BEAN ROOT ROT COMPLEX; ITS MANAGEMENT BY MICROBIAL AGENTS AND PLANT RESISTANCE.

BY

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DEDICATION

To my wife Felistas Muthoni for her love and guidance.

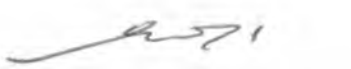
DECLARATION

This thesis is my original work and has not been presented for a degree in any University before.

SAMSON JOSEPH MURIUNGI

21.7.97
DATE

This thesis has been submitted with our approval as University supervisors.



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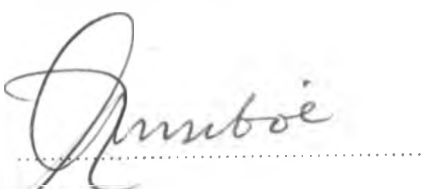

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LIST OF ABBREVIATIONS

GLP - Grain Legume Project

CIAT - Centro Internacional de Agricultura Tropical.

DESIGNATIONS

T3A = *Trichoderma viride*

T13 = *Trichoderma koningii*

F = *Fusarium*

ABSTRACT

The root rot problem which had been described as *Fusarium* yellows because of yellowing of leaves, exhibited hypocotyl and root symptoms characteristic of those caused by *Fusarium* root rot pathogen. No symptoms due to other root rot pathogens were observed in all the 18 samples examined.

Laboratory isolation and identification of the root rot pathogen from representative samples of hypocotyl, and lower roots showing characteristic root rot symptoms and rhizosphere soil collected, confirmed *Fusarium* as the sole pathogen causing the root rot problem. Five *Fusarium* isolates were isolated, three of them were identified as *Fusarium Oxysporum* and two as *Fusarium solani.*

Pathogenicity tests of the five *Fusarium* isolates on large Rose coco (GLP - 2) variety of beans using the root - clip and soil inoculation method revealed that all the five isolates were pathogenic but different in virulence. The mean disease incidence and severity values produced by all the isolates were higher for soil inoculation method than the root clip method. The soil inoculation method was used in all subsequent experiments.

Screening of antagonistic effects of two *Trichoderma isolates* (*Trichoderma viride* and *Trichoderma koningii*) against the *Fusarium* isolates in vitro gave positive results. *Trichoderma viride* produced mean growth reduction of 100% using random sprinkling and 98.2% using equidistant plating on *Fusarium* isolates whereas *Trichoderma koningii* gave mean values of 92% and 56.4% using the two methods respectively. Saprophytic growth and survival of the two *Trichoderma* isolates in autoclaved half broken dehulled rice, sorghum and vermiculite used as food carriers differed in their suitability for the isolates. There was a appreciable increase in the number of conidia per gram of carrier for both *Trichoderma* isolates in rice and sorghum during the first 14 days thereafter levelling off. Growth of the two isolates was very poor in vermiculite with *Trichoderma koningii* giving negligible growth after 18 days of incubation.

Greenhouse tests with the two *Trichoderma* isolates for the management of the root rot of beans gave promising results. *Trichoderma viride* in half broken dehulled rice gave the best control of the disease under greenhouse conditions.

This was followed by *Trichoderma koningii* in sorghum, then *Trichoderma viride* in vermiculite, then *Trichoderma koningii* in rice, then *Trichoderma viride* in sorghum and finally *Trichoderma koningii* in vermiculite. There was some correlation between saprophytic growth of *Trichoderma* isolates in different carriers in the laboratory and their antagonistic activity against the *Fusarium* pathogen in the greenhouse. *Trichoderma koningii* in sorghum and *Trichoderma viride* in rice produced the best growth in the laboratory and also the best management of the disease in the greenhouse.

The two best biocontrol agents (*Trichoderma viride* in rice and *Trichoderma koningii* in sorghum) were compared with a conventional chemical in the management of the *Fusarium* root rot of beans in the greenhouse. A fungicide murtano containing 26% Thiram and 20% Lindane was used to dress Rose coco bean seeds at the recommended rate of 10g per 0.5 kg of seeds.

The two antagonistic fungi were applied at the rate of 1:3, 1:6, and 1:9 (w/w) of colonized carrier to pathogen infested soil. *Trichoderma viride* in rice food carrier when applied at the rate of 1:3 gave the best results. Murtano was the least effective in controlling the disease under greenhouse conditions. Disease level increased with decreasing antagonist colonized carrier.

Five bean varieties and fifty-one bean accessions screened for resistance to the disease showed that all the lines were susceptible with most of them having a disease incidence of 100%.

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CHAPTER ONE

INTRODUCTION.

Beans are the most important pulse crop and second to maize in importance as food crop in Kenya. They constitute an essential source of proteins for human consumption with relatively high amounts of lysine, tryptophan and methionine. Area under bean is estimated to cover more than 320,000 hectares, most of it in the medium potential areas of Central and Eastern Provinces with annual rainfall of 700 to 900 mm per annum distributed in a bimodal pattern (Schonherr and Mbugua 1976). Bean yields are generally low in Kenya varying between 220 - 670 kg per hectare. However sound agronomic packages together with disease and pest control provisions make it possible to increase yield up to 2,200 kg per hectare (Acland 1971). In 1992 and 1993 the area under beans was 502,030 hectares with production of 442,808 metric tonnes and 628,036 hectares with production of 408,731 metric tonnes respectively (Anonymous 1993).

The common varieties grown in Kenya are "Rose coco" (GLP - 2), "Canadian wonder" (GLP - 24), "Red harricot" (GLP - 585) "Mwitemania" (GLP - X - 92) and "Mwezi moja" (GLP -X- 1127); all released by grain legume project (GLP) of the National Horticulture Research Station Thika for different agro-ecological zones of Kenya. However most farmers still continue to grow unimproved indigenous bean varieties probably due to consumer preferences or just diehard traditional habits (Mukunya et al 1975).

1.2 Factors limiting Production.

The production of dry bean (*Phaseolus vulgaris L.*) faces many varied constraints. Diseases, insect pests, soil problems, environmental stress, social economic problems and poor agronomic practises contribute to the large gap between actual and potential yield (Howard et al 1980).

The main biological factors limiting production are fungal, bacterial, viral diseases, nematodes and insect pests. Labour constraints, soil fertility and lack of certified seed are a serious problem in bean production; marketing is not a limiting factor, nor transportation or acceptance by consumers (Mukunya and Keya 1975).

1.3 Diseases as a major Production limiting factor.

Fungal diseases are the most important and of these rusts caused by *Uromyces phaseolus,* root and stem rots caused by various species of soilborne fungi, anthracnose caused by *colletotrichum lindemuthianum* and angular leaf spot caused by *Phaeoisariopsis grieseola* are important world wide (Mukunya and Keya 1975). Bacterial diseases such as Halo blight caused by *Pseudomonas syringe pv phaseolicola*, common blight caused by Xanthomonas phaseoli, fuscous blight caused by *Xanthomonas campestris pv phaseoli* and bacterial brown spot caused by *P. syringae* are also of world wide importance.

Root and stem rot of beans are the most economically important diseases of beans today in Africa and Latin America (Abawi and Pastor 1990). Abawi et al (1989) reported yield reductions of as high as 86% in *Fusarium* infested fields under stressful conditions.

Many root rots are widely distributed and economically important diseases of the common bean *(P. vulgaris L.)* in Africa, Central and Southern America, and Latin America. In Africa severe damage and economic losses resulting from root rots have been reported from Kenya, Burundi, Zaire, Uganda, Rwanda and South Africa (Abawi and Pastor 1990).

In Western Kenya, farmers estimated yield losses of between 30 to 100% in the first season (March-June) of 1992 (Buruchara, personal communication 1992). In 1991, yield loss due to the problem in Kakamega and Vihiga districts was estimated at 45 to 50%. The three varieties of beans widely grown (GLP-2, GLP-24, red haricot) in the two districts are susceptible.

Options available for controlling root rots after planting are very limited and of questionable effectiveness. Thus, a knowledge of the previous history of the particular field such as frequency of the root-rot incidence and severity, identity of causal pathogens, and other information is essential for formulating control programs (Abawi and Pastor 1990).

Proper diagnosis of root rots is essential for developing effective and practical control measures. Although the root rot problem in Western Kenya is a serious one, there are few reports of yield loss assessment, diagnosis of the causal agent and research in control measures. This project was undertaken to identify the causal agent (s) of root rot of beans *(Phaseolus vulgaris L)* in Western Kenya and to look for effective control measures based on microbial antagonism and plant resistance.

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CHAPTER TWO

The specific objectives were;

1. Isolation, identification and pathogenicity test of Root-rot pathogen (s) of beans from Western Kenya.
2. To test two isolates of Trichoderma fungi for antagonism against the bean root rot pathogen (s) in vitro.
3. To identify cheap but effective carriers for the selected antagonists.
4. To control root-rot of beans using the screened antagonists in the greenhouse and to compare its effectiveness with a standard fungicide.
5. To screen locally available bean germplasm and accessions for resistance to the root rot pathogen (s).

3.0 CHAPTER THREE
LITERATURE REVIEW.

3.1 Root rot of beans.

3.1.1 Symptomatology on beans.

Beans are attacked by different pathogens. Root rot of beans caused by *Fusarium solani f. sp phaseoli* appears as reddish lesions or streaks on the hypocotyl and primary root. The disease commonly occurs in Africa and Central America. In Africa it has been reported from Kenya and Malawi (Kraft et al 1981).

Fusarium yellows caused by *Fusarium Oxysporum f. sp phaseoli* occurs on the roots and hypocotyls, usually at wound sites. Initial symptoms appear on lower leaves, which exhibit yellowing and wilting and later progress upward to younger leaves. Naturally or artificially inoculated plants exhibit the diagnostic brown vascular discoloration which becomes evident after the first appearance of foliar symptoms. The disease has been reported from several African countries including Kenya and Central America (Abawi et al 1989).

Rhizoctonia root rot caused by *Rhizoctonia solani* results in seed rot, damping-off, stem canker, root and pod rot. The disease has been reported from several African countries including Kenya and Central America (Anderson 1982). Pythium root rot is caused by various species of pythium (*P. mariotylum, p. irregulare, aphanidermatum*, and *P. ultimum*). It can infect germinating seed cotyledons, terminal buds, radical and hypocotyl tissue. The disease is characterised by elongated water-soaked areas on roots and lower stem (Hendrix et al 1973). Pythium root rot has been reported in several countries in Africa and latin America. In Kenya the disease has been reported from Western Province (Buruchara; personal communication 1993).

Southern blight caused by *Sclerotium rolfsii* produces damping-off, stem blight and root rot. The disease has been reported from several countries in Africa and Central America. In Africa the disease has been reported from Kenya, Uganda, Malawi and many others (Punja 1989).

Black root rot caused by *Macrophomina phaseolina* infects the hypocotyl. Numerous elongated dark, sunken lesions are produced on the stem and root tissues resulting in plant stunting, premature defoliation and eventual plant death. The disease has been reported from several African countries including Kenya and Central America (Dhingra et al 1978).

Texas root rot (Aphanomyces root rot) infects underground plant parts. Above ground symptoms are stunting and sudden wilting at blossom initiation. Plants with Aphanomyces root and hypocotyl rot are stunted, chlorotic and suffer premature defoliation (Crispin et al: 1976).

Likewise, species of plant parasitic nematodes of the genera Meloidogyne, Pratylenchus and others are known to infect beans and cause considerable yield losses (Abawi et al 1990).

3.2.0 **Fusarium Root Rot of Beans.**

3.2.1 **Nomenclature of Fusarium.**

The genus *Fusarium* was erected by link (1809) for species with fusiform, nonseptate spores borne on a stroma and was based on *Fusarium roseum* (Booth 1971). In terms of international botanical code the genus was validated by Fries (1821) who included it in his order Tuberculariae.

In the genus *Fusarium* over 1000 species names have been proposed. Many of these are merely host epithet and usually represent a new record on that host rather than a new species. Present day identification depends upon an assessment of the full range of spore forms, growth rate and pigmentation.

Nectria haematococca, Calonectria rigiscula, Micronnectriella nivalis, Giberella zeae and other perithecia names are for the most part soundly based on a type specimen and these names may be used for species when the perithecial state is known (Booth 1971).

Formae speciales are known to exist within several species of Fusarium.

3.2.2 Identification.

The presence of fusoid macroconidia with a foot cell bearing some kind of heel is now accepted as the most definite character and this foot cell separates it from *Cylindrocarpon*, the most closely related genus. Microconidia and terminal or intercalary chlamydospores may be present or absent. Perithecial state where known, belong to the hypocreals.

As with several other genera of the fungi imperfecti, *Fusarium* species in general can only be identified in pure culture and preferably from cultures started from single spores. Secondly, as *Fusarium* isolates have a remarkable ability for adapting both their form and color in response to the pressure of the culture environment, the cultural medium must be standardized and kept uniform for identification purposes (Booth 1971).

3.2.3 Fusarium morphology and pigmentation.

Spore morphology is the major character in identification of *Fusaria.* Spores may be conidia produced as simple-or polyphialidic slime spores or as enteroblastic spores or chlamydospores. Conidia may occur as 0-10 septate, pyriform, fusoid to oval microconidia through to straight or curved, 0-10 or more septate macrononidia (Booth 1971). Many species of *Fusarium* produce stromatic pustules which may be perithecia initials or develop into true sporodochia.

The development of pigments in respect to light varies in different species. *Fusarium* species also produce pigments over a broad pH range. In general there is a gradual deterioration in growth and morphology when *Fusarium* isolates are maintained on synthetic media due to mutations produced by the selective pressure of the artificial environment and to some extent by nutritional deficiencies (Brown 1926). These cultural forms which arise in the laboratory have led to the term wild type being used for the initial isolate from nature which usually shows optimum growth and sporulation with no abnormalities in conidial shape and size.

3.2.4 Preservation of Fusarium.

As the identification of *Fusarium* is based on their cultural characteristics and their full range of spore morphology, it is necessary to maintain cultures for comparative identification. Miller (1945a) and Cornmack (1951) described the successful maintenance of *Fusarium* species in sterile loam soil. Cornmack (1951) found that *Fusarium avenacium* survived three years in soil at room temperature and upto eight years if the soil is kept under refrigeration at about 5 degrees celsius. Fusarium isolates are preserved in sterile loam soil kept in a refrigerator at 4 degree celsius.

3.2.5 Geographical distribution and importance of the disease.

Fusarium species are widely distributed in soils and or organic substrates (Booth 1971). They abound in cultivated soil both in temperate and tropical regions and are amongst the fungi frequently isolated by plant pathologist. Fusaria also cause diseases of animals and man, and as a major cause of storage rots often produce toxins which contaminate human and animal food.

The predominant interest in the genus has been and still is in the role of Fusarium as plant pathogens. Serious wilts such as Panama disease of bananas caused by *Fusarium oxysporum* f.sp *cubense* are among the most devastating plant diseases in the world. Strains of *F.solani* are also of world-wide occurrence as root rots but may also cause cankers of hardwood trees (Booth 1971).

Fusarium root rot is one of the most prevalent diseases of beans in the world. The disease commonly occurs in Africa, Brazil, Colombia, Peru, Ecuador, Chile, Venezuela, Costa rica Mexico. (Kraft et al 1981). In Africa it has been reported from Kenya and Malawi and undoubtedly occurs in other countries as well.

3.2.6 Symptomatology of Fusarium root-rot on beans.

Initial symptoms of Fusarium root rot appears about 1 to 2 weeks after planting, as longitudinal, narrow, bright-red streaks on hypocotyl and taproot surface (Kraft et al 1981). Each streak continues enlarging and may advance from secondary roots to hypocotyl tissues. With time, the expanding infected areas become numerous, coalesce, and may cover the hypocotyl upto the soil surface. Infected areas become reddish brown, lack definite margins, remain superficial, and may exhibit longitudinal fissures. In severe infections, the primary and lateral roots may die and lower stem tissues become pithy or hollow. Lateral adventitious roots are then produced from stem tissues above infected areas. Severely infected plants are stunted, chlorotic and may defoliate prematurely. The number of pods and seed size may also be reduced on severely infected plants.

