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Biological Control of Rhizoctonia Solani and Sclerotium Rolfsii by Using Local Isolates of Trichoderma Spp

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Contents

List of Tables

List of Figures

List of Appendixes

List of Abbreviations

PDA	Potato Dextrose Agar
TSM	*Trichoderma* Selective Media
PDB	Potato Dextrose Broth
gm	Gram
ml	Milliter
PCNB	Pentachloronitrobenzene
DW	Distilled Water
ALG	Average Linear Growth Rate
L	Liter
R. solani	*Rhizoctonia solani*
S. rolfsii	*Sclerotium rolfsii*
T. harzianum	*Trichoderma harzianum*
T. hamatum	*Trichoderma hamatum*
T. lignorum	*Trichoderma lignorum*
T. viride	*Trichoderma viride*
Ck	Control
CFU	Colony Forming Unit
µm	Micrometer
mm	Millimeter
rpm	Round per minute
cm	Centimeter
hr	Hour
mg	Milligram
IGR	Increased Growth Response
ISR	Induced Systemic Resistance
HUJ	Hebrew University of Jerusalem

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Abstract

The bioagent, *Trichoderma* species are known antagonists of other fungi, and have been shown to be very potent biocontrol agents of several soil borne plant pathogenic fungi under both greenhouse and field conditions. One hundred sixty nine soil samples from irrigated fields in the West Bank were collected for the recovering of *Trichoderma* fungal species using *Trichoderma* selective media (TSM). Forty-seven local *Trichoderma* isolates were isolated.

The antagonistic potential of the local isolates against the phytopathogenic *Rhizoctonia solani* and *Sclerotium rolfsii* was investigated in dual culture and bioassay on bean plants. Application of testing isolates as a conidial suspension ($3*10^6$) greatly reduced the disease index of bean plants caused by *Rhizoctonia solani* and *Sclerotium rolfsii* in different rates by a percentage of 65% and 67%, respectively.

The results revealed that the variation of antagonistic potential between isolates was due to the variation in mycelium-coiling rate, sporulation, fungitoxic metabolites, induced growth response and temperature effect. In dual culture, most effective isolate (Jn14) overgrew the pathogens *R. solani* and *S. rolfsii* at 30 and 25 ^{o}C respectively. In addition, results showed that Jn14 and T36 were the most effective isolates at 25 °C and inhibited *R. solani* and *S. rolfsii* mycelial growth at a

percentage of 79% and 94.7% respectively due to fungitoxic metabolites production. Sporulation of the isolates reached a peak at 30 °C and decreased at 35 °C. The maximum absolute spore production were $1.5*10^9$ spore / ml by the isolate Jn14 at 30 °C. The Effect of *Trichoderma* on bean seedlings growth was obvious; height was nearly doubled (160% - 200%), while fresh and dry weight were increased by 133% - 217% respectively. in plant. Germination of bean seeds treated with *Trichoderma* isolates occurred about four days earlier than those in untreated soil.

Chapter One

Chapter One

1. General Introduction

1.1 The fungus *Trichoderma*

1.1.1 History

The fungus *Trichoderma* was described as early as 1794 by the mycologist Persoon. The potential for using *Trichoderma* as a biocontrol agent was suggested by Weindling (1932), who was the first to demonstrate the parasitic activity the members of this fungus genus to pathogens such as soil borne plant pathogenic fungi e.g., *Rhizoctonia solani*. However, with the increasing interest in biological control, owing to environmental and economic concerns, and with the rapid development of biotechnology, Dennis and Webster (1971b) described the antagonistic properties of *Trichoderma* in terms of antibiotic production and hyphal interactions in the control of *Sclerotium rolfsii.*

Several *Trichoderma* species were formulated in a commercial production for the protection and growth enhancement of a number of crops in several countries such as the United States (Mcspadden & Fravel, 2002).

1.1.2 Biology and Nomenclature

The genus *Trichoderma* belongs to the class Deuteromycetes. It was, for the most parts, classified as an imperfect fungus, in that it has no known sexual stage (Gams & Bisset, 1998).

Rifai (1969) distinguished nine species differentiated primarily by conidiophore branching patterns and conidium morphology based on microscopic characters; *Trichoderma aureoviride, T. hamatum, T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. piluliferum, T. polysporum, T. pseudokoningii*, and *T. viride.*

A sectional classification was proposed for *Trichoderma* recognizing the following five sections; section *Trichoderma*, *Longibrachiatum*, *Saturnisporum*, *Pachybasium* and *Hypocreanum* (Bissett, 1991a). Twenty species were assigned to *Trichoderma* section *Pachybasium.* They were described and differentiated on the basis of conidiophore and conidium morphology (Bissett, 1991b). In the section *Trichoderma,* Persoon (1794) characterized species by narrow and flexuous conidiophores with branches and phialides uncrowded, frequently paired, and seldom in verticals of more than three. In the section *Longibrachiatum,* Bissett (1984) indicated that conidiophores are sparingly and irregularly branched, with irregularly disposed and not usually in whorls or verticals and species in this section produce distinctive greenish yellow pigments in reverse of cultures. In the section *Saturnisporum*, conidiophores have a branching system with branches and phialides uncrowded and frequently paired and compact conidiogenous pustules as in section *Pachybasium.* However, it was differentiated by the bullate or winglike conidial ornamentation. The section *Pachybasium*, have species with highly ramified, broad conidiophores usually arranged in compact pustules or fascicles, and with branches and phialides broad or inflated, relatively short, and disposed in crowded verticals. Some species are characterized by the production of sterile conidiophores. The section *Hypocreanum*, chractarized by effuse, usually spars conidiation, sparingly

branched conidiophores, and cylindrical to subulate phialides frequently borne in Verticillium-like divergent verticals (Bissett, 1991 a).

1.1.2.1 Characteristics of the Genus *Trichoderma*

The genus *Trichoderma* is characterized by rapidly growing colonies bearing tufted or postulate, repeatedly branched conidiophore with lageniform phialides and hyaline or green conidia born in slimy heads (Bissett, 1984).

The primary branches of conidiophore produce smaller secondary branches that also may produce tertiary branch, and so on. The final branches are very simply constructed, with a majority of singly phialides (Rifai, 1969).

Conidiophore may end in sterile appendages with the phialides born on lateral branches in some species. Conidia are hyaline or, more usually, green, smooth – walled or roughened. Hyaline chlamydospores are usually present in the mycelium of older cultures (Domsch *et al*, 1980).

Phialides are ampulliform to lageniform, usually constricted at the base, more or less swollen near the middle, and abruptly near the apex into short subcylindric neck. They are disposed in verticals terminally on branches of the conidiophore, or less frequently singly or in whorls directly beneath septa along the conidiophore and its branches (Bissett, 1991 c).

1.1.3 Growth Medium and Sporulation

Trichoderma species are saprophytic fungi that use a wide range of compounds as carbon and nitrogen sources. The carbon and energy requirements of *Trichoderma* can be satisfied by monosaccharides and disaccharides (Papavizas, 1985). Danielson and Davey (1973a) suggested that ammonium appears to be the most readily utilized source of nitrogen by

Trichoderma spp. in buffered media, and also, they suggested other sources of nitrogen, such as amino acids; urea and nitrate support vegetative growth.

In 1981, Elad, *et al*. found special *Trichoderma* selective media (TSM) that had been recommended for the quantitative isolation of *Trichoderma* from soil. Selectivity was obtained by using chloramphenicol as a bacterial inhibitor and pentachloronitrobenzene (PCNB), and rosebengal as fungal inhibitors.

The colony of *Trichoderma* grows rapidly and matures in 5 days on potato dextrose agar at 25°C, and the colony is wooly and become compact. The colony color is white; as the conidia are formed scattered blue-green or yellow-green patches become visible. These patches may sometimes form concentric rings. They are more readily visible on potato dextrose agar compared to other media-Reverse is pale, tan, or yellowish (Sutton *et al*, 1998; and De Hoog, 2000).

Most species of *Trichoderma* are photosensitive, sporulating on many natural and artificial substrates. Exposure of agar cultures for 20-30 seconds to light of 85-90 lux intensity is usually sufficient to induce some sporulation (Papavizas, 1985). The best photoinduction of phialoconidiogenesis has been obtained with exposure to daylight for three minutes or to near UV radiation (366 nm) for 10 -30 seconds (Betina & Spišiaková, 1976).

Acidic pH levels *in vitro* enhanced the growth of *T. harzianum* and stimulated its conidiophore formation and conidial germination (Chet and Baker, 1980). There has been also considerable interest in CO_2 effects on *Trichoderma* growth *in vitro*. The effect of CO_2 on growth depends on its concentration and medium pH (Danielson and Davey, 1973 b). Pugh and Van

(1969) found that *Trichoderma* growth was rapid at high concentration of CO_2.

Trichoderma species are rarely reported to occur on living plants and have not been founds as endophyte of living plants (Petrini, 1986).

1.1.4 Ecology

Trichoderma species are ubiquitous in the environment, especially in soils. They have been used or encountered in many human activities, including commercial applications in production of enzymes and biological control of plant disease (Samuels, 1996).

Trichoderma species are widely distributed all over the world (Domsch *et al*., 1980), and found in all soils including forest humus layer (Wardle *et al*., 1993) as well as in agricultural orchard soils (Roiger *et al*., 1991) and natural habitats, especially in those containing or consisting of organic matter (Papavizas, 1985). They are also found on root surfaces of various plants and on decaying bark, especially when it is damaged by other fungi; and on sclerotia or propagules of other fungi (Papavizas, 1985). Caldwell (1958) was the first to observe that chlamydospores survive in soil better than conidia.

Trichoderma species can produce chlamydospores on natural substrates, such as oat kernels placed in sterile and natural soils. These structures may play an important role in the survival of the genus in the soil (Henis & Papavizas, 1983). Hypha also survives in soil (Papavizas *et al*., 1984).

Papavizas et al. (1982) found that the conidia of *Trichoderma harzianum* added to soil without nutrient- supplying amendments survived between 110-130 days depending on the isolate.

Davet (1979) determined that, conidia added to soil decreased in number initially, and then was stabilized for two years to about one-tenth the original number added.

Lewis and Papavizas (1984) demonstrated the potential of various *Trichoderma* species aggregates to form chlamydospores readily and in great numbers in natural soil or in fragments of organic matter after the introduction of the fungus to the soil as conidia. Propagation of *Trichoderma* enhanced by the low pH, in the former study indicating that acidification of soil could induce suppressiveness by *Trichoderma* (Chet and Baker, 1980).

Soil moisture enhances the *Trichoderma* conidia to survive longer than in dry soil (Lui and Baker, 1980). *Trichoderma* can tolerate fungicides, such as methyl bromide, captan and maneb (Ruppl *et al*., 1983).

Lewis and papavizas (1984) demonstrated, however, that the number of colony forming units of *T. harzianum* and *T. viride* introduced four days before, or at the time of fumigation with sublethal rate of sodium methane, was significantly less than those in non fumigated soil, and the number of colony forming units of strains introduced four days after fumigation was similar to that in non fumigated soil.

Papavizas (1981) reported that *T. harzianum* did not survive well in the rhizophere of bean and pea seedling when seeds were coated with conidia of the fungus. In addition, he found that the number of colony- forming units recovered per gram of rhizosphere soil including the roots and decaying seed

coats and cotyledons was less than the number of conidia added per individual seed.

The minimal effective amount of *Trichoderma* was found by Chet and Baker (1980) to be around $1*10^6$ CFU/g soil.

1.1.5 Soil Fungistasis

Fungistasis is a natural mechanism of inhibition of growth of fungi imposed by soil microbes and overcome by the nutrient-rich rhizosphere of a host plant, allowing soilborne pathogens to infect the plant. (Papavizas & Lumsden, 1980).

Trichoderma and other potential biocontrol fungi proliferate abundantly in various natural soils when added as young mycelium in intimate contact with food base, but not as conidia with or without bran (Lewis and Papavizasa 1984).

Trichoderma conidia have been found to be either very sensitive to fungistasis (Lockwood, 1977) or to be relatively insensitive, and it is more sensitive than chlamydospores and hyphae less sensitive than conidia (Mitchell and Dix, 1975).

Denielson and Davey (1973 c) found that the sensitivity to fungistasis was higher in neutral or alkaline than in acid soils.

1.1.6 *Trichoderma* Modes of Action

No single mode of action for *Trichoderma* species against fungal plant parasites function alone. There are several mechanisms of action suggested for *Trichoderma* spp.: mycoparasitism, antibiosis, competition for nutrients or

space, tolerance to stress through enhanced root and plant development, induced resistance, solubilization and sequestration of inorganic nutrients, and inactivation of the pathogens enzymes (Samuels, 1996). The first three were the ones by which these fungi have always been considered to function; other mechanisms are suggested but not yet been confirmed (Harman, 2000).

1.1.6.1 Mycoparasitism

Mycoparasitism is considered an important mechanism of biological control and probably depends on the production of lytic enzymes including β-1,3-gluconase, and proteases (Haran *et al*., 1996a). Several chitinolytic enzymes have been reported in *T. harzianum* including endochitinases, exochitinases and 1, 4-β-N-acetylglucosaminidases which are induced during growth in liquid medium containing chitin as carbon source (Haran *et al*., 1996 b).

Mycoparasitism is a complex process including several steps. The initial interaction shows that the hypha of the mycoparasites grows directly towards its host (Chet *et al*., 1981). When the mycoparasite reaches the host, its hypha coils it or attaches to it by forming a hook-like structure. Following these interactions hypha sometimes penetrates the host mycelium, apparently, by partially degrading its cell wall (Elad *et al*., 1983).

The control of *Rhizoctonia solani* and *Pythium ultimum* and by *Trichoderma* species, including *T. harzianum*, may be affected through direct penetration of host hyphae (Dennis & Webster, 1971 b; Benhamou & Chet, 1993). They grow toward hyphae of other fungi, coil about them in a lectin-mediated reaction, and degrade cell walls of the target fungi by the activity of

enzymes, which may be associated with physical penetration of the cell wall (Chet, 1987).

1.1.6.2 Antibiosis

The importance of antibiotics for biocontrol activity was demonstrated in several studies. Dennis and Webster (1971a) found that many *Trichoderma* strains produced volatile and nonvolatile antibiotics. In 1983, Howell and Stipanovic isolated and described a new antibiotic, gliovirin, from *T. virens* that was strongly inhibitory to *Pythium ultimum* and a *Phytophthora* species, but not to *R. solani.*

Lumsden et al. (1992) found that suppressive activity of *T. virens* to damping-off of zinnias, incited by both *R. solani* and *P. ultimum*, was correlated with production of the antibiotic gliotoxin by the biocontrol agent.

Wilhite et al. (1994) used mutation to demonstrate that loss of the antibiotic gliotoxin production in *T. virens* mutants had an adverse effect on biocontrol activity.

Trichoderma spp. produces 43 substances that have antibiotic activity which do not include enzymes (Sivasithamparam and Ghisalbetri, 1998). Of these, alkyl pyrones, isonitriles, polyketides, peptaibols, dikeyopiperazines, sesquiterpenes, and steroids have been associated with biocontrol activity of some species and strains of *Trichoderma* (Howell, 1998).

1.1.6.3 Competition and Rhizosphere Competence

Competition for space or nutrients has long been considered one of the classical mechanisms of biocontrol by *Trichoderma* spp. (Elad *et al*., 1999). The competition for nutrients, primarily carbon, nitrogen, and iron is one of

the methods of the biological control of soilborne plant pathogens (Scher *et al*., 1984).

Trichoderma species are generally considered to be aggressive competitors and the ability of *Trichoderma* to compete is species dependent (Wardle *et al*., 1993). However, the excess nutrient in granules containing *T. harzianum* can be used rapidly by *Phytophthora cinnamomi* when the growth of the antagonist was suppressed by lack of oxygen, and the pathogen increase rather than the antagonist (Kelly 1976).

Competition through rhizosphere competence is a mechanism that has gained adherents in recent years (Howell, 2003). It is an important mechanism because a biocontrol agent cannot compete for space and nutrients if it is unable to grow in the rhizosphere.

Lo et al. (1996) found that a strain of *T. harzianum* (T-22) was strongly rhizosphere competent and able to control several plant pathogenic fungi including *R. solani* causing brown patch, and it reduced the initial disease severity by as much as 71% on a variety of crops.

1.1.6.4 Induced Resistance

Induction of resistance in host plant by treatment with the biocontrol agent *Trichoderma* species is another mechanism in biological control (Howell, 2003). Specific strains of fungi in the genus *Trichoderma* colonize and penetrate plant root tissues and initiate a series of morphological and biochemical changes in the plant, considered to be part of the plant defense response, which finally leads to induced systemic resistance (ISR) in the entire plant (De Meyer *et al*., 1998).

Yedidia et al. (1999) showed that inoculating roots of 7-day-old cucumber seedlings in a hydroponic system with *T. harzianum* (T-203) spores to concentration of 10^5 per ml initiated plant defense responses in both roots and leaves of treated plants. Also they demonstrated that hyphae of the biocontrol fungus penetrated the epidermis and upper cortex of the cucumber root.

The plant response was marked by an increase in peroxidase activity (often associated with the production of fungitoxic compounds), an increase in chitinase activity, and the deposition of callose-enriched wall appositions on the inner surface of cell walls (Howell, 2003).

Yedidia et al. (2000) showed that inoculation of cucumber roots with *T. harzianum* (T-203) induced an array of pathogenesis-related proteins, including a number of hydrolytic enzymes which were similar to plants treated with a chemical inducer (2,6-dichloroisonicotinic acid) of disease resistance displayed defense responses.

Resistance elicitation in plants by *Trichoderma* is becoming a more active field of research. Xylanase and other elicitors were produced by *Trichoderma* spp. and proved to induce resistance (Anderson *et al*., 1993). Some *Trichoderma* strains were inducers of systemic acquired resistance-like responses (SAR) (De Meyer *et al*., 1998).

Cucumber plants were larger in the presence of *Trichoderma*, and the cell wall of roots were strengthened in the area of *Trichoderma* penetration, and both chitinase and peroxidase activities in both root and leaf tissues of treated seedlings were evident (Howell *et al*., 1999). Also, they reported that *Trichoderma virens* mutants that lacked both mycoparasitic ability and the

capacity to produce antibiotics were more effective than the parental strains in biocontrol of *Rhizoctonia solani*. This high level of protection was associated with significantly enhanced levels of the various terpenoid phytalexins known to be involved in disease resistance in cotton.

1.1.6.5 Solubilization and Sequestration of Inorganic Plant Nutrients

Plant nutrients undergo sometimes transitions in soil from soluble to insoluble forms that influence their accessibility and absorption by roots. These transitions may be influenced by microorganisms (Altomare *et al*., 1999). Iron and manganese have been investigated with regard to both microbial solubilization of oxidized forms of these elements and their influence on plant disease (Graham & Webb, 1991). In vitro, strain of *Trichoderma harzianum* produces a large number of chemicals to solubilize rock phosphate, Zn, Mn^{4+}, Fe^{3+}, and Cu^{2+} and increase iron availability and enhance iron uptake (Altomare *et al*., 1999).

A direct role for the nutrient solubilization and chelating abilities of *Trichoderma* has not been demonstrated, but circumstantial evidence of its ability to solubilize iron and make it usable to plants is available (Harman, 2000).

1.1.6.6 Inactivation of the Pathogen Enzymes

Enzymes such as chitinases and /or glucanases produced by the biocontrol agent are responsible for suppression of the plant pathogen. These enzymes function by breaking down the polysaccharides, chitin, and β-glucans that are responsible for the rigidity of fungal cell walls, thereby destroying cell wall integrity (Howell, 2003).

Elad and Kapat (1999) suggested that *T. harzianum* (T39) produce proteases that are capable of degrading the pathogens plant cell wall degrading enzymes, and thereby reducing the ability of the pathogen to infect the plant. They also showed that protease may be directly toxic to germination of the pathogen and also may inactivate its enzymes.

1.1.7 Growth and Formulation of *Trichoderma* for Application as a Biocontrol Agent

In 1975, Backman and Rodriguez used molasses-enriched clay granules as a food base for growing the same antagonist (*Trichoderma harzianum* and *Sclerotium rolfsii*) and a carrier to facilitate dispersal in the field. They also observed a significant decrease in *S. rolfsii* damage to peanuts. Kelley (1976) found that these granules were ineffective when used to control the *Phytophthora cinnamomi* in pine seedlings.

