Title:

EFFICACY OF SEED-DRESSING AND ORGANIC AMENDMENTS AGAINST *FUSARIUM* ROOT-ROT OF FRENCH BEANS (*Phaseolus vulgaris* L. cv. monel.) IN KENYA.

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Declaration by the candidate.

This thesis is a result of my own work except where acknowlgement is in text. It has not been submitted nor is it being concurrently submitted for a degree in any other University or any other award.

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This thesis is dedicated to my beloved wife

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Abstract.

Laboratory, greenhouse and field tests were conducted to (i) compare relative pathogenicity of *Fusarium solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on french beans (*Phaseolus vulgaris* L. cv. Monel), (ii) evaluate the efficacy of seed dressing with various fungicides singly and in combinations against the two pathogens (iii) compare efficacy of organic matter and / or fungicides on root-rot caused by the two pathogens and (iv) compare effects of delay in planting after application of cowdung and *Tithonia diversifolia* on the root-rot. Laboratory experiments were arranged in a completely randomised block design while greenhouse and field experiments were arranged in a randomised complete block design. Five replicates were used in all tests.

In-vitro fungitoxicity potential of fungicides and fungicide combinations was assessed by computing % inhibition of mycelial growth, % spore germination and number of spores produced on fungicide treated dishes relative to that of controls.

Plant growth assessment was based on plant shoot height, dry weights of seeds, shoots and roots, and number of pods produced per plant. Disease severity was determined using mean length of discoloured root tissue (MLDRT) and mean root rot index (MRRI).

The plant growth parameters stated above were significantly (P=0.05) lower on plants infected with the two *Fusarium* spp. than control. However, slightly higher % loss in number of pods and seed dry weight were realised on those plants infected with *F. oxysporum* f.sp. *phaseoli* than *F. solani* f.sp. *phaseoli*. No statistical differences were noted between the effects of the two *Fusarium* spp. on bean growth. Disease severity on plants inoculated with the two pathogens was similar. MLDRT and MRRI, were the same in both cases. However, MLDRT and MRRI were relatively higher on plants infected by *F. oxysporum* f.sp. *phaseoli* than *F. solani* f.sp. *phaseoli*. This suggests that *F.*

oxysporum f.sp. *phaseoli* may have been more pathogenic than *F. solani* f.sp. *phaseoli* under the prevailing experimental conditions.

Fungitoxicity was based on the effect of fungicides on mycelial growth, spore germination and sporulation. Fungicide treatments significantly (P=0.05) inhibited mycelial growth, spore germination and sporulation of both test fungi. However, fungal sporulation was higher in plates treated with fungicides than in controls an indication that fungicides stimulated sporulation. Although no fungicide treatment was seen to be an inhibitor of all the three measures of fungitoxicity, the ranking of the best three fungicide treatments was be, thiram ^{50} + captan ^{50} > triforine > metalaxyl + mancozeb.

Individual fungicide treatments had significantly (P=0.05) better effects on plant growth and disease development than fungicide combination or control. Significantly (P=0.05) higher plant growth parameters and lower MLDRT and MRRI were recorded from single fungicide treatment on both the greenhouse and field grown plants. Significantly (P=0.05) better plant growth, more pods and heavier seed dry weights were recorded from plants obtained from seeds treated with fungicides triforine or metalaxyl.

Integration of cowdung and *T. diversifolia* with the fungicide metalaxyl revealed that cowdung or a combination of cowdung with metalaxyl significantly (P=0.05) reduced disease development and increased plant performance more than *T. diversifolia* or a combination of *T. diversifolia* with metalaxyl. Plants harvested in soils treated with *T. diversifolia* or a combination of *T. diversifolia* with metalaxyl or controls had the poorest seed and pod yields. Germination was low and no pods were harvested from plants grown in these soils.

A delay in planting french bean in soil treated with cowdung significantly (P=0.05) decreased development of root rot when compared to untreated controls. The greatest decrease was obtained with 2 and 4 week delay. Shoot and root dry weights of plants infected by *F. oxysporum* f.sp. *phaseoli* or *F. solani* f.sp. *phaseoli* significantly (P=0.05) increased with delay in planting and were highest where planting was delayed 2 and 4 weeks, respectively. A delay from 0 to 4 weeks in planting french beans in soil treated with crushed leaves of *T. diversifolia* significantly (P=0.05) increased disease severity and reduced plant performance when compared to untreated control.

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CHAPTER 1.

1.0. INTRODUCTION.

Beans (*Phaseolus vulgaris* L.) are ancient food crops, domesticated in the Andean region of south America, and the Mexican-Guatemala region of central America (Purseglove, 1968). Brazil is the leading bean producer in the world (CIAT, 1989) with a mean annual production of $2.2x10^6$ tons. Annual world production is estimated to be 8.5 million metric tons (FAO, 1986), with sub-Saharan Africa accounting for 24.1%.

Phaseolus vulgaris was introduced into East Africa some 300-400 years ago (Purseglove, 1968) and is grown under diverse agricultural production systems (Schwartz and Pastor-Corrales, 1989). Cultivated members of *P. vulgaris* include the common, field, green, snap and wax bean (HCDA, 1995). The snap beans are used for fresh green bean production and have fleshy pods that are harvested when seeds are still immature. Beans provide relatively cheap source of protein, essential amino acids such as lysine, tryptophane and methionine, carbohydrates, vitamin B1, nicotinic acid, calcium and iron (Silbernagel *et al.*, 1991) and are a potential income earner to small scale farmers in countries such as Kenya (HCDA, 1995).

However, bean production is widely limited by various diseases among other limiting factors such as rainfall and temperature (Njuguna *et al.*, 1981; Schwartz and Pastor-Corrales, 1989). In East Africa, bean losses of up to 100% and 84% have been associated with rust and *Fusarium* root rot

respectively, (Allen, 1983). *Rhizoctonia* root rot (*Rhizoctonia solani* Kühn), *Fusarium* dry root rot (*Fusarium solani* (Mart.) Appel and Wollenw. f.sp. *phaseoli* (Burk.) Synder and Hansen) and *Fusarium* yellows (*Fusarium oxysporum* Schlecht. f.sp. *phaseoli* Kend.and Synder

are the most prevalent root-rot diseases in Kenya (Mughogho, 1970; Leakey, 1970; Mukunya, 1974; HCDA, 1995). The diseases depress seedling germination and reduce storage quality of seeds, since the causal agents are seed borne (Neergaard, 1979; Buruchara, 1985).

Effective control strategies against root rot fungal pathogens have not been fully developed (Mutitu *et al.,* 1989). Farmers in Kenya, often use seeds preserved from previous harvest (Rono and Shakoor, 1990), a practice that negates the principle of sanitary practices (such as use of disease free seeds) to control diseases (Buruchara, 1990). Existence of physiological races among root-rotting fungal pathogens complicate efforts to breed for resistant cultivars (Leakey *et al,*1972; Allen, 1983; Stephen and Marcial, 1991). Although chemical control can minimize bean losses (Nevill *et al.,* 1990; Sozzi and Chin, 1990, Urech, 1990) its efficacy has been inconsistent (Nevill *et al.*, 1990). Furthermore, some fungicides are less attractive to farmers because they are too expensive and hazardous to the environment (Carlile, 1988).

Therefore, there is need to develop effective measures for controlling bean root-rot (Papavizas and Lewis, 1979, Burke and Miller, 1983, Sumner *et al.*, 1986a and 1986b). Integration of various control measures such as seed-dressing, soil amendments and host resistance seems a viable alternative. Information is lacking on integrated bean root-rot control.

Therefore, the present study was designed with the following specific objectives:-

1.1. Objectives.

(I) To compare relative pathogenicity of *Fusarium solani* f.sp. *phaseoli* and *Fusarium oxysporum* f.sp. *phaseoli* on french beans.

(II) To conduct *in-vitro, in-vivo* (greenhouse) and field experiments to evaluate efficacy of five selected fungicides when used singly and/ or in combination against *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* root rot fungal pathogens of french beans in Kenya.

(III) To compare effects of organic matter and seed dressing with fungicides on the pathogenicity of *F. solani* f..sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* on french beans.

(IV) To compare effects of delay in planting after application of cowdung and *Tithonia diversifolia* (Hemsl.) Gray on root-rot of french beans caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli.*

CHAPTER 2.

2.0. LITERATURE REVIEW.

2.1. French bean production in Kenya.

French beans, also known as the snap beans, green beans, horticultural haricot beans, (HCDA, 1995) are a major horticultural export crop in Kenya. Earnings from french bean export accounts for about 22% of the total horticultural revenue, placing the crop second to cutflowers in terms of value and export volume (Löhr and Suzzane, 1995). Exports are currently being expanded to the Middle East, South Africa and Japan (HCDA, 1995). They are grown by small scale farmers in Kirinyaga, Mwea-Tebere, Meru, Thika, Athi-River and Naivasha (HCDA, 1995). They are consumed as immature green fiberless pods, dry ripe seeds or as green shelled beans (Purseglove, 1977). The dwarf and the climbing types are the main french bean cultivars grown in Kenya (Purseglove, 1968). Varieties such as Claudia, Gloria, Maasai, Morgan, Espada and Monel are recent introductions to the country (HCDA, 1995). Variety Monel is high yielding and has a longer picking duration, and is grown for fresh export and canning (Omunyin, 1990).

A wide range of soils favour bean production though they do better on well drained friable, medium loam soils with high organic matter (HCDA, 1995). Soil pH of 6.5 to 7.5 is the most favourable for bean production. Harvesting of pods begins 6-8 weeks after planting and continues for about 1.5-2 months depending on cultivar. Picking is done thrice a week to maintain export quality. With proper crop husbandry yields of up to 10 tonnes of marketable

green pods per hectare can be realised (KARI, 1990). However, this level of productivity is never realised due to, in part, to fungal and bacterial pathogens (Owino and Waudo, 1991; Lohr and Susanne, 1995). It is therefore necessary to intensify research and develop appropriate disease control measures.

2.2 . Economic significance of french beans.

French beans provide a source of income to small scale farmers and are a major source of vitamins B1, mineral elements, calcium and iron and essential amino acids such as lysine, tryptophane, methionine and carbohydrates (Silbernagel *et al.*, 1991). They replenish soil nitrogen depleted through leaching, volatization and or denitrification (Dagnew, 1981; Amare and Birhanu, 1984). As legumes, french beans are important tools for sustainability of any agricultural system (Bohlool *et al.*, 1992).

2.3. Diseases of french beans.

Major diseases of french beans are listed in Table 1.

Table 1. Major diseases of French beans.

Disease	Causal agent	Estimated loss	Reference.
Angular leaf spot	*Phaeoisariopsis griseola.* (Sacc.) Ferr.	Major. Loss may be as high 60-80%.	Barros *et al.*, 1957; Ferras, 1980; Schwartz *et al.*, 1981.
Bean common mosaic virus (BCMV).	Bean common mosaic virus	Major. Yield loss estimated 35-98%	Schwartz and Gálvez, 1980.
Halo blight	*Pseudomonas syringae* p.v. *phaseolicola* (Burk). Dows.		
Anthracnose	*Colletotrichum lindemuthianum* (Sacc&Magn) Brit & Cav.	Major. Almost complete crop loss may occur. Over 92% loss estimated in Malawi.	Leakey, 1970.
Rust	*Uromyces appendiculatus* (Pers.) Ung. *appendiculatus*	Major. Complete crop loss may occur.	Zaumeyer and Thomas, 1957; Howland and Macartney, 1966; Ballantyne, 1974.
Root-rot	*Meloidogyne* spp.Major.		
	Fusarium solani f.sp.. *phaseoli*	Up to 84% crop estimated in USA.	Harter& zaumeyer, 1931; Adegbola and Hagedorn, 1969, Hoch *et al.*, 1975.
	Fusarium oxysporum f.sp.*phaseoli.*	Minor.	Bolkan, 1980.
	Rhizoctonia solani Kühn.	Loss of 10% Reported in USA	Zaumeyer and Thomas, 1957; Bolkan, 1980.
	Microphomina phaseolina (Tassi)	Loss of 60-65% reported in USA	Zaumeyer and Thomas, 1957,

	Goid.		1957, Schwartz and Gálvez, 1980.
	Sclerotium rolfsii. Saccardo.		Bolkan, 1980.
Trow.	*Pythium ultimum.*	Minor.	Zaumeyer and Thomas, 1957; Bolkan, 1980.

2.3.2. *Fusarium* 'yellows':

Fusarium yellows is a vascular disease. The causal agent, *Fusarium oxysporum* f.sp.*phaseoli,* penetrates the vascular tissue of a root and hypocotyl and causes systemic necroses. Vascular tissues of infected beans are usually discoloured (Wheeler, 1969). The disease is first noticed as a general chlorosis of the foliage developing into an irreversible wilting of affected plant which may also be stunted.

2.3.3. *Fusarium* dry root rot:

Fusarium dry root rot, caused by *Fusarium solani* f.sp. *phaseoli* is characterised by elongated reddish brown lesions on the primary root within one or two weeks of germination. The lesions may expand to cover the entire root. Longitudinal cracks often appear on the surface of an infected root. Although the pathogen frequently destroys the tap root, secondary roots develop above the initial lesions (Burke and Barker, 1966; Wheeler, 1969). The fungus only invades the vascular tissue in later stages of disease development and it seldom extends in the stem much above the soil line.

2.3.4. Survival of *Fusarium* spp. root rotting fungi.

Chlamydospores serve as the primary inoculum. Chlamydospores are produced within the root cortex. They are released into the soil where they survive (Allen, 1983) and provide the primary inoculum for infection of subsequent crop. The survival of the two *Fusarium* spp. root-rotting fungi

may be enhanced by temporary supplies of nutrients provided by diffusates from non-host plant and crop residues (Schroth and Hendrix, 1962; Papavizas *et al.*, 1968). Chlamydospores germination is stimulated by exudates from both seed and hypocotyl of beans (Schroth and Snyder, 1961), but in the absence of living plant residues, substances produced during natural decomposition of crop residues can also influence their germination (Schroth *et al.*, 1963; Toussoun *et al.*, 1963; Cook and Synder, 1965). *Fusarium* spp. are internally seed-borne and spread in soil as chlamydospores or conidia in surface water and by drainage and irrigation (Kendrick, 1934; Nash and Snyder, 1964). Temporary waterlogging of soil can aggravate root-rot (Miller and Burke, 1977).

2.4. Control of french bean diseases.

Control measures against root-rot include the use of certified seeds, crop rotation, seed dressing using chemicals and the use of resistant varieties (CIAT, 1989). Unfortunately no resistant varieties have been produced specifically for root-rot resistance in the tropics (Stephen and Marcial, 1991). Certified seeds are not widely used because of cost and farmers find the use of chemicals unattractive because of the high cost and health risks associated with pesticide usage. Land scarcity makes use of crop rotation unattractive (Papavizas and Lewis, 1979; Burke and Miller, 1983; Sumner *et al.*, 1986a; 1986b, Mutitu *et al.*, 1995). Therefore, it is imperative to review control strategies against fungal pathogens of beans.

2.4.1. Use of chemicals in controlling plant diseases.

Chemicals have been used to control diseases of plants through soil fumigation, foliar spray and seed-dressing. Seed-dressing provides an effective and efficient way of applying small quantities of fungicides for controlling seed-borne diseases of arable crops (Jeffs, 1978). It has led to an overall

improvement in the health of seeds with a consummate increase in crop yield (Entwistle and Munasinghe, 1981; Salter and West., 1990).

Currently available effective fungicides against soil-borne pathogens are few and must be used carefully to preserve their effectiveness in future (Leadbeater and Nevill., 1990). Widespread use of a chemical can lead to development of resistant strains (Carlile, 1988; Sozzi and Chin, 1990) as is the case of *Fusarium* spp. to benzimidazole and that of *Botrytis cinerea* to Methyl-1-2-benzimidazole carbamate MBC (Locke *et al.,* 1987; Carlile, 1988). There is need to develop anti-resistance strategies to delay evolution of resistant pathogens in order to prolong the useful life of a fungicide (Carlile, 1988; Leadbeater and Nevill, 1990). Such strategies include rotational use of fungicides with different modes of action, limited pesticide application, use of fungicides mixtures (Sozzi and Chin, 1990) and integration of fungicides with cultural practices (Leadbeater and Nevill, 1990). As an example, *Phytophthora megasperma* var. *glycinea* is best managed by use of a combination of field drainage, partial resistant varieties and strategic application of metalaxyl as a soil or seed treatment (Leadbeater and Nevill, 1990)

2.4.2. The use of combinations of control measures in pest management.

The combinations of two or more fungicides with different modes of action in seed dressing programmes can minimise effects of selection pressure on pathogens and enhance chemical efficacy (Carlile, 1988). For instance, *In-vitro* treatment of *Phaseolus vulgaris* seeds with DCT (diazinon + captan + thiophanate) fungicide combination improves germination and reduces seed and hypocotyl infection with *Pythium ultimum* and *Fusarium solani* (Tu and Zheng, 1993). Fungicides have also been successfully integrated with other methods of disease management to control several plant diseases. For instance, seed treatment with a biocontrol agent, *Gliocladium virens* (1 X 10^7 conidia

/ml) combined with Carboxin (0.1%), is highly effective in management of several soilborne plant pathogens including *Sclerotium rolfsii*, *Rhizoctonia solani* and *Fusarium oxysporum* in chickpea, lentil and groundnut (Shrestha and Mukhopadhyay, 1991). Therefore, experiments in this study were conducted to determine the efficacy of using systemic fungicides such as pyrazophos (Afugan EC, 20%), triforine (Saprol EC, 20%) and metalaxyl + mancozeb (Ridomil Mz WP, 63.5%), and protectants fungicides such as captan (Captan WP, 83%) and thiram (Murtano WP,) and organic amendments against root-rot fungi of french beans.

2.5. Use of organic matter in disease control.

The efficacy of organic matter against plant pathogens has been inconsistent for many years (Harry and Peter, 1986). However, the stress on the importance of preservation of our environment requires continued exploration into the area of biological control for suppression of soil-borne plant pathogens (Papavizas, 1968).

Composted organic materials have a positive potential in the management of root-rot diseases (Trutmann and Kaaytare, 1986). In China and Japan, composts have been used in agriculture with beneficial effects (Kelman and Cook,1977). In Africa, the utilization of compost is a relatively recent practice (Harry and Peter, 1986). During the past two decades several reports have discussed suppressive effects of composts to a variety of soilborne plant pathogens (Harry and Peter, 1986). For example, composted larch bark has been used for control of *Fusarium* brown rot of Chinese yam (Sekiguchi, 1977). The decrease in disease was attributed to increase in population of *Trichoderma* spp. an antagonist that was favoured by the composted larch bark (Sekiguchi, 1977). Composts made from waste hard wood and pine bark (Hoitink *et al.,* 1977), are suppressive to several fungal diseases such as *Phytophthora* root rot (*Phytophthora cinnamomi)* (Hoitink, 1980;

Hoitink and Poole, 1980; Spencer and Benson., 1981; Spencer and Benson., 1982) and *Rhizoctonia* root rot (*Rhizoctonia solani)* (Nelson *et al.,* 1983). Coffee hulls and farmyard manure have been found to suppress *Fusarium* 'yellows' of beans when used as organic compost. (Mutitu *et al.,* 1989).

Long term effects of composted municipal sludge (CMS) has been demonstrated against *Sclerotium minor* (Lumsden *et al.*, 1982). Composted municipal sludge incorporated into soil at high rate (10% per weight) controlled *Aphanomyces* root-rot of peas, *Rhizoctonia* root-rot of beans, *Fusarium* wilt of cucumber and *Phytophthora* crown rot of pepper (Lumsden *et al.,* 1983b).

Organic matter has also been used in control of bacteria and nematodes. Population development of *Helicotylenchus* spp. (Hunt *et al.*, 1973) and *Pratylenchus dianthus* (Derrico and Di Maia, 1980) is suppressed by composts prepared from municipal refuse. Organic matter prepared from *Tagetes* spp. and *Datura* spp. reduces the pathogenicity of root-knot nematodes on tomato and okra (Owino *et al.*,1993; Owino, 1994; Owino and Waudo, 1996).

2.5.1. Organic matter and their mode of action.

Organic matter has been used in many parts of the world for several reasons. Costs of composts may be lower than those of peats. Compost amended media, particularly those amended with composted bark, suppress some soilborne plant pathogens, thus reducing plant losses (Spencer and Benson, 1982; Chef *et al.*, 1983; Stephen and Stebbins, 1985), without carrying out steam treatment or fumigation (Daft *et al.*, 1979; Stephen and Stebbins, 1985).

Despite the positive efficacy of composts or organic matter these substances have their own limitations. Differences in efficacy among compost types have been reported (Harry and Peter,

1986). For instance, studies done using chicken manure as organic matter indicated that it enhanced *Fusarium* wilt disease development hence limiting its use in tomato production in areas where *Fusarium* wilt is prevalent (Waudo *et al.*, 1995). Similarly, compost prepared from tree leaves or rice hulls were effective in control of clubroot (*Plasmodiophora brassicae*) of Chinese cabbage, whereas compost prepared from sawdust was less effective (Tamura and Taketani, 1977). It is, therefore, essential to conduct more experiments using organic matter in order to fully understand their role in disease control programmes.

Composted organic wastes affect soilborne diseases in several ways (Lumsden *et al.*,1983a). Some organic matter from *Brassica* spp. suppress nematode and soilborne disease development (Bullock, 1992; Gardner *et al.*, 1992; Owino *et al.*, 1993b), by the production of toxic glucosinolates (Duncan, 1991; Mithen, 1992). Organic amendments affect chemical composition of root tissues rendering plants less suitable hosts (Castagnone-Sereno and Kermarrec, 1991). In other cases, organic matter affects soil antagonists (Owino *et al.*, 1993a; Owino and Waudo, 1996). For instance, it has been shown that organic amendments suppress the activity of the pigeon pea wilt pathogen (*Fusarium udum*) by increasing production of an antibiotic by the antagonist, *Bacillus subtilis* (Vasudeva *et al.*, 1963; Singh and Singh, 1981). The antagonistic potential of *Paecilomyces lilacinus* against root-knot nematodes is also enhanced by organic matter such as chicken manure and *Tagetes* species (Owino *et al.*, 1993a; Owino *et al.*, 1996a).

