CURRENT STATUS OF SUNFLOWER LEAF BLIGHT CAUSED BY *Alternaria helianthi* IN IRAMBA AND HANDENI DISTRICTS IN TANZANIA

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ABSTRACT

Surveys were conducted to investigate the incidence and severity of *Alternaria* blight disease caused by *Alternaria helianthi* in Iramba and Handeni districts. A total of 30 seed samples collected from farmers were tested for infection by *A. helianthi* and other fungi using the Blotter method. Identification of fungi was based on morphological characteristics observed under the stereo and compound microscopes and confirmed by pathogenicity tests done in the screen house. Screen house and field data were analyzed using SAS 2004 and SPSS computer package and mean separation was done by Duncan Multiple Range Test at ≤ 0.05. Results showed that sunflower seed samples were infected by *A. alternata* (47.75 %), *F. palidoroseum* (16.25 %), *F. moniliforme* (12.0 %), M. *phaseolina* (3.75 %), and *A. helianthi* (3.75 %). Seed infection by *A. helianthi* was lower than that of other seed-borne pathogens. However, it was associated with the highest disease incidence (22.7 %) compared to *A. alternata* (13.5 %) and *Fusarium species* (1.5 %), under field conditions. Such results implied that low seed infection by *A. helianthi* can result into high disease incidences under field conditions. The results also showed that *A. helianthi* can be categorized into two groups based on their morphology. The first group had large radial growth, gray mycelia colour and smooth margin and the second group had grayish black appearance and irregular margins on V8 agar medium, implying variations of *A. helianthi*. The results also showed that most of the farmers (70 %) in the study areas were unaware of the disease and 76.7 % of farmers did not apply any control measure for *Alternaria* blight disease. Future studies should therefore, focus on factors that influence high level of disease incidences and severity in the field compared to low level of seed infection. Further studies on the genetic variation between the two groups of *A. helianthi* are also needed. Training of the farmers on identification of *Alternaria* blight disease is of importance for early disease management.

DECLARATION

I, Stella Gamaliel Chirimi, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor being concurrently been submitted for a degree award to any other institution.

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7/10/2013
Date

The above declaration is confirmed by

Prof. R. B. Mabagala
(Supervisor)

07/10/13
Date

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DEDICATION

I dedicate this dissertation to my beloved father, the late Mr. Gamaliel Barsaba Ntenga and my mother Shammedesyo Malema who is behind all academic achievement I have made.

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LIST ABBREVIATIONS AND SYMBOLS

AfSHC	African Seed Health Centre
ARI	Agricultural Research Institute
ANOVA	Analysis of Variance
cm	Centimeter
CRD	Completely Randomized Design
CV	Coefficient of variation
DALDO	District Agricultural and Livestock Development Officer
DMRT	Duncan Multiple Range Test
EPPO	European and Plant Protection Organization
FAOSTA	Food Agricultural Organization Statistics
gm/m^2	Gram per meter square
h	Hour
ha	Hectare
IGN	Iguguno
ISTA	International Seed Testing Association
KM	Kwamatuku
ml	Milliliter
MS	Misima
MT	Mtoa
No	Number
Nos	Numbers
°C	Degree Celsius
PDA	Potato Dextrose Agar

R^2	Coefficient of Determination
SH	Shelui
SIIGG	Singida Iramba Iguguno
SIM	Singida Iramba Mtoa
SIS	Singida Iramba Shelui
SPSS	Statistical Package for the Social Science
SAS	Statistical Analysis System
SUA	Sokoine University of Agriculture
THKWA	Tanga Handeni Kwamatuku
THMIS	Tanga Handeni Misima
THVIB	Tanga Handeni Vibaoni
USA	United States of America
t	Tonne
V8	Vegetable juice
VN	Vibaoni
%	Percent

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Sunflower (*Helianthus annuus* L.) is an important oil seed crop grown globally (FAOSTAT, 2009; Rao and Ramgoap, 2010). Between 2008 and 2009 the world sunflower seed production was 33.3 million tons and the European Union, Russia, Ukraine and Argentina are the major producers of sunflower seed in the world (Kakde *et al.*, 2012). Sunflower is also produced by other countries including France, China, India, Turkey and USA (Dinesh, 2009).

In Tanzania, sunflower is among the major oilseed crops grown by small scale farmers. Other oil seed crops include cotton, groundnuts, sesame and soybeans (David, 2009). The main sunflower growing regions in Tanzania include Singida, Dodoma and Rukwa. Other growing regions include Iringa, Manyara Shinyanga, Mbeya, Morogoro and Tanga (Mpagalile *et al.*, 2008). In 2004 and 2005 these regions produced 98.12 % of the total sunflower production in the country (Ministry of Agriculture, 2008). Sunflower is popular in Tanzania due to its ability to withstand drought conditions, matures early and is high yielding compared to other rainfed crops (David, 2009). In addition, it has high oil content of about 40-50 %. The sunflower industry also provides employment to small and medium scale farmers through manpower absorption in various stages of its marketing and processing (Mpagalile *et al.*, 2008). Extracted oil from sunflower contributes about 40 % of the national cooking oil requirements and the balance of 60 % is met through imports (Mpagalile *et al.*, 2008).

Despite the role sunflower plays in Tanzania, its yield remains low in the country. The average sunflower production by small holder farmers in Tanzania ranges from 0.5 to 0.7 t/ha lowers than the recommended yield of 2.0 t/ha (Kanyeka *et al.*, 2007). Low yields are attributed by several factors such as occasional adverse climatic condition, poor agronomic practices, lack of improved and sufficient seed that forces farmers to use their own-saved seeds, prevalence of diseases and insect pests (Mpagalile *et al.*, 2008, Thebaud *et al.*, 2011).

Sunflower is attacked by a number of seed-borne diseases caused by fungi, bacteria, nematodes and viruses (Mirza and Beg, 1983). Among fungal pathogens reported to infect sunflower worldwide are *Alternaria alternata, A. helianthi, A. zinnie, Aspergillus flavus, A. niger, Macrophomina phaseolina,* various species of *Cladosporium, Curvularia, Fusarium* and *Penicillium* (Raj *et al.*, 2007). These fungi are capable of reducing the viability, seed germination, seedling vigor, and poor crop stand in the field resulting in low yields and quality of the crop (Afzal *et al.*, 2010).

Alternaria blight caused *by A. helianthi* has been considered as one of the major destructive disease of sunflower all over the world, particularly in the tropical and sub-tropical regions (Rao, 2006). The incidence and intensity of *Alternaria* blight disease caused by *A. helianthi* is predisposed by the season, variety grown, and growth stage of the crop, region and climatic conditions (Irum, 2009). The infected plants are characterized by irregular yellow lesions on the leaf margins, stems, and petioles and finally the plant dries (Patel *et al.*, 2010). Since 1950, when the disease was identified in Tanzania, little effort has been employed on the status and management of the *Alternaria* blight disease. Therefore, there is a need to conduct studies on *Alternaria* blight of sunflower in Tanzania to generate relevant data that can be used to manage the disease.

1.2 Problem Statement and Justification

Alternaria blight is a destructive disease which reduces plant height, stem diameter, flower size, head diameter and number of seeds per head that contribute to yield losses (Sasha *et al.*, 2004). The yield losses of 20 to 80 % and oil losses of 20 to 30 % have been reported in the tropical and subtropical regions including Tanzania (Shankergoud *et al.*, 2006; Howard and David, 2007). The seeds and oil losses of 27-80 % and 17-33 %, respectively, have been reported in Brazil (Hiremath *et al.*, 1993; Prasad and Kulshrestha, 1999; Calvet *et al.*, 2005). Disease incidence ranging between 26 and 46 % has been reported in India (Reddy and Gupta, 1977). The *Alternaria* leaf blight disease was reported in 1950 in Tanzania (Wallace and Wallace, 1950; Carson, 1985) and since then, the incidence and severity of the disease have not been studied and documented.

Recently, *Alternaria* blight has continued to be a threat in sunflower production in Tanzania. Complains of the disease on sunflower have been reported from different parts of Tanzania (Mwakitwange, E., Personal Communication, 2011). In Tanzania, acreage under sunflower production is increasing. This is because the crop is used both as a source of oil for human consumption and animal feed in most part of the country thus, an important cash crop for both farmers and consumers. However, productivity is declining because of the diseases (Kanyeka *et al.*, 2007). The study on the causal agent of the disease, *A. helianthi* is of profound importance due to the increasing demand of sunflower and the impact of the disease on yield. Therefore, there is a need to investigate the status of the disease including characterizing the pathogen to distinguish it from other fungal species infecting sunflower and document disease management methods employed by farmers. Such studies will be useful for appropriate planning of effective management of

the disease to minimize losses caused by *A. helianthi,* and effective planning for development of new varieties resistant to *Alternaria* blight disease in Tanzania.

1.3 Research objectives

1.3.1 Overall objective

The main objective was to evaluate the effect of *Alternaria* blight disease of sunflower, characterize causative agent and analyze management options applied to control the disease in Handeni (Tanga) and Iramba (Singida) Districts.

1.3.2 Specific objectives

i. To determine the incidence and severity of *Alternaria* blight disease of sunflower in Handeni and Iramba Districts;

ii. To isolate and characterize the causal agent of *Alternaria* blight disease of sunflower in Handeni and Iramba Districts and

iii. To determine management practices used by farmers in the control of *Alternaria* blight disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin, Distribution and the Importance of Sunflower

Sunflower (*Helianthus annuus* L.) belongs to the family *Asteraceae*. It is one of major oilseed crop grown globally and believed to originate from North America (Hambwe, 1992). Sunflower is now grown in many countries including temperate and tropical areas (Purseglove *et al.*, 1990). In Africa, sunflower is grown in South Africa, Zimbabwe, Sudan, Kenya, Uganda and Tanzania (Raymond and Nalumasi, 1996).

Sunflower seeds contain 40-50 % oil, 23 % protein and it is a source of unsaturated fat acid, fibre, minerals (Copper, Calcium, Magnesium, Phosphorous and Selenium) and vitamins (Vitamin B complex and E). It is thus, used for the manufacturing of edible oil for human consumption and oilcake for animals and poultry (Gonzalez *et al.*, 2002). Sunflower is also used in manufacturing vanishes, paints, detergents and soap and plastics due to its semi-drying properties with linonic acid.

2.2 History, Distribution of the Pathogen and Importance

Alternaria leaf blight disease of sunflower was first reported in 1892 in USA and currently it has been reported in 33 countries worldwide (Kong *et al.*, 1995). In 1943, the pathogen was described causing blackish brown spots on leaves of sunflower in Uganda and it was reported to be caused by *Helminthosporium helianthi* (Mirza *et al.*, 1984). In 1962 the disease was detected in Rhodesia causing breakages in stems of sunflower. In 1969, the pathogen was isolated from diseased leaves of sunflower in Japan and compared with the results of Hansford's specimen of *H. helianthi*, and concluded that, both pathogens were identical and was renamed as *A. helianthi* (Mirza *et al.*, 1984).

Recently, Simmons (2007) designated the pathogen as the affiliate of *Alternariaster* and renamed as *Alternariaster helianthi*, based on the non-existence of conspicuous internal pigmented, circumhilar ring found commonly in *Alternaria* conidia and conidiophores. *Alternaria* blight disease caused by *A. helianthi* has been of importance mainly in the tropical and sub- tropical regions due to high temperatures in combination with high humidity (Kong *et al.*, 1997). It causes seedling decay leading to reduced seed germination and poor crop stand in the field (Srinivas *et al.*, 1998; Rao, 2006; Shanka *et al.*, 2010).

2.3 Morphological Features of the Pathogen

Conidiophores of *A. helianthi* arise singly or in group, they are simple or branched, straight or flexuous, cylindrical, pale to pale brown, smooth, up to 5-septate and sometimes with scars 1-2, up to 130 µm long and 6-12 µm thick (Mesta, 2006). The pathogen produces light gray to gray mycelia growth on seeds and conidia appear sub-hyaline to translucent (Mathur and Kongsdal, 2003). The fungal growth on seeds appears olivaceous brown. The conidia are solitary, cylindrical to obclavate, with rounded ends, pale olivaceous brown or golden brown, smooth with 2-12 transverse septa, sometimes one or few longitudinal septa, prominent constriction at the septa and measure 45-145 x 10-30 µm (Mathur and Kongsdal, 2003).

