# MANAGEMENT OF ASCOCHYTA BLIGHT OF CHICKPEA (*Cicer areientinum* L.) CAUSED BY *Ascochyta rabiei* L. USING FUNGICIDES

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## DECLARATION AND RECOMMENDATION

### DECLARATION

This thesis is my original work and has not been submitted or presented for examination in any other institution for the award of any degree.

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## DEDICATION

This thesis is dedicated to my parents Ann Wambui and the late Francis Nganga for their selfless love. Special dedication to my dear wife Hellen and daughter Neema.

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## ABSTRACT

Chickpea (*Cicer arientinum* L.) is an important dry land legume whose full potential in Kenya has not been realized due to abiotic and biotic stresses. The objectives of this study were to evaluate the use of seed treatment and foliar sprays as disease management options against Ascochyta blight caused by *Ascochyta rabiei* in chickpea. The study was conducted in two sites; Egerton University Njoro and Agricultural Training Centre (ATC) Koibatek. One genotype Chania Desi (ICCV97105) was evaluated in three experiments. The first experiment was conducted both *in vitro* and *in vivo*. In the *in vitro* experiment, seeds from two categories (symptomatic and asymptomatic) were evaluated under a randomized complete block design (RCBD) using the agar plate method to determine germination and *A. rabiei* infection levels. In the *in vivo* experiment seeds within each category were left treated or untreated with either of the fungicides, Azoxystrobin 250g/L, Difenoconazole 250g/L, Azoxystrobin 250g/L+Difenoconazole 125g/L and Metalaxyl 40g/Kg+Mancozeb 640 g/Kg. The emergence of plants and the development of Ascochyta blight (AB) lesions on plants from the two batches were monitored in the greenhouse. The second experiment was to evaluate the efficacy of different fungicides in control of Ascochyta blight. A split-split plot design with four fungicide treatments and four spray regimes, one variety and three replications. The third experiment evaluated one variety and one fungicide only based on the result of the second experiment under a split-split plot design. Data on severity and incidence was collected and subjected to Analysis of Variance (ANOVA) at $P \leq 0.05$ and significant means at the F-test were separated using Fischer's protected LSD test. Seed treatment with azoxystrobin+difenoconazole combination and azoxystrobin alone had the most significant effect in emergence under greenhouse and field conditions. Seed dressing with either of the fungicides used had a significant increase in seedling emergence as compared to non-dressed seeds. Decrease in incidence and severity of ascochyta arising from seed dressing effect was not significant. PDI reduced by 65.5%, 62.25%, 40.55% and 33% in Njoro and 52.8%, 49.63%, 51.41% and 22.64% in Koibatek following application of 6, 5, 4, and 3 foliar sprays respectively compared to control. Combining seed treatment with foliar sprays did not show any superiority over the use of foliar sprays alone at all stages of growth. Upto six foliar sprays with difenoconazole and five sprays with azoxystrobin may be required for control of ascochyta blight in susceptible lines under high disease pressure.

**TABLE OF CONTENTS**

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF PLATES

## LIST OF APPENDICES

## ABBREVIATIONS

| | |
|---|---|
| AB | Ascochyta Blight |
| ATC | Agricultural Training Centre |
| ASALs | Arid and Semi-Arid Lands |
| AUDPC | Area Under Disease Progress Curve |
| CRD | Completely Randomized Design |
| DAE | Days After Emergence |
| DAP | Days After Planting |
| HPR | Host Plant Resistance |
| ICRISAT | International Crop Research Institute for Semi-Arid Tropics |
| IDM | Integrated Disease Management |
| RCBD | Randomized Complete Block Design |
| MOA | Ministry of Agriculture |
| ANOVA | Analysis of Variance |
| DSI | Disease Severity Index |
| PDI | Percentage Disease Index |
| ISO | International Organization for Standization |
| ATP | Adenosine Triphosphate |
| PDA | Potato Dextrose Agar |

# CHAPTER ONE

# INTRODUCTION

## 1.1 Background Information

Chickpea (*Cicer arietinum* L.) is the third most important food legume in the world after dry bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L.). In the West Asia and North Africa (WANA) region, it accounts for more than 27% of the total food legume production. Globally, it is cultivated in 11.67 million ha producing 9.31 million tons of grain worldwide (FAOSTAT, 2008). India accounts for approximately 65% of world chickpea production, followed by Pakistan (9.5%) and Turkey (6.7%) (FAOSTAT, 2008) while in Africa, Ethiopia is the leading chickpea producer. In Kenya annual chickpea production is estimated at 45 M tones from an area of 20-22,000 ha (CRP, 2012). The major chickpea growing areas in Kenya are Eastern Kenya (Embu, Tharaka, Machakos) and Rift Valley region (Bomet and Nakuru) (ICRISAT, 2014). Chickpea is a relatively cheap source of protein (20–23% in the grain), energy (Carbohydrates, 40%), oil (3–6%) (Gil *et al.*, 1996) minerals Mg, K, P, Fe, Zn, and Mn and *β*-carotene in developing world. Chickpea also contributes significantly to sustainability of cereal-legume cropping systems, increasing the yield of cereals through enhancing the soil nitrogen and breaking the disease cycles of important cereal pathogen (Pande *et al*., 2011). Chickpea improved soil fertility by improving maize (*Zea mays L.*) yields by 24-68% in a cereal-legume relay system due to fixing of substantial nitrogen (Cheruiyot *et al*., 2001; Cheruiyot *et al.,* 2002).

As manure chickpea improved soil structure (Wakindiki and Yegon, 2011) of acidic soil in Uasin Gishu, Kenya, as well as reducing passion fruit Fusarium wilt if it preceded passion fruit in the rotation in Egerton-Njoro, Nakuru (Mwangi *et al*., 2009). Currently the crop is gaining popularity among large scale wheat, maize and barley farmers as a rotation crop in the Rift valley dry highlands during the short rains. In arid and semi-arid lands (ASALs), chickpea is also recognized as mitigation strategy towards the prevailing climate change effects (MOA, 2011), mainly due to its early maturity and heat and drought-tolerance characteristics. Hence, promotion of chickpea as a cash and food crop is currently underway in the country.

In Kenya, chickpea is a relatively new crop grown by small scale farmers in both the former Eastern and Rift Valley provinces. It has however spread and is currently adapted to varied agro-ecological zones such as dry highlands, medium altitudes and also in dry lowlands with annual rainfall range of 250-550 mm per annum (Kibe and Onyari, 2007; Onyari *et al.*,

2010). Production of chickpea in Kenya however has been declining over the last 10 years both in acreage and quantity produced. In most chickpea growing regions of the world, production of chickpea has remained stagnant at 400-854 kg/ha for many years against a potential yield of more than 3600 kg/ha. This has been attributed to susceptibility of the crop to insect pests, nematodes and fungi (Barve *et al.,* 2003). Recent efforts in Kenya to introduce improved cultivars from ICRISAT in the dry highlands as a rotation crop has shown significant increase especially with the adoption of new varieties with yields ranging between 1.0-3.5 T $ha^{-1}$(Kimurto *et al.,* 2009; Kimurto *et al.,* 2013).

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labrouse [(teleomorph: *Didymella rabiei* (Kovacheski)] is the most important fungal disease of chickpea (*Cicer arietinum* L.) worldwide (Akem, 2004). Chickpea is traditionally sown on residual moisture after long rains (in rotation with cereals) in Kenya and other major chickpea growing areas of Africa (Ethiopia, Tanzania and Malawi), and as a consequence it experiences terminal drought during the growth period especially in summers (dry seasons) (Kimurto *et al.,* 2013). Millan *et al*., 2006 and Varshney *et al.,* 2009a, reported that similar conditions are experienced in Asia, North Africa and other regions with Mediterranean climate, where chickpea is sown in spring and growth period is in dry summers, resulting in poor biomass development and yield. In both the sub-Sahara Africa and North Africa, sowing earlier during the long rainy season (winter) would reduce terminal drought stress, expand the vegetative growth period and improve the seed yield significantly upto 3 T $ha^{-1}$. However, this is rarely adopted by the farmers because the cool and wet weather, typical for long rainy seasons or Mediterranean winters, favors the development of AB epidemics as in most regions of the world where the crop is commonly grown (Kimurto *et al.,* 2013) like North America, Pakistan, NorthWest India and Australia.

*Ascochyta rabei* survives either on or in seed or plant debris in form of mycelium, pycnidia, and various teleomorphic stages (Kaiser, 1997) and spreads via airborne spores. The sexual (teleomorph) state helps in long-term survival of the pathogen, but there is no work done in Kenya on the presence of this state that is known (Kimurto *et al.,* 2013). Most researchers have concentrated efforts on disease management through host plant resistance (Akem, 1999). No complete immunity to *A. rabiei* has been identified even in highly resistant chickpea lines and pathotypes with greater virulence always have been shown to cause disease regardless of the level of resistance (Cho and Muehlbauer, 2004). Current cultivars only possess low resistance

to the pathogen which can breakdown easily (Gan *et al.,* 2006). This is due to the contribution of the sexual stage (teleomorph: *Didymella rabiei*) of the pathogen to emergence of new races or pathotypes (Barve *et al*., 2003; Bretag *et al*., 2006; Gan *et al*., 2006). Low resistance in these cultivars declines at flowering when weather and crop canopy conditions favour blight development (Chongo *et al.,* 2003). Kimurto *et al.,* (2013) noted that most commercial varieties (LDT065, Chania Desi 1, Saina K 1 and Annigeri) are susceptible to AB with mean disease scores >5.5 when evaluated in dry highlands of Kenya during the long rains. The study noted that genotypes like ICC9755, ICC12324 and ICC8752 which had high resistance and low disease scores between 1.8-2.5 were dark brown to dark brown to dark green seeded and not preferred by consumers. Adoption of chickpea as a food crop therefore remains a challenge especially in the highlands of Kenya.

Integration of host resistance with other cultural practices and minimal chemical control has been suggested. Foliar sprays with fungicides used judiciously, with established minimum rates and frequencies can be effective in slowing down the disease in an integrated disease management program (Akem, 1999; Gan *et al.,* 2006). Temperature and relative humidity affects the growth of the pathogen *A. rabiei*. Development of pseudothecia can be inhibited at temperatures higher than $10^{o}$C or relative humidity lower than 100% (Navas-cortes *et al.,* 1998).The most important factor determining pathogenicity of *A. rabiei* is relative humidity (Cho *et al.,* 2004). There is need to identify the appropriate management strategies in different regions since these environmental factors vary from place to place.

Evaluation of chickpea genotypes in Syria showed that there was significant difference between the time of application of foliar spray (chlorothalonil), severity and incidence of the disease( Akem *et al.,* 2004). Applications made before flowering were found to be most effective than applications made at the reproductive stage. Chongo *et al.,* (2003) reported that fungicide application can complement low resistance to reduce blight severity and increase yield and quality in chickpea. However high or multiple applications appeared to have no effect on yield parameters. Therefore, host plant resistance (HPR), as a major component of integrated disease management (IDM) is the most economical approach to manage this disease since most growers keep their own planting seed. According to Reddy and Singh, 1990 and Pande *et al*., 2005 and IDM strategy would include: use of pathogen-free seed, seed treatment, and crop rotation practice, deep ploughing to bury infested debris, use of disease-resistant genotypes and strategic

application of foliar fungicides (during seedling and early podding). In this study, efficacy of fungicide seed dressing and foliar sprays was evaluated in Koibatek and Njoro, Kenya with the aim of reducing AB severity and incidence thus enhancing adoption and production of chickpea.

**1.2 Statement of the Problem**

Chickpea is an important drought tolerant legume with potential to do well in the ASALs regions and the cool dry highlands of Kenya during the short rains season. It has potential to improve nutrition by providing inexpensive quality proteins, improve soil health, break disease cycle in cereal cropping systems and provide livestock feed therefore enhancing food security. However, the crop is relatively new in these areas and its full adoption and productivity have not been realized due to high yield losses caused by AB which can cause upto 100% yield loss. In the dry cool highlands where chickpea is grown as rotational crop during the short rains, the environmental conditions may change to wet, rainy (high RH >80%) and low temperatures (15-20 $^{o}$C). This favours the development of the pathogen causing total yield losses to farmers frequently. Since AB is spread through infected seed, the intensity of AB infestation could be reduced by maintaining high levels of seed health which involve use of clean seed. Most farmers however plant own-seed which in most cases is infected by AB serving as source of inocula that spreads from one farmer to another over several seasons. Although the most viable and economical control measure against AB is use of host plant resistance, the currently released varieties have low resistance to the pathogen that is easily broken by new pathotypes frequently emerging in these areas. Moreover, the pathogen survives for long period of time in infected debris in the fields due to sexual state (Teleomorph) which favour frequent multiplication and spread of the disease, thus making cultural control measures ineffective. The alternative strategies of integrated disease management (IDM) will include the use of disease free seed, resistant varieties, seed treatment and foliar sprays as the most viable options which have not been evaluated in Kenya yet in management of ascochyta blight of chickpea.

## 1.3 Objectives

### 1.3.1 General Objective

To contribute to effective management of AB in chickpea for increased yield and food security in Kenya's ASALs and dry highlands.

### 1.3.2 Specific Objectives

1. To determine the efficacy of seed treatment fungicides in control of ascochyta blight of chickpea.
2. To determine the efficacy of foliar fungicide on ascochyta blight of chickpea.
3. To determine the effect of timing (application at different times/stages of growth) of spraying of foliar fungicide on ascochyta blight of chickpea.

## 1.4 Hypotheses

1. Seed dressing with fungicides is not effective on reducing incidence and severity of ascochyta blight in the field.
2. Different foliar fungicides are not effective on management of ascochyta blight of chickpea.
3. Application of foliar fungicides at different times is not effective on reduction of ascochyta blight of chickpea.

## 1.5 Justification

Chickpea is an alternative drought tolerant legume that can enhance soil fertility and food security in the ASALs regions of Sub-Sahara Africa. It is also a crop that can utilize residual moisture in the soil during short rains and has potential as a rotation crop with cereals to break disease cycle in these crops. Importance of chickpea continues to increase with increasing food demand in Kenya and export markets in Asia and Europe. Despite tremendous efforts in breeding for improved yields, most current commercial cultivars lack durable resistance hence chickpea farmers frequently experience high yield losses caused by AB.

Breeding as a disease management tool is not sufficient to contain AB. There is need for integrated Ascochyta blight management in Kenya as has been recommended in other chickpea growing regions of the world to reduce yield losses and enhance adoptability. IDM options include the use of treated seeds, tolerant varieties, foliar sprays, deep ploughing and rotation. However, there is limited information on the use of these strategies for AB management in

Kenya. Most farmers do not use fungicides judiciously leading to increased production cost and human and environmental hazards. It is necessary to determine the degree of viability of seed treatment and foliar sprays to facilitate the adoption of the crop by farmers and to allow judicious use of fungicides. This will also allow the shift to sowing into wet long-rainy season for increased yield and productivity (Kimurto *et al.*, 2013).

## REFERENCES


Akem, C. (1999). Ascochyta blight of chickpea: Present status and future priorities. *International Journal of Pest Management*, 45: 2, 131-137.

Akem, C., Kabbabeh S., and Ahmed, S. (2004). Integrating cultivar resistance with a single fungicide spray to manage Ascochyta blight for increased chickpea yields. *Plant Pathology Journal* 3: 2, 105-110.

Barve, M.P., Arie, T., Salimath, S.S., Muehlbauer, F.J., and Peever, T.L. (2003). Cloning and characterization of the mating type (MAT) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a MAT phylogeny of legume-associated Ascochyta spp. *Fungal Genetics and Biology,* 39: 151–167.

Bretag, T. W., Keane, P. J., and Price, T. V. (2006). The epidemiology and control of Ascochyta blight in field peas: a review. *Australian Journal of Agricultural Research,* 57: 883–902.

Cheruiyot, E.K., Mumera, L.M., Nakhone, L.N. and Mwonga, A.M. (2001). Rotational effects of grain legume on maize performance in the Rift valley highlands of Kenya. *African Crop Science Journal,* 9: 667-676.

Cheruiyot, E.K., Mumera, L.M., Nakhone, L.N. and Mwonga, A.M. (2002). Effect of legume-managed fallow on weeds and soil nitrogen in succeeding maize and wheat crops in the Rift valley highlands of Kenya. *Australian Journal of Experimental Agriculture,* 43 (60): 24-34.

Chongo, G., Buchwaldt, L., Gossen, B.D., Lafond, G.P., May, W.E., Johnson, E.N., and Hogg, T. (2003). Foliar fungicides to manage Ascochyta blight [*Ascochyta rabiei*] of chickpea in Canada. *Canadian Journal of Plant Pathology,* 25: 135–142.

CRP (2012). CGIAR Research Program on Grain Legumes. *Leveraging legumes to combat poverty, hunger, malnutrition and environmental degradation.* IITA, ICRISAT, CIAT and ICARDA.

FAOSTAT, 2008. Production year book. Vol 67, pp45. Food and Agriculture Organization of the United Nations, Rome, Italy.

Gan , Y.T., Siddique, K.H.M., MacLeod, W.J. and Jayakumar, P. (2006). Management options for minimizing the damage by Ascochyta blight (*Ascochyta rabiei*) in chickpea (*Cicer arietinum* L.). *Field Crops Research,* 97: 121–134.

Gil, J., Nadal, L.S.D., Moreno, M.T. and Haro, A. (1996). Variability of some physio-chemical characters in *Desi* and *Kabuli* chickpea types. *Journal of Food Science Agriculture* 71: 179-184.

ICRISAT, (2014). International Crops Research Institute for the Semi-Arid Tropics. *Annual Technical Report*, Nairobi, Kenya.

Kaiser, W.J. (1997). Inter-and Intra-national spread of Ascochyta pathogens of chickpea, faba bean, and lentil. *Canadian Journal of Plant Pathology*, 19: 215-224

Kibe, A.M and Onyari, C.N. (2007). Production functions and their use in predicting chickpea biomass yields when grown under varying tillage and sowing dates in Naivasha, Kenya. *Agricultural Journal*, 2: 514-519.

Kimurto, P.K., Towett, B.K., Mulwa, R.M.S., Njogu, N., Jeptanui, L.N., Gangarao, R.O., Silim, S., Kaloki, P., Korir, P., Macharia, J.K. (2013). Evaluation of chickpea genotypes for resistance to ascochyta blight (*Ascochyta rabiei*) disease in the dry highlands of Kenya. *Phytopathologia Mediterranea*, 52 (1): 613-622

Kimurto, P.K., Towett, B.K., Mulwa, R.M.S., Varshney, R.K., Gangarao, Gangarao, R.O., Gaur, Silim, P.S., Cheruiyot, E.K. and Rutto, D. (2009). Improving tolerance to drought and *Helicoverpa armigera* in Chickpeas (*Cicer arientinum* L.) in semi-arid areas of Kenya. In: Proceeding of the International Edible legume conference and 4$^{th}$ World Chickpea congress, 17-22 Durban, South Africa.

Millan, T.J., Clarke, H.J., Kadambot, H., Siddique, M., Buhariwalla, H.K., Gaur, P.M., Kumar, J., Gil, J., Kahl, G. and Winter, P. (2006). Chickpea molecular breeding: New tools and concepts. *Euphytica*, 147: 81-103.

MOA (2011). The Ministry of Agriculture Annual Report at a glance Government Printers, Nairobi, Kenya.

Mwangi, H.G., Gethi, M. and Mumera, L. (2009). Evaluation of strategies for management of Fusarium wilts in passion fruits in central Rift of Kenya. In: Proceedings of KARI Biennual Conference Proceedings, KARI, Nairobi, Kenya, 57-71.

Navas-Cortes, J. A., Trapero-Casas, A., and Jimenez-Diaz, R. M. (1998). Influence of relative humidity and temperature on development of *Didymella rabiei* on chickpea debris. *Plant Pathology*, 47: 57–66

Onyari, C.A.N., Ouma, J.P. and Kibe, A.M. (2010). Effects of tillage method and sowing time on phenology, yield and yield components chickpea (*Cicer arientinum* L.) under semi arid conditions in Kenya. *Journal of Applied Biosciences*, 34: 2156-2165.

Pande S., Siddique, K.H.M., Kishore, G.K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T.W. and Crouch J.H. (2005). Ascochyta blight of chickpea (*Cicer arientinum* L.): a review of biology, Pathogenicity and disease management. *Australian Journal of Agricultural Research*, 56: 317-332.

Pande, S., Sharma, M., Gaur, P.M., Tripathi, S., Kaur, L., Basandrai, A., Khan, T., Gowda, C.L.L. and Siddique, K.H.M. (2011). Development of screening techniques and identification of new sources of resistance to Ascochyta blight disease of chickpea. *Australian Plant Pathology*, 40: 149-156.

Reddy, M.V and Singh K.B. (1990). Management of Ascochyta blight of chickpea through integration of host plant tolerance and foliar spraying of chlorothalonil. *Indian Journal of Plant Protection*, 18: 65-69

Varshney, R.K., Close, T.J., Singh, N.K., Hoising, D.A. and Cook, D.R. (2009). Orphan legume crops enter the genomics era. *Current Opinion Plant Biology*, 12: 202-210.

Wakindiki, I.I.C and Yegon, R. (2011). Effect of decomposition intensity of incorporated chickpea manure on stability and saturated hydraulic conductivity of a clay loam and clay soil. *African Journal of Agricultural Research*, 6(5): 1120-1127.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1 Origin and Importance of chickpea

Chickpea is also known as Bengal, gram or garbanzo bean. It is the most important food grain legume of south Asia and the third most important in the world after common bean (*Phaseolus vulgaris* L.) and field pea (*P. sativum*). Chickpea is a diploid with 2n=2x=16 chromosomes and a genome size of approximately 750 Mbp (Ahmad *et al.,* 2005). The wild annual progenitor of chickpea has been identified as the annual species *C. reticulatum* while *C. anatolium* is the perennial progenitor. It is believed that chickpea originated in the fertile cresent region of southeastern Turkey and adjoining Syria (Ladizinsky and Alder, 1975). From Turkey, chickpea diverged in two directions into the western parts where it is grown in the spring and summer and into the eastern parts where it is grown in the cool dry seasons. Botanically, cultivated chickpea has been split into two; microsperma and macrosperma, corresponding to seed size. Practically, chickpea is also classified into kabuli and desi types. The terms desi and kabuli however do not overlap with microsperma and macrosperma (Ahmad *et al*., 2005).

Globally, chickpea is grown in more than 40 countries with India being the leading producer (FAOSTAT, 2008; ICRISAT, 2008). Africa accounts for only 5%, mostly from Ethiopia, Malawi and Tanzania in Eastern Africa and Morocco in North Africa. Though there has not been much change in the global chickpea area, the production has increased from 7 MT in 1961-1965 to 8.4 MT during 1996-2000 due to enhancement in yield levels from 599 to 791 kg/ha (Gowda and Gaur, 2003). Nutritionally, chickpea has the highest compositions of any dry edible grain legume. On average, it contains 23% protein, 64% carbohydrates, 47% starch, 5% fat (primarily linoleic and oleic acids), 6% crude fiber, 6% soluble sugar and 3% ash (Ahmad *et al*., 2005).