2.6. Control of diseases of beans using resistant plant cultivars.

The potential for controlling diseases of legume crops by host plant resistance was first realised in the USA by Orton, (1902) and several sources of resistance have been identified against fungal diseases of beans such as rust (Ballantyne, 1974; Coyne and Schuster, 1974), anthracnose

(Mastenbroek, 1966; Fouilloux, 1976; Schwartz *et al.*, 1982), *Fusarium* dry root rot (Boomstra *et al.*, 1977; Beebe *et al.*, 1981), and *Fusarium* 'yellows' (McKerral,1923; Pereiro and Hagedorn, 1979a, b). There is of course considerable variation in the expression of resistance due to different mechanisms of operation and genetic control. Some types of resistance provide complete control of diseases while others afford only partial protection. That is either long or short lived resistance. For instance, resistance to curly top derived from a red Mexican type has remained effective in agriculture for over 45 years (Silbernagel and Jafri, 1974), while the hypersensitive resistance, recognised in beans against rust, anthracnose and halo blight is known to be race-specific and has been of short-term benefits (Allen, 1983). In beans, resistance to root-rot is known to be race non-specific (Bravo *et al.*, 1969; Hassan *et al.*, 1971; Boomstra and Bliss 1977; Dickson and Boettger, 1977;). Race non-specific resistance are durable (Ballantyne and

McIntosh, 1977; Schwartz, 1980). Differences in resistance of bean genotypes to race non-specific resistance have been reported (Leakey *et al.*, 1972; Ballantyne, 1974). Host resistance can also be influenced by age, morphological and physiological characteristics of a plant (Patel and Walker, 1963; Patel and Walker, 1966; Coyne and Schuster, 1974; Valladares-Sanchez *et al.*, 1979; Moody *et al.*, 1980). Phytoalexins associated with hypersensitivity have also been detected in bean plants with resistance to *Thielaviopsis basicola, Rhizoctonia solani* and *F. solani* f. sp.*phaseoli* (Pierce, 1971; Smith *et al.*, 1975). However, *F. solani* f sp. *phaseoli* can tolerate and detoxify several phytoalexins (Kühn and Smith,1979; Smith *et al.*, 1980; Kistler and Van Etten, 1981).

Dry root-rot resistance may be correlated with seed size (Wallace and Wilkinson, 1973). This is because large seeds have a large raphe that can suppress seed infection (Zaumeyer and Thomas, 1957; Schuster and Coyne, 1974). Similarly, seed-coat colour influences the response of beans to pathogens (Prasad and Weigle, 1976; Burke and Silbernagel, 1980; Moody *et al.*, 1980). For

instance, the resistance to both *Pythium* and *Rhizoctonia* root-rot is related to seed-coat colour (Deakin and Dukes, 1975; Prasad and Weigle, 1976; Dickson and Boettger, 1977; York *et al.*, 1977; Burke and Silbernagel, 1980). Seeds with black seed-coat are more resistant to *Rhizoctonia solani* (Kühn) than those with white seeds (Prasad and Weigle, 1976; Moody *et al.*, 1980). The resistant black-seeded cultivars produce phenolic compounds that inhibit seed infection (Prasad and Weigle, 1976). Also seed-coat of black seeds do not crack as readily as those of white seeds during germination. Because of this, cotyledons of black seeds are protected against pathogen attack (Prasad and Weigle, 1976).

These inconsistencies confirm that there are no simple indices of durable resistance (Johnson, 1981a, b) and new disease control strategies need to be continuously developed. This is important where reliance on both race and race non-specific resistance has led to the breakdown of resistance. Therefore, the best strategy for a successful management of pathogens of beans in the tropics is likely to be an integrated approach in which the production of clean seed, good crop husbandry, chemical applications and exploitation of genetic diversity will each play a role. Therefore, these studies were conducted to provide information on effects of cropping systems such as the utilisation of chemicals and organic matter on management of bean root-rot caused by *Fusarium* spp.

CHAPTER 3.

3.0. MATERIALS AND METHODS.

In- vitro, greenhouse and field tests were conducted at Kenyatta University, Nairobi, to:

(i) compare relative pathogenicity of *F. solani* f.sp *phaseoli* and *F. oxysporum* f. sp. *phaseoli* on french beans.

(ii) conduct *in-vitro*, greenhouse and field experiments to evaluate efficacy of five selected fungicides when used singly or in combination against *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* root-rot fungal pathogens of french beans in Kenya.

(iii) compare effects of organic matter and seed dressing with fungicides on the pathogenicity of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* on french beans; and

(iv) compare effects of delay in planting after application of cowdung and *Tithonia diversifolia* on root-rot of french beans caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli*

3.1. General methods:

3.1.1. description of sampled regions.

Root-rot fungi were isolated from infected roots of french beans obtained from Meru (Nkubu and Katheri) and Thika (NHRC and Mangu) districts of Kenya. Thika and Meru districts (fig 1) are in different climatic zones of Kenya (District Development Plans, 1994-1996).

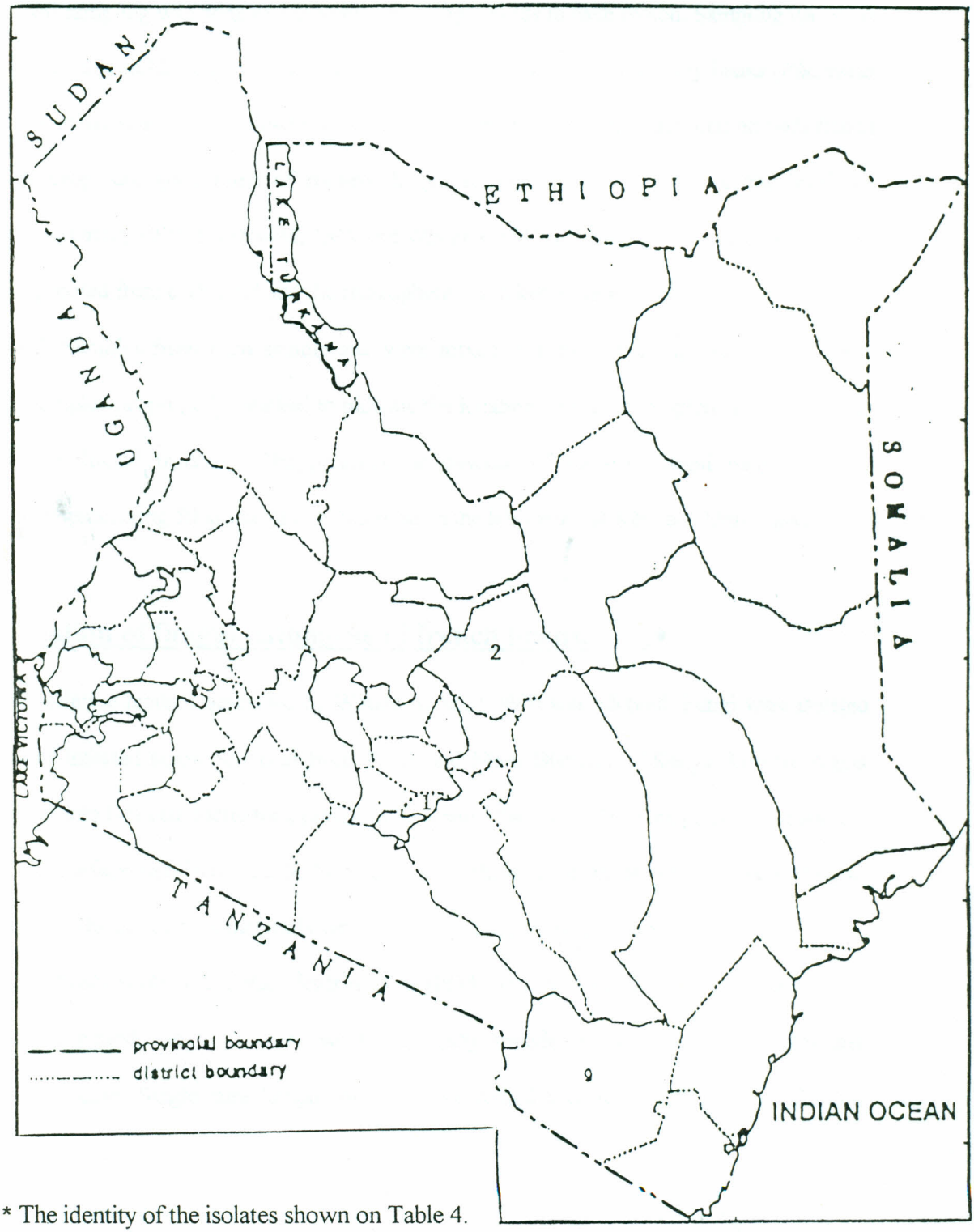
Thika district.

Thika district lies about 1200m above sea level and occupies about 171 sq Km and has savannah type of climate. National Horticultural Research Centre (NHRC) has an annual rainfall of 500mm. The soils vary from well drained, shallow, dark red to yellow red, stony loam sand. The major crops in Thika district are coffee, sunflower, maize, sorghum, sisal, beans, bananas and groundnuts.

Meru district.

Meru district lies between 300m and 5199m above sea level and has an equatorial type of climate. Rainfall pattern is bi-modal with the long rains between mid March and May and short rains between October and December. Nkubu, the region surveyed, receives an annual rainfall of 1800mm and lies 2400m above sea level. Mean maximum temperature is 35°C, while the mean minimum temperature is 23°C. The most dominant soil type is fertile loam. Mixed cropping is commonly practised. The major crops in the district are coffee, tea, cotton, irish potatoes, beans, maize, sorghum and bananas.

Fig 1. A map of Kenya showing Thika and Meru districts of Kenya that were sampled and the number of fungal isolates* from each region.



* The identity of the isolates shown on Table 4.

1 Thika, 7*

2 Meru , 9*

3.1.2. Root and soil sampling.

Root and soil sampling was done by randomly choosing 3 fields in each region. Sampling was done at two sites in each field. The fields had one or more of the following crops: dry beans (*Phaseolus vulgaris* L.), maize (*Zea mays*), tomato (*L. esculentum*) and peas grown in association with french beans. The crops are good hosts of root-rot fungal pathogens, though with varying levels of susceptibility (Orton, 1902; McDonald, 1969 and Wheeler, 1969). Twenty french bean plants were randomly uprooted from each field and the rhizosphere soil taken from a 10-cm-depth using a small hand shovel. Samples from each sample site were mixed to form one composite sample. Each composite sample was properly labelled to indicate the location and areas sampled and taken to the laboratory for fungal isolation. The presence or absence of root-rot fungal pathogens was determined by processing 50 grams of infected roots in the laboratory at Kenyatta University.

3.2.0. Isolation of fungal pathogens of french beans.

The fungal isolation method described by Waudo, *et al.*, (1995) was adopted. Fungi were isolated from roots of infected beans collected from Thika and Meru Districts of Kenya. Infected plants roots were cut into five centimetre long pieces using a pair of scissors. The root pieces were put in a Petridish and surface sterilised with 0.5% sodium hypochlorite for 30 seconds before rinsing in distilled water. The sterile root materials were cut using a sterile razor blade into 1cm long pieces and transferred aseptically onto Potato dextrose agar (PDA) and incubated at 25°C for 4 days. After the incubation period, single colonies were aseptically transferred to fresh PDA plates and incubated for 4 days. Single pure fungal colonies were picked and used in fungal identification exercise.

3.2.1. Fungal identification methods.

Pure fungal cultures growing on PDA were aseptically transferred onto sterile (9-cm-diameter) plastic petridishes (one colony /plate) and incubated at 25°C under alternating cycles of 12 hours of light and 12 hours of darkness for 7 days. Part of the growing fungal mycelium was aseptically transferred onto a sterile slide and suspended in a drop of sterile distilled water. Fungal mycelium were examined under a compound microscope and identification of the fungi was based on their growth habits on PDA and characteristics of the spores. Identification to the order level was based on features such as the nature of mycelia whether coenocytic or septate, the type of reproductive structures, presence or absence of conidia (spores) and position of spore or conidia production. The genera of *Rhizoctonia*, *Pythium* and *Fusarium* which cause damping off or root-rot of *Phaseolus vulgaris* L. were further identified to species level using morphological characters (Barnett and Barry, 1972). *Rhizoctonia* spp. were identified on the basis of sclerotia colouration since the genus lacks spores (Barnett and Barry, 1972). *Pythium* spp. were identified on the basis of sporangia (Barnett and Barry, 1972). *Fusarium* spp. were identified on the basis of morphological characters of micro and macro conidia.

3.2.2. Preparation of fungal inoculum.

Fungal spores and mycelia were used as inocula. To obtain spores petridishes containing 10-day-old isolates of *F. solani* f. *sp. phaseoli* or *F. oxysporum* f. sp. *phaseoli* were flooded with 20ml of sterile distilled water and agitated on the shaker at 200 rpm for 30 minutes. The suspension was then filtered through cheese cloth and centrifuged at 1200 rpm for 15minutes to remove fungal mycelium. Inoculum density was adjusted to 1 $x10^6$ spore/ml of distilled water using a

haemocytometer. Mycelial inoculum was prepared by cutting eight 7-mm-diameter mycelial plugs from the peripheries of 10-day-old fungal cultures of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* using a 7-mm-diameter cork borer. The mycelial plugs were aseptically macerated before using as inoculum (Nigh *et al.*, 1980; Owino *et al.*, 1996).

3.2.3. Inoculation techniques.

Two different inoculation methods were used. Soil infestation method described by Owino *et al.* (1996) was used to inoculate soil contained in 16.5-cm-diameter pots for greenhouse tests. The 7-mm-diameter macerated mycelial plugs were thoroughly mixed with soil contained in 16.5-cm-diameter plastic pots at a rate of eight plugs per pot. Planting was done 10 days after inoculation to allow fungal establishment.

The soil infestation method described by Prasad *et al.* (1976) and Schuster *et al.* (1975) was used to inoculate soil in the field. For inoculation 5 mls of distilled water containing 1. 0 x 10^6 spore /ml were pipetted into planting holes just before planting. Only fresh suspension of spores were used for inoculation to avoid storage effect on viability and virulence (Charles and Craig, 1978).

3.2.4. Disease assessment.

The disease indexing method described by Fernandex *et al.*, (1986) was used to assess disease on bean roots and hypocotyl. Five randomly selected plants per experimental unit were assessed. A rating scale of 1-9 was used where:

1 = no visible disease symptoms on root and hypocotyl of plant.

3 = Light discolouration either without necrotic lesions or with approximately 10% of the hypocotyl and root tissues covered with lesions.

5 = Approximately 25% of the hypocotyl and root tissues covered with lesions but remains firm with deterioration of the root system. Heavy discolouration symptoms may be evident.

7 = Approximately 50% of the hypocotyl and root tissues covered with lesions combined with considerable softening, rotting and reduction of the root system.

9 = Approximately 75% or more of the hypocotyl and root tissues affected with advanced stages of rotting combined with a severe reduction in the root system.

The length of discoloured root and hypocotyl tissue was measured in millimetres(mm) using a ruler and the length was recorded to the nearest mm. The observed values were averaged to obtain a mean length of discoloured root tissue (MLDRT). Disease was assessed at different stages as shown in table 3.

3.2.5. Stages of root-rot evaluation.

Assessment of disease on roots of french bean was done at 3 stages of bean development (Table 2) as outlined by Fernandez *et al.* (1986).

Table 2. Stages of root-rot evaluation and the associated plant growth development characteristics.(Fernandez *et al.*, 1986).

Evaluation stages	Plants characteristics associated with each plant growth developmental stage	Days after planting
V1- Vegetative phase 1:	Emergence from the appearance of cotyledons on the soil surface to the unfolding of the primary leaves.	14
R6- Reproductive phase 6:	Flowering: from the opening of the first flower to the expansion of the ovary after fertilization.	36
R8- Reproductive phase 8:	Pod filling: from the beginning of seed weight and size increase to the development of pigmentation of seeds and onset of leaf senescence.	72

3.2.6. Crop performance assessment.

Effect of different treatments on french beans growth were assessed by measuring shoot height (cm), dry weight of 100 seeds, number of pods per plant, loss in pod and bean yield, dry weight of shoot and root. Shoot heights were measured from the soil surface to the upper most leaf apex.

Dry weights of shoots, roots and of 100 seeds were obtained by drying the materials at 80°C for 72 hours before weighing. Relative yield loss expressed as number of pods and bean dry weight of root-rot infected plants was expressed relative to the yield of healthy control plants based on the direct contribution of each yield components as shown in the equation below:

$$\% \text{ Yield loss} = \frac{Y^1 - Y^2 \times 100}{Y^1}$$

Where,

Y^1 = Yield in grams per experimental unit of the healthy plants (control plant) and

Y^2 = Yield in grams per experimental unit of the infected plants.

3.2.7. Chemical composition of the soil and organic amendments used in this study.

Soil used for all the greenhouse tests had a pH of 5.7 but with varied chemical composition.

The chemical composition of the organic matter used is shown in Table 3.

Table 3. Chemical composition of the organic amendmemts used in this study.

	Composition by %		
Material	C	N	C:N
Cowdung	27	2.75	9.81
Tithonia diversifolia	17	18	0.94

3. 2. 8. Soil sterilisation method.

Soil used in this study was collected from fields that had been left fallow for 3 years. The collected soils were first mixed with sand in 1: 2 (soil : sand) ratio. The mixture was steam sterilised for 16 hours at a temperature of 121°C and 15 atmospheric pressure.

3.3.0. Greenhouse test 1.

3.3.1 Pathogenicity test.

This test was conducted at Kenyatta University, Nairobi, Kenya between November and December, 1996 to compare the relative pathogenicity of *F. solani* f *.sp. phaseoli* and *F. oxysporum* f. sp. *phaseoli* on french beans cv. Monel. The cultivar Monel was chosen because it is widely grown in Kenya and is a good host of the *Fusarium* root-rot pathogens.

The two fungi, *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp *.phaseoli* used in this investigation were isolated from infected roots of french beans collected from farmers fields in Thika and Meru Districts as described in section (3.2.0). Fungal inocula were obtained directly from 10-day-old fungal cultures grown on PDA at 25°C. The inoculum composed of a mass of spores and mycelium, was prepared as described in section (3.2.2). It was applied to sterile soil contained in 16.5-cm-diameter pots at the rate of 8 macerated PDA plugs (7-mm-diameter) per pot. The inoculated soil was left for 10 days to allow inoculum build up before planting. Three disease free seeds (certified seeds) were planted in each plastic pot at a depth of 1-3 cm. Fertilizer (DAP, 18-46-0) was used at planting at a rate of 2g per pot. Seeds planted in plastic pots containing non-infested soil served as control. There were five replicates per treatment arranged in a randomised complete block design. The pots were kept in the greenhouse and watered regularly. After 14, 36 and 72 days (Table 2)

plants were harvested and the mean shoot height (cm), shoot and root dry weights (g), mean length of discoloured root (mm) (MLDRT), mean root rot index (MRRI, 1-9), number of pods per plant and dry weight of 100 seeds were determined. An assessment was also made of the loss in grain and pod yields caused by *F solani* f. sp. *phaseoli* and *F .oxysporum* f. sp. *phaseoli* using the equation shown in section 3.3.6.

3.3.2. *In-vitro* tests: Evaluation of fungicides against the root-rot fungal pathogens, *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.*

Six *in-vitro* tests were conducted between January and February, 1997, at Kenyatta University, Nairobi to determine efficacy of five different fungicides (pyrazophos, thiram, triforine, captan and metalaxyl + mancozeb) and two fungicide combinations (triforine + captan and thiram + captan) against *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli.* Effects of these fungicides on mycelial growth, spore germination and sporulation were assessed, with a view of obtaining data that would assist farmers on the most appropriate fungicides and / or fungicide combinations that can be used effectively for the management of *F. solani* f.sp. *phaseoli and F. oxysporum* f. sp.*phaseoli* the major causal agents for root-rot. The fungicides tested are shown in Tables 4 and 5.

Table 4. List of fungicides tested.

Common name	Trade name	Formulation	Field rates/ Kg of seeds.
• Triforine (Tri)	Saprol	20% EC	10.0ml
• Thiram (Th)	Murtano	WP	0.7g
• Captan (Cap)	Captan 83	83%WP	5.0g
• Pyrazophos	Afugan	30%EC	10.0ml
• Metalaxyl + Mancozeb	Ridomil Mz 63 .5	63.5% WP	8.0g

Table 5. **Fungicides and fungicide combinations tested.**

Fungicide name	Common name	Trade name	Formulation	Field rates/Kg of seeds.
• $Tri^{25}+Cap^{75}$	_	_	5%+ 62.1% granule	
• $Tri^{50} + Cap^{50}$	_	_	10%+41.5% granule	
• $Th^{25} + Cap^{75}$	_	_	-+62.1%WP	
• $Th^{50} + Cap^{50}$	_	_	-+41.5%WP	
• Triforine	Triforine	Saprol	20% EC	10ml
• Captan	Captan	Captan 83	83%WP	5g
• Thiram	Thiram	Murtano	63%	0.7g

$Tri^{25}+Cap^{75}$ refers to where 25 and 50 % of actual field recommended dosage of fungicide has been used, respectively.

$Tri^{50} + Cap^{50}$ refers to where 50 % of actual field recommended dosage the fungicides have been used.

$Th^{25} + Cap^{75}$ refers to where 25 and 50 % of actual field recommended dosage of fungicide has been used, respectively.

$Th^{50} + Cap^{50}$ refers to where 50 % of actual field recommended dosage the fungicides have been used.

3.3.3 *In -vitro* test 1: Effect of fungicides on mycelial growth.

This test was conducted using PDA treated separately with either triforine, thiram, pyrazophos, captan or metalaxyl + mancozeb (Table 4). Fungi were cultured for 10 days as described in section 3.2.0. One mycelial plug from the periphery of a 10 day old fungal culture was transferred aseptically to the centre of each Petri-dish (one plug per plate) containing pyrazophos, triforine, thiram, captan or metalaxyl + mancozeb at dilutions of $0.02cm^3$, $0.02cm^3$, 0.0285g, 0.1g or 0.16g / $20cm^3$. Fungicides were incorporated in PDA by preparing appropriate fungicide dilutions and dispensing in appropriate volume of PDA contained in a conical flask at 45°C and later dispensing approximately 20ml into a petridish as described by Owino, *et al.* (1993). Petri dishes without fungicides acted as control. Each treatment was replicated five times in a randomised complete block design, (CRD).

Diameters of fungal colonies were measured 3, 4, 5, 6 and 7 days after inoculation to the nearest millimetre (mm). The inhibitory activity of fungicides was determined by calculating the inhibition percentage (I%), (Nishijima and Smalley, 1979; Javed, 1980; Fitzell, 1981).

$$I\ \% = \frac{C - T \times 100}{C - M}$$

Where,

C= Diameter (mm) of fungal mycelium on control plates,

T = Diameter (mm) of fungal mycelium on fungicide treated plates

M = Initial diameter (mm) of the mycelial plug.

3.3.4 *In -vitro* test 2: Effect of fungicides on spore germination.

