VIRULENCE OF *Colletotrichum capsici* (syd. Buter and Bisby) IN PEPPER (*Capsicum spp*) VARIETIES IN MAJOR GROWING AREAS OF ETHIOPIA: IMPLICATIONS TO INTEGRATED DISEASE MANAGEMENT (IDM) USING CHEMICALS AND BIOLOGICAL CONTROL AGENTS

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Abstract

Hot pepper (*Capsicum anuum L.)* is the prominent type of *Capsicum* spp grown in Ethiopia, since its introduction in the early 17th century by the Portuguese. It covers 67.98% of all the area under vegetables in Ethiopia. Anthracnose is the most renowned pepper fungal disease in the south western part of the country causing up to 100% loss. Total crop failure due to diseases has been common in the region and farmers are sometimes forced to abandon their production due to excessive infection pressure in the field. The present thesis probes a broad overview of the disease in Ethiopia. The main aim of this study was; thus, to gather scientific information on the characterization of the pathogen and management of the disease it caused in the country. The specific objectives of this study were to assess the magnitude of disease in main chili growing areas of the country; and the level of pathogenesis of the strains of the pathogen using cultural, pathological and morphological tools; evaluate different biological control agents, plant extracts and fungicides in vitro; study the effect of seedling density and phonological stages on development of anthracnose and yield parameters in chili; Search for variability and germplasm tolerance among the existing chili/pepper varieties in Ethiopia; analyze and develop the incidence-severity relationships model; identify economically effective timing and frequency of fungicide spraying programs; and analyze the effect of integrated anthracnose disease management on disease reactions, growth and yield parameters and economic profitability of chili production.

Rigorous survey was conducted in anthracnose prone areas; and the highest and lowest disease spread was observed in Alaba and Shashogo with cumulative incidence of 41.88% and 19.81%, respectively. From the chili farms, the highest incidence was found in Arsi negelle followed by

Alaba with the value of 31.66% and 28.66%, whereas the lowest incidence in farms was found in Humbo and Maraqo with 13.63% and 14.89%. Nurseries with a highest incidence were observed in Humbo and Alaba with values of 13.5% and 13.02%, respectively. The disease incidence was low, 4.13% and 1.28%, in Shashogo and Arsi negelle. Prevalence was higher in upper-kolla agro-ecological zones where the mean was recorded as 21.82% and 7.55% in farms and nurseries, respectively.

The variability study indicated that colonies varied in their cultural behavior ranging from cottony to fluffy, mostly suppressed with regular to irregular margins. Colletotrichum spp pathogen's color ranged between white to grey. Growth rate of isolates on PDA medium was between 22.0 - 69.5 mm. Morphological studies of isolates revealed variations in their color, size, shape, acervuli production, setae size and shape of conidia. Average conidial size varied from 18.00 - 33.3 µm and average setae size varied from 77.2 -181.2 µm. On the basis of disease reaction expressed by differential hosts, eleven groups (races) of *C. capsici* were identified.

The evaluation of fungicides, plant extracts and antagonists showed that all test concentrations of Tilt-250 EC significantly inhibited the mycelial growth of *Colletotrichum capsici*. Among the tested plant extracts, garlic was the best both in reducing the radial mycelial diameter (72.33) and mycelial dry weight (73.33) at the highest concentration of 15 %. There was significant variation among isolates of *Trichoderma* spp and antagonistic activities ranged from 51% to 89% reduction of the mycelial radial growth of *Colletotrichum capsici* on the PDA medium. Among the promising antagonists, the isolate Tri_3 of *Trichoderma harzinum* showed the highest, 89 %, inhibition of mycelial radial growth of *Colletotrichum capsici*.

Experiment on planting densities and seedling phonological stages showed that symptom development was delayed one day in the youngest seedlings compared to the older ones. After the appearance of symptoms, for four consecutive days, the level of leaf disease incidence and severity was consistently lower on the youngest seedlings. Leaf wetness was highly reduced by increasing seedling spacing by at least 15 cm. The highest plant population densities yielded the highest weight of berries per plot.

In multi-locational germplasm screening trial, the majority of the genotypes were moderately resistance to *C. capsici* and none of them was found to be immune at the two locations. Significant variations were also obtained among the genotypes for all yield components, namely percent establishment, dry fruit weight per plant, number of fruit per plant, pulp weight per plant, unmarketable fruits weight per plant, fruit length and days to 50 percent maturity. Total yield per plant was higher at Alaba than Maraqo high level of disease incidence at Alaba. Anthracnose leaf incidence was consistently associated with leaf severity and their relationships can be estimated using the linear function across locations, crop seasons, and genotypes. The economic implication of the timing and frequency of Ridomil application in the current context of fungicide use on chili in Ethiopia was also evaluated. Less frequent applications (3-7 times) starting from flushing successfully prevented the disease development and significantly reduced the incidence of leaf anthracnose.

The lowest plant infection (12.8%), leaf infection per plant (15.2%), percent diseased leaf area(15.2%)and infected fruits per plot (17.4%) was observed on combined application of isolates *Trichoderma* spp, plant extracts and Ridomil in Maraqo fana variety. Regarding the growth parameters, viz. the highest Mean Percent establishment (81.67), mean days to 50% flowering

(65.33), mean days to 50% maturity (82) days to first harvest (106.3) in was observed in T16, T4, and T8, respectively. From the quality parameters, the highest mean number of branches per stem (9), mean canopy diameter (24.8), mean number of flowers per plant (9.6) and mean plant height (61.4) in T10, T15, T6 and T7, respectively. Both negative and positive control showed higher incidence and severity as compared to single and combined application of isolates *Trichoderma* spp, plant extracts and Ridomil. The combined application of Trichoderma, plant extracts and Ridomil performed best in number of fruits/plant, number of seeds/pod, unmarketable fruit, marketable fruit and total yield. This mix up gave a higher benefit-cost-ratio due to reduced production cost indicating that a judicious combination of organic management practice is environment friendly, healthy and sustainable.

Over and above, management of pepper anthracnose disease through integrated means was recommended in Ethiopia.

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List of Abbreviations and Acronyms

AAU	Addis Ababa University
AI	Active ingredient
ANOVA	Analysis of variance
CRD	Completely randomized design
Cc	*Colletotrichum capsici*
CV	Coefficient of variance
DMCM	Department of Microbial Cellular and Molecular Biology
DNA	Deoxyribose nucleic acid
EC	Emulsifiable concentrate
e.g.	Example
EIAR	Ethiopian Institute of Agriculture research
ISSR	Inter simple sequence repeats
LSD	Least significant difference
NGO	Non-governmental organization
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pH	Percentage of hydrogen ions
PROB.	Probability
RAPD	Random amplified polymorphic DNA
SC	Suspension Concentrate
SEM	Standard error mean
USD	United states dollars
VCG	Vegetative compatibility group
WG	Water dispersible granules
WP	Wettable powder

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Pepper/Chilli (*Capsicum annuum* L.) is one of the most widely grown vegetables in the world (Berke, 2002) . However, anthracnose is the major problem for chilli production, processing and quality in tropical and subtropical areas (Sharma *et al*., 2005). Anthracnose, caused by *Colletotrichum capsici* is one of the most serious fungal pathogens of chilli in Asia (Rajput, 2011). In Thailand, *C. capsici* is reported to be an important pathogen in chilli crop production areas (Than *et al*., 2013; Montri *et al*., 2009). This disease produces symptoms on leaves, stem and fruits and causes severe damage to mature fruits in the field, during transit and storage (Mehrotra and Aggarwal, 2003). The virulence degree of disease symptoms on host plants depends on the fungal pathotype. According to Sharma *et al.* (2005), 15 pathotypes of *C. capsici* were identified based on disease symptom development on inoculated fruit of *C. annuum* genotypes.

Anthracnose is one of the diseases that contribute to the low production of hot pepper. The anthracnose disease has been observed to occur in three phase's viz., (i) seedling blight or damping off stage, prevalent in the nursery, (ii) leaf spotting and die back stage which is initiated at different stages of growth and (iii) fruit rot stage in which the ripe fruits are infected. The last phase causes extensive damage to the fruits since the lesions on the fruits considerably reduce the market value of the produce. The disease is both seed borne and air borne and affects seed germination and vigor to a greater extent (Asalmol *et al.,* 2001). Fruit rot upto 32 per cent and dieback up to 29 per cent have been noticed. Seedling decay and seed rot upto 21 per cent were recorded under Central Indian conditions (Rajput, 2011).

Economic losses due to anthracnose disease on chilli are high in tropical and subtropical regions (Pakdeevaraporn *et al.,* 2005). *Colletotrichum capsici* was confirmed as the species responsible for chilli anthracnose in Thailand by pathogenicity test. Pathogenicity studies showed that the behavior of *C. capsici* isolates was homogeneous with regard to disease symptoms. However, variation in virulence or the level of disease (measured quantitatively) within the isolates was observed (Taylor *et al.,* 2007).

Montri *et al.* (2009) showed virulent pathotypes differ within *C. capsici* isolates based on percent lesion size, appearance of necrotic or water-soaked tissue and presence of acervuli. Therefore, information on the distribution of pathotypes or isolates in chilli growing areas and an accurate method for identification and characterization of *C. capsici* is necessary for effective disease management and development of host resistance in breeding programs.

There are different strategies for the control of disease includind anthracnose. These include biological control methods, pesticide applications and good agricultural practices. In Ethiopia, researches on organic management of anthracnose of chilli are very much limited and there is a need for development of information on organic management of chilli anthracnose (Jebessa and Ranamukhaarachchi, 2014). Hence, urgent research intervention should be made in order to overcome the crop losses caused by *C. capsici* on pepper in Ethiopia. Based on the research gap that was observed in the fields of pepper, this research was designed with the following objectives to tackle the problems ofanthracnose of pepper in some of the major growing areas of south-eastern parts of Ethiopia.

Capsicum spp (hot pepper) is one of the most popular vegetable in Ethiopia (Huffnaga, 1961). The genotypes have been widely grown in the country over centuries. More than 100,000 tones (annual average) of dry fruit of hot pepper are produced in the country (Lemma *et al*., 2013); and used for export for worldwide market but substantial amount are consumed locally as spice which exceeds the volume of all other spices put together in the country (EEPA, 2003). Nowadays, there is serious shortage of dry fruits both for export and local markets partly due to very low productivity (0.4 t dry fruit yield/ha) of the crop and disease infection (Lemma *et al*., 2013).

1.2. Objectives

1.2.1. General Objective

To investigate the morphological, cultural and pathological characterizations; virulence and pathogenicity of *Colletotrichum* spp infecting pepper and different control methods of the disease within the context of integrated pest management (IPM) in some parts of south-western Ethiopia.

1.2.2. Specific Objectives

Specific objectives of this thesis are as follows:

- To determine the incidence and severity of the causal agent of anthracnose of chilli in the major pepper growing areas of Ethiopia
- To determine morphology, pathogenicity and virulence of anthracnose (*C.capsici*) isolates of chilli
- To evaluate the effect of fungicides, plant extracts and antagonists *(Trichoderma Spp)* on chili anthracnose *(Colletotrichum Capsici (Syd.)* invitro under laboratory conditions
- To carry out performance evaluation of some chili pepper (*Capsicum* Spp) genotypes for anthracnose (*Colletotrichum capsici* (syd)) resistance in a field
- To study integrated management of anthracnose (Colletotrichum capsici (Syd)) and its implications to disease reactions, quality and growth parameters of three selected genotypes of chili
- To analyse the economic benefits and responses of chili yield to the integrated application of plant extracts and antagonists against the disease

1.2.3. Hypothesis of the PhD Study

- There is non-uniform anthracnose incidence and severity of in pepper growing areas of Ethiopia
- There are morphological and pathological differences among anthracnose (*C.capsici*) isolates of chilli
- Fungicides, plant extracts and antagonists *(Trichoderma Spp)* affect chili anthracnose *(Colletotrichum Capsici (Syd.)*
- There are differences in performances among selected chili pepper (*Capsicum* Spp) genotypes for anthracnose (*Colletotrichum capsici* (syd)) resistance
- Integrated management of anthracnose (*Colletotrichum capsici* (Syd)) has implications to disease reactions, quality and growth parameters of three genotypes of chili
- Integrated application of plant extracts and antagonists against anthracnose (*Colletotrichum capsici* (Syd.)) would give disease reactions, quality, growth parameters, the economic benefits and responses on chili yield in some chili growing areas of Ethiopia

CHAPTER TWO:

LITERATURE REVIEW

2.1. Classification and Nutritional Content of pepper/Chilli

The genus *Capsicum* was originated in the American tropics and has been propagated throughout the world including the tropics, subtropics, and temperate regions. The fruit of *Capsicum* has a variety of names, such as 'chilli', 'chilli pepper' or 'pepper' depending on place (i.e., differences between the English-speaking countries) and type of fruits. The term "chilli" in most of the world refers exclusively to the smaller, hot types of *Capsicum* (Concise Oxford Dictionary, 2010; Wikipedia, 2007). *Capsicum* spp contains approximately 20-27 species, 5 of which are domesticated: *C. annuum*, *C.baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*, and are cultivated in different parts of the world. Among the five species of cultivated *Capsicum*, *C. annuum* is one of the most common cultivated crops worldwide followed by *C. frutescens* (Bosland and Votava, 2003).

Chilli has many culinary advantages. It comprises numerous chemicals including steam-volatile oils, fatty oils, capsaicinoids, carotenoids, vitamins, protein, fibre and mineral elements (Bosland and Votava, 2003). Many chilli constituents are important for fruitritional value, flavor, aroma, texture and color. Chillies are low in sodium and cholesterol free, rich in vitamins A and C, and are a good source of potassium, folic acid and vitamin E. Fresh green chilli peppers contain more vitamin C than citrus fruits and fresh red chilli has more vitamin A than carrots (Marin *et al.*, 2004). Two chemical groups produced by chilli are capsaicinoids and carotenoids. The capsaicinoids are alkaloids that make hot chilli pungent. A large number of carotenoids provide high fruitritional value and the color to chilli (Hornero-Méndez *et al.*, 2002; Pérez-Gálvez *et al.*, 2003).

As a condiment it has become indispensable in every Ethiopian home. It is grown for its pungent fruits which are used both as green and ripe to impart pungency and flavour to the food. Chilli besides imparting pungency and red colour to the dishes is also a good source of vitamin (175 mg/100 g), vitamin A (870 mg/100 g) and vitamin B (0.59 mg/10 g) (AVRDC, 2003). Apart from these, protein, fats, carbohydrates and traces of minerals are also present. The active principle of

pungency which was earlier believed to be a crystalline volatile alkaloid called capsaicin is now found to be a mixture of 20 allied components. The extracted capsaicin is used in pain balms, cosmetics and medicines related to heart diseases. Chilli is also a rich source of red pigments namely capsanthin, capsorubin, cryptoxanthin and related carotenoids which are esters of capsanthin. Oleoresin can also be obtained from chillies and is extensively used in western countries in food preparations, beverage and cosmetics industries and also medicine for treatment of inflammation. Chilli also stimulates saliva and gastric juices and helps in digestion. It is used as a counter irritant in prickly heat powders, skin ointments, cosmetics and pain balms. Chilli extracts are used in wide range of medicines against tonsillitis, diphtheria, loss of appetite, intermittent fever, rheumatism, sore throat, swellings and hardened tumours.

2.2. Production of Chilli

The most important producers and exporters of chilli include China, India, Mexico, Morocco, Pakistan, Thailand and Turkey. India is a leading producer of chilli contributing close to 43 per cent of the world production followed by China (8.6 per cent) and Peru (5.6 per cent) (AVRDC, 2003). Chilli is considered to be one of the most important crops in the tropics. The area cultivated with chilli worldwide is about 1,700,000 ha for producing fresh chilli, and around 1 800,000 ha for producing dried chilli; a total area of 3 729, 900 ha with a total production of 20 million tonnes with an average productivity of 1.62 tonnes per hectare (FAO, 2003).

2.2.1. Production Status in Ethiopia

In Ethiopia the total area under hot pepper production for green pod was to be about 54,376, hectares. However, the area of coverage in the country increased from 54,376 to 81,544 hectares through 2003/04-2005/06 production years (CSA, 2006). In recent years the total production has declined due to various reasons, but there is still enormous potential for its production in the country (MARC, 2005). In Ethiopia, the crop is cultivated at diverse ecological zones from sea level to 2000 m.a.s.l under rain fed and irrigated conditions (Lemma and Edward, 1994.). The crop is one of the most widely grown and plays major role in Ethiopian daily dishes as it has various home and industrial uses as well as good export potential. Whereas sweet pepper and chili are grown in lower altitudes relatively in warmer areas than for hot pepper and is mainly grown in state farms for export. Birds' eye chili, which is the most pungent of all the peppers, is not in great

demand, though few plants are commonly found around the homesteads in high rain fall warmer areas of the country (MARC, 2003).

The dry pod yield estimate in small farmer field was about 4q/ha, in the state farm it ranged from 3 q/ha of dry pod yield and 150 q/ha of green pepper but the dry pod yield in experimental plot ranged between 25-30 q/ha (Lemma and Edward, 1994). This indicates that hot pepper and other vegetable crops need intensive care and management for high return per unit area. Yield is dependent on varieties and varieties themselves are considerably depending on a number of factors. In Ethiopia hot pepper production for dry pod has been low with a national average yield of 0.4 t dry fruit yield/ha (Fekadu and Dandena, 2006).

Much effort has been made and still continued to solve such production constraints nationally and internationally. In collaboration with the universities, EIAR had generated a number of improved varieties, appropriate agronomic practices and crop protection measures for the vegetable production sector that could be grown in the country both under rain fed and irrigated conditions (Fekadu *et al.*, 2008).

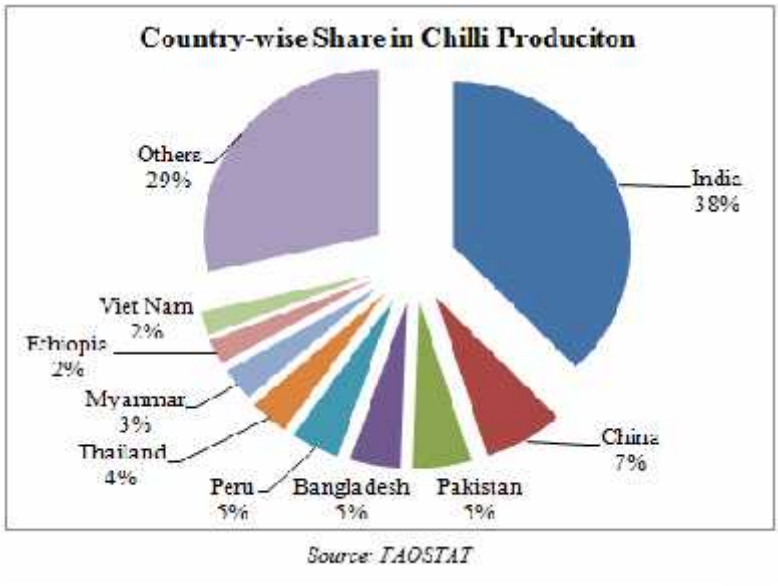

Figure 2.1. Worldwide main pepper producing countries

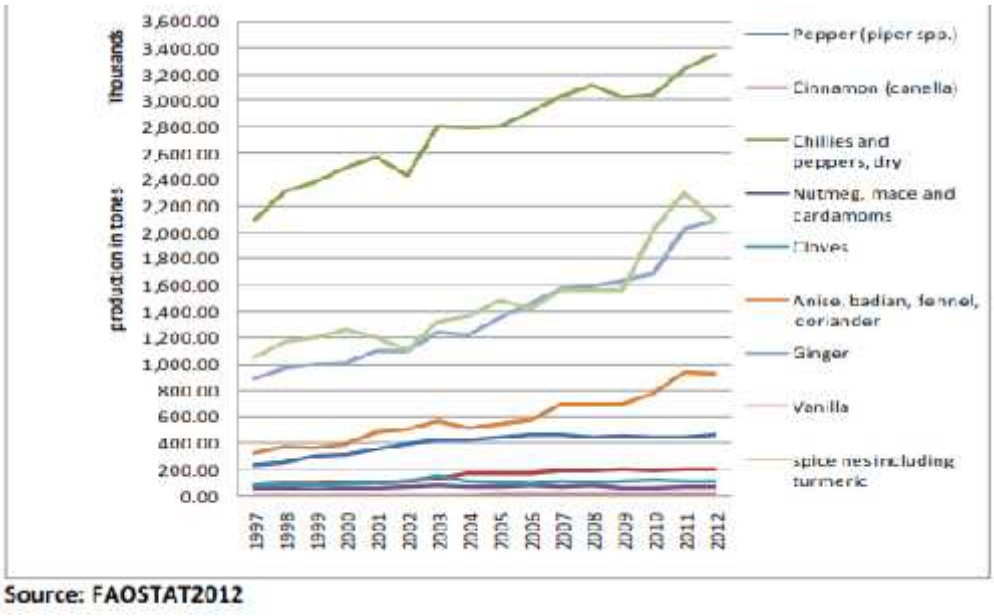

Figure 2.2. Pepper production in Ethiopia in relation to other spice crops in Ethiopia. Source: Ethiopian Institute of Agricultural Research

2.2.2. Varietal Studies and Achievements on Chilli

According to the AVRDC (Asian Vegetable Research and Development Centre), hot pepper is one of its principal crops. At the very beginning, it had a collection comprising 5,177 accessions from 81 countries/territories (Yamamoto and Nawata, 2005). The main emphasis of pepper work is centered on collection, multiplication, conservation, characterization, valuation, documentation, and distribution in comparison with the local varieties which are specific to agro- ecological sites throughout Asia, with the help of evaluation trials, the activity which has not yet been widely and consistently strengthened in this study (AVRDC, 2003).

In Ethiopia, capsicums have been grown for a long time by local farmers and considered as an indigenous vegetable crop and due to a long period of cultivation in different part of the country a great deal of natural hybridization has occurred among different capsicum species. As a result many local genotypes have evolved with various plant and fruit characters as well as disease and pest reactions. Research on *Capsicum* spp. started with minor observation and mass selection from local materials in different experimental stations of Awasa and Bako (MARC, 2003).

Later strong research activities on varietal screening and cultural practices were started at Bako Agricultural Research Center (Lemma and Edward, 1994). Major activities like varietal screening against diseases, adaptation studies and plant selections have been attempted at Nazret and Jimma Research Centers and at different trial cites in Gambella and farmers' fields in Southern Showa (*Mareko*, *Tedele*, *Enseno*) and Bako area. In the last 30 years, extensive research has been conducted mainly on hot pepper in different research centers and in Ambo plant protection centers and Haramaya University. Some improved cultivars from each type have been developed and some management practices like spacing, sowing date, rate of fertilizer, planting method, seeding rate and disease and pest control measures were recommended (MARC, 2003).

Among the selection work conducted earlier in 2002 at Bako and Awasa Research Centers two local selections Mareko Fana and Bako Local cultivars were developed by mass selection, since then they are widely grown in different parts of the country (MARC, 2003). Mareko Fana with larger and pungent pods with highly demanded dark- red color and Bako Local with high pungency content and yield, in which Bako Local was recommended for high rain fall Western part of the country and Bako areas, for *Mareko Fana* was recommended for Southern and Oromiya region and other areas with similar environmental and soil conditions. These cultivars are highly preferred by the local consumers owing to their pungency level, attractive color content and high powder yield and acceptable color. Particularly cultivar *Mareko Fana* is the only cultivar being used for a long time by the local factories for the extraction of capsicum oleoresin for the export market (MARC, 2003). Though hot pepper has been cultivated for centuries in typical tropical climate within Ethiopia, the yield has remained very low due to limited improvement work on the crop. However, in the past three decades, diverse genotypes (more than 300) of the crop have been introduced from different regions of the world and local collections have also been made in the country. The genetic improvement of hot pepper is also lacking in the country due to non availability of requisite genetic information. It is well recognized that the knowledge and understanding of the genetic basis of economic traits is important to enhance the progress in developing new varieties of the crop through breeding (Usman *et al.,* 1991).

The varietal analysis techniques have been found to be the useful tools to obtain precise information about the types of gene actions involved in the expression of various traits and to predict the performance of the progenies in the latter segregating generations. Each variety has its

own significant effect on yield and yield components, and each variety has its own traits that are part and parcel as quality parameters of the crop (shape, size, color, taste and pungency). The most important traits among others include, number of branches per plant (count), plant height, number of fruits per plant, days to maturity (count from days of transplanting), dry fruit yield per plant, fruit length and single fruit weight (Lemma *et al.*, 2008). Even though about a dozen hot pepper cultivar was released, in Ethiopian pepper research history, two cultivars, namely Mareko fana and Bako local, released in 1976, are being extensively produced in the commercial farms and by the peasant sector (Alemu Hailiye and Ermias Abate, 2000) (Appendix 10).

2.2.3. Constraints of Chilli Production

Diseases caused by fungi, bacteria and viruses are the major constraints to chilli production. Among the fungal diseases damping off, anthracnose or fruit rot, powdery mildew and leaf spots are the most prevalent ones. Anthracnose disease caused by *Colletotrichum* species, bacterial wilt caused by *Pseudomonas solanacearum*, and mosaic disease caused by chilli veinal mottle virus (CVMV) or cucumber mosaic virus (CMV) are the most serious destructive diseases of chilli. Anthracnose disease caused by *Colletotrichum* species is one of the most economically important diseases reducing marketable yield from 10% to 80% of the crop production in some developing countries (Poonpolgul and Kumphai, 2007). Anthracnose is mainly a problem on mature fruits, causing severe losses due to both pre- and post-harvest fruit decay (Bosland and Votava, 2003).

2.2.4. Diseases Incidence

According to MoARD (2009) anthracnose or ripe rot (*Collectotrichum capsici*) is one of the main diseases that directly cause the low yield on pepper. The disease causes rotting of the fruits and the underground portion of the stem and in severe conditions causes death, some of them cause small, yellow, slightly raised spots appear on young as well as on older leaves, some attacks the crop at seedling stage, as a result followed by yield loss. Therefore, the control measure includes the use of cultural practices, resistant varieties, rotation of crops, in the severe case application of fungicides are relevant (EARO, 2004).

2.3. Anthracnose Disease

The term anthracnose, derived from a Greek word meaning ‘coal’, is the common name for plant diseases characterized by very dark, sunken lesions, containing spores. Generally, anthracnose disease is caused by *Colletotrichum* species which belongs to the Kingdom Fungi; Phylum Ascomycota, Class Sordariomycetes; Order Phyllachorales; and Family Phyllachoraceae (Halsted, 1890). The teleomorph is *Glomerella* species. Anthracnose of chilli was first reported from New Jersey, USA, by Halsted (1890) in 1890 who described the causal agents as *Gloeopsorium piperatum* and *Colletotrichum nigrum*. These taxa were then considered as synonyms of *C. gloeosporioides* by von Arx (1957).

Anthracnose causes extensive pre- and post-harvest damage to chilli fruits causing anthracnose lesions. Even small anthracnose lesions on chilli fruits reduce their marketable value (Manandhar *et al.,* 1995). Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens. *Colletotrichum* species are the most important pathogens that cause latent infection. Appressoria are known to form adhesive disks that adhere to plant surfaces and remain latent until physiological changes occur in fruits. Appressoria that formed on immature fruits may remain quiescent until ontogenic changes occur in the fruits. Anthracnose disease can occur on leaves, stems, and both pre- and post-harvest fruits. Typical fruit symptoms are circular or angular sunken lesions, with concentric rings of acervuli that are often wet and produce pink to orange conidial masses. Under severe disease pressure, lesions may coalesce. Conidial masses may also occur scatteredly or in concentric rings on the lesions. Many studies have concluded that disease management practices are often inadequate to eliminate the diseases. Breeding to develop durable varieties has also not been successful due to involvement of multiple *Colletotrichum* species in anthracnose infection.

2.4. Economic Losses Due to Anthracnose

Choudhury (1957) reported that this disease was quite serious and wide spread in Assam. The disease has been recorded from the state wherever chilli was grown resulting in a loss of 12 -30 per cent of the fruits. Bansal and Grover (1969) during their studies on *Capsicum frutescens L.* reported that crop losses due to anthracnose disease ranged from 10-35 per cent in 1966 and 20-60 per cent during 1967 in six districts under study. Thind and Jhooty (1985) reported that *C. capsici* was a predominant fungus in causing fruit rot of chilli. Its incidence varied between losses 66-84

per cent. Paul and Behl (1990) reported that, the chilli suffers considerable losses due to fruit rot/dieback/anthracnose caused by *Colletotrichum capsici* in tropical and subtropical areas. Howard *et al.* (1992) reported losses greater than 30 per cent in plant production in United Stated due to anthracnose. Kannan *et al.* (1998) reported under suitable weather condition dieback and fruitrot caused by *Colletotrichum capsici* cause yield loss upto 12 to 15 per cent.

Verma and Sharma (1999) reported that fruit rot of chilli caused by *Colletotrichum capsici* (syd.) Buter and Bisby, was an important disease in field, transit, transport and storage. Pandey and Pandey (2003) reported that yield losses due to anthracnose varied from 10–60% in different parts of India. Bagri *et al.* (2004) reported that several diseases, particularly of fungal origin, attack the chilli crop. Among these, fruit rot, this caused 10-15per cent losses to mature fruits during transit and storage. Singh and Jameel (2007) reported that chilli growing areas were surveyed during the crop season and diseased plant parts showing anthracnose symptoms, particularly dieback and fruit rot were collected. Poonpolgul and Kumphai (2007) have reported that Thailand had encountered severe yield losses up to 80 per cent due to anthracnose (*Colletotrichum* spp).

2.5. Related Causal Agents of Chilli Anthracnose

In the *Colletotrichum* patho-system, different *Colletotrichum* species can be associated with anthracnose of the same host (Cannon *et al.*, 2000). *Colletotrichum* species causing anthracnose of chilli have been reported from different countries and regions. Although these species have been the subject of numerous investigations, there remain many gaps in the knowledge of the disease process and understanding of the complex relationships between the species involved. Kim *et al.* (2004) reported that different species cause diseases of different organs of the chilli plant; such as *C. acutatum* and *C. gloeosporioides* infect chilli fruits at all developmental stages, but usually not the leaves or stems, which are mostly, damaged by *C. coccodes* and *C. dematium*.

Colletotrichum species can survive in and on seeds as acervuli and micro-sclerotia (Pernezny *et al.,* 2003). Survival of mycelia and stomata in colonized chilli seeds had been reported (Manandhar *et al.,* 1995). It has been shown that the pathogen readily colonizes the seed coat and peripheral layers of the endosperm even in moderately colonized seeds. Heavily colonized seeds had abundant inter- and intracellular mycelia and acervuli in the seed coat endosperm and embryo, showing disintegration of parenchymatous layers of the seed coat and depletion of food material

in endosperm and embryo (Chitra and Kannabiran., 2001). Fungal isolates of *C.capsici* can overwinter on alternative hosts such as other solanaceous or legume crops, plant debris and rotten fruits in the field. *Colletotrichum* species naturally produce micro-sclerotia to allow dormancy in the soil during the winter or when subjected to stressful conditions and these micro-sclerotia can survive for many years. During warm and wet periods, conidia from acervuli and micro-sclerotia are splashed by rain or irrigation water from diseased to healthy fruit and foliage. Diseased fruit acts as a source of inoculum, allowing the disease to spread from plant to plant within the field (Roberts *et al.,* 2001). Initial infection by *Colletotrichum* species involves a series of processes including the attachment of conidia to plant surfaces, germination of conidia, production of adhesive appressoria, penetration of plant epidermis, growth and colonization of plant tissue and production of acervuli and sporulation (Prusky *et al.,* 2000). Anthracnose is mainly a problem on mature fruits, causing both pre- and post-harvest fruit decay resulting severe economic losses appressoria that formed on immature fruits may remain quiescent until the fruits mature or ripen (Bosland and Votava, 2003).

Table 2.1. Reported Causal Agents of Chilli Anthracnose (Than *et al.,* 2008)

Country	**Causal Agent**	**Reference**
Australia	*Colletotrichum acutatum, C. atramentarium, C. dematium, C. gloeosporioides var. minor, C. gloeosporioides var. gloeosporioides*	Simmonds, 1965
India	*C. capsici*	Maiti and Sen, 1979; Paul and Behl, 1990
Indonesia	*C. acutatum, C. capsici, C. gloeosporioides*	Voorrips *et al*., 2004
Korea	*C. acutatum, C. gloeosporioides, C. coccodes, C. dematium*	Park and Kim, 1992
Myanmar (Burma)	*Gloeosporium piperatum E. and E., C. nigrum E. and Hals*	Dastur, 1920
Papua New Guinea	*C. capsici, C. gloeosporioides*	Pearson *et al*., 1984
New Zealand	*C. coccodes*	Johnston and Jones, 1997
Taiwan	*C. acutatum, C. capsici, C. gloeosporioides*	Manandhar *et al*., 1995
Thailand	*C. acutatum, C. capsici, C. gloeosporioides*	Than *et al*., 2008
UK	*C. acutatum, Glomerella cingulata*	Adikaram *et al*., 1983
USA	*C. acutatum*	Roberts *et al*., 2001
Vietnam	*C. acutatum, C. capsici, C. gloeosporioides, C. nigrum*	Don *et al*., 2007

2.6. Disease Cycle and Epidemiology of Anthracnose

Environmental factors play a major role in the development of disease epidemics. The relationships among rainfall intensity, duration and crop geometry and the dispersal of inoculum

possibly lead to different levels of disease severity. The effects of temperature often interact with other factors, such as leaf surface wetness, humidity, light or competitive microbiota. The duration of the surface wetness, however, appears to have the most direct influence on the germination, infection and growth of the pathogen on the host. Generally infection occurs during warm, wet weather. Temperatures around 27 °C and high humidity (a mean of 80%) are optimum for anthracnose disease development (Roberts *et al.*, 2001).

Colletotrichum species utilize diverse strategies for invading host tissues, which vary from intracellular hemibiotrophy to subcuticular intramural necrotrophy. *Colletotrichum* species produce a series of specialized infection structures such as germ tubes, appressoria, intracellular hyphae, and secondary necrotrophic hyphae. These pathogens infect plants by either colonizing subcuticular tissues intramurally or being established intracellularly. The preinfection stages of the both are very similar, in which conidia adhere to and germinate on the plant surface, producing germ tubes that form appressoria which in turn penetrate the cuticle directly. Following penetration, the pathogens that colonize the intramural region beneath the cuticle invade in a necrotrophic manner and spread rapidly throughout the tissues. There is no detectable biotrophic stage in this form of parasitism. In contrast, most anthracnose pathogens exhibit a biotropic infection strategy initially by colonizing the plasmalemma and cell wall intracellularly. After the biotrophic state, intracellular hyphae colonize one or two cells and subsequently produce secondary necrotrophic hyphae. These pathogens are therefore regarded as hemibiotrophs or facultative biotrophs (Kim *et al.*, 2004). O′Connell *et al.* (2000) have observed that *C. gloeosporioides* on avocado, chilli and citrus can produce both types of colonizations: intracellular biotrophy at an early stage and intramural necrotrophy later.

Although the mechanisms developed by *Colletotrichum* species appear similar in prepenetration events, there are differences between species in the later mechanisms such as spore adhesion, melanization and cutinization in penetration of the plant cuticle by the appressoria. It has observed that the host-pathogen interaction of *C. acutatum* appears to be more biotrophic than that of some other species such as *C. gloeosporioides* (Wharton and Diéguez-Uribeondo, 2004). Based on studies with *C. acutatum* on specific hosts, four types of interactions or infection strategies were described by Peres *et al.* (2005).

Biotrophic growth of *C. acutatum* with secondary conidiation in which conidia germinate to form appressoria and quiescent infections, and secondary conidia are formed after germination of the appressoria (predominantly biotrophic disease cycle on citrus leaves). Subcuticular intramural necrotrophy with hyphal development within periclinal and anticlinal walls of epidermal host cells which are swollen and wider apart (predominantly necrotrophic disease cycle on strawberry). Hemibiotrophic interaction with infection vesicles and broad primary hyphae within host cells were also identified. Inter- and intracellular hyphal growth could be seen as the subsequent necrotrophic phase (combination of biotrophy and necrotrophy but mostly a biotrophic disease cycle on blueberry fruits).

There are only a few detailed studies on penetration and colonization by *Colletotrichum* species on chilli. Kim *et al*. (2004) have noticed that there was no biotrophic infection vesicle found during the infection process of C. *gloeosporioides* in susceptible chilli during the infection process of C. *gloeosporioides* in susceptible chilli (*C. annuum* cv. *jejujaerae*). Epidermal cytoplasm became condensed and small vacuoles increased and cell destruction extended to the subepidermal cells of the plant, which are likely to be damaged by the pathogen enzymes. At later stages of infection, tissues were colonized inter- and intracellularly by the pathogen. This structural feature indicated that the infection was governed by necrotrophic fungal growth.

2.7. Isolation and Proving Pathogenecity

Gomathi and Kannabiran (2000) reported that fruit samples of chillies showing typical fruit rot symptoms were collected from chilli growing fields. Cultures were identified as *Gloeosporium piperatum* E11 and Ev and *C. capsici* (syd.) Buter and Bisby and tested for their pathogenecity. Suthin Raj *et al.* (2008) as isolated pathogen from infected chilli fruits collected from the Chidambasam. The fungus was purified and identified as *C. capsici* by standard method. Chilli plants of 105 days old were used for inoculation. The plants kept in glass house were sprayed with sterile water followed by conidial suspension using atomizer in the late evening. The control plants sprayed with sterile distilled water. Periodical observations on fruit rot incidence were taken. For pathogenicity test, each isolates of *C. capsici* were cultured on PDA for 3 days. Then 0.7 cm agar plug contained with mycelia of *C. capsici* was placed on pierced area on chili fruit (*Capsicum anuum* L. var. *annuum*) obtained from chilli plantations. All inoculated fruits were incubated in moist plastic chamber, kept at room temperature ($27+1^0$C).Disease severity of

anthracnose infection was recorded at 5 days after incubation by measuring size of diseased lesion on chilli fruit.

Singh *et al.* (2007) collected plant part showing anthracnose symptoms, particularly dieback and fruit rot were collected and brought to laboratory for microscopic examination and isolation of pathogen. Purified cultures were tagged and tentitatively based on location of sample and proved in vitro pathogenicity on detached fruits of chilli cultivar ‘’Kandhari’’. Roat et al. (2009) collected the infected fruits isolated the pathogen and identified. Fresh chilli fruit samples were surface sterilized and incubated for seven days and confirmed pathogenicity. The isolates are used in the inoculation of chilli seedlings and fruits by the detached leaf assay procedure. (i) Conidial suspension (1 x 10^6conidia per ml) of twelve day old PDA grown cultures was sprayed on one-month-old chilli plants. (ii) The inoculated plants are covered with plastic bags for two days to maintain humidity. (iii) The plants were assayed for disease seven days after inoculation and continued to be so for up to 20 days; (iv) the presence of the pathogen was further confirmed by incubating the leaves in moist chambers for 5-7 days at 22 ± 1ºC and observed for the development of fungal growth (Chandra Nayak *et al*., 2009).

Thirty-four isolates of *Colletotrichum* spp. from anthracnose on Bell pepper, pathogenicity tests divided pathogenic potential into low, medium and high virulence groups. It is clearly revealed that *C. capsici* from the three tested hosts expressed the highest virulent isolates. Cross-inoculation of three high virulent isolates of *C. capsici* in accordance with three chilli varieties showed that all isolates could produce anthracnose symptom in the same lesions. All tested isolates developed lesions after co-inoculation of all hosts (Kanchalika *et al.,* 2010).

2.8. Identification of *Colletotrichum* Species

2.8.1. Morphological Characterizations

Accurate identification of ***Colletotrichum*** species along with the knowledge of populations responsible for epidemics is essential for developing and implementing effective disease control strategies. Traditionally, identification and characterization of *Colletotrichum* species have been based on morphological characters, such as size and shape of conidia and appressoria; existence of setae; the teleomorph state and cultural characters such as colony colour, growth rate and texture. These criteria alone, however, are not always adequate for species identification due to overlap in morphological characters and phenotypic variation among species under different environmental conditions. Conidial shape has been applied as a reliable means of discriminating certain species (conidial shape has differentiated *Colletotrichum* species pathogenic to strawberry). However, in other cases, identification can be complicated because of overlapping ranges of conidial morphology and variation in colony characteristics. Correct taxonomic identification is important in disease management such as choosing appropriate fungicides (Whitelaw-Weckert *et al*., 2007). It has been observed that the different responses of *C. acutatum* and *C. gloeosporioides* to benzimidazole-based fungicides (Peres *et al.*, 2004).

2.8.2. Molecular Genetics Approach

To overcome the inadequacies of traditional morphology based identification schemes, DNA sequence analyses have been used to characterize and analyze the taxonomic complexity of *Colletotrichum* (Moriwaki *et al.*, 2002; Du *et al.*, 2005; Photita *et al.*, 2005; Than *et al.*, 2008). Most fungal phylogenetic studies utilized sequences from the ribosomal gene cluster, since they were present in large numbers as tandem repeats and evolved as a single unit. In particular, sequence analysis of the internal transcribed spacer (ITS) regions which lie between the 18S and 5.8S genes. The 5.8s and 28s genes, has proved useful in studying phylogenetic relationships of *Colletotrichum* species because of their comparative variability (Moriwaki *et al.*, 2002; Photita *et al.*, 2005). Apart from ITS region, sequence analysis of protein coding genes such as partial β-tubulin gene, has also been applied to resolve phylogenetic relationships among *C. acutatum* species complexes (Sreenivasaprasad and Talhinhas, 2005). Sequences of introns from two genes (glutamine synthase and glyceraldehyde-3-phosphate dehydrogenase) were also used to evaluate a diverse collection of isolates of *C. acutatum* (Guerber *et al.*, 2003). *Colletotrichum acutatum* isolates clustered into groups (Guerber *et al.*, 2003; MacKenzie *et al.*, 2008; Peres *et al.*, 2008).