3.2.7 Etiology.

The disease is caused by the fungus *Fusarium solani* (Mart.) Appel and Wollen f. sp *phaseoli* (Burk) Snyd. and Hans. It has an ascomycetous sexual state, *Nectria haematococca* Berk. and Br. *Fusarium oxysporum* Schlect f. sp. *Phaseoli* Kendrick and Snyder causes Fusarium yellows of beans. Two pathogenic races of this fungus are known to exist, their distinction being based on the differential reaction of bean germplasm. A recent study suggested that several other races may exist (Kraft et al, 1981). Both pathogens *(F. Solani* and *F. oxyporum*) are morphologically similar to all pathogenic and saprophytic members of the genus *Fusarium*. However they are distinguished by their physiological and pathological adaptation to beans. Like the *Fusarium* wilt fungus *F. solani* also produces macroconidia, microconidia and chlamydospores. The macroconidia differ in shape from those of the *Fusarium* wilt pathogen by being less curved, having one blunt end and being somewhat larger (Kraft et al 1981).

3.2.8 **Fusarium solani group.**

Fusarium solani (Mart.) Sacc., Michelia 2:296, 1881 has a growth rate of 3.2cm: aerial mycelium striate, sparse to dense and floccose, greyish-white (Booth 1971). Agar typically develops a blue to bluish-brown discoloration although occasionally a brownish vinaceous pigmentation is present. Unlike *F. oxysporum* whose microconidia are formed from short microconidiophores with numerous phialides those of *F. solani* are formed from lateral conidiophores which initially may be merely elongated lateral phialides which narrows slightly towards the apex. Microconidia of *F. solani* are also broader and more oval in shape with somewhat thicker walls than those of *F. oxysporum* group (Booth 1971).

Macroconidia which are equilaterally fusoid develops after four to seven days from initially simple but later from short multi-branched conidiophores which soon merge to form effuse sporodochia. Many have a rounded foot cell similar to *Cyclindrocarpon* species but there is always a number of spores in any one isolate which have a well marked-foot cell. The globase to oval chlamydospores may be terminal or intercalary. In the wet tropics perithecia occurs abudantly whereas temperature zones production of perithecia only occurs in cultures (Alexander et al 1966). The distribution of *F. solani* in soil is world -wide. Its true parasitic capabilities are not always clear: apart from causing root rots and stem cankers, it also occurs as a spoilage organism.

Foot rot due to *F. solani* has been known for the past sixty years in Europe as one of the main causes of the failure of pea crop (Buxton 1955b). There is also abundant evidence for the occurrence of *F. solani* as a serious root rot of both broad bean (*Vicia*) and French bean (Phaseolus). Considerable physiological specialization has also been demonstrated.

3.2.9 **Fusarium Oxysporum group.**

Fusarium Oxysporum, apart from being the most economically important member of the genus *Fusarium*, is also one of the most labile and variable species. The average growth rate of cultures is 4.3 cm although in some strains it may reach 6.5 cm. Mycelium is delicate white or peach but usually with a purple tinge, sparse to abundant then floccose becoming felted and sometimes wrinkled in older cultures. Microconidia are borne on simple phialides arising laterally on the hyphae or from short sparsely branched conidiophores. Macroconidia are borne on elaborately branched conidiophores or on surface of *Teberculuria* like sporodochia (Kraft et al 1981)

Fusarium oxysporum is of world - wide in distribution. It occurs chiefly as a soil saprophyte and appears to survive winter in the mycelial or chlamydospore state. Numerous strains of this species are serious wilt pathogens of many crops. The panama disease of bananas caused by *Fusarium oxysporum* f. sp *cubense* is among the most devastating disease in the world.

3 3 0 **Epidemiology.**

Severity of Fusarium root rot has been shown to increase with such stress factors as soil compaction, drought, herbicidal injury, use of ammonium form of nitrogen, toxic metabolites of crop residue decomposition and root parasitism by other plant pathogens (Miller et al 1985). A synergestic interaction was shown to exist between this pathogen and *Pythium ultimum* resulting in severe damage to beans. Growth of the pathogen in agar media is optimal at 29 to 32 degrees celsius, but disease severity is greater at 22 than at 32 degrees celsius. The pathogen is also most damaging in light - textured soils and during drought.

3.3.1 Infection by Fusarium root rot pathogen.

Fusarium solani f. sp *phaseoli* pathogen is capable of directly penetrating bean tissues or through wounds and natural openings (Kraft et al 1981). *Fusarium Oxysporum* f. sp *phaseoli* usually attacks near the root tip, just behind the root cap. Penetration may also occur through wounds and natural openings on other root parts and on stems.

3.3.2 Survival and dissemination.

The dark, thick - walled chlamydospores are the long-term survival structures in soil (Abawi and Pastor 1990). As stated by Nash (1963) *F. solani* occurs in form of chlamydospores in naturally infested soil. Messiaen et al (1968) also stated that chlamydospores were the resting stage of *F. solani* in soil. Alexander et al (1963) demonstrated that sterile soil extracts stimulated chlamydospores production of *F. solani* f. sp *phaseoli* but when the nutrient level was raised in a soil extract by means of glucose no chlamydospores were formed.

Fusarium root rot pathogen also survives in soil by colonising roots of non host crops without causing disease (Abawi and Pastor 1990).

The pathogen is disseminated when infested host tissues or infested soil are moved by water and wind; or on implements, humans, and animals; or through the planting of contaminated seed.

Apart from canker- forming strains, transmission is predominately soil - borne with increasing incidence in cultivated soil with successive crops (Booth 1971).

3.4 Management strategies adopted to control root rot of beans.

3.4.1.0 Biological control of plant pathogens.

More than 100 years after the introduction of soil disinfestation and more than 500 years after Sanford's classical publication on biocontrol . . .. we are frustrated by the large gap between promising results in the greenhouse and failures in the field . . . we no longer aim to achieve absolute control, but rather an economic reduction in disease level.

-J . Katan, 1981.

The scientific basis of biological forms of pest management is very complex; possibilities for their development widen with increase in scientific knowledge, and are exploited in accordance with economic and social needs (Huffaker et al 1976).

The term "biological control" was first used by Smith (1919) to signify the use of natural enemies (whether introduced or otherwise manipulated) to control insect pests. Baker and Cook (1974) defined biological control as the reduction of the amount of inoculum or disease producing activity of a pathogen accomplished by or through one or more organisms other than man. In 1983 the two scientists redefined biological control as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state by one or more organisms accomplished naturally or through manipulation of the environment or host or antagonists or by mass introduction of one or more antagonists. " Disease producing activity" involves growth, infectivity, aggressiveness, virulence and other qualities of the pathogen, or processes that determines infection, symptom development, and reproduction.

Biological control may be accomplished through: cultural practices (habitat management) that creates an environment favourable to antagonists, host plant resistance or both; through plant breeding to improve resistance to pathogen or suitability of the host plant to the activities of antagonists; through the mass introduction of antagonists, non pathogenic strains or other beneficial organisms or agents.

3.4.1.1 Approach to biological control.

Biological control of inoculum, biological protection against infection, cross protection and induced resistance are the three approaches to biological control (Cook 1983). Biological control of inoculum includes destruction of the "propagative units" of the pathogen, prevention of inoculum formation, displacement of the pathogen in crop residues and manipulation of factors inherent in the pathogen. Biological protection against infection involves protection of planting materials such as seeds, transplants, seedpieces, protection of shoots with biological seed treatment, biological protection of foliage and flowers and inoculating of prunning wounds with antagonists. In cross protection and induced resistance, a cross protection is induced by a weak pathogen or a different pathogen.

3.4.1.2 Why biological control?

In recent years the increasing use of potentially hazardous pesticides in agriculture has been the subject of growing concern of both environmentalists and public health authorities. Advantages of biological control are numerous. They include increasing crop production within existing resources, avoiding development of pathogen resistance to chemicals, maintaining relatively pollution and risk - free control and adopting practices compatible with sustainable agriculture (Cook 1983). Most criticisms of biological control have been concerned with its supposedly narrow limitations as a technique of pest suppression and time taken to be effective (Huffaker 1976).

3.4.1.3 **Actual mechanisms and processes of pathogen decline in biological control.**

Antagonism and fungistasis are involved in biological control (Cook and Baker 1974). Antagonism can be looked at from three angles:

(i) Antibiosis: This is a condition where one or more metabolites produced by an organism has harmful effect on one or more organisms.

(ii) Exploitation: This is a condition where one organism inflicts harm on another by Parasitism, predation, mycoparasitism or hyerparasitism. The remarkable amount of natural parasitism and predation of plant pathogenic inoculum by antagonists in soil documents Darwin's "struggle for life". As Darwin said " Everything is born to eat and to be eaten" (Stone 1980).

(iii) Competition: This is a condition whereby two or more species compete for resources that are in short supply. For example *Fusarium* has many strains some pathogenic and others non pathogenic. It has been found that soil amendment with materials that has high carbon to nitrogen ratio prevent infection from *Fusarium* (Cook and Baker 1974). So in presence of such material there is no enough nutrient for the pathogen to germinate.

(iv) Fungistasis: Is the inhibition of spore germination in the soil and it is attributed to the chemical substances or starvation (Baker and Cook 1974).

3.4.1.4 Effect of antagonists on pathogen and disease in soil.

Once an antagonist is selected (through such experiments as in vitro study carried in the laboratory; see section 4.6), it must be tested in soil (Onkar et al 1986). Culture tests help find potential antagonists but give no information on their activity in the soil (Cook and Baker 1974). The effect of the antagonist on the survival, saprophytic growth and the parasitic activity of a pathogen may be tested in the greenhouse or field. The soils for these tests should be sterile, conducive to the pathogen prior to infestation with the antagonists and should be infested with the propagules of the pathogen in which they naturally exist in the soil (Onkar et al 1986). To obtain meaningful data, the quantity of the propagules of the pathogen and antagonist added per unit volume, weight, or area of soil should be controlled.

3.4.1.5 Biological control of root rot pathogens.

The possibility of controlling plant pathogenic fungi by antagonistic micro-organisms added either as a substitute or as an additive to fungicides has been the subject of extensive research. Among the many potentially antagonistic soil inhabitants, members of the genus *Trichoderma* have gained considerable success (Dennis and Webster 1971). With very few exceptions, biological control of plant disease with antagonistic micro-organisms is still restricted to experimental work, despite the large amount of published data on this subject (Wood and Tveit, 1955; Baker and Cook, 1974; Hennis and Chet, 1975; Grindrat, 1978).

A common feature of microbiological agents when compared with chemicals, is the wide spread resistance they encounter from the receiving biotic environment. Breaking or escaping this resistance is a main condition for the success of biological control.

Considerable information is available on the influence of antagonists, competitors, mycoparasites and predators of soilborne fungal and nematode pathogens, including those of the common bean (Backman et al 1975).

Hadar, Anderson and finley (1966) reported mycoparasitism of *Rhizoctonia* and *Fusarium* isolated from bean 'root rot' soil by an actinomycete. Furthermore , reduction of beans -root -rot severity by incorporating organic amendments and the use of crop rotation may well be a result of enhancing beneficial soil-borne micro-organisms. Mutitu, et al (1988) reported considerable reduction of Fusarium yellows severity on beans by use of coffee hulls and farmyard manure.

Hadar et al, (1979) found an isolate of *Trichoderma harzianum* which directly attacked the mycelium of *Rhizoctonia solani*. When applied to soil artificially infested with *R. solani* using wheat bran as a carrier, the antagonistic fungus effectively controlled damping off of bean, tomato and egg plant seedlings in the greenhouse.

One of the most successful examples is a preparation of *T. harzianum* which controls *S. rolfsii* and protected peanuts under field conditions (Martin et al 1985).

Flourescent pseudomonads and non pathogenic isolates of *Fusarium Oxysporum* were effective in inducing suppressiveness to Fusarium wilt of cucumber when added to soil together but ineffective when added separately at nearly neutral to alkaline soils (Park et al 1988).

Cross protection against *F. oxysporum* f. sp *dianthi* was obtained by introducing a non - pathogenic isolates of *F. oxysporum,* isolated from carnation to the plant before inoculation with a pathogenic isolate (Rattink 1989).

In dual culture plate assays indigenous Rhizobium strains isolated from nodules obtained from commercial snap beans in the lower traser valley of British Columbia (Canada) inhibited the radial growth of strains of *Fusarium moniliforme, F. oxysporum*, and *F. solani* f. sp *phaseoli* (Buonassisi et al 1986). Plants growth in pasteurised soil artificially infested with *F.solani* f. sp *phaseoli* from seed receiving varying concentration of Rhizobium showed a significant reduction in root rot. These data suggest that the potential exist for controlling *Fusarium* root rot through seed inoculation with nodulating Rhizobium strains which are also highly antagonistic to *F. solani* f. sp. *phaseoli* (Bounassisi et al 1986).

Reduction of Rhizoctonia root rot severity in beans has been demonstrated with *Trichoderma* species, Binucleate *Rhizoctonia* like fungi, *Gliocladium* species, *Laetisaria arvalis*, and other biocontrol agents (Papavizas 1985). Papavizas (1963) reported substantial reduction of microbial antagonism in bean rhizosphere as a result of soil amendment by oat straw and supplement nitrogen. This could be a result of competition resulting from increased number and type of microorganisms due to this ammendment. In the greenhouse *T. harzianum* plus a reduced dose of methylbromide (equivalent to 200 kg/ha) completely controlled disease incidence of *R. solani* in bean seedlings compared with controls in untreated soils.

T. harzianum also prevented reinfection by *R. solani* in fumigated soils (Strashnow et al 1985). Using light and electron microscopy, *Gliocladium virens* was proved to be a hyperparasite of *R . solani* (TU and Vaartaja 1981). *In vitro, G. virens* effectively inhibited sclerotial formation of *R. solani*. Greenhouse tests showed that the presence of *G. virens* in soil artificially infested with *R. solani* reduced at planting the severity of the Rhizoctonia root rot in white beans (TU and Vaartaja 1982).

T. hamatum, T. harzianum and to a lesser extent *T. koningii*, and *T. viride* have been used against damping off caused by *Rhizoctonia* and *Pythium* in the laboratory, the glasshouse and the field (Papavizas 1985). Commercial preparations are available, though not very widely used.

Simply adding *T. hamatum* to soils can reduce the damping off of peas caused by *pythium, T. harzianum* and *T. koningii* will also do this, but they are not effective against Rhizoctonia (Castanho and Butter 1978). Other workers, with other isolates, have shown effective control of Rhizoctonia and *S. rolfsii* with *T. hamatum* (Campbell 1989).

Control with antagonists can be just as effective as the conventional fungicides and may last much longer, by establishing in the soil and surviving until the next crop (Campbell 1989).

Adding the antagonist as young cultures grown on ground wheat bran produced the most effective control in a series of experiments by lewis and co-workers (1977). Control of Rhizoctonia by two different strains of *T.hamatum* was only effective from growing mycelial bran cultures which gave antagonist populations four to seven time greater control than mycelium with no food source or conidial preparations with or without a food source (Campbell 1989). There are however, complications because other antagonists may not work best from bran cultures. In this study *T. hamatum* still gave much better control from mycelium in bran but *T. viride* and *T. harzianum* did not.

It is worthwhile to screen various food carriers to establish the most suitable. The antagonists may have different optimum temperature from each other and from the pathogens. *T. harzianum* works against *S. rolfsii* and *R. solani* attacking beans in the field at temperatures above 20°c, and the control is best in more acidic soils (Campbell 1989).

Gliocladium is the only fungus which has been widely tested against damping off and its development has gone side by side with that of *Trichoderma* to which it is closely related (Kommedahl et al 1981). It is a mycoparasite and also produces antibiotics.

In this case the best inoculum preparation was conidial preparation, rather than mycelium (Papavizas et al 1989). Apart from use of *Trichoderma* in control of damping off caused by pythium soils suppressiveness to *Pythium* have also been found, Suppressiveness to *Pythium ultimum* was associated with certain finely textured soils in California's san Joaquin Valley. This suppressiveness was transmitted to conducive soils of the same texture usually as a function of the degree of dilution with sand, or mild heat treatment (Hendrix et al 1973).

