

**SCREENING CHICKPEA (*Cicer arietinum* L.) GENOTYPES FOR FUSARIUM WILT
RESISTANCE AND ITS MANAGEMENT USING FUNGICIDES, IN NJORO KENYA**

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the Degree of Master of Science in Agronomy (Crop Protection) of Egerton University**

EGERTON UNIVERSITY

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This is my original work and has not been previously presented for an award of degree in this or any other University.

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DEDICATION

I dedicate this thesis to my family, brothers; Shadrack and Josphat, and my beloved mother.

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ABSTRACT

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceris* is a seed and soil borne disease affecting chickpea, *Cicer arietinum* L. It is widely distributed where chickpea is grown causing yield losses ranging from 12 to 100% depending on the level of resistance of the genotype and the suitability of environmental conditions for disease development. Evolution of new and virulent races of *Fusarium oxysporum* f. sp. *ciceris* necessitates continued screening, breeding and deployment of new resistance genes when hitherto resistance genes succumb to new races of the pathogen. Two experiments were set up in this study. The aim of the first experiment was to screen 20 chickpea genotypes introduced from ICRISAT for resistance to Fusarium wilt under greenhouse conditions in a completely randomized design (CRD). The second experiment was conducted to evaluate efficacy of two fungicides, thiram and carbendazim in managing Fusarium wilt and was in a split plot design. Four rates of each fungicide (0%, 50%, 100% and 150%) of the recommended rate (1.5 g/Kg seed), were used. One resistant variety (Chania 1) and one highly susceptible variety (Chania 2) selected from the greenhouse screening experiment were used in the field experiment. Six genotypes were found to be moderately resistant, ten were susceptible and four were highly susceptible. Carbendazim and thiram rates were effective in reducing wilt incidence in chickpea. The least wilt incidence was observed when 150% rate of either thiram or carbendazim was used. Highest wilt incidence was observed in control treatment plots. There was a positive interaction between variety and fungicide application on dry matter and grain yield. Interaction between moderately resistant Chania 1 and fungicide resulted in significantly lower wilt incidence, higher dry matter production and highest grain yield of 1.4 t/ha. Significantly higher wilt incidence, lower dry matter and grain yield of 1.3 t/ha were observed in the interaction between fungicide treatment and highly susceptible Chania 2 variety. Six moderately resistant varieties; 95423, 97105, 97114, 97125, 97126 and 97406 could be used together with 100% and 150% rate of application of either thiram or carbendazim in order to manage wilt incidence and ensure higher dry matter and grain yield.

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LIST OF ABBREVIATIONS

ABBREVIATION

ASAL:	Arid and semi-arid lands.
ANOVA:	Analysis of variance.
CRD:	Completely randomized design.
DAS:	Days after sowing.
DMRT:	Duncan's multiple range test
DI:	Disease incidence.
DM:	Dry matter.
FW:	Fusarium wilt.
HI:	Harvest index.
HPR:	Host plant resistance.
HS:	Highly susceptible.
ICRISAT:	International Crops Research Institute for the Semi-Arid Tropics
IDM:	Integrated disease management
K.A.L.R.O:	Kenya agriculture and livestock research organization.
LSD:	Least significant difference.
MPEND:	Ministry of Economic Planning and National Development.
PDA:	Potato Dextrose Agar.
PH:	Plant Height
MR:	Moderately Resistant
ROR:	Recommended optimum rate
S:	Susceptible
SSP:	Single Super Phosphate
TN:	Trade Name

CHAPTER ONE

INTRODUCTION

1.1 Background Information

In Kenya, 89% of land is arid and semi-arid (ASAL) (MD&P, 2015). These dry land areas experience problems of moisture stress and various pests and diseases that attack crops. These combined with poor management severely limits crop production and leads to food insecurity. Other constraints to crop production in these areas include poor quality of seed and the suitability of the crop genotype to selected growing environment.

Chickpea (*Cicer arietinum* L.) is an important cool season food legume crop mainly grown in areas with residual soil moisture and has great agronomic potential for use as food grain, salad, snacks like *mandazi* and *chapati* and forage in dry land areas of Kenya (Nielson, 2001; Oweis *et al.*, 2004; Kamithi *et al.*, 2008). It is a hardy crop that grows in dry land areas and yields substantially well. Studies in Naivasha have shown potential yields of between 1.6 and 2.3 tons of grain per hectare for genotype ICCV 95423 (Kabuli) and ICCV 97105 (Desi), respectively (Kibe and Onyari, 2008) and at varying plant population densities and nitrogen levels (Kibe and Kamithi, 2007).

Serious pests and diseases affecting the chickpea crop at various growth stages however limit its productivity. Generally, the pathogens that affect chickpea crop include fungi, bacteria, viruses and mycoplasma. More than 30 pathogens have been reported to affect chickpea in different parts of the world, but only a few of them are economically important in as far as the damage they cause is concerned (Nene *et al.*, 1991). Fungal pathogens affecting roots, stems, leaves, flowers and pods comprise the most devastating group of pathogens of chickpea. The important diseases are Ascochyta blight (*Ascochyta rabiei*); Fusarium wilt, Dry root rot, Stunt, Botrytis gray mould, Collar rot, Black root rot, Phytophthora root rot, Pythium root rot and seed rot (Nene *et al.*, 1991). The complex in which soil borne pathogens occur makes isolated control of one pathogen difficult and therefore necessitates combination of host plant resistance together with fungicidal seed treatment strategies and other cultural practices like crop rotation.

Chickpea is a relatively new crop in Kenya and a lot of yield performance trials are still being undertaken on many genotypes. Some genotypes have been released as varieties and seed bulking in the Rift Valley is going on. Several genotypes that have been found to yield well

across several environments need to be evaluated further for drought tolerance and resistance to pests and diseases. This study was part of the larger ICRISAT research and its aim was to screen 20 genotypes for resistance to Fusarium wilt under greenhouse conditions and later assess the efficacy of carbendazim and thiram as seed dress fungicides in the control of Fusarium wilt in the field.

1.2 Statement of the problem

Soil borne diseases are a problem to sustainable chickpea production in the world. Fusarium wilt causes varying degree of yield losses which can be as high as eighty percent depending on the level of resistance of the genotype. The complex in which soil borne diseases occur necessitates preventive control measures because effective control is hard to achieve once the disease is established. The successful adoption of chickpea could be hampered by diseases and pests which affect the crop. Soil borne diseases occur as a complex and as such control of one disease in isolation is impractical. Host plant resistance (HPR) is one major method used to prevent *Fusarium oxysporum* f. sp. *ciceris* infection in chickpea. Evolution of new races of *Fusarium oxysporum* f. sp. *ciceris* leads to breakdown of host resistance resulting in short lifespan of resistant genotypes. Twenty chickpea genotypes introduced for possible adoption by farmers in Njoro region had to be evaluated for susceptibility/resistance to Fusarium wilt. An integrated disease management approach that entails chemical seed dressing, cultural practices like crop rotation and host plant resistance are used to ensure durability of resistance genes. Fungicides carbendazim and thiram among others; have been used in other parts of the world to control infection of chickpea by *Fusarium oxysporum* f. sp. *ciceris* and other soil borne pathogens. No research has been done in Kenya to assess the efficacy and optimize seed dressing rates of these fungicides for the control of Fusarium wilt in chickpea.

1.3 Objectives

1.3.1 General Objective

To screen chickpea genotypes for resistance to Fusarium wilt and to study the efficacy of fungicides in the management of Fusarium wilt and thus increase in yields.

1.3.2 Specific objectives

- i. To screen twenty chickpea genotypes for resistance to Fusarium wilt under greenhouse conditions.
- ii. To determine the efficacy of varying thiram and carbendazim rates in the control of Fusarium wilt incidence and yield on selected chickpea genotypes under field conditions.
- iii. To determine variety and fungicide interaction effects in the control of Fusarium wilt incidence and on yields of chickpea under field conditions (Sick plot).

1.4 Hypotheses (H₀)

- i. There are no chickpea genotypes resistant to Fusarium wilt.
- ii. Thiram and carbendazim rates are not effective in the control of Fusarium wilt of chickpea.
- iii. There are no interaction effects of fungicide with variety (host plant resistance) in the control of Fusarium wilt and yield of chickpea.

1.5 Justification

Fusarium wilt is a fungal disease which is both seed and soil borne. Yield loss from this disease is variable but it can cause total crop failure when the genotype is susceptible. Most soil borne diseases are mainly controlled through preventive methods like host plant resistance and fungicidal seed treatment. Use of resistant genotypes is the most reliable, environmental friendly, economically affordable method of Fusarium wilt management for small scale farmers. Genotypes of chickpea differ in their levels of resistance to Fusarium wilt. It is important to screen selected genotypes to identify inherent resistance against Fusarium wilt, in each genotype.

This way genotypes introduced from ICRISAT into Kenya had need to be identified for resistance to Fusarium wilt and could be used for further breeding and adoption in farmers' fields. Host plant resistance (HPR) is used together with other methods of control in an integrated management. Fungicidal seed treatment is normally used in conjunction with HPR thus reducing excessive use of fungicides and thus reduces environmental pollution arising from fungicides. Carbendazim and thiram had not been assessed for efficacy against soil borne pathogen *F. oxysporum* f. sp. *ciceris*. This study was conducted to determine the efficacy of carbendazim and thiram fungicidal seed dressing and their interaction with identified varieties possessing various resistance reactions to Fusarium wilt.

CHAPTER TWO

LITERATURE REVIEW

2.1. Chickpea plant

2.1.1 Chickpea production

Chickpea (*Cicer arietinum* L.) is the third most important legume crop in the world after dry beans and peas (Romeis *et al.*, 2004; Kumar *et al.*, 2004). It is second in importance after rice in Asia (ICRISAT, 2005) and is highly adapted to varied agro-ecological zones (Kibe and Onyari, 2008). Annual global production is estimated at 9.24 million tons grown on 12.03 million hectares with average yields of 818 Kg/ha (ICRISAT, 2007) with the major producing areas being India, Pakistan, Turkey, Iran, Myanmar, Ethiopia, Mexico, Australia, Canada and Iraq. Approximately 90% of the global area and 88% of production is concentrated in Asia with India being the leading chickpea growing country taking over 60 % share in acreage and production. In Africa, Ethiopia is the leading grower of chickpea with approximately 37% of the total hectares in Africa and over 48% of production (Daba *et al.*, 2005; Kibe and Kamithi, 2007). In Eastern and Southern Africa, chickpea is an important legume crop, with Ethiopia, Tanzania, Malawi and Sudan being the leading producers (ICRISAT, 2006). In Kenya it is grown by few farmers in Eastern and Rift valley provinces.

Chickpea is an important source of protein for humans which can also be used as animal feed (Oweis *et al.*, 2004). It is a key component in the diets and forms a rich source of essential vitamins, minerals, and important amino acids like lysine and other secondary metabolites (Grusak, 2002). It is also used in snacks like *mandazi* and *chapati* and forms an important salad while large seeded Kabuli type chickpea are sold for canning purposes; with the dry matter being a component of animal feed (Oweis *et al.*, 2004). The crop can fix high amounts of nitrogen in cereal-legume rotation systems, conserves soil moisture through addition of organic matter, act as a break-crop that facilitates control of diseases, pests and weeds and also improves the physical characteristics of various soil types (Pye *et al.*, 1984; Taa *et al.*, 1997; ICRISAT, 2001; Cheruiyot *et al.*, 2001; Cheruiyot *et al.*, 2002).

Chickpea has gained importance in Australia, Canada and USA as a relay and rotational crop with cereals like wheat (ICRISAT, 2008). Desi and Kabuli are the two main types of chickpea. Although Desi type is the most common as compared to the Kabuli type there has been an

increase in farmers' interest on Kabuli because of favorable prices and the extra-large size of beans with test weights of 35 to 40 g/100 seed (Daba *et al.*, 2005). Kabuli beans are whitish/cream coloured while the Desi types are brownish, small seeded with test weights of 15 to 27 g/ 100 seed (Kamithi *et al.*, 2008).

2.1.2 Chickpea production constraints

Biotic and abiotic stresses that affect chickpea are some of the important constraints that limit the production level of chickpea and cause yield loss of about one third (Haware *et al.*, 1992). Abiotic stresses like drought and high temperatures set limits to chickpea production and breeding is usually focused towards these two stresses. Biotic stresses include insect pests and diseases that affect chickpea. Among the insect pests; *Helicoverpa amigera* is the most serious and varieties are developed with resistance to this pest in mind (Singh *et al.*, 2006).

There are numerous diseases of chickpea that cause varying degree of yield loss depending on the pathogen, the resistance of the host and the time of infection with regard to environmental conditions. Ascochyta blight and Botrytis grey mold are foliar diseases of chickpea that are very serious and can cause high degree of yield loss depending on the host's resistance. Use of fungicides to control these two pathogens is not economical because four to six sprays may be necessary (Porta-Puglia *et al.*, 1996) and this necessitates development of resistant genotypes as the only cost effective method of managing these two diseases (Nasir *et al.*, 2000; Pande *et al.*, 2007).

2.2 Diseases of chickpea

Soil borne diseases that commonly affect chickpea include Fusarium wilt, Collar rot, Black root rot and other root rots caused by Pythium. Fusarium wilt is the most serious root disease wherever chickpea is grown (Pande *et al.*, 2007). The pathogen, *Fusarium oxysporum* f. sp. *ciceris* is distributed worldwide (Zamani *et al.*, 2004). Additionally, seed and soil can be infected with the fungus *Botrytis cinerea* (grey mould) and this can attack the plant, causing the base of the chickpea stem to rot and eventually resulting in death of the plant.

2.2.1 Fusarium wilt

Fusarium wilt causes severe losses on most vegetables, flowers, several field crops like cotton and tobacco, and plantation crops such as sugarcane, coffee, plantain, banana and a few shade

trees. *Fusarium* wilt is most severe under warm moist conditions and in greenhouses (Agrios, 2005). *Fusarium oxysporum* is a large cosmopolitan genus of imperfect fungi and is of primary interest because numerous species are important plant pathogens (Austwick, 1982). *Fusarium* species causes vascular wilts, with most of the wilting due to *Fusarium oxysporum* (Agrios, 2005).

Fusarium wilt of chickpea is a major prevalent disease in most chickpea growing areas and is distributed worldwide wherever chickpea is grown (Jalali and chand, 1992). It has been reported to cause over eighty percent yield loss (Singh *et al.*, 2006). The pathogen belongs to the genus *Fusaria*. *Fusarium oxysporum* f. sp. *ciceris* has been identified as causing wilt in chickpea; and these has been accepted worldwide as the causal agent for *cicer* spp (Booth, 1971). *Fusarium oxysporum* f. sp. *ciceris* is a seed and soil borne pathogen that colonizes the xylem vessels and blocks them completely to effect wilting (Bateman *et al.*, 1996). The disease affects crop in all stages and symptoms can be manifested from seedling to maturity. *Fusarium* wilt can cause high degree of yield loss depending on the susceptibility of the cultivar and the race of the pathogen. The disease can cause estimated severe yield loss of 60-70% (Jalali and Chand, 1992) with complete grain loss if the disease occurs at vegetative and reproductive stages (Navas-Cortes *et al.*, 2000).

Management of *Fusarium* wilt has been largely through development of resistant varieties in an integrated approach; but the high pathogenic variability in populations of *Fusarium oxysporum* f. sp. *ciceris* presents problems of sustainable resistance. Two pathotypes and eight races have so far been identified (Jimenez-Diaz *et al.*, 1993). This means that the use of resistant varieties could play an important role in race identification in future. The pathotypes either induce severe wilting or yellowing and gradual wilting on the affected plant (Landa *et al.*, 2004).

The existence of races was first shown by Haware and Nene (1982) who described races 1, 2, 3 and 4. To date; eight races designated as 0, 1A, 1B, 1C, 2, 3 4 5 and 6 (Haware and Nene, 1982; Navas-Cortes *et al.*, 2000) have been described, and the races are divided broadly into two pathotypes of *Fusarium oxysporum* f. sp. *ciceris* (Landa *et al.*, 2004). The first pathotype induce severe leaf chlorosis, flaccidity and plant death by 15-20 days after inoculation (vascular wilt), and the other pathotype induces progressive foliar yellowing, which develops 30-40 days after inoculation, and late death of the plant (vascular yellowing) (Jimenez-Diaz and Trapero-casas,

1990). The regional distribution of these races across the world indicates regional specificity (Kamal and Fred, 2007) for their occurrence.