These groups might represent phylogenetically distinct species of *C. acutatum sensu lato* (Guerber *et al.,* 2003). Because of the high intra-species variability and the low inter-species variability, MAT1-2 mating type sequences gave strong support for branches, allowing differentiation of closely related *Cochliobolus* spp. whose relationships were not resolved by ITS sequences alone. Consequently, Du *et al*. (2005) confirmed that MAT1-2 mating type was useful in differentiating the groups of isolates from the species complexes (*C. graminicola, C. gloeosporioides* and *C. acutatum*). However, there is no report concerning the use of these genes to differentiate between the *Colletotrichum* species involved in chilli anthracnose.

A combined application of molecular diagnostic tools along with morphological characterization is an appropriate and reliable approach for studying *Colletotrichum* species complexes (Cannon *et al.,* 2000). Than *et al.* (2008) differentiated isolates of chilli anthracnose from Thailand into three species: *C. acutatum, C. capsici* and *C. gloeosporioides*, based on morphological characterization, sequencing based on rDNA-ITS region and partial beta tubulin gene and pathogenicity testing. The dendrogram derived from RAPD divided the isolates of *C. capsici* into two clusters. These did not correlate with the data from cultural morphology and virulence patterns. These results are similar to previous studies in which RAPD analysis was shown not to correlate with growth rates in culture and geographic region of different *Colletotrichum* sp. isolates (Sharma *et al*., 2005). However, the RAPD approach has been useful for proper identification and categorization of *Colletotrichum* sp. isolates. *Colletotrichum capsici* consists of variable populations based on cultural morphology, reaction to carbendazim, virulence pattern and RAPD analysis. Molecular phylogenetic grouping obtained by RAPD analysis did not correlate with morphological characteristics and virulence pattern. However, RAPD analysis can be used to classify *C. capsici* more rapidly than these other methods. Therefore, molecular phylogenetic grouping based on RAPD analysis represents a powerful tool for studying genetic diversity in *C. capsici*.

2.8.3. Pathogenic Variability of *Colletotrichum* Species

When any of the progeny exhibits a characteristic that is different from those present in the ancestral individuals or descent individuals, this individual is called a variant (Agrios, 2005). This may involve a change in any conceivable biological characteristic, such as color, shape, growth rate and reproduction rate. In the case of pathogens, changes in host range may occur, i.e., it may be able to infect a variety of the host plant or cultivar not previously infected by the ancestor, or in

virulence, i.e., it may produce a milder or much more severe disease than the ancestors (Agrios, 2005). This is the way that resistance of a plant variety is 'broken down' (Agrios, 2005). The spread of a resistant genotype capable of escaping a current prevalent pathogen will be challenged by a new parasitic strain that harbors a virulent gene which is capable of overcoming that resistance (McDonald and Linde, 2002). Compatibility of plant-pathogen interactions is often governed by the gene-for-gene model in many pathosystems. This suggests that a continuous co-evolutionary change in both host and parasite. Some pathogen populations are known to be pathogenitically diverse, and the diversity seems to be due to continuous generation of novel pathogenic variations (Taylor and Ford, 2007). It has been observed that on pathogen diversity and the geographic distribution of the pathogen population is therefore a prerequisite for accurate assessment of durable resistant germplasm in breeding programs (Abang, 2003). Taylor and Ford (2007) have suggested that knowledge of pathotype diversity is important when choosing the appropriate isolates to screen for resistance in plant breeding programs.

Mongkolporn *et al*. (2004) have studied pathogenic variation among 10 isolates of *C. acutatum* against 7 cultivars including reportedly susceptible species of *Capsicum* (*Capsicum annuum* 'Bangchang') and resistant species such as *Capsicum chinense* 'PBC 932' AVRDC, 1999; 2003; Mongkolporn *et al*., 2004), *Capsicum baccatum* 'PBC 80' and 'PBC 81' in Thailand. This revealed two pathotypes based on qualitative differences in infection of a reported resistant genotype, *C. baccatum* genotype 'PBC 81'. Interestingly, of 10 isolates assayed, the genotype showed complete resistance against 5 isolates tested, whereas it showed a highly susceptible reaction to the other 5 isolates tested. Sharma *et al*. (2005) studied the pathogenic variability in *C. capsici* in India and proposed that 15 pathotypes of *C. capsici* existed among 37 isolates from different chilli growing regions from Himachal Pradesh in India. However, these pathotype differences were based on differences in host reaction.

Taylor *et al*. (2007) defined the pathotype as "a subclass or group of isolates distinguishable from others of the same species by its virulence on a specific host (genotype), i.e., a qualitative difference in disease severity" and the aggressiveness as "the natural variation in virulence or level of disease (measured quantitatively) within the pathogen population." According to Taylor and Ford (2007), there might be confusion as to whether true pathotype differences exist, or whether the differences observed in disease severity are a measure of the natural distribution of

aggressiveness within a population, ranging from low to high. However, the level of aggressiveness of isolates is also an important consideration in resistance breeding programmes and disease control management. Genotypes with partial resistance would result in lower level of infection which eventually will decrease the inoculum amount in the field to limit the potential of epidemics.

Yoon *et al.* (2004) and Than *et al.* (2008) have screened *C. acutatum*, which is the very virulent species against chilli genotypes and found that *Capsicum baccatum* genotype 'PBC 80' is a genetic resource pool for resistance to anthracnose. This genotype is oten used for studying genetics of resistance and practical breeding in chilli pepper. However, it has found that another genotype of *C. baccatum*, 'PBC 81', showed high susceptibility to some *C. acutatum* isolates. This would make it questionable as to whether *C. baccatum* species are a useful genetic resource pool for breeding for resistance to anthracnose. In contrast to *C. baccatum*, susceptibility of the *C. annuum* cultivars has been reported in several studies (Mongkolporn *et al*., 2004b; Park, 2007). In addition to this, *Capsicum chinense* 'PBC 932' has been reported as a resistant variety to *Colletotrichum capsici* and hence has been introgressed with *C. annuum*'Bangchang' to produce F_1 progeny (AVRDC, 2003). However, Yoon *et al*. (2004) and Than *et al.* (2008) found that the 'PBC 932' was highly susceptible to *C. acutatum* isolates. Nevertheless, this information about highly susceptible reaction of 'PBC 932' will help chilli breeders to be aware of the potential for breaking down its resistance to *C. acutatum*.

2.9. Disease Management of Chilli Anthracnose

Agrios (2005) recommended integrated management techniques, as no single specific management program could eliminate chilli anthracnose. Effective control of *Colletotrichum* diseases usually involves the use of a combination of cultural control, biological control, chemical control and intrinsic resistance (Wharton and Diéguez-Uribeondo, 2004).

2.9.1. Cultural Practices

Pathogen-free chilli seed and planting materials should be planted and weeds must be eliminated. Crops should be rotated every 2~3 years with crops that are not alternative hosts of *Colletotrichum sp*. Transplants should be kept clean by controlling weeds and solanaceous volunteers around the transplant houses. The field should have good drainage and be free from infected plant debris. If disease was previously present, crops should be rotated away from solanaceous plants for at least 2 years (Roberts *et al*., 2001). Sanitation practices in the field include control of weeds and volunteer chilli plants. Choosing cultivars that bear fruit with a shorter ripening period may allow the fruit to escape infection by the fungus. Wounds in fruit from insects or other means should be reduced to the extent possibly because wounds provide entry point's for *Colletotrichum* spp. and other pathogens such as bacteria that cause soft rot. At the end of the season, infected plant debris from the field must be removed or deep ploughed to completely cover crop diseases (Agrios, 2005).

2.9.2. Use of Resistant Cultivars

The use of resistant varieties not only eliminated losses from diseases, but also eliminated chemical and mechanical expenses of disease control (Agrios, 2005). Some genetic resources resistant to anthracnose in chilli have been independently reported from different countries and regions (AVRDC, 1999; Yoon and Park, 2001). In particular, some lines of *C. baccatum* show strong resistance to the pathogen, and pathogen inoculation resulted in no or limited lesions on the chilli fruits (Yoon, 2003). However, to date, no strong resistance has been found in *Capsicum annuum*, which is the only species grown worldwide (Park, 2007). Mongkolporn *et al*. (2004a) carried out a genetic study of anthracnose resistance to *C.capsici*, which was expressed in the interspecific cross of Thai susceptible *C. annuum* cv. 'Bangchang' and anthracnose resistant *C. chinense* 'CM 021'. The genetic purity of the F1 was proven by using molecular marker analysis. Voorrips *et al*. (2004) have found one main quantitative trait locus (QTL) with high significance and large effects on resistance and three other QTLs with smaller effects on the F2 population (cross between *C. annuum* and *C. chinense*) on the traits they tested, such as infection frequency, the true lesion diameter and overall lesion diameter after inoculation with *C.gloeosporioides* in the study of resistance to anthracnose disease in Indonesia.

2.9.3. Use of Chemical Fungicides

Chemicals fungicides are the most common and practical method to control anthracnose diseases. However, fungicide tolerance often arises quickly, if a single compound is relied upon too heavily. The fungicide traditionally recommended for anthracnose management in chilli is manganese ethylenebisdithiocarbamate (maneb, mancozeb/scancozeb) (Smith, 2000). The strobilurin fungicides azoxystrobin (Quadris), trifloxystrobin (Flint), and pyraclostrobin (Cabrio) have recently been labeled for the control of anthracnose of chilli (Alexander and Waldenmaier, 2002). The disease can be controlled under normal weather conditions with a reasonable spray program. However, there are numerous reports of negative effects of using chemicals on farmers' income and health, and toxic contamination to the environment, particularly in developing countries (Voorrips *et al*., 2004).

Smith (2000) used manganese (Maneb) against anthracnose of chillies for its management and also recommended traditionally for management of anthracnose. Gopinath *et al.* (2006) tested the efficacy of 3 Triazole fungicides *viz.*, Hexaconazole (0.1%), Propiconazole (0.1%) and Triadimefon (0.1%) against *C. capsici* by poison food technique. Similar results were obtained by Mali and Joi (1985) reported Difolatan (Captafol), Thiram and Vitavax (Carboxin) as most effective against colony growth and sporulation of *C. capsici*. Significant inhibition of mycelial growth was recorded with all three fungicides.

2.9.4. Biological Control

The biological control methods for chilli anthracnose disease have not received much attention. The potential for biological control of *Colletotrichum* species had been suggested as early as in 1976 (Rajput, 2011). The possibilities of biological control of post-harvest fruit diseases by using *Pseudomonas fluorescens* had been discussed. Antagonistic bacterial strains (DGg13 and BB133) were found to effectively control *C. capsici*, the major anthracnose pathogen in Thailand (Intanoo and Chamswarng, 2007). It is also believed that *Trichoderma* species are able to effectively compete for surface area, thereby reducing pathogen infection success (Maymon *et al*., 2004). *Trichoderma* species have been applied to control *Colletotrichum* species in chilli (Boonratkwang *et al*., 2007), strawberries (Freeman *et al*., 2001), and citrus in Belize (Moretto *et al*., 2001) with concomitant disease reduction. Other biological control agents that have been tested for efficacy against *C. acutatum* include *Bacillus subtilis* and *Candida oleophila* (Wharton and Diéguez-Uribeondo, 2004).

Trichoderma viridae, *T. harzianum* and *T. koningi* (Oudem) inhibited mycelial growth of *C. capsici* by 51.7, 56.6 and 42.5 per cent, respectively (Chidanandaswamy, 2001; Rajput, 2011.). Among the four biocontrol agents tested *in vitro* against *C. capsici* causing leaf spot of turmeric, *Pseudomonas fluorescens* Migula was found to be superior in inhibiting the growth of the fungus followed by *T. harzianum* (Rifai) and *T. viride* (Pers) (Chidanandaswamy, 2001). D'Souza *et al.* (2001) screened 8 isolates of *T. harzianum* against *C. capsici* and observed that isolates T1, T2 and T3 of *T. harzianum* had the highest promise as the biocontrol agents under in vitro conditions by over covering the pathogen within 5-6 days. Ramamoorthy and Samiyappan, (2001) reported that *P. fluorescens* isolate pf1 effectively inhibited the mycelial growth of C. *capsici in vitro* and decreased the fruit rot incidence in chilli under greenhouse conditions. Seed treatment plus soil application of talc based formulation of *P. fluorescens* isolate pf1 effectively reduced the disease incidence.

Hegde *et al.* (2002) reported that *P. fluorescens* showed higher antagonistic activity against *C. capsici* recording growth inhibition of 54.38 per cent compared to the control 90.18 per cent, under *in vitro* conditions. Under green house conditions significantly less mortality was obtained on *P. fluorescens* sprayed plants compared to mortality in control plants. Wharton and Dieguez-Uribeondo, (2004) reported that biological control agents that have been tested for efficacy against *Colletotrichum* included *Bacillus subtilis* and *Candida oleophila*. Srinivas *et al*. (2005) reported that antagonist *Pseudomonas fluorescens* as seed treatment and as well as spray @ 10^8 CFU/g-1 were found to be effective against *C. Capsici*. Pratibha Sharma *et al*. (2005) reported that *Trichoderma* species effectively controlled *C. capsici* infection in chilli.

Intana *et al.* (2007) tested three mutant and two wild type strains of *Trichoderma harzianum* for efficacy to inhibit and overgrow mycelia of *Colletotrichum capsici*, a causal agent of anthracnose of chilli on potato dextrose agar (PDA) at room temperature. By using dialysis membrane technique, three mutant strains (T35-co4, T-35-co5 and T-50-co4) could produce antifungal metabolites, which completely inhibited mycelial growth of the pathogen (Intana *et al.*, 2007). Suthin Raj *et al.* (2008) reported that four different chilli seeds infected by *C. capsici* were treated with biocontrol agents. The treated seeds evaluated for per cent reduction of *C.capsici*, seed germination and vigour index. It was found that the pure culture of *P. flourescens* was effective in

reducing *C. capsici* infection fallowed by pure culture of *T. harzianum*. Tiwari *et al.* (2008) reported that antagonistic effect of isolates of *P. flourescens* and *T. harzianum* isolates was tested by dual culture technique against *C. capsici*. Among them three isolates of *T. harzianum* and five isolates of *P. fluorescens* were effective in inhibiting the pathogen.

During the entire course of two years of investigation, Meena *et al*. (2000) did not find any pest and diseases in the crops sprayed with Panchagavya. The Panchagavya contained *Pseudomonas* (45×10^3 cfu/ml) and saprophytic yeasts (35×10^4 cfu/ml), which might have contributed to plant protection because the presence of *Pseudomonas* on plant surfaces have been found to induce the production of pathogensis related protein, siderophores, antibiotics and hydrogen cyanide (HCN) in groundfruit and rice. This enabled its use as a biocontrol agent. Boomiraj and Christopher (2007) conducted experiments to evaluate the impact of organics and souces of neutrients. Higher bacterial and fungal populations were recorded in treatments receving poultry manure + Panchagavya. Salkinkop *et al.* (2008) reported the beneficial microorganisms from Panchagavya and their establishment in the use of compost that improved the plant growth, crop yield and suppressed plant pathogens. Shwetha (2008) found significantly higher microbial activity in treatments given organic manures with fermented organics.

2.9.5. Biofungicides and Organic Products

2.9.5.1. Use of Biofungicides

The control of chilli anthracnose fruit rot has, for many years, relied on chemicals and resulted in many undesirable problems. There is a need to incorporate alternative control components that are effective in field. Among the biofungicides used against the fungus *Colletotrichum* spp on chilli fruit, Chitra (2000) found that the most effective control was sweetflag crude extract when applied in two intervals when the majority of the plants were at the first bloom stage and at the mature bloom stage.

Gomathi and Kannabiran (2000) screened leaf extracts of 23 wild plants against *C. capsici* and reported that leaf extracts of *Solanum torvum*, *Datura metel* L. and *Prospopis juliflora* (Sw.) effectively inhibited the conidial germination and mycelial growth of fungus. *In vitro* evaluation of plant extracts against *C. capsici* causing leaf spot of turmeric showed that parthenium leaf extract was found to be superior in inhibiting the growth of fungus followed by garlic bulb extract

(Chidanandaswamy, 2001). Chitra and Kannabiran (2001) investigated the antifungal activity of fruits and flower extracts of *Datura innoxia* L. against *C. capsici* under *in vitro* condition. They observed that both extracts reduced the fresh and dry weight of *C. capsici* compared with untreated control.

Hegde *et al*. (2002) reported that under in vitro conditions nimbicidine (neem kernel extract) at 0.3 per cent inhibited the growth of *C. capsici* to a greater extent compared to the control plate and under greenhouse conditions significantly less mortality was observed in nimbicidine sprayed plants compared to control plants. Kumaran *et al*. (2003) evaluated the ethanolic extracts of roots of 18 different plant species for their fungitoxic activity against anthracnose in chilli caused by *C. capsici*. They reported that the ethanolic root extracts of Abrus precatorius and Rauvolfia tetraphylla showed significant inhibitory effects on both the conidial germination and radial growth of *C. capsici*.

Bagri *et al.* (2004) tested the antimicrobial properties of Bitter temru (*Diospyros cordifolia*), Datura (*Datura stramonium*), Amaltas (*Cassia fistula*), Brhati (*Solanum indicum*), Sandal (*Santalum album*), Mehandi (*Lawsonia inermis*) and Babool (*Acacia nilotica*) for chilli fruit rot management. Study revealed that in poison food technique maximum growth inhibition of the fungus was observed with Bitter temru extract followed by Datura leaves. Spore germination was also minimum in bitter temru fruit followed by Datura leaves. Tiwari *et al*. (2008) reported that plant extracts Datura leaf, Onion and Garlic bulb extracts completely inhibited the growth and sporulation of *C.capsici*.

Roat *et al.* (2009) reported that bitter temru fruits and datura leaves inhibited the maximum mycealial growth and sporulation of *C.capsici*, while minimum was recorded in sandal seed. The bitter fruits and datura leaves showed maximum reduction in the incidence of fruit rot. The highest disease severity was recorded in *C.capsici* with sandal treated fruits. Bilal *et al*. (2010) evaluated five biopesticides (Achook, Neemgold, Wannis, Spictaf and Neemazal) under *in vitro* and *in vivo* conditions against Colletotrichum lindemuthianum. Among the biopesticides tested at four concentrations, Wanis applied @ 1000 µl/ml caused maximum inhibition of 82.12 per cent followed by Spictaf (52.85%).

More *et al.* (2010) studied the antifungal properties of two plant extracts at different dilutions against anthracnose of chilli caused by *Colletotrichum capsici* under in vitro condition. The antifungal properties of plant species viz., *Callistemon lanceolatus* and *Pongamia pinnata* were tested after extracting in 10 per cent concentration of five solvents viz., acetic acid, acetone, ethanol, petroleum ether and chloroform along with distilled water as sixth solvent. Among the six solvents used for extraction of antifungal properties of *Callistemon lanceolatus, Pongamia pinnata* separately and combination of both *C. lanceolatus* and *P. pinnata* at 1:10, 1:100 and 1:1000 dilutions acetic acid showed complete inhibition of mycelial growth of *Colletotrichum capsici.*

2.9.5.2. Organic Products

Yadav (2008) has reported that dusting of ash, spraying of sour butter milk and spraying of cow urine/goat urine heal to controls the spread of anthracnose of chilli caused by *C. capsici*. Suresh (2008) reported that sprays of cow urine, neem oil, Pseudomonas, panchagava at 2 to 5ml per litre of water alternatively at regular interval control the major disease of leaf curl and anthracnose of chilli. Nagaraja (2009) have reported that seed treatment with organics revealed that, beejamruta was found superior over other with 84 per cent seed germination and highest vigour index of 1211 and there was a gradual reduction in incidence of fruit rot of chilli. Pathak *et al.* (2010) have reported that cowdung showed antipathogenic potential against *Colletotrichum gloeosporioides*. Mode of antipathogenic activity was studied under the microscope and it was found that actinomycetes, identified as *Streptosporangium pseudovulgare* attacked mycelia, cell wall and enter the host. Inside the host it utilizes the host cytoplasm for its multiplication and finally host cell degraded completely.

Bharathi *et al.* (2004) reported that PGPR mixed bioformulation of *P. fluorescens* (pf1) + B. subtilis + neem + chitin was found to be the best for reducing the fruit rot incidence of chilli besides increasing the plant growth and yield parameters under both greenhouse and field conditions. Ekbote (2005) reported that treatment of 40 day old chilli seedlings with *P. fluorescens* solution (1%) reduced the incidence of dieback and fruit rot and increased the yield of chilli compared to the control. The seedling dip treatment for 30 mifruites was found most effective. Yadav (2008) reported that seedling treatment with 20g of *Trichoderma* mixed with one litre of water helped in control of anthracnose of chilli caused by *Colletotrichum capsici*. Benagi

et al. (2009) reported that seed treatment with *T. viridae*-2, *T. hamatum*, TMTD, *T. viride*-3, T. *harzianum*-2 and *P. fluorescens* recorded higher seed germination and effective against anthracnose of chilli and *P. fluorescens* @ 10g/l could be used as seedling dip and sprays for management chilli anthracnose. Mesta *et al*. (2009) tested that seedling dip and spray of Pseudomonas fluorescence @ 10g/l in various combinations with chemicals, viz., carbendazium and hexaconazole. Seed treatment with carbendazim (0.2%) + seedling dip in *P. fluorescence* at 45, 60 DAT + 2 spray Hexaconazole 75 and 90 DAT recorded least disease incidence for fruit rot (20.60 PDI), highest yield (8.0q/ha) higher net returns.

2.10. Current Status of Chilli Anthracnose Disease Management

Management and control of the anthracnose disease are still under extensive research (Yoon *et al*., 2004). Among disease control management, the use of resistant cultivars is the cheapest, easiest, safest and most effective means of controlling the disease. This is not only to eliminate losses from the disease but also decrease the cost of chemical and mechanical control, as well as reduce contamination of the environment from the use of toxic chemicals. However, management of disease through breeding of pathogen-resistant cultivars has only had limited success due to frequent breakdown of resistance under field conditions. Commercial cultivars of *Capsicum annuum* resistant to the pathogens that cause anthracnose have not yet been developed (Park, 2007). Nevertheless, high levels of resistance to the *Colletotrichum* species that infect chilli have been found in some species of *Capsicum*, for instance, *C.baccatum*. Current research is focusing on introgression of this resistance into susceptible commercial cultivars of *C. annuum* (AVRDC, 2003; Pakdeevaraporn *et al*., 2005). Mongkolporn *et al*. (2004) have studied the inheritance of resistance to anthracnose specifically caused by *Colletotrichum capsici*, in a *Capsicum annuum* population established from a cross between accession '83-168' and cv. 'KKU-Cluster', and their progenies. They observed a promising dominant gene responsible for the resistance to *C. capsici*. Voorrips *et al*. (2004) found one main QTL with high significance and strong resistance against *C. gloeosporioides* associated with chilli anthracnose disease.

Although there are currently extensive researches on disease control management including breeding programs for resistant cultivars to anthracnose, the current status of the chilli anthracnose disease still requires improvement. There remain many questions to be answered concerning characterization of *Colletotrichum* species associated with anthracnose; in particular species

present in different countries and regions; pathogenetic or genetic diversity of *Colletotrichum* species worldwide; infection processes and the disease cycle of *Colletotrichum* species leading to effective disease control and resistant plant breeding.

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CHAPTER THREE:

INCIDENCE AND SEVERITY OF ANTHRACNOSE (*Colletotrichum* spp) IN MAJOR CHILI GROWING AREAS OF ETHIOPIA

Abstract

Chili anthracnose is the key constraint that hampers chili production in Ethiopia. But scientific information on the magnitude of the problem was not sufficient. Thus, this survey was aimed to assess the prevalence of chili anthracnose, weaknesses in management and formulate appropriate recommendations. 132 purposively selected chili fields, 106 farms and 26 nurseries throughout the chili livelihoods of Ethiopia had been surveyed. To evaluate the perceptions of farmers, semi-structured questionnaire had been used administered among 132 farmers. Data on incidence, severity and prevalence, and their variation across different locations, seasons and agro-ecological zones, had also been collected. The obtained data had been analysed through descriptive statistics using IBM SPSS 20.0. The highest and lowest disease spread was observed in Alaba and Shashogo with cumulative incidence of 41.88% and 19.81%, respectively. From the chili farms, the highest incidence was found in Arsi negelle followed by Alaba with the value of 31.66% and 28.66%, whereas the lowest incidence in farms was found in Humbo and Maraqo with 13.63% and 14.89%. Nurseries with a highest incidence had been observed in Humbo and Alaba with values of 13.5% and 13.02%, respectively. The disease incidence was low, 4.13% and 1.28%, in Shashogo and Arsi negelle. Prevalence was higher in upper-kolla agro-ecological zones where the mean was recorded as 21.82% and 7.55 in farms and nurseries. The mean incidence of farms was three times higher than nurseries. The spread of the disease was associated with non-hygienic practices in the nurseries as well as the disease inductive irrigation methods in use. Adequate chemical treatment, avoidance of water splashes, disinfection of tools and seedling, removal of sources of contaminations, were recommended to improve the practices in each field and nursery. Based on the information generated on the extent of anthracnose damage, the decision makers, policy experts and researchers will shift their prioritization and embark on controlling chili anthracnose in Ethiopia.

Key Words: Anthracnose, Chili, *Capsicum frutescence L.*, Incidence, Prevalence

3.1 Introduction

Chili (*Capsicum frutescence* L.) is an agriculturally significant crop in Ethiopia and most developing countries providing an important nutrient source and addresses food needs and job creation throughout the crop value chain (Shiferaw and Alemayehu, 2014; Beyene and David, 2007). The total area devoted to hot pepper is estimated 29% (EEPA, 2003). People consume pepper for intake enhancement as well as to supplement the dietary needs. It is also one of the major income generating crops for most households of the pepper producing areas (Roukens, 2005).

Hot pepper is the leading vegetable crop produced in the Ethiopia. The national production of green and dry hot pepper was 2,541,883.97 and 412,503.57 tones with average productivity of 66.88 and 23.31quintals per ha respectively (CSA, 2013). World average green pepper productivity, on the other hand, was 15.5 t ha^{-1} compared to the pepper productivity in Ethiopia (FAO, 2009). Thus, *Capsicum* productivity in Ethiopia is far below the world average that strongly demands immediate productivity improvement. According to Melaku *et al.* (2014) the present situation indicates that in the study area there is no improved hot pepper varieties but there is one local variety named "*Mita Mito*" by local growers, the green pod yield (3 ton per hectare) of this local variety is very low compared to national average yield. As a result, varietal information for the improvement of the crop for high fruit yield and quality in the existing agro-ecology is insufficient (Haileslassie *et al*., 2015).

In spite of its importance, the hot pepper production system for green and dry pod has stayed low due to low input and low output with a national average yield of 7.6 t/ha for green pod whereas it was 1.6 t/ha for the dry pod, respectively (CSA, 2013), which is very low as compared to Thailand's average national yield of hot pepper berries (15 t/ha) (Adams *et al*, 2015).
The decline of hot pepper production is attributed mainly to absence of use of herbicides, lack of improved and good quality varieties, poor agronomic practices, poor disease and pest management, poor harvesting and post harvest practices (Alemu and Ermias, 2000; Fekadu, 2008; Fekadu and Dandena, 2006).

There is limited information on evaluation of hot pepper varieties which enables the growers to select the best performing ones in the country (Melaku *et al.*, 2015). Anthracnose disease,

therefore, is one of the considerations to ease the existing problems of obtaining the desired varieties for which the output of this study was likely to assist and sensitize hot pepper growers and processors, further more the increasing demand for hot pepper to feed the growing human population and supply the ever-expanding pepper industries at national and international level has created a need for the expansion of pepper cultivation in to areas where it has not ever been extensively grown (Fekadu, 2008).

It is reported that assessment of the incidence and severity of plant diseases in general and anthracnose specifically, is important to determine the geographic distribution and status of the disease throughout a region in order to prioritize research themes to the situation (Teklay *et al*., 2015; Eshte *et al*., 2014). However, scientific information on anthracnose disease incidence and severity is not sufficient in the major paper growing parts of the country. Thus, this study was initiated to assess the incidence and severity of anthracnose disease in some major paper growing areas of Ethiopia

3.2. Material and Methods

3.2.1. Experimental Site

This study was carried out in SNNP, Oromiya and Amhara regions, which are the most important pepper growing locations and characterized by range of dry to sub humid climate (Appendix 1 and 2). These locations are hot spot areas for anthracnose (*Colletotrichum* spp) (Shiferaw and Alemayehu, 2015). Table 3.1 shows the coordinates and other geographic characteristics of the survey area.

Table 3.1 General characteristics of trial sites from which anthracnose disease incidence and severity data had been collected in 2013

Trial Name	Latitude	Longitude	Altitude (m)	Region/Zone	AE Zone	Time
ADDIS ABABA						
AAU, 4kilo	9.037354	38.76779	2435.84	Addis Ababa	6	2013 -07-20
HADIYA						
Doisha	7.473552	38.4449	1900.00	Hadiya, SNNPR	1	2013 -09-20
Hossana	7.552825	37.85649	2309.44	Hadiya, SNNPR	2	2013 -08-01
Bonosha Mazoriya	8.042378	38.49944	1840.08	Hadiya SNNPR	3	2013 -08-08
East Badawacho	7.417574	38.19376	1774.78	Hadiya, SNNPR	1	2013 -08-17
Soro	8.142378	38.59944	1840.08	Hadiya SNNPR	3	2013 -08-18
GURAGE & SILTIE						
Sankura	7.353447	38.09112	1823.63	Silti, SNNPR	5	2013 -09-8
Sankura	7.353447	38.09112	1823.63	Silti, SNNPR	3	2013 -09-10
Worabe	7.737333	38.12154	1988.49	Silti, SNNPR	2	2013 -09-09
Alkeso	7.848471	38.18766	2096.36	Silti, SNNPR	4	2013 -09-18
Menaheria	7.918454	38.23706	2306.69	Silti, SNNPR	6	2013 -10-24
Qibet	7.949281	38.26793	2389.2	Gurage, SNNPR	3	2013 -09-21
Mareko-Guraghe Borders	8.024675	38.32799	2120.24	Mareko, SNNPR	3	2013 -10-22
Qoshe Fields	8.01513	38.53197	1872.82	Mareko, SNNPR	4	2013 -10-20
Butajira zuria	7.918454	38.23706	2306.69	Butajira, SNNPR	6	2013 -10-25
Meskan	7.949281	38.26793	2389.2	Butajira, SNNPR	3	2013 -10-23
Azernet-berbere	7.918454	38.23706	2306.69	Silti, SNNPR	6	2013 -10-24
WOLAITA						
Wolaita Sodo	6.852763	37.76414	1997.79	Wolaita, SNNPR	3	2013 -09-11
Humbo Tebella	6.703099	37.7751	1590.79	Wolaita, SNNPR	4	2013 -09-12
Mirab Abaya	6.652763	37.76414	1997.79	Gamogofa, SNNPR	2	2013 -09-13
Boditi (Damot Galle)	7.703099	37.7751	1590.79	Wolaita, SNNPR	4	2013 -09-14
Areka	7.852763	37.76414	1997.79	Wolaita, SNNPR	2	2013 -09-16
KAMBATA & ALABA						
Halaba Field A	7.317574	38.09376	1774.78	Alaba, SNNPR	6	2013 -11-07
Halaba Field B	7.389356	38.1042	1825.77	Alaba, SNNPR	3	2013 -11-08
Hadero	7.989356	38.4042	1825.77	Kambata, SNNPR	3	2013 -11-09
Mazoria	7.889356	38.3042	1815.00	Kambata, SNNPR	3	2013 -11-10
OROMIYA						
Wonji	8.4538411	39.280399	- -	Adama, Oromiya	2	2013 -10-20
Adama zuria	8.5263486	39.2583293	- -	Adama, Oromiya	2	2013 -10-20
Arsi Negelle	7.3610886	38.668713	- -	West Arsi, Oromiya	1	2013 -10-22

Nekemte	9.0893009	36.555386	- -	W. wollega Oromia	1	2013 -10-27
Gute	9.3208484	36.671451	- -	W. wollega Oromia	3	2013 -10-27
Ano	9.0928759	36.959483	- -	W. wollega Oromia	2	2013 -10-28
Bako	9.1248249	37.0588169	- -	W. wollega Oromia	2	2013 -10-29
AMHARA						
Bure	10.708145	37.0668651	- -	E. Gojam, Amhara	3	2013 -10-15
Finote-selam	10.697988	37.176773	- -	E. Gojam , Amhara	2	2013 -10-15

3.2.2. Socio-Economic Survey

The survey was conducted by involving 132 farmers, 26 nurseries managers throughout the main chili producing regions of Ethiopia. The subjects were interviewed during the period from Apr.-June, 2013. A two part, semi-structured questionnaire was used for farmers and nursery managers (Appendix 3.5). Photos of anthracnose disease symptoms were also included to facilitate disease recognition and thus making the communication easier and to avoid possible confusion from other diseases. Other relevant information was noted during the survey, either in the form of additional notes, or as photographic records using a digital camera.

3.2.3. Anthracnose Disease Assessments

132 hot pepper farms including 26 nurseries were visually assessed before and after flowering on permanent field. Of these, 27, 28, 29, 30, 11 and 7 chili farms found in Wolaita zone, Hadiya zone, Gurage/Siltie zone, Oromiya and Amhara regions were assessed, The distance between two nearby randomly surveyed fields per districts was 5 km. Assessments were carried out based on the method recommended by Mekonnen *et al.* (2015) in which, farms were visited diagonally, and the disease incidence was estimated by using 3 m x 3 m quadrant. The number of diseased plants and the total number in each quadrant were recorded. Disease incidence was calculated as the percentage of infected plants in each field at each location. On the other hand, anthracnose severity was assessed as the average leaf/fruit area covered by the symptom of anthracnose among the collected fruits infected with anthracnose in each farm.

3.2.4. Spatial and Temporal Disease Distribution in a Nursery

The role of shedding (type and coverage), watering frequency and method were evaluated through the questionnaire and physical observations. Similarly, the possible role of the microclimate associated with either growth stage (age of the seedlings) or seedling density was also explored. Arising from observations during the survey, further experiments on the effect of seedling density and age on disease development were conducted. Notes on other factors that may facilitate disease spread in the nursery were also taken into consideration. These included handling of the seedlings, transmission routes and the movement of workers. Finally respondents were requested to provide information on temporal distribution of the disease (Shiferaw and Alemayehu, 2014).

3.2.5. Analysis of Data

Quantitative and qualitative data were all tabulated in Microsoft Excel. Disease incidence was computed for each sampled far, nursery and region, totals and means were tabulated. Geographic patterns of the disease prevalence were examined over the Regions, geographic position (GPS data) or agro-ecological zones (Teklay *et al.*, 2015) by overlapping maps of the same scale. Statistical frequencies of particular answers, percentages, totals and or averages and respective standard deviations were computed accordingly. Data on temporal distribution of the disease were analyzed by highlighting in SPSS sheet on the location and respective period (months) of occurrence (SPSS IBM 20.0). Periods of high disease incidence were noted based on frequency of locations with infected seedlings. Similarly, locations of continuous occurrence of the disease were noted over the time.

3.3 Results

3.3.1. Anthracnose Disease Incidence and Severity

The finding showed that none of the farms were completely free and the standard deviation among the location in prevalence, incidence and severity were 14.32, 12.54 and 20.95, respectively (Table 3.1).

Table 3.1. Prevalence Incidence and Severity of Chili seedlings in 132 farms in different regions, zones, kebelles, and locations of Ethiopia (Survey Conducted in 2013)

S. No.	District/Zone/Region	No. of Fields Assessed	Mean Percent of Anthracnose disease:		
			Prevalence	Incidence	Severity
1	Humbo, Wolaita sodo Zuria Mirab Abaya, Boditi (Damot Galle), Areka				
	Mean of Wolaita	27	67.2 %	56.4 %	54 %
2	Hossana zuria (Lemo), Doisha, Shashogo, East Badawacho, Soro				
	Mean of Hadiya	28	75 %	48 %	48 %
3	Mareko-Guraghe Borders, Qosha zuria Butajira zuria, Meskan, Azernet-berbere				
	Mean of Guraghe and siltie	29	58 %	49 %	52 %
4	Alaba, Sankura, Worabie zuria, Hadero, Mazoria				
	Mean of KA	30	80 %	67 %	72 %
5	Adama-Wonji, Mojo-Ziway-Meki, Arsi negelle, Amhara-Bure, Amhara- Finoteselam				
	Mean of Oromiya & Amh.	**18**	**44 %**	**32.8 %**	**14.4 %**
	Standard deviation		**14.32**	**12.54**	**20.96**

The cumulative anthracnose incidence for SNNP Region was as high as 44.29% for transplanted seedlings while it was 42.5%. This was about as twice as the disease incidence recorded from Oromiya and Amhara Regions combined (Table 3.1) (Appendix 3G). Anthracnose incidence means on mature plants as well as seedlings per Region, district and location surveyed are presented in Table 3.2. The overall mean incidence on transplanted-seedlings was higher (13.6 %) than that of direct planted ones (13.6 %) (Table 3.2) (Appendix 3F).

Table 3.2. Percentage of anthracnose infected chili seedlings some selected Districts in Ethiopia (Survey Conducted in 2013)

District	Infection(%) and Number of Seedlings Assessed		Total
	Transplanted (Mn±SD)	**Planted** (Mn±SD)	
Alaba	5194(13.02) ± 67.82	686(28.86) ± 29.77	5880(41.88)
Humbo	726(13.5) ± 7.9	477(13.627) ± 5.04	1203(27.17)
Maraqo	2987(5.48) ±13.73	396(14.89) ± 2.07	3383(20.37)
Shashogo	2507(1.28) ± 2.74	340(18.53) ± 1.87	2847(19.81)
Arsi negelle	436(4.13) ± 1	139(31.66) ± 2.52	575(35.79)
Adama	534(7.87) ±7.02	227(23.34) ± 2.37	761(31.21)
Total	**12384**	**2265**	**14649**
Mean (%)	**229.34 ±25.43**	**41.94 ±11.08**	

Out of( ) = Total number of seedlings assessed; SD= Standard deviation

In a few farms and nurseries in Oromiya and SNNP Regions, seeds are deepen in to a solution of fungicides before sowing, but in most cases they are not. In one case, it was noted that seeded pots, were being sprayed with fungicide (Ridomil) even before seed germination. Very young seedlings, up to five leaf stage, in general, had no symptoms of anthracnose. At more developed stages of the seedlings, when most leaves are severely infected, the first four, at the bottom, tend to show minor expansion of necrotic area. Because this observation has led to the hypothesis of age related seedling tolerance to anthracnose, a specific trial was established.

3.3.2. Infected mature plants and chili Gardens

As indicated in table 3, the mean percent of incidence among transplanted and planted seedlings had been assessed. The mean incidence of Alaba, Humbo, Maraqo, Shashogo, Arsi-negelle and Adama in transplanted seedlings were 21.78, 8.56, 23.2, 23.2, 2 and 3, respectively, whereas the mean incidence values in planted seedlings for these locations were 29.8, 7.4, 3.6, 4.23, 4.4, and 6, respectively (Appendix 3E). For most nurseries (84.4%) there was at least one anthracnose

infected chili at less than 5 kilometers distance. Coffee was occasionally used as shade for the seedlings within the nursery itself. Furthermore, comparison was made among nurseries and gardens, anthracnose incidence; under nurseries in a garden was 69.20% while under the 50% conventional polyethylene shedding, infected seedlings were less than 1%. Therefore, the role of these as source of inoculum was evident. In general, seedlings placed nearby (approximately 1 meter diameter) were highly infected when compared to those located beyond. The chili plots were undersized and include Maraqo types that create a favorable condition for proliferation of anthracnose (Eshte *et al.* 2014). This suggests that the recommended integrated pest and disease management at the nursery (Teklay *et al.*, 2015; Eshte *et al*., 2014; Freire *et al*., 2002) must also be aimed at the nearby chili gardens and nurseries to avoid recurrent infestation.

Table 3.3. Anthracnose severity means on Chili seedlings per regions, zones, kebelles, and locations of Ethiopia (Survey Conducted in 2013)

Order No.	**Regions(Zones)**	**Severity on Seedlings (%)**		
		Transplanted	**Planted**	**Mean**
1.	SNNP, Alaba sp. woreda	21.78±10.64	29.8 ±16.56	34.5 ±16.56
2	SNNP, Wolaita, Humbo	8.56 ±7.02	7.4 ±6.58	11.4 ± 6.03
3	SNNP, Guraghe Mareko sp. district	23.2 ±16.3	3.6 ±2.07	3.6 ±2.07
4	SNNP, Hadiya, Shashogo	23.2 ±16.3	4.23 ±1.14	4.23 ±1.14
5	Oromiya, Lome/ Arsi negelle	2 ± 1.23	4.4 ±2.3	3.2 ±1.15
6	Oromiya, East Shoa, Adama	3 ±1.58	6±2.7	4.5 ±1.7
	Overall (Mean±SD)	**13.6 ±6.7**	**9.24 ± 5.8**	**10.24 ±6.06**

NA=No plants available; SD= Standard deviation

3.4. Discussion

In this survey, the incidence of anthracnose disease in five different farms of Alaba district ranges between 3 % and 100% with the overall mean of 34.5%. In the same district, incidence of diseases on transplanted and directly planted seedlings were 21.78 and 29.8 percent, respectively. The overall mean of these five locations in Alaba district was 34.5%.

In Shashogo district, anthracnose incidence in transplanted seedlings ranged between 2% and 41% while 7% and 9% in planted seedlings. The overall disease anthracnose incidence was 5% and 24% in transplanted and planted seedlings. It was confirmed that the wide distribution of disease throughout the country and provided evidence that SNNP had the highest prevalence of the disease. Probably because the disease is known to be more severe under moderate humid and moderately warm conditions (Teklay *et al*, 2015). SNNP is one of the most chili-conducive regions in the country (Shiferaw and Alemayehu, 2014). The peak of disease was reported to be from January to April. This period coincides with the most rainy and humid period in all parts of the country (Yohannes *et al.*, 2015).