3.4.1.6 **Use of Trichoderma in control of Fusarium species.**

Many studies have shown the potential of *Trichoderma* species as biocontrol agents of soilborne pathogens. Marrois et al (1986) showed the potential of multi-fungus conidial suspension including *T. harzianum* in controlling Fusarium crown rot of tomato.

The direct parasitism of *Trichoderma* on the hyphae of other fungi is one of the significant mechanisms which explains the antagonistic activity of *Trichoderma* species (Dennis and Webster 1971 c). Elad et al (1982b) showed that extracellular lytic enzymes, B, 1-3 - glucanase and chitinase excreted by *T. harzianum* were responsible for cell wall degradation in *Rhizoctonia solani* and *Sclerotium rolfsii*. However excretion of antibiotic compounds (Dennis and Webster, 1971a 1971b) as well as competition (Ahmad and Baker 1986) may both play major roles in the biological control of soilborne pathogens by *Trichoderma* species.

A strain of *T. harzianum* isolated from the rhizosphere of a cotton was found to be an effective biological control agent of *Fusarium oxysporum* f. sp *radicis, iycopersici* on tomatoes and *F. Oxysporum* f. sp. *diveum* on watermelons. Applications of this antagonist under field condition as a wheat bran-peat preparation or as a seed coating decreased disease incidence and significantly increased yields of tomatoes and water melons (Alex and Ilan 1986).

Trichoderma Viride present in the rhizosphere of resistant NP-15 as well as Susceptible T - 21 varieties of pigeon pea was found to be very effective on checking the disease caused by *Fusarium udum* (Vinod et al 1990).

3.4.2. Plant Resistance.

3.4.2.1. Introduction.

Resistance is the ability of a plant to remain relatively free from disease because of its inherent structural or functional properties. Resistance is an active dynamic response of a plant to a pathogen and it excludes passive phenomena like immunity and disease escape. To determine resistance in cultivars, selections or lines a method is used to asses qualitative and quantitative differences in susceptibility. The inoculation method and incubation conditions must be standardized to give reproducible results and produce high levels of disease but not so severe that plants having some resistance are graded as susceptible. The quality and quantity of inoculum, physiological age of the plant, environmental conditions prior to inoculation and incubation of plants after inoculation influence disease establishment (Onkar et al 1986).

3.4.2.2. Use of plant resistance to control root rot of beans.

Efforts at identifying bean germplasm with resistance to root rot pathogens have received considerable emphasis in recent years (Beebet et al 1981). A large number of bean germplasm lines and accessories that usually exhibit resistance to a single root rot pathogen are now available. Not surprisingly, the highest levels of resistance have been idenfied for the so called "specialised soil borne pathogens such as *F- oxysporum solani* and *Sclerotium rolfsii* (Beebe et al 1981).

Greenhouse screening procedures have the advantage of providing constant favarouble conditions for disease development, uniform and high levels of inoculum, and uniform high incidence of infection as opposed to field screening conditions (Dickson et al 1977).

Resistant bean cultivars have been used in the management of Rhizoctonia and Fusarium root rots, charcoal rot and Fusarium yellows (Pastor et al 1987). Hypocotyl tissue of beans (*Phaselous vulgaris* var Red Kidney) was highly susceptible to *Rhizoctonia solani* (isolate P.B.) during the 3rd week and resistant thereafter (Stockwel et al 1987). The changes in resistance was associated with elongation and maturation of the hypocotyl and concomitant changes in the pectic substances and calcium content of cell wall (Bateman et la 1965).

A white seeded french bean line 1273, was found to be highly resistant to the seed decay and pre-emergence damping-off caused by *Pythium ultimum* when tested in artificially infested soil under growth chamber conditions. Resistance was derived from P1 2033958, which has small black seeds. Results indicate that the association between seed coat color and *Pythium* resistance can be broken. There was a strong association between coloured snap bean seed and *Pythium* resistance but no linkage between *Rhizoctonia* resistance and seed color (Dickson et al 1977).

Inheritance of resistance to seed decay and pre-emergence damping off in snap beans caused by *Pythium ultimum* was found to be qulitatively inherited, was associated with seed coat color and it can be broken (York et al 1977).

Root rot resistant (RRR) Wisconsin 77 and Wiscons 83 were derived from a hybrid between the single plant selections WH 72-2 and WA 71-72 with resistance to pythium species (Hagedorn and Rand 1978). Resistant bean cultivars have been used in the management of Rhizoctonia and Fusarium root rots, charcoal rot and Fusarium yellows (Abawi and Pastor 1990).

Currently CIAT and the Ministry of Agriculture are running a joint programme at sabatia and shinyalu (in Kakamega and Vihiga districts of Western Kenya respectively) on use of resistant cultivars to control the disease (personal communication: Vihiga District Agricultural Officer 1993).

3.4.2.3 **Chemical control.**

There are many broad-spectrum and highly specific pesticides that effectively control root rot pathogens and their diseases. Soil fumigants such as methyl bromide, choloropicrin, vortex D-D, are highly effective biocides that kill all the pathogens as well as weed seeds (Munnecke et al 1979).

Briston et al (1973) reported significant reduction in Rhizoctonia root rot of beans by Terraclor at 5 and 10 1b/acre, Thiram (10 1b/acre), folpet (101b) and miller 658 (8 1b).

Root and hypocotyl root of bean caused by *Rhizoctonia solani* and *Fusarium solani* f. sp *phaseoli* was suppressed significantly by preplant incorporation of dinoseb at the rate of 6 - 7 kg/ha as indicated by disease severity decrease and yield increases. Other treatments using dinoseb at 10.1 kg/ha and at the lower rate in combination with trifluralin were also effective, but treatment with trifluralin alone were not as beneficial (Hagedorn et al 1982). Chemical seed treatment with metaxyl + chloroneb increased plant stand and weight in snap bean diseases caused by *Pythium* and *Rhizoctonia solani* (Lewis et al 1983).

Rhizoctonia and *Pythium* species have been controlled by applying 250-500 mg quintozene/100 litre of water 75% formulation; (Maramba 1982). Pentachloro-nitrobenzene (PCNB) at 500 ppm gave good control of *Rhizoctonia solani* on french beans (NIK, et al 1979). Bean seed treatment with DTEH reduced Pythium blight and resulted in significant yield increase (Papavizas, 1977). Seed treatment with fungicides such as Thiram, Benomyl and Captafol has been found to be effective in control of Fusarium root rot of beans (Papavizas et al, 1977).

Metalaxyl is the most recent and effective fungicide in control of pythium root rot of beans. (Abawi et al 1990).

3.4.2.4 **Cultural Methods.**

Effect of tillage practices on root rot severity and yield of snap beans, were reported to give higher yield and lower disease in pots with no tillage compared to pots with conventional tillage (Burke et al 1991). Good disease control was observed when plots were sprayed 4 times with benoyl with a reduction in apparent infection rate and increase of yield. A marked disease reduction observed when no tillage and benomyl application were integrated. Higher plants density showed a better effect in reducing rainsplashing hence root rot severity reduction as compared to lower densities tested.

Pythium ultimum, *Rhizoctona solani* and *Thielaviopsis basicola* infected many plants of beans and peas planted in successive seasons in field where populations of root pathogens had previously accumulated during 15 to 16 years of monoculture of the respective crops; whereas *Fusarium solani* f. sp. *Phaseoli* was prevalent only in the bean field and *F. Solani* f. sp. *Pisii* in the pea field (Burke 1974).

Crop residues of alfalfa, lettuce and cotton gin trash increased root rot severity of Pinto beans caused by *Rhizoctonia solani* and *Fusarium solani phaseoli* whereas residues of barley, sorghum, soybean and Sudan grass reduced it (Maker et al 1965). Tomato, maize, sesame, cotton and bean plant residues either produce inconsistent effects or did not alter the severity.

Cultural practices that increase plant vigor such as decreasing soil compaction, improving soil structure, soil amendment with crop residues having high C:N ratio such as grain crops and crop rotation have been found to reduce Fusarium root rot of beans (summer et al, 1986). Crop rotation and flooding reduces charcoal rot of beans. Deep ploughing, burning infected host residues, improving soil drainage, raised beds, and long crop rotation have been found to reduce disease severity in Rhizoctonia and Sclerotium root rot of beans.

Coffee hulls and farmyard manure were found to be effective in controlling Fusarium yellows in greenhouse experiments in Kenya (Mutitu et al 1988). Cultural practices that increase soil drainage, reduce soil compaction, and increase plant vigor, especially during the seedling stage help reduce damage by *Pythium*.

3 4 2 5. **Integrated control.**

Practical and effective management of bean root - rot disease by using a single control measure has usually been difficult and will probably continue to be so in the future (Abawi and Pastor 1990). Single control measure such as seed or soil treatment with selective fungicides, crop rotation, cover crops and land preparation, have at times and in specific locations increased bean yield or reduced root - rot severity. However, non of those measures has been consistently effective from season to season or over different location and production conditions.

Although progress has been made to identify sources of resistance to individual root - rot pathogens, the development of adapted bean cultivars with a higher level of resistance to multiple root rot pathogens has been very limited to date.

Thus, the difficulty of controlling root rots with a single control measure is not surprising especially in areas where severe root rot pathogens may be involved (Abawi and Pastor 1990).

Good control of Fusarium root rot in bean has been obtained by combining tolerant cultivars, tillage practices to loosen the soil and adjusting the amount and frequency of irrigation (Burke et al 1983).

In 3 of 4 year field study at Salisbury, Maryland, USA, ploughing infested soil to depth of 20 - 25 cm rather than disking to 5 - 7 cm before planting and chemical seed treatment with metalaxyl + chloroneb gave a greater control (yield) of snap bean disease caused by *Pythium* species and *Rhizoconia solani* than that attained when each component was used individually (Lewis et al 1983).

Accordingly, it is more appropriate to combine the most effective and practical control measures available when managing bean root rots.

CHAPTER FOUR

MATERIALS AND METHODS.

4 1 Collection of diseased material.

Bean plants at different stages of growth, and development and those showing characteristic root rot lesions on lower stem and roots were randomly sampled from Bungoma, Kakamega and Vihiga districts of Western Kenya. The sampling was conducted with a bias for those fields showing general symptoms and signs of damage by root rot pathogens. The symptoms included poor seedling establishment (damping-off), stunting and uneven growth, chlorosis, premature defoliation, reduced yield and premature death of infected plants. The plant materials were collected where the observed disease symptoms were clearly and constistently associated with infected plants. At least six different sites per district were visited. Twenty diseased plants per site were collected.

The diseased plants were dug carefully with a shovel and the soil removed with minimal disturbance to the fibrous roots.

Signs of potential pathogens were examined by hand lens. The cropping history of the bean fields sampled and agricultural practices adopted by the farmers were taken. The colleced plants together with soil samples from around the roots of infected plants were placed in paper bags, labelled, and kept away from direct sunlight. The collected samples were carried to the laboratory for pathogen isolation and identification.

4 2 Isolation of the root-rot pathogen from collected samples.

Diseased plants of the samples collected were examined under a dissecting and compound microscope to establish the extent of damage. Three isolation procedures were used which are a modification of the ones given by Onkar et al 1986.

4.2.1 **Method I: Isolation of the root rot pathogen from infected plant tissue.**

Small root and stem sections with early stages of infection were sliced from diseased areas and washed under running tap water for 15 to 30 seconds then surface sterilized with dilute solution (0.5%) of sodium hypochlorite for 2 to 5 minutes.

The working area was kept clean and disinfested by wiping with 70% ethyl alcohol before each isolation or culture transfer. Knives, needles, forceps and other metal tools used were sterilised by dipping in 70% ethyl alcohol for a few seconds and flamed. Potato dextrose agar (PDA) medium was prepared and sterilized at 121°c. Streptomycin at the rate of 0.4 mg/ml was added to the cooled medium at 42°c to suppress bacterial growth. The streptomycin was then mixed thoroughly by shaking before pouring medium into 9- cm petri-dishes. Those sections of sterilized infected tissues were plated in PDA plates prepared earlier. Three plates were made for each sample. Incubation was at room temperature (20 ± 2 °c) and observations were made after 24 hours onwards. Fungal colonies appeared in 2 to 3 days and their numbers were recorded. Hyphal-tip transfer was made from the margin of advancing colonies onto new PDA plates in order to make pure cultures for identification.

Characteristic mycelial growth and reproductive structures of isolated pathogens were recorded.

4.2.2 **Method II: Isolation of the root rot pathogen by serial dilution.**

One gram of surface sterilized infected tissues was placed in 9 ml of sterile distilled water in a test tube and crushed with a sterile glass rod to make a suspension. One millilitre was aseptically transferred to a 9 ml sterile water blank using a sterile pippete to make a 10^{-1} dilution. This was shaken for one minute and 1 ml of the suspension transferred to another 9 ml sterile water blank to make 10^{-2} dilution. This procedure was repeated upto 10^{-6} dilution. From each of the last three dilutions 0.1 ml was plated in PDA plates prepared earlier. A blunt bent glass rod was used to spread the suspension evenly on the surface. Three plates were made for each dilution. Incubation, observation and sub-culturing was as in section 4.2.1.

4.2.3. **Method III: Isolation of the root rot pathogen from the soil.**

Soil dilution plate method.

Isolation was made from the soil samples collected in the rhizosphere of infected plants. Each soil sample (air dried) was mixed thoroughly and passed through a 6-mm mesh to remove stones and large pieces of organic matter.

A sub-sample of 10g soil was placed in a 90 ml sterile water blank and stirred with a magnetic stirrer for 20 to 30 minutes. While the suspension was in motion, 10 ml was withdrawn and added to 90 ml of sterile distilled water in a 250 ml conical flask to make 10^{-1} dilution. This was shaken for 1 minute and 10 ml of the suspension transferred to another 90 ml sterile distilled water blank to make 10^{-2} dilution. The procedure was repeated until a 10^{-7} dilution was obtained. From each of the last three dilutions 0.1 ml of suspension was plated in triplicates in cooled PDA with streptomycin added at the rate of 0.4 mg/ml to suppress bacterial growth. Incubation, observation and sub-culturing was as in section 4.2.1.

The presence of *Pythium* in the samples was checked using Onkar's method (Onkar et al 1986): Two diseased bean seedling roots were placed in 10 ml of sterile distilled water contained in a 9-cm petri dish and six simsim seeds added. Three plates per sample were used and incubated at room temperature (20 ± 2 °c) for four days. The incubated seeds were examined from the 4th day onwards using a microscope. Characteristic mycelial growth and reproductive organs of *Pythium* were checked which includes nonseptic hyphae, fluffy white mycelium, zoopores, oogonia and anthredia.

4.3 **Identification of the fungal isolates.**

Identification was done based on cultural and morphological characteristics of pure cultures of the isolates. First visual and microscopic examinations were done to determine the genus and later a synoptic key was used for species separation.

4.3.1. **Visual and microscopic examination.**

This was done on one week old pure cultures of the isolates grown on PDA. Visual observations on mycelial colour, growth form, smell and other visible characters were done as a preliminary identification. These observations suggested the genus *Fusarium* to be the pathogen of the root rot of bean. Microscopic slides were then prepared from each isolate and mounted under a compound microscope. The specimens were mounted with cotton blue in lactophenol and water under medium power magnification (X 40) and, oil immersion under high power (X 100) and examined for presence or absence of conidia, conidia type and other reproductive and vegetable structures.

4.3.2 **Use of synoptic identification key.**

Fusarium isolates (pure) were examined after culturing them on potato sucrose agar (PSA) and Spezieller nahrstoffarmer agar (SNA) whose compositions were as follows (Anon 1992):

1) PSA

Potato extract	500ml
Sucrose	20.0g
Agar	15.0g
Trace metal solution	1.0ml
Distilled water	500ml

Adjust pH to 6.7 ± 0.1

2.) SNA

Agar	15g
Sugar solution (see below)	100ml
Salt solution (see below)	10ml
Distilled water	1000ml

i) Sugar Solution

K_2	HP 04	50.0g
KNO_3		50.0g
$mgSO_4$	7H20	25.0g
Kcl		25.0g
Distilled water		500ml

ii) Trace Metal Solution

$ZnSO_4$	7 H20	1.0g
$CuSO_4$	5 H20	0.5g
distilled water		100ml

The above values are for 1 litre of the medium. When the medium had set, sterile filter paper (10 x 30mm) were placed on the surface of the solid medium in each petridish.

