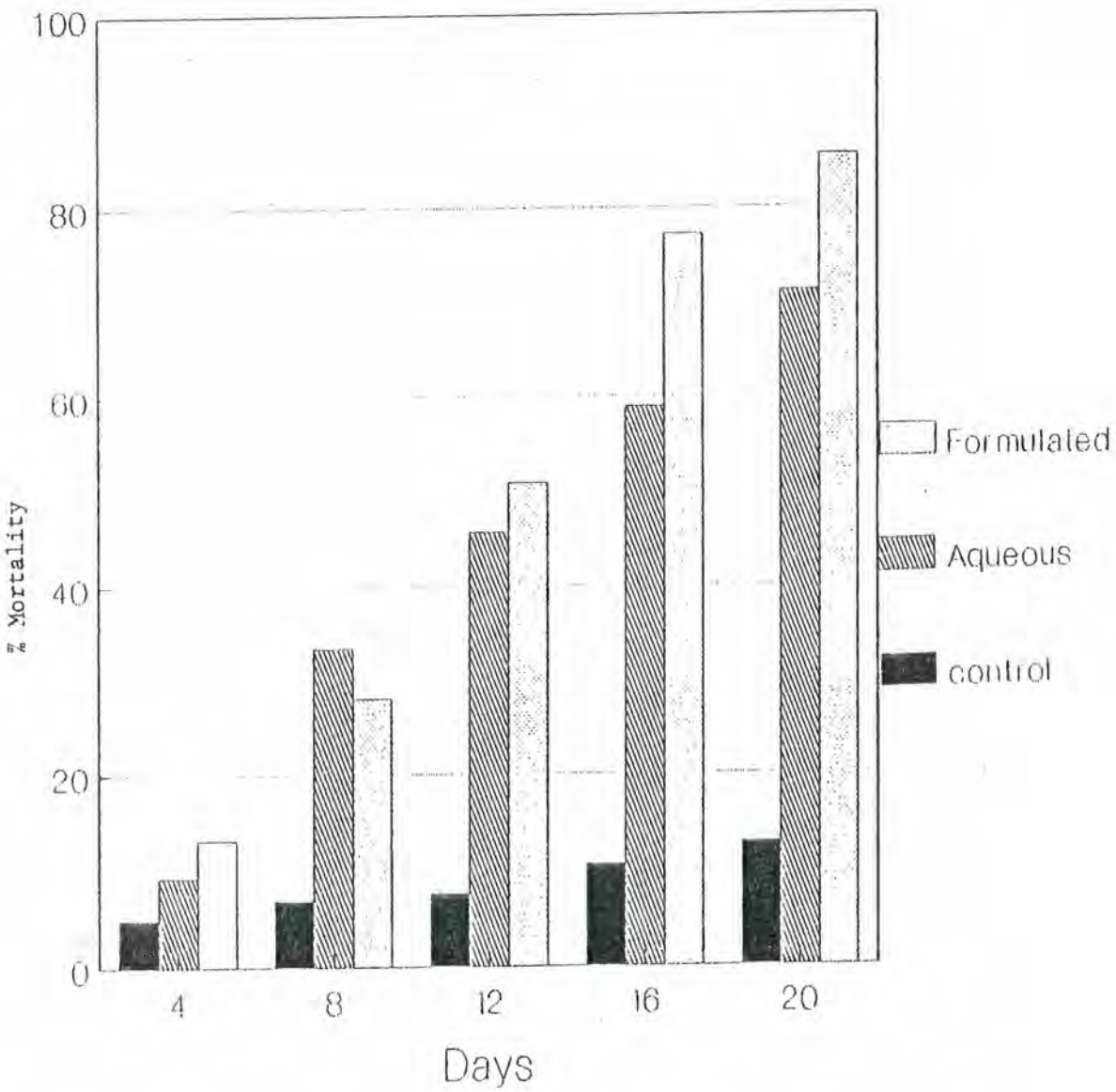

Fig 10. Mortality of *S. Gregaria* infected with *B. bassiana* in field cages

CHAPTER 5

DISCUSSION

Diseased instars of *S. gregaria* showed paralysis before death. The nymphs were inactive as they approached median lethal time (LT_{50}). The inactivity of instars seen in this study compares with that recorded by Paschke (1965) who reported substantial reduction in feeding activity of cereal leaf beetle, *Oulema melanopa* (L.) which had been treated with conidial suspension of *B. bassiana*. The nymphs hardened after death followed by growth of mycelia through the cuticle, with the initial growth. Saprophytic mycelial development occuring between the intersegmental folds then continued gradually until it covered the whole cadaver. Sporulation on the surface of the dead nymphs occurred after 6 days.

Deuteromycetes such as *B. bassiana* are known to infect through mechanical penetration facilitated by enzymatic activity (Vey and Fargues, 1977; Ferron, 1978). There are reports on the ability of the fungus to produce biologically active substances called beauvericins which are known to be toxic to the insects (Pavlyushin, 1969). It can then be concluded that these toxins, together with physiological changes in the haemolymphs resulting from the extensive growth of the fungus in the haemocoel caused the death of the parasitized nymph *S. gregaria*. The toxins could have immobilized the nymphs

by interacting with their life systems causing fatal physiological disorders. It was expected that in *S. gregaria,* the soluble components of the haemolymphs supplied sufficient nutrients for the growth and extensive multiplication of the fungus in the body cavity after penetration. Due to extensive growth of the fungus, death of *S. gregaria* nymphs and adults resulted from exhaustion of nutrients in the haemolymph leading to the hardening of the cadavers of the locusts. According to McCauley *et al.*, (1968), the factors that have been suggested as playing a role in the death of insects parasitized by fungi are toxin production, damaging effect of the mycelium and the pathological changes in the haemolymph.