In 1979, Hadar *et al*., found that agricultural wastes, such as manure, sawdust, and wheat bran proved to be the best media for the growth and sporulation of *T. harzianum*. They also suggested that the fungal preparation contained $2.9*10^9$ conidia/g dry weight; 80% of *T. harzianum* conidia were viable even after storage for 6 months at either 4 ^oC or at room temperature (24-30 ^oC).

In 1982, Elad *et al*., founded new coating technique, in which seeds were treated with either Pelgel (Nitragin, USA) solution (10% w/v) containing $5*10^8$ conidia/ml as an adhesive, or ethylmercury chloride (350g/ton seed) applied as seed treatment with *Trichoderma* for controlling pathogens in naturally infested soil.

In another study, Sivan et al. (1984) found that a mixture of peat: wheat bran (1:1 v/v) with *Trichoderma* used as medium and its pH kept at about 5.5 survived at 25 ^{o}C for 1 year. The preparation of *T. hamatum* based on the wheat-bran/peat mixture controlled *Pythium aphanidermatum* in pea, cucumber, tomato, and pepper, and *R. solani* and *S. rolfsii* in beans.

The wheat bran preparation of *T. harzianum* increased bean plant growth in non infested soil and controlled *S. rolfsii* more efficiently than conidial suspension and reduced significantly bean diseases caused by *S. rolfsii* and *R. solani* in pathogen- infested soil (Chet, 1987).

Harman and Taylor (1988) found that seed treatments with *Trichoderma* are more effective for some crops than others; e.g. only 20% of cucumber seedlings emerged and 80% of treated tomato seedlings emerged when treated seeds were planted in *Pythium* infested soil.

Lumsden et al. (1993) found that *Trichoderma* species have been used in commercial preparations for biological control of fungal-induced plant diseases. *Trichoderma harzianum* is the active ingredient in TRICHODEX™, which is used against post harvest rot of apple (Ricard, 1981).

Aziz et al. (1997) found that application of *Trichoderma lignorum* as a seed coating (8×10^6 conidia/seed) or wheat bran preparation (1×10^6 cfu/g) at a rate of 20 g/kg soil, greatly reduced the number of bean seeds infested by *Rhizoctonia solani,* and the percentage of healthy seeds reached 92%. They also found that germinating conidia of *Rhizoctonia solani* in bean rhizosphere soil were inhibited after soil or seed application with *Trichoderma lignorum*.

1.2 The Fungus *Rhizoctonia Solani*

1.2.1 History and Host Range

The fungus *Rhizoctonia solani* had been described on potato by Kühn (1858). It is a very common soil-borne pathogen, with worldwide distribution and with a great diversity of host plants including bean, alfalfa, peanut, soybean, cucumber, papaya, eggplant, corn and many more (Ogoshi & Ui, 1983).

1.2.2 Biology

The genus *Rhizoctonia solani* belongs to Form Class Deuteromycetes that does not make vegetative spores and can be present as mycelium, sclerotia or basidiospores.

It produces shade of brown, thread-like growth called hypha (Leach and Garber, 1970). It is characterized by the diameter of vegetative hyphae (8-12 μm), constriction at the point of branching, and right angle branching of matured hyphae (Parmeter and Whitney, 1970). The growth rate is very rapid and a typical isolate can grow across a 90-mm Petri plate in three days (Domand and Flentje, 1970).

Sclerotia are undifferentiated aggregations of thick-walled cells, small (1-3-mm diameter); irregular-shaped, brown to black structures (Sherwood, 1970). They are the primary survival structures and important source of inoculums (Guttierez *et al*., 1997), and formed in soil or on plant residues and have been reported to survive for several years (Sherwood 1970). Sclerotia and basidiospores germinate and form mycelium prior to infection and mycelium has a role in dispersal and survival; it can rapidly grow through and colonize soil and is relatively persistent, especially on colonized debris

(Papavisas, 1970). Basidiospores, responsible for a wide range of leaf infection diseases, are very fragile and are therefore not suited for long term survival (Baker, 1970).

R. solani composed of several anastomosis groups distinguished by hyphal anastomosis (Ogoshi, 1972) and also differ morphologically, physiologically, and serologically (Naiki and Ui, 1978).

1.2.3 Sporulation

Teleomorphs of *Rhizoctonia* occur naturally in the field on host plant and /or soil surfaces, and appear to sporulate on surface of host and non-host plants alike (Gunnell, 1986). Anderson (1982) found that teleomorphs can be induced to form on plant, soil, or agar in the greenhouse or laboratory.

Gunnell (1986) suggested that sporulation was favored by maintaining temperatures between 24-35°C and needs 15- 30 days to appear. Sporulation was associated with the change in environmental factors caused by dehydration of the agar. A greater volume of media was used to prolong the dehydration process (Kangatharalingam and Carson, 1988).

Adams and Butler (1983) studied the effects of nutritional and environmental factors on *Rhizoctonia solani* sporulation, such as concentration of nitrogen and glucose, ventilation, relative humidity, and substrate drying affect sporulation.

1.2.4 Epidemiology

Rhizoctonia solani primarily attacks below ground plant parts such as the seeds, hypocotyls, and roots, but is also capable of infecting above ground plant parts (e.g. pods, fruits, leaves and stems) (Ogoshi, 1987). When *R.*

solani hyphae come in contact with the plant, they start to grow over the plant surface and their hypha surrounds the host but they are attached to plant surface after 10 to 12 hours (Armentrout and Downer, 1987).

The attacking hyphae start to follow the anticline walls of epidermal cells (Kousik *et al*., 1990). Some apprised hyphae that change its growing direction grow in an indiscriminate direction over the epidermal cells, and then after 15 hours a side T- shaped branches are formed at right angles (Armentrout and Downer, 1987) which are a determining characteristic for infection structures of *R. solani*. At this point process can deviate in two manners. The side branch either give rise to short swollen hyphae or appressorial structure or to repetitive formation of T-shaped branches from one or several parent hyphae which result in the formation of complex infection structure after 18 hours.

The complex infection structure can be seen as massive dome shaped structure, attached to the epidermis by the hyphae branches and their swollen tips. Next, several of the swollen tips simultaneously form infection pegs, which penetrate the cuticle and epidermal cell wall (Fukutomi and Takada, 1979).

The infection process is promoted by the production of many different extracellular enzymes that degrade various components of plant cell walls (Kolattukudy 1985).

The most common symptom of *Rhizoctonia* disease is referred to as "damping-off" characterized by non germination of severely infected seeds whereas infected seedlings can be killed either before or after they emerge

from the soil. Infected seedlings not killed by the fungus often have cankers, which are reddish-brown lesions on stems and roots (Sneh, 1996).

Doornik (1981) found that the optimum temperature for pathogenicity of *R. solani* is a characteristic of the fungus isolate and is not influenced by temperature or requirements of the host.

Hartill, (1968) suggested that the infection by the fungus *R. solani* slowly increased with increasing soil moisture from about 30 to 70-80% moisture-holding capacity and decreased at higher moisture levels.

1.2.5 Ecology

The fungus can survive as sclerotia in soil and on plant tissue for many years (Sherwood, 1970). Also, it survives as mycelium by colonizing soil organic matter as a saprophyte, particularly as a result of plant pathogenic activity (Ogoshi, 1987).

Sclerotia and/or mycelium present in soil and/or on plant tissue germinate to produce vegetative threads (hyphae) of the fungus that can attack a wide range of food and fiber crops (Guttierez, *et al*., 1997).

Ui et al. (1965) found that population of *R. solani* increases in the field during crop growing season in the presence of the host crop and suitable temperature and increased through July, decreased in August, and increased again in September and October.

1. 3 The Fungus *Sclerotium Rolfsii*

1.3.1 History, Host Range, and Distribution

The fungus had been described by Rolfs (1892), and named *Sclerotium rolfsii* by Saccordo in 1911. It is an economically important pathogen on numerous crops worldwide (Aycock, 1966). It is an omnivorous and destructive parasite of many plants. It has an extensive host range; at least 500 species in 100 families are susceptible, the most common hosts are legumes, crucifers, and cucurbits (Chupp and Sherf, 1960).

It commonly occurs in the tropics, subtropics, and other warm temperate regions (Punja, 1985). Its growth is optimal at 27-30 ºC and rarely occurs where average winter temperatures fall below 0 ºC.

1.3.2 Biology

Sclerotium rolfsii is an imperfect fungus which belongs to Form class Deuteromycetes. It does not produce spores.

Growth of *S. rolfsii* on all organic-based and inorganic synthetic media is accompanied by forming of spherical, brown to tan colored sclerotia measuring 0.3 to 3.0 mm in diameter (Edelstein *et al*., 1983). They are initially developed as white aggregates or knots of mycelium, then it differentiate to form the mature sclerotium within 2-3 weeks. Sclerotia form abundantly on potato dextrose agar (PDA) and can also be produced on a substrate such as autoclaved oat kernels moistened with 1.5% water agar (Punja and Grogan, 1981). These sclerotia resemble those that form in soil (Punja *et al*., 1984).

The prevalence of *S. rolfsii* in warm regions of the world is a reflection of the high temperature optimal for its growth and sclerotial production. The temperature range for hyphal extension and dry weight production is 8-40°C (Zoberi, 1980); maximum growth and sclerotial formation occur at 27-30°C (Mihail and Alcorn, 1984).

Punja (1985) found that exposing sclerotia to temperature above 50°C for extended period was lethal. The linear growth rate of hyphae on agar media at 27 °C range from 0.85-0.97 mm per hour, depending on the isolate.

Invitro Mustafee and Chattopadhyay (1971) found that mycelium growth was reduced with increasing moisture content.

Punja (1985) found that sclerotia fail to germinate when the relative humidity is much below saturation, while it germinates best at relative humidity of 25-35%.

Growth and sclerotial formation were greater under continuous light especially blue light than in continuous darkness (Miller and Liberta, 1977). Humpherson and Cooke (1977) suggested that the sclerotial number of young (1-7 days old) cultures increased after exposure to light more than those of older cultures (8-14 days old).

Punja and Grogan (1981) suggested that the optimum pH range for mycelial growth is 3.0-5.0, and sclerotial germination occurs between 2.0-5.0. Germination of sclerotia is inhibited at a pH above 7.0 (Sharma and Kaushal, 1979).

1.3.3 Epidemiology and Ecology

The fungus attacks all plant parts in contact with the soil under favorable environmental conditions including stems, roots, leaves, and fruits (Farr *et al*., 1989).

Sclerotia serve as the principle primary inoculums for disease (Aycock, 1966), and may exist free in the soil near the soil surface or in association with plant debris (Backman and Brenneman, 1984). The mature sclerotia contain amino acids, sugars, fatty acids and lipids, and its wall contains chitin, laminarin, and ß-1, 3 glucan (Jones *et al*., 1972, Mathur and Sarbhoy 1977).

Disease incidence increase following periods of temperature and moisture fluctuations; cycles of drying and moistening have been reported to stimulate germination of sclerotia (Smith, 1972). Initial infection by the fungus occurs at the soil surface, where sclerotia are most likely to be stimulated to germinate by drying and remoistening (Punja and Grogan, 1981).

Before the pathogen penetrates host tissue it produces a mass of mycelium on the plant surface, a process which can take 2-10 days (Punja and Grogan, 1981, Punja, 1985).

Penetration of host tissues occurs when the pathogen produces an enzyme, which deteriorates the host's outer cell layer (Sadana *et al*., 1983).

S. rolfsii produce extracellular enzymes including pectin methylesterase (Bateman and Beer, 1965), cutinase (Baker and Bateman, 1978), phosphatidase (Kaveriappa, 1979), arabanase (Cole and Bateman, 1969),

galatanase, mannanase, xylanase (Sadana *et al*., 1980), oxalic acid and polygalacturonases (Bateman, 1972).

Punja and Grogan (1981) have described two forms of germination, hyphal or eruptive. Hyphal germination is characterized by production of individual strands of hyphae from the sclerotial surface, while eruptive germination is characterized by plugs or aggregates of mycelium bursting through the sclerotial surface.

1.3.4 The Factors Influencing Survival

Punja (1985) found that the survival of *S. rolfsii* is affected by abiotic and biotic factors. Exposure of sclerotium to temperature above 50 °C for long periods is lethal (Yuen and Raabe, 1984). Beute and Rodriguez (1981) found that the effect of temperature is modified by moisture; while survival of sclerotia is poor in moist than in dry soil.

Abiotic factors include drying (Smith, 1972), heating (Lifshitz *et al*., 1983), deep burial (Punja *et al*., 1984), exposing sclerotia to chemicals (Linderman and Gilbert, 1973) and inducing changes in the integrity of the sclerotial rind (Lifshitz *et al*., 1983).

Antagonistic microorganisms such as *Trichoderma* spp. (Henis *et al*., 1983) and *Aspergillus* can penetrate the rind and destroy the inner sclerotial tissues; for *Trichoderma*, this is facilitated by the production of the enzymes β-1,3 glucanase and chitinase (Elad *et al*., 1984).

1.4 Biological Control of *R. Solani* and *S. Rrolfsii* Using *Trichoderma*

Biological control of plant diseases, especially soilborne plant pathogens, has been the subject of extensive research in the last two decades.

Trichoderma spp. is well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan *et al*., 1984 and Coley-Smith *et al*., 1991). Biological control of soilborne plant pathogens can be achieved by seed treatment with antagonists.

Harman et al. (1980) reported the biocontrol of *Rhizoctonia solani* and *Pythium* spp. by coating radish and pea seed with *Trichoderma hamatum*.

Hadar et al. (1979) and Elad et al. (1980) found that the application of wheat bran colonized by *Trichoderma harzianum* to soils infested by *Rhizoctonia solani* and *Sclerotium rolfsii* reduced the incidence of disease caused by these pathogens in beans.

Control of soil borne plant pathogens including *R. solani* and *S. rolfsii* can be achieved by different fungicides, soil fumigants and bioagents. Because of the concern regarding the toxicity of these compounds, there is a general trend to reduce the amounts applied to soil. Methyl bromide is a typical example of an efficient fumigant about which the public is concerned because its use causes the accumulation of bromide residues, especially in water (Hoffman and Malkomes, 1974).

Harman et al. (1980) found that *Trichoderma* can survive for long periods of time and propagate in soil when applied with a food base or as a seed coating. Its combination with chemical, cultural, or physical methods can achieve a long-term controlling effect on soil borne plant pathogenic fungi (Katan *et al*., 1976).

Integrating biological and chemical control seems a very promising way of controlling pathogens with minimal interference with biological equilibrium (Baker and Cook, 1982). Hadar et al. (1979) applied small non-

effective doses (1-2 µg/kg) of Pentachloronitrobenzene (PCNB) to soil along with a *Trichoderma* preparation (2g/kg) and the incidence of eggplant disease caused by *R. solani* declined from 40 to 13%, while *T. harzianum* reduced disease incidence to 26%.

Combination of heat treatment and *T. harzianum*, both at sublethal doses and under greenhouse conditions, enhanced control of *S. rolfsii* diseases on beans from 90 to 100% (Elad *et al*., 1980).

Lewis and Papavizas (1980) found that integrated management by preventing colonization of plant residues, and combining *Trichoderma* with plowing to a depth of 20-25 cm reduced root rot of beans caused by *R. solani.*

Transplanting tomato plants treated with *T. harzianum* into soil fumigated with methyl bromide reduced the disease incidence caused by *S. rolfsii* and *R. solani* by 93% and increased yield by 160% (Elad *et al*., 1982).

The addition of pregerminated conidia of *Trichoderma lignorum* to soils reduced germination of conidia of *R. solani*. In non-rhizosphere and rhizosphere soil of bean seedlings previously enriched with conidia of *Rhizoctonia solani* and planted with bean seeds, the germination rate of conidia was 21% and 44 %, respectively. However, when *Trichoderma lignorum* was added as seed coating or conidial suspension, the germination rate of *R. solani* was reduced to 30 and 28 %, respectively in rhizosphere soil and to 12 and 8 %, respectively in non-rhizosphere soil (Cook and Baker, 1983).

Hussain et al. (1990) found that the addition of bean seed exudates increased the percentage of germinated conidia of *R. solani* from 33.7% to 39.0% in absence of *T. lignorum*. Similarly, under greenhouse conditions, the

addition of bean exudates to soil infested with *R. solani* and planted with bean reduced the disease control obtained by *T. lignorum* as either seed coating or conidial suspension. The percentage of damping-off increased from 5.7% to 37.7% in seed coating and from 15.3% to 43.7% in conidial suspension. Addition of germinating bean seed exudate increased the percentage of damping-off from 78.3% to 83.3%.

Aziz et al. (1997) found that the application of a wheat bran preparation of *Trichoderma lignorum* conidia (8×10^6 conidia/seed) at a rate of 15 and 20 g/500 g soil decreased greatly the damping-off percentage to 12% and 6%, respectively, as compared to untreated bean seeds. In addition, he found that application of wheat bran preparation of *Trichoderma lignorum* (5×10^6 cfu/g) at a rate of 2.5 g /500 g of soil decreased the damping-off percentage to 45%.

1.5 Objectives of the Current Research

The Present Work, Therefore, was Aimed at:

1. Surveying the existence of the bio-agent *Trichoderma* in Palestinian agricultural fields.
2. Characterizing the *Trichoderma* strains by studying the average linear growth, optimum temperature range, and mode of action.
3. Screening the antagonistic potential of the recovered *Trichoderma* strains against soil-borne phytopathogens, *Rhizoctonia solani* and *Sclerotium rolfsii* by using dual culture and bioassay techniques.
4. Determining the effects of the most effective *Trichoderma* isolates on plant induced growth response (IGR).
5. Studying hyphal interaction on thin films of agar for the most effective isolates.

Chapter Tow

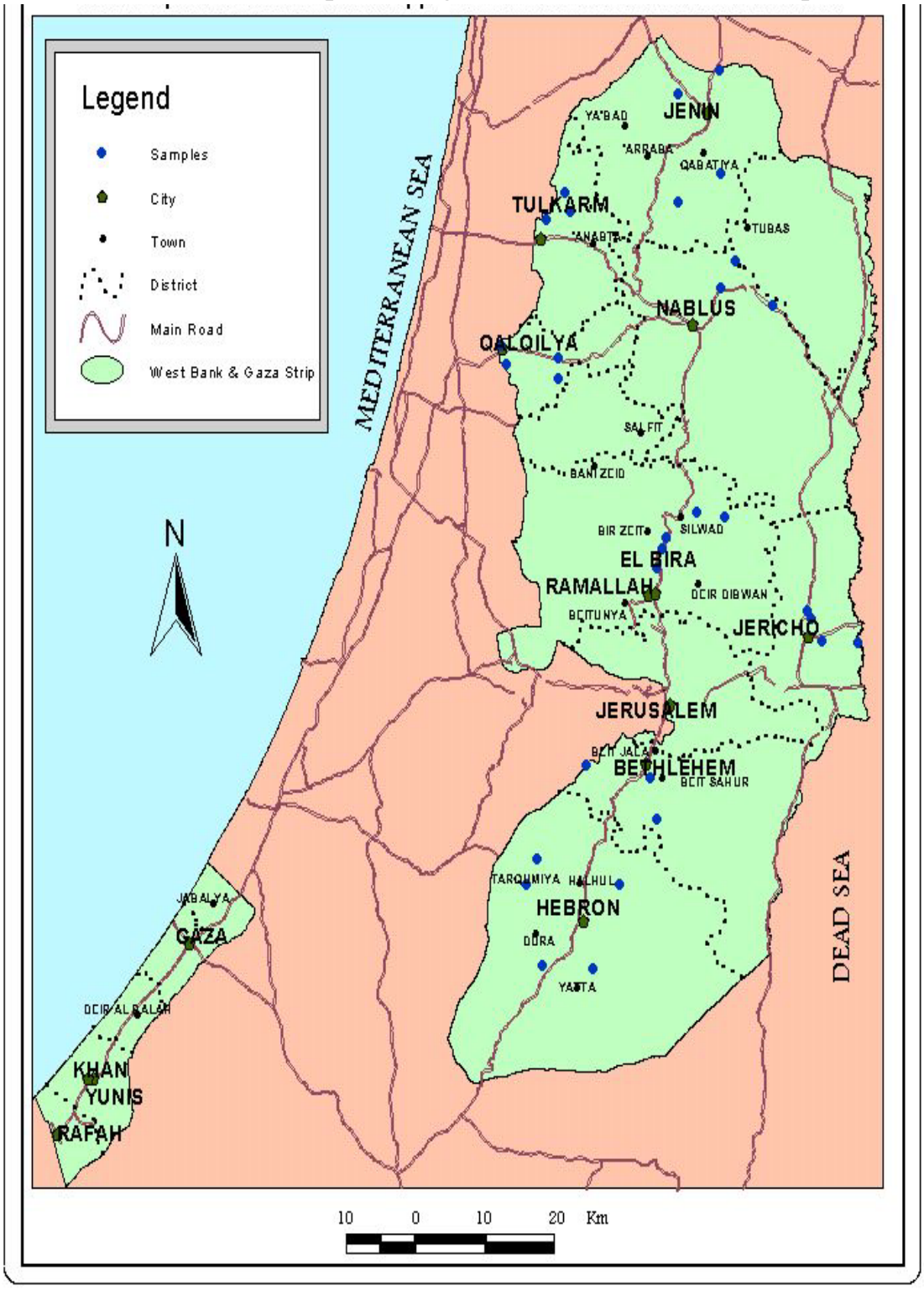
Chapter Tow

2. Materials and Methods

2.1 Collection of Soil Samples

One hundred sixty nine soil samples were collected from thirty locations at eight districts in Palestine-West Bank (Hebron, Jericho, Jenin, Tulkarim, Qalqelia, Ramallah, and Bethlehem) (Figure 2.1). One-liter soil samples were collected from each of irrigated vegetable fields and fruit-tree orchards at a depth of 5-10 cm of soil surface. The samples were collected from open, plastic covered field's solarized or chemically treated (with fungicides such as Dynone and/or Benlate or fumigated with methyl bromide in present or previous agricultural seasons). Each soil sample was placed in a plastic bag and mixed thoroughly by external manipulation by hand through the bag. Twenty-five samples were collected from Hebron area, twenty four from Jericho, thirty two from Jenin, twenty from Qalqilya, eighteen from Tulkarem, fifteen from Nablus, twenty from Ramallah, and fifteen from Bethlehem. Soil samples were collected randomly from the above mentioned areas.

