COMMITTEE DECISION

Distribution, Occurrence and Characterization of Entomopathogenic Fungi in Agricultural Soil in the Palestinian Area

By

Abdel-Basit Basim Moh'd Mara'I

This thesis was defended successfully on 13/12/2000 and approved by:

Committee members	**Signature**
1. Prof. Dr. Mohammed S. Ali-Shtayeh (Supervisor) Professor of Biological Sciences	
2. Dr. Nasser Al-Jaghoub (External Examiner) Entomologist, Research Scientist	
3. Dr. Firas Sawalha (Internal Examiner) Assistant Professor pf Plant Biotechnology	

An-Najah National University
Faculty of Graduate Studies

Distribution, Occurrence and Characterization of Entomopathogenic Fungi in Agricultural Soil in the Palestinian Area

By

ABDEL-BASIT BASIM MOH'D MARA'I

Supervisor

PROFESSOR DR. MOHAMMED S. ALI -SHTAYEH

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science in Biological Sciences, Faculty of Graduate Studies, at An-Najah National University, Nablus, Palestine.

2000

DEDICATION

To

My Dear Mother, Father, Brothers and Sisters

for Their Encouragement, With Love and

Respect

ACKNOWLEDGMENTS

I Would Like To Express Special Thanks and Gratitude to My Supervisor, Professor Dr. Mohammed S. Ali-Shtayeh for His Supervision, Encouragement, Guidance and Help throughout This Study.

I Also Like to Thank My Colleagues Rana M. Jamous, Majdi Bahjat and Suhail Abu-Ghdeib, for their Help and Encouragement Throughout the Work.

Also Special Thanks for my Friends Rabei A. Zayed, Ahmed Salih, Tayseer K. Muraweh, Marwan M. Budair, Abdel-Rahman Salama, Kamal A. Kamel, Mohammad I. Mahassneh, Husam S. Mara'i and Jasser Jasser for their Help in Soil Collection.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Corn Meal agar	CMA
Czapek-Dox agar	CZA
Entomopathogenic fungi	EPF
Galleria - Bait- Method	GBM
Larvae feeding medium	LFM
Potato dextrose agar	PDA
Prophenol oxidase	ProPO
Potato sucrose agar	PSA
Sabouraud dextrose agar	SDA
Ultraviolet	UV
Yeast with sabouraud dextrose agar	YSDA

DISTRIBUTION, OCCURRENCE AND CHARACTERIZATION OF ENTOMOPATHOGENIC FUNGI IN AGRICULTURAL SOIL IN THE PALESTINIAN AREA

Abdel-Basit Basim Moh'd Mara'i

Supervisor
Prof. Mohammed Saleem Ali-Shtayeh

ABSTRACT

The occurrence of entomopathogenic fungi was investigated in 100 irrigated vegetable fields and 40 citrus orchards soils in four geographical locations in the West Bank, using the *Galleria* bait method (GBM). Entomopathogenic fungi were found to occur in 33.57 % (47 of 140) of the soil samples studied, with positive samples yielding 70 fungal isolates, belonging to 20 species in 13 genera. *Conidiobolus coronatus* was the most frequent and abundant entomopathogenic species recovered comprising 31.43 % of the total isolates.

Soil pH, soil moisture content and the geographical location had minor or no effect on the isolation of entomopathogenic fungi in the studied fields. On the other hand, organic matter content of soil, and vegetation type were found to significantly ($P<0.05$) affect the isolation of entomopathogenic fungi from soil, with orchard fields yielding larger numbers of isolates than in the vegetable fields. Using Koch's postulates

the pathogenicity of fungal isolates recovered to *Galleria* larvae was found to range from 16% to 100% (mortality rate). Isolates of *C. coronatus*, proved to be the most pathogenic isolates recovered (87-100% mortality in about 5-8 days after infection).

The effect of media and temperature on mycelial growth rate, conidial production and conidial germination of six entomopathogenic fungal species (one isolate/species) was studied (*C. coronatus, Entomophaga grylli, Erynia castrans, Hirsutella jonesii, Paecilomyces farinosus* and *Sporodinielle umbellata*). Mycelial growth was significantly affected by media and isolates with maximum growth rate obtained with CMA and PDA. Optimum temperature for radial mycelial growth, spore production and spore germination ranged from 20-30°C. Mycelial growth rate, spore production and spore germination were significantly affected by temperature and isolate.

CHAPTER ONE

GENERAL INTRODUCTION

CHAPTER ONE
GENERAL INTRODUCTION

1.1 The development and efficiency of entomopathogenic fungi in biocontrol technology

Dramatic improvements in the production of crop plants have been achieved by the use of developed pesticides. However growing concerns continue to be publicized by environmentalists and public health authorities about the risks associated with use of these chemicals and the ability of pests to develop resistance to them. This has stimulated efforts to develop biological control agents as alternatives or supplements to chemical pesticides (Hajek & St. Leger, 1994).

Interest in entomopathogenic fungi has increased since their pathogenicity to insects was recognized over a century ago (Evans, 1982). They were tested in pioneering biological control experiments against insect pests but poor results reduced the enthusiasm for studying entomopathogenic fungi as biological control agents, which was further eroded by the development of chemical pesticides (Evans, 1982). The application of entomopathogenic fungi onto crops has quite a long history. Traditionally E. Mechnikoff is regarded as the practical founder of the doctrine of biological control of pests, not only elucidating the possible uses of entomopathogenic fungi but also carrying out the first experiments (in Russia as early as 1886) to

test his hypotheses (Samons *et al.*, 1988). The first commercial preparation of an entomopathogenic fungus was available in Paris in 1891 (Van Emden, 1992).

Currently, the interest in entomopathogenic fungi has led to the marketing of several of them (Hajek & St. Leger, 1994). The hyphomycete *Verticillium lecanii* is sold in UK for biocontrol of glasshouse whitefly and thrips (Gillespie & Moorhouse, 1989). A commercial formulation of *Metarhizium anisoplia* has been developed for cockroach control in the US by the Ecoscience Corporation (Andes, 1994), and *M. flavoviride* has been developed for the control of locust and grasshoppers in the Sahel region of Africa (Prior, 1992). The high epizootic efficiency of some *V. lecanii* strains towards some insects has been used to develop commercial mycoinsecticides for the biological control of aphids (Hall, 1981). Nevertheless, of the 750 species of entomopathogenic fungi have been recorded, (Gunde-Cimerman *et al.*, 1998). Only ten species are presently being developed for biological control (Hajek & St. Leger, 1994), and the full potential of entomopathogenic fungi has not been approached.

As a form of pest control, entomopathogens have some quite striking advantages. In contrast to chemical insecticides, they tend to be target specific, leave no toxic residue and are unlikely to stimulate resistance in the target organism (Van Emden, 1992; Vidal *et al*, 1998). In contrast to biological control, many pathogens are compatible with chemical insecticides

and can often be used in combination with them (Van Emden, 1992). Pathogens therefore would appear to be ideal for control programs where it is important to maintain the survival of natural enemies. They are clearly also useful for dealing with cases of post resistance to chemical insecticides, especially where the application of pesticides is restricted, for example close to harvest or on environmental or cost grounds.

Unfortunately, entomopathogens also have very serious disadvantages. The high specificity of emtomopathogenic fungi means that their development has economic limitations, since specificity means market restriction (Hajek & St. Leger, 1994; Van Emden, 1992). They are living organisms, often with a very short life span in nature, and so it can be extremely difficult to produce them on a factory scale and store them while retaining their virulence. Once applied in the field, they may fail if conditions are too dry or hot (Carruthers *et al.*, 1985; Fargues, Goettel *et al.*, 1997) or if the pH conditions on the leaf surface are outside certain limits (Van Emden, 1992). Many of them are also very sensitive to ultraviolet radiation (Smits *et al*, 1997), and so they are rapidly destroyed by sunlight. Another problem is that diseases really spread best where the pest population is reasonably high. There are threshold populations, often above those acceptable to the grower, below which the disease will not spread. Although entomopathogens leave no toxic residues, they do leave the corpses of their victims, and these may adhere firmly to the plant and be very unsightly, forcing the grower into

expensive washing procedures before the product can be sold. Entomapathogens cause great difficulties in the development process in agricultural ecosystems since the direct toxicity which can be demonstrated in the laboratory is perhaps less important than certain behavioral (behavior of larvae that varies by instar and host-host interactions) and biological properties (population density and life span) of their prey in the field, which ultimately determine the contact between the pest and a sprayed pathogen. Entomopathogens are of course developed by multiplying diseases first located in natural insect populations, and this may mean expensive rearing of large numbers of pests to multiply the disease. However many entomopathogens are amenable to multiplication by the modern techniques of biotechnology, and these entomopathogens have received most attention in recent years (Samson *et al.*, 1988).

1.2 Ecological distribution of entomopathogenic fungi

Entomopathogenic fungi are common in the environment, particularly in soil (Chandler *et al.*, 1997). They are distributed in a wide range of habitats. These habitats are varied and can be divided into the following habitats: aquatic habitats, forest habitats, agricultural habitats and pasture habitats (Samson *et at.*, 1988).

1.2.1Aquatic habitats

The entomopathogenic fungi associated with these habitats belong strongly to the lower fungi (Mastigomycotina) because they are able to produce motile spores (Hegedus & Khachatourians, 1995; Samson *et al.*, 1988). Some species of the genus *Coelomomyces* are host specific endoparasites of aquatic larvae of biting flies. Epizootics of *Coelomycidium simulii* and *Lagenidium giganteum* have been recorded in *Anopheles* populations in Europe, Africa and North America (Strand *et al.*, 1977; Sur *et al*, 1999).

The aquatic Entomophthorales, e.g. *Erynia conica,* inhabiting fresh water ponds, streams and rivers in temperate regions (Thaxter, 1888), show remarkable adaptations to the habitat. It has been discovered recently that up to four spore types can be produced by a species to facilitate dispersal and infection, both aerially and aquatically, revealing a great plasticity in both conidial morphology and mode of germination (Descals & Webster, 1984).

Few Deuteromycetes are associated with natural populations of aquatic insects, *Culicinomyces clavisporus,* for example, being recorded until recently only as a laboratory contaminant of mosquito larvae (Samson *et al.*, 1988).

1.2.2 Forest habitat

Most research on entomopathogenic fungi has been directed to agro-ecosystems, conversely no systematic research has been directed to forest

habitats (Evans, 1982). Humid, tropical forests have a rich and varied entomopathogenic mycoflora (Evans, 1982). Most of entomopathogenic fungal species in tropical forests belong in the genus *Corydyceps,* while *Entomophthora* species are poorly represented in tropical forests (Evans, 1982). The ecological pressures in tropical forests may favour the more specialized obligate parasites (e.g, Cordyceps) but this extreme specialization suggests that such fungi will be of limited value in biological control and the most beneficial direction lie in a study of their physiology and biochemistry.

1.2.3 Agricultural habitats

Epizootics caused by entomopathogenic fungi in agricultural habitats are much and more numerous, specially in temperate regions, than those in other habitats. Epizootics have been reported on insect populations in agricultural habitats, caused by a range of genera, including *Beauveria, Paecilomyces, Sporodiniella, Stilbella, Hirsutella, Metarhizium* and *Erynia* (Samson *et al*, 1988; Inglis *et al,* 1996a). In fruit orchards in Israel, it appears that entomophthoralean fungi are important in controlling pest populations (Ben-Ze'sv *et al.*, 1984).

In temperate arable crops, the highest incidence of the entomopathogenic fungi was registered during dry periods and peak incidence was correlated with harvesting, which opened-up the crop canopy and exposed the larvae to soil-borne inoculum.

1.2.4 Pasture habitats

In pasture habitats as in forest habitat no systematic research on entomopathogenic fungi has been directed to it. There is relatively little information available on natural control of pasture grass pests by entomopathogenic fungi. A variety of *Hirsutella* species is involved in natural mortality of eriophyid mite, *Abacarus hystrix,* population in pastures in the UK (Minter *et al.,* 1983). Epizootics on spittlebugs, caused by an Entomophthorales species, have been reported recently in tropical pastures in Ecuador (Evans, 1982). Rath *et al.* (1992) obtained isolates of *Metarhizium anisopliae* from 31% samples of soil taken from pastures across Tasmania.

1.3 Mode of infection of entomopathogenic fungi

The study of fungal pathogen — insect host interactions is very important, since the lack of knowledge in this field has probably contributed to the many failures in the use of fungi as biological control agents of insect pests. Recently the interest in the study of invertebrate diseased has increased. Entomopathogenic fungi infect insects by breaching the host external cuticle (Hajek & St. Leger, 1994). Four stages are involved in the development of an insect mycosis (Samson *et al.*, 1988). These are adhesion of the spore on the host cuticle, germination of the spore, penetration of the host integument by a germ tube and the development of the fungus inside the insect body.

1.3.1 Adhesion of the spore on the host cuticle

The adhesion of a spore to the insect cuticle is the prerequisite for the establishment of a mycosis (Boucias *et al.*, 1988; Hajek & St. Leger, 1994; Samson *et al*, 1988; Hegedus & Khachatourians, 1995; Clakson & Charnley, 1996). The spores of entomopathogenic fungi which produce airborne spores are dispersed passively via air currents and when attach preferentially to the cuticle, produce penetrating germ tubes which breach the host cuticle, and invade the host hemocoel (Boucias *et al.*, 1988). In this case the chances of success depend on climatic conditions, the amount of fungal inoculum and host density. The motile spores of the aquatic entomopathogenic fungi are characterized by their tendency to search for the host via an active chemotactic process; the fungus being chemically attracted by metabolites released by the host (Cerenius & Soderhall, 1984).

The outermost layer (epicuticle) of the host integument is the place for the initial fungus-host interaction. Slimy phialoconidia of *V. lecanii* and *Hirsutella thompsonii* or ballistospores of *Entomophthora mucosae*, are always covered by amorphous mucus that can facilitate the adhesion of the spore to the cuticle (Samson *et al.*, 1998; Eilenberg *et al.*, 1986). The composition of this mucous matrix is unknown in the entomopathogenic fungi as it is known in phytopathogenic fungi, so it requires further study (Samson *et al*, 1988). Lipoproteins which are found on the spore surface facilitate spore attachment to the hydrophobic lipophilic insect epicuticle (Hajek & st. Leger,

1994; Samson *et al.*, 1988; Boucias *et al.*, 1988). The conidia of the entomopathogenic fungi *B. bassiana, M. anisopliae,* and *Nomuraea rileyi* possess a hydrophobic rodlet layer which protect them from dehydration and microbial attack and it provides a means of dispersal in air currents (Boucias *et al.*, 1988). Usually, the attachment of the spores results from an apparently passive mechanisms involving neither the synthesis nor the release of adhesive material (Zaccharuck, 1970). In aquatic entomopathogenic fungi as mentioned above, adhesion usually due to an active chemotactic process consecutive to the recognition of the host by the fungus. Most of the dry conidia are passively attached to the host and may thus be readily removed by rinsing the host with a detergent solution (Samson *et al.*, 1988). Spore adhesion has also been frequently correlated with the host specificity of a fungal species. The conidium cuticle interaction may involve a complex of specific (e.g, glycoprotein) and nonspecific (e.g, electrostatic or hydrophopic) recognition mechanisms (Fargues, 1984).

1.3.2 Germination of the spore

In the germination process the fungal germlings of entomopathogenic fungi respond to the presence of the cuticle which act as an infection barrier, by involving adaptive biochemical processes and cellular differentiation to form a morphological structures such as appressoria (apical swellings on germ tubes for attachment to the host) from which penetration pegs develop that

breach the host epicuticle (Hajek & St. Leger, 1994; St. Leger *et al.*, 1989). The appressoria have been described to be covered with an amorphous mucilaginous sheath that can facilitate the attachment of these structures to the cuticle (Zaccharuk, 1970). Appressoria were produced on a wide range of hard surfaces, suggesting a nonspecific physiochemical stimulus; however there was an apparent preference for the hydrophopic surfaces for the differentiation of gremlins (St. Leger *et al*, 1989).

Spore germination mainly depends on different macro climatic factors, temperature and humidity. Temperature requirements are highly dependent on the ecological niche of the fungus. The optimum temperature for germination of *M. anisopliae* spores was between 25 and 30°C (St. Leger *et al*, 1989). Entomopathogenic fungi require water for germination and extension growth. Therefore, humidity levels act as a switch, making germination possible or not (Hajek *et al.*, 1990). Relative humidity $\geq$ 90% is always necessary to induce germ tube formation and infection initiation (Samson *et al*, 1988).

Spore germination is also dependent on the nutritional environment. Some entomopathogenic fungi conidia germinate very poorly in the absence of nutrients (Boucias & Pendland, 1984), while the mode of germination of *Conidiobolus obscurus* is affected by the quality and quantity of nutrients available (Samson *et al*, 1988). In vivo, entomopathogenic spore germination is highly dependent on both the level and the type of chemicals present on the epicuticle (Samson *et al*, 1988). Chloroform extracts of *Anticarsia*

gemmatalis, containing a heterogenous mixture of lipids, increase the rate and level of conidial germination of *N. rileyi* (Boucias & Pendland, 1984). The inhibitory effect of some cuticular compounds on spore germination has been considered to be due to free short chain fatty acids (FA) (C_6 to C_{12}) (Saito & Aoki, 1983). The saprophytic microbiota associated with the insect cuticle can either stimulate or inhibit the germination of the spore in vivo (Schabel, 1978).

1.3.3 Penetration of the host integument

The arrival of fungal elements inside the host body, a prequisite to infection, depends on the ability of the germ tubes to penetrate the external epicuticle and the procuticle (Samson *et al*, 1988). The external epicuticle in most insects appears to be mechanically fragile and may be penetrated by weak forces (Hajek & St. Leger, 1994). The internal epicuticle (procuticle) consists of chitin embeded in a matrix of lipoprotein fibers stabilized by quinones that implies toughness (Livolant *et al.*, 1978).

The penetration of entomopathogenic fungi to the insect cuticle is considered to occur by both enzymatic digestion and physical process (St. Leger *et al.*, 1986; Vilcinskas & Wedde, 1997; Pekral & Grula, 1979). The cuticular alterations observed during fungal penetration have shown the important role played by enzymes released by the germ tube during perforation of the insect cuticle (Samson *et al.*, 1988). Entomopathogenic

fungi release digestive enzymes such as lipases, chitinases, and proteases during cuticular penetration to overcome the physical barriers (Smith *et al.*, 1981). Proteases seem to play a predominate role in the infection process because up to 70% of the insect cuticle consists of proteins (Griesch & Vilcinskas, 1998).

1.3.4 Development of the fungus inside the host body

In some cases, the internal reaction of the host towards the pathogen can be strong enough to eliminate the pathogen. Once fungi invade the hemocoel, they are potentially exposed to the humoral and cellular defense mechanisms of the infected host (Griesch & Vilicinskas, 1998). These mechanisms include the prophenol oxidase (pro PO) activating system (Hajek & St. Leger, 1994; Soderhall, 1982; Vey & Fargues, 1977). The pro PO cascade plays an essential role in phagocytosis, adherence during nodule formation, microbial killing and cell communication (Ratcliffe *et al.*, 1984). The main reaction in the cellular antifungal defense mechanism is encapsulation of the fungus, which is rapidly melanized (Hajek & St. Leger, 1994). Encapsulation only provides protection for hosts gain weakly virulent pathogens. Inside the granuloma, fungus is always covered by a thick layer of melanin. Quinones and melanins produced from insect phenols besides protease inhibitors can occur in the hemolymph of infected insect and prevent lethal infections (Samson *et al*, 1988).

Entomopathogenic fungi have been developed several mechanisms to overcome host-defense mechanisms. After fungi invade the hemocoel, the host may be killed by some combination of mechanical damage produced by fungal growth, nutrient exhaustion and toxicosis (Gillespie & Claydon, 1989). Several species of Entomophthorales can spontaneously form protoplasts in the hemocoel of the insect and are able to grow in vivo and in vitro in this protoplast stage (Samson *et al.*, 1988). Apparently, soluble components of the hemolymph supply sufficient nutrients for growth and extensive multiplication of the fungus (Pekrul & Grula, 1979). Degradative enzymes of the fungus are probably helpful and could aid growth by solubilizing tissues such as the fat body. Due to extensive growth of the fungus death of larvae could easily result from exhaustion of nutrients in the hemolymph (Pekrul & Grula, 1979). Toxins could also aid the infection by immobilizing the larvae or certain of their life systems (Pekrul & Grula, 1979). Destraxins or cytochalasins (low molecular weight secondary metabolites) of *M. anisopliae* are able to impair the phagocytic activity, attachment, spreading and cytoskeleton formation of plasmatocytes in hemolymph of infected *G. mellonella* larvae (Griesch & Vilcinskas, 1998). Toxin have been claimed also to be responsible for the death of numerous insects infected by Deuteromycetes (Ferron, 1981). At the end of the parasitic phase of fungal development, host will die.

1.4 Behavioral changes of lethally infected hosts

As a result of fungal infection of host insect, some behavioral changes have been documented. The symptoms in infected insects, beginning with a loss of appetite, accompanied by a restlessness in which the movements are weak and uncoordinated (Evans, 1982). In some systems during the period of lethal infection, additional behavioral changes have been observed. Infected carrot flies, *Psila rosae,* do not lay their eggs near food plants, as healthy females do, thereby reducing the chances for egg survival (Eilenberg, 1987). Some diseased ants move away from the center of ant activity (nest) to avoid being eaten and climb herbs and grass, others are invariably hide beneath herb and shrub leaves (Evans, 1982). These behavioral changes could be regarded as altruistic.

1.5 Disease transmission

Dispersal of infective propagules to a new host represents a most important part of the fungal life cycle. Environmental conditions affect the processes of spore production and discharge, spore dispersal, and spore survival and germination (Hajek & St. Leger, 1994). Under favorable environmental conditions most fungi are able to produce resting propagules (Chlamydospores, zygospores or oospores) which allow the fungus to overwinter or to withstand adverse conditions in the absence of the host

(Samson *et al*, 1988). In many, but not all, systems spore production begins after host death. Thus the death of infected insects in elevated positions will favor the dispersal of the infective propagules (Evans, 1982).

The infective propagules of entomopathogenic fungi are dispersed in a variety of ways over varying distances. They are dispersed by movement of infected insects that come into contact with non infected hosts, movement of Oomycetes spores in water, rain splash and by wing (Hajek & St. Leger, 1994, Romoser & Stoffolano, 1998).

1.6 Environmental factors affecting efficacy of entomopathogenic fungi

Understanding the effects of environmental factors on the efficacy of entomopathogenic fungi is necessary to maximize the potential of these fungi (Lacey & Mercadier, 1998). Transmission of fungal diseases is often highly dependent on ambient environmental conditions (Hajek *et al*, 1990). Conidial formation, survival and germination can be influenced by temperature, humidity and ultraviolet radiation.