In Maraqo district, the lowest and highest incidence in transplanted seedlings was 2% and 41% and 4% and 9% incidence was observed in planted seedlings. The overall range of anthracnose incidence was 5.5% and 23.5% for transplanted and planted seedlings. In Humbo, the highest and lowest anthracnose incidences were 23% and 8% in transplanted seedling and 19% and 3% on planted seedlings. The total incidence of anthracnose disease ranges between 6% and 21%. The overall mean of incidence of both transplanted and planted seedlings in Humbo district was 11.4%. Anthracnose incidence on chili farm is estimated between 31.66%, 28.86%, 23.34%, 18.53%, 14.89% and 13.63% in Arsi negelle, Alaba, Adama, Shashogo, Maraqo and Humbo, respectively. But significant variation is found in terms of anthracnose damage on chili seedlings.

In Arsi Negelle, the maximum disease incidences in transplanted and planted seedlings were 4% and 7% while the minimum anthracnose incidence were 1% and 2%, respectively. The overall incidence of the five localities ranged between 2% and 4.5%. This corroborates the results obtained by Shiferaw and Alemayehu (2014) in which 55% of seed beds were infected by seedling diseases. The highest seedling infection (73%) was recorded at Hawassa zuria district followed by Halaba, Lanfro and Dalocha districts (60, 56 and 54%, respectively). Similarly survey results made after transplanting showed that 229 (75 %) of samples were infected at least by one disease. In the same way, survey results of Shiferaw and Alemayehu (2014) made after transplanting showed that 229 (75 %) of samples were infected at least by one disease. The frequency of pathogen growth depicted that 30% of the associated pathogens were different

bacteria, while 21%, 12%, 9% and 3.0% belong to the fungal genera *Fusarium* spp, *Colletotrichum* spp, *Cercospora* spp and *Alternaria* spp respectively.

In Adama, the overall mean of anthracnose incidence was 4.5%. Planted seedlings, having value of 6% and 3%, were found to have as twice higher incidence as transplanted ones. Increased rate of success after treating the seedlings with a fungicide has been observed by the informants. It may be due to fungicide action against other microorganisms or endophytic pathogen. Adoption analysis of previously recommended cultural practices showed 100%, 42%, 31%, 30% and 19% for fertilizer application, engagement in two year rotation, row planting, use of pesticides and improved seed, respectively (Shiferaw and Alemayehu, 2014; Mekonnen *et al.*, 2015) .

Incidence of anthracnose on mature seedlings was almost six times than on young seedlings because of damper microclimate in highly dense arrangement of old plants. Findings from this survey may suggest that matured or old seedlings did not necessarily transmit the *Colletotrichum* spp. to seedlings. The main reason for this could be that infected seedlings may have died long before sprouting and thus not assessed during the study. Subsequent infections on adjacent seedlings may be coming from other aerial sources (Chala, *et al.*, 2010; Merkuz and Getachew, 2012).

3.5. Conclusion and Recommendations

There is a considerably high incidence and severity of leaf anthracnose in the major growing areas of Ethiopia. Transplanted seedlings are severely affected by anthracnose than planted ones. The disease is more prevalent in SNNP than Oromiyaregion. Therefore, it’s highly recommended to provide a manual on chili seedling pests and disease management and use it in a nationwide training program for chili producers.

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CHAPTER FOUR:

DIVERSITY OF CHILI ANTHRACNOSE (*Colletotrichum capsici* (Syd)) ISOLATES FROM MAJOR CHILI GROWING AREAS OF ETHIOPIA

Abstract

Chili had immense dietary and economic importance for Ethiopians. Ironically, it suffers profound losses in yield due to anthracnose caused by *Colletotrichum capsici*. But scientific information on variability of the *Colletotrichum* spp in the country was insufficient. Thus, the present study was undertaken to determine the variability of the pathogen and its biology to design better management practices of the diseases to avoid losses. Morpho-pathological variations were studied in twenty isolates of *C. capsici*, that had been collected from chili farm. The isolates were cultured and identified in Addis Ababa University, College of Natural Sciences, Department of Microbial Cellular and Molecular Biology laboratory. Colony size, color, shape, marginal pattern and characteristics had been identified. For the detached fruit experiment, disease reaction was made on twenty lines originating from ten known chili genotypes. Disease incidence was also calculated. Colonies varied in their cultural behavior ranging from cottony to fluffy, mostly suppressed with regular to irregular margins. Color of colonies ranged between white to grey. Growth rate of isolates was between 22.0-69.5 mm. Morphological studies of isolates revealed variations in their color, size, shape, acervuli production, setae size and shape, conidia. Average conidial size varied from 18.00-33.3 µm and average setae size varied from 77.2-181.2 µm. On the basis of disease reaction expressed by differential hosts, eleven groups (races) of *C. capsici* were identified. The group 1 comprised of isolates AAUCc-1, AAUCc-15 whereas group 2 included the isolate AAUCc-2, AAUCc-6, and AAUCc-16. The AAUCc-3 and AAUCc-10 were included in group-3 whereas group-4 included the isolate AAUCc-5 and AAUCc-11. The group 5 comprised of isolates AAUCc-9, AAUCc-12, AAUCc-13, and AAUCc-20. The group 6 comprised of AAUCc-17 and AAUCc-19. The groups 7, 8, 9, 10 and 11 comprised of isolates AAUCc-4, AAUCc-7, AAUCc-8, AAUCc-14 and AAUCc-18, respectively. In conclusion, the existence of variations among *Colletotrichum capsici* isolates strengthens the present knowledge on the identification and understanding pathogen which has immense contribution for ultimate anthracnose disease management.

Key Words: Chili anthracnose, *Colletotrichum capsici*, Morphological variability, Pathological variability

4.1. Introduction

Chili (*Capsicum frutescence* L.) is an important cash crop grown worldwide (Makari *et al*., 2009; Bosland and Votava, 2000) and in Ethiopia (Seleshi, *et al.,* 2014; EEPA, 2003). Chili is prone to number of fungal bacterial and viral diseases (Devi and Prakasam, 2014) which significantly affect its production and quality. However, huge losses to the crop are incurred mostly by fungal diseases. Of these diseases, dieback and fruit rot has assumed the status of major disease in some important chili growing countries (Makari *et al*., 2009; Sharma *et al.*, 2005). Anthracnose causes extensive pre and post harvest damage to chili fruits causing anthracnose lesions (Mehrotra and Aggarwal, 2003) that reduced their marketable value (Masoodi *et al*., 2013).

Sharma *et al.* (2005) studied the pathogenic variability in *C. capsici* studied and found 15 pathotypes of *Colletotrichium capcisi* that existed from 30 isolates studied in India and proposed 15 pathotypes of *C. capsici* existed among 37 isolates from different chili growing regions in India. In recent years, anthracnose disease has frequently been observed in Ethiopia and is assuming serious proportions causing heavy losses to the crop. Evaluations of pathogens against the causal pathogen are incomplete without variability study (Seleshi, *et al.,* 2014; Tameru *et al.,* 2003; Tameru, 2004). However, there is limited information on the variability of *Colletotrichum capsici* isolates in pepper growing regions of Ethiopia where agro-climatic conditions are entirely different from rest of the country (Tameru *et al.*, 2003; Tameru, 2004). The frequent epiphytotics of the disease in the growing area witnessed during past few years and extent of the damage inflicted by it, has necessitated this study on the diversity of the pathogen.

4.2. Materials and Methods

4.2.1. Sampling Area

Extensive survey was carried out in the South-western, Chili growing parts of Ethiopia during growing season (June-September) 2013 and 2014 for anthracnose disease assessment. Chili fruits from susceptible cultivars exhibiting typical and variable symptoms of *C. capsici* were collected from twelve chili growing locations from four districts of SNNP, two districts of Amhara and two districts of Oromiya viz., Alaba, Maraqo, Shashogo, Humbo-tabala, Adama and Lome districts.

4.2.2. Isolation, purification and preservation of isolates for Cultural Characteristics

Cultural variability among the isolates was studied on the basis of the standard suggested by (Tesfaye and Kapoor, 2007). Five millimeter mycehal discs of 7 days old culture of each isolate was transferred to the centre of sterilized Petri plates containing potato dextrose agar medium and incubated at 25±1 ^{0}C. Colony character viz., color and margins were recorded after 10 days of inoculation by taking two perpendicular measurements and their average calculated.

4.2.3. Morphological Variation

The morphological variation among the various isolates of *C. capsici* was studied on artificial culture in the laboratory. Mono-conidial culture of each isolate was first grown on potato dextrose agar medium and then semi-permanent shades prepared from 10 days old culture, stained with cotton blue in lactophenol. The important characters studied were based on Septation, color and length of hyphae; length and breadth of setae; color and size of acervulli and conidia (Masoodi *et al*., 2013).

4.2.4. Study on Pathogenicity tests

4.2.4.1. Test Capsicum varieties

Twenty cultivars of *C. frutescence* originated from nine known genotypic lines were evaluated for resistance to 20 chosen isolates of *C. capsici.* These included Melka zala, Maraqo fana, Melka shote, Weldele, Bako local, Oda haro, Dube medium, Dube short and Gojeb local from Melkassa Agricultural Research Center (MARC). The genotypes were diverse (two from each) with respect to their collection sites too.

4.2.4.2. Preaparation and Inoculation of Isolates

Fourteen-day-old cultures of *Colletotrichum* spp. grown on PDA under 12 h of alternate light and dark conditions, maintained at 26–28°C were used for making conidial suspension. The plates were flooded with sterile distilled water and gently scrapped by sterile loop to collect the conidia from the culture. The suspension was filtered between layers of muslin cloth and concentration was adjusted to 10^{6} conidia ml^{-1} using a haemocytometer. Locally available fresh chilli fruits of varieties were harvested at pre- and post-ripening stages to study the pathogenicity of Colletotrichum isolates by injection method. The fruits were surface-sterilized with 1% sodium hypochlorite solution for 5 min and rinsed with sterile distilled water for two to three times. Ten micro-liters of a conidial suspension was injected at the centre of each fruit using a

sterile syringe. The fruits were then kept in deep freeze. Un-inoculated but wounded fruits served as control. Five fruits of ripe and unripe stage were considered for studying the pathogenicity of each Colletotrichum isolate. Inoculated fruits were evaluated for anthracnose symptoms after 9 days of incubation on the basis of lesion size relative to overall size of fruit.

The rate of lesion progression on ripe and unripe fruits was measured every day. The disease reaction was scored on the basis of 0-5 point scale modified from Susheela (2012); Where, (+++) no infection, (++) 1-2% fruit area infected, (+) 2.1-5% fruit are infected, (-) 5.1-10% fruit area infected, (- -) 10.1-25% fruit area infected and (- - -) >25% fruit area infected. For pathotype groupings, reaction types of (+++), (++) and (+) or either of these were graded as resistant (+) while those falling in (- - -), (- -) and '-' or either of these were rated as susceptible (-).
The experiment was replicated three times to confirm the results. The pathogens were re-isolated after 10 days using direct isolation and were cultured on PDA to morphologically identify and compare with the original isolate in order to fulfill the Koch's postulates (Masoodi *et al*., 2013).

In order to identify physiological races of *C. capsici*, attempts were made to develop a differential set of capsicum varieties.

4.2.5. Isolation and Purification of *Colletotrichum capsici* Isolates

From a total number of 48 isolates of *C. capsici* obtained, only twenty of them were selected for the experiment. On this basis, 20 different chili growing areas in the 3 surveyed regions (SNNP, Oromiya, and Amhara) in which prevalence was suspected to be high had been considered. Purification had been undertaken in Department of Microbial, Cellular and Molecular Biology laboratory, College of natural and Computational Sciences, Addis Ababa University.

4.2.6. Analysis of data

The data of various experiments were subjected to statistical analysis with the help of computer. The data was subjected to appropriate transformations, wherever needed as suggested by Gomez and Gomez (1984) before analysis.

4.3. Results

As the experimental findings of this research depicts, on the basis of morphological characters, pathogenicity and comparison with the authentic description Kumar *et al*. (2015) the fungus was identified as *Colletotrichum spp*. Diversity amongst the isolates was recorded with respect to cultural, morphological and pathogenic characters.

Table 4.1. Cultural diversity and characteristics of different isolates of *Colletotrichum capsici* on Potato Dextrose Agar medium

Isolate	Source/ Location	Colony Characteristics			Mycelial growth after 7 days (mm)
		Type and Color	Shape (Center)	Margin Pattern	
AAUCC1	Alaba	White	Fluffy	Regular, White	54.60
AAUCC2	Alaba	White	Fluffy, Greyish	Regular, V-Shaped	53.52
AAUCC3	Alaba	White	Cottony	Irregular	52.80
AAUCC4	Alaba	Grey	Whitish , Raised	V-Shaped, Irregular	69.5
AAUCC5	Maraqo	White	Suppressed	V-Shaped, regular	45.5
AAUCC6	Maraqo	White	Fluffy, Greyish	irregular	55.0
AAUCC7	Maraqo	White, Fluffy	Whitish Raised	Suppressed	22.0
AAUCC8	Maraqo	White	Cottony	Regular	58.0
AAUCC9	Humbo	Grey, White	White	Suppressed, White	50.2
AAUCC10	Humbo	White	Cottony	Regular	53.5
AAUCC11	Humbo	Dull White, Fluffy	Brown	Irregular	59.0
AAUCC12	Shashogo	Dull Grey	Suppressed	Regular	52.5
AAUCCl3	Shashogo	Dull White,	Greyish	Irregular	55.5
AAUCC14	Shashogo	Dull White	Light Grey, Fluffy	Regular	54.0
AAUCCl5	Shashogo	White	Fluffy	Regular	52.5
AAUCC16	Arsi Neg.	White, Fluffy	Greyish	Regular, White	55.0
AAUCC17	Wonji	Light Brown	Greyish	Irregular	50.0
AAUCC18	Adama	Light Smokey Grey	White Tinge, Raised	Regular	53.5
AAUCCl9	Amhara	brown,	Grey , Fluffy	Irregular	55.5
AAUCC20	Amhara	smoky grey	White, suppressed	Regular, V-Shaped	53.5

*Mean of 20 observations, AAUCc:= Addis Ababa University *Colletotrichum capsici*

4.3.1. Cultural Variability

Isolates of *Colletotrichum capsici* differed with respect to their cultural characteristics. The characters viz., type and color of colony, growth rate of fungus and pigmentation were recorded.

4.3.1.1. Type and Color of colony

The twenty isolates of *Colletotrichum capsici* had been grown on PDA showed variation in their colony characteristics. Central colony pigmentation varied from light to dark grey with whitish or brownish tinge. Mostly the colonies had cottony or fluffy mycelial growth with regular to irregular margin (Table 1). The fluffy growth was observed in six isolates viz., AAUCc-1, AAUCc-3, AAUCc-6, AAUCc-14, AAUCc-15 and AAUCc-19 whereas cottony growth in three isolates viz., AAUCc-3, AAUCc-8, AAUCc-9 and AAUCc-10. Among the studied colonies, suppressed growth was observed in AAUCc-7 and v-shape pattern was depicted on isolate AAUCc-4 and AAUCc-5. From all the colonies studied, raised type of colonies was observed in AAUCc-4, AAUCc-7 and AAUCc-18 isolates.

Variation had been exhibited among isolates in terms of colony colors (Table 4.1). White colonies were observed in all isolates except three isolates, viz., AAUCc-3, AAUCc-17 and AAUCc-19 which exhibited at least slight greyish color. Isolate AAUCc-4 and AAUCc-17 had shown light brown with white greyish centre. The colony margins varied from regular to irregular (Table 1). Regular margins were observed in eleven isolates viz., AAUCc-1, AAUCc-2, AAUCc-5, AAUCc-8, AAUCc-10, AAUCc-12, AAUCc-14, AAUCc-15, AAUCc-16, AAUCc-18, AAUCc-20 whereas, eight isolates AAUCc-6, AAUCc-3, AAUCc-4, AAUCc-9, AAUCc-11, AAUCc-13, AAUCc-17 and AAUCc-19 had irregular margins. Margins were whitish in AAUCc-1, AAUCc-9, and AAUCc-16 isolates. Besides, V-shaped margins were observed in isolate AAUCc-2, AAUCc-4 and AAUCc-5 and AAUCc-20. In isolates AAUCc-11, AAUCc-13, and AAUCc-14, the margins were had dull white color.

4.3.1.2. Growth Rate of Fungus

The result had shown (as indicated in Table 4.1) considerable variation in the radial growth (in mm) 7 days after inoculation. Least growth rate of 22.0 mm was recorded in AAUCc-7. Isolate AAUCc-4 with mean radial growth 69.5 mm was the fastest followed by AAUCc-11 (59.0%), AAUCc-8 (58.0 mm), AAUCc-13 (55.5 mm) and AAUCc-19 (55.5 mm). Lowest growth was observed in AAUCc-7 (22 mm).

4.3.2. Morphological Variability

Variations were observed amongst the isolates with respect to morphological characters like conidia size, shape, acervuli production, setae size and its characters.

4.3.2.1. Conidial size

The mean conidial size of isolates ranged from 18.19 - 37.30 x 1.00 - 5.31 µm (Table 4.2). The average maximum conidial length (37.30µm) observed in AAUCc-1 was significantly higher than other isolates, whereas minimum length (18.19 µm) in AAUCc-6 was recorded. The mean maximum conidial breadth of 5.31 µm was observed in AAUCc-12 while minimum conidial breadth of 1.00 µm observed in AAUCc-9. The second least conidial length (18.22 µm) and breadth (1.56 µm) was in AAUCc-13 and AAUCc-2, respectively.

4.3.2.2. Conidial shape

It has been revealed in this study that all the isolates had fusiform to falcate type of conidia (Table 2). Nine isolates viz., AAUCc-2, AAUCc-3, AAUCc-5, AAUCc-6, AAUCc-7, AAUCc-8, AAUCc-10, AAUCc-11, AAUCc-13, AAUCc-14, AAUCc-15 were having fusiform conidia and the rest 11 isolates, viz., AAUCc-1, AAUCc-4, AAUCc-9, AAUCc-12, AAUCc-16, AAUCc-18, AAUCc-19, AAUCc-20 having falcate conidia.

Table 4.2. Diversity in conidial shape and size of *Colletotrichum* spp isolates

Isolate	Length (µm)* Range	Length (µm)* Mean	Breadth (µm)" Range	Breadth (µm)" Mean	Shape
AAUCC1	7.2-29.7	37.30	0.78-3.280	2.03	Falcate
AAUCC2	9.8-32.9	30.70	0.25-3.950	1.56	Fusiform
AAUCC3	10.2-42.8	27.20	0.35-2.950	2.50	Fusiform
AAUCC4	11.3-43.00	29.01	0.65-10.50	3.86	Falcate
AAUCC5	10.9-42.00	19.20	0.53-2.100	2.88	Fusiform
AAUCC6	11.5-27.4	18.19	0.55-3.300	3.16	Fusiform
AAUCC7	12.5-43.4	18.40	0.25-11.40	3.56	Fusiform
AAUCC8	10.2-55.8	31.70	0.65-3.980	2.50	Fusiform
AAUCC9	11.2-31.8	28.10	0.42-3.280	1.00	Falcate
AAUCC10	10.1-56.8	27.60	0.72-2.380	2.23	Fusiform
AAUCC11	10.5-35.5	26.40	0.65-3.330	3.30	Fusiform
AAUCC12	12.3-36.8	25.10	3.12-3.660	5.31	Falcate
AAUCCl3	7.9-21.00	18.22	2.65-14.12	3.11	Fusiform
AAUCC14	11.2-32.3	33.60	0.52-15.22	3.33	Fusiform
AAUCCl5	8.2-48.1	24.10	1.32-22.12	5.11	Fusiform
AAUCC16	12.2-32.3	25.10	3.31-14.12	5.44	Falcate
AAUCC17	10.2-49.1	27.09	0.32-13.12	5.21	Falcate
AAUCC18	10.1-37.2	20.10	0.32-11.62	1.00	Falcate
AAUCCl9	8.9-43.00	31.20	0.28-14.6	2.36	Falcate
AAUCC20	9.8-45.00	19.40	2.22-15.7	3.22	Falcate

*Mean of 20 observations, AAUCc:= Addis Ababa University *Colletotrichum capsici*

4.3.2.3. Diveersity in Acervuli Production and Setae Size

Table 3 showed that the variation in setae size and acervuli production. Irrespective of the isolates, setae was measured to have 50.0-193.6 x 3.35 - 6.6 µm. The maximum size of the setae have been observed in isolate AAUCc-5 measuring 102.4 - 193.6 x 4.34-6.6 µm followed by isolate AAUCc-9 having an average measure of 131.22 x 4.66 µm acervuli length and breadth, respectively.

Irrespective of the isolates, Acervuli production ranged from 24 - 57 mm mycelial disc. The least production of acervuli, with 5mm myceliadisc, was recorded in isolate AAUCc-16 (24 mm), followed by AAUCc-20 (26mm) and AAUCc-17 (28mm). To add up, isolates varied significantly in their characteristics (Table 3). AAUCc-2, AAUCc-4, AAUCc-6, AAUCc-8, AAUCc-9, AAUCc-12, AAUCc-15, AAUCc-17, AAUCc-18, and AAUCc-20 were submerged and scattered. Isolates AAUCc-1, AAUCc-5, AAUCc-7, AAUCc-10, and AAUCc-11 were raised and scattered whereas, the rest five isolates named AAUCc-3, AAUCc-13, AAUCc-14, AAUCc-16, and AAUCc-19 were appeared raised with concentric ring.

Table 4.3.Variability in acervuli production and setae size of different isolates of *Colletotrichum capsici* on potato dextrose agar medium

Isolate	Size (µm)" Length (range)	Breadth (range)	No. of acervuli 5mm dia. Mycelial disc	Characteristics
AAUCC-1	77.20 (50.0-109.8)	4.35 (4.14-6.6)	35	Raised, scattered
AAUCC-2	89.80 (64.4-113.6)	3.84 (3.5-5.5)	31	Submerged, scattered
AAUCC-3	96.50 (67.8-119.6)	4.65 (4.4-6.6)	34	Raised, concentric rings
AAUCC-4	86.00 (75.6-119.8)	4.06 (4.04-6.6)	57	Submerged, scattered
AAUCC-5	174.50 (102.4-193.6)	4.40 (4.34-6.6)	43	Raised, scattered
AAUCC-6	118.88 (105.0-139.8)	4.80 (4.34-5.5)	48	Submerged, scattered
AAUCC-7	124.45 (111.0-150.6)	4.35 (3.11-6.6)	43	Raised, scattered
AAUCC-8	79.59 (75.0-102.7)	4.35 (3.14-6.6)	32	Submerged, scattered
AAUCC-9	131.2 (125.6-140.6)	3.48 (4.34-5.5)	49	Submerged, scattered
AAUCC-10	111.59 (114.6-122. 7)	4.80 (4.34-6.6)	45	Raised, scattered
AAUCC-11	133.95 (127.8-139.6)	3.48 (3.34-5.5)	44	Raised, scattered
AAUCC-12	121.11 (118.0-124.8)	4.55 (4.24-6.6)	42	Submerged, scattered
AAUCC-l3	99.49 (98.0-120.7)	4.65 (4.24-6.6)	52	Raised, concentric rings
AAUCC-14	106.12 (105.5-110.6)	4.85 (4.14-6.6)	48	Raised, concentric rings
AAUCC-l5	80.11 (76.0-122.7)	4.48 (4.14-5.5)	45	Submerged, Scattered
AAUCC-16	108.22 (103.0-108.6)	4.88 (4.14-6.6)	24	Raised, concentric rings
AAUCC-17	112.33 (108.8-119.6)	4.88 (4.24-6.6)	28	Submerged, Scattered
AAUCC-18	117.11 (108.6-119.8)	4.75 (4.34-5.5)	32	Submerged, Scattered
AAUCC-l9	131.22 (108.2-140.2)	4.66 (4.24-5.5)	30	Raised, concentric rings
AAUCC-20	93.30 (86.0-100.0)	4.52 (4.14-5.5)	26	Submerged, Scattered

*Mean of 20 observations, AAUCc:= Addis Ababa University *Colletotrichum capsici*

4.3.3. Pathogenecity

The lowest and highest diseases intensity was recorded in AAUCc-12 against OH2 (10.1%) and WD2 (35.5%), correspondingly. Isolate AAUCc-8 had shown the second highest disease intensity against BL1 (33.3%). The second lowest disease intensity was observed on AAUCc-5, AAUCc-18 and AAUCc-5 against LV1 (10.4%), MS2 (10.4%) and MZ1 (10.4%), respectively.

Table 4.4. Severity of *Colletotrichum capsici* isolates on various chili genotypes

Isolate	OH1	WD1	BL1	MF1	MS1	MZ1	DM1	LV1	GL1	DS1
AAUCC1	33.1	26.1	26.8	22.2	18.1	26.3	23.3	17.9	16	14.6
AAUCC2	18.6	22	28.2	29.6	15.6	16.8	25.7	19.4	14.1	16.5
AAUCC3	21	26.6	22.2	27.1	14.2	22.2	14.6	10.6	13.2	19.4
AAUCC4	27	27	28	31.1	27.2	22.1	28.5	12.3	20.3	17.2
AAUCC5	21.1	31.1	31.6	24.5	27.2	31.3	12.4	10.4	22.2	11.2
AAUCC6	22.3	22	21.1	11.10	19.9	31.1	31.5	15	30	21
AAUCC7	17.2	10.6	19.6	13.1	15.8	30	22	19.1	16	15.3
AAUCC8	10.6	17.3	33.3	13.1	12.2	31.3	28.77	15.6	11	18.9
AAUCC9	22.7	25.5	17.5	15.7	25.5	24.4	21.8	19.3	21.2	17.7
AAUCC10	24	26.2	20.2	21.2	24.2	22.7	16.5	19.3	17	26
AAUCC11	25.2	25.5	17.9	23.3	21.1	18.1	24.3	14.6	23.2	31.2
AAUCC12	10.8	16.2	22.2	19.6	26.7	18.2	31.4	11.6	14.6	16
AAUCCl3	27.6	21.1	24.4	15.5	18.6	29.6	30.5	17.8	17.2	21.4
AAUCC14	17.4	19.2	15.7	16.1	19.1	24.3	11.4	21.2	21.2	24
AAUCCl5	11.1	17.2	16.9	11.5	27.8	22.6	11	11.6	25.6	21.1
AAUCC16	15.4	31.8	30.1	22.2	21.1	23.6	17.1	16.6	24.5	22.5
AAUCC17	16.1	23.3	31.6	24.2	21	19.3	17.9	12.1	24.8	26.4
AAUCC18	15.3	22.1	14.5	15.2	21.2	11.1	15.2	20.1	21.9	16.3
AAUCCl9	16.3	20.1	12.6	12.1	25.6	10.4	22.3	18.8	22.2	16.5
AAUCC20	23.3	24.2	25.2	30.0	22.4	22.4	18.6	10.8	21.2	30

*Means of Replications, = Resistance; + = Susceptible; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dube short Types

As depicted in table 4.4, the data of the current study showed that the isolates exhibited at different virulent pattern when inoculated on the 20 chili differential genotypes. In all, 20 virulent isolates were identified based on similarity or dissimilarity in reaction types exhibited by these differential lines. The isolates comprised AAUCc-1, AAUCc-15 showing resistant reaction on 9 differential lines viz., OH1, OH2, DS1, WD2, WD1, BL1, GL1, DS2, LV2 and susceptible reaction on the other 11 differential lines viz., MF1, MF2, MS1, MS2, BL2, MZ1, MZ2, DM1, DM2, LV1, and GL2 (Table 4.4; Appendix 4.4).

Table 4.5. Virulence pattern of *Colletotrichum capsici* isolates on various chili genotypes

Isolate	OH1	OH2	WD1	WD2	BL1	MF1	MF2	MS1	MS2	BL2	MZ1	MZ2	DM1	DM2	LV1	GL1	GL2	DS1	DS2	LV2
AAUCC1	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
AAUCC2	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC3	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
AAUCC5	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC6	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC7	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-
AAUCC8	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-
AAUCC9	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCC10	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC11	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC12	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCCl3	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCC14	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
AAUCCl5	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
AAUCC16	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC17	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC18	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	-
AAUCCl9	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC20	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-

- = Resistance; + = Susceptible; = Resistance; + = Susceptible; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dubeshet Tuma

Three isolates, namely, AAUCc-2, AAUCc-6, AAUCc-16 showing resistant reactions on OH1, WD1, WD2 , MZ1, DM2, GL1, DS1 and susceptible reaction on rest of genotypes. The isolates comprised AAUCc-3 and AAUCc-10 (Group 3) showing resistant response on differential host genotypes OH1, GL1, DS1, DM2 whereas; susceptible reaction was exhibited on rest of cultivars.

Isolates AAUCc-9, AAUCc-12, AAUCc-13 and AAUCc-20 has shown resistant reaction on three differential lines viz., OH1, MF2, MZ2 and susceptible response on rest of the differential host genotypes. The isolates comprised AAUCc-4 showing resistant response on differential host genotypes viz., MF2 and DS1, whereas susceptible reaction exhibited on rest of the cultivars. The isolates comprised of the *Colletotrichum capsici* isolates AAUCc-17, AAUCc-19 showing

resistant reaction on WD2 , DM2, MF2, GL1 chili genotypes and susceptible reaction on rest of genotypes.

The isolates comprised AAUCc-5 and AAUCc-11 (Group 4) showing resistant response on differential host genotypes viz., OH1, OH2, WD2 , MF1, MZ1, DM1, DM2, LV1, GL1, DS1, LV2 chilies whereas the susceptible reaction was exhibited on the rest of the cultivars. Similarly, the isolate AAUCc-7 was clubbed under (Group 8) showing resistant reaction on differential host like OH1, BL1, MF2, MS1, DM1, LV1, and susceptible response on rest of the differential host genotypes. The isolate comprised AAUCc-8 showing the resistant reaction on five differential lines viz., OH1, MF2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes. Isolate comprised AAUCc-14 had shown resistant response on OH1, DM2 and susceptible reactions on rest of the genotypes. The isolate AAUCc-18 shown resistant reaction on six differential lines, viz., OH1, MS2, BL2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes.

Further, as indicated in table 6, eleven pathotype groups were identified based on similarity or dissimilarity in reaction types exhibited by these differential lines. The group-1 comprised of isolates AAUCc-1, AAUCc-15 showing resistant reaction on 9 differential lines viz., OH1, OH2, DS1, WD2 , WD1, BL1, GL1, DS2, LV2 and susceptible reaction on the other 11 differential lines viz., MF1, MF2, MS1, MS2, BL2, MZ1, MZ2, DM1, DM2, LV1, GL2. The group-2 comprised of the AAUCc-2, AAUCc-6, AAUCc-16 showing resistant reaction on OH1, WD1, WD2, MZ1, DM2, GL1, DS1 and susceptible reaction on rest of genotypes. The group 3 comprised of isolates AAUCc-3, AAUCc-10 showing resistant response on differential host genotypes OH1, GL1, DS1, DM2 whereas, susceptible reaction was exhibited on rest of cultivars.

In the same way, the AAUCc-9, AAUCc-12 AAUCc-18, and AAUCc-20, isolate were clubbed under group-5 showing resistant reaction on differential lines viz., OH1, MF2, MZ2 and susceptible response on rest of the differential host genotypes. The group-7 comprised of isolate AAUCc-4 showing resistant response on differential host genotypes viz., DS1, MF2 whereas susceptible reaction exhibited on rest of the cultivars. The group-6 comprised of the *Colletotrichum capsici* isolates AAUCc-17 and AAUCc-19 showing resistant reaction on WD2,

DM2, MS2, and GL1 chili genotypes and susceptible reaction on rest of genotypes. The group-4 comprised of isolates AAUCc-5, AAUCc-11 showing resistant response on differential host genotypes viz., OH1, OH2, WD2 , MF1, MZ1, DM1, DM2, LV1, GL1, DS1, LV2 lines whereas the susceptible reaction was exhibited on the rest of the cultivars.

On the contrary, isolate AAUCc-7, group-8, showing resistant reaction on differential hosts, such as OH1, BL1, MF2, MS1, DM1, LV1, and susceptible response on rest of the differential host. The group 9 comprised of isolate AAUCc-8 showing the resistant reaction on five differential lines viz., OH1, MF2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes. Similarly group 10 comprised of isolate AAUCc-14 that showed resistant response on OH1, DM2 and susceptible reaction on rest of the genotypes. Group 11 comprised isolate AAU Cc-18 showing resistance reaction on six differential lines, viz., OH1, MS2, BL2, DM1, GL1, DS1, and susceptible reaction on rest of the genotypes.

Table 4.6. Pathotype groups of *Colletotrichum capsici* isolates on various chili genotypes

Patho. Gr.	Isolates	OH1	OH2	WD1	WD2	BL1	MF1	MF2	MS1	MS2	BL2	MZ1	MZ2	DM1	DM2	LV1	GL1	GL2	DS1	DS2	LV2
MF1	AAUCC1,15	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
G2	AAUCC2, 6, 16	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
G3	AAUCC3,10	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
G4	AAUCC5, 11	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
G5	AAUCC9, 12,13, 20	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
G6	AAUCC17,19	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
G7	AAUCC4	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-
G8	AAUCC7	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-
G9	AAUCC8	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	+	-	-
G10	AAUCC14	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
G11	AAUCC18	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	-

G= pathotype group; - = Resistance; + = Susceptible; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety;

Table 4.7. Intensity of reactions of *Colletotrichum capsici* isolates on 20 chili genotypes

Isolate	Reaction Types on:-																			
	OH1	**OH2**	**WD1**	**WD2**	**BL1**	**MF1**	**MF2**	**MS1**	**MS2**	**BL2**	**MZ1**	**MZ2**	**DM1**	**DM2**	**LV1**	**GL1**	**GL2**	**DS1**	**DS2**	**LV2**
AAUCC1	+	++	+	+	++	-	-	- -	-	-	-	-	-	-	-	+	-	+	+	+
AAUCC2	+	-	+	+	-	- -	-	-	-	-	++	-	-	+	-	+	-	+	-	-
AAUCC3	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC4	-	-	-	-	-	-	+	-	- -	-	-	-	-	-	-	-	- -	+	-	-
AAUCC5	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC6	++	-	+	++	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC7	+	-	-	-	+	-	+	+	-	-	-	-	+	-	+	-	-	-	-	-
AAUCC8	+	-	-	- -	-	- -	+	-	-	-	-	-	+	-	-	+	-	+	-	-
AAUCC9	+	-	-	-	-	-	+	-	-	- -	-	+	-	-	-	-	-	-	-	-
AAUCC10	+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
AAUCC11	+	+	-	+	-	+	-	-	-	-	+	-	+	+	+	+	-	+	-	+
AAUCC12	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCCl3	+	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-	-
AAUCC14	++	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
AAUCCl5	++	++	++	++	++	-	-	-	-	-	-	-	-	-	-	+	-	++	++	++
AAUCC16	+	-	+	+	-	-	-	-	-	-	+	-	-	+	-	+	-	+	-	-
AAUCC17	- -	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC18	+	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	-	+	+	-
AAUCCl9	-	-	-	+	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-
AAUCC20	+	-	-	-	- -	-	+	-	-	-	-	+	-	-	-	-	- -	-	-	-

+++ = Immune, ++= Highly Resistant,; + = Resistant; - = Susceptible; - - Highly Susceptible; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dube short Types

The intensity of disease reaction has been indicated in table 7. In view of that, isolates AAUCc-1 has shown a highly resistance reaction on OH2 and BL1 genotypes. Isolate AAUCc-15 has shown a highly resistant reaction on OH1, OH2, WD1, WD2, BL1, DS1, DS2 and LV2 genotypes. Isolates AAUCc-2, AAUCc-6, AAUCc-17 and AAUCc-14 had shown a highly resistant reaction on MZ1, OH1 and WD2; OH1and OH1 genotypes, respectively. On the other hand, isolates AAUCc-1, AAUCc-2, and AAUCc-9 had shown a highly susceptibility reaction on single genotypes each, viz., MS1, MF2 and BL2, in order. Isolates AAUCc-4, AAUCc-8, and AAUCc-20 had shown a highly susceptibility reaction on two genotypes each, viz., MS2 and GL2, WD2 and MF1; and BL1 and GL2, respectively.

4.4. Discussion

The present work unraveled that there was variations among isolates of *C. capsici* from different locations of the country. Twenty representative isolates collected from 20 locations of the rift

valley varied in their cultural, morphological, pathogenic characteristics. Isolates of *C. capsici* varied in their cultural characteristics viz., colony type, color, margin, segmentation and growth rate. Colonies were cottony or fluffy and mostly suppressed with color ranging from white to grey. Similarly, several workers have also reported cultural, morphological, pathogenic variability among isolates *Colletotrichum* spp. (Sharma *et al.,* 2005; Masoodi *et al*., 2013).

It has been indicated that conidia and appressions presence or absence of setae, sclerotia, acervuli and teleomorph state and such as colony, growth rate and texture (Photita, *et al.,* 2005; Than, 2008a,b). Isolates of *Colletotrichum capsici* were studied for morphological variation in their setae, conidia and acervuli production (Devi and Prakasam, 2014; Thaung, 2008.

In the present study, great variation in conidial size was observed with maximum length of 33.60µm in AAUCc-14 while minimum length of 19.70 µm in AAUCc-4. Similarly conidia breadth from 4.86 µm in AAUCc-16 among the isolates of *C.capsici* with least breadth in AAUCc-10. Studies on setae revealed the variations in the size and production amongst all isolates. Setae length varied from 115.6-190.6µm in AAUCc-2 and breadth ranged from 3.14-6.6 µm. In AAUCc-5, the maximum setae length (174.21µm) was recorded while the minimum length 77.21µm was recorded in AAUCc-1. Similarly, mean setae breadth varied from 3.35 -4.88 µm. The least was recorded in AAUCc-4 and the maximum was in AAUCc-17. Masoodi *et al.* (2013) and Sharma *et al.* (2005) had similar reports that support the findings of this study. They found that there was a great diversity among *Colletotrichum capsici* isolates in terms of morphological and cultural characteristics.

In this study the acervuli production among the isolates of *C. capsici* ranged from 32-55 µm but acervuli number and dimensions could not be taken with definiteness for determining the relatives virulence of the isolates. However, this may need more investigation in order to establish such relationship among the isolates. The results achieved are in conformity to the works of Masoodi *et al.* (2013); Kumar *et al.* (2015); Sharma *et al*. (2005); and Sangde *et al.* (2011). Isolates of *C. capsici* varied little in their color of colony were nearly whitish, grey, light brown. Twelve isolates whitish, five isolates grey and two isolates were found to be brown. Shape of conidia was another criterion studied for variation among the isolates. Most of the isolates had Fusiform to Falcate conidia with slight differences in their shape. Ten isolates had

fusiform whereas other ten isolates falcate conidia. Similar observations were also observed by Masoodi *et al.* (2013); Sharma *et al*. (2005) and Akhtar and Singh (2007).

The present study on pathological variability among the isolates were made by recording the disease response on a set of 20 chili genotypes, originated from 10 known cultivars, selected arbitrarily from the chili genotype lines, on the basis of their consistent reaction to a few *C. capsici* isolates. These isolates were taken as differential lines for *C. capsic.* Such a selection of differential lines had been adhered to an account of the fact that the differential for pathogenic and variability in isolates of *C. capsici*. In line with this, Sharma *et al.,* (2005) had used the comparable sets of differentials. On the basis of disease reactions expressed by the differential lines, ten groups (races) of *C. capsici* were identified. The group 1 comprised of isolates AAUCc-1, AAUCc-15 whereas group 2 included the isolate AAUCc-2, AAUCc-6, and AAUCc-16. The AAUCc-3 and AAUCc-10 were included in group-3 whereas group-4 included the isolate AAUCc-5 and AAUCc-11. The group 5 comprised of isolates AAUCc-9, AAUCc-12, AAUCc-13, and AAUCc-20. The group 6 comprised of AAUCc-17 and AAUCc-19. The groups 7, 8, 9, 10 and 11 comprised of isolates AAUCc-4, AAUCc-7,AAUCc-8, AAUCc-14 and AAUCc-18, respectively. This corresponds with the findings of Masoodi *et al.* (2013) who found ten patho-gruops.The presence of these eleven different groups or races can account for varied pathogenic response to the different genotypes. The resistant behavior of a chili genotype to *C. capsici* in different parts of the country could also be understood by existence of such a variability occurring among these isolates. The existence of different virulent types of the isolates of *C. capsici* in the area so that the evolved variety shows resistance to all the virulence groups/types of the pathogen as to Masoodi *et al*. (2013)..

Ten isolates of *C. acutatum* against seven cultivars reportedly susceptible species of capsicum (Lin *et al.,* 2004) and resistant species such as capsicum Chinese 'PBC 932' (Lin *et al.,* 2004) . In contrast to *C. baccatum*, susceptibility of the *C. frutescence* cultivars have been reported in several studies (Lin *et al.,* 2004; Park, 2007) while evolving 79 varieties of capsicum for resistance to *C. capsici* in field trials. Kumar *et al.* (2015) have also evaluated 12 chili varieties against anthracnose and found resistance only in one cultivars, whereas, remaining cultivars were either moderately susceptible or highly susceptible.

4.5. Conclusion and Recommendation

The current study indicated that virulence is more severe in local varities than improved ones. The morho-cultural study showed that isolates AAUCc 7 has the lowest growth whereas AAUCc-4 had the highest growth rate. There is also morphological and pathogenic diversity among *C. capsici isolates*. These studies indicated AAUCc2, AAUCc-6 and AAUCc-16 had shown superior pathogenic variability from all the isolates of *C. capsici* considered in this study. In all 20 isolates, pathogenicity was observed on their pathogenic characteristics on 20 chili host genotypes and accessions depending upon their pathogenic behavior. Thus, further molecular characterizations are recommended to confirm the results of the study.