2.4 Sympomatology

Alternaria leaf blight affects all the aerial parts of the plant (Balasubrahmanyam and Kolte; 1980). Infected plants become visible in 40-65 days and show changes in colour, from normal green to pale yellow or yellow with blackish lesions on the leaf margin. The leaves form oval to circular, dark brown to black lesions with concentric rings (Patel *et al.*, 2010). Some lesions have distinct yellow halos on young plants and the

lesions do not cross major leaf veins while becoming angular in shape as the leaf ages. Under severe infection, the lesions on leaves become irregular by coalescing leading to blight and defoliation and death of the plant (Tubaki and Nishihara, 1969). Stem lesions begin as dark flecks that enlarge forming sunken lesions. Large, blackened stem lesions can girdle plants and cause stem breakage (Hiremath *et al.*, 1990; Howard and David, 2007; Singh and Ferrin, 2012). Infection of the disease is greater when the plants weakens and senesce (Allen *et al*,. 1982). It occurs on older leaves of plants before flowering and heaviest infections occur during seed development.

2.5 Epidemiology and Host Range of *Alternaria helianthi*

The sources of pathogen inoculum are contaminated seeds where the pathogen can survive on the outer and inner parts of the seed (Krishnappa and Shetty, 1990). When seeds infected by *A. helianthi* are sown, seedling blight develops indicating the role of seed-borne inoculum (Hiremath *et al.*, 1993) (Fig. 1). Other sources of infection are plant debris and weed hosts. Seedling blight may develop when sunflower plants emerge in rainy weather on *Alternaria* infected land. Hot weather and frequent rains at milk and wax stages of development are more favorable to infection. A combination of high temperature and prolonged wetness alternating with periods of dryness is favorable for sporulation of the fungus (Venkataramana *et al.*, 1995; Cho and Yu, 2000). Conidia are spread by wind and water. Apart from sunflower, other crops affected by *A. helianthi* are safflower (*Carthamus tinctorious* L.), castor (*Ricinus communis*) and cocklebur (*Xanthium* species) (Appaji, 1995; Mesta, 2006).

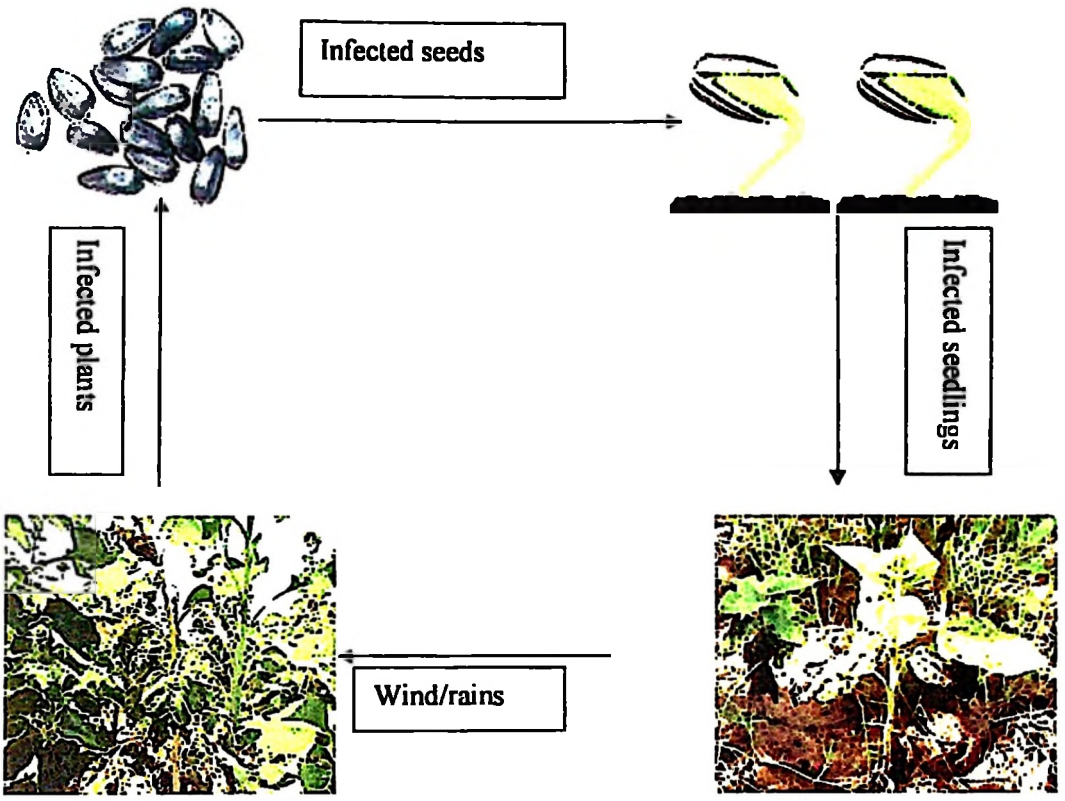

Figure 1: Sketch indicating spread of *Alternaria helianthi* from infected seeds to uninfected field

2.6 Crop Management

2.6.1 Cultural control

Cultural control involves effective management of sunflower crop debris such as deep cultivation that burry crop residues and promote residue decomposition (Chaube and Singh, 2001). Crop rotation over periods that are longer than the period of the pathogen survival in the field may reduce or eliminate the inocula before sunflower is planted (Mesta *et al.*, 2009). Adjustment of sowing dates of sunflower to escape heavy rains during harvesting and use of resistant varieties (Stone *et al.*, 2001).

2.6.2 Chemical control

Different chemicals can be used to control *Alternaria* blight of sunflower. Seed treatment with Thiram and Mancozeb has been used for seed dressing in eradicating or reducing seed borne pathogens (Rao, 2006). Foliar fungicide sprays with a mixture of Dithane M-45 and Endosulfan as soon as the symptoms are observed (OEPP/EPPO, 2000).

2.6.3 Plant extracts

The use of chemicals has been discouraged in the present system of management of disease or pest. The concept of organic farming and eco-friendly management have encouraged plant protection specialist to opt for plant extracts that posses natural substances toxic to many fungi causing plant diseases for the management of diseases and pests (Ramjegathesh *et al.*, 2011). Thus, several plant extracts have been used in the control of *Alternaria* blight of sunflower. Among the plant extracts used, Neem leaf (*Azadirachta indica*) extract, neem kernel extract and garlic bulb (*Allium sativum*) extract are reported be effective against *Alternaria* species (Sivagami, 2003) and can control spore germination to the extent of 30 %.

2.6.4 Integrated pest management

Integration of chemicals, plant extracts and biotic agents for managing plant disease has been considered as a novel approach. It requires low amount of chemicals, reducing the cost of control and pollution hazards while causing minimum interference with biological equilibrium (Rao, 2006). The use of fungicide for seed dressing, bioagents or botanicals with priming agents has become an important method of disease control, particularly in sunflower and in the absence of resistant cultivars (Chattopadhyay, 1999).

2.6.5 Screening for resistant cultivars against *Alternaria* blight of sunflower

Management of the disease through host plant resistance has been of importance. Utilization of resistant varieties in farming is the simplest, effective and economical method in the management of the disease (Mesta, 2006). Sunflower varieties with certain degree of tolerance to *Alternaria* blight have been developed in India and Brazil (Reddy *et al.*, 2006). However, sunflower hybrids have been reported to be more resistant to *Alternaria* blight disease than sunflower cultivars (Nagaraju *et al.*, 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Field Surveys and Collection of Sunflower Seed Samples

The surveys were conducted in Iramba and Handeni districts (Fig. 2) to collect sunflower seeds from farmers in December 2011. The seeds were collected from the wards of Shelui, Mtoa, Iguguno (Iramba District) and Misima, Vibaoni, Kwamatuku (Handeni District) (Fig.1) which were purposively selected as the main producer of sunflower in respective Districts. A total of 30 farmers were randomly selected and seed samples were collected. From each farmer, one kilogram of sunflower seed was collected and packed in paper bags and labeled (name of the farmer, date collected, harvested date, name of collector and village name). Seeds were then sent to the African Seed Health Centre Laboratory (AfSHC) at Sokoine University of Agriculture (SUA), Morogoro for further analysis. The seed samples were stored in a refrigerator at -4 to 10°C until used.

3.1.1 Working seed samples

The conical divider (Mechanical method) was used to obtain working samples (ISTA, 2005). One kilogram of sunflower seed was poured in the conical divider (Chicago ILL. 1912, Seedeburo Equipment Company) and divided into two equal parts. One halve was returned to the original container and another part separated into another two equal parts. This exercise was repeated for several times until the required working seed sample (200g) was obtained.

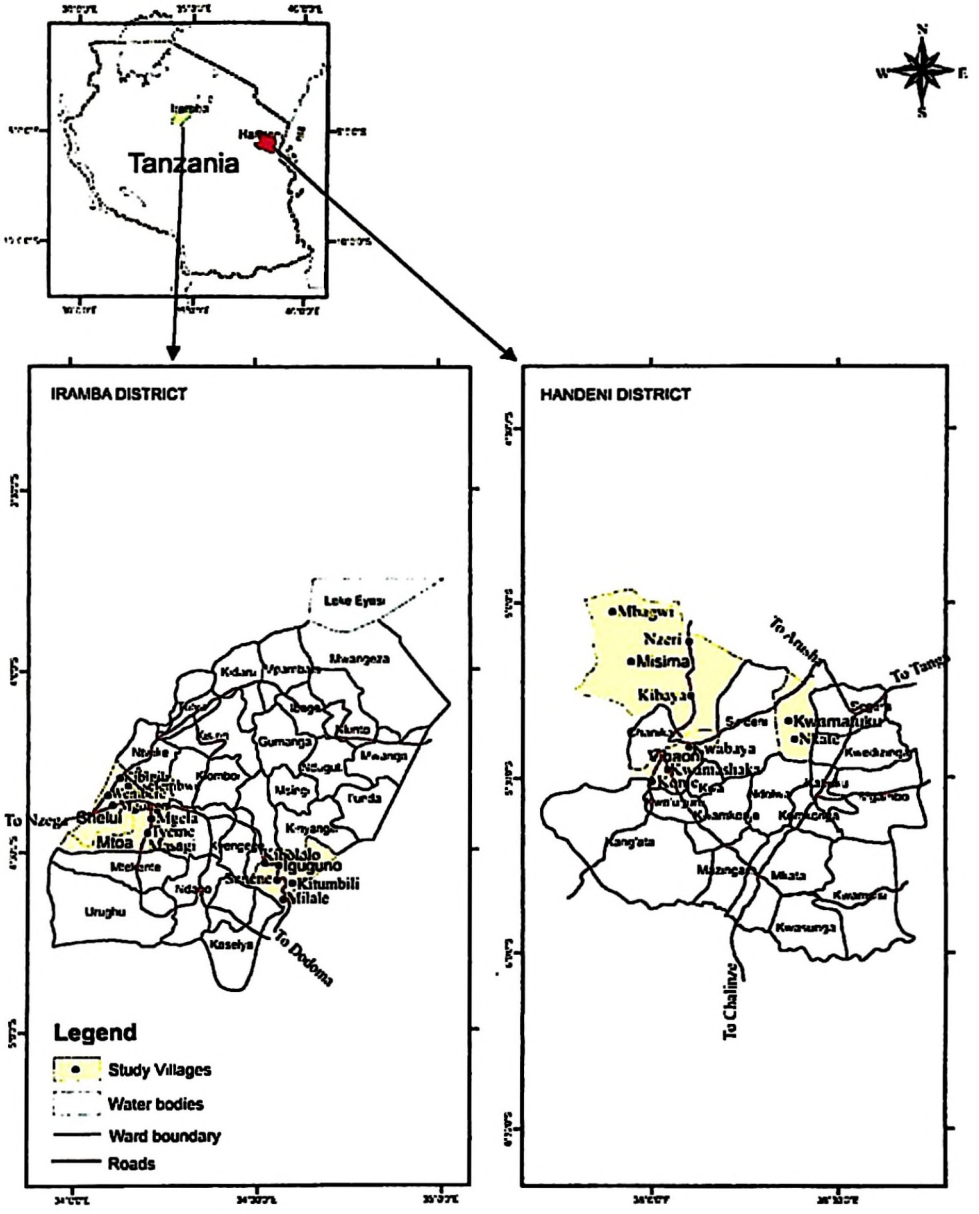

Figure 2: Map of Iramba and Handeni Districts showing wards surveyed for sunflower seed collection and evaluation of incidence and severity of *Alternaria* blight disease

3.1.2 Determination of seed purity from sunflower seed samples

The main objective of purity analysis was to determine and separate pure seeds of sunflower from other various species of seeds and the inert matter that includes sand, sticks, broken seeds and other foreign materials constituting the seed sample (Plate 1). Two hundred gm of each sample was poured on a table and by using spatula, pure seeds were separated from abnormal seeds and inert particles, each component was weighed separately and recorded. All seeds with physical abnormalities, like shriveling of the seed coat, reduction or enlarged seed from normal, and seed discoloration were classified under inert matter. The percentage of each seed component was calculated using the weight of each component divided by the original weight of the seed sample (200 gm) times 100.