1.6.1 Temperature

Temperature is an important environmental factor affecting fungal growth and disease development in insects (Ouedraogo *et al.*, 1997; Ekesi *et*

al., 1999; Fargues, Ouedraogo *et al.*, 1997; Hajek *et al.*, 1990; Moore, Douro-Kpindou *et al.*, 1996). The thermal constrains are not only the result of ambient conditions, but also those achieved through host thermoregulation (Inglis *et al.*, 1996b). For instance insects can elevate their body temperature through habitat selection or basking in the sun and such activity has been shown to reduce disease incidence of *Entomophthora muscae* in house flies, *Nosema acridophaga, Entomophaga grylli* (Ouedraogo *et al.*, 1997) *B. Bassiana* (Inglis *et al.*, 1996b) in acridids. Identifying a fungal strain with a broad temperature range is therefore necessary for rational approach to the management of insects (Ekesi *et al.*, 1999).

1.6.2 Humidity

Humidity is one of the most important environmental factors affecting entomopathogenic fungus to infect and overcome its host (Fargues, Ouedraogo *et al* ., 1997). Some entomophthoralean species produce and discharge primary conidia in abundance only at a constant relative humidity of > 95% (Hajek & St. Leger, 1994). The requirement of high ambient humidity for conidial germination of most species of entomopathogenic fungi and for the infection of many insects by some species has led to the erroneous generalization that use of entomopathogenic fungi as microbial control agents will be severely limited by ambient humidity (Hall & Papierok, 1982). In certain Deuteromycetes, the presence of a film of free water is necessary to

obtain maximum infection levels (Hall, 1981). However, excessively high levels of humidity may be unfavorable for the establishment of some entomophthoralean (Samson *et al.*, 1988) and some Hyphomycetes infections. However, Fargues, Ouedraogo *et al.* (1997) showed that humidity did not affect total mortality or mycosis in adults of *Schistocerca gregaria* treated with *M. flavoviride.*

1.6.3 Ultraviolet radiation (UV)

Infective condida are highly susceptible to the adverse environmental condition of high temperature, low humidity and ultraviolet radiation (Caudwell & Gatehouse, 1996). The conidial germination rate differs with continual exposure to sunlight or dark (Hajek *et al.*, 1990). Ultraviolet radiation rapidly kills or damages conidia (Moor, Higgins e*t al.*, 1996). The most damaging UV wavelength is UVC (Wavelength ranging from 250 to 280 nm). This is largely filtered out by the atmosphere, so conidia are mainly exposed to UVA (320-400 nm) and UVB (280-320 nm) (Moore, Higgins *et al.*, 1996). UV damage is considered to be dependent on dose, period and intensity of irradiation, a short period of high-intensity irradiation will be more damaging than an equal dose given over a much longer period (Moore, Higgins *et al.,* 1996). Exposure of 2h to UV light reduces germination from 49 to 34% (Caudwell & Gatehouse, 1996).

1.7 Soil ecology

A better understanding of soil ecology is essential to the development of entomopathogenic fungi of soil inhabiting insects. Studies of the natural entomopathogenic fungi can be used to help assess which isolates are best suited to a particular environment, determine which factors in that environment may be detrimental to the persistence of the introduced entomopathogenic fungus, and provide environmental data required for registration of the entomopathogenic fungus (Rath *et al.*, 1992).

1.8 Biotechnology of entomopathogenic fungi

As mentioned previously, studies of natural epizootics of entomopathogenic fungi during the latter part of the nineteenth century stimulated man's interest in employing them as mycoinsecticides to control agricultural pests.

The revival of interest in mycoinseticides over the past three decades has led to the large scale production of several promising candidate fungi and to the marketing of the first commercial mycoinsecticides, Mycotal and Vertalec, based on formulations of *V. lecanii* (Chandler, 1997; Caudwell & Gathouse, 1996; Samson *et al.*, 1988; Smits *et al.*, 1997; Stephan & Zimmermann, 1998). Anyone interested in the application of entomopathogenic fungi biotechnology must select the most suitable fungal strain or species to produce cheap, and study the factors that govern the

growth and sporulation of selected fungus and the most appropriate fermenter technology to mass-produce the selected fungi (Samson *et al.*, 1988).

1.8.1 Selection of fungal entomopathogen

The selection of a fungal strain is critical because the aggressiveness of a fungus is highly strain dependent (Samson *et al.*, 1988). Laboratory test is necessary for the assessment of pathogenicity of selected fungal strain (Hall, 1981). Results obtained under laboratory conditions invariably optimise the potentialities of the fungus, so the data should be interpreted carefully. Field experimentation is essential in order to determine if the microclimatic requirements of the fungal entomopathogen and the host coincide (Chandler, 1997). Checking of the pathogenic stability of a strain during repetitive transfers and selection of a suitable media used for these transfers is necessary for best application of selected fungal strain as biocontrol agent (Samson *et al.*, 1988). Selected fungal strain should have wide host range, high sporulation capacity, simple nutritional requirements and high stability (Samson *et al.*, 1988). It should be easy to induce an entomopathogenic fungal infection in insects (Muralimohan *et al*, 1999).

1.8.2 Nutritional requirements

Study of the growth factors of entomopathogenic fungi is essential for mass production. The choice of industrial nutrients will obviously be directly

related to nutritional requirements of the selected fungus, so study of the effect of limited essential components such as carbon and nitrogen-limited cultures would be necessary (Vidal *et al.*, 1998).

Entomopathogenic fungi require oxygen, water, an organic source of carbon and energy, a source of inorganic or organic nitrogen, and additional elements amongst which are minerals and growth factors. Other essential macronutrients are phosphorous, potasium, magnesium and sulpher. Essential microelements usually include calcium, copper, iron, manganese, molybdenum, zinc and water soluble B-complex vitamins especially biotin and thiamine (Samson *et al.*, 1988). Nutritional factors have been known to play an important role in sporulation when the nutrient requirement is higher than that needed for growth. There seems to be no clear relationship between nutrition content and sporulation on solid substrate. However, a lower protein content appears to encourage higher sporulation, as indicated by the corn leaf material, rice straw, fresh corn and sorghum grains substrates (Zhang & Watson, 1997).

1.8.3 Mass production

Mass production technology is one important way for improving the quality of the entomopathogenic inocula (Vidal *et al.*, 1998). Liquid media and solid media have been used to produce sufficient amounts of inoculum of various entomopathogenic fungi used for biological control (Zhang &

Watson, 1997). Several fermenter designs could be used to attain more efficient mass-production of entomopathogenic fungi. In liquid media three types of fermenters are commonly used (Samson *et al.*, 1988), the stirred tank, the tower and the loop fermenters (Samson *et al.*, 1988). In solid media mass production of entomopathogenic fungi is usually undertaken using primitive fermenters in which the solid media are stirred only weakly, or not at all. The substrates used industrially are mainly cereal grains, broken or not, supplemented with specific nutrients or inert clays (Samson *et al.*, 1988).

Fermentation in liquid media is preferred because of the availability of technology and the scale-up process is relatively easy (Hall & Papierok, 1982). But solid media fermentation also provides sufficient conidia for some of biocontrol agents that do not sporulate in liquid culture such as *Cercospora rodmanii* Conway (Zhang & Watson, 1997).

1.8.4 Formulation

The wide spread acceptance and use of mycoinsecticides will depend on improvements in the development of formulations that will enhance virulence, extend the shelf life of the pathogen, improve efficiency of application, and prolong field persistence (Butt *et al.*, 1998). Formulating agents should have spreader-sticker qualities and protect the organism from desiccation and solar degradation, before and during germination of the spores on the target insect (Samson *et al.*, 1988).

A typical formulation is a mixture of the active ingredient, typically spores; a diluent and / or dispersent; a wetting agent and a sticker (Samson *et al.*, 1988). Three types of dry formulation exist; dusts, granules (bait substrate) and wettable powders (Inglis *et al.*, 1996a; Samson *et al.*, 1988). Usually entomopathogenic fungi are formulated in water or oil carrier. The formulation of conidia in oil was consistently more efficacious than in water. Possible advantages of the use of oil rather than water as a carrier of fungal propagules include the infection at lower humidities, stimulated germination, longer duration of viability, decreased sensitivity to high temperature in storage, decreased sensitivity to UV radiation and enhanced attachment to the hydrophobic surfaces of insect integument (Inglis *et al.*, 1996 a; Ibrahim *et al.*, 1999).

1.8.5 Storage

One fundamental objective with biological pesticides is long-term storage with no loss of product viability; at least 18 months; shelf-life has been suggested (Couch & Ignoffo, 1981), but less could be acceptable for specific control operations (Moore, Douro-Kpindou *et al.*, 1996). The survival capacity of a spore is directly dependent upon its moisture contents: a slow dehydration of the spore being favorable for the conservation of Deuteromycete conidia (Samson *et al.*, 1988). For short-term storage (months), moisture content of up 10% may be acceptable under cool

conditions (Hedgecock *et al.*, 1995). For long-term storage, lower temperatures would be greatly beneficial, along with desiccation (Hedgecock *et al.*, 1995). At room temperature, most fungi rapidly lose viability often in less than one month (Samson *et al.*, 1988).

1.9 Objectives

The present work was aimed at:

1. Investigating the biodiversity of entomopathogenic fungi in agricultural fields in the northern part of the West Bank in relation to abiotic factors including pH, moisture content and organic matter, site and vegetation (cropping practices);
2. Investigating the effect of different standard agar media on mycelial growth of selected isolates of the common entomopathogenic fungal species recovered; and
3. Investigating the effect of temperature on mycelial growth, spore production and spore germination of selected isolates of the common entomopathogenic fungal species recovered.

CHAPTER TWO

MATERIALS AND METHODS

CHAPTER TWO
MATERIALS AND METHODS

2.1 Insect rearing

Insect rearing was carried out as follows (Zimmermann, 1986; Vilicinskas & Wedde, 1997; Ali-Shtayeh *et al.*, 1998; Mahassneh, 1999).

1- Adults of wax moth *Galleria mellonella* were placed in 2L glass jars and left at 25°C in darkness. Ninety millimeters Petri dishes containing sterile sugar solution (10%) were placed in jars for feeding the adults.

2- Eggs were collected on folded tissue paper by placing the paper in the jar containing *Galleria* moths for 7-10 day.

3- Tissue paper with eggs were removed cut into small pieces (1 x 1cm), and then sterilized by immersing them in 10% formalin solution for 1 hour followed by washing under running tap water for 1 hour.

4- The tissue paper pieces with eggs were then removed and placed separately on dry tissue paper and were allowed to dry in a laminar flow desk.

5- A suitable number of pieces of tissue paper with eggs were transferred to the bottom of autoclaved 1-L glass jars, and covered with larvae feeding medium (LFM) composed of honey 200g, dry yeast 47g, antibiotic nipagene 4g, glycerine 183g, and wheat bran 320g. The medium was prepared as follows. Honey and wheat bran were autoclaved separately.

Dry yeast, nipagene, glycerine and honey were mixed, and the wheat bran was added to the mixture and mixed thoroughly.

6- Jars containing eggs covered with LFM were then incubated at 31°C in darkness for about 3 weeks until the larvae reached last instar. Additional LFM was added when needed.

7- The LFM was kept in the refrigerator until use.

2.2 Sampling sites

One hundred and forty samples of soil were collected from irrigated fields in the northern part of the West Bank (Figure 2.1). Based on cropping practices the fields surveyed can be divided into fields under tree fruits, vegetables (Table 2.1).

2.3 Collection of soil samples

Four 0.5 liter soil samples were collected from a depth of 5-20 cm with a trowel from an area of 16m2. Samples were placed in clear plastic bags (31cm x 20cm), sealed with a rubber band and were brought back to the laboratory. The composite sample was mixed thoroughly and two 50g aliquots of soil were weighted out of the soil sample to determine soil moisture content, 10g of soil sample were weighed to determine soil pH, and about 250g of soil sample were left to dry aerially for the determination of

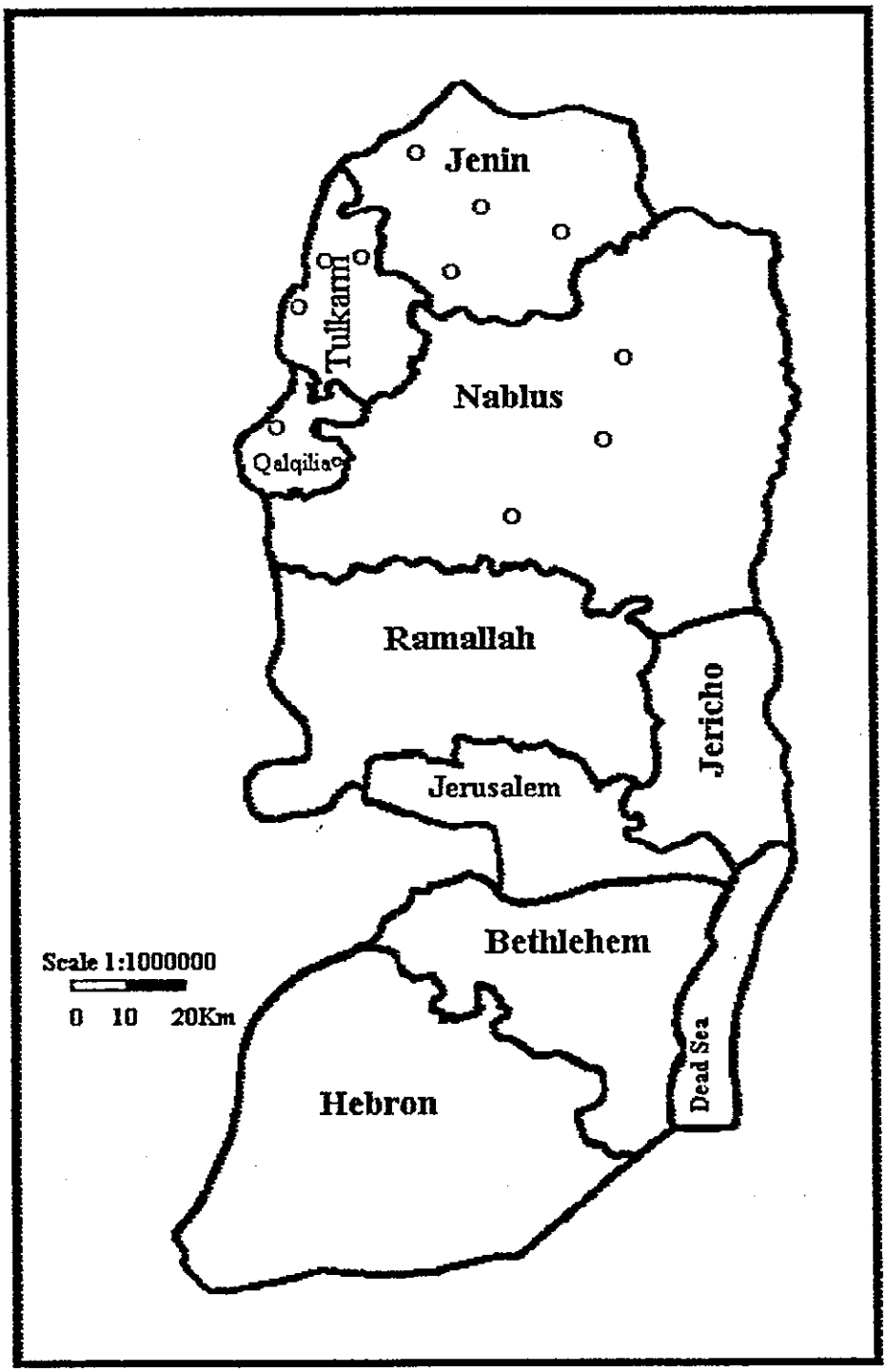

Figure 2.1: West Bank map showing the locations of the sampling sites (o).

Table 2.1: Field characteristics (Jenin area)

Field No	Sample #	Location	Vegetation	pH	% Organic matter	% Moisture content	No. of isolates recovered
Irrigated Citrus Orchards							
58	J1 - 10.8 . 99	Kufor-Dan	Citrus	7.43	0.28	20	0
58	J2 - 10.8 . 99	Kufor-Dan	Citrus	7.36	1.14	23	0
58	J3 - 10.8 . 99	Kufor-Dan	Citrus	7.49	2.61	24	4
58	J4 - 10.8 . 99	Kufor-Dan	Citrus	7.22	1.62	22	0
58	J5 - 10.8 . 99	Kufor-Dan	Citrus	7.13	2.34	24.6	0
58	J9 - 10.8 . 99	Kufor-Dan	Citrus	7.37	0.68	20	2
68	J1 - 1.11 . 99	Anin	Citrus	7.61	3.20	26	2
69	J1 - 4.12 . 99	Anin	Citrus	7.25	0.28	17.4	0
69	J2 - 4.12 . 99	Anin	Citrus	7.25	0.26	20	0
70	J2 - 4.12 . 99	Anin	Citrus	7.26	2.61	22.6	2
Irrigated Soils under Vegetables							
5	J1 - 27.5. 99	Qabatia	Tomato	7.27	0.75	24.6	2
6	J2 - 27.5. 99	Qabatia	Cucumber	7.20	0.30	24.6	0
6	J3 - 27.5. 99	Qabatia	Egyptian cucumber	6.93	0.37	28.4	0
6	J4 - 27.5. 99	Qabatia	Cucumber	7.00	0.42	28	0
7	J5 - 27.5. 99	Qabatia	Bean	7.18	0.62	28	2
7	J6 - 27.5. 99	Qabatia	Bean	7.28	0.30	27	0
7	J7 - 27.5. 99	Qabatia	Cowpea	7.24	0.30	28	0
9	J1 - 6.6. 99	Kufor-Dan	Thyme	6.99	0.42	22.6	0
9	J2 - 6.6. 99	Kufor-Dan	Thyme	6.99	0.82	24.4	0
9	J3 - 6.7. 99	Kufor-Dan	Jew's mallow	6.99	0.68	22	0
10	J4 - 6.6. 99	Kufor-Dan	Cucumber	6.93	0.30	25.4	0
9	J5 - 6.6. 99	Kufor-Dan	Parsley	7.00	0.68	27.4	2
11	J6 - 6.6. 99	Kufor-Dan	Aubergine	7.40	0.28	25.6	0
11	J7 - 6.6. 99	Kufor-Dan	Aubergine	6.83	1.14	27	0
13	J8 - 6.6. 99	Kufor-Dan	Pepper	7.31	0.34	25	0
12	J9 - 6.6. 99	Kufor-Dan	Cowpea	7.37	0.25	23	0
11	J10 - 6.6. 99	Kufor-Dan	aubergine	7.19	0.25	28	0
10	J11 - 6.6. 99	Kufor-Dan	Cucumber	6.93	0.30	25	1
59	J6 - 10.8. 99	Kufor-Dan	Cauliflower	7.30	0.25	17.6	0
59	J7 - 10.8. 99	Kufor-Dan	Cauliflower	7.34	0.56	16.4	0
59	J8 - 10.8. 99	Kufor-Dan	Cauliflower	7.27	0.46	17	1
66	J1 - 27.10. 99	Arraba	Cabbage	6.69	3.69	28	0
66	J2 - 27.10. 99	Arraba	Cabbage	7.11	3.69	29	1
66	J3 - 27.10. 99	Arraba	Cabbage	7.02	4.21	33	3
67	J2 - 1.11. 99	Arraba	Cabbage	7.59	0.56	16	0

Table 2.1 Field characteristics (Nablus area)

Field No	Sample #	Location	Vegetation	pH	% Organic matter	% Moisture content	No. of isolates recovered
Irrigated Citrus Orchards							
3	N1-15.4 . 99	Wadi-Qana	Citrus	7.06	6.21	22.6	0
3	N2-15.4 . 99	Wadi-Qana	Citrus	6.8	7.36	28.6	0
4	N1-22.4 . 99	Tell	Citrus	7.28	0.51	14.6	1
53	N1 - 2.8 . 99	Al-Fara'	Citrus	7.03	9.69	34.6	1
53	N2 - 2.8 . 99	Al-Fara'	Citrus	7.51	3.52	27	1
53	N3 - 2.8 . 99	Al-Fara'	Citrus	7.41	3.36	18.6	1
53	N4 - 2.8 . 99	Al-Fara'	Citrus	7.54	1.42	22.6	0
53	N5 - 2.8 . 99	Al-Fara'	Citrus	7.55	5.16	28	2
53	N6 - 2.8 . 99	Al-Fara'	Citrus	7.41	7.6	20.6	2
21	N11-21.6.99	Al-Fara'	Citrus	7.29	2.34	31	2
Irrigated Soils under Vegetables							
18	N1 - 21.6 . 99	Al-Fara'	Aubergine	7.10	1.42	22	1
19	N2 - 21.6 . 99	Al-Fara'	Tomato	7.28	2.09	30	0
18	N3 - 21.6 . 99	Al-Fara'	Aubergine	7.18	2.21	14	0
22	N4 - 21.6 . 99	Al-Fara'	Cauliflower	7.21	1.22	22	1
22	N5 - 21.6 . 99	Al-Fara'	Cauliflower	6.92	0.97	25	0
19	N6 - 21.6 . 99	Al-Fara'	Tomato	7.28	0.89	18	2
19	N7 - 21.6 . 99	Al-Fara'	Tomato	7.42	1.42	24	1
18	N8 - 21.6 . 99	Al-Fara'	Aubergine	7.18	1.62	17	0
20	N9 - 21.6 . 99	Al-Fara'	Jew's mallow	7.53	1.42	25	1
20	N10- 21.6 . 99	Al-Fara'	Jew's mallow	7.34	1.42	26	0
28	N1 - 3.7 . 99	Asira Qiblia	Aubergine	7.24	6.21	23	0
28	N2 - 3.7 . 99	Asira Qiblia	Aubergine	7.25	5.36	18	0
28	N4 - 3.7 . 99	Asira Qiblia	Aubergine	7.00	9.69	19	0
28	N5 - 3.7 . 99	Asira Qiblia	Aubergine	7.20	5.46	19	1
54	N1 - 8.8 . 99	Al-Badan	Tomato	7.21	1.32	28.6	0
57	N2 - 8.8 . 99	Al-Badan	Pepper	7.62	0.68	31	0
57	N3 - 8.8 . 99	Al-Badan	Zucchini	7.48	0.25	24	0
55	N4 - 8.8 . 99	Al-Badan	Cucumber	7.46	0.46	26	1
56	N5 - 8.8 . 99	Al-Badan	Aubergine	7.57	0.28	25	0
56	N6 - 88 . 99	Al-Badan	Aubergine	7.44	0.62	21	0
56	N7 - 8.8 . 99	Al-Badan	Aubergine	7.34	0.42	25	0
55	N8 - 8.8 . 99	Al-Badan	Cucumber	7.36	0.37	27.4	0
57	N9 - 8.8 . 99	Al-Badan	Muskmelon	7.48	0.56	30	0
54	N10 - 8.8 . 99	Al-Badan	Tomato	7.21	0.82	29.4	1
55	N11 - 8.8 . 99	Al-Badan	Cucumber	7.31	0.75	30	0