2.2.1 Symptomatology of Fusarium wilt

The symptoms that are observed on the chickpea plant infected and affected by Fusarium wilt are generally classified according to growth stage. It is worthwhile to note that symptoms observed may vary according to the pathotypes or race of the pathogen. As mentioned earlier, there are eight races of *Fusarium oxysporum* f. sp. *ciceris*; and the symptoms observed are classified into two categories; those that cause instant wilting, and those that induce yellowing and progressive wilting (Landa *et al.*, 2004). Based on crop stage, the following symptoms can be observed on infected plants. The infection process is influenced by the environment specifically temperature and inoculum load.

2.3 Seedling stage

The disease can be observed within three weeks of sowing. Whole seedlings (3 to 5 weeks after sowing) collapse and lie flat on the ground. These seedlings retain their dull green colour. When uprooted, they usually show uneven shrinking of the stem above and below the collar region (soil level). The shrunken portion maybe about 2.5cm or longer. Affected seedlings do not rot on the stem or root surface. However, when split open vertically from the collar downwards or cut transversely, dark brown to black discolouration of the internal stem tissues is clearly visible and in the seedlings of highly susceptible cultivars which die within 10 to 15 days of emergence, the black discolouration may not be clearly visible. However, internal browning from the root tip upwards is clearly seen (Nene *et al.*, 1991).

2.4 Adult stage

The affected plants show typical wilting symptoms i.e. drooping of the petioles, rachis and leaflets followed by a yellowing of foliage and premature senescence. Drooping is clearly visible initially in the upper part of a plant but within a day or two; the entire plant droops and the affected leaves are chlorotic but most of the other leaves drop while still green. Gradually; however, all the leaves turn yellow and then light brown or straw coloured (Agrios, 2005). Dried leaflets or infected plants are not shed at maturity. Affected plants when uprooted and examined before they are completely dry show no external rotting or root discolouration. When stem is split open vertically, internal discolouration can be seen. Around the collar region above and

below the xylem in the central and inner portion (pith and part of the wood) is discoloured dark brown or black (Nene *et al.*, 1979).

In the initial stages of wilting the discolouration may not be continuous. Discolouration also extends several centimeters above the collar region into the main stem and branches. If the collar region is cut transversely with a sharp razor blade, black discoloration of both pith and xylem can be seen. Sometimes only a few branches are affected resulting in partial wilt in certain cultivars. The lower leaves dry up before the plant wilts. Wilt incidence is generally higher when chickpea is grown in warmer and drier climates ($>25^{\circ}\text{C}$) and when crop rotations are not practiced (Nene *et al.*, 1991).

2.5 Control measures

2.5.1 Control of soil borne diseases of chickpea

The complexity in the control of established soil borne diseases makes it necessary to apply integrated disease management (IDM) strategies. Some IDM measures commonly used involve the utilization of host plant resistance, fungicidal seed dressing methods, biological suppression of the pathogens and other cultural methods in a compatible manner. Preventive methods of Fusarium wilt management are more effective because the chlamyospores can persist in the soil for indefinite periods of time (Haware *et al.*, 1986; Agrios, 2005).

2.5.2 Cultural control of Fusarium wilt

Cultural management strategies like avoiding plant stress (poor fertility, water logging, drought, herbicide injury) which increase the risk of root rot problems should be avoided where possible. Using healthy seed with high germination is important because vigorous seedlings have a better chance to outgrow early-season infection. Planting crops when the weather conditions are likely to be non-conducive for the pathogen can also ensure a healthy crop (Agrios, 2005).

Cultural control mainly entails having a stress free crop and reliance of unfavourable weather conditions, but once the inoculum has been introduced into the soil, it remains there for long periods of time of over 6 years (Haware *et al.*, 1986) and this method may then cease to be effective. Practices like crop rotation can therefore be ineffective (Haware *et al.*, 1990) due to the persistence of *Fusarium oxysporum* chlamyospores in the soil and hence long term control can only be achieved by use of resistant cultivars (Nene and Haware, 1980; Haware *et al.*, 1992).

Integrated management combination of sowing date, partially resistant genotypes, seed and soil treatments with bio-control agents in field micro-plots infested with *Fusarium oxysporum* f. sp. *ciceris* race 5 showed that advancing sowing date from early spring to winter significantly delayed disease onset, reduced final disease intensity and increased yield (Landa *et al.*, 2004).

Some of these soil borne diseases may be effectively managed through alteration of sowing date such that an unsuitable environmental conditions of the pathogen to develop are created. The long term control strategy however is the use of resistant or partially resistant varieties (Landa *et al.*, 2004).

2.5.3 Biological control of Fusarium wilt

Biological management of Fusarium wilt of chickpea has been addressed using bacterial and fungal antagonists. Isolates of *Pseudomonas* spp., *Bacillus* spp., *Peanibacillus* spp., and non-pathogenic isolates of *F. oxysporum* have been found to be effective in suppressing Fusarium wilt under controlled conditions (Landa *et al.*, 2004). Mycorrhizal fungi can be used to effectively manage soil borne pathogens. Kumar *et al.* (2004) demonstrated by a pot research the efficacy of mycorrhizal fungi in controlling soil borne plant pathogens. He found that Mycorrhizal inoculation suppressed the incidence of wilt and root rot diseases by fifty-four and sixty-two percent, respectively.

Biological control therefore offers potential suppression of Fusarium wilt under field conditions especially if used in combination with partial host plant resistance. The drawback of this management option was the short life span of the micro-organisms and the over reliance on conducive environmental conditions which might not always be possible.

2.5.4 Fungicidal control of soil borne diseases

The value of Fungicide seed treatments includes protection of seed viability and inhibition of diseases like seed rot and seedling blight. Seed treatments protect the seed by controlling fungi present either on the seed surface or carried internally in the seed and by controlling fungi present in the soil, or on crop residue in the soil (Pesticide News, 2002). Fungicides can only be used in a limited scale, for example as seed dress in an integrated disease management approach (Wagara, 2005). Fungicide seed treatments such as Apron (metalaxyl), Agrox, Captan Flowable (captan) and Thiram 75WP (thiram) can be used to protect the seedlings in early stages of plant establishment. In a study to evaluate the efficacy of different fungicide treatments, 50 seeds of

chickpea cultivar Pant G-186 were subjected to standard blotter tests. The fungicide treatments were carbendazim + thiram and benomyl + thiram (each applied at 1 g/ kg seed) and carbendazim, benomyl, captan, thiram, and indofil M-45 (Mancozeb + thiophanate-methyl) and Difolatan (captafol) (each applied at 2 g/kg seed) in controlling seed pathogens. Seed germination was highest with carbendazim (Singh *et al.*, 2004).

Based on this research, it would be easy to advocate for use of carbendazim for seed treatment. But it may not be possible to directly extrapolate the results to our local conditions and it is necessary to try out our own studies to see if our results as regards carbendazim will tally with this. In a different study, seven fungicides (thiram, Bavistin (carbendazim) Blitox (copper oxychloride), Captaf (captan), Indofil M-45 (Mancozeb + thiophanate-methyl), Ridomil MZ (Mancozeb + Metalaxyl) and Kitazin (iprobenfos) were evaluated against chickpea wilt *in-vitro* and *in-vivo* (seed treatment) and as soil drench. Thiram and Bavistin proved the most effective *in-vitro* by decreasing disease incidence and increasing grain yield under field conditions, (Singh and Jha, 2003). Based on the above two studies, it can be concluded that both carbendazim and thiram seem to be the best as regards fungicidal prevention of seed and seedling infection by soil borne pathogens.

2.5.4.1 Carbendazim

Carbendazim (methyl benzimidazol-2-acylcarbamate) is a systemic benzimidazole fungicide. It is used to control a broad range of diseases on cereals, fruits, cotton, tobacco, turf, ornamentals and vegetables (Pesticides News, 2002). It is also used in post-harvest food storage, as a seed pre-planting treatment and as a timber treatment fungicide. In addition to being a fungicide in its own right, carbendazim is a metabolite of thiophanate-methyl. Thiophanate breaks down rapidly in the environment to carbendazim and the use of thiophanate-methyl can lead to residues of carbendazim in treated commodities. It is frequently sold in combination with other fungicides, such as triazoles, dithiocarbamates and dicarboximides.

Carbendazim works by inhibiting the development of fungi probably by interfering with spindle formation at mitosis (cell division). It has extensive applications worldwide, with the global market worth over \$200 million at user level, equivalent to over 12000 tones active ingredient. Over the years, there has been a gradual reduction in carbendazim use and in 1996 just over two million hectares were treated with carbendazim in Great Britain, compared to nearly 1.8 million

hectares in 1999 and 821,000 hectares in 2000. Modern conazole and strobilurin fungicides are more efficacious. Application rates of 0.6-0.8 g/kg seed are effective for control of Fusarium (Pesticides News, 2002).

2.5.4.2 Thiram

Thiram belongs to the class dimethyl dithiocarbamate and is widely used as a seed dress fungicide to prevent crop damage in the field and to protect harvested crops from deterioration in storage or transport. It is mostly used to protect fruits, vegetables, ornamentals and turf crops from a variety of fungal diseases. Thiram is available as dust, flowable, wettable powder, water dispersible granules, and water suspension formulations and in mixtures with other fungicides. It is classified as toxicity class III- slightly toxic. Thiraflo is a broad spectrum flowable seed treatment fungicide and contains thiram as the active ingredient. It is registered for the control of damping off (*Pythium* spp.) as well as other seed and soil borne diseases of chickpea including Botrytis grey mould (*Botrytis cinerea*). Thiraflo acts by controlling and protecting the plant from all these diseases and thus increases early establishment of the chickpea crop (Hannaford, 2007).

2.6 Integrated management of Fusarium wilt in chickpea.

Integrated disease management (IDM) is important for sustainable legume farming. Fusarium wilt epidemics cause significant annual losses that may reach 100% (Landa *et al.*, 2004) under conditions favourable for disease development. Management of Fusarium wilt is difficult to achieve and no single control strategy is effective. The use of resistant varieties of chickpea is the most practical and economically efficient means of controlling Fusarium wilt. Good progress has been made in developing high yielding well adapted chickpea genotypes with complete or partial resistance to Fusarium wilt (Landa *et al.*, 2004). The evolution of new and virulent races of *Fusarium oxysporum* f. sp. *ciceris* to hitherto resistant varieties could curtail wilt management using resistance mechanisms.

Crop rotation, soil solarization, pathogen free seed and fungicide seed treatment have been used to control Fusarium wilt in an integrated approach. A proper integrated package will vary according to the prevailing environmental conditions and the nature of the genotype in terms of resistance or susceptibility.

2.7 Host plant resistance

One of the aims of plant breeding is developing resistant cultivars. Host plant resistance is stable, durable environmentally friendly and is technically feasible at the farmers' level (Pastor-Corrales *et al.*, 1998). It is one of the methods of preventing Fusarium wilt and other soil-borne diseases. Plant screening techniques include use of sick plot technique and green house screening through inoculation (Pande *et al.*, 2006). Screening is geared towards identification of genotypes that are resistant to some of these important diseases during research stage. Breeding for resistance has led to the introduction of early maturing and Fusarium wilt resistant chickpea varieties culminating into dramatic change in productivity.

2.8 Screening chickpea germplasm for Fusarium wilt resistance

An essential component is screening and assessment in which presence or absence of a resistant character or the degree to which it is expressed is determined. A great deal of effort goes into elimination of susceptible plant material early in the breeding programme using large scale, free choice evaluations, often in field plots or greenhouses (Miklas *et al.*, 2005). Material selected in these initial experiments is then assessed more vigorously in smaller groups that usually include susceptible genotypes as well as identifying useful sources of resistance for breeding. The more detailed approach also provides the basis of breeding genotypes during cultivar development.

Before individual cultivars are finally released, large scale field assessments are carried out to confirm the performance of variety under more realistic field conditions. Evolution of new races of *Fusarium oxysporum* f. sp. *ciceris* necessitates continuous screening and assessment of chickpea germplasm to identify resistance genes which can still resist the pathogen and those that have succumbed to these new races. Screening methodologies have been evolving over time, from the conventional pots experiments, field sick plot experiments and lately genetic markers have been used to identify resistance genes in plants. Screening method adopted should be able to incite a disease such that it is possible to identify the susceptible from the resistant varieties. The methodology should ensure proper exposure of the pathogen inoculum to the plant. Pot screening is an effective methodology when the roots of the plant are injured, thereby creating an entry point for the pathogen. Field sick plot screening is effective but the inoculum level has to be high enough through repeated planting and testing of susceptible genotypes. Where genetic markers are used, field tests are still useful in ascertain the results of the lab screening.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Experimental site description

K.A.L.R.O Njoro, Kenya lies at a latitude of 0° 23' south, longitude of 35° 35' East and altitude of 2,238 meters above sea level. This area falls in agro-ecological zone Lower Highland 3 (Jaetzold and Schmidt, 1983) and the soils at the site are vitric mollic Andosols. The site receives annual mean rainfall of 930 mm. The temperatures in the field are in the range of 19°C-22°C (mean maximum) and 5-8°C (mean minimum) (Jaetzold and Schmidt, 1983). Two experiments were set up in this study. The first experiment was aimed at screening 20 chickpea genotypes for resistance to *Fusarium* wilt. The experimental set up was in pots in a glasshouse at K.A.L.R.O Njoro in the year 2009. The second was done in K.A.L.R.O Njoro, field 4. The aim of the second experiment was to determine the efficacy of two fungicides; thiram and carbendazim in the control of *Fusarium* wilt and the effect of the treatments on the yield of chickpea. The experiment was setup in a wilt sick-plot at K.A.L.R.O Njoro.

3.2 Pathogen isolation, purification and plant inoculation

The pathogen; *Fusarium oxysporum* f. sp. *ciceris* was cultured and sub-cultured through single spore isolation/streaking (to purify) in the department of Biological sciences laboratory at Egerton University using potato dextrose agar (PDA). The initial culture was obtained from diseased plant tissues collected from Bomet, K.A.L.R.O Njoro and Gilgil where the crop had been grown previously in order to have all the representative races of the pathogen.

3.3 Screening chickpea genotypes for resistance

A total of 20 genotypes were planted in pots inside a greenhouse in K.A.L.R.O Njoro, Kenya during July to October season of 2009 and during the January-March season of 2010. The plants were grown in the greenhouse in pots in a completely randomized design and the level of resistance to *Fusarium* wilt assessed. Randomization was achieved using simple random numbers selection by shaking and picking from a container with the pot initials. The pathogen *Fusarium oxysporum* f. sp. *ciceris* causing *Fusarium* wilt was confirmed as present through pathological studies of identification, culture and subculture and plant re-infection chickpea crop at experimental sites of K.A.L.R.O Njoro. The study was repeated for a second season. The Soil

was sterilized at 121 °C at 15 pounds of pressure for one hour in an autoclave and placed in sterile plastic pots. Ten seedlings were raised in each pot; the experimental design was completely randomized design (CRD). The Ten-day old seedlings of the 20 chickpea genotypes were inoculated by dipping their roots in a suspension (5.1×10^6) of the pathogen and the seedlings transplanted into the pots containing the sterilized soil. The greenhouse experiment was replicated three times giving 60 pots in total.

During the plant growth duration, periodic scoring for the Fusarium wilt was performed on all the pots. This was done by monitoring weekly for symptoms of Fusarium wilt. The wilted plants were counted on each pot and scored on a record sheet. Data obtained was computed into excel data sheet and the wilt incidence determined. The wilt incidence was determined as follows, (Neupane *et al.*, 2007)

$$\% \text{ wilt incidence} = (\text{number of wilted plants/Initial number of plants}) \times 100$$

Further data analysis was done using Genstat to determine whether the observations were significantly different from each other. The genotypes were then classified as resistant (less than 10% incidence), moderately resistant (11-20% incidence), susceptible (21-50% incidence) and highly susceptible (over 50% incidence) (Neupane *et al.*, 2007).

The statistical model for the greenhouse experiment was:

$$y_{ij} = \mu + t_i + e_{ij}$$

where, y_{ij} was the area under disease in the i^{th} chickpea genotype at the j^{th} replicate.

μ was the overall mean

t_i was the i^{th} chickpea genotype effect

e_{ij} was the random error component

3.4 Evaluation of fungicides rates in control of Fusarium wilt (sick plot)

The experiment was set up in a sick plot at KARI Njoro where other screening efforts were on going. One moderately resistant genotype (MR) and one highly susceptible (HS) genotype selected from the greenhouse experiment was used in the experiment and two fungicides (carbendazim and thiram). Four levels/rates; 0, 50, 100 and 150% of ICRISAT's recommended dosage (1.5 g/kg seed) of each fungicide was used. The experimental design was split-plot replicated three times in two seasons. The main plot factors were two varieties of chickpea (ICCV 97105 and ICCV 92944) while the sub plot factors were the two fungicides (Ft and Fc)

thiram and carbendazim both at rates of 50, 100 and 150% randomized within the main plot. There was one sub plot of control (0%) in each main plot; which was not treated with any fungicide.

