THE EFFECT OF VESICULAR – ARBUSCULAR MYCORRHIZA AND RHIZOSPHEREPSEUDOMONADS ON CHICKPEA WILT IN ZAMBIA.

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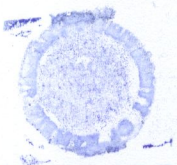
by

BERNARD KAKUMBI, B.Sc. Ed (UNZA)

A DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN PLANT PATHOLOGY



UNIVERSITY OF ZAMBIA

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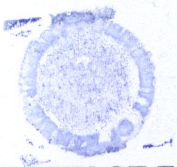

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DECLARATION

I, BERNARD KAKUMBI hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other university.

.....
Signature

17/03/2011
.....
Date

APPROVAL

THIS DISSERATATION BY BERNARD KAKUMBI IS APPROVED AS FULFILLING THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF PLANT PATHOLOGY BY THE UNIVERSITY OF ZAMBIA.

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DEDICATION

To my wife Mwangala, for introducing me to the truth, loving me unfailingly and enduring my absence from our home.

To my children Mulenga, Mubita, Mwamba and Anita for their patience when I was away from them at the time they needed me most.

To Jehovah God who created me.

So be it.

ABSTRACT

The effect of vesicular arbuscular mycorrhiza (VAM) and rhizosphere pseudomonads on chickpea (*Cicer arietinum* L.) wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Matuo and Sato) in Zambia was investigated. Vesicular arbuscular mycorrhiza was isolated from three soils obtained from Kasama area of Lusaka. Rhizosphere pseudomonads were isolated from a chickpea farm in the same area. Physical and chemical characteristics of the soils were analyzed. Both VAM and pseudomonads were characterized and identified.

The mycorrhizal flora of the three soils consisted of *Glomus etunicatum, Gigaspora nigra* and *Acaulospora scrobiculata.* This composite mycorrhizal flora and rhizosphere pseudomonads were used to determine their effect on fusarium wilt of chickpea. Their effect on growth and productivity of the crop was also investigated. The study was carried out between December 2006 and April 2007 in a greenhouse at the School of Agriculture of the University of Zambia.

The VAM flora was amplified for a period of eight weeks by planting surface sterilized seeds of maize variety MMV 600 in heat sterilized soil. Vesicular arbuscular mycorrhiza soil inoculums was mixed in proportions of 3 sterilized soil to 1 VAM soil portion (1 portion equals 1.5kg amplified VAM soil) for use in two of the four treatments. A complete randomized block experimental block design was used with four treatments and four replications. Surface disinfected SPGR-4869 chickpea seeds were planted in the soil mixtures in plastic pots in a greenhouse. The four treatments consisted of the following:

A. A combination of fusarium wilts pathogen, sterilized soil and chickpea seeds.
B. Fusarium wilt pathogen, pseudomonads, sterilized soil and chickpea seeds.
C. Fusarium wilt pathogen, VAM, sterilized soil and chickpea seeds.
D. Fusarium wilt pathogen, pseudomonads, VAM, sterilized soil and chickpea seeds.

A conidial suspension of 2 cm^3 containing 5.07 x 10^4 micro conidia of *Fusarium oxysporum* f.sp. *ciceri* were inoculated per plant at the time of sowing. For treatments C and D colony forming

units (cfu) of 10^5 of pseudomonad bacterial extract (turbidity 27.20 mg/L at 430nm) was inoculated per plant. The plants were watered with tap water at intervals of 48 hours.

Chickpea plants were examined at fifteen days intervals from 15 to 90 days after sowing (DAS) to measure plant height, the number of leaves, flowers and pods per plant. At 90 (DAS) seed weight per plant, fresh shoot weight, fresh root weight and disease incidence were recorded.

The results show that there was 52% reduction of fusarium wilt in chickpea inoculated with VAM and pseudomonads (dual inoculation) compared to the control. Single inoculation of VAM showed 50% reduction of fusarium wilt in chickpea plants. The analysis of variance (ANOVA) of the measured parameters indicated that the dual inoculation (VAM and pseudomonads) enhanced growth and development of chickpea plants by 16% compared to the control and produced taller plants with more leaves, flowers and a high shoot and root weight than either of the single inoculation of VAM or pseudomonads.

Results of the best subset regression analysis of climatic characteristics (independent variables) on mean numbers of growth, productivity and disease incidence parameters (dependent variables) revealed that the most important climatic characteristic that accounted for most of the observed variation was the photoperiod which was responsible for the high temperature in the greenhouse.

ACKNOWLEDGEMENTS

I greatly and sincerely thank my supervisors, Prof R.G. Kapooria and Prof. J.N Zulu. The former, for his unwavering patience, firm but constructive criticism, his wonderful selection of words of advice and the photographs of the potted plants. The later, for his guidance during the course of study and in particular for suggestions that encouraged me to go on. Very special thanks go to Prof. K. Mbata for his assistance in the statistical analysis of this work.

I would also like to thank Mr. H. Zulu for his technical assistance and co-operation he offered while I worked in the Plant Pathology Laboratory.

Further thanks go to the members of the Plant Protection Department at Mt. Makulu, especially Mr. G. Kaula and Mr. H. Tembo for their technical assistance in the laboratory.

Finally, I thank all other individuals, too numerous to mention, including friends who directly or indirectly played a part to the success of this research, however small. Bravo to you all.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
C.V	Coefficient of variation
BS	Bonferroni Significance
d.f.	Degree of freedom
DAS	Days after Sowing
FAO	Food and Agriculture Organization
Fpr	Frequency probability
Ms	means of square
PDA	Potato Dextrose Agar
PDI	Percent Disease Incidence
Ppm	Parts per million.
VAM	Vesicular-arbuscular mycorrhiza
Photoperd	Photo period
Ps	Pseudomona
ss	sum of squares
PGPR	Plant growth promoting Rhizobacteria

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I. CHAPTER ONE

A Introduction

Chickpea (*Cicer arietinum* L.) is the third most important pulse crop in the world. It is grown on a large scale in Asia, South America and Africa. In Zambia it is grown in isolated areas in the Eastern Province, particularly in the Districts of Petauke, Chipata and Lundazi. A little is also grown in Southern, Lusaka and the Copperbelt Provinces. It is a drought tolerant herbaceous crop planted during the cool dry season in Asia and Africa. After germination, it develops a taproot and an extensive lateral root system that usually bears numerous nodules. It plays a vital role in many farming systems because it fixes atmospheric nitrogen into the soil through symbiosis with rhizobium (Singh and Saxena, 1999).

The types of chickpea commonly grown in Africa are "Kabuli" and "Desi". These types have a maturation period of 85 to 110 days, depending on the cultivar. The crop does well in cool dry weather. For this reason, it is grown from the months of April to July in the African Southern hemisphere and September to November in the northern hemisphere (Singh and Saxena, 1999).

The chickpea plant has an indeterminate and branched growth habit. Plants may be erect or spreading. They have compound leaves, which are pubescent in appearance. The chickpea plant leaflets are ovate, elliptical or obovate and have serrated margins. Flowers are self

pollinated, solitary and cleistogamous (Nene *et al.,* 1981). The flowers are 1.5cm to 2cm long and come in purple, white, pink or blue colour depending on the variety. Each flower produces a short pubescent pod which is 1.5cm to 2.5cm long and appears inflated and encloses one or two large seeds. The number of pods varies from a few to over 300 per plant. The seeds vary in colour (white, red, brown or black) and shape. Chickpea has a deep tap root system.

The nutritional value of chickpea is high. It contains 23.0% protein, 6.3% oil, 63.5% carbohydrates and 6.3% fibre (Summerfield and Robert, 1985). Therefore, it provides a fair amount of protein in the diets of those who are vegetarian and those who cannot afford expensive animal protein. The digestibility of chickpea protein is estimated to be 76-90%, which is one of the highest among pulses (Haware and Nene, 1982). Chickpea is unique among the legumes because it combines well with all kinds of cereal-dominated diets. It is used in the preparation of many more dishes than any other food legume. Because of its nutritive value, chickpea is a potentially important crop. Its cultivation is receiving increased attention in Zambia (Nkhoma, 2006: personal communication).

Chickpea is attacked by more than 52 pathogens, some of which are *Fusarium oxysporum* f. sp. *ciceri, Ascochyta rabiei, Botrytis cineria* and Leaf roll virus (Nene *et al.,* 1981). Principally, rainfall and temperature determine the distribution and importance of chickpea diseases. Collectively, plant diseases are probably the main cause of the instability that characterizes the yields of the chickpea crop. One of the major diseases of chickpea is Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *ciceri.* Fusarium wilt of chickpea is

widespread and occurs in India, Burma, Russia, USA, Mexico, Peru, Tunisia, Ethiopia, Malawi and Zambia (Summerfield and Robert, 1985). Crop losses due to diseases may be up to 80% in Africa and 10% - 15% in India (Nene, 1980).

Fusarium oxysporum f. sp. *ciceri* is a soil-borne pathogen and can survive for more than four years in organic matter (Nene, 1980). It is a systemic pathogen and has been isolated from all parts of infected plants, including seeds. The symptoms of fusarium wilts become visible on the chickpea plant after twenty-one to thirty days from sowing. The leaves and the leaflets nearest to the ground turn grey-green and then progressively become chlorotic from the leaf edges inwards (Haware and Nene, 1982).

The infection then progresses upwards on a plant. The infected plant gradually becomes dull yellow, weak and finally wilts and dies. The etiology of this pathogen shows that it consists of branched, septate and hyaline mycelium. On an artificial medium the mycelium turns rose yellow or purple (Booth, 1977). Fusarium also infects other grain legumes, such as pigeon pea, lentils and pea (Haware and Nene, 1982). Once established in any soil, it is difficult to eradicate *F. oxysporum* f. sp. *ciceri* due to its soil-borne nature and the ability to survive in the soil. Crop rotation is relatively ineffective in eradicating this pathogen (Haware, *et al.*, 1978 and Nene, *et al.*, 1980).

Mycorrhiza is a symbiotic association of fungi and roots of higher plants (Azcon-Aguilar and Barea, 1996). They are either endomycorrhiza and classified as Zygomycetes or

ectomycorrhiza and classified as Basidiomycetes (Sieverding, 1991). Vesicular-arbuscular mycorrhiza (VAM) is the most common type of association of crop plants in the tropics. Hackskaylo (1972) states that mycorrhizal relationships with plant roots are physiologically balanced reciprocal parasitism. The reproductive structures of VAM are the zygospores, which are the largest spores among the fungi with a very high survival potential. Vesicular arbuscular mycorrhiza can survive and retain infectivity in infected root fragments, clumps of hyphae or mycelium in the soil for at least one year (Hayman, 1982).

The presence of mycorrhiza in plant roots contributes to plant's well being in many ways. It enhances absorption of mineral ions by plant roots and the fungus also benefits by obtaining soluble organic nutrients from the soil. Vesicular arbuscular mycorrhiza fungi are widely distributed in soil. They are inter and intracellular and form finely branched arbuscles. They also form apical or intercalary large food storing swellings called vesicles. The infective propagules of VAM may be spores, sporangia or vesicles occurring in the previously infected root residues (Stan, 1981). Several genera of VAM exist and are known as *Acaulospora, Entrophospora, Gigaspora, Glomus, Sclerocystis and Scutellospora* (Sieverding, 1991).

The colonization of roots by VAM fungi is known to increase plant growth and vigour and plant tolerance to many root-borne diseases (Dodd and Thomson., 1992; Linderman, 1994 and Sieverding, 1991).

The rhizosphere is a zone surrounding the immediate vicinity of roots where microbial communities abound and exist in a dynamic state. It is a heterogeneous continuous natural

zone in which many interactions occur between soil microbes and plant roots. The beneficial plant-microbe interactions occurring in the rhizosphere are the primary determinants of plant health and soil fertility (Jeffries *et al.,* 2003). Arbuscular mycorrhizal fungi are one of the most important and common microbial symbionts for the majority of plants. Under phosphate-limited soil conditions, VAM fungi influence plant community development, nutrient uptake, water relations and above ground plant productivity. They also act as bioprotectants against pathogens and enable plants to stand toxic stresses (Jeffries *et al.,* 2003). Vesicular arbuscular mycorrhiza also interacts with different kinds of bacteria in the rhizosphere. These interactions can occur at all stages of the VAM life. Vesicular arbuscular mycorrhiza is active from spore formation and germination to root colonization and to the production of external hyphae (Bianciotto & Bonfante, 2002; Bianciotto *et al.,* 1996, Toljander *et al.,* 2006). The nature of these interactions may be inhibitory or stimulatory, competitive or mutuality to each other. This can affect plant physiology differently.

Different functional groups of bacteria, such as N_2-fixing bacteria (Secilia & Bagyaraj, 1987), plant growth-promoting rhizobacteria (von Alten, Lindermann & Schonbeck, 1993), phosphate-solubilizing bacteria (Toro *et al.,* 1996) exist together with antagonists of plant pathogens (Citernesi *et al.,* 1996; Budi *et al.,* 1999). This shows the diversity and richness of the rhizosphere of different plants colonized by VAM. Some bacteria have also been found to be associated with VAM fungal structures such as external hyphae (Toljander *et al.,* 2006), spores and spore walls (Mayo *et al.*, 1986). Bacteria have also been reported to live inside the spores of certain VAM fungal isolates (Bianciotto *et al.,* 1996). Thus a variety of fungi and bacteria occur in the rhizosphere. Of the bacteria occurring in the rhizosphere, those that belong to the Family *Pseudomonadaceae* are reported to confer a growth-promoting effect on

plants (Pandey and Upadhyay, 2000). The number of pseudomonads has been found to be many times higher than the non-rhizosphere microorganisms. The increase in microbial number and their activities are referred to as the 'rhizosphere effect' (Schippers *et al.*, 1987). This effect is caused by growth promoting substances present in the root exudates of the plant (Schippers *et al.*, 1987).

The members of the bacterial Family *Pseudomonadaceae* consist of gram–negative, polarly flagellated, straight or curved aerobic rods that do not form spores. The metabolic and physiological properties of pseudomonads are simple. In their metabolism they use a wide range of sugars, both aromatic and heterocyclic which other genera of bacteria do not use. Pseudomonads therefore, are ubiquitous because they have very simple requirements for their sustenance. Many pseudomonads can be easily recognized by their production of water-soluble-yellow-green fluorescing pigments like Pterin (Suslow, 1982). Pseudomonads exist in chickpea rhizosphere and the majority of them are non-pathogenic and strongly antagonistic to fungi (Dube, 2001). The rhizosphere antagonistic pseudomonads have been shown to increase plant tolerance to root pathogens (Sharma and Champawat, 2004).

Fusarium wilt is a serious disease of chickpea (Nene *et al.*, 1981). Its causal pathogen is soil-borne and difficult to eradicate if it is already established in the soil. Its management through crop rotation has proved ineffective (Singh and Saxena, 1999). Vesicular arbuscular mycorrhiza has been reported to improve plant growth and productivity (Harley and Smith, 1983). Rhizosphere pseudomonads have been found to suppress Fusarium wilts of chickpea (Yuen *et al.*, 1985). The occurrence of pseudomonads in the myco-rhizosphere of chickpea

and its effect on fusarium wilt has not been studied in Zambia. The present study was undertaken to investigate the effect of pseudomonads and VAM on chickpea plant growth and the incidence of fusarium wilt in chickpea grown in a greenhouse.

A. Objectives

The objectives of the study were to investigate, under greenhouse conditions, the effect of:

1. Vesicular arbuscular mycorrhiza (VAM) on fusarium wilt of chickpeas.
2. Pseudomonads on fusarium wilt of chickpea plants.
3. A combination of VAM and pseudomonads on the incidence of fusarium wilt in chickpeas.
4. Vesicular arbuscular mycorrhiza and pseudomonads on growth and productivity of chickpea plants.