The dense growth of the fungal conidia observed in *S. gregaria* nymphs at the intersegmental folds of the integument had been reported in earlier studies on different insects (McCauley, *et al.*, 1968;). It suggested the easy access which these channels provided for the fungus because of their thin membrane which required minimum pressure and enzymatic action to penetrate them. These folds are favoured sites of infection since conidia could adhere within them unlike on the smooth surfaces of sclerites where they would easily be removed. The micro-climate within these folds also maintain enough humidity which is conducive to conidial germination as reported in (Charnley, 1984).

The mode of action of *B. bassiana* made it necessary that stage-susceptibility of *S. gregaria* was examined. All nymphal and adult stages of the desert locust were susceptible to the pathogen, but the degree of

susceptibility varied with stage. First and second nymphal instars were most vulnerable to the pathogen and it was found that there is no significant difference ($P < 0.01$) in varying the concentration as both stages produced high mortalities. This may be due to their smaller size and reduced ability to resist the impact of spraying as well as shifts in micro-environmental conditions in the cages.

Mortality in the third and fourth stages were lower than the first two. However, between them, the difference was minimal. The fifth stage and adults were less susceptible as compared to the earlier stages. On the basis of these results, the third stage was considered ideal for both the laboratory and the field studies. The findings are similar to those reported in *Trichoplusia ni* by (Getzin, 1961) where instar susceptibility to muscardine disease decreased with age. Similar observations have been reported by (Fargues 1972, Feng *et. al.* 1985). In this study and the cases cited above, differences in susceptibility could be attributed to the process of mechanical penetration, where the early instars had weaker cuticle compared to the tough cuticle of later instars and adults. It was also possible that the extracellular enzymes produced by the fungus were not effective against the tough cuticle of later nymphs and adults as reported by (Hall, 1981). These results suggested that *B. bassiana* applications against *S. gregaria* should be synchronized with the most susceptible and easily targeted stage such as the third and fourth.