## 2.2 Biology and Life Cycle of *Ascochyta rabiei*

The pathogen *A. rabiei* (Passarini) Labrouse [(teleomorph: *D. rabiei* (Kovacheski)] was first described on chickpea in 1911 (Akem, 1999). Labrousse first described the fungus in 1930 as *Phyllosticta rabiei* because he saw no bicellular spores on the host though a few were observed in culture. One year later he suggested that the fungus be called *A. rabiei* as it produces 2-4% single septate spores on artificially inoculated plants (Shahid *et al.,* 2008). Passarini from France was the first to describe the teleomorph *D. rabiei* in 1867. He first named the fungus as

*Zythia rabiei*. The teleomorph was later described by Kovacheski as *D. rabiei* (Akem, 1999). The pathogen is well adapted for survival and can over-winter on infested chickpea residue or infected seed (Wiese *et al.*, 1995).

The fungus has two reproduction cycles each producing distinct spores; sexual and asexual. The asexual spores called conidia are produced within fruiting bodies called pycnidia embedded in diseased tissues while the sexual spores are produced within pseudothecia on over-wintering chickpea residues (Wiese *et al.,* 1995). The sexual stage (teleomorph: *Didymella rabiei*) which arise from mating of compatible strains may contribute to development of new strains or pathotypes (Gan *et al.,* 2006). The spores are spread by agents such as water splash, wind, debris and seed onto established crop where they germinate and continue to reproduce asexually (conidia). Lesions and characteristic symptoms of concentric rings of pycnidia appear on stems, leaves and pods. The seeds shrivel and discolor if they are infected (Faye *et al.,* 2010).

*A. rabiei* (teleomorph *Didymella rabiei*) is a directly penetrating, necrotrophic fungus that infects all above ground parts of chickpea (Jayakumar *et al*., 2005). The pathogen survives by over seasoning on plant residues and on infected or contaminated seeds as well as in infected volunteer chickpea plants (Wiese *et al.,* 1995; Moore *et al.,* 2011). The pathogen is highly variable due to potential for sexual recombination. Cultivar resistance can therefore break easily (Gan *et al*., 2006). Over wintered infected chickpea residue produces spore-bearing structures pycnidia (asexual) or pseudothecia (sexual). Conidia are produced in pycnidia while ascospores are borne in pseudothecia.

### 2.3 Epidemiology of *Ascochyta* rabiei

Chickpea is the only known host that suffers economic damage from *A. rabiei* (Wiese *et al.,* 1995). However, other alternative hosts have been reported including; faba beans, alfalfa (lucerne), lentils, and field peas among other legumes. Ascospores produced from the sexual stage developing on stubble or seed are airborne and can be spread over long distances. In favourable conditions i.e. at least two hours of wetness on the leaves, the ascospores germinates but the likelihood of establishment increases if the wetness continues for more than six hours (Markell *et al.,* 2008). The germinated spore forms an appressorium from which it penetrates the plant within 12-24hrs. Once the fungus has successfully invaded the plant, it starts to kill plant tissues and within 4-6 days, the disease lesions are visible (Faye *et al.,* 2010).

The disease develops rapidly in cool wet conditions. Hot, dry conditions have been said to delay disease development though spread can continue once conditions become favorable (Markell *et al.*, 2008; Moore *et al.,* 2011). The pathogen can develop over a wide range of temperatures (5-30$^{o}$C). However the disease develops faster when temperatures are between 15-25$^{o}$C and relative humidity is high (Navas-cortes *et al.,* 1998; Cho and Muehlbauer, 2004; Moore *et al.,* 2011). Regular seasonal occurrences of epidemics of Ascochyta blight suggest the existence of efficient mechanisms for the survival of *D. rabiei* from one season to the next. The spread of the disease has been attributed to the conidiospores produced at the foci of primary infection, either through crop debris or infected seed (Akem, 1999). Seed borne inocula as well as infected or contaminated seeds have been shown to be responsible for the introduction of Ascochyta blight (Gan *et al.,* 2006; Markell *et al.,* 2008; Moore *et al.,* 2011). Infected seed lead to a random distribution of infected seedlings, which serve as the initial foci from which the pathogen spreads in the field.

**2.4 Symptoms of Ascochyta blight**

Ascochyta blight first appears as gray areas on the leaves, stems or pods that quickly turn into brown lesions with dark borders. As the disease progresses, small, circular, brown-black dots (pycnidia) develop in the center of the lesions, and are frequently arranged in concentric circles and resemble a bull's-eye (Markell *et al*., 2008). Infections usually begin low in the crop canopy during periods of cool, wet weather (Wiese *et al.,* 1995). The pale green/yellow discolouration on the leaves is often referred to as "ghosting" (Moore *et al*., 2011). These symptoms become visible in 4-6 days (Faye *et al*., 2010). Concentric rings of pycnidia are the most diagnostic characteristic of the disease. Infected seed may be discolored, shrunken or shriveled and in severity, lesions with dark pycnidia may be present on the seed (Markell *et al*., 2008; Moore *et al*., 2011). Lesions often girdle stems, weaken and break branches and petioles and kill all plant parts above the lesion (Wiese *et al.,* 1995).

A

B

**Plate 1A and B: A) Girdling symptoms on stems of plants growing in the greenhouse at Egerton University Njoro and B) Concentric rings on chickpea pods. Source, Author.**

## 2.5 Management of Ascochyta Blight

### 2.5.1 Seed Treatment

Seed borne inocula as well as infected or contaminated seeds have been shown to be responsible for the introduction of Ascochyta blight (Akem *et al.,* 1999; Gan *et al*., 2006; Markell *et al.,* 2008; Moore *et al.,* 2011). Seed for commercial production of chickpea should be tested for infection level by an accredited laboratory, so that appropriate seed treatment can be followed (Gan *et al.,* 2006). Broad spectrum fungicides can be used in seed treatment to limit fungal pathogens that may be present on the seed or in the soil. Small-sized seeds usually have a higher level of ascochyta infection than large-sized seeds. This is because diseased pods typically produce seed with a smaller size. Use of large seed screened from a seed lot may reduce the risk of ascochyta infection (Gan *et al*., 2006). However research has shown that seed dressings will only protect emerging seedling from seed borne ascochyta and seed borne botrytis. Seed dressing will not protect the emerging seedling from rain-drop splashed ascochyta or wind borne botrytis (Markell *et al*., 2008).

### 2.5.2 Fungicides

The use of fungicides is not cost effective if severity is low. The best time for application recommended is during flowering or at podding stages under such circumstances (Shahid *et al.,* 2008). Evaluation of chickpea genotypes in Syria by Akem *et al*., (2004) showed that there was significant difference between the time of application of foliar spray (chlorothalonil), severity and yield of the disease. Applications made before flowering were found to be most effective than applications made at the reproductive stage. Further studies by Chongo *et al.,* (2003) indicated that fungicide application can complement low resistance to reduce blight severity and increase yield and quality in chickpea. However high or multiple applications appeared to have no effect on yield parameters. Preventive fungicides should be applied prior to flowering and before disease develops in a field, that is before the rains (Markell *et al*., 2008; Moore *et al*., 2011). Fungicides that have been found to be effective for seed treatment include carbathiin, thiabendazole, azoxystrobin and metalaxyl among other which can help minimize seed-borne disease in both kabuli and desi varieties. An additional seed treatment with metalaxyl-based product (e.g., Apron) is recommended for kabuli varieties which are susceptible to seed rot diseases. The products to be used in this study have been reviewed below.

**Azoxystrobin**

Azoxystrobin is the International Organization for Standardization (ISO) approved name for Methyl(E)-2-{2[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3methoxyacrylate (IUPAC) (Giza and Sztwiertnia, 2003). It is a broad spectrum fungicide. It's a synthetic strobilurin a synthetic analogue of naturally occurring fungal metabolites the strobilurins and oudemansins (Giza and Sztwiertnia, 2003). It disrupts mitochondrial respiration through inhibitition of electron transfer in fungal mitochondria by binding at a specific site on cytochrome b. The results in the cessation of normal energy production (ATP production) within the cell thus cell dies (Sundravadana *et al*., 2007).

According to Syngenta group (2005), azoxystrobin has the following chemical and physical properties; the molecular formula is $C_{22}H_{17}N_3O_5$ and molecular weight 403.4. It has a melting point of 116$^{o}$C. Chemical stability is 14 days in pure sterile water and 3 days in natural river water. It is stable at environmental pH and temperature. It is non-volatile from soil or plant surface. The chemical is rapidly dissipated in the terrestrial environment with a lab metabolism of 57-136 days and a Koc factor of 300-1690. The breakdown products/metabolites of azoxystrobin are readily degraded/mineralized to carbon dioxide and thus are only present at low levels and do not accumulate in soil (soil half life is 14 days). Azoxystrobin and its breakdown products do not leach to groundwater due to a combination of their degradation rates and relatively low mobility in soil. Toxicity studies show that azoxystrobin is of low toxicity to terrestrial organisms, including birds, mammals, bees and other insects, and earthworms: birds oral LD50 > 2000mg/kg dietary LC50>5200 ppm; Bees and other non-target arthropods, LD50>200 ug/bee; Earthworms LC50 284mg/kg soil. It's highly to moderately toxic to aquatic life, fish LC50 470-2160 ug/L, EC 50 aquatic invertebrates 55>4000 ug/L, EC 50 aquatic plants/algae EC 57-10000ug/L.

**Difenoconazole**

Syngenta group (2005) classifies difenoconazole as a triazole fungicide that has protective, curative, and systemic activity against wide spectrum of fungal diseases. IUPAC name for difenoconazole is cis, trans-3-chloro-4-[4-methyl-2-(1H-1,2,4-triazol-1-ylmethyl)-1,3-dioxolan-2-yl]phenyl 4-chlorophenyl ether. The mode of action of difenoconazole is that it is a sterol demethylation inhibitor which prevents the development of the fungus by inhibiting cell

membrane ergosterol biosynthesis by leaf surface treatment or seed treatment. Difenoconazole show permanent protection on beet cercospora, leaf spot, leaf blight, rust disease and mildew, potato early blight, leaf spot of peanut, net spot disease, apple black spot disease, grape white powder. Difenoconazole is a broad spectrum fungicide that controls a wide variety of fungi including members of the Ascomycetes, Basidomycetes and Deuteromycetes families. It acts as a seed treatment, foliar spray and systemic fungicide. It is taken up through the surface of the infected plant and is translocated to all parts of the plant. It has a curative effect and a preventative effect. Difenoconazole can be applied to winter wheat, oil seed rape, brussels sprouts, cabbage, broccoli/calabrese and cauliflower. It controls various fungi including *Septoria tritici*, brown rust, light leaf spot, leaf spot, pod spot, ring spot and stem canker. It also prevents ear discolouration in winter wheat. Difenoconazole is generally slowly absorbed and metabolized. In most cases, particularly for parts of the plant directly exposed to the treatment, the parent difenoconazole is the dominant part of the residue. The residue in parts of the plant not directly exposed is more likely to contain a residue dominated by a mobile water-soluble metabolite such as triazolylalanine (Mensik, 2008).

### 2.5.3 Host Plant Resistance (HPR)

Current cultivars only possess low resistance to the pathogen which can breakdown easily (Gan *et al.,* 2006). This is due to the contribution of the sexual stage (teleomorph) of the pathogen to emergence of new races or pathotypes (Barve *et al*., 2003; Bretag *et al*., 2006; Gan *et al*., 2006). Additionally, partial resistant in these cultivars decline at flowering when weather and crop canopy conditions often favor blight development (Chongo *et al.,* 2003). Resistance begins to break shortly after flowering and pod formation thus the need to seek alternative measures after this period (Shahid *et al.,* 2008).

Additionally indiscriminate use of fungicides in resistant varieties under low disease pressure may not be cost effective. Varieties with disease resistance have been recorded. They include Sanford, Evans, Dwelly and Myles (Wiese *et al.,* 1995). Most variety selection has concentrated efforts in improving markets driven traits (such as yield). However there exist moderately resistant varieties such as small Kabuli/desi-type chickpeas which can make disease management easier (Markell *et al*., 2008). Monitoring blight development at regular interval has been recommended (Wiese *et al*., 1995). This can help recognize disease symptoms at an initial stage which is essential in blight control.

### 2.5.4 Integrated Disease Management (IDM)

Integration of host resistance with other cultural practices and minimal chemical control has been suggested. Foliar sprays with fungicides used judiciously, with established minimum rates and frequencies can be effective in slowing down the disease in an integrated disease management program (Akem, 1999; Gan *et al*., 2006). According to Pande *et al*., (2005) and Reddy and Singh, 1990, IDM strategy would include: use of pathogen-free seed, seed treatment, crop rotation practice, deep ploughing to bury infested debris, use of disease-resistant genotypes (e.g. ICL 482) and strategic application of foliar fungicides during seedling and early podding.

Ascochyta blight is effectively managed through integrated approaches. These strategies include rotation with non-host crops, not planting chickpea more frequently than 3-4 years, use of disease free seeds, destruction of plant debris and selection of fields without a previous history of blight (Shahid *et al.,* 2008). This is possible because *A. rabiei* only affects chickpea. Shahid *et al*., 2008 also suggested tillage practices like deep burial of infected residue and controlling volunteer chickpeas. The uses of inspected and certified seeds have been recommended (Wiese *et al*., 1995).

## REFERENCES


Ahmad, F., Gaur, P., & Croser, J. (2005). Chickpea (Cicer arientinum L.). In R. Singh, & P. Jauhar, *Genetic resources, chromosome engineering and crop improvement* (Vol. 1, pp. 188-189). New York: Taylor and Francis.

Akem, C. (1999). Ascochyta blight of chickpea: Present status and future priorities. *International Journal of Pest Management,* 45: 2, 131-137.

Akem, C., Kabbabeh S., and Ahmed, S. (2004). Integrating cultivar resistance with a single fungicide spray to manage Ascochyta blight for increased chickpea yields. *Plant Pathology Journal* 3: 2, 105-110.

Barve, M.P., Arie, T., Salimath, S.S., Muehlbauer, F.J., and Peever, T.L. (2003). Cloning and characterization of the mating type (MAT) locus from *Ascochyta rabiei* (teleomorph: *Didymella rabiei*) and a MAT phylogeny of legume-associated Ascochyta spp. *Fungal Genetics and Biology,* 39: 151–167.

Bretag, T. W., Keane, P. J., and Price, T. V. (2006). The epidemiology and control of Ascochyta blight in field peas: a review. *Australian Journal of Agricultural Research,* 57: 883–902.

Cho, S. and Muehlbauer, F.J. (2004). Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (*Cicer arietinum* L.). *Physiological and Molecular Plant Pathology,* 64: 57–66.

Chongo, G., Buchwaldt, L., Gossen B.D., Lafond, G.P., May, W.E., Johnson, E.N., and Hogg, T. (2003). Foliar fungicides to manage Ascochyta blight [*Ascochyta rabiei*] of chickpea in Canada. *Canadian Journal of Plant Pathology,* 25: 135–142.

FAOSTAT (2008). Production year book. Vol 67, pp45. Food and Agriculture Organization of the United Nations, Rome, Italy.

Faye, D.B., Sean, M., Penny, M. and Ray, M. (2010). Scouting and management of Ascochyta blight in chickpea. Website: http://www.agriculture.gov.sk.ca/Ascochyta blight of chickpea. Accessed on 14th June, 2013.

Gan , Y.T., Siddique, K.H.M., MacLeod, W.J., and Jayakumar, P. (2006). Management options for minimizing the damage by Ascochyta blight (*Ascochyta rabiei*) in chickpea (*Cicer arietinum* L.). *Field Crops Research,* 97: 121–134.

Giza, I. and Szwiertnia (2003). Gas Chromatographic determination of azoxystrobin and tifloxystrobin residues in apples. *Acta Chromatographica, 13,* 226-229.

Gowda, C., and Gaur, P. (2003). Global scenario of chickpea research:Present status and future thrusts. *Proceedings of National Symposium on Pulses for crop Crop Diversification and Natural resource Management.* Kanpur, India: The Indian Society of Pulses Research and Development.

ICRISAT (2008). International Crops Research Institute for the Semi-Arid Tropics. *Annual Technical Report*. Nairobi, Kenya.

ICRISAT, (2014). International Crops Research Institute for the Semi-Arid Tropics. *Annual Technical Report*, Nairobi, Kenya.

Syngenta Group (2005). *ENVIROfacts.* Greensboro, NC 27419-8300: Syngenta Group.

Jayakumar, J., Gossen, B.D., Gan, Y.T., Warkentin, T.D., and Banniza, S. (2005). Ascochyta blight of chickpea: infection and host resistance mechanisms. *Canadian Journal of Plant Pathology*, 27: 499–509.

Ladizinsky, G., & Alder, A. (1975). The origin of chickpea as indicated by seed protein electrophoresis. *Israel Journal of Botany, 24*, 183.

Markell, S., Wise, K., McKay, K., Goswami, R., and Gudmestad, N. (2008). Aschochyta blight of chickpea. Plant Disease Management. NDSU Extension service.

Mensik, B.W.J.G. (2008). Environmemtal risk limits for difenoconazole. *Letter report*, National Institute for Public Health and the Environment, Bilthoven, Netherlands.

Moore, K., Ryley, M., Cumming, G., and Jenkins, L. (2011). Chickpea: Ascochyta blight Management. Northern Pulse Bulletin. PA 2011: 9.

Navas-Cortes, J. A., Trapero-Casas, A., and Jimenez-Diaz, R. M. (1998). Influence of relative humidity and temperature on development of *Didymella rabiei* on chickpea debris. *Plant Pathology*, 47: 57–66.

Pande S., Siddique, K.H.M., Kishore, G.K., Bayaa, B., Gaur, P.M., Gowda, C.L.L., Bretag, T.W. and Crouch J.H. (2005). Ascochyta blight of chickpea (*Cicer arientinum* L.): a review of biology, Pathogenicity and disease management. *Australian Journal of Agricultural Research*, 56: 317-332.

Shahid, A. A., Husnain, T., Riazuddin, S. (2008). Ascochyta blight of chickpea: Production of phytotoxins and disease management. *Biotechnology Advances,* 26: 511–515.

Sundravadana, S., Alice, D., Kuttalam, S., & Samiyappan, R. (2007). Efficacy of azoxystrobin on Colletotrichum gloeosporiodes Penz growth and on controlling Mango Anthracnose. *Journal of Agricultural and Biological Science, 2* (3), 10-15.

Wiese, M. V., Kaiser, W. J., Smith, L.J., Muehlbauer, F. J. (1995). Ascochyta blight of chickpea. University of Idaho College of Agriculture. Ag communications center. CIS 886.

## CHAPTER THREE

# Efficacy of Foliar Fungicide and Seed Treatment on Percentage Disease Incidence (PDI) Disease Severity Index (DSI) and Area Under Disease Progress Curve (AUDPC) of Ascochyta Blight (*Ascochyta rabiei* L.)


## ABSTRACT

Ascochyta blight, caused by *Ascochyta rabiei L.*, is the most devastating foliar disease of chickpea in the dry highlands of Kenya and need effective strategies of control. The efficacy of Azoxystrobin, Difenoconazole, Azoxystrobin+Difenoconazole and Metalaxyl+Mancozeb was evaluated in field experiments conducted in two sites (ATC-Koibatek and Egerton University, Njoro) over the period of two years. Preliminary studies in the laboratory to determine the percentage of infection in two seed lots; symptomatic and asymptomatic seeds were conducted. This was followed by laboratory, greenhouse and field screening of the four fungicides for their efficacy in reducing incidence and severity of ascochyta blight. The experiments were laid out in a completely randomized design (laboratory assays), completely randomized block design (greenhouse screening) and split-split plot design in the field. Data on incubation period, percent infection and germination in the laboratory and greenhouse trials and percent disease incidence (PDI) and disease severity index (DSI) in the field trials were taken and subjected to analysis of variance following PROC GLM procedure in SAS. Significant means at F-test separated using Tukey's test statistics at $P \leq 0.05$. Seed dressing with fungicides significantly increased percent germination by upto 14.73% and incubation period (days to development of first symptoms) by upto 6 days (for asymptomatic seeds) compared to control at $P \leq 0.05$. Foliar application of fungicides was more effective than seed dressing in reducing incidence and severity of ascochyta blight in all trials. The findings suggest that seed dressing may improve seed germination as well as reduce disease severity to compliment the more effective foliar spray application.

## INTRODUCTION

Ascochyta blight (AB), caused by *Ascochyta rabiei* (Pass.) Labrouse [(teleomorph: *Didymella rabiei* (Kovacheski)], is the most devastating fungal disease of chickpea globally (Akem *et al.,* 2004). The fungus *A. rabiei* infects all aerial parts of the plant producing characteristic necrotic lesions on stems, leaves and pods causing subsequent dropping of leaf tips and breakage of stems (Markell *et al.,* 2008). The fungus infections are favoured by temperatures of 5-30$^{o}$ C, with an optimum temperature of 20$^{o}$ C and relative humidity of > 80% (Shtienberg *et al.,* 2006). *A. rabei* survives either on or in seed or plant debris in form of mycelium, pycnidia, or various teleomorphic stages (Kaiser, 1997) and spreads via airborne spores. The sexual (teleomorph) state helps in long-term survival of the pathogen, but there is no work done in Kenya on the presence of this state that is known. Management of AB in most chickpea growing regions is mainly through fungicide sprays and growing of tolerant varieties. However, most commercial varieties grown in Kenya [LDT068, Chania Desi (1, 2 and 3), Saina K 1, Ngara Local and Annigeri] are susceptible to AB with mean disease scores above 5.5 (Kimurto *et al.,* 2013). Chickpea is traditionally sown on residual moisture after long rains (in rotation with cereals) in Kenya and other major chickpea growing areas of Africa (Ethiopia, Tanzania, Malawi), and as a consequence it experiences terminal drought during the growth period especially dry seasons/summer (Kimurto *et al.,* 2013). Similar conditions are experienced in Asia, North Africa and other regions with Mediterranean climate; where chickpea is sown in spring and growth period is in dry summers, resulting in poor biomass development and yield (Millan *et al*., 2006; Varshney *et al*., 2009). In both the sub-Sahara Africa and North Africa, sowing earlier during the long rainy season (winter) would reduce terminal drought stress, expand the vegetative growth period and improve the seed yield significantly upto 3 T ha$^{-1}$. However, this is rarely adopted by the farmers because the cool and wet weather, typical for long rainy seasons or Mediterranean winters, favours the development of AB epidemics as in most regions of the world where the crop is commonly grown (Kimurto *et al.,* 2013) like North America, Pakistan, Northwest India and Australia.