II. CHAPTER TWO

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A. REVIEW OF LITERATURE

1. Influence of Vesicular arbuscular mycorrhiza on Plant Growth and Yield.

Two relationships occur naturally between higher plants and soil microorganisms. These include mycorrhiza and rhizosphere microorganisms (Schippers *et al.*, 1987). Of these, mycorrhizal association is concerned with the uptake of nutrients. Among these nutrients, phosphorus is the most important element (Crush, 1974). It has been generally recognized that plant growth is considerably improved if plant roots are colonized by VAM hyphae (Suslow, 1982). Vesicular arbuscular mycorrhiza hyphae explore the soil outside the nutrient depletion zone and absorb phosphorus ions from the rhizosphere soil and transfer them to plant roots and subsequently to the whole plant system (Hayman and Mosse, 1972). It is reported that inoculation of plant roots with VAM fungi can stimulate nodulation and nitrogen fixation by legumes (Abbott and Robson, 1977; Hayman, 1980). Vesicular arbuscular mycorrhiza fungi are beneficial to plants and improve plant nutrition and growth especially in phosphorus deficient soils (Harley and Smith, 1983). In addition, arbuscular mycorrhizal fungi also improve plant growth, increase hormone production and make it easier for bacteria to fix atmospheric nitrogen (Hayman, 1986). Wright *et al.,* (1998) reported that plants with VAM attain consistently higher rates of photosynthesis and exhibit a higher specific leaf area compared to non-mycorrhizal plants. Mycorrhiza is also reported to increase seed production in soybean, barley, tomato and oats (Stanley *et al.*, 1993 and Koide *et al.*, 1988). Dwivedi (2004) reported that winter wheat plants inoculated with *Glomus*

mosseae resulted in increased growth. He also reported that all growth parameters of wheat plants studied were statistically higher in VAM inoculated than in control plants.

A. Disease Control

Methods of control of soil-borne diseases are variable and include crop rotation, use of certified seed and resistant plant varieties and direct control with fungicides or soil fumigation. However, many problems are associated with controlling pathogens with persistent survival structures due to difficulties in reducing pathogen inoculums and lack of good sources of plant resistance. Soil fumigants are not allowed in certain countries; where they are used, the most commonly used compound is methyl bromide which is highly toxic and depletes the stratospheric ozone layer (Gan *et al.*, 1997). This has stimulated research in trying to find alternative methods of control of soil-borne diseases. Manipulation of soil microorganisms has been found to enhance plant protection from many soil-borne pathogens (Grosch *et al.*, 2005). This method enables the treatment of seeds as well as soil. The beneficial microorganisms are antagonistic bacteria such as *Pseudomonas fluorescens*, *Bacillus subtilis* and some fungi like VAM and *Trichoderma* species. These organisms compete with plant pathogens for nutrients and space, produce antibiotics, parasitize pathogens, and induce resistance in the host plants. These organisms have been used for biocontrol of many pathogens (Berg *et al.*, 2007).

1. Influence of VAM on Plant Pathogens

VAM fungi play an important function in the reduction of plant pathogens (st-Arnaud *et al.*, 1995; Azcon-Aguilar and Barea, 1996; Whipps, 2004). Many researchers have observed an antagonistic effect of VAM against some fungal pathogens such as *Fusarium oxysporum* (Dehne and Schonbeck, 1979; Caron *et al.*, 1986; St-Arnaud *et al.*, 1997; Filion *et al.*, 1999), different *Phytophtora* species (Davis and Menge, 1980; Cordier *et al.*, 1996), *Rhizoctonia solani* (Yao *et al.*, 2002 and *Pythium ultimum* (Calvet *et al.*, 1993) attacking various crops. Vesicular arbuscular mycorrhiza fungi have also been shown to reduce bacterial diseases (Dehne, 1982). For example, *Glomus intraradices* suppresses *Fusarium sambucinum*, the causal organism of potato dry rot (Niemira *et al.,* 1996) while *Glomus etunicatum* suppresses *Rhizoctonia solani* in potato (Yao *et al.*, 2002). The mode of action of VAM biocontrol activity is assumed to be the direct interaction between VAM and pathogens, but mycorrhizal-mediated triggering of plant defence reactions have also been proposed (Azcon-Aguilar and Barea, 1996; Whipps, 2004). Dehne (1977) reported that *Fusarium oxysporum* infections are reduced in legume roots by mycorrhizal fungi. Levy and Krikvin (1980) also made a similar observation. Linderman (1994) reported that mycorrhiza can successfully manage many root diseases. He further suggested that VAM fungi are able to control many root diseases by involvement of many mechanisms such as changes in nutrient status of plants, biochemical changes in plant tissues, anatomical changes in host plant cells, stress alleviation, microbial changes in the rhizosphere and changes to host root-system morphology.

2. Influence of Pseudomonads on Fusarium Wilt

The bacteria that colonize plant roots have been termed rhizobacteria (Kloepper and Schroth, 1978). They are primarily strains of pseudomonads which are believed to have co-evolved with their host plants. The term rhizobacteria has been used to accentuate their intimate association with plant roots (Suslow, 1982). These non-pathogenic and root-colonizing pseudomonads are beneficial to plant growth (Edwards *et al.*, 1998). The pseudomonads have been called Plant Growth Promoting Rhizobacteria (PGPR). Most PGPR are fluorescent pseudomonads and include *Pseudomonas fluorescens* and *Pseudomonas putida* and some non-fluorescent pseudomonads like *Bacillus subtilis* and *Serratia* species. The pseudomonads produce *alkaligenes*, which inhibit pathogen colonization and delay pathogen development and onset of wilt symptoms. The pathogen inhibition is mediated by the production of siderophores (Yuen *et al.*, 1985). *Bacillus subtilis* is used as a plant growth bacterium and yield enhancing agent. This effect is achieved by seed bacterization. It is sold under the name Quantum 4000 (Weller, 1988). Ahmad and Jha (1977) and Gaur (1979) reported an increase in yield of chickpea and soybean respectively when pseudomonads were used as phosphate solubilizing agents. Besides enhancing growth and yield, pseudomonads also act as biocontrol agents especially when they have been isolated from disease suppressive soil as reported by Smith and Read,(1997).

3. Fluorescent Pseudomonads in Biological Control

The fluorescent pseudomonads are known to suppress plant disease and also promote plant growth. Pseudomonads have been reported to suppress many plant pathogens. They are well-known in the biocontrol of "take-all" disease of wheat caused by *Gaeumannomyces graminis* f. sp. *tritici* and act as an antagonist to this pathogen (Cook and Rovira, 1976). The pseudomonads can reduce plant diseases by antagonizing soil-borne pathogens through various mechanisms. These mechanisms include:

1. Rhizosphere competence.
2. Production of antibiotics.
3. Production of lytic enzymes.
4. Competition for iron.
5. Rhizobacteria-mediated induced systemic resistance (ISR).

Rhizosphere competence takes into consideration both root colonization and soil colonization. It refers to the bacterial capacity to multiply and to outnumber and dominate other microorganisms, and at the same time keep pace with the growing roots in the field by the introduced bacteria (Ahmad and Baker, 1987). Bacterial-rhizosphere-competence is also a relative term and can be quantified by measuring its population on a root by determining the length and number of roots colonized. Thus, different strains of pseudomonads can be compared for their rhizosphere competence (Pandey and Upadhyay, 2000).

Competition for iron in oxygenated and weakly acidic, neutral or alkaline soil occurs as insoluble iron complexes, Fe (OH_3). It becomes unavailable to the plant and thus serves as a limiting factor for growth. To sequester the scarcely available iron, Bakker *et al.*, (1993) showed that microorganisms produce low molecular weight compounds called siderophores which bind to iron. Many bacteria and fungi produce siderophores which give a competitive edge to the organisms producing stronger siderophores with high stability constant. Specific siderophores and their organism receptors can bind siderophores of other organisms. The ability of *Pseudomonas* strains to utilize siderophores produced by other strains of rhizobacteria increases their competitiveness in the rhizosphere. Raaijmaker *et al.*, (1995) demonstrated that siderophores mediated competition for iron between *P. fluorescens* and *P. putida* strains can decrease the root colonization by pathogens.

Fluorescent pseudomonads produce secondary metabolites with antibiotic properties (Kumar and Dube, 1992). Many such pseudomonads have been implicated in suppression of soil-borne diseases. Phenazine-1-carboxylic acids (PCA), 2, 4–diacetylphlroglucinol (DAPG), oomycin–A, pyocyanine, pyoluteorin and pyrrolnitrin have all been implicated in suppression of soil-borne diseases (Lemanceau and Alabouvette, 1993). Under natural conditions, antibiotics are synthesized in response to environmental signals such as high cell densities and nutrient depletion. Raaijmakers *et al.*, (1995) found that a minimum of 105 cfu/g of root was required for *Pseudomonas fluorescens* to suppress *Fusarium oxysporum* f. sp. *raphini* on radish through competition for iron. Similar levels of antibiotics appear to be required for protection against some diseases.

Several bacteria produce lytic enzymes which are also important in the control of many fungal pathogens. These bacteria can parasitize pathogenic fungi and kill them. The enzymes produced by such bacteria are chitinases, proteases and lipases. The growing hyphal tips burst as a result of chitinase action. Chitinases produced by *Serratia marcescens* have been shown to be associated with biocontrol of fungal diseases of pea and bean (Dube, 2001).

The disease suppressing activity of biocontrol bacteria is not limited to antagonism against the pathogens; it can also operate through its action on plants. Rhizobacteria can induce systemic resistance in plants. Dube (2001) reported that when biocontrol bacteria and a foliar pathogen were applied at spatially separated locations on the same plant, a form of plant resistance developed and the disease was suppressed. Induced systemic resistance has been demonstrated against many pathogens of fungi, bacteria and viruses in a number of plants such as bean, carnation, cucumber, radish, tobacco and tomato (Dube, 2001).

III. CHAPTER THREE

A. MATERIALS AND METHODS

1. Soil Collection and isolation of Vesicular arbuscular mycorrhiza Flora.

Three soil samples from points ten metres apart, along a 50m transect, collected from the New Kasama area of Lusaka, were analysed for physical and chemical properties at the department of soil sciences, School of agriculture of the University of Zambia. The New kasama area was chosen for soil samples because of its proximity to the laboratory facilities at the University of Zambia. It also provided an easily accessible undisturbed piece of land.

A sterile augur was used to extract the soil samples from a depth of 15-20 cm. The three-soil samples of approximately 6 Kg were bulked to make a composite soil mixture from which Vesicular arbuscular mycorrhiza (VAM) propagules were isolated. Wet sieving and decantation method of Gerdemann and Nicolson (1963) was used to isolate VAM spores.This method involved mixing 6kg of composite soil into a 20-litre container of tap water and stirring it vigorously for 5 minutes to dissolve the soil. The mixture was then left to settle for another 5 minutes after which it was stirred once again and poured over a series of sieves of different mesh size. The sieve size ranged from 2mm to 50μm. The residue was slowly washed from the last sieve with the smallest mesh size. At this stage the residue appeared dark brown in colour and was collected on to a blotter paper for drying. The residue so collected was examined for the presence of vesicular arbuscular mycorrhiza (VAM).The

characterization of VAM propagules was done microscopically at a magnification of X400 using a photo-micrographic binocular light microscope (Canon) following the features described by Gerdermann and Nicolson (1963) as keys and photographs.

2. Isolation and Characterization of Pseudomonads.

Closely associated pseudomonads with the rhizosphere of roots infected with Fusarium wilt of chickpeas were isolated on King's B medium. This medium consists of:

Peptone (20.0g/litre),
Heplahydrated magnesium sulphate (1.50g/litre),
Potassium hydrogen phosphate (1.50g/litre) and
bacteriological agar(15.0g/litre).

It is recommended for the isolation of rhizosphere pseudomonads (Murray, 1974).Appearing colonies of pseudomonads are seen as circular and their elevation on a media is described as low convex and yellow in colour. The colony edges are entire while the size ranges from 0.15μm to 10μm.

Soil samples were taken from a chickpea field in New Kasama area of Lusaka. Ten chickpea plants showing symptoms of Fusarium wilt were up-rooted together with the adhering soil. The roots were vigorously shaken to collect 1g of soil. To 1g of soil from diseased plants $99cm^3$ of sterile distilled water was added to obtain a soil suspension of $1/99cm^3$. The suspension was mixed thoroughly and left to stand for 15 minutes and then re-shaken. Using a sterile graduated pipette, $1cm^3$ soil suspension was transferred to a glass tube containing 9

cm^3 sterile distilled water to obtain 1:1000 dilution. This procedure was repeated until one in a million dilution was reached which was then used as a working solution. Petri dishes containing King's 'B' medium were inoculated with $1cm^3$ working solution by gently releasing it on to the medium using a sterile pipette and quickly covering each Petri dish. The inoculated dishes were incubated at room temperature (± 25 ° C) for 7 days. Characterization of pseudomonads was based on the description given by Murray (1974).

3. Isolation and Purification of *Fusarium oxysporum* f. sp. *ciceri*, the wilt pathogen.

One hundred grammes of suspected chickpea seeds from the Southern African Development Community (SADC) Plant Genetic Resources Center (PGRC), Lusaka were used to isolate the Fusarium wilt pathogen. The seeds were surface sterilized with 5% hypochlorite (JIK) and then rinsed twice in sterilized water. The seeds (Three per Petri dish) were then aseptically incubated on twenty dishes, using a blotter method for seven days at room temperature (± 25 ° C) under conditions of 12 hrs of near ultra violet (NUV) light and 12 hrs of darkness. The dishes were periodically examined for pathogen growth. On the 8^{th} day, fungal mycelium became visible. Identification of *Fusarium oxysporum* f.sp. *ciceri* was done using the monograph by Booth (1977).Fig 1a and b.

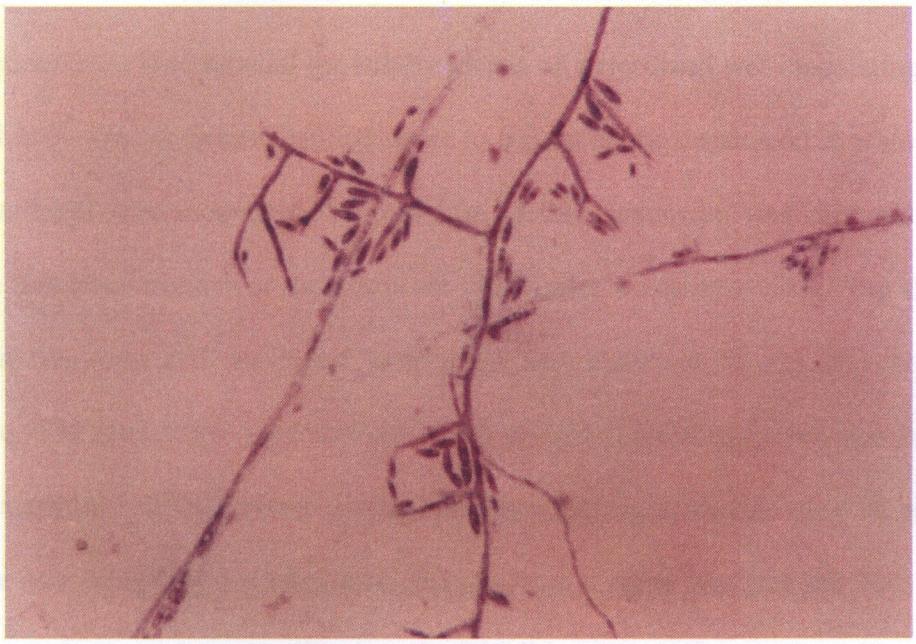


Figure 1a. Conidiophore and conidia of *Fusarium oxysporum* f. sp.*ciceri*, the cause of Fusarium wilt of chickpea. X400.

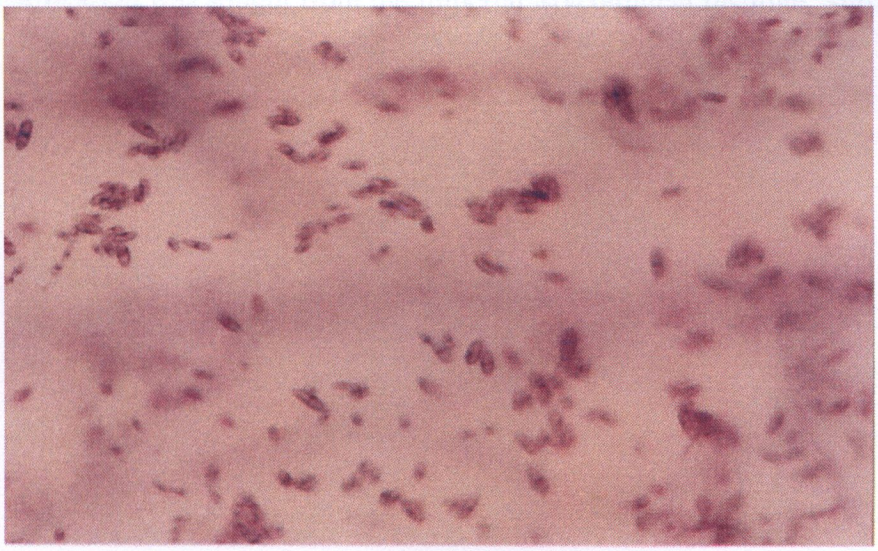


Figure 1b. Microconidia of *Fusarium oxysporum* f. sp *ciceri*. X400.