Dose-mortality bioassays showed that mortality rates of *S. gregaria* were a function of conidial concentration. Mortality responses observed were

proportional to concentrations of the suspension. Similar results have been reported in *O. surinamensis* exposed to wheat treated with *B. bassiana* conidiospores (Searle and Doberski, 1984). The dose-response results suggested that higher concentrations of *B. bassiana* could be used for the rapid kill against developing hopper bands of *S. gregaria* in the field, while applications at lower dosages could be used during recession periods.

The study revealed that the efficacy of sublethal dose ($5x10^5$ conidia/ml) of *B. bassiana* could be improved through several steps of *in vivo* passages within *S. gregaria*. Passaged conidia produced enhanced sporulation abilities and subsequent high mortality in *S. gregaria* when compared to the non-passaged conidial suspension. Mortality improved after the fifth passage onwards. Pathogen activity can therefore be maintained through such serial pathogen inductions, especially if the pathogen has to be mass produced *in vitro* which could result into loss of virulence. The low mortality in adults reported earlier in stage susceptibility tests could likewise be improved through similar passages from one generation to another. Reduction in virulence for *M. anisopilae* and *B. bassiana* following repeated *in vitro* subculturing have been reported elsewhere (Schaerffenburg, 1964; Latch, 1965;). Culture age also determine potency and subsequent reduction in virulence as reported when *B. bassiana* harvested from 1-4 months old cultures were found to have only 14% viability compared to freshly harvested conidia which showed >90% viability (Weiser, 1982). This reduction in viability resulted from decreased pathogen

activity due to accumulation of toxic metabolic wastes which decrease fungal activity as reported in (Pavlyushin, 1969). The main reason, however, for serial passages, is that it serves to reduce the effect of the host humoral and cellular responses by consistently introducing virulent strains on the pathogen against the host which (Pavlyushin, 1969). Serial passages maximized the biocidal potential of the *B. bassiana* against the desert locust.

Temperature was a major environmental factor that influenced the pathogenicity of *B. bassiana* against *S. gregaria*. The present study indicated that the pathogen infected the host at all temperature regimes from 25°C to 45°C. The most rapid fungal sporulation and subsequent host mortality occurred at 25°C and 30°C. Outside this range, disease development was retarded. These findings were similar to those of Roberts and Yendol (1971) who observed that the limits for growth of *B. bassiana* generally fall between 35-50°C. Soares and Pinnock (1984) had likewise recorded that blastospores and conidia of *Tolypocladium cylindrosporum* (Gamps) germinated in *Aedes sinensis* Ludlow larvae between 12°C and 30°C but growth was reduced beyond these units. They further noted that LT50 values were reduced from 22.7 days at 12°C to 5.6 days at 25°C. In *S. gregaria* temperatures above 35°C delayed the growth and infection of mycelia even when humidity is favourable. High temperature inactivate the primary conidia through excess loss of water hampering growth and sporulation (West and Briggs, 1968).

In this study, it was also shown that relative humidity as an

environmental parameter, influenced *B. bassiana* activity. The biocidal activity of *B. bassiana* was low at low RH levels as shown in the low mortalities produced at 40% RH, the situation is reversed when RH is increased. Such influence of RH have been reported by Ramoska (1984) who demonstrated that *B. bassiana* was pathogenic to the Chinch bug *Blissus leucopterus* (Say) at 0, 30, 50, 75 and 100% RH, but conidial germination was faster above 65% RH. In *S. gregaria*, although pathogenicity was directly proportional to the levels of RH, mortality was still achieved at less than 50% RH, however a significant delay in growth potential of *B. bassiana* was observed at low RH hampering saprophytic growth. These results could be taken in light of Kramer (1980) who suggested that a constant micro-climate existed around the host cuticle which was conducive for the primary infection of the fungal pathogen, but which might not necessarily favour extensive growth afterwards.