The production area under chickpea in Kenya has been declining steadily over the last decade from 51,772 ha in 2000 to only 190 ha in 2013. Similarly, yield per hectare has declined from 4.5 t/ha to 2.6 t/ha over the same period (FAOSTAT, 2014). However, recent efforts in breeding work have increased chickpea production to about 45-50,000 tonnes and area to 20,000

ha (Kimurto *et al*., 2013; ICRISAT, 2014). High yield losses experienced in Kenya as well as other chickpea growing regions of the world, which in most cases exceed 50%, are attributed to ascochyta blight (Barve *et al.,* 2003). Devastating ascochyta blight epidemics can be attributed to farmer's practice of recycling their own seed over the years. In Australia for example, 1998 and 1999 epidemics, as a result of growing highly susceptible cultivars with high seed infection in weather patterns favourable to disease spread caused reduction in area sown to chickpea from 95,000 ha in 1999 to less than 5500 ha in 2004 (Shtienberg *et al.,* 2006).

An integrated disease management (IDM) strategy would involve the combination of host resistance, cultural practices and fungicides applied as seed dressing or foliar sprays. Fungicide seed treatment, especially when used on seeds of low vigour or infected seed lot, remains the most effective means of increasing seedling emergence and delay early foliar infections reducing disease severity significantly (Mancini & Romanazzi, 2014). This can help increase farmers yields by reducing epidemics and reducing seed inocula especially since most farmers use their own seed (Shtienberg *et al.,* 2006). When seeds are grown for seed production or where good quality seed with a low fungal infection is required, it becomes even more important to dress seeds with fungicides as a means of eradicating or reducing seed borne pathogens. However, the vigilant use of seed-dressing and foliar fungicide is rarely practical for on-farm seed and grain production especially in intensive production areas and on susceptible cultivars (Shtienberg *et al*., 2006). Host resistance has been identified as the most economical option in management of AB and it remains a major objective in chickpea growing areas of the world (Akem *et al.,* 2004). However, pathotypes with greater virulence have been shown to cause disease regardless of the level of resistance (Cho and Muehlbauer, 2004; Gan *et al.,* 2006). This is due to the contribution of the sexual stage (teleomorph: *Didymella rabiei*) of the pathogen to emergence of new races or pathotypes (Barve *et al*., 2003; Bretag *et al*., 2006).

The aim of this study were; 1. To determine the efficacy of the fungicides foliar sprays (azoxystrobin, difenoconazole, azoxystrobin+difenoconazole and Metalaxyl-Mancozeb) in suppressing severity of ascochyta blight. 2. To determine the efficacy of seed dressing chickpea seeds with fungicides against control of ascochyta blight.

## MATERIALS AND METHODS

### 3.2 Determination of the level of infection in the seed

This experiment involved the evaluation of the efficacy of seed treatment before planting. This experiment was conducted both *in vitro* and *in vivo*. The *in vitro* experiment was carried out at the department of biological sciences laboratories at Egerton University, Njoro Kenya. Seeds used in this experiment were obtained from a lot that was previously affected by Ascochyta blight and evaluated for seed treatment efficacy as described by Wise *et al.*, (2009) with modifications. Seeds were sorted manually into two categories, asymptomatic and symptomatic seeds based on visual observations. Asymptomatic seeds were those with uniform size and colour without any discoloration, shriveling or lesions. Symptomatic seeds were those that had visible lesions of disease.

The seeds from both categories were evaluated under a completely randomized design (CRD) in the laboratory using the culture plate method (Haware *et al.*, 1986) to determine germination and *A. rabiei* infection levels. Seeds were surface disinfested by soaking in 2.5% sodium hypochlorite (NaOCl) solution for 2 min with constant agitation followed by a thorough rinse in sterile distilled water. Ten seeds per plate, for a total of 20 plates per seed category were placed onto potato dextrose agar (PDA) in petri dishes (100 × 15 mm) to which streptomycin sulphate 1g/L was added. Seeds were incubated at 20 ± 2 $^{0}$C in the dark for 10 days in a cycle of 12h light followed by 12 h darkness. Each seed was scored for germination and *A. rabiei* infection. A seed was considered germinated if the radicle was as long as the diameter of the seed. Seeds infected with *A. rabiei* were confirmed by examining conidia of symptomatic samples at 100× magnification under a microscope. The individual plates served as replicates (20 replicates total per seed category), and the experiment was repeated twice.

### 3.3 Pathogen isolation and disease inoculation

The pathogen for inoculating green house plants was isolated from infected leaves and stems of chickpea. The portions of the infected area were excised with a scapel and washed in water to remove dust and soil. The portions were then dipped in 2.5% NaOCl for 2 minutes and then into 70% ethanol for 30 seconds followed by a rinse in distilled water for 5 minutes with constant agitation. The portions were then placed on PDA containing antibiotics (streptomycin), at 1g/L, under a laminar flow using a sterile needle. The plates were incubated at 20$^{o}$C for 14 days 12 hr diurnal light. *A. rabiei* colonies were identified under a microscope using (100x) the

cellotape technique. Positively identified colonies were sub-cultured in PDA for multiplication of the pure culture. Pathogenicity tests were conducted by inoculating seedlings of chickpea in the greenhouse followed by observation for development of characteristic symptoms. The multiplied inocula were used to inoculate plants in the greenhouse for screening of the efficacy of seed treatment. The conidia on the resulting cultures were scrapped with a sterile scapel and washed with sterile distilled water. The suspension was standardized in a hymacytometer to create an initial concentration of $5x10^5$ spores/ml. This suspension was sprayed onto chickpea seedlings soon after emergence using a hand held atomizer in the evening. Experimental plots were monitored periodically starting at emergence to determine the time of disease onset and the first disease symptoms were recorded and analysed to determine significant differences in the incubation period between the different treatments.

**3.4 Determination of seedling emergence under green house conditions**

Seeds were treated with slurries of the fungicides in a zipper-seal type plastic bag and shaken vigorously for about 2 minutes for uniform coating. To compare emergence of plants and the development of ascochyta blight lesions on plants from the two batches i.e. asymptomatic and symptomatic seeds; a greenhouse trial was conducted. Ten chickpea seeds per pot were planted in plastic pots with potting mix and placed in a green house. The pots were watered as needed throughout the experiment. Each pot contained seeds of a single treatment (seed category by seed treatment). The pots were covered with a modified plastic mini-dome inverted upon pots so as to ensure uniform and high level of humidity. The four fungicides used in this experiment Azoxystrobin 250g/L, Difenoconazole 250g/L , Azoxystrobin 250g/L – Difenoconazole 125g/L and Metalaxyl-M 40g/kg – Mancozeb 640g/kg.

Plants were monitored daily for 30 days for the development of ascochyta blight lesions, and the number of days after planting (DAP) that a lesion was first observed was recorded. The number of emerged seedlings was determined after 30 days. A randomized complete block design was used for this experiment with seed category, genotype and fungicide treatments as the components of the model.

$Y_{ijkl} = \mu + \alpha_i + B_j + G_k + T_l + \varepsilon_{ijkl}$

Where; $\mu$ = overall mean

$\alpha_i$ = effect due $i^{th}$ seed category

$B_j$ = effect due to $j^{th}$ block

$G_k$= effect due to *k*th variety

$T_l$ = effect due *k*th fungicide treatment

$\varepsilon_{ijkl}$ = random error component

### 3.5 Determination of fungicide efficacy in management of AB in the field

#### 3.5.1 Site description

Two fungicide trials were conducted at two sites Egerton University, Njoro and ATC Koibatek in the 2013-2014 long rains season. ATC-Koibatek (latitude 1$^o$ 35' S, and longitude 36$^o$ 66' E) lies at altitude 1890m above sea level (a.s.l) in the agro-ecological zone upper midlands (UM4), with low agricultural potential. Average annual rainfall is 767 mm while mean temperatures ranges between 18.2-24.3$^o$C. The mean annual minimum and maximum temperature are 10.9$^o$C and 28.8$^o$C respectively. Soils are vitric andosols with moderate to high soil fertility, well drained deep to sandy loam soil (Jaetzold and Schimdt, 1983). Egerton University, Njoro (0$^o$ 23'S and 35$^o$ 35'E) lies at altitude 2265m a.s.l in lower highland (LH2-LH3) agro-ecological zone and has a sub-humid modified tropical climate. The annual average rainfall is 931mm and mean temperature ranges between 16-19.1$^o$C. The mean maximum and minimum temperature are 22.7$^o$C and 7.9$^o$C, respectively. Fields selected at both sites were known to have high inocula load of ascochyta blight since they had been under chickpea crop for more than three years.

#### 3.5.2 Test germplasm

A susceptible chickpea (*C. arientinum L.*) genotype Chania desi 1 (ICCV97105) was selected from a seed lot that was sorted and visually diseased seeds removed. Clean seeds were screened in a split-split plot design in the field. The main plot were the four types of fungicides, in the sub plot, the plot was left sprayed or not sprayed with either of the fungicides in the main plot four times during the growth period of the crop. In the sub-sub plot, the plot was left treated or untreated with either of the fungicide in the main-plot.

### 3.6 Fungicide treatments

**Table 1. Product names, active ingredients, manufacturers and standard rates of chemicals used in ascochyta blight control experiments carried out in Njoro and Koibatek, Kenya**

| Product name | Active ingredient | Manufacturer | Standard rate of Product |
|---|---|---|---|
| **Ortiva SC** | 250g/L azoxystrobin | Syngenta | 1.00 ml/L |
| **Amistar Top 325 SC** | Azoxystrobin 250g/L – Difenoconazole 125g/L | Syngenta | 1.00 ml/L |
| **Score 250 EC** | Difenoconazole 250g/L | Syngenta | 1.00 ml/L |
| **Ridomil Gold MZ 68 WG** | Metalaxyl-M 40g/kg – Mancozeb 640g/kg | Syngenta | 50.00 g/L |

Fungicides were applied at four stages of chickpea growth at seedling (22 DAE), vegetative (45 DAE), 50% flowering and 50 % podding. The plot size was measuring length 2.5m x width 2m with 0.5 m pathway between the sub-sub plots and 1m between main plots. The seeds were sown by hand in open furrows spaced 40cm apart at 10cm intra row spacing to give a plant population of 25 plants per row, five rows per plot. Fungicides were applied using a 20 L capacity manual backpack sprayer. The crop was exposed to natural inocula from the field.

### 3.7 Data collection

#### 3.7.1 Disease assessment

Data on severity was collected by scoring the percentage of damage on individual plant using the 1-9 scale as described by Jan and Wiese, (1991), Chen and Muehlbauer, (2003) and Pande *et al*., (2011), where 1=no visible symptoms; 2=minute lesions prominent on the apical stems; 3=lesions up to 5-10 mm in size and slight drooping of apical stems; 4=lesions obvious on all plant parts and clear drooping of apical stems; 5=lesions on all plants parts, defoliation initiated, breaking and drying of branches slight to moderate; 6=lesions as in 5, defoliation, broken, dry branches common, some plants killed; 7=lesions as in 5, defoliation, broken, dry branches very common, up to 25% of plants killed; 8= symptoms as in 7 but up to 50% of the plants killed and 9=symptoms as in 7 but up to 100% of the plants killed. A total of 75 plants in three middle rows were scored for severity in each plot. Disease severity index (DSI) similar to that one used by Montaser (2011) was calculated for each plot as follows.

DSI= Σd/ (d **max** X n) x 100

Where: d is the disease rating of each plant

d max is the maximum disease rating and

n is the total number of plants examined in each plot.

### 3.7.2 Area under disease progress curve

The disease severity index assessments at each interval of recording (every 14 days) were used to calculate the area under disease progress curve (AUDPC) for each plot as per the equation suggested by (Wilcoxson *et al*., 1975) as follows.

$$AUDPC = 1/2 \sum_{i=1}^{N} [(Y_{i+1} + Y_i)(T_{i+1} - T_i)]$$

Where, $Y_i$ = the proportion of the host tissue damaged at $i^{th}$ observation

$T_i$ = the time (day) after appearance of the disease at $i^{th}$ observation

$N$ = the total number of observations

### 3.7.3 Percentage disease incidence

Percentage disease incidence (PDI) was measured based on the number of diseased plants per plot in three middle rows. Percent incidence was calculated as the ratio of the diseased plant to the total plant multiplied by 100%. The following formula was used to calculate the disease incidence of each plot as adopted from Amadioha (2003).

$$PDI = ((\text{No of diseased plants})/(\text{Total no of plants})) \times 100\%.$$

### 3.8 Data Analysis

Data analyses were conducted using SAS 9.1.3 data analysis software. Pair-wise testing between means was done using the PROC GLM procedure and significant means at F-test separated using Tukey's test statistics at $P \leq 0.05$. The following model was used for experimental analyses.

$$Y_{ijklm} = \mu + E_i + B_{j(i)} + T_k + ET_{ik} + S_l + ES_{il} + ETS_{ikl} + \varepsilon_{m(ijklm)}$$

Where μ= overall mean

$E_i$ = effect due to $i^{th}$ location/environment

$B_{k(ij)}$ = effect due to $j^{th}$ block within $i^{th}$ environment

$T_k$ = effect due to $k^{th}$ fungicide

$ET_{il}$ = effect due to interaction of $i^{th}$ environment and $k^{th}$ fungicide

$ET_{ik}$ = effect due to $i^{th}$ environment and $k^{th}$ fungicide

$S_l$= effect due to $l^{th}$ spray application

$ES_{il}$ = effect due to $i^{th}$ environment and $l^{th}$ spray application

$ETS_{ikl}$ = effect due to $i^{th}$ location $k^{th}$ fungicide and $l^{th}$ spray application

$\varepsilon_{m(ijklm)}$ = random error component

### 3.9 Results

#### 3.9.1 Determination of infection level and emergence

There was a significant difference in percentage infection and emergence between asymptomatic and symptomatic seed lots. The asymptomatic seed lot used in the first season (trial 1, Table 2) indicated upto 11.5% infection compared to 64.5% infection for the symptomatic category. In the second season (trial 2, Table 2) seed lot, the asymptomatic seed lot showed 20% infection while the symptomatic seed lot indicated 96.5% infection. Preliminary germination tests conducted in the laboratory in each season indicated there was significance difference in emergence between asymptomatic and symptomatic seed categories. Seed from the asymptomatic category showed 61.5% germination compared to 32.5% in the symptomatic category in season one (seeds from the first lot). In the second season (seeds from the second lot), emergence in the asymptomatic seed lot was relatively higher at 84.5%. However, in the symptomatic category, emergence decreased to 15.5% (Table 2). Seeds from first and second lots were sourced from different locations and were therefore analysed separately.

**Table 2: Percent seed infection and emergence of two seed categories, asymptomatic and symptomatic, in the laboratory**

| Seed Category | Trial | %Infection †† | %Emergence†† |
|---|---|---|---|
| Asymptomatic | First Seedlot | 11.5±2.6 | 61.5±4.1 |
| | Second seedlot | 20±3.7 | 84.5±2.5 |
| Symptomatic | First Seedlot | 64.5±2.6 | 32.5±3.4 |
| | Second seedlot | 96.5±1.5 | 15.5±2.8 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.2 Effect of fungicide seed treatment on emergence and incubation period of ascochyta blight

Treatment of seeds with fungicides had a high significant effect on percent germination and incubation period (days to development of first symptoms) compared to control at $P \leq 0.05$ in experiments conducted under greenhouse conditions. The highest percent emergence under the asymptomatic category was observed under seeds treated with azoxystrobin+difenoconazole and azoxystrobin which had 65.6% and 64.4% respectively in the greenhouse (Table 3). However, the percentage emergence under metalaxyl+mancozeb was as high (61.1%, Table3) and not significantly different from either fungicide. The lowest percent emergence under asymptomatic category, in the greenhouse, was under difenoconazole at 58.9%. Percentage emergence in symptomatic seeds was significantly lower compared to the asymptomatic ones for each individual treatment. The highest emergence was achieved under azoxystrobin+difenoconazole (53.3%) while the lowest was under metalaxyl+mancozeb (35.6%).

The days to first symptoms were significant at $P \leq 0.05$ for asymptomatic seeds treated with any of the four fungicides used in this experiment (Table 3). Results from this experiment indicated that it took significantly longer period (days) for first symptoms to appear under treated seeds compared to control. Incubation period in the symptomatic seeds was significantly lower in comparison to the asymptomatic category. The longest incubation period under this category was 6.56 days under seeds treated with azoxystrobin+difenoconazole.

**Plate 2: Arrangement of treatments in the greenhouse, partly shown is the polythene dome for modification of humidity and polythene covering (top left) immediately after spraying the suspension containing *A. rabiei*. Source, Author.**

**Table 3: Mean percent emergence and incubation period of two seed categories, asymptomatic and symptomatic for selected fungicides in the greenhouse**

| Treatment | Seed Category | Percent Emergence†† | Incubation (days) †† |
|---|---|---|---|
| Azoxystrobin + Difenoconazole | Asymptomatic | 65.6±5.6 | 18.0±0.8 |
| | Symptomatic | 53.3±3.7 | 6.56±0.4 |
| Azoxystrobin | Asymptomatic | 64.4±3.4 | 19.44±0.9 |
| | Symptomatic | 44.4±2.9 | 5.4±0.5 |
| Difenoconazole | Asymptomatic | 58.9±5.1 | 18.1±1.1 |
| | Symptomatic | 44.4±4.8 | 6.4±0.4 |
| Metalaxyl + Mancozeb | Asymptomatic | 61.1±4.6 | 19.4±1.2 |
| | Symptomatic | 35.6±6.9 | 5.6±0.5 |
| Control | Asymptomatic | 50.83±3.8 | 12.6±0.5 |
| | Symptomatic | 32.5±5.1 | 4.7±0.3 |

†† The values are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$).

**Plate 3: Two seed categories, symptomatic seeds on the left and asymptomatic seeds on the right. Source, Author.**

**Table 4: Effect of fungicide seed treatment on percent emergence of chickpea seeds in the field.**

| Fungicide | Seed Treatment | % Emergence†† |
|---|---|---|
| Azoxystrobin | Dressed | 67.1±2.2 |
| | Not dressed (Control) | 48.2±3.5 |
| Difenoconazole | Dressed | 57.3±4.2 |
| | Not dressed (Control) | 50.6±4.2 |
| Azoxystrobin + Difenoconazole | Dressed | 68.1±4.4 |
| | Not dressed (Control) | 52.1±3.9 |
| Metalaxyl + Mancozeb | Dressed | 53.7±5.1 |
| | Not dressed (Control) | 46.3±3.9 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

Dressing of seeds with any of the four fungicides in the field had a significant increase in percent emergence compared to undressed seeds (Table 4). Seed dressing with azoxystrobin+difenoconazole showed the highest percent emergence (68.1%) which was not significantly different from azoxystrobin (67.1%). The lowest fungicide effect in the field was metalaxyl+mancozeb (53.7%) that did not vary significantly from dressing with difenoconazole (57.3%).

### 3.9.3 Effect of fungicides on percentage disease incidence (PDI) in the field

The percent disease incidence (PDI) was significantly higher at Njoro than in Koibatek in the season 2013 at $P \leq 0.05$. The PDI means for Njoro showed as high as 73.8% incidence compared to the highest at Koibatek 43.1% (Table 5). The effectiveness of spraying schedule was higher in Koibatek than Njoro. The mean PDI under sprayed treatments for all fungicides at Koibatek was 24.9% which was significantly different from control treatment means at 41.5% (Table 5). In Njoro however, PDI was higher with sprayed treatments showing a mean of 62.9%. Nevertheless, this mean was significantly lower compared to control treatments that were not sprayed (72.9%). Seed dressing had a significant effect on reducing PDI at both sites. The effect due to dressing seeds with fungicides had a similar response to the spray schedule with Koibatek showing the largest difference between dressed and undressed seeds. The percent disease incidence increased from seedling to podding at both sites. PDI at Njoro increased from 4.2% at seedling to 100% at podding compared to 4.2% to 62.8% in the same period.

**Plate 4: Field trial showing varying levels of AB severity depending of control efficacy at Field 7, Egerton University Njoro. Source, Author.**

**Table 5: Effect of fungicide spray, spray schedule, seed treatment and stage of growth on percentage disease incidence (PDI) of ascochyta blight of chickpea in 2013**

| | Percent Disease Incidence (PDI) | |
|---|---|---|
| **Fungicide** | **Njoro**†† | **Koibatek** †† |
| Azoxystrobin | 65.4±5.0 | 27.9±3.4 |
| Difenoconazole | 68.3±5.0 | 32.7±3.6 |
| Azoxystrobin+Difenoconazole | 64.0±4.9 | 29.4±3.3 |
| Metalaxyl+Mancozeb | 73.8±4.8 | 43.1±4.1 |
| **Spray Schedule** | | |
| Not sprayed (Control) | 72.9±3.4 | 41.5±2.8 |
| Sprayed | 62.9±3.5 | 24.9±2.1 |
| **Seed Treatment** | | |
| Dressed | 64.9±3.6 | 28.9±2.5 |
| Not dressed (Control) | 70.8±3.4 | 37.6±2.7 |
| **Stage** | | |
| Early vegetative | 52.7±2.8 | 11.7±1.5 |
| Flowering | 97.6±0.9 | 51.2±3.3 |
| Late vegetative | 85.1±2.8 | 36.1±2.6 |
| Podding | 100±0 | 62.8±3.5 |
| Seedling | 4.2±0.5 | 4.4±0.7 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.4 Effect of seed dressing and spray combination on percent disease Incidence (PDI) in the field

There was no significant difference between dressed and none dressed treatments for all fungicides (Table 6). However, there was significantly low PDI under treatments sprayed with azoxystrobin+difenoconazole and azoxystrobin (53.0% and 50.8% for dressed treatments respectively), compared to none sprayed treatments at $P \leq 0.05$. The superimposed effect of seed dressing followed by the spray schedule was mostly non-significant at Njoro except where azoxystrobin, seed dressing plus foliar spray, was applied (50.8% dressed and 63.9% none dressed) (Table 6).

**Table 6: Effect of the combination of fungicide sprays and seed treatment on percentage disease index of ascochyta blight at Njoro and Koibatek in 2013**

| Fungicide | Spray Schedule | Seed Treatment | Percent Disease Incidence (PDI) | |
|---|---|---|---|---|
| | | | Njoro†† | Koibatek†† |
| Azoxystrobin + Difenoconazole | Control | Dressed | 69.1±10.2 | 31.39±7.7 |
| | | Control | 71.5±9.9 | 46.21±7.6 |
| | Sprayed | Dressed | 53.0±9.9 | 19.47±3.8 |
| | | Control | 62.4±9.6 | 20.55±3.9 |
| Azoxystrobin | Control | Dressed | 71.1±10.4 | 32.07±7.8 |
| | | Control | 75.9±9.7 | 47.58±8.3 |
| | Sprayed | Dressed | 50.8±10.1 | 13.74±2.9 |
| | | Control | 63.9±9.6 | 18.03±3.3 |
| Difenoconazole | Control | Dressed | 69.9±10.2 | 34.82±7.5 |
| | | Control | 72.5±9.9 | 49.78±8.7 |
| | Sprayed | Dressed | 60.8±10.7 | 19.78±4.2 |
| | | Control | 69.9±9.9 | 26.24±5.3 |
| Metalaxyl + Mancozeb | Control | Dressed | 76.1±10.1 | 40.66±8.2 |
| | | Control | 77.3±9.1 | 49.72±7.9 |
| | Sprayed | Dressed | 68.9±10.7 | 39.41±8.8 |
| | | Control | 72.9±9.7 | 42.43±8.4 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

Application of azoxystrobin foliar spray on seed dressed treatments significantly reduced disease incidence by 25.2 % compared to control and by 12% compared to spraying alone. The mean PDI reduced by 18.5%, 11.7% and 8.4% for azoxystrobin+difenoconazole, difenoconazole and mancozeb+metalaxyl respectively for the same treatment compared to control in Njoro.