Pathogen purification was obtained by single spore isolation method as follows. Conidia were picked up from the mycelial growth by means of a sterilized wet inoculating loop. This was placed in 15 cm^3 of sterile distilled water to form a spore suspension in a storage bottle. Using a sterilized wire inoculating loop, a film of the suspension was captured within the loop. With this suspension four strokes were made on a water agar dish. Four dishes were prepared in this way and incubated over night and observed 24 hrs later for signs of germination. The petri dishes were then observed under a dissecting microscope at X40 using the light transmitted from beneath (Olympus-Binocular light microscope). A single germ tube visible as a translucent emerging outgrowth from a spore was located. The agar block was lifted by sliding a heat sterilized scalpel blade and transferred to a plate containing potato sucrose agar (PSA) medium. The inoculated plates were incubated at room temperature to allow for fungal growth and sporulation. The characteristic features of *Fusarium oxysporum* f.sp.*ciceri* were microscopically confirmed. Confirmation of the pathogen identification was sought from the Imperial Mycological Institute, Surrey, England.

4. Vesicular arbuscular mycorrhiza multiplication

Maize seeds of cultivar MMV 600 (a known trap plant for the amplification of VAM inoculum) were surface sterilized with 5% hypochlorite (commercial JIK). Twelve plastic pots of 15 litre capacity were procured for the purpose of VAM multiplication. 150kg of soil from the University of Zambia farm was sterilized using an electric soil sterilizer (Industrial autoclave machine) for 24hours at the Soil sciences department of the school of agricultural sciences, University of Zambia. The soil was sterilized to kill off all other organisms that may interfere with the results. Each of the 12 pots was filled with 12kg of sterilized soil and 10 planting stations of 2cm deep were made per pot. Ten sterilized maize seeds were placed in each pot, but before covering the seeds, fifteen grammes of extracted VAM spores were suspended in 2 litres of sterile water. Two cubic centimeters of the VAM suspension was poured over each maize seed and covered with the sterilized soil. Ten seedlings were raised per pot for three weeks, after which the roots of growing maize plants were periodically examined microscopically to confirm colonization by VAM. The first positive examination was done five weeks after planting maize. One plant was carefully uprooted from each pot at random and examined. When evidence of root colonization became evident fine feeder roots of plants less than 2mm in diameter and 2cm long were removed and washed carefully. The roots were then completely submerged in a glass vial containing 10% Potassium hydroxide (KOH) and held for three days at room temperature ($\pm$ 25° C). At the end of this, KOH was decanted and the roots were washed three times in running tap water. They were now submerged in alkaline hydrogen peroxide (H_2O_2) for 20min. The alkaline H_2O_2 was decanted and the root pieces were washed with water. The root pieces were then placed in 10% hydrochloric acid (HCl) to neutralize the KOH. The vial with roots were shaken and left in

HCl for 15min. The HCl was decanted and the roots left in vials in a solution of 0.5% trypan blue in lacto phenol for three days at room temperature (± 25° C). The trypan blue in lacto phenol was later replaced with lacto phenol only to retain the blue stain. The root pieces processed in this way were microscopically examined at a magnification of X400 (Photo-micrographic binocular light microscope, Canon). When root colonization had been confirmed, maize shoots in all the pots were cut off and discarded. Vesicular arbuscular mycorrhiza colonized roots and soil from the pots were mixed thoroughly and softened ready for use in the main experiment as (VAM) inoculum.

All the materials for the study of fusarium wilt on chickpea were ready. The chickpea seeds from the SADC plant genetic resource centre, Lusaka; the VAM inoculums already multiplied at the University of Zambia greenhouse; sterilized soil; the pseudomonads and the pathogen, *Fusarium oxysporum* f.sp.*ciceri*, the cause of fusarium wilt.16 plastic pots were procured for this next experiment.

Each of the 16 plastic pots of 18 litre capacity was filled with sterilized soil. Seven chickpea seeds surface sterilized with 5% hypochlorite (commercial JIK) were sown per pot. At each sowing station in a pot, a 2 cm^3 Conidial suspension of *Fusarium oxysporum* f. sp. *ciceri* containing 5.07 x 10^4 microconidia of the pathogen was placed in each 2 cm deep station. In 8 of the 16 pots, 10^5 colony forming units (cfu) of pseudomonad extract of turbidity 27.20mg/L at 430 nm was poured at each station at the time of sowing. Eight pots were inoculated with VAM. Four pots had neither pseudomonads nor VAM. This was the control experiment. Initially, seven seedlings were raised. These were subsequently thinned to five

per pot 10 days after planting. The plants were observed and measurements of plant height and number of leaves per plant taken at 15days after planting. Thereafter, plants were observed at 30, 45, 60, 75 and 90 days after planting. At 45 days after planting, the parameter of flowers per plant was taken and at 75 days after planting, pods per plant parameter were also taken. The fresh root weight, fresh shoot weight and seed weight parameters were taken at 90 days after planting.

5. Study Design.

A complete randomized block experimental design was used with four treatments and four replications. The treatments were as shown below.

Treatment	Combinations
A	Sterile soil + pathogen + chickpea seeds (Control).
B	Sterile soil + pseudomonads +pathogen + chickpea seeds.
C	Sterile soil + VAM + pathogen + chickpea seeds.
D	Sterile soil + pseudomonads + VAM + pathogen + chickpea seeds.

The above four treatments were used to establish the individual and combined effects of VAM and rhizosphere pseudomonads on growth, productivity and incidence of Fusarium wilt on chickpea plants. All experiments were conducted in a greenhouse at the University of

Zambia (UNZA) during the months of November 2006 to March 2007. UNZA was selected because of its easy access and availability of a usable greenhouse. For each treatment a total of four pots per block were used and each treatment was replicated four times.

The four replications for each inoculation treatment were prepared as follows: (A) pathogen + sterile soil + chickpea seeds (control); (B) Pathogen + pseudomonads + sterile soil + chickpea seeds; (C) Pathogen + VAM + sterile soil + chickpea seeds; (D) Pathogen + pseudomonads + VAM + sterile soil + chickpea seeds. The test plants were maintained for sixteen weeks and at the end of this period seven parameters (pods per plant, seed weight, fresh shoot weight, fresh root weight, and leaves per plant, plant height and flowers per plant) were measured. The plants were maintained in a greenhouse at a temperature range of 29° C to 35° C and were irrigated with one litre tap water every two days.

6. Fusarium Wilt Disease Score

Fresh roots of each plant were weighed and the weight was used to score the disease intensity as shown below.

Root weight.	Degree of disease incidence.
5g.	0.
4g.	1.
3g.	2.
2g.	3.
1g.	4.
<0.1g	5.

Key 0 = no disease.

5 = high disease incidence (maximum).

A Percent Disease Incidence (PDI) was calculated as per FAO methods of plant disease assessment (FAO, 1971).

$$PDI = \frac{\text{Sum of numerical values of fresh root weight X 100.}}{\text{Total numbers of plants measured X maximum grading (5).}}$$

7. DATA ANALYSIS

a).Determination of the Effect of VAM and Rhizosphere Pseudomonads on Chickpea Wilt.

All data analyses were done using STATISTIX® for windows (1985, 96) computer software (version 1.0).

One way analysis of variance ANOVA was applied to data generated from observation made on;

(i).growth parameters (plant height, leaves per plant and fresh shoot weight),

(ii).productivity parameters (flowers per plant, pods per plant and seed weight),

(iii) disease incidence parameter, (fresh root weight).

This was to determine if there were significant differences in growth, productivity and disease on different effects of VAM with or without pseudomonads on the chickpea plant. The Bonferroni significance test (STATISTIX® for windows (1985, 96) was employed to separate the means in the analysis.

Best subset regression analysis of treatment combinations (control – pathogen only, pathogen + pseudomonads, pathogen + VAM and pathogen + VAM + pseudomonads) on mean numbers of disease incidence, growth and productivity parameters were done to determine;

1. How treatment combinations related to plant disease incidence.
2. The effect of climatic factors on disease incidence, growth and productivity of chickpea.

IV. CHAPTER FOUR

A. RESULTS

1. Soil Analysis for Physical and Chemical Properties

The soil collected from the Kasama area of Lusaka was analyzed at the University of Zambia soil science laboratory of the School of Agriculture. All the three samples had a sandy-clay-loamy texture. The soils were acidic and had low levels of Nitrogen, Potassium and Phosphorus. The levels of Calcium and Manganese were relatively high (Table. 1).

Table 1. Physical and Chemical Properties of Soil Samples Collected from new Kasama area of Lusaka.

Samples	ST	pH	N %	K %	Ca %	Mg %	C %	OM %	S %	Zn ppm	Mn ppm	Fe Ppm	P ppm
1	SCL	4.94	0.28	0.23	3.3	0.8	0.44	2.5	Tr	2.6	23.1	13.4	10.2
2	SCL	4.32	0.70	0.41	2.7	0.7	0.12	2.2	Tr	0.8	20.6	38.2	10.1
3	SCL	6.02	0.42	0.22	4.9	0.9	0.40	1.6	Tr	1.1	13.6	7.5	8.4

Key: OM = (Organic matter)

SCL = (Sandy-clay-loam)

ST = (Soil texture)

Tr = (Trace)

ppm = (Parts per million

2. VAM *colonization.*

In the vesicular arbuscular mycorrhiza (VAM) colonization experiment, the study showed positive evidence after 35 days of VAM inoculation. The appearance of the uncolonized and the colonized roots are shown in figures 2a, b and c.

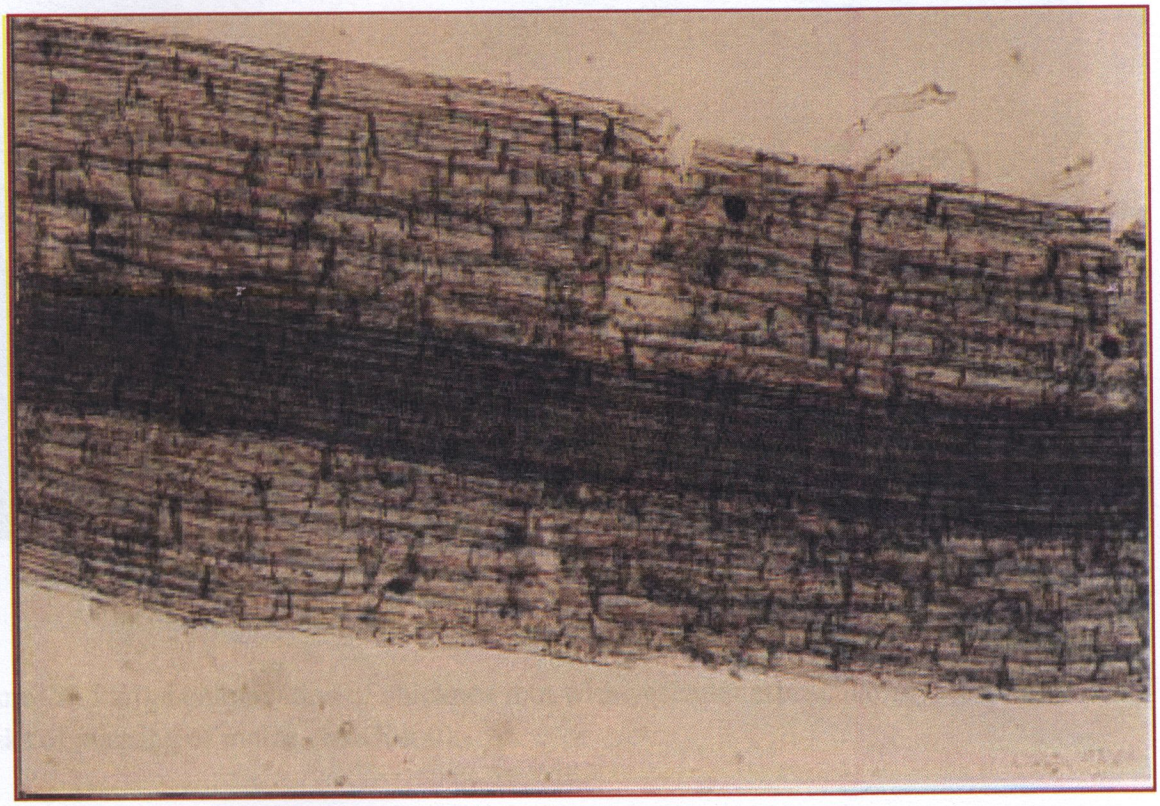


Figure 2a Photomicrograph of a healthy chickpea root showing no VAM colonization. (X400)

Figure 2b. Partial colonization of chickpea root with vesicular arbuscular mycorrhiza 5 weeks of planting of maize seed(X400).

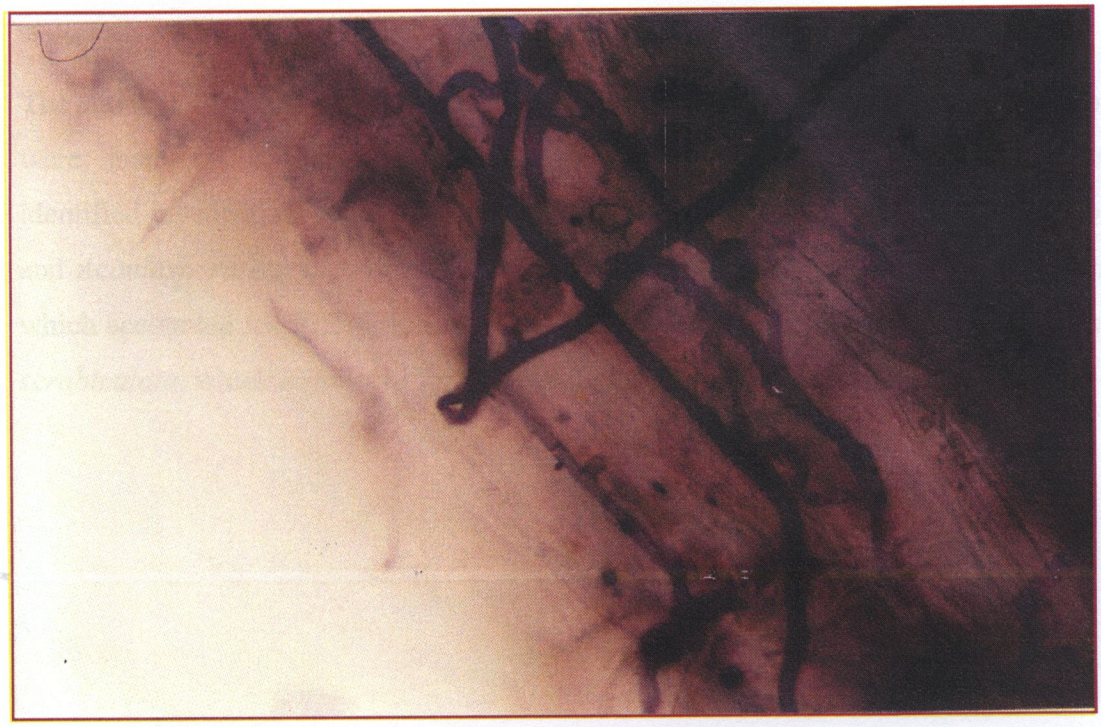
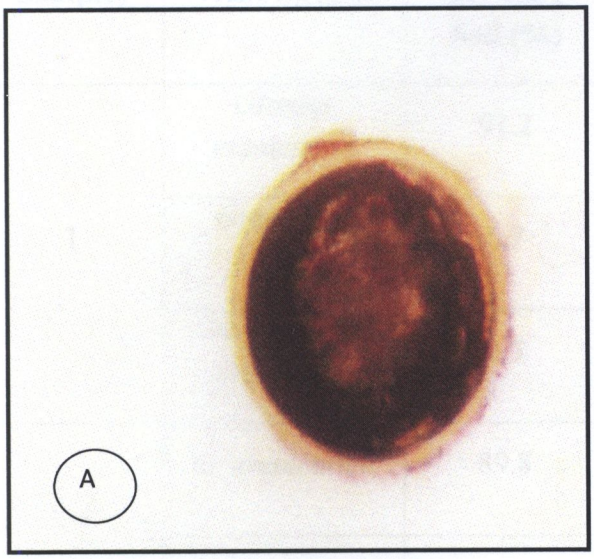


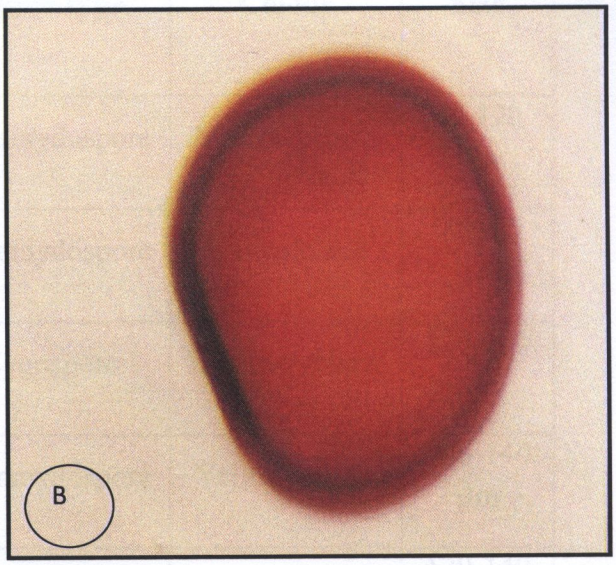
Figure 2c. Advancing colonization of chickpea root by vesicular arbuscular mycorrhiza hyphae after 50 days of plant age. (X400).