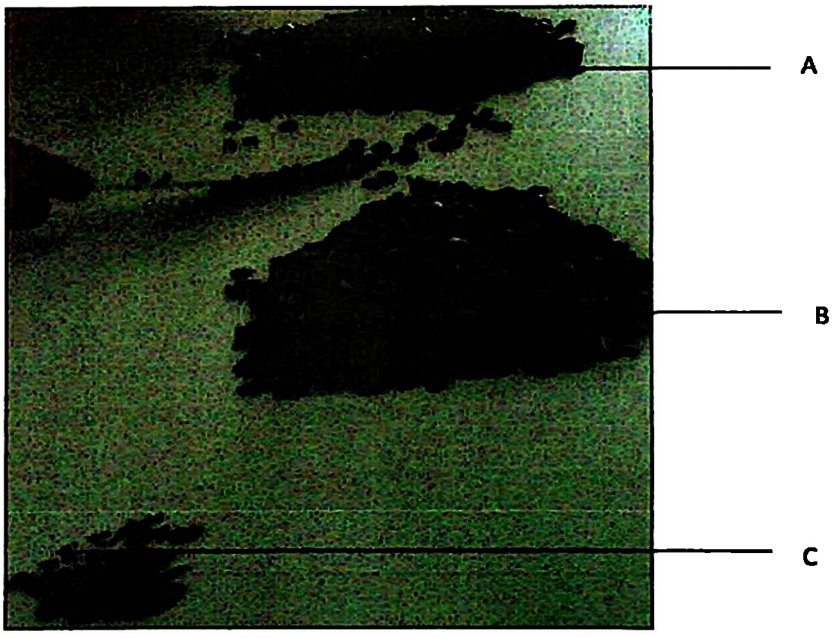

Plate 1: Purity analysis of sunflower seed sample No 4 collected from Mgongo in Iramba District. A = Clean sunflower seed, B = Sunflower seeds mixed with inert matter and C = Inert mater

3.1.3 Detection and identification of microorganisms from sunflower seed samples

The Blotter method was used to detect fungal microorganisms from sunflower seeds as described by Mathur and Kongsdal (2003). Four hundred seeds from each sample were plated in three moist layers of blotter papers poured on Petri dishes. The plates were incubated for 7 days at 20-25 °C under alternating cycles of 12 h light and 12 h darkness (Mathur and Kongsdal, 2003, Gowder *et al.*, 2007). Fungi that developed on each seed were examined under different magnifications on a stereo microscope and identified based on the growth habit, colony characters and conidia which were observed under a compound microscope. The seeds that developed fungal colonies were counted and recorded. Seed infection was calculated using a formula described by Sharfun (2006).

$$\% \text{ seed infection} = \frac{\text{Number of seeds infected with the fungal species}}{\text{Total number of tested seeds}} \times 100 \quad \text{.....(1)}$$

The fungi were isolated from infected seeds using a sterile needle technique (Mathur and Kongsdal, 2003) and grown on Potato Dextrose Agar (5ml) media for purification. The pure culture obtained were maintained on Potato Dextrose Agar (PDA) slant and preserved in a refrigerator at 4 °C for further characterization.

3.1.4 Evaluation of morphological characters of *Alternaria helianthi*

A single germinating conidium of *A. helianthi* grown on PDA slant at 4 °C was transferred to petridishes containing Vegetable juice (V8) medium using inoculating loop. The petri dishes were incubated at room temperature for 10 days. The fungal growth was then examined visually to determine colour, margin and the development of pigments or crystals in the V8 medium following the method described by Barry and Themis (2002). Growth and sporulation for each isolate was recorded by measuring the growth diameter of each isolate using a ruler.

3.1.5 Preparation of inocula for inoculation of sunflower seedlings in the screen house

The pure mycelia of *A. helianthi* isolated from sunflower seed sample cultured on PDA slant were used in the preparation of inocula. The isolates were grown on V8 medium which was prepared using 800 ml distilled water, 400 ml V8 juice, 3 gm Caco3 and 20 gm general Agar. The medium was stirred thoroughly using magnetic stirrer and sterilized in the autoclave at a temperature of 121°C for 15 minutes and allowed to cool. Then 15 mls of the V8 medium was poured into petri dishes and thereafter, inoculated with 5 mm of the fungal hyphae at the center of the petri dish and incubated for ten days in 12 hrs light and 12 hrs darkness at room temperature in the incubation room.

3.1.6 Pathogenicity tests

Pathogenicity tests were done in the screen house in order to confirm whether the isolated fungi from sunflower seeds were able to cause disease symptoms on sunflower plants following procedures described by Lelliot and Stead (1987). The inoculum prepared from the isolates (Table 1) of *A. helianthi* obtained from 30 samples collected from Handeni and Iramba districts were used in this test. The experimental design used was Completely Randomized Design (CRD) (Gomes and Gomez, 1984) with three replications. Fungal isolates were used as treatments. The inocula suspensions contained 1 x 10^3 spores/ml and were determined using Haemocytometer (Depth 1.00 mm, No. BS 748 by Weber England). Using sterile distilled water the fungal suspensions were sprayed-inoculated (using a hand atomizer) onto 28 day-old sunflower seedling, cultivar Record grown in containers with dimension of 30 cm x 32 cm containing 20 kg of sterile compost soil: manure (4:1 ratio, respectively). The inoculated plants were covered with polyethylene bags at 25 °C for 2 days in a screen house.

Table 1: Isolates of *Alternaria helianthi* used for pathogenicity tests in the screen house

Isolates	Pathogen	Ward	Village
SIS_1	*A. helianthi*	Shelui	Kibigiri
SIM_6	*A. helianthi*	Mtoa	Mgela
SIM_7	*A. helianthi*	Mtoa	Tyeme
SIM_8	*A. helianthi*	Mtoa	Masagi
SIM_9	*A. helianthi*	Mtoa	Tyeme
$THMIS_{23}$	*A. helianthi*	Misima	Kibaya
Control	Distilled water		

SIS_1 –Sample from Singida Iramba Shelui in Kibigiri village, SIM_6 – Singida Iramba Mtoa in Mgela village, SIM_7 – Singida Iramba Mtoa in Tyeme village, SIM_8 – Singida Iramba Mtoa in Masagi, SIM_9 – Singida Iramba Mtoa in Tyeme village, $THMIS_{23}$ – Tanga Handeni Misima in Kibaya village

3.1.7 Incidence and severity of *Alternaria* blight in the screen house

The disease incidence and severity of inoculated sunflower plants were recorded at the interval of 14 days until the seed filling stage (61 days). The lower, middle and upper leaves with disease symptoms were counted and recorded at 14, 28, 42 and 61 days after inoculation. Disease severity assessment was done based on a 0-9 score scale as described by Mirza and Hoes (1996); where 0 – no infection, 1 – few small lesions, 2 – lesions on few leaves with no stem lesions, 3 – lesion on few leaves with superficial stem lesions, 4 – few well formed leaf lesions or superficial stem lesions, 5 – few well formed lesions or enlarged stem lesions, 6 – many large leaf lesions or more than 50 % defoliation, 7 – over 75 % of plant area affected or defoliated, 8 – plant highly defoliated, leaves and stem with abundant sporulating lesions and 9 – plant dead. Disease incidence was calculated using the formula below as described by Kutama *et al.* (2009).

$$\text{Incidence} = \frac{\text{total number of diseased plants}}{\text{Number of plants examined}} \times 100 \quad \text{.....(2)}$$

3.1.8 Sunflower seed yield and yield component

The experiment on detecting the effect of *Alternaria helianthi* on sunflower seed yield in the screen house was designed as describes in section 3.1.6. The effect was observed on yield and yield components of sunflower plants inoculated with *A. helianthi* isolates and recorded. The parameters recorded on selected plants were plant height (in centimeters), head size (diameter of sunflower heads in centimeter) and yield of sunflower seeds weight in grams using weighing balance type Sartorius CP1200 IS.

3.2 Determination of Disease Incidence and Severity under Farmer's Fields

Disease incidence and severity were observed in farmers' fields at flowering stage. The assessment was done in farmer fields who previously supplied seeds for the current work. The farmers' fields were divided into two diagonal parts then disease assessment was done on ten plants at ten meters intervals. The disease incidence was done by counting the infected plants among the selected 10 divided by 10 times 100 in order to obtain the percentage of infected plants. The exercise was repeated ten times making a total of 100 plants per field/farmer. This calculation was done using the formula below as described by Kutama *et al.* (2009).

$$\text{Incidence} = \frac{\text{total number of diseased plants}}{\text{Number of plants examined}} \times 100 \quad \text{.....(3)}$$

Disease severity was calculated as described under section 3.1.7. The same plants used for the calculation of disease incidence were used in the determination of disease severity. In addition, infected leaf samples were collected and packed in perforated bags and brought to the African Seed Health Centre (AfSHC) for further isolation of the pathogens. Isolation of pathogens from the leaves was done by chopping the infected leave into small piece based on location. Ten chopped small pieces of leaves from each ward were plated on petri dishes with three moist blotter papers and incubated in the incubation room for 7

days at 20-25 °C under alternating cycles of 12 h light and 12 h darkness (Mathur and Kongsdal, 2003, Gowder *et al.*, 2007). Fungi that developed from each piece were examined under different magnifications on a stereo microscope and identified based on the growth habit, colony characters and conidia which were observed under a compound microscope. This was done to compare the incidence of *A. helianthi* on infected seeds and leaves collected from farmers' fields.

3.3 Determination of Management Strategies Applied by Farmers

A simple checklist was administered to provide information on the perception and knowledge of the farmers on *Alternaria* blight disease and management strategies employed by the farmers against the disease. The area of interest being farmers awareness on *Alternaria* blight disease and its common name or local name if known, history of the field, source of the seeds, losses caused by the disease, ability of the farmer to purchase improved seeds and *Alternaria* blight disease control strategies applied by the farmers. A simple checklist questions was used as guidance (Appendix 1).

3.4 Data Analysis

Data collected in the laboratory such as seed purity analysis and percentage seed infection were calculated using procedures described by ISTA (2005). Percentage seed infection was calculated by counting the number of infected seeds obtained from each seed sample x 100 divided by total seeds observed (400). Data collected in the screen house, such as disease incidence and severity, plant height, head size and seed weight, and disease incidence from farmers fields were compiled and analyzed using SAS computer package (version 9.1) for Analysis of Variance (ANOVA) and mean separation test was done using the Duncan Multiple Range Test. Results from interviewed farmers on awareness of management practices employed on *Alternaria* blight disease and other parameters assessed were analyzed using SPSS (version 16) computer package.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Purity of Sunflower Seeds Collected from Iramba and Handeni Districts

The results of purity analysis for pure seeds, inert matter and seeds from other crops separated from sunflower seed samples are presented in Table 2. The result indicate that seed sample Nos 12, 13, 22 and 24, obtained from Senene and Kitumbili (Iguguno) in Iramba District and from Konje and Kwamashaka (Vibaoni) in Handeni District had the highest seed purity (98.1 %, 98.0 % 99.3 % and 98.5 %. Sunflower seed sample Nos 1 and 10 (local varieties) collected from Kibigiri and Masagi (Shelui and Mtoa) in Iramba district had low seed purity of 55.9 % and 73.6 %. High proportion of inert matter (43.6 %, 23.5 %) was recorded from seed sample collected from Kibigiri and Masagi village in Iramba District (Table 2). High proportion of inert matter may be attributed by poor harvesting and threshing techniques. Furthermore, seed samples with high proportion of inert matter are not preferred by farmers because impurities increase cost and quantity of required seeds for sowing. According to Jonar *et al.* (2011), high amount of inert matter content may provide suitable environment for pathogen survival and development.