This experimental design gave a total of seven treatment combinations per main plot factor, 14 treatments per rep and an overall total of 42 micro-plots. Weekly scoring for wilt incidence was done on all treatment plots and the experiment was repeated in another season. Overall wilt incidence was calculated as a percentage of the original population using standard method described by Nasir *et al.* (2000) and Neupane *et al.* (2007). The data was tabulated and analyzed using Genstat software. The linear model fitted this field experiment was as follows.

$$Y_{jk} = \mu + V_j + V_{ij} + F_k + (FV)_{jk} + F_{ik} + \epsilon_{jk}$$

$j = 1, 2; k = 1, 2 \dots 7$

where, μ was Grand mean, V_j is j^{th} chickpea genotype effect, V_{ij} was main plot error (error a), F_k was effect of k^{th} fungicide combination, $(FV)_{jk}$ was interaction effect of the j^{th} genotype and the k^{th} fungicide combination, F_{jk} was subplot error (error b); while ϵ_{jk} was the residual effect error.

3.5 Land preparation and Planting

Land preparation was done before planting by clearing the weeds followed by deep ploughing in order to create a suitable tilth for planting. Harrowing was done using a forked jembe and leveled with a rake. Seeds of the selected genotypes were planted at an intra-spacing of 10cm and while the interspacing was 40cm, thereby achieving a plant population of 250,000 plants/ha.

3.6 Agronomic practices

Debris and stubble were removed before sowing. Supplementary watering was done to the pots in the greenhouse using a watering-can before planting and thereafter at a 14-day interval to avoid plant water stress. The pots were kept weed and pest free through monitoring for pests like *Helicoverpa amigera*, and weeding. First weeding was done after 21 DAS and subsequently, on need basis. For the field study, weeding was done by uprooting and hoeing out the weeds in the field to achieve clear fields.

3.6.1 Fungicide and Pesticide application

The seeds were treated uniformly at each of the four rates (0%, 50%, 100% and 150%) for each fungicide to protect against Fusarium wilt. Computation of the actual rate was done based

ICRISAT recommended rates which on average is 1.5 g/kg seed for each fungicide. Knapsack sprayer was used to apply Bulldock Star at 0.5 Litres/ha for control of *Helicoverpa amigera* and other pests. No other fungicide was applied.

3.7 Crop attributes measured (Field experiment)

3.7.1 Fusarium wilt incidence

Disease score for Fusarium wilt incidence following the standard procedure (described by Nasir *et al.*, 2000; Neupane *et al.*, 2007) was done from the 8th DAS until maturity on weekly basis for field experiment. Observations on wilt incidence were recorded and the data was tabulated into growth intervals and analyzed using Genstat statistical software. The results were interpreted as resistant (less than 10% incidence), moderately resistant (11-20% incidence), susceptible (21-50% incidence) and highly susceptible (over 50% incidence) (Neupane *et al.*, 2007). Further data analysis to separate means of treatments was performed using Genstat software version 14.

3.7.2 Plant growth attributes and yield parameters (Field experiment)

1. Height of the plant: The height of the plant was determined at 30, 60, 90 and 120 days after sowing (DAS). The vertical height of 4 randomly sampled plants was measured with a tape measure from ground level to the highest upper part and their average computed.
2. Periodic dry matter: Periodic dry matter was determined at intervals of 30, 60, 90 days after sowing and final harvesting. This was done using destructive sampling where above ground parts in 0.5m² was harvested and oven dried at 60°C to a constant weight after which the dry matter weight was measured with electronic balance. Sampling was done randomly.
3. Number of pods per plant: This was obtained by sampling randomly 8 plants within each micro-plot and counting the number of pods per plant, and then calculating the average.
4. Grain Yield: This was established by harvesting the seed from each plot, drying and taking their weights.
5. Harvest Index: The relationship between grain (seed) yield and the total dry matter (harvesting) was established.

3.8 Data analysis

Data obtained was analysed for ANOVA and mean separation performed using LSD. Genstat version 14 statistical software was used to analyse data.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Screening chickpea genotypes for resistance to Fusarium wilt

The first visible wilt symptoms appeared two weeks after inoculation with the pathogen. Fusarium wilt incidence varied between the 20 genotypes. It was observed that in the moderately resistant genotypes, there was no wilting in the first 30 days after sowing (DAS). In the susceptible and highly susceptible genotypes, wilting was observed beginning 30 DAS. Of the 20 genotypes assessed, 6 were moderately resistant (MR), 10 were susceptible (S) and the remaining 4 were highly susceptible (HS). None was completely resistant (R) (Table 1).

Moderately resistant genotypes identified were 95423, 97105, 97114, 97125, 97126 and 97406. In these MR chickpea genotypes, Fusarium wilt incidence at 30 DAS was 0-3.7%, and then increased to a range between 11.4-14.8% by the 45th DAS and 13.6-15.9% at 60 DAS. Chickpea genotypes susceptible (S) to Fusarium wilt had a disease incidence range of 0-20.6% by 30 DAS, 10.3-36.7% by 45 DAS and 21.5-42.7% at 60 DAS (Table 1). The susceptible genotypes were 00108, 00302, 00305, 92311, 92318, 95311, 97031, 96329, 97201 and Ngara local. Chickpea genotypes that were highly susceptible (HS) were 00402, 92944, 97110 and 97306. The HS genotypes had wilt incidence in the range of 11.1-14.2% at 30 DAS, 33.2-65.4% at 45 DAS and 66.3-83.8% at 60 DAS (Table 1). Chaudry *et al.* (2007) screened 196 accessions and found no single immune or highly resistant genotype.

Screening work by Muhammad *et al.* (2010) found genotypes 92944, 00108, and 00305 were resistant to wilt, but in this study were found susceptible. Genotype 97126 which this study found to be moderately resistant was also found to be resistant by Muhammad *et al.* (2010). The variations could be due to different races found in different geographic regions and the evolution of new races. In the moderately resistant chickpea (MR) genotypes, wilting incidence between 45 and 60 DAS rose from 14.8 to 15.9% only, i.e., 1.1%. Susceptible (S) genotypes revealed a wider variation in wilt incidence of up to 6% (that is, 36.7 to 42.7% (Table 1) during the same period of growth. The smaller range of increase in wilt incidence amongst the MR genotypes is attributed to their genetic make-up (host plant resistance).

Table 1: Periodic wilt incidence for 20 chickpea genotypes inoculated with *Fusarium oxysporum* f. sp. *ciceris* under greenhouse conditions

Code	Genotype	30 DAS	45 DAS	60 DAS	Rating (See legend below)
1	ICCV 00108	20.67	31.58	41.88	S
2	ICCV 00302	13.75	36.70	39.88	S
3	ICCV 00305	12.23	23.32	42.73	S
4	ICCV 92311	11.77	28.63	33.55	S
5	ICCV 92318	10.55	21.10	41.08	S
6	ICCV 95311	7.32	13.40	23.90	S
7	ICCV 96329	12.32	22.25	41.32	S
8	ICCV 97201	6.62	13.33	24.08	S
9	ICCV 97031	11.08	26.33	40.42	S
10	Ngara local	10.58	21.93	31.47	S
11	ICCV 00402	11.18	33.25	66.30	HS
12	ICCV 92944	12.68	65.48	83.80	HS
13	ICCV 97110	12.30	33.07	70.12	HS
14	ICCV 97306	14.27	41.67	71.22	HS
15	ICCV 95423	0.00	12.42	14.37	MR
16	ICCV 97105	0.00	11.43	13.60	MR
17	ICCV 97114	0.00	14.83	15.72	MR
18	ICCV 97125	0.00	14.63	15.90	MR
19	ICCV 97126	0.00	11.78	13.98	MR
20	ICCV 97406	3.78	13.48	15.22	MR
Lsd ($P \leq 0.05$)		0.91	1.91	2.15	
S.E		0.79	1.65	1.86	
C.V (%)		9.30	6.70	5.0	

Legend: Disease score rating (based on % disease incidence): 0-10 Resistant, 11-20 Moderately resistant 21-50 Susceptible, Over 51 Highly susceptible (Neupane *et al.*, 2007)

Previous studies by Haware and Nene (1980) reported that yield of chickpea was significantly affected by the time of wilting; with more losses being experienced when early wilting occurs. Early wilting could result in low population which consequently leads to low yield. Late wilting also contributes to yield losses (Haware and Nene, 1980). It was apparent that susceptible and highly susceptible genotypes experienced high Fusarium wilt incidence from the 30th DAS and peaking at 60 DAS. Resistance to wilt is a complicated mechanism and is governed by either monogenes or oligogenes. Late wilting is due to oligogenic resistance mechanism which delays disease symptoms and slow development of disease after pathogen reaction takes place (Sharma and Muehlbauer, 2007).

Models were developed to relate wilt incidence to plant growth for each classification of resistance as shown in Tables 2, 3 and 4. The functions of the models for the susceptible chickpea genotypes are given in Table 2.

Table 2: Functional relationship of Fusarium wilt incidence with time (DAS) from 30 DAS to 60 DAS for susceptible (S) chickpea genotypes

Genotype (ICCV)	Function	Goodness of fit (R^2)
92311	$y = 10.89x + 2.87$	0.91
92318	$y = 15.27x - 6.29$	0.97
97201	$y = 8.73x - 2.79$	0.98
00302	$y = 13.07x + 3.98$	0.84
Ngara local	$y = 10.44x + 0.44$	0.99
00305	$y = 15.25x - 4.40$	0.97
95311`	$y = 8.29x - 1.71$	0.97
97031	$y = 14.67x - 3.39$	0.99
96329	$y = 14.50x - 3.71$	0.96
00108	$y = 10.61x + 10.16$	0.99

These models could be used in predicting the wilt incidence in susceptible, highly susceptible and moderately resistant genotypes of chickpea, respectively. It was observed from the models that wilt incidence increased at a higher rate in the susceptible and the highly susceptible chickpea genotypes. It can be inferred that these susceptible genotypes would need management strategies to prevent economic damage from Fusarium wilt given the high rate of disease progression under ideal pathogenic conditions. It is evident from these models that in MR genotypes, though infection occurred, the rate of disease development was slow which was reflected by a low wilt incidence.

Table 3: Functional relationship of Fusarium wilt incidence with time (DAS) from 30 DAS to 60 DAS for highly susceptible (HS) chickpea genotypes

Genotypes (ICCV)	Function	Goodness of fit (R^2)
00402	$y = 27.56x - 18.21$	0.99
97110	$y = 28.91x - 19.32$	0.97
97306	$y = 28.48x - 14.57$	0.99
92944 (Chania 2)	$y = 35.56x - 17.13$	0.93

Table 4: Functional relationship of Fusarium wilt incidence with time (DAS) from 30 DAS to 60 DAS for moderately resistant (MR) chickpea genotypes

Genotype (ICCV)	Function	Goodness of fit (R^2)
97105 (Chania 1)	$y = 6.8x - 5.26$	0.87
97126	$y = 6.99x - 5.39$	0.87
95423 (Saina 1)	$y = 7.19x - 5.44$	0.85
97406	$y = 5.72x - 0.61$	0.86
97114	$y = 7.86x - 5.54$	0.79
97125	$y = 7.95x - 5.72$	0.81

In the moderately resistant (MR) genotypes, it was observed that the rate of increase in wilt incidence ranged from 5.7 to 7.9% increase per day (Table 4). This was a lower rate of increase in wilt incidence and had goodness of fit (R^2) ranging from 0.80 to 0.87. In the susceptible genotypes, wilt incidence increased in the range of 8.3 to 15.3% per day (Table 2) which was higher than the rate of increase observed in the moderately resistant genotypes. In the highly susceptible (HS) genotypes, the range of wilt incidence was from 27.6 to 35.6% increase per day (Table 3). These results were in agreement with findings of Muhammad *et al.* (2010) who reported that disease development in resistant genotypes was slow as compared to susceptible genotypes. Sharma and Muehlbauer (2007) also reported that in resistant genotypes, disease progression was somewhat low due to the genetic response of the genotype that delayed symptom development.

4.2 Effect of thiram and carbendazim rates on Fusarium wilt in chickpea (Sick plot)

Control of Fusarium wilt was assessed by determining the disease incidence on chickpea plants during the growth period (Table 5). Highly susceptible genotype (92944, i.e., variety Chania 2) and moderately resistant genotype (97105, i.e., Chania 1) identified in the greenhouse study (Table 1) were used in the trial. Chickpea genotype 92944 was released as variety Chania 2 by Egerton University in 2013 while moderately resistant genotype 97105 was released as Chania 1 in the year 2011. All fungicide treatments were significantly ($P \leq 0.05$) lower in wilt incidence as compared to the control treatment (no fungicide). Application of fungicide at all rates was observed to significantly reduce wilt incidence across all stages of growth.

4.2.1 Effect of variety on Fusarium wilt incidence

Highly susceptible Chania 2 showed significantly ($P \leq 0.05$) higher wilt incidence than MR Chania 1 across all growth stages (Table 5). This means that the pathogen readily established itself after invading the highly susceptible Chania 2 than Chania 1. Wilt incidence in Chania 2 ranged from 4.6 to 23.8% while in Chania 1, the range was between 2.6 and 11.9. Wilt incidence was regressed over time in Figure 1 to determine the rate of change in wilt incidence with growth stage. Fusarium wilt (FW) incidence of both chickpea genotypes grown in the field was explained by the quadratic functions given in Figure 1. It was evident from the models in Figure 1 that Fusarium wilt incidence after the 30th DAS increased at the rate of 2.2% for the moderately resistant variety Chania 1 and 4.0% for the HS Chania 2. Compared to the green

house data, the rates for HS genotypes and MR genotypes were over 35% and 5.7% (Table 3 and 4), respectively. In the field, other environmental conditions might affect the development of Fusarium wilt. Therefore, greenhouse wilt incidence cannot be reliably extrapolated to predict response of field grown chickpea. The greenhouse had perfect conditions for disease development. Using the models, it can be inferred that complete wilting would take up to 75 days for MR and 54 days for HS genotypes. This shows that MR Chania 1 can resist the pathogenic attack for a prolonged time as compared to the highly susceptible genotypes Chania 2. In figure 1 Chania 2 had low wilt incidence at 60 DAS. This low incidence is an advantage because it means such genotypes would require less foliar fungicide for management of Fusarium wilt incidence.

Table 5: Effect of variety and fungicide rates on Fusarium wilt incidence of chickpea under field conditions

Treatments	Disease incidence (%) (Season I & II)					
	30 DAS (I)	30 DAS (II)	45 DAS (I)	45 DAS (II)	60 DAS (I)	60 DAS(II)
Chania 2	4.70 a	4.60 a	13.68 a	12.79 a	23.83 a	21.41 a
Chania 1	2.67 b	2.58 b	9.60 b	8.53 b	13.16 b	11.99 b
LSD	0.50	0.88	0.80	2.10	3.19	2.63
C.V	3.90	7.0	2.0	5.60	4.90	4.50
No fungicide	5.65 a	5.90 a	19.90 a	19.68 a	30.20 a	27.57 a
Thiram _{50%}	4.70 b	4.15 b	15.95 b	13.38 b	22.88 b	21.32 b
Thiram _{100%}	3.27 c	3.30 c	9.05 c	8.82 c	17.12 c	15.60 c
Thiram _{150%}	1.83 e	1.80 d	5.18 d	4.87 d	8.92 d	7.87 d
Carbendazim _{50%}	4.82 b	4.42 b	16.48 b	13.82 b	23.12 b	21.67 b
Carbendazim _{100%}	3.35 c	3.43 c	9.45 c	8.95 c	18.17 c	14.57 c
Carbendazim _{150%}	2.15 d	2.11 d	5.45 d	5.10 d	9.08 d	8.33 d
LSD	0.27	0.41	0.93	0.78	1.15	1.18
C.V	6.10	9.60	6.70	6.10	5.20	7.70

Means followed by the same letter(s) in the same column are not significantly different at $P \leq 0.05$ using LSD

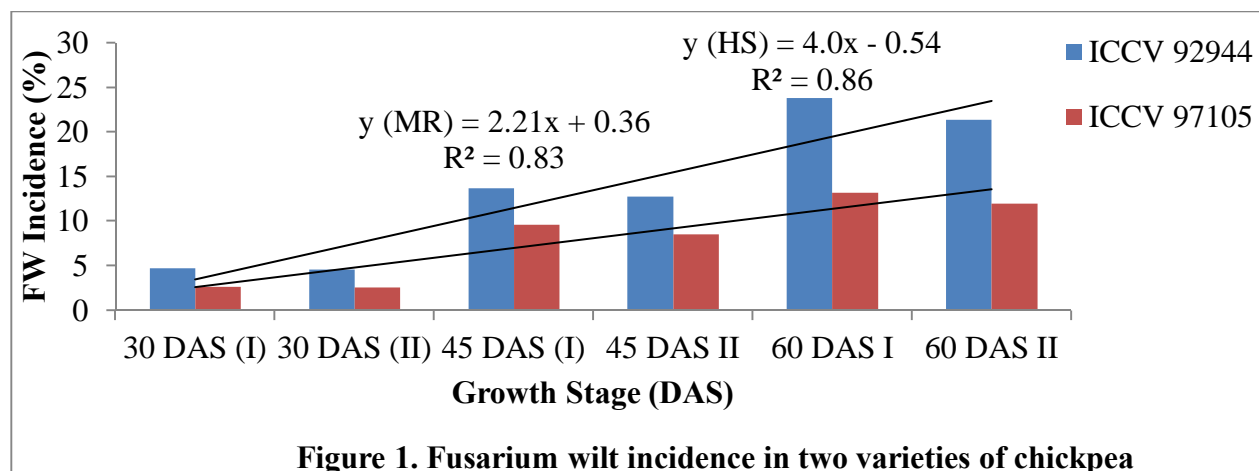


Figure 1. Fusarium wilt incidence in two varieties of chickpea

4.2.2 Effect of varying carbendazim rates in control of Fusarium wilt of chickpea

Significant ($P \leq 0.05$) differences in wilt incidences were observed between carbendazim rates used. Treatment of chickpea with 150% carbendazim resulted in low wilt incidence of 2.1% at 30 DAS, in both season I and II (Table 5). At 45 DAS, treatment of chickpea with 150% carbendazim resulted in wilt incidence of 5.4% and 5.1% in season I and II, respectively. At 60 DAS plots treated with 150% carbendazim had wilt incidence of 9.0% and 8.3% during season I and II, respectively. At 30 DAS treatment plots with 100% carbendazim had 3.3% wilt incidence in season I and 3.4% in season II. At 45 DAS, 9.4% and 8.9% wilt incidence was observed in 100% carbendazim treatment, for season I and II, respectively.