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CHAPTER FIVE:

INVITRO EVALUATION OF FUNGICIDES, PLANT EXTRACTS AND ANTAGONISTS (*Trichoderma* Spp) ON CHILI ANTHRACNOSE (*Colletotrichum Capsici* (Syd.)

Abstract

Chili anthracnose caused by *Colletotrichum spp* is one of the most devastating diseases that deter chili production in Southern Ethiopia. This study was conducted in 2014 with the aim to judiciously manage *Colletotrichum capsici* causing anthracnose of Chili. Six fungicides, namely, Benomyl, Tilt-250 EC, Vitavax-200, Rovral 50 WP, Dithane M-45 and Ridomil at concentration of 150, 250 and 300 ppm; and leaf extracts of garlic, ginger, onion and neem at three different concentrations (15%, 10% and 5%) were evaluated against the radial growth and mycelial dry weight of *Colletotrichum capsici (*AAUCc-4 isolate). Concurrently, 20 *Trichoderma* isolates formulated with residues of different crops were also tested against *Colletotrichum capsici* by using dual culture technique. In all these three experiments the treatments were arranged in CRD and data was analyzed through ANOVA. All the treatments were statistically different from the untreated check. From the six fungicides, all test concentrations of Tilt-250 EC significantly inhibited the mycelial growth of *Colletotrichum capsici*. Vitavax-200 and Royal 50WP gave 55.33-77.33 and 59.67-83.67; and 20.33-68.33mm and 20.67-73.67g, radial growth and mycelial dry weight of the test pathogen, respectively. Dithane M-45 and Cupravit were found to be significantly inferior to the rest of the fungicides. Among the tested plant extracts, garlic at the highest concentration (15 %) was found to be best in the reduction both the radial mycelial diameter (72.33) and mycelial dry weight (73.33) followed by Onion (15%) with 53.33 and 58% of radial mycelial diameter and mycelial dry weight, respectively. There was significant variation among isolates of *Trichoderma* sppand antagonistic activities ranged from 51 to 89% reduction of the mycelial radial growth of *Colletotrichum capsici (*AAUCc-4 isolate). Among the promising antagonists, the isolate Tri_3 of *Trichoderma harzinum* showed the highest, 89 %, inhibition of mycelial radial growth of *Colletotrichum capsici (*AAUCc-4 isolate). The wise use of these plant extracts, fungicides and antagonists must be enhanced to curtail chili anthracnose, In light with this study, there will be gradual shift from "routine fungicide use" to "apply when necessary" regime owing to these cheap and effective tactics ensuing in sustainable chili production.

Key Words: Anthracnose, *Colletotrichum capsici*, Fungicides, Plant Extract and *Trichoderma harzianum*

5.1. Introduction

Chili (*Capsicum frutescence* L.) has received a great deal of attention all over the world as important cash crop in general, and particularly in Ethiopia. Chili suffers from many diseases among them anthracnose caused by *Colletotrichum capsici* is the predominant one (Freema, 2000; Farr *et al.*, 2009; Pakdeevaraporn *et al.*, 2005). There are a few studies on yield losses due to diseases of Chili is available in the country.

Though chemical fungicides have been extensively used worldwide to control various pathogens, their use has many associated problems, such as environmental pollution, deteriorating human health, development of pathogen resistance to fungicides, and phytotoxicity (Tesfaye and Kapoor, 2007; 2012). To minimize these problems, researchers have sought to develop biological control agents for soil-borne plant pathogens that might be more environment-friendly. Phytopathogens have been used to suppress different fungal diseases, including *Trichoderma* spp.

Methods for complete control of Chili seed borne diseases are yet to be developed. Management strategies for these diseases include use of presumed disease free seeds, resistant cultivars and fungicidal sprays. Seed treatment is one of the best methods to manage seed-borne diseases. The continuous and indiscriminate use of chemicals to manage the crop disease results in accumulation of harmful chemical residues in the soil, water and grains. In recent years, considerable success has been achieved by introducing antagonists to control seed-borne fungal pathogens (Tesfaye and Kapoor, 2010). A considerable work has been done in controlling seedling diseases of many crops caused by *Rhizoctonia solani, Botrytis gladiolorum, Botrytis fabae, Fusarium xylarioides* and *Sclerotium rolfsii* both *in vitro* and pot culture experiments by using *Trichoderma* (Tesfaye and Kapoor, 2007; 2010, Haider, 2005). Complete elimination of *Colletotrichum capsici* from Chili and other crops is very difficult by any single approach of control. Some plant extracts also found to be most effective in reducing the mycelial growth and development of *Colletotrichum* spp (Raihan *et al.*, 2003; Haider, 2005; Serawit and Tesfaye, 2014).

To develop a sustainable integrated control strategy against the anthracnose of chili, it is essential to evaluate the efficacy of antagonists, such as *Trichoderma* spp, fungicides and some plant extracts under the context of integrated pest management (IPM). The present study was, therefore, undertaken with the objective to evaluate the efficacy of fungicides, plant extracts and *Trichoderma* spp against the major anthracnose (*Colletotrichum capsici)* of chili.

5.2. Materials and Methods

5.2.1. Study Area

The experiment was conducted to control *Colletotrichum capsici* causing anthracnose of chili by using fungicides, plant extracts and *Trichoderma* in the Department Microbial, Cellular and Molecular biology, Addis Ababa University.

5.2.2. Sources of plant extracts, Fungicides and *Trichoderma* spp isolates

Collectively, 20 isolates of *Trichoderma* spp (with 6 categories in three formulations each) were previously isolated from rhizosphere and rhizoplane soils of bean, barley, tomato, sweet potato, chili, cauliflower, sweet pepper, and wheat field of SNNPR and Oromiya and farmer's fields of Amhara by using the standard methods. A representative was taken from each category.

Six fungicides namely Ridomil, Benomyl, Dithane M-45, Rovral 50WP, Tilt 250EC and Vitavax-200 were tested in an *in vitro* to evaluate their antagonistic effect on mycelial growth of *Colletotrichum capsici* following poison food technique. All fungicides were used at 150 ppm, 250 ppm and 300 ppm. Fungicidal suspensions of different concentrations were prepared by dissolving required amount of each fungicide in warm PDA at 40-45 ^{0}C. The fungicides were thoroughly mixed with the medium by shaking with hands before autoclaving. Twenty ml of sterilized medium was poured in each 9 cm sterilized petridish. After solidification, the plates were inoculated by placing 5 mm discs of 3 days old PDA cultures of *Colletotrichum capsici.* Three replicated plates were used for each concentration of each fungicide. Three replicated PDA plates received without fungicides served as control. The inoculated plates were incubated at 28^{o}C and data on the radial mycelial diameter was recorded after 4-5 days of incubation when the growth of the control plates completely covered the petri plate. The flasks were placed inside a clean bench for cooling ambient temperature. The flasks were inoculated with mycelial discs of 5 day's old culture of *Colletotrichum capsici*. The discs were cut with a flame sterilized cork

borer (5 mm). Inoculation was done by putting one mycelial disc per flask with a flame-sterilized needle. Additional three flasks containing the broth (PDB) receiving no fungicides were used as control. The inoculated flasks were incubated at room temperature for 15 days. At the end of incubation, the cultures in all flasks were filtered separately through pre-weighted filter paper. Dry weight of mycelium was obtained by subtracting weight of filter paper from weight of filter paper and mycelium. Diameter of the colonies on PDA with and without fungicide was measured from the bottom side of the petri dishes. Inhibition of radial growth was computed based on mycelial diameter on control plate using the following formula:

% Inhibition = $\frac{X-Y}{X}$ * 100; Where, X= Growth of control plate; Y= Growth of fungicide treated plate.

5.2.3. *In Vitro* Evaluation of plant extracts on the mycelia growth of *C. capsici*

In-vitro test was conducted to determine the effect of plant extracts on radial mycelial growth of *Colletotrichum capsici* following poison food technique. Water extracts of garlic (*Allium sativum*), ginger (*Zingiber officinale*), onion (*Allium cepa*) and neem (*Azadirachta indica)* leaves were tested. Stalk solutions of the materials were prepared by blending 100g of each plant material in 100 ml of sterilized water in a blender. PDA medium was amended with individual extract at 0, 5, 10 and 20% (v/v). Required amount of individual plant extract was added to the 100 ml conical flask with PDA medium to have concentrations of 0, 5, 10 and 20% (w/v) based on the procedure coined by Kabir *et al*. (2015). After thorough mixing with plant extracts the medium was autoclaved and approximately 15 ml of melted PDA mixed with extracts was poured into each 90 mm Petri dish. To each plate, 5 mm discs of 3 days old PDA cultures of *C. capsici* (AAUCc-4 isoate). Similar procedure was followed for in vitro evaluation of fungicides against the growth of *Colletotrichum capsici* (AAUCc-4 isoate). Inhibition of radial mycelia growth was computed based on mycelial diameter on control plate using the same method.

5.2.4. *InVitro* Evaluation of *T. harzianum* Isolates against chili Anthracnose

Twenty isolates of *Trichoderma* spp. having different formulations and origins had been used for this experiment. The isolates of *Trichoderma* were purified in acidified agar (pH 4.5) using hyphal tip technique. After purification they were maintained as stock culture in PDA slants at

4 ^{o}C for future study. An *in vitro* screening experiment was conducted to find out the antagonistic effect of all the isolated 20 *T. harzianum* isolates against isolated pathogen on PDA by using dual culture technique. Discs of mycelium (5mm diameter) of each of the selected fungal isolates were cut from the edge of an actively growing fungal mycelia with a cork borer. Test plates were prepared by pouring 20 ml of PDA per plate. After solidification, one mycelial disc of individual isolate of *T. harzianum* and one disk of test fungal pathogen was placed simultaneously on the edge of the each PDA Petri plate at opposite direction. Three replicated plates were used for each isolate of *Trichoderma* and test pathogen. The plates were arranged on the laboratory desks following completely randomized design. The plates received only mycelial discs of the test pathogens served as control. The plates were incubated in the laboratory having ambient temperature of 25±3 C until mycelium growth of the test pathogens (*Colletotrichum capsici)* cover the whole control plate. Thereafter, inhibition percentages of *Colletotrichum capsici* was calculated based on the growth of the pathogen on PDA plates.

5.2.6. Experimental Design and Data Analysis

The experiments were conducted following Completely Randomized Design (CRD) with three replications. Data were analyzed by using IBM SPSS version 20.0 program. The significant difference, if any, among the means were compared by Least Significance Difference (LSD).

5.3. Results and Discussion

5.3.1. *InVitro* Evaluation of Fungicide against chili anthracnose

In the present study, six fungicides, namely, Tilt-250 EC, Vitavax-200, Dithane M-45, Cupravit and Royal 50WP gave noticeable inhibition in mycelia radial growth and mycelial dry weight as compared to control (Table 5.1). Among six fungicides all concentrations Tilt-250 EC completely inhibit the mycelial growth of *Colletotrichum capsici* Vitavax-200 and Royal 50WP gave 55.33-77.33 and 59.67-83.67; and 20.33-68.33mm and 20.67-73.67g, radial growth and mycelial dry weight of the test pathogen, respectively. Dithane M-45 and Cupravit were appeared to be significantly inferior in comparison to other fungicides in inhibiting the mycelial growth.

Table 5.1. In vitro Evaluation of Fungicides on the growth of chili anthracnose (*Colletotrichum capsici* (AAUCc-4 isoate).

Fungicides	Concentration (ppm)	% Inhibition		Fungi-cides	Concentration (ppm)	% Inhibition	
		Radial Growth (mm)	Mycelial dry weight (g)			Radial Growth (mm)	Mycelial dry weight (g)
Ridomil	150	18.67b	70.67e	Vitavax-200	150	76.33d	82.67f
	250	20.33b	71.33e		250	77.33d	83.67f
	300	23.33b	24.33bc		300	55.33c	59.67d
Benomyl	150	23.4b	28.67c	Royal 50WP	150	65.33cd	70.33e
	250	26.9b	83.33f		250	68.33d	73.67e
	300	99.33e	99.33g		300	20.33b	20.67b
Tilt-250EC	150	99.33e	99.33g	Dithane M-45	150	23.33b	24.33b
	250	99.33e	99.33g		250	26.67b	23.67b
	300	68.33d	75.67ef		300	20.33b	19.67b
Control		7.33a	0.37a	CV=15.8		LCD=10.6	LCD=6.4

*Values with same letter are not significantly different

Mycelial dry weight was reduced by 83.33% and 73.67 with the second highest concentrations of Vitavax-200 and Royal 50WP, respectively. But Vitavax 200 was significantly superior to Rovral 50WP but significantly inferior to Tilt-250EC. Dithane M-45 at 300 ppm inhibited only 19.67 % mycelial dry weight and statistically similar to Royal 50% WP but superior to Cupravit which was appeared to be significantly inferior in comparison to all other fungicides. The trends in efficacy of all fungicides at all concentrations were almost similar as observed in case of inhibition of radial mycelial growth of the fungus (Table 5.1).

Among six tested fungicides, Tilt-250 EC appeared to be the best one in inhibiting the hyphal growth of *Colletotrichum capsici* which was followed by Vitavax-200, Rovral 50WP, Dithane M-45 and Cupravit. The present results are in partial agreement with other investigators who observed that Tilt 250 EC, Vitavax-200 and Rovral 50 WP were most effective against *Colletotrichum* spp. in different crops (Islam, 2002). Dithane M-45 was noted as poor performing fungicides against *Colletotrichum capsici* (Table 5.1). Its poor performance against some *Colletotrichum* spp. was also reported by Islam *et al.*(2002) and Sharif (2005).

5.3.2. *InVitro* Evaluation of Plant Extracts on the Mycelial Growth of Chili Anthranose

Reduction of mycelial diameter and dry mycelium weight by anthracnose was found by all the treatments compared to control and the highest reduction was measured from the extracts of garlic followed by onion.

Table 5.2. *In vitro* evaluation of plant Extracts on chili anthracnose (*Colletotrichum capsici*)

Plant Extract	Concentration (%)	% Inhibition		Plant Extract	Concentration (%)	% Inhibition	
		Radial Growth (mm)	Mycelial dry weight(g)			Radial Growth (mm)	Mycelial dry weight(g)
1.Garlic	5	42e	44.67f		15	39.67e	42.67e
	10	51f	51.67g		5	21.67b	23.33b
	15	72.33g	73.33 i	4.Neem	10	25.67bc	28.67c
2.Onion	5	37.33de	40.33e		15	32.67d	34.33d
	10	48.67f	53.33gh	5.Control		7.67a	0.34a
	15	53.33f	58h				
3.Ginger	5	42e	41.67e				
	10	36.67d	39.33e		CV = 14.7	LSD = 5.5	LSD = 4.2

*Values with same letter are not significantly different

From the tested plant extracts garlic at 15 % v/v concentration was showed best in the percentage of reduction both the radial mycelial diameter (72.33) and mycelial dry weight (73.33) followed by Onion at 15 % v/v that gave (53.33) and (58%), respectively. The rate of reduction was corroborated with its concentrations in case of all the tested plant extracts. Ginger at the highest concentration gave 40 % and 43 % reduction in mycelial diameter and dry mycelium weight, respectively, and significantly inferior to onion but superior to neem extract at all the concentrations. The results indicated by Kabir *et al*. (2015) with different botanicals in combination for controlling alternaria blight disease of broccoli are also in strong evidence with the present findings.

Neem extract gave the highest 32.67% reduction in mycelial diameter and 34.33% reduction in mycelium dry weight at the highest 15% concentrations (Table 5.2) and significantly inferior to all other extracts. The result of the experiment showed that the most effective material is garlic, which was followed by onion, ginger and neem. The result was in conformity to the findings of Serawit and Tesfaye (2014) who recommended that neem extract was very effective in

controlling the anthracnose pathogen *Colletotrichum* spp. on coffee. This result is also in corroboration with some previous research works (Kabir *et al.,* 2015; Nashwa and Abo-Elovousr, 2012; Ademe *et al.,* 2013).

5.3.3. *InVitro* Evaluation of *T. harzanum* against chili anthracnose

The results of the screening of 20 isolates of *T. harzianum* against *Colletotrichum capsici* on PDA plates are presented in Table 5.3.

Table 5.3. Inhibition of radial Growth of *Colletotrichum capsici* by selected *Trichoderma harzanium* isolates in dual plate techniques

Isolate	% inhibition of radial Growth(mm)	Isolate	% inhibition of radial Growth(mm)	Isolate	% Inhibition of radial Growth(mm)
Tri-1	62.33 e	Tri-8	64f	Tri-15	60de
Tri-2	67.33 g	Tri-9	52.67b	Tri-16	53.33bc
Tri-3	89 h	Tri-10	52.67b	Tri-17	61e
Tri-4	58d	Tri-11	52 b	Tri-18	63.33 ef
Tri-5	66.33 ef	Tri-12	64.67 f	Tri-19	51.33 b
Tri-6	55 c	Tri-13	57.33cd	Tri-20	51 b
Tri-7	62e	Tri-14	50.33 b	Control	10a
				CV=16.1%	LSD=3.5

*Values with same letter are not significantly different(p=0.05)

Among the selected isolates Tri_3 was appeared to be most effective against the test pathogens showing 89 % inhibition of mycelial growth and significantly higher compared to the other isolates. Identically higher inhibition of radial growth against *Colletotrichum capsici* was recorded with the isolates Tri-2 and Tri-5. The isolate Tri-8 inhibited 64 % radial growth of the pathogen but significantly inferior to the isolate Tri-2 and Tri-5.

Isolate Tri-14, though, had shown the lowest inhibition in radial growth against *Colletotrichum capsici,* was statistically not different to Tri-9, Tri-10, Tri-11, Tri 16, Tri-19 and Tri-20. The finding was in conformity with the reports of D'Souza *et al*. (2001); and Rajathilagam and

Kannabiran, (2001) who observed significant reduction of the growth of chili anthracnose (*Colletotrichum capsici)* mycelia growth by using *T. harzianum*. All stuff considered, the effectiveness of the antagonist *Trichoderma* spp against Chili anthracnose (*Colletotrichum* spp) was worth appreciation to design a control option. The results of the current *in vitro* evaluation of twenty *Trichoderma* isolates against *Colletotrichum capsici* of chili satisfied the required criteria for the selection of specific antagonist and isolate to pave the for the development of safer disease management of anthracnose of Chili. *Trichoderma* species were found more effective in suppressing the mycelial growth of *Colletotrichum capsici* when compared to plant extracts.

5.4. Conclusion and Recommendations

From the results of the current *in vitro* evaluation of fungicides, it was shown that Tilt gave the most superior result while garlic gave superior inhibition percentage among the plant extracts. From the seven *Trichoderma* isolates applied against *Colletotrichum capsici* (isolate AAUCc-4) Tri-3 treated plates gave lowest dry mycelia weight. Thus, fungicides, plant extract and antagonist fungal isolates are recommended for further field research against anthracnose before use.

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CHAPTER SIX:

INFLUENCE OF PLANT PHENOLOGY AND DENSITY ON DEVELOPMENT OF CHILI ANTHRACNOSE (*Colletotrichum capsici (syd*)) LEAF SYMPTOMS

Abstract

Chili (*Capsicum frutescence* L.) seedlings of three phenological categories, viz, young, intermediate and old plants, were arranged in a completely randomized design (CRD). The seedlings were inoculated by the anthracnose pathogen. On the youngest seedlings, symptom development was delayed one day compared to that on the older ones. After the appearance of symptoms, for four consecutive days, the level of leaf disease incidence and severity was consistently lower on the youngest seedlings. Therefore, the application of fungicides on seedlings is recommended only after four days of exposure to inoculum and for seedlings with six or more leaves. In another trial, inoculated seedlings were placed in RCBD experiment with five treatments: 0, 15, 30, 45 and 60 cm spacing between plants to provide different planting densities. Leaf wetness was highly reduced by increasing seedling spacing by at least 15 cm. The key finding was that the highest plant population densities yielded the highest weight of berries per plot. The different densities had no significant effect on mean weights of the berries, mean plant height, mean width of plant canopy and mean number of secondary lateral branches per plant. Prophylactic application of fungicides on pepper seedlings should commence only from the phenological stage of six leaves and as the seedlings grow, spacing must be widened to minimize both plant-to-plant inoculation and locally conducive micro-climatic conditions for infection. This approach can reduce the cost and risk of transplanting infected pepper seedlings.

Key Words*:* Age, Anthracnose, *Colletotrichum* spp., Days of wetness, Density

6.1 Introduction

Anthracnose (*Colletotrichum capsici* (syd.) Buter and Bisby) is the most common disease of pepper seedlings (Belete *et al.,* 2012). At least ten hours of air saturation (Freire *et al.,* 2002) or four hours of wetness (Agrios., 2005) are required for Colletotrichum infection to take place. The water is important in dilution of gelatinous self-inhibitory conidial substances (Agrios, 2005; Freire *et al.,* 2002) and relative humidity is the most important factor during the infection process (Adams *et al.,* 2015, Freire *et al.,* 2002).

A study by Skeete *et al.* (2004) in Barbados found that population density had the greatest impact on yield which was increased 67% by doubling plant density. Adams *et al.* (2011), also in Barbados, concluded that a population density of 40,000 plants/ha produced a yield higher than the farmer practice (9,570 plants/ha) by 123%. The higher densities of the hot pepper cultivar, West Indies Red, did not affect berry sizes and shapes. O'Keefe and Palada (2002), in St Croix, found that different optimal intra-row spacing for three hot pepper varieties produced the highest yields of berries as follows: West Indies Red (18,436 kg/ha) at 91 x 41 cm (27,222 plants/ha); Habanero (18,753 kg/ha) at 91 x 61 cm (18,150 plants/ha); and Yellow Scotch Bonnet (14,717 kg/ha) at 91 x 61 cm (18,150 plants/ha)(Adams, *et al*. 2015). Anthracnose symptoms are observed mainly on aerial parts of the plant and more frequently on the leaves (Freire *et al.,* 2002). Lesions are initially water-soaked and later become orange brown to light reddish with age and sporulation of the fungus (Freire *et al.,* 2002). In cases of a severe attack defoliation of leaves may occur or even seedling mortality (Agrios, 2005). Yield can be doubled or tripled through the use of higher plant population densities coupled with good agricultural practices (Adams *et al.,* 2011). Optimal plant population densities for the commercial landraces of chili in Ethiopia will lead not only to increases in yields but also to a reduction in the cost of production since the closer spaced plants will shade out weeds more effectively and lower the rate of evapo-transpiration thus consuming less water.

It is well known, however, that appropriate selection of plantlet or organ age or position enhances accuracy in screening programs: Contrasts in sensitivity to a pathogen, may be erroneously attributed to differences between genotypes or treatments if arbitrary selection is made (Visker *et al.*, 2003). This is because plants resist pathogen infection through age related

structural and chemical mechanisms. Natural defense mechanisms are poorly developed in immature tissues. These must either be preformed (cuticle and cell wall) or induced upon infection (reactive oxygen species, cell wall strengthening, phytoalexin biosynthesis and accumulation of pathogenesis–related defense proteins respectively (Rivera *et al*., 2002; Romero *et al*; 2013). Currently, the devastating capacity and the high disease incidence and severity of anthracnose have been observed in major growing areas of Ethiopia. Therefore, this study has been initiated to evaluate the effects of seedling age and density, and spacing on seedling wetness, infection, incidence and severity of anthracnose (*Colletotrichum* spp).

6.2 Material and Methods

6.2.1 Study Area

Three trials were undertaken at Shashogo, Alaba and Maraqo Sp. Districts of SNNPR in Ethiopia, between April and June, 2014. Daily climatic data collected from the nearest (one kilometer) Alaba Station included rainfall, temperature and relative humidity.

6.2.2 Effect of Seedling Phenological age on Anthracnose Disease Development

Seedlings of found at different phonological age (Young, Intermediate and Old) were raised concomitantly from a highly disease susceptible genotype (Maraqo fana variety) 15cm X 28 cm in a glass house which then was transplanted to the field. Operationally, Young seedlings were defined as those with less than 5 leaves and aged one and half weeks; intermediate seedlings with 6 to 14 leaves and aged two and half weeks; and the old ones with 15 or more leaves and aged four weeks. Then, anthracnose asymptomatic seedlings were visually categorized into three groups, corresponding to different numbers of leaves and days after planting. Twenty pots of seedlings of each phenological age category were uniformly inoculated with *Colletotrichum* spp (AAUCc-4 isolate) cultured and purified in Department of Molecular, Cellular and Molecular Biology, Addis Ababa University. The three treatments were arranged in CRD with four replicates of each containing five plants per/pot. The cultural practices were applied evenly to all treatments including the following components of an updated production system (Adams *et al*., 2007).

6.2.3 Study on effect of seedling spacing on Anthracnose Disease Development

To evaluate the effect of seedling density, 80 uniform seedlings, were grown a nursery and transplanted to the main field at the five leaf phenological stage. The seedlings were inoculated with *Colletotrichum* spp (AAUCc4 isolates) at a density of $1x10^6$ through spraying teachniques three days later. The seedlings were arranged in RCBD with a four replicate (Adams, *et al*. 2015; Petersen, 1994). Five different spacing were considered as treatments: 0 (Broadcasting), 15, 30, 45 and 60 cm between pots. Each plot consisted of five seedlings. The inter-raw spacing was kept as recommended by EIAR, i.e., 70 cm (EARO, 2004). The effect of planting density on days of wetness was intuitively recorded (as wet and not wet dichotomy) each day for 20 days and analyzed by using the regression analysis.

6.2.4. Collected Data

Developmental traits of chili plants were recorded at each picking from five random plants per plot. These traits were Mean plant height (cm), Mean width of plant canopy (cm), Mean number of lateral branches and the mean berry weight (g) was recorded from ten random berries per plot at each picking. Additionally, observations were made on the leaves to determine the effect of age and spacing on anthracnose disease incidence and severity were recorded based on Sidduiqi *et al.,* (2008). During each picking the berries were graded in the field by placing those damaged in a separate container. This was followed by a further discarding the non-marketable berries during the bagging and weighing process. The yields of marketable berries from the respective plots were recorded.

6.2.5. Data Analysis

Incidence and severity data were statistically analyzed using GenStat (2003) for windows program for the seedling age trial. One-way analysis of variance (ANOVA) was used to test for differences between the three (young, intermediate and old) seedling ages. The data was acceptably normal with heterogeneous treatment variances, thus age means were separated using Fishers protected t-test least significant difference (LSD) at the 1% level of significance. Simple regression analysis was carried out for density and days of wetness.

6.3. Results

6.3.1. Effect of Seedling Leaf Age on Incidence and severity of Anthracnose

All seedlings were disease free for one day after they had been placed under the infected tree. However, they showed appearance of anthracnose disease symptoms within the intermediate and old seedling catagories two days later (Table 6.1). The youngest seedlings showed symptoms three days later. When the youngest seedlings acquired one additional leaf, during the period from the second to the third day, highly significant differences on anthracnose incidence as well as severity were registered. However, incidence was significantly lower on the youngest seedlings than the other catagories on the last scoring day (Table 6.1).

Table 6.1. Number of Infected leaves, disease incidence and severity means on *Colletotrichum capsici* (AAUCc-4 isolate) inoculated seedlings starting from four DAI at Alaba, Ethiopia

Statistics & Days	Seedling Pheno-stage	Mean Assessed Variate			**Statistics & Dayse**	**Seedling Pheno-stage**	Mean Assessed Variate		
		No. of Inf leaves	% dis. Incidence	% dis. Severity			No. of Inf. leaves	% dis. Incidence	% dis. Severity
1	**Young**	0a	0	0	**5**	**Young**	6.75a	0.5	0.5
	Intermediate	1a	0	0		**Intermediate**	8.75b	2.2	0.7
	Old	1b	0	0		**Old**	14.25c	0.89	0.9
LSD(5%)		0.12			**LSD(5%)**		1.8	1.4	0.8
CV(%)		0.1			**CV(%)**		3.5	10.4	9.7
2	**Young**	5.7a	0	0	**6**	**Young**	7a	0.7	0.6
	Intermediate	8.45a	1.67	0.016		**Intermediate**	8.75b	2.2	0.8
	Old	14.45c	2.17	0.04		**Old**	14.5c	1.2	0.9
LSD(5%)		0.99	0.81	0.002	**LSD (5%)**		2.1	1.2	0.22
CV(%)		5.4	14.1	14.1	**CV (%)**		3.5	6.9	13.5
3	**Young**	6.25a	0.2	0.1	**7**	**Young**	7.25a	0.8	0.8
	Intermediate	8.5b	2.1	0.5		**Intermediate**	8.75b	2.3	0.9
	Old	14.75c	1.7	0.6		**Old**	14.25c	1.8	1
LSD(5%)		1.2	1.0	0.1	**LSD (5%)**		2.5	1.3	0.4
CV(%)		19.00	9.50	12.4	**CV (%)**		3.2	8.2	10.9
4	**Young**	6.5a	0.4	0.3	**8**	**Young**	7.5a	1	1
	Intermediate	8.5b	2.2	0.6		**Intermediate**	8.75b	2.3	1.1
	Old	14.25c	1.89	0.8		**Old**	14.25c	2	
LSD(5%)		2.6	1.5	0.8	**LSD (%)**		1.9	1.1	0.9
CV(%)		6.62	14.5	16.8	**CV (%)**		31.00	15.2	11.12

*Means with same letters are not significantly different; LSD = Least significant difference and CV = Coefficient of variance

The mean number of infected leaves on the youngest seedlings increased two units (five in days 1 to seven in day 10). However, these remained less than the number of leaves on the intermediate and old seedlings which did not increase almost throughout the whole experimental period (Table 6.1). Anthracnose incidence under the described conditions, increased from zero to a maximum mean of 7.5-14.25% while the severity reached a maximum mean of 1.2 and 1.3 (less than 1% leaf area necrotic) depending on the age of the seedlings (Table 6.1).

6.3.2. Effect of seedling spacing on Incidence and severity of chili Anthracnose

There is a statistical difference among the four spacing categories (Table 6.2).

Table 6.2. Frequency of wet leaves, disease incidence and severity means in *Colletotrichum capsici* (AAUCc-4) inoculated seedlings: per assessment date at Alaba, Ethiopia

Statistics & Days of Wet	Plant Spacing in cm	Mean Assessed Variate			Statistics & Days of Wet	Plant Spacing in cm	Mean Assessed Variate		
		Freq of wet leaves	% dis. Incidence	% dis. Severity			Freq of wet leaves	% dis. Incidence	% dis. Severity
1	0	6.25cd	0	0	5	0	6.75e	5.2c	5.2e
	15	4.75bc	0	0		15	5.5d	0.5a	0.5b
	30	4.25ab	0	0		30	5c	2.2b	0.7c
	45	3.25b	0	0		45	3.5b	0.89a	0.9d
	60	1.5a	0	0		60	1.5a	0.22a	0.3a
LSD(%)		1.12			LSD(%)		1.4	1.1	0.11
CV(%)		4.42	3.6	4.12	CV(%)		4.6	4.1	8.2
2	0	6.75d	5.32b	0.9c	6	0	7	5.5c	5.1b
	15	5.75c	6.5bc	0.2b		15	5.5c	0.7a	0.6a
	30	5.25b	1.67a	0.016a		30	4.75b	2.2b	0.8a
	45	2.25a	2.17a	0.04a		45	4a	1.2a	0.9a
	60	1a	1.226a	0.012a		60	3a	0.9a	0.88a
LSD(%)		1.4	1.53	0.11	LSD(%)		1.2	0.9	1.0
CV(%)		55.1	14.1	14.1	CV(%)		4.2	4.1	18
3	0	6.25e	3.2c	4.7e	7	0	7.25d	4.6c	4.9c
	15	5.25d	0.2a	0.1a		15	5bc	0.8a	0.8a
	30	4.75c	2.1bc	0.5c		30	4.5b	2.3b	0.9a
	45	4b	1.7b	0.6d		45	3.75a	1.8a	1b
	60	2.5a	0.1a	0.3b		60	2.75a	0.2a	0.3a
LSD(%)		1.33	1.1	0.2	LSD(%)		2.1	1.9	2.2
CV(%)		3.12	8.3	12.4	CV(%)		40.1	42.1	32.2
4	0	6.5e	4.5e	5.1e	8	0	7.5c	4.59c	4.38d
	15	4.5d	1.89c	0.8bc		15	6b	2.8b	2.9c
	30	3.5c	2.2d	0.6a		30	5.5b	2.3b	1.1b
	45	2.5b	1.5b	1.6d		45	4a	2b	0.99b
	60	1.25a	1.2a	1.1c		60	2.75a	0.1a	0.1a
LSD(%)		0.8	0.6	0.4	LSD(%)		2.2	1.8	1.5
CV(%)		5.5	6.4	6.2	CV(%)		38.1	32.4	35.2

*Means with same letters are not significantly different; LSD = Least significant difference and C V = Coefficient of variance

There is strong negative relationship between seedling spacing and incidence and severity of chili anthracnose (Figure 6.2). Prolonged wetness was observed with highest frequency on seedlings whose plots were directly next to each other (0 cm between seedlings). By moving the seedlings just 15 cm away from one another, the frequency of prolonged wetness was reduced almost three times and so on at 30, 45 and 60 cm (Fig. 6.1a-e). It can, therefore, be assumed that seedlings planted closely will remain wetter for longer periods of time.

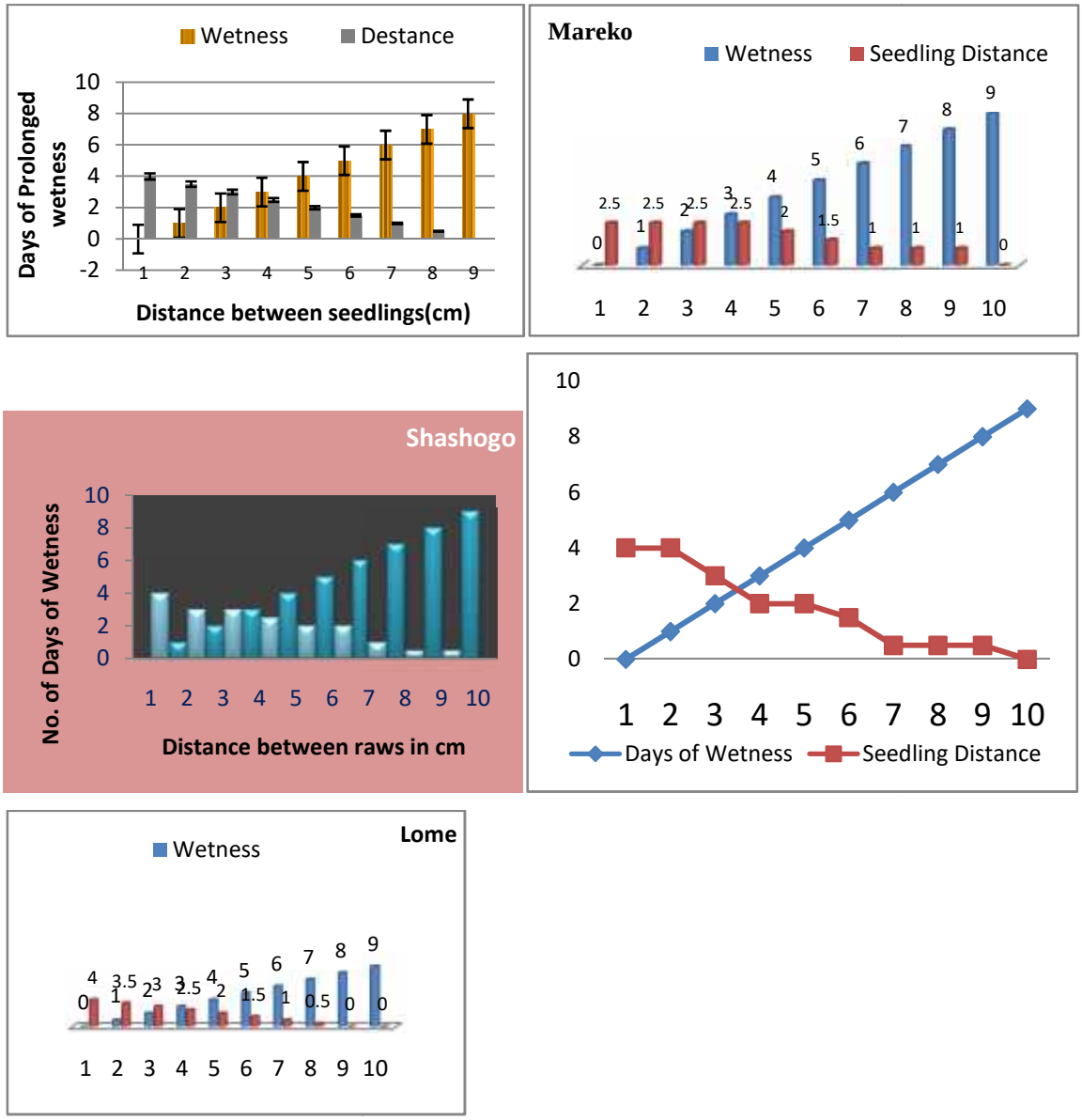

Figure 6.1. Effect of pepper seedling raw distance on the number of days of wetness at Adama (First raw left), Maraqo (first raw right), Shashogo (second raw left), Alaba (second raw right) and Lome (third raw) 2013 out of 9 days of mist.

6.3.3. Effect of seedling Density on Days of Wetness in chili farms in different Districts

The influence of seedling density in purposively selected nurseries and small gardens of chili in Adama, Shashogo, Maraqo and Lome districts had been observed with the objectives of identifying enabling environmental conditions essential for anthracnose disease incidence (Appendix 6.1a-e). The result showed that days of wetness were negatively correlated to spacings in all locations. When the inter-raw spacings increased, wetness declined. In Alaba, the highest and lowest days of seedling wetness was observed at 1 and 10 cm spacings with 4 and 3.8 days of wetness. The mean days of wetness ranges between 0 to 2.5, 0 to 4, 0 to 4 and 0 to 4, in Maraqo, Shashogo, Alaba-2, and Lome area, respectively.

Regression analysis between planting density and Days of wetness, in nurseries at Adama, Maraqo, Shashogo and Lome districts showed that there were strong negative associations with regression formula of y=0.4727x +3.9273, y=0.4727x +3.9273, y=0.4727x +3.9273, and y=0.4727x +3.9273; and (R^2 values) of 0.94, 0.89, 0.97 and 0.99, respectively (Appendix 6.2a-d).

6.4. Discussion

The findings of this research revealed that there is a positive correlation between plant age and infection. Infection is found to be higher at younger plants as compared to those seedlings at juvenile stage. A similar research disclosed researches directly related to the effects of plant population density on hot pepper of the same species (*Capsicum chinense* Jacq.) that is commercially grown in the Caribbean (Adams *et al.,* 2015). It is different from the chilies (*Capsicum frutescence* L.) mostly grown in Mexico and other parts of the world and which dominate the global hot pepper trade (Campodonico, 2002).

Closer intra-row spacing was optimal for the West Indies Red variety unlike the wider spacing for Habanero and Yellow Scotch Bonnet. In Trinidad, Indalsingh and Antoine (2006), using the cultivar ‘Local Red’, showed that 27,777 plants/ha yielded 150,000 kg/ha as compared to 45,800 kg/ha for the lowest plant population density. At the same time, berry quality was not affected. Skeete (2009) planted at a population density four times that of the average farmer in Barbados and obtained a yield higher by 2.5 times at the first picking.

All the studies agreed that hot pepper yields increased with higher plant population densities. Similar conclusions were arrived at by Adams *et al.,* (2015) working with *C. annuum* L. and *C. chinense* Jacq. in other regions of the world. However, Moirangthem *et al.* (2012) in India concluded that the best spacing for the cultivar Bhoot (*C. chinense* Jacq.) with regards to growth parameters and yield components was 105 x 105 cm which did not agree with the findings of this study in which highest yields were obtained from closer spacings. Motsenbocker (1996) in Louisiana, USA, working with *C. annuum var annuum* L. CV 'Golden Greek', stated that generally plants grown at the narrowest spacings produced the lowest fruit yield per plant but the most fruit per hectare. Cavero *et al*. (2001) studied direct seeded paprika pepper (*C. annuum var annuum* CV 'Agridulce SIA') and concluded that fruit number and weight per plant decreased with increasing plant population densities. However, the increase in fruit yield per hectare as plant population density increased was as a result of the larger number of fruits per hectare. Therefore there is general agreement between researchers on this phenomenon (Adams *et al.,* 2015).

In this trial, a high level of anthracnose incidence was found on leaves (95%) contrasting with a low severity rating of less than 1%. This opposes the finding from adult pepper where anthracnose increases rapidly in both number (incidence) and size (severity) of lesions whenever climatic conditions and host phenology is favorable (Adams *et al*., 2011).

In this study it has been observed that the first four leaves are perpendicularly inserted to the stem allowing relatively lower periods of wetness or surrounding air saturation due to fast evaporation. Contrarily, the subsequent leaves were oblique to the stem and therefore exhibited propensity towards harvesting and retaining more wetness and therefore induce fungal spores to germinate. Initial four leaves were almost permanently dull and developed relatively small lesions upon *Colletotrichum* infection when compared to better developed leaves. Pepper leaf development takes 21 days to reach maturity or full green color (Adams *et al.,* 2015) but the initial four take less. The researcher also noted that during seedling leaf development a dull and a shiny area can be distinguished. Shiny area was associated with gradual cuticle formation and thus found to be more susceptible to disease. It was found from this experiment that high density of seedlings supported prolonged leaf wetness. Observations on adult revealed that such

conditions are highly conducive to infection by anthracnose (Adams *et al.,* 2015) and pruning or providing a wide spacing between plants at planting is recommended (Ikisan, 2000).