PSA was used in order to observe such aspects as colony margin, colour and appearance of the aerial mycelium, colony colour (aerial and reverse), smell , growth at 37°c and colony diameter.

With the exception of colony diameter which was determined on 4th day of incubation at 20 ± 2°c on PSA, all other aspects above were determined on the 7th day of incubation (at 20 ± 2°c) on SNA. Conidia type, production and shape of conidia, chylamydospore production and sporodochial colour were determined on SNA on the 7th day of incubation. Preparation of microscopic slides and their examination was as in section 4.3.1. Using this key the identity of various isolates were arrived at by taking each isolate at a time and going through all the above parameters eliminating those characters which do not fit it at every stage. Eventually only the characters with lead to a single species only were left. A representative sample of each isolate was preserved.

List of Species for the synoptic identification key:

1. *F. acumunatum* chemotype 1
2. *F. acumunatum* chemotype 2
3. *F. aquaeductuum*
4. *F. avenaceum*
5. *F. cerelis*
6. *F. chlamydosporum*
7. *F. ceoruleum*
8. *F. compactum*
9. *F.culmorum*
10. *F. dimerum*
11. *F. equiseti* chemotype 1
12. *F. equiseti* chemotype 1
13. *F. flocciferum*
14. *F. graminearum*
15. *F. lateritium*
16. *F. merismoides*
17. *F. oxysporum* (group)
18. *F. pallidoroseum*
19. *F. poae*
20. *F. proliferatum*
21. *F sacchari*
22. *F sambucinum*
23. *F solani* (group)
24. *F. sporotrichioides*
25. *F. tabacinum*
26. *F. torulosum*
27. *F. tricinctum*
28. *F. verticillioides*

Only one type of conidia (macroconidia) : 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 22, 25, 26.

More types of conidia (micro and macrocononidia) : 4, 6, 17, 18, 19, 20, 21, 23, 24, 27, 28.

Microconidia produced :

in chains : 20, 28

i clusters, "heads" : 17,19, 20, 21, 23, 24, 27,

Singly: 4, 6, 18, 24, 27

Microconidia, shape:

globose (1 - 2 celled) : 19.

citrus, pearshaped (1 - 2 celled) : 19, (20) 24 27

oval, elliposoide, reinform (1 - 2 celled) : 6, 17, (19), 20 21 23 24 27 28

fusoid, cigarshaped (3-5 celled) : 4, 18.

clavate, clunshaped (1 - 2 celled) : (6), (17), 20, 21,(23), (24), (27), 28.

Macroconidia, shaped :

falcate: 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 12, 13, 14, 17, 18, 20, 21, 22, 24, (25), 26, 27, 28

sausage - shaped: 7, 9, 15, 16, (17), 19, 23, (25).

needle shaped, thin: 1, 2, 4.

hunched (asymetrically curved): 1, 2, (5) , 8, 11, 12, 13, (14), 18, (22), (26).

cylindrical: 25

Chlamydospores: 1, 2, 3, (5), 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 22, 23, 24, 25, 26, 27.

Polyphialides: (4), 6, 18, 20, 21, 24.

Sporodochia, colour:

light, clear, creme: 1, 2, 4, 7, 9, 11, 12, 13, 15, 17, 20, 21, 23, 24, 28

orange: 1, 2, 4, 6, 8, 9, 11, 12, 13, 14, 15, 18, 21, 22, 24, 26, 27.

red- brown: 4, 5, 9, 14

blue - green: 7, 17, 23

blue - violent: (7), 17, 23

Pionnote cultures, colour:

creme, yellow: 10 (16), 25.

orange: 3, 10, 16

rose. " salmon": 3, 25.

Colony diameter on PSA (4 days 25° c)

< 9 mm: 3, 16

10 - 19 mm: 3, 7, 10, 16, 25

20 - 29 mm: 7, 10, 13, 15, 23, 25, 26

30 - 39 mm: 4, 13, 15, 17, 20, 21, 23, 26, 27, 28

40 - 49 mm: 1, 4, 6, 11, 12, 17, 18, 19, 20, 21, 22, 23 (26) 27, 28

50 - 59 mm: 1, 4, 6, 8, 11, 12, 17, 18, 19, 20, 21, 22, 23, 27, 28

60 - 69 mm: 2, 5, 6, 8, 11, 12, 18, 19, 24

79 - 79 mm: 2, 5, 8, 9, 14, 19, 24

> 80 mm: 2, 5, 9, 14, 19, 24.

Growth on PSA 37° c (7 days): 1,2.6.8.12.17.18, 19, 20, 21, (22), 23, 24, (26), 28

Growth on TAN 25° c (7 days, colony diameter > 1 mm): 1, 2, 3, 4, 6, 7, 8, 10, 11, 12, 13, 15, 17, 18, 20, 21, 22, 23, 24, 25, (26), 27, 28,

Pigmentation on PSA, 25° c

light, beige, yellow: 2, 3, 7, 10, 11, 12, 15, 16, 17, 18, 19, 20, 21, 22, 23, 25, (26), 27, 28

orange, ochreous, brownish: 2, 4, 8, 11, 12, 13, 15, 17, 18, 20, 21, 22, 27, 28

rose, red, burgundy: 1, (2), 4, 5, 6, 8, 9, 13, 14, 17, 19, 20, 21, 22, 23, 24, 26, 27, 28

dark bluish red, violet: 17, 20, 21, (23), (26), 28.

bluish (no reddish tinge) , 7, (15), (17), (28).

Odours (PSA and /or YES cultures:

like flowers (lilac) : 17, 20, 26

fruity, sweedish: (17), 19, 20, (21)

musty, earthly: 11: 12

Secondary metabolities:

antibiotics Y: 1, 2, 4, 6, 13, 15, 26, 27

butenolide: 1, 5, 9, 14, 24, 27

chlamydosporol: 1, 4

deoxynivalenol, fusarenon X + derivatives: 5, 9, 14

diacetoxyscirpenol: (2_, 11 (12), 22, (24)

equisetin: 11, 12, 18

fusarochromanone: 11

fusarubin, solaniol + derivatives: 7, 17, 20, 21, 23, 28

fumonisins: 20, 28

fusariums C: 4, 5, 9, 14, 19, 20, 24, 28

moniliformin: 4, (20), 28

nectriafurone: 7, 17

T - 2 toxin: 2, (19), 24

worrmannin: 26

zaeralenone: 5, 9,11, 12,14,18

4.4 Maintenance of the Fusarium isolates.

Fusarium isolates were preserved using Booth's method (Booth 1971). A few grams of sieved loam soil was placed in universal bottles (about 2/3 full). The bottle and soil were then autoclaved twice at 121°C and 15 PSI for 15 minutes at two interval. A spore suspension was prepared by flooding the plate with about 5 ml of sterile distilled water and gently scrapping the surface of the colonies with an edge of a glass slide to dislodge the spores. Two millilitres of this suspension was poured over the sterilized soil in the universal bottles aseptically. The tops with their rubber liners were left loosely screwed for two weeks at room temperature to allow the fungus to grow. The caps were then tightened and the culture stored in the refrigerator at 4°C. Retrieval was by placing a few particles of soil from the bottle and sprinkling them onto PDA plates. Sub-culturing was done as soon as Fusarium grew out from the soil particles. Most of the cultures used in the subsequent experiments were retrieved this way.

4.5 Pathogenicity test.

Pathogenicity test of all the isolates was done on a susceptible bean cultivar (Large Rosecoco variety : GLP -2) obtained from bean Research Project, University of Nairobi, Kabete Campus.

4.5.1 Preparation of inocolum.

Fusarium inoculum was produced by growing it on PDA plates for one week at 20° C. A spore suspension from the pure culture were prepared by adding 5 ml of sterile distilled water to each PDA plate and scraping the surface of the culture with a glass slide. The suspension was then passed through four layers of cheesecloth to remove mycelial fragments and the spore concentration determined by the viable count method as described in section 4.5.2 below. Spore suspensions prepared using this method consisted mostly of macroconidia, microconidia and a few chlamydospores.

4.5.2 **Determination of spore concentration.**

Dilution of up to 10^{-7} was prepared by serial dilution as described in section 4.2.2. From the dilutions of 10^{-5} to 10^{-7}, 1 ml of the well agitated suspension was transfered to a sterile petri dish using a 1 ml pippette. Into each seeded petri dish, about 12 ml of cooled PDA at 42°C was poured and swirled in both anti-clockwise and clockwise direction by hand to ensure thorough mixing. The plates were incubated at room temperature for one week and Fusarium colonies that developed were counted. Total number of colonies in the three plates at dilutions of 10^{-5} to 10^{-7} were counted and the average number of colonies for each dilution worked out. This gave the number of fungal propagules per ml of the particular dilution. To get the number of propagules per ml of the stock suspension used, each of the three averages were multiplied by the reciprocal of the dilutions 10^{-5}, 10^{-6} and 10^{-7} respectively.

4.5.3 **Inoculation of Bean Seedlings.**

Two methods of inoculation both adopted from Abawi et al (1990) were used.

The two experiments were laid down as completely randomised design with five replicates and non-inoculated plants as control.

4.5.3.1 **Method I: Root - clip inoculation.**

One week-old seedlings grown in steam sterilized soil mixture (Soil: Sand: Ballast: Manure = 2:1:1:1 V/V) in 20 cm - diameter polythene sleeves were carefully dug up , their roots were washed in running tap water and 1 cm segments cut from the tip of the roots. The plants were then root dipped for 5 minutes in a spore suspension (1×10^{6} conidia / ml) of Fusarium pathogen. The inoculated seedlings were replanted in their former polythene sleeves and maintained normally in the greenhouse for 6 weeks . The experiment was replicated 5 times for each of the five isolates and the control. A total of sixty pots were used in the experiment. Four bean seedlings were used in each of the 10 cm-diameter polythene sleeves.

Since above ground symptoms are usually not diagnostic for most root-rot pathogens and are often delayed in appearance, the root-rot incidence and severity was determined by underground symptoms. The procedure for disease severity estimation was adopted from Campell et al 1980. This assessment was done on the seventh week after inoculation by carefully uprooting all the plants and washing their roots in running tap water. The roots were then examined for any visible infection.

Disease incidence was determined by counting the number of infected plants per treatment and their percentage calculated.

Disease severity was assessed by linear measurements of the characteristic reddish brown lesions on the hypocotyl and lower roots. The proportion of the surface covered by lesion was determined. The average lesion length and the percentage infected area was calculated for each isolate.

4 5.3.2 **Method II. Soil inoculation.**

A spore suspension was prepared as in section 4.4.1.

A spore suspension of each isolate was mixed into steam sterilized soil at a rate of 30000 conidia/g of soil (15 ml of spore suspension containing 10^6 spores/ml used in each 1/2 kg plating soil mixer).

Four Rosecoco (GLP-2) bean seeds were planted in pots filled with steam sterilized soil mix and then covered with 2 to 4 cm of infected soil. The experimental design and disease assessment are as in section 4.4.3.1.

4.6 **The Effect of Conidia concentration on Disease development.**

This experiment was done to determine the most effective spore concentration for development of the root rot disease under artificial inoculatiion. Rosecoco (GLP 2) variety of beans grown in the steam sterilized soil put in the 9 - cm diameter polythene sleeves were inoculated with conidia suspension (inoculum was a mixture of the five Fusarium isolates) of concentrations 1×10^2 ; 1×10^3 ; 1×10^4 ; 1×10^5 ; 1×10^6 ; 1×10^7 ; 1×10^8 using soil inoculation procedure.

The experiment was laid down as a completely randomized design with non-inoculated pots as a control. Each concentration constituted an experimental unit and was replicated eight times. Six plants were used per pot. Four plants were removed from each treatment at the end of 1st, 2nd, 3rd, 4th, 5th, 6th, 7th and 8th weeks after inoculation to asses disease severity and incidence as in the pathogenicity test. A disease progress curve was developed to show disease progress over time for the various treatments.

4.7 Screening for antagonism against the pathogen in vitro.

In this experiment two species of the antagonistic fungi *Trichoderma* designated as T3A and T13 obtained from Dr. E.W. Mutitu of the Dept. of Crop Science, University of Nairobi were used against the Fusarium isolates. Trichoderma is a fungus whose species are known to have antagonistic effect on a wide range of other fungal species. The two *Trichoderma* isolates were tested for their affect on the growth of the five *Fusarium* isolates in culture. The experiment was carried out on PDA medium in 9-cm diameter petridishes. Ten millilitres of the medium was used in 9-cm culture plates. Two methods of screening adopted from Onkar et al (1986) were used.

4.7.1 Method I: Random sprinkling.

The two Trichoderma isolates were grown on PDA plates for one week at room temperature before they were used for this experiment. Each of the biocontrol isolate was seeded on PDA plates using 5mm discs of the fungus at four equidistant points, 1 cm from the plate periphery and the entire surface of each plate immediately flooded with 15 ml spore suspension of the pathogen isolate. The Trichoderma discs were removed from one week old cultures using 5mm tube. This was repeated for each antagonist against each Fusarium isolate. Plates with Fusarium alone were used as control. Each plate was replicated 5 times and incubated at room temperature ($20 \pm 2^{\circ}$ C). Observations were made daily up to the seventh day to check the effect of the antagonist on Fusarium growth.

4 7.2 **Method II: Equidistant plating.**

Each of the potential antagonist was seeded at three to four sites, 1cm from the plate periphery and the pathogen isolate seeded in the center using 5 mm square discs of the Pathogen.

This was repeated for each of the potential biocontrol agents against each Fusarium isolate. Each plate was replicated 5 times. Incubation was done at 20 ± 2° C and observations made daily for seven days. As the two organisms grew towards each other, the reduction of growth of the pathogen at a distance from the periphery of the potential biocontrol agent indicated inhibition of the pathogen growth. On the 7th day the diameter of each of the Fusarium isolates was measured with a ruler and the average calculated for each antagonist. Reduction in growth of Fusarium as compared to the control was an indication of inhibition by the antagonist.

4 8 **Assessment of growth and survival of Trichoderma in three carriers.**

The method of assessment adopted from Onkar et al 1986 was used.

The experiment was carried out in the laboratory using the two Trichoderma isolates (T3A and T13) and three different carriers. Half broken dehulled rice grains (broken using a mortar), sorghum seed and vermiculite.

Broken rice grains and sorghum seeds were each soaked in tap water (enough to cover the grains completely) in a beaker for 1 to 2 hours. Water was decanted and the grains autoclaved for 1 hour for 2 consecutive days at 121° C and 15 PSI. After cooling the grains were spread in a 3-cm thick layer in sterile metallic trays and a spore suspension (1×10^{-6} conidia/ml) uniformly sprayed on the surface using an atomiser until they were wet. The trays were covered with aluminium foil and incubated at room temperature (20 ± 2° C). The experiment was carried out in triplicate for each *Trichoderma* isolate and carrier. To obtain uniform colonization for the granules by the antagonist, the substrate was stirred every other day with a sterile glass rod. Spore concentration per gram of carrier was determined on 7th, 14th and 18th day. Color of the carrier was noted as part of the assessment.

4 8.2 **Vermiculite.**

Vermiculite granules were spread in a 3-cm thick layer in metallic trays and the trays covered with aluminium foil and autoclaved for one hour. After cooling spore suspension (1 x 10^{-6} conidia/ml) was uniformly sprayed over the surface of the granules as in section 4.7.1 and incubated at room temperature.

To obtaom uniform colonization of the granules, they were stirred every other day. Assessment of growth and survival was as in section 4.7.1.

4.9 **Isolation and enumeratiion of Trichoderma Propagules in the carriers.**

This was done on the 7th, 14th and 18th day after inoculation. The soil dilution plate method was used. Ten grams of Trichoderma colonised carrier was suspended in 90 ml of sterile distilled water and shaken for 30 minutes on a shaker 300 -500 rpm. While the suspension was in motion, 10 ml was withdrawn and added to 90 ml of sterile water blank in a conical flask. The procedure was repeated up to 10^{-6} dilution. From each of the last three dilutions, 0.1ml of the suspension were mounted under a microscope and spore concentration determined by use of a haemocytometer. Three samples were taken per replicate and the mean spore concentration calculated by multiplying the mean squares concentration by the reciprocal of the dilution used.