At 60 DAS, treatment of chickpea with 100% carbendazim resulted in wilt incidence of 18.1% and 14.5% for season I and II, respectively (Table 5). At 30 DAS control treatment had wilt incidence of 5.6% and 5.9% in season I and II, respectively. At 45 DAS, control treatment had wilt incidence of 19.9% and 19.6% in season I and II, respectively and at 60 DAS, the same treatment had 30.2% and 27.5% wilt incidence in seasons I and II, respectively. From these results, it was observed that significant ($P \leq 0.05$) variations occurred between carbendazim rates used in terms of control of wilt incidence. The least wilt incidence was observed when 150% of carbendazim was used, with values ranging from 2.1% to 9.0% across all the growth stages. It can be inferred that the best fungicide treatment rate in the management of Fusarium wilt was 150%.

Fungicides act by eradicating the pathogen in the soil or on the seed (Pesticide news, 2002), thereby reducing chances of wilt development. Muhammad (2010) reported a direct correlation

between inoculum density and wilt severity. Carbendazim at 100% was the next best level of treatment in the control of Fusarium wilt. The treatment that was least effective was the control. This shows that if chickpea is not treated with seed dress fungicide, under suitable environmental and host conditions, high wilt incidence is likely to be observed. *In vitro* studies on the inhibition of *F. oxysporum* f. sp. *ciceris* by various fungicides indicate that carbendazim at varying rates is effective against this pathogen (Maitlo *et al.*, 2014). This study demonstrated that treatment of chickpea with rates of over 100% (1.5g/kg seed) carbendazim significantly reduced plant mortality (lower wilt incidence) which was in agreement with studies by Maitlo *et al.* (2014) and Nikam *et al.* (2007). Fungicide application protects the seed from soil and seed borne *F. oxysporum* f. sp. *ciceris* by mechanism like the eradication of the pathogen from the seed or the impact of fungicide on the inoculum in the rhizosphere.

4.2.3 Effect of varying thiram rates in control of Fusarium wilt of chickpea

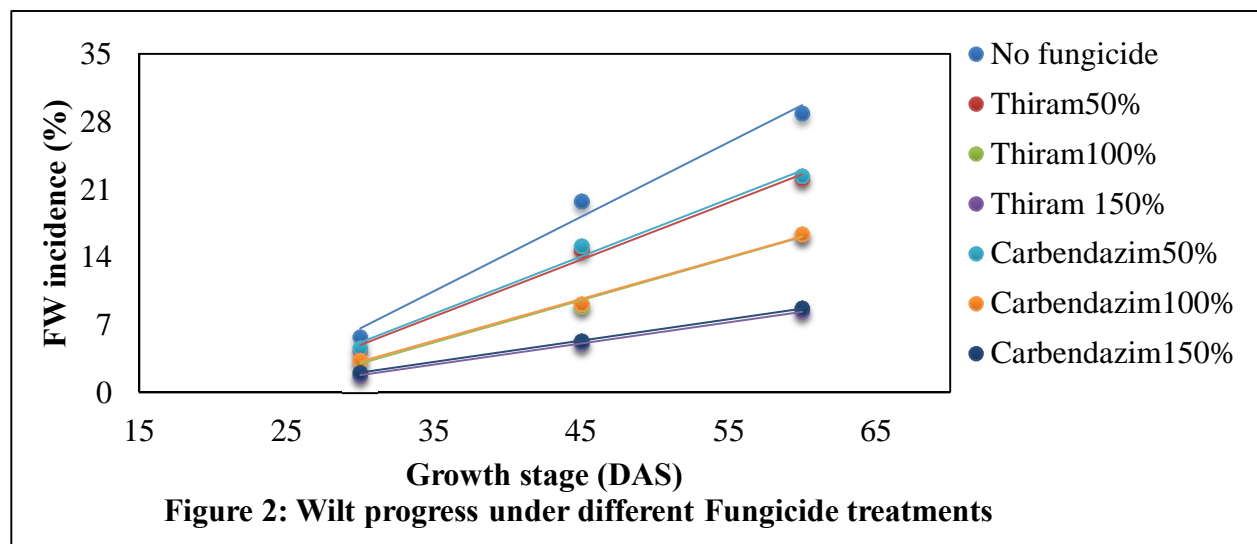
Treatment using varying rates of thiram resulted in significant ($P \leq 0.05$) differences in the management of Fusarium wilt at all the growth stages (Table 5). Application of thiram at the rate of 150% gave the best control of FW incidence at 30th growth stage with Fusarium wilt incidence of 1.8% being observed in season I and II. At 45 DAS, treatment with 150% thiram resulted in 5.2% and 4.9% wilt incidence in season I and II, respectively (Table 5). At 60 DAS, thiram at 150% resulted in wilt incidence of 8.9% and 7.9% for season I and II, respectively. Treatment of chickpea with 100% thiram gave wilt incidence of 3.3% at 30 DAS in both seasons. At 45 DAS, 100% treatment with thiram gave 9.0% wilt incidence and 8.8% wilt incidence in season I and II, respectively. At 60 DAS, treatment with 100% thiram gave 17.1% and 15.6% wilt incidence in season I and II, respectively. Treatment of chickpea with 50% thiram at 30 DAS resulted in wilt incidence of 4.7% and 4.1% in season I and II, respectively. At 45 DAS, treatment of chickpea with 50% thiram resulted in 15.9% and 13.4% wilt incidence in season I and II, respectively. At 60 DAS, chickpea plots treated with 50% thiram had wilt incidence of 22.8% and 21.3% for season I and II, respectively. Control treatment plots had wilt incidence of 5.6% and 5.9% in season I and II, respectively, at 30 DAS (Table 5). At 45 DAS, no fungicide treatment had wilt incidence of 19.9% and 19.6% in season I and II, respectively.

At 60 DAS, control treatments had 30.2% and 27.5% wilt incidence in seasons I and II, respectively (Table 5). It was apparent that application of thiram had significant control on

Fusarium wilt incidence of chickpea. The least wilt incidence was observed when thiram at 150% was used with values ranging from 1.8% to 8.9% across all growth stages. This was followed by application of thiram at 100% with wilt incidence ranging from 3.2% to 17.1% across all growth stages. Thiram at 50% treatment rate followed 100% treatment rate with wilt incidence values ranging from 4.1% to 22.8% across all growth stages. No-fungicide treatment had significantly ($P > 0.05$) higher wilt incidence ranging from 5.6% to 30.2% across all growth stages (Table 5).

Verma (1976) showed that seed dress fungicides were absorbed into the plant; translocated in the plant and protected the seedlings in the field for 30 days or more. This study found that better wilt management was achieved with higher fungicide rate. The results are in conformity with findings of Maitlo *et al.* (2014) who reported that increase in fungicide rate reduced wilt incidence. Muhammad (2010) reported a direct correlation of wilt severity to inoculum density.

Fungicide application reduced the inoculum level and hence low wilt incidence. The rate of increase in wilt incidence over time was high under no fungicide treatment (Figure 2) as shown by the steep slope. It was observed that in the other curves of 50% rate of thiram and carbendazim, the rate of wilt progression was lower. The lowest rate of increase in wilt incidence was portrayed by 150% application of either thiram or carbendazim in Figure 2. This fact is reflected further by the functional relationships in Table 6. Under no-fungicide treatment, wilt incidence increased by a 0.77 factor per day which was higher compared to the other treatments (Table 6).



Thiram and carbendazim at 50% treatment rates had 0.6 factors while treatments at 100% rate of application of either carbendazim or thiram had a 0.4 factor of increase in wilt. The least factor 0.2 of increase in wilt incidence was observed in treatment with 150% of carbendazim or thiram (Table 6).

Table 6: Functional relationships between Fusarium wilt incidence over time and fungicide treatment in chickpea

Treatment	Function	Goodness of fit (R^2)
No fungicide	$y = 0.77x - 16.5$	0.98
Carbendazim _{50%}	$y = 0.59x - 12.6$	0.98
Thiram _{50%}	$y = 0.58x - 12.7$	0.99
Carbendazim _{100%}	$y = 0.43x - 9.8$	0.99
Thiram _{100%}	$y = 0.43x - 10.0$	0.99
Carbendazim _{150%}	$y = 0.22x - 4.5$	0.99
Thiram _{150%}	$y = 0.22x - 4.8$	0.99

The linear graphs in figure 2 are supported by the respective production functions in Table 6 which indicate that the rate of wilt development was dependent on the fungicide treatment. The highest rate of increase in wilt incidence of 0.77 per day of growth was observed under no fungicide treatment. This shows that application of fungicide reduced disease incidence significantly in chickpea. This rate declined by 0.18% (i.e. 0.59%), -0.34% (i.e. 0.43% x) and 0.55% (0.22x) when fungicides were applied at 50%, 100% and 150%, respectively. These genetic coefficients are true for the genotypes.

Further linear regression models were fitted on the data to evaluate the relationship between FW incidence and fungicide application (Table 7) at specific growth stages (DAS). It was observed that increase in rates of thiram treatment from 0 to 150% (of the recommended 1.5 g/kg seed), suppressed Fusarium wilt incidence at the rate of -1.3% for every unit (%) of applied thiram by

the 30th DAS. With regard to carbendazim treatment, a relatively lower rate but similar trend in change of disease incidence per unit change of fungicide treatment was observed. The rate of Fusarium wilt was suppressed at the rates -1.2% per unit (%) increase in rate of carbendazim applied by the 30th DAS (Table 7). At 45 DAS, thiram treatments rates related negatively with wilt incidence declining at the rate of -5.1 per increase in 1% of thiram applied, while for carbendazim, FW incidence declined at the rate of -5.0 per 1% increase in carbendazim (Table 7). At 60 DAS, wilt incidence declined at the rate of -7.4 per 1% of applied thiram and at -7.3% per 1% of applied carbendazim.

These models are useful in predicting the pattern of decline in Fusarium wilt following treatment with fungicide and can influence other management decisions on wilt control. The models conform to the research findings of Maitlo *et al.* (2014) who reported that increasing fungicide rates reduced wilt incidence of chickpea thereby promoting growth of chickpea plants. The results indicate that treatment using either thiram or carbendazim resulted in significant reduction in Fusarium wilt incidence of chickpea across all growth stages as compared to the control treatment. The least wilt incidence was observed when 150% of either thiram or carbendazim was used across all growth stages.

Table 7: Relationship between Fusarium wilt at different growth stages and Fungicide treatment

Treatment	Function	Goodness of fit (R^2)
Thiram 30 DAS	$y = -1.3x + 7.1$	0.98
Carbendazim 30 DAS	$y = -1.2x + 6.9$	0.98
Thiram 45 DAS	$y = -5.1x + 25.3$	0.98
Carbendazim 45 DAS	$y = -5.0x + 25.4$	0.98
Thiram 60 DAS	$Y = -7.4x + 39.0$	0.99
Carbendazim 60 DAs	$Y = -7.3x + 39.1$	0.99

The degree of Fusarium wilt suppression varied for the different fungicide rates as shown in Table 5. Increasing rate of fungicide application achieved better control of Fusarium wilt was

achieved. De *et al.* (1996) reported that coating seeds with 0.2% carbendazim was more effective in reducing wilt and increasing yield in chickpea. Maitlo *et al.* (2014) also reported that increase in dosage of fungicide; as long as it was not phytotoxic, resulted in better wilt management. Nikam *et al.* (2007) working on Fusarium wilt management using *in vitro* and field studies found that thiram and carbendazim were the most effective in suppressing Fusarium wilt. Kovacicova (1970) reported that seed treatment with thiram at 2 g (133.3%) per kg seed gave the best protection against the Fusarium wilt of chickpea caused by *F. oxysporum* f. sp. *ciceris*.

In vitro chemical studies found carbendazim to suppress *F. oxysporum* to different degrees based on the relative concentrations of the chemical. Other studies found that carbendazim suppressed *F. oxysporum* f. sp. *ciceris* by 70.0% at 0.3%; 50.6 at 0.2% and 25.9% suppression at 0.1% of carbendazim (Animisha *et al.*, 2012). A direct correlation between inoculum density and disease severity was reported, although the genotype resistance and environmental conditions also play a role (Muhammad, 2010). Fungicide treatment reduces the amount of inoculum required for severe Fusarium wilt development. Further research should be focused on alternative and optimum foliar fungicides for management of Fusarium wilt. The economics (costs: benefits) of foliar applications rates used should also be included in future studies.

4.3 Interaction effects of fungicide rates and variety on Fusarium wilt incidence

The genetic resistance (host) to Fusarium wilt interacted significantly ($P \leq 0.05$) with the fungicide treatments in both seasons (Table 8). Control treatments had higher wilt incidences for both varieties compared with the fungicide treatments for both seasons. In field sick plot experiment, higher Fusarium wilt incidences were observed in HS Chania 2 as compared to the MR Chania 1 across all growth stages. As the thiram or carbendazim rate of application was increased, wilt incidence reduced significantly ($P \leq 0.05$) in both varieties and at all growth stages.

4.3.1 Interaction between treatments on Fusarium wilt incidence at 30 DAS

Significant ($P \leq 0.05$) interactions between the varieties and fungicides treatment were observed (Table 8). Chania 1 had lower wilt incidence as compared to Chania 2. At 30 DAS, Chania 2 under control treatment resulted in the highest wilt incidence of up to 7.1% and 7.7% in Seasons I and II, respectively which was higher than those observed from the other fungicide treatments.