Figure 2.1. West Bank map showing the location of the soil sample collections



2.2 Isolation of *Trichoderma* species From Soil

Trichoderma were isolated from soil samples, as follows: 25 g of soil samples was suspended in 250 ml of 0.1% agar water. Samples were shaked for 20-30 minutes on a rotary shaker at 250 rpm. Serial dilutions 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} were made for each soil sample and aliquot 0.1 ml of soil suspension dispensed onto *Trichoderma* - selective media (TSM) surface with a glass rod (Elad *et al*., 1981). The plates were incubated at 25 ^{0}C for 5-7 days.

After incubation, *Trichoderma* were identified from other fungi based on color, size, shape, and appearance of colony on surface of TSM and then it was transferred to a potato dextrose agar (PDA) medium for purification and further identification. For each soil sample and suspension concentration, 5 plates were considered as replicates.

2.3 Identification of *Trichoderma* Isolates

Trichoderma isolates were grown on PDA in 90-mm Petri dishes, and plates incubated at 25 °C for 7 days. Isolates were placed into groups according to colonial morphology, conidiation color, and color of reverse colony. Sterile glass cover slips, 50 x 25 mm, were held with forceps and immersed in autoclaved melted water agar (20g Difco agar / 1L distilled water) at about 45°C for 1-2 second, and allowed to drain. The cover slip was then laid singly on the surface of 2% solidified water agar in center of 90-mm diameter Petri dishes, so that a thin film of agar set on the upper surface (Laing & Deacon, 1991). Each plate was inoculated with a 5 mm plug cut from the edge of 7 days old growing colonies of each *Trichoderma* isolates on potato dextrose agar (PDA). The plug is then placed 2cm apart of the cover

slip placed on the agar surface, so that *Trichoderma* colony would grow across the coated cover slips. Plates containing coated cover slips were incubated at 25 ± 2 °C and inspected daily for four days for *Trichoderma* mycelium growth. Each cover slip was removed carefully without damaging the fungus mycelium, and then it was inverted on sterile microscopic slide (24.4 x 76.2) and sealed by nail varnish to prevent drying. The growing isolates were studied using fresh direct mounts in Lactephenol cotton blue under medium and high magnifications, x 20, and x40, respectively. The top of the cover slip was cleaned, and microscopic observations were made throughout the coated cover slip and thin film of agar. Three replicates were used for each *Trichoderma* isolate.

2.4 Isolates Mycelial Growth Rate

The method was based on that of Elad et al. (1981). Mycelial growth rate of *Trichoderma* strains was observed on plates containing potato dextrose agar (PDA) as follows: Four Petri dishes (90mm diameter) containing PDA were centrally inoculated with a 5-mm of agar plugs from 7-day-old PDA cultures of each *Trichoderma* isolate to determine the average linear growth of each isolate. Plates were incubated at 25 ±2°C under continuous light and inspected daily for three consecutive days. Radial mycelial growth was recorded every 24 hours during this period.

The colony diameter was measured as the mean of two perpendicular diameters measured at the third day minus the diameter at first day. Average linear growth rates (ALG) were calculated by using formula (Elad *et al*., 1981): **ALG (mm /day) = [C3 – C1] /T**

Where C3: colony diameter in mm after three days, C1: colony diameter in mm after one day of incubation and T: the difference in time (day).

The experimental design used was a completely randomized one with four replicates (plates) for each treatment.

2.5 Dual Culture Interaction

Dual culture interaction between the two pathogenic fungi, *R. solani*, *S. rolfsii* and the mycoparasites *Trichoderma* isolates (47 isolates) were studied using the following method.

A 5-mm diameter mycelial block 7-days-old cut from the margin of each *Trichoderma* isolates and of two pathogens colonies on potato dextrose agar (PDA) placed 3 cm apart on the PDA surface. Five Petri dishes 90 mm diameter were incubated at 25 ±2°C under continuous light and inspected daily for approximately 8-9 consecutive days for mycoparasitism. The fungi colony margins would meet 2-3 days after inoculation. The area interaction of mycoparasite and the host was measured every 24 hours after contact (Dennis & Webster, 1971 b). The experiment was conducted as completely randomized design with five replicate (Petri dishes) used for each host-parasite interaction and the entire experiment was repeated twice.

2.6 Preparation of *Rhizoctonia Solani* Inoculum

Fifty milliliters of potato dextrose broth (PDB) in 250ml Erlenmeyer flasks was inoculated with two 7mm-diameter mycelial disks from 7- days-old PDA cultures of *Rhizoctonia solani* and incubated at 27°C for 10 days.

After incubation, the upper solid layers that grew were washed and air-dried with tissue paper layers.

The amount needed from this preparation (0.9 g dry *Rhizoctonia*/ 0.5 kg soil) was calculated for the whole experiment. The air-dry mycelium in autoclaved 250ml Erlenmeyer flasks was shreded by Ultra thorax in distilled water and then it was passed through a 2-mm sieve to obtain inoculums pieces of 1-2 mm in diameter.

2.7 Preparation of *Sclerotium Rolfsii* Inoculum

PDA media were dispensed (8-10ml) in 90-mm diameter Petri dishes and were centrally inoculated with 5mm-agar plug of 7- day-old PDA cultures of *S. rolfsii* obtained from the lab. of Prof. Ilan Chet (Hebrew University of Jerusalem). The plates were incubated at 27°C for three weeks; the sclerotia were collected from the plates and dried for three days in incubator at 30°C. One hundred (100 mg) dried sclerotia of *S. rolfsii* was used per 0.5 kg of soil.

2.8 Evaluation of Antagonistic Potential in Bioassay

The screening for antagonistic potential was studied using the method of Mihuta and Rowe (1986).

Forty-seven local isolates of *Trichoderma* and another five isolates (three isolates from Egypt and two isolates from the Hebrew University) were tested in growth chamber for antagonisms to *R. solani* and *S. rolfsii*. They were grown on potato dextrose agar (PDA) at 25°C under continuous fluorescent light. After 7-10 days of incubation, conidia were harvested from cultures by flooding the plates with 10 ml of sterile distilled water and removed by agitation with sterile bent glass rod and poured into sterile test

tubes and agitated for 15 sec. with vibrating agitator. The resulting suspensions were filtered through a layer of sterile tissue papers and conidial concentration in the suspension was determined with a haemacytometer and sterile distilled water was added to bring concentration to $3*10^6$ conidia / ml (Mihuta & Rowe, 1986). Four milliliters of each suspension was added to pots containing a 0.5-kg sand soil previously autoclaved at 121°C for 1 hr on three successive days.

The inoculated sand soil was incubated for seven days at 25 °C and then mixed thoroughly with a 0.9 g *R. solani* preparation and 0.1g sclerotia of *S. rolfsii*, respectively.

Each plastic pot (10 cm diameter) filled with non-autoclaved sand to 2/3 of pot volume and then seeded with six bean seeds. The final mixture containing pathogens and bioagent was filled the last 1/3 volume of pots which were previously seeded with bean seeds. The experimental design used was completely randomized with five replicates (pots) for each *Trichoderma* isolate and pathogens. The control soil containing pathogens without the bioagent (*Trichoderma* isolates).

Plants were harvested after three weeks of seeding in growth chamber at 25 ±2°C under a 12-hr photoperiod. All seedlings were uprooted, and hypocotyls were evaluated for infection by *R. solani* and *S. rolfsii* on a scale from 1 to 4: (1= symptomless, 2= small lesions with no hypocotyl constriction, 3= large lesions with some hypocotyl constriction, and 4= hypocotyl girdled).

These ratings were converted to a disease index (DI) value for each pot by using the formula: **DI = 30 * (1A + 2B + 3C +4D) / N**

were A, B, C, and D represent the number of seedlings rated as 1, 2, 3, or 4 respectively; 30 –the number of seeds planted; and N – the number of seedlings rated after three weeks.

2.9 Effect of Temperature on *Trichoderma* Mycelial Growth and Spore Production

The effect of temperatures on linear growth for *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q27, T33, T36, N38, R42, and Egy.52) on PDA under continuous fluorescent light supplemented with Chloramphenicol (25mg/L) was evaluated. Ninety mm diameter Petri dishes were inoculated with 5mm agar plugs from 5-day-old PDA cultures of isolates mentioned above. Plates were randomly incubated at different temperatures including 10, 15, 20, 30, 35, and 40 °C with four replicates per treatment. Radial mycelial growth was measured as the mean of two perpendicular diameters after 48 hours minus that after 24 hours of mycelial growth. Radial mycelial growth rate was calculated in cm^2/day.

At the end of mycelial growth study, the plates of all *Trichoderma* isolates were incubated for 1 more week and used for the assessment of spore production. Spores were harvested by flooding the plates with 10 ml of distilled water and then agitated with a glass rod. The resulting suspensions were filtered through a layer of sterile filter papers and spore concentrations from the four plates/temperature were counted using a haemacytometer under microscopic power of 40X (Zhang & Watson, 1997).

2.10 Effect of Temperature on Antagonism in Dual Culture

Dual culture interaction between the *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q27, T33, T36, N38, R42, and Egy.52) and pathogenic fungi (*R.*

solani, *S. rolfsii*) was studied based on the method of Prasun & Kanthadai (1997). Four replicates of 90 mm diameter PDA plates were inoculated with 7mm mycelial discs of 7-day-old *Trichoderma* isolates in addition to *R. solani* or *S. rolfsii* at a distance of 3 cm apart from each other. The plates were incubated at 15, 25, 30, and 35 °C for 3-5 days in closed polyethylene bags lined with moist tissue papers (to prevent desiccation of the media), and observed regularly for ability of one fungus to restrict the growth, or to overgrow the other.

The experimental design was completely randomized with four replicates (plates) for each treatment.

2.11 Effect of Temperature on Production of Toxic Metabolites by *Trichoderma* Isolates (Antibiosis)

The ability of *Trichoderma sp*. to inhibit the mycelial growth of *R. solani* and *S. rolfsii* through production of fungitoxic metabolites at different temperatures was tested according to the method mentioned by Dennis & Webster (1971 c).

Fifty ml of potato dextrose broth (PDB) pH 6 in 250 ml Erlenmeyer flasks was inoculated with 7mm-agar disk from 7-day-old PDA cultures of three *Trichoderma* isolates J8, Jn14, and T36 and incubated at 15, 20, 25, 30, and 35 °C without shaking. After 10 days of incubation, the cultures were filtrated through Millipore membrane filter (0.45 μm) and were autoclaved at 121°C for 15 minutes.

The culture filtrate (1.2 ml) was placed in Petri dishes (90-mm diameter) and approximately 12 ml of PDA was added and mixed with the filtrate (10% v/v). The filtrate-amended PDA plates were then centrally

inoculated with 7-mm mycelial plugs of *R. solani* and *S. rolfsii*. Plates were incubated at 15, 20, 25, 30, and 35 °C with four replicates; unamended PDA served as control. The linear growth rate of *R. solani* and *S. rolfsii* was measured after 48 hour as cm^2 /day and percent inhibition was calculated.

The experimental design was completely randomized with four replicates (plates) for each treatment.

2.12 Hyphal Interaction on Thin Films of Agar

This procedure was done according to that of Laing & Deacon (1991). Hyphal interaction was made on sterile glass cover slips coated with 2% water agar (20g Difco agar / 1L distilled water). Each cover slip was immersed for 1-2 sec in autoclaved melted water agar at about 45 °C, allowed to drain and then placed on the surface of 2% solidified water agar in a 90mm diameter Petri dishes, so that a thin film of agar set on the upper surface.

Five mm disk of one week old growing colonies cut from the margin of each of *R. solani*, *S. rolfsii* and *Trichoderma* isolates were placed 3 cm apart on the agar surface and then incubated at 25 ±2°C. Cultures were inspected daily for mycoparasitism; host and mycoparasite colony margins should meet across the coated coverslips in less than three days.

Each cover slip was removed carefully without damaging the mycelial contact and then it was inverted on a sterile microscopic slide (25.4 x 76.2). Microscopic examination was carried out through the coated cover slip using fresh direct mounts in Lactephenol cotton blue under medium and high magnifications x 20, x40, respectively. Specimens were always sealed by nail varnish to prevent drying.

Mycoparasitism was indicated by hyphal coiling and internal colonization of the host hyphae (*R. solani* and *S. rolfsii*) by the mycoparasite *Trichoderma*.

2.13 Effect of *Trichoderma* on Plant Increased Growth Response (IGR)

The ability to induce and increase the emergence of seedling, increase plant height, and dry weight were tested. Fifteen *Trichoderma* isolates (H2, H3, H4, J8, J9, Jn14, Jn18, Jn21, Q26, Q27, T36, T37, N38, R42, and B47) were used. The strains were grown on plate's (90-mm in diameter) containing potato- dextrose agar for 10 days at 25 °C under illumination. Conidia were harvested from the cultures, washed several times in water, and suspended in 0.001% Tween 20 (Polyoxyethylenesorbitan, Sigma- aldrich. Com.) (Chang *et al*., 1986). These suspensions were added to the peat moss growth substrate at a concentration of $5x10^6$ conidia per gram of soil and incubated for 14 days at 25 ± 2 °C. After 14 days, six bean (*Phaseolus vulgaris*) seeds were sown per pot and five replicates were employed. Plants were grown in a growth chamber at 25 ± 2 °C for 4 weeks. The various measurements of plant growth responses were made including number and time of emergence of seedlings. Plant heights were measured from soil surface to apical buds. Concerning fresh and dry weights, plants were washed under running tap water to remove soil from roots; plants were then dried at 80 °C in drying oven after recording fresh weights. After 72hr, plant dry weights were determined (Shenker *et al*., 1992).

2.14 Statistical Analysis

The results of all experiments were analyzed statistically using One-Way Analysis of Variance (ANOVA) to test for significance, and the Fisher Test was used for mean separations by SigmaStat Software (1999).

Chapter Three

Chapter Three

3. Results

3.1 Isolation of *Trichoderma* Species from Soil

Forty-seven isolates of *Trichoderma* species were recovered from 169 soil samples collected from different locations in the Palestinian areas of the West Bank during the period between April and September 2000 (Table 3.1).

The numbers of *Trichoderma* isolates recovered were 7, 3, 13, 7, 7, 3, 3, and 4 *Trichoderma* from Hebron, Jericho, Jenin, Qalqilya, Tulkarem, Nablus, Ramallah, and Bethlehem, respectively, obtained from the rhizosphere of different soil types (Table 3.1). The average percentage of *Trichoderma* isolates recovered to soil samples collected was 27.8%.

Trichoderma isolates were recovered according to Elad et al (1981) method using *Trichoderma* selective medium (TSM). Cultures were then subcultured on PDA for purification.

The percentage of isolates recovered to soil samples in respect to location were 28%, 12.5%, 40.6%, 35%, 38.9%, 20%, 15%, and 26.7% in Hebron, Jericho, Jenin, Qaqilya, Tulkarem, Nablus, Ramallah, and Bethlehem, respectively (Figure 3.1). Of the 47 isolates recovered, Forty-four isolates were recovered from irrigated vegetable fields fertilized with manure and solarized in current or previous season; two isolates were recovered from fields planted with grape and banana in Jericho, and one isolate from field planted with thyme in Ramallah.

In addition, fourteen isolates out of the forty four isolates were collected from soil fumigated with methyl bromide and/or drenched with fungicides Dynone and Benlate in present or previous agricultural seasons (H2, H3, J9, Jn12, Jn17, Jn18, Q27, Q28, Q29, Q30, T31, N38, B46, and B47) (Table3.2).

Eleven isolates were recovered from greenhouses (H2, H3, H7, Jn12, Q27, Q28, Q29, Q30, N38, B46, and B47) (Table 3.2).

The *Trichoderma* isolates were named as (X B). Where X: the first letter of the name of district area and B: the serial number of the isolate (Table 3.2).

Table 3.1 Number of *Trichoderma* isolates recovered from Soil samples collected from different locations in the West Bank

Area	**Site**	**# of Soil Samples Collected**	**# Of Isolates Recovered**	**Local *Trichoderma* Isolates Recovered**
Hebron	Al- Majnunah	5	3	H1*, H2, H3
	Tarqumia	5	1	H4
	Biet aula	5	1	H5
	Al- Majur	5	0	-
	Wad Fraides	5	2	H6, H7
Jericho	Ketif Al-Wad	6	1	J8
	Maghtas St.	6	0	-
	Qaser Hisham St.	6	1	J9
	Ayne Al-Sultan	6	1	J10
Jenin	Kufrathan	8	0	-
	Al Jalameh	8	3	Jn11, Jn12, Jn13
	Qabatya	8	4	Jn14, Jn15, Jn16, Jn17
	Al-Jarbaah	8	6	Jn18, Jn19, Jn20, Jn21, Jn22, Jn23
Qalqilya	Hableh	5	4	Q24, Q25, Q26, Q27
	Azon Atmeh	5	1	Q28
	Qalqilya	5	0	-
	Azon	5	2	Q29, Q30
Tulkarem	Shwakeh	6	4	T31, T32, T33, T34
	Deir Al-Ghson	6	0	-
	Attel	6	3	T35, T36, T37
Nablus	Al-Bathan	5	1	N38
	Al-Nassareyeh	5	1	N39

Area	**Site**	**# of Soil Samples Collected**	**# Of Isolates Recovered**	**Local *Trichoderma* Isolates Recovered**
	Al-Faraah	5	1	N40
Ramallah	Ayne Samyah	5	1	R41
	Ayne Senya	5	1	R42
	Dura Al-Qareh	5	1	R43
	Mazraah Al-Qebleyah	5	0	-
Bethlehem	Artas	5	2	B44, B45
	Wad Fuken	5	0	-
	Tequa	5	2	B46 , B47

* X B: where X: the first letter of the name of district area and B: the serial number of the isolate.

Table 3.2 *Trichoderma* isolates recovered from soil in different districts and sites in the West Bank.