3. Vesicular arbuscular mycorrhiza spore composition and density.

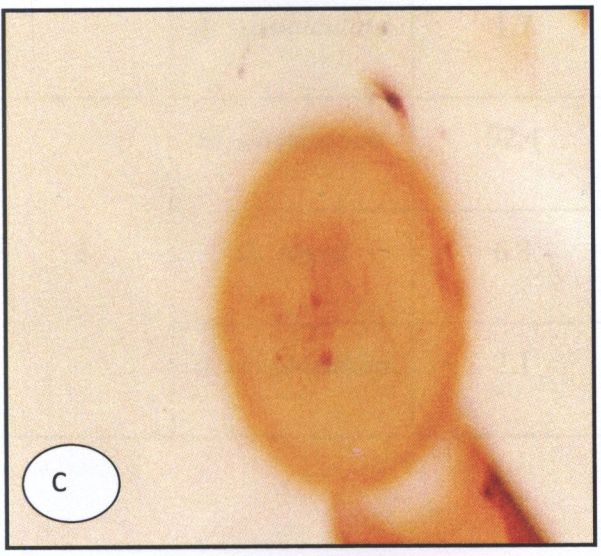
The number of VAM spores isolated from the three sites of new Kasama area of Lusaka were; (sample 1.) 12.565, (sample 2.) 19,620; (sample 3.) 20,901 per 100g soil. The identified mycorrhizal species were *Glomus etunicatum, Glomus mosseae, Gigaspora nigra,* and *Acaulospora scrobiculata* (Fig.3). The most prominent species was *Glomus etunicatum* which accounted for 90.2%, followed by *Gigaspora nigra* accounting for 7.4%, *Acaulospora scrobiculata,* which accounted for 2.3% and *glomus mosseae* at 0.1% (Table.2).



A- *Acaulospora nigra.*(X400.)



B- *Acaulospora scrobiculata.* (X400.)



C - Glomus etunicatum

D - *Glomus mosseae.* (X400.)

igure 3. Species of *Acaulospora* and *Glomus* showing characteristic spore morphology.

Table 2. VAM Spore Density and Species Composition in the three Soil Samples Collected from New Kasama, Lusaka.

Samples	VAM Species	Composition per 100g Soil (%)	Spore type	Colour	Size
1	*Glomus etunicatum*	91.2	Chlamydospore	Yellow/brown	80-120 µm
	Gigaspora nigra	6.7	Chlamydospore	Brown/black	90-120 µm
	Acaulospora scrobiculata	2.3	Zygospore	Hyaline/yellow	120-140 µm
2	*G. etunicatum*	89.8	Chlamydospore	Yellow/brown	120-140 µm
	G. nigra	9.2	Chlamydospore	Brown/black	120-140 µm
	A. scrobiculata	1.7	Zygospore	Hyaline/yellow	120-140 µm
3	*G. etunicatum*	92.4	Chlamydospore	Yellow/brown	120-140 µm
	G. nigra	6.3	Chlamydospore	Brown/black	120-140 µm
	A. scrobiculata	2.1	Zygospore	Hyaline/yellow	120-140 µm

4. Effect of VAM and Pseudomonads on Chickpea Growth and Productivity.

Fifteen days after sowing, plants in all treatments exhibited no significant differences in both height and number of leaves per plant (Fig. 4). Significant differences ($p = 0.05$) were noticed (Ninety days after sowing) in the mean numbers of each of the following parameters per plant (pod numbers, seed weight, number of flowers, plant height, fresh shoot weight, fresh root weight and leaves per plant (Tables 3 and 4). Maximum crop growth was observed in treatment involving the pathogen, pseudomonads and VAM. This treatment showed the highest mean numbers of height (Fig. 5), leaves per plant (Fig. 6) and fresh shoot weight (Fig. 7) which were significantly different from the control and those treatments involving the pathogen and pseudomonads in one case and VAM in another. However, the mean fresh root weight of the treatment involving pathogen and VAM was not significantly different from treatments involving the pathogen, pseudomonads and VAM. It is noted from Table 3 that chickpea plants that received a dual inoculation of VAM and pseudomonads were 50% taller than those that had no additions (P only or control). The plants inoculated with VAM and pseudomonads showed 75% more leaves than the control. Plants inoculated with VAM and pseudomonads had the highest fresh root weight per plant. These roots weighed 75% more than the control and those inoculated with pseudomonads.

Figure 4 Chickpea (*Cicer arietinum* L.) plants growing in a greenhouse 15 days after sowing, indicating no sign of disease in any treatment.

Key

P = Pathogen

P + Ps = Pathogen + Pseudomonads

P + V = Pathogen + Vam

P + Ps + V = Pathogen + Pseudomonads + Vam.

Table 3. Performance of chickpea (*Cicer arietinum* L.) under different experimental treatments in relation to growth and development.

Treatment	Plant Height In (cm)	Leaves Per Plant	Fresh root Weight In (g)	Fresh Shoot Weight in (g)
P only(control)	11.505[a]	12.900[a]	0.788[a]	5.530[a]
P+Ps	12.165[a]	15.450[a]	1.038[a]	9.400[a]
P+Vam	17.840[b]	36.700[b]	2.486[b]	12.820[a]
P+P+Vam	22.680[b]	59.300[b]	1.734[b]	18.385[b]
Mean	16.048	31.088	1.512	11.534
BS(0.05)	2.037	5.444	0.787	1.177
CV	2.709			

Key

P = Pathogen

P + Ps = Pathogen + Pseudomonads

P + Vam = Pathogen + Vam

P + Ps + Vam = Pathogen + Pseudomonads + Vam.

Means followed by the same letter are **not** significantly different from each other at P=0.05, by Bonferroni significant (BS) test.

Table 4. Performance of chickpea (*Cicer arietinum* L.) under different experimental treatments in relation to pods, flowers and seed weight per plant.

Treatment	Pods per Plant	Flowers per plant	Seed weight per plant
P only(control)	1.750[a]	1.150[a]	0.195[a]
P+Ps	2.750[a]	3.100[b]	0.775[a]
P+Vam	8.200[b]	12.350[c]	2.010[b]
P+Ps+Vam	11.250[b]	12.750[c]	2.010[b]
Mean	5.988	7.338	1.248
BS(0.05)	2.452	2.774	0.537
CV	2.709		

Key

P = Pathogen

P + Ps = Pathogen + Pseudomonads

P + Vam = Pathogen + Vam

P + Ps + Vam = Pathogen + Pseudomonads + Vam.

Means followed by the same letter are **not** significantly different from each other at P=0.05, by Bonferroni significant (BS) test.

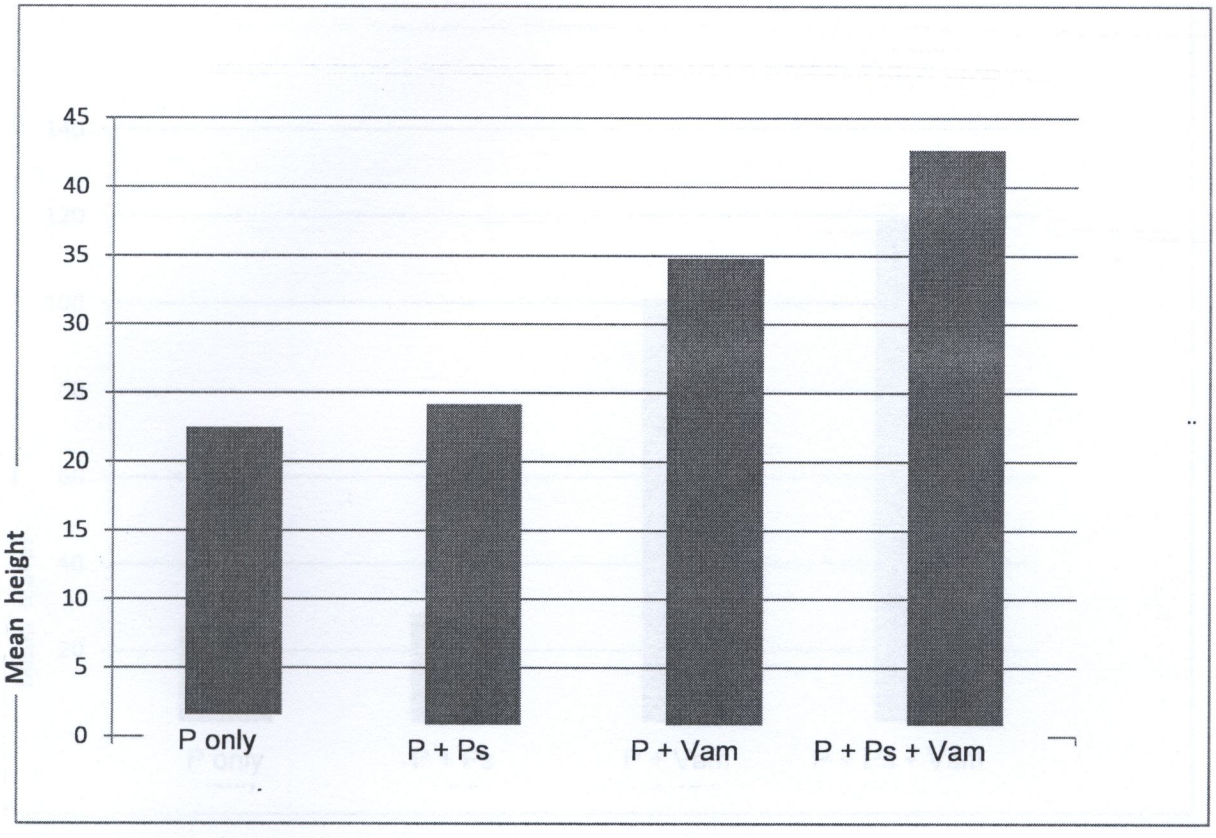


Figure 5. A comparison of plant height per plant for each treatment in chickpea (*Cicer arientinum*. L.) plants.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

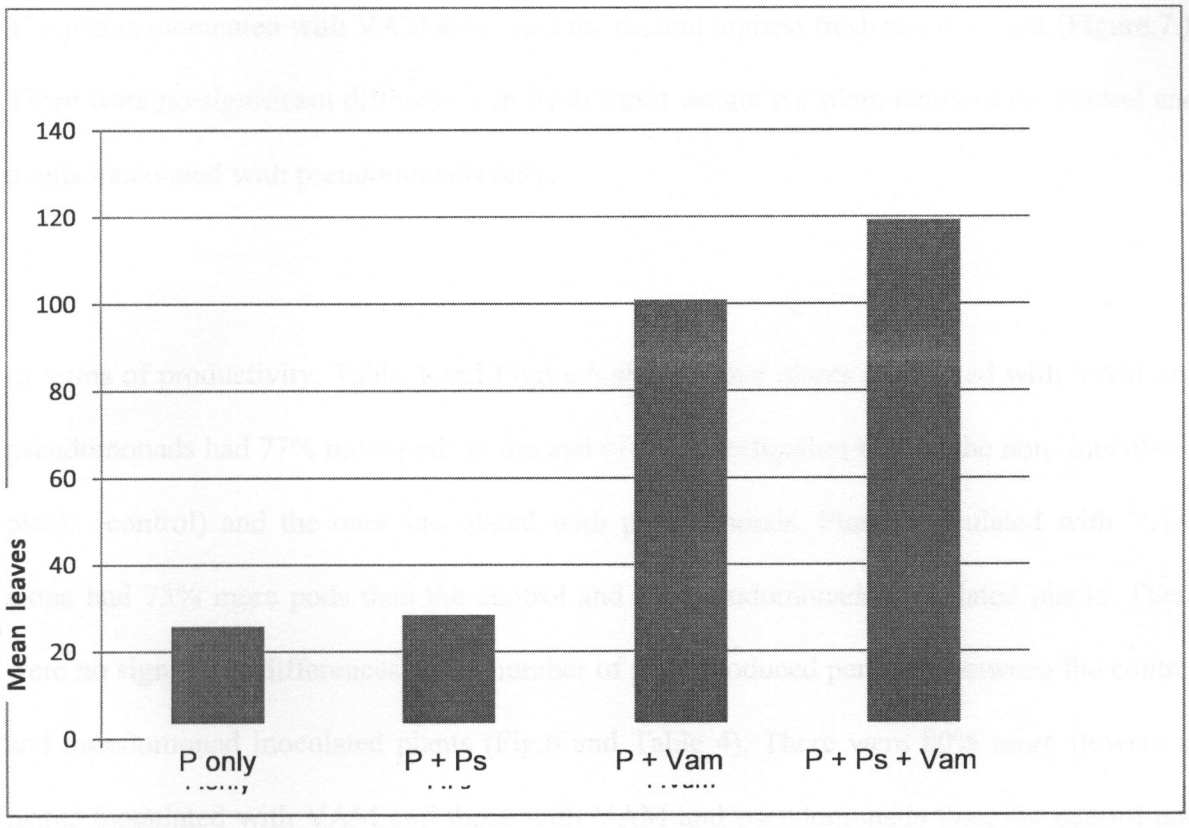


Figure 6. A comparison of leaves per plant for each treatment in chickpea (*Cicer arientinum*. L.) plants.

Key

P = Pathogen

P + Ps = Pathogen + Pseudomonads

P + Vam = Pathogen + Vam

P + Ps + Vam = Pathogen + Pseudomonads + Vam.

The plants inoculated with VAM alone had the second highest fresh shoot weight (Figure.7.). There were no significant differences in fresh shoot weight per plant between the control and plants inoculated with pseudomonads only.

In terms of productivity, Table 4 and Figure 6 showed that plants inoculated with VAM and pseudomonads had 77% more pods at the end of the investigation than in the non- inoculated plants (control) and the ones inoculated with pseudomonds. Plants inoculated with VAM alone had 73% more pods than the control and the pseudomonads inoculated plants. There were no significant differences in the number of pods produced per plant between the control and pseudomonad inoculated plants (Fig.6 and Table 4). There were 80% more flowers in plants inoculated with VAM and those with VAM and pseudomonads than the control and those inoculated with pseudomonads (Fig. 7). No significant differences were observed in the number of flowers per plant between the control and pseudomonad inoculated plants either. The highest amounts of seed weight were recorded in plants inoculated with VAM and with both VAM and pseudomonads and this was 82% more than the rest of the treatments (Fig. 8). A summary of how all parameters compared under all the treatments is given in Figure 9.