Regression analysis indicated that strong negative correlation existed between raw spacing and days of wetness. Further, statistical analysis revealed there is no significant (P=0.001) difference among the varying, density treatments on yield components. Several previous studies disagree with this finding stating that hot pepper yields steadily increased as in- row spacing decreased (Adams *et al.*,2001;2015; O'Keefe and Pallada, 2002; Skeete *et al.*, 2004; Indalsingh and Antoine, 2006; and Skeete, 2009).

6.5. Conclusion and Recommendation

High seedling density, accompanied by both lower spacing and high leaf number, increase prolonged cumulative wetness and subsequent plant propensity to infection by anthracnose pathogen. It was also found that intermediate and old seedlings with six or more leaves showed inferior incidence and severity of anthracnose disease. Thus, it's recommended to transplant after six leaves' development stage and at spacing of 30 cm and above to reduce incidence and severity of chili anthracnose.

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CHAPTER SEVEN:

PERFORMANCE EVALUATION OF SOME CHILI PEPPER (Capsicum Spp) GENOTYPES FOR ANTHRACNOSE (*Colletotrichum capsici* (syd)) RESISTANCE

Abstract

This research evaluated 20 chili (*Capsicum* spp) varieties through screening for resistance to anthracnose (*Colletotrichum capsici*) in field experiments at two locations, *Alaba* and *Maraqo*. Starting seven days after transplanting, plants in each plot were monitored for diseases symptoms and infection. Data on incidence and severity of anthracnose were collected. Besides the area under disease progress curve (AUDPC) were calculated. Yield components were also recorded before and after harvest. Anthracnose disease and yield parameters differed significantly among the tested genotypes at both locations. The majority of the genotypes were moderately resistance to susceptible to *C. capsici* none of the genotypes was found to be immune at both locations. Significant variations were also obtained among the genotypes for all yield components, namely percent establishment, dry fruit weight per plant, number of fruit per plant, pulp weight per plant, unmarketable fruits weight per plant, fruit length and days to 50 percent maturity. Total yield per plant were higher at Alaba than Maraqo. This variation is related to the level of disease intensity, which was higher at Maraqo. Thus, after thorough multi-locational researches, the identified sources of resistance are recommended to be utilized in future pepper breeding programs.

Key words: Anthracnose, Chili, *Colletotrichum capsici*, Resistance, Screening

7.1 Introduction

Chili pepper (*Capsicum frutescence* L.*)* is the leading vegetable crop produced in Ethiopia. The national production of green and dry hot pepper was 2,541,883.97 and 412,503.57 tones with average productivity of 66.88 and 23.31tones ha^{-1}, respectively (CSA, 2013). Notwithstanding the importance of pepper to the economy of Ethiopia, low yields are constantly being recorded by the local farmers, leading to a decline in production of the crop. Thus, *Capsicum* productivity in Ethiopia is far below the world average that strongly demands immediate productivity improvement. People consume pepper for intake enhancement as well as to supplement the dietary needs. It is also one of the major income generating crops for most households of the pepper producing areas and it plays a vital role in food security in SNNP and Ethiopia (Roukens, 2005). Hot pepper is the main parts of the daily diet of most Ethiopian societies. The fine powdered pungent product is an indispensable flavoring and coloring ingredient in the common traditional sauce "*Wot*", whereas the green pod is consumed as a vegetable with other food items. The average daily consumption of hot pepper by Ethiopian adult is estimated 15g, which is higher than tomatoes and most other vegetables (MARC, 2004). The total area devoted to hot pepper worldwide is estimated at four million hectare with an average annual increase of 5% (Weiss, 2002).

In spite of its importance, hot pepper production system for green and dry pod has stayed as low input and low output with a national average yield of 7.6 tons ha^{-1} for green pod whereas it was 1.6 tones ha^{-1} for the dry pod, respectively (CSA, 2006). The decline of hot pepper production in the country is also attributed to lack of improved, good quality and well adapted varieties, nutrient depletion (poor soil fertility), inappropriate fertilizer utilization (due to an increase in the price of fertilizers), poor agronomic practices, poor disease and pest management and poor harvesting and post-harvest practices (Fekadu and Dandena, 2006; Alemu and Ermias, 2000).

Chili anthracnose, the most important fungal disease, drastically reduces yield, deteriorates the quality of fruit, and hence gives low returns to the farmers. Thus, it is one of the major pests of economic importance to chili production in Ethiopia and it has been reported that pre-harvest and post harvest losses account for more than 50 percent in severe cases (Pakdeevaraporn *et al*., 2005). Many studies have indicated that disease management practices are often inadequate to

control the diseases. Moreover, pesticide residue has become the major constraint in an approach to meet the stringent requirements of the importing countries. Hence, most economical way to minimize the crop losses is to cultivate resistant varieties/hybrids.

However, there is no reliable method to identify anthracnose resistant varieties/hybrids of chili. Currently, resistance of chili variety/hybrid against anthracnose pathogen is measured employing fruit puncture method and sprays inoculation methods, at lab and field conditions, respectively, without considering the mode of anthracnose development on fruit surfaces. Therefore, development of an effective ideal screening method is an important requirement to minimize crop losses by identifying the anthracnose resistant chili varieties/ hybrids.

The present situation indicates that in South west area there is limitation of well adapting hot pepper varieties including both improved and the local ones. As a result, varietal information for the improvement of the crop for high fruit yield in the existing agro-ecology is insufficient. No sufficient research work on evaluation of hot pepper which enables the growers to select best performing varieties in the study area. Evaluating selected varieties for their agronomic performance is one of the most important considerations to ease the existing problems of obtaining best adaptable varieties for which the output of this study was likely to assist and sensitize hot pepper growers and processors. Therefore, this research has been initiated in order to evaluate the performance of some pepper genotypes for anthracnose (*Colletotrichum* species) resistance in SNNP region of the country.

7.2 Materials and Methods

7.2.1 Description of Experimental Sites

This field study was carried out at two important pepper growing locations (Alaba and Maraqo) whereas identification of the *Colletotrichum* spp was undertaken in Department of Microbial, Cellular and Molecular Biology laboratory, College of Natural and Computational Sciences, Addis Ababa University. Alaba and Maraqo have altitude of 1680 and 1800 m above sea level, respectively, and both locations are characterized by dry sub humid climate. Alaba has monthly mean minimum and maximum air temperature of 15°C and 29.5°C, respectively, and rain fall of 900-1300mm/year. On the other hand, Maraqo has annual rain fall of 1500-1850 mm/year and

minimum and maximum temperature of 8°C and 26.5°C, respectively. Both locations are hot spot areas for anthracnose (*Colletotrichum* spp) (Belete *et al*,. 2012; Tameru *et al*., 2003; Simon *et al*., 2009).

7.2.2. Treatments and Experimental Design

The current investigation was carried between April 2013 and January, 2014 using 20 genotypes of the known pepper types in the country. These included Melka zala, Maraqo fana, Melka shote, Weldele, Bako local, Oda haro, Dube medium, Dube short and Gojeb local from Melkassa Agricultural Research Center (MARC). The rest were selections collected from the farmers. The genotypes were diverse with respect to their collection sites too. For comparison, seeds of the local races were obtained from the local farmers. Seedlings were raised in seed beds and transplanted to an open field at the 4-5 leaves stage. The experiment was conducted in randomized complete block design (RCBD) with three replications. The plot size was 4.2m X 4m with four rows to accommodate 56 plants per plot. Intera-row spacing of 0.3m and inter-row spacing of 0.70m were used for the experiments (EARO, 2004). Crop management practices were carried out as per needed or recommended. Moreover, during flowering, single flower caging (50 mesh net of 0.78X0.26 hole size) was practiced to prevent the chance of out crossing (Belete *et al*,. 2012; Fekadu *et al*., 2008).

7.2.3. Data Collection and Analysis

7.2.3.1. Disease Intensity and Genotype Reactions

Starting seven days after transplanting, plants in each plot were monitored for diseases symptoms and infection. Anthracnose incidence on each experimental plot was recorded by counting number of diseased plants and calculating as the proportion of the diseased plant over the total number of stand count on the plot. Each plant within each plot was visually evaluated for percent foliar infection (severity). Further, disease severity data were converted to a rating scale according to affected leaf area, proposed by Ngugi, *et al.*, (2002); where: For foliar diseases, severity was assessed on a 1 to 9 scale where 1 = no disease, 2 = disease affecting 1 to 4% area of top 5 leaves, 3 = 5 to 9%, 4 = 10 to 19%, 5 = 20 to 29%, 6 = 30 to 44%, 7 = 45 to 59%, 8 = 60 to 75%, and 9 = >75% of leaf area affected.

7.2.3.2. Yield Components

The fruits were harvested when they reached full maturity in November, 2013. Harvesting started at the end of December 2013 and lasted to the end of January, 2014 (the investigated genotypes have the same harvest periods). Measurements were done on 10 plants, which had been randomly chosen from the middle row of each plot, and the mean values were used to represent each experimental unit. The traits recorded include: number of fruits per plant (count), dry fruit yield per plant (g), fruit length (cm), single fruit weight (g), and pulp weight per plant (g) and non-marketable fruit yield per plant (g). Weighing has been done with 12% moisture.

7.2.3.3. Data Analysis

The data on disease severity were converted to area under disease progress curves (AUDPC), mean value of disease incidence, disease severity and yield components were subjected to repeated measures of analysis of variance (ANOVA) to evaluate treatments effect. The analysis was done using the general linear model of statistical analysis using SAS computer package (SAS Inc., 2003). Means for different treatments were compared using least significant difference test at 5% significance level (LSD 5%).

7.3. Results

7.3.1. Reaction of Capsicum genotypes to *Colletotrichum capsici*

Results of the current study demonstrated that the intensity of Anthracnose differed significantly ($p<0.05$) among the tested genotypes at both location (Table 7.1). Infections of the pathogens were first observed in the most susceptible materials. The examination of all 20 tested genotypes indicated a continuum of reactions to Anthracnose, ranging from highly resistant to highly susceptible. In 2013, the incidence of diseased plant ranged from 13.51% (BL2) to 50.86% (BL1) and from 25.00 (OH2) to 50.71% (BL1) at Alaba and Maraqo, respectively. In 2014, the incidence of diseased plant ranged from 22.14% (DS2) to 49.43% (GL1) and from 23.71 (OH2) to 49.43% (GL1) at Alaba and Maraqo, respectively. Generally, at Maraqo, the incidence of Anthracnose was significantly high as compared to Alaba. In addition, the disease occurred at the early stage in Maraqo as compared to Alaba site. Severity data also ranging from 21.5 (OH2) to

52.3% (DS2) and 16.3(BL2) to 48.7% (MF2) at Alaba and Maraqo, respectively the incidence and severity were closely related data for most plant genotypes.

Table 7.1. Mean disease incidence and severity of *C. capsici* on pepper cultivar during 2013 and 2014 at Alaba and Maraqo

Gen. Code	Alaba						Maraqo					
	Incidence		Severity		AUDPC		Incidence		Severity		AUDPC	
Year	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
OH1	15.14b	19.29a	22.67b	21.00b	253.50	322.50	27.86b	32.29e	32.67g	21.00 b	447.50	526.00
OH2	22.14c	23.57b	21.33b	24.00de	360.00	375.00	25.00a	23.71a	28.33e	24.00e	400.00	376.00
WD1	48.29ef	47.57g	26.33f	19.67a	798.00	790.50	47.14ef	47.14 i	36.33j	19.67 a	782.50	787.50
WD2	46.43d	47.43g	26.33g	21.00b	776.50	794.50	47.43f	47.43 i	38.67k	21.00 b	794.50	794.50
BL1	50.86g	50.71h	38.67 l	22.67c	855.50	852.50	50.71g	43.00g	38.67k	22.67 c	852.50	743.50
BL2	13.51a	23.00b	34.67 k	41.67p	228.50	363.50	28.00b	35.14f	44.67m	41.67 o	446.00	596.00
MF1	22.14c	26.43cd	38.33 l	38.67 o	360.00	425.00	27.14ab	32.14de	36.33j	38.67 n	432.50	530.00
MF2	46.86d	46.86g	28.67 h	27.00i	783.00	783.00	45.71e	42.29g	36.33j	37.00 m	770.00	716.00
MS1	47.43de	47.43g	22.33 b	34.00n	787.00	787.00	47.43f	47.43i	29.00f	34.00 k	787.00	787.00
MS2	49.29f	49.3h	30.67 i	25.67g	843.50	843.50	49.29f	48.86i	42.33 l	35.67 l	843.50	842.00
MZ1	13.71a	23.71bc	39.67 m	32.00 l	228.50	363.50	29.43b	28.00c	32.33 g	32.00 j	471.00	438.50
MZ2	22.14c	25.71c	33.33 j	26.33 h	360.00	425.00	25.71a	30.00cd	43.33 m	26.33 g	425.00	502.50
DM1	46.86d	42.57f	25.67 f	25.00 f	783.00	718.00	42.14d	42.14g	35.00 i	25.00 f	715.00	715.00
DM2	46.86d	46.86g	29.00 h	32.67 m	783.50	783.50	46.86e	46.86i	27.00 d	22.67 c	783.50	783.50
GL1	49.43f	49.43h	25.67 f	44.00 q	848.50	848.50	49.43f	49.43ij	26.33 c	24.00 e	848.50	848.50
GL2	13.71a	28.71d	22.33 b	23.67 d	228.50	446.00	34.43c	30.14d	28.00 e	23.67 d	546.00	481.00
DS1	22.14c	22.14b	22.33 b	28.00 j	360.00	360.00	27.86b	25.71ab	26.00 c	28.00 h	440.00	432.50
DS2	46.29d	46.29g	24.33 e	30.00 k	773.00	773.00	45.86e	46.00hi	34.00 h	30.00 i	770.00	771.00
LV1	46.43d	46.43g	23.33 d	23.67 d	776.50	776.50	47.43f	47.86i	25.67 b	23.67 d	787.00	792.50
LV2	49.30f	49.29h	21.67 ab	24.67 f	842.50	842.50	48.57f	44.43h	22.67 a	25.00 f	835.00	748.50
LSD(5%)	1.44	2.54	0.59	0.54	-	-	2.56	2.11	0.55	0.509	-	-
CV%	9.14	14	14	18.1	-	-	6.8	7.8	1.44	1.77	-	-

*Means followed by the same letter in the same column are not significant difference at P<0.05; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dube short Types

Most genotypes were moderately resistance to susceptible to *C. capsici*, while the disease response of controls remained constant at each test (Table 7.2). At both locations maximum disease incidence was recorded on GL1. At Maraqo, 5, 10, 50, 20 and 15 % of the tested genotypes were found to be highly resistant, resistant, moderately resistant, susceptible and highly susceptible to Anthracnose, respectively (Table 7.2) while at Alaba, 5, 5, 45, 35 and 10% were highly resistant, resistant, moderately resistance, susceptible and highly susceptible to anthracnose , respectively (Table 7.2).

Table 7.2. Number and percent of resistance types on pepper cultivars evaluated against *C.capsici* in 2013at Alaba and Maraqo

Disease Reaction	Alaba	Maraqo
Highly susceptible	2(10%)	3(15%)
Susceptible	7(35%)	4(20%)
Moderately resistance	9(45%)	10(50%)
Resistant	1(5%)	2(10%)
Highly Resistant	1(5%)	1(5%)

7.3.2. Yield and Yield Components of Chili Pepper Genotypes

In Alaba experimental Site, 20 genotypes were evaluated in two consecutive years (2013 and 2014 cropping seasons) and there were significant variations among the tested genotypes in terms of the number of fruits per plant; dry fruit weight per plant; non marketable fruit weight per plant; fruit length; single fruit weight; and pulp weight per plant (Table 7.3). The results showed a range of 27.00 (DM1) to 58.67 (WD2) for number of pods per plant and 30.67(DS2) to 50.0(MS2) ; 25.3 g (MS2) to 50.67 g (DM2) and 20.67g(MS2) and 37.33g(BL1)for dry fruit weight per plant; 4.47 g (DS2) to 6.04 g (MF1) and 3.68 g (G2) to 4.35 g (MZ1) for non marketable fruit weight per plant; 2.17 g (OH2 and BL1) to 6.0 g (GL2 and DS1) and 3.27(BL1) and 5.67(DM2) for marketable yield; 5.0 cm (MS2) to 12.0 cm (DM1) and 5.33cm (MS1) and 11.67cm (GL1) for

fruit length; and 5.67 q/ha (GL2) to 11.33 q/ha (MF 1, MF1,MZ1) and 4.33q/ha(BL1) and 11.0q/ha(OH2) for total yield (q/ha) in 2013 and 2014, respectively.

Table 7.3. Yield and Yield Components of Pepper accessions at Alaba, 2013 and 2014

Gen. code	No. of Fruits/pl		Dry fruit wt/pl(g)		Unmarketable Fruit(q/ha)		Marketable Fruit(q/ha)		Fruit Length(cm)		Total Yield(q/ha)	
Year	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
OH1	42.33 d	46.33 c	45.67 h	30.67 f	4.65	4.16a	3 b	4.67e	11.67 i	9 ef	11.33 h	9.67 ef
OH2	40.67 e	39.67 e	27.67c	32.67 g	4.5	3.68a	2.17 b	3.33 a	12 j	6.67 b	9 e	11 m
WD1	38.33 f	42.67 d	49.67i	23 ab	4.98	4a	4 c	4.33 d	11 h	5.67 a	9 e	11m
WD2	58.67 a	41.33de	57.67 l	24 b	4.91	3.74a	2.5b	4.67 e	9 f	8.33 de	10.33 h	8.67 gh
BL1	51.33 b	33.33fg	51.67 ij	37.33 k	6.04	4.12a	2.17 b	3.27 a	5.67 b	8 d	8.67 d	4.33 a
BL2	33.67 h	32gh	26.33bc	35.33 j	5.69	3.73a	1.47a	4.33 d	7.67 d	8.67 e	9 e	8 fg
MF1	47.67 c	31gh	42.67 g	32.67 g	5.96	4.23a	4.17 ef	3.83 bc	8 e	7.67 cd	11.33 h	7.33 de
MF2	42.33 d	41de	32de	26.67 cd	5.09	3.94a	4.67 fg	4.33 d	9 f	8.33 de	9.67 fg	9 hi
MS1	31.67 i	49b	41.67 g	27.67 cd	4.53	4.13a	5.17 h	3.67 b	8 e	5.33 a	9 e	6.33 c
MS2	51 b	50b	25.33b	33 i	5.24	4.29a	5.33 h	4.17 cd	5 a	6.33 b	9.33 ef	10.33 kl
MZ1	35 h	54.33a	29cd	37 k	5.79	4.35ab	5.5 hi	3.33 a	7 bc	7.33 c	11.33 h	7.33 de
MZ2	37.33 g	42.67d	19a	25.67 c	5.49	4.08a	5.33 h	4.33 d	11 h	10.67 h	7 ab	5.33 b
DM1	27 j	45.67c	47 hi	33 i	4.75	3.98a	3.67 e	3.67 b	12 j	10 g	7 ab	7.67 ef
DM2	49.67 b	33.33fg	50.67 ij	37 k	5.15	4.03a	5.67 i	5.67 g	9.67	11.33 i	7.67 c	6.33 c
GL1	43.33 d	31.33gh	31.67d	21.67 a	4.96	4.06a	4.67 fg	3.67 b	9 f	11.67 i	7.67 c	7 d
GL2	42 d	32gh	35f	20.67 a	4.51	4.06a	6 ij	4 c	7.67 d	9.33 fg	5.67 a	10 jk
DS1	41 de	35.33f	23.67b	29.67 f	4.57	4.22a	6 ij	5 f	7.33 cd	9 ef	6 a	8.33 g
DS2	39 f	30.67hi	36.67f	24.67b	4.71	4.29a	5.83 i	4 c	4.67 a	8 d	10 gh	8.33 g
LV1	33.67 h	39e	27.67c	36.33 jk	4.54	4.12a	4.33 f	4.67 e	12 j	5.67 a	9.33 ef	9 hi
LV2	35 h	38.33e	35f	25.33 j	4.47	4.4b	5.67 i	4.33 d	11.67 i	7.33 c	9 e	6.33 c
LSD(5%)	1.55	2.5	2.91	1.55	2.09ns	0.66	0.38	0.3	0.53	0.45	0.57	0.57
CV%	5.4	12.7	19	5.4	3.78	6	7.7	4.9	7.3	5.6	8.3	9.1

* Means with the same letters are not significantly different; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dube short Types

In Maraqo experimental site, these 20 genotypes were evaluated in two consecutive years (2013 and 2014 cropping seasons) and there were significant variations among the tested genotypes in terms of the number of fruits per plant; dry fruit weight per plant; non marketable fruit weight per plant; fruit length; single fruit weight; and pulp weight per plant (Table 7.4). The results showed a

range of 7.33 (MS1) to 16.00 (LV1) for number of fruits per plant and 7.67(LV1) to 14.33 (DM1) ; 12.67 g (DM1) to 22.67 g (OH1) and 12.0g (MS2) and 22.0 g(OH1)for dry fruit weight per plant; 2.0 g (MF2) to 7.0 g (LV1) and 1.67 g (MF2) to 8.0 g (DS1) for non marketable fruit weight per plant; 1.8 (BL2) to 6.17 (DS2) and 2.17 g (BL2 and MF2) to 5.17 g (WD2) for marketable yield; 7.67 cm (DS2) to 13.33 cm (MZ1) and 8.0 cm (OH1) and 12.0 cm (MZ2) for fruit length; and 6.0 q/ha (WD2) to 14.0 q/ha (DS2) and 6.0q/ha(BL1) and 11.0q/ha(OH1) for total yield (q/ha) in 2013 and 2014, respectively.

Table 7.4. Yield and Yield Components of Pepper accessions at Maraqo, 2013 and 2014

Gen. code	No. of fruits/pl		Dry fruit wt/pl(g)		Unmarketable fruit(q/ha)		Marketable fruit (q/ha)		Fruit Length(cm)		Total Yield(q/ha)	
year	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
OH1	12.33 gh	10.00 d	18.67 a	22.00 i	4.83d	7.17 g	5.33e	3.83de	11.67 ij	10.00cd	12.67k	7.00bc
OH2	13.67 i	11.00e	22.00 hi	19.67 gh	2.83ab	3.83 c	2.50 ab	4.00 e	10.67gh	8.00 a	8.33cd	11.00i
WD1	15.00 jk	8.67 b	21.33 h	16.67 e	6.67ef	2.17 a	4.33 d	4.17 ef	12.67 kl	11.67ef	10.67h	10.67h
WD2	11.67 fg	9.00 bc	20.67 gh	20.33h	3.67bc	4.50 c	2.50 ab	5.17 g	8.00 ab	9.67 c	6.00a	8.00de
BL1	13.33 hi	10.33 de	19.00 ef	17.00ef	3.67bc	6.17 f	2.33 a	3.83 de	12.00 j	11.33 e	9.00ef	6.00a
BL2	11.00 ef	10.00 d	19.00ef	13.33bc	4.50cd	4.67 d	1.80 a	2.17 a	10.67gh	8.67ab	10.33gh	8.33ef
MF1	11.33 hi	8.33 ab	19.67 fg	18.33fg	4.33c	3.83 c	3.33 c	4.33 f	8.67 bc	11.00de	10.00g	8.00de
MF2	13.00 h	9.67 cd	22.00 hi	13.67c	2.00a	1.67a	5.00 de	2.17 a	9.00 c	8.33 a	9.00ef	8.67f
MS1	7.33 a	11.67 ef	22.67 i	20.00h	5.00d	7.33 gh	5.85 f	2.67 ab	12.67 kl	8.67ab	11.33j	8.67f
MS2	10.00 de	9.00 bc	19.33 f	12.00a	3.07b	5.67 ef	6.00 f	4.83 fg	9.67 e	9.67 c	10.67h	10.33h
MZ1	9.67 cd	13.00 hi	20.67 gh	13.00b	5.67e	5.17 de	5.67 ef	3.67cde	13.33m	10.00cd	12.33k	6.33ab
MZ2	10.67 de	10.00 d	17.67 d	14.33cd	5.17d	3.40 c	3.00 bc	4.67 f	9.67 e	12.00f	8.67de	7.67cd
DM1	13.00 h	14.33 j	12.67 a	17.33f	2.50a	5.50 e	4.33 d	4.33 f	10.00 f	12.67g	11.00ij	7.33c
DM2	9.33 b	8.67 b	15.00 c	19.67gh	4.00c	5.67 ef	5.50 e	5.83 g	9.00 cd	14.00 g	10.33gh	8.67f
GL1	10.67 de	13.00 hi	14.33 b	12.67b	5.33de	3.33 c	5.00 de	4.00 e	8.67 bc	11.67ef	7.67b	6.00a
GL2	9.33 b	10.67 e	18.00 de	18.00f	4.33c	4.50 d	4.67 d	3.17 bc	13.00 l	9.33bc	10.67h	9.33g
DS1	14.33 ij	8.00 a	17.67 d	15.67de	4.67d	8.00 h	5.17 e	4.00 e	9.33 de	12.33fg	9.33f	10.33h
DS2	10.33 de	12.33 gh	19.00 ef	14.00c	2.17a	2.33ab	6.17 f	4.33 f	7.67 a	11.33 e	14.00m	8.33e
LV1	16.00 k	7.67 a	18.33 e	12.33ab	7.00f	4.67 d	4.67 d	4.33 f	11.00 f	9.00b	10.33gh	6.33ab
LV2	13.33 hi	12.00 fg	17.00 d	16.00e	6.00e	4.67 d	5.33e	4.67 f	10.67gh	10.67 d	13.33 l	8.67f
LSD(5%)	1.00	0.89	1.23	1.50	0.85	0.80	0.80	0.73	0.64	0.83	0.58	0.69
CV%	1.08	9.7	9.8	5.7	10	7.8	7.8	8.2	8.4	5.5	9.6	6.9

*Means followed by the same letter in the same column are not significant difference at P<0.05; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dube short Types

On other multi-locational, consecutive trial conducted in Alaba and Maraqo, there were significant variations among the tested genotypes in terms of the percent establishment, days to 50% maturity and days to first harvest (Table 7.5). The result showed a range of 77 (DS2) to 88.33(MZ1) and 78.0(MF1) to 86.33 (BL1) for percent establishment; 53.0(MZ1) to 65.33(LV1)

and 51.67(MF2) to 67.33(GL2) for days to 50% maturity; 98.33(LV1) to 108.0(WD2) and 96.67(OH1) to 107.67(GL2) for days to first harvest.

Table 7.5. Description of mean performance of Hot pepper varieties in 2014 and 2013 cropping season

Gen. code	Alaba						Maraqo					
	Establishment %		Days to 50% maturity		Days to First Harvest		Establishment %		Days to 50% maturity		Days 1st Harvest	
Year	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014	2013	2014
OH1	78.33a	82.00 d	63.67g	57.67ef	106.00d	99.67b	81.67b	85.00d	57.00d	54.00c	103.33d	106.67g
OH2	81.67b	85.33fg	56.00b	52.67ab	102.00b	96.67a	83.67bc	83.00c	54.67bc	46.67a	103.00d	100.33ab
WD1	82.00b	82.67d	61.00e	58.67fg	99.67a	100.33b	82.33b	85.67d	57.67de	54.00c	100.67ab	99.00a
WD2	79.33ab	82.00d	62.00f	65.33k	108.00f	102.67cd	80.00a	83.33cd	68.00h	54.00c	108.33g	106.00f
BL1	81.00b	86.33g	54.00a	60.67hi	102.00b	103.33d	82.33b	82.33cd	56.67d	50.00b	104.33ef	100.33ab
BL2	85.33d	79.33ab	54.00a	54.00cd	102.67c	100.67b	88.67e	92.00h	50.00a	47.67a	102.33bc	99.67a
MF1	78.67a	78.00a	59.67d	63.00j	101.00b	106.33gh	82.00b	85.33de	60.67g	60.67f	102.67cd	98.67a
MF2	79.00a	81.33c	61.67f	51.67a	106.33de	103.67de	79.67a	83.00c	61.00g	59.67ef	106.00f	102.33cd
MS1	86.67e	79.67b	63.00f	56.33e	106.33de	100.00b	93.67f	87.00ef	55.67c	57.67de	104.33ef	102.67d
MS2	85.00d	84.33ef	60.67de	57.33e	107.00ef	104.00e	91.67f	88.33f	58.00ef	60.00f	104.67f	102.00c
MZ1	88.33f	80.33bc	53.00a	59.67gh	106.00de	104.67ef	85.00c	88.33f	60.33g	56.33d	104.33ef	102.67d
MZ2	79.00a	80.33bc	58.00d	58.00f	98.33a	100.00b	85.67cd	89.00f	56.00cd	57.00d	102.33bc	100.33ab
DM1	80.00b	86.67g	56.00b	56.00de	103.33c	105.00f	86.67cd	86.67e	56.33d	55.33c	104.33ef	106.00f
DM2	85.67de	85.33fg	63.00f	59.67gh	101.67b	105.67f	82.33b	85.67e	60.33g	58.67e	104.00e	104.33e
GL1	87.33ef	82.67d	64.00gh	64.00jk	102.00b	105.00f	86.00d	79.33ab	59.67fg	61.00fg	105.33f	106.33fg
GL2	82.00b	85.67fg	61.33e	67.33kl	106.33d	107.67h	83.00bc	89.67fg	59.00f	60.00f	108.33g	107.33g
DS1	83.67c	81.67cd	56.00b	62.33ij	103.33c	103.33d	85.67cd	85.67e	61.00g	62.67g	106.00f	105.67ef
DS2	77.00a	83.00de	60.67de	53.00bc	102.33bc	105.67fg	79.00a	82.33bc	53.33b	60.67f	103.33d	105.33e
LV1	82.67bc	82.00d	65.33h	65.33k	98.33a	101.33bc	86.00bc	82.67c	59.00f	62.67g	99.67a	101.67bc
LV2	84.00cd	84.33ef	62.67f	56.00de	101.67b	105.00f	80.67ab	77.33a	53.67b	56.00cd	103.67de	106.33f
LSD(5%)	2.58	1.51	1.78	2.32	1.60	1.42	2.43	2.14	2.32	2.12	1.41	1.62
CV%	1.01	2.84	6.7	8.7	3.1	2.5	8.9	6.8	7.4	1.00	2.4	3.2

*Means followed by the same letter in the same column are not significant difference at P<0.05; OH=Oda Haro;BL=Bako Local; WD=Weldele; MS=Melka shoal; LV=local variety; MF=Maraqo Fana; GL=Gojeb local; MZ= Melka Zala; DM=Dube medium; DM=Dube short Types

In line with this, in Maraqo's experiment, the result showed a range of 79.0(DS2) to 93.67(MS1) and 77.33(LV2) to 92.0(BL2) for percent establishment; 53.33(DS2) to 68.0(WD2) and 46.67(OH1) and 62.67(DS1 and LV2) for days to 50% of maturity; 99.67(LV2) to 108.33(WD2 and GL2) and 98.67(MF1) to 107.33(GL2) for days to first harvest, in 2013 and 2014, respectively.

7.4. Discussion

The current study showed that half of the tested chili genotypes were found to be resistant while the rest half were susceptible to anthracnose. Susceptibility of the varieties has been bigger at Alaba than Maraqo in which, none of the 20 genotypes were highly resistant to Anthracnose. Genetic resources for a cultivated species are generally regarded as a gene pool of cultivars, species and genera that can be utilized as sources of additional genetic variation for crop improvement. The study also unleashed that anthracnose disease caused by *C. capsici* was recorded more or less throughout the year. The incidence and severity of disease depends on local agronomical conditions pepper genotypes, cultural practices and season. The results presented here shows that highest incidence recorded at Maraqo as compared to Alaba. Several researches indicated that the average disease incidence varied in different locations in different genotypes owing to varied agro climatic conditions and inoculum potential (Belete *et al.* 2012; Dikshit *et al.* 2006; Chakravorty *et al.* 2003; Sudheendr, 2005).

C. capsici isolates from SNNP and Oromiya regions posed epidemiological and management challenges for broad ranges of crops. This agrees with the general description of *C.capsici* as having a very large host range (Sharma *et al.,* 2014). All sweet pepper hybrids are classed as susceptible to *C.capsici* Torres-Calzada *et al.,* (2011) A more or less similar result were obtained by Belete *et al,* (2012) , who reported range of resistance classes among 17 hot pepper genotypes. At Alaba, out of 17 genotypes, none were highly resistant, while 5.8, 23.5, 58.8, and 5.8% were resistant, moderately resistance, susceptible and highly susceptible to Anthracnose, respectively. In Mareqo, 0, 5.8, 17.7, 47.1 and 29.4% of the tested genotypes were found to be highly resistant, resistant, moderately resistant, susceptible and highly susceptible to Anthracnose, respectively.

In this study, none of the 20 genotypes were immune which strengthens the results from Belete, *et al.* (2012) and Park (2007) who reported that commercial cultivars of *Capsicum annuum* resistant to the pathogens that cause anthracnose have not yet been developed. Current research is focusing on evaluation of resistance onto susceptible commercial cultivars of *C. annuum* based on the methods coined by AVRDC, (2003); and Pakdeevaraporn *et al*., (2005). Mongkolporn *et al.* (2004) have studied the inheritance of resistance to anthracnose specifically caused by *Colletotrichum capsici,* in a *Capsicum annuum* population established from a cross between accession '83-168' and cv. 'KKU-Cluster', and their progenies. They

observed a promising dominant gene responsible for the resistance to *C. capsici*. Voorrips *et al*. (2004) found one main QTL with high significance and strong resistance against *C. gloeosporioides* associated with chilli anthracnose disease. No genotypes were reported as immune (Belete, *et al.* 2012).

Belete *et al.* (2012) determined the relationship between characters affecting optimum output is very important for increasing yield components in pepper genotypes. Larger fruit dimensions are desirable for both farmers and consumers. To this end, this study has revealed the relationships between yield and yield components of the pepper. Number of pod per plant; dry fruit weight per plant; non marketable fruit weight per plant; fruit length; single fruit weight; and pulp weight per plant was the most influential factors in this relation. Generally numbers of pods per plant were higher at Alaba than Maraqo. This variation may be related to the level of disease intensity, which was higher at Maraqo. According to Fekadu *et al*. (2003) and Shaban (2007), number of pods per plant differences was mainly based on genotypic variations and less influence by environment. The yield may be highly affected by insufficient cultural practices and especially environment factors. Yield alone may not be sufficient criteria to describe the performance of a certain genotype, since it does not indicate the relative performance with other genotypes over different environments (Zewdie and Poulos, 1995). So, it is essential to grow these types at different locations to explore genotype x environment interaction effects. Solanki *et al*.(1986) and Basavaraj (1997) have reported that fruit length, fruit width, number of fruits per plant and total fruit weight have strong positive correlations with yield. Therefore, the results obtained from this work will advance plant breeding practices by reducing the negative effect of disease and research on yield components by guiding *Capsicum* breeders in selecting the best plant characters in peppers production. In conclusion, this will lead to an increase in desirable yield values by decreasing the number of studied characters, which will in turn increase selection efficiency in pepper production.

7.5. Conclusion and Recommendations

The results obtained from this work will advance plant breeding practices by reducing the negative effect of disease and research on yield components by guiding *Capsicum* breeders in selecting the best plant characters in peppers production. In conclusion, this will lead to an

increase in desirable yield values by decreasing the number of studied characters, which will in turn increase selection efficiency in pepper production.

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CHAPTER EIGHT:

INCIDENCE-SEVERITY RELATIONSHIPS ON CHILI ANTHRACNOSE (*Colletotrichum capsici* (Syd.) Bisby and Butler)

Abstract

The incidence-severity relationship for chili anthracnose, caused by *Colletotrichum* spp., was studied on ten released Chili/pepper genotypes to determine the feasibility of using disease incidence to estimate indirectly disease severity in order to establish the potential damage caused by this disease in southern region, Ethiopia. Data were statistically analyzed by regression. Anthracnose leaf incidence was consistently associated with leaf severity and their relationships can be estimated using the linear function across locations, crop seasons, and genotypes. Thus, the use of easily assessed incidence data had been recommended for determination of severity as well as epidemic comparisons, genotype and seasonal evaluation in chili anthracnose management. This study will pave the way for chili producing farmers for cheaper and efficient approach tailoring it for determination of economic threshold level and launch opportune management practices.

Key words: Anthracnose, Chili, Incidence, i-s-Relationship, Severity

8.1. Introduction

Chili anthracnose, caused by *Colletotrichum capsici*(Syd.), is one of the most important chili disease in pepper growing regions of Ethiopia (Belete *et al.*, 2012), which manifests its symptoms in both leaves and young fruits (Freire *et al.,* 2002). At its ceiling level, severity may lead to permanent wilting and senescence of leaves on mature plant leading to defoliation during shoot development, chlorosis of inflorescences and afterward necrosis and falling of young fruits (Cardoso *et al.*, 2000; 2004). Chili anthracnose symptoms and the impact of the disease were barely described in Ethiopia (Belete *et al.,* 2012). Quantification of anthracnose disease is one of the most challenging tasks. The assessment of disease incidence (i.e., the proportion of diseased plants in a population) is an apparently simple counting task. The accurate and precise estimation or measurement of disease severity (i.e., the area of plant tissue that is symptomatic) formidable task (Groth *et al.*, 1999; Campbell and Madden, 1990). It is tedious, time consuming and physically discomforting task.

As Campbell and Maden (1990) clearly described incidence and severity, the distinction between these two measures is not always as apparent as the definitions suggest. Usually the definitions of incidence or severity rely on the sampling unit used during disease measurement (Seem, 1984). In chili anthracnose quantification, this distinction is made even more difficult by the fact that disease may be assessed at different levels (field, plot, plant, pod, green fruit, and ripened fruit) within a spatial hierarchy. The incidence–severity association represents a relationship between disease intensity measured at different levels (Seem, 1984).

Relationships between incidence and severity at different levels of a spatial hierarchy have been developed for several pathosystems (Xu *et al.*, 2002). Various factors determine the relationships and differ from one pathosystem to another. The cultivar and plant organ assessed, time of disease assessment during an epidemic, growing season, location, and treatment applied to the assessed plots are some of the determinant factors (Seem, 1984). Thus, it would be imprudent to use these models to describe the relationship between incidence and severity of Chili anthracnose of chili prior to thoroughly evaluating them over multiple years and locations under a range of cropping/management scenarios to ascertain which model provides consistently strong relationships between incidence and severity of this disease (Paul *et al.*, 2005).

The relationship between measures of anthracnose intensity at different levels of a spatial scale has been studied by Xu *et al.* (2002). The former studies focused mainly on the comparison of year-to-year repeatability of disease incidence, diseased fruit severity (mean proportion of infected fruits per infected branch, or equivalently, mean relative area of infected fruit with symptoms), and disease index (equivalent to the standard definition of severity (Campbell and Madden, 1990), as used here as measures of components of resistance to *Colletotrichum* spp. Xu *et al.* (2002) did not fully explore the relationship between incidence and severity from the standpoint of its practical application in disease quantification and surveys, and how it may be influenced by sampling. While the work of Xu *et al.* (2002) did address issues related to practical application of the relationship between incidence and severity of Colletotrichum leaves, as was the case in the study conducted by Xu *et al.* (2002), the epidemiological conditions under which this study was conducted were different from those occurring commonly in many areas. Information concerning the chili severity-incidence relationship was inadequate in major chili growing areas of SNNP, Oromia and Amhara regions of Ethiopia. Chili cultivars planted in SNNP differ from the rest of the country; the composition of the *Colletotrichum* complex inciting *Colletotrichum* spp in SNNP is generally different from that found in Oromia and Amhara regions, the latter being predominantly *Colletotrichum* spp and severity was quantified in the aforementioned studies differs from the way it is commonly done in SNNP. These factors may all influence the relationship between measures of disease intensity Xu *et al.* (2002).

Typically, damage assessment can be done for fungicide or germplasm screening after identification of the causative pathogen carried out (Cardoso *et al.*, 2004) or epidemiological investigations (Cardoso *et al.*, 2004; McRoberts *et al.*, 2003). In any of the above approaches, terminology such as disease incidence, disease severity, disease density and others are commonly used to measure the disease. Relative advantages and practical applications of their relationships have been discussed (McRoberts *et al.*, 2003). However, realistic limitations ensuing from discrepancy of the associations across locations, stage of the epidemic, host genotype and crop cycle have been depicted (Agrios, 2005). Simple, consistent and useful relationships in different pathosystems had been developed (Cardoso *et al.*, 2004). As a result, tedious and time consuming work associated with severity measurement has been replaced by the easily measured incidence (Cardoso *et al.*, 2004; Uaciquete, 2013).

The objectives of this study were to (i) determine if there was a significant and consistent relationship between incidence and severity of chili anthracnose (*Colletotrichum* spp) in SNNP, Oromia and Amhara regions; (ii) determine whether severity could be predicted reliably from disease incidence data; and (iii) determine the effects of sampling for incidence on the precision of estimates of severity.

8.2. Material and Methods

8.2.1. Study Area

The study had been conducted in southern Ethiopia namely, Alaba (at 7.317574 latitude, 38.1042 longitude, with altitude of and 1825.77 masl), and Maraqo (at 8.024675 latitude, 38.32799 longitude, and with altitude of 2120.24 masl). The area is known for high prevalence of anthracnose disease. The plants were irrigated and cropping practices consisted of weeding and application of fungicides against anthracnose was carried out.

8.2.2. Assessment of Chili anthracnose incidence and severity in different locations

Two districts viz. Alaba and Maraqo in SNNP region, Ethiopia were selected for the survey. Assessments were carried out between June-August, 2013 and November- January, 2014 using 20 genotypes of the known pepper types in the country. These included Melka zala, Maraqo fana, Melka shote, Weldele, Bako local, Oda haro, Dube medium, Dube short and Gojeb local from Melkassa Agricultural Research Center (MARC). They were planted using a 70X30 inter and intra-raw spacing. They were replicated thrice in randomized block design. Two central plants were selected at random from each plot for the assessment and only the leaves of the top 2 whorls were assessed. They were visually classified on a scale of one to six, respectively for 0%, 0-20%, 21-40% 41 - 60%, 61 - 80% and 81 - 100% of the leaf area infected for computation of S. When there was complete defoliation of leaves, it was considered to be 100% infected (Siddiqui *et al.*, 2008).