Ultra-violet radiation has been shown to lower viability of *B. bassiana* and subsequently the mortality rate in *S. gregaria*. Exposure of conidia to UV for varying times produced marked differences in pathogenicity. Longer exposures proved deleterious to the pathogen. A gradual drop in viability was recorded from the first hour onwards upto 48h. In a similar experiment, Daoust and Pereira (1986) reported reduction in potency of *B. bassiana* conidia exposed to UV light and then sprayed on *Trypodendron linnet* (Oliv). It has been reported that UV produces mutants which interferes with the DNA sythesis (Usenko *et al.*, 1973; Nikolayev, 1974).

The successful use of *B. bassiana* as a microbial control agent against *S. gregaria* will ultimately depend on the degree to which the upper and lower limits of pathogen activity can be extended through formulation and application techniques. In this study, conidial suspensions were formulated in order to improve the stability of propagule under the varying abiotic factors. Non-volatile oils and UV-protectants were used as spray supplements to overcome premature desiccation resulting from high temperatures and to screen the conidia from deliterious UV irradiation. Viability tests to determine sensitivity of conidia to the formulation agents were conducted and proved that the oils and UV protectants did not interfere with the potency of conidia. Corn oil was found to have the least effect on the germination potential of *B. bassiana* conidia as compared to coconut and mineral oils.

The oil based formulation rated higher in causing mortality in *S. gregaria*. In an experiment performed by Boucious *et al.*, (1981) oil formulated *M. flavovoride* conidia applied to detached *S. gregaria* cuticle showed, that germ tubes, appressoria and penetration structures are formed despite the presence of an oil film, confirming that oil did not impede the infection process. The various oils increased inoculum threshold and there was a prolonged stability of the conidia. It is speculated that the increased infectivity of the pathogen in oil was due to greater adhesiveness to the lipophilic insect cuticle, whereas in water formulations the adhesiveness does not occur long enough for effective infection and germination (Prior *et. al.* 1991). Soper and Ward (1981) also reported

variation in tolerance of *M. anisopliae* to various Kaolins. Formulation with oils therefore opens up the possibility of achieving rapid pest mortality with lower doses of conidia under high temperatures and low humidity. Recent reports on field trials using *M. anisopliae* have further shown that oil formulations applied at Ultra Low Volume (ULV) rates with hand-held sprayers can cause upto 90% mortality to *S. gregaria* in less than 10 days at 70% RH in the coastal areas of Benin and at 15% RH in the Sahel (Bateman *et al.*, 1992).

UV protection of canidia similarly produced marked improvement in tolerance to UV radiation. Fungal potency was maintained over longer periods as compared to controls. Results indicated a significant stability in conidial viability and induction of mortality in *S. gregaria* unlike in situations where protectants were not used. Molasses showed greater promise in effectiveness among the protectants because in addition to its UV protection role, it provided nutrients which produced aggressive fungal growth. It also acts as a bait for locusts, constituting the best candidate as a UV protectant. The formulated suspensions provided a system to *B. bassiana* for interactions with the environment, involving the target host, dose-mortality responses and the abiotic factors.

The performance of *B. bassiana* had to be verified in the field since it has been known that the environmental extremes existing in the field have great influence on the activity of any pathogen (Clerk and Madelin, 1965). In addition, stress is said to disrupt the homeostasis of insects and make them respond

variably to diseases. Thus a fungus can sometimes infect a host in the laboratory, but would not, or only with difficulty infect the same host in the field (Madelin, 1963). It was therefore necessary to evaluate the actual potential of *B. bassiana* in the field.