4 10 **Effect of the screened antagonists in the control of the Fusarium root rot disease of beans in the greenhouse.**

The procedure is a modification of the one given by Onkar et al 1986.

This experiment was carried out in the greenhouse using Rose coco (GLP - 2) bean variety, two isolates of the Trichoderma fungi and the three types of carriers (half broken dehulled rice, sorghum seed and vermiculite) previously tested in the laboratory.

The experimental design was a completely randomized design (CRD) with two factors (antagoist and carriers) at two and three levels respectively hence making up six treatment combinations. Each treatment combination (each antagonist and carrier) was replicated five times and five untreated pots were used as control.

The tests were carried out in steam sterilized soil in 10cm-diameter polythene sleeves. The soil was infested with Fusarium using soil inoculation procedure as described in section 4.5.2.2 at the ratio of 1:3 respectively. The antagonist inoculum in each of the carriers was mixed thoroughly with the infested soil using both hands such that the entire root system would be in contact with the antagonist. The carrier and the soil mix were mixed at the ratio of 1:3 respectively and put into polythene sleeves. Four bean seeds were planted per polythene sleeve. The planted sleeves were watered normally. Disease incidence and severity were measured as in section 4.4.2.1.

4.11 Evaluation of biocontrol of bean root rot with Trichoderma using a fungicide as a standard.

The procedure used was adapted from Summer (1987), Cardoso et al (1990), and Buonassisi et al (1986). The experiment was carried out to compare the two biocontrol agents in the two best carriers as shown from the previous experiment with a conventional chemical control. From the previous experiments, T3A in half broken dehulled rice gave the best control of the Fusarium root rot disease of beans in the greenhouse followed by T13 in sorghum and the two were used in this experiment. Steam sterilized soil in polythene sleeves was inoculated with Fusarium pathogen at a rate of 30,000 conidia/g of pot mix using soil inoculation procedure described in section 4.4.3.2. Six bean seeds (later thinned to four) of Rose coco (GLP - 2) variety were grown on the potted soil in the greenhouse. Bean seeds were dressed with a chemical called murtano (trade name) which contains 20% Thiram (fungicide) and 20% Lindane (insect repellant) at the recommended rate of 10g per 1/2 kg of seed before planting them in the inoculated soil.

Each of the two biocontrol agents above in their respective carriers were applied at the rates of 1:3, 1:6 and 1:9 of the antagonist inoculated carrier : pathogen inoculated soil.

The experiment was laid out in a completely randomised design with 5 replicates. Pots with no control agents were used as control. The pots were maintained normally in the greenhouse and disease assessment done on the 7th week using disease incidence and severity.

4.12 Screening bean lines and accessions for resistance to the Fusarium root rot.

The screening procedure was adopted from Onkar et al (1986).

This experiment was carried out in the greenhouse using fifty six (56) locally available bean accessions from local germplasm. The bean lines included "Rosecoco "(GLP - 2), "canadian wonder"(GLP - 24), "Red Harricot" (GLP - 585) "Mwitemania" (GLP X 92), "Mwezi Moja" (GLP - X - 112) and fifty one (51) other lines from the bean project - department of crop science (provided by Dr. Mwang'ombe.).

Spore suspension from the 5 Fusarium isolates were harvested and all mixed in one suspension and adjusted to 1×10^{6} conidia/ml. This inoculum was added to steam sterilized soil put into polythene sleeves (see section 4.4.2.2) at the rate of 30,000 conidia per gram of soil. Six bean seeds were planted into the potted soil and later thinned to 4 after emergence. The experimental design was CRD with 5 replicates for each line. The pots were watered regularly and disease development assessed on the seventh week. Disease incidence and severity were determined as in section 4.4.2.1. The following disease scoring key adopted from Abawi et al (1990) was used to categorise various infection levels:

Scale	Descriptive
1.	No visible symptoms
3.	From light discoloration and necrotic lesions to a maximum of 10% of hypocotyl and root tissues with lesions.
5.	Approximately 25% of hypocotyl and root tissues have lesions, but the tissue remain firm. There is little decay or damage to the root system.
7.	Approximately 50% of hypocotyl and root tissues have lesions. The root system suffers considerable decay and reduction. Fungal structure are visible.
9.	Approximately 70% or more of the hypocotyl and root tissues have lesions. The root system suffers advanced stages of decay and considerable reduction. There is extensive fungal growth.

The reaction of the different bean accessions and cultivars to the Fusarium root rot Pathogen was categorised as following:-

Grade / scale	Mean disease severity (%)		Disease Reaction
1		0	Immune
3	≈	10	Resistant
5	≈	25	Intermediate
7	≈	50	Susceptible
9	≥	70	Highly Susceptible.

This following five hypothesis were tested using the above results at both 5% and 1% level significance.

1. There is no germplasm which is resistent to the root rot pathogen.
2. That there is no difference in resistance among the germplasms tested.
3. The antagonist have no effect on disease severity.
4. There is no difference among Trichoderma isolates in different carries on diseases severity.
5. Biological control is as effective as convectional chemical control.

5. CHAPTER FIVE

RESULTS.

5.1 OBSERVATIONS IN THE FIELD.

Visits to farmers fields in Kakamega, Vihiga and Bungoma districts in Western Kenya and discussions with farmers, divisional and district agricultural extension officers showed that root rots had become a major and serious problem in the three districts. Plants observed were at different stages of growth but mainly between two and four weeks old. The disease occured after germination when plants were two to four weeks old and was associated with heavy rains. Symptoms exhibited were longitudinal fissures, bright-red streaks on hypocotyl and tap root surfaces. Infected areas were reddish brown, lacked definite margins, remained superficial and exhibited longitudinal fissures. Severely infected plants were stunted, chlorotic and mostly defoliated prematurely. Farmers estimated yield losses of between 40 and 100% in the first (March - June) season of 1993. Three bean varieties (GLP-2, canadian wonder and red harriot) widely grown in the districts were susceptible with disease incidences of approximately 60%, 50% and 30% respectively.

The root rot disease which had been described as Fusarium yellows because of yellowing of leaves, exhibited hypocotyl and root disease symptoms characteristic of those caused by Fusarium species. No symptoms due to other root rot Pathogens were observed in all samples examined from 9 divisions of the three districts.

Table 1: The occurrence and % incidence of root rot of beans in Western Kenya.

Symptoms observed

District	Site; Area	% Disease Incidence	Associated Root rot Symptoms.
Bungoma	Kimilili Sirisia Kanduyi Sangalo Mjini Chwele	60 % of the field infected	Infected plants were chlorotic, stunted and exhibited reddish brown lesions on hypocotyls and lower roots.
Kakamega	Mumias Shinyalu Butere Shibale Bukhungu Chavakali	30 % of the field infected	Infected plants were Chlorotic, stunted and exhidited reddish brown lesions on hypocotyls and lower roots.
Vihiga	Sabatia Emuhaya Majengo Lwanda Kaimosi Mbale	50% of the field infected	Infected plants were chlorotic, stunted and exhibited reddish brown lesions on hypocotyls and lower roots.

5.1.1 **Symptoms.**

In the farms visited the root rot of beans occurred as follows: Germination occurred well (about 80% germination) after planting but when plants started forming the first or second trifoliate leaves, premature yellowing of the leaves started, the affected plants got stunted and wilted without flowering. In most cases these plants died before maturity. Some plants simply wilted and died without yellowing. The hypocotyl and lower stems died before secondary roots formed. The affected plants exhibited reddish brown longitudinal lesions (plates 1).

Plate 1. Chlorosis and premature senescence observed on two week old bean plant at Sabatia in Vihiga district of Western Kenya.

Plate 2. Chlorosis and premature senescence observed on bean plants at different stages of growth at Kimilili in Bungoma district of Western Kenya.

Plate 3. A dead plant (about 2 weeks old) observed in a highly infected farm at Shinyalu in Kakamega district of Western Kenya.

5.2 ISOLATION.

Isolations were done from infected plant tissues and infested rhizosphere soil.

5.2.1. Method I: Isolation of the root rot pathogen from infected plant tissues.

After 2 to 3 days of incubation at room temperature hyphae of the fungi grew out into the agar, producing colonies around each infected segment. The number of fungal colonies per plate were recorded as they became evident within a week. After sub-culturing three times at one week interval, five pure isolates were obtained and were designated as isolate I to V (Table 2 and Plate 4). They displayed a mycelium tinged with a light purple to purple or dark blue color characteristic of *Fusarium solani* and *Fusarium oxysporum.*

Attempt to trap any *Pythium* using simsim seeds were not successful.

5.2.2 Method II: Isolation of the root rot pathogen by serial dilution of infected plant Sap.

Dilutions of 10^{-4} to 10^{-6} of infected plant tissue gave well spread fungal colonies which were purified. Five different fungal isolated were obtained by this method (Table 2 and Plate 4).

5.2.3 Method III: Isolation of root rot pathogen from rhizosphere soil.

Dilutions of 10^{-5} to 10^{-7} of infested rhizosphere soil gave well spread colonies of soilborne fungi within 4 days and their number was recorded. In this isolation procedure 4 fungal isolates were obtained all similar to those obtained in method I and II (Table 2 and Plate 4).

Table 2: Identity of the five Fusarium isolates and their source.

Isolate	Identity	District collected	Source of isolation
I	*Fusarium*	Bungoma	Soil
	Solani	Kakamega	Plant tissue
II	*Fusarium*	Bungoma	Soil
	Oxysporum	Vihiga	Plant tissue
III	*Fusarium*	Bungoma	Soil
	Solani	Kakamega	Plant tissue
IV	*Fusarium*	Kakamega	Plant tissue
	Oxysporum	Vihiga	
V	*Fusarium*	Bungoma	Soil
	Oxysporum	Vihiga	Plant tissue.

Plate 4: One week old cultures (on PDA) of five Fusarium isolates obtained from infected plant tissues and soil samples.

I - *Fusarium solani*

II - *Fusarium oxysporum*

III - *Fusarium solani*

IV - *Fusarium oxysporum*

V - *Fusarium oxysporum*

5.3.0 **Preliminary Identification.**

This was done based on cultural and morphologic characteristic of the pure isolates grown on PDA.

On PDA all the five isolates displayed aerial mycelium tinged with a light purple to purple or dark-blue color characteristic of the the genus *Fusarium*. They sporulated within a week to produce both micro and macroconidia. Also in some isolates terminal or intercalary chlamydospores were present. The presence of Fusoid macroconidia with foot cell bearing some kind of a heel confirmed their identity as *Fusarium*. Further identification to species level was done based on a synoptic key.

3.3.1 **Use of Synoptic identification key.**

This was used to differentiate the five *Fusarium* isolates into different species and was done on pure cultures of the isolates grown on PSA and SNA. The results are presented in Table 3.

Table 3: Synoptic key characterization and identification of Fusarium species associated with root rot of beans.

ISOLATE	I	II	III	IV	V
Type of conidia	Both micro and macroconidia	Both macro and microconidia	Both macro and microconidia	Both macro and microconidia	Both maro and microconidia
Micro conidia produced	Clusters	Clusters	Clusters	Clusters	Clusters
Microconidia, shape	Oval	Oval	Oval	Oval	Oval
Macroconidia shape	Sausage-shaped	Sausage-shaped	Sausage-shaped	Sausage-shaped	Sausage-shaped
Chlamydospores	Present	Present	Present	Present	Present
Polyphialides	Absent	Absent	Absent	Absent	Absent
Sporodachia colour	Clear	Light	Light	Clear	Clear
Colony diameter on PSA (4 days 25°C	25mm	34mm	42mm	22mm	30mm
Growth on PSA 37°C (7 days)	No	No	No	No	No
Pigmentation on PSA, 25°C	Yellow	Orange	Yellow	Purple	Purple
Odour (PSA)	-	Fruity	-	Fruity	Lilac
Identity	Fusarium solani	Fusarium oxysporum	Fusarium solani	Fusarium oxysporum	Fusarium oxysporum

5.4.0 PATHOGENICITY TEST.

5.4.1 Method I: Root - clip inoculation.

Assessment of the disease incidence and severity for each isolate was done on the 7th week of inoculation and was based on underground infection. The plants were carefully dug up and their roots washed in running tap water for about 5 minutes. All the 5 isolates were found to incite disease but with varying degree of virulence. The most virulent was isolate II with disease severity of 70% and incidence of 80% and the least isolate V with disease severity of 39% and incidence of 30%.

Disease incidence which was assessed based on the proportion of infected plants per treatment also gave different values for the 5 isolates (Table 5) with isolate II producing the highest incidence (85%) and isolate V the lowest (30%).

Disease severity was done based on the proportion of hypocotyl and tap root surfaces showing the characteristics reddish brown lesion and length of these characteristic lesion. The mean values were found to be different for each isolate. Isolate III produced the highest disease severity value (70%) and mean lesion length of 2.78 cm, followed by isolate II (65%) and mean lesion length of 2.46 cm, then isolate I (54%) and the mean lesion length of 1.6, then isolate IV (53%) and mean lesion length of 1.26, isolate V (39%) and the mean lesion length of 0.76 cm and finally the uninfected control (Plate 6).

All the isolates were found to be significantly different from the control at both 5% and 1% level of significance.

However most of the isolates were not significantly different from each other at both 5% and 1% level of significance. The mean disease incidence produced by various isolates at both 5% and 1% level of significance were in the following order II > III > I > IV > V > control. Isolate II and III were found to be more virulent than isolate I, IV and V.

Table 4 **Root rot % incidence and % severity produced by various *Fusarium* isolates on Rose coco (GLP-2) beans after 7 weeks of inoculation using root clip method (p = 0.05).**

	Isolate	% root rot incidence	% root rot Severity (%)	Root rot Severity (cm)
1.	I (*F. solani*)	65 a	54 b	1.6 b
2.	II (*F. oxysporum*)	85 c	65 ab	2.46 ab
3.	III (*F. solani*)	80 ab	70 a	2.78 a
4.	IV (*F. oxysporum*)	45 b	53 b	1.26 c
5.	V (*F. oxysporum*)	30 d	39 c	0.76 cd
6.	Control	00	00 d	00 d

The values represent a mean of 5 replicates

	0.05	0.01
LSD (% incidence)	25.6	34.7
LSD (severity (%))	13.1	17.8
LSD (severity (cm))	0.9	1.2

Values followed by the same letters are not significantly different at 5% according to Duncan's multiple range test.

5.4.2 **Method II. Soil inoculation.**

Disease development occurred as in Method I but symptoms were more pronounced in this method. Disease incidence and severity were also found to be higher in this inoculation method than the root dip inoculation (Table 6).

Both disease incidence and severity values for all the isolates were found to be significantly different from each other at both 1% and 5% level of significance. As in root-dip method disease incidence and severity values for isolate II and III were higher than those for isolate I, IV and V.

Table 5: Root rot incidence and severity produced by various isolates on Rose coco (GLP-2) beans using soil infestation method.

	Isolate	% root rot incidence	% root rot severity (%)	Root rot severity (cm)
1.	I	75 abc	59c	1.1a
2.	II	100 a	82 a	4.1 c
3.	III	85 b	61 b	2.0 b
4.	IV	70 c	53 bc	1.2 a
5.	V	50 d	47 d	1.0 a
6.	VI	00 e	00 e	00 d

The values represent a mean of 5 replicates

	0.05	0.01
LSD (% INCIDENCE)	24.92	33.8
LSD (severity (%))	10.8	14.6
LSD(severity (cm))	0.6	0.8

Values followed by the same letters are not significantly different at 5% according to Duncan's multiple range test.

Plate 5: Chlorosis produced on five week old bean plants in the greenhouse during pathogenicity test.

Plate 6: Root rot symptoms produced by various Fusarium isolates on bean plants (7 weeks old) during Pathogenicity test.