Isolate No.	Area	Site	Crop	Soil treatment
H1	Hebron	Al- Majnunah	Cabbage & Cauliflower (Open field)	Non-treated
H2	Hebron	Al- Majnunah	Cucumber (Greenhouse)	Irrigated by fungicide; Dynone in present season and fumigated with methyl bromide in previous season
H3	Hebron	Al- Hejreh	Cucumber (Greenhouse)	Irrigated by fungicide; Dynone in present season
H4	Hebron	Tarqumia	Tomato (Open field)	Non-treated
H5	Hebron	Biet aula	Tomato (Open field)	Non-treated
H6	Hebron	Al- Majur	Squash (Open field)	Non-treated
H7	Hebron	Wad Fraides	Cucumber (Greenhouse)	Non-treated
J8	Jericho	Ketif Al-Wad	Grape (Open field)	Non-treated
J9	Jericho	Qaser Hisham St.	Jews mallow (Open field)	Irrigated by fungicide; Dynone in previous season
J10	Jericho	Ayne Al-Sultan	Banana (Open field)	Non-treated
Jn11	Jenin	Al-Jalameh	Cucumber (Open field)	Non-treated
Jn12	Jenin	Al-Jalameh	Cucumber (Greenhouse)	Irrigated by fungicides; Dynone and Benlate in present season
Jn13	Jenin	Al-Jalameh	Pepper (Open field)	Non-treated
Jn14	Jenin	Qabatya	Cucumber (Open field)	Non-treated
Jn15	Jenin	Qabatya	Tomato (Open field)	Non-treated
Jn16	Jenin	Qabatya	Eggplant (Open field)	Non-treated
Jn17	Jenin	Qabatya	Pepper & Bean (Open field)	Irrigated by fungicide; Dynone in present season

Isolate No.	Area	Site	Crop	Soil treatment
Jn18	Jenin	Al-Jarbaah	Cauliflower (Open field)	Irrigated by fungicide; Dynone in present season
Jn19	Jenin	Al-Jarbaah	Eggplant (Open field)	Non-treated
Jn20	Jenin	Al-Jarbaah	Tomato (Open field)	Non-treated
Jn21	Jenin	Al-Jarbaah	Pea (Open field)	Non-treated
Jn22	Jenin	Al-Jarbaah	Bean (Open field)	Non-treated
Jn23	Jenin	Al-Jarbaah	Pepper (Open field)	Non-treated
Q24	Qalqilya	Hableh	Bean (Open field)	Non-treated
Q25	Qalqilya	Hableh	Tomato (Open field)	Non-treated
Q26	Qalqilya	Hableh	Pepper (Open field)	Non-treated
Q27	Qalqilya	Hableh	Cucumber (Greenhouse)	Irrigated by fungicide; Dynone in present season
Q28	Qalqilya	Hableh	Tomato (Greenhouse)	Irrigated by fungicides; Dynone and Benlate in present season
Q29	Qalqilya	Azon Atmah	Cucumber (Greenhouse)	Irrigated by fungicide; Dynone in present season and fumigated with methyl bromide before four years.
Q30	Qalqilya	Azon	Cucumber (Greenhouse)	Irrigated by fungicide; Dynone in present season
T31	Tulkarem	Ezbet Shufiah	Jews mallow (Greenhouse)	Irrigated by fungicide; Dynone in present season.
T32	Tulkarem	Shwakeh	Eggplant (Open field)	Non-treated
T33	Tulkarem	Shwakeh	Tomato (Open field)	Non-treated
T34	Tulkarem	Shwakeh	Pepper (Open field)	Non-treated

Isolate No.	Area	Site	Crop	Soil treatment
T35	Tulkarem	Attel	Cauliflower (Open field)	Non-treated
T36	Tulkarem	Attel	Bean (Open field)	Non-treated
T37	Tulkarem	Attel	Squash (Open field)	Non-treated
N38	Nablus	Al-Bathan	Bean (Open field)	Fumigated with methyl bromide in present season.
N39	Nablus	Annassareyeh	Pepper (Open field)	Non-treated
N40	Nablus	Al-Faraah	Cucumber (Open field)	Non-treated
R41	Ramallah	Ayne Samyah	Thyme (Open field)	Non-treated
R42	Ramallah	Ayne Senya	Eggplant (Open field)	Non-treated
R43	Ramallah	Dura Al-Qareh	Bean (Open field)	Non-treated
B44	Bethlehem	Artas	Bean (Open field)	Non-treated
B45	Bethlehem	Artas	Bean (Open field)	Non-treated
B46	Bethlehem	Tequa	Tomato ,Cucumber (Greenhouse)	Fumigated with methyl bromide before three years.
B47	Bethlehem	Tequa	Tomato (Greenhouse)	Fumigated with methyl bromide in present season

3.2 Identification of *Trichoderma* Isolates

Most effective Local *Trichoderma* fungal isolates (H2, J8, Jn14, Jn17, Jn21, Q28, T33, T36, N38, R42, and B47) were placed into groups according to their conidial morphology, color and texture, and growth characteristics. Microscopic examination was carried out according to Bissett (1984, 1991 a, b, c) classification method (Table 3.3).

The isolate (H2), *Trichoderma lacteal* was characterized by limited aerial mycelium, white in color and arachnoid; culture reverse was dull yellow in color. Conidiation effuse, and white; conidiophores hyaline, straight and stout, wide near the base, unbranched in the lower part, irregularly branched toward the apex. Phialides subulate, arising singly or in whorls. Conidia ellipsoid to ovoid, it belongs to *Trichoderma* section *Hypocreanum*.

The isolates (J8, Jn17, Jn21, T33, R42 and B47) belonged to *Trichoderma* section *Longibrachiatum.* Species in this section were characterized by sparsely branched conidiophores having a high proportion of solitary phialides, in addition to the production of characteristic bright yellow-green pigments visible in the colony reverse. The isolates (T33 and B47) were identified as *T. citrinoviride;* conidia were ellipsoid and smaller than in the other species, conidiation formed yellow-green shades or was dark olive in older cultures. The isolate (Jn17) identified as *T. atroviride,* has relatively large, ellipsoidal conidia and a very sparse branching system with curved or sinuous conidiophores branches and phialides. The isolate (J8) identified as *T. viride* has conidiophores with side branches relatively long and rebranched several times; branches often curved or sinuous.

The isolate (Jn21) identified as *T. pseudokoningii* has conidiophores branching similar to *T. citrinoviride;* colonies with conidial areas widely effused and not forming pustules; conidiation form bluish-green shades and not darkening appreciably in age. *T. koningii* (isolate R42) was characterized by phialides arising predominately in false whorls and does not produce intercalary phialides.

The isolates (Jn14, Q28 and T36) belonged to section *Pachybasium* which is characterized by broad or inflated conidiophores elements and phialides, which give the conidiophores a stout or rigid appearance. Phialides were ampulliform, divergent, and arranged in crowds on terminal branches of conidiophore that are repeatedly branched and rebranched at an indefinite number of levels. In addition, many species have conspicuous, sterile elongation of the conidiophore main axis.

Table 3.3 Identification of the local *Trichoderma* isolates (H2, J8, Jn14, Jn17, Jn21, Q28, T33, T36, N38, R42, and B47).

Isolate	***Trichoderma* species**
H2	*Trichoderma lacteal*
J8	*Trichoderma viride*
Jn14	*Trichoderma harzianum*
Jn17	*Trichoderma atroviride*
Jn21	*Trichoderma pseudokoningii*
Q28	*Trichoderma hamatum*
T33	*Trichoderma citrinoviride*
T36	*Trichoderma hamatum*
R42	*Trichoderma koningii*
B47	*Trichoderma citrinoviride*

3.3 Isolates Mycelial Growth Rate (Average Linear Growth)

Average linear growth rates (ALG) were calculated by using the formula: ALG (mm /day) = [C3 – C1] /T. The mean of average linear growth rate for isolates ranged from 10.67 mm/day for isolate T34 and

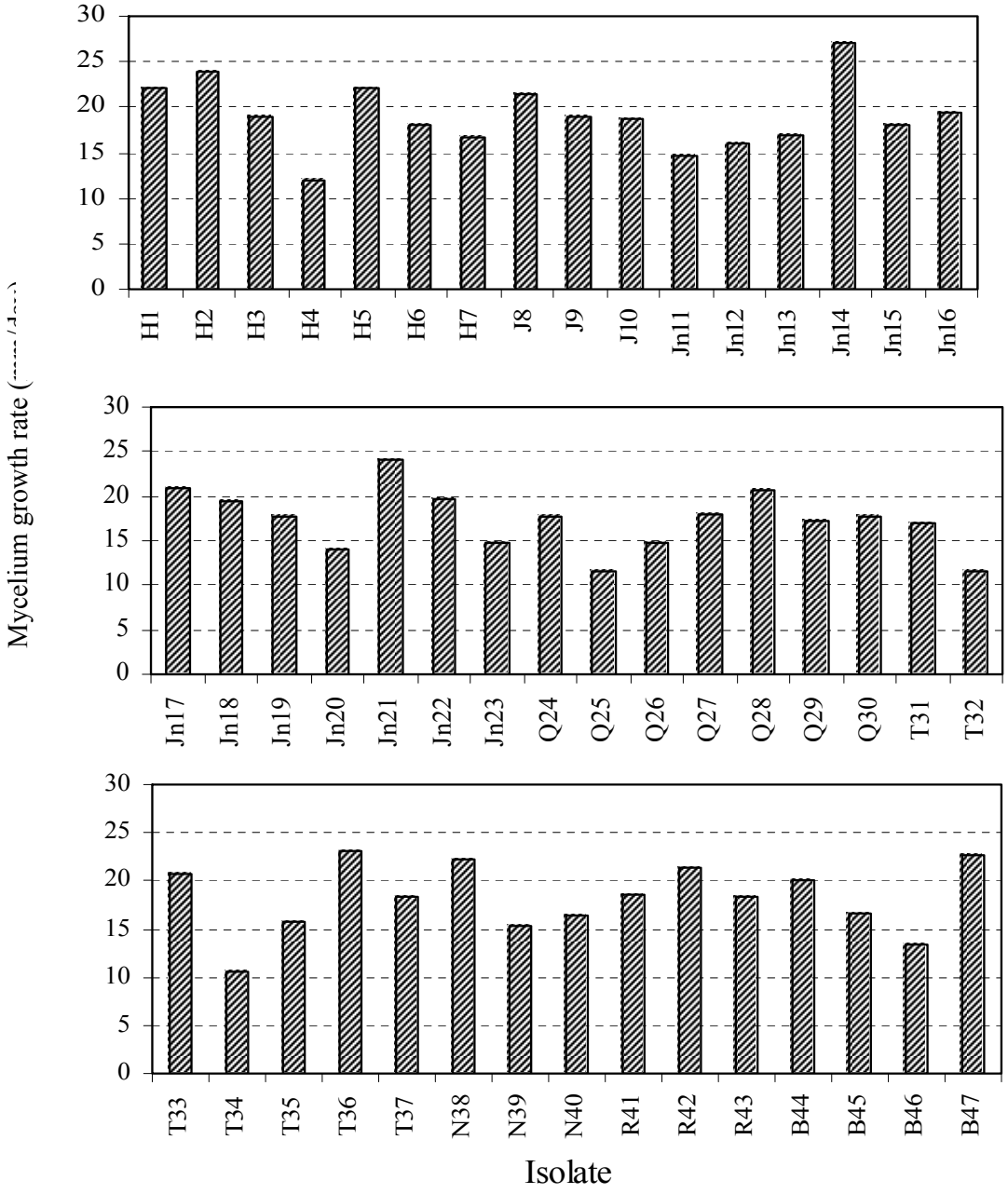
27.0 mm/day for isolate J14. Results of radial mycelial growth rate of the local *Trichoderma* isolates growing on PDA are presented in Table 3.4 and Fig. 3.1. The radial mycelial growth rate in mm/day between local *Trichoderma* isolates varied according to the isolate tested. The highest radial mycelium growth rate was 27.0 mm/ day for the isolate Jn14 later identified as *Trichoderma harzianum.*

Table 3.4 *Trichoderma* isolates average linear growth rate (mm/day) growing on PDA after 4-days of incubation at 25°C

Isolate	Mycelium Growth Rate (mm/day)	Isolate	Mycelium Growth Rate (mm/day)
Jn14	27.00* ± 1.00 a	**H6**	18.00 ± 1.00 g h i j k l m
H2	24.00 ± 1.00 b	**Jn15**	18.00 ± 3.61 g h i j k l m
Jn21	24.00 ± 1.00 b	**Jn19**	17.67 ± 2.52 g h i j k l m
T36	23.00 ± 1.00 b c	**Q30**	17.67 ± 2.52 g h i j k l m
H5	23.00 ± 1.00 b c	**Q24**	17.67 ± 1.53 g h i j k l m
B47	22.67 ± 1.16 b c d	**Q29**	17.33 ± 0.58 g h i j k l m n
N38	22.33 ± 2.52 b c d	**T31**	17.00 ± 2.00 h i j k l m n
J8	21.33 ± 1.56 b c d e	**Jn13**	17.00 ± 1.00 h i j k l m n
R42	21.33 ± 1.53 b c d e	**B45**	16.67 ± 1.53 i j k l m n o
H1	21.17 ± 1.44 c d e	**H7**	16.67 ± 2.08 i j k l m n o
Jn17	21.00 ± 1.00 c d e f	**N40**	16.33 ± 1.53 j k m n o
Q28	20.67 ± 2.08 c d e f	**Jn12**	16.00 ± 1.00 k l m n o p
T33	20.67 ± 0.58 c d e f	**T35**	15.67 ± 2.08 l m n o p
B44	20.00 ± 1.00 d e f g	**N39**	15.33 ± 1.53 m n o p
Jn22	19.67 ± 0.58 d e f g h	**Jn11**	14.67 ± 1.53 n o p
Jn16	19.33 ± 1.53 e f g h i	**Q26**	14.67 ± 1.53 n o p
Jn18	19.33 ± 2.52 e f g h i	**Jn23**	14.67 ± 2.52 n o p
H3	19.00 ± 1.00 e f g h i j	**H4**	14.00 ± 2.00 o p q
J9	19.00 ± 1.00 e f g h i j	**Jn20**	14.00 ± 1.00 o p q
R41	18.67 ± 0.58 e f g h i j k	**B46**	13.33 ± 1.53 p q
J10	18.67 ± 1.53 e f g h i j k	**Q25**	11.67 ± 2.08 q r
R43	18.33 ± 2.08 f g h I j k l	**T32**	11.67 ± 2.52 q r
T37	18.33 ± 0.58 f g h I j k l	**T34**	10.67 ± 2.08 r
Q27	18.00 ± 2.00 g h i j k l m		

* Mean of three replicates ± standard deviation followed by the same letters within columns are not statistically significant according to Fisher LSD method (LSD= 2.714, *P*=0.05)

Figure 3.1. Mycelium growth rate (mm/day) of local *Trichoderma* isolates collected from Palestinian area growing on PDA culture and incubated at 25 ºC.



3.4 Mycoparasitism in Dual Culture

The ability of local *Trichoderma* isolates to inhibit the mycelial growth of *Rhizoctonia solani* and *Sclerotium rolfsii* in dual culture was determined on PDA medium. Results of mycoparasitism in dual culture for

all *Trichoderma* isolates are presented in (Table 3.5, 3.6 and Fig. 3.2). By 24 hr after interaction between mycelia of the bioagent *Trichoderma* isolates and the pathogens (*R. solani* & *S. rolfsii*) mycelia, a clear zone of interaction was formed on PDA media.

The results of interactions between the local *Trichoderma* isolates mycelia and the pathogen *R. solani* mycelia indicate that there is a statistically significant difference in the overgrowth rate between the different isolates on the *R. solani* (LSD= 0.867; F= 31.055; P <0.05) (Table 3.5 and Fig. 3.2) (See appendix B for ANOVA tables).

The overgrowth rate (mm/day) was measured during 10 days after contact between isolates and pathogens. The results also indicate that overgrowth rate of *Trichoderma* isolates on *R. solani* is faster three times at least than *S. rolfsii*. The mean of overgrowth rate (mm/day) of *Trichoderma* isolates on *R. solani* ranged from 9.9 for *Trichoderma* isolate Jn17 (*Trichoderma atroviride*) and 2.4 for *Trichoderma* isolate H5. Results showed that the most effective *Trichoderma* isolates against *R. solani* were (Jn17, J8, J9, T36, Jn18, Jn14, Jn21, and H1).

However the mean overgrowth rate of *Trichoderma* isolates on *S. rolfsii* ranged from 3.25 for isolate J10 and 0.35 for isolate Jn22 and the most effective isolates were (J10, T36, and T33).

The results of interactions showed that there is a statistically significant difference in the overgrowth rate between the different isolates on the *S. rolfsii* (LSD= 0.445; F= 25.26; P <0.05) (Table 3.6 and Fig. 3.2) (See appendix B for ANOVA tables).

Table 3.5 Mycelium Overgrowth Rate (mm/day) of *Trichoderma* isolates on phytopathogen *R. solani* in dual culture both growing on PDA medium and incubated at 25°C.

Isolate	Mycelium Overgrowth Rate (mm/day)	Isolate	Mycelium Overgrowth Rate (mm/day)
Jn17	9.90* ± 0.52 a	**B44**	7.30 ± 0.76 j k l
J8	9.75 ± 0.31 a b	**Jn20**	6.95 ± 0.93 k l m
J9	9.50 ± 1.29 a b c	**B45**	6.75 ± 0.47 l m n
Jn18	9.30 ± 0.67 a b c d	**Jn23**	6.75 ± 0.95 l m n
T36	9.30 ± 1.02 a b c d	**Q27**	6.65 ± 0.68 l m n o
Jn14	9.25 ± 0.73 a b c d	**B46**	6.40 ± 0.38 m n o p
Jn21	9.10 ± 0.91 a b c d e	**H3**	6.30 ± 1.20 m n o p
H1	9.05 ± 0.48 a b c d e	**Jn11**	6.20 ± 0.78 m n o p
H2	8.95 ± 0.33 b c d e f	**H4**	6.05 ± 1.34 n o p
N40	8.85 ± 0.46 c d e f g	**N39**	6.00 ± 1.08 n o p q
R42	8.60 ± 0.14 d e f g h	**J10**	5.90 ± 0.22 n o p q r
H7	8.55 ± 0.67 d e f g h	**B47**	5.85 ± 0.93 o p q r s
Jn15	8.35 ± 0.58 e f g h i	**T35**	5.60 ± 0.68 p q r s t
T33	8.30 ± 0.54 e f g h i	**Q29**	5.56 ± 0.60 p q r s t
Q28	8.30 ± 0.93 e f g h i	**T31**	5.15 ± 0.55 q r s t
T37	8.30 ± 0.74 e f g h i	**T32**	5.15 ± 0.42 q r s t
Jn12	8.10 ± 0.34 f g h i j	**T34**	5.05 ± 0.60 r s t
N38	8.05 ± 0.54 f g h I j	**Jn19**	5.00 ± 0.75 s t
Jn13	8.00 ± 0.35 g h i j	**H6**	4.95 ± 0.54 t u
R41	7.95 ± 0.41 h i j	**Q25**	4.95 ± 0.60 t u
R43	7.85 ± 0.60 h i j	**Q26**	4.90 ± 0.58 t u
Q30	7.75 ± 0.64 h i j k	**Q24**	4.10 ± 0.34 u
Jn22	7.65 ± 0.84 I j k	**H5**	2.4 ± 0.22 v
Jn16	7.50 ± 0.43 I j k l		

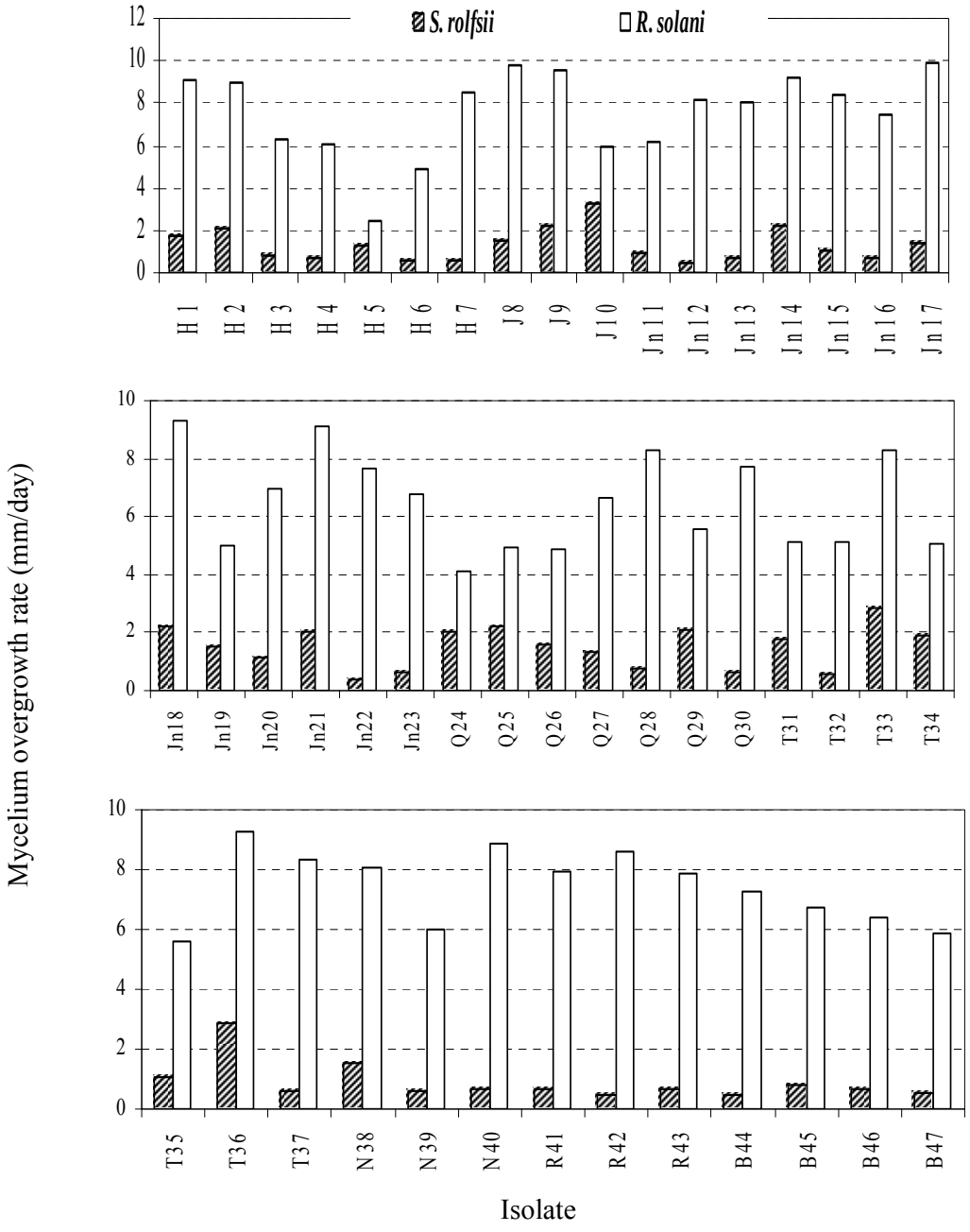
* Mean of five replicates ± standard deviation followed by the same letters within columns are not statistically significant according to Fisher LSD method (LSD= 0.867, *P*=0.05)

Table 3.6 Mycelium Overgrowth Rate (mm/day) of *Trichoderma* isolates on phytopathogen *S. rolfsii* in dual culture both growing on PDA medium and incubated at 25°C.