Safety tests of *B. bassiana* were conducted prior to the field cage tests in which two candidate organisms; honey bee which represented a beneficial insect, while the rat served as a test for mammalian toxicity. *B. bassiana* was not harmful to either the honey bees or the rats. When incubated in a moist chamber, fungal growth was not recorded on the honey bee cadavers. This confirmed that any mortality was due to handling or natural factors enhanced by stress as a result of the confinement of the bees in small cages. Likewise rats administered with the pathogen neither died nor showed signs of fungal infection. Serological tests confirmed that conidia did not occur in the blood. These results confirmed previous studies that showed *B. bassiana* fungal pathogens as not harmful to non-target organisms (Goettel and Roberts, 1991). Although *B. bassiana* can infect 700 different insect species, individual pathotypes show a high degree of specificity due to unique interactions between host cuticle and the pathogen (Charnley, 1984). Hence, differences in cuticle biochemistry have been reported to determine specificity and this specificity reduces the danger that a particular pathotype will attack non-target organisms (Vandenberg, 1990)

Infectivity of the pathogen to *S. gregaria* was clearly demonstrated in the field cages, where significant mortality ($P>0.01$) occurred in the treated cages

as opposed to the controls. Mortality rate was however very slow with only 33.33% in the aqueous suspension and 27.93% in formulated suspensions. While the low level of mortality in the formulated at LT50 could not be explained, the delayed activity of the pathogen could have been due to the multiplicity of environmental factors which might be influencing both pathogen and the host. For instance, due to the marked increase in activity of the locust which resulted from the increased space in the field cages and the abundant supply of fresh food as compared to laboratory cages, vulnerability to the pathogen during the initial phases of disease development could have been reduced. The activity level of the pathogen in the field-cages test was found to extend over a longer period of time as shown by the paralysis and eventual death of the host extending upto 20 days post-inoculation. In contrast, laboratory findings showed a distinct level of mortality after only 14 days. This disparity could have been due to a number of reasons; the non-existence of extended periods of extreme temperatures (>35°C), low humidity and only limited UV exposure since the locusts could hide under the vegetation and thus avoiding direct exposure. These factors could have resulted into the viability of the pathogen being maintained for a longer time. On the other hand, other disease causing organisms such as bacteria and protozoa could gradually have contributed to the death of the locusts weakened by mycosis as was reported by Beaver (1966), who demonstrated that natural regulation of *S. scolytus* populations was due to pathogens, parasites and predators.

Formulated propagules of *B. bassiana* gave higher mortalities in *S.*

gregaria under field cage conditions. This indicated that the formulations developed and tested under laboratory condition could similarly produce enhanced activity in the field. These findings compare to those by Delgado *et al.*, (1991) in Mali where oil-based *B. bassiana* used in the field against grasshoppers produced higher infections. Results of subsequent trials in Cape Verde using the same conidia and oil preparations as in Mali by (Delgado *et al.*, 1991) indicated that formulations were important particularly under field conditions.

Oil based formulations have both the advantage of excellent adhesion to the hydrophobic cuticle of the pest, as well as the reduced volume of application associated with ULV sprays (Aguldelo and Falcon, 1983). For instance, conidia formulated in oil allowed a much larger number of conidia to reach the intersegmental membranes as compared to the aqueous suspension. The results indicated that the effectiveness of conidial suspension may be determined as much by the application medium as by the susceptibility of the host or virulence of the pathogen. A conidial suspension in oil may therefore be more effective for field application because its non drying properties would allow application of droplets smaller than those at which evaporation rate becomes a limiting factor such as in water based formulation. It has been shown that a given dose of particle is distributed to a higher proportion of the target population if it is distributed among more numerous smaller droplets so that the number reaching the target is increased (Graham-Bryce, 1977). Such evidence

suggests that oil based formulations of *B. bassiana* may be more effective in the control of the desert locusts because droplet sizes between 30-50µm in diameter could be delivered in adequate lethal quantities to an insect as large as *S. gregaria* through ULV application without raising concentrations to impractically high levels. This could minimize quantities which have to be used in water deficient habitats where *S. gregaria* occurs (Steedman, 1988). At the same time storage and handling of such materials would be easier.