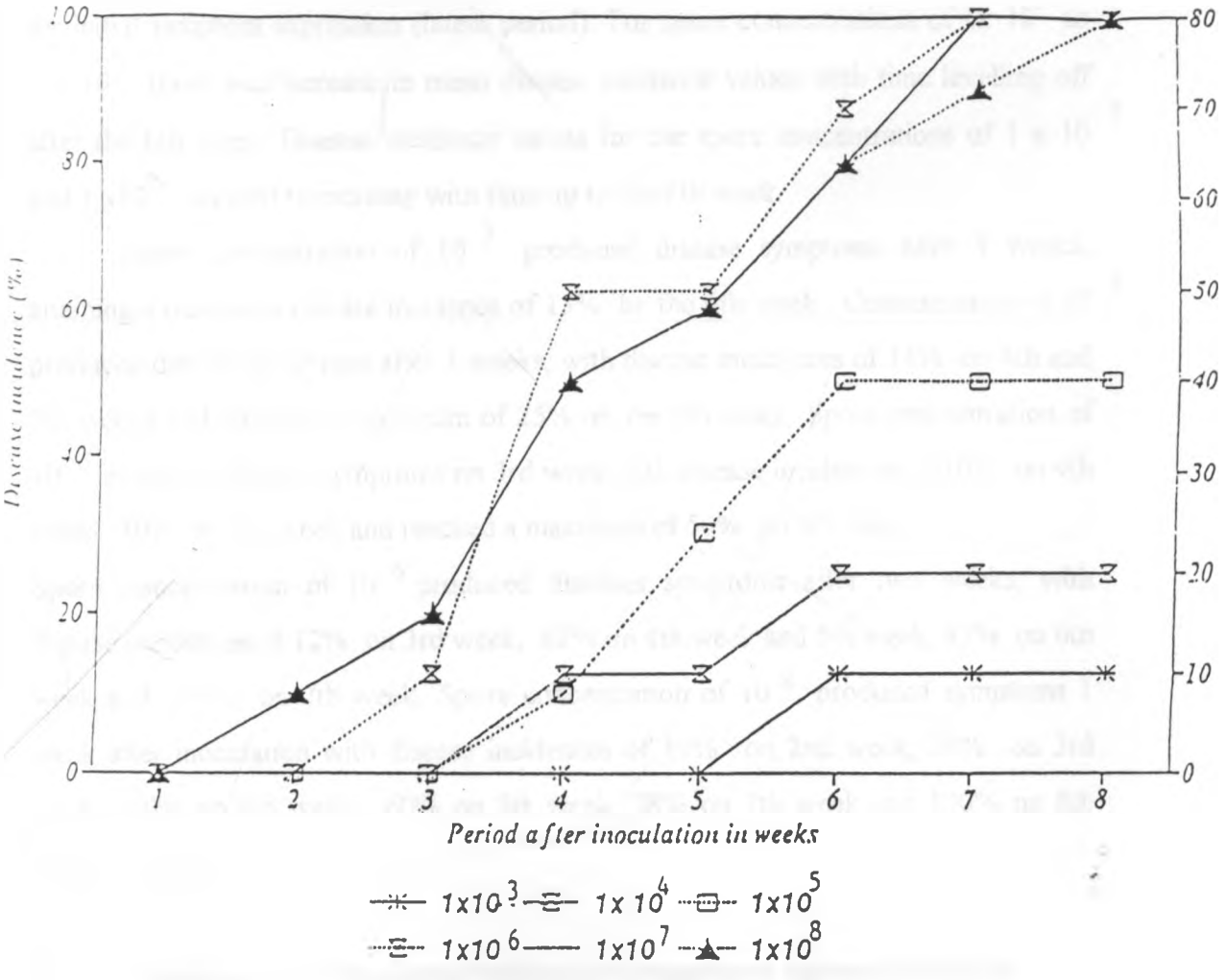

Figure 1 Progress of *Fusarium* bean root rot incidences with time for various inoculum spore concentrantions.

5.5 Effect of conidia concentration on disease development.

There was a positive relationship between spore concentration and mean disease incidence values. The higher the spore cocentration the shorter the time taken for initial symptom expression (latent period). For spore concentrations of $1x\ 10^5$ to 1×10^3 there was increase in mean disease incidence values with time levelling off after the 6th week. Disease incidence values for the spore concentrations of 1×10^7 and 1×10^8 seemed to increase with time up to the 8th week.

Spore concentration of 10^3 produced disease symptoms after 5 weeks, attaining a maximum disease incidence of 13% by the 6th week. Concentration of 10^4 produced disease symptoms after 3 weeks, with disease incidences of 14% on 4th and 5th weeks and attained a maximum of 25% on the 6th week. Spore concentration of 10^5 produced disease symptoms on 3rd week with disease incidences of 10% on 4th week, 30% on 5th week and reached a maximum of 50% on 6th week.

Spore concentration of 10^6 produced diseases symptoms after two weeks, with disease incidences of 12% on 3rd week, 62% on 4th week and 5th week, 87% on 6th week and 100% on 7th week. Spore concentration of 10^8 produced symptoms 1 week after inoculation with disease incidences of 10% on 2nd week, 20% on 3rd week, 50% on 4th week, 60% on 5th week, 78% on 7th week and 100% on 8th week (Figure 1).

5.6 Screening of Trichoderma isolates for antagonism against Fusarium isolates in vitro.

In this experiment two species of fungi *Trichoderma* designated as T3A and T13 were tested for their antagonistic activity against Fusarium isolates in culture. The screening was done on PDA plates using two methods.

5.6.1 **Random sprinkling method.**

There was total (100%) suppression of Fusarium growth by T3A in all the plates by the seventh day of incubation (Plate 7 (a)). In presence of T13 Fusarium showed very poor growth with some plates having no growth at all (Plate 8(b)). The three treatment (T13 and T3A against Fusarium plus the untreated control) were found to be significantly different at 5% significant level (Table 6). This shows that the two *Trichoderma* isolates have varying antagonistic activity on Fusarium growth.

Table 6 Inhibition of Fusarium growth by T3A and T13 in cultures using equidistant planting method (P = 0.05).

Treatment	Mean radial growth of Fusarium	% growth reduction
1. T13 against Fusarium	0.4 a	91 a
2. T3A " "	0.00 b	100 b
3. Control	9.00 c	0.00 c

The values represent a value of 5 replicates.

Values followed by same letters are not significantly different.

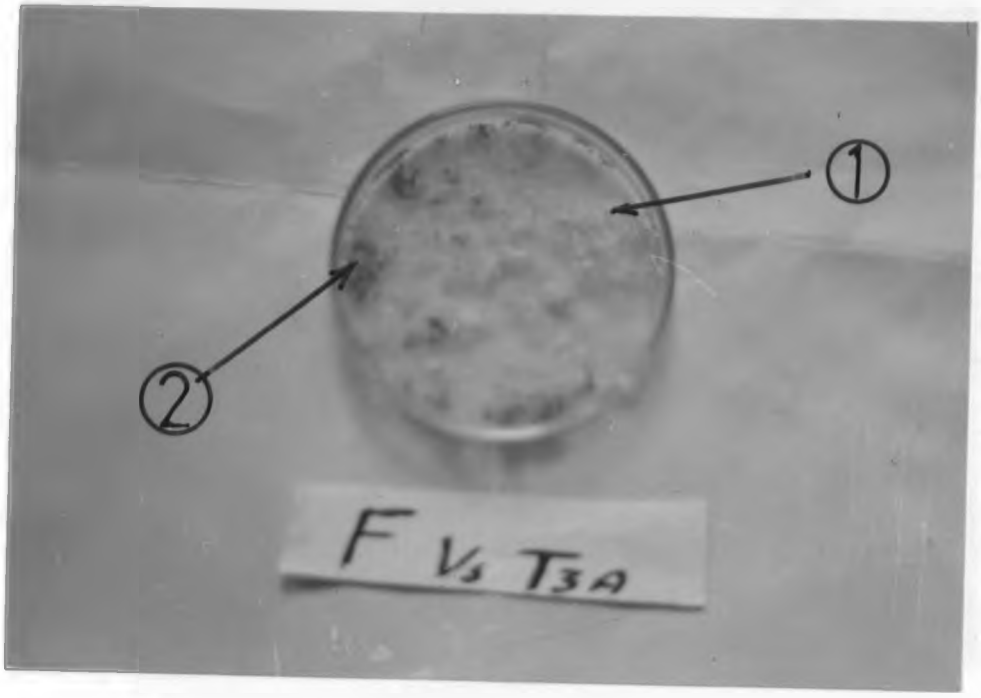

Plate 7. Suppression of *Fusarium* groth by T3A using random sprinkling method.

1. -*Fusarium*
2. -*Trichoderma viride.*

Plate 8. Suppression of *Fusarium* growth by T13 using random sprinkling method.

F -*Fusarium*

T13 - *Trichoderma koningii.*

5.6.2 **Equidistant plating.**

The size of Fusarium colony was reduced by T3A and T13 in all the plates by the seventh day of incubation as shown in Plates 9. The radial growth of Fusarium was smaller in plates with T3A (0.08 cm) than those with T13 (1.9 cm). This indicates that T3A has greater effect (higher percentage growth reduction of 98.2%) on Fusarium growth than T13 (56.40%) (Table 7).

Table 7 Inhibition of Fusarium growth by T3A and T13 in cultures (P = 0.05).

Treatment	Mean radial growth of Fusarium	% growth reduction of Fusarium
1. T13 against Fusarium	1.9 a	56.4 b
2. T3A " "	0.08 b	98.2 a
3. Control	9.00 c	0.00 c

LSD	(growth)	0.05	0.01
		0.35	0.48

The values represent a mean of 5 replicates.

Values followed by the same letters are not significantly different at 5% according to Duncan's multiple range test.

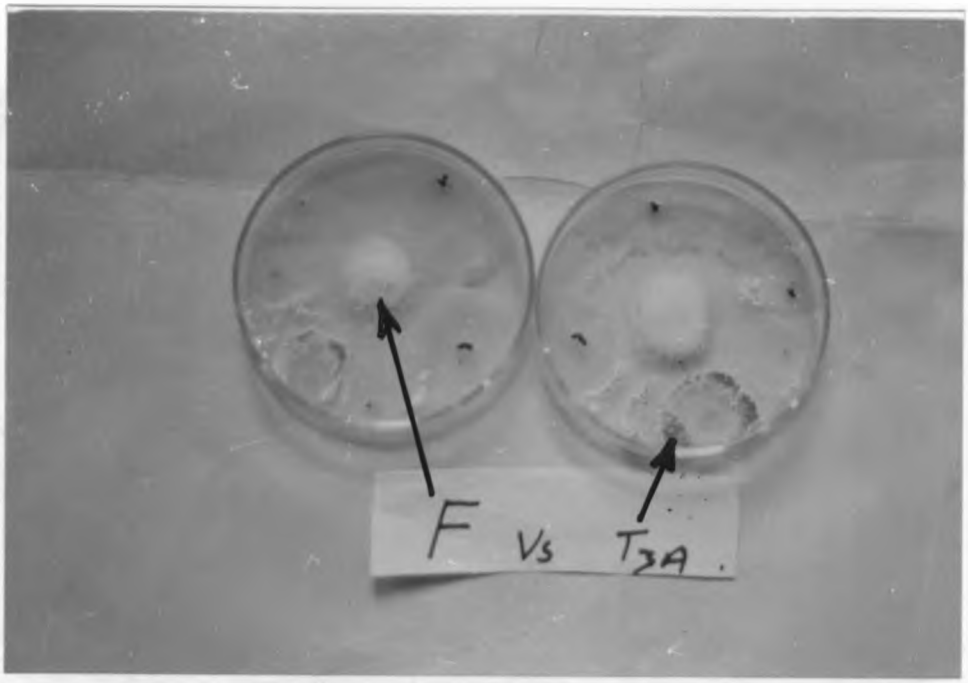

Plate 9: Growth inhibition produced by T3A on *Fusarium* isolates using equidistant plating method.

F - *Fusarium*

T3A - *Trichoderma viride.*

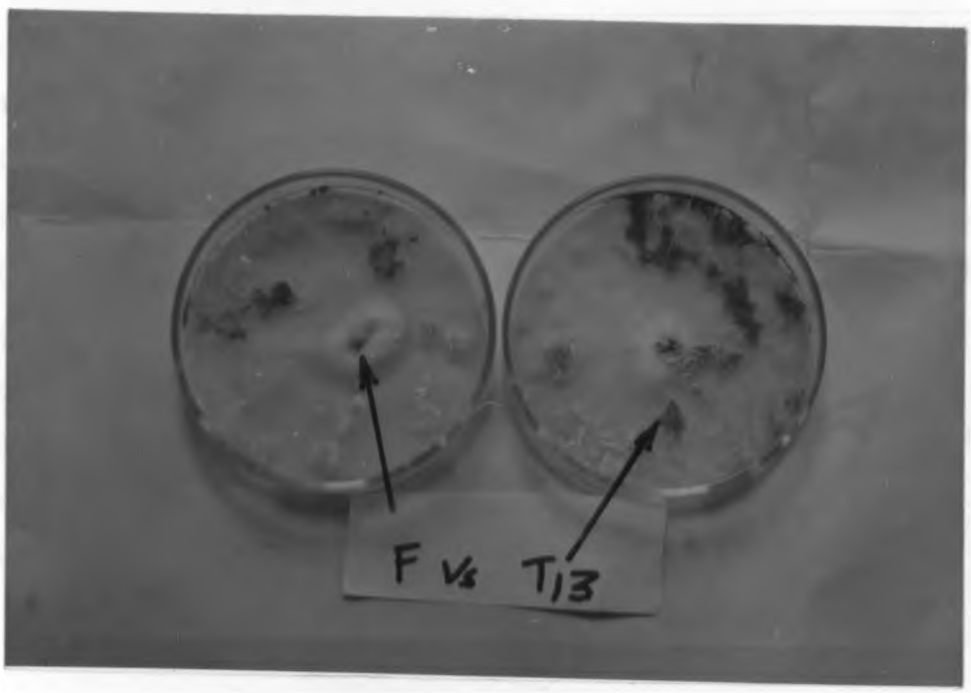

Plate 10: Growth inhibition produced by T13 on *Fusarium* isolates using equidistant plating method.

F - *Fusarium*

T13 -*Trichoderma koningii.*

5.7 Assessment of growth and survival of Trichoderma in three different carriers.

In this experiment the two *Trichoderma* isolates (T3A and T13) were cultured in sterile half broken dehulled rice grain, sorghum and vermiculite as carriers in the laboratory. Isolation and enumeration of *Trichoderma* spores in the carriers was done on the 7th, 14th and 18th day of incubation using the normal soil dilution plate method. Also colour of the media (carrier) was recorded as part of the assessment.

The spore concentration of each *Trichoderma* isolate per gram of carrier was found to increase with time after inoculation but not indefinitely. Half broken dehulled rice was found to be an excellent medium for growth of both *Trichoderma* isolates as shown by mean number of conidia per gram of carrier (55,500 conidia for T3A and 12,500 conidia for T13 after 7 days and 25,000,000 conidia/g for T3A and 24,000,000 conidia/g for T13 after 18 days of incubation). Sorghum was moderately good (13,000,000 and 22,000,000 conidia/g for T3A and T13 respectively after 14 days of incubation) and vermiculite very poor (1400 and 100 conidia/g for T3A and T13 respectively after 14 days of incubation). Except T3A in vermiculite which grew steadily during the whole incubation period, the growth of the two fungal isolates in the carriers increased steadily with time levelling off after 14 days of incubation (Table 8).

Table 8 Mean number of Trichoderma conidia per gram fo carrier after 7, 11, 14 and 18 days of incubation.

	Incubation period (days)			
	7	11	14	18
Treatment				
1. T3A in rice	55,500	2,000,000	25,000,000	25,000,000
2. T13 in rice	12,500	447,500	14,000,000	14,000,000
3. T3A in sorghum	47,200	47,666.7	13,333,333	13,000,000
4. T13 in sorghum	606	95,000	22,000,000	22,000,000
5. T3A in vermiculite	17	497	1,400	2,000
6. T13 in vermiculite	240	106.7	1,000	1,000

LSD (growth (day 7))	78998.4
LSD (growth (day 11))	4784035.6
LSD (growth (day 14))	3911313.5
LSD (growth (day 18))	726271

*The values represents a mean of 3 replicates

Values followed by same letters are not significantly different at 5% according to Duncan's multiple range test.