Isolate	Mycelium Overgrowth Rate (mm/day)	Isolate	Mycelium Overgrowth Rate (mm/day)
J10	3.25* ± 0.20 a	**Jn11**	0.95 ± 0.27 j k l m n o
T33	2.85 ± 0.22 a	**H3**	0.85 ± 0.14 k l m n o p
T36	2.85 ± 0.34 a	**B45**	0.80 ± 0.11 l m n o p q
Jn14	2.25 ± 0.35 b	**H4**	0.75 ± 0.18 m n o p q
J9	2.25 ± 0.20 b	**Q28**	0.75 ± 0.18 m n o p q
Jn18	2.19 ± 0.69 b c	**B46**	0.69 ± 0.13 n o p q
Q25	2.19 ± 0.24 b c	**N40**	0.69 ± 0.24 n o p q
H2	2.15 ± 0.40 b c	**R41**	0.65 ± 0.22 n o p q
Q29	2.06 ± .024 b c d	**R43**	0.65 ± 0.14 n o p q
Q24	2.05 ± 0.33 b c d	**Jn13**	0.65 ± 0.14 n o p q
Jn21	2.00 ± 0.53 b c d e	**Jn23**	0.65 ± 0.14 n o p q
T34	1.88 ± 0.63 b c d e f	**Q30**	0.65 ± 0.14 n o p q
H1	1.75 ± 0.59 c d e f g	**Jn16**	0.65 ± 0.29 n o p q
T31	1.75 ± 0.25 c d e f g	**N39**	0.63 ± 0.14 n o p q
Q26	1.60 ± 0.22 d e f g h	**H7**	0.63 ± 0.14 n o p q
N38	1.56 ± 0.38 e f g h	**T37**	0.60 ± 0.14 n o p q
J8	1.50 ± 0.47 f g h i	**H6**	0.56 ± 0.24 n o p q
Jn19	1.50 ± 0.59 f g h i	**B47**	0.56 ± 0.38 o p q
Jn17	1.40 ± 0.49 g h i j	**T32**	0.55 ± 0.11 o p q
Q27	1.30 ± 0.65 g h i j k	**Jn12**	0.44 ± 0.13 p q
H5	1.25 ± 0.25 h i j k l	**B44**	0.44 ± 0.13 p q
Jn20	1.15 ± 0.14 h i j k l m	**R42**	0.44 ± 0.24 p q
T35	1.05 ± 0.11 i j k l m n	**Jn22**	0.35 ± 0.22 q
Jn15	1.00 ± 0.35 j k l m n o		

* Mean of five replicates ± standard deviation followed by the same letters within columns are not statistically significant according to Fisher LSD method (LSD= 0.445, *P*=0.05).

Figure 3.2. Mycelium overgrowth rate (mm/day) of local *Trichoderma* isolates on *Sclerotium rolfsii* and *Rhizoctonia solani* on dual PDA culture incubated at 25 ºC.



3.5 Evaluation of Antagonistic Potential in Bioassay

Forty seven of local *Trichoderma* isolates and five more isolates (three from Egypt and two isolates from Hebrew University) were tested in growth chamber as antagonists for biocontrol of *Rhizoctonia solani* and *Sclerotium rolfsii* on bean (*Phaseolus vulgaris)*.The tested *Trichoderma* isolates reduced disease index of bean plants caused by *R. solani* and *S. rolfsii* in different rates; means of disease index ranged from 38.2 to 111for *Trichoderma* isolates used against *R. solani* and from 37.8 to 114 for *Trichoderma* isolates used against *S. rolfsii* (Table 3.7, 3.8 and Fig. 3.3).

Results indicates that most of *Trichoderma* isolates significantly reduced the index of Rhizoctonia damping-off on bean (LSD= 19.588; F= 6.595; P≤0.05). Of the 52 isolates Forty four *Trichoderma* isolates (Jn14, R42, Jn21, T33, T36, H2, N38, J8, Q28, J9, H3, H4, T37, N39, Q26, Jn17, Jn18, B47, GH11, Egy52, Q24, Q29, J10, Q27, Egy50, Q30, R43, Jn20, Q25, Jn11, Egy20, Jn19, Jn23, Y, H7, T35, T31, R41, H5, T32, H6, Jn15, Jn12, and Jn16) significantly reduced disease index by 18.2%- 65.6%.

Disease index on bean seedlings under the most effective *Trichoderma*-treated soils Jn14, R42, Jn21, T33, T36, and H2, were reduced by 65.6%, 60.5%, 56%, 54.9%, 54.8%, and 53.4% respectively as compared to the control. The other eight isolates (T34, N40, Jn13, B46, B44, B45, Jn22, and H1) reduced disease index by 1.5%-16.4%.

Furthermore, *Trichoderma* isolates significantly reduced disease index caused by *S. rolfsii* (LSD= 23.585; F= 5.518; P≤0.05). Forty three *Trichoderma* isolates (Jn14, Jn21, H2, Egy52, T33, N38, T36, Q28, J8,

R42, Jn23, Q26, H4, J9, T37, B47, H3, R43, R41, Q27, Jn17, Jn13, N39, GH11, Jn18, Q29, Jn16, H6, H7, B45, B44, H1, T34, Q24, N40, B46, Egy50, Q25, T32, T31, Jn22, Egy20, and Jn20) significantly reduced disease index by 20.8%-66.8%. The most effective isolates Jn14 (*Trichoderma harzianum*), Jn21 (*Trichoderma pseudokoningii*), and H2 (*Trichoderma lactea*) reduced disease index by 66.8%, 65.8%, and 63% respectively. The other nine isolates (Jn12, Jn19, Jn15, Q30, T35, Y, Jn11, J10, and H5) reduced disease index by 1.3%-18.5%.

Results in this study showed that most effective local *Trichoderma* isolate (Jn14) (*Trichoderma harzianum*) reduced disease index more than those *Trichoderma* isolates from Hebrew University (GH11) and Egypt (Egy52); isolate GH11 reduced disease index caused by *R. solani* and *S. rolfsii* by 40% and 45.7% respectively, and isolate Egy52 reduced disease index caused by *R. solani* and *S. rolfsii* by 39.7% and 58.9% respectively.

Table 3.7 The effect of *Trichoderma* isolates on *Rhizoctonia* damping- off of bean plants measured as disease index after 14 days of sowing the seeds in the bioassay studies.

Isolate	Disease Index (DI)	Isolate	Disease Index (DI)
Ck	111.0* ± 13.42 w	**Q30**	73.3 ± 6.26 f g h i j k l m n o
T34	109.3 ± 11.40 v w	**Egy50**	73.0 ± 4.47 e f g h i j k l m n
N40	102.7 ± 12.10 u v w	**Q27**	71.0 ± 6.86 d e f g h i j k l m
Jn13	101.5 ± 13.18 t u v w	**J10**	70.4 ± 26.7 d e f g h i j k l m
B46	98.7 ± 3.07 s t u v w	**Q29**	70.3 ± 11.2 d e f g h i j k l
B44	96.0 ± 12.07 r s t u v w	**Q24**	70.0 ± 20.99 d e f g h i j k l
B45	95.0 ± 16.58 q r s t u v w	**Egy52**	66.9 ± 13.40 c d e f g h i j k
Jn22	93.2 ± 4.60 p q r s t u v w	**GH11**	66.6 ± 4.67 c d e f g h i j k
H1	92.8 ± 13.69 o p q r s t uv w	**B47**	66.3 ± 26.97 c d e f g h i j
Jn16	90.8 ± 21.46 n o p q r s t u v	**Jn18**	65.5 ± 24.52 c d e f g h i j
Jn12	90.8 ± 11.88 n o p q r s t u v	**Jn17**	63.0 ± 12.37 b c d e f g h i j
Jn15	90.0 ± 22.85 m n op qr st uv	**Q26**	62.9 ± 21.04 b c d e f g h i j
H6	89.0 ± 23.28 l m n op qr s tu	**N39**	62.1 ± 12.71 b c d e f g h i
T32	88.8 ± 10.94 l m n op q rst u	**T37**	62.0 ± 28.03 b c d e f g h i
H5	87.6 ± 12.76 l m n op q r t u	**H4**	61.3 ± 8.42 b c d e f g h i

Isolate	Disease Index (DI)	Isolate	Disease Index (DI)
R41	86.7 ± 13.52 l m n op q rst u	**H3**	60.2 ± 16.68 b c d e f g h
T31	86.2 ± 23.8 k l m n op q rst u	**J9**	59.5 ± 7.53 b c d e f g h
T35	82.2 ± 12.3 j k l m n op qrs t	**Q28**	57.9 ± 19.26 b c d e f g
H7	80.9 ± 4.25 i j k l m n opqr s	**J8**	54.6 ± 5.55 a b c d e f
Y	80.7 ± 12.9 i j k l m n opqr s	**N38**	53.6 ± 12.30 a b c d e
Jn23	78.6 ± 10.4 h i j k l m n opqr	**H2**	51.7 ± 23.58 a b c d
Jn19	78.2 ± 24.1 h i j k l m nopq r	**T36**	50.2 ± 12.62 a b c
Egy20	77.4 ± 12.8 g h i jkl m nopqr	**T33**	50.1 ± 9.81 a b c
Jn11	75.5 ± 18.7 g h i jkl m nop q	**Jn21**	48.8 ± 11.10 a b c
Q25	75.2 ± 29.3 g h i j k l mnop	**R42**	43.8 ± 9.34 a b
Jn20	75.2 ± 12.2 g h i j k lmno p	**Jn14**	38.2 ± 5.57 a
R43	74.7 ± 18.7 g h i jk lm no p		

* Mean of five replicates ± standard deviation followed by the same letters within columns are not statistically significant according to Fisher LSD method (LSD= 19.588, *P*=0.05).

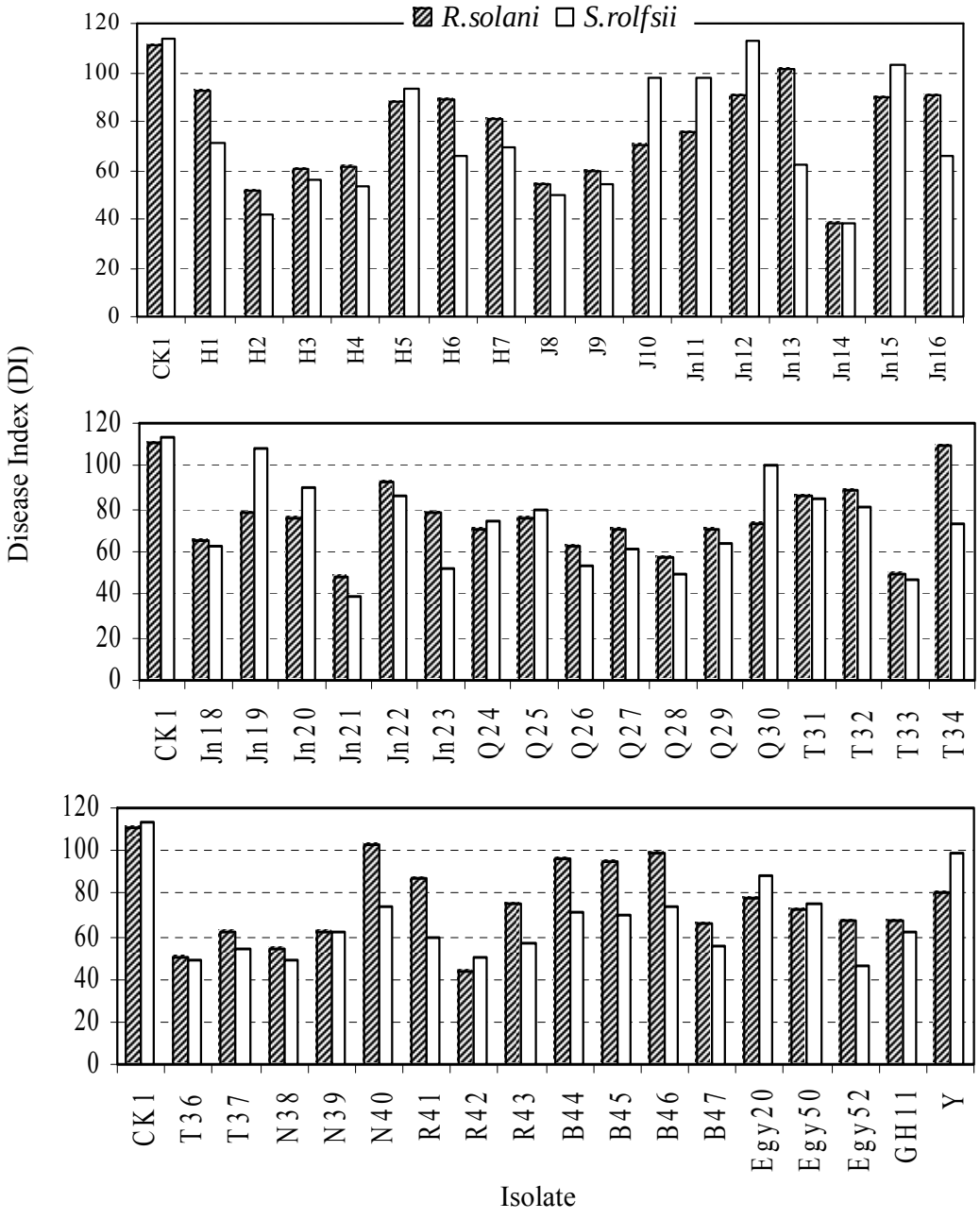
Table 3.8 The effect of *Trichoderma* isolates on *Sclerotium* rot of bean plants measured as disease index after 14 days of sowing the seeds in the bioassay studies.

Isolate	Disease Index (DI)	Isolate	Disease Index (DI)
Ck	114.0* ± 13.42 w	**Q29**	64.0 ± 30.7 c d e f g h i j k l
Jn12	112.5 ± 13.77 v w	**Jn18**	62.7 ± 12.43 c d e f g h i j k
Jn19	108.0 ± 16.43 u v w	**GH11**	61.8 ± 13.01 b c d e f g h i j k
Jn15	103.5 ± 17.10 t u v w	**N39**	61.8 ± 5.75 b c d e f g h i j k
Q30	100.5 ± 18.06 s t u v w	**Jn13**	61.8 ± 22.61 b c d e f g h i j k
T35	99.0 ± 15.17 r s t u v w	**Jn17**	61.5 ± 13.18 b c d e f g h i j k
Y	99.0 ± 23.36 r s t u v w	**Q27**	61.4 ± 24.7 a b c d e f g h i j k
Jn11	98.0 ± 19.56 q r s t u v w	**R41**	59.8 ± 6.58 a b c d e f g h i j
J10	97.6 ± 13.28 p q r s t u v w	**R43**	56.5 ± 17.82 a b c d e f g h i
H5	92.9 ± 14.4 o p q r s t u v w	**H3**	56.4 ± 5.37 a b c d e f g h i
Jn20	90.3 ± 7.68 n o p q r s t u v	**B47**	55.2 ± 9.63 a b c d e f g h
Egy20	88.5 ± 28.3 m n o p q r s t u	**T37**	54.6 ± 11.05 a b c d e f g h
Jn22	86.4 ± 15.97 l m n opq r s tu	**J9**	54.2 ± 18.17 a b c d e f g h
T31	84.7 ± 14.5 k l m n opq r s tu	**H4**	53.5 ± 11.94 a b c d e f g h
T32	80.9 ± 22.6 jk l m n o pq r s t	**Q26**	53.4 ±15.36 a b c d e f g h
Q25	79.0 ± 26.3 i jk l m n o pq r s	**Jn23**	52.7 ± 16.78 a b c d e f g h
Egy50	75.5 ± 28.4 h i jk l m n o pqr	**R42**	50.4 ± 15.06 a b c d e f g
B46	74.5 ± 11.5 h i j k l m n o pq	**J8**	49.9 ± 8.31 a b c d e f g
N40	74.4 ± 16.6 h i j k l m n o pq	**Q28**	49.6 ±11.54 a b c d e f g
Q24	74.2 ± 28.52 h i j k l m n o p	**T36**	49.2 ± 6.22 a b c d e f g
T34	72.8 ± 17.24 g h i j k l m n o	**N38**	48.9 ± 11.40 a b c d e f
H1	71.1 ± 25.66 fgh i jk l m n o	**T33**	47.4 ± 7.47 a b c d e

Isolate	Disease Index (DI)	Isolate	Disease Index (DI)
B44	70.7 ± 30.7 e fgh i jk l m n o	**Egy52**	46.8 ± 19.63 a b c d
B45	69.6 ± 4.16 d e fgh i jk l m n	**H2**	42.2 ± 10.62 a b c
H7	69.0 ± 17.1 d e fgh i jk l m n	**Jn21**	39.0 ± 10.39 a b
H6	66.2 ± 11.9 d e f g h i j k l m	**Jn14**	37.8 ± 3.03 a
Jn16	66.1 ± 34.6 d e f g h i j k l m		

* Mean of five replicates ± standard deviation followed by the same letters within columns are not statistically significant according to Fisher LSD method (LSD= 23.585, *P*=0.05).

Figure 3.3. The effect of *Trichoderma* isolates on diseases caused by *R. solani* and *S. rolfsii* on bean plants.



3.6 Effect of Temperature on *Trichoderma* Mycelial Growth and Spore Production

Radial mycelial growth rate was measured under six temperature treatments including 10, 15, 25, 30, 35, and 40 °C with four replicates/temperature combination on PDA supplemented with chloramphenicol (25mg/L) and under continuous light.