To estimate “I”, the total number of leaves and the total number of diseased leaves in the 2 selected plants were recorded. Assessments were made in June-August, 2013 and November-January, 2014 about 2 weeks after, the rain had started. Disease incidence was calculated by dividing the number of infected leaves by the total number of leaves and expressed as a

percentage. For the estimation of S the sum of percentage area damaged by the pathogen was divided by the total number of leaves which included both infected and non-infected.

Spearman's Rank Correlation was employed to determine as to what extent the ratio between the index of resistance and susceptibility of different clones corresponded in different seasons and different locations In respect of each of the indices of incidence and severity. Two locations, Alaba where the incidence was generally low and Maraqo where the incidence was generally high were selected. Data obtained from these two locations in two seasons viz. June-August, 2013; which had low incidence and November-January, 2014 which had high incidence were considered for analysis. The selection of locations and seasons were made in order to have a better contrast of the computed ratio.

8.2.3. Relationship between Incidence and severity

For determination of the relationship between I and S of the disease, data was considered from 10 resistant clones and 10 susceptible clones of Melka zala, Maraqo fana, Melka shote, Weldele, Bako local, Oda haro, Dube medium, Dube short and Gojeb from each of the two locations, Alaba and Maraqo and in two different seasons, June-August, 2013 and November-January, 2014. The regression analysis was carried out by using the linear regression model S= a+bi where S=disease severity, I = disease incidence and a and b = regression parameters.

8.3. Results

8.3.1. The *I-S* Relationship

A highly significant relationship between incidence and severity of *Colletotrichum* spp was observed for all data sets at each location in each year. Despite the variation in severity at a given incidence, the relationship was fairly consistent among data sets. The model based on *log ln*-transformation of incidence and severity (equation : y=a+bx) performed consistently well on all data sets, explaining between 15.2 and 98% of the variation in severity on a *log ln* scale. The squared correlation between *S* and predicted *S* was between 0.5 and 0.92. As expected, severity was estimated more precisely at lower incidence values than at higher values, based on the width of the severity prediction interval. It should be noted that a significant relationship does not necessarily mean that precision is high enough (e.g., that the prediction interval is narrow enough) for a model to be used for predictions, since achieved significance level is highly influenced by number of observations.

Table 8.1. Regression Equations of Incidence (I) on severity(s) of anthracnose under different Environments and Different Chili Genotypes 2013-2014

linear function		**S = a+bi**					
Location	**Germplasm**	**Year**	**a**	**b**	**R^2**	**p-value**	**SE**
Maraqo	Melka zala	2013	1.1501	0.051	0.7571	0.05834	2.444
		2014	1.8769	0.1808	0.9703	0.0216	0.5697
	Maraqo fana	2013	7.0794	0.1397	0.4399	0.0279	2.311
		2014	1.3731	0.1047	0.8465	0.0754	0.6135
	Melka shote	2013	0.8941	0.0507	0.7782	0.2618	0.7071
		2014	0.1537	0.0131	0.9862	0.0122	0.0401
	Weldele	2013	0.6403	0.0799	0.8501	0.489	0.8578
		2014	1.8655	0.0781	0.8791	0.0534	0.7414
	Bako local	2013	2.0747	0.0549	0.7982	0.0376	0.7392
		2014	0.5779	0.0231	0.6407	0.1943	0.3857
	Oda Haro	2013	0.8796	0.0349	0.9127	0.0039	0.1742
		2014	1.3667	0.0952	0.8531	0.1205	0.7312
	Dube medium	2013	2.418	0.1056	0.7637	0.0498	0.9395
		2014	9.4386	0.3419	0.9218	0.0033	1.7988
	Gojeb local	2013	0.3527	0.0391	0.9352	0.2236	0.254
		2014	9.6733	0.153	0.8613	0.0009	1.3777
	Dube short	2013	-0.1293	0.1262	0.522	0.9687	3.143
		2014	1.8032	0.0192	0.8563	0.0003	0.2047
	Local LR	2013	11.5823	0.5563	0.9882	0.0007	1.5694
		2014	0.9392	0.0889	0.632	0.606	1.7114
Alaba	Melka zala	2013	0.3115	0.0901	0.9536	0.193	0.207
		2014	1.1815	0.435	0.9403	0.3441	1.1311
	Maraqo fana	2013	0.7648	0.0683	0.8791	0.118	0.4056
		2014	1.5966	0.3444	0.9909	0.0168	0.4531
	Melka shote	2013	6.6.61	0.2967	0.628	0.308	5.8314
		2014	5.9905	0.2585	0.8663	0.07167	2.6292
	Weldele	2013	-1.6292	0.2542	0.9257	0.4168	1.8414
		2014	0.0885	0.1608	0.9666	0.9123	0.7642
	Bako local	2013	-0.2317	0.0863	0.932	0.7258	0.6245
		2014	16.492	0.3509	0.9535	0.0005	2.0771
	Oda Haro	2013	1.5668	0.0561	0.8836	0.00269	0.2827
		2014	12.355	0.2579	0.8192	0.0007	1.6826
	Dube medium	2013	1.10278	0.0664	0.7794	0.0972	0.5413
		2014	0.6895	0.3036	0.8852	0.6978	0.6895
	Gojeb local	2013	0.9766	0.0675	0.9956	0.0003	0.1132
		2014	20.859	0.3353	0.7764	0.0060	4.5665
	Dube short	2013	1.535	0.02486	0.6622	0.0209	0.4621
		2014	0.3161	0.1982	0.9543	0.7906	1.1286
	Local LR	2013	0.1543	0.0664	0.6178	0.9135	1.351
		2014	0.2951	0.2132	0.8887	0.8857	1.9517
Overall mean			**3.0878**	**0.1567**	**0.836**	**0.244**	**3.088**
Alaba Mean			**3.391**	**0.1967**	**0.863**	**0.317**	**1.3958**
Maraqo Mean			**2.8005**	**0.1168**	**0.809**	**0.1664**	**1.006**

= Regression equation of incidence applied for each location: s=a+bi, SE= Standard Error of observation, R= coefficient of determination, b= incidence, a= constant.*multiply, For all locations p***<0.001

In this study, severity described the percentage of necroticised leaf area while incidence reflected the percentage of diseased leaves out of the total evaluated (Cardoso *et al.*, 2004; Alamdarloo and Aghajani 2015). Later in each crop season, anthracnose incidence on the

fruits was also assessed as percentage of symptomatic immature fruits/panicle/plant. Disease scores were initially processed to return plant mean scores (Uaciquete, 2013).

Regression analysis of incidence and severity from untransformed data were performed using SAS (2003) package for windows. Variables means over date and treatment were computed to fit a linear function Alamdarloo and Aghajani (2015). Incidence was the response variant and severity the explanatory (Cardoso *et al.*, 2004; Alamdarloo and Aghajani, 2015). Furthermore, leaf severity and incidence were used as explanatory to the incidence on pods. Daily rainfall data were obtained from the closest district directorate of agriculture of each site. Weekly sums were computed and graphically represented for each location.

The relationship between incidence and severity on the chili leaf anthracnose pathosystem as consistently best characterized by the restricted exponential function ($P<0.001$): $s = a+ix$ across locations, crop seasons, and chili genotype (Table 8.1). In this function, '*I*' stands for incidence, '*S*' for severity, *b* for intercept and *a* stands for constant.

8.3.2. The incidence-severity relationships across locations on chili anthracnose disease

A negative correlation between chili anthracnose disease incidence and severity with locations was observed. In 2013 at Alaba site, the relationship between disease incidence and severity could be expressed by the equation $Y= -0.762X + 30.606$ ($R^2 =0.0367$), where X = incidence and Y = disease severity (Figure 8.1a).

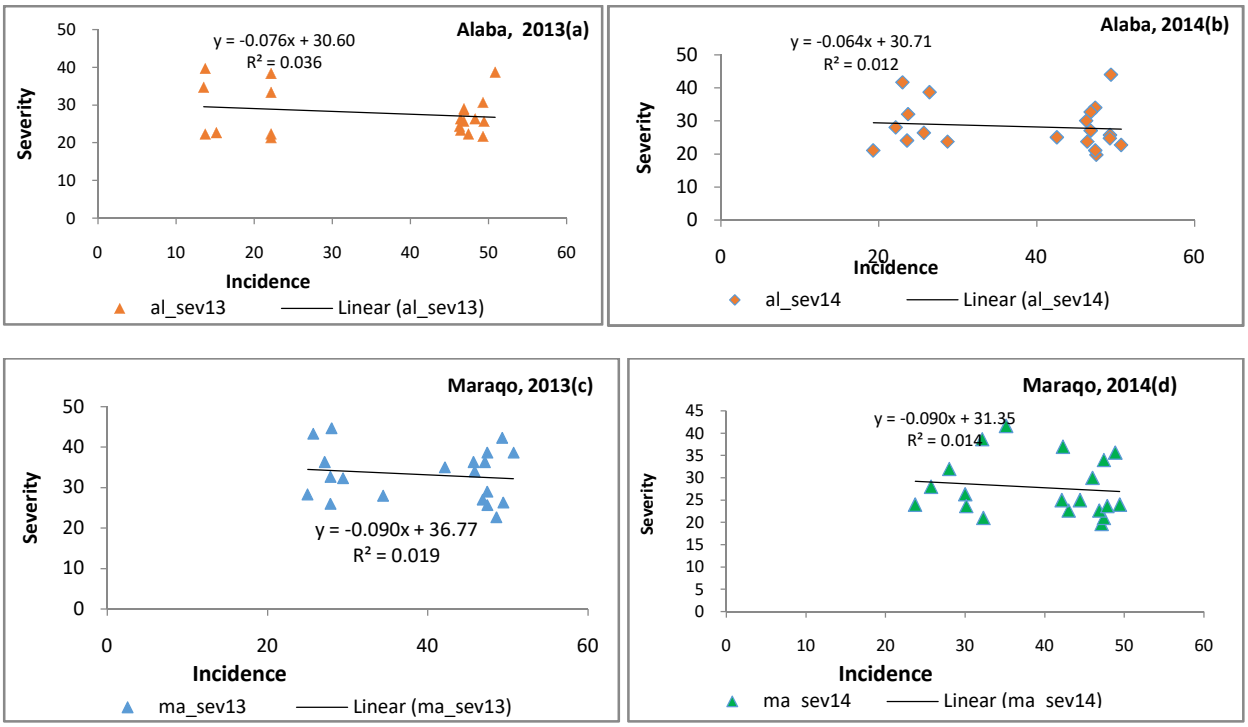

Figure 8.1. Relationship between severity and incidence of chili anthracnose (*Colletotrichum* spp) in 2013 (a & b) and 2014 (c & d) cropping seasons.

The R^2 value indicates that the contribution of locations was 3.67% on the incidence of chili anthracnose of chili. On the other hand, at Alaba in 2014, the relationship between disease severity and incidence could be expressed by the equation Y= -0.0641X + 30.714(R^2 =0.0124), where X = incidence and Y = disease severity. Here, the R value indicates that the contribution of incidence was 1.24% on the severity of chili anthracnose of chili (Figure 8.1b).

A negative correlation between chili anthracnose disease incidence and severity with locations was observed. In 2013 at Maraqo site, the relationship between disease incidence and severity could be expressed by the equation Y= -0.0906X + 36.777 (R^2 =0.0196), where X = incidence and Y = disease severity (Figure 8.2c).

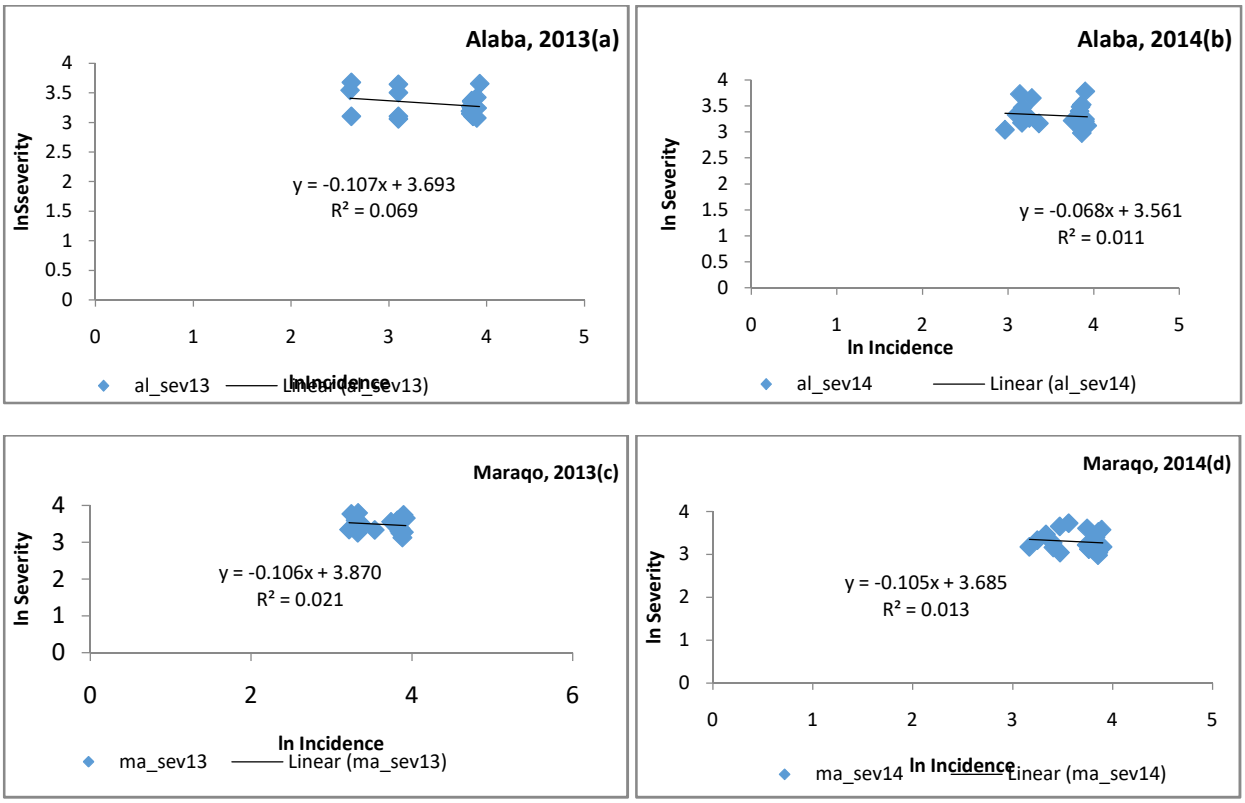

Figure 8.2. Relationship between severity (a and b) and incidence (c and d) of chili anthracnose (*Colletotrichum* spp) at Alaba and Maraqo with log (ln) transformation.

The R^2, value indicates that the contribution of locations was 6.92% on the incidence of chili anthracnose of chili. On the other hand, at Maraqo in 2014, the relationship between disease severity and incidence could be expressed by the equation Y= -0.0904X + 31.357(R^2 =0.0148), where X = incidence and Y = disease severity. Here, the R value indicates that the contribution of incidence was 1.3% on the severity of chili anthracnose of chili (Figure 8.2d).

8.3.3. *ln* incidence-severity relationships on chili anthracnose disease

A negative correlation between chili anthracnose disease ln incidence and ln severity with locations was observed. In 2013 at Alaba site, the relationship between disease ln incidence and ln severity could be expressed by the equation Y= -0.1075X + 3.6931 (R^2 =0.0692), where X = incidence and Y = disease ln severity (Figure 8.2a). The R^2 value indicates that the contribution of locations was 6.92% on the ln incidence of chili anthracnose of chili. On the other hand, at Alaba in 2014, the relationship between disease ln severity and incidence could be expressed by the equation Y= -0.0685X + 3.5612(R^2 =0.0111), where X = ln incidence and Y = disease *ln*

severity. Here, the R value indicates that the contribution of incidence was 1.11% on the severity of chili anthracnose of chili (Figure 8.2b).

Negative correlation between chili anthracnose disease ln incidence and ln severity with locations was observed. In 2013 at Maraqo site, the relationship between disease ln incidence and ln severity could be expressed by the equation Y= -0.1061X + 3.8709 (R^2 =0.0219), where X = incidence and Y = disease ln severity (Figure 8.2c).

The R^2 value indicates that the contribution of locations was 6.92% on the ln incidence of chili anthracnose of chili. On the other hand, at Maraqo in 2014, the relationship between disease ln severity and incidence could be expressed by the equation Y= -0.1055X + 3.6851(R^2 =0.013), where X = ln incidence and Y = disease ln severity. Here, the R value indicates that the contribution of incidence was 1.3% on the severity of chili anthracnose of chili (Figure 8.2d).

8.3.4. Temporal, pooled incidence-severity relationships on chili anthracnose disease

In 2013, a positive correlation between chili anthracnose disease incidence and severity with seasons was observed. The relationship between disease incidence and seasons could be expressed by the equation Y= 0.3142X + 3.374 (R^2 = 0.1546), where X = seasons and Y = disease incidence. Here, the R value indicates that the contribution of seasons was 15.46% on the incidence of chili anthracnose of chili (Figure 8.3).

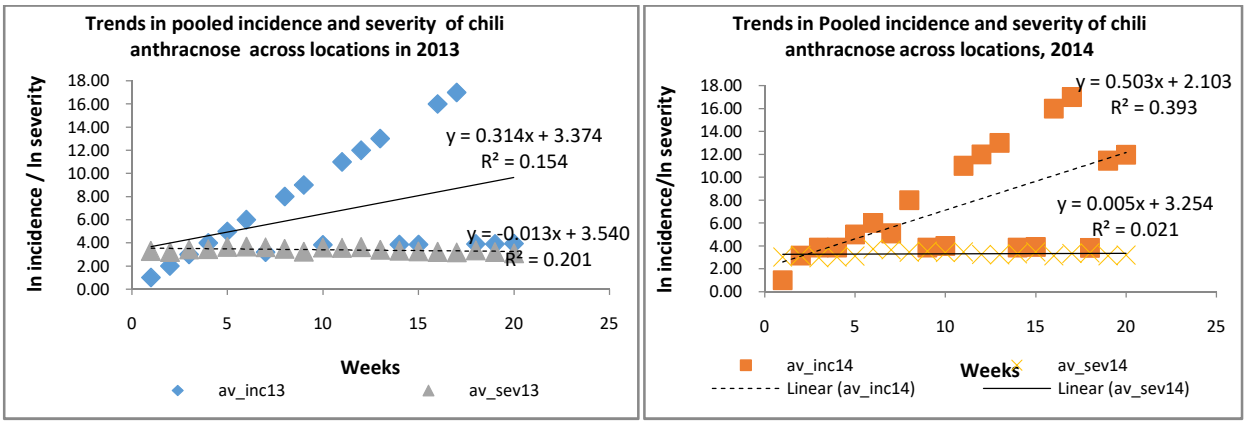

Figure 8.3. Trends in Pooled incidence (solid line) and severity (broken line) of chili anthracnose (*Colletotrichum* spp) across locations in 2013(left) and 2014(Right)

On the other hand, the relationship between disease severity and seasons could be expressed by the equation Y= 0.0139X + 3.5407 (R^2 =0.2016), where X = seasons and Y = disease severity. Here, the R^2 value indicates that the contribution of seasons was 20.16% on the severity of chili anthracnose of chili (Figure 8.3).In 2014, a positive correlation between chili anthracnose disease incidence and severity with seasons was observed. The relationship between disease incidence and seasons could be expressed by the equation Y= 0.5035X + 2.103 (R^2 = 0.3938), where X = seasons and Y = disease incidence. Here, the R value indicates that the contribution of seasons was 39.38% on the incidence of chili anthracnose of chili (Figure 3b). On the other hand, the relationship between disease severity and seasons could be expressed by the equation Y= 0.0015X + 3.2546 (R^2 =0.021), where X = seasons and Y = disease severity. Here, the R^2 value indicates that the contribution of seasons was 21.00% on the severity of chili anthracnose of chili (Figure 8.3b).

8.3.5. Spatial, pooled incidence-severity relationships on chili anthracnose disease

In Maraqo, a positive correlation between chili anthracnose disease severity with seasons was observed. The temporal, pooled relationship between disease locations could be expressed by the equation Y= 0.0293X + 3.0032 (R^2 = 0.9459), where X = seasons and Y = disease severity. Here, the R value indicates that the contribution of seasons was 94.59% on the severity of chili anthracnose of chili (Figure 8.4a). On the other hand, in Alaba, the relationship between disease severity and seasons could be expressed by the equation Y= 0.0282X + 3.0955 (R^2 =0.944), where X = location and Y = disease severity. Here, the R^2 value indicates that the contribution of seasons was 94.44% on the severity of chili anthracnose of chili (Figure 8.4a).

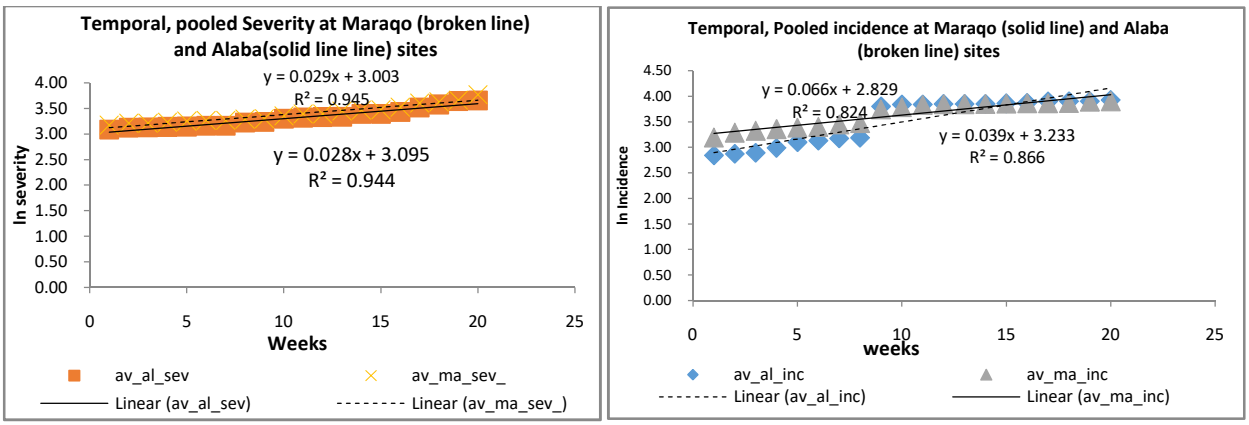

Figure 8.4. Temporal, pooled severity (left) and incidence (right) of chili anthracnose (*Colletotrichum* spp) in Maraqo and Alaba Sites

Furthermore a positive correlation between chili anthracnose disease incidences with seasons was observed. The relationship between disease incidence and seasons could be expressed by the equation Y= 0.0665X + 2.8291 (R^2 = 0.8246), where X = seasons and Y = disease incidence. Here, the R value indicates that the contribution of seasons was 82.46% on the incidence of chili anthracnose of chili (Figure 8.4b). On the other hand, the relationship between disease severity and locations could be expressed by the equation Y= 0.0396X + 3.233 (R^2 = 0.866), where X = location and Y = disease incidence. Here, the R^2 value indicates that the contribution of seasons was 86.6% on the incidence of chili anthracnose of chili (Figure 8.4b).

8.4. Discussion

In this study, the relationship between incidence and severity on chili leaf anthracnose non-transformed data, best fitted the restricted exponential group model. This model curve was previously used by Cardoso *et al.* (2004) on two different pathosystems (McRoberts *et al.,* 2003). Numerous publications have dealt with the incidence-severity relationship of various pathosystems (Cardoso *et al.,* 2004). Various models have been produced and their application and limitations were reviewed (Cardoso *et al.,* 2004; McRoberts *et al.,* 2003). Limitations associated with practical use of incidence-severity relationships are essentially derived from their inconsistency in relations to location, season, epidemic stage, crop management systems and host genotype variations (Cardoso *et al.,* 2004). Once the model has proven robust across all these, one may opt to use the easily measured parameter (incidence) Cardoso *et al.* (2004). Therefore we recommend the use of leaf incidence in place of severity in genotype and season trials, describing models for economic thresholds or epidemics studies of chili leaf anthracnose. However, caution is needed since the chili leaf anthracnose severity or incidence link to the fruit anthracnose incidence/severity has not been established. This is in conformity with previous finding in Brazil where severity of anthracnose was coupled with rainfall and flushing of chili (Cardoso *et al.,* 2000; 2004)

In the model, severity was considered as dependent variable and incidence as the independent in contrary to Uaciquete (2013). Since anthracnose is a polycyclic disease (Agrios, 2005). Changes in incidence over time are determined by the dynamics of severity or sources of inoculum at initial stages of epidemics (Cardoso *et al.,* 2004). By exploring the regression curve minimum and maximum limits derived from the incidence-severity relationship, the propensity of the

environment for the disease epidemics across different sites, crop seasons, genotype combinations and production system had been assessed by Uaciquete (2013). Anthracnose spread was clearly associated with rainfall during the first week of July. In general, this coincided with the flushing peak for most clones involved in the trials. This in agreement with knowledge that dispersion of anthracnose inoculum is by rain splashes (Freire *et al.*, 2002).

The relationships between pairs of incidence and severity are mathematically expressed and consistent at multiple locations or environments, data from individual sites can be pooled into a summary equation without prejudice to proper interpretation (Cardoso *et al*., 2004). The overall means for essential coefficients such as transformed '*S*' and '*I*' were used to generate the summary equation that explained the relationships between anthracnose incidence and severity across different environments (Uaciquete, 2013). To add up, the data indicated that very low levels of severity are associated with increased infection, which is evident in the works of Uaciquete (2013).

In this model, both incidence and severity were found to increase in time. When the incidence approaches a maximum of 98%, the severity is around 37%. Then, only severity continues to increase up to a maximum of 45%. This pattern of post-maximum incidence increase has been discussed by Cardoso *et al.* (2004). The spread of the disease may be limited because severely infected senescent leaves tend to drop off and the un-infected ones (30%) may reach maturity inhibiting fungal penetration.

The result of this study was adopted the scale developed by Alamdarloo and Aghajani (2015) that had initially been used for scelerotina leaf rot. In previous studies, chili leaf anthracnose was assessed based on whole plant scores (Cardoso *et al.*, 2004) without standardized pictorial diagrams thus making it difficult to use by other workers. The use of diagrammatic scale decreased the absolute error of disease estimations by raters in the case of chili leaf and fruit blight patho-system (Menge, 2013a; b).

All chili genotypes, including the local varieties, grown extensively towards the end of the rainy season and reproductively when the temperature declines which was in direct conformity with

the findings of Alamdarloo and Aghajani (2015). Seedlings and young tend to grow continuously Alamdarloo and Aghajani (2015). Thus when the environment is favorable, two peaks of the disease epidemic may be observed in a year (Cardoso *et al.*, 2000; 2004). This study was annual-crop, young-leaf-based and had the advantage of being able to estimate the epidemics accurately. The authors cannot guarantee that if this method, whatsoever, be applied in all other crops with two flushes per year.

Estimation of chili anthracnose damage through its incidence on young leaves has proven to be a more effective, faster, more accurate and user friendly method than severity scores. This is in line with Alamdarloo and Aghajani (2015) who found incidence data to be simpler to collect and less subjective than severity and thus recommended for larger scale surveys.

However, special attention may be necessary when assessing chili anthracnose where other similar but distinguishable leaf diseases such as leaf blight (Sijaona, 2001; Sijaona *et al*., 2005) and Pestalotiopsis (Uaciquete, 2013) are present. Furthermore, a recent review (Lewis and Ward, 2013; Lopez, and Lucas, 2010; Estrada *et al.*, 2000; Wiik and Ewaldz, 2009), indicates that epidemiological data analysis could be improved through multivariate regression modeling.

8.5. Conclusion and Recommendations

The results of the current study establish that by simply sampling and counting the number of diseased plants in a chili farm, it is possible to estimate anthracnose severity. An acceptable model for estimating levels of anthracnose severity based upon data from different levels of disease pressure, different chili genotypes, different season, and locations at several stages of epidemic progress was obtained. Further studies on gummosis damage to obtain models to describe economic thresholds, host genetic reactions and effectiveness of disease management practices will be facilitated by the findings presented this study.

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CHAPTER NINE:

EFFECTS OF TIMING AND FREQUENCY OF FUNGICIDE APPLICATIONS ON CHILI ANTHRACNOSE (Colletotrichum capsici (Syd.)) IN SNNP REGION, ETHIOPIA

Abstract

Chili anthracnose is posing a severe menace to chili production in southern Ethiopia. Study on fortnightly treatments of the fungicide Ridomil starting at different chili phenological stages had been carried out so as to devise a strategy for appropriate frequency and timing for spraying against anthracnose diseases on chili. Maraqo Fana and Oda Haro varieties were treated with four different timings and frequency of ridomil application, viz., flushing, flowering, fruiting and control.. The treatments were replicated four times in which Completely Randomized Block Design was employed. Data on incidences of anthracnose on leaves severity on fruits were collected. The financial impact and profitability of frequent spraying was computed. The economic implication of high frequency application of Ridomil in the current context of fungicide use on chili in Ethiopia was evaluated. A maximum of 3-7 applications starting from flushing successfully prevented the disease development and significantly reduced the incidence of leaf anthracnose. The best economic profit was obtained with 7 applications of Ridomil for chili with potential yield of at least 29 kilograms per season per plot. In light of this research, small scale chili farmers will be able to lessen pesticide treadmill thereby escalating ecological safety and profitability.

Key Terms: Chili Anthracnose, Financial Impacts, Frequency, Fungicide, Ridomil

9.1. Introduction

Chili (*Capsicum frutescence* L.) is a crop of increasing importance in the economies of sub-Saharan Africa (Nsabiyera *et al.,* 2012). However, what is being produced has been below the potential that this area is capable of producing (Nsabiyera *et al.,* 2012; Lemma *et al.,* 2008). For instance, over the past many years, hot chili production in Ethiopia has stagnated at 0.4 tonnes per ha, with yields remaining lower than the average global production of 28 metric tones (Lemma *et al.,* 2008). Moreover, the quality of the produce realized does not meet the stringent standards of the international markets, where most countries face fierce competition from major producing countries such as India and China (Thampi, 2003). The poor quality of the produce is largely attributed to biotic and abiotic stresses in the field and the poor quality cultivars grown by farmers (Tusiime *et al.,* 2010). Particularly, attack by different pest infestations or infections can cause significant losses in chili production (Ochoa-Alejo and Ramirez-Malagon, 2001). The most common diseases of most chili peppers are phytopathogenic fungi, bacteria, and viruses. By and large, from 50% (Pakdeevaraporn *et al.,* 2005) to 100% loss had been was observed due to chili anthracnose (Melanie and Sally, 2004); causing severe defoliation of plants, resulting in reduced yield and loss of quality of harvested fruit when severe damage occurs on enlarging fruits (Melanie and Sally, 2004).

Control and/or prevention of these diseases and their vector populations are usually through use of chemical sprays on diseased plants and use of various cultural practices. In developing countries such as Uganda and Ethiopia, farmers largely use pesticides for disease and pest control on hot chili (Karungi *et al.,* 2010). Ever-increasing loads of disease management via chemicals accompanied by rising cost make pesticides non-affordable for small scale farmers (Lemma *et al.,* 2008). Application of pesticides arbitrarily over time has also resulted in a buildup of resistance among target pests and pathogens (Flint, 1999). Pests and diseases are best managed using host resistance, which is a cheap option for farmers (Duveiller *et al.,* 2007).

However, in cases where disease-resistant cultivars are not available, fungicide control would be the best control strategy. In some countries like Brazil, fungicides such as copper oxychloride, copper hydroxide, zinc + manganese, carbamate, captafol, benomyl, dithianon, anilazine, bitertanol, tridimenol and triforine, are used to control the disease (Freire *et al.,* 2002). However,

the choice of fungicides depends on various factors such as biological efficiency, crop sensitivity (Uaciquete, 2013), economic feasibility (Freire *et al.,* 2002), environmental aspects (Sijaona, *et al.,* 2001), previous exposure to a certain fungicide or group (Siddiqui *et al.,* 2001) and local regulations for registration (Arauz, 2000). In general, research information on the frequency of fungicides application is very limited in Ethiopia. Thus this paper was initiated with the aim to determine the efficacy of various fungicides rate and frequency against chili anthracnose in south western regions of the country.

Typically copper oxychloride is used to control chili anthracnose in many countries. Other fungicides such as 5% trifloxystrobin WG, 25% difeconazole EC and 25% azoxystrobin EC were proven also to be effective (Uaciquete, 2013). However, these fungicides have not been widely adopted due to similar constraints as described in Brazil, i.e. extensive area planted but low productivity and the high cost of these fungicides (Freire *et al.,* 2002). In Ethiopia, the cost is increased because other and timely asynchronized series of fungicide applications are required for anthracnose control.

The incidence of anthracnose is higher in the southern areas as compared to the other, surveyed parts of Ethiopia. The recommended frequency of fungicide application was as often as three times though the farmers seldom trail it. Copper fungicides are very old, have a residual build up in soils and were found to be detrimental to avocado phylloplane microorganisms with natural suppressive effect on *Colletotrichum spp*) (Freire *et al.,* 2002; Uaciquete, 2013; Sijaona, *et al.,* 2001). Alternatively, Ridomil is widely used in chili control but it has shown no significant reduction on anthracnose severity. The current study was, thus, conducted to determine the effectiveness of Ridomil against chili anthracnose through changes in timing and frequency of applications at different stages.

9.2. Material and Methods

9.2.1. Location, Plants and Experimental Design

The present experiment was conducted during 2013 and 2014 cropping seasons in a FTC farm in Shashogo District, which is located at 8.042378 latitudes, 38.49944 longitudes, and with altitude of 1840.08**.** The farm is located just about 24 km avert from Addis-Hossana road which is about 200 kms in from the capital. The experiments were carried out for two consecutive seasons

starting from May/June till December each year. The sites were chosen on the basis of safety, disease confinement and focusing on absence of interlocking branches with adjacent shades.

Two factor factorial experiments in randomized complete block design (RCBD) were adopted with 4 replications. Factor A was the genotypes (A1= Maraqo fana and A2= Oda Haro) obtained from Melkassa Agricultural Research Center). Factor B was spray times at which Ridomil were initiated. The first treatment aimed at protecting the plants from the true leaf stage onwards (B1); the second treatment was aimed at protecting the plant from flowering production stage onwards (B2) and the third, from fruiting(B3) and then a fourth treatment that consisted of un-sprayed served as control (B4) (Table 9.1). Experimental plot consisted of five plants and the plot was separated from another by at least one row in each direction. The applied dose was 0.5 ml of active ingredient per . Experimental site measured was measure as 674.4m^2 [length: 1.4m x 10m x 8 = 112.4 m and the width: (0.3mx5) + (1m+4) = 6m].

9.2.2. Spraying procedure

Spraying was carried out fortnightly using a hand held knapsack mist blower operated by a trained person. The rate of Ridomil application was determined based on the recommendations of Uaciquete (2013). Detailed procedures such as speed of the operator, wind challenge and rain coverage had been avoided for uniformity (Uaciquete, 2013; Sijaona, *et al.*, 2001).

9.2.3. Data collection

Both anthracnose leaf incidence and severity on flowers were recorded fortnightly basis (Table 2) in purposively selected ten young leaves per plot. A scale (0-6) developed by Siddiqui *et al*. 2008) for coverage on leaf surface were adopted. The area covered in the scale corresponds well with the necroticised area in the case of anthracnose infection and designated as Healthy (0); 1-5% of mature leaves with necrotic and chlorotic (*Colletotrichum* spp.) symptoms (1); 6–15% of mature leaves with necrotic and chlorotic symptoms (2); 16–50% of young shoots and stem with water soaked lesions and minor shoot die back (3); 51–95%water-soaked lesions with abundant mycelia growth and fructification, and extensive shoot dieback (4); Dead plant (5) (Siddiqui *et al*. (2008). Three to four observations were made in order to cover the peak of disease intensity as observed from the untreated.

9.2.4. Statistical analysis

Data on disease incidence and severity (D-S) were transformed using the square root function (S=√ (X+1) so as to normalize variability. The transformed data were subjected to analysis of variance using GenStat computer package (GenStat 9.2) to examine variations among frequencies. For both anthracnose, individual plant score means for shoots and fruits were annually calculated in the field forms as detailed before (Sijaona, *et al.,* 2001). Then, plant means, over the years, were used to run the statistical analysis for both anthracnose incidence and severity. Analysis of variance (ANOVA) was used to determine differences between the treatment effects on means. The data was acceptably normally distributed with heterogeneous treatment variances. Means were therefore separated using Fisher's protected t-test least significant difference (LSD) at 5% level of significance (Petersen, 1994; Gomez and Gomez, 1984).

9.3. Results

9.3.1. Effect of Frequency of Ridomil Application on Anthracnose Incidence and Severity on different plant parts

The finding of this study indicated that fungicide applications aimed at protecting emerging seedlings from flushing stage, in which total number of 7 sprays were made, had shown minimum incidence and severity of anthracnose on fruits of Maraqo Fana variety, with 2.5 and 2.53; and 2.17 and 1.83 in 2013 and 2014 seasons; and respectively (Table 9.2). On Oda haro variety, the minimum incidence and severity of anthracnose on fruits was 2.43 and 1.37; and 1.8 and 3.37 in 2013 and 2014, respectively. Overall, the severity of anthracnose on leaves of Maraqo fana was lower than Oda haro variety in Ridomil treated plots in 2013. On the contrary, the percent severity on fruits was higher in Oda haro than Maraqo fana variety in untreated check the same season. The severity on leaves and fruits had been lower in variety Maraqo fana than Oda Haro in Ridomil treated plots in 2014. Regardless of when the sprayings started, severity mean scores for two consecutive seasons were significantly lower on Ridomil treated compared to untreated controls (Table 9.2). Furthermore, the percent incidence and severity on parts of chili plant had been compared. In fact, no symptoms developed on flowers in all plots. However, high disease incidence were observed on leaves than fruits in 2013 season while percent severity was higher on fruits than leaves in 2014 (Table 9.2).

Table 9.1. Date of Application of Ridomil per treatment, during 2013 and 2014 pepper crop, Shashogo District, SNNP

Date of application (2013)	Treatments at:	16-Jun	23-Jun	30-Jun	7-Jul	18-Jul	25-Jul	1-Aug	8-Aug	15-Aug	23-Aug	23-Aug	29-Aug	7-Sep	15-Sep
Maraqo fana & Oda haro	Flushing	*	-	*	-	*	-	*	-	*	-	*	-	*	-
	Flowering	-	-	-	*	-	*	-	*	-	*	-	*	-	*
	Fruiting	-	-	-	-	-	-	-	-	-	-	-	*	*	*
	Untreated	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Date of application (2014)	Treatments at:	10-Jun	17-Jun	25-Jun	2-Jul	9-Jul	16-Jul	23-Aug	30-Aug	1-Aug	23-Aug	30-Aug	8-Aug	16-Sep	1-Sep
Maraqo fana & Oda haro	Flushing	-	*	-	*	-	*	-	*	-	*	-	*	-	*
	Flowering	-	-	-	-	*	-	*	-	*	-	*	-	*	-
	Fruiting	-	-	-	-	-	-	-	-	-	-		*	*	*
	Untreated	-	-	-	-	-	-	-	-	-	-	-	-	-	-

*= Application of Ridomil, - = No application

9.3.2. Effect of Frequency of Ridomil on Incidence and severity of chili anthracnose

As indicated in Table 9.2, there is a significant variation in incidence and severity of chili anthracnose among genotypes and frequency of applications across seasons. In 2013 cropping season, leaf severity and incidence of anthracnose on both Maraqo Fana and Oda Haro genotypes ranged between 1.23 and 3.83 and 1.67 and 4.31 percents, respectively; whereas fruit severity and incidence of anthracnose on Maraqo Fana and Oda Haro genotypes ranged between 1.23 and 3.83; and 1.67 and 4.31 percents. In 2014 cropping season, leaf severity and incidence of anthracnose on both Maraqo Fana and Oda Haro genotypes ranged between 3.2 and 10.63; and 5.63 and 8.93 percents, respectively; whereas fruit severity and incidence of anthracnose on Maraqo Fana and Oda Haro genotypes ranged between 1.83 and 12.93; and 1.8 and 8.43 percents, respectively. In each season, variety and plant parts, the maximum incidence and severity were observed on untreated control (no application at all) while the minimum incidence and severity of anthracnose on leaves and fruits was observed when application is started at flushing.