The results show that 10 out of 30 sunflower seed samples tested contained seeds of other crops (Table 2). Seed sample Nos 18, 19 and 22 collected from Nzeri, Kibaya and Kwamashaka (Handeni District) had the lowest amount (0.1%) while the seed sample No 6 collected from Mgela (Iramba District) had high (0.8%) amount of seeds of other crops. The presence of other seeds in sunflower seed samples implies that most farmers grow several crops in the same field, harvested at the same period and stored in the same place without sorting and grading seeds for the next season.

Table 2: Purity of sunflower seeds collected from Iramba and Handeni Districts in December 2011

Sample No	Ward	Village	Variety	Pure seed	Inert matter	Other seeds
1	Shelui	Kibigiri	Punda milia	55.9	43.6	0.0
2		Nselembwe$_1$	Serena	92.4	6.9	0.2
3		Wembere	Punda milia	96.7	2.3	0.5
4		Mgongo	Punda milia	97.9	1.9	0.0
5		Nselembwe$_2$	Punda milia	96.8	2.7	0.2
6	Mtoa	Mgella	Serena	94.5	4.5	0.8
7		Tyeme$_1$	Punda milia	95.9	3.8	0.0
8		Masagi$_1$	Punda milia	94.8	5.0	0.0
9		Tyeme$_2$	Mixed variety	93.4	6.5	0.0
10		Masagi$_2$	Mixed variety	73.6	25.3	0.4
11	Iguguno	Kibololo	Lulu	92.9	6.9	0.0
12		Senene	Serena	98.1	1.8	0.0
13		Kitumbili	Sedico	98.0	1.8	0.0
14		Milale	Kenya Fedha	96.8	3.0	0.0
15		Iguguno	Mixed variety	94.0	5.9	0.0
16	Misima	Misima$_1$	Record	91.3	8.1	0.0
17		Misima$_2$	Kenya Fedha	97.9	1.8	0.0
18		Nzeri	Record	95.0	4.7	0.1
19		Kibaya	Pannar	96.2	4.4	0.1
20		Mbagui	Mixed variety	90.6	9.4	0.0
21	Vibaoni	Kwabaya$_1$	Record	97.4	2.6	0.0
22		Konje	Local variety	99.3	0.4	0.1
23		Kwabaya$_2$	Record	97.4	2.5	0.0
24		Kwashaka$_3$	Local variety	98.5	1.2	0.0
25		Vibaoni	Record	90.1	9.3	0.3
26	Kwamatuku	Kmatuku$_1$	Record	96.6	3.8	0.3
27		Nkate$_1$	Record	95.6	4.2	0.0
28		Ktuku$_2$	Record	95.9	3.7	0.0
29		Nkate$_2$	Record	86.4	13.1	0.0
30		Nkate$_3$	Record	96.5	3.3	0.0

Each value is a percent proportion in 200 grams of sunflower seeds tested Kbaya = Kwabaya, Kshaka = Kwamashaka, Ktuku = Kwamatuku

4.1.1 Seed-borne fungal pathogens isolated from sunflower seed samples

Results of fungal identification obtained using Blotter method show that all seed samples tested were contaminated with various fungal pathogens (Table 3). The fungal pathogen identified included *A. helianthi*, *A. alternata*, *Macrophomina phaseolina*, *Phoma* species, *F. palidoroseum* and *F. moniliforme*. *Alternaria alternata* was the most abundant occurring fungus in all samples tested except one samples collected from Shelui (Iramba District) (Table 3). The highest incidence of *A. alternata* was observed from seed samples collected from the Wards of Misima, Vibaoni and Shelui with the incidence ranging from 31.00 % to 47.25 % (Table 3). The least incidence (1.50 %) of *A. alternata* was recorded in sunflower seed sample No 12 collected from Senene (Iguguno) in Iramba District. Such results indicate that *A. alternata* is an important fungus associated with sunflower in the surveyed area. For a long time, *A. alternata* was not considered an important pathogen due to its association with declining plants (Kolte, 1985). However, it has become a serious pathogen on various crops due to its ability to produce host-specific toxins during pathogenesis. Lagopoli and Thanassoulopoulous (1998) reported that *A. alternata* can be as damaging to sunflower as *A. helianthi* and can reduce the number of seeds produced per head from 16-69 %. *Alternaria alternata* had also been reported to cause yield losses of about 61.9 % in Iraq and India (Chattopadhyay, 1999).

It was also observed that *Macrophomina phaseolina* caused high seed infection (3.75 % and 4.25) in seed sample Nos 3 and 23 collected from Wembere in Iramba District and Kwabaya (Handeni District), respectively (Table 3). The least seed infection (0.05 %) was recorded in sunflower seed sample No 24 collected from Kwamashaka in Handeni District (Table 3). Such results implied that sunflower seeds collected from surveyed areas were infected by *M. phaseolina*. The fungal pathogen *M. phaseolina* has been reported to cause 10-90% yield loss (Bokor, 2007; Khan, 2007).

Results derived from the study found that sunflower seed samples used in this study were contaminated with *F. moniliforme*. The highest seed incidence (12.0%) due to this pathogen was observed in seed sample No 2 (local cultivar) collected from Nselembwe$_2$ (Iramba District) (Table 3). The least incidence (0.25%) of *F. moniliforme* was recorded from sunflower seed sample No 8 collected from Masagi (Iramba District). *Fusarium moniliforme* is a wide spread pathogen which has also been reported in maize (Saleem *et al.*, 2012). Nahar and Mushtaq (2006) reported *Fusarium* as the main causal agent of wilt, seedling collar and stem rots, stunting and tip burn disease in sunflower.

Fusarium palidoroseum was also among the pathogens detected in sunflower seed samples tested in the current study. The results indicate high seed infection of 16.25 % for the seed sample collected from Nkate village in Handeni District while the least (0.5 %) seed infection was observed from sample No 8 of Masagi village in Iramba district (Table 3).

Table 3: Incidence of seed-borne fungal pathogens detected in sunflower seeds collected from Iramba and Handeni Districts

			Incidence of different pathogens detected from sunflower seeds (%)						
District	Wards	Sample	A.h	A.a	Mac.ph	P.spp	F.pali	F.moni.	Bact
Iramba	Shelui	SH1	2.25	0.00	0.00	0.00	0.00	0.00	0.50
		SH2	0.00	38.5	0.00	0.00	3.25	12.00	0.00
		SH3	0.00	38.75	3.50	0.00	0.00	0.00	0.00
		SH4	0.00	31.00	2.25	0.00	3.25	0.00	0.00
		SH5	0.00	17.50	2.50	0.00	0.00	2.50	0.00
	Mtoa	MT6	2.00	4.50	2.50	2.50	0.75	0.00	1.50
		MT7	2.25	7.00	3.75	0.00	1.50	3.00	0.75
		MT8	3.75	6.00	1.00	0.00	0.50	0.25	0.00
		MT9	3.00	6.25	2.50	0.00	0.75	0.00	0.00
		MT10	0.00	13.75	2.25	0.00	3.75	0.00	0.00
	Iguguno	IGN11	0.00	3.25	0.00	0.00	0.00	0.00	0.25
		IGN12	0.00	1.50	0.00	0.00	0.00	2.75	0.00
		IGN13	0.00	3.00	0.00	0.00	0.00	2.50	0.00
		IGN14	0.00	14.00	0.00	0.00	0.00	2.50	0.25
		IGN15	0.00	4.25	0.00	0.00	0.00	1.50	0.00
Handeni	Misima	MS16	0.00	14.25	0.00	0.00	0.00	2.00	0.00
		MS17	0.00	8.75	0.00	0.00	3.00	1.00	0.75
		MS18	2.00	13.00	0.00	0.00	1.25	5.25	0.00
		MS19	0.00	9.50	0.00	0.00	2.75	0.00	0.00
		MS20	0.00	47.25	0.00	0.00	1.25	0.00	0.00
	Vibaoni	VN21	0.00	28.00	1.50	0.00	9.00	9.00	0.00
		VN22	0.00	41.25	1.50	0.50	7.75	1.50	0.00
		VN23	0.00	34.00	4.25	0.00	7.25	8.50	0.00
		VN24	0.00	6.75	0.50	0.00	7.75	7.25	0.00
		VN25	0.00	23.00	3.25	0.00	8.00	1.00	0.00
	Kwamatuku	KM26	0.00	5.75	0.00	0.00	6.00	5.00	0.00
		KM27	0.00	4.50	1.75	0.00	7.25	5.75	0.00
		KM28	0.00	11.00	1.0	0.00	5.75	6.00	0.00
		KM29	0.00	5.50	1.75	0.00	16.25	5.00	0.50
		KM30	0.00	8.75	0.75	0.00	9.75	4.75	0.00

Ah = *Alternaria helianthi*, Aa = *Alternaria alternata*, Mp = *Macrophomina phaseilina*, Ps = *Phoma* species, Fp = *Fusarium palidoroseum*, Fm = *Fusarium moniliforme*, Bact = Bacteria.

* = The highest incidence (%) of the pathogen.

400 seeds per each sample were subjected to blotter methods for pathogen detection.

Percent incidence was calculated by counting infected seeds for each pathogen divided by total seeds tested (400) times 100.

The results also show that two out of 30 seed samples tested i.e. sample Nos 6 and 22 were infected by *Phoma* species (Table 3) with the incidence of 2.5 % and 0.5 %, respectively. *Phoma* species is a wide spread pathogen but causes minimal sunflower yield losses in Pakistan although 20 % yield losses had been reported in Iran (Irum, 2008). However, several *Phoma* species are of quarantine significance causing serious problems to organizations involved in plant health quarantine (Aveskamp *et al.*, 2008). The seed-borne pathogens detected from the sunflower seed samples have also been reported by Raj *et al.* (2007); Abdullah and Al-Mosawi (2010) and Rathod and Chavan (2010).

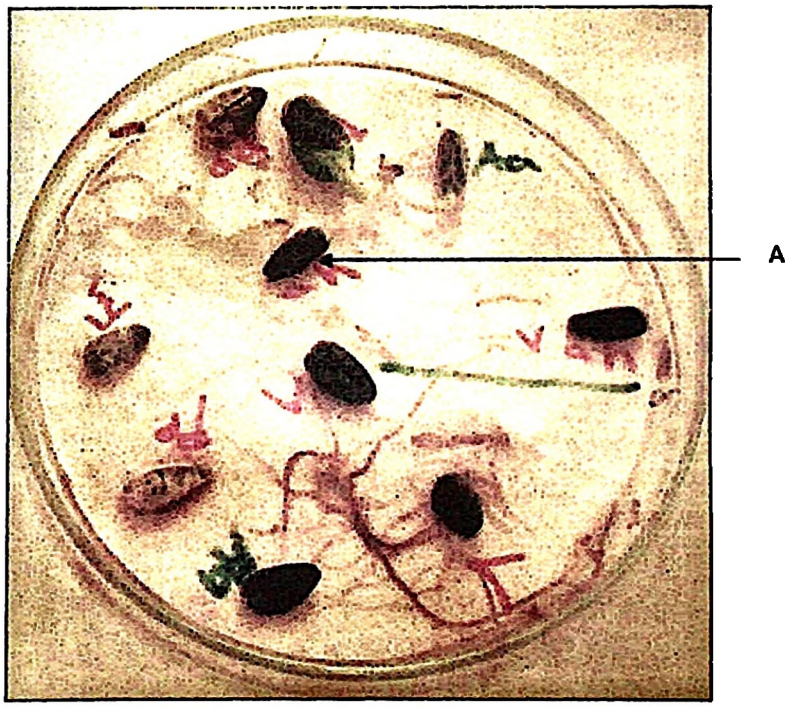

Plate 2: Detection of fungi from sunflower seed samples using the blotter method, A = sunflower seed with colonies of *A. helianthi*

Furthermore, the results show that a number of sunflower seed samples collected from Iramba and Handeni Districts were infected by *A. helianthi* (Table 3). Sunflower seed sample No. 8 collected from Masagi village in Iramba District had high seed infection (3.75 %) compared to other samples used in this study. Seed infection levels for sunflower sample Nos 1, 6, 7, 9 from Kibigiri, Mgela, Tyeme and Masagi villages in Iramba District and No 18 from Kwabaya village in Handeni district are shown in Table 3. These results indicate that the level of seed infection by *A. helianthi* was lower compared to most other pathogens detected from sunflower seed samples. The low level of seed incidence might be attributed by masking effect of *A. alternata* (Udaya *et al.*, 2010). Venette *et al.* (1996) reported that any level of seed contamination can contribute to initial infection and one infected seed in 10 000 was sufficient to cause bacterial disease epidemics in dry beans.