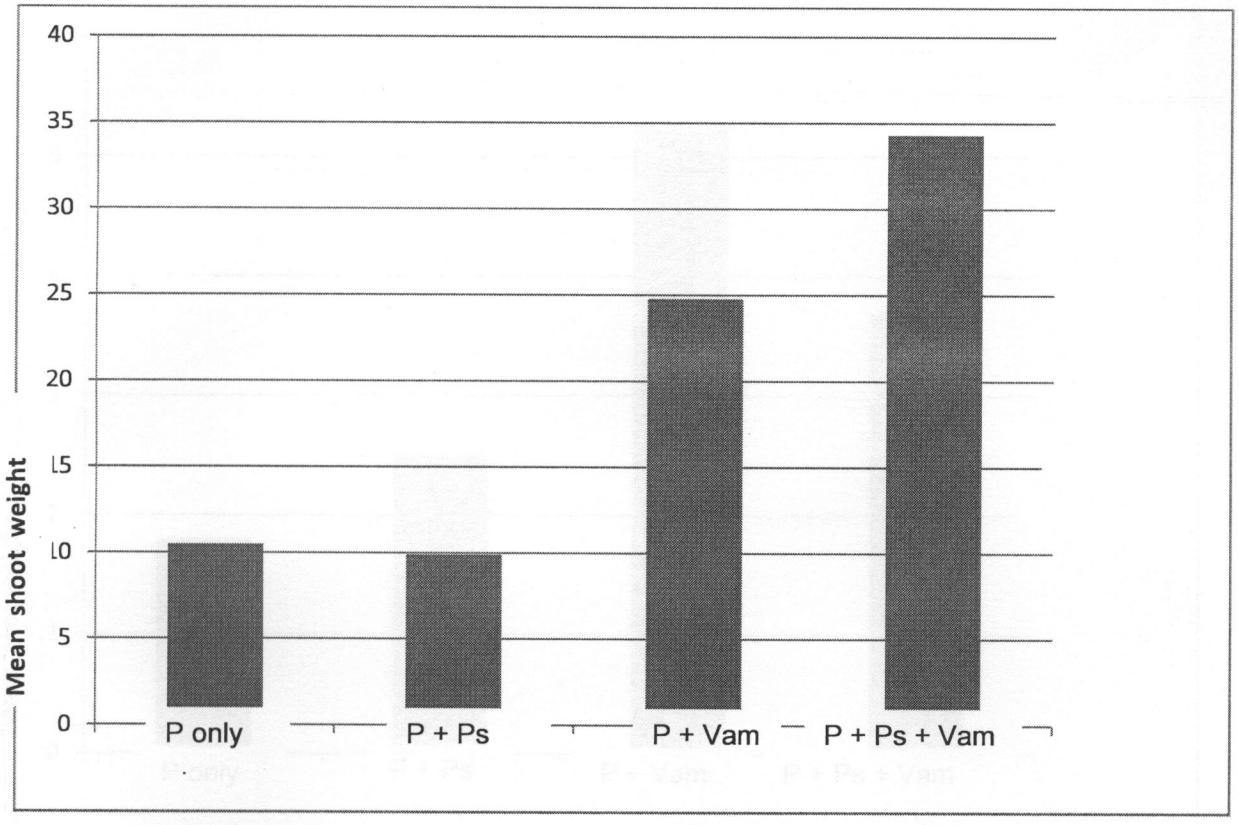


Figure 7. A comparison of fresh shoot weight per plant for each treatment in chickpea (*Cicer arientinum*. L.) plants.

Key

P = Pathogen

P + Ps = Pathogen + Pseudomonads

P + Vam = Pathogen + Vam

P + Ps + Vam = Pathogen + Pseudomonads + Vam.

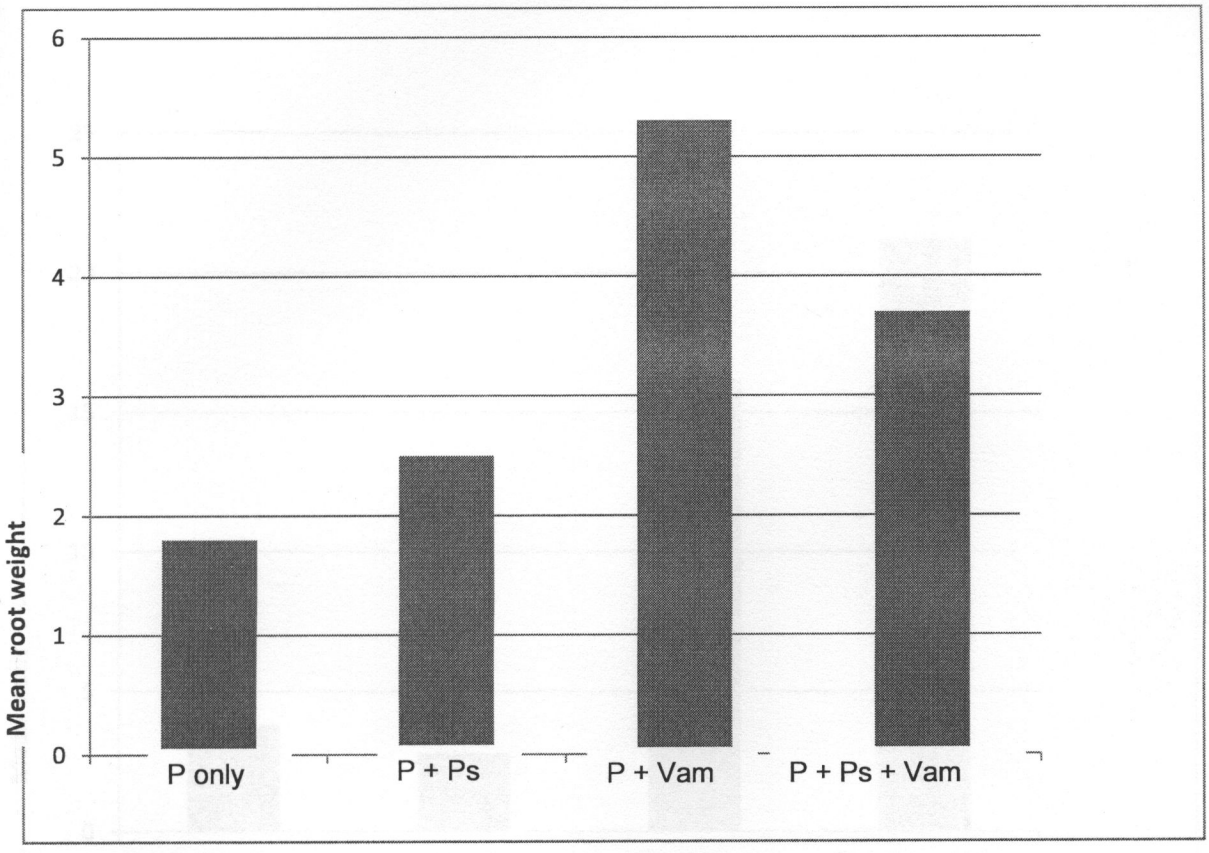


Figure 8. A comparison of fresh root weight per plant for each treatment in chickpea (*Cicer arientinum*. L.) plants.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

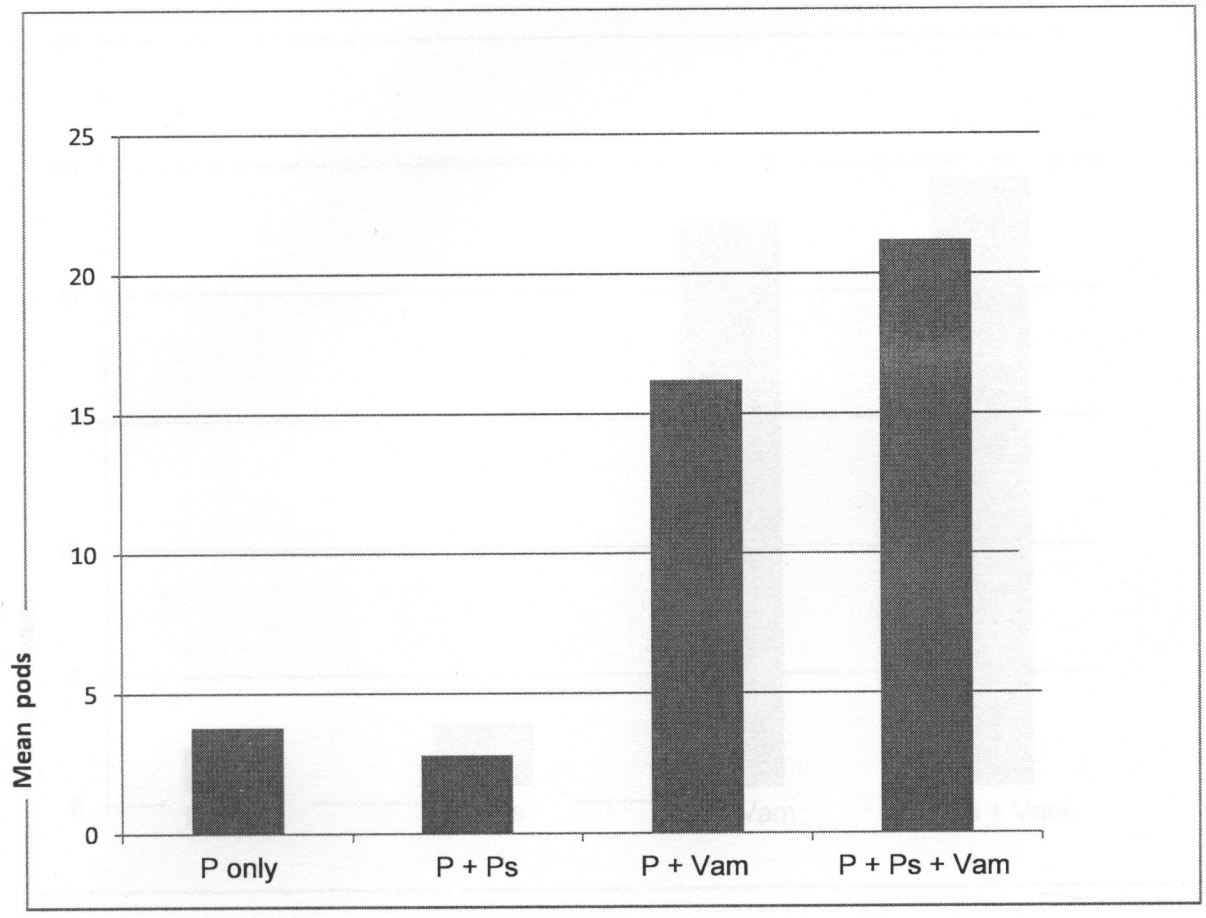


Figure 9. A comparison of the number of pods per plant for each treatment in Chickpea (*Cicer arientinum* L.) plants.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

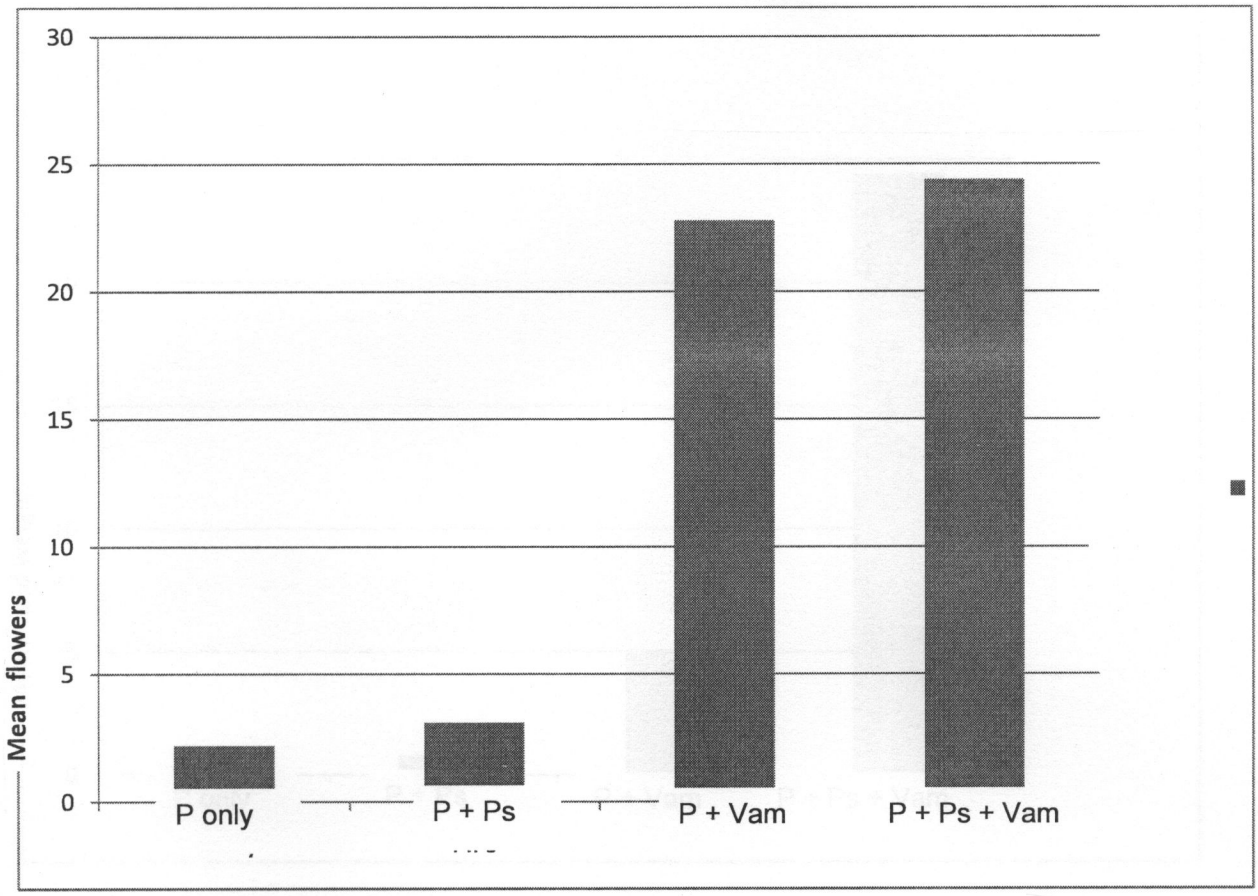


Figure 10. A comparison of the number of flowers per plant for each treatment in Chickpea (*Cicer arietinum* L.) plants.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

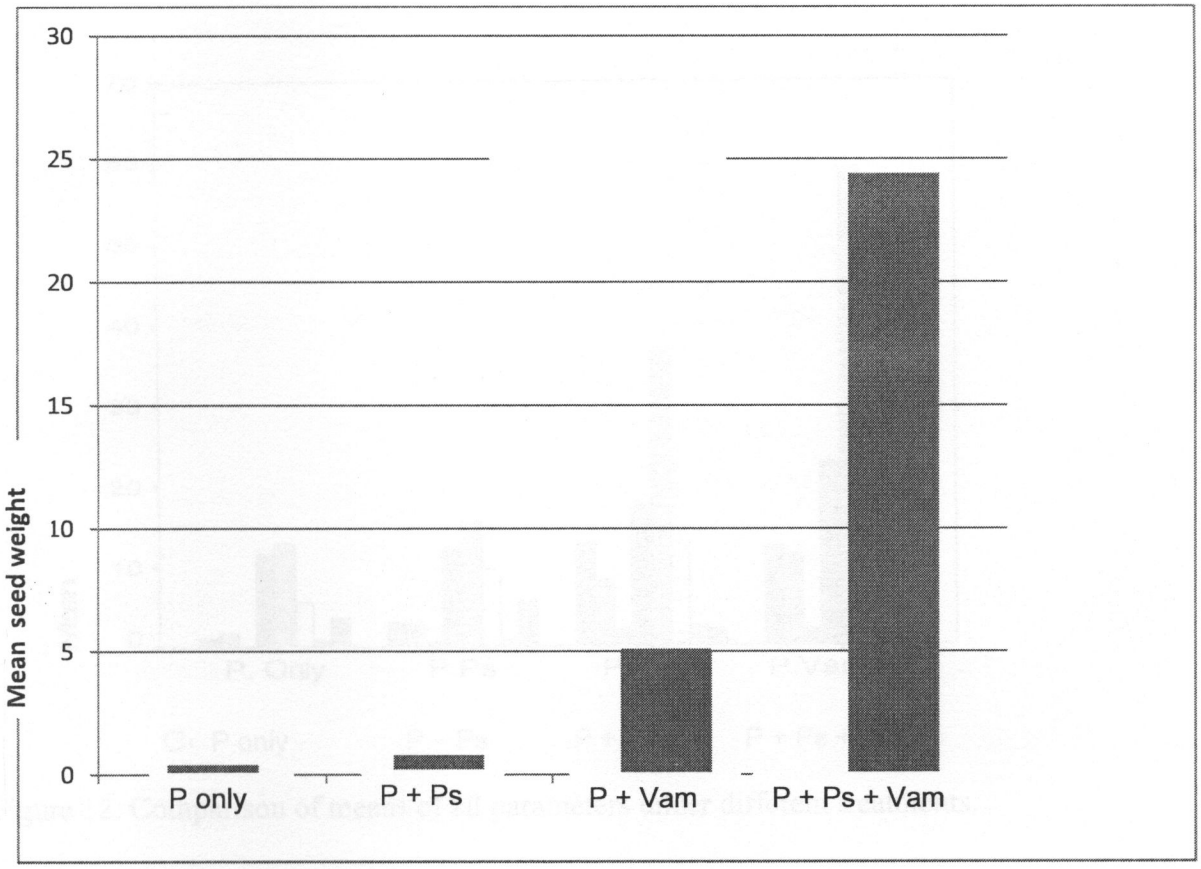


Figure 11. A comparison of seed weight per plant for each treatment in chickpea (*Cicer arietinum* L.) plants.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

5. Disease incidence on chickpea plants.

At twenty-one days after sowing, plants in all treatments exhibited the onset of disease symptoms. After 45 days, advancing disease symptoms of chlorosis on the lower leaves on each plant were now very visible, but at varying degrees (Fig. 13).