Table 9.2. Effect of Fortnightly application of Ridomil for the control of anthracnose during 2013 and 2014 cropping season in Shashogo, Ethiopia

Genotype	First Application at:	2013				2014			
		Severity (%) on:		Incidence (%) on:		Severity (%) on:		Incidence (%) on:	
		Leaves	Fruits	Leaves	Fruits	Leaves	Fruits	Leaves	Fruits
Maraqo Fana	Flushing	1.23a*	2.17a	2.3a	2.50a	3.20a	1.83a	5.97a	2.53b
	Flowering	1.3a	1.43a	2.5a	2.33ab	9.63d	3.77b	5.90a	3.67c
	Fruiting	1.3a	1.53a	1.67a	2.33ab	10.63e	5.87c	6.00a	6.00c
	control	2.9b	2.90ab	5.35d	5.43d	12.90f	8.90d	8.70c	9.63f
Oda Haro	Flushing	1.57a	1.37a	2.57ab	2.43b	6.27c	3.37b	5.83a	1.80a
	Flowering	1.53a	1.80a	2.3a	2.60b	5.00b	9.80d	5.63a	2.30b
	Fruiting	1.57a	1.47a	2.23a	1.93a	6.00c	9.47d	8.93c	6.93d
	control	3.83c	4.27c	4.31cd	5.10c	10.63e	12.93e	7.63b	8.43e
	CV (%)	16.75	11.5	13.6	15.2	14.1	17.6	16.6	14.77
	LSD	1.22	0.96	1.37	0.53	0.85	1.11	0.78	0.55

*Values with same letter are not significantly different (P<0.05)

9.3.3. Effect of Frequency of Ridomil on chili Yield

As indicated in Table 9.3, there is a variation among frequency of applications in yield/plot/season during 2013 cropping season. At poor performance-per-plot scenario, the minimum and maximum yield/plot/season was observed in untreated control (zero application) and flushing (7 times application) with 0.25 and 10, respectively. At average performance-per-plot scenario, the minimum and maximum yield/plot/season was observed in untreated control (zero application) and flushing (7 times application) with 1.00 and 19.5, respectively. At best performance-per-plot scenario, the minimum and maximum yield/plot/season was observed in untreated control (zero application) and flushing (7 times application) with 1.5 and 29, respectively (Table 9.3). Equivalent findings had been obtained in 2014 cropping season at poor, average and best yield-per-plot scenarios with a slight variations in price of the product (Table 9.4).

9.4. Discussion

In this trial, leaf and fruit anthracnose spread (incidence) on chili young shoots at Shashogo farm during two crop seasons, was significantly reduced by four to seven applications of Ridomil starting at flowering as compared to untreated. The same fungicide application plan was also highly effective against severity. Simultaneous management of anthracnose on mango has been reported in a 25 times application plan of fungicides per season (Sijaona *et al.,* 2001; Arauz, 2000). When to initiate the spraying is a critical aspect. In Brazil, using a 0 to 4% leaf area damage scale and chemical action is recommended when 2% of the assessed leaf area has developed necrosis (Uaciquete, 2013; Cardoso *et al.,* 2004). Adopting an action point of two percent disease severity implies starting applications at flowering. In the current study, this notion coincided with the most profitable timing of applications. Therefore, confirming the findings of Cardoso *et al.* (2004). In many countries including Ethiopia, disease surveillance is not common, due to logistics, high rate of disease spread (Karungi *et al.*, 2010; Topper *et al.*, 1998) endemic nature of the disease (Sijaona *et al.,* 2001), phenologically heterogeneous farms (Freire, 2002) and time and labor consuming for scouting procedure. The study also showed that spraying service providers instead, start sprayings following the plant phenology (flowering phase) rather than scouting individual plants. However the use of a measurable action point avoids un-necessary sprays with all ecological concerns and development of resistance by the pathogen as referred by Cardoso *et al.* (2004).

9.5. Conclusion and Recommendation

This study showed that effective anthracnose diseases management were achieved by spraying Ridomil fungicide seven times, starting at flushing phenological phase of the crop and therefore the fungicide application plan is highly recommended.

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CHAPTER TEN:

INTEGRATED DISEASE MANAGEMENT OF ANTHRACNOSE (*Colletotrichum capsici* (Syd)): IMPLICATIONS TO DISEASE REACTIONS, QUALITY AND GROWTH PARAMETERS OF THREE GENOTYPES OF CHILI

Abstract

Anthracnose (*Colletotrichum* spp) is one of the most important diseases that decimate chili production in Ethiopia. The efficacy of three Trichoderma isolates viz., AAU-37, AAU-Th and AAU-69 with aqueous leaf extracts Onion, Garlic, Neem and *Cassia* spp were harmoniously applied on Oda Haro, Mareko Fana and Melka zala pepper varieties, with the aim to examine the influence of IDM of anthracnose on disease reactions, quality and growth parameters of chili. The treatments were arranged in RCBD replicated thrice. Data on disease reaction, quality and growth characteristics of chili had been collected. Analysis of data was carried out using ANOVA. The lowest plant infection (12.8%), leaf infection per plant (15.2%), percent diseased leaf area(15.2%)and infected fruits per plot (17.4%) was observed on combined application of isolates *Trichoderma* spp, plant extracts and Ridomil in Maraqo fana variety. Regarding the growth parameters, viz. the highest Mean Percent establishment (81.67), mean days to 50% flowering (65.33), mean days to 50% maturity (82) days to first harvest (106.3) in was observed in T16, T16, T4, and T8, respectively. From the quality parameters, the highest mean number of branches per stem (9), mean canopy diameter (24.8), mean number of flowers per plant (9.6) and mean plant height (61.4) in T10, T15, T6 and T7, respectively. Both negative and positive control showed higher incidence and severity as compared to single and combined application of isolates *Trichoderma* spp, plant extracts and Ridomil.Therefore, integrated use of *Trichoderma* spp and plant extracts can be recommended. Conventional fungicides will be replaced by antagonists and botanicals. This will improve crop quality and growth; maximize profitability of chili and ultimate sustenance.

Key Terms: Anthracnose, infection, Quality, Growth Parameters, Integrated Disease Management, Plant Extracts

10.1. Introduction

Chili (*Capsicum frutscenes L.*) is a highly profitable cash crop popular among farmers and their markets (Amusa *et al.,* 2004), but its production poses significant risks. Peppers continue to grow under substantial pressure from pests and diseases. It is widely consumed in home, fresh seasoning, and as a cooking ingredient. Farmers mainly produce their crop for commercial marketing. Ethiopia's share in the world, however, is insignificant (5%) compared to India (36%) and China (11%) with a production of 1.25, 0.39 and 0.17 million tones ((Faisal and Mohammad, 2011). The decline of hot pepper production (0.4 tones fruit yield/ha) is attributed to the prevalence of fungus among others (Amusa *et al.,* 2004; Fekadu and Dandena, 2006). The reason for decline of hot pepper production is attributed to poor varieties, poor cultural practices, the prevalence of fungal (blights) and bacterial as well as viral diseases (Fekadu and Dandena, 2006). It is a serious threat to crop productivity during rainy weather (Amusa *et al.,* 2004). This can be doubled or tripled through in the absence of appropriate disease management coupled with good agricultural practices. According to Melaku *et al.*(2014), the present situation indicates that in the study area there is no improved hot pepper varieties but there is one local variety named "*Mita Mito*" by local growers, the green pod yield (3 ton per hectare) of this local variety is very low compared to national average yield. As a result, information for the improvement of the crop for high fruit yield and quality in the existing agro-ecology is insufficient (Haileslassie, *et al.,* 2015). There has also been no research on evaluation of hot pepper which enables the growers to select the best performing varieties in the study area (Melaku *et al.,* 2014). Conversely, the dose and frequency of fungicides being applied to control the disease is costing too much for the small scale farmers often without significant benefit.

The effectiveness of fungicides against chili anthracnose was lower or comparable to antagonists and botanicals (Deeksha *el al.,* 2002; Ekbot, 2002; Khoda *et al.,* 2003; Rahman *et al.* 2004). Even though botanicals are safe, cheap and obtainable (Serawit and Tesfaye, 2014), their application is far below it was supposed to be. However, limited efforts have been made to screen plants that are suspected to possess antimicrobial properties for effect against *C. capsici*. Higher plants may contain secondary compounds that could effectively control plant diseases (Nduagu and Nwankiti, 2008). Yet, options have been identified to address these challenges. Conventional synthetic fungicides need to be replaced by bio-fungicides as the former lost their effectiveness

due to pesticide treadmill (Rashid *et al*. 2015; Fekadu and Tesfaye, 2013). Environment friendly control tactics gained impetus due to growing socio-economic concerns. In pursuit of finding replacement of toxic pesticides scientists working in these lines started trying botanical extracts and such other substitutes. In the past decades, therefore, quite limited number of published scientific information became available on the problem under investigation. This research was, therefore, initiated with objective of managing chili anthracnose through integrated use of antagonists and plant extracts under field conditions.

10.2. Materials and Methods

10.2.1. Experimental site

The experiment was conducted at the farmers' training center (FTC) site in Alaba which is one of the most important pepper growing locations that have altitude of 1680 above sea level, is characterized by dry sub humid climate during June, 2013. Alaba has monthly mean minimum and maximum air temperature of 15°C and 29.5°C, respectively, and rain fall of 900-1300mm/year. This location is a hot spot area for anthracnose (*Colletotrichum* spp) (Belete *et al.*, 2012; Tameru *et al*., 2003; Simon *et al*., 2009).

10.2.2. Treatments

A total of 16 treatments, viz., Untreated (T0), combination of all (Extracts + *Trichoderma* spp.+ Ridom)(T1) Neem (T2), *Cassia* spp (T3), Onion (T4), Garlic (T5), Neem + Onion+ Cassia spp (T6), Neem + Onion + Garlic (T7), Ridomil (T8), AAU-Th (T9), AAU-37 (T10), AAU-69(11), AAU-Th + Garlic (T12), AAU-69 + AAU-Th + Onion(T13), AAU-37 + AAU-Th +Neem (T14), AAU-69 + AAU-37 +Neem(T15), and AAU-69 + AAU-37 + AAU-Th (16) had been used in this experiment.

10.2.3. The Fungal Pathogen

Sweet pepper fruits with anthracnose lesions were collected from farmers' fields in main chili growing areas in September 2012. Sections of 3-5 mm were cut from the margin of the infected lesions and sterilized for one minute in 1.0% sodium hypochlorite solution and rinsed in three changes of sterile distilled water (SDW). The sterile pieces were blotted dry using sterile filter papers and placed on Potato Dextrose Agar (PDA) in 9cm Petri dishes. The dishes were incubated at ambient conditions of light and temperature (30 ± 2°C) for 7 days after which cultures with salmon-pink sporulation typical of *Colletotrichum* spp were sub-cultured to obtain

pure cultures (Nduagu and Nwankiti, 2008). Culture identification was confirmed by microscopes and comparison with reference cultures. From these, virulent isolate of *C. capsici*, A38, was obtained from the Laboratory of the Department of Microbial, and Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia. The fungal pathogen was maintained on potato dextrose agar (PDA) slants at 4^{O} C (Fekadu and Tesfaye, 2013).

10.2.4. Preparation of *Trichoderma* spp isolates for the Experiment

Three isolates of *Trichoderma* spp with high biocontrol efficacies were cultured in Potato dextrose broth (PDB) and cells were collected by centrifugation at 3,000 rpm for 20 min. *Trichoderma* cells were washed twice with sterile distilled water and re-suspended.

10.2.5. Layout and Design of the Experiment

The experiment was laid in Randomized Complete Block Design (RCBD) and divided into three blocks and each block was divided into 17 plots. The plot size was 5m x 1.2m. Treatment was assigned to each block at random. The space between the blocks and between the plots was 1.00 m and 0.50 m, respectively. The first spray was given on the 21^{st} days after transplanting (DAT) as leaf spots could be located the day before. All the treatments (T0-T17) were administered as foliar spray. Plant extracts were formulated at 1:10 suspensions, T6 and T7 as 1:10 suspension cocktail (Serawit and Tesfaye, 2014). Ridomil (T8) was administered @ 0.2% suspension. Usually in the evening calm weather light spraying was done so that neighboring plots cannot share a wrong treatment.

10.2.6. Data collection

Number of infected plants per plot, Number of infected leaves per plant, number of spots per leaf, Percent diseased leaf area (DLA %), were data collected on disease reactions. Data collected on Growth Parameters included Plant height, days to 50% flowering, number of flowers per plant, Days to first harvest, Canopy diameter (cm), Number of branches per stem and Dry weight content per plant. Data collected on Quality factors were fruit pericarp thickness (mm), Fruit dry weight content (g), fruit length (cm), and fruit diameter (cm).

10.2.7. Analysis of data

The data were statistically analyzed with the help of Analysis of variance (ANOVA). To compare the means, Fisher's Least significance Difference (FLSD) was used to compare the effect of the treatment at $p<0.05$ (Obi, 2002).

10.3. Results

The obtained data on different parameters are presented in the Tables 10.1, 10.2, and 10.3. The effect differences of the treatments significantly varied from one another and gave a clear picture about the effects on disease reaction, quality and growth factors.

10.3.1. Effect of IDM on Disease reaction

10.3.1.1. Plant Infection

In this study, infection by the anthracnose of chili disease was significantly reduced compared to the control treatment. In all the observed varieties the control (T0) plots had the highest percent plant infection. The highest percentage of plants showing anthracnose of chili symptoms on Maraqo Fana was 85.33 in T0 (Control) and it was the lowest (12.80) in combinations of *Trichoderma* spp isolates, plant extracts and Ridomil (T1). Though numerically different, percent plant infection in T16 (AAU-69+AAU-37+AAU-Th) was in a statistically similar level of significance with T1 (combination of *Trichoderma* spp isolates, plant extracts and Ridomil). On Oda Haro variety, the highest percent plant infection was found in To (control) which was 89.6 and the lowest percent plant infection was found in T1 (*Trichoderma* spp isolates, plant extracts and Ridomil) which was 15.33. On Bako local variety, the highest (85.2) and the lowest (19.6) percent plant infection was observed in T0(control) and T1(*Trichoderma* isolates, plant extracts

and Ridomil). The AAU-Th and AAU-37 *Trichoderma* spp isolates (T9 and T10) in the same level of significance with 22.6 and 23.6, respectively. Overall, percent plant infection was found to be higher in aqueous plant extracts than isolates of *Trichoderma* spp. The infection in *Trichoderma* spp treated plots, on the other hand, was significantly lower than Ridomil (T8) treatment.

Table 10.1. Percent plant and Leaf infection per plant due to at Anthracnose of chili at different days after transplanting as in influenced by some management practices

Treat-Ments	Percent Plant Infection*			% Diseased Leaf Area (%DLA)			% Fruit Infection Plant		
	MF	OH	BL	MF	OH	BL	MF	OH	BL
T0	85.33k	89.6 i	85.2k	87.4i	89.9 f	87.4g	89.9f	85.33f	81.8g
T1	12.8a	15.33a	19.6a	15.2a	17.4a	19.9a	17.4a	19.9a	15.33a
T2	71.66j	73.7 i	78.66j	32.8e	35.33d	39.6f	36.33d	49.6e	23.7d
T3	27.62f	44.54g	54.2i	33.66e	33.7c	38.66e	35.7cd	48.66de	24.54de
T4	44.44i	49h	53.25i	30.62d	34.54cd	36.2d	35.54c	46.2d	29f
T5	40.66h	46.22gh	51.23i	34.44e	39e	39.25ef	39.11e	49.25e	26.22e
T6	33.14g	35.88f	41.56gh	30.66d	36.22d	38.23e	37.22d	48.23d	25.88e
T7	27.66f	32.31ef	42.2h	32.14de	35.88d	36.56d	37.88de	46.56d	22.31cd
T8	22.43d	30.6e	37.2g	34.66e	32.31c	32.2b	36.31d	42.2c	20.6b
T9	23de	19.19b	22.6a	31.43d	30.6b	37.2de	36.6d	47.2d	19.19b
T10	18.3c	19.9b	23.6ab	24b	29.19b	32.6bc	29.99b	42.6c	19.9b
T11	22.6d	26.53d	29.9cd	27.3c	29.9b	33.6c	29.9b	43.6c	26.53e
T12	27.6f	29.9de	31.8d	33.6e	36.53de	39.9f	37.53d	49.9e	20.9b
T13	17.7c	20.3c	25.6bc	25.6b	30.9bc	31.8b	36.9d	41.8c	20.3b
T14	21.6d	30e	35.5fg	25.7bc	30.3b	35.6cd	36.3d	45.6cd	20b
T15	24e	28.2d	32.2df	28.6cd	30b	35.5c	38e	45.5c	21.2bc
T16	15ab	20.15bc	25.6bc	23b	30.2b	31.2b	33.2bc	38.2b	26.33e
LSD (5%)	2.25	4.2	4.4	3.59	3.3	2.2	2.6	3.4	2.3
CV	15.8	16.1	10.6	14.3	18.1	15.4	18.7	18.5	21.7

* Significant at 5% level; Means followed by the same letter (s) in a column did not differ at 5% level by LSD, MF=Maraqo Fana, OH=Oda Haro, BL= Bako local

Statistically identical results were recorded in Maraqo Fana plots in T10 (AAU-37) and T13 (AAU-69+Th + Onion) with values of 17.7 and 18.3, respectively. The next highest percent plant infection in Oda Haro was observed inT9 and T10, 19.19 and 19.9, respectively. Moderate infection was observed in T13(with value of 20.3) and T16 (with 20.15). In Bako local variety, the second lowest (29.9, 25.6 and 25.6) was observed in T11 (AAU-69), T13(AAU-69+AAU-Th+Onion) and T16 (AAU-69+AAU-Th+Onion).But treatment T8 (Ridomil) was chemical

which was not environment friendly which also significantly differed from To(Control), had a value of 22.43, 30.6 and 37.2 percent plant infection, in Maraqo fana, Oda Haro and Bako local varieties. This higher infection may be due to the fact that Ridomil is a very toxic and injurious to human health and not an environment-friendly product it would have destroyed the beneficial microorganisms too.

10.3.1.2. Leaf Infection

Effect of different treatments on percent leaf infection are had revealed that there is statistical difference among the treatments at $p<0.05$. On Maraqo Fana variety, the highest percentage of leaf infection 87.4 % was observed in the treatment T0 (control). The result clearly indicated that the treatments had significant effect on percent leaf infection. The lowest (14) percent leaf infection was observed in treatment T10(AAU-37), T2 (combination of Trichoderma isolates, plant extracts and Ridomil), T13(AAU-69+AAU-Th+Onion), T14(AAU-37+AAU-Th+Neem) and T16 (AAU-69 + AAU-37+ AAU-Th) with values of 15.2, 15.6, 15.7 and 17 percent leaf infection, respectively (Table 10.1).

On Oda Haro variety, the highest percentage of leaf infection, 89.9, was observed in the treatment To (control) and this was significantly higher than under any other treatments. The result clearly indicated that the treatments had significant effect on percent leaf infection. The lowest (17.4) percent leaf infection was observed in treatment T2 (combination of Trichoderma isolates, plant extracts and Ridomil). The second lowest(19.19 and 19.9) percent leaf infection was observed in T10 (AAU-37) and T11(AAU-69) which were statistically similar to T13(AAU-69+AAU-Th+Onion), T15(AAU-69+AAU-37+Neem), T16 (AAU-69+AAU-37 + AAU-Th), T14 (AAU-69+AAU-Th+Neem), T9 (AAU-Th) and T8 (Redomil) having of 20.9%, 20, 20.2, 20.3, 20.6 and 22.31% respectively (Table 10.1).

The result clearly indicated that, on Bako local variety, the treatments had significant effect on percent leaf infection. The highest leaf infection, 85.2% , was observed in treatment To (control). The lowest (19.9 %) percent leaf infection was recorded in treatment T2 (combination of isolates *Trichoderma* spp, plant extracts and Ridomil). Percent leaf infection was statistically similar in T8 (Ridomil) and T_{10} (AAU-37) having of 22.2% and 22.6%, respectively. Regarding the second lowest infection, T16 (AAU-69+AAU-37 + AAU-Th), and T_{11} (AAU-69)

were statistically similar having 23.2% and 23.6%, respectively. The rest of the treatments had shown statistically significant effect (Table 10.1).

10.3.1.3. Percent of Diseased Leaf Area (%DLA)

The result clearly indicated that the treatments had significant effect on percent diseased leaf area. On Maraqo Fana variety, the highest percent diseased leaf area was observed in To (control) having value 87.4 % and the lowest diseased leaf area was in T2 (combination of Trichoderma isolates, plant extracts and Ridomil). Many treatments had fallen in the second statistical significance category. Treatment T16 (AAU-69+AAU-37 + AAU-Th), T10 (AAU-37), T13 (AAU-69+AAU-Th+Onion) and T14 (AAU-69+AAU-Th+Neem) showed statistically similar significant effect having values 23.0 %, 24.0 % , 25.6% and 25.7 %, respectively. The rest of the treatments also showed significant effect (Table 1). Single (T9-T11) and combined (T9-T16) treatments of *Trichoderma* spp, that includes AAU-Th, AAU-37and AAU-69; and plant extracts which include extracts of Neem, Onion and Garlic as mixture spray was found more efficient than Ridomil. The relative efficiencies of treatments suffered only a slight change on other varieties probably due to environmental factors and the growth stage physiology of the plants. The disease development in the infected leaves at this period was rather low Maraqo fana compared to the Oda Haro variety.

On Oda Haro variety, the highest percent diseased leaf area was observed in To (control) having value 89.9 % and lowest diseased leaf area was in T1 (combination of isolates of *Trichoderma* spp, plant extracts and Ridomil) having value 17.4 %. On the other hand, Treatment T9 (Th), T10 (37), T11 (69), T13 (69+Th+O), T14 (37+Th+N), T15(69+37+N)and T16 (69+37+Th) showed statistically similar effect having values 30.6 %, 29.19 %, 29.19%, 30.9%, 30.3%, 30% and 30.2 % respectively. The rest of the treatment also showed significant effect (Table 10.1).

On Bako local variety, the highest percent diseased leaf area was observed in T0 (control) having value 87.4 % and the diseased leaf area was in T1(combination of Trichoderma isolates, plant extracts and Ridomil) having value 19.9 %. The second least infection was observed in Treatment 16 (AAU-69+AAU-37 + AAU-Th), T13 (AAU-69+AAU-Th+Onion),T10 (AAU-37) and T8 (Ridomil) with statistically similar effect having values 31.2 %, 31.8%, 32.6% and 32.2 %, respectively. The rest of the treatment also showed significant effect (Table 10.1).

10.3.1.4. Infected Fruits per plot

Effect of different treatments on percent fruit infection had shown that in Maraqo fana variety, all the treatments showed statistically significant effect on reducing percent fruit infection compared to control. The highest percent of fruit infection was observed in control plot To (89.9%) and the lowest percent of fruit infection was recorded in T1(17.4%).The treatments 16 (AAU-69 + AAU-37 + AAU-Th), T11(AAU-69) and T10 (AAU-37) had shown statistically similar significant effect with values of 32.2%, 29.9% and 29.99%, respectively (Table 10.1).

On Oda Haro variety, the treatments also showed statistically significant effect on percent fruit infection. The highest percent of fruit infection was observed in control plot To (85.33%) and the lowest percent of fruit infection was recorded in T1 (19.9%).The treatments 16 (AAU-69 + AAU-37 + AAU-Th) had shown the second lowest infection with value of 38.2%. On Bako local variety, the highest percent of fruit infection was observed in control plot To (81.8 %) and the lowest percent of fruit infection was recorded in T1(15.33%). The treatments T8 (Ridomil), T9 (AAU-Th), T10 (AAU-37), T12 (AAU-Th + Garlic), T13 (AAU-69 + AAU-Th + Onion), T14 (AAU-37 + AAU-Th + Neem), T15(AAU-69+ AAU-37+Neem) and T16 (AAU69+ AAU-37 + AAU-Th) showed statistically similar effect having values 20.6 %, 19.19 %, 19.9%, 20.9%, 20.3%, 20% and 21.2 %, respectively (Table 10.1).

Control ($T_{0)}$ where no treatment was given, the % fruit infection on Bako local became less as compared to the situation observed on Maraqo fana and Oda Haro varieties. But the plants which received treatment, whichever it may be, the spread of the disease on the fruit was considered low. The highest fruit infection was observed in T_0 (Control) and significantly small difference in other treatments.

From the tested Trichoderma spp isolates applied singly or in combination, the lowest infection was observed on AAU-69+AAU-37+AAU-Th. However, the efficacy of antagonists, extracts and fungicides, i. e, AAU-Th + Neem + Ridomil, appeared to be compatible and effective.

Among the botanical extracts the strongest anti-*Colletrotrichum capsici* (Syd) reaction has been shown in terms of percent fruit infection by Garlic leaf extract (39.11, 49.25 and 26.25, on Maraqo Fana, Oda Haro and Bako local varieties, respectively), which was lower than *Cassia* spp

extract (35.7, 48.66 and 24.54, on Maraqo Fana, Oda Haro and Bako local varieties, respectively). Ridomil's effect in reducing % fruit infection was not as anticipated. It was lower than the antagonists and leaf extracts.

Throughout the experiment in all the parameters taken into account Integrated use of Antagonists, plant extracts and 0.2% application of Ridomil as foliar spray performed excellent. This was the expected result too. Ridomil treatment was a positive control treatment as no treatment was considered as a control treatment. The aim was to compare the effects of the antagonist (*Trichoderma* spp isolates) and organic (botanicals) treatments with both. The results and their analyses revealed that the test treatments, antagonists and botanical extracts, are capable of reducing anthracnose of chili in the cultivars Maraqo fana, Oda haro and Bako local quite significantly even when applied in combination and singly. However, combination of antagonists and botanical extracts were more strong and effective even to a level that with minimum risk such treatment can replace a highly effective chemical fungicidal treatment.

10.3.2. Effect of IDM on Growth Performance of Hot pepper

There were significant variations among the tested genotypes in terms of the percent establishment, days to 50% maturity and days to first harvest (Table 2). The result showed a range of 48.67 (T0) to 82.67(T11), 48.00 (T0) to 83.33(T11) and 49.67(T0) to 89.67(T4) for percent establishment; 43.0(T0) to 67.33(T13), 41.00 (T0 and T4) to 53.33(T10) and 36.33(T0) to 53.67(T5) for days to 50% flowering; 62.0(T0) to 82.0(T4), 65.33 (T0) to 79.67(T13) and 40.67(T0) and 81.0 (T14) for days to 50% of maturity; 78.67(T0) to 106.0(T8), 52.67 (T0) to 108.33(T13) and 51.67(T0) to 107.33(T13) for days to first harvest for Maraqo fana, Oda haro and Bako local varieties, respectively (Table 10.2).

Table 10.2. Effect of IDM on growth parameters of Hot pepper varieties in 2013 cropping season

Var.	*Establishment %			Days to 50% Maturity			Days 1st Harvest		
Treat	MF	OH	BL	MF	OH	BL	MF	OH	BL
T 0	48.67a	48.00a	49.67a	62.00a	65.33a	40.67a	78.67a	52.67a	51.67a
T 1	69.33b	72.00b	73.00bc	70.00b	73.33cd	78.00e	100.00e	101.33b	102.00c
T 2	71.00bc	81.33d	74.00c	72.33bc	72.33b	76.67d	98.00cd	104.33c	100.33b
T 3	75.33d	77.33c	74.00c	78.67g	72.00b	70.00b	97.67c	102.33b	99.67b
T 4	74.67d	77.00c	89.67e	82.00h	75.33d	80.67g	98.67d	102.67bc	98.67b
T 5	74.00cd	82.33d	71.67b	79.67g	73.00bc	71.00bc	98.67d	106.00d	102.33c
T 6	81.67f	73.67b	73.00bc	73.67cd	77.00f	75.67d	97.67c	104.33c	102.67c
T 7	81.00f	81.33d	70.67b	81.67h	78.33g	78.00e	100.0e	104.67cd	102.00c
T 8	81.33f	81.33d	73.00bc	75.00de	78.33g	80.33g	106.3f	104.33c	102.67c
T 9	77.00de	82.33d	78.00d	75.67e	79.00g	76.00d	97.00c	102.33b	100.33b
T 10	81.00f	81.67d	76.00d	76.67f	76.67ef	76.33d	95.33b	104.33c	106.00c
T 11	82.67f	83.33f	73.00bc	72.33bc	75.67e	80.33g	98.67d	104.00c	104.33c
T 12	81.33f	81.67d	74.00c	76.00e	79.33g	79.67fg	101.0e	105.33d	106.33c
T 13	80.00e	80.67d	71.33b	73.00c	79.67gh	79.00ef	100.0e	108.33e	107.33cd
T14	80.67ef	82.67e	76.00d	75.67e	75.67e	81.00gh	102.7e	106.00de	105.67c
T 15	72.00c	82.00d	70.67b	79.00g	72.33b	73.33c	100.6e	104.33c	105.33c
T 16	81.67f	80.00d	75.33cd	76.00ef	72.67b	79.00ef	102.7e	101.67b	101.67bc
LSD(5%)	3.11	2.51	2.78	1.43	1.14	1.32	1.32	2.41	5.62
CV%	11.1	28.4	16.7	18.9	16.8	17.4	11.00	22.4	23.2

*Means followed by the same letter in the same column are not significant difference at P<0.05; MF= Maraqo Fana, OH= Oda Haro, BL= Bako local

Quality parameter trial, the result revealed that significant variations existed among the tested genotypes in terms of number of branches per stem, canopy diameter (cm), number of flowers per plant and plant height (Table 3). The result showed a range of 1.0 (T0) to 9.0(T10), 1.0 (T0) to 8.0(T4) and 2.0 (T0 and T13) to 8.0 (T11) for branches per stem; 5.80(T0) to 24.8(T15), 4.8 (T0) to 12.8 (T11) and 5.6 (T0) to 12.6 (T2) for canopy diameter (cm); 5.26 (T12) to 9.4 (T2), 4.7 (T1) to 9.6(T3) and 5.8(T0) to 10.8(T4) for number of flowers per plant, 13.9 (T0) to 61.4 (T7), 15.5 (T0) to 78.8 (T15) and 12.8 (T0) to 68 (T8) for plant height, on Maraqo Fana, Oda Haro, and Bako local varieties, respectively.

Table 10.3. Effect of IDM Quality Parameters of three chili accessions at Alaba 2013(P-I)

Treat-ments	Number of Branches Per Stem*			Canopy Diameter (cm)			Number of Flowers Per Plant			Plant Height (cm)		
	MF	OH	BL	MF	OH	BL	MF	OH	BL	MF	OH	BL
T0	1a	1a	2a	5.8a	4.8a	5.6a	6.7b	4.7a	5.8a	13.9a	15.5a	12.8a
T1	4. 3b	7de	6. 3c	12b	11.6c	12.6e	9.4e	4.7a	7.3ab	47.6d	61i	67.3g
T2	7d	2a	2.1a	13.8b	10.44b	11.3c	6.8b	8.2d	9.7cd	26.5b	47.2d	49.5d
T3	5.3c	2a	2.2a	12.8b	10.6b	11.1b	8.5d	9.6d	6.6a	37.5c	49.1e	40.7c
T4	2.5b	8ef	2.2a	24.6f	10.9b	10.7b	9.6e	8.2d	10.8d	37.1c	30.5b	58.9f
T5	3.6b	5bc	6bc	19.9d	11.4bc	10.89b	6.87b	7.1c	7.5b	36.7c	52.7f	57.99e
T6	4.7bc	4b	2.3a	16.8c	12.5d	10.84b	6.9bc	6.5bc	8.7bc	60.6h	59.4h	67.34g
T7	9e	7de	2.1a	19.6d	12.4d	12.1d	5.6a	7.9d	9.5c	61.4h	38c	60f
T8	2.6b	2a	3a	21.3de	12.6d	11.9d	6.7b	6.1b	9.1c	58.8g	49.3e	68g
T9	6.7cd	6cd	2.3a	18.7cd	11.8c	11.6c	7.6cd	7.6cd	7.8b	54.9e	69.4k	49.2d
T10	9e	9f	7.4c	19.6d	12.3d	11.7cd	6.3ab	8.2d	6.3a	55.8f	38.6c	49.9d
T11	3.33b	6cd	8cd	11.8b	12.8d	11.6c	6.7b	6.4b	7.8b	56.9f	49.5e	48d
T12	6c	5bc	4.2b	13.8b	11.9cd	11.8d	5.26a	7.3c	6.6a	59.2gh	57.2fg	39.1b
T13	8de	7de	2a	22.7e	11.3b	10.9b	5.44a	5.6a	7.5b	58.7g	67.4j	56.5e
T14	6c	6cd	3.6ab	16.8c	11.9c	12.2de	8.7de	5.8ab	8.2b	58.8g	47.6d	58ef
T15	5c	6cd	5.6b	24.8f	11.68c	10.96b	6.2a	7.8de	7.6b	56.4f	78.8m	58.76f
T16	4b	4b	6bc	23.3ef	11.7c	11.2bc	5.5a	8.5d	9.3c	57.1fg	72 l	59.7f
LSD(5%)	2.2	1.4	2.1	2.33	0.94	0.59	1.3	1.2	1.6	2.4	1.3	2.1
CV%	18.1	19.6	16.2	10.3	14.5	17.3	10.1	12.4	13.7	18.5	16.7	17.8

*Means followed by the same letter in the same column are not significant difference at P<0.05, MF=Maraqo Fana, OH=Oda Haro, BL= Bako local

10.3.3. Effect of IDM on Quality Performance of Hot pepper

On another quality parameter trial, the result revealed that significant variations existed among the tested genotypes in terms of fruit diameter(in cm), Fruit pericarp thickness (mm), fruit length (cm) and fruit dry weight content (g) (Table 3). The result showed a range of 5.25 (T0) to 9.7(T4), 4.69 (T1) to 9.59 (T3) and 6.3(T10) to 10.79(T4) for fruit length (cm); 4.9(T9) to 9.2 (T12), 6.74 (T13) to 11.0 (T1) and 6.5 (T13) to 10.7 (T3) for fruit dry weight content (g) on Maraqo Fana, Oda Haro, and Bako local varieties, respectively (Table 10.4).

Table 10.4. Effect of IDM on Quality Parameters of three chili accessions at Alaba 2013

	Fruit Length (cm)			Fruit dry weight Content (g)		
Treat.	**MF**	**OH**	**BL**	**MF**	**OH**	**BL**
T0	6.7b	6.4b	7.8c	6.9b	9.5cd	8ab
T1	9.4d	4.7a	7.3b	7.6cd	11d	7.3a
T2	6.8b	8.2d	9.7ef	6.5b	7.24a	9.5b
T3	8.5c	9.6e	6.6b	7.5c	9.1bc	10.7c
T4	9.6d	8.2d	10.8f	7.1bc	10.5d	8.9b
T5	6.87b	7.1c	7.5b	6.7b	8.27b	7.99a
T6	6.9bc	6.5bc	8.7d	6.6b	9.4c	7.34a
T7	5.6a	7.9d	9.5e	6.4b	8b	10bc
T8	6.7b	6.1b	9.1de	5.8ab	9.3c	8ab
T9	7.6c	7.6c	7.8c	4.9a	9.4c	9.2b
T10	6.3ab	8.2d	6.3a	5.8ab	8.6b	9.9b
T11	6.7b	6.4b	7.8c	6.9b	9.5cd	8ab
T12	5.26a	7.3c	6.6b	9.2d	7.25a	9.1b
T13	5.44a	5.6a	7.5b	8.7d	6.74a	6.5a
T14	8.7cd	5.8ab	8.2cd	8.8d	10.6d	8ab
T15	6.2a	7.8cd	7.6bc	6.4b	7.88ab	8.76b
T16	5.5a	8.5de	9.3e	7.1bc	7.2a	9.7b
LSD(5%)	1.11	1.12	1.1	1.4	1.23	2.21
CV%	20.1	18.4	14.5	13.5	14.7	19.8

*Means followed by the same letter in the same column are not significant difference at P<0.05, MF=Maraqo Fana, OH=Oda Haro, BL= Bako local

10.4. Discussion

This study showed that antagonists and plant extract had very effective strong fungicidal effect. In some cases, the reported effect is more than that of chemical pesticides. Out of the many such reported promising plants, on the simple basis of availability Garlic, Neem, Onion and Cassia have been selected to assess their combat ability against *Collectrotrichum capsici*, that is the ability against anthracnose of chili. Different integrated practices like A total of 16 treatments, viz., Untreated (T0), combination of all (Extracts + *Trichoderma* spp + Ridomil) (T1) Neem (T2), Cassia spp (T3), Onion (T4), Garlic (T5), Neem + Onion+ *Cassia* spp (T6), Neem + Onion + Garlic (T7), Ridomil (T8), AAU-Th(T9), AAU-37 (T10), AAU-69(11), AAU-Th + Garlic (T12), AAU-69 + AAU-Th + Onion (T13), AAU-37 + AAU-Th +Neem (T14), AAU-69 + AAU-37 + Neem (T15), and AAU-69 + AAU-37 + AAU-Th(16) had been used in this experiment. Data on disease reaction, quality and growth parameters had been collected. Plant infection, leaf infection, leaf area diseased, and fruit infection, were the parameters for disease reaction. The highest

average fruit yields however were observed under the treatment T_6 and T_7 and the lowest under the treatment *To*. As a result low yield was found in control plots and high in the plots where combined treatments were applied. The combined treatment had a highly significant effect on the fruit yield. The results obtained by Serawit and Tesfaye (2014), Ngullie, *et al*., (2010), Nashwa and Abo-Elyousr, (2012) with the use of garlic and Kabir *et al*. (2014) with Neem *(Azadirachta indica)* leaf and Neem seed extracts nicely corroborate with the present findings.

The results also showed organic treatment was more profitable than a chemical fungicide treatment of all the fruit collection from the respective plots. The chili test plants which have received Ridomil @ 0.2% had the lowest disease intensity as well as severity parameters. Plot treated with antagonists, plant extracts and fungicides (T_1) has shown the highest disease control potential but significantly superior to the control(To). The plant extract treatments have shown significantly better control than the T8, though they varied widely amongst themselves. The combined treatments T_6(Neem + Onion + Cassia) and T_7 (Neem + Onion + Garlic) have shown very strong response though lesser than the T8 (Ridomil) in controlling anthracnose of chili. This is in conformity with Ademe *et al*. (2013) and Rashid *et al.* (2015).

The highest average fruit diameter however were observed under the treatment T1 and T10 and the lowest under the treatment To. As a result, low diameter was found in control plots and high in the plots where combined treatments were applied. The combined treatment had a highly significant effect on the fruit diameter. The results obtained by Kabir *et al*. (2014) with the use of garlic and Serawit and Tesfaye (2014) with Neem *(Azadirachta indica)* leaf and Neem seed extracts nicely corroborate with the present findings.

10.5. Conclusion and Recommendation

The results of this experiment indicated that incidence and severity of Anthracnose of chili disease can significantly be reduced by the combined use of (*Trichoderma* spp + Plant Extracts) 1: 10 dilution suspension foliar spray in order to have a higher profitable yield and eventual higher economic return . Therefore, the farmers may be advised to take an integrated use of antagonists and plant extracts.

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CHAPTER ELEVEN

ECONOMIC BENEFITS AND RESPONSES OF CHILI YIELD TO INTEGRATED APPLICATION OF PLANT EXTRACTS AND ANTAGONISTS AGAINST ANTHRACNOSE (*Colletotrichum capsici* (Syd.)) IN SNNP, ETHIOPIA

Abstract

Chili (*Capsicum frutescenes* L) is a well-acclaimed commercial crop in Ethiopia. But its production significantly stagnated primarily due to *Colletotrichum capsici* (Syd.).Thus, this research was initiated with the objective of managing chili anthracnose on three varieties. Three Trichoderma isolates, four leaf extracts and Ridomil were were arranged in RCBD in 2013on-season FTC farm in Alaba Special District, SNNP Region, Ethiopia. The treatments were replicated thrice. Data on yield and yield components of chili had been collected. Analysis of data was carried out using ANOVA and simple correlation. The finding showed that plots treated with Trichoderma isolates and plant extracts gave higher yield compared to both positive (Ridomil) and negative control (no treatment). Though the 0.2% Ridomil gave the yield of chili, the combined application of Trichoderma, plant extracts and Ridomil performed best in number of fruits/plant, number of seeds/pod, unmarketable fruit, marketable fruit and total yield. This mix up gave a higher benefit-cost-ratio due to reduced production cost indicating that a judicious combination of organic management practice is environment friendly, healthy and sustainable.

Key Terms: BCR, *Colletotrichum* spp, IDM, Plant Extracts, *Trichoderma* spp, yield

11.1. Introduction

Chili (*Capsicum frutscenes L*.) is a highly profitable cash crop popular with farmers and their markets (Beyene and David, 2007), but its production poses significant risks (Mitiku Tesso and Ranamukhaarachchi. 2005). Ethiopia's share in the world, however, is insignificant (5%) compared to India (36%) and China (11%) with a production of 1.25, 0.39 and 0.17 million tones (Faisal and Mohammad, 2011; CSA, 2012). The decline of hot pepper production (0.4 tones fruit yield/ha) is attributed to the prevalence of fungus among others (Fekadu and Dandena, 2006; Fekadu, 2008). Peppers are widely consumed in home, fresh seasoning, and as a cooking ingredient. Farmers mainly produce their crop for commercial marketing (Beyene and David, 2007),. However, yields are quite low, averaging 0.38 t/ha (Fekadu, 2008), in contrast to more than 5 t/ha yields in developed countries (Faisal and Mohammad, 2011). Peppers continue to grow under substantial pressure from pests and diseases. Anthracnose has become the most prominent fungal disease in Ethiopia, and incidence continues to expand. It is a serious threat to crop productivity during rainy weather (Yohannes *et al*, 2015). Diseases attacking pepper constitute one of the key challenges to increasing productivity. Pesticides are applied sometimes twice weekly, and as frequently as 40 times over the course of the crop, often without significant benefit. These losses can be reduced by the use of pesticides but as these have residual toxic effects, alternative bio- control agents should be sought (Mitiku Tesso and Ranamukhaarachchi. 2005) Jeffries and Koomen (1992) reported that the use of biological approaches for control of *Colletotrichum* diseases had not been developed partly because the fungus often has a quiescent phase in the life cycle when it may appear to be protected from microbial antagonism.

Chemical control of anthracnose, (*Collelotrichum capsici*(syd.)) on urdbean *(Vigna mungo*) were investigated. Seed treatment followed by two prophylactic sprays of Bavistin (Carbenxazim) or tilt (propiconazole) (at 0.10% each) at 15 days interval showed minimum disease severity and maximum grain yield followed by Contaf (0.10%) and Indofil M-45 (Mancozeb +Thiophanate-methyl) (0.20%) sprayed plots (Deeksha *el al.* 2002).