Table 8: Interaction of thiram or carbendazim with two chickpea varieties on Fusarium wilt incidence at 30, 45 and 60 DAS

	30 DAS_I		30 DAS_II		45 DAS_I		45 DAS_II		60 DAS_I		60 DAS_II	
Treatment	92944	97105	92944	97105	92944	97105	92944	97105	92944	97105	92944	97105
Control	7.1a	4.2c	7.7a	4.0c	23.0a	16.7c	21.1a	18.2b	41.2a	19.1cd	36.9a	18.2c
Thiram ₅₀	5.9b	3.4d	5.2b	3.1d	18.1b	13.7d	16.3bc	10.4d	29.2b	16.5e	27.0b	15.6d
Thiram ₁₀₀	4.3c	2.2e	4.2c	2.3e	11.1e	6.9f	11.3d	6.3e	21.2c	13.0f	19.1c	12.0e
Thiram ₁₅₀	2.3e	1.3f	2.3e	1.2f	6.1f	4.2g	6.0e	3.6f	10.8fg	7.0h	10.8e	4.9f
Carbendazim ₅₀	6.1b	3.5d	5.5b	3.3d	18.8b	14.1d	17.1b	10.4d	29.7b	16.5e	27.7b	15.5d
Carbendazim ₁₀₀	4.4c	2.3e	4.4c	2.4e	11.7e	7.1f	11.2d	6.6e	22.7c	13.6f	16.7cd	12.3e
Carbendazim ₁₅₀	2.6e	1.6f	2.6e	1.6f	6.7f	4.1g	6.3e	3.9f	12.0f	6.1h	11.4e	5.2f
LSD	0.4		0.7		1.2		1.6		2.4		2.4	

Means followed by the same letter(s) in the same column are not significantly different at $P \leq 0.05$ using LSD

*Chania 2 is 92944, while 97105 is Chania 1

Interaction of Chania 2 with either thiram 100% and or carbendazim 100% resulted in significantly ($P \leq 0.05$) lower wilt incidence of 4.3% and 4.4%, respectively. This was lower than 50% fungicide treatments and the control treatment of Chania 2 in season I. However, these interactions were not significantly different from the 4.2% wilt incidence observed for Chania I at 0% (control) fungicide treatment (Table 8). Chania I interaction with thiram 50% and carbendazim 50% treatment rates had wilt incidence values of 3.4% and 3.5%, respectively. It was evident that significantly ($P \leq 0.05$) lower wilt incidence was observed when the MR genotype Chania I was seed dressed with either 50% thiram or carbendazim as compared to HS Chania 2 when dressed with either of the two fungicides at 100% at 30 DAS (Table 8).

Treatment of Chania 2 with 150% of thiram or carbendazim resulted in lower wilt incidence of 2.3% and 2.6, respectively (Table 8) in Season I. Chania 2 at 150% fungicide treatments interactions were statistically similar to the interactions of Chania I with thiram and or carbendazim at 100% rate, with wilt incidence of 2.2% and 2.3%, respectively in season I being observed. The same trend was observed in Season II (Table 8). This means that the resistance level of Chania 1 interacted with fungicide treatment rate and hence low wilt incidence as compared to Chania 2 which needed 50% more of the fungicide to achieve a similar wilt incidence. The lowest wilt incidence was observed when Chania I interacted with either thiram or carbendazim treatments at 150% rate, with values of 1.3% and 1.6%, respectively (Table 8). The same trend was observed in Season II.

It is therefore that farmers treat chickpea seed with either thiram or carbendazim at the rate of 150% (2.25 g/kg seed) in order to achieve the best management (2.4%) of FW when growing either HS or S genotypes. However, if MR cultivar Chania 1 is grown, farmers could use lower rates of 100% of either thiram or carbendazim to achieve similar Fusarium wilt management levels of less than 2.3% incidence by the 30 DAS. This would be comparatively a better management option than growing the HS chickpea variety Chania 2 and treating seed with 150% fungicide rates at this growth stage.

4.3.2 Interaction between treatments on Fusarium wilt incidence 45 DAS

At 45 DAS, the highest wilt incidence 23.1% and 21.1% was observed with Chania 2 under control treatment for season I and II, respectively. This wilt incidence was significantly ($P \leq 0.05$) higher from the other fungicide treatments. In season I, Chania 2 under thiram or

carbendazim at 50% rate of application resulted in 18.1% and 18.8% wilt incidence, respectively in season I. This interaction was significantly ($P \leq 0.05$) different from the others (0%, 100% and 150%) but not from each other (Table 8). In season I, Chania 1 control treatment was significantly ($P \leq 0.05$) superior to Chania 2 at either treatment with 50% thiram or 50% carbendazim with value of 16.7% wilt incidence. In Season II, interaction of carbendazim or thiram 50% treatment and Chania 2 was not significantly different from Chania I control treatment (Table 8). We can infer from these results a possible environmental influence that could have affected the interaction of Chania 1 under control treatment.

Treatment of Chania I with either thiram or carbendazim at 50% resulted in significantly ($P \leq 0.05$) lower wilt incidence of 13.7% and 14.1%, respectively in season I. The interactions of Chania 1 with 50% thiram or carbendazim were lower to application of the same rate (50%) of fungicides to Chania 2. A similar trend was observed in Season II. Interaction of Chania 2 with thiram or carbendazim at 100% resulted in lower wilt incidence values of 11.1% and 11.7% which were significantly ($P \leq 0.05$) different from other treatment interactions. Seed treatment of Chania 2 with 150% of either thiram or carbendazim resulted in 6.1% and 6.7% Fusarium wilt incidence, respectively. This was similar to application of thiram or carbendazim at 100% to Chania 1 which resulted in 6.9% and 7.1% Fusarium wilt incidence, respectively (Table 8). A similar trend was observed in Season II at 45 DAS.

The lowest Fusarium wilt incidence was observed when Chania 1 was treated with either thiram or carbendazim at 150% which resulted in 4.2% and 4.1% Fusarium wilt incidence, respectively in season I. During season II, lower Fusarium wilt incidence of 3.6% and 3.9% were observed in Chania 1 treated with thiram and carbendazim, respectively (Table 8).

4.3.3 Interaction between treatments on Fusarium wilt incidence 60 DAS

At 60 DAS, Chania 2 under control treatment had 41.2% and 36.9% Fusarium wilt incidence in seasons I and II, respectively. Significant ($P \leq 0.05$) interaction occurred between Chania 2 and thiram or carbendazim at 50%, with values 29.2% and 29.7% wilt incidence, respectively in season I. (Table 8). Treatment of Chania 2 with 100% of either thiram or carbendazim resulted in a lower wilt incidence of 21.2% and 22.7%, respectively. These values were similar to the Fusarium wilt incidence of Chania I under control treatment. Application of thiram or carbendazim at 50% to Chania I resulted in significantly ($P \leq 0.05$) lower wilt incidence, with

values of 16.5% and 16.5% being observed, respectively (Table 8). This trend was consistent in season II. Treatment of Chania 2 with either thiram or carbendazim at 150% resulted in lower wilt incidence compared to treating the same variety with the other treatment rates (0%, 50% and 100%), with Fusarium wilt incidence of 10.8% and 12.0% being observed for thiram and carbendazim, respectively at 60 DAS. These incidences were similar to Chania I treated with either thiram or carbendazim at 100% giving wilt incidence values of 13.0% and 13.6% for thiram and carbendazim, respectively in season I (Table 8). From these results, it was inferred that MR Chania 1 needed less chemical treatment as compared to the HS Chania 2 at 60 DAS.

The interaction between Chania 1 with either thiram or carbendazim at 150% resulted in lower Fusarium wilt incidence of 7.0% and 6.1%, respectively in season I; and 4.9% and 5.2%, respectively in season II at 60 DAS (Table 8). It was observed that MR Chania 1 needed significantly lower fungicide rate as compared to Chania 2 to achieve a similar level of wilt incidence. It is possible to infer that planting of MR Chania 1 will require lesser chemical treatment to control Fusarium wilt as compared to growing the HS Chania 2 across all growth stages. Fungicide treatments reduce wilt incidence resulting in a healthy plant that yield more. Increasing fungicide rate reduces wilt incidence (Maitlo *et al.*, 2014). The results have demonstrated that combining host plant resistance with higher rate of fungicide resulted in lower wilt incidence as the two disease management options worked in synergy against the invasive pathogen.

4.4 Effects of variety and fungicide in periodic dry matter yield of chickpea

4.4.1 Effect of variety on Dry matter yield of Chickpea

Chickpea DM accumulation followed a sigmoid curve, showing an initial lag phase between sowing and 55 DAS. After the 55th DAS, rapid growth phase followed up to 90th DAS after which the rate of DM production declined as the crop approached maturity. First flowering was observed about 45 DAS while 50% flowering was at 58 DAS. Higher dry matter accumulation depends primarily on the genotype potential in terms of production and pest resistance and secondly on the management practices. Moderately resistant Chania 1 had high DM due to its genetic potential and wilt resistance than HS Chania 2 (Table 8). This is further supported by the negative correlation between DM and wilt incidence of -0.95 at 45 DAS and -0.83 at 60 DAS (Table 14).

At 30 DAS, dry matter (DM) yield for Chania 1 and 2 were not significantly different (Table 9). At 60 DAS, significant ($P \leq 0.05$) differences were observed in DM of the two varieties, with Chania 2 yielding 2.4 g/plant and Chania 1 yielding 2.8 g/plant in season I. During season II, Chania 2 had 2.5 g/plant DM yield while Chania 1 had 2.8 g/plant at 60 DAS (Table 9). At 90 DAS, Chania 1 had 7.8 g/plant DM yield in season I and II, while Chania 2 had 7.6 g/plant in season I and II. At 120 DAS, DM yield of 11.1 g/plant and 10.8 g/plant were observed in Chania 1 and Chania 2, respectively in season I.

MR Chania 1 had significantly ($P \leq 0.05$) higher DM yield as compared to HS Chania 2, which was evident from the 60th DAS onwards (Table 9). Higher incidences of wilt in chickpea have been reported to result in up to 100% yield loss under ideal conditions (Navas-Cortes *et al.*, 2000). In highly susceptible (HS) Chania 2, lower DM values were observed because of higher wilt incidence. Negative correlation between Fusarium wilt incidence and DM yield was observed at 45 DAS and 60 DAS, respectively (Table 14). From this negative correlation it can be inferred that if higher wilt incidences are observed in the field at 45 DAS and 60 DAS, lower DM yields of chickpea would be experienced. This observation was in agreement with Haware and Nene, (1980) who reported that early wilting caused more damage as compared to late wilting in chickpea.

The negative correlation between wilt and DM could explain the variation in terms of DM yield observed between the two varieties, MR Chania 1 and HS Chania 2 as Chania 1 had low wilt incidence as compared to Chania 2. The highest DM was realized with variety Chania I (97105) at 11.1 g/plant and 11.2 g/plant for season I and II, respectively, at 120 DAS. These DM values for Chania 1 were significantly ($P \leq 0.05$) higher than for Chania 2 which had 10.8 g/plant and 10.9 g/plant, respectively, for season I and II at 120 DAS (Table 9).

4.4.2 Effect of fungicide rates on DM yield of chickpea at 30 DAS

Chickpea DM was lowest in plots under control treatment on both seasons. In season 1, control treatment had 0.6 g/plant which was significantly ($P \leq 0.05$) lower compared to all the other fungicide rates (Table 9). There was no significant difference between the fungicide rates 50%, 100% or 150% in terms of DM at 30 DAS.

Table 9: Effect of variety, thiram or carbendazim rates on periodic dry matter (g/plant) of chickpea at 30, 60, 90 and 120 DAS

Treatments	30 DAS g/plant	30 (SII) DAS	60 DAS g/plant	60 (S II) DAS	90 DAS g/plant	90 (SII)DAS	120 DAS g/plant	120 (SII)DAS g/plant
Chania 2	0.6 a	0.6 a	2.4 a	2.5 a	7.6 a	7.6 a	10.8 a	10.9 a
Chania 1	0.6 a	0.6 a	2.8 b	2.8 b	7.8 b	7.8 b	11.1 b	11.2 b
LSD	0.01	0.02	0.02	0.03	0.02	0.02	0.04	0.03
C.V	0.3	0.4	0.3	0.3	0.1	0.1	0.1	0.1
Control	0.64 a	0.65 a	2.30 a	2.31 a	7.45 a	7.46 a	10.25 a	10.27 a
Thiram _{50%}	0.66 b	0.66 b	2.44 b	2.49 b	7.61 b	7.61 b	10.37 b	10.59 b
Thiram _{100%}	0.67 c	0.67 b	2.61 c	2.76 c	7.76 c	7.77 c	11.38 c	11.38 c
Thiram _{150%}	0.68 c	0.68 b c	3.03 d	3.05 d	8.02 d	8.03 d	11.72 d	11.78 d
Carbendazim _{50%}	0.66 b	0.67 b	2.43 b	2.49 b	7.60 b	7.61 b	10.37 b	10.59 b
Carbendazim _{100%}	0.67 c	0.68c	2.60 c	2.77 c	7.73 c	7.76 c	11.38 c	11.38 c
Carbendazim _{150%}	0.68 c	0.68 c	3.03 d	3.05 d	8.01 d	8.02 d	11.71 d	11.78 d
LSD	0.01	0.01	0.03	0.02	0.02	0.03	0.05	0.03
C.V	1.1	1.1	0.8	0.7	0.3	0.3	0.3	0.2

*Means followed by the same letter(s) in the same column are not significantly different at $P \leq 0.05$ using LSD

Thiram and carbendazim at 150% treatments resulted in DM of 0.7 g/plant for both treatments. No significant ($P \leq 0.05$) differences were observed between thiram and carbendazim at 150% rate, 30 DAS. A similar trend was observed during the second season with the control treatment being the lowest in terms of DM yield while the fungicide treatments had higher DM values, though a slight difference between the rates of fungicides was observed (Table 9).

4.4.3 Effect of fungicide rates on DM yield of chickpea at 60 DAS

Control treatment plots had 2.3 g/plant DM which was significantly lower ($P \leq 0.05$) compared to all the other treatments in season I (Table 9). Treatment plots with thiram at 50% and carbendazim at 50% had 2.4 g/plant DM in season I and 2.5 g/plant in season II. There was no significant ($P \leq 0.05$) difference between the thiram and carbendazim at this rate 50%. Treatment of plots with thiram at 100% and carbendazim at 100% resulted in 2.6 g/plant DM yield. The difference between thiram and carbendazim at 100% was not significant ($P \leq 0.05$) (Table 9). Plots treated with thiram and carbendazim at 150% had 3.0 g/plant DM in season I. There was no significant difference ($P \leq 0.05$) between the two treatments (thiram 150% and carbendazim 150%). In season two, a similar trend was observed, with greater DM coming from plots treated with thiram and carbendazim at 150% (of active ingredient) each.

4.4.4 Effect of fungicide rates on chickpea DM yield 90 DAS and 120 DAS

Dry matter of chickpea increased with fungicide treatment (Table 9). The least DM 90 DAS was obtained under control treatments, with 7.4 and 7.5 g/plant DM being realized in season I and II, respectively which was significantly ($P \leq 0.05$) lower when compared to other fungicide rates. There were no significant differences between thiram and carbendazim fungicides, however, significant differences were observed within the fungicide rates (0%, 50%, 100% and 150% active ingredient 1.5 g/kg seed). The best treatment that yielded higher DM 90 DAS was either thiram or carbendazim at 150% (active ingredient 1.5 g/kg seed) with DM yield of 8.0 g/plant, in season I and II. Treatment of chickpea varieties with either thiram or carbendazim at 100% resulted in DM values of 7.7 g/plant in season I and II, at 90 DAS (Table 9). This treatment rate of 100% resulted in significantly ($P \leq 0.05$) higher DM than 50% rate of either thiram or carbendazim, but no significant differences were observed between thiram and carbendazim at 100% in both season I and II, 90 DAS.

Treatments of chickpea varieties with 50% of either thiram or carbendazim resulted in DM of 7.6 g/plant in season I. The same DM value of 7.6 g/plant in season II at 90 DAS was observed. These DM values were significantly higher as compared to the control treatment which had 7.4 g/plant in season I and II, 90 DAS (Table 9). It was observed that DM increased across all growth stages and with increasing fungicide rate.

At 120 DAS, significantly ($P \leq 0.05$) higher DM was realized in plots treated with either thiram or carbendazim at 150%, with 11.7 g/plant in season I and II (Table 9). Chickpea varieties treated with 100% of either thiram or carbendazim resulted in 11.3 g/plant DM yield in season I and II. The 100% rate was lower than 150% at 120 DAS. Treatment rate of 50% using either thiram or carbendazim resulted in DM which was lower than the 150% and 100% rates of treatments. At 50% rate of application of thiram and carbendazim, DM yield of 10.3 g/plant were observed in season I, while DM yield of 10.5 g/plant were observed for both thiram and carbendazim in season II, 120 DAS (Table 9). Control treatment plots had significantly ($P \leq 0.05$) low DM yield at 120 DAS, with 10.2 g/plant in season I and II. It was observed that application of fungicide increased the DM yield of chickpea from a range of 5.9% (at 30 DAS) up to 14.6% (at 120 DAS). It can be observed that the selection of either thiram or carbendazim did not significantly ($P \leq 0.05$) affect the DM realized across all growth stages.

The best treatments rate was 150% irrespective of the fungicide used (thiram or carbendazim). This was followed by 100% rate of either thiram or carbendazim. Previous studies found that carbendazim and thiram were effective in reducing wilt incidence and increasing yield (Kamdi *et al.*, 2012), while De *et al.* (1996) found that coating seeds with carbendazim was more effective in reducing wilt and resulted in yield of chickpea. Other studies have also found that plants protected from the pathogen through chemical seed dress and or genetic resistance had improved yield (Maitlo *et al.*, 2014). Singh *et al.* (2004) also reported highest seed germination with carbendazim. This high germination could affect plant population and hence dry matter.