The results also reveal that sunflower seed samples used in this study were contaminated by other microorganisms (Table 4). Some of the common fungal saprophytes detected were *Aspergillus favus*, *A. niger*, *Curvularia lunata*, *Cladosporium* species, *Rhizopus* species and *Penicillium* species. The same saprophytes were also reported by Abdullah and Al-Mosawi (2010). Some of the saprophytic fungi such as *Aspergillus* species have been reported to produce different groups of aflatoxins which are natural toxin and hazardous to human and animals (Abdullah and Al-Mosawi, 2010). Furthermore, the presence of saprophytic fungi on sunflower seeds may lower the seed quality (Nahar *et al.*, 2005). The high levels of infection by fungal saprophytes observed in this study might be attributed by poor storage conditions (David, 2009).

Table 4: Other microorganisms detected from sunflower seeds used in this study

Wards	Variety	Source	Name of microorganisms
Shelui	Pmilia	Farmer	*Aspergillus. flavus, A.niger, Penicillium Curvularia lunata*
	Serena	Farmer	*Penicillium* spp *A. niger, A. flavus, C. lunata* and *A.niger*
	Pmilia	Farmer	*Penicillium* spp
	Pmilia	Farmer	*A. flavus, A. niger, Penicillium* spp, *Cladosporium* spp
	Pmilia	Farmer	*Rhizopus* spp
Mtoa	Serena	Farmer	*Penicillium* spp., *Cladosporium* spp
	Pmilia	Farmer	*Rhizopus* spp., *Cladosporium* spp
	Milia	Farmer	*C. lunata, Penicillium* spp.
	M/variety	Farmer	*Cladosporium* spp., *Rhizopus* spp.
	M/variety	Farmer	*C. lunata, Penicillium* spp.
Iguguno	Lulu	Farmer	-
	Serena	Farmer	*Penicillium* spp., *Cladosporium* spp
	Sedico	Farmer	-
	K/fedha	Stockiest	*Rhizopus* spp., *Cladosporium* spp.
	M/variety	Farmer	-
Misima	Record	Farmer	-
	K/fedha	Stockiest	*A. flavus, A. niger,*
	Record	Farmer	*Actinomycetes* spp., *Peniciliium* spp *A.flavus* and *A.niger*
	Pannar	Stockiest	*Peniciliium* spp, *A.flavus and A.niger*
	M/variety	Farmer	*A.flavus, A. niger, Penicillium spp,*
Vibaoni	Record	Stockiest	*A. flavus, A. niger,*
	Local var	Farmer	*Actinomycetes* spp., *Peniciliium* spp *A.flavus* and *A.niger*
	Record	Stockiest	*Actinomycetes* spp., *Peniciliium* spp, *A.flavus* and *A.niger*
	Local var	Farmer	*A. niger, A. flavus, Rhizopus* spp., *Penicillium* spp
	Record	Stockiest	A. *flavus, A. niger* and *Rhizopus* spp
Kmatuk u	Record	Farmer	*A. niger, A. flavus, Rhizopus spp, Penicillium* spp.
	Record	Farmer	*Peniciliium* spp, *A.flavus* and *A.niger*
	Record	Stockiest	*A.niger* and *A. flavus*
	Record	Farmer	*Actinomycetes* spp., *Penicillium* spp
	Record	Stockiest	*A. niger, A. flavus, Rhizopus, Penicillium* spp

400 seeds were used in each sample for identification of the microorganisms.

4.1.2 Fungal microorganisms isolated from sunflower leaf samples

The results of isolations from sunflower leaves collected from Handeni District show that, *A. helianthi* was the most prevalent fungus in the leaf samples tested (Table 5). High overall mean incidence of 22.7 % due to *A. helianthi* was observed from both districts compared to *Alternaria alternata* (13.5 %) (Table 5). Based on Ward level, the results indicate that the sunflower leaf samples collected from Misima, Vibaoni and Kwamatuku have the highest mean disease incidence (50.8, 39.8, and 38.4 %, respectively) by *A. helianthi* (Table 5). Such results indicate that low inoculum of *A. helianthi* found in seed samples may result in high disease incidence under favorable field conditions. Reddy *et al.* (2006) reported that, the incidence and intensity of the disease are influenced by season, stage of the crop, region and climatic conditions. Other fungi recorded from sunflower leaves included species of *Fusarium*, *Curvularia*, *Aspergillus* and *Cladosporium* (Table 5).

Table 5: Fungi detected from leaf samples collected from Iramba and Handeni Districts

Wards	Ah	Aa	Az	Asp	Csp	Cl	Fsp
Shelui	0	9	0	2	1	0	1
	1	8	0	1	0	0	1
	0	8	0	0	0	1	0
	0	9	0	0	0	0	0
	2	0	1	1	0	0	0
Mean	0.6	6.8	0.2	0.8	0.2	0.2	0.4
Mtoa	0	7	0	0	2	0	0
	2	7	0	0	0	0	0
	3	6	0	0	0	2	2
	3	6	0	1	1	0	1
	0	3	0	0	0	0	0
Mean	1.6	5.8	0.0	0.2	0.6	0.4	0.6
Iguguno	0	4	0	0	0	0	0
	0	3	0	0	1	0	0
	0	2	0	0	0	0	0
	0	3	0	0	0	0	0
	0	2	0	0	0	0	0
Mean	0.0	2.8	0.0	0.0	0.2	0.0	0.0
Misima	46	11	0	0	20	0	10
	50	20	0	0	20	0	0
	50	10	0	0	0	0	10
	30	20	0	0	0	0	20
	23	17	0	0	0	0	0
Mean	39.8	15.6	0.0	0.0	8.0	0.0	8.0
Vibaoni	36	10	0	0	10	0	0
	48	0	0	0	16	0	0
	26	10	0	0	10	0	0
	16	12	0	0	16	0	0
	66	24	0	0	22	0	0
Mean	38.4	11.2	0.0	0.0	14.8	0.0	0.0
Kwamatuku	32	62	0	0	6	0	0
	70	38	0	10	10	0	0
	80	0	0	0	10	0	0
	40	34	0	0	10	0	0
	32	60	0	10	12	0	0
Mean	50.8	38.8	0.0	4.0	9.6	0.0	0.0
Overall Mean	22.7	13.5	0.3	5.4	0.8	0.1	1.5

Ah = *Alternaria helianthi*, Aa = *Alternaria alternata*, Az = *Alternaria zinnie*, Csp = *Curvularia* species, Asp = *Aspergillus* species, Cl = *Cladosporium* species and Fsp = Fusarium species

4.1.3 Growth and morphological features of *Alternaria helianthi* isolated from sunflower seeds

The results show that, the morphology of *A. helianthi* grown on V8 medium varied among fungal isolates (Table 6). The mean colony diameter ranged from 2.1 mm to 6.4 mm (Table 6). Fungal isolate SIS_1 from sunflower seed collected from Kibigiri in Iramba District had the largest mean diameter (6.4 mm) compared to other isolates (Table 6). Isolates SIM_6, SIM_7, SIM_8 and SIM_9 collected from Mgella, Tyeme1, $Masagi_1$ and $Tyeme_2$ villages (Mtoa) in Iramba District had mean diameters of 5.7, 5.2, 4.7 and 4.3 mm, respectively. Isolate $THMIS_{23}$ from $Kwamashaka_2$ (Vibaoni) in Handeni District had the least diameter (2.1 mm) (Table 6). The conidia of *A. helianthi* were solitary and borne on simple conidiophores (Plate 3). Under high magnification (x 40) the conidia were cylindrical with rounded ends and four to nine transverse septa (Plate 6). The results on morphological characteristic agree with descriptions reported by Ellis (1977) and Mathur and Kongsdal (2003).

Table 6: Colony characteristics and growth features of *Alternaria helianthi* on V8 media

Isolates	Colony colour	Margin	Colony size (mm)
$THMIS_{23}$	Grayish black	Irregular	2.1
SIS1	Gray	Smooth	6.4
SIM6	Gray	Smooth	5.7
SIM7	Gray	Smooth	5.2
SIM8	Gray	Smooth	4.7
SIM9	Gray	Smooth	4.3

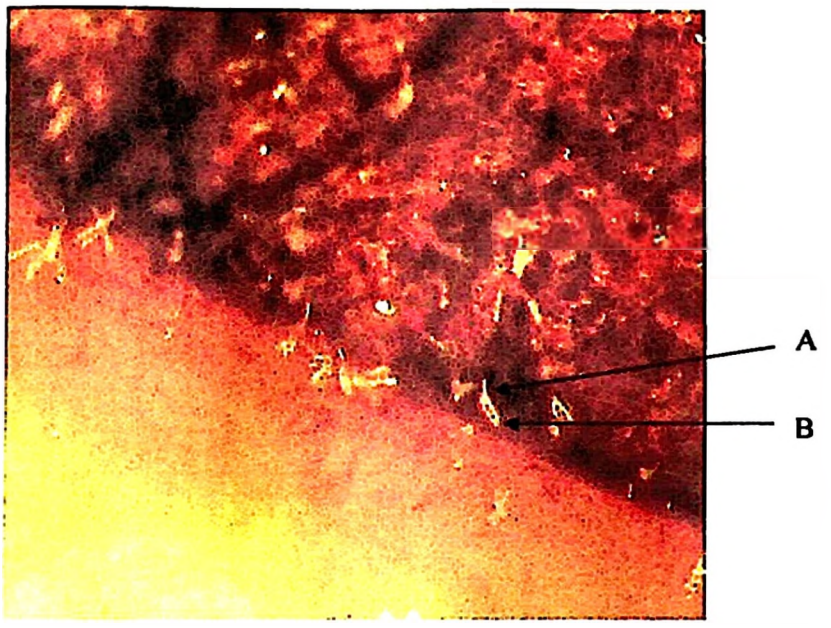

Plate 3: Micrograph showing conidiophores and conidia of *Alternaria helianthi* on infected sunflower leaf collected from Handeni District (x 40); A = Conidiophores, B = Conidia

The colony colour of *A. helianthi* showed variation among isolates. Isolate $THMIS_{23}$ produced grayish black colour (Plate 4) while the rest had gray colony colour. Similarly, isolate $THMIS_{23}$ had irregular margin (Plate 4) while the rest of the isolates that gave smooth margin (Plate 5). Differences on growth diameter and colony colour observed may be an important parameter pointing to variation among the isolates. Thus, this study groups *A. helianthi* into two morphological categories (gray and grayish black colour). In addition, the grayish groups were fast in radial growth compared to the slow grayish-black-looking *A. helianthi* mycelia with irregular margins on V8 medium. Variation in colony colour, growth habit and margins might be attributed with diversification among *A. helianthi* species. Morphological variations in *A. helianthi* have been reported by Babu *et al.* (2000); Mesta, (2006); Kumar (2008) as an important tool in identification and classification of the pathogen.