In-vitro test was conducted to evaluate the effect of fungicides on spore germination. Spore suspension was prepared by flooding 10-day-old cultures of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* in Petri dishes with 20 ml of sterile distilled water and agitating the mixture on a shaker at a speed of 200 rpm for 30 minutes. The suspension was filtered through cheese cloth to remove fungal mycelium as described in 3.2.2 (Nigh *et al.*, 1980).

About 1.0 ml of the spore suspension carrying approximately 1.0×10^6 spore/ml was spread evenly on PDA treated with different fungicides at dilutions $0.02cm^3$, $0.02cm^3$, 0.0285g, 0.1g or 0.16g /20ml of PDA. Petri-dishes containing PDA without the fungicides served as control. There were five replicates per treatment arranged in a completely randomised design. After 24 or 48 hours, 5 ml of distilled water was added in each of the petri-dishes to suspend the spores. The spore suspension was transferred into a haemocytometer and germinating spores (visible germ tubes) were counted under a light microscope (Fitzell, 1981). The germinated spores were expressed as a percentage of the total number of spores counted per 1ml of spore suspension as shown in the equation below:-

% Spore germination = $\frac{\text{Total number of germinating spores in 1ml}}{\text{Total number of spores counted in 1ml of spore suspension}} \times 100$

3.3.5. *In -vitro* test 3: Effect of fungicides on sporulation.

In-vitro test was conducted using PDA treated with the fungicides triforine, thiram, pyrazophos, captan or metalaxyl + mancozeb. Fungi were cultured for 10 days as described in section 3.2.0., and one mycelial plug from the periphery of a 10-day-old fungal culture was transferred aseptically to a petri-dish, one plug per plate, containing pyrazophos, triforine, thiram, captan or metalaxyl + mancozeb at dilutions of $0.02cm^3$, $0.02cm^3$, 0.0285g, 0.1g and 0.16g / $20cm^3$ of PDA. Fungicides were incorporated in PDA by preparing appropriate fungicide dilutions and dispensing in appropriate volume of PDA contained in a conical flask at 45°C and later dispensing approximately 20ml into a petridish as described by Owino, *et al.* (1993). Inoculated PDA plates were incubated for 10 days in total darkness inside a wooden hood which was insulated with an opaque material (aluminium foil). They were then exposed to alternate room light for 10 hours per day for four days at room temperature. Petri dishes without fungicides acted as control. Each treatment was replicated five times in a randomised complete block design, (CRD).

After the incubation period each plate was flooded (washed) with 20 ml of sterile distilled water and the number of suspend spores/ml was counted using a haemocytometer (Biratu *et al.*, 1990). Sporulation was determined by comparing the number of spores produced on fungicide treated PDA to the untreated control.

3.3.6. *In -vitro* test 4: Evaluation of fungicide combinations against root-rot fungal pathogens of french beans.

In-vitro test 4 was carried out to assess effects of two fungicides combinations, triforine + captan and thiram + captan on root-rot fungal pathogens. For each combination, fungicides in proportions 0. 25 + 0.75 and 0.50 + 0.50 of the actual recommended dosage were prepared and their effect on fungal mycelial growth, spore germination and sporulation assessed as described in *in-vitro* tests 1, 2 and 3, respectively (3.3.2).

3.3.7. Greenhouse tests 2 and 3.

Greenhouse tests 2 and 3 were conducted simultaneously at Kenyatta University, Botany Department between March and July, 1997 to:

(i) Investigate effects of seed dressing with triforine, pyrazophos, captan, thiram and metalaxyl + mancozeb on the development of *Fusarium* 'yellows' and *Fusarium* dry root rot of french beans (greenhouse test 2), and

(ii) Investigate the comparative effects of seed dressing with two fungicide combinations, triforine + captan and thiram + captan, and that of single fungicides on the development of *Fusarium* 'yellows' and *Fusarium* dry root-rot of french beans (greenhouse test 3).

3.3.8. Greenhouse test 2: Evaluation of efficacy of five fungicides against fungal isolates in sterile soil.

Soil used in this test was collected from the field that had been left fallow for three years and was sterilised as described in section 3.2.8. Sterilised soil was transferred into 16.5-cm-diameter plastic

pots at a rate of 1800 g per pot. It was then artificially infested with *F. solani* f.sp. *phaseoli* isolate or *F. oxysporum* f. sp. *phaseoli* at a rate of 8 macerated PDA plugs (7-mm-diameter) per pot as described in section 3.2.2 and left for 10 days before planting to allow inoculum build up. Each pot was then planted with four-fungicide treated seeds. Fertilizer (DAP, 18-46-0) was used at planting at a rate of 2 g per pot. Control pots were planted with untreated seeds. Each treatment was replicated five times and arranged in a randomized block design. Plants were harvested at stages V1, R6 and R8 (Table 2) which in terms of days corresponded to 14, 36 and 72 days after planting, respectively, as described in section 3.2.5. Plant performance was evaluated by measuring shoot height (cm), shoot and root dry weights (g), length of discoloured root tissue, root-rot index (1-9), number of pods per plant and dry weight of 100 randomly selected seeds per treatment.

3.3.9 Greenhouse test 3: Evaluation of fungicides when used in combinations.

Effects of different fungicide combinations on the development of root-rot of french bean were investigated in greenhouse test 3. Steam sterilised field soil contained in 16.5-cm-diameter pot was artificially inoculated with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f. sp. *phaseoli* as described in section 3.2.3. Two combinations, triforine + captan and thiram + captan were used to dress seeds before planting, one combination per seed-treatment. Treated seeds were planted at a depth of 1-3 cm at a rate of four seeds per pot. Fertilizer (DAP, 18-46-0) was used at planting at a rate of 2 g per pot. Other treatments included seeds dressed with triforine, captan or thiram. Untreated seeds served as control. Each treatment was replicated five times in a randomised complete block design, (CRD).

Plants were harvested and plant growth parameter mentioned in section 3.6.6 were assessed 14, 36 and 72 days after planting.

3.4.0 Greenhouse test 4: Comparative efficacy of seed dressing and organic matter application against root rot fungal isolates.

Comparative efficacy of fungicide seed-dressing and organic matter on *Fusarium* root-rotting fungi was investigated in this test. *T. diversifolia* and cowdung were used as organic residues. *Tithonia* leaves were sun dried for 48 h and oven dried at 80°C for 72 h. Dried materials were ground into 20µm particles using Wiley's grinding mill (Waceke and Waudo, 1993). Sun dried cowdung was sieved through a 2000µm pore sieve to remove large lumps. A 200 g subsample of each organic material was then analysed for mineral element content (Day, 1965) shown in Table 3. About 200g of organic material was incorporated in steam sterilised field soil in 16.5-cm-diameter plastic pots at a ratio of 1:5 (w/w). Pots without organic material served as controls. Two *Fusarium* spp. isolates cultured on PDA were then added separately to each pot at a rate of eight macerated PDA plugs (7-mm-diameter) per pot and mixed (Owino *et al.*, 1993). Four treated seeds were planted in each pot 10 days after inoculation. Fertilizer (DAP, 18-46-0) was used at planting at a rate of 2 g per pot. Non-treated seeds were also planted to serve as controls. All treatments were replicated five times in a randomised block design. Root rot evaluation was done at stages V1, R6 and R8 which occurred 7, 36 and 72 days after planting and plant performance parameters were assessed as described in greenhouse test 2 (section 3.3.0).

3.4.1 Greenhouse test 5: Effect of decomposition period on efficacy of organic matter on *Fusarium* root rot of french beans.

The fungi used in this test were isolated from infected french bean roots and cultured on PDA in 9-

cm-diameter Petri dishes for 10 days at 25° C. Pure cultures were used in the test. Cowdung or *T. diversifolia* organic matter was incorporated into steam sterilised field soil contained in 16.5-cm-diameter plastic pots at the ratio of 1:8 (w/w) (organic amendment: soil). Soil without organic matter served as control. *F .solani* f.sp. *phaseoli* or *F. oxysporum* f. sp. *phaseoli* was immediately added into each pot at a rate of eight macerated PDA plugs (7-mm-diameter) per pot and thoroughly incorporated into the soil. Soil without fungus was also included as a treatment. Cowdung and *T. diversifolia* were left to decompose for 0, 2, 3 and 4 weeks before planting with disease-free seeds. Seeds were planted at a rate of three seeds per pot. Fertilizer DAP (18-46-0) was used at planting at a rate of 2 g per pot. Each treatment was replicated five times in a randomised complete block design, (CRD).

The test was terminated 40 days after the planting date and shoot height (cm), shoot and root dry weights (g), length of discoloured root tissue, root rot index (1-9, where 1 = no symptoms on the root and 9 = 75 - 100% of the root system is infected) and number of pods per plant assessed.

3. 4. 2 Field tests 1 and 2.

Field tests 1 and 2 were conducted between May and July, 1997, to investigate effects of seed dressing with five fungicides (triforine, pyrazophos, metalaxyl + mancozeb, captan and thiram) and two fungicide combination (triforine + captan and thiram + captan) on the development of root rot diseases of french beans caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli*. The experiments were conducted in the field at the Botany Research farm, Kenyatta University.

3.4.3 Field test 1: Evaluation of relative efficacy of fungicides when used singly.

The relative efficacy of triforine, pyrazophos, metalaxyl + mancozeb, captan and thiram against *F. solani* f.sp. *phaseoli* or *F .oxysporum* f. sp. *phaseoli* was determined in field test 1. The inoculum potential of the field soil was boosted by artificially infesting the soil with the fungus by pipetting 5 mls of spore suspension containing 1.0 X 10^6 spores per ml of distilled water into a 1-mm-deep planting hole just before planting. French bean seeds were treated with the fungicides triforine, pyrazophos, metalaxyl + mancozeb, captan and thiram at concentrations 0.02 cm^3, 0,02 cm^3. 0.16g, 0.1g or 0.0285g. Treated seeds were then planted at a depth of 1-3cm at the rate of 4 seeds per hole. Intra-and-inter-row spacing was 10cm and 50cm, respectively. A single three meter long row constituted an experimental unit. D.A.P fertilizer (18-46-0), was used at planting at a rate of 1g per hole. Plants were watered regularly using a portable watering can during dry weather. Untreated seeds grown in field soil served as control for the experiment. A randomised block design with five replicates was used. Thinning was done 14 days after seeding to leave one plant per hole. Root rot evaluation was done at stages V1, R6, and R8 (Table 2) using a 1-9 rating scale, (Fernandez *et al.*, 1986 as described in section 3.2.4. Shoot height (cm), number of pods/plants, length of discoloured vascular system (mm) of the root system, root and shoot dry weights (g) were assessed as described by Waudo *et al.* (1995).

3.4.4 . Field test 2: Evaluating effects of fungicides when used in combinations.

Field test 2 was conducted by treating 100 french bean seeds with triforine + captan or thiram + captan fungicide combination. These combinations were chosen because they were the most

effective against the root rot fungal pathogens as revealed by results of the *in-vitro* test 4 (section 3.3.6). Field soil was artificially infested separately with each of the two *Fusarium* spp. isolate before planting seeds. Treated seeds were planted at a rate of 4 seeds per hole at a depth of 1-3 cm. Intra-and-inter-row spacing was 10 cm and 50 cm, respectively. Seeds treated with triforine, captan or thiram alone were also included as treatment for comparison purposes. A single three meter row constituted an experimental unit. Plants grown from untreated seeds served as controls. All treatments were replicated five times in a completely randomised block design. Antifungal activity of the different fungicides combinations was also evaluated at stages V1, R6 and R8 using a scale of 1-9 (Fernandez, *et al.*, 1986). Plant growth parameters described in field test 1 (section 3.4.3) were also measured. Bean yield was assessed by measuring and comparing seed dry weight and number of pods /plot size.

3.4.5 Data analysis.

Data obtained from the 10 tests were subjected to a two-way analysis of variance (ANOVA). Treatment means were compared using Duncan's Multiple Range Test (Duncan, 1955) at 5% (P = 0.05) probability level. A split-split plot analysis of variance was used to determine the effect of time (days), fungicides, isolates and the interaction of days and fungicides (days x fungicides), isolate and fungicides (isolate x fungicides) and days, isolate and fungicides (days x isolate x fungicides) on *in-vitro* growth of fungal mycelium. Linear tests were also used to determine relationships between different variables.

CHAPTER 4.

4.0 RESULTS.

4.1. Occurrence of fungal species detected from root and soil samples collected from Meru Central and Thika Districts in Kenya.

A total of seven genera were recorded from the root and soil sample (Table 6) that were collected between the months of October and December, 1997, in Meru (Nkubu, Katheri) and Thika (Mangu, NHRC, National Horticulture Research Centre) Districts of Kenya. *Fusarium* spp. were the most dominant in the samples. Out of 12 samples. *F. oxysporum* was recorded from 6 samples, *F. solani* from 4, *F. equisite* from 7, *Rhizoctonia* from 1, *Pythium* spp..from 4, *Rhizopus* spp. from 2, *Phoma* spp. from 2, *Aspergillus* spp. from 5, *Penicillium* from 2. Among the fungi only *F. oxysporum*, *F solani* and *R. solani* are known to cause root rot disease of french beans. *F. equisite* is sometimes associated with the legume crops (Norse, 1974, Kannaiyan and Nene, 1979b). Only *F. solani* and *F. oxysporum* were utilised in various experiments in this study.

Table 6. Number of fungi and percentage occurrence (in parentheses) in french bean root and soil samples collected from french bean fields in Thika and Meru Districts of Kenya.

Fungal species detected	Thika.	Meru.
1. *F. solani*	2 (20.89)	2 (13.54)
2. *F. oxysporum*	4 (25.37)	2 (15.79)
3. *F. equisite*	2 (16.47)	5 (32.30)
4. *Phoma* spp.	0 (0.00)	2 (7.12)
5. *Aspergillus* spp.	2 (16.00)	3 (22.90)
6. *Rhizopus* spp.	1 (7.30)	1 (6.20)
7. *R. solani*	0 (0.00)	2 (21.41)
8. *Penicillium* spp.	2 (19.51)	2 (17.42)
9. *Pythium* spp.	2 (14.22)	2 (15.17)
Total occurrence	**15**	**21**

4.1.1 Greenhouse test 1.

4.1.2. Pathogenicity test.

Pathogenicity test was carried out between November and December, 1997, at Kenyatta University, Nairobi, Kenya to compare the relative pathogenicity of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on french beans c.v. Monel, a good host of the *Fusarium* root-rot fungal pathogens (HCDA, 1995).

Mean shoot heights and shoot, root and seed dry weights, and mean number of pods produced per plant were significantly (P=0.05) lower in plants inoculated with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* than those of control plant at all the stages of french beans development

(Table 7-9). The two fungal isolates had similar effects on shoot height and dry root weight (Tables 7-8). Plants inoculated with *F. solani* f.sp. *phaseoli* had significantly (P=0.05) longer length of discoloured root tissue than that of plants treated with *F. oxysporum* f.sp. *phaseoli* 14 days after inoculation (Table 7). Effect of these two fungi on length of discoloured root tissue was similar 36 and 72 days after inoculation (Tables 8 and 9). Significantly shorter length of discoloured root tissue was recorded in control plants. The most prominent symptoms produced by these two fungal isolate was root rot of both young and mature plants. Sometimes, a few seedlings in the control exhibited root browning symptoms.

Root-rot index was significantly (P=0.05) higher in inoculated plants than control. No significant difference in root rot index was noted among plant treated with the two fungal isolates at all stages of bean development (Tables 7-9).

Table 7. Effect of *F solani f.sp. phaseoli* and *F oxysporum f.sp. phaseoli* on growth of french beans, LDRT[1] and Mean root rot index[2] at development stage V1[3] 14 days after inoculation and planting. Greenhouse test 1.

Fungal species	Mean* shoot height (cm)	Mean* shoot dry weight (g)	Mean* root dry weight (g)	Mean* L.D.R.T[1] (mm)	Mean* root rot index[2] (1-9)
F.s.f.sp phaseoli	13.60b**	0.284b	0.021b	16.60a	2.60a
F.o.f.sp phaseoli	15.20b	0.275b	0.027b	8.20b	2.00a
Control	24.66a	0.658a	0.035a	0.40c	1.00b

*Mean of five replicates.

**Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

[1] L.D.R.T, Length of discoloured root tissue.

[2] Based on percentage of root infection where, 1 = no symptoms, 3 =10, 5 = 25, 7 = 50 and 9 = 75% of the root was infected.

[3] Development stage V1 refers to appearance of cotyledons on the soil surface to the unfolding of primary leaves as described by Fernandez *et al* ., 1986.

Table 8. Effect of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on growth of french (snap) beans, L.D.R.T[1] and mean root rot index[2] at development stage R6[3] 36 days after inoculation and planting. Greenhouse test 1.

Fungal species	Mean* shoot height (cm)	Mean* shoot dry weight (g)	Mean* root dry weight (g)	Mean* L.D.R.T[1] (mm)	Mean* root rot index [2] (1-9)
F.s.f.sp. phaseoli	40.20b**	1.430b	0.130b	99.70a	6.25a
F.o.f.sp. phaseoli	47.80b	1.630b	0.140b	133.60a	7.00a
Control	65.50a	2.940a	0.210a	1.72b	1.00b

*Mean of five replicates.

**Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

[1] L.D.R.T, Length of discoloured root tissue.

[2] Based on percentage root infected where, 1 = no symptoms,

3 = 10, 5 = 25, 7 = 50 and 9 = 75% of the root infected.

[3] Development stage R6 refers to the opening of the first flower to the expansion of the ovary after fertilisation as described by Fernandez *et al.*, 1986.

Table 9. Effect of *F. solani* f sp. *phaseoli* and *F. oxysporum.* f sp. *phaseoli* on growth of french beans, L.D.R.T[1] and mean root rot index[2] at development stage R8[3] 72 days after inoculation and planting. Greenhouse test 1.

Treatment	Mean* shoot height (cm)	Mean* Shoot dry weight (g)	Mean* root dry weight (g)	Mean* L.D.R.T[1] (mm)	Mean* root rot index[2]	Number of pods /plant	Mean* dry weight of 100 seeds
F s f.sp phaseoli	44.75c*	3.25b	0.167b	97.5a**	7.00a	6.0b	25.11b
F .o f.sp phaseoli	64.50b	3.87b	0.225b	143.2a	7.25a	4.0b	23.38b
control	78.60a	6.52a	0.402a	10.5b	1.40b	24.0a	33.49a

*Mean of five replicates.

**Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

[1] L.D.R.T, Length of discoloured root tissue.

[2] Based on percentage root infected where, 1 = no symptoms,

3 = 10, 5 = 25, 7 = 50 and 9 = 75% of the root infected.

[3] Development stage R8 refers to the beginning of pods to fill with seeds and size increase to the development of pigmentation of seeds as described by Fernandez *et al.*, 1986.

Overall relationships.

There were great variations among plant responses to *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* infection. Regression coefficients, regression equations and correlation co-efficients showing the relationship between the level of root rot and plant performance are depicted in (Table 10; Fig 2and 3). Significant regression co-efficient of -1.218 and -1.52 were obtained from the regression of the number of pods per plant and dry weight of seeds on the level of root rot caused by *F. oxysporum* f.sp. *phaseoli,* respectively (Table 10; Fig 2 and 3). The negative slopes revealed an inverse relationship between the number of pods produced per plant, seed dry weight and the level of root rot (Table 10). The slope of -1.218 was highly significant (P=0.01) indicating that increase in *F. oxysporum* f.sp. *phaseoli* root rot index suppressed pod set and seed dry weight. Up to 83.40% and 30.2% loss in pods and seed dry weight, respectively, were associated with *F. oxysporum* f.sp. *phaseoli* infection (Figs 4 and 5).

Table 10. Relationship between the level of root rot and the performance of french bean inoculated with *F. solani* f.sp. *phaseoli* and *F. oxysporum* f sp. *phaseoli*.

Treatment	Dependent variable	Independent variable	Regression equation	Regression co-efficient (b)	Days after inoculation	Correlation Coefficient R
F.s f sp phaseoli	Number of pods	level of root rot	Y=15.26-1.218X	-1.218*	72	-0.992*
	Dry seed weight	level of root rot	Y=34.75-1.52X	-1.520*	72	-0.982*
F o.f sp.phaseoli	Number of pods	level of root rot	Y=14.61-1.266X	-1.266*	72	-0.957*
	Dry seed weight	level of root rot	Y=33.70-1.244X	-1.244*	72	-0.965*

*Regression and correlation co-efficients significant at P = 0.05.

Mean number of pods produced per plant and mean dry weight of seeds had an inverse relationship with the level of root rot obtained from plants infected with *F. solani* f.sp. *phaseoli* as revealed by the significant (P=0.05) negative slopes of -1.266, (r= 0.957) and -1.224, (r=0.956), respectively (Table 10). The figures indicate that increase in *F. solani* f.sp. *phaseoli* root-rot severity was accompanied by reduction in both the mean number of pods produced per plant and mean dry weight of seeds, respectively. This is further confirmed by a root-rot index of 7.8 where pod set and seed dry weight was suppressed by 75.00% and 22.23%, respectively (Figs 4 and 5).