Further biotests and field experimentation are necessary in the actual locust habitat to establish the relative effectiveness of the pathogen under open field conditions. This would possibly help to develop effective delivery methods for release of *B. bassiana*. Life tables and survivorship curve analysis provided useful information on the extent to which each of the environmental factors has determined pathogen activity in the laboratory, giving an indication of their influence in the field. The impact of environmental parameters is clearly marked and even an increase in pathogen concentration could not increase mortality without optimum conditions of temperature humidity and UV radiation.

It is therefore essential to maximize the effect of the pathogen through optimization of the conditions which facilitate fungal development particularly in the initiation of germination where the balance of temperature and humidity conditions must be achieved. On a field scale this would involve formulating the propagules in the most effective way, timing of application to coincide with the right developmental stage, and Spraying when conditions favour fungal

development. *B. bassiana* had previously not been extensively studied as a biocontrol agent to *S. gregaria*, although it has widely been used for the control of other crop pests (Ferron, 1977; CAB, 1989). In this regard, *S. gregaria* presents new challenges by being a migratory pest (Waloff, 1976; Farrow, 1990), which requires that any fungal biocontrol agent developed should act like a contact pesticide and maximizes the kill from the initial spray, especially during the non-flying stages in the field.

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Appendix 1. Analysis of variance of the susceptibility of first instar *S. gregaria* to different concentrations of *B. bassiana*

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	7	83.3745	11.9106	991.85	0.0001
Error	10	0.1200	0.0120		
Total	17	83.4946			

R-Square	C.V.
0.9985	1.4382

Appendix 2. Analysis of variance of susceptibility of the second nymphal instar *S. gregaria* to different *B. bassiana* concentration

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	7	73.4628	10.4946	736.78	0.0001
Error	10	0.1424	0.0142		
Total	17	73.6052			

R-Square	C.V.
0.9980	1.5870

Appendix 3. Analysis of variance of susceptibility of the third nymphal instar *S. gregaria* to different *B. bassiana* concentration

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	5	71.27	10.18	2098.21	0.001
Error	10	0.05	0.005		
Total	17	71.32			

R-Square	C.V.
0.999320	0.981377

Appendix 4. Analysis of variance of susceptibility of the fourth nymphal instar *S. gregaria* to different *B. bassiana* concentration

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	7	60.0673	8.5810	462.46	0.001
Error	9	0.1669	0.03447		
Total	16	60.2343			

R-Square	C.V.
0.9972	2.0842

Appendix 5. Analysis of variance of susceptibility of the fifth nymphal instar *S. gregaria* to different *B. bassiana* concentration

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	7	51.9889	7.4269	215.43	0.001
Error	10	0.1669	0.03447		
Total	17	52.3337			

R-Square	C.V.
0.9934	2.9583

Appendix 6. Analysis of variance of susceptibility of the Adult *S. gregaria* to different *B. bassiana* concentration

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	7	45.1794	7.4542	518.40	0.001
Error	10	0.1245	0.03447		
Total	17	45.3039			

R-Square	C.V.
0.9972	1.8585

Appendix 7. Analysis of variance of susceptibility of the *S. gregaria* to the control

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	7	0.7198	11.9106	13.36	0.0002
Error	10	0.0769	0.0120		
Total	17	0.7967			

R-Square	C.V.
0.9034	3.3001

Appendix 8. Analysis of variance of mortality of III instar *S. gregaria* to varying concentrations of *B. bassiana*

Source of variation	DF	SS	MS	F-value	Pr>F
Conc	113	1962.2986	11.9106	104.46	0.0001
Error	221	36.7408	0.1662		
Total	334	1999.0395			

R-Square	C.V.
0.9816	8.1241

Appendix 9. Analysis of variance of mortality caused by serial passaged and non-passaged *B. bassiana* against *S. gregaria*.