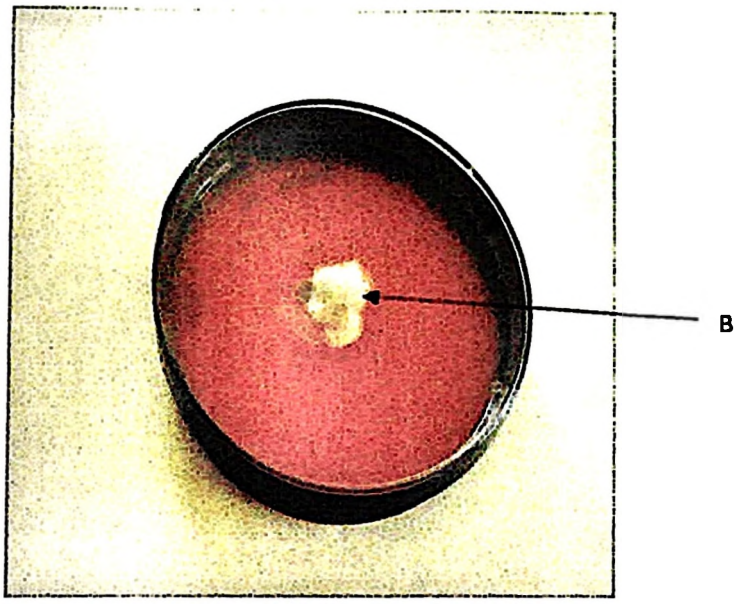

Plate 4: Ten-day old colony of *Alternaria helianthi* grown on V8 agar, B = irregular margins of *Alternaria helianthi*

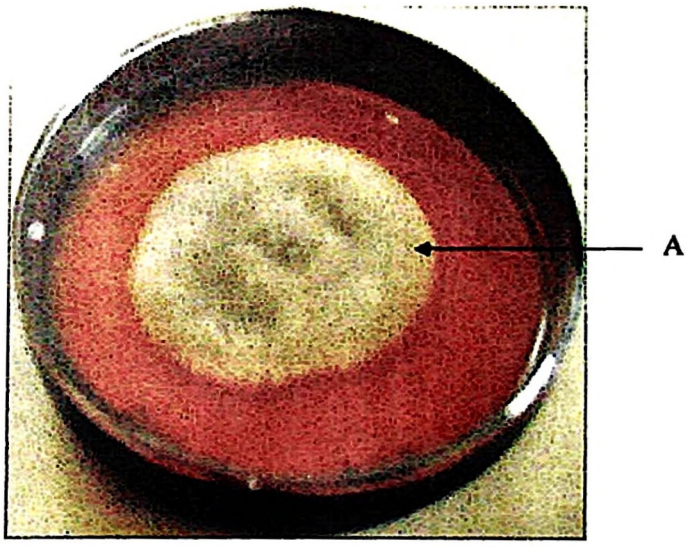

Plate 5: Ten-day old colony of *Alternaria helianthi* grown on V8 agar, A = Smooth margins of *Alternaria helianthi*

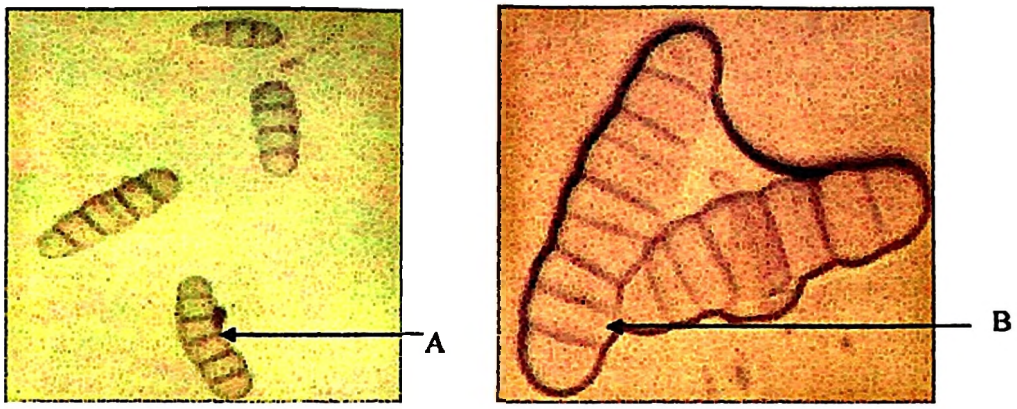

Plate 6: Micrographs of *Alternaria helianthi* conidia at x 40 magnification A = isolate of *Alternaria helianthi* obtained from the seed collected from Iramba and B = isolate of *Alternaria helianthi* obtained from sunflower leaf sample collected from Handeni District

4.1.4 Pathogenicity test of fungal isolates obtained from Iramba and Handeni districts

Pathogenicity tests using fungal microorganisms isolated from sunflower seeds revealed that all isolates obtained from Iramba and Handeni Districts produced disease symptoms as those observed in the field. *Alternaria* blight symptoms were observed on the lower leaves as circular spots surrounded by yellow hallo measuring 1-2 mm in diameter (Plate 7). These spots eventually coalesced forming leaf blight (Plate 7). Such observations imply that the pathogen inoculated was indeed *A. helianthi.*

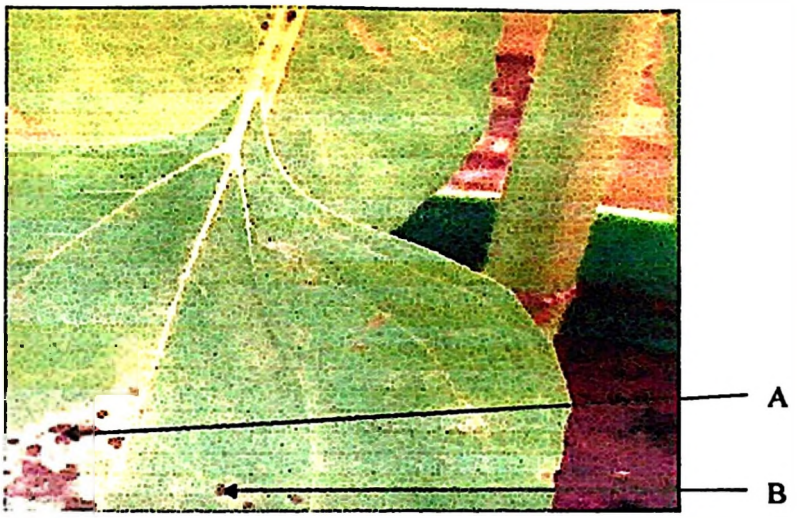

Plate 7: Disease symptoms on sunflower leaves inoculated with *Alternaria helianthi* in the screen house, A = Coalesced leaf spots on sunflower plant, B = Brown leaf spot

4.1.5 Incidence and severity of *Alternaria* blight of sunflower in the screen house

The results on disease incidence in the screen house were statistically significant different ($P \leq 0.001$) among plants inoculated with *A. helianthi* (Table 7). Sunflower plants inoculated with sterile distilled water (control) did not develop *Alternaria* blight symptoms. Such results imply that crops free from diseases can be raised if health seeds free from seed-borne microorganisms are used. At 14 day after inoculation the highest disease incidence (41.7 %) was recorded in plants inoculated with isolate SIM_6 collected from Mgella. Plants inoculated with SIM_7 and $THMIS_{23}$ collected from Tyeme and Kwabaya in Iramba and Handeni Districts had the disease incidence of 25.0 % (Table 7). The disease incidences of 8.3 % to 33.3 % were recorded for isolates SIM_8, SIM_9 and SIS_1 (Table 7). The results also indicate that isolate SIM_6 was more virulent on sunflower plants compared to other isolates in that it caused the highest disease incidence of 41.7 % compared with 16.7 % and 8.3 % recorded in plants inoculated with isolates SIM_8 and SIM_1, respectively (Table 7).

Sunflower plants inoculated with sterile distilled water (control) did not show any *Alternaria* blight disease symptoms. Such results imply that, the fungal isolates obtained from sunflower seeds were pathogenic. The results in this study also indicate that, sunflower plants were more tolerant to *Alternaria* blight at vegetative growth and become susceptible in later stages of growth that have also been reported by Anikumar *et al.* (1974).

Table 7: Incidence and severity levels of *Alternaria* blight recorded under controlled condition

	Disease incidence				Disease severity			
Days	14	28	42	61	14	28	42	61
Isolates								
SIS1	8.3	41.7d	83.3c	100.0a	1.0e	3.3b	4.7a	5.3a
SIM6	41.7a	66.7a	91.7b	100.0a	3.0a	3.0c	3.7d	4.0e
SIM7	25.0c	58.3b	91.7b	100.0a	2.7b	3.7b	4.0c	4.7c
SIM8	16.7d	50.0c	75.0d	100.0a	2.3c	3.7b	4.7a	5.3a
SIM9	33.3b	66.7a	83.3c	100.0a	2.7b	3.3b	4.0c	4.3d
THMIS23	25.0c	58.3b	100.0a	100.0a	2.0d	2.7d	4.3b	5.0b
Control	0.0f	0.0e	0.0e	0.0b	0.0f	0.0e	0.0e	0.0f
Mean	21.4	48.8	75.0	85.7	2.0	3.0	3.6	4.1
F Test	***	***	***	***	***	***	***	***

Control = Distilled water; disease severity was based on 1-9 scale

Results of *Alternaria* blight disease severity were statistically significant different ($P \leq 0.001$) among plants inoculated with different fungal isolates (Table 7). At 14 days, the highest (3.0) disease severity was recorded in sunflower inoculated with SIM_6. The least (1.0) disease severity was shown on SIS_1 isolates. Sunflower plants inoculated with isolates SIM_7, SIM_8, SIM_9 and $THMIS_{23}$ had disease severity levels between 2.0 and 2.7. The highest disease severity was recorded at 28, 42 and 61 days after inoculation in both isolates except on plants inoculated with sterile distilled water (control) (Table 7).

Generally, the results imply that the age of sunflower plants played a significant role in disease infection. During vegetative growth (14 to 28 days) both disease incidence and severity were low compared to later stages of plant growth (Days 42 to 61) (Table 7). Calvet *et al.* (2005) reported that *Alternaria* blight under natural infection occurs only sporadically on the older lower leaves of plants before flowering and that the heaviest infection occurs during seed development in the later stages of growth.

4.1.6 Effects of *Alternaria* blight disease on sunflower yield and yield parameters

Yield and yield parameters of sunflower plants inoculated with the isolates of *A. helianthi* were statistically significant different ($P \leq 0.001$) (Table 8). Large heads (3.40 cm) were recorded from plants inoculated with SIM_8 and the least (2.73) from $THMIS_{23}$. Head size of sunflower plants inoculated with SIS_1 and SIM_9 were 3.23 cm and 3.30 cm (Table 8). Such results indicate the effect caused by *Alternaria* bight disease that resulted in formation of small heads. However, the plants inoculated with distilled water (control) resulted with large head size (5.77 cm) compared to those inoculated with *A. helianthi*.

Table 8: Evaluation of the effect of *Alternaria* blight disease on yield and yield parameters of sunflower plants in the screen house

Isolates	Head Size (cm)	Plant Height (cm)	Seed weight (gm/m^2)
SIS_1	3.23cb	188.00a	1.40cb
SIM_6	2.67c	189.50a	1.40cb
SIM_7	3.70b	192.03a	1.67b
SIM_8	3.40cb	196.10a	1.17cb
SIM_9	3.30cb	190.10a	1.57cb
$THMIS_{23}$	2.73cb	197.50a	0.97c
Control	5.77a	189.97a	3.33a
Mean	3.54	187.63	1.64
F Test	***	**	***
CV	15.73	4.09	23.65

Means followed by the same letters within the column are not significantly different $P \leq 0.05$ based on Duncan's Multiple Range Test (DMRT); Control = Distilled water.

The results of sunflower seeds recorded from the screen house are as shown on Table 8. There were statistically significant differences ($P \leq 0.001$) between seed weight among sunflower plants inoculated with different isolates of *A. helianthi.* Low seed weight ranging between 0.9 gm/m^2 to 1.57 gm/m^2 was recorded. The lowest seed weight of 0.9 gm/m^2 was recorded from sunflower plants inoculated with $THMIS_{23}$ (Table 8). High seed weight of 3.3 gm/m^2 was recorded from plants inoculated with sterile distilled water (negative control) (Table 8). Low seed weight obtained from sunflower plants in the screen is an indication of the losses that can be perceived when infected seeds by *A. helianthi* are used.

4.2 *Alternaria* Blight Disease Incidence and Severity in Farmers' Fields

The results indicate that there were statistically significant differences ($P \leq 0.01$) in disease incidence between wards covered in this study (Table 9). The highest disease incidence (88.67) and the next highest (71.40%) were observed at Vibaoni and Kwamatuku wards in Handeni districts (Table 9). The disease incidences in other wards were as indicated in Table 9. The results also show that there were significant differences ($P \leq 0.001$) between *Alternaria* blight disease severity in different wards (Table 9). The disease severity was higher (4.17 and 4.00) at Vibaoni and Misima in Handeni District, respectively than Shelui in Iramba District (1.80) (Table. 9). Results for disease severity for other wards are presented in Table 9.