Table 2.1: Field characteristics (Tulkarm area)

Field No	Sample #	Location	Vegetation	pH	% Organic matter	% Moisture content	No. of isolates recovered
Irrigated Citrus Orchards							
23	K1 - 27.6 . 99	Illar	Citrus	7.02	3.86	28	0
50	K11 - 24.7 . 99	Atteel	Citrus	7.30	3.36	21	0
50	K12 - 24.7 . 99	Atteel	Citrus	7.35	0.97	24.6	2
50	K13 - 24.7 . 99	Atteel	Citrus	7.00	4.96	26	4
50	K14 - 24.7 . 99	Atteel	Citrus	7.16	5.16	24	0
50	K15 - 24.7 . 99	Atteel	Citrus	7.31	0.62	24	0
50	K16 - 24.7 . 99	Atteel	Citrus	7.17	2.09	27	1
50	K17 - 24.7 . 99	Atteel	Citrus	6.61	5.16	27	1
50	K18 - 24.7 . 99	Atteel	Citrus	7.19	3.69	21	1
50	K19 - 24.7 . 99	Atteel	Citrus	7.25	2.48	21	0
Irrigated Soils under Vegetables							
8	K1 - 1.6 . 99	Talkarm	Peanut	7.27	0.97	26	1
8	K2 - 1.6 . 99	Talkarm	Peanut	7.31	2.75	24.4	0
8	K3 - 1.6 . 99	Talkarm	Peanut	6.61	0.82	27	0
8	K4 - 1.6 . 99	Talkarm	Peanut	7.35	0.97	24.6	0
8	K5 - 1.6 . 99	Talkarm	Peanut	7.40	4.77	26.2	0
8	K6 - 1.6 . 99	Talkarm	Peanut	7.28	0.75	25.4	0
29	K1 - 11.7 . 99	Talkarm	Jew's mallaw	7.42	1.05	21	0
29	K2 - 11.7 . 99	Talkarm	Jew's mallaw	7.34	1.22	23	1
30	K3 - 11.7 . 99	Talkarm	Aubergine	7.30	1.05	12.6	0
31	K4 - 11.7 . 99	Talkarm	Cabbage	7.31	2.21	21.6	0
31	K5 - 11.7 . 99	Talkarm	Cabbage	7.27	1.05	17	2
24	K2 - 27.6. 99	Illar	Bean	7.19	1.32	14	0
25	K3 - 27.6 . 99	Illar	Couliflower	7.72	1.52	20	0
26	K4 - 27.6 . 99	Illar	Cucumber	7.10	2.21	17	1
27	K5 - 27.6 . 99	Illar	Potato	7.07	4.21	16	0
49	K1 - 24.7 . 99	Atteel	Cucumber	7.41	1.05	21	0
51	K2 - 24.7 . 99	Atteel	Cabbage	7.24	1.05	24.6	0
47	K3 - 24.7 . 99	Atteel	Cauliflower	7.27	1.73	26	1
48	K4 - 24.7 . 99	Atteel	Aubergine	7.30	0.68	22	0
47	K5 - 24.7 . 99	Atteel	Couliflower	7.17	0.97	26	0
51	K6 - 24.7 . 99	Atteel	Cabbage	7.28	0.56	25.4	0
47	K7 - 24.7 . 99	Atteel	Couliflower	7.22	1.73	22	1
51	K8 - 24.7 . 99	Atteel	Cabbage	7.25	1.52	20	0
52	K9 - 24.7 . 99	Atteel	Tamato	7.13	0.62	22.6	0
52	K10 - 24.7 . 99	Atteel	Tomato	7.31	0.89	26	0

Table 2.1: Field characteristics (Qalqilia area)

Field No	Sample #	Location	Vegetation	pH	% Organic matter	% Moisture content	No. of isolates recovered
Irrigated Citrus Orchards							
1	Q1 - 6.4 . 99	Qalqilia	Citrus	7.11	0.54	20	0
2	Q2 - 6.4 . 99	Qalqilia	Fig	7.31	1.32	16	2
37	Q1 - 12.7 . 99	Qalqilia	Citrus	7.05	1.14	27.4	0
40	Q4 - 12.7 . 99	Qalqilia	Citrus	6.52	4.58	30	0
36	Q10 - 12.7 . 99	Qalqilia	Citrus	6.94	0.56	20	0
37	Q20 - 12.7 . 99	Qalqilia	Citrus	7.35	1.22	27	0
64	Q6 - 2.10 . 99	Qalqilia	Fig	6.87	1.32	14	1
65	Q7 - 2.10. 99	Qalqilia	Citrus	7.02	1.22	21	0
65	Q8 - 2.10. 99	Qalqilia	Citrus	7.02	1.22	22	0
64	Q9 - 2.10 . 99	Qalqilia	Citrus	6.87	1.42	13	0
Irrigated Soils under Vegetables							
14	Q2 - 16.6 . 99	Azzoon	Tomato	6.99	6.66	16	2
15	Q3 - 16.6 . 99	Azzoon	Pepper	7.75	6.21	20	0
16	Q4 - 16.6 . 99	Azzoon	Zucchini	7.09	2.48	13	1
17	Q5 - 16.6 . 99	Azzoon	Cowpea	7.01	5.46	12	0
32	Q2 - 12.7 . 99	Q-D.C.O	Okra	7.24	1.05	22	0
38	Q3 - 12.7. 99	Qalqilia	Cabbage	6.64	0.97	25	0
35	Q5 - 12.7 . 99	Q-D.C.O	Corn	7.00	3.2	26	1
38	Q6 - 12.7 . 99	Qalqilia	Cabbage	7.15	0.46	23.6	0
41	Q7 - 12.7 . 99	Qalqilia	Cowpea	6.55	0.34	21.6	0
42	Q8 - 12.7 . 99	Qalqilia	Tomato	6.71	0.25	20	0
33	Q9 - 12.7 . 99	Q-D.C.O	Aubergine	7.39	0.68	20	1
33	Q11 - 12.7 . 99	Q-D.C.O	Aubergine	6.66	0.42	20	1
34	Q12 - 12.7 . 99	Q-D.C.O	Tomato	6.74	0.82	24.6	0
34	Q13 - 12.7 . 99	Q-sufin	Tomato	6.54	0.84	26	0
46	Q14 - 12.7 . 99	Q-sufin	Aubergine	6.75	0.68	27	0
45	Q15 - 12.7 . 99	Q-sufin	Cabbage	6.69	1.22	24	0
45	Q16 - 12.7 . 99	Q-D.C.O	Cabbage	6.68	1.42	29.6	2
39	Q17 - 12.7 . 99	Qalqilia	Cabbage	6.70	0.75	28.6	0
43	Q18 - 12.7 . 99	Qalqilia	Bean	6.63	1.05	28	1
44	Q19 - 12.7 . 99	Qalqilia	Peper	6.76	0.56	20	0
60	Q1 - 2.10 . 99	Qalqilia	Thyme	7.60	1.05	20	0
61	Q2 - 2.10 . 99	Qalqilia	Parsley	6.98	1.05	20	0
62	Q3 - 2.10 . 99	Qalqilia	Jew's mallow	6.87	0.37	13	0
63	Q4 - 2.10 . 99	Qalqilia	Aubergine	7.04	1.14	20	0
62	Q5 - 2.10 . 99	Qalqilia	Pepper	6.83	0.89	13	1

soil organic matter. The remaining composite soil sample was used for the isolation of entomopathogenic fungi. All soil samples were processed within 24 hour of collection.

2.4 Isolation of entomopathogenic fungi: *Galleria* bait method

Fungi were isolated from soil samples using a "*Galleria* bait method" (GBM)(Zimmermann, 1986). The remaining composite soil sample was divided into two equal parts (replicates) (about 400 ml). Coarse debris (pebbles, twigs) was removed from each part. Each part was placed in 500 ml-plastic cup with cover and three last instar (250-350 mg) *Galleria mellonella* L. larvae were added to each cup. The cups were laterlly perforated for the ventilation, and incubated up-side down at 25°C in darkness. Cups were turned and agitated every day to ensure that the larvae came into maximum contact with the soil (Mietkiewski *et al.*, 1997). The larvae were inspected once every two days for a period of two weeks. Dead larvae were removed and surface-sterilized in 1% sodium hypochlorite for 3min, then washed three times in sterile distilled water (Chandler *et al.*, 1997), and finally plated on malt extract agar (MEA) supplemented with Rifampicin (75mg/L) (media used in this work are presented in Appendix A). The cadavers were scored for overt mycosis (sporulation) and the plates were incubated at 25°C in darkness (Poprawski *et al.*, 1998). If external fungal

growth was already visible, larvae were only rinsed in sterile distilled water (to remove soil) (Mietkiewski *et al.*, 1997) before being plated on MEA.

2.5 Preliminary pathogenicity test (Koch's Postulates)

The isolated fungus was first grown on MEA medium for 10-12 days until the fungus reached sporulation stage. The last instar larvae of *G. mellonella* were held with forceps and the dorsal larval surface was rolled across a sporulating culture (Hajek *et al.*, 1993). Infected larvae were transferred into a 5-cm diameter Petri dishes with a moistened filter paper to encourage spore germination on the insect cuticle. Then the lids of the Petri dishes were sealed with strips of parafilm to maintain suitable relative humidity, and placed in an incubator at 25°C in darkness. Petri dishes with larvae were inspected daily until larval death or pupation. Fungus recovery was attempted using the above mentioned procedures and the experiment was carried out three times to confirm the pathogenicity of the fungus.

2.6 Identification of entomopathogenic fungal isolates

Entomopathogenic fungi recovered by "*Galleria* bait method" were first grown on four media: MEA, sabouraud's dextrose agar (SDA), potato dextrose agar (PDA) and potato sucrose agar (PSA) and agar plates were incubated for 10 days at 25°C. The isolates were then placed into groups on the basis of their colonial morphology, color and texture, and growth

characteristics. Microscopic examination was carried out using fresh direct mounts in lactophenol cotton blue under high powers (X40, X100). Cultures which did not produce any reproductive structures, were incubated in the light for two days and then examined under the microscope. Identification of isolates were made with the aid of several taxonomic references including: Booth (1977), Domsch *et al.* (1980), Onions *et al.* (1981), Samson *et al.* (1988), and Waterhouse & Brady (1982).

Fungal isolates were preserved by growing them on PDA slants supplemented with 75mg/L Rifampicin in 30-ml sterile screw- caped culture tubes and keeping them in a refrigerator at 4°C. They were subcultured monthly.

2.7 Characterization of common entomopathogenic fungal isolates

2.7.1Effect of nutrient media on mycelial growth

Mycelial growth of fungal isolates was observed on different agar media. The media tested were MEA, PDA, SDA, yeast with sabouraud's dextrose agar (YSDA), corn meal agar (CMA) and Czapek-Dox agar (CZA). Media were dispensed in 90-mm diameter Petri dishes and were centrally inoculated with 8mm agar plugs from 7-day-old PDA cultures of fungal isolates. Three replicate dishes of each medium for each fungal isolate were incubated for 7 days at 25°C in darkness (Zhang & Watson, 1997). Radial

mycelial growth during this period was recorded as the mean of two perpendicular diameters minus the diameter of the inoculum plug (8mm). Radial mycelial growth rate was calculated in mm/day.

2.7.2 Effect of temperature on mycelial growth and spores production

Radial mycelial growth was measured following the procedure outlined above for six temperatures including 10, 15, 20, 25, 30, and 37°C with three replicates/temperature combination on SDA supplemented with chloramphenicol (50mg/L) in darkness.

At the end of mycelial growth study, plates of all isolates were incubated for 1 more week and then assessed for spores production. Spores were harvested by flooding the plates with 10ml of distilled water and scraping the surface of the colonies with a glass slide. The resulting suspensions were filtered through a layer of sterile tissue papers and spore concentration in the suspension from three colonies/temperature was determined using a haemacytometer (Zhang & Watson, 1997).

2.7.3 Effect of temperature on spore germination

The effect of temperature on spore germination was tested by spread-plating 0.1ml of spore suspension containing $3x10^6$ spore ml^{-1} on MEA plates in Petri dishes (Ekesi *et al.*, 1999). Inoculated plates were sealed with strips of parafilm and incubated at 10, 15, 20, 25, 30, and 37°C in complete darkness.

There were 18 dishes prepared for each isolate/temperature combination. After 24, 48 and 72h, six dishes of each isolate/temperature treatment combination were removed, and germination rates were determined by examination of a minimum of 100 spores per replicate dish (Fargues, Ouderaogo *et al.*, 1997). A spore was considered germinated if the germ tube was at least as long as the swollen conidium.

2.8 Determination of physical factors of soil

2.8.1 Determination of soil moisture content

Two 50g aliquots of fresh soil were weighed out of each sample, each was placed in 100mm diameter glass Petri dish. The Petri dishes were then placed in a hot-air oven at 105°C for 24 hours, soil samples were removed from the oven, allowed to cool and reweighed. Dry weight was recorded and soil moisture content was calculated as follows:

% moisture content = [(wt. of fresh soil - wt. of dry soil) / (wt. of fresh soil)] x 100%

2.8.2 Determination of soil pH

The method of Schofield & Tayler (1955) was used. Approximately 10g of soil were weighed and placed in a 50ml beaker. Twenty ml of 0.1M $CaCl_2$ solution were added to the sample and stirred several times over a period of 30 minutes. The suspension was allowed to stand for a further 30 minutes, then the soil pH was measured using a pH meter.

2.8.3 Determination of organic matter content

The method of Weber (1977) was used. Air-dried portions of soil samples were ground finely and sieved to a particle size of 0.5 mm. About 1.5g of soil were weighed and placed into 250-ml Erlenmeyer flask. Smaller samples may be required for soils with a very high organic matter content, and larger samples may be required for soils with a very low organic content. Twenty ml of 4N $K_2Cr_2O_7$ solution were poured into the flask followed by the addition of 20-ml of 18M H_2SO_2 by using a pipette equipped with a suction bulb. Twenty ml of 4N $K_2Cr_2O_7$ solution were added to 20-ml 18M H_2SO_4, the preparation of a blank reference (reagents only). The flask was mixed vigorously for 1 min in a fume hood, and left to stand for 1 hour on an asbestos sheet to allow complete oxidation of the organic matter. One hundred ml of deionized water were then added, mixed and allowed to stand overnight to permit soil particles to sediment. About 10ml of the supernatant liquid were drawn out using a pipette, without disturbing the sediment, and transferred to an absorption cell. Percent of transmittance was measured at 625 nm using a photoelectric colorimeter (Spectronic 21, Bousch & Lomb, made in USA). The percent organic matter content of the unknown soil sample was determined using the following equation:

$$\% \text{ organic matter} = 14.97 - 0.3448\ (\%T) + 0.00201\ (\%T)^2$$

where T is the transmittance at 625 nm.

2.9 Statistical analysis

The occurrence of entomopathogenic fungi was analyzed on No. of isolates basis in 140 soil samples in conjunction with each factor using analysis of variance (ANOVA) with multiple range Duncan's test, and univariante analysis of variance (UNIANOVA). Readings of pH were grouped at 0.25 unit intervals, organic matter content readings were grouped at 2 unit intervals, and readings of moisture content were grouped at 6 unit intervals.

The results of all characterization experiments were analysed using ANOVA with multiple range Duncan's test, and UNIANOVA. All analysis was conducted using SPSS version 9.0 software program.

CHAPTER THREE

RESULTS

CHAPTER THREE
RESULTS

3.1 Occurrence of entomopathogenic fungi

Entomopathogenic fungi were found to occur in 33.57% (47 of 140) of the soil samples studied (Table 3.1, 3.2), with positive samples yielding 70 fungal isolates belonging to 20 species and 13 genera (Tables 3.1 - 3.3).

Conidiobolus coronatus was the most frequent and abundant entomopathogenic species recovered comprising 31.43% of the total isolates, followed by *Fusarium semitectum* (10%), *Mucor flavus* (10%), *F. solani* (7.14%), *Aspergillus niger* (7.14%), *Sporodiniella umbellta* (7.14%), *Nectria ventricosa* (5.71%), *F. oxysporum* (2.8%) and *Gliocladium viride* (2.86%) (Table 3.3). Other species were isolated only once from soil samples (Table 3.3).

3.2 Preliminary pathogenicity test of fungal isolates

Test isolates showed varied degrees of pathogenicity to *Galleria* larvae using Koch's postulates. Pathogenicity (% mortality) ranged from 16% to 100% (Table 3.4). Of the seventy fungal isolates recovered, isolates of *Conidiobolus coronatus* proved to be most virulent (87-100%) followed by

Table 3.1 Distribution and frequency of occurrence of entomopathogenic fungi in soil by geographical Location

Fungall Species	Jenin		Nablus		Tulkarm		Qalqilia		Total	
	Number of isolates (%)	Number of positive samples (%)	Number of isolates (%)	Number of positive samples (%)	Number of isolates (%)	Number of positive samples (%)	Number of isolates (%)	Number of positive samples (%)	Number of isolates (%)	Number of positive samples (%)
All species	22 (62.85)	11 (31.4)	19 (54.28)	15 (42.9)	16 (45.71)	11 (31.4)	13 (37.14)	10 (28.57)	70 (50)	47 (33.57)
Absidia cylindrospora (Hagem var. cylindrospora)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Aspergillus niger (Van Tieghem).	2 (5.71)	1 (2.86)	0 (0)	0 (0)	1 (2.86	1 (2.86)	2 (5.71)	2 (5.71)	5 (3.57)	4 (2.86)
A. ochraceus (Wilhelm)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Conidiobolus coronatus (Constatin) Batko	10 (28.57)	5 (14.29)	12 (34.28)	9 (25.71)	0 (0)	0 (0)	0 (0)	0 (0)	22 (15.71)	14 (10)
Entomophaga grylli (Fres,) Batko	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Erynia castrans (Batko & weiser) Remaudiere & Keller	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	1 (0.71)	1 (0.71)
Fasrium heterosporum (Nees ex Fr.)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
F. moniliforme var. *subglutinans* (Wollenw & Reink)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	1 (0.71)	1 (0.71)
F. nivale (Fr.) Ces., Robehn	1 (2.86)	1 (2.86)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
F. oxysporum (Schlecht)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	1 (2.86)	1 (2.86)	2 (1.43)	2 (1.43)
F. semitectum (Berk & Rav)	2 (5.71)	2 (5.71)	0 (0)	0 (0)	2 (5.71)	2 (5.71)	3 (8.57)	2 (5.71)	7 (5)	6 (4.28)
F. solani (Mart.) Sacc.	0 (0)	0 (0)	3 (8.57)	3 (8.57)	2 (5.71)	2 (5.71)	0 (0)	0 (0)	5 (3.57)	5 (3.57)
Gliocladium viride (Martr.)	2 (5.71)	2 (5.71)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (1.43)	2 (1.43)
Hirsutella jonesii (Speare) Evans & Samson	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Mucor flavus (Bainier)	4 (11.42)	2 (5.71)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	2 (5.71)	2 (5.71)	7 (5)	5 (3.57)
M. piriformis (Fischer)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	1 (0.71)	1 (0.71)
Nectria ventricosa (C. Booth)	1 (2.86)	1 (2.86)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	2 (5.71)	2 (5.71)	4 (2.88)	4 (2.88)
Paecilomyces farinosus (Holm) Brown & Smith	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Sporodiniella umbellata (Boedijn)	0 (0)	0 (0)	0 (0)	0 (0)	5 (3.57)	3 (8.57)	0 (0)	0 (0)	5 (3.57)	3 (2.14)
Verticillium nigrescens (Pethybr)	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.86)	1 (2.86)	0 (0)	0 (0)	1 (0.71)	1 (0.71)

-Total number of soil samples= 140 (35 from each of 4 geographical locations)

Table 3.2 Distribution and frequency of occurrence (% positive samples) of entomopathogenic fungi in soil by crop

Fungal species	Crop				Total	
	Vegetable fields N= 100		Orchard fields N= 40			
	No. of isolates (%)	No. of +ve sample (%)	No. of isolates (%)	No. of +ve sample (%)	No. of isolates (%)	No. of +ve sample (%)
All species	38 (38)	29 (29)	32 (80)	18 (45)	70 (50)	47 (33.57)
Absidia cylindrospora	1 (1)	1 (1)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Aspergillus niger	4 (4)	3 (3)	1 (2.5)	1 (2.5)	5 (3.57)	4 (2.86)
A. ochraceus	1 (1)	1 (1)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Conidiobolus coronatus	9 (9)	7 (7)	13 (32.5)	7 (17.5)	22 (15.71)	14 (10)
Entomophaga grylli	1 (1)	1 (1)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Erynia castrans	0 (0)	0 (0)	1 (2.5)	1 (2.5)	1 (0.71)	1 (0.71)
Fusarium heterosporum	0 (0)	0 (0)	1 (2.5)	1 (2.5)	1 (0.71)	1 (0.71)
F. moniliforme var *subglutinans*	1 (1)	1 (1)	0 (0)	0 (0)	1 (0.71)	1 (0. 71)
F. nivale	1 (1)	1 (1)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
F. oxysporum	1 (1)	1 (1)	1 (2.5)	1 (2.5)	2 (1.43)	2 (1.43)
F. semitectum	7 (7)	6 (6)	0 (0)	0 (0)	7 (5)	6 (4.28)
F. solani	4 (4)	4 (4)	1 (2.5)	1 (2.5)	5 (3.57)	5 (3.57)
Gliocladium viride	0 (0)	0 (0)	2 (5)	2 (5)	2 (1.43)	2 (1.43)
Hirsutella jonesii	1 (1)	1 (1)	0 (0)	0 (0)	1 (0.71)	1 (0.71)
Mucor flavus	4 (4)	2 (2)	3 (7.5)	3 (7.5)	7 (5)	5 (3.57)
M. piriformis	0 (0)	0 (0)	1 (2.5)	1 (2.5)	1 (0.71)	1 (0.71)
Nectria ventricosa	3 (3)	3 (3)	1 (2.5)	1 (2.5)	4 (2.88)	4 (2.88)
Paecilomyces farinosus	0 (0)	0 (0)	1 (2.5)	1 (2.5)	1 (0.71)	1 (0.71)
Sporodiniella umbellata	0 (0)	0 (0)	5 (12.5)	3 (7.5)	5 (3.57)	3 (2.14)
Verticillium nigrescens	0 (0)	0 (0)	1 (2.5)	1 (2.5)	1 (0.71)	1 (0.71)

Table 3.3 Isolation frequency of fungal isolates

Fungal species*	Number of isolates	% F**
Absidia cylindrospora	1	1.43
Aspergillus niger	5	7.14
A. ochraceus	1	1.43
Conidiobolus coronatus	22	31.43
Entomophaga grylli	1	1.43
Erynia castrans	1	1.43
Fusarium heterosporm	1	1.43
F. moniliforme	1	1.43
F. nivale	1	1.43
F. oxysporum	2	2.86
F. semitectum	7	10
F. solani	5	7.14
Gliocladium viride	2	2.86
Hirsutella jonesii	1	1.43
Mucor flavus	7	10
M. piriformis	1	1.43
Nectria ventricosa	4	5.71
Paecilomyces farinosus	1	1.43
Sporodiniella umbellata	5	7.14
Verticillium nigrescens	1	1.43
Total	70	100

* Total number of species = 20.