4.5 Interaction between fungicide and variety on periodic dry matter production

Significant ($P \leq 0.05$) interactions were observed between the treatments and varieties at 60, 90 and 120 DAS (Table 10). The least DM was observed in Chania 2 with control treatment, across all growth stages, with values ranging from 2.1 g/plant at 60 DAS to 10.1 g/plant at 120 DAS.

All fungicide treatments of 50%, 100% and 150% had significantly ($P \leq 0.05$) higher DM yield than the control (0%).

4.5.1 Interaction between fungicide and chickpea variety on DM at 60 DAS

Significant ($P \leq 0.05$) interactions between the fungicide treatments and varieties were observed on the DM at 60 DAS (Table 10). The least DM was observed in Chania 2, under no fungicide, with DM of 2.1 g/plant and 2.2 for seasons I and II, respectively. At 50% thiram or carbendazim treatment significantly higher DM of 2.3 g/plant was realized in season I and II for both fungicides (Table 10). Treatment of Chania 2 with either thiram or carbendazim at 100% resulted in significant higher DM yield of 2.4 g/plant and 2.3 g/plant, respectively, in season I which was higher than either fungicide at 50% in season I with Chania 2. Chania 1 under control treatment had a significantly ($P \leq 0.05$) higher DM yield of 2.4 g/plant than Chania 2 under either thiram or carbendazim at 100% (Table 10) in season I.

In season II, 2.4 g/plant DM was observed in Chania 1 under control treatments which was significantly ($P \leq 0.05$) lower to Chania 2 and either thiram or carbendazim at 100% treatments which had 2.6 g/plant. This suggests an environmental influence on Chania 1 during season II which might have resulted in slightly higher Fusarium wilt incidences, hence the low yield. The results show that with higher disease incidence (low or no fungicide), low DM will be realized and this will eventually affect grain yield. This is further supported by the significant ($P \leq 0.01$) negative correlation; -0.95 between Fusarium wilt incidence at 45 DAS and DM 120 DAS (Table 14). The highest DM of 2.90 g/plant was observed in Chania1 under 150% treatment with either thiram or carbendazim (Table 10).

Previous studies reported that treatment of seed with fungicides significantly checks wilt incidence and enhances plant growth and yield (Maitlo *et al.*, 2014). Kamdi *et al* (2012) found that seed treatment with carbendazim and thiram resulted in increased germination, reduced wilt and increased yield. Muhammad (2010) reported a direct correlation between inoculum density and wilt severity in chickpea. Fungicide treatment reduces the amount of inoculum in the rhizosphere and these results in a healthy crop which explains the observed increase in dry matter with increase in fungicide rate.

Table 10: Effects of interaction between fungicide rates and variety on periodic dry matter (g/plant) 60, 90 and 120 DAS

Treatment	60 DAS_I		60 DAS_II		90 DAS_I		90 DAS_II		120 DAS_I		120 DAS_II	
	Chania	Chania	Chania	Chania	Chania	Chania	Chania	Chania	Chania	Chania	Chania	Chania
	2	1	2	1	2	1	2	1	2	1	2	1
Control	2.18a	2.42d	2.21a	2.41c	7.32a	7.57c	7.35a	7.57c	10.13a	10.37c	10.12a	10.42b
Thiram ₅₀	2.29b	2.58e	2.30b	2.68d	7.45b	7.76e	7.45b	7.76d	10.25b	10.49d	10.45b	10.71c
Thiram ₁₀₀	2.36c	2.85f	2.66d	2.85e	7.62d	7.88f	7.61c	7.91e	11.31e	11.44f	11.10d	11.65f
Thiram ₁₅₀	2.87f	3.18g	2.91f	3.18g	7.91f	8.12g	7.92e	8.14f	11.52g	11.91h	11.61f	11.94g
Carbendazim ₅₀	2.28b	2.57e	2.30b	2.67d	7.44b	7.76e	7.45b	7.76d	10.24b	10.49d	10.45b	10.71c
Carbendazim ₁₀₀	2.34c	2.85f	2.67d	2.85e	7.58c	7.88f	7.60c	7.92e	11.30e	11.44f	11.09d	11.65f
Carbendazim ₁₅₀	2.87f	3.17g	2.91f	3.19g	7.89f	8.13g	7.89e	8.15f	11.52g	11.90h	11.61f	11.94g
LSD	0.034		0.034		0.034		0.039		0.062		0.041	

Means followed by the same letter(s) in the same column are not significantly different at $P \leq 0.05$ using LSD

Figures 3 and Figure 4 show the relationship between DM 60 DAS with wilt incidence 30 DAS, for Chania 2 and Chania 1, respectively. From the linear models in figure 3 and 4, DM yield declined with increasing wilt incidence. These models can be used to predict DM loss in chickpea in relation to development of Fusarium wilt. From these results it can be inferred that at 60 DAS, MR Chania 1 yielded more DM with comparatively less fungicides than HS Chania 2. The results are linked to the wilt incidence observed in Chania 1 and 2. High wilt incidence led to low yield due to unhealthy plants whose vascular systems have been invaded by the pathogen. Khan *et al.* (2004) reported the production of phytotoxins by the wilt pathogen which led to chlorosis in affected chickpea. Chlorophyll content determines the amount of dry matter through photosynthesis. Effective management of Fusarium wilt using fungicide treatments and resistant varieties therefore led to increased dry matter as less pathogen invasion occurred in fungicide treated chickpea compared to the control. Also, it was observed that combination of genetic resistance by choice of resistant variety with fungicide treatment was effective in controlling Fusarium wilt and improving yield, this was in agreement with Maitlo *et al.* (2014) who reported yield increase with increasing fungicide rates.

4.5.2 Interaction effects between treatments on DM at 90 DAS

Significant ($P \leq 0.05$) interactions between varieties and fungicide treatments occurred on DM at the 90 DAS. Higher DM yield values in the range of 7.867.9 g/plant were observed when Chania 1 interacted with 150% of either thiram or carbendazim (Table 10). The least DM yield was obtained with Chania 2 under control treatment with DM yield of 7.3 g/plant for season I and II, 90 DAS. This was followed by the combination of Chania 2 with either 50% of thiram or carbendazim, resulting in DM yield of 7.4 g/plant in season I and II. Chania 1 combination with control treatment was the next best, in both seasons I and II, with DM yield of 7.5 g/plant in both season I and II. Chania 1 interacted significantly with thiram or carbendazim at 100% to give DM yield which was equivalent to Chania 2 yield under thiram or carbendazim at 150% (Table 10) in both seasons. It can be inferred that to achieve a similar yield in Chania 2, significantly higher (+50%) application of thiram or carbendazim is necessary to achieve the same yield as MR Chania 1. The highest DM yield was obtained in the interaction between Chania 1 and either thiram or carbendazim at 150% giving 8.1 g/plant in season I and II.

Figure 5 and Figure 6 show the relationship between DM 90 DAS with wilt incidence (45 DAS) for Chania 2 and Chania 1, respectively. The linear functions indicate declining DM 90 DAS for both varieties as wilt incidence increases, with high coefficient of determination, R^2 of 0.93 and 0.89 for Chania 2 and Chania 1, respectively. With the high values of goodness of fit, we can predict accurately DM loss in chickpea when we observe wilt incidence in chickpea at 45 DAS.

4.5.3 Interaction between fungicide and variety on DM at 120 DAS

The highest DM yield 120 DAS was obtained in Chania 1 treated with either thiram or carbendazim at 150%, yielding DM of 11.9 g/plant in season I and II for both fungicides (Table 10). Chania 2 under thiram or carbendazim at 150% resulted in DM yield of 11.5 g/plant for both fungicides in season I and 11.6 g/plant for both fungicides in season II. The least DM yield was obtained with Chania 2 under control treatment, giving DM yield of 10.1 g/plant for seasons I and II. Treatment of Chania 2 with 100% thiram or carbendazim resulted in DM yield of 11.3 g/plant in season I and 11.1 g/plant in season II for both fungicides (Table 10).

DM yield of Chania 2 at 100% fungicide was significantly ($P \leq 0.05$) higher than treatment with 50% of either fungicide, but lower than the yield of Chania 1 under 100% rate of thiram or carbendazim. Chania 1 with 100% treatment of thiram or carbendazim resulted in 11.4 g/plant DM in season I and 11.6 g/plant in season II for both fungicides. MR Chania 1 had significantly higher yield than Chania 2 under control treatment. Treatment of MR Chania 1 with either fungicide resulted in higher DM yield as compared to treatment to HS Chania 2, across all treatment rates (50%, 100% and 150%). It is therefore recommended that farmers adopt MR Chania 1 genotype as opposed to HS Chania 2 so as to reduce fungicide treatment rates and realize higher DM yields.

Combination of resistance with fungicide treatment led to increased DM yield as the crop had better health. Figure 7 shows the relationship between DM 120 DAS and wilt incidence 60 DAS in Chania 2, while Figure 8 shows the relationship between DM 120 DAS and wilt incidence 60 DAS in Chania 1. In Figure 7 and 8, increase in wilt incidence resulted in reduced DM. Based on the vascular nature of the pathogen, invasion of xylem tissues deprives the plant of water uptake which affects other processes like photosynthesis and nutrient mineral absorption. Padwick (1941) reported that some plants may look apparently healthy while they have been invaded by the pathogen. In severe cases, the pathogen produces phytotoxins which causes wilting and leaf

burning (Khan *et al.*, 2004). It was observed that MR Chania 1 was able to yield more DM with lower fungicide treatment as compared to the HS Chania 2. Higher DM in MR Chania 1 is due to the lower wilt incidence (Table 8) which resulted in a healthy crop and hence higher DM yield.

Studies on the effect of fungicide on yield of soybean showed that the yield increase was more related to the disease control aspect of the fungicide that led to a healthy crop yielding more (Catherine and Palle, 2009). This study observed that combining resistance with fungicide treatment led to increasing yield as the wilt incidence was reduced. Further, there was a positive correlation between DM and grain yield (Table 14). Figure 9 and Figure 10 shows the relationship between grain yield and final dry matter (120 DAS) in Chania 2 and Chania 1. Grain yield increased with increasing dry matter both in Chania 1 and 2. It was observed that grain yield increased by a factor of 0.1 increase in dry matter in both varieties. The goodness of fit was 0.99 in Chania 2 and 0.88 in Chania 1. This means that there were other contributing factors towards grain yield especially in Chania 1 than in Chania 2.

Mallu *et al.* (2015) reported a positive and significant correlation between biomass and grain yield. Management practice that enhances dry matter accumulation therefore leads to higher grain yield. These include management options like treatment of chickpea with fungicides to control wilt. Fusarium wilt has been reported to result in reduced chlorophyll content in chickpea (Khan *et al.*, 2004) through the production of phytotoxins that can cause leaf wilting, chlorosis or complete plant wilting depending on the race of the pathogen. This way, wilt incidence leads to decline in dry matter of chickpea by affecting processes of photosynthesis and water uptake by blocking xylem vessels.