Figure 2. Regression of mean number of pods produced per plant on mean root rot index of french bean plants after inoculation with *F. oxysporum f.sp. phaseoli* and *F. solani f.sp phaseoli.*

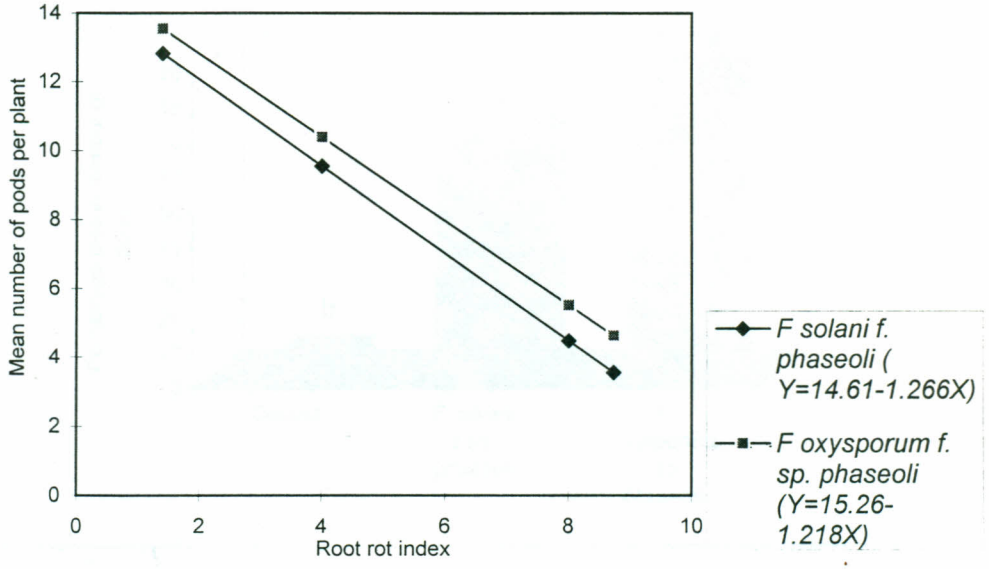


Figure 3. Regression of mean dry weight of 100 seeds on mean root rot index of french bean plants after inoculation with *F. solani* f. sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.*

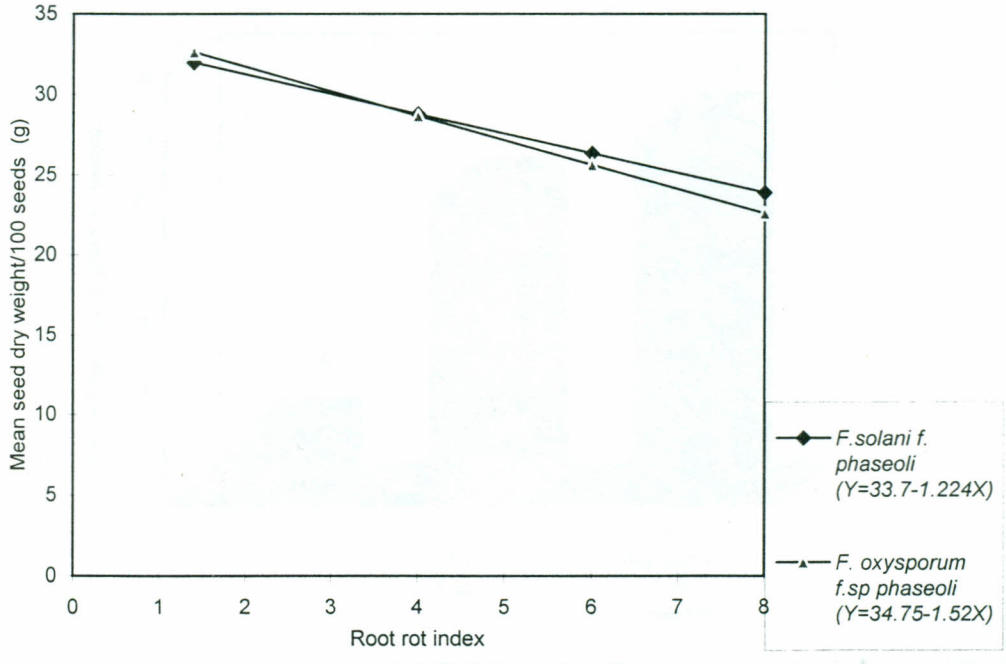
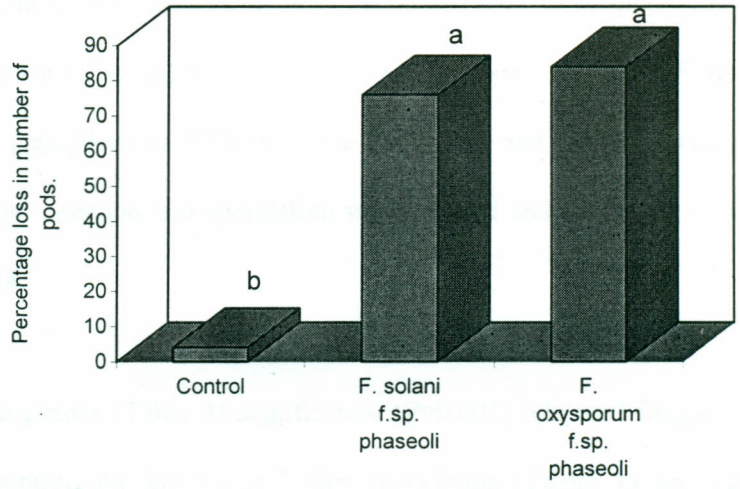
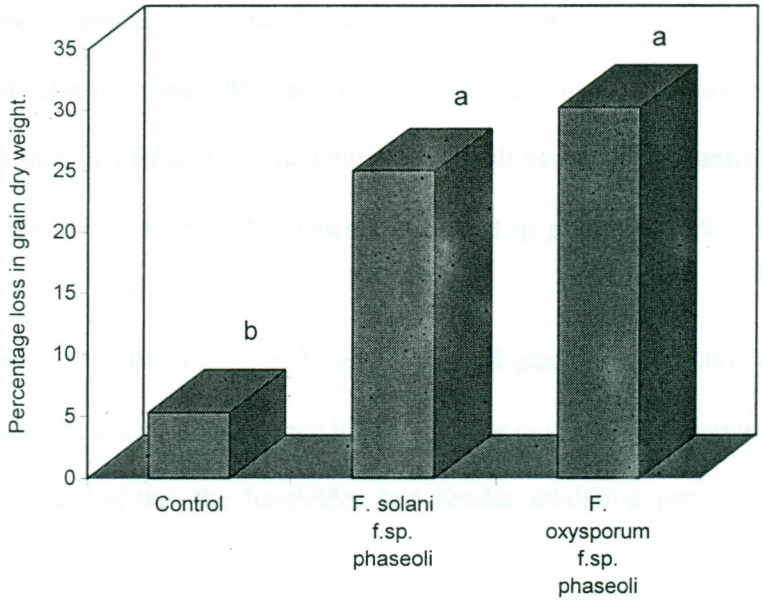


Figure 4. Effect of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on % yield loss in number of pods produced per plant. Greenhouse test 1.



Bars with different letters are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

Figure 5. Effect of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on % yield loss in grain dry weight.



Bars with different letters are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

4.2.1. *In-vitro* test 1. Effect of fungicides on mycelial growth.

Six *in-vitro* tests were conducted to determine efficacy of five different fungicides (Pyrazophos, Thiram, Triforine, Captan and Metalaxyl + Mancozeb) and two fungicide combinations (Triforine + Captan and Thiram + Captan) against root rot of french beans caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.* Effects of the fungicides and fungicide combinations on mycelial growth, spore germination and sporulation was assessed and results are depicted in Tables 11-15 and figures 6-18.

All the five fungicides (Table 4) significantly (P=0.001) inhibited fungal mycelial growth when compared to controls on day 3 and 7 after inoculation (Tables 11 and 12),. The overall mean inhibition % of mycelial growth by triforine, metalaxyl, thiram, captan and pyrazophos were 95.01, 86.74, 93.53, 62.18 and. 45.84% and 96.31, 99.29, 90.91, 88.99 and 51.78% for *F. oxysporum* f.sp. *phaseoli* and *F. solani* f.sp. *phaseoli*, respectively. Thiram and pyrazophos caused the highest and lowest *F. solani* f.sp. *phaseoli* mycelial inhibition by the day 3 after inoculation. Pyrazophos also caused the lowest inhibition in the *F. oxysporum* f.sp. *phaseoli* mycelial growth 3 days after inoculation. Metalaxyl caused 100% inhibition in mycelial growth of *F. oxysporum* f.sp. *phaseoli* 3 days after inoculation. (Table 11). The inhibition overall % was significantly (P=0.001) higher on *F. oxysporum* f.sp. *phaseoli* (85.41%) than on *F. solani* f.sp. *phaseoli* (76.98%).

Mycelial growth of the two fungal pathogens increased gradually from day 3 to day 7 of the test (Fig 6 and 7), but the growth in each individual fungicide in some cases were not statistically different, an indication that the fungicides had similar inhibition potential throughout the test period.

In general, triforine was the most effective fungicide, it inhibited up to 95.66% (Fig 8) on fungal growth of the two isolates though, its effect was not significantly different (P=0.001) from those obtained with metalaxyl, captan or thiram (Fig 8). These fungicides were second to triforine in inhibition potential (Fig 8). Out of all the fungicide treated plates, petridishes treated with pyrazophos had the lowest (P=0.05) mean overall inhibition % of 49.61 (Fig 8).

Out of the five screened fungicides, triforine, metalaxyl and thiram had among the highest inhibitory % effect on *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli*, (Fig 9). Throughout the 5 days of the test, triforine and metalaxyl treated plates maintained the highest mean inhibitory % on *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli,* respectively (Fig 9). Effect of time on the inhibitory effects of the two fungicides on *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* are depicted in Figs 10 and 11, respectively.

The inhibitory potential of triforine on *F. solani* f.sp. *phaseoli* increased with time and was highest 7 days after inoculation (Fig 10). The opposite was true for *F. oxysporum* f.sp. *phaseoli*. No significant variation on inhibition % with time were noted where metalaxyl was used (Fig 10). The latter fungicide inhibited growth of *F. oxysporum* f.sp. *phaseoli* significantly (P=0.05) by up to 100% (Fig 10).

In general, fungal growth was significantly (P=0.05) affected by the fungicides and time taken after inoculation.

Table 11.

Effect of fungicides on mean mycelial inhibition % of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on day 3 after inoculation. *In-vitro* test 1.

Treatments	Inhibition %	
	F. solani f.sp. *phaseoli*	*F. oxysporum* f.sp. *phaseoli*
Triforine	92.72d*	98.40c
Metalaxyl	86.70d	100.00c
Thiram	96.20d	92.00c
Pyrazophos	47.78b	52.90b
Captan	71.84c	85.88c
Control	0.00a	0.00a

*Means followed by the same letter within each column are not significantly different at P = 0.05 level by Duncan's Multiple Range Test.

Table 12. Effect of fungicides on mean mycelial inhibition % of *F . solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on day 7 after inoculation. *In-vitro* test 1.

Treatment	Inhibition %	
	F. solani f.sp. *phaseoli*	*F. oxysporum* f.sp. *phaseoli*
Triforine	95.84e*	93.98cd
Metalaxyl	85.71d	98.68d
Thiram	89.09de	89.74c
Pyrazophos	37.92b	48.56b
Captan	48.05c	89.40c
Control	0.00a	0.00a

*Means followed by the same letter within each column are not significantly different at P = 0.05 level by Duncan's Multiple Range Test.

Figure 6. Effect of fungicides on mycelial growth of *F. solani* f.sp. *phaseoli* from day 3 to day 7 after inoculation.*In-vitro* test 1.

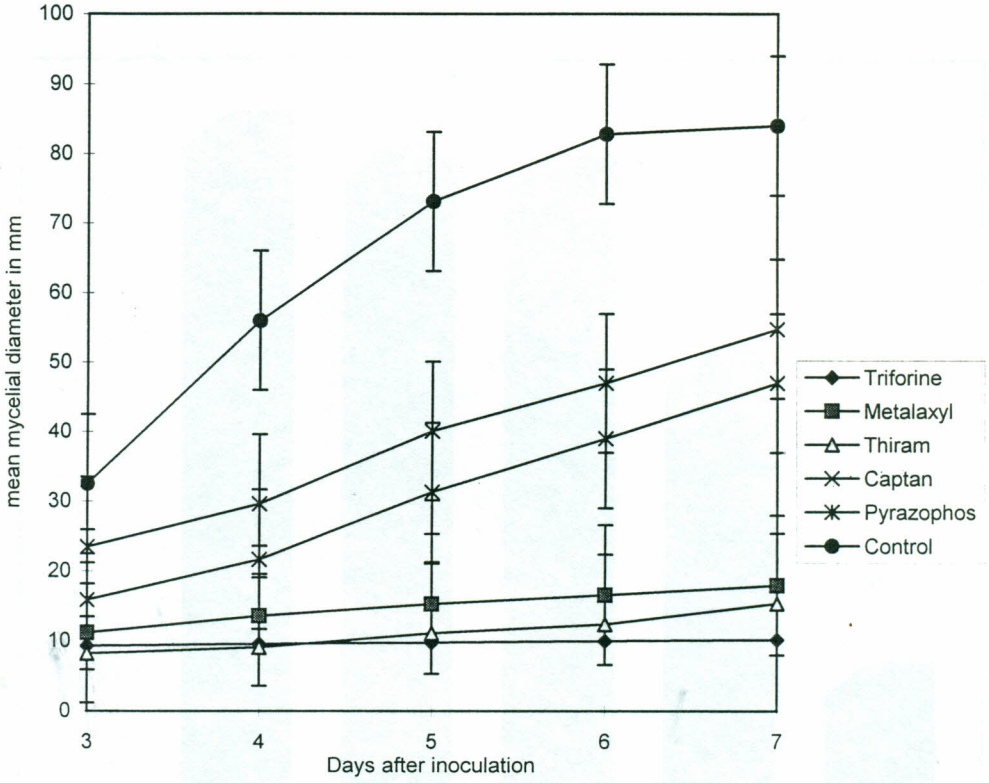


Figure 7. Effect of fungicides on mycelial growth of *F. oxysporum* f.sp. *phaseoli* from day 3 today 7 after inoculation.*In-vitro* test 1

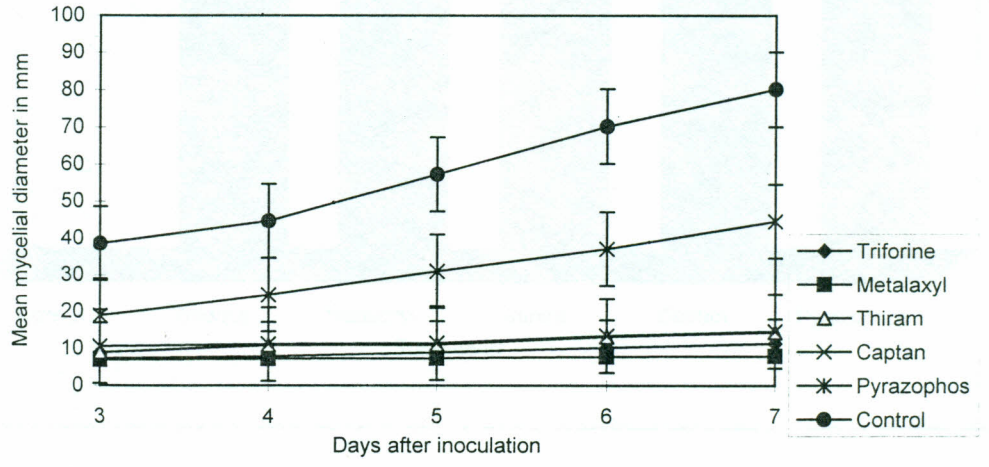
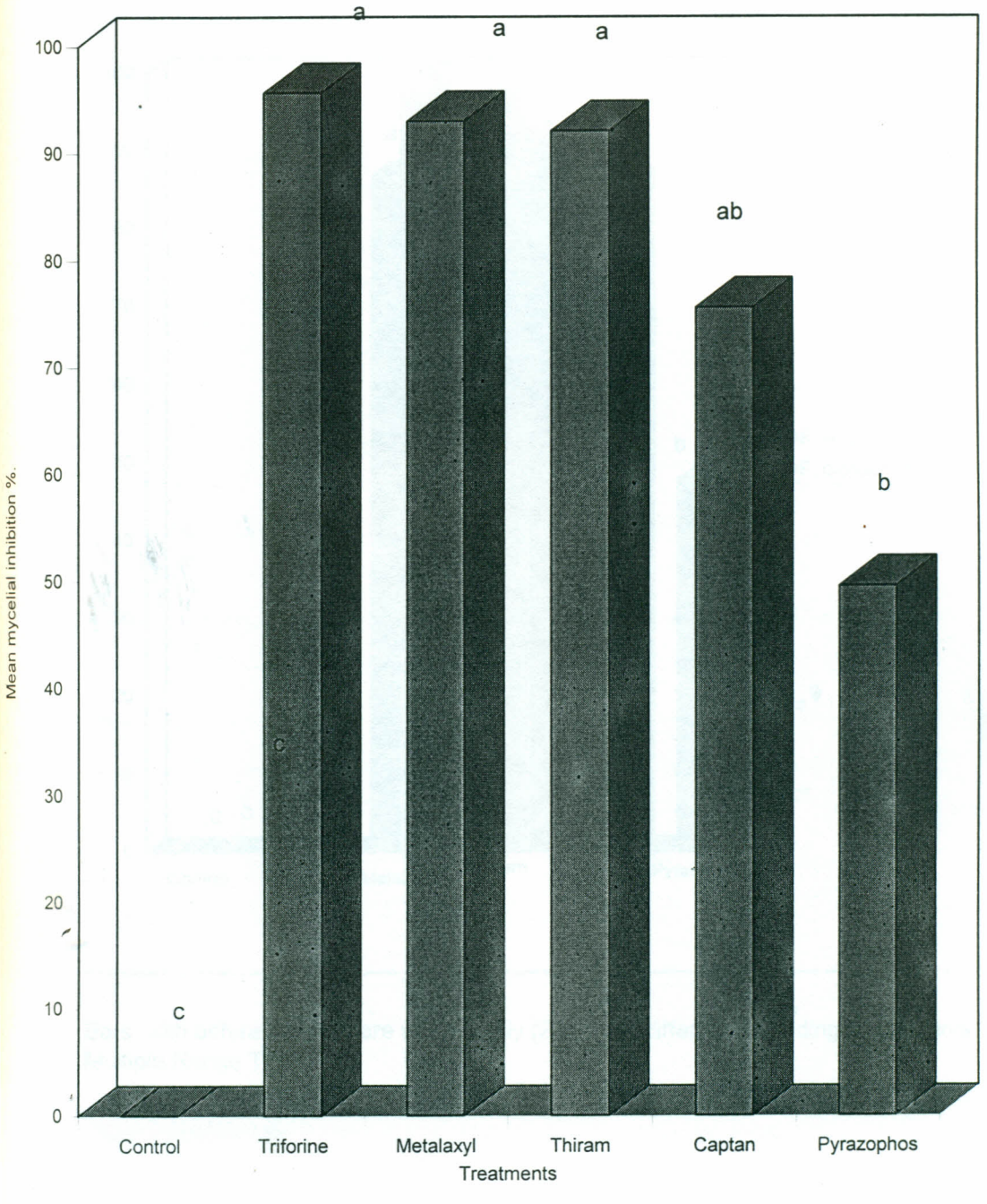
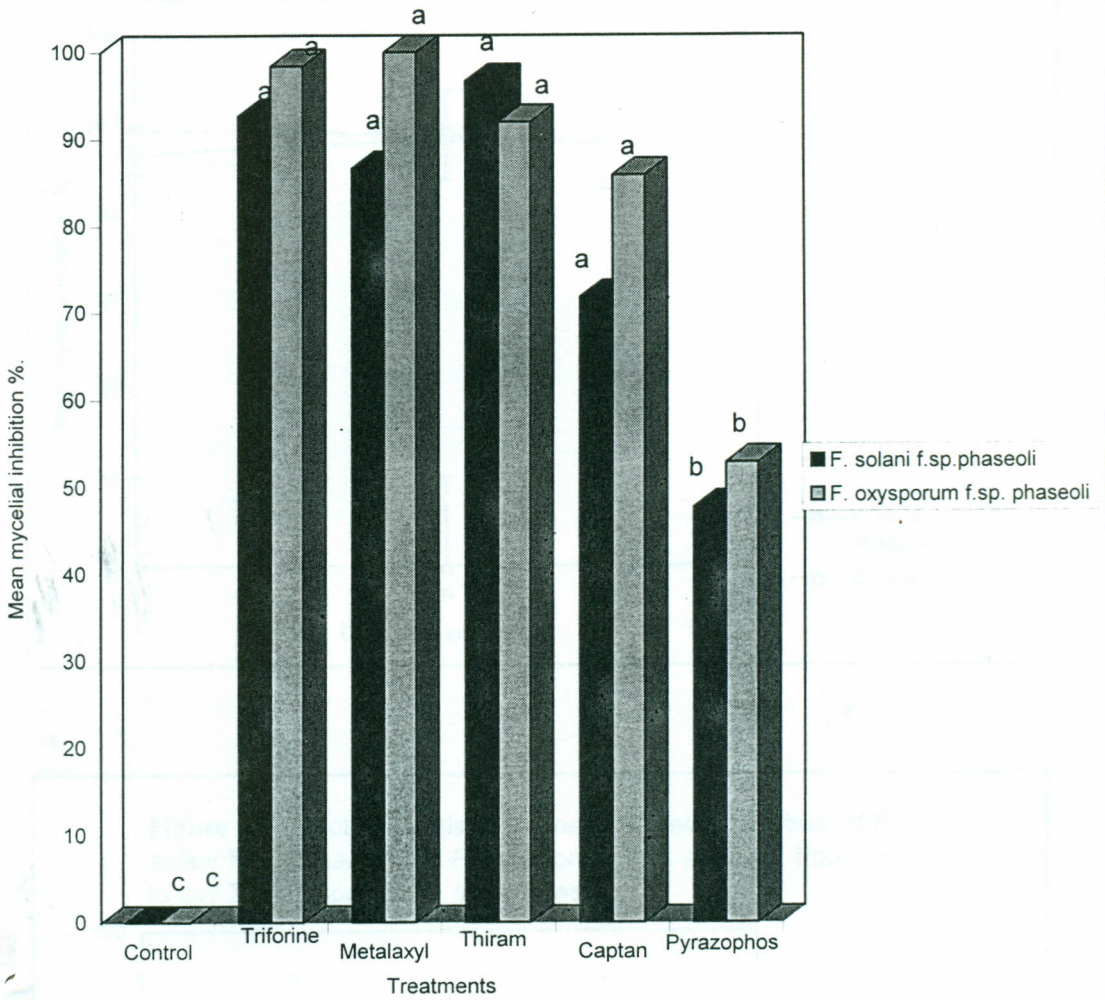


Figure 8. Overall effects of five fungicides on mean mycelial inhibiton % of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli*. *In-vitro* test 1.



rs with different letters are significantly (P = 0.05) different according to Duncan's ltiple Range Test.

Figure 9. Mean inhibitory % effect of five fungicides on mycelial growth of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* over a 5 days period. *In-vitro* test 1.



Bars with different letters are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

Figure 10. Effect of triforine on mean mycelial inhibition % of *F. solani* f.sp. *phaseoli* and *F. solani* f.sp. *phaseoli* from day 3 to day 7 after inoculation. *In-vitro* test 1.