High incidences and severities of *Alternaria* blight disease in the surveyed wards may be attributed by different factors such as favorable climatic conditions; as reported by Mesta, (2006) to be among factors that influence the incidence and intensity of *Alternaria* blight of sunflower. Even it could also be attributed by poor cultural practices e.g. improper destruction of plant debris after crop harvest and use of contaminated sunflower seeds.

Several workers have reported that continuous cropping of sunflower in farmers' fields pave the ways for continuous survivability of the pathogen (Kolte, 1985; Venkataramana *et al.* 1995; Amaresh and Nargund, 2000; Khan, 2007). Thus, the infected sunflower plant debris left in the field serve as a major source of infection causing epidemics throughout the season. At District level, the disease incidence of 57.7 % and 15.2 % were recorded in Handeni and Iramba Districts while severities were 3.93 and 2.1, respectively (Fig. 3).

Disease symptoms of *Alternaria* blight of sunflower included brown, oval to circular spots which were observed on leaves, petals, sepals (Plate 8) and stem lesion (Plate, 9) and sometimes the plants became stunted (Plate 10). However, *Alternaria helianthi* infects sunflower plants in all stages of growth (Plate 11 and Plate 12). Under severe infections, disease lesions became irregular and coalesced that lead to wilting of the leaves (Plate 11) and finally death of the plants (Plate 13).

Table 9: Incidence and severity of *Alternaria* blight disease of sunflower in the surveyed wards of Iramba and Handeni Districts

Ward	Incidence	Severity
Shelui	19.20b	1.80c
Mtoa	16.80b	2.60bc
Iguguno	9.60b	2.00c
Misima	9.60b	4.00a
Vibaoni	88.67a	4.17a
Kwamatuku	71.50a	3.50ab
Mean	35.90	3.01
CV	40.37	26.95
F Test	***	***

Means followed by the different letters within the column are significantly different $P<0.05$ based on Duncan's Multiple Range Test (DMRT).

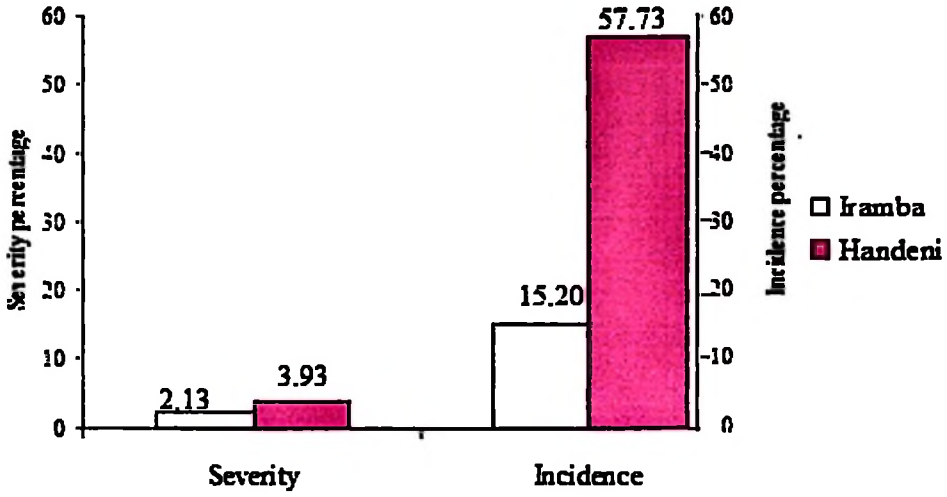

Figure 3: Incidence and severity of *Alternaria* blight disease in Iramba and Handeni Districts

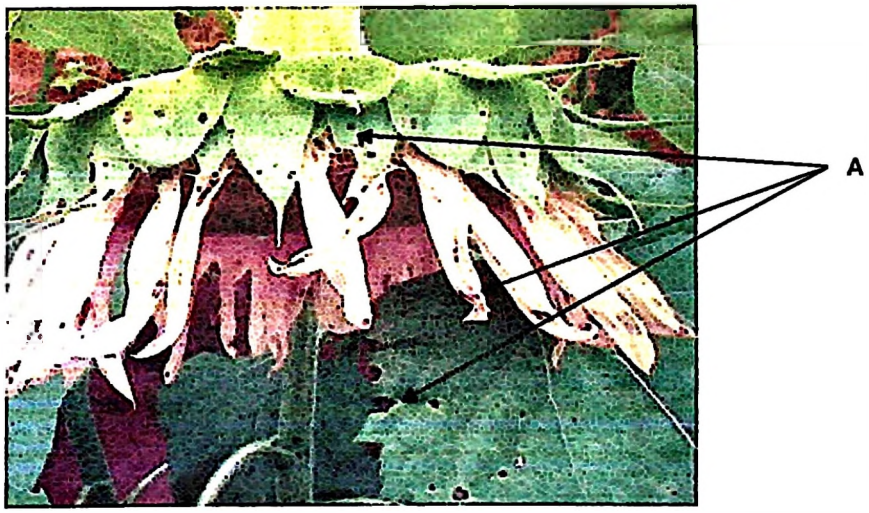

Plate 8: Leaves, petals and sepals of sunflower plant infected by *Alternaria* bight disease in Misima at Handeni District

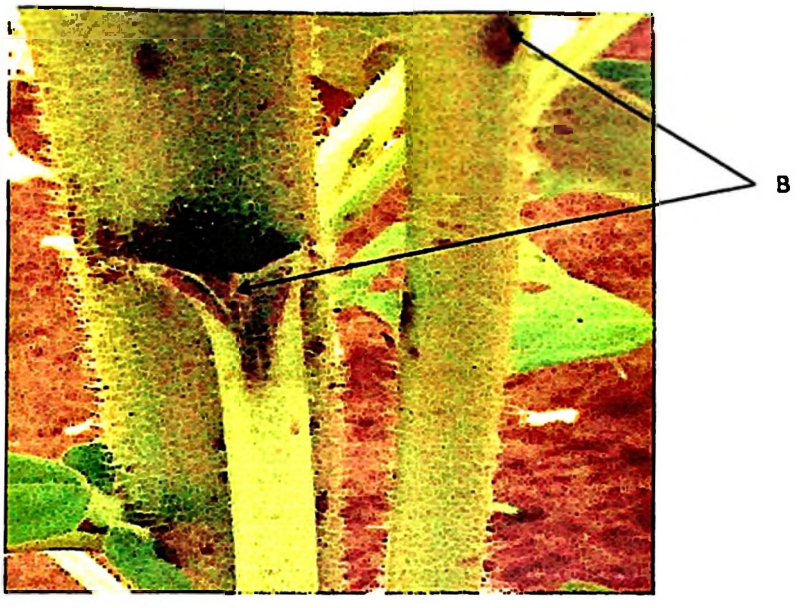

Plate 9: Stem and petiole of sunflower plant infected by *Alternaria* blight disease in Misima at Handeni District

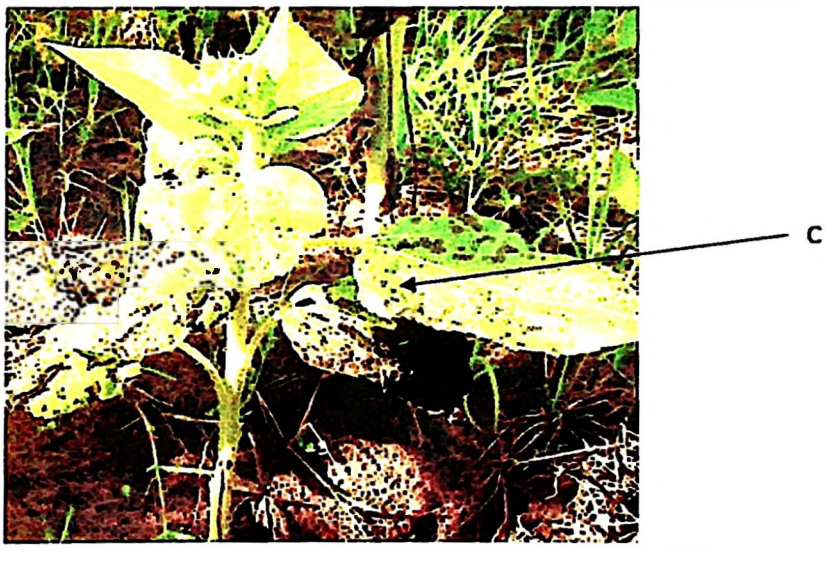

Plate 10: A stunted sunflower plant with symptoms of *Alternaria* blight disease at Mtoa (Iramba District)

Plate 11: Lower and upper leaves of sunflower plants infected by *Alternaria* blight disease in Vibaoni in Handeni District

Plate 12: Well developed and matured sunflower head infected by *Alternaria* blight disease in Misima at Handeni District

Plate 13: Sunflower field with premature plants infected by *Alternaria* blight in Mtoa, Iramba District

4.3 Management Practices Applied by Farmers to Control *Alternaria* Blight Disease

4.3.1 Farmer's knowledge on *Alternaria* blight disease of sunflower

The results of the farmers' knowledge on *Alternaria* blight disease show that, 70 % of farmers were unaware of the disease (Fig. 5). Thirty percent of the farmers who recognized the disease associated it with drought stress hence naming it as Ukungu and Kyusa (Table 10). Little awareness on *Alternaria* blight disease among farmers in the surveyed district may be due to poor extension services on identification of the disease. The ignorance on the disease recognition may accelerate the dissemination of the pathogen through exchange of infected farmers saved seeds (Raj *et al.*, 2007).

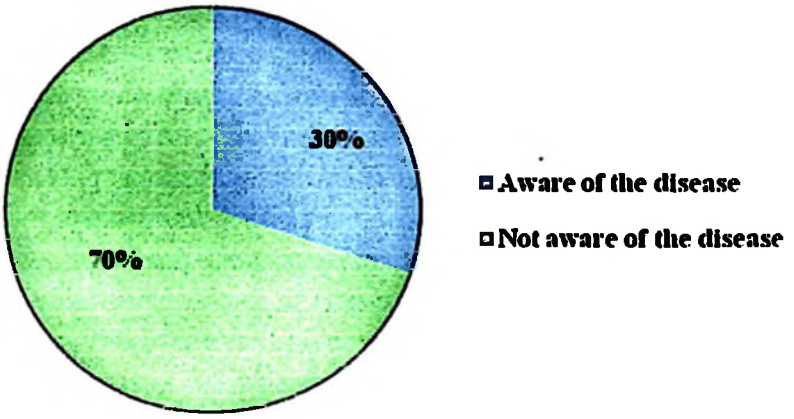

Figure 4: Farmers' awareness on *Alternaria* blight disease of sunflower in Iramba and Handeni Districts

Table 10: Local names used by the farmers in Iramba and Handeni District to identify *Alternaria* blight disease of sunflower

Name of the disease	Frequency	Percentage
Ukungu	5	16.6
Kyusa	2	6.7
Unknown	23	76.7
Total	30	100.0

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

4.3.2 Farmer's opinion on occurrence and the cause of *Alternaria* blight disease

The result showed that, the farmers from Handeni and Iramba Districts had different opinion on *Alternaria* blight disease occurrence (Table 11). Fifty percent of the farmers from both districts showed that the disease occurred after heavy rains followed by drought spell, while 6.7 % of farmers associated the disease with late sown crops. The remaining percentage of the farmers (43.3 %) had no idea about the disease (Table 11). Such results imply that farmers were not sure of the etiology of the disease.

Table 11: Farmers understanding on *Alternaria* blight disease occurrence in Iramba and Handeni Districts

Disease occurrence	Frequency	Percentage
Excessive rainfall/drought	15	50.0
Late sown	2	6.7
Not aware	13	43.3
Total	30	100.0

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

Most of the farmers (56.7 %) in the surveyed area were aware on the effect caused by *Alternaria* blight disease while 43.3 % were unaware of the problem (Table 12). Such results indicate that the disease is a problem in these districts that needs attention.