At 90 days after sowing, maximum disease incidence was reached. In the control treatment and the one inoculated with pseudomonads only, the plant were stunted and wilting (Fig. 14).

The percent disease incidence (PDI) of Fusarium wilt was higher in the plants inoculated with the pathogen only or control and those inoculated with pathogen and pseudomonads (P + Ps) as seen from figure 15. No significant difference in PDI was noticed between these two treatments (Table 5). Plants inoculated with VAM alone (single inoculation) and those with dual inoculation of VAM and pseudomonads showed the least PDI. The PDI in plants inoculated with VAM alone (P + VAM) was 44% while in those inoculated with VAM and pseudomonads (P + Ps + VAM), was 41% (Table 5).Therefore, the disease incidence in VAM inoculated plants and those inoculated with VAM and pseudomonads was reduced by 50% and 52% respectively (Fig 15.).

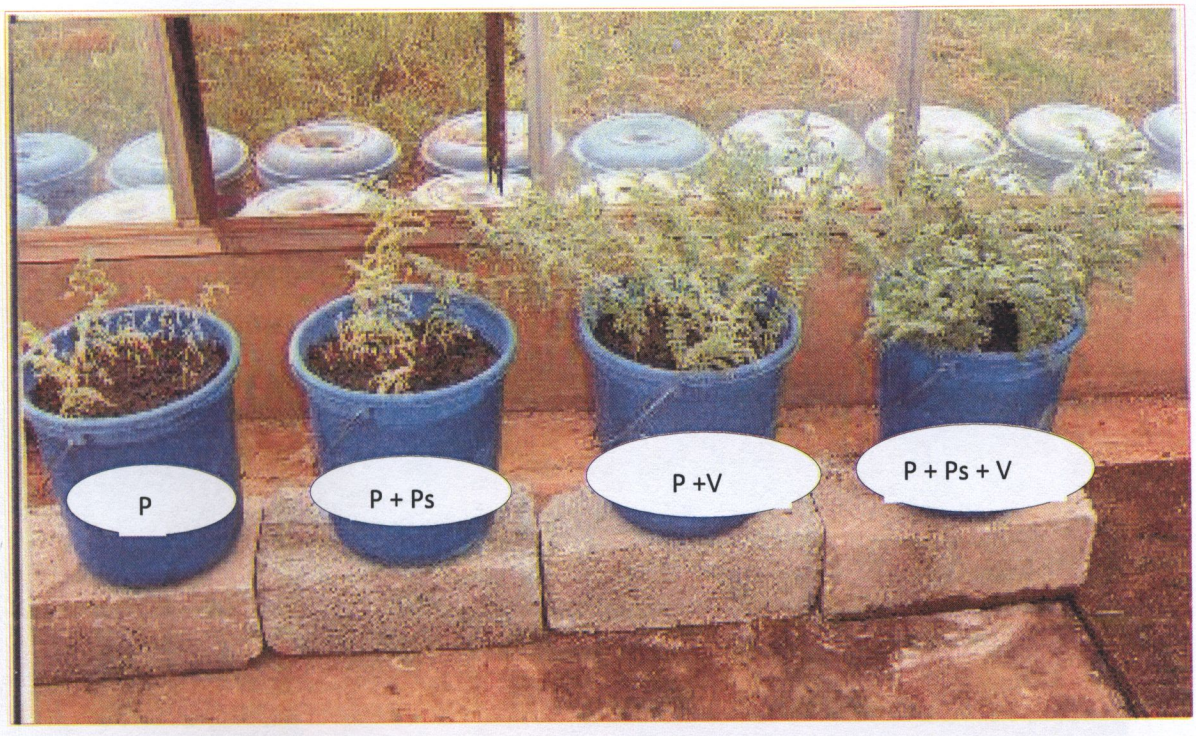


Figure 13 Fusarium wilt symptoms on chickpea (Cicer arietinum L.) plants growing in a greenhouse 45 days after sowing.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

Figure 14 Fusarium wilt symptoms on Chickpea in a greenhouse 90 days after sowing.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

Table 5. Performance of chickpea (*Cicer arietinum* L.) under different experimental treatments in relation to Fusarium wilt disease incidence.

Treatment	Disease incidence
P. only(control)	7.500[a]
P + Ps	7.300[a]
P + Vam	4.400[b]
P + Ps + Vam	4.100[b]
Mean	5.825
BS(0.05)	0.740
CV	2.709

Key

P = Pathogen

P + Ps = Pathogen + Pseudomonads

P + Vam = Pathogen + Vam

P + Ps + Vam = Pathogen + Pseudomonads + Vam.

Means followed by the same letter are not significantly different from each Other at P = 0.05, by Bonferroni Significance (BS) test.

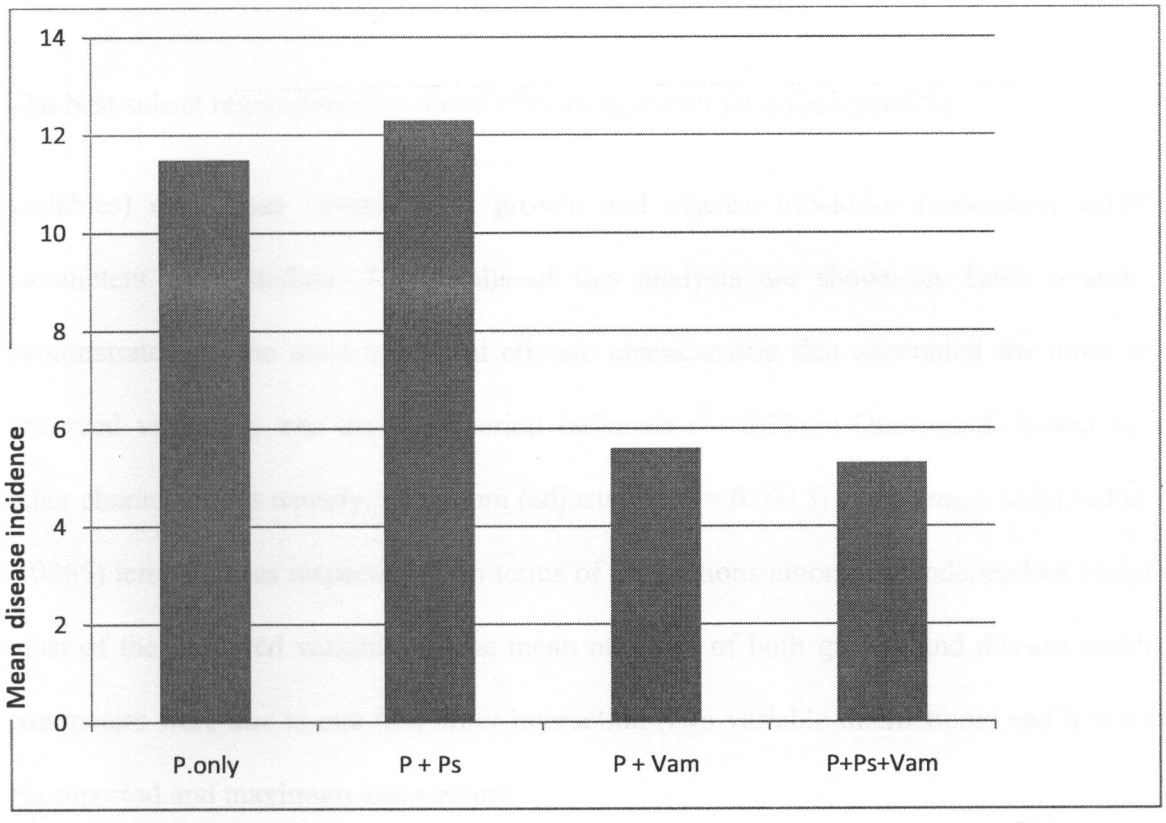


Figure 15. Incidence of *Fusarium oxysporum* f. sp. *ciceri* (Matuo and Sato) disease per treatment in Chickpea (*Cicer arientinum* L.) plants.

Key

P	=	Pathogen
P + Ps	=	Pathogen + Pseudomonads
P + Vam	=	Pathogen + Vam
P + Ps + Vam	=	Pathogen + Pseudomonads + Vam.

6. Regression Analysis

The best subset regression analysis of climate characteristics (independent variables) on mean numbers of growth and disease incidence (dependent variables) parameters were studied. The results of this analysis are shown in Table 6 and they demonstrate that the most important climate characteristic that accounted for most of the observed variations was the photoperiod (adjusted r^2= 0.2153).This was followed by two other characteristics namely, maximum (adjusted at r^2 = 0.0313) and average (adjusted at r^2 = 0.0265) temperatures respectively. In terms of interactions among the independent variables, most of the observed variables in the mean numbers of both growth and disease incidence parameters were due to one first order interaction (two-variable interactions) and it involved photoperiod and maximum temperature.

Table 6. Best subset regression models for fresh root weight per plant for chickpea (*Cicer arietinum* L.)

Model Variables	Mallows Cp Statistic	Adjust r^2	r^2	Residual Sum of squares (RSS)
Intercept only	21.4	0.0000	0.0000	99.0602
Photoperd	2.0	0.2052	0.2153	77.7366
Max temp	20.3	0.0189	0.0313	96.4376
Av temp	20.7	0.0140	0.0265	76.9111
Photoperd*Min Temp	3.2	0.2034	0.2236	76.9111
Photoperd*Min Temp	3.7	0.1976	0.2179	77.4766
Photoperd*Av, Temp	3.9	0.1955	0.2159	77.6778
Photoperd*Min Temp*Max Temp	3.1	0.2148	0.2446	74.8320
Photoperd*Min Temp*Av Temp	3.7	0.2083	0.2384	75.4463
Photoperd*Max Temp*Av Temp	4.6	0.1986	0.2291	76.3703
Photoperd*Min Temp*Max Temp*Av Temp	5.0	0.2050	0.2452	74.7659

Av =temp=Average temperature; photoperd = photoperiod; Max.temp. = Maximum temperature; Min temp = Minimum temperature

V. CHAPTER FIVE

A. DISCUSSION.

Microorganisms in the rhizosphere are influenced by plant roots and the plants have the ability to modify rhizosphere microbes during their growth and exudates. The effect of rhizosphere microorganisms on plants can be detrimental to the host plant as shown by pathogens, or they can exert a beneficial effect on plant growth like VAM and plant growth promoting bacteria (PGPR). The composition and activities of microorganisms in the rhizosphere are thus greatly affected by plants and plant roots.

Vesicular-arbuscular mycorrhiza exerts inhibitory effect on root pathogens and stimulatory effect on plants (Smith and Read, 1997). In recent year's plant growth promoting bacteria (PGPR) have been identified from the rhizosphere which plays a useful role in plant disease control (Bianciotto and Bonfante, 2002). Considering the various interactions that can possibly occur in chickpea (*Cicer arietinum* L.) plants, the following model (Fig. 16) is instructive (Bharadwaj 2007).

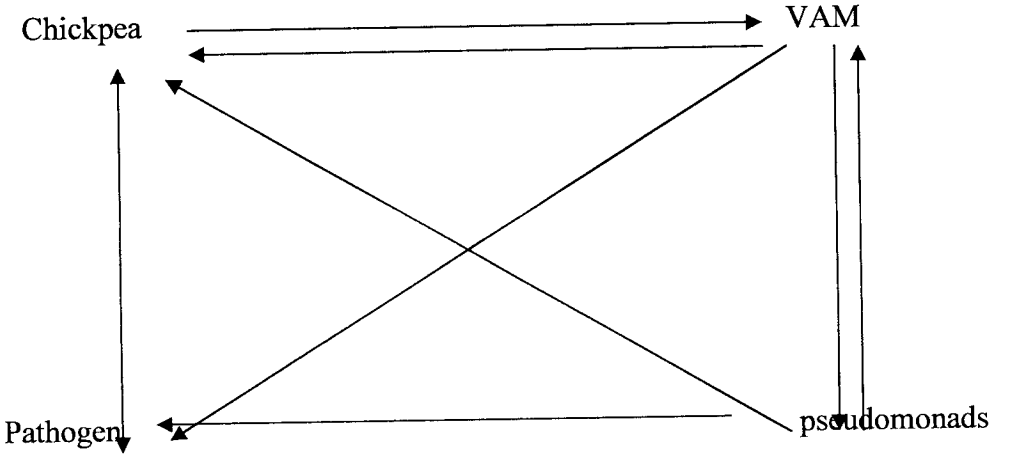


Figure 16. An outline of possible interactions that may occur in Chickpea plants

The study on the effect of mycorrhiza and rhizosphere pseudomonads on chickpea (*Cicer arietinum* L.) wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (Matuo and Sato), in a greenhouse showed that their interactions produced significant improvements in plant growth. The growth and productivity of chickpea plants produced a positive effect on the incidence of chickpea wilts on sixteen week old plants raised in a greenhouse. All plants in all treatments were of similar height and produced the same number of leaves fifteen days after sowing (DAS). None of the plants showed any visible disease symptom in any of the treatments. This could be the incubation period of Fusarium oxysporum f.sp. ciceri (Matuo and Sato), the cause of Fusarium wilt in chickpea. However, forty-five days after sowing the treatment C inoculated with the pathogen and VAM (P + VAM) and the treatment D (P + Ps + VAM) showed higher plant height and also produced many more leaves in comparison to plants inoculated with the pathogen only or the control. The treatments C and D in which four growth parameters (plant height, shoot weight, root weight and number of leaves per plant) were studied, plants showed a significantly higher growth. The control plants and plants inoculated with pseudomonads alone became stunted and their leaves withered due to severe attack of *Fusarium oxysporum* f. sp. *ciceri* (Matuo and Sato), in them.

Chickpea plants inoculated with VAM and pseudomonads produced significantly more pods per plant than any other treatment. The treatment D (P+Ps+VAM) had the highest mean number of pods. Similar observations were made by Ahmad and Jha (1977) who reported an increase in chickpea yield in the presence of pseudomonads. Stanley *et al*; (1993) and Dwivedi, (2004) also reported that seed production of soybean increased in the presence of VAM. In these studies, chickpea plants inoculated with VAM and pseudomonads produced more leaves per plant. This could be explained on the basis of increased photosynthesis

shown by plants. The chickpea plants inoculated with VAM and pseudomonads also produced more roots per plant. The roots were longer in this treatment (D) in comparison to the other treatments. This improvement in plant growth and plant productivity could be attributed to the enhancement of the plant to absorb more nutrients such as phosphates by an increase in the absorbing surface area of roots.

The chickpea plants inoculated with VAM alone and those inoculated with both VAM and Pseudomonads exhibited the least percent disease incidence. Sharma and Champawat (2004) observed that pseudomonads increased plant tolerance to root pathogens due to their antagonistic behaviour. The dual inoculation of VAM and pseudomonads seem to have exerted beneficial effects by enhancing better growth and antagonizing the pathogen in the soil. This modification of pathogen behavior mediated by VAM and pseudomonads seem to have increased plant resistance against the wilt pathogen. Chickpea plants inoculated with pseudomonads alone showed complete wilting after 45 days and consequently pod formation could not occur. Apparently, rhizosphere bacteria failed to induce resistance in chickpea plants and therefore enabled the plants to develop fusarium wilt unchecked. This failure of rhizosphere pseudomonads could be explained on the basis of the absence of antagonistic strains against *Fusarium oxysporum* f. sp. *ciceri* (Matuo and Sato).