The results indicate that the most effective *Trichoderma* isolates reached a peak in mycelial growth rate at 30 °C and was completely inhibited at 40 °C (Table 3.9 and Fig. 3.4). The variability between the isolates was significantly observed at 30 °C (LSD= 0.979; F= 100.245; P <0.05). The isolate Jn14 (*Trichoderma harzianum*) recorded a growth of 16.75 cm^2 /day at 30 °C and was clearly the highest while the lowest measurements were recorded by the isolate Q28 (*Trichoderma hamatum*) at 10 °C (Table 3.9 and Fig 3.10).

Spore production occurred at temperatures between 15 and 35 °C. Spore production was significantly affected by temperatures (LSD= 1.186; F= 53.871; P <0.05). Sporulation of the isolates reached a peak at 30 °C and declined at 35 °C. The highest spore production rate ($1.44*10^8$) spore / ml was recorded by the isolate Jn14 (*Trichoderma harzianum*) at 30 °C and the lowest value was recorded by the isolate Egy52 at 35°C ($0.74*10^8$)spore / ml (Table 3.10 and Fig 3.5).

Table 3.9 Mycelium growth rate (cm^2/day) of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) growing on PDA medium at different Temperatures (10, 15, 25, 30, 35, and 40 ^{0}C).

isolate	10 ^{0}C	15 ^{0}C	25 ^{0}C	30 ^{0}C	35 ^{0}C	40 ^{0}C
H2	0.04*± 0.02 v	0.49±0.11 s t u v	4.56± 0.69 f g	11.22±2.33 b	3.21±0.58 h i j kl	0.00±0.00 v
J8	0.07±0.03 u v	2.24±0.19 l m n o	4.49±0.68 f g	7.61±0.63 d	2.28±0.9 k l m n o	0.00±0.00 v
Jn14	0.11±0.06 t u v	0.81±0.5 r s t u v	7.81±1.23 d	16.75±1.90 a	3.67±0.62 g h i	0.00±0.00 v
Jn21	0.05±0.02 v	0.48±0.11 s t u v	2.04±0.2 m nopq	7.14±1.43 d	2.97±0.28 i j kl m	0.00±0.00 v
Q28	0.02±0.00 v	2.11±0.07 m n op	5.69±0.73 e	7.98±1.23 d	3.25±0.28 h i j k	0.00±0.00 v
T33	0.03±0.01 v	1.16±0.16 p q r s	3.48±0.29 h i j	7.10±0.79 d	1.74±0.2 n o p q r	0.00±0.00 v
T36	0.12±0.06 t u v	2.31±0.4 k l m n o	4.54±1.32 f g	9.86±1.64 c	4.16±0.46 g h	0.00±0.00 v
N38	0.15±0.08 t u v	2.69±0.3 i j kl m n	7.39±1.44 d	10.82±0.54 b c	0.06±0.03 v	0.00±0.00 v
R42	0.15±0.05 t u v	2.86±0.3 i j kl m	3.43±0.45 h i j	5.42±1.14 f	1.05±1.00 r s t u	0.00±0.00 v
Egy52	0.09±0.04 u v	1.56±0.32 o p q r	2.66±0.2 j kl m n	3.14±0.28 i j kl	1.08±0.33 q r s t	0.00±0.00 v

* Mean of four replicates ± standard deviation; values followed by the same letter within columns or rows are not significantly different according to Fisher LSD test (LSD= 0.979, $P \leq 0.05$).

Table 3.10 Spore production (10^{8}[62] spore/ml) of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) growing on PDA medium after 15 day of incubation under continuous light at different temperatures (10, 15, 25, 30, 35, and 40 ^{0}C).

Isolate	15 ºC	25 ºC	30 ºC	35 ºC
H2	3.94*±0.82 o p q	7.97±0.34 f f g h	7.03±0.19 h i	3.84±0.41 p q
J8	1.57±0.98 s t	9.78±0.90 d	11.6±0.85 c	3.00±1.08 q r
Jn14	7.22±1.22 g h i	12.88±0.25 b	14.45±0.67 a	1.08±0.61 t
Jn21	2.56±0.56 r s	6.63±0.95 i j	11.35±0.54 c	7.18±1.53 g h i
Q28	4.57±0.7 m n o p	5.46±0.34 j k l m	9.39±0.43 d e	4.33±0.8 m nop
T33	5.01±0.8 l m no p	9.68±2.40 d	8.70±0.44 d e f	5.21±0.91 klmn
T36	8.26±0.36 e f g	4.28±0.6 m n o p	6.34±1.19 i j k	3.98±0.34 o p q
N38	5.41±0.41 k l m	4.72±0.4 m n o p	6.19±0.52 I j kl	8.78±1.38 d e f
R42	4.62±1.1 m n o p	8.11±0.44 f g h	5.26±0.33 k lm	5.11±0.64 lmno
Egy52	4.52±0.3 m n o p	7.86±1.53 f g h	4.03±0.25nopq	0.74±0.43 t

* Mean of four replicates ± standard deviation; values followed by the same letter within columns or rows are not statistically significant different according to Fisher LSD test (LSD= 1.186, $P \leq 0.05$).

Figure 3.4. Mycelium growth rate (mm^2/day) of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) growing on PDA medium and incubated at different temperatures (10, 15, 25, 30, 35 and 40ºC).

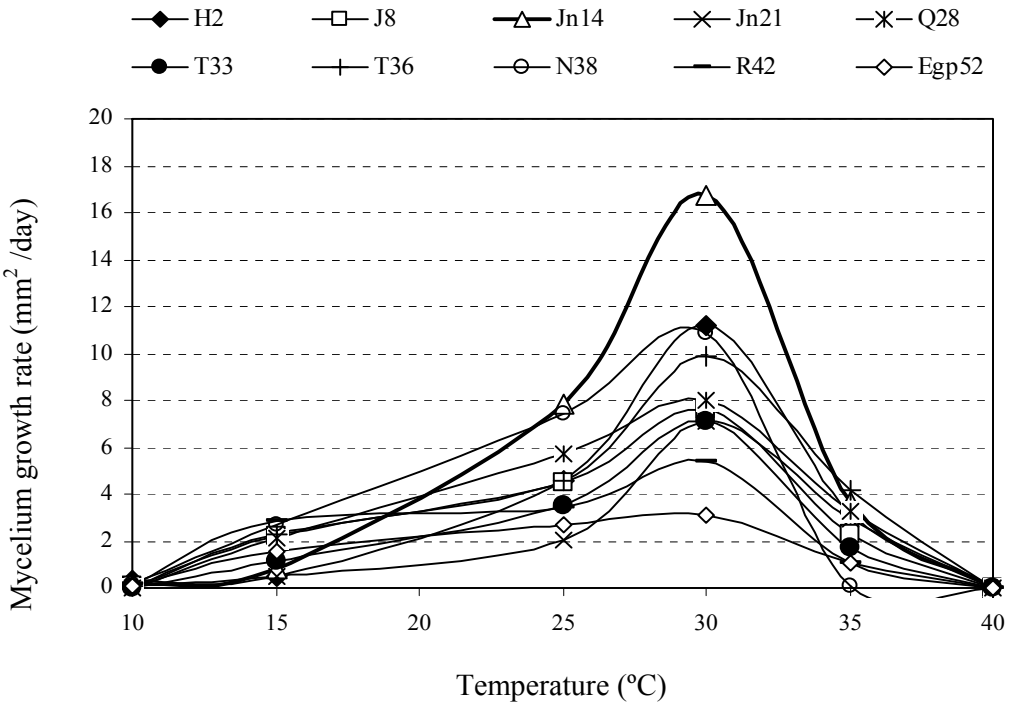
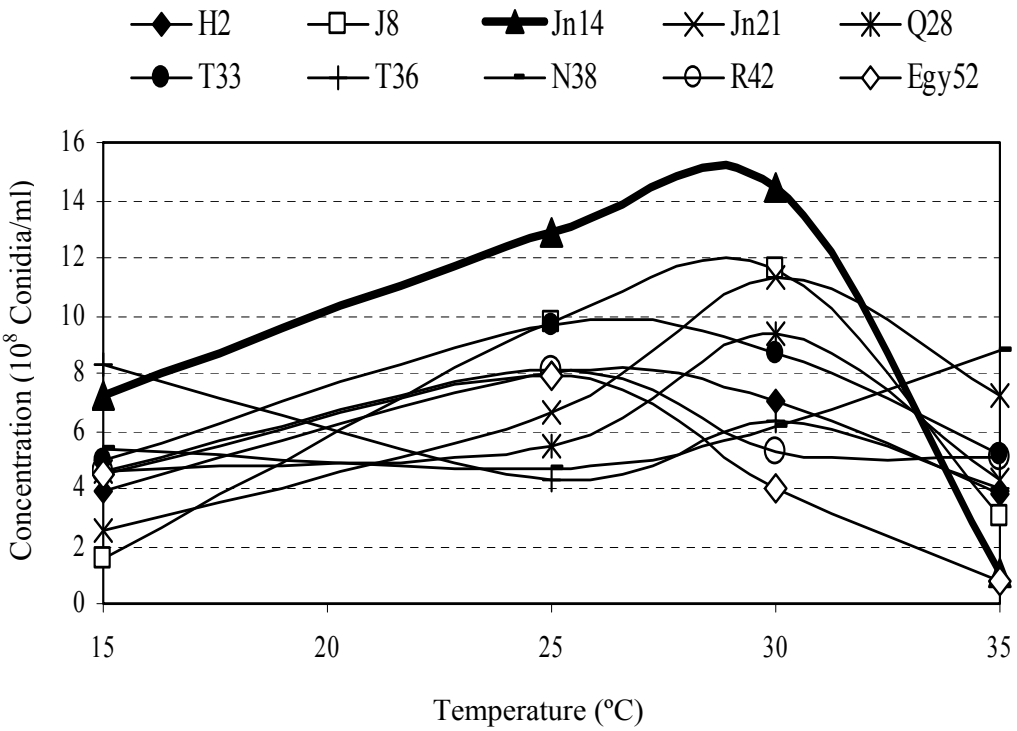


Figure 3.5. The effect of temperature on sporulation of the *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42 and Egy52) growing on PDA medium.



3.7 Effect of Temperature on Antagonism in Dual Culture

Results of the effect of different temperatures on the interaction between *Trichoderma* isolates mycelia and the pathogens (*R. solani & S. rolfsii*) mycelia showed that there was a statistically significant difference between groups (LSD= 1.635; F= 182.557; P <0.05) and (LSD= 0.279; F= 28.883; P <0.05) respectively (Table 3.11, 3.12 and Fig. 3.6) (See appendix B for ANOVA tables).

The interaction between *R. solani*, *S. rolfsii* and the *Trichoderma* isolates studied were highly dependent on temperature. The overgrowth rates (mm/day) of *Trichoderma* isolates growing on PDA medium were measured during 96 hours of interaction at 15-35 °C. The mean overgrowth rate of *Trichoderma* isolates on *R. solani* ranged from 1.42 mm/day for the

isolates Q28 (*Trichoderma hamatum*), R42 (*Trichoderma koningii*) at 15 °C and 24.0 mm/day at 30 °C for the isolate Jn14 (*Trichoderma harzianum*) (Table 3.11). However, the mean overgrowth rate of *Trichoderma* isolates on *S. rolfsii* ranged from 0.17 mm/ day for isolates N38 and Egy52at 35 °C and 30 °C, respectively, and 2.4 mm/day for the isolate Jn14 (*Trichoderma harzianum*) at 25 °C (Table 3.12).

A clear zone of inhibition was formed between the two fungal colonies at 25 and 30 °C after 48 hours of contact in the isolate N38 when used against *R. solani* and *S. rolfsii.*

Table 3.11 Overgrowth rates of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) on *R. solani* growing on PDA medium in dual culture at different temperatures (15, 25, 30, and 35 ^{0}C).

Isolate	**15 °C**	**25 °C**	**30 °C**	**35 °C**
H2	2.83* ± 0.6 m n	12.50 ± 1.92 i	19.00 ± 1.41 cd	0.00 ± 0.00 o
J8	2.75 ± 0.42 m n	13.25 ± 0.96 h i	16.50 ± 0.58 e f	0.00 ± 0.00 o
Jn14	3.83 ± 0.58 l m	14.50 ± 2.65 gh	24.00 ± 1.41 a	0.00 ± 0.00 o
Jn21	2.33 ± 0.94 m n	6.50 ± 1.29 k	23.75 ± 1.71 a	0.00 ± 0.00 o
Q28	1.42 ± 0.32 n o	7.75 ± 0.96 k	13.50 ± 0.58 h i	0.00 ± 0.00 o
T33	2.50 ± 0.69 m n	15.50 ± 1.29 f g	17.50 ± 1.00 de	0.00 ± 0.00 o
T36	2.83 ± 0.33 m n	16.00 ± 1.2 e fg	20.25 ± 0.96 bc	0.00 ± 0.00 o
N38	2.17 ± 0.58 n	15.75 ± 2.87 f g	21.50 ± 2.38 b	0.00 ± 0.00 o
R42	1.42 ± 0.57 n o	4.50 ± 1.29 l	10.50 ± 1.00 j	0.00 ± 0.00 o
Egy52	2.58 ± 0.69 m n	2.00 ± 0.82 n	9.50 ± 2.65 j	0.00 ± 0.00 o

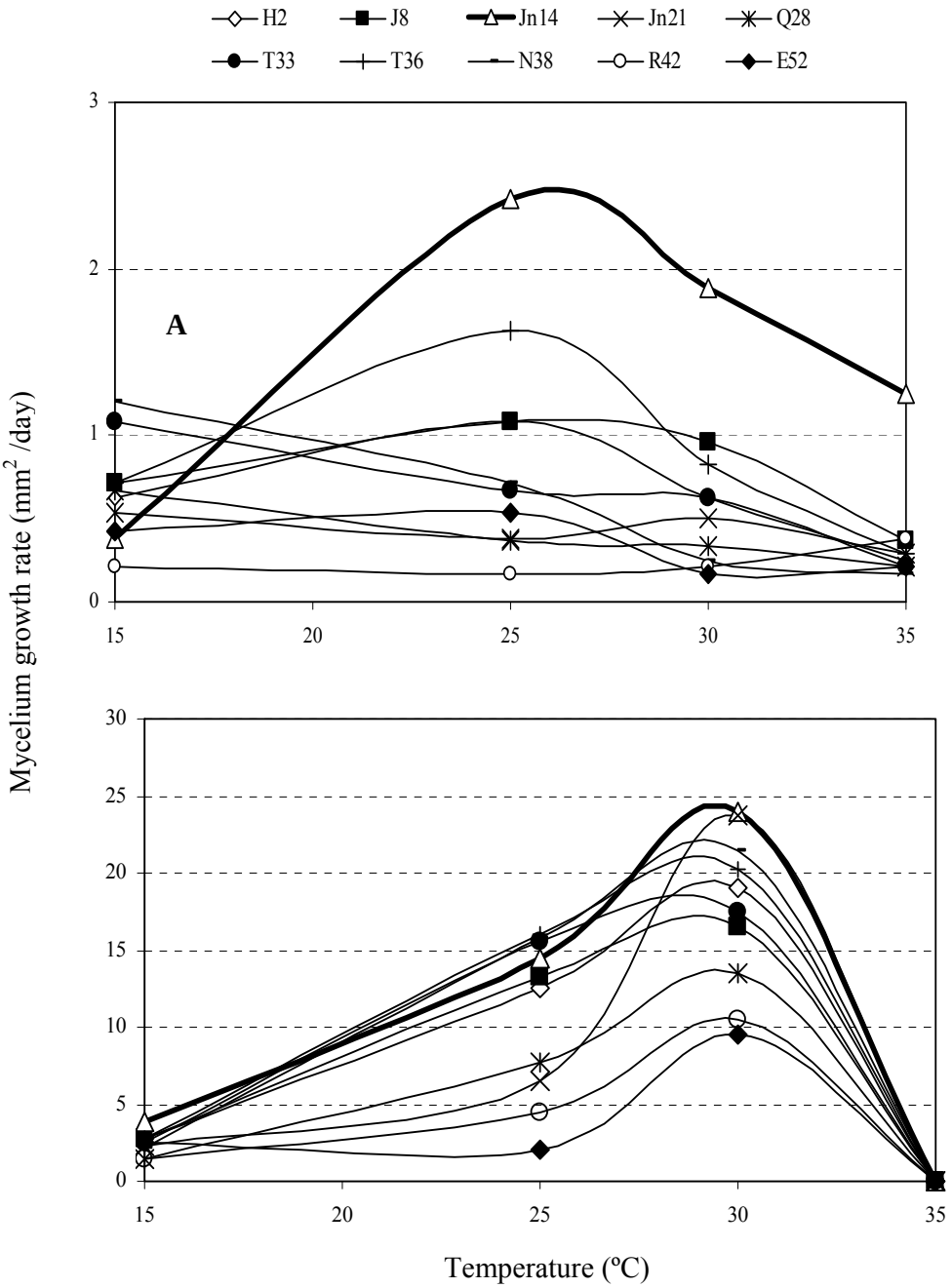
* Mean of four replicates ± standard deviation; values followed by the same letter within columns or rows are not significantly different according to Fisher LSD test (LSD= 1.635, $P \leq 0.05$).

Table 3.12 Overgrowth rates of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) on *S. rolfsii* growing on PDA medium in dual culture at different temperatures (15, 25, 30, and 35 ^{0}C).

Isolate	15 °C	25 °C	30 °C	35 °C
H2	0.63* ± 0.1 g h ij	1.08 ± 0.2 c de	0.63 ± 0.2 g h i j	0.25 ± 0.1 l m
J8	0.71 ± 0.4 f g h	1.08 ± 0.2 c de	0.96 ± 0.4 d e f	0.37 ± 0.1 jklm
Jn14	0.38 ± 0.4 j kl m	2.42 ± 0.4 a	1.88 ± 0.3 b	1.25 ± 0.3 c
Jn21	0.54 ± 0.1 h i j k	0.38 ± 0.2 jklm	0.50 ± 0.1 hi j kl	0.29 ± 0.1 klm
Q28	0.67 ± 0.2 g h i	0.37 ± 0.1 jklm	0.33 ± 0.1 k l m	0.21 ± 0.1 m
T33	1.08 ± 0.2 c d e	0.67 ± 0.2 g hi	0.63 ± 0.2 g h i j	0.21 ± 0.1 m
T36	0.71 ± 0.2 f g h	1.63 ± 0.2 b	0.83 ± 0.4 e f g	0.29 ± 0.2 klm
N38	1.21 ± 0.4 c d	0.71 ± 0.2 f gh	0.25 ± 0.1 l m	0.17 ± 0.0 m
R42	0.21 ± 0.1 m	0.17 ± 0.0 m	0.21 ± 0.1 m	0.38 ± 0.2 jklm
Egy52	0.42 ± 0.1 i jklm	0.54 ± 0.1 hijk	0.17 ± 0.0 m	0.21 ± 0.1 m

* Mean of four replicates ± standard deviation; values followed by the same letter within columns or rows are not significantly different according to Fisher LSD test (LSD= 0.279, $P \leq 0.05$).

Figure 3.6. Mycelium overgrowth rate (mm^2/day) of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) on *S. rolfsii* **(A)** and *R. solani* **(B)** growing on PDA medium and incubated at different temperatures (15, 25, 30, and 35ºC).



3.8 Effect of Temperature on Production of Toxic Metabolites by *Trichoderma* Isolates (Antibiosis)

The growth rate of *R. solani* was reduced significantly in amended media at 20 °C and 25 °C (LSD = 0.942; F= 59.33; P <0.05) (See Appendix B for recipes). The variations between isolates were observed significantly at 25 °C. *R. solani* growth rate inhibition percentages were 42%, 78%, and 50.5% when isolates were grown on PDA medium amended with 10% of PDB containing metabolites produced by the isolates T36, Jn14, and J8, respectively. Results showed that Jn14 was the most effective isolate at all temperatures (15 °C, 20°C, and 25 °C), and reduced *R. solani* mycelial growth by percentages of 76%, 66%, and 78%, respectively (Table 3.13 and Fig. 3.7).