** % F, Percentage frequency = number of isolates of a species / total number of isolates of all species (70).

Table 3.4 Pathogenicity test (Koch's Postulates) results

Species	No. of isolates	Time (Days) after infection	Mortality* (%)
Absidia cylindrospora	1	5	56
Aspergillus niger	5	5-7	66-78
A. ochraceus	1	5	20
Conidiobolus coronatus	22	5-8	87-100
Entomophaga grylli	1	5	45
Erynia castrans	1	8	60
Fusarium heterosporum	1	10	18
F. moniliforme var. *subglutinans*	1	6	30
F. nivale	1	6	56
F. oxysporum	2	6	30-33
F. semitectum	7	5-7	16-33
F. solani	5	5-10	28-44
Gliocladium viride	2	6	78-84
Hirsutella jonesii	1	10	19
Mucor flavus	7	5-9	22-33
M. piriformis	1	7	56
Nectria ventricosa	4	5-6	22-44
Paecilomyces farinosus	1	6	28
Sporodiniella umbellata	5	7-9	45-63
Verticillium nigrescens	1	9	37

* Number of larvae replicates = 9

Gliocladium viride (78-84%), *Aspergillus niger* (66-78%), *Sporodiniella umbellata* (45-63%) and *Erynia castrans* (60%).

3.3 Effect of edaphic factors, location, and vegetation type on the occurrence of entomopathogenic fungi in soil

3.3.1 Soil pH

The isolation of entomopathogenic fungi from soils was not significantly (df = 4; F = 0.426; P = 0.79) affected by the pH of soil with soils in the range of 7.25-7.50 giving higher percent of entomopathogenic isolates (Table 3.5).

Table 3.5 Effect of pH on the isolation of entomopathogenic fungi from soil

pH	Number of samples	Number of Positive samples	% Positive samples	Number of isolates recovered	% Isolates recovered
6.5-6.75	15	4	26.6	5	33.33
6.75-7.00	22	7	46.66	12	54.55
7.00-7.25	43	14	32.56	18	41.86
7.25-7.50	49	18	36.73	29	59.18
7.50-7.75	11	4	36.36	6	54.55
Total	140	47	33.57	70	50

3.3.2 Organic matter content

The isolation of entomopathogenic fungi from soils was significantly affected (df = 4; F = 2.916; p = 0.024) by the organic matter content of the soil with soils in the range of 4-6% giving higher percent of entomopathogenic isolates (Table 3.6).

Table 3.6 Effect of organic matter content on the isolation of entomopathogenic fungi from soil.

% Organic matter	Number of samples	Number of Positive samples	% Positive samples	Number of isolates recovered	% Isolates recovered
0-2	100	27	27	36	36
2-4	21	12	57.14	18	85.71
4-6	11	5	45.54	11	100
6-8	6	2	33.33	4	66.67
8-10	2	1	0.5	1	50
Total	140	47	33.57	70	50

3.3.3 Soil moisture content

The isolation of entomopathogenic fungi in soil was not significantly (df= 3; F= 2.198; P = 0.091) affected by the moisture content of the soil. Soils in the range of 30-36% giving higher percent of entomopathogenic isolates (Table 3.7).

Table 3.7 Effect of soil moisture content on the isolation of entomopathogenic fungi from soil.

% Moisture content	Number of samples	Number of positive samples	% Positive samples	Number of isolates recovered	% Isolates recovered
12-18	23	9	39.13	12	52.17
18-24	53	15	28.30	22	41.51
24-30	60	20	33.33	30	50
30-36	4	3	75	6	150
Total	140	47	33.57	70	50

3.3.4 Geographical location

The isolation of entomopathogenic fungi from soils varied with location (d f = 3; F= 0.622; P= 0.602) (Table 3.1, 3.8). The highest number of entomopathogenic isolates was in Jenin followed by Nablus, Tulkarm, and Qalqilia.

Table 3.8 Effect of geographical location on the isolation of entomopathogenic fungi from soil.

% Geographical location	Number of samples	Number of positive samples	% Positive samples	Number of isolates recovered	% Isolates recovered
Jenin	35	11	31.42	22	62.85
Nablus	35	15	42.85	19	54.28
Tulkarm	35	11	31.42	16	45.71
Qalqilia	35	10	28.57	13	37.14
Total	140	47	33.57	70	50

3.3.5 Vegetation type

The isolation of entomopathogenic fungi from soils was significantly affected (df= 1; F= 7.731; p= 0.006) by vegetation type, with orchards soils yielding the highest percent of entomopathogenic isolates (Table 3.2, 3.9).

Table 3.9 Effect of vegetation (Cropping practices) type on the isolation of entomopathogenic fungi

Vegetation type	Number of samples	Number of Positive samples	% Positive samples	Number of isolates recovered	% Isolates recovered
Orchards	40	18	45	32	80
Vegetables	100	29	29	38	38
Total	140	47	33.57	70	50

3.3.6 Interactions between different variables

A univariate analysis of variance (UNIANOVA) of the results revealed that there was no significant interactions between different variables (Table 3.10). Overall they did not have any statistically significant effect on the isolation of entomopathogenic fungi from soils.

Table 3.10 Interactions between different variables based on UNIANOVA

Variables interaction	df	F	P
Location *Vegetation type	2	0.811	0.449
Location* pH	6	0.365	0.898
Vegetation type* pH	2	1.007	0.371
Location * organic matter content	4	0.998	0.416
Vegetation type * organic matter content	1	0.036	0.850
Location * moisture content	4	0.938	0.448
Vegetation type * moisture content	2	1.427	0.248
pH * moisture content	4	1.399	0.245
Location * pH * moisture content	2	0.595	0.555
Organic matter content * moisture content	2	0.003	0.997

3.4 Characterization of selected isolates of the common entomopathogenic fungal species recovered

3.4.1 Effect of different standard agar media on mycelial growth rate (mm day^{-1})

The radial mycelial growth rates of entomopathogenic fungal species varied with the different media. The highest radial mycelial growth rate was supported by CMA. Higher radial growth rates was supported by CMA, followed by PDA, MEA, YSDA, SDA, and CZA (Table 3.11), however, differences between media were not statistically significant ($df = 5$; $F = 0.753$; $p = 0.585$).

Table 3.11 Overall effect of nutrient media on mycelial growth rates of fungal species

Media	* Growth rate (mm day^{-1} +SE)
SDA	4.04 + 0.45
YSDA	4.82 + 0.42
MEA	4.86 + 0.58
CMA	5.00 + 0.42
CZA	4.01 + 0.69
PDA	4.98 + 0.57

* Mean growth rate for all fungi

Comparisons of radial mycelial growth rates of different fungi on medium indicated that there were significant differences between these fungal isolates on different media: SDA ($df = 5$; $F = 90.43$; $P < 0.0001$), YSDA ($df = 5$; $F = 514.67$; $p < 0.0001$), MEA ($df = 5$; $F = 321.27$; $P < 0.0001$), CMA ($df = 5$; $F = 948.74$; $P < 0.0001$), CZA ($df = 5$; $F = 1863.66$; $p < 0.0001$), and PDA ($df = 5$; $F = 771.28$; $p < 0.0001$) (Table 3.12; Figure 3.1).

Comparisons of radial mycelial growth rates for each fungal isolate on different media indicated significant differences: for *Conidiobolus coronatus* ($df = 5$; $F = 306.44$; $P < 0.0001$), *Entomophaga grylli* ($df = 5$; $F = 250.17$; $P < 0.0001$), *Erynia castrans* ($df = 5$; $F = 9.25$; $P < 0.0001$), *Hirsutella jonesii* ($df = 5$; $F = 41.53$; $P < 0.0001$) *Paecilomyces farinosus* ($df = 5$; $F = 1438.3$; $P < 0.0001$) and *Sporodiniella umbellata* ($df = 5$; $F = 137.89$; $P < 0.0001$) (Table 3.12).

Table 3.12 Effect of nutrient media on the mycelial growth of selected isolates of entomopathogenic fungi

Fungal species	Isolate #	Mean growth rate (mm day $^{-1}$) ± SE					
		SDA	YSDA	MEA	CMA	CZA	PDA
Conidiobolus coronatus	J11a-6.6	5.09±0.20ab	5.23±0.09ab	5.43±0.01a	5.43 ±0.02a	0.71±0.08c	5.00±0.08b
Entomophaga grylli	N4b1-21.6	5.43 ± 0.01b	7.56±0.09a	4.60±0.02d	5.43±0.07b	5.00±0.05c	4.80±0.2cd
Erynia castrans	Q2a-6.4	3.9 ± 0.37c	5.14±0.14ab	4.43±0.07cd	4.86±0.03abc	4.62±0.14bc	5.43±0.01a
Hirsutella jonesii	K3a1-24.7	4.29±0.29b	3.57±0.06cd	3.81±0.06c	5.00±0.07a	2.57±0.03e	3.33±0.02d
Paecilomyces farinosus	N2a-2.8	0.1±0.005d	1.86±0.02a	1.43±0.005c	1.71±0.005b	1.70±0.02b	1.86±0.03a
Sporodiniella umbellata	K13a3-24.7	5.43±0.04c	5.6±0.02c	9.5±0.34a	7.6±0.11b	9.5±0.02a	9.5±0.17a

Means within a row followed by the same letter are not significantly different by Student-Newman-Keuls (SKN) test ($p > 0.05$).

The relative radial mycelial growth rate was significantly affected by fungal isolates ($df = 5$; $F = 53.44$; $p < 0.0001$). The maximum absolute

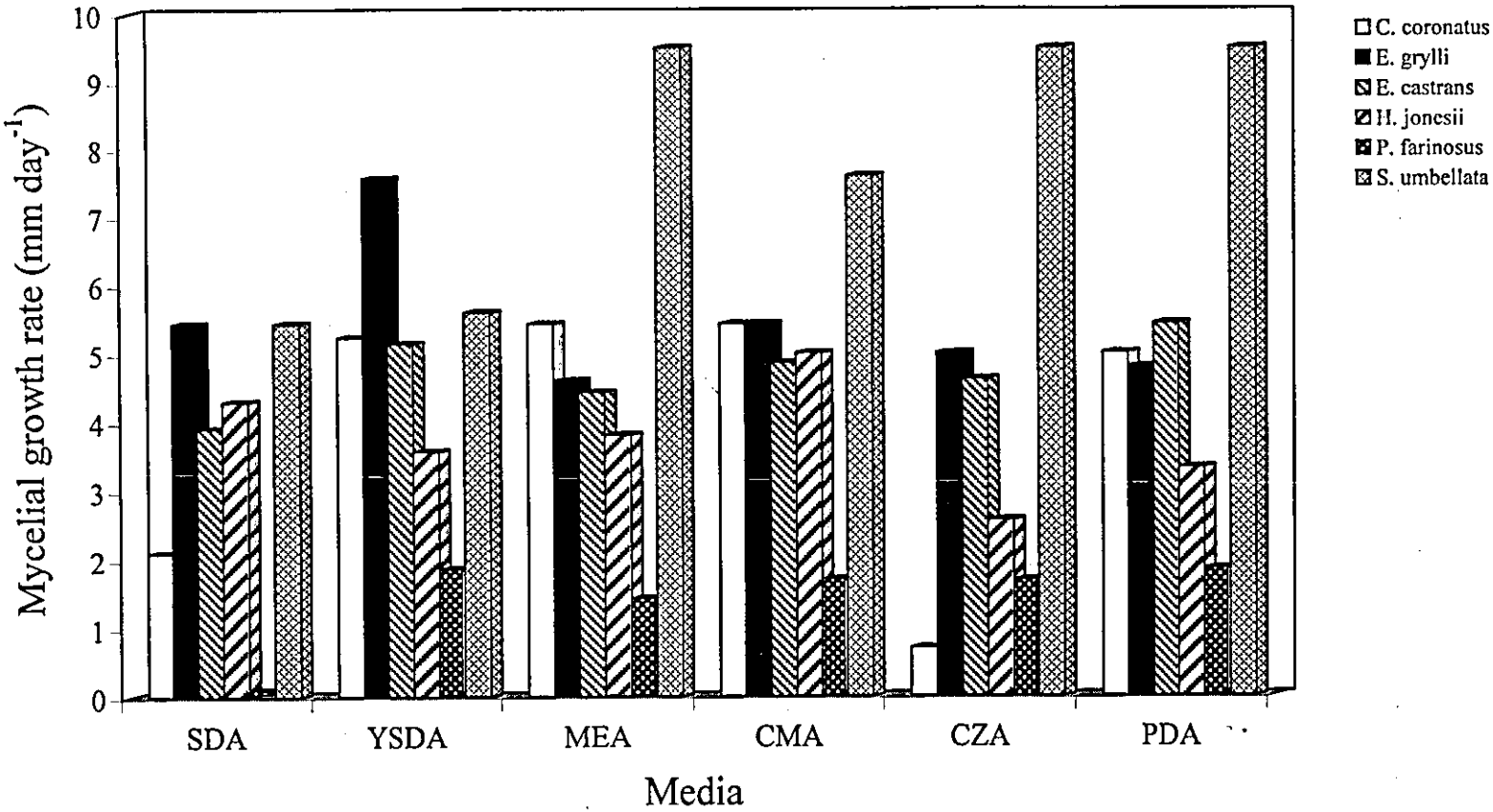

Figure 3.1 Effect of media on the mycelial growth of six selected species of entomopathogenic fungi

growth rates among fungal isolates ranged from 1.44 ± 0.15 for *Paecilomyces farinosus* to 7.85 ± 0.43 mm day^{-1} for *Sporodiniella umbellata* (Table 3.13).

Table 3.13 Effect of fungal species isolates on mycelial growth rates on different media tested

Fungal species	Isolate #	* Growth rate (mm day^{-1}) ± SE
Conidiobolus coronatus	J11a-6.6	4.48 ± 0.41 cd
Entomophaga grylli	N4b1-21.6	5.47 ± 0.24 b
Erynia castrans	Q2a-6.4	4.73 ± 0.13 bc
Hirsutella jonesii	K3a1-24.7	3.76 ± 0.19 d
Paecilomyces farinosus	N2a-2.8	1.44 ± 0.15 e
Sporodiniella umbellata	K13a3-24.7	7.85 ± 0.43 a

Means within a column followed by the same letter are not significantly different by (SKN) test ($p > 0.05$)
* Mean growth rate on all media

3.4.2 Effect of temperature on mycelial growth

Radial mycelial growth occurred at all temperatures tested, but was slower at 10 and 37°C as compared to 25 and 30°C (Table 3.14). Relative mycelial growth rate was significantly affected by temperature ($d f = 5$; $F = 20.64$; $p < 0.001$) and isolate ($d f = 5$; $F = 9.152$; $p < 0.001$). The maximum absolute growth rates among the isolates ranged from 0.73 ± 0.14 mm day^{-1} for *Paecilomyces farinosus* to 5.29 ± 0.63 mm/d for *Sporodiniella umbellata* (Table 3.16).

Table 3.14 Overall effect of temperature on mycelial growth rates of fungal species.

Temperature (C°)	* Growth rate (mm day^{-1}) ± SE
10	0.25 + 0.06 d
15	3.53 ± 0.37 c
20	4.23 ± 0.48 bc
25	5.21 ± 0.56 ab
30	5.97 ± 0.51a
37	3.22 ± 0.43 c

Means within a column followed by the same letter are not significantly different by SNK test ($p > 0.05$).
* Mean growth rate for all fungi.

Comparisons of relative mycelial growth rates at each temperature indicated that there were significant differences between isolates, at 10 °C (d f = 5; F= 20.57; $p < 0.001$), 15 °C (df = 5; F = 148.66; $P < 0.001$), 20 °C (df = 5; F = 115.81; $P < 0.001$), 25 °C (df = 5; F = 323.68; $p < 0.001$), 30 °C (df = 5; F = 296.12; $p < 0.001$)) and at 37 °C (df = 5; F = 265.62; $p < 0.001$) (Table 3.15).

Comparisons of relative mycelial growth rates for each fungal isolate indicated that there were significant differences between temperatures, for *Conidiobolus coronatus* (df = 5; F = 291.98; $p < 0.001$), *Entomophaga grylli* (df = 5 ; F = 187.35; $p < 0.001$), *Erynia castrans* (d f = 5; F= 286.48; $p < 0.001$), *Hirsutella jonesii* (Df= 5; F = 397.90; $P < 0.001$), *Paecilomyces farinosus* (df = 5; F = 249.33; $p < 0.001$) and for *Sporodiniella umbellate* (df = 5 ; F = 330.49 ; $P < 0.001$) (Table 3.15; Figure 3.2).

Table 3-15 Effect of temperature on mycelial growth rates of selected isolates of entomopathogenic fungi

Fungal species	Isolate #	Mean growth rate (mm day^{-1}) ± SE					
		10 °C	15 °C	20 °C	25 °C	30 °C	37 °C
Conidiobolus coronatus	Jlla-6.6	0.01 ± 0.00 e	3.6 ± 0.23d	4.6 ± 0.20 c	6.40 ± 0.05b	7.40 ± 0.00a	3.80 ± 0.20 d
Entomophaga grylli	N4bl-21-6	0.70 ± 0.10e	5.20±0.30b	5.80 ± 0.15c	7.60 ± 0.20 a	4.70±0.20c	1.70 ± 0.05d
Erynia castrans	Q2a-6.4	0.50±0.11d	3.00±0.10d	3.33±0.17cd	3.60±0.10c	7.40±0.05a	4.19±0.19b
Hirsutella jonesii	K3al-24.7	0.01±0.00e	3.60±0.05d	4.00±0.25c	5.00±0.10b	7.00±0.05a	5.30±0.05b
Paecilomyces farinosus	N2a - 2.8	0.15±0.00d	0.60±0.05c	0.70±0.05c	1.10±0.00b	1.80±0.05a	0.06±0.003d
Sporodiniella umbellata	K13a3-24.7	0.15±0.00e	5.20±0.15c	7.00±0.11b	7.60±0.23a	7.53±0.23a	4.3±05d

Means within a row followed by the same letter are not significantly different using SNK test ($p>0.05$)

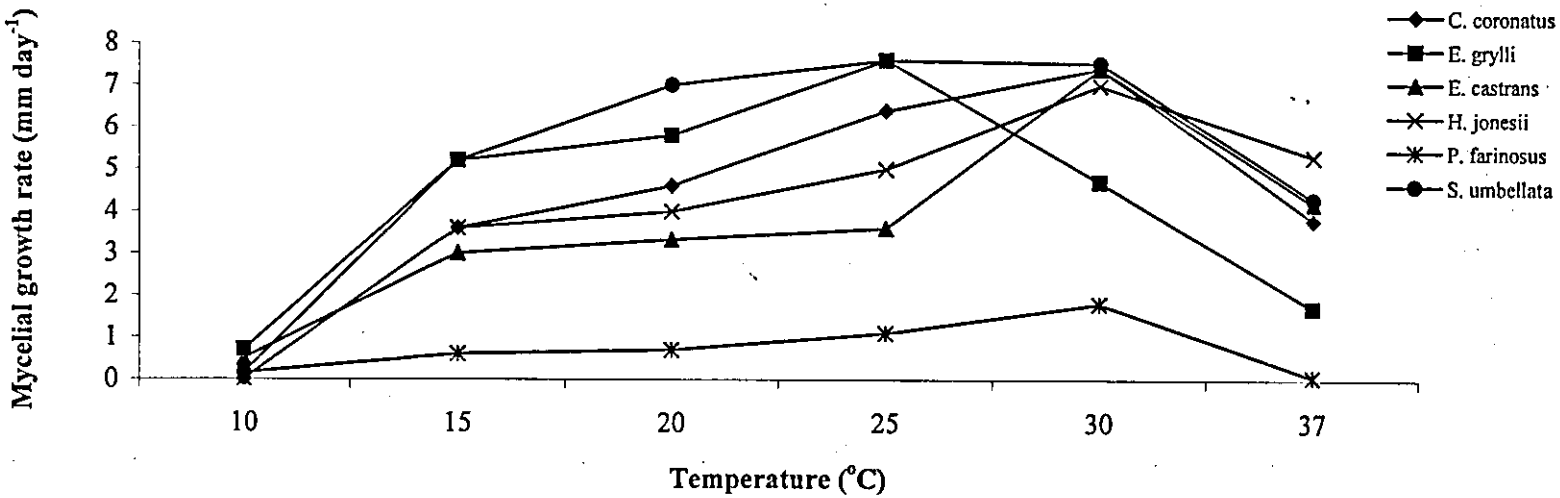

Figure 3.2 Effect of temperature on mycelial growth of selected isolates of entomopathogenic fungi.

Table 3.16 Effect of fungal species on mycelial growth at all temperatures

Fungal species	Isolate #	Mean growth rate±SE
Conidiobolus coronatus	Jlla-6.6	4.30 ±0.57 ab
Entomophaga grylli	N4b1-21.6	4.28±0.57ab
Erynia castrans	Q2a-6.4	3.67±0.49b
Hirsutella jonesii	K3al-24.7	4.15±0.52ab
Paecilomyces farinosus	N2a-2.8	0.73±0.14c
Sporodiniella umbellata	K13a3-24.7	5.29±0.63a

Means within a column followed by the same letter are not significantly different by SNK test at ($p > 0.05$).

3.4.3 Effect of temperature on spore production

Spore production occurred between 10 and 37 °C (Table 3.17). Spore production was significantly affected by temperature ($df=5$; $F= 4.179$; $p = 0.002$) and fungal isolate ($Df=5$; $F = 6.682$; $p < 0.0001$). The maximum absolute spore production rates among isolates ranged from $(0.18\pm0.04) \times 10^6$ spore/plate for *Hirsutella jonesii* to $(6.95\pm0.8) \times 10^6$ spore/plate for *Paecilomyces farinosus* (Table 3.19).