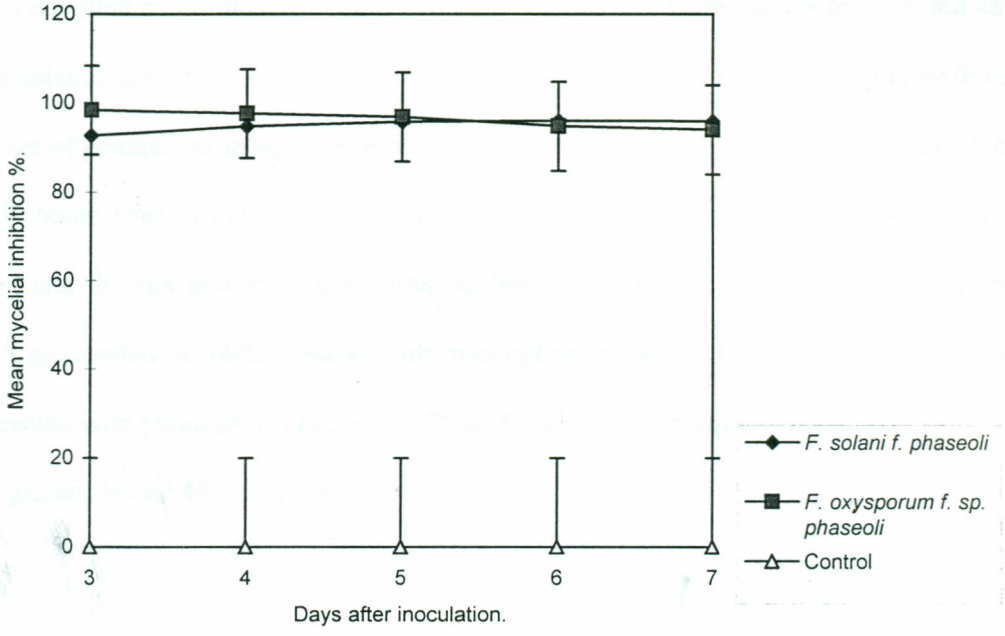
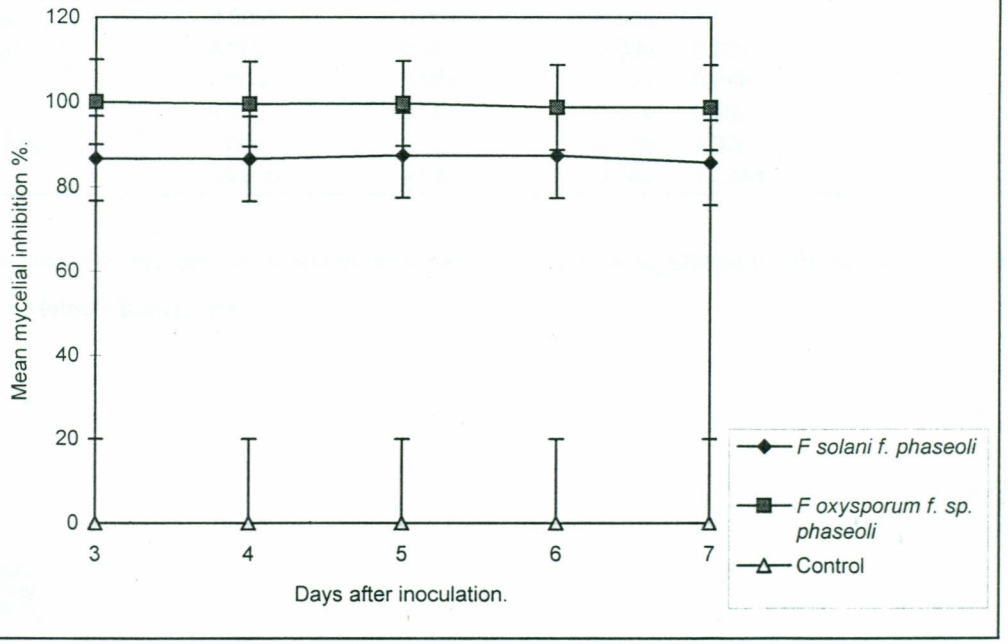


Figure 11. Effect of metalaxyl on mean mycelial inhibition of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* from day 3 to day 7 after inoculation. *In-vitro* test 1.



4.2.2. *In-vitro* test 2. Effect of fungicides on spore germination.

The germination of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* spores was significantly (P=0.05) inhibited by all the five fungicides when compared to untreated controls 24 and 48 hours after inoculation and incubation (Table 13). Untreated controls had significantly (P=0.05) higher percentage of germinated spores. Spore germination increased with time and was highest on control plates 48 hours after inoculation. (Table 13). Spores of *F. solani* f.sp. *phaseoli* were completely inhibited and did not germinate even after 48 hours. In contrast, spores of *F. oxysporum* f.sp. *phaseoli* germinated on plates treated with pyrazophos, thiram and triforine, and was highest in plates treated with pyrazophos (Table 13). Thus, *F. solani* f.sp. *phaseoli* was fully inhibited by the five fungicides 24 and 48 hours after inoculation.

Table 13. *Mean % spore germination of *F . solani* f. sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* on five different fungicides 24 and 48 hours after inoculation.

	Isolates			
Treatments	*F.solani.fsp. phaseoli*		*F.oxysporum f.sp.phaseoli*	
	24 hours	48 hours	24 hours	48hours
Triforine	0.00a*	0.00a	0.01a	2.62c
Metalaxyl	0.00a	0.00a	0.00a	0.00a
Thiram	0.00a	0.00a	0.00a	0.69b
Captan	0.00a	0.00a	0.00a	0.00a
Pyrazophos	0.00a	0.00a	0.03b	3.02c
Control	49.05b	81.81b	0.08c	12.24d

*Mean followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan's Multiple Range Test.

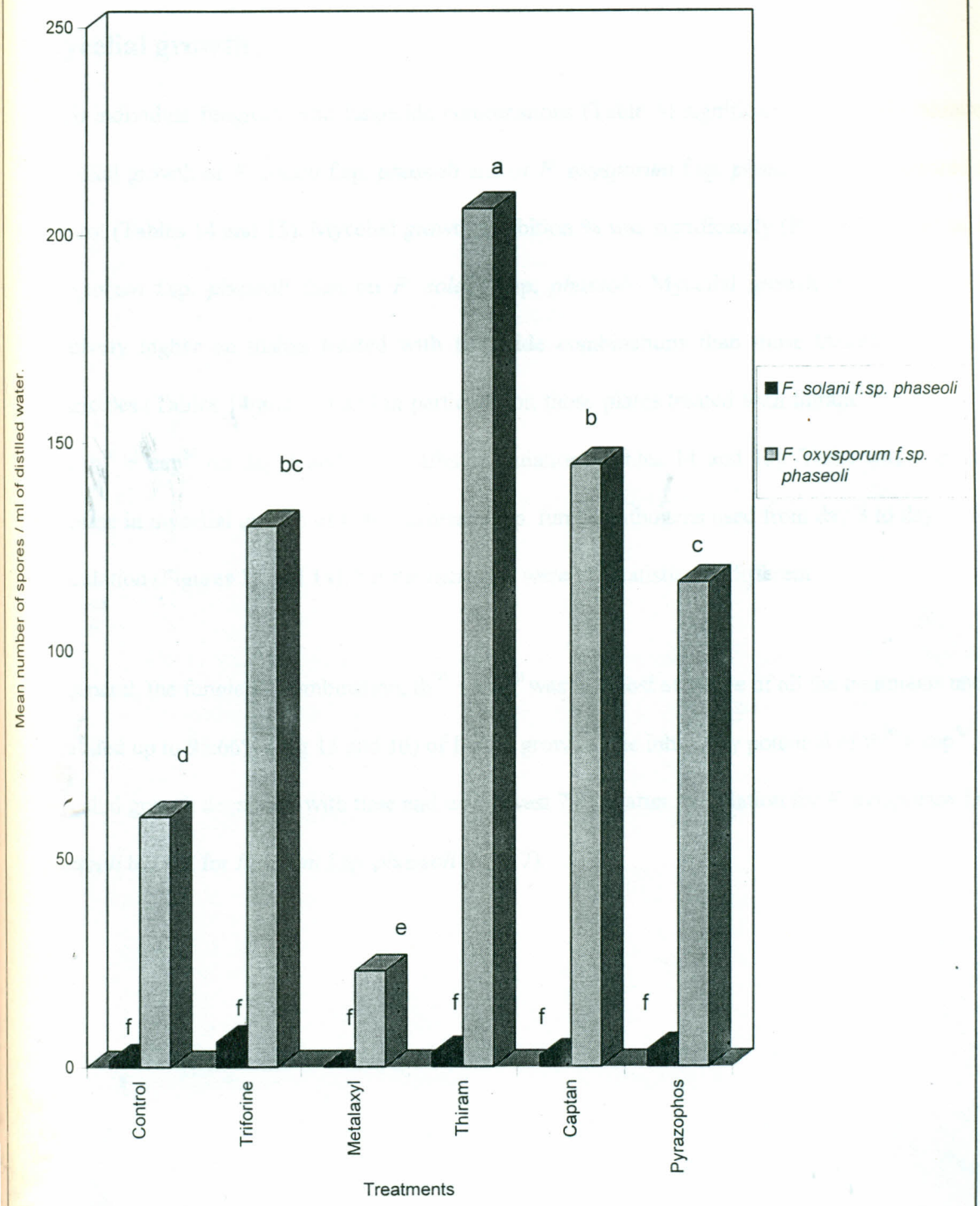
4.2.3. *In-vitro* test 3. Effect of fungicides on sporulation.

All the fungicides significantly (P = 0.05) stimulated sporulation of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* (Fig 12) when compared to control, except metalaxyl which inhibited sporulation. The stimulatory effects of the four fungicides on sporulation was significantly (P=0.05) different. *F. solani* f.sp. *phaseoli* sporulated better in triforine while of *F. oxysporum* f.sp. *phaseoli* in did better in thiram.Sporulation in untreated controls was significantly low (Fig 12).

F. oxysporum f.sp. *phaseoli* sporulated significantly (P=0.001) higher in fungicides than *F. solani* f.sp. *phaseoli* did (Fig 12). *F. oxysporum* f.sp. *phaseoli* had the highest sporulation in thiram treated plates, with approximately 2062 X 10^4 spores / ml (Fig 12). Sporulation of the two fungal isolates was significantly (P =0.05) lowest in metalaxyl treated plates in which *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* produced approximately 1.2 X 10^4 and 228.4 X 10^4 spores/ml, respectively (Fig 12).

Overall sporulation of *F. oxysporum* f.sp. *phaseoli* in fungicides was significantly (P=0.001) higher than that of *F. solani* f.sp. *phaseoli* (Fig 12). On average, 29.53 X 10^4 and 1133.70 X 10^4 spores / ml were produced on *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* treated plates, respectively, these indicates that fungicide treatments significantly (P = 0.05) stimulated sporulation of *F. oxysporum* f.sp. *phaseoli* than of *F. solani* f.sp. *phaseoli*.

Figure 12. Comparative effect of five fungicides on sporulation of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* 13 days after inoculation. *In-vitro* test 3.



Bars with different letters are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

4.2.4. *In-vitro* test 4. Effect of fungicides and fungicide combinations on mycelial growth.

Each individual fungicide and fungicide combinations (Table 5) significantly (P=0.001) inhibited mycelial growth of *F. solani* f.sp. *phaseoli* and/or *F. oxysporum* f.sp. *phaseoli* when compared to control (Tables 14 and 15). Mycelial growth inhibition % was significantly (P=0.001) higher on *F. oxysporum* f.sp. *phaseoli* than on *F. solani* f.sp. *phaseoli*. Mycelial growth inhibition % was relatively higher on dishes treated with fungicide combinations than those treated with single fungicides (Tables 14 and 15) and in particular on those plates treated with thiram$^{0\ 25}$ + captan$^{0\ 75}$ and th^{50} + cap^{50} on day 3 and day 7 after inoculation (Tables 14 and 15). There was a gradual increase in mycelial growth of both *Fusarium* spp. fungal pathogens used from day 3 to day 7 after inoculation (Figures 13 and 14), but the increases were not statistically different.

In general, the fungicide combination, th^{50} + cap^{50} was the most effective of all the treatments and it inhibited up to 95.66% (Fig 15 and 16) of fungal growth. The inhibitory potential of th^{50} + cap^{50} on mycelial growth decreased with time and was lowest 7 days after inoculation for *F. oxysporum* f.sp. *phaseoli* but not for *F. solani* f.sp. *phaseoli* (Fig 17).

Table 14. Effect of fungicides and fungicide combination on mean mycelial inhibition % of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on day 3 after inoculation. *In-vitro* test 4

	Inhibition %	
	F. solani f.sp. *phaseoli*	*F. oxysporum* f.sp. *phaseoli*
$Tri^{25}+Cap^{75}$	84.04b*	95.60a
$Tri^{50}+Cap^{50}$	89.50ab	97.20a
$Th^{25}+Cap^{75}$	96.50a	98.41a
$Th^{50}+Cap^{50}$	96.90a	98.80a
Triforine	91.30a	98.00a
Thiram	95.21a	93.28ab
Captan	86.08ab	84.98b
Control	0.00c	0.00c

* Means followed by the same letter within each column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

Table 15. Effect of fungicides and fungicide combination on mean mycelial inhibition % of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* on day 7 after inoculation. *In-vitro* test 4

	Inhibition %	
	F. solani f.sp. *phaseoli*	*F. oxysporum* f.sp. *phaseoli*
Tri25+Cap75	78.6b*	86.21a
Tri50+Cap50	72,78b	88.20a
Th25+Cap75	97.46a	93.72a
Th50+Cap50	98.22a	95.09a
Triforine	96.00a	92.04a
Thiram	90.75a	89.28a
Captan	61.26c	87.74a
Control	0.00c	0.00b

* Means followed by the same letter within each column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

Figure 13. Effect of fungicides and fungicide combinations on mycelial growth of *F. oxysporum* f.sp. *phaseoli* from day 3 to day 7 after inoculation. *In-vitro test* 4.

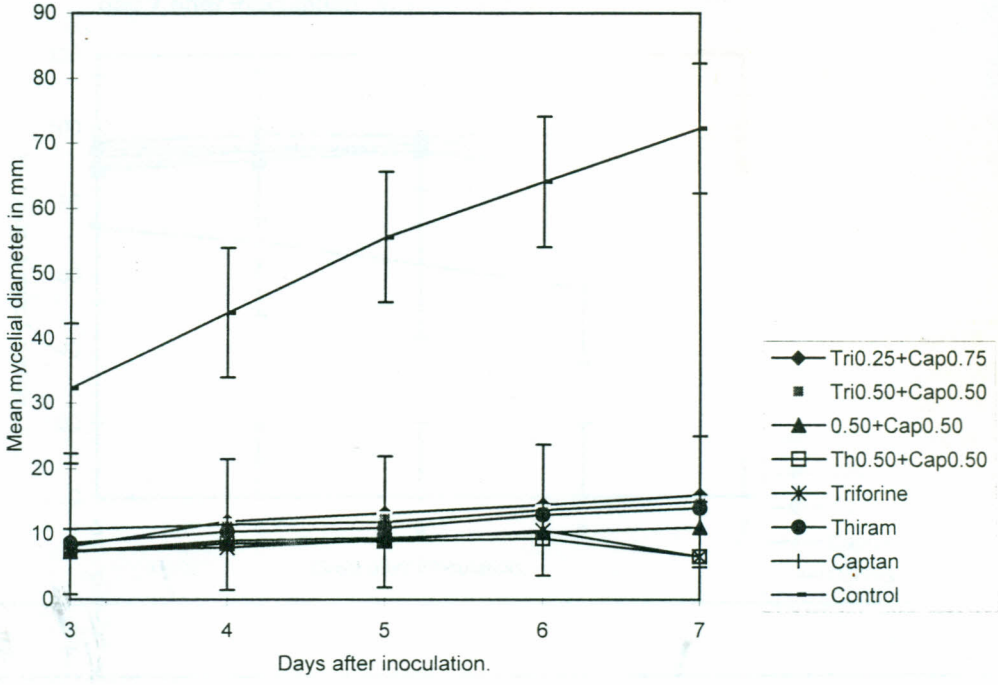


Figure 14. Effect of fungicides and fungicide combination on mycelial growth of *F. oxysporum* f.sp. *phaseoli* from day 3 to day 7 after inoculation.*In-vitro* test 4.

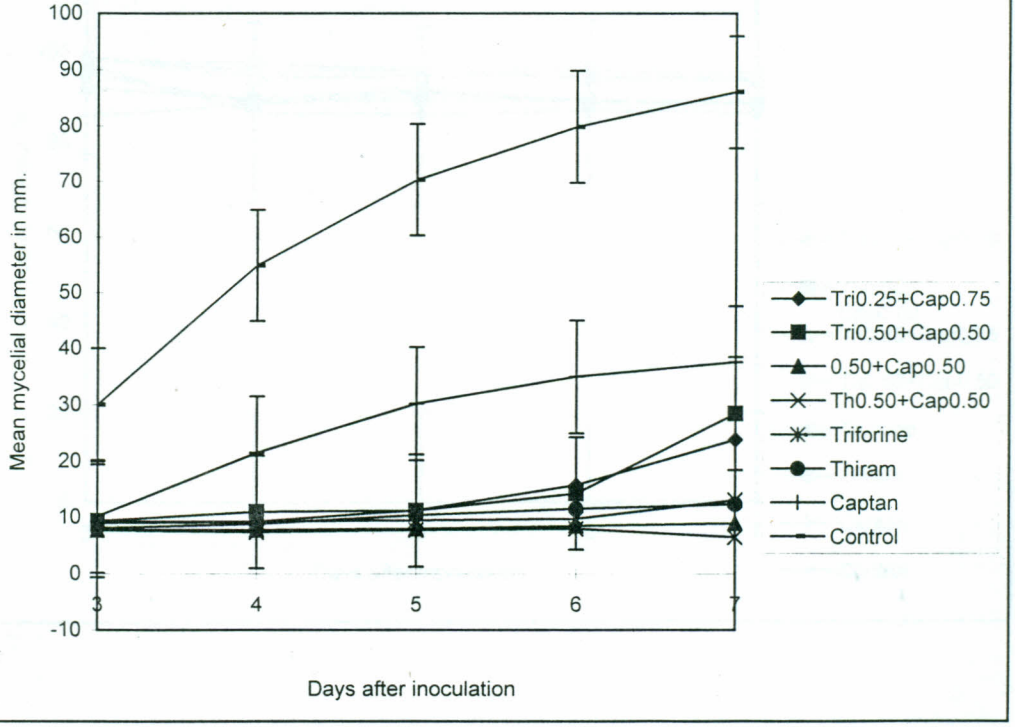


Figure 15. Effect of fungicides and fungicide combinations on mean mycelial inhibition % of *F. solani* f.sp. *phaseoli* from day 3 to day 7 after inoculation. *In-vitro* test 4.

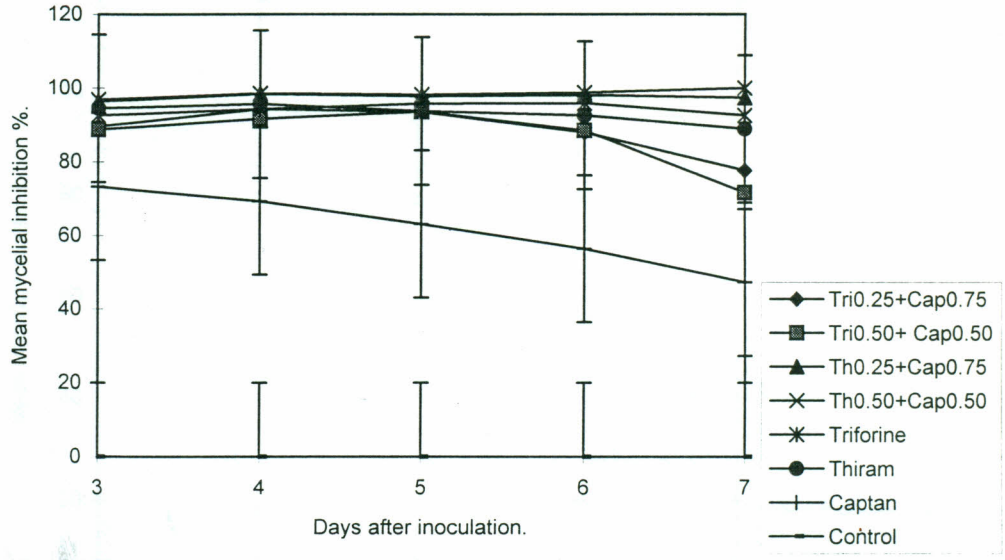


Figure 16. Effect of fungicides and fungicide combinations on mean mycelial inhibition % of *F. oxysporum* f.sp. *phaseoli* from day 3 to day 7 after inoculation. *In-vitro* test 4.

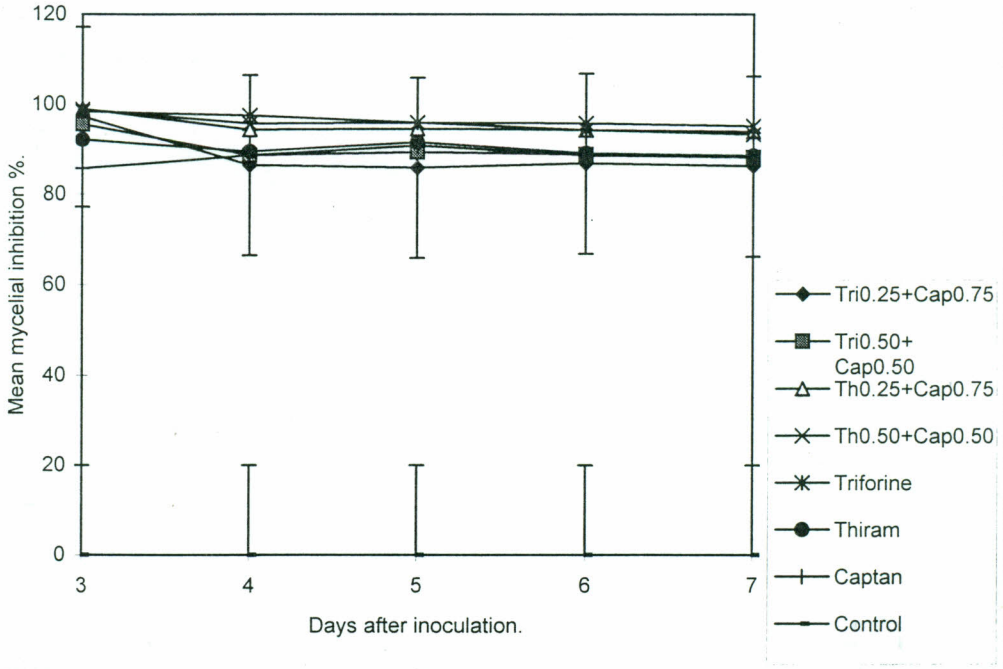
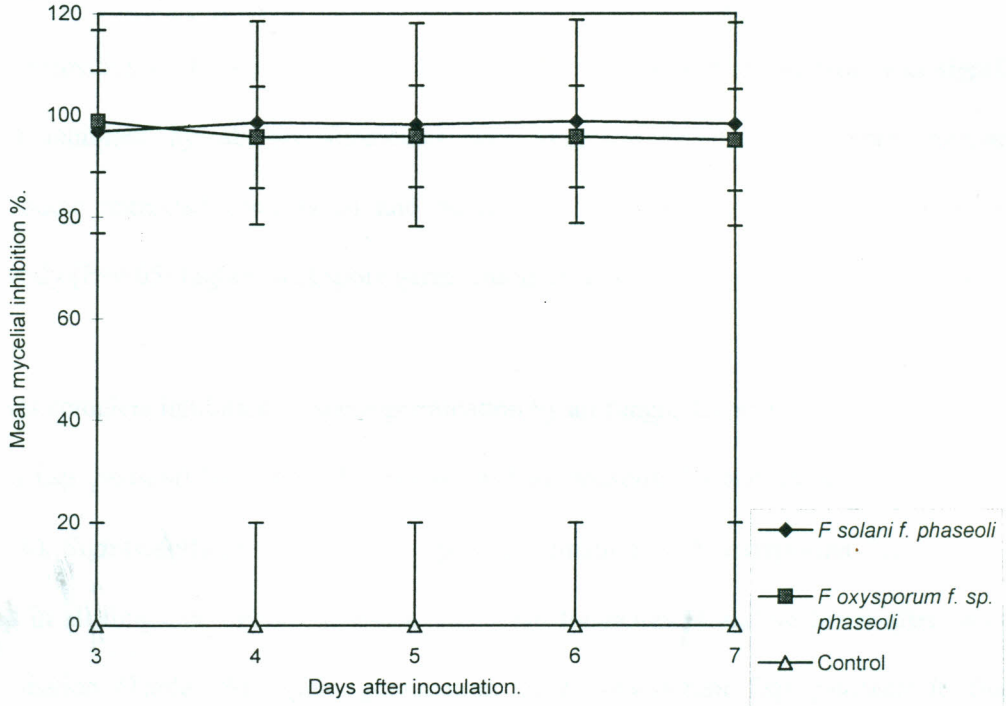


Figure 17. Effect of fungicide combination thiram ^{50} + captan ^{50} on mean mycelial inhibition % of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* from day 3 to day 7 after inoculation. *In-vitro* test 4.