Table 12: Response of farmer on the effect of *Alternaria* blight disease in Iramba and Handeni Districts

Response	Frequency	Percentage
Yes	17	56.7
No	13	43.3
Total	30	100.0

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

Among the farmers interviewed during the survey, 56.7 % indicated that their fields have been planted with sunflower consecutively for more than two seasons (Table 13), while 30.0 % of the fields have been planted within the last two year. Thus, planting of sunflower on the same fields for more than two seasons without proper field sanitation i.e. destruction of plant debris and volunteering/alternative plants, may provide good environment for survival of *A. helianthi*. *Alternaria helianthi* can survive in plant debris

for 13 months (Amaresh and Nargund, 2000) that promotes the existence of the disease in the field.

Table 13: Evaluation of the intervals used by farmers for sunflower cultivation in the same fields in Iramba and Handeni Districts

Period	Frequency	Percentage
Above two years	17	56.7
Two years	9	30.0
One year	4	13.3
Total	30	100.0

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

4.3.3 Estimated yield loss caused by *Alternaria* blight disease of sunflower

The results indicate that 73.3 % of the farmers in the surveyed Districts do not keep farm records on sunflower yields (Table 14). Thus, it was difficult to estimate yield losses caused by *Alternaria* blight disease based on farmers' records. However, the available information indicate that 10 % of the interviewed farmers estimated yield losses of 10-25 % and 6.7 % reported losses of 45-50 % due to *Alternaria* blight disease (Table 14). Losses of 1-10% were reported by 10 % of the farmers. This implies that, it is difficult to implement disease control measures where the farmers do not feel the impact of the disease through yield losses encountered. The yield loss of 80 % due to *Alternaria* blight disease has been reported in India (Howard and David, 2007).

Table 14: Evaluation of yield loss caused by *Alternaria* blight disease of sunflower in Iramba and Handeni Districts

Estimated loss	Frequency	Percentage
10-25%	3	10.0
45-50%	2	6.7
1-10%	3	10.0
Not observed	22	73.3
Total	30	100.0

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

4.3.4 Sources of sunflower seeds and purchasing power of the farmers in surveyed area

The current study indicate that 33.3 % of the interviewed farmers use their own saved seeds from their previous harvests or bought from their neighbors, while 16.7% of the farmers purchased seeds from stockiest (Table 15). The infected farmers saved seeds may lead to multiplication of *Alternaria helianthi* and other micro-organisms. Such practices may also lead to dissemination of *A. helianthi* to areas where the seeds will be sown (Sheppard, 2003).

Table 15: Sources of sunflower seeds used by farmers and their ability to purchase the seeds in Handeni and Iramba Districts

Seed source	Frequency	Percentage
Farmer using saved seeds	10	33.3
Farmers using purchased seeds from stockiest	5	16.7
Farmers capable to purchase sunflower seeds	6.5	21.7
Farmers with low purchasing power for seeds	8.5	28.3
Total	30	100

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

Furthermore, the results of the current study also reveal that 28.3 % of the farmers in the surveyed Districts had low purchasing power thus, could not manage to pay for high prices of sunflower seeds. The remaining 21.7 % (Table 15) of the farmers were able to purchase sunflower seeds. The ability of these farmers to purchase seeds for planting was due to subsides provided by Muungano wa Ujasiriamali Vijijini (MUVI) project especially in Handeni District (Mujuni, P. personal communication, 2012). The results imply that low purchasing power of the farmers might contribute to disseminate the pathogen in disease free areas through contaminated farmer's saved seeds.

4.3.5 Management strategies applied by farmers in controlling *Alternaria* blight disease of sunflower

Results of interviewed farmers indicate that 76.7 % of the farmers in Handeni and Iramba Districts do not apply any control measure for *Alternaria* blight disease (Table 16). This is because farmers were unaware of the disease and its effects (Fig. 5). The percentage of farmers applying some form of managerial practices such as crop rotation, early sowing and early weeding is shown in Table 16. The use of crop rotation reduces disease incidence in the field (Amaresh and Nargund, 2000). It has been reported that under continuous cultivation of sunflower, nutrients may be exhausted from the soil and survival of the pathogen may dominate that may result to epidemic (Chaube and Singh, 2001; Mesta *et al.*, 2009). However, only 3.3 % of farmers used fungicide for seed dressing (Table 16). Seed treatment by chemicals reduces initial inoculum on seed hence suppressing *A. helianthi* (Rao, 2006). In other parts of the world, farmers use Mancozeb as foliar spray once disease symptoms are observed in the field (Chattopadhyay, 1999, OEPP/EPPO, 2000).

Table 16: Control measures applied by farmers for management of *Alternaria* blight in Iramba and Handeni Districts

Applied control measures	Frequency	Percentage
Do not use any control measure	23	76.6
Crop rotation	2	6.7
Early sowing	2	6.7
Early weeding	2	6.7
Seed dressed with chemicals	1	3.3
Total	30	100.0

Percentages were based on 30 farmers involved in the interview and the percentages calculated using number of farmers responded to the question (frequency) divided by total farmers (30) times 100.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

(i) The results of the current study showed that sunflower seeds used in this study were infected by several fungal microorganisms. Some of the fungi detected were pathogenic to sunflower and others were saprophytes. The pathogenic fungi included *Alternaria alternata* (47.75 %), *Fusarium palidoroseum* (16.25 %), *Fusarium moniliforme* (12.0 %), *Macrophomina phaseolina* (3.75 %), and *Alternaria helianthi* (3.75 %). Saprophytic fungi identified in this study included, *Aspergillus favus*, *Aspergillus niger*, *Curvularia lunata*, *Penicillium* spp., *Cladosporium* spp. and *Rhizopus* spp.

(ii) This study has shown that, the incidence of *A. helianthi* causing *Alternaria* blight disease was low (3.75 %) in seeds compared to other fungal microorganisms. However, the leaf samples tested had high incidence of *A. helianthi* (22.7 %). Such results imply that low inoculum of *A. helianthi* from the seeds can result into high disease incidence in the field.

(iii) Results indicate that incidences and severities of *Alternaria* blight were high under farmers' fields. The highest disease incidence was recorded at Vibaoni (88.7 %) and Kwamatuku (71.5) in Handeni District while high disease severity was recorded in Vibaoni (4.17) and Misima (4.00). These disease incidences were higher than that of Iramba District implying that *Alternaria* blight disease is a threat in Handeni District that needs attention.

(iv) Characterization of *A. helianthi* isolates based on morphological features resulted into two categories of the pathogen. First group with large radial growth, grey mycelia colour and smooth margin were faster in growth on V8 medium. The group was isolated from the seeds sample collected from Iramba District. The second group from seed sample collected from Handeni District was grayish black, slower in growth and has irregular margins. Such differences of morphological features between *A. helianthi* isolates reveal that there is genetic variation among *A. helianthi* populations infecting sunflower in the study area.

(v) It was evidenced that 70 % of the interviewed sunflower farmers in the study area were unaware on *Alternaria* blight disease while 30 % of them were aware about the disease naming it Ukungu and Kyusa. It was also noted that most of the farmers used farmers' saved seeds (66.7 %) infected by *Alternaria helianthi* that accelerated the spread of the disease in their fields. However, only 7 % of the interviewed farmers applied different managerial practices including crop rotation, early sowing and weeding and used fungicides for seed dressing to control *Alternaria* blight disease. There is therefore, a need of creating awareness to farmers on *Alternaria* blight disease of sunflower for early identification of the disease and its management and to discourage use of farmers' saved seeds.

5.2 Recommendations

(i) Most of sunflower seed samples tested had low levels of seed infection by *Alternaria helianthi*. However, high levels of *Alternaria* blight incidences and severity were recorded from farmers' fields. There is therefore, a need

of more research to find out other factors that might cause high incidences and severity of *Alternaria* bight disease under field condition.

(ii) Based on location, Handeni District had higher levels of disease incidence and severity than Iramba District. Thus, there is a need of introducing different cultural management practices that are affordable to most of the farmers. Farmers' saved seeds should be discouraged and advised to use sunflower seeds treated with fungicides that may provide initial control of *Alternaria* blight of sunflower.

(iii) This study identified two groups of *A. helianthi* based on morphological characters. There is therefore, a need to conduct more morphological studies to establish whether there are genetic variations between the two groups. There is also a need for breeding for resistance against the disease.

(iv) The majority of farmers covered by study were unaware of *Alternaria* blight disease. There is a need to conduct awareness seminar and workshops to sunflower farmers on identification and early management of the disease.

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APPENDICES

Appendix 1: Check list questions to determine management practices applied by farmers to control *Alternaria* blight of sunflower

1) Do you know this disease (*Alternaria* blight)?
2) What is the local name of the disease?
3) Do you think the disease is a major problem?
4) When does this disease occur?
5) What is the estimated loss caused by the disease?
6) How long the disease has been prevailing in their area?
7) How long does the farm have been used continuously
8) Is there any measure known to control the disease?
9) If any, what are the control measures?
10) Have you applied any of control measure?
11) What are the source of the seeds, from stockiest or owned?
12) Do the improved seeds available, Yes/No
13) If available, you afford to purchase seeds, Yes/No

Appendix 2: Sketch of experimental layout in the screen house

4 15	7 16	5 17	1 18	4 19	3 20	3 21

5 14	5 13	6 12	7 11	6 10	2 9	7 8

1 1	3 2	6 3	2 4	2 5	1 6	4 7

NB: 1-21: Number of plots; 1-7: Number of treatments

Appendix 3: The Analysis of variance (ANOVA) of Disease Incidence recorded in the Screen House

Variable	Source	df	SS	MS	F	P
Day 14	Model	17	7857.14	462.18	Infty	0.0001
	Error	3	0.00	0.00		
	Total	20	7857.142			
	CV%					0
	R^2					1.00
Day 28	Model	17	13095.24	770.31	Infty	0.0001
	Error	3	0.00	0.00	Infty	
	Total	20	13095.24			
	CV%					
	R^2					
Day 42	Model	17	25000.00	1470.59		0.0001
	Error	3	0.00	0.00		
	Total	20	25000.00			
	CV%					0.00
	R^2					1.00
Day 61	Model	17	25714.29	1512.61	Infty	0.0001
	Error	3	0.00	0.00		
	Total	20	25714.29			
	CV%					0.00
	R^2					1.00

Appendix 4: Analysis of variance of Disease Severity Recorded in the Screen House

Variable	Source	df	SS	MS	F	P
Day 14	Model	19	26.95	1.42	Infty	0.0001
	Error	1	0.00	0.00		
	Total	20	26.95			
	CV%					0
	R^2					1.00
Day 28	Model	19	31.81	1.67	Infty	0.0001
	Error	1	0.00	0.00		
	Total	20	31.81			
	CV%					0
	R^2					1.00
Day 42	Model	19	56.95	2.99	Infty	0.0001
	Error	1	0.00	0.00		
	Total	20	56.95			
	CV%					0
	R^2					1.00
Day 61	Model	19	69.81	3.67	Infty	0.0001
	Error	1	0.00	0.00		
	Total	20	69.81			
	CV%					0.00
	R^2					1.00

Appendix 5: Analysis of variance of sunflower seed yield and yield parameters recorded in the screen house

Variable	Source	df	SS	MS	F	P
Head size	Model	6	19.70	3.28	10.58	0.0002
	Error	14	4.35	0.31		
	Total	20	24.05			
	CV%					15.73
	R^2					0.82
Plant height	Model	6	2894.25	482.37	8.21	0.0006
	Error	14	823.02	58.79		
	Total	20	3717.27			
	CV%					4.09
	R^2					0.79
Seed weight	Model	6	10.99	1.83	12.14	0.0001
	Error	14	2.11	0.15		
	Total	20	13.11			
	CV%					23.65
	R^2					0.84

Appendix 6: The Analysis of variance of disease incidence and severity recorded from farmers' fields

Variable	Source	df	SS	MS	F	P
Disease Severity	Model	9	29.60	3.29	4.92	0.0015
	Error	20	13.37	0.67		
	Total	29	42.97			
	CV%					26.95
	R^2					0.69
Disease Incidence	Model	9	33064.93	3673.88	16.95	0.0001
	Error	20	4334.53	216.73		
	Total	29	37399.46			
	CV%					40.37
	R^2					0.88