Table 3-17 Overall effect of temperature on spore production of fungal species.

Temperature	* Number of conidia/plate ($X10^6$) ±SE
10	0.025 ± 0.009 b
15	2.995 ± 0.9 b
20	2.174 ± 0.7 b
25	1.52 ± 0.32b
30	6.643 ± 1.2a
37	0.965 ± 0.2b

Means within a column followed by the some letter are not significantly different by SNK test ($p > 0.05$).
* Mean number of conidia for al fungi.

Optimum temperatures for spore production were for *Conidiobolus coronatus* 20°C, for *Entomophaga grylli* 30°C, for *Erynia castrans* 30°C, for

Hirsutella jonesii 25°C, for *Paecilemyces farinosus* 30°C and for *sporodiniella ambellata* 25°C (Table 3.18; Figure 3.3).

Comparisons of spore production rates for each fungal isolate indicated that there were significant differences in relation to temperatures, for *Conidiobolus coronatus* (df=5; F=1378.745; $p < 0.0001$), *Entomophaga grylli* (df = 5; F = 760.182; $p < 0.0001$), *Erynia castrans* (df=5; F = 50.275; $p < 0.0001$), *Hirsutella jonesii* (df = 5; F 55.628; $p < 0.0001$), *Paecilomyces farinosus* (df = 5 ; F = 444.917; $p < 0.0001$), and *Sporodiniella umbellata* (df = 5 ; F = 187.412; $p < 0.0001$) (Table 3.18).

Comparisons of spore production rates at each temperature indicated that there were significant differences between isolates, at 10°C (df= 5; F = 57.239; $p < 0.0001$), 15°C (df = 5; F = 383.796; $p< 0.0001$), 20°C (df = 5; F = 3955.528; $p< 0.0001$), 25°C (df = 5; F = 1187.89; $p< 0.0001$), 30°C (df = 5; F = 498.361; $p< 0.0001$), 37°C (df = 5; F = 11.661; $p< 0.0001$) (Table 3.18).

Table 3-18 Effect of temperature on spore production of selected isolates of entomopathogenic fungi

Fungal species	Isolate #	Mean number of conidia / plate (X 10^6) ± SE					
		10 °C	15 °C	20 °C	25 °C	30 °C	37 °C
Conidiobolus coronatus	J11a-6.6.99	0.000001 ± 0.0f	6.40 ± 0.057b	8.6 ± 0.057a	3.5 ± 0.057e	5.0 ± 0.11c	4.0 ± 0.11 d
Entomophaga grylli	N4bl-21-6	0. 01 ± 0.0d	0.08±0.01d	1.74 ± 0.06c	3.20 ± 0.05b	4.80±0.15a	0.12 ± 0.01d
Erynia castrans	Q2a-6.4	0.04±0.005c	0.18±0.02c	0.48±0.06b	0.12±0.01c	1.43±0.1a	1.20±0.05a
Hirsutella jonesii	K3al-24.7	0.000001±0.0d	0.07±0.01 cd	0.12±0.00c	0.50±0.05a	0.28±0.01ab	0.10±0.005c
Paecilomyces farinosus	N2a - 2.8	0.1±0.01d	11±0.57b	2.0±0.057c	0.55±0.01cd	28±1.15a	0.06±0.01d
Sporodiniella umbellata	K13a3-24.7	0.000001±0e	0.24±0.02c	0.11±0.03d	1.25±0.02a	0.35±0.06b	0.31±0.02bc

Means within a row followed by the same letter are not significantly different by SNK test ($p > 0.05$)

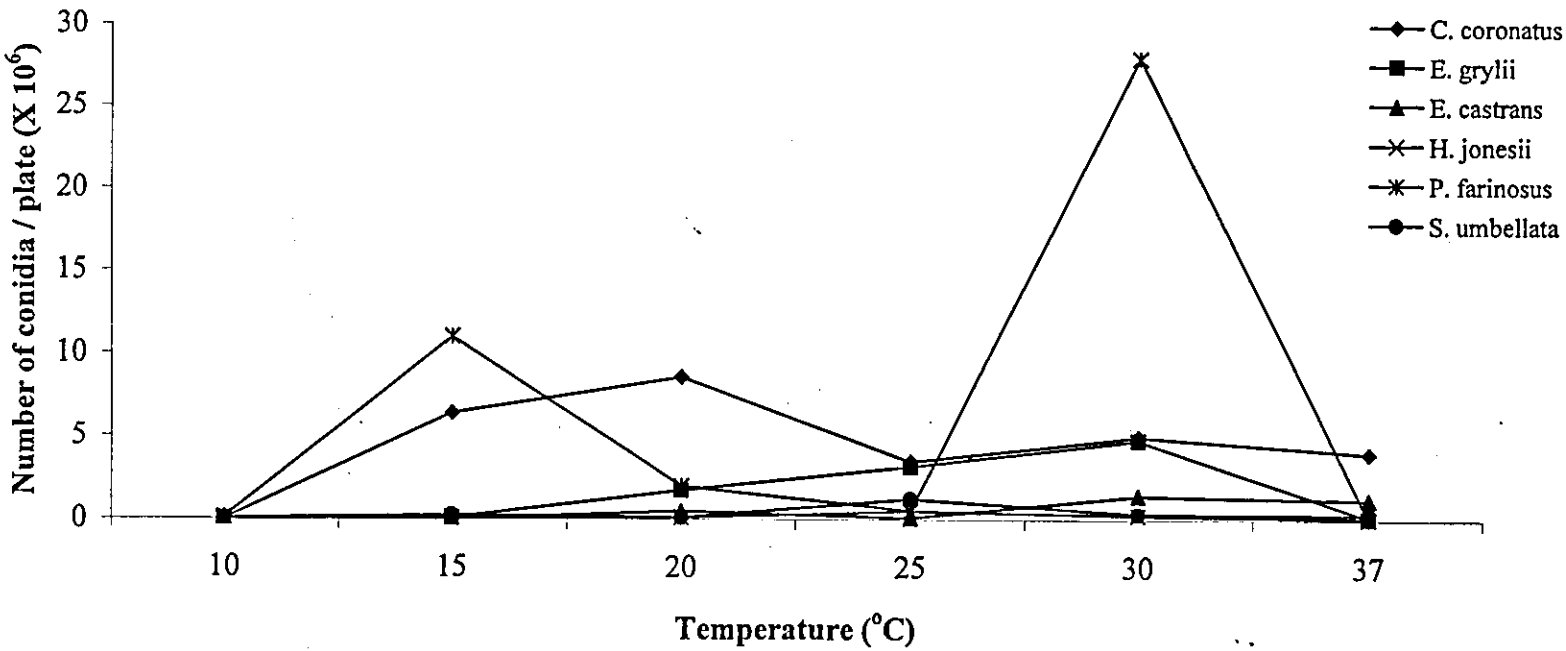

Figure 3.3 Effect of temperature on conidia production of selected isolates of entomopathogenic fungi.

Table 3.19 Effect of fungal species on spore production at all temperatures.

Fungal species	Isolate #	Mean number of conidia / plate (X 10^6) ± SE
Conidiobolus coronatus	Jlla-6.6	4.59 ±0.64 ab
Entomophaga grylli	N4b1-21.6	1.66±0.44bc
Erynia castrans	Q2a-6.4	0.57±0.13c
Hirsutella jonesli	K3al-24.7	0.18±0.04 c
Paecilomyces farinosus	N2a-2.8	6.95±0.8a
Sporodiniella umbellata	K13a3-24.7	0.38±0.09c

Means within a column followed by the same letter are not significantly different by SNK test (p>0.05).

3.4.4 Effect of temperature on spore germination

Spore germination was significantly affected by temperature (df = 5; F = 63.952; p< 0.0001), isolate (df = 5; F = 20.616; p< 0.0001), and time (df = 5; F = 7.701; p< 0.0001), and isolate * temperature (df = 25; F = 11.846; p< 0.0001), time * temperature (df = 10; F = 2.119; p< 0.023), and isolate * time * temperature (df = 50; F = 63.946; p< 0.0001), interactions (Table 3-20).

There was no significant effect with isolate * time interactions (df = 10; F = 0.274; p< 0.986). Temperatures above 10 °C and below 37 °C greatly influenced germination rates with an optimum range of 20-30°C. Prolonging germination for 48-h and 72-h period improved germination rates distinctly under the harsher temperature conditions.

Spore germination of the different isolates was significantly affected by temperature (isolate * temperature interactions) after a 24-h (df = 25; F = 170.57; p < 0.0001), 48-h (df = 25; F = 344.99 ; p < 0.0001), and 72-h (df = 25; F = 105.37 ; p < 0.0001) incubation periods (Figure 3.4).

Table 3.20 Effect of temperature on spore germination of six selected entomopathogenic fungal isolates.

Fungal species	Isolate number	Incubation time (h)	Mean spore germination (percent) ± SD according to temperature (°C)					
			10	15	20	25	30	37
Conidiobolus coronatus	J11a-6.6	24	0.1 ± 0.0e	20 ± 5.29c	4 ± 1.7d	99.66 ± 0.57a	74 ± 6.08b	0.1 ± 0.0e
		48	2 ± 0.1d	26 ± 2c	30 ± 2.6c	99.33 ± 1.15a	100 ± 0.0a	65 ± 3.46b
		72	4 ± 1d	65 ± 2.64b	34.66 ± 5.68c	100 ± 0.0a	99.33 ± 0.57a	95 ± 2.64a
Entomophaga grylli	N4b1-21.6	24	0.1± 0.0c	56± 6b	96 ± 0.0a	99.66 ± 0.5a	99 ± 1a	0.1 ± 0.0c
		48	0.1± 0.0b	97± 1a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		72	5± 1c	100± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	98 ± 2.64b
Erynia castrans	Q2a-6.4	24	0.1± 0.0d	71.33± 12c	88 ± 3.46b	100 ± 0.0a	100 ± 0.0a	97 ± 1.52a
		48	0.1± 0.0b	98 ± 2a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		72	24 ± 4b	97 ± 0.57	97 ± 0.57a	100 ± 0.0a	99.33 ± 1.15a	98 ± 1a
Hirsutella jonesii	K3a1-24.7	24	0.1± 0.0e	6± 2d	15 ± 1.73b	25 ± 4.6a	18 ± 2.6ab	10 ± 2c
		48	0.1± 0.0d	20± 2c	25 ± 2.64bc	40 ± 6ab	48 ± 3.46a	32 ±2b
		72	0.1± 0.0d	36± 3bc	40 ± 2b	47 ± 4.35ab	55 ± 7a	38 ± 3.46b
Paecilomyces farinosus	N2a-2.8	24	0.1± 0.0d	52± 2c	75 ± 4.35b	96 ± 2a	100 ± 0.0a	97 ± 2a
		48	0.1± 0.0b	100± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
		72	0.1± 0.0b	100± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a
Sporodiniella umbellata	K13a3-24.7	24	0.1± 0.0c	84± 8.7b	96 ± 2.0a	100 ± 0.0a	98 ± 0.0a	4 ± 0.0c
		48	6± 2b	100± 0.0a	99.66 ± 0.57a	100 ± 0.0a	100 ± 0.0a	98 ± 0.0a
		72	16± 6b	100± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a	100 ± 0.0a

Means within a row followed by the same letter are not significantly different by SNK test ($p < 0.05$).

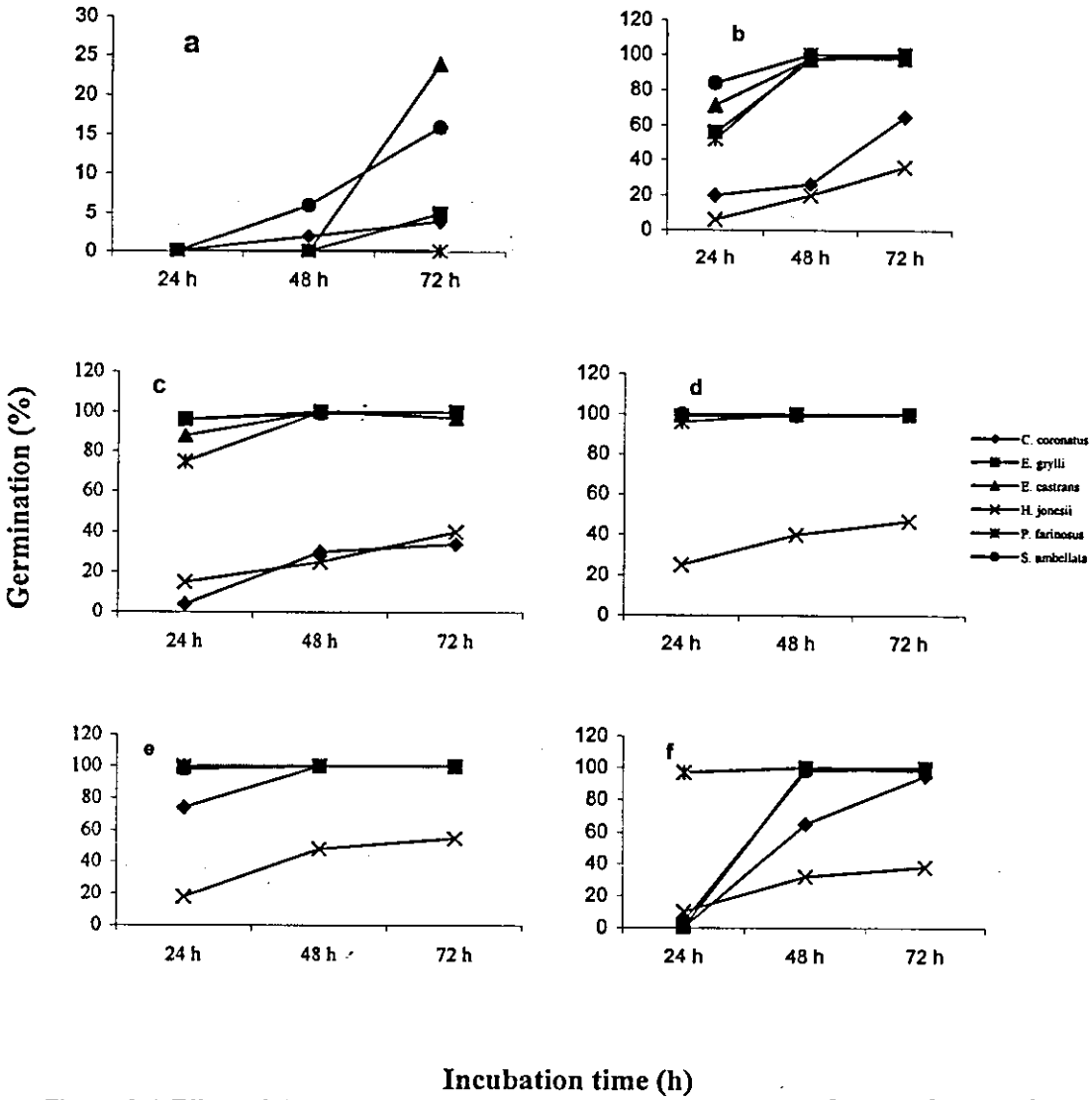

Figure 3.4 Effect of time of incubation on spore germination at a: 10°C, b: 15°C, c: 20°C, 30°C, f: 37°C.

Spore germination of the different isolates differed significantly with isolate * time interactions at 10 °C (df = 10; F = 23.315; p < 0.0001), 15 (df = 10; F = 18.105; p < 0.0001), 20 (df = 10; F = 26.476; p < 0.0001), 25 (df = 10; F = 13.224; p < 0.0001), 30 (df = 10; F = 39.533; p < 0.0001), and 37 °C (df = 10; F = 479.214; p < 0.0001), (Figure 3.5).

Also, spore germination of some isolates differed significantly with time * temperature interactions for *Conidiobolus coronatus* (df = 10; F = 133.32; p < 0.0001), *Entomophaga grylli* (df = 10; F = 220.879; p < 0.0001), *Erynia castrans* (df = 10; F = 18.834; p < 0.0001), *Hirsutella jonesii* (df = 10; F = 13.368; p < 0.0001), *Paecilomyces farinosus* (df = 10; F = 209.379; p < 0.0001), and *Sporodiniella umbellata* (df = 10; F = 183.852; p < 0.0001), (Figure 3.5).

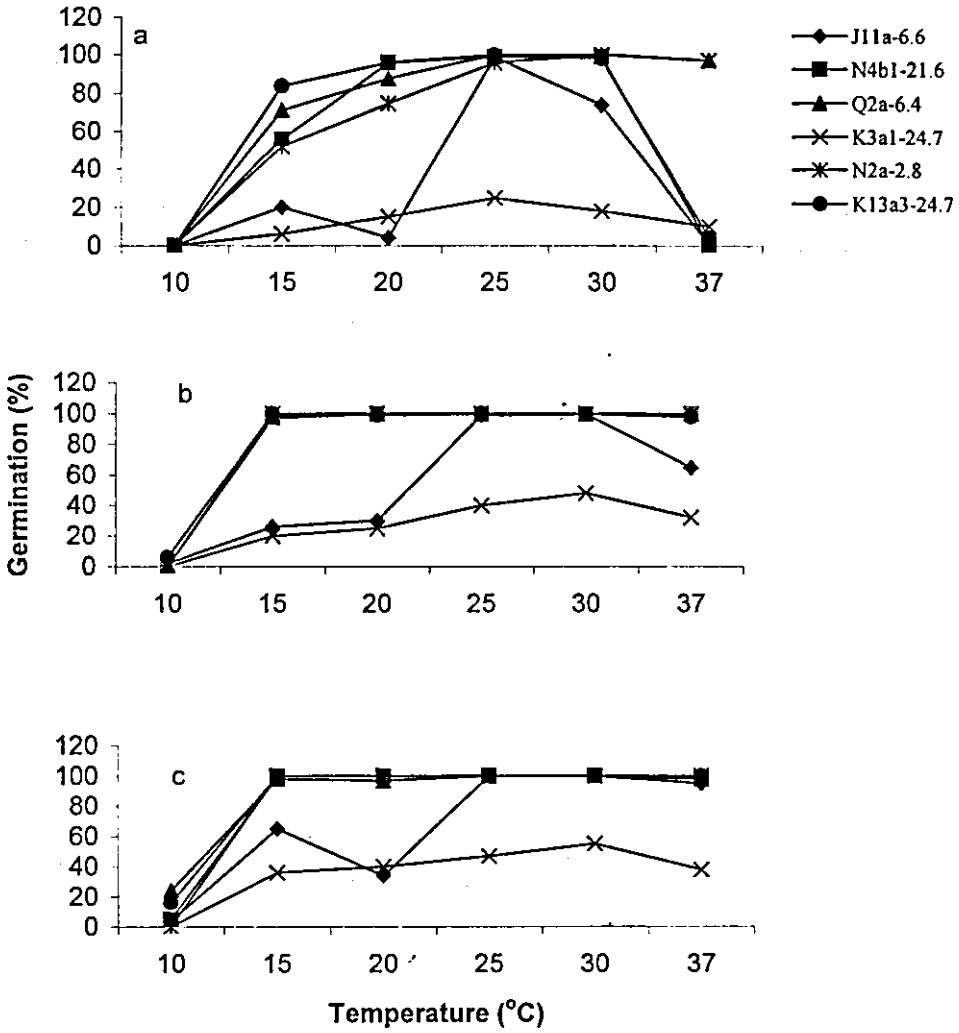

Figure 3.5 Effect of temperature on spore germination of selected isolates of entomopathogenic fungi after a 24 h, b 48 h, and c 72 h.

CHAPTER FOUR

DISCUSSION

CHAPTER FOUR
DISCUSSION

Zimmerman (1986) and Chandler *et al.* (1997) recommended the use of the *Galleria* bait method for the detection of entomopathogenic fungi in soil. In this study, the technique was simple to operate and allowed a large number of samples to be handled concurrently.

In this study, entomopathogenic fungi were found to occur in 33.57% of the soil samples studied. No previous detailed studies have been conducted on the occurrence of soil dwelling entomopathogenic fungi in the Palestinian area. However, Mahassneh, (1999), studied the occurrence of these fungi in only a few (76) soil samples taken randomly from Hebron (25), Bethlehem (32), Nablus (30), and Gaza strip (16). Seventeen fungal isolates belonging to 7 species in 6 genera were recovered and characterized by Mahassneh, (1999). Universally, several studies on the occurrence of entomopathogenic fungi in soil have been conducted. Chandler *et al.* (1997) isolated entomopathogenic fungi from 17.5% of samples of soil taken from different habitats in Warwickshire, UK. Rath *et al.* (1992) obtained isolates of *Metarhizium anisopliae* from 31% of samples of soil taken from pastures across Tasmania. Vanninen *et al.* (1989) isolated entomopathogenic fungi from 32% of soil samples collected from different cultivated habitats across Finland.

The rarity of a species in a given habitat does not necessarily mean a reduction in the number of infective propagules at the sites where the species does occur. The proportion of infected larvae alone may not, however, be the best way to measure the abundance of infective propagules in soil samples; the time required to kill the baits should also be taken into account. Bait larvae move differently in soils of different structure and particle size and therefore may not be infected equally quickly in all soil types (Vanninen, 1995).

The biodiversity of entomopathogenic fungi in field soils is evident. A total of 20 species of entomopathogenic fungi were recovered in our study. Mahassneh, (1999) recorded a different pattern of species abundance for entomopathogenic fungi taken from soils of defined locations in the southern part of the West Bank. In total, seven species of entomopathogenic fungi were recovered. In contrast, a much greater diversity of entomopathogenic fungi (~ 750 species) has been recorded from tropical habitats, particularly tropical rainforest (Evans, 1982; Gunde-Cimerman *et al*, 1998).

In this study, two species of *Aspergillus, A. niger* and *A. ochraceus* have been recovered as entomopathogenic fungi. *Aspergillus* species have been found associated with a large number of insects such as *Vespula vulgaris, (A. niger) Anopheles stephensi, (A. niger), Troglophilus neglectus (A. niger, A. ochraceus),* queen larvae of bees *(A. niger)* and

Culex pipiens (*A. ochraceus*) (Sur *et al.*, 1999; Gunde-Cimerman *et al.*, 1998; Domsch *et al.*, 1980).

Conidiobolus coronatus was the most frequently isolated species in field soil comprising 31.43% of total isolates recovered. It is probably the best known member of the genus *Conidiobolus*. This fungus is common in soil and can easily be grown in culture. It has also been found to be pathogenic to the mosquitoes *Culex pipiens, quinquefasciatus* and *Aedes taeniorhynchus,* the Guadeloupean parasol-ant *Acromyrmex octospinosus,* the root maggots *Phorbia brassicae* and *Ph. platura* (Domsch *et al.*, 1980), spittlebugs (*Aeneolomiai albo-fasciata* and *Prosapia simulans*) (Samson *et al.*, 1988) and *Metopolophium dirihodum* (Ben-Ze'ev, 1993).