4.2.5. *In-vitro* test 5. Effect of fungicides and fungicide combinations on spore germination.

Spore germination of *F. oxysporum* f.sp. *phaseoli* and *F. solani* f.sp. *phaseoli* was significantly (P=0.05) inhibited by all the fungicides and fungicide combinations when compared to corresponding untreated controls 24 and 48 hours after inoculation (Table 16). Controls had significantly (P=0.05) higher % of spore germination, than that of fungicide treatments (Table 16).

There was complete inhibition of spore germination by all fungicides and fungicide combination on *F. solani* f.sp. *phaseoli* but not on *F. oxysporum* f.sp. *phaseoli*, 24 and 48 hours after inoculation (Table 16). Significantly (P=0.05) higher spore germination of *F. oxysporum*.f.sp. *phaseoli* was recorded in all fungicide treatments except in captan fungicide 24 and 48 hours after inoculation and incubation (Table 16). Spore germination of *F. oxysporum* f.sp. *phaseoli* in fungicide combinations was relatively lower than in corresponding single fungicide treatments (Table 16).

Table 16.
***Mean % spore germination of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* in fungicides and fungicide combinations 24 and 48 hours post inoculation.**

	Isolate			
	F solani f.sp. phaseoli		*F oxysporum f.sp. phaseoli*	
Treatments	24 hours	48 hours	24 hours	48 hours
$Tri^{25}+Cap^{75}$	0.00b*	0.00b	0.02a	1.33c
$Tri^{50}+Cap^{50}$	0.00b	0.00b	0.02a	1.15c
$Th^{25}+Cap^{75}$	0.00b	0.00b	0.00c	0.43d
$Th^{50}+Cap^{50}$	0.00b	0.00b	0.00c	0.39d
Triforine	0.00b	0.00b	0.01c	2.31b
Thiram	0.00b	0.00b	0.00c	0.69d
Captan	0.00b	0.00b	0.00c	0.00e
Control	43.00a	79.22a	0.10b	11.29a

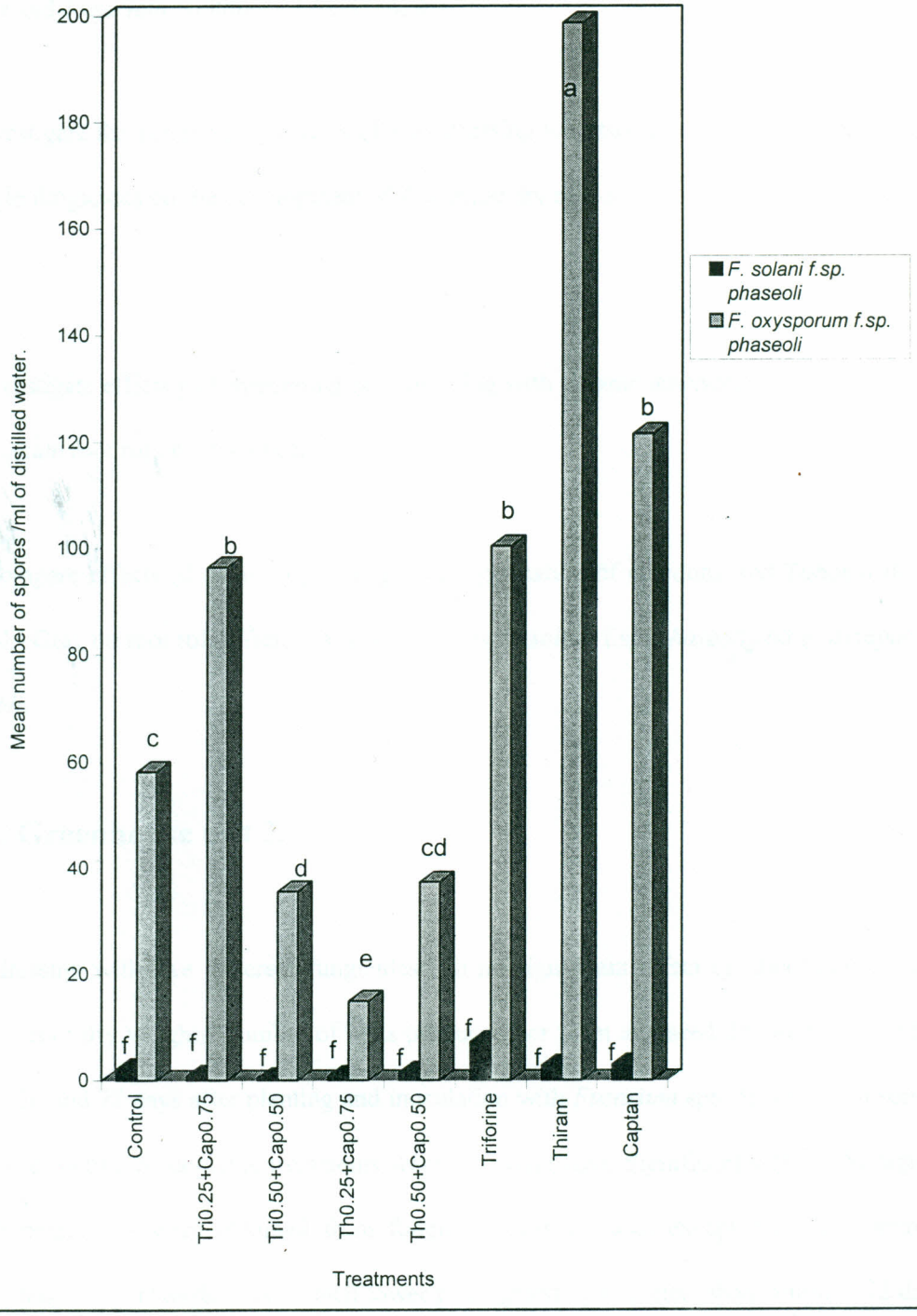
*Mean followed by the same letter within each column are not significantly different at P = 0.05 level by Duncan's Multiple Range Test.

4.2.6. *In-vitro* test 6. Effect of fungicide and fungicide combinations on sporulation.

Most of the individual fungicide combinations (Table 5) had significantly (P=0.05) higher inhibitory effects on sporulation of *F. oxysporum* f.sp. *phaseoli* and *F. solani* f.sp. *phaseoli* than single fungicide treatments and corresponding controls. (Fig 18). Single fungicides had stimulatory effect on sporulation of both *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.* Of the two *Fusarium* spp. pathogens used, sporulation of *F. oxysporum* f.sp. *phaseoli* in fungicides and fungicide combinations was significantly (P=0.001) higher than that of *F. solani* f.sp. *phaseoli* (Fig 18).

The highest inhibitory effect of fungicide treatments on sporulation of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* was recorded from dishes treated with fungicide combinations tri^{50} + cap^{50} and th^{25} + cap^{75}, respectively (Fig 18).

Figure 18. Comparative effect of fungicides and fungicide combinations on sporulation of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* 13 days after inoculation. Mean number of spores /ml X 10^5. *In-vitro* test 6.



Bars with different letters are significantly (P = 0.05) different according to Duncan's Multiple Range Test.

4.3.0. Greenhouse tests.

Four greenhouse tests were conducted between the months of March and July, 1997 to:

(I) Investigate the effects of seed dressing with five fungicides on the development of *Fusarium* dry root rot and *Fusarium* 'yellows' of french beans.

(II) Investigate the comparative effects of seed dressing with two fungicide combinations with that of single fungicides on the development of *Fusarium* dry root rot and *Fusarium* 'yellows' of french beans.

(III) Investigate efficacy of integrating seed dressing with organic amendments on the development of *Fusarium* root rot of french beans.

(IV) Compare effects of delay in planting after application of cowdung and *Tithonia diversifolia* (Hemsl.) Gray on root rot of french beans caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli*.

4.3.1. Greenhouse test 2.

Seed dressing with five different fungicides had no significant effect on shoot height, shoot dry weights, root dry weights, number of pods produced per plant and seed dry weights (Tables 17 to 22) 14, 36 and 72 days after planting and inoculation with *Fusarium* spp. However, in some cases, differences between fungicide treatments were not significant. Significantly (P=0.05) higher plant growth parameters were obtained from fungicide treated plants except for plants treated with pyrazophos which recorded even much lower plant growth parameters than controls, 72 days after planting and inoculation with either of *Fusarium* spp. (Tables 21, 22).

Plants treated with fungicides had relatively shorter mean lengths of discoloured root tissues (MLDRT) or lower mean root rot index (MRRI), 14, 36 or 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli*. Significantly (P=0.05) shorter mean lengths of discoloured root tissues (MLDRT) and lower mean root rot index (MRRI) were obtained from plants treated with triforine and metalaxyl than control 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Table 21) or *F. oxysporum* f.sp. *phaseoli* (Table 22).

Triforine and metalaxyl accounted for significantly (P=0.05) the lowest mean root rot index (MRRI), heavier seed dry weight and more pods per plant 72 days after planting and inoculation with *F. solani* f.sp. phaseoli or *F. oxysporum* f.sp. *phaseoli* (Tables 21, 22).

TABLE 17. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and mean root rot index[2] at development stage VI[3] 14 days after planting in soils inoculated with F. *solani* f.sp.*phaseoli*. Greenhouse test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean shoot dryweight (g)	Mean L.D.R.T.index[2]	*Mean root rot (1-9 mm
Triforine	17.22**	0.395	0.053	0.00b	1.00
Metalaxyl	15.40	0.380	0.042	0.00b	1.00
Thiran	16.81	0.373	0.046	4.25ab	1.50
Captan	14.20	0.323	0.039	5.00ab	2.00
Pyrazophos	16.00	0.334	0.039	6.00ab	2.00
Control	13.50	0.284	0.034	11.14a	3.14
	Ns	Ns	Ns		Ns

*Means of five replicates.
**Mean values followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test. (DMRT).

TABLE 18. Effect of seed-dressing with different fungicides on growth of french beans, LDRT[1] and mean root rot index[2] at development stage VI[3] 14 days after planting in soils inoculated with *F.oxysporum* f.sp.*phaseoli* Greenhouse test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean shoot dryweight (g)	Mean L.D.R.T[1].index[2]	*Mean root rot (1-9 mm
Triforine	15.00**	0.380a	0.056	3.33b	1.67
Metalaxyl	17.50	0.355ab	0.051	0.00b	1.00
Thiran	14.35	0.313ab	0.059	3.57b	1.71
Captan	15.71	0.282ab	0.048	3.57b	1.71
Pyrazophos	15.63	0.229ab	0.045	4.21b	2.00
Control 1	4.55	0.166b	0.044	0.25a	2.20
	Ns		Ns		Ns

*Means of five replicates.
**Means followed by the same letter within the same column are not significantly different at P= 0.05 level by Duncan Multiple Range Test (DMRT)

TABLE 21. Effect of seed-dressing with different fungicides on growth of french beans, LDRT[1] and mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F.solani* f.sp.*phaseoli* Greenhouse test 2.

Treatment	Mean shoot height (cm)	Mean shoot dry weight (g)	Mean root dry weight (g)	Mean L.D.R.T (mm)	Mean root rot index1 (1-9)	Dry weight of 100 seeds	Number of pods/plant
Triforine	63.6a**	6.433a	1.685a	33.3b	2.86bc	31.94a	25.0a
Metalaxyl	54.3a	6.029a	1.610a	20.0b	2.00c	29.52a	24.0a
Thiran	58.7a	6.091a	1.594a	35.0b	3.25c	28.36a	22.0a
Captan	51.0a	5.209ab	1.328a	55.0ab	5.33abc	28.88b	10.0b
Pyrazophos	49.0a	3.843ab	0.276b	93.3a	7.00ab	22.88b	6.0b
Control	42.7a	3.253b	0.167b	103.3a	7.99a	21.21b	4.0b

*Means of five replicates.
**Means followed by the same letter within the same column are not significantly different at P= 0.05 level by Duncan Multiple Range Test (DMRT)

TABLE 22. Effect of seed-dressing with different fungicides on growth of french beans, LDRT[1] and mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F.oxysporum* f.sp.*phaseoli* Greenhouse test 2.

Treatment	Mean shoot height (cm)	Mean shoot dry weight (g)	Mean root dry weight (g)	Mean L.D.R.T (mm)	Mean root rot index1 (1-9)	Dry weight of 100 seeds	Number of pods/plant
Triforine	61.0**a	5.367a	1.567a	28.3d	2.30b	29.13a	24.0a
Metalaxyl	69.7a	5.737a	1.615a	36.7cd	3.00b	30.41a	23.0a
Thiran	64.5a	4.202ab	1.350a	33.0cd	3.30b	26.52a	21.0a
Captan	52.0a	4.609ab	1.358a	75.0bc	3.25b	28.08a	10.0b
Pyrazophos	46.7a	1.931c	0.188b	100.0ab	7.00a	22.42b	4.0c
Control	46.2a	2.20bc	0.275b	125.0a	8.00a	21.58b	4.0c

*Means of five replicates.
**Means followed by the same letter within the same column are not significantly different at P= 0.05 level by Duncan Multiple Range Test

TABLE 19. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and mean root rot index[2] at development stage R6[3] 36 days after planting in soils inoculated with F. *solani* f.sp.*phaseoli*. Greenhouse test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean shoot dryweight (g)	Mean L.D.R.T.index[2]	*Mean root rot (1-9 mm
Triforine	61.0**	2.79	1.217a	25.57c	2.33b
Metalaxyl	46.3	2.48	1.207a	11.00d	2.00b
Thiran	43.3	1.40	1.132a	30.72c	2.86b
Captan	44.0	1.64	1.132b	38.72c	4.06b
Pyrazophos	44.0	1.32	0.121b	92.3a	5.13a
Control	38.3	1.32	0.121b	92.3a	6.0a
	Ns	Ns	Ns		Ns

*Means of five replicates.
**Mean followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test. (DMRT).

TABLE 20. Effect of seed-dressing with different fungicides on growth of french beans, LDRT[1] and mean root rot index[2] at development stage R6[3] 36 days after planting in soils inoculated with *F.oxysporum* f.sp.*phaseoli* Greenhouse test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean shoot dryweight (g)	Mean L.D.R.T.index[2]	*Mean root rot (1-9 mm
Triforine	58.7	2.90	1.231a**	15.7b	1.80b
Metalaxyl	62.7	4.26	1.257a	13.4b	1.70b
Thiran	58.0	2.68	1.217a	26.0b	2.61b
Captan	48.0	2.68	1.218a	26.0b	6.76b
Pyrazophos	44.3	1.52	0.137b	98.9a	6.76a
Control	42.7	1.52	0.133b	100.5a	7.96a
	Ns	Ns			

*Means of five replicates.
**Means followed by the same letter within the same column are not significantly different at P= 0.05 level by Duncan Multiple Range Test (DMRT)

4.3.2. Greenhouse test 3.

Treatment of french bean seeds before planting with fungicides or fungicide combinations had significant (P=0.05) effect on shoot height, shoot dry weights, root dry weight, length of discoloured root tissues, root rot indices, seed dry weights and number of pods produced per plant 14, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 23 to 28).

Plants treated with fungicide combinations, $th^{50} + cap^{50}$, $tri^{25} + cap^{75}$ or $tri^{50} + cap^{50}$ against *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* had relatively heavier root dry weights and shorter length of discoloured root tissues than those plants treated with individual fungicides and control, 14 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Table 23) or *F. oxysporum* f.sp. *phaseoli* (Table 24). However, this enhanced plant performance by fungicide combinations decreased 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Tables 25, 27) or *F. oxysporum* f.sp. *phaseoli* (Tables 26, 28).

Plants treated with individual fungicides had significantly (P=0.05) better bean growth, shorter lengths of discoloured root tissues or lower root rot index than those treated with fungicide combinations and control 36 and 72 days planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 25, 26, 27, 28).

Plants treated with individual fungicides had significantly (P=0.05) shorter mean lengths of discoloured root tissues and lower mean root rot index than those treated with fungicide combination or control 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or

F. oxysporum f.sp. *phaseoli* (Tables 25, 26, 27, 28).

Significantly (P=0.05) heavier seeds, more number of pods and low disease development were recorded from plants treated with individual fungicides than fungicide combinations or control (Tables 27, 28). Seed treatment with triforine gave plants that had better plant growth and reduced disease development 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 27, 28). The relatively high *in-vitro* potency of th^{50} + cap^{50} fungicide combination was inconsistent with the greenhouse results.

TABLE 23. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and means of root rot index[3] at development stage VI[3] 14 days after planting in soils inoculated with *F solani*.f.sp.*phaseoli*. Greenhouse test 3

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T	*Mean root rot index[1] (1-9)
$Th^{50}+Cap^{50}$	14.60abc**	0.315	0.056	3.00d	1.10b
$Tri^{25}+Cap^{75}$	12.66cd	0.300	0.063	5.00c	1.63b
Thiram	15.90ab	0.382	0.047	6.54b	1.87b
Triforine	17.00a	0.391	0.061	0.00e	1.00b
Captan	13.85bcd	0.361	0.041	7.17b	2.00b
Control	12.33d	0.302	0.032	21.3a	5.40a

*Mean of five replicates.

**Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test.

TABLE 24. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and means of root rot index[3] at development stage VI[3] 14 days after planting in soils inoculated with *F oxysporum*.f.sp.*phaseoli*. Greenhouse test 3

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T	*Mean root rot index[1] (1-9)
$Th^{50}+Cap^{50}$	15.80	0.408	0.055	5.67b**	1.80b
$Tri^{25}+Cap^{75}$	17.50	0.465	0.050	6.00b	1.75b
Thiram	15.34	0.352	0.047	4.79b	1.62b
Triforine	16.30	0.402	0.059	0.00c	1.00b
Captan	15.60	0.321	0.044	5.13b	1.68b
Control	15.33	0.315	0.042	46.00a	6.00a

*Mean of five replicates.

**Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test.

TABLE 25. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and means of root rot index[3] at development stage R6[3] 36 days after planting in soils inoculated with *F solani*.f.sp.*phaseoli*. Greenhouse test 3

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T	*Mean root rot index[1] (1-9)
$Th^{50}+Cap^{50}$	25.5bc**	0.797c	0.463b	107.5a	9.0a
$Tri^{25}+Cap^{75}$	20.0c	0.653c	0.590b	100.5a	9.0a
Thiram	45.0ab	2.140	1.397a	32.0b	3.76b
Triforine	59.0a	2.621a	1.513a	29.3b	2.46c
Captan	42.7ab	1.371b	1.313a	41.0b	4.32b
Control	25.4bc	0.567c	0.063c	92.0a	9.0a

*Mean of five replicates.
**Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test.

TABLE 26. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and means of root rot index[3] at development stage R6[3] 36 days after planting in soils inoculated with *Foxysporum*.f.sp.*phaseoli*. Greenhouse test 3

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T	*Mean root rot index[1] (1-9)
$Th^{50}+Cap^{50}$	33.6bc**	0.605c	0.077b	101.25a	9.0a
$Tri^{50}+Cap^{50}$	23.50c	0.709c	0.089b	105.00a	9.0a
Thiram	55.21ab	2.680a	1.198a	33.00b	3.33b
Triforine	61.32a	3.120a	1.214a	21.42b	2.00b
Captan	50.19a	2.990a	1.353a	30.00b	3.21b
Control	36.33a	1.190b	0.112b	103.33a	8.33a

*Mean of five replicates.
**Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test.

TABLE 27. Effect pf seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F.solani* f.sp.*phaseoli*. Greenhouse test 3.

Treatment	*Mean * shoot height (cm)	Mean shoot dry weight (g)	*Mean root dry drt weight (g)	*Mean L.D.R.T[1] (mm) (1-9)	*Mean root rot index[1]	*Mean number of pods per plant	*Mean dry weight of 100 seeds
Th.^{50}+Cap^{50}	55.0b**	5.54ab	0.251d	107.5a	9.0a	3.00c	21.10b
Tri.^{25}+Cap^{75}	45.0c	3.182c	0.209d	105.0a	9.0a	4.00c	20.90b
Thiram	61.3a	6.217ab	1.512b	42.2b	3.92b	22.00a	29.50a
Triforine	64.3a	7.134a	1.711a	31.2b	2.96c	24.00a	31.29a
Captan	56.1a	6.00ab	0.437c	47.2b	4.73b	9.00b	28.35a
Control	41.7c	3.839bc	0.123e	100.0a	9.0a	4.00c	25.07a

* Mean of five replicates

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test

TABLE 28. Effect pf seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F.oxysporum* f.sp.*phaseoli*. Greenhouse test *3*.