The effect of seed dressing alone at Koibatek was significant compared to non dressed treatments for all fungicides. Seed dressing with azoxystrobin had the highest reduction in PDI (15.5%) against control as compared to dressing with any of the other three fungicides. The effect of spraying alone was highly significant compared to none sprayed treatments at Koibatek for all fungicides except metalaxyl+mancozeb. The effect of combining seed dressing and foliar sprays was significant for azoxystrobin and difenoconazole fungicides at $P \leq 0.05$. Seed dressing followed by foliar spray with azoxystrobin reduced PDI at Koibatek by a margin of 33.8% compared to control. This is in comparison to 30%, 26.7% and 10.3% margin reduction against

controls for difenoconazole, azoxystrobin+difenoconazole and mancozeb+metalaxyl at the same location.

### 3.9.5 Effect of fungicide spray schedule on PDI at different stages of chickpea growth

The PDI means at seedling, early vegetative, late vegetative and flowering were significantly different from controls for both locations at $P \leq 0.05$ (Table 7). At seedling, only treatments sprayed with metalaxyl+mancozeb at Njoro had significantly different mean PDI from control treatments. The mean PDI of sprayed treatments at Njoro were significantly different from controls at early vegetative, late vegetative and flowering stages for all fungicides used. At podding however mean PDI for all sprayed treatments among the four fungicides had reached 100% and there was no significant difference from controls. At Koibatek however, the mean PDI for azoxystrobin+difenoconazole, azoxystrobin and difenoconazole at early vegetative (5.7%, 6.1% and 8.9% respectively), late vegetative (20.8%, 14.0% and 22.2% respectively), and flowering (29.9%, 25.0% and 34.1% respectively) through podding (38.9%, 25.0% and 34.1% respectively) were significantly different from control treatments at $P \leq 0.05$. Azoxystrobin was most efficacious in reducing PDI at early vegetative and flowering stages at Njoro with a mean difference of 33.1% and 10.1% against control at the two growth stages respectively. However, the mean PDI was not significantly different from azoxystrobin+difenoconazole at early vegetative and flowering (35.9% and 91.10% respectively). Similarly at Koibatek, azoxystrobin was more efficacious in suppressing incidence (at all stages of growth except early vegetative) followed by azoxystrobin+difenoconazole and difenoconazole in that order. There was a reduction of 6.3%, 37.7%, 26.8%, 47.9% and 1.1% in mean PDI at early vegetative, flowering, late vegetative, podding and seedling stages for the fungicide azoxystrobin against the controls in Koibatek.

The application of metalaxyl+mancozeb at Koibatek was significant from controls seedling to late vegetative stages. This efficacy was low compared to that achieved by azoxystrobin, difenoconazole and the combination of the two fungicides. This response was also observed at Njoro where the mean PDI of Metalaxyl+Mancozeb were significantly higher compared to azoxystrobin, difenoconazole and azoxystrobin+difenoconazole from early vegetative through flowering (53.8%, 96.1% and 100%). At late vegetative stage, azoxystrobin+difenoconazole in Njoro had the least mean PDI (58.2%) though it was not significantly different from mean PDI of azoxystrobin (61.3%).

**Table 7: Effect of fungicide spray schedule and stage of application on PDI at Njoro and Koibatek stations in 2013.**

| Fungicide | Spray Schedule | Stage | Percent Disease Incidence (PDI) | |
|---|---|---|---|---|
| | | | Njoro†† | Koibatek†† |
| Azoxystrobin | control | Seedling | 3.8±1.3 | 4.3±2.1 |
| Azoxystrobin | control | Early vegetative | 65.2±5.6 | 12.3±5.8 |
| Azoxystrobin | control | Late vegetative | 98.7±0.6 | 40.8±5.9 |
| Azoxystrobin | control | Flowering | 100.0±0.0 | 62.7±5.3 |
| Azoxystrobin | control | Podding | 100.0±0.0 | 78.9±6.8 |
| Azoxystrobin | Sprayed | Seedling | 3.7±1.4 | 3.3±1.0 |
| Azoxystrobin | Sprayed | Early vegetative | 32.0±6.5 | 6.1±1.8 |
| Azoxystrobin | Sprayed | Late vegetative | 61.3±7.9 | 14.0±2.0 |
| Azoxystrobin | Sprayed | Flowering | 89.9±4.2 | 25.0±3.5 |
| Azoxystrobin | Sprayed | Podding | 100.0±0.0 | 31.1±1.9 |
| Azoxystrobin + Difenoconazole | control | Seedling | 3.2±1.2 | 4.6±2.2 |
| Azoxystrobin + Difenoconazole | control | Early vegetative | 60.2±7.5 | 13.6±4.7 |
| Azoxystrobin + Difenoconazole | control | Late vegetative | 88.1±5.4 | 43.3±6.4 |
| Azoxystrobin + Difenoconazole | control | Flowering | 100.0±0.0 | 56.7±9.4 |
| Azoxystrobin + Difenoconazole | control | Podding | 100.0±0.0 | 75.7±6.5 |
| Azoxystrobin + Difenoconazole | Sprayed | Seedling | 3.4±1.4 | 4.7±2.3 |
| Azoxystrobin + Difenoconazole | Sprayed | Early vegetative | 35.9±2.7 | 5.7±0.7 |
| Azoxystrobin + Difenoconazole | Sprayed | Late vegetative | 58.2±7.6 | 20.8±3.4 |
| Azoxystrobin + Difenoconazole | Sprayed | Flowering | 91.1±3.1 | 29.9±2.6 |
| Azoxystrobin + Difenoconazole | Sprayed | Podding | 100.0±0.0 | 38.9±2.8 |
| Difenoconazole | control | Seedling | 3.9±1.1 | 4.2±2.2 |
| Difenoconazole | control | Early vegetative | 56.6±1.8 | 14.7±4.4 |
| Difenoconazole | control | Late vegetative | 95.7±4.1 | 50.2±5.7 |
| Difenoconazole | control | Flowering | 100.0±0.0 | 63.7±6.9 |
| Difenoconazole | control | Podding | 100.0±0.0 | 78.8±7.6 |
| Difenoconazole | Sprayed | Seedling | 2.7±1.3 | 4.7±2.3 |
| Difenoconazole | Sprayed | Early vegetative | 42.2±7.9 | 8.9±1.6 |
| Difenoconazole | Sprayed | Late vegetative | 82.7±5.7 | 22.2±5.6 |
| Difenoconazole | Sprayed | Flowering | 99.5±0.4 | 34.1±5.9 |
| Difenoconazole | Sprayed | Podding | 100.0±0.0 | 45.2±4.6 |
| Metalaxyl+Mancozeb | control | Seedling | 7.9±2.1 | 7.1±2.6 |
| Metalaxyl+Mancozeb | control | Early vegetative | 5.5±3.7 | 19.2±6.5 |
| Metalaxyl+Mancozeb | control | Late vegetative | 100.0±0.0 | 54.2±5.5 |
| Metalaxyl+Mancozeb | control | Flowering | 100.0±0.0 | 69.6±7.3 |
| Metalaxyl+Mancozeb | control | Podding | 100.0±0.0 | 75.9±6.3 |
| Metalaxyl+Mancozeb | Sprayed | Seedling | 4.5±1.6 | 1.9±0.7 |
| Metalaxyl+Mancozeb | Sprayed | Early vegetative | 53.8±6.4 | 13.5±3.2 |
| Metalaxyl+Mancozeb | Sprayed | Late vegetative | 96.1±2.5 | 43.7±4.7 |
| Metalaxyl+Mancozeb | Sprayed | Flowering | 100.0±0.0 | 67.9±9.3 |
| Metalaxyl+Mancozeb | Sprayed | Podding | 100.0±0.0 | 77.6±7.1 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.6 Effect of fungicide seed treatment on percent disease incidence at different stages of growth

The effect of seed dressing on PDI was more pronounced at seedling stage. The mean PDI for all fungicides used at both locations were significantly different from means of control treatments (none dressed) at seedling and early vegetative stages at $P \leq 0.05$ (Table 8). The mean PDI at seedling stage reduced by 4.4%, 4.7%, 4.6% and 7.5% for fungicides azoxystrobin+difenoconazole, azoxystrobin, difenoconazole and metalaxyl+mancozeb respectively in Njoro and 2.1%, 3.2%, 6.9% and 6.1% respectively in Koibatek. Subsequently, seed dressing with metalaxyl+mancozeb recorded the highest reduction of mean PDI at seedling stage at both locations Njoro and Koibatek. However, the response beyond early flowering was varied with some fungicides showing significant differences while others did not. Azoxystrobin indicated the highest efficacy in seed dressing with significant differences of PDI means of seed dressed treatments against controls at all stages of growth (except at podding) in both locations. Metalaxyl+Mancozeb had the least efficacy in seed dressing with only significant differences shown at seedling stage in Njoro and at seedling and vegetative stages in Koibatek.

**Table 8: Effect of seed treatment on percentage disease incidence at different stages of growth of chickpea in 2013.**

| Fungicide | Seed Treatment | Stage | Percent Disease Incidence (PDI) | |
|---|---|---|---|---|
| | | | Njoro†† | Koibatek†† |
| Azoxystrobin | control | Seedling | 6.1±0.9 | 5.4±1.8 |
| Azoxystrobin | control | Early vegetative | 58.5±7.8 | 15.2±5.1 |
| Azoxystrobin | control | Late vegetative | 87.4±6.5 | 34.7±8.5 |
| Azoxystrobin | control | Flowering | 97.9±2.0 | 47.3±9.4 |
| Azoxystrobin | control | Podding | 100.0±0.0 | 61.4±12.9 |
| Azoxystrobin | Seed Dressed | Seedling | 1.4±0.6 | 2.2±1.1 |
| Azoxystrobin | Seed Dressed | Early vegetative | 38.7±9.2 | 3.2±0.7 |
| Azoxystrobin | Seed Dressed | Late vegetative | 72.6±11.7 | 20.2±4.3 |
| Azoxystrobin | Seed Dressed | Flowering | 91.8±4.4 | 40.4±9.5 |
| Azoxystrobin | Seed Dressed | Podding | 100.0±0.0 | 48.6±9.9 |
| Azoxystrobin+Difenoconazole | control | Seedling | 5.5±1.1 | 5.7±1.9 |
| Azoxystrobin+Difenoconazole | control | Early vegetative | 48.3±6.3 | 13.9±4.5 |
| Azoxystrobin+Difenoconazole | control | Late vegetative | 83.8±5.2 | 40.7±7.5 |
| Azoxystrobin+Difenoconazole | control | Flowering | 97.1±1.8 | 47.4±8.9 |
| Azoxystrobin+Difenoconazole | control | Podding | 100.0±0.0 | 59.3±10.7 |
| Azoxystrobin+Difenoconazole | Seed Dressed | Seedling | 1.1±0.3 | 3.6±2.4 |
| Azoxystrobin+Difenoconazole | Seed Dressed | Early vegetative | 47.8±9.1 | 5.4±0.8 |
| Azoxystrobin+Difenoconazole | Seed Dressed | Late vegetative | 62.6±10.2 | 23.5±4.2 |
| Azoxystrobin+Difenoconazole | Seed Dressed | Flowering | 93.9±3.6 | 39.2±9.1 |
| Azoxystrobin+Difenoconazole | Seed Dressed | Podding | 100.0±0.0 | 55.4±8.3 |
| Difenoconazole | control | Seedling | 5.6±0.9 | 7.9±2.3 |
| Difenoconazole | control | Early vegetative | 55.7±5.2 | 15.3±4.2 |
| Difenoconazole | control | Late vegetative | 95.1±3.6 | 41.0±9.7 |
| Difenoconazole | control | Flowering | 99.8±0.1 | 56.6±9.2 |
| Difenoconazole | control | Podding | 100.0±0.0 | 69.3±10.2 |
| Difenoconazole | Seed Dressed | Seedling | 1.0±0.4 | 1.0±0.0 |
| Difenoconazole | Seed Dressed | Early vegetative | 43.1±6.7 | 8.2±1.6 |
| Difenoconazole | Seed Dressed | Late vegetative | 83.3±6.3 | 31.3±6.1 |
| Difenoconazole | Seed Dressed | Flowering | 99.6±0.4 | 41.3±7.9 |
| Difenoconazole | Seed Dressed | Podding | 100.0±0.0 | 54.7±8.2 |
| Metalaxyl+Mancozeb | control | Seedling | 9.9±1.3 | 7.6±2.5 |
| Metalaxyl+Mancozeb | control | Early vegetative | 66.7±4.0 | 20.9±5.9 |
| Metalaxyl+Mancozeb | control | Late vegetative | 98.8±1.2 | 52.3±4.2 |
| Metalaxyl+Mancozeb | control | Flowering | 100.0±0.0 | 71.0±8.3 |
| Metalaxyl+Mancozeb | control | Podding | 100.0±0.0 | 78.5±6.8 |
| Metalaxyl+Mancozeb | Seed Dressed | Seedling | 2.5±1.1 | 1.5±0.3 |
| Metalaxyl+Mancozeb | Seed Dressed | Early vegetative | 62.6±9.2 | 11.7±3.5 |
| Metalaxyl+Mancozeb | Seed Dressed | Late vegetative | 97.3±2.4 | 45.5±6.4 |
| Metalaxyl+Mancozeb | Seed Dressed | Flowering | 100.0±0.0 | 66.5±8.3 |
| Metalaxyl+Mancozeb | Seed Dressed | Podding | 100.0±0.0 | 75.0±6.5 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.7 Effect of fungicides on disease severity index (DSI) and area under disease progress curve (AUDPC) in the field

**Table 9: Effect of fungicides on disease severity index of ascochyta blight at Njoro and Koibatek in 2013.**

| | Disease Severity Index (DSI) | |
|---|---|---|
| **Fungicide** | **Njoro**†† | **Koibatek**†† |
| Azoxystrobin+Difenoconazole | 40.3±2.2 | 28.7±1.9 |
| Azoxystrobin | 37.1±2.3 | 25.8±1.9 |
| Difenoconazole | 37.9±2.4 | 26.6±2.2 |
| Metalaxyl+Mancozeb | 44.9±2.8 | 33.9±2.5 |
| **Spray Schedule** | | |
| Not Sprayed (Control) | 45.5±1.9 | 31.1±1.7 |
| Sprayed | 34.6±1.4 | 26.3±1.4 |
| **Seed Treatment** | | |
| Dressed | 38.6±1.7 | 27.0±1.5 |
| Not Dressed (Control) | 41.5±1.7 | 30.5±1.6 |
| **Stage** | | |
| Early vegetative | 28.2±1.0 | 23.9±1.7 |
| Flowering | 54.9±2.8 | 36.1±1.4 |
| Late vegetative | 40.4±1.4 | 31.2±1.4 |
| Podding | 63.3±2.7 | 44.5±1.9 |
| Seedling | 13.5±1.6 | 7.9±1.7 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

Disease severity index (DSI) was significantly higher in Njoro than Koibatek in season one (2013). The foliar spray application schedule indicated significant differences ($P \leq 0.05$) compared to controls at both sites with DSI means of 34.6% in Njoro and 26.3% Koibatek for the sprayed treatments compared to 45.5% and 31.1% in none sprayed (control) treatments respectively (Table 9). Seed dressing application was also significant at both locations with Njoro having 41.5% under none dressed and 38.64% dressed DSI and Koibatek having 30.5% and 27% none dressed and dressed treatments respectively. Severity was highest (63.3% Njoro and 44.5% Koibatek) at podding in both locations and lowest at seedling stage (13.5% Njoro and 7.9% Koibatek).

### 3.9.8 Effect of following seed treatment with fungicide sprays on disease severity index (DSI)

There was significant difference in DSI between foliar sprayed and none sprayed treatments. Seed dressing as well as combination of spraying and seed dressing was also significant ($P \leq 0.05$) (Table 10). Azoxystrobin+difenoconazole, azoxystrobin and difenoconazole had significantly low DSI (37.3%, 29.5% and 32.3% respectively) compared to their respective controls (47.7%, 49.3% and 43.2% respectively) while metalaxyl+mancozeb did not show significant difference compared to control (45.4% sprayed and 47.3% none sprayed) when applied as foliar sprays in Njoro. Foliar sprays alone with azoxystrobin+difenoconazole, azoxystrobin, difenoconazole and metalaxyl+mancozeb reduced the mean disease severity by 10.5%, 19.8%, 10.9% and 1.9% respectively compared to control treatments in Njoro. In Koibatek however, only azoxystrobin and difenoconazole indicated such significant difference. The effect of seed dressing was only significant for azoxystrobin (39.8%) compared to control (49.3%) in Njoro and 24.4% and compared to control (35.4%) in Koibatek. Seed dressing with azoxystrobin+difenoconazole and metalaxyl+mancozeb followed by foliar spray application had significantly lower DSI (31.9% and 39.7% respectively) compared to seed dressing alone (44.22% and 47.43% respectively) or spraying alone (37.9% and 45.4%) in Njoro. Subsequently, following seed dressing with foliar sprays with the fungicides azoxystrobin+difenoconazole, azoxystrobin difenoconazole and metalaxyl+mancozeb reduced mean disease severity by 15.8%, 19.4%, 12.2% and 7.7% respectively compared to controls in Njoro.

Following seed dressing with foliar sprays at Koibatek showed significantly low DSI compared to seed dressing alone. Azoxystrobin in this case yielded the lowest (21.5%) DSI followed by difenoconazole (23.1%) though it was not significantly different from azoxystrobin+difenoconazole and difenoconazole, while metalaxyl+mancozeb had the highest (30.5%) in this category. The mean DSI for the fungicides azoxystrobin+difenoconazole, azoxystrobin difenoconazole and metalaxyl+mancozeb reduced by 7.4%, 13.9%, 7.3% and 4.4% respectively compared to controls in Koibatek.

**Table 10: Effect of combining fungicide spray and seed treatment on disease severity index at Njoro and Koibatek in 2013.**

| Fungicide | Spray Treatment | Seed Treatment | Disease Severity Index (DSI) | |
|---|---|---|---|---|
| | | | Njoro†† | Koibatek†† |
| Azoxystrobin+Difenoconazole | control | Seed Dressed | 44.2±4.7 | 31.5±3.8 |
| Azoxystrobin+Difenoconazole | control | control | 47.7±5.9 | 31.7±5.1 |
| Azoxystrobin+Difenoconazole | Sprayed | Seed Dressed | 31.9±2.9 | 24.3±2.8 |
| Azoxystrobin+Difenoconazole | Sprayed | control | 37.3±3.4 | 27.2±3.2 |
| Azoxystrobin | control | Seed Dressed | 39.8±5.5 | 24.4±4.5 |
| Azoxystrobin | control | control | 49.3±5.6 | 35.4±4.7 |
| Azoxystrobin | Sprayed | Seed Dressed | 29.9±2.8 | 21.5±2.6 |
| Azoxystrobin | Sprayed | control | 29.5±2.9 | 21.8±2.6 |
| Difenoconazole | control | Seed Dressed | 45.0±5.7 | 28.5±4.3 |
| Difenoconazole | control | control | 43.2±5.6 | 30.4±4.7 |
| Difenoconazole | Sprayed | Seed Dressed | 31.1±3.5 | 23.1±4.3 |
| Difenoconazole | Sprayed | control | 32.3±3.3 | 24.3±4.4 |
| Metalaxyl+Mancozeb | control | Seed Dressed | 47.4±6.2 | 32.2±5.2 |
| Metalaxyl+Mancozeb | control | control | 47.3±5.9 | 34.9±5.8 |
| Metalaxyl+Mancozeb | Sprayed | Seed Dressed | 39.7±5.6 | 30.5±4.9 |
| Metalaxyl+Mancozeb | Sprayed | control | 45.4±4.9 | 37.8±3.9 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.9 Effect of fungicide sprays at different stages of chickpea growth

The effect of spraying with azoxystrobin+difenoconazole and difenoconazole on AB severity was significantly different from control ($P \leq 0.05$) at all stages except at seedling in Njoro and Koibatek (Table 11). Azoxystrobin on the other did not show significant difference at both seedling and early vegetative compared to control. The efficacy of metalaxyl+mancozeb was not consistent across the locations with significant differences from controls at flowering and late vegetative in Njoro and early vegetative and podding in Koibatek. The most efficacious fungicide was azoxystrobin which had the lowest DSI means at flowering (33.0% and 23.5%), late vegetative (33.6% and 21.0%) and podding stages (40.5% and 26.5%) in Njoro and Koibatek respectively. Metalaxyl+Mancozeb had lowest DSI at seedling (8.6% and 7.9% in Njoro and Koibatek respectively) while difenoconazole had lowest means at early vegetative (22.5% and 14.4%) in Njoro and Koibatek respectively.