Source of variation	DF	SS	MS	F-value	Pr>F
Model	41	699.6228	17.0639	124.44	0.0001
Error	77	10.5586	0.1371		
Total	118	710.1815			

R-Square	C.V.
0.9851	6.1582

Appendix 10. Analysis of variance of effect of temperature on pathogenicity of *B. bassiana* to *S. gregaria*

Source of variation	DF	SS	MS	F-value	Pr>F
Model	31	276.410	8.9164	113.52	0.0001
Error	58	4.5558	0.0785		
Total	89	280.9659			

R-Square	C.V.
0.9837	4.2806

Appendix 11. Analysis of variance for the effect of RH on pathogenicity of *B. bassiana* to *S. gregaria*

Source of variation	DF	SS	MS	F-value	Pr>F
Model	25	246.3415	9.8536	537.02	0.0001
Error	46	0.8440	0.0183		
Total	71	247.1855			

R-Square	C.V.
0.9965	2.0177

Appendix 12. Analysis of variance of effect of UV on pathogenicity of *B. bassiana* to *S. gregaria*

Source of variation	DF	SS	MS	F-value	Pr>F
Model	25	196.9503	7.8380	92.44	0.0001
Error	46	0.0847			
Total	71	199.8506			

R-Square	C.V.
0.9804	4.6363

Appendix 13. Analysis of variance for the effect of formulation on pathogenicity of *B. bassiana* to *S. gregaria* at varying temperatures

Source of variation	DF	SS	MS	F-value	Pr>F
Model	49	476.3783	9.7220	339.98	0.0001
Error	94	2.6879	0.0285		
Total	143	479.0663			

R-Square	C.V.
0.9943	2.5096

Appendix 14. Analysis of variance for effect of formulation on pathogenicity of *B. bassiana* to *S. gregaria* at varying relative humidities

Source of variation	DF	SS	MS	F-value	Pr>F
Model	25	195.9503	9.7220	92.98	0.0001
Error	46	3.9003	0.0847		
Total	71	199.8506			

R-Square	C.V.
0.9804	4.6362

Appendix 15. Analysis of variance for the effect of formulation on pathogenicity of *B. bassiana* to *S. gregaria* at varying UV radiation.

Source of variation	DF	SS	MS	F-value	Pr>F
Model	49	444.9879	90813	598.08	0.0001
Error	94	1.4273	0.0151		
Total	143	446.4152			

R-Square	C.V.
0.9804	4.6362

Appendix 16. Analysis of variance for the effect of combined corn oil/molasses formulation on pathogenicity of *B. bassiana* to *S. gregaria* at different temperatures

Source of variation	DF	SS	MS	F-value	Pr>F
Model	58	469.0442	8.0869	185.03	0.0001
Error	112	4.8950	0.0437		
Total	170	473.9392			

R-Square	C.V.
0.9896	3.1044

Appendix 17. Analysis of variance for the effect of corn oil-molasses formulation on pathogenicity of *B. bassiana* to *S. gregaria* at different relative humidities

Source of variation	DF	SS	MS	F-value	Pr>F
Model	49	484.1861	9.8813	374.92	0.0001
Error	94	2.4774	0.0263		
Total	143	486.6636			

R-Square	C.V.
0.9949	2.3797

Appendix 18. Analysis of variance for effect of corn oil-molasses formulation on pathogenicity of *B. bassiana* to *S. gregaria* at different UV exposure times

Source of variation	DF	SS	MS	F-value	Pr>F
Model	49	452.2194	9.2289	270.91	0.0001
Error	94	3.2022			
Total	143	455.4216			

R-Square	C.V.
0.9929	2.7562

Appendix 19. Analysis of variance for mortality (%) of *S. gregaria* nymphs exposed to *B. bassiana* in field cages.

Source	DF	SS	MS	F-Value	Pr>F
Treatment	2	11594.78	5797.40	26.55	0.0001
Days	4	14628.44	3657.11	16.75	0.0001
Rep	2	1067.19	533.60	2.44	0.1011