Treatment	*Mean * shoot height (cm)	Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T (mm)	*Mean root rot index[1] (1-9)	*Mean number of pods per plant	*Mean dry weight of 100 seeds
Th.^{50}+Cap^{50}	51.00c**	3.000ab	0.220b	103.7a	9.00a	5.0bc	23.27b
Tri.^{50}+Cap^{50}	47.33cd	2.526ab	0.199b	107.3a	9.00a	2.0cd	11.05c
Thiram	61.03ab	4.188a	1.373a	42.2b	3.97b	22.0a	29.39a
Triforine	64.97a	5.132a	1.545a	33.4b	2.63c	23.0a	30.65a
Captan	55.48b	4.317a	1.388a	69.1ab	4.02b	9.0b	30.65a
Control	42.00d	1.231b	0.142b	107.73a	9.00a	4.0c	25.69ab

* Mean of five replicates

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test

4.3.3. Greenhouse test 4.

Soil treatment with organic amendments or combinations of either cowdung or *Tithonia diversifolia* with metalaxyl in soils inoculated with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* had significant (P=0.05) effect on plant growth and disease development 14, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 29 to 34).

Cowdung or combination of cowdung with metalaxyl significantly (P=0.05) improved plant growth and reduced disease development. However, *T. diversifolia* alone or combination of *T. diversifolia* with metalaxyl supported poor plant growth and high disease development as indicated by the relatively higher mean length of discoloured root tissues and high mean root rot indices 14, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 29 to 34).

Cowdung alone or a combination of cowdung with metalaxyl significantly (P=0.05) increased seed dry weight and pod yield more than *T. diversifolia* or a combination of *T. diversifolia* with metalaxyl including the control 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Table 33) or *F. oxysporum* f.sp. *phaseoli* (Table 34). *T. diversifolia* alone and combination of *T. diversifolia* with metalaxyl suppressed seed germination and pod set by both *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* infected plants.

There was no significant difference between the effects of using organic amendments alone with that of combining organic amendments with metalaxyl. Cowdung alone supported plants that had

better plant performance, shorter lengths of discoloured root tissues and lower root rot indices on both plants infected by *F. solani* f.sp. *phaseoli* (Table 33) and *F. oxysporum* f.sp. *phaseoli* (Table 34) than those obtained from combination of cowdung with metalaxyl. *T. diversifolia* alone or a combination of *T. diversifolia* with metalaxyl supported plants that had poor plant growth and high disease development as indicated by high mean root rot indices on both plants infected by *F. solani* f.sp. *phaseoli* (Table 33) and *F. oxysporum* f.sp. *phaseoli* (Table 34). Effects of *T. diversifolia* alone or combination of *T. diversifolia* with metalaxyl on plant growth and disease development were not significantly different from the control.

TABLE 29. Effect of organic amendments and their combination with fungicide on growth, LDRT[1] and mean root rot index[2] at development stage VI[3] 14 days after planting in soils inoculated with *F.solani* f.sp *phaseoli*

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight(g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Cowdung(Cd) +Fungi	17.75**	0.560ab	0.069	19.00b	2.13ab
Cd+Metalaxyl +Fungi	16.50	0.508ab	0.060	25.00b	2.22ab
Tithonia (Td)+ Fungi	14.75	0.289b	0.069	40.00a	4.00ab
Td+Metalaxyl +Fungi	14.50	0.324b	0.061	55.00a	5.31a
Fungi alone	13.80	0.288b	0.059	22.5b	2.20ab
+Cd -fungi	18.00	0.688a	0.074	0.00c	1.00b
+Td	18.25	0.732a	0.099	0.00c	1.00b
	Ns		Ns		

*Means of five replicates.

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test.

TABLE 30. Effect of organic amendments and their combination with fungicide on growth, LDRT[1] and mean root rot index[2] at development stage VI[3] 14 days after planting in soils inoculated with *F.oxysporum f.sp phaseoli*

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight(g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Cowdung(Cd) +Fungi	17.66a**	0.491ab	0.060b	25.00ab	2.19abc
Cd+Metalaxyl +Fungi	17.50a	0.302b	0.057b	20.00b	2.00bc
Tithonia (Td)+ Fungi	14.50a	0.278b	0.055b	46.25ab	4.61ab
Td+Metalaxyl +Fungi	16.25a	0.323b	0.066a	41.00ab	5.23a
Fungi alone	15.97a	0.293b	0.35b	57.00a	3.99ab
+Cd -fungi	17.81a	0.701a	0.069a	0.00b	1.00c
+Td	17.93a	0.761a	0.104a	0.00b	1.00c

*Means of five replicates.

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan Multiple Range Test.

TABLE 31. Effect of organic amendments and their combination with fungicide on growth, LDRT[1] and mean root rot index[2] at development stage R6[3] 36 days after planting in soils inoculated with *F.solani* f.sp *phaseoli*

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight(g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Cowdung(Cd) +Fungi	43.8ab**	1.84bc	1.470a	28.00b	2.30b
Cd+Metalaxyl +Fungi	40.5ab	1.71bc	1.128a	43.00ab	4.30ab
Tithonia (Td)+ Fungi	33.7ab	1.36bc	0.151b	50.00ab	5.71a
Td+Metalaxyl +Fungi	23.0b	1.01c	0.140b	70.00a	6.91a
Fungi alone	41.3ab	1.51bc	0.122b	83.00a	6.00a
+Cd -fungi	55.2a	3.17ab	0.323b	5.00c	1.00c
+Td	30.4a	1.23c	0.497b	3.00c	1.00c

*Means of five replicates.

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 32. Effect of organic amendments and their combination with fungicide on growth, LDRT[1] and mean root rot index[2] at development stage R6[3] 36 days after planting in soils inoculated with *F.oxysporum* f.sp *phaseoli*

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight(g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Cowdung(Cd) +Fungi	47.0ab**	1.401b	0.154bc	43.12b	4.10b
Cd+Metalaxyl +Fungi	47.7ab	1.730b	0.252abc	40.00b	4.00b
Tithonia (Td)+ Fungi	25.0b	0.663c	0.096c	90.00a	8.20a
Td+Metalaxyl +Fungi	40.0ab	1.04b	0.096	93.75a	8.50a
Fungi alone	45.7ab	1.49b	0.124bc	99.43a	8.60a
+Cd -fungi	57.6a	3.49a	0.351ab	3.71c	1.00c
+Td	30.4a	30.9a	1.14b	0.473a	1.00c

*Means of five replicates.

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 33. Effect of organic amendments and their combination with fungicide on growth, LDRT[1] and mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F.solani* f.sp *phaseoli*

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean* L.D.E.T[1] (mm)	*Mean root rot index[2] (1-9)	*Mean number pods per plant	*Mean dry weight of 100 seeds
Cowdung(Cd) +Fungi	63.0a	4.84b	1.398a	30.bc	3.13c	22.0a	25.05a
Cd+Metalaxyl +Fungi	58.0a**	4.26b	1.166b	50.0abc	5.41b	21.0a	19.02b
Tithonia (Td)+ Fungi	54.5a	3.36b	0.232d	62.1ab	6.23b	5.0b	23.57b
Td+Metalaxyl +Fungi	35.0a	1.03c	0.014e	90.0ab	8.00a	0.0c	0.00c
Fungi alone	49.0a	3.12b	0.167e	97.5a	7.25a	4.0bc	21.37a
+Cd -fungi	72.0a	7.84a	0.733c	6.11c	1.00d	22.0a	32.17a
+Td	49.3a	2.31c	0.796c	5.43c	1.00d	4.0bc	32.85a

*Means of five replicates.

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 34. Effect of organic amendments and their combination with fungicide on growth, LDRT[1] and mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F.oxysporum* f.sp *phaseoli*

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean* L.D.E.T[1] (mm)	*Mean root rot index[2] (1-9)	*Mean number pods per plant	*Mean dry weight of 100 seeds
Cowdung(Cd) +Fungi	61.7a**	6.89a	1.178a	51.7ab	4.00b	22.0a	28.07ab
Cd+Metalaxyl +Fungi	55.0a	6.89a	1.245a	50.0ab	5.00ab	22.0a	26.23abc
Tithonia (Td)+ Fungi	27.5a	1.39c	0.099c	99.1a	9.00a	0.0-c	0.00d
Td+Metalaxyl +Fungi	46.0a	1.43c	0.100c	99.00a	9.00a	0.0c	0.00d
Fungi alone	61.2a	3.07b	0.187c	100.2a	7.20a	5.0b	24.56bc
+Cd -fungi	71.4a	7.92a	0.777b	7.10b	1.00c	21.0a	32.04a
+Td	37.4a	2.47bc	0.781b	6.40b	1.00c	6.0b	22.97c

*Means of five replicates.

** Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

4.3.4. Greenhouse test 5.

A delay in planting french beans in soils treated with cowdung and *T. diversifolia* had significant (P=0.05) effect on plant growth and root-rot disease development 40 days after planting (Tables 35, 36, 37). Delay in planting french beans in soils treated with cowdung improved plant growth and reduced disease development. In contrast, 2-4 weeks delay in planting french beans in soils treated with *T. diversifolia* suppressed seed germination and increased disease severity on both *F. oxysporum* f.sp. *phaseoli* and *F. solani* f.sp. *phaseoli* infected plants (Table 37). Although, seeds planted 2-4 weeks in soils amended with *T. diversifolia* and inoculated with *F. solani* f.sp. *phaseoli* germinated, their shoot and root dry weights were significantly (P=0.05) lower than those of seeds planted 0 weeks (Tables 35, 36).

Significant (P=0.05) better plant growth and low disease development were recorded in soils in which cowdung was left to decompose before planting and inoculation with *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* (Tables 35, 36, 37). Increased shoot dry weights, root dry weights, and low root rot indices were significantly (P=0.05) better as planting in cowdung was delayed and were greatest 2 and 4 weeks delay on *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* (Tables 35, 36), respectively.

Regression and correlation statistics (Tables 38 and 39) reveal that delay in time of planting in soils amended with cowdung from zero to 4 weeks resulted in reduction of both *Fusarium* dry root rot and *Fusarium* 'yellows', however, a delay in planting in soils amended with *T. diversifolia* from zero to 4 weeks resulted in increase in *Fusarium* dry root rot and *Fusarium* 'yellows'. Variations in time of planting had no significant effect on french bean growth, length of discoloured root tissues

and root rot indices in soils not amended with cowdung or *T. diversifolia.*

In absence of the two root rot fungal pathogens, cowdung was seen to stimulate plant growth, whereas, *T diversifolia* inhibited seed germination and supported poor plant growth as indicated by low shoot and root dry weights (Tables 35 and 36). This, may suggest that decomposition products of *T diversifolia* may have been phytotoxic.

Table 35. Mean dry shoot weight (g) of french bean plants planted in sterile greenhouse soil over a 4-week period after treatment with cowdung and *Tithonia diversifolia* and inoculation with *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.*

Treatments	Time of planting (Weeks)							
	0		2		3		4	
	F. s	*F .o*	*F.s*	*F.o*	*F.s*	*F.o*	*F.s*	*F.o*
T .diversifolia + Fungi	2.12	1.65	0.84	0.00	0.00	0.00	0.00	0.00
Cowdung + *Fungi*	3.36	3.10	7.91	3.12	4.30	4.52	4.40	6.74
Control	1.43	1.57	1.88	1.59	1.81	1.42	1.76	1.55
L.S.D (0.05)	0.91	0.70	2.21	2.32	2.40	3.10	3.00	3.31

F.s refers to *F. solani* f.sp. *phaseoli*
F.o refers to *F.oxysporum* f.sp. *phaseoli*

Table 36. Mean dry root weight (g) of french bean plants planted in sterile greenhouse soil over a 4-week period after treatment with cowdung and *Tithonia diversifolia* and inoculation with *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.*

Treatments	Time of planting (Weeks)							
	0		2		3		4	
	F. s	F .o	F.s	F.o	F.s	F.o	F.s	F.o
T .diversifolia + Fungi	1.09	1.07	0.22	0.00	0.00	0.00	0.00	0.00
Cowdung + *Fungi*	2.45	1.20	3.40	1.58	2.17	2.28	2.36	2.61
Control	1.20	1.13	1.88	1.23	1.47	1.45	1.61	1.60
L.S.D (0.05)	0.55	0.004	2.42	0.65	1.17	1.27	1.38	1.65

F.s refers to *F. solani* f.sp. *phaseoli*
F.o refers to *F.oxysporum* f.sp. *phaseoli*

Table 37. Mean root-rot indices of french bean plants planted in sterile greenhouse soil over a 4-week period after treatment with cowdung and *Tithonia diversifolia* and inoculation with *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli.*

Treatments	Time of planting (Weeks)							
	0		2		3		4	
	F. s	F .o	F.s	F.o	F.s	F.o	F.s	F.o
T .diversifolia + Fungi	7.93a	8.12a	9.00a	9.00a	9.00a	9.00a	9.00a	9.00a
Cowdung + *Fungi*	4.39b	4.75b	3.01c	4.20b	3.32c	3.90c	3.44c	2.80c
Control	7.37a	5.67b	6.73b	5.39b	6.58b	6.03b	6.88b	5.42b
L.S.D (0.05)	3.46	2.89	3.38	5.97	3.33	2.99	3.28	3.64

F.s refers to *F. solani* f.sp. *phaseoli*
F.o refers to *F.oxysporum* f.sp. *phaseoli*

Table 38. Regression co-efficients for the relationships between time of planting french beans in soil inoculated with *F. solani* f.sp. *phaseoli* alone or in combination with cowdung and *Tithonia diversifolia* and shoot dry weight (MSDW), root dry weight (MRDW) and root rot index (MRRI).

Treatments	MSDW		MRDW		MRRI	
	b	r	b	r	b	r
T .diversifolia + F. solani	-0.57*	- 0.97*	-0.28	-0.94	0.27	0.93
Cowdung + *F. solani*	0.16	0.14	-0.07	0.22	-0.24	0.70
Control	0.08	0.73	0.10*	0.98*	-0.14	0.73

* Significant at P = 0.05.

Table 39. Regression co-efficients for the relationships between time of planting french beans in soil inoculated with *F. oxysporum* f.sp. *phaseoli* alone or in combination with cowdung and *Tithonia diversifolia* and shoot dry weigh (MSDW), root dry weight (MRDW) and root rot index (MRRI).

Treatments	MSDW		MRDW		MRRI	
	b	r	b	r	b	r
T .diversifolia + F. oxy	-0.43	-0.88	-0.27	- 0.878	0.23	0.88
Cowdung + *F. oxy*	0.87	0.86	0.36*	0.96*	-0.45	0.93
Control	-0.02	0.40	0.12*	0.95*	-0.01	0.06

* Significant at P = 0.05.
F. oxy refers to *F. oxysporum f.sp. phaseoli*

4.4.0. FIELD TESTS.

Two field tests were carried out. Field tests 1 and 2 were duplications of the greenhouse tests 2 and 3, respectively (sections 3.3.7 and 3.3.8). Field tests 1 and 2 were conducted between the months of May and July, 1997, to:

(i) Investigate the effects of seed dressing with five fungicides on the development of *Fusarium* dry root rot and *Fusarium* 'yellows' of french beans.

(ii) Investigate the comparative effects of seed dressing with two fungicide combinations with that of single fungicides on the development of *Fusarium* dry root rot and *Fusarium* 'yellows' of french beans.

4.4.1. FIELD TEST 1.

Treatment of french bean seeds before planting with five fungicides had significant (P=0.05) effect on shoot height, shoot dry weights, root dry weights, number of pods, length of discoloured root tissues, root rot indices and seed dry weights, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 42 to 45). At 14 days a positive effect of seed-dressing was evident though not significantly different from control (Tables 40, 41).

Plants treated with fungicides had significant (P=0.05), inhibitory effects on reduction in both *Fusarium* dry root rot (Table 44) or *Fusarium* 'yellows' (Table 45) disease development than control 72 days after planting and hence enhanced plant growth. Although most fungicide

treatments were not significant different in terms of effects on french bean growth or disease

development, plants treated with triforine, metalaxyl or thiram against *Fusarium* dry root rot (Table 44) or *Fusarium* 'yellows' (Table 45) had relatively better effects on french bean growth and disease reduction than other fungicide treatments or controls.

Plants treated with fungicides had significantly (P=0.05) shorter mean length of discoloured root tissues (MLDRT) or lower mean root rot indices (MRRI) 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Table 44) or *F. oxysporum* f.sp. *phaseoli* (Table 45) indicating suppressive effect on disease development. Significantly (P=0.05) more number of pods, heavier seeds dry weights, shorter mean lengths of discoloured root tissues and lower root rot indices were obtained from plants treated with triforine or metalaxyl than control 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Table 44) or *F. oxysporum* f.sp. *phaseoli* (Table 45).

In conclusion, better french bean growth and lower disease development on plants infected with *Fusarium* dry root rot or *Fusarium* 'yellows' were supported by triforine and metalaxyl , respectively 72 days after planting.

TABLE 40. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and mean root rot index[2] at development stage VI[3] 14 days after planting in soils inoculated with *F.solani* f.sp.*phaseoli*. Field test 1

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Triforine	16.6**	0.798	0.201	2.20	1.60
Metalaxyl	16.4	0.772	0.230	8.00	2.00
Thiram	18.3	0.700	0.181	11.20	2.80
Captan	17.2	0.723	0.168	10.80	2.80
Pyrazophos	15.4	0.653	0.103	12.20	3.00
Control	15.3	0.501	0.017	24.60	4.40
	Ns	Ns	Ns	Ns	Ns

*Means of five replicates.

**Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 41. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and mean root rot index[2] at development stage VI[3] 14 days after planting in soils inoculated with *F.oxysporum* f.sp.*phaseoli*. Field test 1

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Triforine	15.4**	0.626	0.096	6.00	2.00
Metalaxyl	16.8	0.658	0.106	2.50	1.80
Thiram	14.6	0.663	0.117	7.60	2.00
Captan	13.4	0.627	0.088	10.57	2.60
Pyrazophos	13.6	0.511	0.080	15.57	3.00
Control	13.2	0.222	0.051	26.60	4.60
	Ns	Ns	Ns	Ns	Ns

*Means of five replicates.

**Means followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 42. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and mean root rot index[2] at development stage R6[3] 36 days after planting in soils inoculated with F.solani f.sp.phaseoli. Field test 1

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Triforine	59.5**	14.50a	1.628a	32.50b	3.21b
Metalaxyl	54.5	9.32a	1.606a	30.00b	3.17b
Thiram	55.1	4.55bc	1.388a	42.67b	4.8ab
Captan	52.2	4.48bc	1.384a	42.50b	4.8ab
Pyrazophos	45.3	3.93bc	0.309b	45.00b	5.00ab
Control	35.0	2.31c	0.239b	80.00a	7.02a
	Ns				

*Means of five replicates.

**Mean followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 43. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and mean root rot index[2] at development stage R6[3] 36 days after planting in soils inoculated with *F.oxysporum* f.sp.*phaseoli*. Field test 1

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
Triforine	55.1	9.61ab**	1.564a	37.33b	3.41b
Metalaxyl	73.0	12.31a	1.624a	32.00b	3.33b
Thiram	67.1	10.21ab	1.617a	34.17b	3.81b
Captan	48.5	8.37b	1.533a	40.23b	4.63b
Pyrazophos	46.0	3.77c	0.377b	50.50b	6.00ab
Control	44.3	2.89c	0.293b	78.33a	7.50a
	Ns				

*Means of five replicates.

**Mean followed by the same letter within the same column are not significantly different at P=0.05 level by Duncan's Multiple Range Test.

TABLE 44. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and Mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F. solani* f.sp. *phaseoli*. Field test 1

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)	*Mean number of pods per plant	*Mean dry weight of 100 seeds (g)
Triforine	53.8**	16.698a	1.775a	40.33b	4.14c	20.0a	31.89
Metalaxyl	60.5	11.993ab	1.614a	41.20b	4.13c	20.0a	30.93
Thiram	52.0	6.752ab	1.462a	43.57b	4.60bc	18.0a	28.34
Captan	51.4	7.876ab	1.451a	46.5b	5.50abc	11.0b	29.55
Pyrazophos	43.8	5.032b	0.403b	59.6ab	6.25ab	5.0c	27.38
Control	45.2	5.173b	0.328b	89.3a	7.53a	5.0c	25.51
	Ns						Ns

*Means of five replicates

**Means followed by the same letter within the same column are not significantly different at P= 0.05 level by Duncan's Multiple Range Test.

TABLE 45. Effect of seed-dressing with different fungicides on growth of french beans LDRT[1] and Mean root rot index[2] at development stage R8[3] 72 days after planting in soils inoculated with *F. oxysprum* f.sp. *phaseoli*. Field test 1

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)	*Mean number of pods per plant	*Mean dry weight of 100 seeds (g)
Triforine	52.8**	10.14	1.571a	40.8b	4.00b	20.0ab	30.66
Metalaxyl	64.6	14.16	1.737a	37.6b	4.46b	23.0a	31.12
Thiram	64.0	11.69	1.576a	38.0b	4.60b	14.0bc	30.04
Captan	60.0	9.66	1.535a	45.6b	4.53b	9.ocd	30.16
Pyrazophos	49.9	6.27	0.444b	53.8b	5.63b	6.0cd	27.04
Control	49.1	6.12	0.442b	93.1a	7.86a	4.0d	25.39
	Ns	Ns					Ns

*Means of five replicates

**Means followed by the same letter within the same column are not significantly different at P= 0.05 level by Duncan's Multiple Range Test.

4.4.2. FIELD TEST 2.

Treatment of french bean seeds before planting with individual fungicides or fungicide combination had significant effects on shoot height, shoot dry weight, root dry weight, length of discoloured root tissues, root rot index, number of pods per plant and seed dry weight 14, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. phaseoli (Tables 46 to 51). Control plants had significantly (P=0.05) poor plant growth, higher length of discoloured root tissues and root rot indices than other plants.

Although no significant differences were observed between fungicide treatments, plants obtained from seeds treated with single fungicides had relatively better growth and low disease development than those obtained from fungicide combinations and control as indicated by heavier shoot and root dry weights, shorter mean lengths of discoloured root tissues and low mean root rot indices 14, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 46 to 51).

In contrast to the greenhouse test 3, the improved plant performance by fungicide combinations, th^{50} + cap^{50}, tri^{25} + cap^{75} or tri^{50} + cap^{50} on french bean growth, 14 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 46, 47) were inconsistent with the field test 2 results. Individual fungicide treatments supported plants that had relatively better bean growth and lower disease development than those treated with the three fungicide combinations or control 14, 36 and 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 46 to 51).

In general, triforine fungicide was the most effective in suppressing disease development and improving french bean growth than other fungicide treatments including controls. This fungicide, was slightly more effective in boosting pod production and increasing seed dry weights 72 days after planting and inoculation with *F. solani* f.sp. *phaseoli* (Table 50) or *F. oxysporum* f.sp. *phaseoli* (Table 51).