The growth and survival of the two isolates was affected by the type of carriers. The colour of half broken dehulled rice and sorghum carriers changed with time assuming the characteristics greenish yellow and whitish colour of the respective fungal isolates after 14 days of inoculation (Table 9).

Table 9: Colour of various carriers after 14 days of incubation.

Carrier	T3A	T13
Half broken dehulled rice	Moderately to dense whitish fungal outgrowth	Moderately to dense greenish fungal outgrowth
Sorghum	very dense white fungal outgrowth	very dense greenish fungal outgrowth
Vermiculite	No visible outgrowth	No visible outgrowth

5.8 **Effect of the Screened antagonist in the control of the Fusarium root rot disease of beans in the greenhouse.**

The management of the bean root rot disease in the greenhouse by the biocontrol agents varied with the agent and the carrier used. The best control of the disease as determined by the disease severity (%) on the seventh week was achieved by T3A in rice (32%) carrier. This was followed by T13 in sorghum (61%), T13 in rice (67%), T3A in sorghum (69%), T3A in vermiculite (73%), T13 in vermiculite (74%) and finally untreated check (75%) (Table 10).

The mean disease severity value produced by T3A in rice as a carrier as determined by measurement of the lesion length on hypocotyl and lower root (mean lesion length of 0.9 cm) area was less than half the mean value (2.4cm) of the second best (T13 in sorghum).T3A in rice carrier produced very low disease incidence (15%) followed by T13 in rice (20%), T13 in sorghum (30%), then T3A in vermiculite (45%), then T3A in sorghum (65%), then T13 in vermiculite (85%) and finally untreated check (95%). As in disease severity T3A in rice and T13 in sorghum produced very low disease severity (15 and 30% respectively).

Considering the mean values of disease incidence 15% and severity 32% for T3A in rice as determined by the proportion of hypocotyl and lower root surfaces are very low, it implies that good management of *Fusarium* root rot of beans can be attained by using this *Trichoderma* isolate and rice as a carrier (Plate 12).

Table 10: Root rot incidence and severity produced by T3A and T13 in various carriers on Rose coco variety of beans (GLP-2) (P = 0.05).

Treatment	% root rot severity	% root rot incidence	Root rot severity(cm)
1) Untreated check	75 a	95 a	3.78 a
2) T13 in vermiculite	74 a	85 a	3.88 a
3) T3A in vermiculite	73 a	45 c	3.12 b
4) T3A in sorghum	69 b	65 b	2.9 b
5) T13 in rice	67 b	20 e	2.72 c
6) T13 in sorghum	61 c	30 d	2.4 c
7) T3A in rice	32 d	15 e	0.9 d

The values represent a mean of 5 replicates.

	0.05	0.01
LSD severity (%)	19.31	
LSD severity (cm)	1.71	2.309

Values followed by the same letters are not significantly different at 5% according to Duncan's multiple range test.

Plate 11. One week old cultures of T3A and T13.

Plate 12. Management of *Fusarium* root rot disease in the greenhouse using T3A and T13 in various carriers.

T3A/R = *Trichoderma* isolates 3A in rice carrier

T13/R = *Trichoderma* isolates 13 in rice carrier

T3A/S = *Trichoderma* isolates 3A in sorghum carrier

T13/S = *Trichoderma* isolates 13 in sorghum carrier

T13/V = *Trichoderma* isolates 13 in vermiculite

T3A/V = *Trichoderma* isolates 3A in vermiculite.

5.9: Evaluation of biocontrol of bean root rot with trichoderma using a fungicides as a standard.

From the previous experiment on biological control, T3A in half broken dehulled rice gave the best control of the disease in the greenhouse followed by T13 in sorghum as a carrier and these were evaluated against fungicide as a standard in the control of the disease. Disease assessment was done as in section 5.8.

Effectiveness on the bean root rot management in the greenhouse was found to vary with the control agent used and the application rate of the antagonistic inoculum. Higher application rate of the antagonistic inoculum resulted in better disease control as indicated by lower disease severity and incidence values. The two biological agents at all the three application rates gave better management of the disease than the standard fungicide. T3A in rice at 1:3 produced disease severity of 45%, mean lesion length of 2.1 cm and disease incidence of 45%, this was followed by T13 in sorghum at 1:3 with disease severity of 60%, mean lesion length of 2.32cm and incidence of 45%, then murtano with disease severity of 60%, mean lesion length of 4.85 cm and incidence of 75%, then T3A in rice at 1:6 with disease severity of 80% mean lesion length of 4.02cm and incidence of 70%, then T13 in sorghum at 1:6 with severity of 85% and mean lesion length of 3.12 cm and incidence 64%, then T3A in rice at 1:9 with severity of 90%, mean lesion length of 5.4 cm and incidence of 72%, then T13 in sorghum at 1:9 with severity of 100%, mean lesion length of 3.68 cm and incidence of 62% and finally untreated check with a severity 95%, mean lesion length of 4.04cm and incidence of 73% . T3A in rice produced the best management of the disease followed by T13 in sorghum and finally Murtano (Table 11).

Table 11: Root rot incidence and severity produced on Rosecoco variety of beans by various control agents (P = 0.05).

Treatment	% root rot severity	% root rot incidence	Root rot severity (cm)
1. T3A in rice at 1:3	45 c	45 b	2.1 d
2. T3A in rice at 1: 6	80 ab	70 a	4.02 ab
3. T3A in rice at 1: 9	90 b	72 a	4.54 ab
4. T13 in sorghum 1: 3	60 bc	45 b	2.32 cd
5. T13 in sorghum 1:6	85 b	64 a	3.12 bcd
6. T13 in sorghum 1: 9	100 a	62 a	3.68 abc
7. Seed dressed with Murtano	60 bc	75 a	4.85 a
8. Untreated control	95 a	73 a	4.04 ab

The values represent a mean of 5 replicates.

	0.05	0.01
LSD incidence	28.58	19.63
LSD (severity (%))	15.6	19.6
LSD (" (cm))	1.3	1.8

Values followed by the same letters are not signficantly different at 5% according to Duncan's multiple range test.

6.0: Screening bean germplasm for resistance to the Fusarium root rot in the greenhouse.

The degree of resistance to the root rot within the germplasm was evaluated on the seventh week by determining the disease incidence and severity. None of the bean varieties was found to be immune or resistant to the disease. Fifteen germplasms were highly susceptible, thirty nine susceptible and two intermediate susceptible to the pathogen. Reaction of various lines and accessions to the *Fusarium* root rot disease was classified as immune, intermediate, susceptible and highly susceptible.

The germplasm reflected a wide range of disease severity as shown by table 12. This implied a wide range of susceptibility of the bean varieties to the disease.

Generally there was no notable difference in disease severity between the commonly grown bean lines Mwitemania. Rosecoco, Mwezi moja, Canadian Wonder and White Harricot and the 51 bean accessions tested.

The greenhouse screening procedures provided the advantages of constant favourable conditions for disease development, uniform and high levels of inoculum and uniform and high incidence of infection as opposed to field screening conditions.

Table 12: Reaction of various bean germplasm to the Fusarium root rot pathogen.

Bean Germplasm	Mean disease severity (%)	Disease Reaction
1	74	HS
2	62	S
3	59	S
4	80	HS
5	71	HS
6	69	S
7	78	HS
8	77	HS
9	79	HS
10	62	S
11	58	S
12	72	HS
13	66	S
14	61	S
15	65	S
16	71	HS
17	62	S
18	62	S
19	58	S
20	75	HS
21	48	S
22	49	S
23	68	S
24	57	S

25	65	S
26	55	S
27	60	S
28	77	HS
29	77	HS
30	62	S
31	69	S
32	66	S
33	71	HS
34	68	S
35	62	S
36	49	S
37	57	S
38	26	I
39	48	S
40	19	I
41	63	S
42	64	S
43	70	HS
44	59	S
45	40	S
46	46	S
47	52	S
48	58	S
49	53	S
50	63	S
51	75	HS
52	55	S

53	54	S
54	47	S
55	56	S
56	83	HS

* The values represent a mean of 5 replicates

* Germplasm 1 - 10 represents bean accessions E1 - E10

Germplasm 11 - 30 " M11 - M30

Germplasm 31 - 50 " L31 - L50

Germplasm 51 " No 1b

Germplasm 52 " line large Rosecoco

Germplasm 53 " Mwezi moja

Germplasm 54 " Canadian wonder

Germplasm 55 " White Harricot

Germplasm 56 " Mwitemania

E = Early maturity

M = Medium maturing

L = Late maturing

Key :

I - Intermediate

S - Susceptible

HS - Highly susceptible.

Plate 13: Root rot symptoms produced by Fusarium Pathogen on the common bean lines.

R C = Rose coco

M M = Mwezi Moja

C W = Canadian wonder

M T M = Mwitemania.

6.00 CHAPTER SIX

DISCUSSION.

The bean root rot problem prevalent in Kakamega, Vihiga and Bungoma districts of Western Kenya is an important disease and merits urgent efforts to control it. During the survey for this study in November 1993 it was noted that the problem was widespread in all the 18 sites visited. The severity of the disease had made farmers in areas like Kimilili in Bungoma district and Shinyalu in Kakamega districts abandon bean growing. The problem which was referred to as *Fusarium* yellows by the local people and extension officers because of yellowing of bean plant leaves and identified as Pythium root rot by technical staff from CIAT exhibited hypocotyl and root symptoms characteristic of those caused by *Fusarium* species. No symptoms due to other root rot pathogens were observed.

Isolation from infected plant materials and the rhizosphere soil from all the sites visited gave five *Fusarium* isolates and no other root rot pathogens.

Results from pathogenicity tests showed that all the five isolates were pathogenic but differed in virulence. The disease incidence and severity produced by various isolates were significantly different from the untreated control at both 5% and 1% level of significance. The disease incidence and severity produced by the soil inoculation method were generally higher than for those produced by the root clip method. This indicated that the former is a more effective method of inoculation than the later.

In the root clip method plants were inoculated when they were 7 days old. By this time probably some lignification had occured which tends to confine the infection on the outer region preventing it from reaching the vascular system. Such an effect would be minimal in the soil infestation method where inoculation occurs before any meaningful lignification occurs. Stockwell et al (1987) reported similar findings when he established that increase in resistance of bean plants to *Rhizoctonia solani* as the hypocotyls advances in age was due to increase in deposition of calcium in the cell wall which forms cross links between adjacent carboxyl groups of the pectic molecules. The pathogen *Sclerotium rolfsii* which is capable of attacking older bean hypocotyl has a capacity to produce oxalic acid which complexes with calcium component in the pectic compounds thus decreasing resistance of this old hypocotyl to this pathogen.

Burke et al (1966) reported a great reduction in plant weights and seed yields of beans *(Phaseolus vulgaris)* in Fusarium infested fields, whether the plants were grown in islands of non infested soil which protected the tap root and hypocotyls from root rot until senescence or grown without this local protection. This confirms that soil infestation is a very effective method of inoculation with *Fusarium.*

From this study conidia concentration of 1×10^6 /ml was found to be optimum in inciting disease within six weeks of inoculation. Abawi et al (1990) reported that conidia concentration of 1×10^6 / ml of *Fusarium* root rot Pathogen was capable of inciting disease in susceptible bean cultivars within two weeks. This indicated that conidia concentration of 10^6 conidia/ml was effective for the greenhouse experimentation.

6.01 **Assessment of growth and survival of Trichoderma in carriers.**

From this study, it was found that there was an increase in conidia production of the two *Trichoderma* isolates grown in different food carriers with time but not indefinitely. The spore count increased upto day 14 after inoculation thereafter levelling off except for *Trichoderma* 3A in vermiculite whose growth remained constant over time. This indicates that two weeks are enough to give maximum spore concentration of the two fungal isolates in these two carriers. The best performance was given by *Trichoderma* 3A in rice, followed by Trichoderma 13 in sorghum, then Trichoderma 3A in sorghum, then Trichoderma 13 in rice and lastly by the two Trichoderma isolates in Vermiculite. The results indicated that Trichoderma 3A grew better in both rice and sorghum food carriers as compared to Trichoderma T13. Vermiculite was found to be a very poor growth media for the two Trichoderma isolates.

Food carriers vary in their suitability for growth and survival of a given antagonist. For the cultivation of an antagonist on a relatively large scale for greenhouse or field experiments, any organic matter on which the antagonist grows and sporulate can be used. Powdered crop residues, grain and grain sand mixers have been used for this purpose (Onkar et al 1986).

Wells et al (1972) used 1: 10 : 1 (v/v) mixer of ground seeds of annual rice grass, sand and water for *Trichoderma harzianum* Campell (1989) reported that control of Rhizoctonia by two different strains of *T. hamatum* was only effective when bran was used as a carrier and gave antagonists populations in the soil four to seven times greater than mycelium added with no food source or conidial preparation without a food source. There were however complications because whereas *T. hamatum* always gave much better control from mycellium in bran, *T. viride* and *T. harzianum* gave good control from conidia preparations.

Alex and Ilan (1986) reported an effective biological control of *Fusarium oxysporum* f.s.p *radicis,* lycopersici on tomatoes by a strain of *T.harzianum* applied under field conditions as a wheat bran-peat preparations.

Selections of the food carriers is dependent on many factors which includes pH, temperatures and water holding capacity of the medium. Thus it is worthwhile to screen various food carriers before using them. This would help to establish the most suitable carrier for each isolate.

6.02 **Biological control of bean root rot pathogens.**

From this study the two *Trichoderma* isolates tested were found to be antagonistic to Fusarium pathogens both in vitro and in vivo. Among treatments when T13 was applied to Fusarium culture using both random sprinkling and equidistant plating, its antagonistic activity on Fusarium was lower than that of T3A. Future field experimentations should target use of T3A than T13 in the biocontrol of fusarium root rot of beans.

The inhibition of Fusarium growth by Trichoderma in vitro indicated the production of antibiotics inhibitory to Fusarium growth which diffused through the growth medium. Parasitism and competition might also be involved in the growth inhibition process as is usual with *Trichoderma.*

Reports by other workers indicate inhibition of the root rot pathogen in culture by antagonistic fungi including those of the genus *Trichoderma.* Bounassisi et al (1986) reported inhibition of the radial growth of strains of *Fusarium monoliforme, F. Oxysporum* and *F. solani* f.sp. *phaseoli* in dual culture plate assays by indigenous *rhizobium* strains isolated from nodules obtained from commercial snap beans.

Using light and electron microscopy, *Gliocladium virens* was proved to be a hyperparasite of *F. solani* (Tu and Vaartaaja 1981). In vitro, *G. virens* effectively inhibited sclerotial formation of *R. solani*. *Trichoderma hamatum, T. harzianum* and to a lesser extent *T. koningii* and *T. viride* have been used against damping off caused by *Rhizoctonia* and *pythium* in the laboratory, glasshouse and the field (Papavizas 1985).

Hadar et al (1978) found an isolate of *Trichoderma hazianum* which directly attacked the mycellium of *Rhizoctonia solani.*

The greenhouse experiment established to determine the effectiveness of the two *Trichoderma* isolates on two carriers sorghum and rice, showed that T3A in rice carrier was the most effective system both in the laboratory and in the green house. This study also established that there is a positive correlation between the perfomance of *Trichoderma* in the carriers and in the greenhouse. *Trichoderma viride* in rice and *T. koningii* in sorghum sporulated well in the carriers and also gave the best management of the disease under greenhouse conditions.

This indicates that preliminary assessment of food carriers for their suitability in the laboratory prior to using them in the field can give some information on their subsequent effectiveness.

Wells et al 1972 found dehulled broken rice grain to be an excellent growth and delivery medium for the *Trichoderma harzianum* and *Trichoderma koningii* in the control of *Sclerotium rolfsii*. A strain of *T. harzianum* isolated from the rhizosphere of a cotton plant was found to be an effective biocontrol agent of *Fusarium oxysporum* f. sp *radicis, lycopersicion* tomatoes and of *F. oxysporum* f. sp *niveum* on water melons. Application of this antagonist under field conditions as a wheat bran-peat preparation or as a seed coating decreased disease incidence and significantly increased yield of tomatoes and water melons (Alex and Ilan, 1986).