**Table 11: Effect of fungicide sprays at different stages of chickpea growth at Njoro and Koibatek in 2013.**

| Fungicide | Spray Schedule | Stage | Disease Severity Index (DSI) | |
|---|---|---|---|---|
| | | | Njoro†† | Koibatek†† |
| Azoxystrobin | control | Seedling | 18.4±6.7 | 8.0±5.1 |
| Azoxystrobin | control | Early Vegetative | 25.9±3.7 | 20.9±6.8 |
| Azoxystrobin | control | Late Vegetative | 43.2±3.6 | 33.0±3.7 |
| Azoxystrobin | control | Flowering | 61.1±8.6 | 39.2±4.1 |
| Azoxystrobin | control | Podding | 74.2±8.1 | 48.4±4.7 |
| Azoxystrobin | Sprayed | Seedling | 12.7±3.9 | 12.4±5.6 |
| Azoxystrobin | Sprayed | Early Vegetative | 28.8±1.6 | 24.9±1.6 |
| Azoxystrobin | Sprayed | Late Vegetative | 33.6±4.0 | 21.0±1.1 |
| Azoxystrobin | Sprayed | Flowering | 33.0±3.6 | 23.5±3.2 |
| Azoxystrobin | Sprayed | Podding | 40.5±4.8 | 26.5±4.8 |
| Azoxystrobin+Difenoconazole | control | Seedling | 17.6±5.6 | 5.6±5.6 |
| Azoxystrobin+Difenoconazole | control | Early Vegetative | 32.8±1.4 | 31.7±2.7 |
| Azoxystrobin+Difenoconazole | control | Late Vegetative | 43.6±3.4 | 33.2±2.9 |
| Azoxystrobin+Difenoconazole | control | Flowering | 64.2±9.4 | 36.7±3.5 |
| Azoxystrobin+Difenoconazole | control | Podding | 71.8±7.4 | 50.9±3.0 |
| Azoxystrobin+Difenoconazole | Sprayed | Seedling | 18.2±4.3 | 11.5±5.2 |
| Azoxystrobin+Difenoconazole | Sprayed | Early Vegetative | 28.3±2.8 | 24.1±5.1 |
| Azoxystrobin+Difenoconazole | Sprayed | Late Vegetative | 38.3±3.2 | 28.5±1.8 |
| Azoxystrobin+Difenoconazole | Sprayed | Flowering | 41.2±3.1 | 33.5±1.6 |
| Azoxystrobin+Difenoconazole | Sprayed | Podding | 46.9±6.2 | 31.0±3.3 |
| Difenoconazole | control | Seedling | 9.1±3.6 | 0.0±0.0 |
| Difenoconazole | control | Early Vegetative | 30.9±1.2 | 29.5±1.9 |
| Difenoconazole | control | Late Vegetative | 41.9±3.0 | 33.0±0.5 |
| Difenoconazole | control | Flowering | 63.8±9.4 | 35.0±1.2 |
| Difenoconazole | control | Podding | 74.9±7.7 | 49.9±2.9 |
| Difenoconazole | Sprayed | Seedling | 12.4±4.7 | 8.1±5.2 |
| Difenoconazole | Sprayed | Early Vegetative | 22.6±4.0 | 14.4±6.7 |
| Difenoconazole | Sprayed | Late Vegetative | 33.9±3.9 | 24.2±5.1 |
| Difenoconazole | Sprayed | Flowering | 41.6±3.6 | 30.8±1.7 |
| Difenoconazole | Sprayed | Podding | 48.1±3.1 | 41.2±4.9 |
| Metalaxyl+Mancozeb | control | Seedling | 11.0±3.7 | 7.9±5.0 |
| Metalaxyl+Mancozeb | control | Early Vegetative | 26.7±4.0 | 17.8±5.9 |
| Metalaxyl+Mancozeb | control | Late Vegetative | 47.6±5.3 | 39.0±3.8 |
| Metalaxyl+Mancozeb | control | Flowering | 72.9±8.4 | 45.8±4.1 |
| Metalaxyl+Mancozeb | control | Podding | 78.7±6.6 | 57.5±2.9 |
| Metalaxyl+Mancozeb | sprayed | Seedling | 8.6±3.8 | 9.9±6.3 |
| Metalaxyl+Mancozeb | sprayed | Early Vegetative | 29.8±1.6 | 28.7±1.8 |
| Metalaxyl+Mancozeb | sprayed | Late Vegetative | 40.8±3.2 | 37.5±4.2 |
| Metalaxyl+Mancozeb | sprayed | Flowering | 61.8±7.2 | 44.3±4.1 |
| Metalaxyl+Mancozeb | sprayed | Podding | 71.6±7.5 | 50.6±3.8 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$).

### 3.9.10 Effect of fungicide seed treatment on DSI at different stages of growth

The effect of seed dressing with fungicides had significant reduction of DSI at various stages of growth in both locations Njoro and Koibatek (Table 12). Seed dressing with difenoconazole did not show significant differences at all stages compared to control treatments at Njoro. Dressing with azoxystrobin+difenoconazole was only significant at podding in Njoro and at seedling, early vegetative and flowering in Koibatek. Azoxystrobin on the other hand showed significance at early vegetative, flowering and late vegetative in both locations. The effect of metalaxyl+mancozeb was significant at seedling in Njoro and at seedling, late vegetative and podding in Koibatek. Azoxystrobin had the lowest DSI means early vegetative, late vegetative, flowering and podding (24.1%, 40.3%, 35.7% and 56.6%) in Njoro and at early vegetative, late vegetative and podding (15.8%, 23.2% and 36.0%) in Koibatek. Metalaxyl+Mancozeb had the lowest DSI means in both sites at seedling stage (4.3% and 3.7% in Njoro and Koibatek respectively) while difenoconazole had the lowest DSI in Koibatek at early vegetative state (22.8%).

**Table 12: Effect of fungicide seed treatment at different stages of chickpea growth at Njoro and Koibatek in 2013.**

| | | | Disease Severity Index (DSI) | |
|---|---|---|---|---|
| **Fungicide** | **Seed Treatment** | **Stage** | **Njoro**†† | **Koibatek**†† |
| Azoxystrobin | control | Seedling | 13.4±5.4 | 7.4±4.7 |
| Azoxystrobin | control | Early vegetative | 30.7±1.2 | 30.0±2.1 |
| Azoxystrobin | control | Late vegetative | 41.1±3.9 | 30.8±4.3 |
| Azoxystrobin | control | Flowering | 53.7±8.5 | 36.0±5.2 |
| Azoxystrobin | control | Podding | 58.1±8.3 | 38.9±7.4 |
| Azoxystrobin | Dressed | Seedling | 17.6±5.7 | 12.9±5.9 |
| Azoxystrobin | Dressed | Early vegetative | 24.1±3.6 | 15.8±5.1 |
| Azoxystrobin | Dressed | Late vegetative | 35.7±4.1 | 23.2±2.3 |
| Azoxystrobin | Dressed | Flowering | 40.3±6.5 | 26.7±3.9 |
| Azoxystrobin | Dressed | Podding | 56.6±8.4 | 36.0±6.2 |
| Azoxystrobin+Difenoconazole | control | Seedling | 18.1±5.8 | 3.7±3.7 |
| Azoxystrobin+Difenoconazole | control | Early vegetative | 32.1±0.7 | 31.0±1.1 |
| Azoxystrobin+Difenoconazole | control | Late vegetative | 42.2±3.6 | 31.5±3.1 |
| Azoxystrobin+Difenoconazole | control | Flowering | 55.8±8.2 | 37.3±3.5 |
| Azoxystrobin+Difenoconazole | control | Podding | 64.3±8.3 | 43.4±6.2 |
| Azoxystrobin+Difenoconazole | Dressed | Seedling | 17.8±4.0 | 13.3±6.1 |
| Azoxystrobin+Difenoconazole | Dressed | Early vegetative | 29.0±3.1 | 24.7±5.8 |
| Azoxystrobin+Difenoconazole | Dressed | Late vegetative | 39.7±3.2 | 30.2±1.9 |
| Azoxystrobin+Difenoconazole | Dressed | Flowering | 49.6±7.3 | 32.8±1.2 |
| Azoxystrobin+Difenoconazole | Dressed | Podding | 54.4±6.9 | 38.5±4.3 |
| Difenoconazole | control | Seedling | 13.1±4.6 | 8.1±5.2 |
| Difenoconazole | control | Early vegetative | 26.3±3.6 | 21.1±6.8 |
| Difenoconazole | control | Late vegetative | 37.0±4.2 | 27.8±5.6 |
| Difenoconazole | control | Flowering | 50.3±7.3 | 33.1±2.0 |
| Difenoconazole | control | Podding | 62.0±7.1 | 46.8±4.8 |
| Difenoconazole | Dressed | Seedling | 8.3±3.7 | 0.0±0.0 |
| Difenoconazole | Dressed | Early vegetative | 27.0±2.8 | 22.8±4.9 |
| Difenoconazole | Dressed | Late vegetative | 38.8±3.2 | 29.3±1.7 |
| Difenoconazole | Dressed | Flowering | 55.1±8.3 | 32.7±1.4 |
| Difenoconazole | Dressed | Podding | 61.0±7.2 | 44.3±4.0 |
| Metalaxyl+Mancozeb | Dressed | Seedling | 4.3±2.5 | 3.7±3.7 |
| Metalaxyl+Mancozeb | Dressed | Early vegetative | 28.3±3.3 | 22.9±4.8 |
| Metalaxyl+Mancozeb | Dressed | Late vegetative | 44.8±5.9 | 35.0±3.3 |
| Metalaxyl+Mancozeb | Dressed | Flowering | 68.2±8.3 | 44.8±3.6 |
| Metalaxyl+Mancozeb | Dressed | Podding | 72.3±7.3 | 51.9±3.3 |
| Metalaxyl+Mancozeb | control | Seedling | 15.4±4.1 | 14.1±6.4 |
| Metalaxyl+Mancozeb | control | Early vegetative | 28.2±2.9 | 24.3±5.1 |
| Metalaxyl+Mancozeb | control | Late vegetative | 43.7±2.5 | 41.5±4.1 |
| Metalaxyl+Mancozeb | control | Flowering | 66.5±7.7 | 45.9±4.6 |
| Metalaxyl+Mancozeb | control | Podding | 78.1±6.9 | 56.1±3.9 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.11 Effect of fungicide application on area under disease progress curve (AUDPC) in the field

There was significant difference in application of fungicides as seed dressing or foliar sprays on AUDPC in both locations Egerton-Njoro and ATC-Koibatek (Table 13). AUDPC was significantly higher in Egerton-Njoro than ATC-Koibatek. The spray schedule also had significantly lower AUDPC compared to effect of seed dressing in both locations. Metalaxyl+Mancozeb had significantly higher AUDPC in both locations. The lowest AUDPC in Njoro was achieved under azoxystrobin (3632.6) though this was not significantly different from AUDPC under difenoconazole (3766.8) and azoxystrobin+difenoconazole (3816.4). In ATC-Koibatek, the lowest AUDPC was under azoxystrobin (1150.1) which was not significantly different from difenoconazole (1161.5). It was however different from AUDPC under azoxystrobin+difenoconazole (1298.2).

**Table 13: Effect of fungicides on Area under Disease Progress Curve at Njoro and Koibatek in 2014.**

| | Area Under Disease Progress Curve (AUDPC) | |
|---|---|---|
| **Fungicide** | **Njoro**†† | **Koibatek**†† |
| Azoxystrobin+Difenoconazole | 3816.4±241.4 | 1298.2±75.0 |
| Azoxystrobin | 3632.6±291.9 | 1150.1±108.1 |
| Difenoconazole | 3766.8±266.4 | 1161.5±93.5 |
| Metalaxyl+Mancozeb | 4395.4±227.7 | 1468.9±105.2 |
| **Spray Schedule** | | |
| Not Sprayed | 4583.8±118.8 | 1363.2±63.9 |
| Sprayed | 3221.8±129.1 | 1176.2±74.1 |
| **Seed Treatment** | | |
| Dressed | 3836.2±194.9 | 1184.2±61.3 |
| Not Dressed | 3969.4±180.9 | 1355.2±77.0 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 3.9.12 Effect of integrating seed dressing and foliar spray on AUDPC

Application of azoxystrobin as a foliar spray alone had significantly lower AUDPC compared to azoxystrobin+difenoconazole (2861.2 and 3257.9 respectively) (Table 14). There was no difference however between azoxystrobin and difenoconazole in Egerton-Njoro (2861.17 and 2992.9 respectively). Metalaxyl+mancozeb had significantly higher AUDPC compared to the other three fungicides in both locations (3948.1 and 1681.1 in Njoro and Koibatek respectively). Seed dressing alone was not as efficacious in reducing AUDPC as foliar sprays. The lowest AUDPC in Egerton Njoro was achieved under azoxystrobin (4010.9) though it was not significantly different from azoxystrobin+difenoconazole (4243.3). The highest was in metalaxyl+mancozeb (4935.4) which was not significantly different from difenoconazole (4786.14). In ATC-Koibatek, the lowest AUDPC for seed dressed treatments alone was achieved under azoxystrobin (992.9) which was significantly different from response achieved in the other three fungicides. The highest AUDPC was in azoxystrobin+difenoconazole (1429.3) which was not significantly different from Metalaxyl+Mancozeb (1364.2).

The effect of combined seed dressed followed by foliar spray was significantly different from application of fungicides (all fungicides) as seed dress alone in both locations. However, there was variable response in comparison to application of foliar sprays alone. Application of azoxystrobin+difenoconazole as seed dress followed by foliar sprays was significantly different

(3024.0 and 1071.1) from application as foliar spray alone (3257.9 and 1286.7) in Egerton-Njoro and Koibatek respectively. However, the other fungicides did not show significant difference between spraying alone and spray plus seed dressing in locations. The lowest AUDPC under this integrated approach was achieved under azoxystrobin, 2763.9 in Egerton-Njoro, and 971.2 in ATC-Koibatek while the highest was in Metalaxyl+Mancozeb, 3907.6 Egerton-Njoro and 1359.9 in ATC-Koibatek.

**Table 14: Effect of combination of fungicide spray and seed treatment on Area under Disease Progress Curve at Njoro and Koibatek in 2014.**

| Fungicide | Spray Schedule | Seed Treatment | Area under disease progress curve(Audpc) | |
|---|---|---|---|---|
| | | | Njoro†† | Koibatek†† |
| Azoxystrobin+ Difenoconazole | control | Dressed | 4243.3±477.4 | 1429.3±111.8 |
| | control | control | 4740.2±209.5 | 1405.6±164.7 |
| Azoxystrobin+ Difenoconazole | Sprayed | Dressed | 3024.0±132.3 | 1071.1±190.0 |
| | Sprayed | control | 3257.9±71.3 | 1286.7±88.7 |
| Azoxystrobin | control | Dressed | 4010.9±431.4 | 992.9±274.6 |
| | control | control | 4894.4±89.7 | 1595.2±145.4 |
| Azoxystrobin | Sprayed | Dressed | 2763.9±315.6 | 971.2±146.1 |
| | Sprayed | control | 2861.8±198.6 | 1040.9±75.7 |
| Difenoconazole | control | Dressed | 4786.1±28.9 | 1287.9±62.3 |
| | control | control | 4269.9±498.1 | 1359.6±18.9 |
| Difenoconazole | Sprayed | Dressed | 3018.2±194.6 | 996.6±160.5 |
| | Sprayed | control | 2992.9±220.3 | 1002.0±329.3 |
| Metalaxyl+ Mancozeb | control | Dressed | 4935.4±208.2 | 1364.2±125.9 |
| | control | control | 4790.5±322.6 | 1470.7±334.1 |
| Metalaxyl+ Mancozeb | Sprayed | Dressed | 3907.6±696.9 | 1359.9±150.2 |
| | Sprayed | control | 3948.1±254.2 | 1681.1±243.5 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

## DISCUSSION

The findings of this study show that there was a significantly high rate of infection in seeds used for this study for all experiments. Seed lot that was selected on basis of being clean based on visual symptoms apparently also showed very high infection percentage (upto 20%) (Table 2). This is an indication that even seemingly healthy seeds may contain significant quantities of inocula that can serve as primary source of infection of ascochyta blight in farmers' fields. Seedling emergence between asymptomatic and symptomatic seed categories varied significantly in all experiments. Infected seeds usually have less weight and size which affects their germination capacity. As seen in this study seedling emerging from infected seeds may also suffer higher rate of mortality than those emerging from healthy seeds. Dressing seeds with Metalaxyl+mancozeb, azoxystrobin and difenoconazole significantly increased the incubation period and percent germination in both seed categories (symptomatic and asymptomatic).

Seed treatment with azoxystrobin+difenoconazole combination and azoxystrobin alone had the most significant effect in emergence under greenhouse and field conditions. There was upto 18.9% and 16.1% increase in seedling emergence when seeds were dressed with azoxystrobin and azoxystrobin+difenoconazole respectively. Seed dressing with either of the fungicides (azoxystrobin, azoxystrobin+difenoconazole, difenoconazole and metalaxyl+mancozeb) had a significant increase in seedling emergence as compared to non-dressed seeds. There was significant decrease in incidence and severity of ascochyta arising from seed dressing effect. The mean PDI at seedling stage reduced by 4.4%, 4.7%, 4.6% and 7.5% for fungicides azoxystrobin+difenoconazole, azoxystrobin, difenoconazole and metalaxyl+mancozeb respectively in Njoro and 2.1%, 3.2%, 6.9% and 6.1% respectively in Koibatek. Subsequently, seed dressing with metalaxyl+mancozeb recorded the highest reduction of mean PDI at seedling stage at both locations Njoro and Koibatek. This suggests that seed dressing may compliment foliar application of fungicides in integrated disease management programs. However, the effect may not be sufficient in the absence of foliar fungicide application especially under high disease pressure (favourable environmental conditions). In addition, phytotoxic nature of some fungicides has been reported to affect germination and seedling vigour adversely (Sharafeh and Banihashemi, 1992; Gan *et al*., 2006). Further studies focused on nature of interactions of Metalaxyl+mancozeb, azoxystrobin and difenoconazole with seed germination and vigour would be needed to establish their effectiveness as seed dressers.

Disease incidence and severity were significantly higher at Egerton-Njoro location than at ATC-Koibatek throughout the crop growth period. Azoxystrobin was most efficacious in reducing PDI at early vegetative and flowering stages at Njoro with a mean difference of 33.1% and 10.1% compared to control at the two growth stages respectively. Similarly at Koibatek, azoxystrobin was more efficacious in suppressing incidence (at all stages of growth except early vegetative) followed by azoxystrobin+difenoconazole and difenoconazole in that order. There was a reduction of 6.26%, 37.66%, 26.83%, 47.89% and 1.05% in mean PDI at early vegetative, flowering, late vegetative, podding and seedling stages for the fungicide azoxystrobin against the controls in Koibatek. The high humidity and low temperature at Egerton-Njoro could have favoured this response. These results agree with other findings by Chongo *et al* (2003) on foliar fungicides to manage ascochyta blight which reported low severity of ascochyta under drier conditions compared to wet ones. Following seed dressing with foliar spray reduced incidence and severity of ascochyta blight when azoxystrobin and difenoconazole were used (Table 6 and Table 10). However, disease incidence and severity increased steadily from seedling to maturity regardless of the treatments suggesting a response typical of susceptible varieties. Control efficacy of fungicides application programs depends on host resistance, efficacy of fungicide, foliar coverage achieved with fungicide application, disease pressure and weather conditions under which the foliar fungicides are applied (Gan *et al*., 2006)

Disease incidence reduced significantly compared to controls when treatments were sprayed at early vegetative, late vegetative and flowering stages of chickpea growth in Njoro and early vegetative, late vegetative, flowering and podding in Koibatek. Azoxystrobin and azoxystrobin+difenoconazole were most efficacious in reducing disease incidence at early vegetative and flowering stages in Njoro and at all stages in Koibatek. These results agree with Akem *et al*., (2004) on integrating of host resistance with fungicide spray to manage ascochyta blight where they established that foliar spray application at the early stages of chickpea growth provided the greatest disease control and highest grain yield. Findings by Chongo *et al* (2003) reported that azoxystrobin applied at early and mid flowering reduced final disease severity. Application of azoxystrobin+difenoconazole and difenoconazole as foliar sprays was effective at all stages of chickpea growth except at seedling in both locations. Azoxystrobin did not indicate significant differences at seedling and early vegetative compared to controls. Metalaxyl+mancozeb application showed highly variable efficacy in the two locations with some

significant differences reported at flowering, vegetative and podding stages across the two sites. Azoxystrobin reported the lowest DSI means when sprayed at flowering, late vegetative and podding stages at the two locations and was thus more effective among the four fungicides at these stages. Foliar sprays alone with azoxystrobin+difenoconazole, azoxystrobin difenoconazole and metalaxyl+mancozeb reduced the mean disease severity by 10.5%, 19.8%, 10.9% and 1.9% respectively compared to control treatments in Njoro. Following seed dressing with foliar sprays with the fungicides azoxystrobin+difenoconazole, azoxystrobin difenoconazole and metalaxyl+mancozeb reduced mean disease severity by 15.8%, 19.4%, 12.2% and 7.7% respectively compared to controls in Njoro.

The mean DSI for the fungicides azoxystrobin+difenoconazole, azoxystrobin difenoconazole and metalaxyl+mancozeb reduced by 7.4%, 13.9%, 7.3% and 4.4% respectively compared to controls in Koibatek. The three fungicides azoxystrobin, difenoconazole and azoxystrobin+difenoconazole were more efficacious in Egerton Njoro while azoxystrobin and difenoconazole were more effective in Koibatek. Metalaxyl+mancozeb were consistently inferior in protection against foliar blight in both locations except when used as a seed dresser. There was no significant difference in following seed dressing of the clean seed categories with foliar application of azoxystrobin and difenoconazole at Koibatek. Azoxystrobin was the most effective while applied as foliar spray at Koibatek in reduction of foliar blight. Metalaxyl+mancozeb were least effective in both locations when applied both as a seed dress or foliar spray. Although the combination of seed dressing did not yield very promising results against severity of ascochyta blight, the effect of seed coating with other groups of fungicides might be greater and so are worth testing.

Area under disease progress curve (AUDPC) was generally very high in both locations with upto to values of 4583.8 and 1468.9 recorded at Egerton and Koibatek respectively. These results agree with findings by Dermici *et al*., 2011 which reported ineffectiveness of fungicides in vivo under high infection pressure. They are also in line with findings of Nene and Reddy, (1987) who reported that rapidity of disease spread can make it difficult to follow an application schedule in susceptible cultivars. However, AUDPC at ATC-Koibatek was significantly lower than AUDPC at Egerton Njoro. Azoxystrobin recorded the lowest AUDPC in both locations followed by azoxystrobin+difenoconazole and difenoconazole in that order. There was no significant reduction in AUDPC among the four fungicides when applied as seed dress followed

by foliar sprays except under azoxystrobin+difenoconazole where the combination of seed dress and foliar sprays was superior to application of foliar sprays alone. The efficacy of the fungicides in reducing incidence, severity and AUDPC was not promising owing to the high disease pressure experienced across the two locations over the period of study.

Damage caused by ascochyta blight in chickpea can be economically minimized by use of moderately resistant cultivars (Collard *et al*., 2001; Akem *et al*., 2004). Foliar and seed application of fungicides effective against ascochyta blight are, however still required for reliable production of partially resistant chickpea cultivars, especially in seed production/breeding programs (Gan *et al*., 2006). When multiple applications are required, alternating different types of fungicides will minimize the probability of the development of fungicide resistance. Azoxystrobin and difenoconazole could therefore be used in management programs together with other classes of fungicides for control of ascochyta blight in Kenya.

## CONCLUSION

This study found that seed dressing with azoxystrobin, azoxystrobin+difenoconazole or metalaxyl+mancozeb increased the incubation period of ascochyta blight by delaying infection in the presence of the causative agent *A. rabiei* and also improved seedling emergence. This delay was only useful when the crop (*C. arientinum*) was followed with foliar sprays upon the expiry of the protection period from seed dressing. Failure to do this rendered the practice of seed dressing to protect against AB insignificant. The study established that the most significant fungicide management for AB is foliar application beginning vegetative (22-30 DAE) where seeds were dressed, or at seedling stage (less than 20 DAE) where seeds were not dressed with systemic fungicides. Combining of seed dressing and foliar spray when clean seeds are used was as good (no significant difference) as using foliar application alone and therefore seed dressing may not be necessary if seeds are sorted to remove symptomatic/diseased seeds. This is highly recommended for farmers who use (recycle) their own seeds.