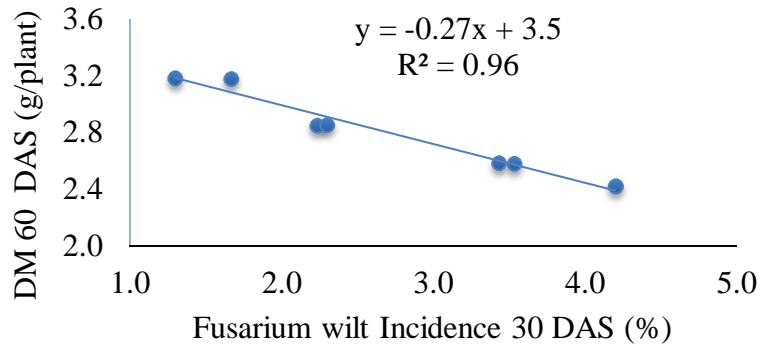


Figure 3. Relationship between FW 30 DAS and DM 60 in Chania 1

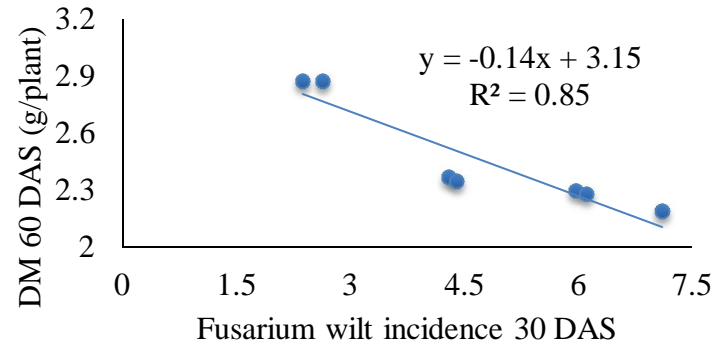


Figure 4. Relationship between FW 30 DAS and DM 60 DAS in Chania 2

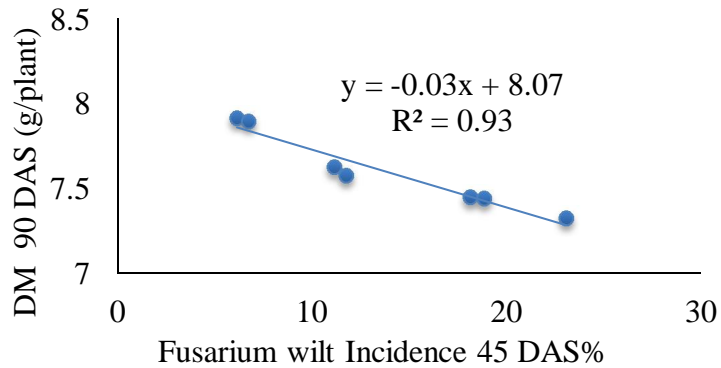


Figure 5. Relationship between FW and DM 90 DAS in Chania 2

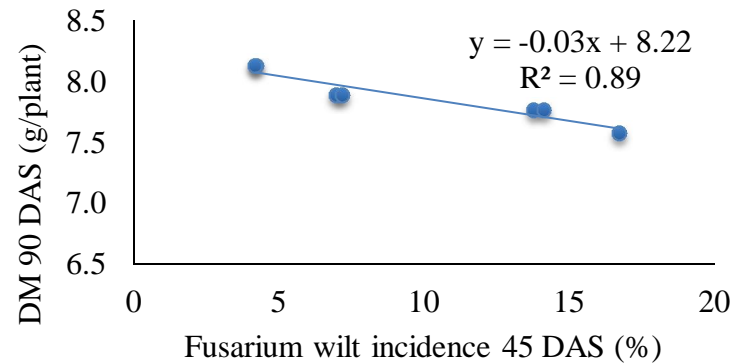
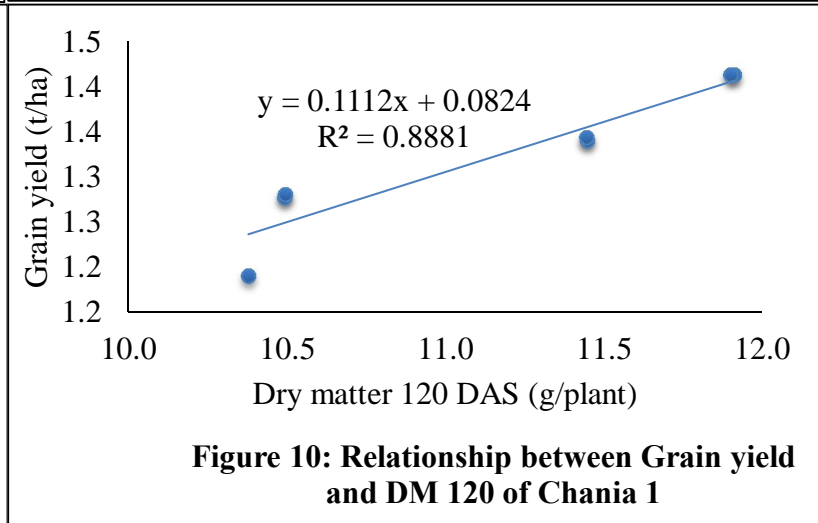
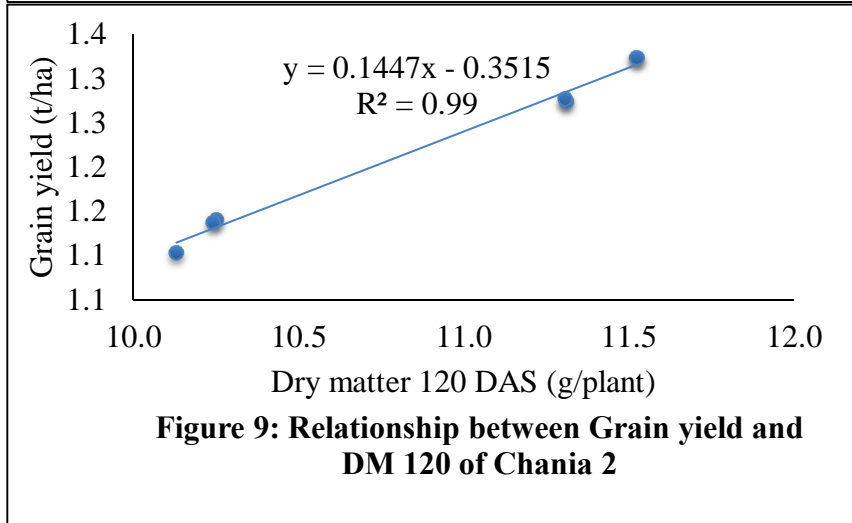
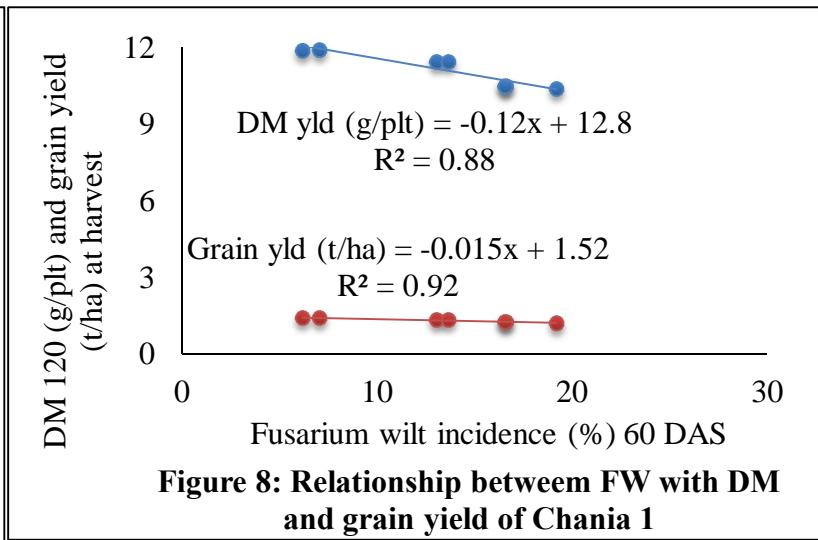
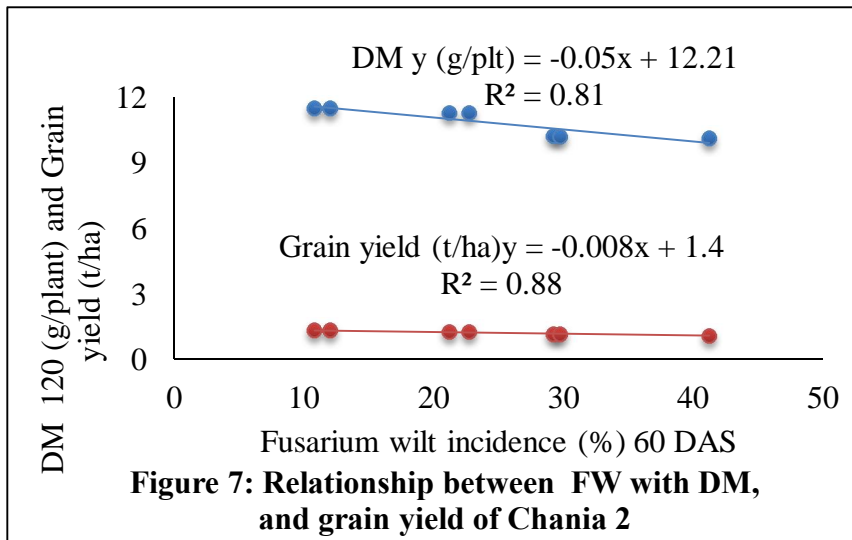


Figure 6. Relationship between FW and DM 90 DAS in Chania 1



4.6 Effect of variety and thiram or carbendazim on chickpea plant height (PH)

4.6.1 Effect of variety on plant height

There were significant ($P \leq 0.05$) differences in the plant height (PH) (Table 11) between the two varieties. At 30 DAS, Chania 1 had a height of 10.8cm while Chania 2 had 10.1cm in season I. In season II, Chania 1 had 11.1cm while Chania 2 had 10.1cm at 30 DAS. At 60 DAS, Chania 1 had plant height (PH) 24.3cm while Chania 2 had 22.3cm (Table 11). The differences were significant ($P \leq 0.05$). The same trend was observed in season II, with Chania 1 obtaining PH of 24.4cm while Chania 2 had 22.8cm. At 90 DAS, Chania 1 had plant height of 28.4cm while Chania 2 had 26.2cm in season I. In season II, Chania 1 had 28.7cm while Chania 2 had 26.3cm. The differences between the two varieties at 90 DAS in terms of PH were significant (Table 11). At 120 DAS, Chania 1 had a PH of 32.3cm while Chania 2 had 30.0cm in season I. In season II, Chania 1 had 32.7cm while Chania 2 had 30.19cm. The difference between the two varieties was significant ($P \leq 0.05$) (Table 11).

Moderately resistant Chania 1 had higher plant height across all growth stages in contrast with HS Chania 2, across all growth stages. Table 12 shows a high positive correlation of 0.92 between grain yield and plant height. It is possible to infer that MR variety Chania 1 would yield more grain as compared to HS Chania 2 as a result of better plant height. Figure 11 shows that grain yield increases at the rate of 0.02 for every unit increase in plant height. Genetic resistance of a variety reduces the negative impacts of that pathogen on that variety. It has been reported in other studies that a healthy crop results from management of *Fusarium oxysporum* f. sp. *ciceris* (through fungicides) and had higher shoot length (Maitlo *et al.*, 2014).

It also observed that between the two varieties, Chania 1 and 2 significant differences in terms of plant height occurred. Due to the nature of the pathogen, it can be inferred that HS Chania 2 was affected by the pathogen, which could have resulted in blockage of its xylem vessels, thereby leading to reduced plant vigor and subsequently low plant height. This is supported by Khan *et al.* (2004) who reported stunting in infected chickpea plants.

4.6.2 Effect of fungicide treatment on plant height

Plants treated with fungicide were significantly taller in height to the control treatment at all growth stages (Table 11). At 30 DAS, control treatment had lower plant height (PH) which was significant ($P \leq 0.05$) than all the other fungicide treatments with PH of 9.3cm and 9.1cm for

seasons I and II, respectively. Treatment with thiram or carbendazim at the rates of 50% to 150% rates gave significantly higher PH values in the range of 10.1 to 11.3cm to the control. A similar trend was observed in season II. At 60 DAS, control treatment had significantly ($P \leq 0.05$) lower PH of 20.0cm and 20.1cm for season I and II, respectively. Treatment with either thiram or carbendazim at 50% gave PH values of 21.5cm and 21.6cm in season I, respectively, while in season II, 22.5cm was observed for either of thiram and carbendazim. Treatment with 100% of either thiram or carbendazim gave PH of 24.3cm and 24.1cm, respectively, in season I at 60 DAS. In season II, treatment of chickpea with 100% of either thiram or carbendazim yielded PH values of 24.5cm and 24.3cm, respectively (Table 11) at 60 DAS.

The highest PH was obtained by treating chickpea with either thiram or carbendazim at 150% which yielded 25.7cm and 26.3cm, respectively in season I, while in season two, 25.7cm and 25.8cm was obtained for the two fungicides, respectively (Table 11). At 90 DAS, significantly ($P \leq 0.05$) low plant height was observed in the control treatment giving PH of 23.1cm and 22.6 cm for Season I and II respectively. At 100% and 150% treatment with thiram or carbendazim, PH values in the range of 28.3cm to 30.8cm were observed 90 DAS. The highest PH 90 DAS was obtained by treating with thiram or carbendazim at 150% with PH of 30.5cm and 30.8cm, respectively (Table 11). The results of this study are in agreement with other studies which found that fungicide treatments increased plant height as compared to the control (Khalil *et al.*, 2002; Maitlo *et al.*, 2014). Khan *et al.* (2004) reported that phytotoxins released by the pathogen induce leaf wilting, chlorosis and sometimes stunting.

Muhammad (2010) found a direct correlation between wilt severity and inoculum density. It can therefore be inferred that fungicidal seed treatment inhibits seed or soil borne pathogens creating a rhizosphere free from biotic stress of the pathogen and this could result in vigorous seedlings. At 120 DAS control treatments had significantly ($P \leq 0.05$) low PH of 25.1cm and 24.7cm for seasons I and II, respectively (Table 11). Treatment of chickpea with either thiram or carbendazim at 50% rate gave PH value of 29.0cm and in season II, 29.3cm. Significantly higher PH was obtained by treating chickpea with either thiram or carbendazim at 100% or 150% with PH values in the range of 32.7cm to 35.5cm 120 DAS (Table 11). No interactions were observed between treatments. In figure 13 the relationship between PH and grain yield is illustrated. Grain yield increases by 0.02 per increase in PH. According to Mallu *et al.* (2015) the correlation between plant height and dry matter led to more dry matter partitioning into grain yield.

Table 11: Effect of thiram or carbendazim rates on periodic plant height of two chickpea varieties

Treatment	Height 30_I	Height 30_II	Height 60_I	Height 60_II	Height 90_I	Height 90_II	Height 120_I	Height 120_II
92944	10.09 a	10.14 a	22.38 a	22.86 a	26.24 a	26.33 a	30.05 a	30.19 a
97105	10.85 b	11.14 b	24.38 b	24.43 b	28.43 b	28.71 b	32.38 b	32.76 b
LSD	0.73	0.71	0.35	1.28	0.73	0.2	0.82	0.35
C.V	2.0	1.9	0.4	1.5	0.8	0.2	0.7	0.3
Control	9.33 a	9.17 a	20.00 a	20.17 a	23.17 a	22.67 a	25.17 a	24.67 a
Thiram ₅₀	10.50bc	10.67 b	21.50 b	22.50 b	25.50 b	25.50 b	29.00 b	29.33 b
Thiram ₁₀₀	10.50bc	10.50 b	24.33 c	24.50 c	28.33 c	28.50 c	32.67 c	33.00 c
Thiram ₁₅₀	11.33c	11.50 b	25.67 d	25.67 c	30.00 c	30.50 de	35.17 d	35.50 d
Carbendazim ₅₀	10.17 ab	10.67 b	21.67 b	22.50 b	25.50 b	25.83 b	29.00 b	29.33 b
Carbendazim ₁₀₀	10.50bc	10.67 b	24.17 c	24.33 c	28.33 c	28.83 cd	33.00 c	33.33 c
Carbendazim ₁₅₀	11.00bc	11.33 b	26.33 d	25.83 c	30.50 c	30.83 e	34.50 cd	35.17 d
LSD	0.65	0.77	0.78	0.96	1.31	1.18	1.15	1.07
C.V	5.3	6.1	2.8	3.4	4.1	3.6	3.1	2.9

Means followed by the same letter(s) in the same column are not significantly different at $P \leq 0.05$ using LSD

Studies by Maitlo *et al.* (2014) indicated that plants treated with fungicides had enhanced growth and had higher shoot length values. The eradication of the pathogen by fungicide treatment led to healthy plants devoid of negative effects of phytotoxins released by the wilt pathogen after it invades chickpea as reported by Khan *et al.* (2004). Treatment of seeds with fungicides protects the plant from invasion by soil borne pathogen and consequently a healthy crop grows. This explains why seed treated fungicides had significantly higher plant heights as opposed to untreated plants. Other studies have found that treatment of chickpea with fungicides and other plant extracts resulted in increased root and shoot weight (Khalil *et al.*, 2002; Animisha *et al.*, 2012; Maitlo *et al.*, 2014).

It can be inferred that as a result of increased fungicide rate, the negative effect of rhizosphere pathogens was reduced leading to a healthy crop, hence the observations on increasing plant height with fungicide rate increase. This is in agreement with Maitlo *et al.* (2014) who found that increasing dosages of carbendazim were effective in reducing wilt incidence and improving shoot height. Plant height has a positive correlation to grain yield as reported by Mallu *et al.* (2015). Table 14 shows a high positive correlation (r) of 0.92 between grain yield and plant height. These results are in agreement also with Halila and Strange (1997) who reported that plants infected by *Fusarium oxysporum* f. sp. *ciceris* were occasionally stunted and seed yield was correlated to plant height.

4.7 Effect of variety and fungicide treatments on yield parameters of chickpea

4.7.1 Effect of variety and fungicide treatments on number of pods per plant

Significant ($P \leq 0.05$) differences in the number of pods per plant were observed between the two varieties. Chania 2 had 114.1 while Chania 1 had 123.4 pods/ plant (Table 12). The number of pods per plant is correlated to grain yield, with grain yield increasing by a factor of 0.01 for every increase in pods per plant (Figure 14). Pods per plant also had a high positive correlation (r) of 0.93 to grain yield (Table 14). These results are in agreement with Mallu *et al.* (2015) who found a positive relationship between number of pod per plant and grain yield. Significantly low number of pods/plant of 101.8 was observed in the control treatment. In chickpea treated with either thiram or carbendazim at 50%, 113.8 pods per plant were observed (Table 12).

Treatment of chickpea with 100% of either thiram or carbendazim gave 122.7 and 123 number of pods/plant, respectively. The highest number of pods per plant was obtained when either thiram

or carbendazim was used at 150% recommended active ingredient, which gave 128.3 and 128.1 number of pods per plant, for thiram and carbendazim, respectively (Table 12). Number of pods per plant was positively correlated to both DM and grain yield with correlation coefficients of 0.87 and 0.93, respectively (Table 14). This means that for a farmer to achieve high yield (DM or grain yield), it is necessary; one to choose a variety like Chania 1 which had significantly ($P \leq 0.05$) higher pods /plant of 123.4, and two, adopt a wilt management plan of 150% application which also gave the highest number of pods per plant at 128.3 (Table 12).

4.7.2 Effect of treatments on 100 seed weight and Harvest Index

Significant ($P \leq 0.05$) differences between varieties were observed with Chania 1 having 24.2 g/100 seeds, while Chania 2 had 19.7 g/100 seeds (Table 12). Figure 15 shows the relationship between seed weight and grain yield, whereby grain yield increased by a factor of 0.01 seed weight. This low relationship could be attributed to the fact that there are only one or two seeds per pod in chickpea hence lesser influence on grain yield. Control treatment had significantly low seed weight at 20.3 g/100 seeds. Treatment of chickpea with either thiram or carbendazim at 50% gave seed weight of 21.2 g/100 seeds and 21.3 g/100 seed, respectively, which were significantly higher than the control treatment (Table 12).

Seed treatment with either thiram or carbendazim at 100% gave test weight of 22.3 g/100 seeds. Treatment of chickpea with 150% of either thiram or carbendazim gave significantly higher test weight of 23.1 g/100 seed for both fungicides. Fusarium wilt has been shown to affect the seed weight resulting in lighter and shriveled seeds of dull colour (Haware and Nene, 1980). As fungicide rate was increased, inoculum density was reduced as a result of the negative effect of fungicide. This led to a healthy plant and hence better seed weight which explains the variation observed in the seed weight. Muhammad (2010) reported a direct correlation between wilt severity and inoculum density.

4.7.3 Effects of variety and fungicide treatments on harvest index

Results on harvest index (H.I) showed significant difference ($P \leq 0.05$) between varieties Chania 1 and Chania 2, with 48.1% for Chania 1 and 45.6% for Chania 2 (Table 12). The higher harvest index in Chania 1 could be a result of higher DM and grain yield as compared to Chania 2. Fungicide control treatment showed the least harvest index of 44.7% which was lower than all

the other fungicide treatments. The highest harvest index was obtained at carbendazim 100% which was significantly higher at 49.2% followed by carbendazim at 150% at 48.7% (Table 12).

4.7.4. Effect of variety on Grain Yield

Grain yield was significantly ($P \leq 0.05$) different between the two varieties with Chania 1 giving a higher grain yield of 1.3 t/ha while Chania 2 gave a mean grain yield of 1.2 t/ha (Table 12). These variations could be due to the genetic differences between the two varieties.

4.7.5 Effect of fungicide treatments on Grain yield

Fungicide rates had a significant ($P \leq 0.05$) effect on grain yield of chickpea. Highest grain yield of 1.4 t/ha was recorded when either thiram or carbendazim was seed dressed at 2.25 g/kg seed (150%). This was followed by applying either carbendazim or thiram at 100% which had a mean grain yield of 1.3 t/ha. The lowest grain yield of 1.1 t/ha was observed when no fungicide treatment was used (Table 12).