Reports suggest that VAM can improve plant nutrition by absorbing available soil nutrients and thus contribute to better growth and development of VAM inoculated plants (Abbott and Robson, 1977; Hayman, 1980). The effect of VAM alone on chickpea plants, treatment C, (P + VAM), was outstanding. All growth parameters indicted that VAM improved plant

nutrition and increased photosynthesis. This agrees with the findings of Abbott & Robson (1977).

In the parameter (fresh root weight) used to calculate the percent disease incidence (PDI), all treatments inoculated with VAM had very elaborate tap roots and their masses were higher than treatments A(P only) and B(P + Ps).

Thus, plants inoculated with VAM escaped early infection by either direct antagonistic interactions between VAM and the pathogen, or mycorrhiza-mediated triggering of plant defences (Whipps, 2004).

Rhizosphere pseudomonads act as plant growth promoting rhizobacteria (PGPR) and promote plant growth; they also act as antagonists to many root pathogens of plants by the involvement of different mechanisms (Cook and Rovira, 1976). Meyer & Linderman (1986) found that pseudomonads may inhibit development for some pathogens, but yet may allow others to flourish. This variation in effects on pathogens may depend on the amount and type of isolates of the bacteria used, the time of inoculation and the time of harvest. This might have been the case in the present study where pseudomonads of the chickpea rhizosphere did not show any antagonism to *Fusarium oxysporum* f.sp.*ciceri* (Matuo and Sato), when they were applied to chickpea soil alone. This might also have been due to incompatibility between pseudomonads and the chickpea vascular wilt pathogen. Similarly, VAM plays an important function in the reduction of plant pathogens (St-Arnaud *et al.*, 1995). Many

workers have observed an antagonistic effect of VAM against some fungal pathogens such as *Fusarium oxysporum* (Caron *et al.*, 1986; Dehne & Schonbeck, 1979).

In 1999 Budi *et al.,* postulated that bacteria such as pseudomonads in association with VAM also had the potential to control plant pathogens. Other studies also indicated that VAM-associated bacteria have the potential to control different plant pathogens such as *Pythium* sp., *Fusarium* sp. and *Rhizoctonia solani* (Secilia and Bagyaraj, 1987). A joint inoculation of VAM and pseudomonads on Fusarium wilt of chickpea was investigated. The effect of this co-inoculation was positive. All growth parameters measured under this treatment showed high growth and productivity and reduced disease incidence.

There seem to be strong antagonistic responses against the pathogen due to the interaction between VAM and pseudomonads. Therefore, the arbuscular mycorrhizal – bacterial combination studied here show to have functions both as growth promoting as well as pathogen antagonists. The mechanisms behind these observed effects can be multifold. They can produce an array of several enzymes such as chitinases, cellulases and proteases, and several extracellular metabolites like siderophores and indole acetic acid (IAA). All these are considered to be involved in nutrient acquisition, colonization competence and biocontrol ability as mentioned earlier.

VI. CHAPTER SIX

A. CONCLUSION.

The results of this investigation demonstrate that VAM fungi can reduce Fusarium wilt of chickpea, while rhizosphere pseudomonads alone cannot. A dual inoculation of VAM fungi and rhizosphere pseudomonads show that both can enhance growth and improve plant productivity and also reduce Fusarium wilt of chickpea in greenhouse grown plants. Plants under dual inoculation resulted in better growth, gave higher yield and also successfully reduced disease incidence in comparison to single inoculation with pseudomonads and control.

A. RECOMMENDATIONS.

The use of VAM fungi and rhizosphere pseudomnads in the control of Fusarium wilt of chickpeas is effective. However, more work is required to establish exact dosages per plant and under varying ecological conditions. This technique can be applicable in both greenhouses and field cultivation as long as the indigenous VAM levels and species effectiveness are known.

VII. REFERENCES

1. **Abbott, L. K.** and **Robson, A. D.** 1977. Growth stimulation of subterranean Clover, with vesicular-arbuscular mycorrhiza. *Australian journal of Agricultural Research* **28:**691-49.

2. **Ahmad, J. S.** and **Baker, R.** 1987. Competitive saprophytic ability and Cellulolytic activity of rhizosphere-competent mutants of *Trichoderma harzianum. Phytopathology* 7:358-362.

3. **Ahmad, N.** and **Jha, K. K.** 1977. Effect of inoculation with phosphate Solubilizing organisms on the yield of phosphate uptake of gram *Journal of Indian society of soil sciences* **25**: 391-393.

4. **Azcon-Aguilar, C.** and **Barea, J. M.** 1996. Arbuscular mycorrhiza and biological control of soil-borne plant pathogens: an overview of the mechanisms involved. *Mycorrhiza* **6**:457-464.

5. **Bakker, P. A. H. M., Lamers, J. G., Bakker, A. W., Marugg, J. D.,Weisbeek, P. J. and Schippers, B.** 1993. The role of siderophores in Potato tuber yield increase by *Pseudomonas putida* in short rotation of Potato. *European journal of plant pathology* 92: 249-256.

6. **Bharadwaj, D. P.** 2007. *The Plant – Arbuscular Mycorrhizal Fungi – Bacteria Pathogen System. Multifunctional Role of AMF Spore Associated Bacteria.* Ph. D. dissertation. Swedish University of Agricultural Sciences, Uppsala.

7. **Berg, G., Grosch, R.** and **Scherwinski, K.** 2007. Risk assessment for microbial Pantagonists: Are there effects on non-target organisms? *Gesunde Pflanzen.* 10.1007/s10343-007-0155-1.

8. **Bianciotto, V., Bandi, C., Minerdi, D., Sironi, M., Tichy, H.V. and Bonfante, P.** 1996. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. *Applied and environmental Microbiology* **62**: 3005-3010.

9. **Bianciotto, V.** and **Bonfante, P.** 2002. Arbuscular mycorrhizal fungi: a specialized niche for rhizospheric and endocellular bacteria Antonie Leeuwenhoek **81**: 365-371.

10. **Booth, C.** 1977. *Fusarium: Laboratory Guides to the Identification of Major Species.* Commonwealth Mycological Institute. Kew, Surrey, England.

11. **Budi, S.W., van Tuinen, D., Martinoti, G.** and **Gianinazzi, S.** 1999. Isolation from the sorghum bicolor mycorrhizosphere of a bacterium compatible with Arbuscular mycorrhiza development and antagonistic toward soil borne fungal pathogens. *Applied and Environmental Microbiology* **65**:5148-5150.

12. **Calvet, C., Pera, J.** and **Barea, J. M.** 1993. Growth response of marigold (*Tagetes erecta*) to inoculation with *Glomus mosseae*, *Trichoderma aureoviride* and *Pythium ultimum* in a peat-perlite mixture. *Plant and Soil* **148**: 1-6.

13. **Caron, M., Fortin, J.A.**, and **Richard, C**. 1985. Influence of substrate on the interaction of *Glomus intraradices* and *Fusarium oxysporum* f. sp.*radias-lycopersici* on tomato. *Plant Soil* **87**:233-239.

14. **Caron, M., Richard, C**. and **Fortin, J.A.** 1986. Effects of preinfestation of the soil by a Vesicular arbuscular mycorrhizal fungus, *Glomus intraradices*, on *Fusarium* crown and root rot of tomatoe *Phytoprotection* **67**: 15-19.

15. **Citernesi, A. S., Fortuna, P., Filippi, C., Bagnoli, G and Giovannetti, M.** 1996. The occurrence of antagonistic bacteria in *Glomus mosseae* pot cultures. *Agronomie* **16**:671-677.

16. **Cordier, M., Gianinazzi, S.**, and **Gianinzzi-Pearson, V**. 1996. Colonization patterns of root tissue by *Phytophtora nicotinae* var. *parasitica* relate to reduced disease in mycorrhizal plants. *Plant and Soil* **185**: 223-232.

17. **Crush, J. R**. 1974. Plant growth responses to Vesicular-arbuscular mycorrhiza.VII. Growth and nodulation of some herbage legumes. *New Phytologist* **73**:743-749.

18. **Cook, R. J.** and **Rovira, A. D**. 1976. The role of bacteria in the biological control of *Gaeumannomyces graminis* by suppressive soil. *Soil Biology. Biochemistry* **8**:269-273.

19. **Davis, R. M.** and **Menge, J. A.** 1980. Influence of *Glomus fasciculatus* on *Phytophtora* root rot of citrus. *Phytopathology* **70**: 447-452.

20. **Dehne, H. W**. 1977. Untersuchunge uber den einfluss der endotrophenmychorrhiza auf die Fusrium-Welke on tomate and Gurke. Diss. Bon (Cited by F. Schonbeek in "*Soil-borne Plant Pathogens*" edited by B.Schippers and W. Gams.) 1979.

21. **Dehne, H. W.** 1982. Interaction between vesicular arbuscular mycorrhizal fungi and plant

pathogens. *Phytopathology* **72**: 1115-1119.

22. **Dehne, H. W.** and **Schonbeck, F.** 1979. Untersuchungen zum Einfluss der endotrophen Mycorrhiza auf Pflanzenkrankheiten 1. Ausbreitung von *Fusarium oxysporum* f. sp. *Lycopersici* in Tomaten.*Phytopothologische Zeitschrift* **95**: 105-110.

23. **Dodd, J. C.** and **Thomson, B. D.** 1992. The screening and selection of inoculants in arbuscular mycorrhizal and ectomycorrhizal fungi. *The International Symposium on Management of Mycorrhiza in Agriculture.Horticulture and Forestry.* 28 September to 2 October 1992. Perth.Western Australia. pp.96.

24. **Dube, H. C.** 2001. Rhizobacteria in biological control and plant growth promotion. *Journal of Mycology and Plant Pathology* **31**:9-21.

25. **Dwivedi, O. P.** 2004. Effects of VA-mycorrhiza on winter wheat genotype C-306: *Journal of Mycology and Plant Pathology* **34**(2): 623-625.

26. **Edwards, S. G., Young, J. P. W.** and **Fitter, A. H.** 1998. Interactions between *Pseudomonas fluorescens* biocontrol agents and *Glomus mosseae*, an arbuscular mycorrhizal fungus, within the rhizosphere. *FEMS Microbiology Letters* **166**: 297-303.

27. **Filion, M., St-Arnaud, M.** and **Fortin, J. A.** 1999. Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere micro-organisms. *New Phytologist* **141**: 525-533.

28. **Food and Agricultural Organization of the United Nations** (1971). Crop Loss Assessment Methods; FAO manual on the evaluation and prevention of losses by pests' diseases and weeds. Rome: FAO pp. 200.

29. **Gan, J. Yates, S. R., Spencer, W. F., Yates, M.V. and Jury, W. A.** 1997.Laboratory scale measurements and simulations of effect of application methods on soil methyl bromide emission. *Journal of environmental quality* **26**: 310-317.

30. **Gaur, A. C.** 1979. Role of Phosphate solubilizing microorganisms increase phosphate nutrition and crop yields. *Krishi Chanekya* **1**:7-10.

31. **Gerdermann, J. W.** and Nicolson, T. H. 1963. Spores of Mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Transactionsof British Mycological Society* **46**:235-244.

32. **Grosch, R., Lottmann, J., Faltin, F.** and **Berg, G.** 2005. Use of bacterial antagonists to control disease caused by *Rhizoctonia solani. Gesunde Pflanze.* **57**: 199-205.

33. **Hackskaylo, E.** 1972. Mycorrhiza: The ultimate in reciprocal parasitism. *Bio-Science* **22**:577-582.

34. **Harley, I. R.** and **Smith, S. E.** 1983. *Mycorrhizal symbiosis*. Academic Press.London.

35. **Haware, M. P., Nene, N. L.** and **Rajeshwari,** R. 1978. The pulses.*Phytopathology* **68**:1364-7.

36. **Haware, M. P.** and **Nene, Y. L.** 1982. International Chickpea. *Newsletter***6**:17-18

37. **Haware, M. P.** and **Nene, N. L.** 1982. Fusarium wilts. *Plant Disease Reporter***66**:250-251.

38. **Hayman, D. S.** 1982. Influence of soils and fertility on activity and survival of vesicular-arbuscular mycorrhizal fungi. *Phytopathology* **72**:1110-1125.

39. **Hayman, D. S.** 1980. Mycorrhiza and crop production. *Nature* **287**:487-488.

40. **Hayman, D. S.** 1986. Mycorrhiza of nitrogen fixing legumes. MIRCEN *Journal* **2**:319-357.

41. **Hayman, D. S.** and **Mosse, B.** 1972. Plant growth responses to Vesicular-Arbuscular mycorrhiza. III: Increased uptake of labile Phosphorus from soil. *New Phytologist* **71**:41-47.

42. **Jeffries, P., Gianinazzi, S., Perotto, S., Tumau, K,** and **Barea. J.M.** 2003.The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biology and Fertility of Soils* **37**: 1-16.

43. **Koide, R. T., Li, M., Lewis, J.** and **Irby, C.** 1988. Role of mycorrhizal Infection in the growth and reproduction of wild versus cultivated plants. Wild versus cultivated oats. *Oecologia* 77:537-543.

44. **Kloepper, J. W.** and **Schroth, M. N.**1978. Plant growth-promoting hizobacteria on radishes. *In: proc. 4th International Conference. Plant pathogenic bacteria. Angers,* France pp. 879-882.

45. **Kumar, B. S. D.** and **Dube, H. C.** 1992. Seed bacterization with a fluorescent Pseudomonad for enhanced plant growth, yield and disease control. *Soil Biology and Biochemistry* **24**(6): 539-542.

46. **Lemanceau, P.** and **Alaboouvette, C.** 1993. Suppression of Fusarium wilts by fluorescent Pseudomonads: mechanisms and applications. *Biocontrol Science and Technology* **3**: 219-234.

47. **Levy, J.** and **Krikvin, J.** 1980. Effect of Vesicular-arbuscular mycorrhiza on citrus Jambhiri water relations. *New phytology* **85**:25-31.

48. **Linderman, R. G.** 1994. Role of vam fungi in biocontrol. (Mycorrhiza and Plant Health). *The American Phytopathological Society.* St. Paul, Minnesota.pp.1-25.

49. **Mayo, K., Davis, R. E.** and **Motta, J.** 1986. Stimulation of germination of spore of *Glomus versiforme* by spore-associated bacteria. *Mycologia* **78**: 426-431

50. **Meyer, J. R.** and **Linderman, R. G.** 1986a. Response of subterranean clover to dual-inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonas putida. Soil Biology and Biochemistry* **18**: 185-190.

51. **Murray, R. G. E.** 1974. Division II. *The bacteria.* In Bergey's manual of Determinative Bacteriology. The Williams and Wilking Company. Baltimore, pp. 1268.

52. **Nene, Y. L., Haware, M.** P. and **Reddy, M. V.** 1981. Chickpea disease resistance screening techniques. *ICRISAT. Information Bulletin* **10**:1-10.

53. **Nene, Y. L.** 1980. *Pulse Pathology, Progress Report 8.* Hyderabad, India:ICRISAT.

54. **Nene, Y. L., Kannaiyan, J., Haware, M. P.** and **Reddy, M. V.** 1980. InProceedings of the Consultant's Group Discussion on the Resistance to Soil-Borne Disease Of Legumes.Hyderabad, India: ICRISAT, 3-35.

55. **Niemira, B. A., Hammerschmidt, R.** and **Safir, G. P.** 1996. Post-harvest suppression of potato dry rot *(Fusarium sambucinum)* in pre-nuclear minitubers by arbuscular mycorrhizal fungi inoculums. *American Potato Journal* **73**: 509-515.

56. **Pandey, K. K.** and **Upadhyay, J. P.** 2000. Microbial Population from Rhizosphere and non-rhizosphere soil of pigeon pea: screening for resident antagonists and mode of mycoparasitism. *Journal of Mycology and Plant Pathology* **30**:7-10.

57. **Raaijmakers, J. M., Leeman, M., van Oorschot, M. M. P., van der Sluis, I.,Schippers, B. and Bakker, P. A. H. M.** 1995. Dose-response relationships in biological control of Fusarium wilt of radish by *Pseudomonas* spp. *Phytopathology* **85**: 1075-1081

58. **Schippers, B., Bakker, A. W.** and **Bakker, P. A. H. M.** 1987. Interactions ofdeleterious and beneficial rhizosphere microorganisms and the effect of cropping practice. *Annual Review of Phytopathology* **25**: 339-358.