## REFERENCES


Ahmad, F., Gaur, P., & Croser, J. (2005). Chickpea (*Cicer arientinum* L.). In R. Singh, & P. Jauhar, *Genetic resources, chromosome engineering and crop improvement* (Vol. 1, pp. 188-189). New York: Taylor and Francis.

Akem, C. (1999). Ascochyta blight of chickpea: Present status and future priorities. *International Journal of Pest Management*, 45 (2): 131-137.

Akem, C., Kabbabeh, S., & Ahmed, S. (2004). Integrating Cultivar Resistance with a single fungicide spray to manage ascochyta blight for increased chickpea yields. *Plant Pathology, 3* (2): 105-110.

Amadioha, A.C. (2003). Evaluation of some plant leaf Extract against *Colletotrichum lindemuthianum* in Cowpea. *Acta Phytopathological et Entomologica Hungarica*, 38 (3-4**)**: 259 – 265.

Barve, M., Arie, T., Salimath, S., Muehlbauer, F., & Peever, T. (2003). Cloning and characterization of the mating type (MAT) locus from Ascochyta rabiei (teleomorph: Didymella rabiei) and a MAT phylogeny of legume-associated Ascochyta spp. *Fungal Genetics and Biology, 39*: 151-167.

Bretag, T. W., Keane, P. J., and Price, T. V. (2006). The epidemiology and control of Ascochyta blight in field peas: a review. *Australian Journal of Agricultural Research,* 57: 883–902.

Chen, W. and Muehlbauer, F.J. (2003). An improved technique for virulence assay of ascochyta rabiei on chickpea. *International Chickpea and Pigeonpea Newsletter*, 10: 31-33

Cho, S., & Muehlbauer, F. (2004). Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (Cicer arietinum L.). *Physiological and Molecular Plant Pathology, 64*: 57–66.

Chongo, G., Buchwaldt, L., Gossen, B.D., Lafond, G.P., May, W.E., Johnson, E.N., and Hogg, T. (2003). Foliar fungicides to manage Ascochyta blight [*Ascochyta rabiei*] of chickpea in Canada. *Canadian Journal of Plant Pathology,* 25: 135–142.

Collard, B.C.Y., Ades,P.K., Pang, E.C.K., Brouwer, J.B., and Taylor, P.W.J. (2001). Prospecting for sources of resistance to ascochyta blight in wild *Cicer* species. *Australian plant pathology*, 30: 271-276.

Dermici, F., Bayraktar, H., Babaliogullu, I., Dolar, F. S., and Maden, S. (2003). In vitro and In vivo effects of some fungicides against the chickpea blight pathogen, Ascochyta rabiei. *Journal of Phytopathology, 151*, 519-524.

FAOSTAT (2014). The state of food and agriculture, Food Agriculture Organization of the United Nations Report, Rome Italy.

Gan, Y.T., Siddique, K.H.M., MacLeod, W.J. and Jayakumar, P. (2006). Management options for minimizing the damage by Ascochyta blight (*Ascochyta rabiei*) in chickpea (*Cicer arietinum* L.). *Field Crops Research*, 97: 121–134.

Haware, M. P., Nene, Y. L., & Mathur S, B. (1986). *Seed Borne Diseases of Chickpea. Technical Bulletin No. 1.* Danish Governement Institute of seed Pathology for Developing countries Copenhagen, Denmark and ICRISAT for Semi Arid Tropics, India.

ICRISAT, (2014). International Crops Research Institute for the Semi-Arid Tropics. *Annual Technical Report*, Nairobi, Kenya.

Jaetzold, R., & Schimdt, H. (1983). *Farm management handbook of Kenya-Natural conditions and farm management information* (Vol. 2B). Nairobi, Kenya: Ministry of Agriculture.

Jan, H. and Wiese, M.V. (1991). Virulence forms of *Ascochyta rabiei* affecting chickpea in the Palouse. *Plant disease*, 75: 904-906

Kaiser, W.J. (1997). Inter-and Intra-national spread of Ascochyta pathogens of chickpea, faba bean, and lentil. *Canadian Journal of Plant Pathology*, 19: 215-224.

Kimurto, P.K., Towett, B.K., Mulwa, R.M.S., Njogu, N., Jeptanui, L.N., Gangarao, R.O., Silim, S., Kaloki, P., Korir, P., Macharia, J.K. (2013). Evaluation of chickpea genotypes for resistance to ascochyta blight (*Ascochyta rabiei*) disease in the dry highlands of Kenya. *Phytopathologia Mediterranea*, 52 (1) : 613-622.

Mancini, V., & Romanazzi, G. (2014). Seed treatments to control seedborne fungal pathogens of vegetable crops. *Pest Management Sci*ence, *70*: 860–868.

Markell, S., Wise, K., McKay, K., Goswami, R., & Gudmestad, N. (2008). *Plant Disease Management NDSU Extension service.* North Dakota State University Fargo, North Dakota 58105.

Millan, T., Clarke, H., Kadambot, H., Siddique, M., Buhariwalla, H. and Gaur, P.(2006). Chickpea Molecular breeding: New tools and concepts. *Euphytica, 147*: 81-103.

Montaser, F. A. (2011). Integrated management of damping-off, root and/or stem rot diseases of chickpea and efficacy of the suggested formula. *Not Sci Biol*, *3* (3): 80-88.

Nene, Y. L., & Reddy, M. V. (1987). *Chickpea diseases and their control.* (M. C. Singh, Ed.) Oxforshire: CAB (Commonwealth Agricultural Bureaux).

Pande, S., Sharma, M., Gaur, P.M., Tripathi, S., Kaur, L., Basandrai, A., Khan, T., Gowda, C.L.L., and Siddique, K.H.M. (2011). Development of screening techniques and identification of new sources of resistance to ascochyta blight disease of chickpea. *Australian Plant Pathology*, 40:149-156.

Sharafeh, M. and Banihashemi, Z. (1992). Study of chickpea blight and its control in Fars Province. *Iranian Journal of Plant Pathology*, 28 (1-4): 1, 35-48.

Shtienberg, D., Kimber R, B. E., McMurray, L., & Davidson, J. A. (2006). Optimisation of the chemical control of ascochyta blight in chickpea. *Australian Plant pathology, 35*: 715-724.

Varshney, R.K., Close, T.J., Singh, N.K., Hoising, D.A. and Cook, D.R. (2009). Orphan legume crops enter the genomics era. *Current Opinion Plant Biology,* 12: 202-210.

Wilcoxson, R., Skovmand, B., and Atif, H. (1975). Evaluation of wheat cultivars ability to retard development of stem rust. *Annual Applied Biology*, 80: 275-282.

Wise, A. K., Henson, A. R., & Bradley, A. C. (2009). Fungicide seed treatment effects on seed-borne Ascochyta rabiei in chickpea. *HortTechnology*, *19* (3), 533-537.

# CHAPTER FOUR

## Optimization of the Spray Schedule in Management of Ascochyta Blight (*Ascochyta rabiei* L.) of Chickpea (*Cicer arientinum* L.) in the dry highlands of Kenya


### ABSTRACT

Ascochyta blight, caused by the fungus *Ascochyta rabiei* L., is the most limiting biotic factor in chickpea production in Kenya. Field trials were conducted at ATC-Koibatek and Egerton Njoro in cropping season 2014 to evaluate the control of ascochyta blight by seed dressing followed by multiple spray schedules of two fungicides; azoxystrobin and difenoconazole. The experiments were laid out in a split-split plot design with the two fungicides in the main plots, spray schedule in the subplot and seed dressing in the sub-sub plots. Plots were sprayed 2 to 6 times in the sub plots at five stages of chickpea growth; seedling, early vegetative, late vegetative, flowering and podding stages. In the sub-sub plots, asymptomatic chickpea seeds were left treated or untreated with either azoxystrobin or difenoconazole fungicides. The treatments were replicated in three blocks in RCBD arrangement. Data on disease incidence and severity were collected and subjected to analysis of variance following PROC GLM procedure in SAS. Significant means at F-test separated using Tukey's test statistics at $P \leq 0.05$. Fungicides applied as foliar sprays were more effective in suppressing disease incidence and severity than seed dressing alone. Seed dressing was effective in delaying initial disease incidence and reducing severity at early stages of development but lacked advantage over foliar spraying alone in later stages of chickpea growth. Growing chickpea at Njoro (when conditions are extremely wet and humid) requires more than six foliar sprays with either azoxystrobin or difenoconazole while five foliar sprays are needed for the two fungicides in Koibatek. Evaluating tolerant or resistant varieties is recommended since it would reduce the number of sprays in both locations drastically. Economic analysis to determine the optimal spraying regimes is also recommended.

## INTRODUCTION

Ascochyta blight (AB), caused by *Didymella rabiei* (Kovacheski) var. Arx [Anamorph *Ascochyta rabiei* (Pass) Labr.], is one of the most important diseases of chickpea (*Cicer arientinum* L.) and has been reported in most chickpea growing countries of the world (Kaiser, *et al*., 2000). In Kenya, the disease has been reported to cause yield losses of upto 100% on susceptible cultivars and upto 10% on resistant ones. The disease development and spread is favored by cool and wet conditions common during long rains in the Kenya and winters in the temperate regions (Kimurto *et al*., 2013; Akem *et al*., 2004). The disease is primarily spread by ascospores discharged from pseudothecia during periods of rains but can also be spread by splash dispersal and seed (Shtienberg *et al*., 2006). Seed borne inocula has been responsible for introduction of ascochyta blight in new regions of the world such as Australia, Canada, Iran and the USA (Gan *et al*., 2006). Seed infected with *A. rabiei* is usually discolored, shrinkled with low seed weight.

The fungus produces characteristic necrotic lesions on all foliage parts causing collapse of tissue, stem breakage and plant death (Pande *et al*., 2005). The disease is particularly problematic to control because of the high variability of the *A. rabiei* caused by emergence of new races or pathotypes arising from sexual recombination of the mating types (Trapero-Casas and Kaiser, 1992). Resistant cultivars have been employed as the first line of defense against ascochyta blight management (Singh and Reddy, 1996). However, owing to the high variability of the pathogen, resistant cultivars alone cannot provide adequate management of the disease (Akem *et al*., 2004). Moderately resistant to resistant cultivars at seedling stage become susceptible at older stages of growth as resistance declines over time (Chongo and Gossen, 2001). In the light of this, fungicides applied either as foliar spray or as a seed dress in an integrated scheme remain an option in the management of AB in chickpea. Cultural practices such as planting disease free seeds, crop rotation and destruction of infected stubble can also reduce introduction and spread of AB.

Many fungicides have been tested and used for foliar application in chickpea as well as seed dressing, but efficacy varies from region to region and season to season. Some of the effective fungicides that have been used include azoxystrobin, difenoconazole, chlorothalonil, mancozeb, boscalid, pyclastrobin and tebuconazole (Gan *et al*., 2006). There has been reported various levels of efficacy of fungicide control depending on the level of host resistance,

fungicide efficacy, disease pressure, foliar coverage and weather conditions. Effective control of AB has been achieved by multiple applications of foliar fungicides of upto five times during the period of chickpea growth (Akem *et al*., 2004). Limited resistance in existing chickpea germplasm in Kenya has prompted the integration of judicious application of fungicides and host tolerance in integrated disease management (IDM) of ascochyta blight.

The objective of this study was therefore, to (a) determine the efficacy of azoxystrobin and difenoconazole and their timing effects to control ascochyta blight and (b) determine effect of combining seed dressing and foliar sprays in a fungicide management program. This information is important for integrated ascochyta blight disease management strategies on susceptible chickpea cultivars especially in Kenya and the East Africa region.

## MATERIALS AND METHODS

### 4.2 Study sites

Field trials were conducted at Egerton University, Njoro and ATC-Koibatek (Agricultural Training Centre). ATC-Koibatek (latitude 1$^{o}$ 35’ S, and longitude 36$^{o}$ 66’ E) lies at altitude 1890m above sea level (a.s.l) in the agro-ecological zone upper midlands (UM4), with low agricultural potential. Average annual rainfall is 767 mm while mean temperatures ranges between 18.2-24.3$^{o}$C. The mean annual minimum and maximum temperature are 10.9$^{o}$C and 28.8$^{o}$C respectively. Soils are vitric andosols with moderate to high soil fertility, well drained deep to sandy loam soil (Jaetzold and Schmidt, 1983). Egerton University, Njoro (0$^{o}$ 23’S and 35$^{o}$ 35’E) lies at altitude 2265m a.s.l in lower highland (LH2-LH3) agro-ecological zone and has a sub-humid modified tropical climate. The annual average rainfall is 931mm and mean temperature ranges between 16-19.1$^{o}$C. The mean maximum and minimum temperature are 22.7$^{o}$C and 7.9$^{o}$C, respectively (Jaetzold and Schmidt, 1983). Fields selected at both sites were known to have high inocula load of ascochyta blight since they had been under chickpea crop for more than three years. The experiments were carried out during the 2014/2015 cropping seasons. A susceptible chickpea variety (ICCV 97105), currently released by Egerton University (2013) and selected for its agronomic adaptation and high yield production in the dry highlands of Kenya was used (KEPHIS, 2013).

**4.3 Experimental design**

A split-split plot design was used in the experiments at all locations with the fungicides as the main plots, seed treatment as sub plots and spray schedule (timing) as sub-sub plots in a randomized complete block design with three replications. Seed were hand planted at a depth of 3-5 cm a spacing of 40 cm × 10 cm in five rows on plots measuring 3 m long. The following schedules of fungicide application were evaluated at the two locations: (1) Untreated control – no fungicide applications; (2) Two times spray – two fungicide applications at 22 days after emergence (DAE) and 36 DAE (3) three times spray – three fungicide applications at 22, 36 and 50 DAE (4) four times spray – four fungicide applications at 22, 36, 50 and 64 DAE (5) five times spray – five fungicide applications at 22, 36, 50, 64 and 78 DAE and (6) six times spray – six fungicide applications at 22, 36, 50, 64, 78 and 92 DAE. The fungicides azoxystrobin and difenoconazole were sprayed at recommended rates appropriately using a knapsack back sprayer delivering about 200 $Lha^{-1}$. Normal agronomic practices for chickpea including fertilizer use and weed control were followed. The experimental model used is as shown below;

$$Y_{ijklm} = \mu + E_i + B_{j(i)} + G_k + EG_{ik} + T_l + ET_{il} + GT_{kl} + EGT_{ikl} + t_m + Et_{im} + Gt_{km} + Tt_{lm} + EGTt_{iklm} + \varepsilon_{n(ijklm)}$$

Where μ= overall mean

$E_i$ = effect due to $i^{th}$ location/environment

$B_{j(i)}$ = effect due to $j^{th}$ block within $i^{th}$ environment

$G_k$ = effect due to $k^{th}$ genotype

$EG_{ik}$ = effect due to interaction of $i^{th}$ environment and $k^{th}$ genotype

$T_l$ = effect due $l^{th}$ fungicide

$ET_{il}$ = effect due to $i^{th}$ environment $l^{th}$ fungicide

$GT_{kl}$ = effect due to $k^{th}$ genotype and $l^{th}$ fungicide

$EGT_{ikl}$ = main treatment error

$t_m$= effect due to $m^{th}$ time of fungicide application

$Et_{im}$ = effect due to $i^{th}$ environment and $m^{th}$ time of fungicide application

$Gt_{km}$ = effect due to $k^{th}$ genotype and $m^{th}$ time of fungicide application

$Tt_{lm}$ = effect due interaction of $l^{th}$ fungicides and $m^{th}$ time

$EGTt_{iklm}$ = effect due to interaction of $i^{th}$ environment, $k^{th}$ genotype, $l^{th}$ fungicide and $m^{th}$ time of fungicide application.

$\varepsilon_{n(ijklm)}$ = random error component

**4.4 Disease assessment**

Data on severity was collected by scoring the percentage of damage on individual plant using the 1-9 scale (Jan and Wiese, 1991); (Chen and Muehlbauer, 2003) and (Pande *et al*., 2011), where 1=no visible symptoms; 2=minute lesions prominent on the apical stems; 3=lesions up to 5-10 mm in size and slight drooping of apical stems; 4=lesions obvious on all plant parts and clear drooping of apical stems; 5=lesions on all plants parts, defoliation initiated, breaking and drying of branches slight to moderate; 6=lesions as in 5, defoliation, broken, dry branches common, some plants killed; 7=lesions as in 5, defoliation, broken, dry branches very common, up to 25% of plants killed; 8= symptoms as in 7 but up to 50% of the plants killed and 9=symptoms as in 7 but up to 100% of the plants killed. A total of 75 plants in three middle rows were scored for severity in each plot. Disease severity index (DSI) similar to that one used by (Montaser, 2011) was calculated for each plot as follows.

$DSI = \Sigma d/(d\ max \times n) \times 100$

Where: d is the disease rating of each plant

d max is the maximum disease rating and

n is the total number of plants examined in each plot.

Percentage disease incidence (PDI) was measured based on the number of diseased plants per plot in three middle rows. Percent incidence was calculated as the ratio of the diseased plant to the total plant multiplied by 100%. The following formula was used to calculate the disease incidence of each plot as adopted from Amadioha (2003).

$PDI = [(\text{No of diseased plants})/(\text{Total no of plants})] \times 100\%.$

Disease severity index (DSI) and percent disease incidence (PDI) data were subjected to analysis of variance using the glm procedure in SAS (SAS 9.1.3 version, SAS Institute Inc.), to determine main, sub plot and sub-sub plot effects. Mean separations were performed using Tukey HSD test statistic.

## 4.5 Results

### 4.5.1 Effect of azoxystrobin and difenoconazole on ascochyta blight incidence

The PDI at Njoro was significantly higher than PDI at Koibatek (P≤0.05) (Table 15). Azoxystrobin was more efficacious than difenoconazole in reducing disease and severity incidence in both locations. Foliar application of fungicides multiple times (3 to 6 foliar sprays) significantly reduced disease incidence and severity in both locations except for application of only two foliar sprays in Njoro. There was no significant difference in seed dressing with the two fungicides in both locations. There was significant difference in disease incidence and severity at different stages of growth with the highest incidence and severity recorded at 106 DAE, 97.54% and 74.10% in Njoro and Koibatek respectively and the lowest at 22 DAE, 2.88% and 1.01% in Njoro and Koibatek respectively.

**Table 15: Effect of fungicides spray schedule on ascochyta blight incidence and severity in Njoro and Koibatek in 2014.**

| | Percent Disease Incidence (PDI) | | Disease Severity Index (DSI) | |
|---|---|---|---|---|
| **Fungicide** | **Njoro††** | **Koibatek††** | **Njoro††** | **Koibatek††** |
| Azoxystrobin | 74.95±2.94 | 40.96±2.30 | 49.77±2.52 | 30.63±2.14 |
| Difenoconazole | 81.33±2.72 | 51.26±2.42 | 55.35±2.49 | 45.12±2.46 |
| **Spray regime** | | | | |
| 2times | 96.44±4.48 | 59.31±4.62 | 81.35±3.95 | 51.04±4.52 |
| 3times | 83.04±5.32 | 43.62±3.90 | 54.06±4.07 | 27.32±2.80 |
| 4times | 72.54±5.39 | 32.08±3.31 | 46.51±4.08 | 22.27±2.84 |
| 5times | 61.08±4.49 | 34.55±2.98 | 24.81±2.26 | 24.05±2.60 |
| 6times | 53.62±3.97 | 29.14±2.72 | 21.56±2.04 | 20.88±3.61 |
| control | 100±0 | 77.95±4.14 | 87.06±3.29 | 73.68±4.34 |
| **Seed treatment** | | | | |
| No treated (Control) | 79.01±2.90 | 46.46±2.36 | 53.46±2.52 | 36.83±2.31 |
| Treated | 77.28±2.77 | 45.76±2.40 | 51.66±2.50 | 38.91±2.38 |
| **stage** | | | | |
| INCID22 | 17.70±2.39 | 2.88±0.43 | 4.60±0.82 | 1.01±0.16 |
| INCID36 | 48.36±6.69 | 18.28±3.11 | 30.70±4.94 | 8.97±1.81 |
| INCID50 | 83.29±5.85 | 29.39±3.65 | 47.93±4.79 | 23.46±3.75 |
| INCID64 | 100±0 | 53.47±3.52 | 70.15±3.58 | 46.08±4.08 |
| INCID78 | 94.54±1.45 | 67.31±3.58 | 70.66±3.57 | 53.55±3.98 |
| INCID92 | 96.31±1.03 | 73.57±3.20 | 69.78±3.55 | 63.06±3.76 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

### 4.5.2 Effect of Fungicide Spray Schedules on Percent Incidence at Different Growth Stages

The spray regimes with either azoxystrobin or difenoconazole indicated significant differences ($P \leq 0.05$) in PDI compared to control at different growth stages in both locations (Table 16). Spraying twice with azoxystrobin in Njoro showed significantly low PDI compared to control at seedling stage (22 DAE). However, there was no difference in PDI at seedling stage for the same treatment in Koibatek. Spraying twice with difenoconazole on the other hand indicated significant differences at 22 DAE in Njoro and at 22, 36, 50 and 64 DAE in Koibatek. The three sprays schedule with azoxystrobin indicated significantly low PDI compared to control at 22 DAE and 36 DAE in Njoro and at all stages in Koibatek. Difenoconazole at the same treatment also had significantly low PDI at 22 DAE and 36 DAE in Njoro and at 36, 50, 64, 78 and 92 DAE in Koibatek. In both fungicides, there was consistently low PDI at Koibatek compared to Njoro.

Spraying four times with azoxystrobin in Njoro showed significantly lower PDI compared to control at 22, 36 and 50 DAE. Percent disease index was low at all stages for the same treatment in Koibatek. Difenoconazole on the other hand showed significant differences at 22 and 36 DAE in Njoro, one less stage than azoxystrobin while there were significance at all stages in Koibatek except at 22 DAE. The five spray schedule with azoxystrobin yielded significant differences compared to control at all stages in Njoro and Koibatek. Difenoconazole however indicated significant difference at 22, 36 and 50 DAE in Njoro and at all stages except 22 DAE in Koibatek. Under the maximum spray schedule of six sprays, both fungicides indicated significant differences from control at all stages in both locations except at 22 DAE for difenoconazole in Koibatek.

Azoxystrobin had significantly lower PDI (Table 16) than difenoconazole under the five spray schedules in Koibatek as follows; at 22 DAE under two times spray schedule; at 22, 64, 78 and 92 DAE under three times spray schedule, at 64, 78 and 92 DAE under four spray schedule; 36, 50, 64, 78 and 92 DAE under five times spray schedule and at 36, 50, 64 and 92 under six times spray schedule. In Njoro, azoxystrobin showed similarly lower PDI compared to difenoconazole as follows; at 36, 64, 78 and 92 DAE under the five times spray schedule and at 22, 36, 50 and 92 DAE under the six times spray schedule.