The growth rate of *S. rolfsii* was reduced significantly at 20 °C and 25 °C (LSD = 1.008; F= 355.69; P <0.05) (See Appendix B for recipes). Growth rate inhibition percentages of *S. rolfsii* at 25 °C were 37.4%, 90.5% and 94.7% when isolates were grown on PDA amended medium with 10% of PDB containing metabolites produced by the isolates J8, Jn14, and T36, respectively. Looking at variation in inhibition of mycelial growth of *S. rolfsii*, T36 was the most effective isolate and reduced the pathogen mycelium growth at the temperatures 15 °C, 20 °C, and 25°C by 64.5%, 84.1%, and 94%, respectively (Table 3.14 and Fig.3.7).

Table 3.13 Effect of temperature on antibiosis of *Trichoderma* isolates (J8, Jn14, and T36) on *R. solani*, 48 hours after incubation.

Isolate	**15 ^{0}C**	**20 ^{0}C**	**25 ^{0}C**
Ck	3.25* ± 0.52 c	6.10 ± 0.00 e	9.65 ± 0.90 f
J8	1.58 ± 0.15 a b	5.43 ± 1.43 d e	5.60 ± 0.00 d e
Jn14	0.78 ± 0.15 a	2.05 ± 0.66 b	2.03 ± 0.38 b
T36	2.48 ± 0.68 b c	2.48 ± 0.47 b c	4.78 ± 0.87 d

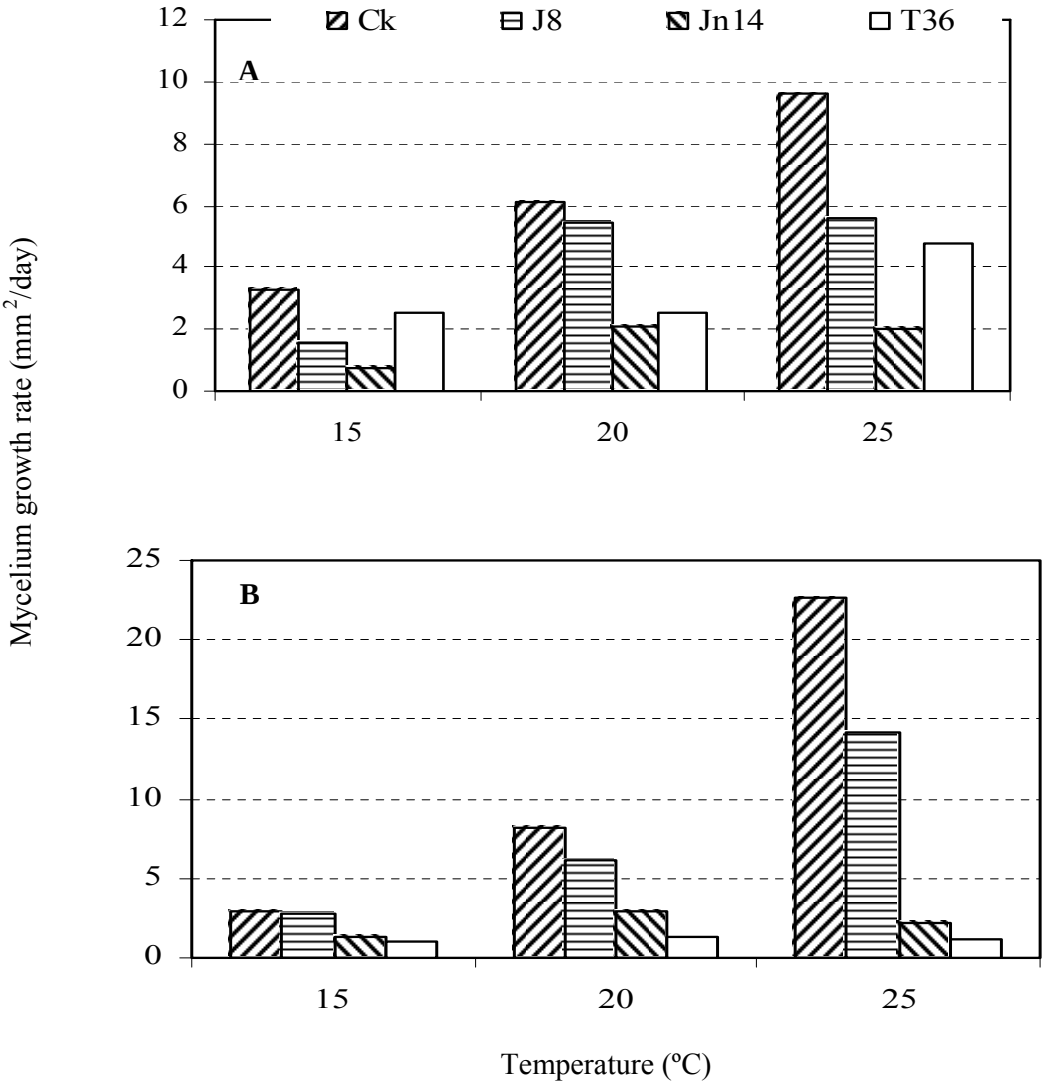
* Mean of four replicates ± standard deviation; values followed by the same letter within columns or rows are not significantly different according to Fisher LSD test (LSD= 0.942, $P \leq 0.05$).

Table 3.14 Effect of temperature on antibiosis of *Trichoderma* isolates (J8, Jn14, and T36) on *S. rolfsii*, 48 hours after incubation.

Isolate	**15 ^{0}C**	**20 ^{0}C**	**25 ^{0}C**
Ck	2.90* ± 0.25 c	8.20 ± 0.85 e	22.66 ± 0.86 g
J8	2.85 ± 0.5 c	6.13 ± 1.44 d	14.19 ± 1.06 f
Jn14	1.30 ± 0.28 a b	2.90 ± 0.42 c	2.15 ± 0.37 b c
T36	1.03 ± 0.43 a	1.30 ± 0.28 a b	1.20± 0.55 a b

* Mean of four replicates ± standard deviation; values followed by the same letter within columns or rows are not significantly different according to Fisher LSD test (LSD= 1.008, $P \leq 0.05$).

Figure 3.7. Mycelium overgrowth rate (mm^2/day) of *R. solani* **(A)** and *S. rolfsii* **(B)** growing on PDA medium amended with metabolites produced by the *Trichoderma* isolates (J8, Jn14, T36) and incubated at different temperatures (15, 20, and 25 ºC).



3.9 Hyphal Interaction on Thin Films of Agar

The *Trichoderma* isolates (H2, J8, Jn14, Jn17, Jn21, T33 and T36) showed identical mode of action during interacting with *Rhizoctonia solani* and *Sclerotium rolfsii* on water agar films. After contact, the hyphae of *Trichoderma* grew along the pathogens hyphae; sometimes the main hyphae coiled around the host or produced short branches that tightly surrounded the host hyphae.

Dense coiling around host hyphae and internal growth within the host mycelium had commonly been seen during interaction between hosts (*R. solani* and *S. rolfsii*) and *Trichoderma*; disintegration of the host cell wall was observed as well.

When *Trichoderma* hyphae reached the older portions of the pathogen colony, they were seen to be growing inside the host hyphae. At this stage most of the host cells were already lacking cytoplasm (Inbar *et al*., 1996).

Microscopic examination had also revealed that the isolate Jn14 (*T. harzianum*) was found to be a very efficient mycoparasite during interaction on thin film of agar. Coiling of *Trichoderma harzianum* (Jn14), *T. hamatum* (T36), and *T. Pseudokoningii* (Jn21) hyphae around *R. solani* and *S. rolfsii* hyphae was noticed on 40x microscopic magnification. Internal colonization by local *Trichoderma* isolates Jn14, T33, and J8 was abundant and well-developed throughout the *R. solani* and *S. rolfsii* mycelium. *Trichoderma* isolate H2 also made extensive coiling around *R. solani* hyphae. The isolate Jn14 hyphae could be seen to be growing inside the host hyphae of *R. solani* and the host cells were lacking cytoplasm.

Five types of hyphal interactions were observed in this study: 1) Coiling around the host hyphae; 2) Extensive coiling around the host hyphae; 3) Penetration of the host hyphae; 4) Penetration of the host hyphae and lacking cytoplasm of the host cells; and 5) Subsequent lysis of the infected hyphae.

3.10 Effect of *Trichoderma* on Bean Increased Growth Response (IGR)

Trichoderma isolates were applied to soil as conidial suspension at a concentration of ($5x10^6$ cfu/ g soil).

The results showed that there was a significant increase for each of the parameters measured (plants emergence, plant height, plant fresh, and plant dry weight) in bean (*Phaseolus vulgaris*) seedlings, 4 weeks after sowing compared to the non-treated seedlings (Table 3.15 and Table 3.16); for plant height (LSD= 4.19; F = 8.630; P <0.05) (Fig. 3.8), plant emergence (LSD= 0.998; F = 22.087; P <0.05) (Fig. 3.9), plant fresh weight (LSD= 2.232; F = 4.999; P <0.05), and plant dry weight (LSD= 0.223; F = 4.976; P <0.05) (Fig. 3.10) (See appendix B for recipes).

Bean seedlings treated with *Trichoderma* isolates J8 and T36 increased in height by 160 to 200%, respectively (Fig 3.8). In addition, seedlings treated with the isolates Jn14 and Jn21 increased in fresh weight in the range of 133 % to 217%, respectively (Fig 3.9). There was no significant difference, however, in respect to plant dry weights within treatments (Fig 3.9).

Germination of bean seeds planted in soils treated with *Trichoderma* isolates mentioned above occurred about four days earlier than those planted in untreated soil. Germination of seeds planted in treated soil

increased in the range of (16.7% - 55.6%) compared to the control. The enhancement was induced by the isolates Jn14 and T36 (55.6%) after the 7 days.

Table 3.15 Effect of local *Trichoderma* isolates on bean plant growth response (PGR).

Isolate	Plant height	Plant weight (Fresh)	Plant weight (dry)
Ck	18.3* ± 1.6 f	5.9 ± 1.0 h	0.59 ± 0.1 h
H2	29.9 ± 4.7 d e	10.4 ± 1.6 c d e f g	1.04 ± 0.2 c d e f g
H3	31.0 ± 2.7 c d e	10.6 ± 1.3 b c d e f g	1.07 ± 0.1 bcdefg
H4	34.7 ± 4.7 a b c	11.1 ± 2.2 a b c d e	1.11± 0.2 a b c d e
J8	29.8 ± 1.8 d e	8.9 ± 0.8 e f g	0.89 ± 0.1 e f g
J9	29.8 ± 3.8 d e	8.8 ± 2.4 f g	0.88 ± 0.2 f g
Jn14	36.7 ± 1.1 a b	12.9 ± 1.7 a	1.28 ± 0.2 a
Jn18	29.7 ± 3.4 e	11.2 ± 1.8 a b c d	1.12 ± 0.2 a b c d
Jn21	32.1 ± 2.7 c d e	8.4 ± 1.9 g	0.84 ± 0.2 g
Q27	32.5 ± 2.1 b c d e	11.8 ± 1.5 a b c	1.18 ± 0.2 a b c
Q28	33.4 ± 2.9 a b c d e	11.9 ± 1.3 a b c	1.19 ± 0.1 a b c
T36	36.8 ± 3.0 a	12.7 ± 1.9 a b	1.27 ± 0.2 a b
T37	29.7 ± 2.6 e	9.5 ± 1.5 d e f g	0.95 ± 0.2 d e f g
N38	34.7 ± 1.0 a b c	11.0 ± 2.7 a b c d e f	1.10 ± 0.3 a b cd e f
R42	34.7 ± 1.0 a b c	9.8 ± 2.2 c d e f g	0.98 ± 0.2 c d e f g
B47	34.0 ± 7.1 a b c d	10.9 ± 0.9 a b c d e f	1.09 ± 0.1 a b c de f

* Mean of five replicates ± standard deviation; values followed by the same letter within columns are not significantly different according to Fisher LSD test ($P \leq 0.05$).

LSD Plant height = 4.190
Plant weight (Fresh) = 2.232
Plant weight (Dry) = 0.223

Table 3.16 Effect of *Trichoderma* isolates on bean seedlings (*Phaseolus vulgaris*) emergence.

Isolate	3 days	7 days
Ck	0.00* ± 0.00 f k	3.6 ± 1.14 b g h
H2	1.6 ± 1.14 d j	4.6 ± 0.55 a g
H3	1.5 ± 0.58 e i j	4.75 ± 0.50 a g
H4	1.6 ± 0.55 d j	4.4 ± 0.55 b g
J8	1.75 ± 0.50 d j	4.25 ± 0.5 b g h
J9	1.0 ± 0.71 e j	4.2 ± 0.84 b g h
Jn14	3.4 ± 1.14 c h	5.6 ± 0.55 a
Jn18	2.0 ± 1.0 d j	4.2 ± 0.84 b g h
Jn21	1.8 ± 0.84 d j	5.2 ± 0.84 a g
Q27	1.6 ± 0.89 d j	4.8 ± 0.84 a g
Q28	1.6 ± 0.55 d j	4.8 ± 0.84 a g
T36	2.2 ± 0.84 d i	5.6 ± 0.25 a
T37	0.6 ± 0.55 e k	4.6 ± 1.14 a g
N38	2.2 ± 0.84 d	5.2 ± 0.84 a g
R42	2.4 ± 1.14 d i	5.2 ± 0.45 a g
B47	1.2 ± 0.84 e j	4.6 ± 0.89 a g

* Mean of five replicates ± standard deviation; values followed by the same letter within columns or rows are not significantly different according to Fisher LSD test (LSD= 0.998, $P \leq 0.05$).

Figure 3.8. Increased Growth Response (height in cm) in bean plant induced by the *Trichoderma* isolates (H2, H3, H4, J8, J9, Jn14, Jn18, Jn21, Q27, Q28, T36, N38, R42, and B47) incubated in growth chamber at 25 ± 2 ºC.

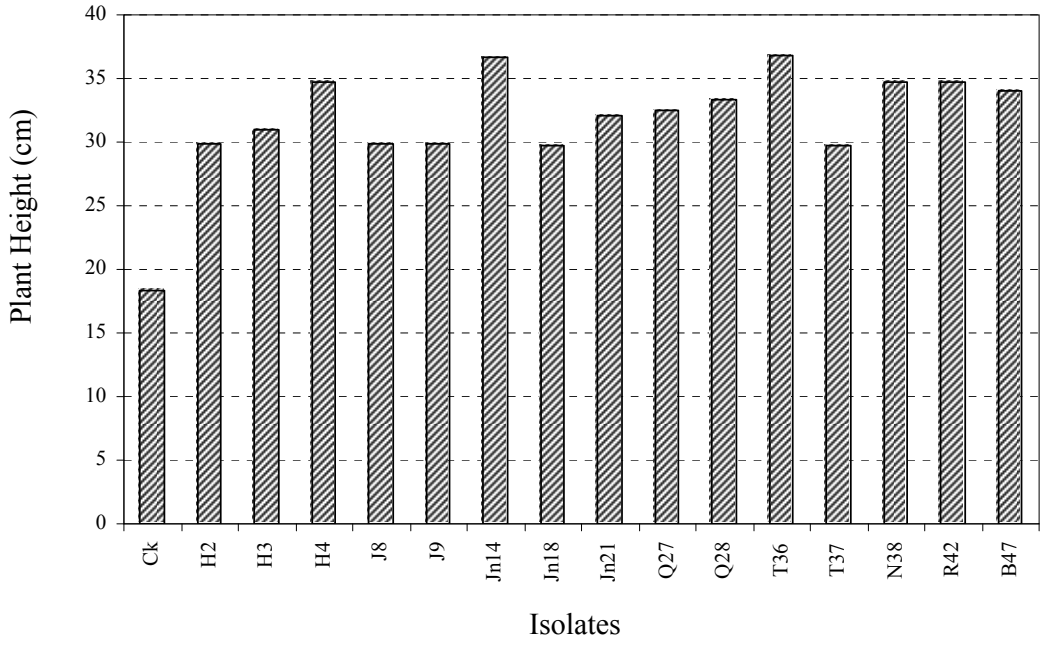


Figure 3.9. Increased Growth Response (fresh and dry weight in grams) of bean plant induced by *Trichoderma* isolates (H2, H3, H4, J8, J9, Jn14, Jn18, Jn21, Q27, Q28, T36, N38, R42, and B47) incubated in growth chamber at 25 ± 2 ºC.

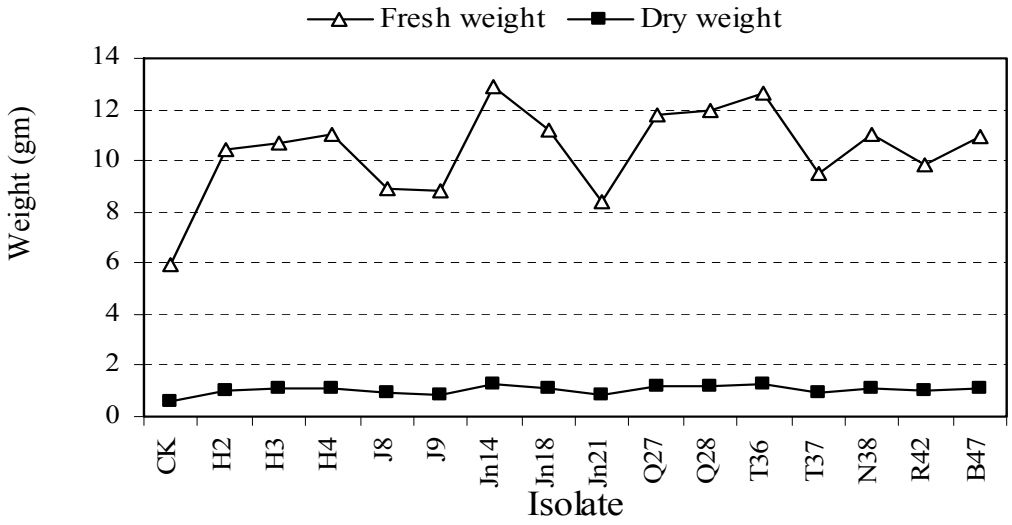
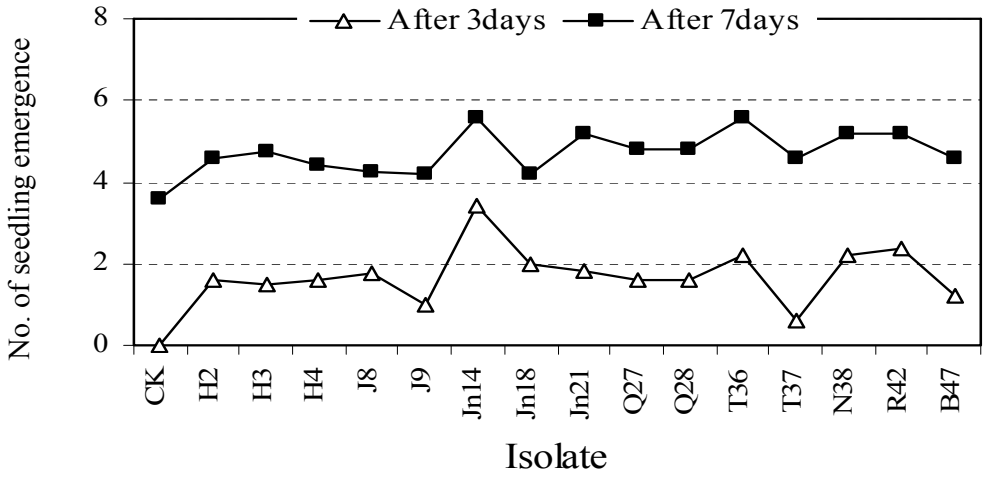


Figure 3.9. The effect of different *Trichoderma* isolates (H2, H3, H4, J8, J9, Jn14, Jn18, Jn21, Q27, Q28, T36, N38, R42, and B47) on bean seedlings emergence after incubation in growth chamber at 25 ± 2 ºC



Chapter Four

Chapter Four

Discussion

Fungal species belonging to the genus *Trichoderma* are easily isolated from soil, decaying wood and other forms of plant organic matter (Howell, 2003).

The present study demonstrated the presence of *Trichoderma* spp. in the Palestinian studied areas that are irrigated and cultivated with different crops (Tomato, cucumber, bean, eggplant, pepper, squash, cauliflower, cabbage, and Jews mallow, grape, banana, and Thyme). Forty-seven *Trichoderma* isolates were obtained from 169 soil samples collected from different locations in Palestinian districts of the West Bank.