Conidiobolus coronatus is characterized by the rapid killing of its hosts and high toxin activity (Samson *et al.*, 1988). A formulation of *C. coronatus* was used as a biological control agent against pasture spittlebugs in Mexico (Samson *et al.*, 1988).

Excluding the isolates of *Conidiobolus*, the genus *Fusarium* had a high incidence of isolation (24%). Species in the genus *Fusarium* display a great diversity of life strategies including a variety of types of associations with insects. *Fusarium* species can act as weak to virulent entomopathogens. They live as saprophytes on insect cadavers or as nonpathogenic insect associates (Hajek *et al.*, 1993). Many *Fusarium* species may kill insect hosts through the activity of fungal-produced

toxins (Cupta *et al.*, 1991). Beauvericin produced by *Fusarium* spp (e.g. *F. semitectum, F. moniliforme* var. *subglutinans*) was found to be toxic to the Colorado potato beetle (Cupta *et al.*, 1991), the blowfly, *Calliphora erythrocephala* and mosquito, *Aedesu egypti* (Hegedus & Khachatourians, 1995). *Fusarium heterosporum* has been reported from long-horned beetle larvae (Cerambycidae) (Claydon & Grove, 1984); *F. moniliforme* var. *subglutinans* from *Pyrausta nubialis* larvae (Claydon & Grove, 1984); *F. nival* from *Tenebrio molitor* larvae (Claydon & Grove, 1984); *F. oxysporum* and *F. semitectum* from *Aedes cantans* and *Anopheles stephensi* larvae (Sur *et al.*, 1999); and *F. solani* from Bark beetles (Claydon & Grove, 1984). Both *F. solani* and *F. semitectum* have also been recorded by Mahassneh (1999) as soil entomopathogenic fungi from Hebron, Gaza strip and Bethlehem.

In this study *Entomophaga grylli* was isolated once from the soil samples studied. *Entomophaga grylli* is considered to be a significant natural control agent of locusts in Africa and North America and this has been confirmed by monitoring population changes of locusts *(Oxyahyla)* in rice fields in Asia (Samson *et al.*, 1988). *E. grylli* has been found associated with large varieties of insects such as *Troglophilus neglectus* (Gunde-Cimerman *et al.* 1998) and *Cammula pellacida* (Hajek & St. Leger, 1994). Annual epizootics of *E. grylli* in populations of grasshopper (*C. pellacida*) are dependent on high host density and humidity, they do

appear to play a primary role in regulating grasshopper populations (Samson *et al.*, 1988).

Erynia castrans was isolated once from the soil samples studied. *E. castrans* has been reported from *Hylemya* flies (Anthomyiidae), serious pests of vegetables, in the USA and Europe (Samson *et al.*, 1988). *E. castrans* is a highly adapted parasite which produces its spores internally and disseminates them through a hole in the abdomen of the living insect (Samson *et al.*, 1988).

Gliocldium viride was isolated twice from the studied field soils. *Gliocladium* species have been reported from *Aedes cantans* and *Anopheles stephensi* larvae (Sur *et al.*, 1999). Mahassneh (1999) has also recorded *Gliocladium roseum* (Bain) as soil entomopathogenic fungi in Palestine from Nablus area.

Hirsutella jonesii was isolated once from the studied field soils. *H. jonesii* has been reported from leafhopper *(Nephotettix* species) (Samson *et al.*, 1988). In Florida, *Hirsutella* species have been used against the citrus rust mite (Atlas, 1988). In Cuba and Mexico, whiteflies and mites have been naturally controlled by *Hirsutella* species (Samson *et al.*, 1988).

Mucor species were isolated with high frequencies from studied field soils. They comprised 11.43% of the total isolates recovered. These species are known for their pathogenic and biotoxic activity on humans,

cattle, pigs, poultry and insects such as *Lepidoptera*, *Coleoptera* and *Diptera* species (Reiss, 1993). An essential saprophytic or weakly pathogenic fungus may at times becomes associated as the causal agent of epizootics in predisposed insects. Fast growing saprophytes with toxigenic properties like *Mucor* species may cause infections of injured or by mounting weakened insects when they are normally not pathogenic to healthy one (Gunde-Cimerman *et al.*, 1998).

Paecilomyces farinosus was isolated once from studied field soils. The most important *Paecilomyces* species include *P. farinosus* and *P. fumosoroseus,* which have been isolated from numerous insect hosts worldwide. *P. farinosus* is a common fungal pathogen of codling moth sometimes causing high mortality especially in mixtures with *Beauveria bassiana* (Cross *et al.* 1999). *P. farinosus* has been reported from spruce budworm *(Choristoneura fumiferana)* in Canadian forests (Samson *et al.*, 1988) and from *Culex pepiens* from the USSR (Sur *et al.*, 1999). It is usually found on over wintering larvae (Cross *et al.*, 1999). Jaworska (1992) found that isolates of *P. farinosus* and *P. fumosoroseus* gave 100% mortality of apple sawfly larvae in the laboratory. As with other entomopathogenic fungi, high humidities and temperatures are required for infection (Cross *et al.*, 1999).

Nectria ventricosa was recovered as entomopathogenic fungal species comprising 5.71% of the total isolates. It is occasionally isolated

from soil. *Nectria* species have been reported from Diaspid scale *(Ischnaspsis* species) (Samson *et al.*, 1988).

Sporodiniella umbellata was isolated only from orchards of Tulkarm area and comprised 7.145% of the total isolates. It has been reported from treehoppers (Membracidae; *Umbonia* species) (Samson *et al.*, 1988).

Verticillium nigrescens was isolated once in the present study. *Verticillium psalliotae* has also been isolated once by Mahassneh (1999) from Nablus area. *Verticillium* species have been isolated from adult cadavers of *T. neglectus*. (Cunde - Cimerman *et al.*, 1998), *Aphis gossypii, Myzus persicae* and *Rhopalosiphum padi* (Ben-Ze'ev, 1993).

Preliminary testing for pathogenicity showed that this character varied with fungal isolates. This could be related to the disparate modes of pathogenesis, conidia dosage, toxins and germination behavior (Vestergaard *et al.*, 1995; De La Rosa *et al.*, 1997).

It is not surprising that pH has little effect on the distribution and abundance of entomopathogenic fungi, as the fungi are able to germinate over a wide pH range. Vanninen *et al.* (1989) and Rath *et al.* (1992) also observed that the occurrence of entomopathogenic fungi was not related to soil pH.

Higher frequency of isolation of entomopathogenic fungi seemed to be associated with higher soil organic matter. This finding is in

agreement with that of Milner (1989) who also found increased soil organic matter to aid infection with entomopathogenic fungi. Mietkiewski *et al.* (1997) also observed that the isolation of *Beauveria bassiana* from arable soils in the UK was normally associated with natural, undisturbed habitats which were high in organic matter.

In our study the isolation of entomopathogenic fungi was slightly affected by soil moisture with no significant differences between soils with different moisture content. High humidity or free water is generally required by entomopathogenic fungi for germination (Samson *et al.*, 1988). In the soil, water is probably not limiting, as many fungi can still utilize the available soil moisture at water potentials far lower (-20 MPa) than permanent wilting point (-1.5 MPa) (Yanagita, 1980). Further insects surviving in soil probably give fungi the high humidity required for germination through their respiration and the closeness of their contact with the soil. Rath et al. (1992) however, observed that the occurrence of entomopathogenic fungi was not related to soil moisture.

The isolation of entomopathogenic fungi was slightly influenced by geographical location. This may be related to variations in climatic conditions, cultivation processes and sampling times.

Entomopathogenic fungi were more frequently isolated from soils under fruit trees than from soils under vegetables. Tentatively, therefore, the frequency of occurrence of entomopathogenic fungi would appear to

be related to the permanence of the habitat, possibly because permanent habitats support larger, more diverse and stable communities of insects. Ploughing of cultivated soils may also prevent the build-up of high populations of insect pathogens by disrupting infection foci, exposing pathogens to adverse environmental conditions on the surface of soil or by burying them away from potential hosts (Chandler *et al.*, 1997). Vanninen *et al.*, (1989) also showed the frequency of entomopathogenic fungi in intensively cultivated soils is lower than that in forest soils in Finland. Similar results were obtained by Mietkiewski *et al.* (1991) in Poland and by Chandler *et al.* (1997) in UK.

The current results demonstrated that, under laboratory conditions, the optimal radial mycelial growth, conidial production and conidial germination of entomopathogenic fungi were dependent on culture media and temperature. Radial mycelial growth was best on CMA for *Conidiobolus coronatus* and *Hirsutella jonesii;* on YSDA for *Entomophaga grylli*; on PDA and YSDA for *Paecilomyces farinosus*; on PDA for *Erynia castrans* and on CZA and MEA for *Sporodiniella umbellata.* Radial mycelial growth for all selected fungal isolates therefore appeared to be best on CMA and PDA. These findings were consistent with those of Mahassneh (1999), who also found that radial mycelial growth of entomopathogenic fungi was best on CMA, CZA and PDA. The variations in mycelial growth rate of fungal isolates on

different media could be related to their different nutritional requirements.

The present work has shown that the optimum temperature of mycelial growth, conidial production and conidial germination of the selected fungal isolates tested, range between 20-30°C.

This finding is in concordance with that obtained by many other entomopathogenic fungi workers (e.g., Hall & Papierok, 1982; Hajek *et al.*, 1990; Fargues *et al.*, 1997; Ouedrago *et al.*, 1997; Ekesi *et al.*, 1999; Sztejnberg *et al.*, 1997).

The optimal temperatures for growth for the selected fungal isolates we studied were as follows: for *Conidiobolus coronatus* 30°C, *Entomophaga grylli* 25°C, *Erynia castrans* 30°C, *Hirsutella jonesii* 30°C, *Paecilomyces farinosus* 30°C, and *Sporodiniella umbellata* 25°C. The optimal temperature for all selected fungal isolates studied therefore appeared to lie between 25-30°C. These results are in agreement with those obtained by Ekesi *et al.* (1999) and Mahassneh (1999), who also found that optimum temperature for radial mycelial growth of *Metarhizium anisopliae, Beauveria bassiana* and other entomopathogenic fungal species ranged between 25-30°C.

Temperature may have affected sporulation directly by influencing conidial production or indirectly by influencing colony development which later provided a large colony area for the further production of

conidia. The optimal temperatures for sporulation of the selected fungal isolates we studied were as follows; for *C. coronatus* 20°C, *E. grylli* 30°C, *E. castrans* 30°C, *H. jonesii* 25°C, *P. farinosus* 30°C and *S. umbellata* 25°C. The optimal temperature for all selected fungal isolates studied therefore appeared to lie between 20-30°C which was consistent with radial mycelial growth studies carried out by Mahassneh (1999).

The present work demonstrates differences among the selected fungal isolates with relation to the effect of temperature on their conidial germination. The optimal temperatures for conidial germination for the selected fungal isolates appear to range between 25°C (*C. coronatus, E. grylli, E. castrans, S. umbellata*) and 30°C (*H. jonesii, P. farinosus*). This finding is consistent with studies carried out by Hajek *et al.* (1990), Mahassneh (1999), Ekesi *et al.*, (1999) and Fernando *et al.* (2000), who also found that optimum temperature for conidial germination of *Entomophaga maimiga, Beauveria bassiana* and *Metarhizium anisopliae* ranged between 25-30°C.

The fungi described in the present study are probably very small of potential species. This study has shown that the isolation rate of entomopathogenic fungi is little affected by differences in soil environment. This study also indicates that biological control of soil-dwelling pests by resident entomopathogenic fungi is likely to be more effective on perennial crops (orchards) than annual crops (vegetables).

Entomopathogenic fungi are common inhabitants of the soil biota in Palestine, but the diversity of species is low, with few species occurring frequently.

The effective exploitation of entomopathogenic fungi for the biological control of pests in agricultural fields has been limited by the humid warm condition required for infection. Such conditions occur sporadically and only transiently on the surfaces of leaves of trees and vegetables. The isolation of entomopathogenic fungi, provides an opportunity to develop them as biocontrol agents for agricultural pests in the future.

The results obtained in the present study also indicate that temperature differences of several degrees at the upper thermal threshold can have a considerable effect on the efficacy of the fungus and the extent of this effect would vary according to the isolate used (Table 3.14; 3.17; 3.19). Consequently, thermal tolerance must be considered when selecting an isolate for development as a control agent of thermoregulating insects. Moreover, since acridids primarily elevate their body temperatures only during periods of sunlight, lower thermal limits must also be taken into consideration (Inglis *et al.*, 1996 b).

Laboratory studies which simulate varying field conditions can provide quantitative data that may be useful to predict field results (Samson *et al.*, 1988). The optimum temperature of between 25-30 °C for

sporulation, germination and growth of the entomopathogenic fungi compared fovourably with the optimum temperature of 27-29 °C for high thrips numbers under field conditions (Alghali, 1991). This implies that the insects would be susceptible to infection by the pathogens at temperatures at which entomopathogenic fungal isolates are active in the field. We conclude that entomopathogenic fungal isolates with broad temperature range of activity have good potential for management in the field.

In the light of our results, *C. coronatus* could be the species suited for inculative use in pest control in agricultural soils, as it has wide tolerance to agricultural practices, it has been frequently isolated from both vegetable and orchard fields, and also it is characterized by higher mycelial growth rate, conidial production and conidial germination. *S. umbellata* may also be a good candidate for use in orchard soils as biological control agent (it has been isolated from orchard soils in Tulkarm area), but its ecology in the other areas needs to be studied more closely before any specific recommendations for its use can be given.

Future research on this subject may include the following:

- Studying the biodiversity, distribution, and seasonal variation of entomopathogenic fungi in the rest of Palestinian area including uncultivated areas (forests, pastures) and the effect of soil type,

conductivity, temperature and rainfall on the occurrence of entomopathogenic fungi;

- Collection of cadavers of insects for studying natural hosts of entomopathogenic fungal species;
- Studying the behavior of lethally infected insects;
- Application of the most common entomopathogenic fungal isolates as a biological control agents in the field; and
- Developing new strategies for producing entomopathogenic fungi as commercial products and for long term storage or product shelf life of spore powder.

REFERENCES

REFERENCES

Alghali, A. M. (1991). The effects of some argometeorological factors on the fluctuation of flower thrips *Megalurothrips sjostedti* Trybom (Thysanoptera: Thripidae), on two cowpea varieties. *Journal of Plant Protection in the Tropics, 8*, 63-68.

Ali-Shtayeh, M. S., Jamous, R. M., & Yaghmour, R. M-R. (1998). *Mycology manual*. Nablus: Authors.

Andes, M. (1994). The biopath cockroach control chamber uses nature to control nature's pests. *Pest Control, 62*, 44-48.

Atlas, R. M. (1988). *Microbiology: fundamentals and applications* (2nd ed.) New York: Macmillan Publishing Company.

Ben-Ze'ev, I., Kenneth, R. G., Bitton, S. & Soper, R. S. (1984). The Entomophthorales of Israel and their arthropod hosts: seasonal occurrence. *Phytoparasitica, 12*, 167-176.

Ben-Ze'ev, I. S. (1993). Check-list of fungi pathogenic to insects and mites in Israel, Updated through 1992. *Phytoparasitica, 21* (3), 213-237.

Booth, C. (1977). *Fusarium laboratory guides to the identification of the major species*. England: Commonwealth Mycological Institute, Key, Survey.

Boucias, D. G., & Pendland, J. C. (1984). Nutritional requirement for conidial germination of several host range pathotypes of the entomopathogenic fungus *Nomuraea rileyi. Journal of Invertebrate Pathology, 43*, 288-292.

Boucias, D. G., Pendland, J. C., & Latge, J. P. (1988). Nonspecific factors involved in attachment of entomopathogenic Deuteromycetes to host insect cuticle. *Applied and Environmental Microbiology, 54* (7), 1795-1805.

Butt, T. M., Harris, J. G., & Powell, K. A. (1998). Microbial biopesticides: The European Scene. In F. R. Hall & J. Menn (Eds.) *Methods in biotechnology, Biopesticides: Use and Delivery* (Vol. 5-Chaptr 3). Totowa, N. J: Humana Press.

Carruthers, R. I., Feng, Z., & Roberts, D. W. (1985). In vivo temperature-dependant development of *Beauveria bassiana* (Deuteromycotina: Hyphomycetes) mycosis of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *Journal of Invertebrate Pathology, 46*, 305-311.

Caudwell, R. W., & Catehouse, A. G. (1996). Laboratory and field trails of bait formulations of the fungal pathogen, *Metarhizium flavoviride,* against a tropical grasshopper locust. *Biocontrol Science and Technology, 6*, 561-567.

Cerenius, L., & Soderhall, K. (1984). Chemotaxis in *Aphanomyces astaci*, and arthropod-parasitic fungus. *Journal of Invertebrate Pathology, 43*, 278-281.

Chandler, D. (1997). Selection of an isolate of the insect pathogenic fungus *Metarhizium anisoplia* virulent to the lettuce root aphid, *Pemphigus bursarius. Biocontrol Science and Technology, 7*, 95-104.

Chandler, D., Hay, D., & Reid, A. P. (1997). Sampling and occurrence of entomopathogenic fungi and nematodes in UK soils. *Applied Soil Ecology, 5*, 133-144.

Clarkson, J. M., & Charnley, A. K. (1996). New insights into the mechanisms of fungal pathogenesis in insects. *Trends in Microbiology, 4* (5), 197-203.

Claydon, N., & Grove, F. (1984). *Fusarium* as an insect pathogen. In M.O. Moss & E. Smith (Eds.) *The applied mycology of Fusarium* (pp. 115-123). New York: Cambridge University Press.

Couch, T. L., & Ignoffo, C. M. (1981). Formulation of insect pathogens. In H. D. Burges (Ed.) *Microbial control of pests and plant diseases 1970-1980* (pp. 621-634). London & New York: Academic Press.

Cross, J. V., Solomon, M. G., Chandler, D., Jarrett, P., Richardson, P. N., Winstanley, D., Bathon, H., Huber, J., Keller, B., Langenbruch, G. A., & Zimmermann, G. (1999). Biocontrol of pests of apples and pears in northern and central Europe: 1. Microbial agents and nematodes. *Biocontrol Science and Technology, 9,* 125-149.

Cupta, S., Krasnoff, S. B., Underwood, N. L., Renwick, J. A. A., & Roberts, D. W. (1991). Isolation of beauverecin as an insect toxin from *Fusarium semitectum* and *Fusarium moniliforme* var. *subglutinans. Mycopathologia, 115,* 185-189.

De La Rosa, W., Alatorre, R., Trujillo, J., & Barrera, F. (1997). Virulence of *Beauveria bassiana* (Deuteromycetes) strains against the coffee berry borer (Coleoptera; Scolytidae). *Economic Entomology, 90* (6), 1534-1538.

Descals, E., & Webster, J. (1984). Branched aquatic conidia in *Erynia* and *Entomphthora sensulato. Trans. Br. Mycol. Soc., 83*, 669-682.

Domsch, K. H., Gams, W., & Anderson, T. (1980). *Compendium of soil fungi* London: Academic Press.

Eilenberg, J. (1987). Abnormal egg-laying behaviour of femal carrot flies (Psilarosae) induced by the fungus *Entomophthora muscae. Entoml. Exp. Appli, 43*, 61-65.

Eilenberg, J., Bresciani, J. P., & Latge, P. (1986). Ultrastructural studies of primary spore formation and discharge in the genus *Entomophthora. Journal of Invertebrate pathology, 48*, 318-324.

Ekesi, S., Maniania, N. K., & Ampong-Nyarko, K. (1999). Effect of temperature on germination, radial growth and virulence of *Metarhizium anisopliae* and *Beauveria bassiana* on *Megalurothrips sjostedti. Biocontrol Science and Technology, 9*, 177-185.

Evans, H. C. (1982). Entomogenous fungi in tropical forest ecosystems: an appraisal. *Ecological Entomology, 7*, 47-60.

Fargues, J. (1984). Adhesion of the fungal spore to the insect cuticle in relation of pathogenecity. In D. W. Roberts & J. R. Aist (Eds.) *Infection processes of fungi* (pp. 90-110). Italy: Rockefeller Foundation Study Center.

Fargues, J., Ouedraogo, A., Goettel, M. S., & Lomer, C. J. (1997). Effect of temperature, humidity and inoculation method on susceptibility of *Schistocerca gregaria* to *Metarhizium flavoviride. Biocontrol Science and Technology, 7*, 345-356.

Fargues, J., Goettel, M. S., Smits, N., Quedaogo, A., & Rougier, M. (1997). Effect of temperature on vegetative growth of *Beauveria bassiana* isolates from different origins. *Mycologia, 89* (3), 383-392.

Fernando, T. H. P. S., Jayasinghe, C. K., & Wijesundera, R. L .C. (2000). Factors affecting spore production, germination and viability of *Colletotrichum acutatum* isolates from *Hevea brasiliensis. Mycological Research, 104* (6), 681-685.

Ferron, P. (1981). Pest control by the fungi *Beauveria* and *Metarhiziam.* In H. D. Burges (ed.) *Microbial control of pests and plant diseases 1970-1980* (pp. 465-482). London & New York: Academic Press.

Gillespie, A. T., & Claydon, N. (1989). The use of entomogenous fungi for pest control and the role of toxins in pathogenesis. *Pesticide Science, 27*, 203-215.

Gillespie, A. T., & Moorhouse, E. R. (1989). The use of fungi to control pests of agricultural and horticultural importance. In J. M. Whipps & R. D.

Lusmsden (Eds.). *Biotechnology of fungi for improving plant growth* (pp. 85-126). Cambridge, UK: Cambridge University Press.

Grunde-Cimerman, N., Zalar, P., & Jerm, S. (1998). Mycoflora of cave cricket *Troglophilus neglectus* cadavers. *Mycopathologia, 144,* 111-114.

Griesch, J., & Vilcinskas, A. (1998). Proteases released by entomopathogenic fungi impair phagocytic activity, attachment and spreading of plasmatocytic isolated from haemolymph of greater wax moth *Galleria mellonella. Biocontrol Science and Technology, 8,* 517-531.

Hajek, A. E., & St. Leger, R. J. (1994). Ineractions between fungal pathogens and insect hosts. *Annu. Rev. Entomol., 39,* 293-322.

Hajek, A. E., Carruthers, R. I., & Soper, R. S. (1990). Temperature and moisture relation of sporulation and germination by *Entomophaga maimaiga* (Zygomycetes: Entomophthoraceae), a fungal pathogen of *Lymantria dispar* (Lepidoptera: Lymontriidae). *Environmental Entomology, 19* (1), 85-90.