**Table 16: Effect of fungicides spray schedule at different stages of chickpea growth at Njoro and Koibatek in 2014.**

| Spray Schedule | Stage | Percent Disease Incidence (PDI) | | | |
|---|---|---|---|---|---|
| | | Azoxystrobin | | Difenoconazole | |
| | | Njoro†† | Koibatek†† | Njoro†† | Koibatek†† |
| 2times | INCID22 | 15.48±5.52 | 3.50±1.12 | 14.39±6.13 | 5.50±1.52 |
| | INCID36 | 100.00±0.00 | 10.67±3.22 | 100.00±0.00 | 17.50±3.77 |
| | INCID50 | 100.00±0.00 | 18.00±5.55 | 100.00±0.00 | 23.17±4.94 |
| | INCID64 | 100.00±0.00 | 76.00±11.29 | 100.00±0.00 | 81.67±6.01 |
| | INCID78 | 100.00±0.00 | 94.33±5.67 | 100.00±0.00 | 100.00±0.00 |
| | INCID92 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |
| 3times | INCID22 | 11.23±5.50 | 5.33±2.16 | 16.60±6.65 | 1.00±0.68 |
| | INCID36 | 11.16±3.53 | 7.83±2.15 | 59.67±24.54 | 10.17±4.17 |
| | INCID50 | 87.68±12.32 | 22.67±4.02 | 100.00±0.00 | 25.17±8.56 |
| | INCID64 | 100.00±0.00 | 38.33±6.74 | 100.00±0.00 | 52.83±6.78 |
| | INCID78 | 100.00±0.00 | 58.50±13.63 | 100.00±0.00 | 80.50±12.37 |
| | INCID92 | 100.00±0.00 | 65.17±11.51 | 100.00±0.00 | 83.50±10.44 |
| 4times | INCID22 | 8.50±4.71 | 2.67±2.08 | 11.70±4.39 | 1.00±0.52 |
| | INCID36 | 4.98±1.53 | 5.50±3.07 | 6.47±2.08 | 6.17±2.43 |
| | INCID50 | 35.13±7.06 | 10.00±4.74 | 85.14±19.08 | 11.17±6.40 |
| | INCID64 | 100.00±0.00 | 24.50±3.26 | 100.00±0.00 | 42.83±12.76 |
| | INCID78 | 100.00±0.00 | 38.83±4.73 | 100.00±0.00 | 57.83±11.56 |
| | INCID92 | 100.00±0.00 | 44.83±3.96 | 100.00±0.00 | 71.83±11.08 |
| 5times | INCID22 | 11.57±2.87 | 2.50±1.63 | 7.66±2.63 | 4.83±1.54 |
| | INCID36 | 7.33±1.11 | 3.00±1.15 | 10.15±1.40 | 12.50±3.82 |
| | INCID50 | 32.50±14.56 | 15.33±3.22 | 60.56±9.08 | 21.33±5.44 |
| | INCID64 | 68.51±13.34 | 32.83±4.59 | 100.00±0.00 | 41.17±5.83 |
| | INCID78 | 77.78±7.38 | 43.17±3.78 | 100.00±0.00 | 54.33±7.33 |
| | INCID92 | 88.67±5.48 | 51.83±6.99 | 100.00±0.00 | 71.00±9.49 |
| 6times | INCID22 | 6.79±2.27 | 1.67±0.80 | 7.94±3.55 | 2.00±0.93 |
| | INCID36 | 5.99±2.23 | 4.67±1.69 | 11.01±3.38 | 16.17±4.83 |
| | INCID50 | 29.58±10.81 | 9.83±3.30 | 48.55±7.97 | 19.33±4.98 |
| | INCID64 | 64.42±11.37 | 20.33±3.82 | 63.94±6.94 | 46.17±9.70 |
| | INCID78 | 84.00±7.38 | 29.17±1.30 | 76.00±6.41 | 51.00±8.78 |
| | INCID92 | 89.78±4.84 | 38.00±2.48 | 81.11±4.86 | 56.67±7.41 |
| Control | INCID22 | 44.76±9.52 | 1.00±0.45 | 55.83±11.15 | 3.50±2.19 |
| | INCID36 | 100.00±0.00 | 41.83±18.81 | 100.00±0.00 | 83.33±5.27 |
| | INCID50 | 100.00±0.00 | 85.00±15.00 | 100.00±0.00 | 91.67±5.27 |
| | INCID64 | 100.00±0.00 | 93.33±6.67 | 100.00±0.00 | 91.67±5.27 |
| | INCID78 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |
| | INCID92 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$).

### 4.5.3 Effect of Azoxystrobin and Difenoconazole on Disease Severity Index (DSI)

**Table 17: Effect of azoxystrobin and difenoconazole on disease severity index at Njoro and Koibatek in 2014.**

| | Disease Severity Index (DSI) | |
|---|---|---|
| **Fungicide** | **Njoro**†† | **Koibatek**†† |
| Azoxystrobin | 49.77±2.52 | 30.63±2.14 |
| Difenoconazole | 55.35±2.49 | 45.12±2.46 |
| **Spray Schedule** | | |
| 2times | 81.35±3.95 | 51.04±4.52 |
| 3times | 54.06±4.07 | 27.32±2.80 |
| 4times | 46.51±4.08 | 22.27±2.84 |
| 5times | 24.81±2.26 | 24.05±2.60 |
| 6times | 21.56±2.04 | 20.88±3.61 |
| control | 87.06±3.29 | 73.68±4.34 |
| **Seed Treatment** | | |
| Not dressed (Control) | 53.46±2.52 | 36.83±2.31 |
| Dressed | 51.66±2.50 | 38.91±2.38 |
| **Stage** | | |
| DSI22 | 4.60±0.82 | 1.01±0.16 |
| DSI36 | 30.70±4.94 | 8.97±1.81 |
| DSI50 | 47.93±4.79 | 23.46±3.75 |
| DSI64 | 70.15±3.58 | 46.08±4.08 |
| DSI78 | 70.66±3.57 | 53.55±3.98 |
| DSI92 | 69.78±3.55 | 63.06±3.76 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

The mean disease severity index (DSI) for the two fungicides; azoxystrobin and difenoconazole were significantly different at $P \leq 0.05$ (Table 17). The spray schedules and stage of crop growth also indicated significant differences in DSI among the various treatments. There was however no significant difference in mean DSI of seed dressing against control. Azoxystrobin had significantly lower mean DSI (49.77%) than difenoconazole (55.35%) in Njoro and 30.63% and 45.12% respectively. Disease severity was lowest under the most spray schedule (6 times) in both locations. In both locations, there were significant differences in mean DSI for all schedules against control. The six times schedule had the lowest mean DSI, 21.56% and 20.88% in Njoro and Koibatek respectively while disease severity was highest under the two times spray schedule, 81.35% and 51.04% in Njoro and Koibatek respectively. Disease severity was highest at 78 DAE in Njoro and 92 DAE in Koibatek and lowest at 22 DAE in both locations.

### 4.5.4 Effect of azoxystrobin and difenoconazole seed treatment and foliar spray on disease severity

**Table 18: Effect of fungicide spray schedule combined with seed treatment on disease severity index at Njoro and Koibatek in 2014.**

| | | Disease Severity Index (DSI) | | | |
|---|---|---|---|---|---|
| | | Azoxystrobin | | Difenoconazole | |
| Spray Schedule | Seed Treatment | DSI Njoro†† | Koibatek†† | Njoro†† | Koibatek†† |
| 2times | Control | 77.88±8.52 | 50.02±9.16 | 83.75±7.73 | 57.09±9.68 |
| | Treated | 78.29±8.38 | 40.73±8.09 | 85.50±7.35 | 56.31±9.37 |
| 3times | Control | 44.37±8.01 | 23.46±4.59 | 62.57±7.84 | 33.83±6.71 |
| | Treated | 49.02±8.17 | 21.65±4.75 | 60.29±8.42 | 30.33±6.01 |
| 4times | Control | 45.67±8.93 | 17.29±3.52 | 54.11±8.90 | 25.03±6.12 |
| | Treated | 40.04±7.68 | 13.64±3.28 | 46.22±7.34 | 33.11±7.85 |
| 5times | Control | 19.26±3.67 | 19.04±3.84 | 28.51±4.77 | 28.09±5.71 |
| | Treated | 22.90±4.71 | 13.38±2.69 | 28.57±4.81 | 35.70±6.57 |
| 6times | Control | 28.35±5.39 | 14.29±3.04 | 21.70±3.72 | 30.57±8.02 |
| | Treated | 19.99±4.09 | 16.83±4.78 | 16.23±2.39 | 38.85±8.43 |
| control | Control | 86.77±6.62 | 64.92±9.39 | 88.56±6.40 | 78.39±8.16 |
| | Treated | 84.74±7.20 | 72.30±9.31 | 88.18±6.49 | 79.11±8.02 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

There was little significant difference in disease severity between plots that were seed dressed followed with foliar sprays schedules and those that were only sprayed (Table 18). This was the case even for the control (none sprayed) treatment. However, it was observed that disease severity at the four times and five times spray schedules in Koibatek and six times schedule in Njoro were significantly lower where seed dressing was followed by foliar spray of azoxystrobin compared to none dressed treatments. There was similar occurrence under six times schedule in Njoro and four times schedule in Koibatek where difenoconazole was applied. However, at Koibatek under four times schedule sprayed with difenoconazole, there was inconsistent significantly higher severity where seed dressing was followed by foliar spray an indication that the few significant occurrences of seed dressing combined with foliar sprays, might have been chance occurrence or as a result of experimental error.

### 4.5.5 Effect of azoxystrobin and difenoconazole on disease severity at different stages of chickpea growth

There was significantly low disease severity index (DSI) ($P \leq 0.05$) at all stages of crop growth in plots sprayed with either azoxystrobin or difenoconazole compared to control in both locations (Table 19 and figures 1-4). Under 2 times spray schedule with azoxystrobin, severity at seedling (22 DAE) in Njoro and at all stages in Koibatek was significantly different from control with the lowest mean severity (4.02%) recorded at 22 DAE. There was similarly significantly low DSI where difenoconazole was sprayed twice compared to control except at 50, 64, 78 and 92 DAE in Njoro and 22 and 92 DAE in Koibatek. Severity in the 3 times, 4 times, 5 times and 6 times schedule was mostly significantly lower compared to control for both fungicides at the two locations except in few instances. The highest reduction in severity against control was achieved in Njoro under azoxystrobin with means as follows; 9.24% and 25.98% for 2 sprays program 22 and 36 DAE respectively; 11.56%, 84.6%, 71.73%, 29.63%, 27.78% and 25.93% for 3 spray program 22-92 DAE respectively; 11.66%, 86.03%, 91.51%, 31.48%, 29.63%, and 29.63% for 4 spray program 22-92 DAE respectively; 10.89%, 85.39%, 89.23%, 68.22%, 69.8% and 67.58% for 5 spray program 22-92 DAE respectively; 11.8%, 85.73%, 89.78%, 69.11%, 71.75% and 72.52% for 6 spray program 22-92 DAE respectively.

**Table 19: Effect of fungicide spray schedule on disease severity at different stages of chickpea growth at Njoro and Koibatek in 2014.**

| | | Disease Severity Index (DSI) | | | |
|---|---|---|---|---|---|
| | | Azoxystrobin | | Difenoconazole | |
| Spray Schedule | Stage | Njoro†† | Koibatek†† | Njoro†† | Koibatek†† |
| 2times | DSI22 | 4.02±1.62 | 1.23±0.45 | 3.46±1.66 | 2.05±0.57 |
| | DSI36 | 61.06±17.83 | 4.07±1.29 | 88.89±5.74 | 8.12±2.32 |
| | DSI50 | 81.48±11.71 | 9.58±3.36 | 100.00±0.00 | 16.25±4.28 |
| | DSI64 | 100.00±0.00 | 58.15±9.59 | 100.00±0.00 | 83.46±5.48 |
| | DSI78 | 100.00±0.00 | 70.49±9.71 | 100.00±0.00 | 90.74±4.46 |
| | DSI92 | 100.00±0.00 | 83.33±6.25 | 100.00±0.00 | 98.15±1.85 |
| 3times | DSI22 | 1.70±0.71 | 1.90±0.85 | 3.63±1.43 | 0.30±0.20 |
| | DSI36 | 2.44±0.61 | 2.99±1.02 | 18.99±8.08 | 4.12±1.92 |
| | DSI50 | 28.27±9.14 | 12.25±2.59 | 75.93±8.32 | 14.27±5.30 |
| | DSI64 | 70.37±8.45 | 20.42±4.26 | 83.33±7.45 | 35.11±6.14 |
| | DSI78 | 72.22±8.49 | 30.57±8.08 | 83.33±7.45 | 47.09±7.89 |
| | DSI92 | 74.07±8.45 | 40.94±8.48 | 77.78±9.51 | 58.12±10.00 |
| 4times | DSI22 | 1.60±0.97 | 0.89±0.71 | 2.32±0.87 | 0.35±0.18 |
| | DSI36 | 1.01±0.45 | 2.27±1.39 | 1.41±0.46 | 2.22±1.06 |
| | DSI50 | 8.49±2.06 | 6.10±3.70 | 30.74±7.66 | 6.35±3.80 |
| | DSI64 | 68.52±9.69 | 14.25±3.93 | 83.33±4.76 | 29.90±10.85 |
| | DSI78 | 70.37±8.92 | 20.77±4.48 | 81.48±4.68 | 41.28±11.36 |
| | DSI92 | 70.37±8.92 | 25.73±4.65 | 72.22±6.25 | 57.19±11.89 |
| 5times | DSI22 | 2.37±0.64 | 0.81±0.55 | 1.46±0.53 | 1.78±0.64 |
| | DSI36 | 1.65±0.48 | 0.89±0.34 | 2.25±0.31 | 5.56±2.56 |
| | DSI50 | 10.77±6.81 | 7.38±1.97 | 17.65±2.01 | 13.75±4.23 |
| | DSI64 | 31.78±6.01 | 18.47±3.26 | 41.41±3.04 | 29.85±6.52 |
| | DSI78 | 30.20±7.01 | 20.42±2.42 | 48.89±4.80 | 43.93±8.84 |
| | DSI92 | 32.42±6.38 | 31.63±6.03 | 41.33±5.86 | 55.62±6.74 |
| 6times | DSI22 | 1.46±0.55 | 0.59±0.28 | 1.38±0.61 | 0.59±0.28 |
| | DSI36 | 1.31±0.68 | 1.38±0.50 | 2.32±0.77 | 5.68±2.22 |
| | DSI50 | 10.22±5.70 | 4.96±2.00 | 11.60±2.30 | 9.48±8.09 |
| | DSI64 | 30.89±6.20 | 12.30±3.45 | 28.20±4.33 | 18.79±14.24 |
| | DSI78 | 28.25±6.61 | 19.06±4.62 | 27.19±3.73 | 31.95±14.36 |
| | DSI92 | 27.48±8.28 | 30.59±8.03 | 27.65±3.94 | 45.44±14.18 |
| control | DSI22 | 13.26±3.07 | 0.37±0.20 | 18.59±5.37 | 1.26±0.86 |
| | DSI36 | 87.04±8.80 | 20.27±9.68 | 100.00±0.00 | 50.00±3.80 |
| | DSI50 | 100.00±0.00 | 71.11±15.50 | 100.00±0.00 | 100.00±0.00 |
| | DSI64 | 100.00±0.00 | 92.22±7.78 | 100.00±0.00 | 100.00±0.00 |
| | DSI78 | 100.00±0.00 | 96.30±3.70 | 100.00±0.00 | 100.00±0.00 |
| | DSI92 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 | 100.00±0.00 |

††Figures shown are the Means ± SE of three replicates. Figures within a column whose SE values do not overlap are statistically different at ($P \leq 0.05$)