TABLE 46. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root[2] rot index at development stage VI[3] 14 days after planting in soils inoculated with *F. solani* f.sp. *phaseoli*. Field test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
$Th^{50}+Cap^{50}$	13.5a**	0.696a	0.118ab	11.5a	3.00ab
$Tri^{25}+Cap^{75}$	11.8a	0.554a	0.107b	13.5a	3.21ab
Thiram	13.8a	0.746a	0.103a	10.0a	3.00ab
Triforine	14.3a	0.826a	0.142a	4.14a	1.30b
Captan	13.8a	0.823a	0.118ab	13.2a	3.10ab
Control	13.5a	0.213b	0.074c	25.0a	5.00a

*Means of five replicates.

Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

TABLE 47. Effect of seed-dressing with different fungicides and fungicide combinations on growth ot french beans, LDRT[1] and mean root[2] rot index at development stage V1[3] 14 days after planting in soils inoculated with *F. oxysporum* f.sp. *phaseoli*. Field test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
$Th^{50}+Cap^{50}$	16.0ab**	0.521	0.113a	6.3b	2.10b
$Tri^{50}+Cap^{50}$	13.5b	0.352	0.099a	11.0a	3.00ab
Thiram	17.3ab	0.586	0.103a	7.5b	2.00b
Triforine	18.2a	0.675	0.117a	5.0b	2.00b
Captan	17.0ab	0.535	0.108a	10.0b	2.50ab
Control	15.0ab	0.289	0.077b	37.0a	5.50a
		Ns			

*Means of five replicates.

**Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

TABLE 48. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root[2] rot index at development stage R6[3] 36 days after planting in soils inoculated with *F. solani* f.sp. *phaseoli*. Field test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)
$Th.^{50}+Cap^{50}$	44.9**	3.690bc	1.367a	40.0ab	3.97b
$Tri^{25}+Cap^{75}$	42.8	2.195c	1.264a	55.0ab	5.20ab
Thiram	47.0	5.800bc	1.340a	40.1b	3.88b
Triforine	54.0	16.50a	1.633a	37.5b	3.81b
Captan	50.9	3.140bc	1.300a	41.8b	4.00b
Control	35.0	2.517c	0.200b	80.0a	7.21a
	Ns				

*Means of five replicates.

**Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

TABLE 49. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root[2] rot index at development stage R6[3] 36 days after planting in soils inoculated with *F. oxysporum* f.sp. *phaseoli*. Field test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot1 index[2] (1-9)
$Th^{50}+Cap^{50}$	45.9**	3.08	1.445a	57.0ab	5.41ab
$Tri^{50}+Cap^{50}$	44.2	3.28	1.442a	55.0ab	5.30ab
Thiram	59.0	9.41	1.533a	38.0b	3.92b
Triforine	52.1	10.00	1.600a	31.0b	3.51b
Captan	58.0	5.82	1.500a	41.0b	4.00b
Control	44.1	2.08	0.225b	86.1a	7.14a
	Ns	Ns			

*Means of five replicates.

Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

TABLE 50. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root[2] rot index at development stage R8[3] 72 days after planting in soils inoculated with *F. solani* f.sp. *phaseoli*. Field test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] (mm)	*Mean root rot index[2] (1-9)	*Mean number of pods per plant	*Mean dry weight of 100 seeds
Th^{50} +Cap^{50}	52.8**	8.58	1.478ab	97.7a	6.20a	8.0b	26.33ab
Tri^{25}+Cap^{75}	51.1	5.83	1.378ab	83.3a	6.69a	6.0b	23.55b
Thiram	51.9	10.67	1.496ab	46.7b	4.67b	17.0a	28.91ab
Triforine	54.7	17.85	1.692a	44.0b	4.00b	19.0a	31.49a
Captan	52.1	8.42	1.479ab	444.0b	4.40b	13.0ab	28.95ab
Control	40.8	5.21	0.228b	89.1a	7.33a	6.0b	25.43ab
	Ns	Ns					

*Means of five replicates.

Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

TABLE 51. Effect of seed-dressing with different fungicides and fungicide combinations on growth of french beans, LDRT[1] and mean root[2] rot index at development stage R8[3] 72 days after planting in soils inoculated with *F. oxysporum* f.sp. *phaseoli*. Field test 2.

Treatment	*Mean shoot height (cm)	*Mean shoot dry weight (g)	*Mean root dry weight (g)	*Mean L.D.R.T[1] . (mm)	*Mean root rot index[2] (1-9)	*mean number of pods per plant	*Mean dry weight of 100 seeds
Th^{50}+Cap^{50}	51.0**	6.52	1.483a	88.8a	7.38a	8.1b	25.96ab
Th^{50}+Cap^{50}	51.0	4.92	1.305a	87.1a	7.50a	4.0c	20.36b
Thiram	63.3	11.97	1.546a	40.1b	5.00b	18.0a	29.70a
Triforine	56.0	11.16	1.628a	42.5b	4.00b	20.0a	31.42a
Captan	60.5	10.89	1.670a	47.5b	4.30b	9.0b	30.73a
Control	42.0	5.99	0.301b	96.1a	7.97a	4.0c	24.46ab
	Ns	Ns					

*Means of five replicates.

**Means followed by the same letter within the same column are not significantly different at P = 0.05 level by Duncan Multiple Range Test.

CHAPTER 5.

Discussion, Recommendations and Conclusion.

5.1 DISCUSSION.

The relatively low occurrence of root rot fungal pathogens (Table 6) in the areas sampled could be attributed, in part, to variations in rainfall distribution (Buruchara, 1990). The roots and soil samples were collected from crops grown during the short rains, and reduced rainfall has been found to limit the development of root rot (Buruchara, 1990; Tu *et al.*, 1992). The severity of root rot is also influenced by variations in temperatures (BuerKert and Marschner, 1992), an indication that higher fungal inocula could be obtained in the same regions if environmental conditions were more conducive. Experiments to quantify the relationship between climatic factors and the occurrence of root rot disease may clarify this hypothesis. Among the isolates obtained, *Fusarium* spp. were recorded in most of the samples from the two districts, an indication that this species was predominant and widespread. Similar results have been reported elsewhere (Buruchara, 1990). The occurrence of *F. equisite* at relatively greater frequencies than *F. solani* and *F. oxysporum* is interesting and may be due to soil contamination. Intercropping of maize and french beans is a common practice particularly in Meru district where the samples were collected, and the fungus (*F. equisite*) has been associated with maize and beans in many places (Richardson, 1979).

The significantly (P=0.01) high mean root rot index obtained from plants treated with *F. solani* f. sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* (Table 7, 8 and 9) reveals that these fungi are equally pathogenic to french beans. Although high fungal inoculum levels were used in this study much lower inoculum load has been found to exhibit the same root rot symptoms (Buruchara, 1990). The high infection levels achieved with these two pathogens elsewhere (Buruchara, 1990) and in this study (Table 7 and 9) regardless of the inoculum density is a clear indication that *Fusarium* spp. is a destructive pathogen of french beans world-wide.

The relatively longer length of discoloured root tissue obtained from plants infected with *F. solani* f.sp. *phaseoli* than those infected by *F. oxysporum* f.sp. *phaseoli* at developmental stage V1 may be attributed to their inherent variations in the mode of infection (Bailey, 1991). *F. solani* f. sp. *phaseoli* is known to infect bean plants directly, through stomata and wounds, whereas, infection by *F. oxysporum* f. sp. *phaseoli,* occurs mainly through wounds (Christou and Snyder, 1962; Ribeiro and Hagedorn, 1979a). Therefore, it appears that the reduced pathogenicity of *F. oxysporum* f.sp. *phaseoli* at developmental stage V1 (Table 7) was limited by its inability to penetrate unwounded root tissue. Pathogenicity test reveals that the bean development stage is crucial in determining the appropriate time of disease control. *Fusarium* dry root-rot was much severe at development stage V1 whereas, the severity of *Fusarium* yellows increased with maturity. Thus, disease control strategy should be more durable in order to cope up with disease development in the soils. The results generally support the concept that most *Fusarium* spp. associated with crown and root rot of legumes lack the ability to initiate root-rot on their own (Leah and Kendall, 1978).

Although *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* vary in their modes of infection (Bailey, 1991), the two fungal pathogens caused root rot on beans in all tests (Tables 7, 8 and 9), and there were no significant differences in the root-rot indices between plants infected by the two

fungal species (Tables 7, 8 and 9). This is an indication of similar levels of pathogenicity between the two fungal pathogens. These findings are further confirmed by the non significant differences in % yield loss. The number of pods produced per plant and % loss in grain dry weight between plants infected by *F. solani* f. sp. *phaseoli* and *F. oxysporum* f. sp. *phaseoli* were similar (Table 9) in both cases. The number of pods produced per plant and dry weight of seeds decreased with the increase in root-rot severity (*F. solani*, r= 0.957 pods, r= 0.965 dry weight; *F. oxysporum* , r=0.992 pods, r=0.982 dry weight, (Fig 2 and 3), an indication that root rot affected pod set and seed size regardless of the pathogen (Table 10). Similar findings were also found by Gorfu (1993).

Although the two fungi used in the current study appear to be mildly destructive on the french beans under the pot experimental conditions (Table 9) they cause considerable reduction in pod production and grain dry weight in the field (Gorfu, 1993). Even though the greenhouse results do not reflect the exact extent of damage caused in the field, the data supports earlier findings that *Fusarium* spp causes significant reduction in pod production and grain dry weight (Leakey, 1970a; Mughogho, 1970; Mukunya, 1974).

Effects of fungicides on fungal growth.

The significantly (P=0.05) short mycelial diameters and high mycelial inhibition % obtained from Petri dishes treated with single fungicides and fungicide combinations, when compared to control, indicate that fungicide treatment significantly inhibited mycelial growth of *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* (Tables 11, 12, 14, 15). However, the relatively higher inhibitory effects of fungicides on the growth of *F. oxysporum* f.sp. *phaseoli* than that of *F. solani* f.sp. *phaseoli* suggests that *F. oxysporum* f.sp. *phaseoli* was more sensitive or susceptible to fungicide treatments. Such differences may reflect inherent variations in accessibility of the active toxicants within the fungal systems (Lukens, 1971). Variations in responses of the pathogens of the same

species to chemical treatments is an attribute that could be exploited in Pest Management Programmes (**IPM**), particularly at gene level. Pathogenic isolates could be manipulated and made more susceptible to chemical treatment.

The lack of statistical differences between day 3 and day 7 of fungicide treatments on mycelial diameters and inhibitory effects indicates that all the fungicide treatments maintained their level of potency throughout the test period. This consistency in potency underscores possible value of these fungicides in possibly controlling initial inoculum of the pathogen. However, the effect of time (age) should not be underestimated, since age has been found to increase or decrease sensitivity of fungal cells to toxicants (Oster, 1934; Dimond and Dugger, 1941; Dimond *et al.*, 1941).

The relatively high inhibitory effect of triforine on mycelial growth of *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* might have been, in part, due to the ability of this fungicide to inhibit ergosterol biosynthesis (Sherald and Sisler, 1973; Sherald *et al.*, 1973; Carlile, 1988). Sterols have two major roles in fungi (Hendrix, 1970). Firstly, they are major structural components of cell membrane where they contribute to their selective permeability properties. In the absence of sterols, vegetative growth is much reduced and in some cases non-existent, and the fungal cells tends to be leaky (Child *et al.*, 1969). Exogenous sterols are an absolute requirement for reproductive growth (whether sexual or asexual) (Nelson *et al.*, 1967). Similarly high inhibitory effect of fungicide combination, th^{50} + cap^{50} in inhibiting mycelial growth of the two test pathogens might, in part be attributed to multi-site inhibitory effect or cumulative effects of the integrated different modes of action of the different fungicides (Carlile, 1988). The exact mode of action of this fungicide combination is, however, unknown and may need further investigation.

Effects of fungicides on spore germination and sporulation.

The significantly (P=0.05) low germination of *F. oxysporum* f.sp. spores *phaseoli* obtained from dishes treated with single fungicides and fungicide combinations indicated that fungicides inhibited germination. Fungicides suppress spore germination by targeting on one or more of the biochemical or physical processes responsible for spore germination (McCallan, 1930), an attribute that makes them important tools in pest management. It is not known if fungicides can also suppress spore germination through other means and this may require further investigation.

Spore germination of *F. solani* f.sp. *phaseoli* was completely inhibited by all the fungicides, however spores of *F . oxysporum* f.sp. *phaseoli* germinated in some fungicides. This indicates that spores from *F. solani* f.sp. *phaseoli* were more susceptible to fungicide treatments. This can be ascribed to inherent differences in accessibility of the active toxicants between the two fungal pathogens.Variations in percentage spore germination of both fungal species from the time of inoculation (Tables 13, 16) could be ascribed to changes in the quality and quantity of fungicide decomposition products. Decomposition products enhance but can also reduce fungal activity. However, the breakdown of triforine in water or plants does not appear to give rise to such antifungal products (Fuchs and Ost, 1976).

The ability of the two fungi to sporulate in plates treated with fungicides was not un-usual as Griffith (1981) and Biratu *et al.* (1990) found a higher conidia production of *C. coffeanum* on fungicides treated berries than untreated. This was attributed in part, to low growth rate, nitrogen depletion (Righelato *et al.*, 1968), temperature and oxygen (Anderson and Smith, 1971).

The suppressive effect of metalaxyl + mancozeb on sporulation of *F. oxysporum* f.sp. *phaseoli* or *F. solani* f.sp. *phaseoli* might be due to the existence of multi-site action of this fungicide mixture (Gozzo *et al.*, 1984). This fungicide suppresses synthesis of nucleic acid (RNA) (KerKennar, 1981) and also inactivates enzyme function (Davisde, 1981; Carlile, 1988). The fungitoxicity of metalaxyl is a property of ester, since the free acid is not active (unpublished data of Pinkas and Edgington cited by Edgington, 1981).

Effects of fungicides on root-rot and french bean development in greenhouse and in the field.

Efficacy of fungicides or fungicide combinations in controlling root rot caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* in the greenhouse and in field tests was indicated by their ability to reduce infection (MLDRT, MRRI) and increase yields. The enhanced plant growth and reduced root rot infection by the two pathogens with fungicide treatments indicate that these fungicides enhanced plant growth and suppressed fungal infectivity than in controls. The effects of fungicide treatments on root infection and plant growth may be attributed to the direct impact of these compounds, their breakdown products or both.

The high plant growth and pod yield associated with triforine or metalaxyl treatment in the greenhouse and field tests could be ascribed to the ability of these fungicides to delay and (or) impair the infection process by these two fungi. Despite the high *in-vitro* potency of th^{50} + cap^{50} combination, its effects appear to have been diluted under greenhouse and field conditions. This may be attributed to the short life span of this fungicide mixture and / or breakdown of the active compound(s) into a non-potent product(s). However, further investigations should be done to ascertain whether adjusting the rates would maximise plant performance at the same time

improving disease control while using less chemicals. This fungicide mixture has opened new horizons and posed a challenge to the fungicide manufacturers to formulate a new fungicide that would mimic the combined modes of action of these two fungicides, in addition to, maintaining low cost for the sake of the resource poor farmers in the developing countries. Finally, it is important to select chemicals that have multiple disease and (or) pest control attributes to realise total benefit in agriculture.

Effects of organic matter on french bean growth and root rot development.

The superior performance of plants treated with cowdung compared to plants treated with *T. Diversifolia* may be a reflection of differences in nutrient contents of the materials (Table 3). The differences in nutrient quality and quantities must have also influenced the differential disease development among cowdung or *T. diversifolia* plants observed in this study.

The high (P=0.05) plant growth and pod yield associated with cowdung treatments on both *Fusarium* dry root rot and *Fusarium* 'yellows' infected plants may be ascribed to the ability of cowdung to promote plant growth. Host resistance might have resulted from enhanced host nutritional status. The disease escape phenomenon may also have played a role, particularly in the presence of phosphorus which is known to facilitate rapid root development (Clark, 1942; Ko and Kao, 1989). These possibilities, and the actual role of cowdung on pathogenicity of *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* need further investigation.

The poor (P=0.05) plant growth and high mean root rot index associated with *T. diversifolia* on plants infected by *F. solani* f.sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* may indicate that decomposition products of *T. diversifolia* were phytotoxic on plant growth and stimulated fungal

infection than other treatments. The stimulatory effects of *T. diversifolia* might have been due to formation of decomposition products containing Ammonium compounds. The ammonium compounds have been found to enhance pathogenicity of *Fusarium oxysporum* (Barna *et al.*, 1983; Sarhan and Sharrif, 1983). These effects, however, need further investigation.

Although cowdung was less effective than metalaxyl or combination of cowdung with metalaxyl in reducing *Fusarium* dry root rot or *Fusarium* 'yellows' infection , it would still be desirable in root rot control programme because of its stimulatory effect on plant growth. In addition, the use of organic matter in farming systems does not require special technology or expertise (Owino and Waudo, 1994). Furthermore, cowdung is widely available in Kenya and would be more economical than the toxic chemicals. Although cowdung may compete with fungicides, this is outweighed by the cost effects of these fungicides. Cowdung was less toxic and resulted in better plant growth than *T. diversifolia* or fungicide treated plants. These attributes might make it more attractive for use in Integrated Pest Management (**IPM**) programme when compared to *T. divesifolia* or metalaxyl fungicide. In contrast to chemical control, the action of organic matter against target pest is not rapid, but they provide more stable and long lasting control (Barker and Cook, 1974). Integration of organic amendments and chemicals would be safer, cheaper and more effective (Owino and Waudo, 1994). Further experiments, however, need to be conducted to evaluate the effectiveness of such integration under different conditions.

Effects of delay in planting after application of cowdung and *T. diversifolia* in soils on growth of french beans and root rot development.

Cowdung and *T. diversifolia* influenced the pathogenicity of *F. solani* f. sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* differently. Disease suppression occurred soon after amendments with *T.*

diversifolia in soil infected with *F. solani* f. sp. *phaseoli* or *F. oxysporum* f.sp. *phaseoli* (Tables 37 and 38). However, suppression of *Fusarium* dry root rot and *Fusarium* 'yellows' diseases after amendment with cowdung increased with increase in the decomposition period from 0 to 4 weeks.

The high MRRI, long MLDRT and poor seed germination in soil amended with *T. diversifolia* indicate that this treatment had a stimulatory effect on fungal development and / or was phytotoxic to seed germination and plant growth. *Fusarium* dry root rot and *Fusarium* 'yellows' were made more severe by *T. diversifolia* with time. These results suggest that time allows either for removal and or change of decomposition product from amended soils thus increasing pathogen aggressiveness (Lumsden *et al.*, 1983b). In addition, the concentration and levels of toxic products from decomposing organic amendments change with the decomposition period (Sayre *et al.*, 1965; Johnson, 1974; Owino and Waudo, 1996). Perhaps these long term effects might also apply to diseases caused by *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* which were decreased by cowdung. Although *T. diversifolia* suppressed french bean germination and supported poor plant growth, these does not rule out its possible use in integrated pest management (**IPM**) programmes and perhaps its beneficial role in suppressing other plant diseases.

The ($P=0.05$) low mean root rot index, short length of discoloured root tissues (MLDRT) and high pod yield from soil amended with cowdung indicate that this treatment had an inhibitory effect on fungal development and / or improved plant growth. The increase in french bean plant performance, low mean root rot index and short mean length of discoloured root tissues with delay in planting could be attributed to changes in the quality and quantities of decomposition products and / or nutrients that enhanced nutrition (Sayre *et al.*, 1965). However, whether cowdung used in this study stimulated plant growth and / or inhibited fungal activities is not clear.

In general, only th^{50} + cap^{50} retarded fungal growth decisively followed by triforine, metalaxyl and thiram. Although spore germination was also reduced by some of these fungicides, triforine and thiram enhanced sporulation. This indicates that the fungicides suppressed root rot mainly through the inhibition of the developing fungus. The low MLDRT might be attributed to this phenomena. Reduced cell invasion due to chemical treatment was also reported by (Biratu *et al.,* 1990). The significantly low sporulation, germination and disease development associated with fungicide combination suggests that the use of fungicides with different modes of action in spray and seed dressing programmes may reduce and counteract selection pressure that may be exerted on pathogens (Carlile, 1988). It is for this reason that fungicides have also been marketed as mixtures for the control of a wide range of diseases (Carlile, 1988), and this trend is increasing. For instance co-formulation and reliant of metalaxyl in mixture with the protectant fungicide mancozeb for control of downy mildew and potato blight have proved successful (Carlile, 1988). Therefore, the excellent performance of mixture th^{50} + cap^{50} in suppressing *in-vitro* growth of the two root rot fungal pathogens (Tables 14 and 15) is not unusual. Intensive studies are currently needed to outline the exact mode of action these two fungicides were able to complement one another in this fungicide mixture.

5.2 Recommendations.

Fungicides were able to control root-rot of french beans in the greenhouse and field experiments, but they would be of more value to farmers when used in an integrated pest management (**IPM**) program. There efficacy in an **IPM** set up need to be evaluated with regards to cost and benefits.

Although the efficacy of fungicide mixtures was lower in most cases, its use should be encouraged. Fungicide mixtures are useful in checking against the development of resistant strains (Carlile,

1988). Further tests to evaluate efficacy of more fungicide mixtures against root-rot of french beans need to be conducted.

Cowdung suppressed *Fusarium* root-rot and increased plant performance in sterile greenhouse soils, but the results may not give a reflection of what would be attained in the field. It is essential that additional tests be carried out in natural soils to subject this soil amendment to natural decomposition processes. In addition, more field tests should be conducted to determine the role of cowdung in enhancement of antagonistic micro-organisms or enhancement of competition between the micro-organisms and *Fusarium* root-rot fungi. Without such information, it would be difficult to effectively rank cowdung with other potential organic amendments for the management of *Fusarium* root-rot.

Although this study reveal that chemicals have a positive potential in management of *Fusarium* root-rot other more viable alternative measures for disease control should be developed particularly with the use of resistant cultivars and biological control agents. This is because of their potential in sustainability and durability.

5.3 Conclusion.

Results from this study reveal that triforine and metalaxyl were the most effective in reducing growth, spore germination of the two test fungi and infection of beans with *F. solani* f.sp. *phaseoli* and *F. oxysporum* f.sp. *phaseoli* in either *in-vitro*, greenhouse and field tests. Although fungicide combination th^{50} + cap^{50} gave the best results *in-vitro*, its effect in the greenhouse and field tests may have been reduced by environmental factors.

Cowdung was the most superior in enhancing french bean growth. Root-rot index and length of discoloured root tissue were significantly low in soil amended with cowdung than *T. diversifolia.* Delaying the time of planting french beans in soils amended with cowdung significantly reduced root-rot index, whereas the opposite was true for plants grown in soil amended with *T. diversifolia.* The treatments might have acted on the fungi directly, however, more work on fungi-organic amendment interaction is, therefore, needed in order to fully understand this relationships for an effective integrated root-rot control.

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