Among the Forty-seven *Trichoderma* isolates that were recovered from soil samples, five isolates (H2, Q29, N38, B46 and B47) were isolated from soil fumigated with methyl bromide in the same or previous agricultural season. Similar results were observed by Munnecke *et al*., 1981 who reported that *Trichoderma* spp. is the most common antagonist to appear after soil fumigation with methyl bromide and it can reproduce rapidly. It was shown that *T. harzianum* prevented reinfestation of soil by *R. solani* and *S. rolfsii* (88% reduction) in peanut field under both controlled-environment and field conditions. This agrees again with the work of Strashnov et al. (1985). They found that combination of *T. harzianum* and methyl bromide enhanced proliferation and establishment of the antagonist in soil. The isolates (H2, H3, J9, Jn12, Jn17, Jn18, Q27, Q28, Q29, Q30, and isolate T31) were recovered from soil irrigated by fungicide Dynone in

the same or previous agricultural season, while the isolates Jn12 and Q28 recovered from soil irrigated by fungicides Dynone and Benlate. The rest isolates were recovered from non treated soils.

Dual culture interaction between the two pathogenic fungi, *R. solani* and *S. rolfsii* and *Trichoderma* isolates (47 isolates) were studied in *vitro*. In this study, the objective was a rapid evaluation of the antagonistic capacity of *Trichoderma* isolates against *R. solani* and *S. rolfsii.*

Results showed that the contact between *Trichoderma* isolates and *R. solani* were after 2 days and 3 days for *S. rolfsii*. The overgrowth rate of *Trichoderma* isolates on *R. solani* was faster three times than that on *S. rolfsii.* All *Trichoderma* isolates grew over the *R. solani* colony and degraded its mycelium, while not all isolates grew on *S. rolfsii* colony except the isolates (H1, H2, J8, J9, Jn14, Jn21, T34, and N38). The isolates (Q24, Q25, Q26, Q29, and T31) grew toward *S. rolfsii* colony and stopped its growth before reaching it, forming a clear zone.

This study revealed that *Trichoderma* isolates (H2, J8, Jn14, Jn17, Jn21, T33 and T36) parasitized the hyphae of *Rhizoctonia solani* and *Sclerotium rolfsii*. *Trichoderma* isolates hyphae of (Jn14, Jn21, and T36) grew over those of the pathogens and formed branches that coiled around them. Dense coiling of *Trichoderma* isolate (H2) around *R. solani* hyphae was observed. Light microscopy revealed the penetration and growth of *Trichoderma* isolates (Jn14, T33, and J8) inside the hyphae of *R. solani* and *S. rolfsii*. Similar observations have been reported for the *Trichoderma harzianum* and *Sclerotinia sclerotiorum* interaction by Inbar et al. (1996).

Trichoderma spp. is well known mycoparasites (Elad & Chet, 1983). Mycoparasitism is a complex process including successive steps. The initial visible interaction shows that the hypha of the mycoparasite grows directly toward its host (Chet *et al*., 1981). When the mycoparasite reach the host, its hyphae coil around it or it attached to it by forming hook-like structures. Following these interactions, the mycoparasite penetrates the host mycelium, apparently, by partially degrading its cell wall.

Rhizoctonia solani and *S. rolfsii* are considered important soilborne pathogenic fungi capable of causing severe damage to agricultural crops such as bean, potato, and cotton (Lartey *et al*., 1991). *Rhizoctonia solani* are capable of attacking a tremendous range of host plants causing seed decay, damping-off, stem cankers, root rot, fruit decay, and foliage disease (Elad *et al*., 1980).

The antagonistic ability of *Trichoderma* isolates is highly variable (Chet *et al*., 1979), as was shown in this study in which only 11.54% and 5.77% of the *Trichoderma* isolates tested were effective in controlling *R. solani* and *S. rolfsii,* respectively, in the bioassay studies done in the growth Chamber. The most effective isolates were (Jn14, Jn21, T33, T36, H2, and R42). These isolates were recovered from soils generally not treated with chemicals, and planted with cucumber, pea, tomato, bean, and eggplant. However the isolate H2 was isolated from soil that were drenched with the fungicide Dynone in that very season and fumigated with methyl bromide in the year before.

Trichoderma spp., are well documented as effective biological control agents of plant diseases caused by soilborne fungi (Sivan *et al*., 1984; Coley-Smith *et al*., 1991). In the present study, application of

Trichoderma isolates as a conidial suspension greatly reduced disease index by 65.6% and 66.8% caused by *R. solani* and *S. rolfsii,* respectively.

The ability of *Trichoderma* to reduce diseases caused by soil borne pathogens is well known and it is related to the antagonistic properties of *Trichoderma*, which involve parasitism and lysis of pathogenic fungi and /or competition for limiting factors in the rhizosphere mainly iron and carbon (Sivan & Chet, 1986). Another mechanism has been suggested by kleifeld and Chet (1992) and related to *Trichoderma*-induced resistance in host plants to fungal attack.

Furthermore, Aziz et al. (1997) reported the effect of plant exudate on the inhibition of conidia germination *in vitro* and on the suppression of *Rhizoctonia* damping-off of bean *in vivo* when *Trichoderma lignorum* was applied. In the presence of bean exudates, the reduction in *Rhizoctonia* damping-off of bean by *Trichoderma lignorum* was obvious.

Increased growth response of several plants including vegetables, following the application of *Trichoderma* to pathogen-free soil has been documented (Baker, 1989; Chang *et al*., 1986; Kleifeld and Chet, 1992).

In this study, bean seeds which were planted in *Trichoderma* treated soils germinated earlier by 4 days than those planted in nontreated soils in addition to better emergence rate. In relation to this, Yedidia et al. (2001) suggested that a 30% increase in cucumber seedling emergence was observed up to 8 days after sowing when soil was amended with *T. harzianum* propagules. This can be explained as expensed by (Kleifeld and Chet, 1992) by the ability of *Trichoderma* to inhibit minor pathogens in the rhizosphere which might induce seed rots and preemergence damping off.

Furthermore, seedlings grown in *Trichoderma* treated soils recorded higher values of plant heights and weights. This agrees again with the work of (Kleifeld and Chet, 1992). They found that dry weights of cucumber plants grown in autoclaved sandy loam soils treated with a conidial suspension (10^6 CFU / gm soil) was increased by 26% compared with 43 % in autoclaved soil. This variation is explained by the authors that the increased growth response of plants depends on the ability of the fungus (*Trichoderma*) to survive, develop in the rhizosphere, and varies as well with the substrate.

Some investigators reported that the increased growth response caused by *Trichoderma* isolates resulted in large increase in root area and root lengths and may be related to the effect on root system. These results were similar to the results revealed by (Yedidia *et al*., 2001) who showed that treatment of cucumber plants in soil with *T. harzianum* (T-203) resulted in large increase in root area and cumulative root lengths, and significant increase in dry weight, shoot length and leaf area over that of the untreated control.

Yedidia et al. (2001) suggested a direct role for *T. harzianum* in mineral uptake by the plant at a very early stage of the fungal-plant association. In addition, Harman (2000) established that *Trichoderma* spp. are opportunistic plant colonizers that affect plant growth by promoting abundant and healthy plant roots, possibly via the production or control of plant hormones. Increased growth response has been demonstrated by several other investigators (Altomare *et al*., 1999; Anusuya and Jayarajan, 1998). They demonstrated the ability of *T. viride* and *T. harzianum* to solubilize insoluble tricalcium phosphate *in vitro*.

The effect of temperatures on radial mycelium growth and spore production were studied for the most effective *Trichoderma* isolates. The results showed that the optimum temperature for mycelium growth rate and spore production of isolates was 30 ^{o}C and the mycelium growth rate ranged from 0.02 cm^2 for the isolate (Q28) and 16.75 cm^2 for the isolate (Jn14). These results agree with the work of Prasun and Kanthdai (1997). They found that the optimum temperature for *Trichoderma* sp. was 25-30^{o}C. In addition, Chet (1990) reported that the optimal temperature for *Trichoderma* growth is around 28 ^{o}C and growth is very slow below 18 ^{o}C.

The effect of temperature on the interaction between the bioagent and the pathogens was evaluated as well. *Trichoderma* isolates overgrew and killed the pathogen (*R. solani*) in dual culture at the temperatures 15-35^{o}C. The overgrowth rate of *Trichoderma* isolate (Jn14) reached a peak at temperature 30 ^{o}C. However, the mean overgrowth rate of *Trichoderma* isolate (Jn14) reached a peak at temperature 25 ^{o}C. Similar results were demonstrated by Prasun and Kanthdai (1997). They found that *Trichoderma* overgrew *S. rolfsii* at 25 ^{o}C and 30 ^{o}C in dual culture.

In contrast to the interaction in dual culture, results of this study revealed that the growth rate of *R. solani* and *Sclerotium rolfsii* was reduced due to the production of fungitoxic metabolites produced by *Trichoderma* isolates at different temperatures. The growth rate of *R. solani* and *S. rolfsii* was reduced significantly at 20 °C and 25 °C by the *Trichoderma* isolate (Jn14). *Trichoderma* isolate (T36) was the most effective isolate when used against *S. rolfsii*; it reduced the pathogen mycelium growth at temperatures 15 °C, 20 °C, and 25°C. Similar results were observed by Brasun and Kanthadai (1997) who reported that

Trichoderma (isolate Td-1) produced higher concentration of fungitoxic metabolites at higher temperatures and it effectively suppressed the growth of *S. rolfsii* at or below 33 °C.

The importance of antibiotics for biocontrol activity was established in several studies. In 1983, Howell and Stipanovic isolated and described a new antibiotic, gliovirin produced from *Trichoderma virens* that was strongly inhibitory to *Pythium ultmum* and *Phytophthora* species, but not to *R. solani*, *Phymatotrichum omnivorum*, *Rhizopus arrhizus*, or *Verticillium dahlia*.

Conclusions

Forty-seven of *Trichoderma* isolates were isolated from 169 soil samples collected from different locations in Palestinian districts of the West Bank. The present study demonstrated the presence of *Trichoderma* spp. in the Palestinian studied areas that were irrigated and cultivated with different vegetable, horticultural, and medical crops.

In vivo bioassay studies showed that disease index of bean plants caused by *Rhizoctonia solani* and *Sclerotium rolfsii* was reduced at different rates by a percentage of 65% and 67%, respectively.

In dual culture, the most effective isolate (Jn14) overgrew the pathogens *R. solani* and *S. rolfsii* at 30 and 25 ^{o}C, respectively. In addition, results showed that Jn14 and T36 were the most effective isolates at 25 °C and inhibited *R. solani* and *S. rolfsii* mycelial growth rate at a percentage of 78% and 94.7%, respectively.

The increased growth response of plant, caused by *Trichoderma* was studied. Plant heights increased approximately twice (160% _ 200%) compared to the control, and (133% _ 217%) in plant fresh and dry weight. Germination of bean seeds treated with *Trichoderma* isolates occurred about four days earlier than those in untreated soil.

Two isolates (Jn14 = *Trichoderma harzianum* and T36 = *Trichoderma hamatum*) isolated from non-treated soil in Jenin and Tulkarem, respectively, showed great success in several parameters evaluate in respect to biological control and can be considered seriously for future implementation and practical studies in biocontrol of soil-borne pathogenic fungi.

Future research on this subject may include the following:

- The mechanisms employed by biological control agents to affect biological control of plant diseases are many and complex and their use varies with the kind of biocontrol agent, pathogen, host plant, soil type, temperature, PH, moisture of the plant, soil environment, and other members of the microflora. Therefore, it is important to obtain *Trichoderma* with expanded host, temperature, and moisture parameter, and strains with better storage qualities by using hybridization.

- Results show *Trichoderma* promise as part of a replacement strategy for toxic fungicides and soil fumigants and because of the toxicity and environmental impacts as results of applying large amount of fungicides. It is important to study the compatibility of *Trichoderma* isolates with low doses of pesticide, which may be able to lead to a synergistic effect resulting from suppression of competitive soil microflora.

- Studying the relationship between *Trichoderma* and plant growth regulators in plant increased growth response (IGR).

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Appendix A. *Trichoderma* Selective Media (Tsm) (Gram/Liter Of Distilled Water)

$MgSO_4 .7H_2O$	0.2 g
K_2HPO_4	0.9 g
KCl	0.15 g
NH_4NO_3	1.0 g
Glucose	3.0 g
Chloramphenicol	0.25 g
Dexon 60%	0.3 g
PCNB	0.2 g
Rose-bengal	0.15 g
Agar	20 g
Microelements	1 ml

Appendix B. Microelements Used in *Trichoderma* Selective Media (Gram/100ml of Distilled Water)

$FeSO_4 .7H_2O$	1.0 g
$MnSO_4 H_2O$	0.6 g
$ZnSO_4 .7H_2O$	0.9 g

Appendix C. Anova Tables

Table 3.4 *Trichoderma* isolates average linear growth (mm/day) growing on PDA after 4-days incubation at 25°C

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	46	1670.26	36.31	12.95	< 0.05
Within groups	94	263.50	2.80		
Total	140	1933.76			

Table 3.5 Mycelium Overgrowth Rate (mm/day) of *Trichoderma* isolates on phytopathogenic *R. solani* in dual culture both growing on PDA medium and incubated at 25°C.

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	46	689.928	14.998	31.055	< 0.05
Within groups	188	90.797	0.438		
Total	234	780.725			

Table 3.6 Mycelium Overgrowth Rate (mm/day) of *Trichoderma* strains on phytopathogenic *S. rolfsii* in dual culture both growing on PDA medium and incubated at 25°C.

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	46	118.22	2.57	25.26	< 0.05
Within groups	172	17.50	0.10		
Total	218	135.72			

Table 3.7 Effects of the application *Trichoderma* isolates on *Rhizoctonia* damping- off of bean plants measured as disease index after 14 days of sowing the seeds in bioassay method.

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	53	86301.294	1628.326	6.595	< 0.05
Within groups	216	53334.900	246.921		
Total	269	139636.194			

Table 3.8 Effects of the application *Trichoderma* isolates on *Sclerotium* rot of bean plants measured as disease index after 14 days of sowing the seeds in bioassay method.

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	53	104685.574	1975.200	5.518	< 0.05
Within groups	216	77318.800	357.957		
Total	269	182004.374			

Table 3.9 Mycelium growth rate (cm^2/day) of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) growing on PDA medium at different Temperatures (10, 15, 25, 30, 35, and 40 ^{0}C).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	59	2914.433	49.397	100.245	< 0.05
Within groups	180	88.698	0.493		
Total	239	3003.131			

Table 3.10 Spore production (10^8 spore/ml) of *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) growing on PDA medium after 15 day of incubation under continues light at different temperatures (10, 15, 25, 30, 35, and 40 ^{0}C).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	39	1506.695	38.633	53.871	< 0.05
Within groups	120	86.056	0.717		
Total	159	1592.751			

Table 3.11 Overgrowth rates of most effective isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) on *R. solani* growing on PDA medium in dual culture at different temperatures (15, 25, 30, and 35 ^{0}C).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	39	9708.855	248.945	182.557	< 0.05
Within	120	163.639	1.364		

groups					
Total	159	9872.494			

Table 3.12 Overgrowth rates of most effective *Trichoderma* isolates (H2, J8, Jn14, Jn21, Q28, T33, T36, N38, R42, and Egy52) on *S. rolfsii* growing on PDA medium in dual culture at temperatures (15, 25, 30, and 35 ^{0}C).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	39	44.648	1.145	28.883	< 0.05
Within groups	120	4.756	0.0396		
Total	159	49.404			

Table 3.13 Effect of temperature on antibiosis of *Trichoderma* isolates (J8, Jn14, and T36) on *R. solani,* 48 hours after incubation.

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	11	281.777	25.616	59.333	< 0.05
Within groups	36	15.543	0.432		
Total	47	297.320			

Table 3.14 Effect of temperature on antibiosis of *Trichoderma* isolates (J8, Jn14, and T36) on *S. rolfsii,* 48 hours after incubation.

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	11	1932.660	175.696	355.692	< 0.05
Within groups	36	17.782	0.494		
Total	47	1950.443			

Table 3.15 Effect of local *Trichoderma* isolates on bean plant height (**A**).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	15	1421.836	94.789	8.630	< 0.05
Within groups	62	680.988	10.984		
Total	77	2102.824			

Table 3.15 Effect of local *Trichoderma* isolates on bean plant fresh Weight (**B**).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	15	233.821	15.588	4.999	< 0.05
Within groups	63	196.445	3.118		
Total	78	430.266			

Table 3.15 Effect of local *Trichoderma* isolates on bean plant dry Weight (**C**).

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	15	2.319	0.155	4.976	< 0.05
Within groups	63	1.957	0.0311		
Total	78	4.276			

Table 3.16 Effect of *Trichoderma* isolates on bean (*Phaseolus vulgaris*) seedling emergence

Source	D.F	Sum Square	Mean of Square	F. Ratio	P
Between groups	31	435.381	14.045	22.087	< 0.05
Within groups	124	78.850	0.636		
Total	155	514.231			

جامعة النجاح الوطنية
كلية الدراسات العليا

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اشراف
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قدمت هذه الأطروحة استكمالا لمتطلبات درجة الماجستير في العلوم البيئية بكلية الدراسات العليا في جامعة النجاح الوطنية في نابلس، فلسطين.
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الملخص

فطر التريكودرما من الفطريات القاطنة في التربة، ينتمي الى مجموعة الفطريات الناقصة الرمية. اوضحت الأبحاث العلمية امكانية استخدامه في المكافحة الحيوية للامراض النباتية القاطنة في التربة.

في هذه الدراسة تم الحصول على 47 عزلة مختلفة من فطر *Trichoderma* عزلت من 169 عينة تربة جمعت من الحقول الزراعية المروية والمزروعة بالمحاصيل المختلفة باستخدام طريقة Dilute Plate Technique على وسط غذائي خاص بفطر التريكوديرما.

تم دراسة مقدرة العزلات المختلفة من فطر *Trichoderma* لمكافحة مرضي عفن الجذور الريزوكتوني (*R. solani*) ومرض العفن الأبيض السكليروشيني (*S. rolfsii*) باستخدام طريقة التداخل الفطري (Dual Culture) ومعاملة نباتات الفاصولياء الكاملة (Bioassay). اظهرت نتائج دراسة التداخل الفطري بين عزلات فطر التريكوديرما وفطر ال *R. solani* ان اقوى العزلات كانت العزلة Jn17 وكان معدل النمو 9.9 ملم^2 / يوم. وعند استخدام العزلات مع فطر*S. rolfsii* اظهرت الدراسة ان اقوى العزلات J10 ومعدل نموها 3.25 ملم^2 /يوم. اظهرت نتائج استخدام عزلات التريكوديرما كمعلق يحتوي ابواغ الفطر بتركيز ($3x10^6$ /g soil) تم اضافتها الى التربة لمكافحة مرضي عفن الجذور الريزوكتوني ومرض العفن السكليروشيني على نباتات الفاصولياء حيث ادت اقوى العزلات

Jn14 الى تقليل شدة الاصابة بمرض العفن الريزوكتوني بنسبة 65% والعفن السكلورشيني بنسبة 67%. واوضحت نتائج دراسة تاثير الحرارة على عملية التداخل بين اقوى العزلات والفطريات الممرضة بان العزلة Jn14 كانت اقوى العزلات على درجات الحرارة 25، 30 درجة مئوية.

تم فحص قدرة العزلات على انتاج المضادات الحيوية في الوسط الغذائي (PDB) الممزوج مع الوسط الغذائي PDA بنسبة 10% حيث اظهرت النتائج ان العزلة Jn14 ثبطت نمو فطر *R. solani* بمعدل 78% اما العزلة T36 ثبطت نمو فطر *S. rolfsii* بمعدل 94.7% على درجة حرارة 25م°. واظهرت النتائج ان عزلة التريكوديرما Jn14 انتجت أبواغ وصلت الى الحد الاعلى وكانت بتركيز ($1.5x10^9$ spore/ml) على درجة حرارة 30م°. اظهرت نتائج دراسة تاثير التريكوديرما على انبات بذور نبات الفاصولياء المعاملة قبل 4 ايام من البذور الغير معاملة والى زيادة طول النباتات 160%-200% وزيادة الوزن 133%-217%.