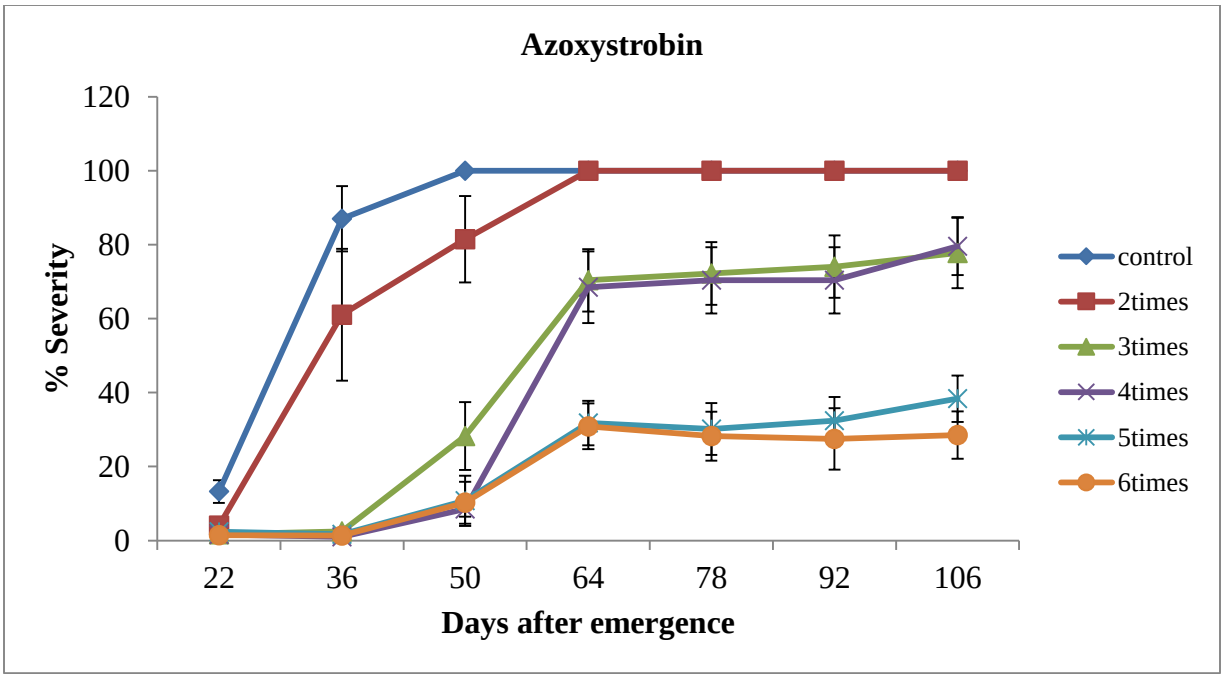


**Figure 1: Disease progress curve for azoxystrobin application at Egerton Njoro in 2014**

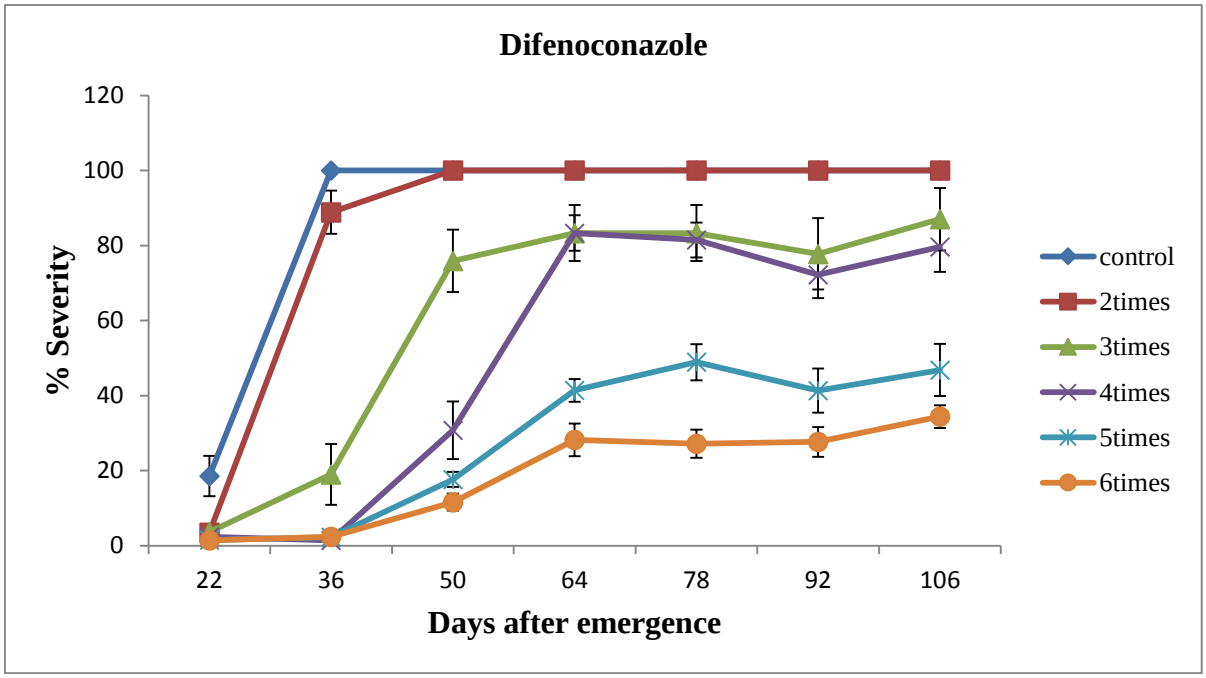


**Figure 2: Disease progress curve for application of difenoconazole at Egerton, Njoro in 2014**

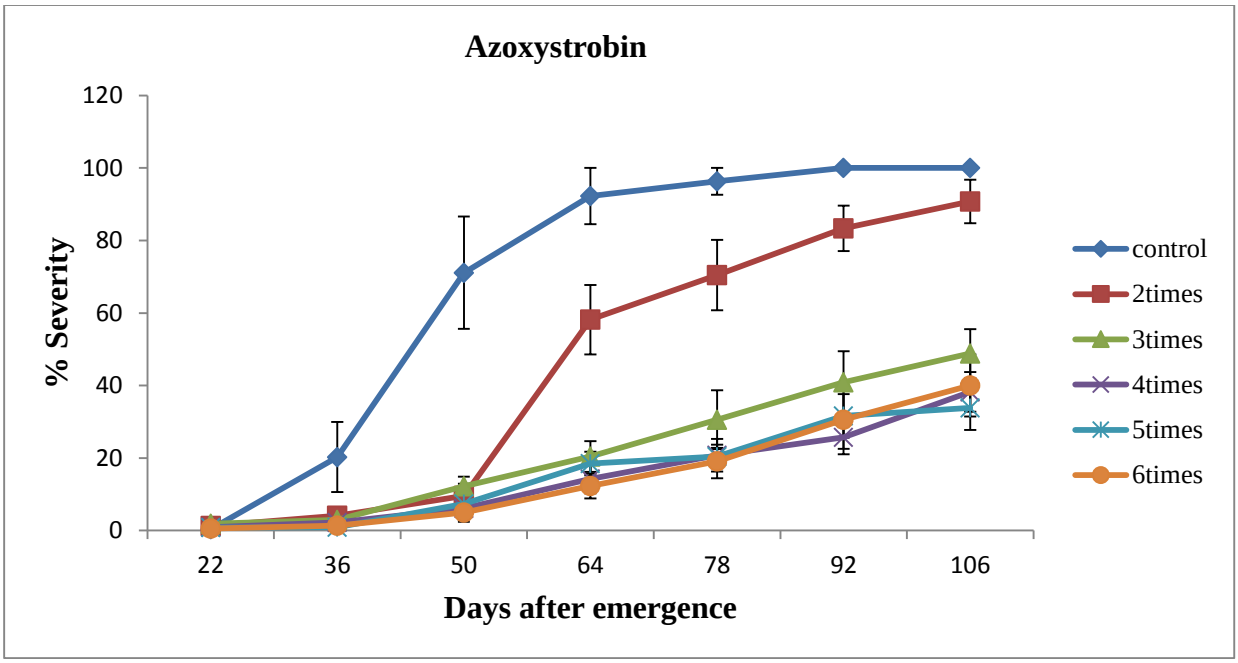


**Figure 3: Disease progress curve for application of azoxystrobin at ATC, Koibatek in 2014.**

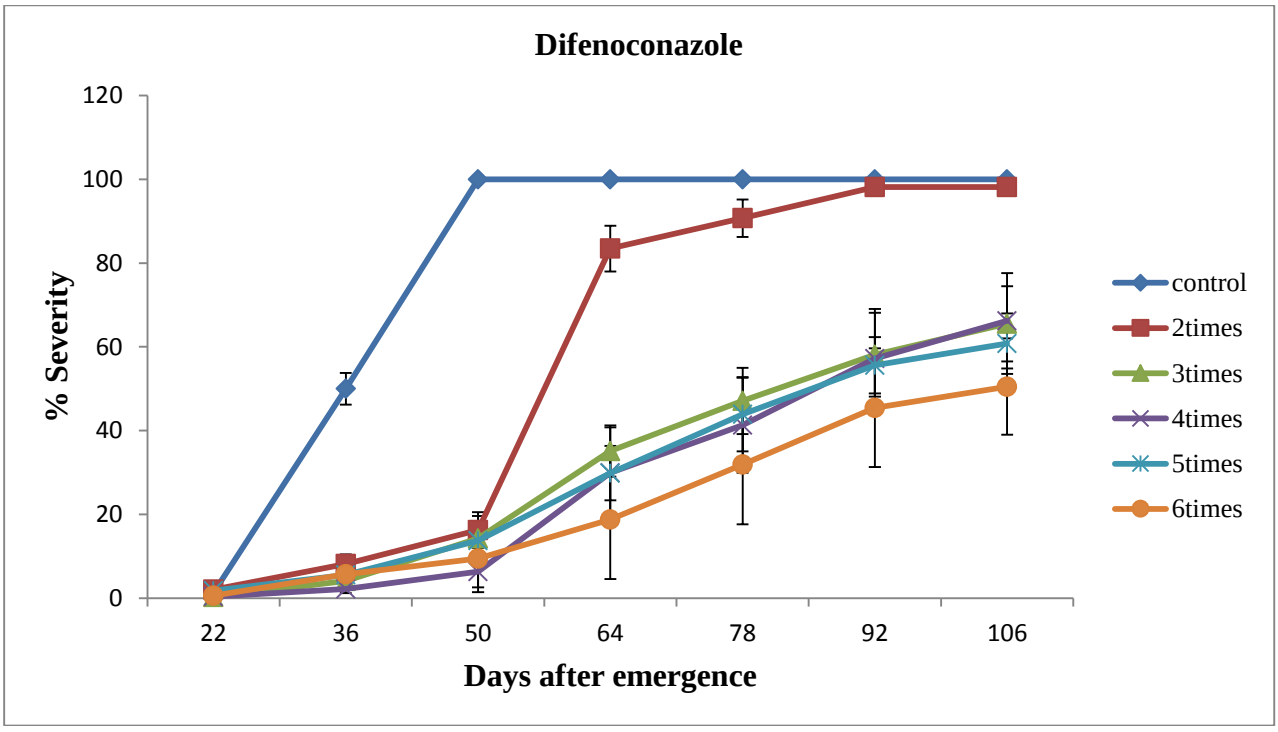


**Figure 4: Disease progress curve for application of difenoconazole at ATC, Koibatek in 2014.**

## DISCUSSION

Ascochyta blight was significantly high compared to controls in these experiments, causing extensive girdling of stems and subsequent breakage and death. Both disease incidence and severity were higher at Egerton-Njoro than Koibatek. This was favored by the higher rainfall and cooler temperatures that characterizes Egerton-Njoro. Other findings have found that high rainfall as well as low temperatures favors spread and development of ascochyta blight (Kimurto, *et al*., 2013; Shtienberg *et al*., 2006; Akem *et al*., 2004). Disease severity and incidence increased steadily with time across the two locations and reduced with increasing number of sprays for both fungicides. There was no significant difference in severity compared to control when fungicides were sprayed two times at Egerton-Njoro. Additionally, there was higher than 50% severity at the same location when three sprays were applied. At Koibatek however, a three spray application of either fungicide was able to maintain disease severity below 30%. Koibatek is relatively drier and temperature is relatively higher (mean ranges 18.2-24.3$^{o}$C) favoring slow development of disease compared to Njoro whose temperatures mean are lower (16-19.1$^{o}$C) favouring fast disease development. Application of two or three fungicide sprays of either azoxystrobin or difenoconazole may not be effective in controlling ascochyta blight at the two locations.

Azoxystrobin was superior to difenoconazole in reducing ascochyta severity and incidence at all growth stages, except seedling, across the two locations. Its effectiveness in controlling this disease has been reported in other findings (Wise *et al*., 2009; Dermici *et al*., 2003). Application of fungicides made at seedling and early vegetative stages (two or three sprays) may delay early disease incidence at Njoro but they are not effective in reducing severity throughout the period of growth. (Chongo and Gossen, 2001) reported that plant age did not affect disease progress in a susceptible chickpea variety UC27. Other studies by (Trapero-Casas and Kaiser, 1992) also reported the same. In contrast, partially resistant varieties are highly resistant at seedling and vegetative stages but resistance declines as they approach flowering (Chongo and Gossen, 2001; Nene and Reddy, 1987). Results in this study indicate that upto five foliar sprays at Egerton-Njoro and upto four sprays at Koibatek with azoxystrobin are needed for effective control of ascochyta blight while six sprays at Egerton-Njoro and five sprays at Koibatek with difenoconazole are needed for effective control under high disease pressure on susceptible varieties. Difenoconazole may have better control of *A. rabiei in vitro* (Dermici *et al*.,

2003) but it appeared to be slightly inferior in the field as compared to azoxystrobin. The strobilurin component has been found to have to have strong activity on *T. discolor* (Andrew *et al*., 2010) in almonds, and in brown spot (*Corynespora cassiicola*) and black spot (*Asperisporium caricae*) compared to triazole compound including difenoconazole (Vawdrey *et al*., 2008).

Seed dressing with either of the fungicide was mostly insignificant in reducing severity of AB compared untreated controls. In addition, the effect of combining seed treatment with foliar sprays did not show any advantage over the controls at all stages of growth. The most important effect in reducing AB severity was that of foliar application of the fungicides evaluated in this study. Seed in this study were selected from a lot that were visually symptomless. The objective of seed dressing is to protect seedling from seed borne ascochyta. Seed dressing may not protect emerging seedlings from rain-drop splashed or wind borne inocula (Markell *et al*., 2008). Selection of large sized seeds of chickpea, which are symptomless or conducting preliminary seed health tests should be carried out before planting chickpea especially in seed multiplication/breeding programs so as the appropriate fungicide may be applied.

## CONCLUSION

This study concludes that AB incidence and severity in Chania Desi 1 increases progressively from seedling to maturity. A successful control strategy should therefore target to prevent introduction of AB in the field or control disease immediately after infection regardless of the growth stage of the crop. Seed dressing as a control strategy with either of the fungicides used did not prove to be very effective in managing AB in the absence of foliar sprays. Additionally, using fungicides to dress clean seeds (visually asymptomatic) and following the treatment with foliar sprays may not be economical since foliar sprays alone were sufficiently effective the number of sprays being constant. Azoxystrobin applied four to five times and difenoconazole applied five to six times at seedling to flowering stages of chickpea growth are needed for effective control of AB in Egerton-Njoro and ATC-Koibatek.

## REFERENCES


Akem, C., Kabbabeh, S., and Ahmed, S. (2004). Integrating Cultivar Resistance with a single fungicide spray to manage ascochyta blight for increased chickpea yields. *Plant Pathology, 3* (2): 105-110.

Amadioha, A.C. (2003). Evaluation of some plant leaf Extract against *Colletotrichum lindemuthianum* in Cowpea. *Acta Phytopathological et Entomologica Hungarica*, 38 (3-4**)**: 259 – 265.

Andrew, H., Trevor, W., and Doug, W. (2010). Field evaluation of fungicides for the control of rust, brown rot, shot hole and scab in almonds. *Australasian Plant Pathology, 39*: 112-119.

Chen, W., and Muehlbauer, F. J. (2003). An improved technique for virulence assay of Ascochyta rabiei on chickpea. *Int Chickpea Pigeonpea Newsletter, 10*, pp. 31-33.

Chongo, G., and Gossen, B. D. (2001). Effect of plant age on resistance to Ascochyta rabiei in chickpea. *Canadian Journal of Plant Pathology, 23*: 358-363.

Dermici, F., Bayraktar, H., Babaliogullu, I., Dolar, F. S., and Maden, S. (2003). In vitro and In vivo effects of some fungicides against the chickpea blight pathogen, Ascochyta rabiei. *J. Phytopathology, 151*: 519-524.

Gan, Y., Siddique, K., MacLeod, W., and Jayakumar, P. (2006). Management options for minimizing the damage by ascochyta blight (Ascochyta rabiei) in chickpea (Cicer arietinum L.). *Field Crops Research, 97*: 121–134.

Jaetzold, R., and Schmidt, H. (1983). *Farm Management Handbook of Kenya* (Vol. Volume II/B). Nairobi, Kenya: Ministry of Agriculture, Kenya, in cooperation with Germany Agricultural Team (GAT) of Germany Agency for Technical Cooperation (GTZ).

Jan, H., and Wiese, M. V. (1991). Virulence forms of Ascochyta rabiei affecting chickpea in the Palouse. *Plant Disease, 75*: 904-906.

Kaiser, W. J., Ramsey, M. D., Makkouk K, M., Bretag, T. W., Acikgoz, N. and Kumar, J. (2000). Foliar diseases of cool season legumes and their control. *In 'linking research and marketing opportunities for pulses in the 21st century.'* (pp. 437-455). Dordrecht: Kluwer Academic Publishers.

KEPHIS, (2013). Kenya Plant Health Inspectorate Service. National Performance Trials for Variety Release in Kenya. *Annual reports*, Government of Kenya Printers, Nairobi, Kenya.

Kimurto, P. K., Towett, B. K., Mulwa, R. S., Njogu, N., Jeptanui L, J. and Gangarao, N. R. (2013). Evaluation of Chickpea genotypes for resistance to Ascochyta blight (Ascochyta rabiei) disease in the dry highlands of Kenya. *Phytopathologia Mediterranea, 52* (1): 613-622.

Montaser, F. A. (2011). Integrated management of damping-off, root and/or stem rot diseases of chickpea and efficacy of the suggested formula. *Not Sci Biol , 3* (3): 80-88.

Nene, Y. L., and Reddy, M. V. (1987). *Chickpea diseases and their control.* (M. C. Singh, Ed.) Oxforshire: CAB (Commonwealth Agricultural Bureaux).

Pande, S., Sharma, M., Gaur, P. M., Tripathi, S., Kaur, L. and Basandrai, A. (2011). Development of screening techniques and identification of new sources of resistance to Ascochyta blight disease of chickpea. *Australian Plant Pathology, 40*: 149-156.

Pande, S., Siddique, H. M., Kishore, G. K., Bayaa, B., Gaur, P. and Gowda, C. L. (2005). Ascochyta blight of chickpea (Cicer arientinum L.): a review of biology, pathogenecity and disease management. *Australian Journal of Agricultural Research, 56*: 317-332.

Shtienberg, D., Kimber R, B. E., McMurray, L., and Davidson, J. A. (2006). Optimisation of the chemical control of ascochyta blight in chickpea. *Australian Plant pathology, 35*: 715-724.

Singh, K. B., and Reddy, M. V. (1996). Improving chickpea yield by incorporating resitance to Ascochyta blight. *Theory of Applied Genetics, 92*: 509-515.

Trapero-Casas, A., and Kaiser, W. J. (1992). Influence of temperature, wetness period, plant age and inoculum concentration on infection and development of ascochyta blight of chickpea. *Phytopathology, 82*: 589-601.

Vawdrey, L. L., Grice, K. R., & Weterhuis, D. (2008). Field and laboratory evalutions of fungicides for the control of brown spot (Corynepora cassiicola) and black pot (Asperisporium caricae) of papaya in far north Queensland, Australia. *Australian Plant Pathology, 37*: 552-558.

Wise, A. K., Henson, A. R., & Bradley, A. C. (2009). Fungicide seed treatment effects on seed-borne Ascochyta rabiei in chickpea. *HortTechnology, 19* (3): 533-537.

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1 CONCLUSIONS

Seed dressing with azoxystrobin, azoxystrobin+difenoconazole and Metalaxyl+mancozeb may significantly increase seedling emergence and delay ascochyta blight incidence and subsequently severity in chickpea production. Foliar spray with azoxystrobin, difenoconazole and azoxystrobin+difenoconazole was effective in reducing ascochyta disease incidence and severity especially under low disease pressure (Koibatek). To contain ascochyta blight, upto four foliar sprays are required under low disease environments while five foliar application of either azoxystrobin or difenoconazole may be needed when disease pressure is high. The most important stages in management of ascochyta blight in Chania Desi 1 are in the early vegetative and flowering stages of growth.

## 5.2 RECOMMENDATIONS

1. Following seed dressing with multiple applications of foliar fungicide sprays may not be economical when using clean seeds in chickpea production in low disease pressure environments. However, the practice may be useful when there is high infection with ascochyta in the planting materials or when producing chickpea under high disease pressure environments (Njoro).
2. Production of chickpea under high disease environment such as Njoro may not be economically viable even with multiple applications of foliar fungicides.
3. Further screening of broad spectrum fungicides with different modes of action is necessary to strengthen the pool of available fungicides for integrated programmes in ascochyta disease management.
4. More efforts in breeding for resistance is necessary to replace the existing commercial varieties whose resistance is not sufficient.

## APPENDICES

Appendix 1. Mean squares for the percentage disease incidence (PDI) and disease severity index (DSI) for fungicides (azoxystrobin, difenoconazole, azoxystrobin+difenoconazole and metalaxyl) application as seed dressing and foliar spray at different stages of chickpea growth in Egerton-Njoro and ATC-Koibatek in the year 2013.

| Source of Variation | df | PDI | | DSI | |
|---|---|---|---|---|---|
| | | Njoro | Koibatek | Njoro | Koibatek |
| **Rep** | 2 | 566.79*** | 2654.48*** | 769.25** | 227.32 ns |
| **Fungicide** | 3 | 1119.37*** | 2808.03*** | 703.69** | 796.51*** |
| **Spray regime** | 1 | 6108.57*** | 16473.56*** | 17212.71*** | 1394.81*** |
| **Seed treatment** | 1 | 2046.00*** | 4488.83*** | 305.75 ns | 713.39** |
| **Stage** | 4 | 78025.05*** | 30014.33*** | 34270.41*** | 9167.33*** |
| **Fungicide*Spray regime** | 3 | 397.71*** | 1089.86*** | 346.48 ns | 215.14 ns |
| **Fungicide*Seed treatment** | 3 | 104.23 ns | 65.40 ns | 243.51 ns | 74.29 ns |
| **Fungicide*Stage** | 12 | 293.19*** | 302.58** | 632.36*** | 185.23* |
| **Spray regime*Seed treatment** | 1 | 570.85*** | 1463.23*** | 28.88 ns | 14.86 ns |
| **Fungicide*Spray regime*Seed treatment** | 3 | 20.88 ns | 41.59 ns | 212.54 ns | 172.82 ns |
| **Spray regime*Stage** | 4 | 1517.63*** | 1726.24*** | 2995.27*** | 607.59*** |
| **Fungicide*Spray regime*Stage** | 12 | 144.84** | 306.84** | 271.99 ns | 144.06 ns |
| **Seed treatment*Stage** | 4 | 299.76*** | 85.93 ns | 120.21 ns | 31.54 ns |
| **Fungicide*Seed treatment*Stage** | 12 | 88.43 ns | 48.43 ns | 157.50 ns | 116.21 ns |
| **Spray regime*Seed treatment*Stage** | 4 | 125.83* | 44.39 ns | 247.47 ns | 90.62 ns |
| **Fungicide*Spray regime*Seed treatment*Stage** | 12 | 50.54 ns | 29.57 ns | 67.06 ns | 55.20 ns |
| **Error** | 158 | 49.31 | 107.60 | 156.41 | 97.32 |
| **$R^2$** | | 0.98 | 0.91 | 0.88 | 0.77 |
| **CV** | | 10.34 | 31.20 | 24.32 | 34.33 |

*, **, *** Significant at ($P \leq 0.05$), ($P \leq 0.01$) and ($P \leq 0.001$) respectively
ns, not significant

Appendix 2. Mean squares for the percentage disease incidence (PDI) and disease severity index (DSI) for fungicides (azoxystrobin and difenoconazole,) application as seed dressing and foliar spray at different stages of chickpea growth in Egerton-Njoro and ATC-Koibatek in the year 2014.

| Source of Variation | df | PDI | | DSI | |
|---|---|---|---|---|---|
| | | Njoro | Koibatek | Njoro | Koibatek |
| **Rep** | 2 | 2235.93** | 7263.23*** | 5073.73*** | 4257.39*** |
| **Fungicide** | 1 | 5121.99*** | 13361.16*** | 3915.04*** | 26449.63*** |
| **Spray regime** | 5 | 31234.34*** | 30453.18*** | 63648.91*** | 34982.95*** |
| **Seed treatment** | 1 | 374.93 ns | 62.16 ns | 404.83 ns | 543.56 ns |
| **Stage** | 6 | 78103.47*** | 62279.8*** | 50358.96*** | 51817.44*** |
| **Fungicide*Spray regime** | 5 | 840.34 ns | 477.71 ns | 910.27*** | 855.16** |
| **Fungicide*Seed treatment** | 1 | 86.75 ns | 1423.43* | 42.12 ns | 1841.58** |
| **Fungicide*Stage** | 6 | 1465.80* | 553.67* | 600.77** | 1367.20*** |
| **Spray regime*Seed treatment** | 5 | 291.21 ns | 145.08 ns | 343.47 ns | 821.31** |
| **Fungicide*Spray regime*Seed treatment** | 5 | 1370.21* | 220.00 ns | 75.01 ns | 537.96* |
| **Spray regime*Stage** | 30 | 5806.42*** | 1965.87*** | 2788.84*** | 2114.08*** |
| **Fungicide*Spray regime*Stage** | 30 | 588.86 ns | 291.28 ns | 213.06 ns | 374.74* |
| **Seed treatment*stage** | 6 | 47.17 ns | 68.08 ns | 101.54 ns | 46.49 ns |
| **Fungicide*Seed treatment*Stage** | 6 | 66.01 ns | 53.70 ns | 31.09 ns | 122.96 ns |
| **Spray regime*Seed treatment*Stage** | 30 | 198.59 ns | 60.85 ns | 52.04 ns | 88.94 ns |
| **Fungicide*Spray regime*Seed treatment*Stage** | 30 | 379.62 ns | 57.20 ns | 9.75 ns | 63.62 ns |
| **Error** | 334 | 456.63 | 245.16 | 181.64 | 223.36 |
| **$R^2$** | | 0.85 | 0.89 | 0.92 | 0.89 |
| **CV** | | 27.35 | 33.95 | 25.63 | 39.46 |

*, **, *** Significant at ($P \leq 0.05$), ($P \leq 0.01$) and ($P \leq 0.001$) respectively
ns, not significant

Appendix 3. Mean squares for the Area Under Disease Progress Curve (AUDPC) for fungicides (azoxystrobin, difenoconazole, azoxystrobin+difenoconazole and Metalaxyl) application as seed dressing and foliar spray at different stages of chickpea growth in Egerton-Njoro and ATC-Koibatek in the year 2013.

| Source of Variation | df | Njoro | Koibatek |
|---|---|---|---|
| **Rep** | 2 | 885292.30 ns | 82828.31 ns |
| **Fungicide** | 3 | 1366430.00 ** | 266059.70 ns |
| **Spray regime** | 1 | 22263375.00 *** | 419254.10 ns |
| **Seed treatment** | 1 | 212942.90 ns | 351576.30 ns |
| **Fungicide*spryreg** | 3 | 285512.50 ns | 116040.80 ns |
| **Fungicide*Seed treatment** | 3 | 379067.40 ns | 52131.06 ns |
| **Spray regime*Seed treatment** | 1 | 26058.25 ns | 3924.08 ns |
| **Fungicide*Spray regime*Seed treatment** | 3 | 231979.40 ns | 96683.69 ns |
| **Error** | 30 | 273117.57 | 105471.40 |
| **$R^2$** | | 0.79 | 0.64 |
| **CV** | | 13.39 | 25.57 |

*, **, *** Significant at ($P \leq 0.05$), ($P \leq 0.01$) and ($P \leq 0.001$) respectively
ns, not significant

Appendix 4. Mean squares for the Area Under Disease Progress Curve (AUDPC) for fungicides (azoxystrobin and difenoconazole,) application as seed dressing and foliar spray at different stages of chickpea growth in Egerton-Njoro and ATC-Koibatek in the year 2014.

| Source of Variation | df | Njoro | Koibatek |
|---|---|---|---|
| **Rep** | 2 | 6868959** | 5816469** |
| **Fungicide** | 1 | 5375799* | 36506191*** |
| **Spray regime** | 5 | 86641846*** | 48622024*** |
| **Seed treatment** | 1 | 528112.8 ns | 768447.9 ns |
| **Fungicide*Spray regime** | 5 | 1275457 ns | 1172229 ns |
| **Fungicide* Seed treatment** | 1 | 64276.6 ns | 2505842 ns |
| **Spray regime* Seed treatment** | 5 | 475278.3 ns | 1137023 ns |
| **Fungicide*Spray regime* Seed treatment** | 5 | 97006 ns | 750095.6 ns |
| **Error** | 46 | 883950.70 | 1108772.80 |
| **$R^2$** | | 0.92 | 0.86 |
| **CV** | | 18.29 | 28.34 |

*, **, *** Significant at ($P \leq 0.05$), ($P \leq 0.01$) and ($P \leq 0.001$) respectively

ns, not significant