Hajek, A. E., Nelson, P. E., Humber, A. A., & Perry, J. L. (1993). Two *Fusarium* species pathogenic to gypsy moth, *Lymontria dispar. Mycologia, 85* (6), 937-940.

Hall, R. A., & Papeirok, B. (1982). Fungi as biological control agents of arthropods of agricultural and medical importance. *Parasitology, 84,* 205-240.

Hall, R. A. (1981). The fungus *Verticillium lecanii* as a microbial insecticide against aphids and scales. In H. D. Burges (ed.) *Microbial control*

of pest and plant diseases 1970-1980, (pp. 483-498). London & New York: Academic Press.

Hedgecock, S., Moore, D., Higgins, P. M., & Prior, C. (1995). Influence of moisture content on temperature tolerance and storage of *Metarhizium flavoviride* conidia in an oil formulation. *Biocontrol Science and Technology, 5*, 371-377.

Hegedus, D. D., & Khachatourians, G. G. (1995). The impact of biotechnology on Hyphomycetous fungal insect biocontrol agents. *Biotechnology Advances, 13*, (3), 455-490.

Ibrahim, L., Butt, T. M., Beckett, A., & Clark, S. J. (1999). The germination of oil formulated conidia of the insect pathogen *Metarhizium anisopliae. Mycological Research, 103* (7), 901-907.

Inglis, G. D., Johnson, D. L., & Goettel, M. S. (1996a). Effect of bait substrate and formulation on infection of grasshopper nymphs by *Beauveria bassiana. Biocentrol Science & Technology, 6*, 35-50.

Inglis, G. D., Johnson, D. L., & Goettel, M. S. (1996b). Effects of temperature and thermoregulation on mycosis by *Beauveria bassiana* in grasshoppers. *Biological Control, 7,* 131-139.

Jaworska, M. (1992). Biological control of *Hoplocampa testudinea* Klug. (Hymenoptera: Tenthredinidae). *Acta Phytopathologica et Entomologica Hungarica, 27,* 311-315.

Lacey, L. A., & Mercadier, G. (1998). The effect of selected allelochemicals on germination of conidia and blastospores and mycelial growth of the entomopathogenic fungus, *Paecilomyces fumosoreseus* (Deuteromycotina: Hyphomycetes). *Mycopathologia, 142*, 17-25.

Livolant, F., Giraud, M. M., & Bouligand, Y. (1978). A goniometric effect observed in sections of twisted fibrous material. *Biol. Cell., 31*, 159-168.

Mahassneh, M. I. (1999). *Characterization of entomopathogenic fungal isolates recovered from soil of the Palestinian area.* MSc. thesis, An-Najah National University, Nablus, Palestine.

Mietkiewski, R. T., Pell, J. K., & Clark, S. J. (1997). Influence of pesticide use on the natural occurrence of entomopathogenic fungi in arable soils in the UK: field and laboratory comparisons. *Biocontrol Science and Technology, 7*, 565-575.

Mietkiewski, R. T., Zurek, M., Tkaczuk, C., & Balazy, S. (1991). Occurrence of entomopathogenic fungi in arable soil, forest soil and litter. *Roczniki Nauk Rolniczych*, Seria E, *21*, 61-68.

Milner, R. J. (1989). Ecological Considerations in the use of *Metarhizium* for control of soil-dwelling pests. In L. N. Robertson & P. G. Allsoppl (Eds.), *Proceedings of a soil-invertebrate workshop,* (pp. 10-13). Queensland Department of Primary Industries conference and workshop series QC 89004: Indooroopilly, Queensland.

Minter, D. M., Brady, B. L., & Hall, R. A. (1983). Five hyphomycetes isolated from eriophyid mites. *Trans. Br. Mycol. Soc., 81*, 455-471.

Moore, D., Douro-Kpindou, O. K., Jenkins, N. E., & Lomer, C. J. (1996). Effects of moisture content and temperature on storage of *Metarhizium flavoviride* conidia. *Biocontrol Science and Technology, 6*, 51-61.

Moore, D., Higgins, P. M., & Lomer, C. J. (1996). Effect of simulated and natural sunlight on the germination of conidia of *Metarhizium flavoviride* gams and rozsypal and interactions with temperature. *Biocontrol Science and Technology, 6*, 63-76.

Muralimohan, C. H., Lakshmi, K. A., & Devi, K. U. (1999). Laboratory evaluation of the pathogenecity of three isolates of the entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuillemin on the Amarican Cockrooch (*Periplaneta amiricana). Biocontrol Science and Technology, 9*, 29-33.

Onions, A. H., S., Allsopp, D., & Eggins, H. O. W. (1981). *Smith's introduction to industrial mycology* (7th ed). London: Edward Arnold.

Ouedraogo, A., Fargues, J., Goettel, M. S., & Lomer, C. J. (1997). Effect of temperature on vegetative growth among isolates of *Metarhizium anisopliae* and *M. flavoviride. Mycopathologia 137*, 37-43.

Pekrul, S., & Grula, E. A. (1979). Mode of infection of the corn earworm (*Heliothis zea*) by *Beauveria bassiana* as revealed by scanning electron microscopy. *Journal of Invertebrate Pathology, 34*, 238-247.

Poprawski, J. J., Legaspi, J. C., & Parker, P. E. (1998). Influence of entomopathogenic fungi on *Serangium parcesetosum* (Coleoptera: Coccinellidae), an important predator of white flies (Homoptera: Aleyrodidae). *Biological Control, 27* (3), 785-795.

Prior, C. (1992). Discovery and characterization of fungal pathogens for locust and grasshopper control. In C. J., Lomer & C. Prior (Eds.). *Biological control of locusts and grasshoppers* (pp. 159-180). UK: CAB International, Wallinyford.

Ratcliffe, N. A., Leonard, C., & Rowley, A. F. (1984). Prophenoloxidase activation: non-self recognition and cell coorperation insect immunity. *Science, 226*, 557-559.

Rath, A. C., Koen, T. B., & Yip, H. Y. (1992). The influence of abiotic factors on the distribution and abundance of *Metarhizium anisopliae* in Tasmanian pasture soils. *Mycological Research, 96*, 378-384.

Reiss, J. (1993). Biotoxic activity in the *Mucorales. Mycopathologia, 121*, 123-127.

Romoser, W. S., & Stoffolano, J. G. Jr. (1998). *The science of entomology* (4th ed). Singapore: Mc Graw-Hill Companies.

Saito, T., & Aoki, J. (1983). Toxicity of free fatty acids on the larval surface of two lepidopterous insects towards *Beauveria bassiana* (Bals.) Vuill. and *Paecilomyces fumosoroseus* (Wize) Brown et Smith (Deutoromycetes: Moniliales) *Appl. Ent. Zool. 18*, 225-233.

Samson, R. A., Evans, H. C., & Latge, P. (1988). *Atlus of entomopathogenic fungi*. Berlin, Germany: Springer-Verlag.

Samson, R. A., Mccoy, C. W., & O'Donnell, K. L. (1980). Taxonomy of the parasite *Hirsutella thompsonii. Mycologia, 72*, 359-377.

Schabel, H. G. (1978). Percutaneous infection of *Hylobius pales* by *Metarhizium anisopliae. Journal of Invertebrate Pathology, 31*, 180-187.

Schofield, R. K., & Taylor, W. (1955). The measurements of soil pH. *Soil Society Preceedings,* pp. 164-167.

Smith, R. J., Pekrul, S., & Grula, A. A. (1981). Requirement for sequential enzymatic activities of the corn earworm *(Heliothis zea). Journal of Invertebrate Pathology, 38*, 335-344.

Smits, N., Fargues, J., & Rougier, M. (1997). Modelling the persistence of quiescent conidia of the entomopathogenic Hyphomycetes *Paecilomyces fumosoroseus* exposed to solar radiation. *Biocontrol Science & Technology, 7*, 365-373.

Soderhall, K. (1982). Prophenol oxidase activating system and melanization- a recognition mechanism of arthropods. *Areview. Dev. Comp. Immun. 6*, 601-611.

St. Leger, R. J., Butt, T. M., Goettell, M. S., Stoples, R. C., & Robertts, D. W. (1989). Production in vitro of appressoria by the entomopathogenic fungus, *Metarhizium anisopliae. Experimental Mycology, 13*, 274-288.

St. Leger, R. J., Cooper, R. M., & Charnley, A. K. (1986). Cuticle degrading enzymes of entomopathogenic fungi. Regulation of production of chitinolytic enzymes. *Journal of General Microbiology, 132*, 1509-1517.

Stephan, D., & Zimmermann, G. (1998). Development of spray-drying technique for submerged spores of entomopathogenic fungi. *Biocontrol Science and Technology, 8*, 3-11.

Strand, M. A., Bailey, C. H., & Laired, M. (1977). Pathogens of Simuliidae (black-flies). *WHO Bulletin, 55*, 213-237.

Sur, B., Bihari, V., Sharma, A., & Basu, S. K. (1999). Survey of termite-inhabited soil and mosquito breeding sites in Lucknow, India for potential mycopathogens of *Anopheles stephensi. Mycopathologia, 144*, 77-80.

Sztejnberg, A., Doron-Shloush, S., & Gerson, U. (1997). The biology of the acaropathogenic fungus, *Hirsutella Kirchner. Biocontrol Science and Technology, 7*, 577-590.

Thaxter, R. (1888). The Entomophthoraceae of the United States. *Memoirs of the Boston Society of Natural Histury, 4*, 133-201.

Van Emden, H. F. (1992). *Pest control* (2nd ed.). Great Britain: University Press.

Vanninen, I. (1995). Distribution and occurrence of four entomopathogenic fungi in Finland: effect of geographical location, habitat type and soil type. *Mycological Research, 100*, (1), 93-101.

Vanninen, I., Husberg, G, B., & Hokkanen, H. M. T. (1989). Occurrence of entomopathogenic fungi and entomoparasitic nematodes in cultivated soils in Finland. *Acta Entomologica Fennica, 53*, 65-71.

Vestergaard, S., Gillespie, A. T., Butt, T., Schreiter, G., & Eilenberg, J. (1995). Pathogenicity of the Hyphomycetes fungi *Verticillium lecanii* and *Metarhizium anisopliae* to western flower thrips, *Frankliniella accidentalis. Biocontrol Science and Technology, 5*, 185-192.

Vey, A., & Fargues, J. (1977). Histological and ultrastructural studies of *Beauveria bassiana* infection in *Liptinotarsa decemlineata* larvae during ecdysis. *Journal of Invertebrate pathology, 31*, 207-215.

Vidal, C., Fargues, J., Lacey, L. A., & Jackson, M. A. (1998). Effect of Various liquid culture media on morphology, growth, propagule production and pathogenic activity to *Bemisia argentifolii* of the entomopathogenic Hyphomycete *Paecilomyces fumosoroseus. Mycopathologia, 143*, 33-46.

Vilcinskas, A., & Wedde, M. (1997). Inhibition of *Beauveria bassiana* proteases and fungal development by inducible protease inhibitors in the heamolymph of *Galleria mellonella* larvae. *Biocontrol Science and Technology, 7*, 591-601.

Waterhouse, G. M., & Brady, A. K. (1996). Key to the species of *Entomophthora* sensulato. *Bull. Br. Mycol. Soc. 16*, 113-143.

Weber, J. B. (1977). Soil properties, herbicide sorption, and model soil systems. In R. Bryan (ed.) *Research and methods in weed science* (2nd ed) (pp. 59-72). USA: Southern, T. Weed Science Society.

Yanagita, T. (1990). *Natural microbial communities.* Berlin, Germany: Springer-Verlag.

Zaccharuck, R. Y. (1970). Fine structure of the fungus *Metarhizium anisopliae* infecting three species of larval *Elateridae* (Coleoptera) II. Conidial germ tube and appressoria. *Journal of Invertebrate Pathology, 15*, 81-91.

Zhang. W. M., & Watson, A. K. (1997). Characterization of growth and conidia production of *Exserohilum monoceras* on different substrates. *Biocontrol Science and Technology, 7*, 75-86.

Zimmermann, G. (1986). The *"Galleria* bait method" for detection of entomopathogenic fungi in soil. *Z. Angew. Entomol. 102*, 213-215.

APPENDICES

APPENDIX A

Media and stains

Corn Meal Agar (CMA)

Ground maize kernels	60 g
Agar	15 g

Directions:-

Boil 60 g freshly ground maize kernels in 1-liter water for 1 hour. Strain the suspension through two layers of muslin. Add the agar and heat until dissolved. Autoclave at 121°C for 15 min.

Czapek-Dox Agar (CzA)

Sodium nitrate	2 g
Potassium chloride	5 g
Magnessium glycerophosphate	0.5 g
Ferrous sulphate	0.01 g
Potassium sulphate	0.35 g
Sucrose	30 g
Agar	15 g

Directions:-

Suspend 53 g of the powder in 1-liter of distilled water, mix thoroughly, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes.

Malt Extract Agar (MEA)

Maltose	12.75 g
Glycerol	2.35 g
Dextrin	2.75 g
Pancreatic Digest of Gelatin	0.78 g
Agar	15 g

Directions:-

Suspend 33.6 g of the powder in 1-liter of distilled water, mix thoroughly, heat with frequent agitation and boil for 1 minute to completely dissolve the powder. Autoclave at 121°C for 15 minutes.

Potato Dextrose Agar (PDA)

Potato extract	4 g
Dextrose (Glucose)	20 g
Agar	15 g

Directions:-

Suspend 39 g of the powder in 1-liter of distilled water, mix thoroughly, and heat with frequent agitation to completely dissolve the powder. Autoclave at 121 °C for 15 minutes.

Sabouraud's Dextrose Agar (Oxoid) (SDA)

Dextrose	40 g
Peptone	10 g
Agar	15 g

Directions:-

Suspend 65 g of the powder in 1-liter of distilled water, mix thoroughly and heat with frequent agitation to completely dissolve the powder. Autoclave at 121°C for 15 minutes.

Yeast - Sabouraud's Dextrose Agar (YSDA)

Dextrose	40 g
Peptone	10 g
Yeast extract	10 g
Agar	15 g

Directions:-

Suspend the above mentioned ingredients in 1-liter of distilled water, mix thoroughly and heat with frequent agitation to completely dissolve the ingredients. Autoclave at 121°C for 15 minutes.

Lactophenol-Cotton Blue Stain

Distilled water	20 ml
Lactic acid	20ml
Phenol crystals	20g
Glycerol	40ml.
Aniline blue	0.05g

Directions:-

Lactophenol can be prepared by warming the phenol with the water until dissolved, and then adding lactic acid, glycerol, and aniline blue.

APPENDIX B

Table B. 1 Fungi isolated from field soils using *Galleria*-bait method.

Fungal Species	Isolate#	Location	Vegetation
Absidia cylindrospora (Hagem var cylindrospora)	K1a-1.6	Tulkarm	Peanut
Aspergillus niger (Van Tieghem)	J1a-27.5	Qabatia	Tomato
	J1b-27.5	Qabatia	Tomato
	Q2a1a-16.6	Azzun	Tomato
	Q4a-16.6	Azzun	Zucchini
	K12ba-24.7	Atteel	Citrus
A. ochraceus (Wilhelm)	K4a-27.6	Ilar	Cuccumber
Conidiobolus coronatus (Constatin) Batko	J5b2-27.5	Qabatia	Bean
	N1b-21.6	Al-Fara'	Aubergine
	N6a-21.6	Al-Fara'	Tomato
	N6b-21.6	Al-Fara'	Tomato
	N9b-21.6.99	Al-Fara'	Jew's mallow
	N11a-21.6	Al-Fara'	Citrus
	N11b-21.6	Al-Fara'	Citrus
	J5a-6.6	Kufor-Dan	Parsley
	J5b-6.6	Kufor-Dan	Parsley
	J11a-6.6	Kufor-Dan	Cuccumber
	N1b1a-2.8	Al-Fara'	Citrus
	N5a1-2.8	Al-Fara'	Citrus
	N6a1a-2.8	Al-Fara'	Citrus
	N6b-2.8	Al-Fara'	Citrus
	N3b1a-2.8	Al-Fara'	Citrus
	N4b-8.8	Al-Badan	Cuccumber
	J3a-10.8	Kufor-Dan	Citrus
	J3b1-10.8	Kufor-Dan	Citrus
	J3b2-10.8	Kufor-Dan	Citrus
	J3b3-10.8	Kufor-Dan	Citrus
	J9a1-10.8	Kufor-Dan	Citrus
	J9b1-10.8	Kufor-Dan	Citrus
Entomophaga grylli (Fres.) Batko	N4b1-21.6	Al-Fara'	Cauliflower
Erynia castrans (Batko & Weiser) Remaudiere & Keller	Q2a-6.4	Qalqilia	Fig
Fusarium heterosporum (Nees ex. Fr.)	K18b-24.7	Atteel	Citrus
F. moniliforme var. *subglutinas* (Wallenw. & Reink)	Q11a-12.7.99	Qlqilia-D	Aubergine
F. nivale (Fr.) Ces., Robehn	J2b-27.10	Arraba	Cabbage

Table B1/ continued

Fungal Species	Isolate#	Location	Vegetation
F. oxysporum (Schlecht)	K12b2-24.7	Atteel	Citrus
	Q5a1-2.10	Qalqilia	Pepper
F. semitectum (Berk & Rav)	J5b1-27.5	Qabatia	Bean
	Q9a-12.7	Qalqilia-D	Aubergine
	Q16b1-12.7	Sufeen	Cabbage
	Q16b2-12.7	Sufeen	Cabbage
	K7b1-24.7	Atteel	Cauliflower
	J8a1a-10.8	Kufor-Dan	Cauliflower
	K5a1-11.7	Tulkarm	Cabbage
F. solani (Mart.) Sacc.	N5b-3.7	Asira-Q	Aubergine
	K2a-11.7	Tulkarm	Jew'smallow
	K5b2a-11.7	Tulkarm	Cabbage
	N5a2-2.8	Al-Fara'	Citrus
	N10b-8.8	Al-Badan	Tomato
Gliocladium viride (Martr.)	J1b-1.11	Anin	Citrus
	J3a1-4.12	Anin	Citrus
Hirsutella jonesii (Speare) Evans & Samson	K3a1-24.7	Atteel	Cauliflower
Mucor flavus (Bainier)	Q2b-16.6	Azzun	Tomato
	J3b1-27.10	Arraba	Cabbage
	J3b2-27.10	Arraba	Cabbage
	J3b3-27.10	Arraba	Cabbage
	J3a2-4.12	Anin	Citrus
	N1b1-22.4	Tell	Citrus
	Q6a-2.10	Qalqilia	Citrus
M. piriformis (Fischer)	Q2b-6.4	Qalqilia	Fig
Nectria ventricosa (C. Booth)	N7a1-21.6	Al-Fara'	Tomato
	Q5b1-12.7	Qalqilia-D	Corn
	Q18a1-12.7	Qalqilia	Bean
	J11a-1.11.99	Anin	Citrus
Paecilomyces farinosus (Holm) Brown & Smith	N2a-2.8	Al-Fara'	Citrus
Sporodiniella umbellata (Boedijin)	K13a2-24.7	Atteel	Citrus
	K13a3-24.7	Atteel	Citrus
	K13b1-24.7	Atteel	Citrus
	K16a-24.7	Atteel	Citrus
	K17b1-24.7	Atteel	Citrus
Verticillium nigriscens (Pethybr)	K13a1-24.7	Atteel	Citrus

Table B2. Preliminary pathogenicity test of fungal isolates to *Galleria mellonella* larvae using Koch's Postulates.