Trichoderma hamatum applied as a coating of spores and the seed was found to protect peas and radish against seed decay caused by *Pythium* (Harman et al 1980, 1981). *Trichoderma harzianum, T. Zeae* and *T. hamatum* have been found to protect snap beans against Rhizoctonia root rot (Cardoso et al 1987).

Hadar et al (1978) found an isolate of *Trichoderma harzianum* which directly attacked mycellium of *Rhizoctonia solani* and effectively controlled damping off of bean, tomato and egg plant seedlings in the greenhouse when applied to soil artificially infested with *R. solani* using wheat bran as a carrier. Bounassisi et al (1986) showed a significant reduction in root rot of snap beans grown in pasteurised soil artificially infested with *Fusarium solani* f. sp *Phaseoli* receiving varying concentration of rhizobium. Preparations of *T.harzianum* has been found to control Sclerotium rolfsii and protect peanuts under field conditions (Abawi et al 1990).

The direct parasitism of *Trichoderma* on hyphae of other fungi is one of the significant mechanisms which can explain the antagonistic activity of *Trichoderma* species (Dennis and Webster 1971c). Elad et al (1982b) showed that extracellular lytic enzymes (B -1, 3 - glucanase and Chitinase) excreted by *T. harziaum* were responsible for cell wall degradation in *Rhizoctonia solani* and *Sclerotism rolfsii*. However, excretion of antibiotic compounds (Dennis and Webster, 1971a: 1971b) as well as competition (Ahmad and Baker 1986) may both play major roles in the biological control of soil borne plant pathogens by *Trichoderma* species. This study has shown that a potential exists for controlling *Fusarium* root rot disease using the *Trichoderma* isolates on food carriers. The need for sustainable agriculture will be met in part by adoption of such biological control systems as a disease management strategy.

6.03 Evaluation of biological control of bean root rot using a chemical as a standard.

Trichoderma isolate T3A in half broken dehulled rice applied at the rate of 1:3 of carrier to pathogen infested soil gave the best control of the root rot under greenhouse conditions. Chemical control using murtano gave the poorest control of this disease. For the two Trichoderma isolates in their respective carriers there was a general increase in disease incidence and severity with decreasing antagonist to pathogen ratio. The best ratio was 1:3 of carrier to pathogen inoculated soil. The larger ratio of carrier to planting soil mixture implied that the antagonist was present in high concentrations. This increased the number of initial "hits" of the pathogen propagules by the antagonist. Van der Plank (1975), reported similar findings .

Continued infections and destruction of the pathogen propagules by a hyperparasite in soil might be expected to follow a pattern of infected foci (Van der plank 1975), but with the amount of secondary spread of the hyperparasite among propagules being limited or non existence owing to greater distance between the propagules. Pathogenic propagules tend to become more uniformely distributed in soil over time which increases their chance for contacting a host root but also maximises their chance to escape an antagonist growing or spreading from other infected propagules. A model of Coy M.L. and Powelson (1974) for the relationship between the nearest propagules uniformely distributed in soil indicates distances of 0.8 and 1.1 mm for 2,000 and 1,000 propagules per gram respectively, but a much greater distance as the propagules drops below 1000/g. Applying the antagonists just as the inoculum is formed or released might help circumvent this advantage of escape otherwise gained by the pathogen when distributed more uniformely and sparsely in the soil.

Several fungicides have been used in the management of bean root rot disease in the greenhouse. *T. harzianum* plus reduced doses of methylbromide (equivalent to 200 kg/ha) completely controlled disease incidence of *R. solani* in bean seedlings compared with controls in untreated soils (Munnecke et al 1979).

Crossman et al (1969) reported significant reduction of *Rhizoctonia* root rot of french beans by Terraclor at 5 and 10lb/acre, Thiram (10lb/acre) folpet (10 1b / acre) and Miller 658 (8lb). Briston et al (1973) reported significant reduction in *Rhizoctonia* root rot of beans severity in seedlings of snap beans treated with pentachloronitrobenzene (PCNB) as compared to those not previously exposed to the fungicide. Seed treatment with fungicides such as Thiram, Benomyl and Captafol has been found to be effective in the control of *Fusarium* root rot of beans (Abawi and Pastor 1990).

From the foregoing examples it is clear that bean root rot disease has been managed using fungicides. However there is no available information on comparative studies of these fungicides and others with biological agents. From this study the two *Trichoderma* isolates in their respective carriers were found to be more effective in the management of *Fusarium* root rot of beans under greenhouse conditions compared to the chemical murtano.

The reason could be that the biological agents established themselves in the soil immediately they were introduced thus giving constant protection of the bean plants against the *Fusarium* pathogen during growth. The activity of the fungicide might have been affected by the soil environment and thus failed to give sustained control.

Seed treatment with effective fungicides like Thiram (Thiram 70s), Benomyl (Benlate), and Captafol (Difolatan) are only partially effective in reducing damage because infection occurs at fibrous roots at a distance far from the seed placement (Munnecke et al 1979).

In contrast to biological agents whose activity in the soil increase with time as a result of their multiplication, chemicals become less effective with time due to degradation by mircoorganisms, sunlight, floculation, evaporation and binding by chelating agents in the soil (Backman 1978).

Advantages of biological control are numerous. They include high levels of control at low costs, self perpetuation at little or no cost following the initial efforts, absence of harmful effects on man, his cultivated plants, and domesticated animals, wildlife and other beneficial organisms on land or in the sea, and the utility of some types as biotic insecticide (Huffaker et al 1976). Critics of biological control have been focusing on its supposedly narrow spectrum of activity and the fact that it is a slow process of pest management. However although resistance to antibiotics produced by a given antagonist has been found, when antibiotics are formed in situ by microorganism, more than one antibiotic may be produced or some other biological process such as parasitism and competition may be involved (Cook 1983). Thus the proposotion of biocontrol being a narrow method of pest management is more imaginary than real. There is need to popularize and introduce biological control of plant diseases to farmers. The antagonistic *Trichoderma* fungi can be packaged and sold to farmers as biological pesticides. For the cultivation of the fungi on large scale, farmers could be trained on how to multiply them on their farm on cheap organic matter such as manure and rice husks. Such a farmer participatory programme would receive little resistance in Vihiga and Kakamega districts where farmers are already growing snap beans under similar guidance. Snap beans were introduced in the three districts especially Kakamega and Vihiga in early 80s.

They are grown on contract whereby each farmer is supplied with seeds and other inputs and a package of recommendations. This include planting on ridges, fertilizer and spraying against foliar pathogens mainly rust. Farmers are then paid for their harvest delivered less the cost of inputs. Also one or two variety of snap beans are grown rather than the varietal mixtures as in common beans.

6.04 **Screening for resistance.**

The disease incidence qualitatively determined by assessing the proportion (%) of infected plants showed that all the 56 accessions in the germplasm were susceptible to the disease in varying degrees.

However resistant bean cultivars have been used in the management of *Rhizoctonia* and *Fusarium* root rots, charcoal rot and *Fusarium* yellows (Abawi and Pastor 1992). Dickson et al (1977, reported a strong association between coloured snap bean and pythium resistance but no linkage between *Rhizoctonia* resistance and seed coat.

Root rot resistant (RRR) Wisconsin 77 and Wisons 83 were derived from a hybrid between the single plant selection WH 72-2 and WA 71-72 with resistance to *Pythium* species (Hagedorn and Rand 1978). This meant that through plant breeding, bean lines resistant to fusarium root rot can be developed. York et al (1977) reported that resistance to seed decay and pre-emergence damping off caused *Pythium ultimum* was qualitatively inherited, associated with seed coat color and it could be broken. From the foregoing examples it can be seen that a possibility exists for developing bean varieties resistant to Fusarium root rot through plant breeding. The lack of resistance in the 5 bean lines and 51 accessions screened could be due to low levels of tolerance in parental germplasm, low heritability of tolerance factors and difficulty in combining tolerance to distinct pathogens. This could be improved by testing a larger germplasm for root rot resistance.

Three to four varieties are grown in the three districts, included in this study, the most popular being GLP - 2 (Rosecoco) followed by GLP - 24 (Canadian Wonder), GLP - 585 (Red Harricot) and GLP - X 92 (Mwezi Moja). All those lines were susceptible to the root rot. For successful screening and breeding for resistance against root rot, a large germplasm would be required.

A large number of bean lines and accessions that have polygenic resistance to root rots are available. Unfortunately, the development of adapted and accepted bean cultivars with root rot resistance has been difficult and very slow, partly because of the low level of tolerance in parental germplasm used, low heritability of tolerance factors, and difficulty in combining tolerances to several distinct pathogens (Dickson et al 1977). Nevertheless, research efforts aimed at the selection and development of multiple root rot resistance bean cultivars need to be expanded and intensified.

7.0 CHAPTER SEVEN

CONCLUSION AND RECOMMENDATIONS.

This study has shown that *Fusarium* is the major cause of root rot of beans in Westerm Kenya. Most of the research should now be directed towards effective management of the disease. The two *Trichoderma* isolates were found to have a great effect on the saprophytic growth and the parasitic activity of the pathogen both in the laboratory and under greenhouse conditions. Biological control of the disease by *Trichoderma* antagonist (T3A) in half broken dehulled rice as food carrier gave a good control of the disease under greenhouse conditions. This control was found to be more effective than the conventional chemical murtano and may last much longer.

Use of resistant bean cultivars could provide the most practical, economical and long lasting root rot management strategy. Unfortunately the 5 bean lines and 51 accession tested were all found to be susceptible to the disease.

Nevertheless, research efforts aimed at the selection and development of multiple root rot resistance bean cultivars need to be expanded and intensified. The need for a sustainable agriculture will be met in part by the adoption of biological control strategies, and the development of disease resistance cultivars. Research on biological control however lags seriously behind that of chemical control of plant pathogens (Cook 1983).

From the results of this study I would recommend the following strategies for the management of bean root rot in Western Kenya:

1. Antagonistic or biocontrol agents like *Trichoderma* with appropriate carriers should be used for management of root rot of beans.
2. Further evaluation of possible sources of resistance in local germplasms would be useful. Introduction of new bean lines into Kenya is vital.
3. Integrated control combining the most effective and practical control measures should be applied. This should involve combining biocontrol methods with compatible chemical control, resistance cultivars or cultural methods.

4. Farmer participatory approach in development and evaluation of root rot management methods would save time since it considers the farmers views during the process.
5. Technical assistance in diagnosing and monitoring the root rots particularly in areas most prone to the problem should be provided.

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APPENDICES.

Appendix 1a: Root rot incidence produced by various Fusarium isolates on Rose coco variety (GLP-2) of beans after 7 weeks of inoculation using root clip method.

ANOVA TABLE.

Source	df	SS	MS	Fvalue
Isolate ***	5	26354.17	527.83	13.38
Error	24	2250		
Total	29	35604.17		

*** Highly significant at both 5% and 1% levels

Appendix 1b: Root rot severity (determined by measuring external length of lesion) produced by various Fusarium isolates on Rose coco variety (GLP-2) of beans after 7 weeks of inoculation using root clip method.

ANOVA TABLE.

Source	df	SS	MS	Fvalue
Isolate ***	5	27.11	5.24	12.09
Error	24	10.76	0.45	

Appendix 1c: Root rot severity (determined by proportion of the external root infected) produced by various Fusarium isolates on Rose coco variety (GLP - 2) of beans after 7 weeks of inoculation using root clip method.

ANOVA TABLE

Source	df	SS	MS	Fvalue
Isolate ***	5	16054.17	3210	31.8429
Error	24	2420	110.83	
Total	29	18474.1		

*** Highly significant at both 5% and 1% levels.

Appendix 2a: Root rot incidence produced by various Fusarium isolates on Rose coco variety (GLP - 2) of beans using root infestation method.

ANOVA TABLE.

Source	df	SS	MS	Fvalue
Isolate ***	5	30916.67	6183.33	16.96
Error	24	8750.76	364.58	

Appendix 2b: Root rot severity (determined by external length of lesion) produced on Rose coco variety (GLP - 2) of beans using root infestation method.

ANOVA TABLE.

Source	df	SS	MS	Fvalue
Isolate ***	5	49.15	9.83	45.82
Error	24	5.15	0.21	
Total	29	54.29		

*** Highly significant at both 5% and 1% levels

Appendix 2c: Root rot severity (determined by proportion of root infected) produced on Rose coco variety of beans (GLP - 2) by various Fusarium isolates using soil infestation method.

ANOVA TABLE.

Source	df	SS	MS	Fvalue
Isolate ***	5	18716.67	3743.33	55.11
Error	24	16.30	67.92	
Total	29	20346.67		

Appendex 3: Zones of Growth inhibition produced on Fusarium isolates by T3A and T13 using equidistant planting method.

ANOVA TABLE.

Source	df	SS	MS	Fvalue
Treatment	4	70.30	17.58	247.54 ***
Error	20	1.42	0.071	
Total	24	71.7216		

*** Highly significant at both 5% and 1% levels.

Appendix 4: Mean number of Trichoderma conidia per gram of carriers over a period of time.

ANOVA TABLE.

(i) Day 7

Source	df	SS	MS	Fvalue
Treatment	5	99233.51	19846.70	1.91 ns
Error	12	124164.4	10410.95	
Total	17	224164.4		

ns not significant at 5% level.

ANOVA TABLE.

(i) Day 11

Source	df	SS	MS	Fvalue
Treatment	5	7709784.33	154196.87	3.12 *
Error	12	5927331.11	493944.26	
Total	17	13637115.44		

* Significant at 5% level.

ANOVA TABLE

(i) Day 14

Source	df	SS	MS	Fvalue
Treatment	5	72310168.18	15862033.64	318.36 ***
Error	12	597881.11	49823.49	
Total	17	13637115.44		

*** Highly significant at 5% level

ANOVA TABLE

(i) Day 18

Source	df	SS	MS	Fvalue
Treatment	5	26909162.88	15921832.56	5332.85 ***
Error	12	35827.34	2985.6	
Total	17			

Appendix 5a: Effect of Biocontrol agents on the Fusarium root rot of bean incidence in the greenhouse.

ANOVA TABLE

Source	df	SS	MS	Fvalue
Treatment	6	30107.14	5017.86	13.39 ***
Error	28	10500	375	
Total	34	40607.14		

*** Highly significant at 5% level

Appendix 5b: Effect of Biological agents on Fusarium root rot of bean (GLP - 2) severity (determined by external lengthy of lesion on root) in the greenhouse.

ANOVA TABLE

Source	df	SS	MS	Fvalue
Treatment	6	30.07	5.01	2.98 *
Error	28	48.91	1.75	
Total	34			

*** Highly significant at 5% and 1% level

Appendix 5c: Effect of Biocontrol agents on Fusarium root rot of bean (GLP - 2) severity (determined by the proportion of root infected) in the greenhouse.

ANOVA TABLE

Source	df	SS	MS	Fvalue	
Treatment	6	7330	1221.67	5.50	***
Error	28	622	222.14		
Total	34	13550			

*** Highly significant at 5% and 1% level.

Appendix 6a: Evaluation of the effect of the Biocontrol agents against fungicides (murtano) on Fusarium root rot of bean (GLP - 2) incidence in the greenhouse.

ANOVA TABLE

Source	df	SS	MS	Fvalue	
Treatment	7	13484.38	1926.34	3.19	***
Error	32	15750	492.19		
Total	39	29234.38			

*** Highly significant at 5% and 1% level.

Appendix 7a: Fusarium root rot incidence on 5 bean lines and 51 accessions.

ANOVA TABLE

Source	df	SS	MS	Fvalue	
Germplasm	55	29828.57	525.97	1047	***
Error	224	11250	50.22		
Total	279	40178.57			

*** Highly significant at 5% and 15 levels.

Appendix 7b: Fusarium root rot severity (determined by external length of lesion) on 5 bean lines and 51 accessions.

ANOVA TABLE

Source	df	SS	MS	Fvalue	
Germplasm	55	290.96	5.29	4.04	***
Error	224	292.62	1.31		
Total	279	583.58			

*** Highly significant at 5% and 15 levels.

Appendix 7c: Fusarium root rot severity (determined by external length of lesion) on 5 bean lines and 51 accessions.

ANOVA TABLE

Source	df	SS	MS	Fvalue	
Germplasm	55	42071.07	764.93	5.18	***