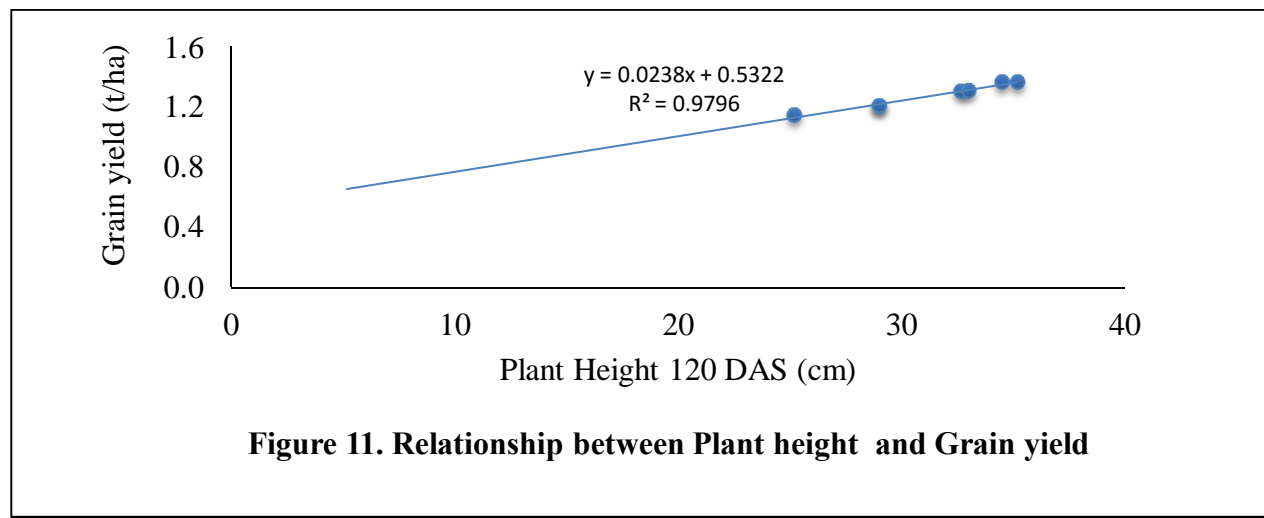
Table 12: Effect of variety and fungicide rate on yield, pods/plant, seed weight and harvest index

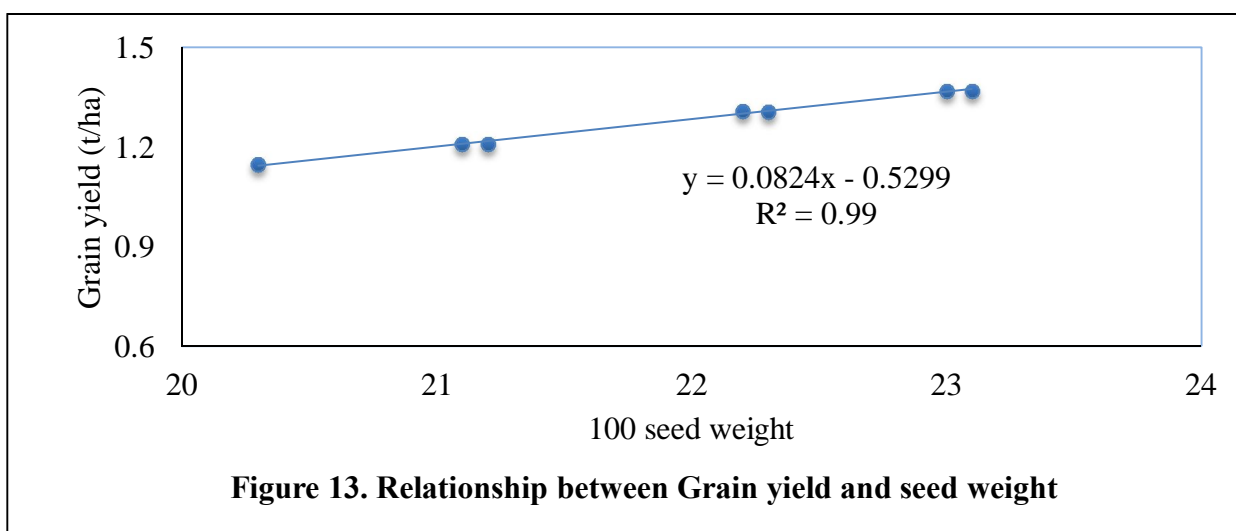
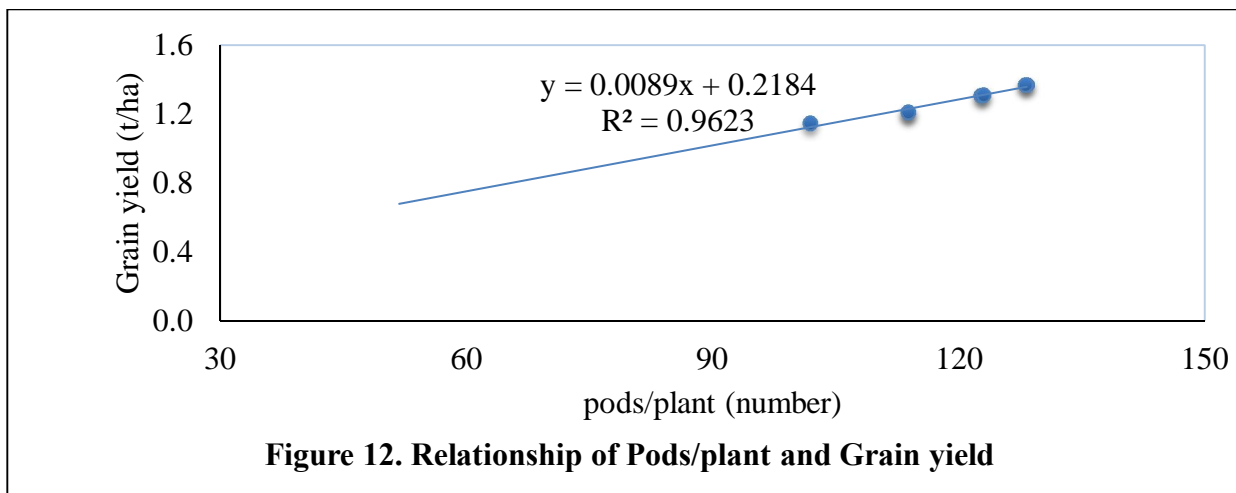
Treatments	Pods per plant	100 seed weight (g)	Harvest Index (%)	Grain Yield (T/ha)
Chania 2	114.19 a	19.7 a	45.68a	1.2 a
Chania 1	123.43 b	24.2 b	48.15b	1.3 b
LSD	2.5	3.9	0.8	0.02
C.V	0.6	0.5	0.5	0.5
Control	101.8 a	20.3 a	44.72a	1.15 a
Thiram _{50%}	113.8 b	21.2 b	46.29b	1.21 b
Thiram _{100%}	122.6 c	22.3 c	45.85b	1.31 c
Thiram _{150%}	128.3 d	23.1d	46.68b	1.37 d
Carbendazim _{50%}	113.8 b	21.3 b	46.87b	1.21 b
Carbendazim _{100%}	123.0 c	22.3 c	49.29d	1.31 c
Carbendazim _{150%}	128.2 d	23.1 d	48.70c	1.34 d
LSD	2.9	3.7	0.7	0.02
C.V	2.1	1.4	1.3	1.2

Means followed by the same letter(s) in a column are not significantly different at $P \leq 0.05$ LSD

These results corroborated those of De *et al.* (1996) who noted that coating seeds with 0.2% carbendazim was more effective in reducing wilt and increasing yield. Kamdi *et al.* (2012) reported low wilt incidence and maximum grain yields after applying carbendazim at 2 g/kg seed (i.e., 133.3% rate). Khalil *et al.* (2002) reported that fungicide treatments increased grain yield in wheat as compared to untreated plots. Maitlo *et al.* (2014) reported that fungicide treatment remarkably checked disease development and subsequently increased plant growth and yield as compared to untreated plants. It can be inferred that treatment of chickpea with 150% and 100% thiram or carbendazim was very effective in controlling wilt incidence. The wilt pathogen releases phytotoxins (Khan *et al.*, 2004) which leads to wilting, stunting, and chlorosis hence low dry matter. Application of fungicide protects the crop from the pathogen which results in taller healthy plants, higher DM yields and better yield of chickpea.

There was a positive correlation between grain yield and plant height, number of pods and seed weight. Chickpea height was shown to increase seed/grain yield by a factor of 0.0238 t/ha per cm increase in chickpea height (Figure 11). In pods per plant, 0.0089 t/ha increase for every increase in pod, while 0.082 t/ha increase in gain yield was observed for every single increase in 100 seed weight (Figure 12 and Figure 13, respectively).





4.8 Interaction effects of varieties with fungicide treatments on Grain yield

Significant ($P \leq 0.05$) interactions between varieties and fungicide treatments were observed (Table 13) on grain yield of chickpea. Chania 1 interacted with either thiram or carbendazim applied at 150% (of recommended optimum rate -ROR) yielding highest grain yield of 1.4 t/ha. Chania 2 interacted with thiram and carbendazim applied at the rate of 2.25 g/kg seed yielding grain yield in the range of 1.3 t/ha grain yield (Table 13). The lowest grain yield was obtained with Chania 2 under control treatment with grain yield of 1.1 t/ha (Table 13). It was observed that lower grain yield was realized in the treatments that had higher incidence of Fusarium wilt (Table 8). There was a negative correlation of -0.95 and -0.83 between grain yield and wilt incidence at 45 DAS and 60 DAS (Table 14). Figure 7 and Figure 8 shows the relationship between grain yield with wilt incidence for Chania 2 and Chania 1, respectively.

Table 13: Interaction of variety with thiram or carbendazim on Grain yield of chickpea

Treatment	Grain Yield (T/ha)	
	92944	97105
Control	1.10a	1.19c
Thiram ₅₀	1.14b	1.27d
Thiram ₁₀₀	1.27d	1.34e
Thiram ₁₅₀	1.32e	1.41f
Carbendazim ₅₀	1.13b	1.28d
Carbendazim ₁₀₀	1.27d	1.34e
Carbendazim ₁₅₀	1.32e	1.41f
LSD	0.03	

The relationships show that as the wilt incidence increased, grain yield declined at -0.01 and -0.02 for Chania 1 and 2, respectively with R^2 values of 0.88 and 0.92 for Chania 2 and Chania 1, respectively. Application of fungicide as seed dress to MR Chania 1 resulted in a synergistic effect with less pathogen establishment and this resulted in a healthier crop as compared to the lower fungicide rates and HS Chania 2. Thus, the healthy crop from Chania 1 interactions resulted in higher grain yield. Muhammad (2010) reported a direct correlation between wilt severity and inoculum density. Inoculum density is adversely affected by fungicide application and use of resistant genotype.

4.9 Correlation analyses of yield attributes

There were positive correlations between chickpea pods per plant and 100 seed weight. Grain yield was positively correlated ($R=0.90$) with dry matter at 120 DAS (final harvest). Plant height was positively correlated to dry matter and Grain yield. The number of pods per plant was positively correlated to grain yield with a high correlation coefficient $R = 0.93$ (Table 14). This means that a healthy (wilt free) chickpea crop would have tall plants of 32.6-35.5 cm as a result of absence of the inhibiting phytotoxins released by the pathogen (Khan *et al.*, 2004). The healthy crop would also have more pods per plant in the range of 122.6 - 128.3; a higher rate of DM accumulation throughout the crop growth cycle, which would consequently translate into higher grain yield of over 1.3 t/ha.

Grain yield was positively correlated to harvest index with a coefficient of 0.73 (Table 14). This observation was in agreement with Halila and strange (1997). Grain yield was negatively correlated to Fusarium wilt disease incidence, with correlation coefficient r of -0.94 and -0.95 at 45 DAS and 60 DAS, respectively (Table 14). Fusarium wilt incidence should be controlled early in order to increase grain yield because early wilting leads to more yield loss (Haware and Nene, 1980). Plant height was positively correlated to dry matter. Dry matter was also positively correlated to number of pods per plant and harvest index (Table 14). Dry matter 120 DAS was negatively correlated to Fusarium wilt incidence both at 60 DAS and 45 DAS.

It was observed that as wilt incidence increased, DM yield was reduced due to low plant stand and low weight in infected plants (Table 14). Number of pods per plant was positively correlated to harvest index and plant height with r values of 0.66 and 0.93 respectively both of which were significant ($P \leq 0.01$). Number of pods was negatively correlated with Fusarium wilt incidence at 60 DAS and 45 DAS (Table 14). Harvest Index (H.I) was positively correlated with plant height with an r value of 0.60, but negatively correlated with Fusarium wilt incidence both at 60 DAS and 45 DAS with r values of -0.70 and -0.60, respectively (Table 14).

Table 14: Correlation between key crop attributes, yield parameters and Fusarium wilt incidence

	DM 120	Grain yield	Pods/plant	Harvest index	Plant height	DI 60 DAS
Dry matter 120						
Grain yield	0.90**					
Number of Pods	0.87**	0.93**				
Harvest Index	0.50**	0.73**	0.66**			
Plant Height	0.90**	0.92**	0.93**	0.60**		
DI 60 DAS	-0.83**	-0.94**	-0.91**	-0.70**	-0.87**	
DI 45 DAS	-0.95**	-0.96**	-0.94**	-0.61**	-0.95**	0.92**

** Significant at $P \leq 0.01$; * significant at $P \leq 0.05$. $r_{(24,0.05)} = 0.388$, $r_{(24,0.01)} = 0.496$

The positive correlation between plant height and harvest index is supported by the relationship between plant height and grain yield, which showed that as plant height increased, grain yield also increases (Figure 13). Studies by Halila and strange (1997) and Mallu *et al.* (2015) showed a positive correlation between plant height and grain yield. Wilt incidence in chickpea leads to low dry matter which in turn leads to low grain yield (Figure 7 and 8), hence low H.I values. Wilt incidence leads to low population and stunted crop which culminates into low yield (Singh and Jha, 2003). Plant height was negatively correlated with Fusarium wilt incidence at 60 DAS and 45 DAS giving r values of -0.87 and -0.95, respectively (Table 14). These findings are in agreement with research studies done by Halila and Strange (1997) who found a positive correlation between seed yield, plant height and seed weight. Muhammad (2010) found a direct correlation between inoculum density and wilt severity.

These results were in agreement with Haware and Nene (1980) who reported stunting in infected plants. Muhammad (2010) reported a direct correlation between wilt severity and pathogen inoculum density. This means that as fungicide rate was increased, plant height was improved due to low infection by the pathogen. Maitlo *et al.* (2014) also found that increasing fungicide rates improved shoot height by reducing wilt incidence. Fusarium wilt incidence at 60 DAS was positively correlated with Fusarium wilt incidence at 45 DAS with an r value of 0.92 which was significant ($P \leq 0.01$) (Table 14). This means that wilt incidence observed at 45th DAS was likely to incite more wilt incidence at 60th DAS and therefore alternative management options should be adopted.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

Six genotypes were found to be moderately resistant (MR), ten were susceptible (S) and the remaining four were highly susceptible (HS). None was completely resistant (R). The moderately resistant genotypes identified were 95423, 97105, 97114, 97125, 97126 and 97406.

Under varying rates of carbendazim and thiram, Fusarium wilt incidence (%) was lowest when chickpea was seed dressed with 150% of the fungicides. Highest grain yields of 1.4 t/ha were recorded when carbendazim and thiram were seed dressed at 150% (2.25 g/kg seed).

Positive interactions between variety and fungicide treatments were observed on wilt incidence of chickpea. Chania 1 needed significantly lower fungicide treatment as compared to Chania 2 to achieve a similar level of disease incidence. This means that to achieve effective wilt management, it is appropriate to use resistant or moderately resistant varieties and at least 100% rate of either fungicide.

5.2 RECOMMENDATIONS

1. Screening for resistance/tolerance should be a continuous effort as the pathogen evolves new races over time, which could be virulent to hitherto resistant/tolerant chickpea varieties.
2. The moderately resistant genotypes identified in this study should be deployed for use in integrating Fusarium wilt resistance in popular chickpeas varieties and thus, management of Fusarium wilt disease.
3. Trials should be done to determine the optimum timing with respect to wilt incidence on field crop, the rate and number of alternative fungicide spray(s) required for effective management of Fusarium wilt in chickpea.
4. Further studies to determine the most economic rates of applying thiram and or carbendazim for Fusarium wilt management and increased yields for various agro ecological zones of Kenya is recommended.
5. Exploit resistant genes in MR genotypes in molecular mapping to assist in future screening and breeding through gene tagging.

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