59. **Secilia, J.** and **Bagyaraj, D.J.** 1987. Bacteria and actinomycetes associated with pot cultures of vesicular arbuscular mycorrhiza. *Canadian Journal of Biology* **33**: 1069-1073

60. **Sharma, R. N.** and **Champawat, R. S.** 2004. Rhizosphere and rhizoplane microorganisms in the control of wilt disease of Jojoba (*Simmondsia chinensis*) caused by *Fusarium oxysporum.* (Abstr.) *Journal Mycology and Plant Pathology* **34**: 522-3.

61. **Sieverding, E.** 1991. Vesicular-arbuscular mycorrhiza management in tropical agro system. Schriftenreiheder Gtz No.224, Federal Republic of Germany.

62. **Singh, K. B.** and **Saxena, M. C.** 1999. Chickpeas. *The Tropical Agriculturalist*, PP 110-114.

63. **Smith, S. E.** and **Read, D. J.** 1997. *Mycorrhizal symbiosis*. Academic Press.San Diego.

64. **St – Arnaud, M., Hamel, C., Caron, M.,** and **Fortin, J.A.** 1995. Inhibition of *Pythium ultimum* in roots and growth substrate of mycorrhizal *Tagetes patula* colonized with *Glomus intraradices*. *Canadian Journal of Plant Pathology* **16**: 187-194

65. **St – Arnaud, M., Hamel, C., Vimard, B., Caron, M**, and **Fortin, J.A.** 1997.Inhibition of *Fusarium oxysporum* f. Sp. Dianthi in the non-VAM species *Dianthus caryophyllus* by the co-culture with *Tagetes patula* companion plants colonized by *Glomus intraradices*. *Canadian Journal of Botany* **75**: 998-1005.

66. **Stan, N.** 1981. Anatomy and physiology of Vesicular-arbuscular and non-mycorrhizal roots. *Phytopathology* **72**:1102.

67. **Stanley, M. R., Koide, R. T.** and **Shumway, D. L.** 1993. Mycorrhizalsymbiosis increases growth, reproduction and recruitment and *Abutilon theophratic* medic. In the field. *Ecologia* **94**: 30-35.

68. **Statistix For Windows (1985, 96).** Analytical Software. Version 1.0. P.O. Bo 12185, Tallahassee FL 32317, USA.

69. **Summerfield, R. J.** and **Robert, E. H.** 1985. *Grain legume crops*. William Collins and Sons Co. Ltd. London.

70. **Suslow, T. V.** 1982. Role of root-colonizing bacteria in plant growth. In: M. S.Mount and G. H. Lacy (Eds). *Phytopathogenic Prokaryotes. Vol. 1.* Academic Press. New York. pp. 123-187.

71. **Toro, M., Nedialkova, K., Azron, R.** and **Barea, J.M.** 1996. Establishment of two rock phosphate solubilizing bacteria in the rhizosphere of mycorrhizal onion plants and their effect on plant growth in a microcosm. In Mycorrhizas in integrated systems: From genes to plant development (EUR 16728). Edited by C. Azcon – Aguilar and J. M.Borea. European Commission, Luxemburg, Luxemburg. pp. 665-668.

72. **Toljander, J. F., Arthursson, V., Paul, L. R., Jansson, J. K.** and **Finlay, R.** 2006. Attachment of different soil bacteria to arbuscular mycorrhizal fungal extra radical hyphae is determined by hyphal vitality and fungal species. *FEMS letters* **254**(1):34-40.

73. **Von Alten, H., Lindermann, A.** and **Schonbeck, F.** 1993 Stimulation of vesicular-arbuscular mycorrhiza by fungicides or rhizosphere bacteria. *Mycorrhiza* **2**: 167-173.

74. **Weller, D. M.** 1988. Biological Control of soil-borne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* **26**: 379-407.

75. **Whipps, J.M.** 2004. Prospects and Limitations for Mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany* **82**: 1098-1127.

76. **Wright, D. P., Scholes, J. D.** and **Read, D. J.** 1998. VA mycorrhizal sink strength influences the carbon allocation of *Trifolium repens*. *Journal of Experimental Botany* **49**: Supplement, 60.

77. **Yao, M.K., Tweddell, R.J.** and **Desilets, H.** 2002. Effect of Two vesicular-arbuscular

mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. *Mycorrhiza* **12**: 235-245.

78. **Yuen, G. Y., Schroth, M. N.** and **McCain, A. H.** 1985. Reduction of Fusarium Wilt of Carnation with Suppressive soils and antagonistic bacteria.*Plant Disease* **69**: 1071-1075.

VIII. Appendix

Appendix 1 Maximum and Minimum temperature (oC) recorded from the green house.

Month Parameter	Dec 2006	Jan 2007	Feb 2007	Mar 2007	April 2007
Min (range)	22 – 40	21 – 40	22 – 34	22 – 31	24 – 33
Min (mean)	31	29	28	26	29
Max (range)	37 – 48	29 – 46	29 – 46	26 – 45	29 – 44
Max (mean)	44	39	38	36	37

Appendix 2: SUNRISES AND SUNSETS FOR LUSAKA

Location EO 28:19 S15:25

Rise and Set for the sun for 2006/2007

Zone 2 hr. East of Greenwich

	Dec (2006)		Jan		Feb		Mar		April	
Day	Rise	Set	Rise	Set	Rise	Set	Rise	Set	Rise	Set
	h:m	h:m	h:m	h:m	h:m	h:m	h:m	h:m	h:m	h:m
01	05:26	18:25	05:39	18:41	05:57	18:43	06:07	18:31	06:12	18:09
02	05:27	18:25	05:40	18:42	05:57	18:43	06:07	18:30	06:12	18:08
03	05:27	18:26	05:40	18:42	05:58	18:43	06:08	18:30	06:12	18:08
04	05:25	18:27	05:41	18:42	05:58	18:43	06:08	18:29	06:13	18:07
05	05:27	18:27	05:42	18:42	05:59	18:42	06:08	18:28	06:13	18:06
06	05:28	18:28	05:42	18:43	05:59	18:42	06:08	18:28	06:13	18:05
07	05:28	18:28	05:43	18:43	06:00	18:42	06:08	18:27	06:13	18:05
08	05:28	18:29	05:43	18:43	06:00	18:42	06:09	18:26	06:13	18:04
09	05:28	18:29	05:44	18:43	06:01	18:41	06:09	18:26	06:13	18:03
10	05:29	18:30	05:45	18:43	06:01	18:41	06:09	18:26	06:13	18:03
11	05:29	18:31	05:45	18:44	06:01	18:40	06:09	18:25	06:14	18:02
12	05:29	18:31	05:46	18:44	06:09	18:24	06:09	18:24	06:14	18:01
13	05:30	18:32	05:46	18:44	06:02	18:39	06:10	18:24	06:14	18:01
14	05:30	18:32	05:47	18:44	06:03	18:39	06:10	18:23	06:14	18:00
15	05:31	18:33	05:48	18:44	06:03	18:39	06:10	18:22	06:14	17:59
16	05:31	18:33	05:48	18:44	06:03	18:38	06:10	18:21	06:14	17:59
17	05:31	18:34	05:49	18:44	06:04	18:38	06:10	18:20	06:14	17:58
18	05:32	18:34	05:49	18:45	06:04	18:37	06:10	18:19	06:15	17:58
19	05:32	18:35	05:50	18:45	06:04	18:37	06:10	18:19	06:15	17:57
20	05:53	18:36	05:51	18:45	06:05	18:36	06:11	18:18	06:15	17:56
21	05:33	18:36	05:51	18:45	06:05	18:36	06:11	18:17	06:15	17:56
22	05:34	18:37	05:52	18:45	06:05	18:35	06:11	18:16	06:15	17:55
23	05:34	18:37	05:52	18:45	06:06	18:35	06:11	18:16	06:16	17:55
24	05:35	18:37	05:53	18:45	06:06	18:34	06:11	18:15	06:16	17:54
25	05:35	18:38	05:53	18:44	06:06	18:33	06:11	18:14	06:16	17:53
26	05:36	18:38	05:54	18:44	06:06	18:33	06:11	18:13	06:16	17:53
27	05:36	18:39	05:54	18:44	06:07	18:32	06:12	18:13	06:16	16:53
28	05:37	18:39	05:55	18:44	06:07	18:32	06:12	18:12	06:17	17:52
29	05:37	18:40	05:55	18:44			06:12	18:11	06:17	17:51
30	05:38	18:40	05:56	18:44			06:12	18:11	06:17	17:51
31	05:39	18:40	05:56	18:44			06:12	18:10		

Appendix 3: One-way analysis of variance (ANOVA) for the number of pods per plant produced by chickpea (*Cicer arietinum* L.)

Source	df	ss	ms	f	p
Between pods	03	1220.54	406.85	49.68	0.060
Within pods	76	622.45	8.19		
Total	79	1842.99			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

Appendix 4: One-way analysis of variance (ANOVA) for seed weight per plant produced by chickpea (*Cicer arietinum* L.)

Source	df	ss	ms	f	p
Between seeds	03	49.88	16.63	42.28	0.00
Within seeds	76	29.88	0.39		
Total	79	79.76			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

Appendix 5: One-way analysis of variance (ANOVA) for leaves per plant produced by chickpea (*Cicer arietinum* L.)

Source	df	ss	ms	f	p
Between leaves	03	28055.2	9351.75	231.57	0.00
Within leaves	76	3069.2	40.39		
Total	79	31124.4			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

Appendix 6: One-way analysis of variance (ANOVA) for plant height produced by chickpea (*Cicer arietinum* L.)

Source	df	ss	ms	f	p
Between heights	03	1658.22	552.74	97.79	0.00
Within heights	76	429.58	5.65		
Total	79	2087.80			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

Appendix 7: One-way analysis of variance (ANOVA) for the number of flowers per plant produced by chickpea (*Cicer arietinum* L.)

Source	df	ss	ms	f	p
Between flowers	03	2213.24	737.74	70.38	0.00
Within flowers	76	796.65	10.48		
Total	79	3009.89			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

Appendix 8: One-way analysis of variance (ANOVA) for shoot weight produced by chickpea (*Cicer arietinum* L)

Source	df	ss	ms	f	p
Between shoots	03	1783.84	594.61	314.96	0.00
Within shoots	76	143.48	10.89		
Total	79	1927.32			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

Appendix 9: One-way analysis of variance (ANOVA) for root weight produced by chickpea (*Cicer arietinum* L.)

Source	df	ss	ms	f	p
Between roots	03	34.92	11.64	13.79	0.00
Within roots	76	64.13	0.84		
Total	79	99.06			

df = degree of freedom; ss = sum of squares; ms = mean square; f = f-ratio; p = probability

.Appendix.10: Best subset regression models for pods per plant of chickpea (*Cicer arietinum* L.)

Model Variables	Mallows Cp Statistic	Adjust r^2	r^2	Residual Sum of squares (RSS)
Intercept only	107.4	0	0	1842.99
Photoperd	2.6	0.5706	0.576	781.393
Max temp	90.5	0.0904	0.1019	16.55.12
Av Temp	99.6	0.0408	0.0529	17.45.48
Photoperd*Min Temp	4.1	0.5679	0.5788	776.249
Photoperd*Min Temp	4.3	0.5665	0.5766	778.77
Photoperd*Av, Temp	31	0.5656	0.5766	780.407
Photoperd*Min Temp*Max Temp	4.9	0.579	0.595	746.48
Photoperd*Min Temp*Av Temp	4.9	0.5688	0.5852	764.438
Photoperd*Max Temp*Av Temp	5.9	0.563	0.5796	774760
Photoperd*Min Temp*Max Temp*Av Temp	5	0.5739	0.5955	745.49

Av. Temp= Average temperature; photoperd = photoperiod; Max.temp = Maximum temperature; Min temp= Minimum temperature

Appendix.11: Best subset regression models for seed weight per plant of chickpea (*Cicer arietinum* L.)

Model Variables	Mallows Cp Statistic	Adjust r^2	r^2	Residual Sum of squares (RSS)
Intercept only	97.5	0	0	79.7595
Photoperd	0	0.5611	0.5667	34.5632
Max temp	85.8	0.066	0.0778	73.5561
Av Temp	89.3	0.046	0.0581	75.1282
Photoperd*Min Temp	1.9	0.5562	0.5675	34.4986
Photoperd*Min Temp	2	0.5555	0.5667	34.5562
Photoperd*Av, Temp	2	05554	0.5667	34.5623
Photoperd*Min Temp*Max Temp	3.5	0.553	0.57	34.3
Photoperd*Min Temp*Av Temp	3.8	0.5509	0.568	34.4572
Photoperd*Max Temp*Av Temp	4	0.5498	0.5669	34.5451
Photoperd*Min Temp*Max Temp*Av Temp	5	0.5498	0.5726	34.0884

Av. temp = Average temperature; photoperd = photoperiod; Max.temp = Maximum temperature; Min temp= Minimum temperature

Appendix.12: Best subset regression models for plant height of chickpea (*Cicer arietinum* L.)

Model Variables	Mallows Cp Statistic	Adjust r^2	r^2	Residual Sum of squares (RSS)
Intercept only	138.3	0	0	3009.89
Photoperd	1.1	0.639	0.6435	1072.9
Max temp	123.8	0.0642	0.0076	2781.11
Av Temp	124.3	0.0621	0.0739	2787.37
Photoperd*Min Temp	2.9	0.635	06443	1070.75
Photoperd*Min Temp	3.1	0.6343	0.6436	1072.79
Photoperd*Av, Temp	3.1	0.6	0.5766	780.407
Photoperd*Min Temp*Max Temp	3.9	0.6353	0.6491	1056.04
Photoperd*Min Temp*Av Temp	4.9	0.6304	0.6445	1070.14
Photoperd*Max Temp*Av Temp	5.1	0.6297	0.6437	1072.35
Photoperd*Min Temp*Max Temp*Av Temp	5	0.6347	0.6532	1043.82

Av. Temp= Average temperature; photoperd = photoperiod; Max.temp = Maximum temperature; Min temp= Minimum temperature

Appendix.13: Best subset regression models for number of leaves per plant of chickpea (*Cicer arietinum* L.)

Model Variables	Mallows Cp Statistic	Adjust r^2	r^2	Residual Sum of Squares (RSS)
Intercept only	22.8	0	0	311.4
Photoperd	1.8	0.7544	0.7575	7547.2
Max temp	198.9	0.1385	0.1494	26473.4
Av Temp	211.2	0.0934	0.1049	27858.8
Photoperd*Min Temp	2.4	0.7556	0.7618	7414.52
Photoperd*Min Temp	3.4	0.7526	0.7589	7504.07
Photoperd*Av, Temp	3.7	0.7516	0.7579	7535.6
Photoperd*Min Temp*Max Temp	3	0.757	0.7662	7275.9
Photoperd*Min Temp*Av Temp	3.4	0.7556	0.7649	7318.4
Photoperd*Max Temp*Av Temp	3.6	0.755	0.7643	7336.16
Photoperd*Min Temp*Max Temp*Av Temp	5	0.7538	0.7662	7275.69

Av. Temp= Average temperature; photoperd = photoperiod; Max.temp = Maximum temperature; Min temp= Minimum temperature

Appendix.14: Best subset regression models for number of flowers per plant of chickpea (*Cicer arietinum* L.)

Model Variables	Mallows Cp Statistic	Adjust r^2	r^2	Residual Sum of squares
Intercept only	287.7	0.0000	0.0000	1927.32
Photoperd	3.1	0.7807	0.7835	417.322
Max temp	244.4	0.1120	0.1233	1689.74
Av Temp	261.3	0.0652	0.0771	1778.78
Photoperd*Min Temp	3.1	0.7837	0.7891	406.412
Photoperd*Min Temp	5.0	0.7783	0.7839	416.467
Photoperd*Av, Temp	5.0	0.7781	0.7838	416.761
Photoperd*Min Temp*Max Temp	3.0	0.7866	0.7947	395.607
Photoperd*Min Temp*Av Temp	3.5	0.7853	0.7934	398.098
Photoperd*Max Temp*Av Temp	4.4	0.7826	0.7909	403.031
Photoperd*Min Temp*Max Temp*Av Temp	5.0	0.7838	0.7948	395.571

Av. Temp= Average temperature; photoperd = photoperiod; Max.temp = Maximum temperature; Min temp = Minimum temperature