Fungal Species	Isolate#	Time (day) after infection	% Mortality *
Absidia cylindrospora	K1a-1.6	5	56
Aspergillus niger	J1a-27.5	5	66
	J1b-27.5	6	67
	Q2a1a-16.6	6	73
	Q4a-16.6	7	76
	K12ba-24.7	6	78
A. ochraceus	K4a-27.6	5	20
Conidiobolus coronatus	J5b2-27.5	5	100
	N1b-21.6	7	88
	N6a-21.6	6	93
	N6b-21.6	6	95
	N9b-21.6.99	5	89
	N11a-21.6	6	88
	N11b-21.6	7	89
	J5a-6.6	6	98
	J5b-6.6	6	96
	J11a-6.6	5	100
	N1b1a-2.8	7	98
	N5a1-2.8	7	89
	N6a1a-2.8	7	93
	N6b-2.8	6	94
	N3b1a-2.8	7	88
	N4b-8.8	5	87
	J3a-10.8	8	91
	J3b1-10.8	7	90
	J3b2-10.8	6	90
	J3b3-10.8	7	92
	J9a1-10.8	7	89
	J9b1-10.8	8	91
Entomophaga grylli	N4b1-21.6	5	45
Erynia castrans	Q2a-6.4	8	60
Fusarium heterosporum	K18b-24.7	10	18
F. moniliforme var. *subglutinas*	Q11a-12.7.99	6	30
F. nivale	J2b-27.10	6	56
F. oxysporum	K12b2-24.7	6	33
	Q5a1-2.10	6	30
F. semitectum	J5b1-27.5	5	33
	Q9a-12.7	6	28

Table B2/ continued

Fungal species	Isolate#	Time (day) after infection	% Mortality *
F. semitectum	Q16b1-12.7	5	24
	Q16b2-12.7	5	24
	K7b1-24.7	7	16
	J8a1a-10.8	7	18
	K5a1-11.7	6	17
F. solani	N5b-3.7	6	44
	K2a-11.7	5	28
	K5b2a-11.7	6	33
	N5a2-2.8	6	44
	N10b-8.8	10	33
Gliocladium viride	J1b-1.11	6	84
	J3a1-4.12	6	78
Hirsutella jonesii	K3a1-24.7	10	19
Mucor flavus	Q2b-16.6	5	32
	J3b1-27.10	8	28
	J3b2-27.10	9	28
	J3b3-27.10	9	30
	J3a2-4.12	8	23
	N1b1-22.4	6	22
	Q6a-2.10	6	33
Mucor piriformis	Q2b-6.4	7	56
Nectria ventricosa	N7a1-21.6	5	28
	Q5b1-12.7	6	44
	Q18a1-12.7	7	44
	J1a-1.1199	6	22
Paecilomyces farinosus	N2a-2.8	6	28
Sporodiniella umbellata	K13a2-24.7	7	63
	K13a-24.7	8	62
	K13b1-24.7	7	49
	K16a-24.7	7	45
	K17b1-24.7	8	50
Verticillium nigriscens	K13a1-24.7	9	37

*Percent mortality was the average of nine replicate larvae

APPENDEX C
ANOVA TABLES

Table C1. Effect of pH on the isolation of entomopathogenic fungi.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.183	4	0.296	0.426	0.790
Within groups	93.817	135	0.695		
Total	95.0	139			

No. of soil samples = 140 (df= 139), No. of pH intervals = 5 (df=4), error df = 139-4= 135

Table C2. Effect of organic matter content on the isolation of entomopathogenic fungi

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	7.555	4	1.889	2.916	0.024
Within groups	87.445	135	0.648		
Total	95.00	139			

No. of organic matter intervals = 5 (df = 4), error df = 135

Table C3. Effect of moisture content on the isolation of entomopathogenic fungi

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	4.393	3	1.464	2.198	0.091
Within groups	90.607	136	0.666		
Total	95.0	139			

No. of moisture content intervals = 4 (df = 3), error df = 136

Table C4. Effect of vegetation type on the isolation of entomopathogenic fungi

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	5.040	1	5.040	7.731	0.006
Within groups	89.960	138	0.652		
Total	95.0	139			

Types of vegetation = 2 (df =1), error df = 138

Table C5. Effect of geographical location on the isolation of entomopathogenic fungi

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.286	3	0.429	0.622	0.602
Within groups	93.714	136	0.689		
Total	95.0	139			

No. of locations = 4 (df = 3), error df = 136

Table C6. Effect of different variable interactions on the isolation of entomopathogenic fungi

Source	Type III sum of squares	df	Mean squares	F	*P*
Location * vegetation type	0.864	2	0.432	0.811	0.449
Location * pH	1.167	6	0.194	0.365	0.898
Vegetation type * pH	1.073	2	0.536	1.007	0.371
Location * organic matter	2.127	4	0.532	0.998	0.416
Vegetation type * organic matter	0.019	1	0.019	0.036	0.850
Location * moisture content	1.999	4	0.500	0.98	0.448
Vegetation type * moisture content	1.520	2	0.760	1.427	0.248
pH * moisture content	2.981	4	0.745	1.399	0.245
Location * pH * moisture content	0.634	2	0.317	0.595	0.555
Organic matter * moisture content	0.00287	2	0.001437	0.003	0.997
Error	31.429	59	0.533		
Total	130.00	140			

Table C7. Overall effect of nutrient media on the isolation of entomopathogenic fungi species.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	19.526	5	3.905	0.753	0.585
Within groups	528.723	102	5.184		
Total	548.250	107			

No. of media = 6 (df = 5), No. of fungal species = 6, total No. of readings = 6x6x3= 108 (df = 107), error df = 102

Table C8. Effect of nutrient media on mycelial growth of *Conidiobolus coronatus*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	51.560	5	10.312	306.449	0.000
Within groups	0.404	12	0.0336		
Total	51.964	17			

No. of media = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C9. Effect of nutrient media on mycelial growth of *Entomophaga grylli*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	17.478	5	3.496	250.178	0.000
Within groups	0.168	12	0.0139		
Total	17.645	17			

No. of media = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C10. Effect of nutrient media on mycelial growth of *Erynia castrans*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	4.398	5	0.880	9.257	0.001
Within groups	1.140	12	0.09502		
Total	5.538	17			

No. of media = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C11. Effect of nutrient media on mycelial growth of *Hirsutella jonesii*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	10.374	5	2.075	41.539	0.000
Within groups	0.599	12	0.04995		
Total	10.974	17			

No. of media = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C12. Effect of nutrient media on mycelial growth of *Paecilomyces farinosus*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	6.872	5	1.374	1438.317	0.000
Within groups	0.01147	12	0.09556		
Total	6.883	17			

No. of media = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C13. Effect of nutrient media on mycelial growth of *Sporodiniella umbellata*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	57.446	5	11.489	137.899	0.000
Within groups	1.000	12	0.08332		
Total	58.446	17			

No. of media = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12.

Table C14. Effect of fungal species on mycelial growth on media (all media collectively).

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	396.799	5	79.360	53.448	0.000
Within groups	151.451	102	1.485		
Total	548.250	107			

No. of fungal species = 6 (df = 5), No. of media = 6, total No. of readings = 6x6x3 = 18 (df = 107), error df = 102

Table C15. Effect of fungal species on mycelial growth on SDA.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	61.738	5	12.348	90.433	0.000
Within groups	1.638	12	0.137		
Total	63.377	17			

No. of fungal species = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C16. Effect of fungal species on mycelial growth on YSDA.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	56.257	5	11.251	514.673	0.000
Within groups	0.262	12	0.02186		
Total	56.519	17			

No. of fungal species = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C17. Effect of fungal species on mycelial growth on MEA.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	104.922	5	20.984	321.273	0.000
Within groups	0.784	12	0.06532		
Total	105.706	17			

No. of fungal species = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C18. Effect of fungal species on mycelial growth on CMA.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	53.920	5	10.784	948.74	0.000
Within groups	0.136	12	0.01137		
Total	54.056	17			

No. of fungal species = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C19. Effect of fungal species on mycelial growth on CZA.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	149.197	5	29.839	1863.665	0.000
Within groups	0.192	12	0.01601		
Total	149.389	17			

No. of fungal species = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C20. Effect of fungal species on mycelial growth on PDA.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	99.367	5	19.873	771.283	0.000
Within groups	0.309	12	0.02577		
Total	99.676	17			

No. of fungal species = 6 (df = 5), total No. of readings = 6x3 = 18 (df = 17), error df = 12

Table C21. Overall effect of temperature on mycelial growth rates of fungal species.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	357.787	5	71.557	20.64	0.000
Within groups	353.619	102	3.467		
Total	711.405	107			

No. of temperatures = 6 (df = 5), No. of fungal species = 6, total No. of readings = 6x6x3= 108 (df = 107), error df = 102

Table C22. Effect of temperature on mycelial growth of *Conidiobolus coronatus*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	99.762	5	19.952	291.987	0.000
Within groups	0.820	12	0.0633		
Total	100.582	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C23. Effect of temperature on mycelial growth of *Entomophaga grylli*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	101.485	5	20.297	187.357	0.000
Within groups	1.300	12	0.108		
Total	102.785	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C24. Effect of temperature on mycelial growth of *Erynia castrans*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	74.398	5	14.880	286.488	0.000
Within groups	0.623	12	0.05194		
Total	75.021	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C25. Effect of temperature on mycelial growth of *Hirsutella jonesii*

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	82.896	5	16.579	397.902	0.000
Within groups	0.500	12	0.0416		
Total	83.396	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C26. Effect of temperature on mycelial growth of *Paecilomyces farinosus*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	6.254	5	1.251	249.339	0.000
Within groups	0.0602	12	0.05017		
Total	6.314	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C27. Effect of temperature on mycelial growth of *Sporodiniella umbellata*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	122.101	5	24.420	330.498	0.000
Within groups	0.887	12	0.07389		
Total	122.987	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C28. Effect of fungal species on mycelial growth at all temperatures tested.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	220.319	5	44.064	9.152	0.000
Within groups	491.087	102	4.815		
Total	711.405	107			

No. of fungal species = 6 (df = 5), No. of temperatures = 6, No. of total readings = 6x6x3 = 108, error df = 102

Table C29. Effect of fungal species on mycelial growth at 10°C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.200	5	0.240	20.578	0.000
Within groups	0.140	12	0.0116		
Total	1.340	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C30. Effect of fungal species on mycelial growth at 15°C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	43.360	5	8.672	148.663	0.000
Within groups	0.700	12	0.05833		
Total	44.060	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C31. Effect of fungal species on mycelial growth at 20°C.

Source	Sum of squares	Df	Mean squares	F	*P*
Between groups	70.776	5	14.155	115.815	0.000
Within groups	1.467	12	0.122		
Total	72.243	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C32. Effect of fungal species on mycelial growth at 25°C.

Source	Sum of squares	Df	Mean squares	F	*P*
Between groups	97.105	5	19.421	323.68	0.000
Within groups	0.720	12	0.06		
Total	97.825	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C33. Effect of fungal species on mycelial growth at 30°C.

Source	Sum of squares	Df	Mean squares	F	*P*
Between groups	79.789	5	15.958	296.126	0.000
Within groups	0.647	12	0.05389		
Total	80.436	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C34. Effect of fungal species on mycelial growth at 37°C.

Source	Sum of squares	Df	Mean squares	F	*P*
Between groups	57.198	5	11.440	265.62	0.000
Within groups	0.517	12	0.04307		
Total	57.715	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C35. Overall effect of temperature on spore production of fungal species.

Source	Sum of squares	Df	Mean squares	F	*P*
Between groups	4.68E+14	5	9.36E+13	4.179	0.001
Within groups	2.28E+15	102	2.24E+13		
Total	2.75E+15	107			

No. of temperatures = 6 (df = 5), No. of fungal species = 6, total No. of readings = 6x6x3= 108 (df = 107), error df = 102

Table C36. Effect of temperature on spore production of *Conidiobolus coronatus*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.26E+14	5	2.53E+13	1378.745	0.000
Within groups	2.20E+11	12	1.83E+10		
Total	1.27E+14	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C37. Effect of temperature on spore production of *Entomophaga grylli*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	5.95E+13	5	1.19E+13	760.18	0.000
Within groups	1.88E+11	12	1.57E+10		
Total	5.97E+13	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C38. Effect of temperature on spore production of *Erynia castrans*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	5.34E+12	5	1.07E+12	50.275	0.000
Within groups	2.55E+11	12	2.13E+10		
Total	5.60E+12	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C39. Effect of temperature on spore production of *Hirsutella jonesii*.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	5.01E+11	5	1.00E+11	55.628	0.000
Within groups	2.16E+10	12	1.80E+09		
Total	5.22E+11	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C40. Effect of temperature on spore production of *Paecilomyces farinosus.*

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.86E+15	5	3.72E+14	444.917	0.000
Within groups	1.00E+13	12	8.35E+11		
Total	1.87E+15	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C41. Effect of temperature on spore production of *Sporodeniella umbellata.*

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	3.00E+12	5	6.00E+11	187.412	0.000
Within groups	3.84E+10	12	3.20E+09		
Total	3.04E+12	17			

No. of temperatures = 6 (df = 5), total No. of readings = 6x3= 18 (df = 17), error df = 12

Table C42. Effect of fungal species on spore production at all temperatures.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	6.79E+14	5	1.36E+14	6.682	0.000
Within groups	2.07E+15	102	2.03E+13		
Total	2.75E+15	107			

No. of fungal species = 6 (df = 5), No. of temperatures = 6, No. of total readings = 6x6x3 = 108, error df = 102

Table C43. Effect of fungal species on spore production at 10 °C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	2.38E+10	5	4.77E+09	57.239	0.000
Within groups	1.00E+09	12	83333333		
Total	2.48E+10	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C44. Effect of fungal species on spore production at 15 °C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	3.25E+14	5	6.49E+13	383.796	0.000
Within groups	2.03E+12	12	1.69E+09		
Total	3.27E+14	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C45. Effect of fungal species on spore production at 20 °C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.59E+14	5	3.17E+13	3955.528	0.000
Within groups	9.63E+10	12	8.02E+09		
Total	1.59E+14	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C46. Effect of fungal species on spore production at 25 °C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	3.23E+13	5	6.45E+12	1187.890	0.000
Within groups	6.52E+10	12	5.43E+09		
Total	3.23E+13	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C47. Effect of fungal species on spore production at 30 °C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	1.72E+15	5	3.43E+14	498.361	0.000
Within groups	8.26E+12	12	6.89E+11		
Total	1.72E+15	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C48. Effect of fungal species on spore production at 37 °C.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	3.4775E+13	5	6.96E+12	11.661	0.000
Within groups	7.1570E+12	12	5.96E+11		
Total	4.1932E+13	17			

No. of fungal species = 6 (df = 5), No. of total readings = 6x3 = 18 (df = 17), error df = 12

Table C49. Effect of fungal species on spore germination at all temperatures.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	132189.15	5	26437.829	20.616	0.000
Within groups	407802.56	318	1282.398		
Total	539991.71	323			

No. of fungal species = 6 (df = 5), No. of temperatures = 6, No. of times of incubation = 3, total No. of readings = 6x6x3x3 = 324 (df = 323), error df = 318

Table C50. Effect of time on spore germination of fungal species at all temperatures.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	24724.096	2	12362.048	7.701	0.001
Within groups	515267.61	321	1605.195		
Total	539991.71	323			

No. of times of incubation = 3 (df = 2)

Table C51. Effect of temperature on spore germination of fungal species.

Source	Sum of squares	df	Mean squares	F	*P*
Between groups	270740.05	5	54148.009	63.952	0.000
Within groups	269251.66	318	846.703		
Total	539991.71	312			

No. of temperatures = 6 (df = 5)

Table C52. Effect of fungal species * time interactionss on spore germination at all temperatures.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	132189.15	5	26437.829	7.600	0.000
Time	24724.096	2	12362.048	1078.901	0.00
Fungal species * time	3400.971	10	340.097	21.307	0.986
Error	379677.50	306	1240.776	9.963	
Total	1878666.4	324		0.274	

No. of fungal species = 6 (df = 5)

Table C53. Effect of fungal species * temperature interactions on spore germination after all times.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	132189.15	5	26437.829	112.678	0.00
Temperature	270740.05	5	54148.009	230.779	0.00
Fungal species* temperature	69488.753	25	2779.550	11.846	0.00
Error	67573.764	288	234.631		
Total	1878666.4	324			

Table C54. Effect of time * temperature interactions on spore germination at all temperatures.

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	24724.096	2	12362.048	16.541	0.00
Temperature	270740.05	5	54148.009	72.454	0.00
Time * temperature	15839.891	10	1583.989	2.119	0.023
Error	228687.68	306	747.345		
Total	187866.4	224			

Table C55. Effect of fungal species * time * temperature interactions on spore germination at all temperatures.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	132189.15	5	26437.829	3822.337	0.000
Time	24724.096	2	12362.048	1787.284	0.000
Temperature	270740.05	5	54148.009	7828.628	0.000
Fungal species * time	3400.971	10	340.097	49.171	0.000
Fungal species * temperature	69488.753	25	2779.550	401.863	0.000
Time * temperature	15839.91	10	1583.989	229.010	0.000
Fungal species * time * temperature	22114.807	50	442.296	63.946	0.000
Error	1494.000	216	6.917		
Total	1878666.4	324			

Table C56. Effect of time * temperature interactions on spore germination of *Conidiobolus coronatus*.

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	10211.791	2	5105.896	650.279	0.000
Temperature	66302.236	5	13260.447	1688.831	0.000
Time * temperature	10468.160	10	1046.816	133.321	0.000
Error	282.667	36	7.852		
Total	227.780.06	54			

Table C57. Effect of time * temperature interactions on spore germination of *Entomophaga grylli*.

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	4072.669	2	2036.335	662.422	0.000
Temperature	75574.716	5	15114.943	4916.909	0.000
Time * temperature	6789.993	10	678.999	220.879	0.000
Error	110.667	36	3.074		
Total	355774.09	54			

Table C58. Effect of time * temperature interactions on spore germination of *Erynia castrans*.

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	1038.496	2	519.248	51.167	0.000
Temperature	59687.570	5	11937.514	1176.324	0.000
Time * temperature	1911.257	10	191.126	18.834	0.000
Error	365.333	36	10.148		
Total	426033.06	54			

Table C59. Effect of time * temperature interactions on spore germination of *Hirsutella jonesii*.

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	5174.333	2	2587.167	229.404	0.000
Temperature	9279.408	5	1855.882	164.561	0.000
Time * temperature	1507.667	10	150.767	13.368	0.000
Error	406.000	36	11.278		
Total	50917.090	54			

Table C60. Effect of time * temperature interactions on spore germination of *Paecilomyces farinosus*.

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	2151.148	2	1075.574	598.773	0.000
Temperature	68705.312	5	13741.062	7649.664	0.000
Time * temperature	3761.074	10	376.107	209.379	0.000
Error	64.667	36	1.796		
Total	410733.09	54			

Table C61. Effect of time * temperature interactions on spore germination of *Sporodeniella umbellata.*

Source	Type III sum of squares	df	Mean squares	F	*P*
Time	5476.629	2	2738.315	372.466	0.000
Temperature	60679.556	5	12135.911	1650.728	0.000
Time * temperature	13516.546	10	1351.655	183.852	0.000
Error	264.667	36	7.352		
Total	407429.03	54			

Table C62. Effect of fungal species * temperature interactions on spore germination after 24h.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	54349.557	5	10869.911	921.468	0.000
Temperature	93374.430	5	18674.886	1583.114	0.000
Fungal species * temperature	50302.317	25	2012.093	170.570	0.000
Error	849.333	72	11.796		
Total	493973.24	108			

Table C63. Effect of fungal species * temperature interactions on spore germination after 48h.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	46925.901	5	9385.180	3167.498	0.000
Temperature	100691.53	5	20138.306	6796.678	0.000
Fungal species * temperature	25554.994	25	1022.200	344.992	0.000
Error	213.333	72	2.963		
Total	669275.12	108			

Table C64. Effect of fungal species * temperature interactions on spore germination after 72h.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	34314.659	5	6862.932	1145.590	0.000
Temperature	92513.980	5	18502.796	3088.566	0.000
Fungal species * temperature	15746.248	25	629.850	105.137	0.000
Error	431.333	72	5.991		
Total	715418.06	108			

Table C65. Effect of fungal species * time interactions on spore germination at 10 °C.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	573.940	5	114.788	29.101	0.000
Time	681.240	2	340.620	86.354	0.000
Fungal species * time	919.640	10	91.964	23.315	0.000
Error	142.000	36	3.944		
Total	2881.360	54			

Table C66. Effect of fungal species * time interactions on spore germination at 15 °C.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	44134.833	5	8826.967	480.500	0.000
Time	11870.778	2	5935.389	323.096	0.000
Fungal species * time	3325.889	10	332.589	18.105	0.000
Error	661.33	36	18370		
Total	312553.00	54			

Table C67. Effect of fungal species * time interactions on spore germination at 20 °C.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	61558.833	5	12311.767	2408.824	0.000
Time	2826.778	2	1413.389	276.533	0.000
Fungal species * time	1353.222	10	135.322	26.476	0.000
Error	184.000	36	5.111		
Total	348891.00	54			

Table C68. Effect of fungal species * time interactions on spore germination at 25 °C.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	29131.481	5	5826.296	1278.943	0.000
Time	188.481	2	94.241	20.687	0.000
Fungal species * time	602.407	10	60.241	13.224	0.000
Error	164.000	36	4.556		
Total	460316.00	54			

Table C69. Effect of fungal species * time interactions on spore germination at 30 °C.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	25454.148	5	5090.830	851.098	0.000
Time	1282.481	2	641.241	107.204	0.000
Fungal species * time	2364.630	10	236.463	39.533	0.000
Error	215.333	36	5.981		
Total	451020.00	54			

Table C70. Effect of fungal species * time interactions on spore germination at 37 °C.

Source	Type III sum of squares	df	Mean squares	F	*P*
Fungal species	40824.661	5	8164.932	2308.410	0.000
Time	23714.228	2	11857.114	3352.273	0.000
Fungal species * time	16949.990	10	1694.999	479.214	0.000
Error	127.333	36	3.537		
Total	303005.06	54			

بسم الله الرحمن الرحيم

توزيع الفطريات الممرضة للحشرات في تربة الحقول الزراعية في فلسطين ودراسة بعض خواصها

عبد الباسط باسم محمد مرعي

المشرف

بروفيسور محمد سليم علي اشتية

تمت دراسة توزيع الفطريات الممرضة للحشرات في تربة الحقول الزراعية (خضروات مروية، بيارات حمضيات) موزعة على أربع مناطق جغرافية (جنبن، نابلس، طولكرم، قلقيلية) في الضفة الغربية، وذلك باستخدام طريقة الطعوم بيرقات عثة الشمع (*Galleria*-bait method). أظهرت النتائج وجود الفطريات الممرضة للحشرات في 33.57 % من عينات التربة (47 من 140) التي تمت دراستها. وكان عدد العزلات التي تم عزلها من هذه العينات 70 عزلة تابعة ل 20 نوعاً و 13 جنساً. وكان الفطر *Conidiobolus coronatus* هو الأكثر وجوداً وتكراراً من الفطريات الممرضة للحشرات التي تم عزلها إذ شكلت عزلاته ما نسبته 31.43 % من العدد الكلي للعزلات. لقد تم دراسة تأثير عوامل التربة مثل درجة الحموضة القلوية (pH)، والرطوبة ونسبة المادة العضوية في التربة وكذلك تأثير الموقع الجغرافي ونوع النمو الخضري للحقل الذي أخذت منه العينات على وجود الفطر في التربة. وقد أظهرت النتائج أن تأثير الحموضة القلوية ونسبة الرطوبة في التربة على توزيع هذه الفطريات في الحقول المدروسة غير معنوي ($p> 0.05$) في

حين كان تأثير مستوى المواد العضوية ونوع الغطاء النباتي معنويـــا ($p< 0.05$) علـــى توزيـــع الفطريات المذكورة حيث كان عدد عزلات الفطر أعلى في الحقول ذات المحتوى الأعلى من المادة العضوية، وكذلك أعلى في البيارات منها في الخضار المروية. وأظـــهرت النتـــائج أيضـــاً قـــدرة الفطريات المعزولة على إحداث المرض ليرقات عثة الشمع ومـــوت 16–100 % منـــها وكـــانت عزلات الفطر *C. coronatus* هي الأكثر قدرة على إحداث المرض حيث كانت قادرة على قتل ما نسبته 87–100% من اليرقات خلال 5 إلى 8 أيام بعد العدوى. كذلك أظهرت نتائج دراسة تـــأثير نوع الوسط المغذي والحرارة على النمو وإنتاج الأبواغ وإنباتها على عزلة واحدة مـــن كـــل مـــن الفطريات التاليـــة: *C. coronatus*, و *Entomophaga grylli* و *Erynia castrans* و *Hirsutella jonesii* و *Paecilomyces farinosus* و *Sporodinielle umbellata* ، أن الوسطين المغذيين CMA و PDA قد أعطيا أعلى معدل من نمو الغزل الفطري. كما تبيـــن أن هناك فروقاً ذات دلالة إحصائية ($p< 0.05$) بين معدلات النمو للعزلات المختلفة علـــى البيئـــات الغذائية المختلفة. وبينت الدراسة المخبرية لتأثير درجة الحرارة على العزلات الست المنتخبـــة؛ أن أكبر نمو للغزل الفطري وأعلى إنتاج للأبواغ وأعلى نسبة لاستنباتها تكون ما بين درجة 20 إلـــى 30 درجة مئوية. وتبين أيضاً أن هناك فروقاً ذات دلالة إحصائية ($p< 0.05$) بين معدلات النمـــو ومعدلات إنتاج الأبواغ واستنباتها للعزلات المختلفة وعلى درجات الحرارة المختلفة.