The efficacy of Copper hydroxide at 0.10, 0.15, 0.20 and 0.25%; Chlorothalonil at 0.20%; and Carbendazim at 0.10% against fruit rot of chili *(Capsicum annuum)* caused by *Colletotrichum capsici* had been evaluated. The lowest disease index (30.47%) resulted from the application of 0.10% Carbendazim (30.47%) followed by 0.25% Copper hydroxide in

controlling *Colletotrichum capsici* to avoid the development of resistance of the pathogen to carbendazim (Ekbot, 2002). Foliar fungicides were used to control Alternaria blight of cauliflower seed crop. The maximum reduction in severity of Alternaria blight and the highest increase in seed yield over control were achieved with Royal 50 WP (0.21%) followed by Dithane M-45 (0.25%) (Khoda *et al.,* 2003). In another trial conducted in Bangladesh, no die-back symptom appeared in Bion (0.05%0 treated seeds but was recorded in Azoxystrobin and Carboxin treated seeds. Lesion size, leaf infection and leaf area damage were less in plants grown from Bion and Azoxystrobin treated seeds. Bion treated plots resulted in moderate resistance of plant to anthracnose, where Azoxystrobin and Carboxin treatment resulted in susceptibility of the crop to the disease (Rahman *et al.* 2004).

Commercial cultivars carrying immune resistance to the diseases have not yet been released. Some cultural practices lead to disease development. Recommended fungicides for anthracnose control are often ineffective, with excessive pesticide sprays (up to 100, averaging 40) applied to the crop. Yield losses of 50% in local and open pollinated varieties have been reported. This research was, therefore, initiated with objective of determining the economic benefits of managing chili anthracnose through integrated use of antagonists and plant extracts under field conditions.

11.2. Materials and Methods

11.2.1. Experimental site

The pot experiment was conducted in the Addis Ababa University, College of Natural and Computational Sciences, Department of Microbial, Cellular and Molecular Biology glass house in 2013. The field experiment was conducted at the FTC site in Alaba during November, 2013 to January, 2014. This study was carried out at two Alaba, which is one of the most important pepper growing locations that have altitude of 1680 above sea level, is characterized by dry sub humid climate. Alaba has monthly mean minimum and maximum air temperature of 15°C and 29.5°C, respectively, and rain fall of 900-1300mm/year. This location is a hot spot area for anthracnose (*Colletotrichum* spp) and wilts (Belete *et al.*, 2012; Tameru *et al*., 2003; Simon *et al*., 2009).

11.2.2. Treatments

A total of 16 treatments, viz., Untreated (T0), combination of all (Extracts + Tricho.+ Ridomil) (T1), Neem (T2), *Cassia* spp (T3), Onion (T4), Garlic (T5), Neem + Onion+ *Cassia* spp(T6), Neem + Onion + Garlic (T7), Ridomil (T8), AAU-Th(T9), AAU-37(T10), AAU-69 (T11), AAU-Th + Garlic (T12), AAU-69 + AAU-Th + Onion(T13), AAU-37 + AAU-Th +Neem (T14), AAU-69 + AAU-37 + Neem(T15), and AAU-69 + AAU-37 + AAU-Th (16) had been used in this experiment.

11.2.3. The Fungal Pathogen

Isolate of *C. capsici*, A38, was obtained from the Laboratory of the Department of Microbial, and Cellular and Molecular Biology, College of Natural and Computational Sciences, Addis Ababa University, Addis Ababa, Ethiopia. The fungal pathogen was maintained on potato dextrose agar (PDA) slants at 4^{O} C.

11.2.4. Pot experiment against *Colletotrichum capsici* (Syd.)

The experiment was arranged in a completely randomized design (CRD) with 17 treatments and replicated thrice. There were five seedlings per pot and fifteen seedlings per treatment were considered as replication. Seedlings of three chili varieties (Maraqo Fana, Oda Haro and Bako local) were planted in plastic tube. Mycelium of *C. capsici* was ground in mortar and mixed with sterilized water to make up a liquid mixture. A hole of an inoculation site was made by needle puncture in the middle of the fruit. Three isolates of *Trichoderma* spp (AAU-Th, AAU-37 and AAU-69) and aqueous extracts of Onion, neem, garlic, and *Cassia* spp were used against anthracnose by using *in vivo* tests (He *et al.,* 2003). Chili leaves (no wound or scar on the surface) from untreated pots were selected for the experiments. They were surface-sterilized with 0.5% NaCl for 5 min and then washed with tap water. After air-drying, the chili leaves were treated with 70 % ethanol. Each fruit was wounded by using a sterile cork-borer (0.6 mm in diameter and 1mm in depth), one wound per fruit. Isolates of *Trichoderma* spp and with high IDM efficacies, selected based on data from the *in vivo* tests, were cultured in Potato dextrose broth (PDB) and cells were collected by centrifugation at 3,000 rpm for 20 min. *Trichoderma* cells were washed twice with sterile distilled water and re-suspended. Then 20 µl of cell suspension of each strain of *Trichoderma* at concentration of $5x10^6$ cells/ml was added to the wound of 17 x 3 x 2 = 102 treated chili leaves. After air drying, 20 µl of *C. capsici*, $5x10^6$ cells/ml was added to the wound. Chili leaves were detached and put on a plastic tray and stored

at 28 ^{o}C. Disease severity, as indicated by increased wound diameter, was counted after 5 days of inoculation. The effect of integrated use of plant extracts and *Trichoderma* spp strain on yield parameters and economic impacts was observed and compared.

11.2.3. Layout and Design of the field Experiment

The experiment was laid out in a Randomized Complete Block Design (RCBD). The experiment was laid and divided into three blocks and each block was divided into 17 plots. The plot size was 5m x 1.2m. Treatment was assigned to each block at random. The space between the blocks and between the plots was 1.00 m and 0.50 m, respectively. The first spray was given on the 21st DAT as leaf spots could be located the day before. All the treatments (T0- T16) were administered as foliar spray. Plant extracts were formulated at 1:10 suspensions, T6 and T7 as 1:10 suspension cocktail. Ridomil (T8) was administered @ 0.2% suspension. Usually in the evening calm weather light spraying was done so that neighboring plots cannot share a wrong treatment. As the symptom bearing leaves / infected plants serve as a source of inoculum, field sanitation was used as a treatment (T2). In this case all the fallen or hanging diseased leaves were collected removed and destroyed.

11.2.4. Data collection

- **Number of fruits per plant:-** Mean number of red ripe fruits of individual plants from central rows for each plot at each harvest will be recorded.
- **Average number of seed per pods**:- Seeds of randomly picked ten marketable pods from sample plants will be counted and recorded.
- **Seed weight (g):-** Seed extracted from ten marketable pods will be weighed using sensitive balance and mean values will be calculated.
- **Marketable yield (t/ha):-** The marketable yield of nine sample plants will be determined at each harvesting by sorting dried fruits according to color, shape, shininess, firmness and size of the fruits. After drying, the dried marketable fruits will be separated; the weight of the respective categories will be recorded and converted to t/ha.
- **Unmarketable yield (t/ha):-** Is the yield which will be obtained by sorting the diseased, discolored, shrunken shape and small sized, totally unwanted pods by consumers from marketable dried pods will be recorded at each harvest and converted to t/ha .

- **Total dry fruit yield (t/ha):-** Weight of total (marketable and unmarketable) fruits harvested at each successive harvesting from the sample plants will be recorded and summed up to estimate yield per hectare.
- **Cost-Return Analysis:-**The cost production was analyzed in order to find out the most economic treatment of different integrated management practices. All input costs, including the cost for lease land and interest on running capital were considered for computing the cost of production. The interests were calculated at 13% per year for 6 months. The benefit cost ratio (BCR) was calculated as follows:

$$\text{Benefit Cost Ratio (BCR)} = \frac{Gross\ Return\ per\ ha}{Total\ Cost\ of\ Production\ Per\ ha}$$

11.2.5. Analysis of data

For each measured response variables analysis of variance (ANOVA) mean separation procedure will be carried out. After fitting ANOVA model for those significant interactions or main effects a mean assumption procedure using LSD mean methods will be carried out at required levels of probability. All the statistical analysis will be carried out using SAS-9.2 statistical soft ware package (SAS Institute Inc., 2008).

11.3. Results

11.3.1. Effect of IDM on Yield and Yield Components of Chili in Pot Experiment

In the pot experiment conducted in AAU glass house, there were significant variations among the tested genotypes in terms of the number fruits per/pl, Average number of seeds/pod, Unmarketable fruit (q/ha), Marketable fruit(q/ha) and Total yield (q/ha) (Table 11.1). The result showed a range of 10.33 (T0) to 28.7(T8) and 11.67 (T0) to 28.7(T16) for number fruits per/pl; 11.67 (T0) to 21.67 (T4, T9 and T11) and 11.0 (T0) to 21.67(T2 and T3) for average number of seeds/pod; 1.94(T0) to 6.67(T11) and 1.33 (T0) to 6.67(T14) for Unmarketable fruit(q/ha), 1.85 (T0) to 6.33 (T1) and 1.67(T0) to 6.67(T9) for Marketable fruit(q/ha); 0.41(T0) to 1.66(T16) and 0.32(T0) and 1.6 (T7) for days to 50% for Total yield (q/ha) on Maraqo fana and Oda Haro varieties, respectively.

Table 11.1. Effect of different treatments on the yield and yield components as affected by anthracnose of chili (pot Experiment, in 2013, at AAU)

Treatments	*No. of Fruits/Pl		Average No. Seeds/Pod		Unmarketable Fruit(Q/Ha)		Marketable Fruit (Q/Ha)		Total Yield(t/Ha)	
	MF	OH	MF	OH	MF	OH	MF	OH	MF	OH
T0	10.33a	11.67a	11.67a	11a	1.94a	1.33a	1.85a	1.67a	0.41a	0.32a
T1	15.9b	14.8b	17.67d	20d	5.83de	6.17cd	6.33d	4.83bc	1.52bc	1.44b
T2	14.6b	24.7d	21ef	21.67e	3.83b	5.83c	3.5b	5c	1b	1ab
T3	17.7c	26.5f	20.33e	21.67e	5.67d	3.17b	5.33c	5.17c	1.25b	1.33b
T4	22.6e	28.7f	21.67f	21.33de	5.67d	5.5c	3.5b	4.17b	1.24b	1.1b
T5	22.8e	26.8f	18d	19cd	4.67c	6.17cd	3.33b	4.83bc	1.45b	1.28b
T6	21.7de	27.4f	17cd	18.33c	5.5d	5.67c	2.8ab	3.17a	0.99b	1.45b
T7	26.5g	20.6c	18.67de	17.33bc	6.33e	4.83c	4.33bc	3.33b	1.24b	1.6b
T8	28.7hi	21.3c	20e	17.67c	3b	2.67ab	4b	3.17a	1.06b	1.52b
T9	26.8gh	25.6e	21.67f	21d	4bc	6.33d	4.85c	6.67d	1.21b	1.32b
T10	27.4h	23.2d	18.33d	19cd	4.07c	4.67c	7d	3.83b	1.29b	1.17b
T11	20.6d	26.3ef	21.67f	18c	6.67e	3.17b	6.67d	4.67b	1.24b	1.11b
T12	21.3d	20.7c	16.67c	16.33b	5.37d	4.4b	5c	5.67cd	1.16b	0.98b
T13	15.9d	24.8de	14.67bc	17.33bc	3.5b	4.5bc	5.33c	3.33b	1.8c	0.87b
T14	24.6f	24.7d	13ab	18.67c	5cd	6.67d	4.5c	4.83bc	1.37b	1.23b
T15	27.7h	26.5f	12.33a	14.67b	5.63e	5.33c	4b	3a	1.24b	0.9b
T16	22.6e	28.7f	17cd	19cd	6.33e	5.5c	5.67cd	4.17b	1.66c	0.99b
LSD(5%)	1.5	1.62	2.43	2.55	1.05	1.8	1.6	1.73	0.55	0.78
CV%	18.1	17.7	18.8	15.7	11	13.8	18	12	16	19

*Means followed by the same letter in the same column are not significant difference at P<0.05, MF=Maraqo Fana, OH=Oda Haro, BL= Bako local

11.3.2. Effect of IDM on Yield and Yield Components of Chili under field Condition

Effect of different treatments on number of fruit of chili in terms of dry weight was determined and presented in Table 2. In Maraqo Fana variety, the highest mean number of fruit was recorded in treatment T1 plot (19.4) and the lowest number of fruit was recorded in control plot To (9.33). Treatment 5 and Treatment 11 were found in the same level of statistical significance and had the second highest number of fruits per plant. Fruit number of Oda Haro variety greatly varied from treatment to treatment which ranged from 8.67(T0) to 18.9 (T2). The second highest yield was observed in T2 and T13 (18.9). The treatments T7 showed similar level of statistical significance. As compared to control, extracts and antagonists had the highest fruit number that was observed in the T2 and/or T13 (Antagonist + extract + Ridomil) treatment (Table 11.1). From above, it is revealed that fruit production is inversely proportional to fruit infection. It is interesting to find in Table 11.1 that the least number of fruits was obtained in treatment T0 (non-treated plot). In Bako local variety, the highest mean number of fruit was recorded in treatment T13 plot (19.9) and the

lowest number of fruit was recorded in control plot To (8.34). The second highest yield was observed in T2 (19.8), which showed similar level of statistical significance with T13. Further interesting matter was that the plants which received Ridomil treatment produced lesser quantity of fruit than those under T2and T13 which had antagonists and extracts in combination that probably promoted production physiology. The possible reason may be Ridomil might have adversely influenced the reproduction physiology of the chili plants.

In addition to that, there were significant variations among the tested genotypes in terms of the average number of seeds/pod, unmarketable fruit (q/ha), marketable fruit (q/ha) (Table 1). The result showed a range of 9 (T0) to 21.77 (T11), 9 (T0) to 21.77 (T11) and 8.0 (T0) to 21.5(T1 and T9) for average number of seeds/pod; 1.32(T0) to 6.8(T11), 1.2 (T0) to 7.17 (T2) and 0.71 (T0) to 1.91(T13) for unmarketable fruit(q/ha); 0.57 (T0) to 6.5 (T10), 0.99(T0) to 5.77 (T4) and 0.68(T0) to 9.91(T13) for marketable fruit(q/ha); 0.33(T0) to 1.55(T13) and 0.39(T0) and 1.61 (T7) for total yield (q/ha); on Maraqo fana, Oda Haro and Bako local varieties, respectively.

Effect of different treatments on total yield of Maraqo fana, Oda Haro and Bako local varieties has shown a significant difference at $p < 0.05$. In Maraqo fana variety, the highest yield was recorded in treatment T6 plot (1.55 ton/ha) and the lowest yield was recorded in control plot (To) (with the values of 0.33 ton/ha). Yield of Maraqo fana variety profoundly varied from one treatment to another treatment. Plant extracts and antagonists gave a comparable yield to Ridomil treated plots. The second highest yield was observed in T5, T10 and T8 (with values of 1.38, 1.37 and 1.32 ton/ha). The rest of the treatments had shown statistically similar effect. In Oda Haro variety, the highest yield was observed in T7 with the value of 1.6 ton/ha (Table 11.1). The second highest yield was observed in T8 (Ridomil) (1.52) that had similar level of statistical significance. In Bako local variety, the highest yield was observed in T7 with the value of 1.61 ton/ha (Table 11.1). The second highest yield was observed in T8 (Ridomil), 1.51, that had similar level of statistical significance. The finding implied that when number of fruits increased then yield of chili increased, conversely when increased fruit number then decreased yield of chili.

Table 11.2. Effect of different treatments on the yield and yield components as affected by anthracnose of chili (under field condition, at Alaba, 2013).

Treat-ments	*No. of Fruits/pl			Average No. seeds/pod			Unmarketable fruit(q/ha)			Marketable fruit (q/ha)			Total Yield(t/ha)		
	MF	OH	BL	MF	OH	BL	MF	OH	BL	MF	OH	BL	MF	OH	BL
T0	9.33a	8.67a	8.34a	9a	8a	8.5a	1.32a	1.2a	8.71a	0.57a	0.99a	7.68a	0.33a	0.39a	0.35a
T1	19.4f	14.7cd	13.6cd	17.67c	21.5c	13.7cd	5.83b	7.17de	13.5cd	5.33cd	3.83bc	15.6cd	1.11b	0.95a	1.00ab
T2	10.5a	18.9e	19.8e	20d	17.67b	19.5e	4.53b	4.53c	17.9e	2.5b	4.5c	19.9e	1.15b	1a	1.00ab
T3	12.4b	13.6c	14.5c	20.33d	15.67b	13.6c	5.67b	3.17b	14.6c	4.33c	4.87c	14.6c	1.13b	1.33b	1.33b
T4	14.7cd	13.1bc	14.2bc	21.67e	21.33c	13.3bc	4.67b	2.5ab	14.1bc	2.5b	5.77d	14.1bc	1.21b	1.1b	1.10b
T5	18.9ef	12.8b	13.7b	17c	16b	12.8b	5.67b	4.17bc	11.8b	2.33ab	3.53b	13.8b	1.38bc	1.28b	1.28b
T6	13.6bc	16.5d	15.6d	18cd	15.33b	16.5d	5.5b	3.67b	15.5d	1.8a	3.57b	17.5d	1.55c	1.45b	1.45b
T7	13.1b	18.3de	19.4de	19.67d	16.33b	18.4de	5.33b	4.83	17.3de	3.33b	4.83c	19.3de	1b	1.6b	1.60b
T8	12.8b	17.9d	18.8d	21de	15.67b	17.8d	4.5b	3.67b	16.9d	5.5d	3.57b	18.9d	1.32b	1.52b	1.52b
T9	16.5de	11.5b	12.6b	20.67d	21.5c	11.4b	4.5b	4.33c	10.5b	5.85d	2.57ab	12.5b	1.22b	1.32b	1.32b
T10	18.3e	10.5ab	11.6ab	17.33c	16.7b	10.4ab	4.57b	3.67b	9.5ab	6.5d	4.93c	11.5ab	1.37b	1.17b	1.17b
T11	17.9e	12.4b	13.4b	21.67e	15.8b	12.5b	6.67b	4.17bc	11.4b	5.67d	4.87c	13.4b	1.21b	1.11b	1.11b
T12	11.5ab	14.7cd	15.7cd	16.67c	16.33b	14.8cd	6.17b	6.4d	13.7cd	3.5bc	3.57b	15.7cd	0.88ab	0.98a	0.98a
T13	14.6c	18.9e	19.9e	14.67bc	18.33b	18.8e	6.5b	5.5c	17.9e	4.33c	3.63b	19.9e	0.91b	0.87a	0.87a
T14	13.6bc	13.6c	14.6c	13b	14.67b	13.5c	6b	5.67cd	12.6c	5.5d	5.83d	14.6c	1.13b	1.23b	1.23b
T15	12.7b	13.1bc	14.2bc	13.33b	17.67b	13.2bc	6.33b	3.33b	12.1bc	5.5d	4.5c	14.1bc	0.89b	0.9a	0.90a
T16	10.6a	12.8b	13.9b	17c	19.4bc	12.7b	6.33b	4.5c	11.8b	4.5c	5.17cd	13.8b	0.94b	0.99a	0.99a
LSD(5%)	2.63	2.89	2.90	3.53	5.5	2.91	2.85	1.8	2.86	1.8	1.73	2.99	0.58	0.69	0.67
CV%	12.5	13.85	18.55	14.55	15.7	19.00	10.9	17.8	14.88	14.8	8.2	10.27	10	6.9	11.33

*Means followed by the same letter in the same column are not significant difference at P<0.05, MF=Maraqo Fana, OH=Oda Haro, BL= Bako local

11.3.3. Benefit Cost Ratio (BCR)

As indicated in Tables 11.3- 11.5, weather the treatments are profitable or not had been determined. Using cost and gross return data, the benefit cost ratio (BCR) was calculated. This is an abstract factor originating from virtual appropriately transformed data. BCR values recorded on the Table 2 are expressing some very important facts.

Table 11.3. Cost and Return of Maraqo Fana variety due to different integrated management practices

Treatment	Yield (T/Ha)	Gross Return (Br/ha)	Total Cost of Production (Br/ha)	Net Return (Br/ha)	Cost-Benefit Ratio (CBR)
To	0.33	3300.00	1300.00	2000.00	0.65
T1	1.11	11100.00	7062.00	4038.00	1.75
T2	1.15	11500.00	8000.00	3500.00	2.29
T3	1.13	13300.00	9600.00	3700.00	2.59
T4	1.21	12100.00	8700.00	3400.00	2.56
T5	1.38	13800.00	8500.00	5300.00	1.60
T6	1.55	15500.00	8000.00	7500.00	1.07
T7	1.00	10000.00	8400.00	1600.00	5.25
T8	1.32	13200.00	8100.00	5100.00	1.59
T9	1.22	12200.00	9200.00	3000.00	3.07
T10	1.37	13700.00	9103.00	4597.00	1.98
T11	1.21	12100.00	7011.00	5089.00	1.38
T12	0.88	8800.00	6000.00	2800.00	2.14
T13	0.91	9100.00	5200.00	3900.00	1.33
T14	1.13	11300.00	6200.00	5100.00	1.22
T15	0.89	8900.00	8700.00	200.00	43.50
T16	0.94	9400.00	5420.00	3980.00	1.36

Tables 11.3 – 11.5 present the fact that no treatment (Control) is also a profitable practice. However, in this experiment all the treatments, costly (T15) or cheap (T7), have been in practice and found highly profitable with respective BCR value of 43.5 (highest) and 5.25(the second highest) in Maraqo fana variety. The integrated treatment T16 worked so splendidly in minimizing anthracnose of chili disease and its ill effects on the yield contributing factors, yield and while maximizing the yield contributing factors, yield of chili in the best possible amount among all the treatments (T1 through T16).The precise intention to pick up T2 treatment and place by the side of T7 is simple to compare with a single plant extract which has been able to show its efficacy only significantly higher than the control (To) and similar to another combined treatment with (Neem, Onion and Cassia) extract T7 (5.25). All the combine treatments are more or less having high BCR values (1.07 - 43.50) (Table 3). In Oda Haro variety, the highest BCR (29.00) and the lowest BCR (4.00) was observed in T15 and T2, respectively (Table 11.4). In Bako local variety, the maximum BCR was observed in T15 (with value of 29.0) and the minimum in T 1 and T2 (with value of 4.0) (Table 11.5). A judicious selection of treatments and their integrated application will ensure a healthy but profitable crop.

Table 11.4. Cost and Return of Chili due to Different Integrated Management Practices Oda Haro varieties

Treatment	Yield (T/Ha)	Gross Return (Br/ha)	Total Cost of Prod.(Br/ha)	Net Return (Br/ha)	Cost-Benefit Ratio (CBR)
T0	0.39	3900.00	1300.00	2600.00	0.50
T1	0.95	9500.00	7062.00	2438.00	2.90
T2	1.00	10000.00	8000.00	2000.00	4.00
T3	1.33	13300.00	9600.00	3700.00	2.59
T4	1.10	11100.00	8700.00	2400.00	3.63
T5	1.28	12800.00	8500.00	4300.00	1.98
T6	1.45	14500.00	8000.00	6500.00	1.23
T7	1.60	16000.00	8400.00	7600.00	1.11
T8	1.52	15200.00	8100.00	7100.00	1.14
T9	1.32	15200.00	9200.00	6000.00	1.53
T10	1.17	11700.00	9103.00	2597.00	3.51
T11	1.11	11100.00	7011.00	4089.00	1.71
T12	0.98	9800.00	6000.00	3800.00	1.58
T13	0.87	8700.00	5200.00	3500.00	1.49
T14	1.23	12300.00	6200.00	6100.00	1.02
T15	0.90	9000.00	8700.00	300.00	29.00
T16	0.99	9900.00	5420.00	4480.00	1.21

Table 11.5. Cost and Return of Chili due to Different Integrated Management Practices on Bako local varieties

Treatment	Yield (T/Ha)	Gross Return (Br/ha)	Total Cost of Production (Br/ha)	Net Return (Br/ha)	Cost-Benefit Ratio (CBR)
T0	0.35	3500.00	2000.00	1500.00	1.33
T1	1.00	10000.00	8000.00	2000.00	4.00
T2	1.00	10000.00	8000.00	2000.00	4.00
T3	1.33	13300.00	9600.00	3700.00	2.59
T4	1.10	11000.00	8700.00	2300.00	3.78
T5	1.28	12800.00	8500.00	4300.00	1.98
T6	1.45	14500.00	8000.00	6500.00	1.23
T7	1.60	16000.00	8400.00	7600.00	1.11
T8	1.52	15200.00	8100.00	7100.00	1.14
T9	1.32	13200.00	9200.00	4000.00	2.30
T10	1.17	11700.00	9103.00	2597.00	3.51
T11	1.11	11100.00	7011.00	4089.00	1.71
T12	0.98	9800.00	6000.00	3800.00	1.58
T13	0.87	8700.00	5200.00	3500.00	1.49
T14	1.23	12300.00	6200.00	6100.00	1.02
T15	0.90	9000.00	8700.00	300.00	29.00
T16	0.99	9900.00	5420.00	4480.00	1.21

11.4. Discussion

Chili is a profitable cash crop in Ethiopia. In this country, chili is a popular member of species and condiments for its special taste, flavor, color and even long-term storage ability etc. The most serious diseases of chili, anthracnose is the number one of the devastating diseases. It is understood that chemical fungicides are harmful to human health and environment; essential steps are being made, all over the world, to utilize plant extracts to control against plant diseases. Thus, as in other sections of medicine, Plant Pathologist around the world is making efforts to replace the use of toxic chemicals by more environment friendly less harmful substances.

By this time some plant extract have already been found having very effective strong fungicidal effect. In some cases, the reported effect is more than that of chemical pesticides. Out of the many such reported promising plants, on the simple basis of availability Garlic, Neem, onion and Cassia have been selected to assess their combat ability against, *Collectrotrichum capsici*, that is the ability against anthracnose of chili. Different integrated practices like (i) Control, (no treatment) (ii) Combination of Plant extract + Antagonist (*Trichoderma* spp) and Fungicide (Ridomil), foliar sprays of (iii) Neem, (iv) Onion, (v) Cassia, (vi) Garlic, (vii) Neem+Garlic + Cassia, (viii) Neem + Onion + Garlic extracts and (ix) Ridomil @0.2% (x), AAU-69(xi), AAU-Th + Garlic (xii), AAU-69 + AAU-Th + Onion(xiii), AAU-37 + AAU-Th + Neem (xiv), AAU-69 + AAU-37 + Neem(xv), and AAU-69 + AAU-37 + AAU-Th(xvi) were applied.

Yield and yield components, viz., Yield per plot (g) and yield ton/ha and cost benefit analysis were the parameters for data collection on three observations: Maraqo Fana, Oda Haro and Bako local were made, fruit disease was observed on the last two observation and the yield data was a cumulative of all the fruit collection from the respective plots.

The highest average fruit yields however were observed under the treatment T_6 and T_7 and the lowest under the treatment *To*. As a result low yield was found in control plots and high in the plots where combined treatments were applied. The combined treatment had a highly significant effect on the fruit yield. The results obtained by Rashid *et al*. (2015) with the use of

garlic and Achium and Schloesser (1992) with Neem *(Azadirachta indica)* leaf and Neem seed extracts nicely corroborate with the present findings.

The results obtained through this experiment indicated that a judicially designed combined organic treatment even may be profitable than a chemical fungicide treatment of all the fruit collection from the respective plots. The chili test plants which have received Ridomil at 0.2% had the lowest disease intensity as well as severity parameters. The sanitation (T_1) has shown the weakest disease control potential but significantly superior to the control (To). The plant extract treatments have shown significantly better control than the T_1, though they varied widely amongst themselves. The combined treatments T_6 (Neem + Onion + Cassia) and T_7 (Neem + Onion + Garlic) have shown very strong response though lesser than the T8 (Ridomil) in controlling anthracnose of chili.

The highest average fruit yields however were observed under the treatment T_6 and T_7 and the lowest under the treatment T_o. As a result, low yield was found in control plots and high in the plots where combined treatments were applied. The combined treatment had a highly significant effect on the fruit yield. This result corroborates the findings of Serawit and Tesfaye (2014) with the use of garlic and Ngullie *et al*. (2010) with Neem *(Azadirachta indica)* leaf and Neem seed extracts nicely corroborate with the present findings. The result from the benefit cost ratio analysis revealed that highest financial benefit was obtained from the combined treatment T15 where the BCR (43.0) was the highest and the second highest BCR was from T7 (5.25) while the lowest BCR (0.65) was observed in control plot. It was evident from the obtained results that comparatively low yields were responsible for the lower gross return and a lower BCR against each treatment. (Nashwa and Abo-Elyousr, 2012; Ademe *et al*., 2013)

11.5. Conclusion and Recommendation

This study indicated that combined organic treatment was profitable than a chemical fungicide treatment. Thus it can be concluded that IPM of chili by using Antagonists and plant extracts significantly increase yield. It can also be shown that be reduced by the combined use of (Trichoderma + Plant Extract + Fungicide) 1: 10 dilution suspension foliar spray in order to have a higher profitable yield and eventual higher economic return with minimum health risk as well as environmental pollution. Therefore, the farmers may be advised to take an integrated

approach which should to raise a profitable production without polluting the environment and adding toxins in the food chain.

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CHAPTER TWELVE:

SUMMARY, CONCLUSION AND RECOMMENDATIONS

12.1 Summary

The present thesis probes a broad overview of anthracnose disease in Ethiopia. The main aim of this study was; thus, to gather scientific information on the characterization of the pathogen and management of this disease in the country and through experimentation, generate recommendations that help farmers and decision makers to mitigate the disease pressure. The specific objectives of this study were to assess the magnitude of disease in main chili growing areas of the country; enhance current knowledge on the identity of Ethiopian pathogen isolates using cultural, pathological, physiological and morphological tools; evaluate antagonists, plant extracts and fungicides in vitro; study the effect of seedling density, phonological stages, and climatic variability on anthracnose development and yield parameters in chili; Search for variability and germplasm tolerance among the existing chili/pepper varieties in Ethiopia; analyze and develop the incidence-severity relationships model; identify economically effective timing and frequency of fungicide spraying programs; and analyze the effect of integrated anthracnose disease management on disease reactions, growth and yield parameters and economic profitability of chili production.

Rigorous survey was conducted in anthracnose prone areas; and the highest and lowest disease spread was observed in Alaba and Shashogo with cumulative incidence of 41.88% and 19.81%, respectively. From the chili farms, the highest incidence was found in Arsi negelle followed by Alaba with the value of 31.66% and 28.66%, whereas the lowest incidence in farms was found in Humbo and Maraqo with 13.63% and 14.89%. Nurseries with a highest incidence had been observed in Humbo and Alaba with values of 13.5% and 13.02%, respectively. The disease incidence was low, 4.13% and 1.28%, in Shashogo and Arsi negelle. Prevalence was higher in upper-kolla agro-ecological zones where the mean was recorded as 21.82% and 7.55 in farms and nurseries. The mean incidence of farms was three times higher than nurseries.

The variability study indicated that colonies varied in their cultural behavior ranging from cottony to fluffy, mostly suppressed with regular to irregular margins. Color of colonies ranged between white to grey. Growth rate of isolates was between 22.0 - 69.5 mm. Morphological studies of isolates revealed variations in their color, size, shape, acervuli production, setae size and shape, conidia. Average conidial size varied from 18.00 - 33.3 µm and average setae size varied from 77.2 -181.2 µm. On the basis of disease reaction expressed by differential hosts, eleven groups (races) of *C. capsici* were identified.

In invitro evaluation of fungicides, plant extracts and antagonists, all test concentrations of Tilt-250 EC significantly inhibited the mycelial growth of *Colletotrichum capsici*. Among the tested plant extracts, garlic at the highest concentration (15 %) was found to be best in the reduction both the radial mycelial diameter (72.33) and mycelial dry weight (73.33). There was significant variation among isolates of *Trichoderma* sppand antagonistic activities ranged from 51 to 89% reduction of the mycelial radial growth of *Colletotrichum capsici.* Among the promising antagonists, the isolate Tri_3 of *Trichoderma harzinum* showed the highest, 89 %, inhibition of mycelial radial growth of *Colletotrichum capsici*.

Experiment on planting densities and seedling phonological stages showed that symptom development was delayed one day in the youngest seedlings compared to the older ones. After the appearance of symptoms, for four consecutive days, the level of leaf disease incidence and severity was consistently lower on the youngest seedlings. Leaf wetness was highly reduced by increasing seedling spacing by at least 15 cm. The highest plant population densities yielded the highest weight of berries per plot.

In multi-locational, germplasm screening trial, the majority of the genotypes were moderately resistance to *C. capsici* and none of them was found to be immune at the two locations. Significant variations were also obtained among the genotypes for all yield components, namely percent establishment, dry fruit weight per plant, number of fruit per plant, pulp weight per plant, unmarketable fruits weight per plant, fruit length and days to 50 percent maturity. Total yield per plant were higher at Alaba than Maraqo. This variation is related to the level of disease intensity was higher at Maraqo. Anthracnose leaf incidence was consistently associated with leaf severity and their relationships can be estimated using the linear function across locations, crop seasons,

and genotypes. The economic implication of the timing and frequency of Ridomil application in the current context of fungicide use on chili in Ethiopia was also evaluated. A maximum of 7 applications starting from flushing successfully prevented the disease development and significantly reduced the incidence of leaf anthracnose.

The lowest plant infection (12.8%), leaf infection per plant (15.2%), percent diseased leaf area(15.2%)and infected fruits per plot (17.4%) was observed on combined application of isolates *Trichoderma* spp, plant extracts and Ridomil in Maraqo fana variety. Regarding the growth parameters, viz. the highest Mean Percent establishment (81.67), mean days to 50% flowering (65.33), mean days to 50% maturity (82) days to first harvest (106.3) in was observed in T16, T16, T4, and T8, respectively. From the quality parameters, the highest mean number of branches per stem (9), mean canopy diameter (24.8), mean number of flowers per plant (9.6) and mean plant height (61.4) in T10, T15, T6 and T7, respectively. Both negative and positive control showed higher incidence and severity as compared to single and combined application of isolates *Trichoderma* spp, plant extracts and Ridomil. The combined application of Trichoderma, plant extracts and Ridomil performed best in number of fruits/plant, number of seeds/pod, unmarketable fruit, marketable fruit and total yield. This mix up gave a higher benefit-cost-ratio due to reduced production cost indicating that a judicious combination of organic management practice is environment friendly, healthy and sustainable.

Over and above, through analyzing and integrating the obtained result on the subject, it had been successfully addressed issues that concerned previously inaccessible information from those that reflect insufficient scientific knowledge. The study accurately resolved the bottle-neck problems of chili anthracnose (*Colletotrichum capsici* (syd.)) and integrated disease management practices of pepper disease in Ethiopia. Subsequently, the information obtained will be used to develop a national strategic framework for research and extension in the country. Areas where scientific information lacked were identified.

12.2 Conclusion

The survey conducted in anthracnose prone areas showed that the highest and lowest disease spread was observed in Alaba and Shashogo with cumulative incidence of 41.88% and 19.81%, respectively. From the chili farms, the highest incidence was found in Arsi negelle followed by

Alaba with the value of 31.66% and 28.66%, whereas the lowest incidence in farms was found in Humbo and Maraqo with 13.63% and 14.89%. Nurseries with a highest incidence were observed in Humbo and Alaba with values of 13.5% and 13.02%, respectively. The disease incidence was low, 4.13% and 1.28%, in Shashogo and Arsi negelle. Prevalence was higher in upper-kolla agro-ecological zones where the mean was recorded as 21.82% and 7.55% in farms and nurseries, respectively.

Diversity study indicated that the collected isolated varied in their cultural pigmentation ranging from cottony to fluffy types, mostly suppressed with regular to irregular margins. *Colletotrichum* spp isolates' central color ranged between white to grey. Growth rate of isolates on PDA medium was between 22.0 - 69.5 mm. Morphological studies of isolates revealed variations in their color, size, shape, acervuli production, setae size and shape of conidia. Average conidial size varied from 18.00 - 33.3 µm and average setae size varied from 77.2 -181.2 µm. On the basis of disease reaction expressed by differential hosts, eleven groups (races) of *C. capsici* were identified.

Experiment on evaluation of fungicides, plant extracts and antagonists showed that all test concentrations of Tilt-250 EC significantly inhibited the mycelial growth of *Colletotrichum capsici*. Among the tested plant extracts, garlic was the best both in reducing the radial mycelial diameter (72.33) and mycelial dry weight (73.33) at the highest concentration of 15 %. There was significant variation among isolates of *Trichoderma* spp and antagonistic activities ranged from 51% to 89% reduction of the mycelial radial growth of *Colletotrichum capsici* on the PDA medium. Among the promising antagonists, the isolate Tri_3 of *Trichoderma harzinum* showed the highest, 89 %, inhibition of mycelial radial growth of *Colletotrichum capsici*.

Experiment on planting densities and seedling phonological stages showed that symptom development was delayed one day in the youngest seedlings compared to the older ones. After the appearance of symptoms, for four consecutive days, the level of leaf disease incidence and severity was consistently lower on the youngest seedlings. Leaf wetness was highly reduced by increasing seedling spacing by at least 15 cm. The highest plant population densities yielded the highest weight of berries per plot.

In multi-locational germplasm screening trial, the majority of the genotypes were moderately resistance to *C. capsici* and none of them was found to be immune at the two locations. Significant variations were also obtained among the genotypes for all yield components, namely percent establishment, dry fruit weight per plant, number of fruit per plant, pulp weight per plant, unmarketable fruits weight per plant, fruit length and days to 50 percent maturity. Total yield per plant was higher at Alaba than Maraqo high level of disease incidence at Alaba. Anthracnose leaf incidence was consistently associated with leaf severity and their relationships can be estimated using the linear function across locations, crop seasons, and genotypes. The economic implication of the timing and frequency of Ridomil application in the current context of fungicide use on chili in Ethiopia was also evaluated. Less frequent applications (3-7 times) starting from flushing successfully prevented the disease development and significantly reduced the incidence of leaf anthracnose.

The lowest plant infection (12.8%), leaf infection per plant (15.2%), percent diseased leaf area(15.2%)and infected fruits per plot (17.4%) was observed on combined application of isolates *Trichoderma* spp, plant extracts and Ridomil in Maraqo fana variety. Regarding the growth parameters, viz. the highest Mean Percent establishment (81.67), mean days to 50% flowering (65.33), mean days to 50% maturity (82) days to first harvest (106.3) in was observed in T16, T4, and T8, respectively. From the quality parameters, the highest mean number of branches per stem (9), mean canopy diameter (24.8), mean number of flowers per plant (9.6) and mean plant height (61.4) in T10, T15, T6 and T7, respectively. Both negative and positive control showed higher incidence and severity as compared to single and combined application of isolates *Trichoderma* spp, plant extracts and Ridomil. The combined application of Trichoderma, plant extracts and Ridomil performed best in number of fruits/plant, number of seeds/pod, unmarketable fruit, marketable fruit and total yield. This mix up gave a higher benefit-cost-ratio due to reduced production cost indicating that a judicious combination of organic management practice is environment friendly, healthy and sustainable.

12.3 Recommendations

From the outcome of this study, the following recommendations are forwarded.

- Incidence and severity of chili anthracnose is high across the country. Thus, research centers, regional bureau of agriculture, the decision makers, policy experts and development agents shall revisit their prioritization shift from other less economic diseases and embark on controlling chili anthracnose in Ethiopia.
- Morphological identification of fungal plant pathogens is time taking and also potentially erroneous as it depends on nuance structural differences. While molecular identification methods are more accurate and quick, there are limitations in laboratory facilities and access to the molecular recipes in Ethiopia. Therefore, an in-depth molecular diagnosis shall be carried out to characterize anthracnose pathogen.
- Wise use of plant extracts, fungicides and antagonists must be enhanced to curtail chili anthracnose. In light with this study, there will be gradual shift from "routine fungicide use" to "apply when necessary" regime owing to these cheap and effective tactics ensuing in sustainable chili production.
- Prophylactic application of fungicides on pepper seedlings should commence only from the phenological stage of six leaves and as the seedlings grow, spacing must be widened to minimize both plant-to-plant inoculation and locally conducive micro-climatic conditions for infection. This approach can reduce the cost and risk of transplanting infected pepper seedlings.
- To continually renovate and implement an integrated control strategy, analyzing the efficacy of the individual component of an integrated measure against the test pathogen is vital. The results of the current *in vitro* evaluation of fungicides, plant extracts and *Trichoderma* isolates are recommended against *Colletotrichum capsici* of chili to develop an eco-friendly integrated disease management of anthracnose of chili in the field.
- Sources of resistance are recommended to be utilized in future pepper breeding programs. On the other hand, spraying shall be done starting at flushing phonological phase of the crop and therefore the fungicide application plan is highly recommended. Otherwise, integrated use of *Trichoderma spp* and plant extracts can be recommended. Conventional fungicides will ultimately be replaced by antagonists and botanicals. This will improve crop quality and growth; maximize profitability of chili and fundamental sustenance.

- Future work should also focus on gene marking of tolerant genotypes as a way through for germplasm introductions. Continous characterization of *C. capsici* (syd.) isolates and comparison by cross inoculation in a variety of cultivars and environmental conditions can be of practical value in development of local or global disease management strategies.

Academic contributions include seven publications in an International Journal of Sciences: Basic and Applied Research, International Journal of Basic and Applied Sciences, International Journal of Life Sciences, and American Journal of Engineering and sciences (Appendices 7.1 - 7.9). One article was published in edited proceedings of Plant Protection Society of Ethiopia and two other articles are currently under process for publication in Biological Society of Ethiopia. In conclusion, the present study provided a significant contribution towards reduction of pepper losses caused by anthracnose in Ethiopia and has opened new avenues for future chili research.

APPENDICES

Appendix 2.1: The Pepper Map of Ethiopia

Chilli peppers are cultivated especially in the higher lands of SNNP (10) and Oromia (8) regions.

Ginger and turmeric are grown in the lower lands of SNNP (10), Oromia (8) and Afar (2).

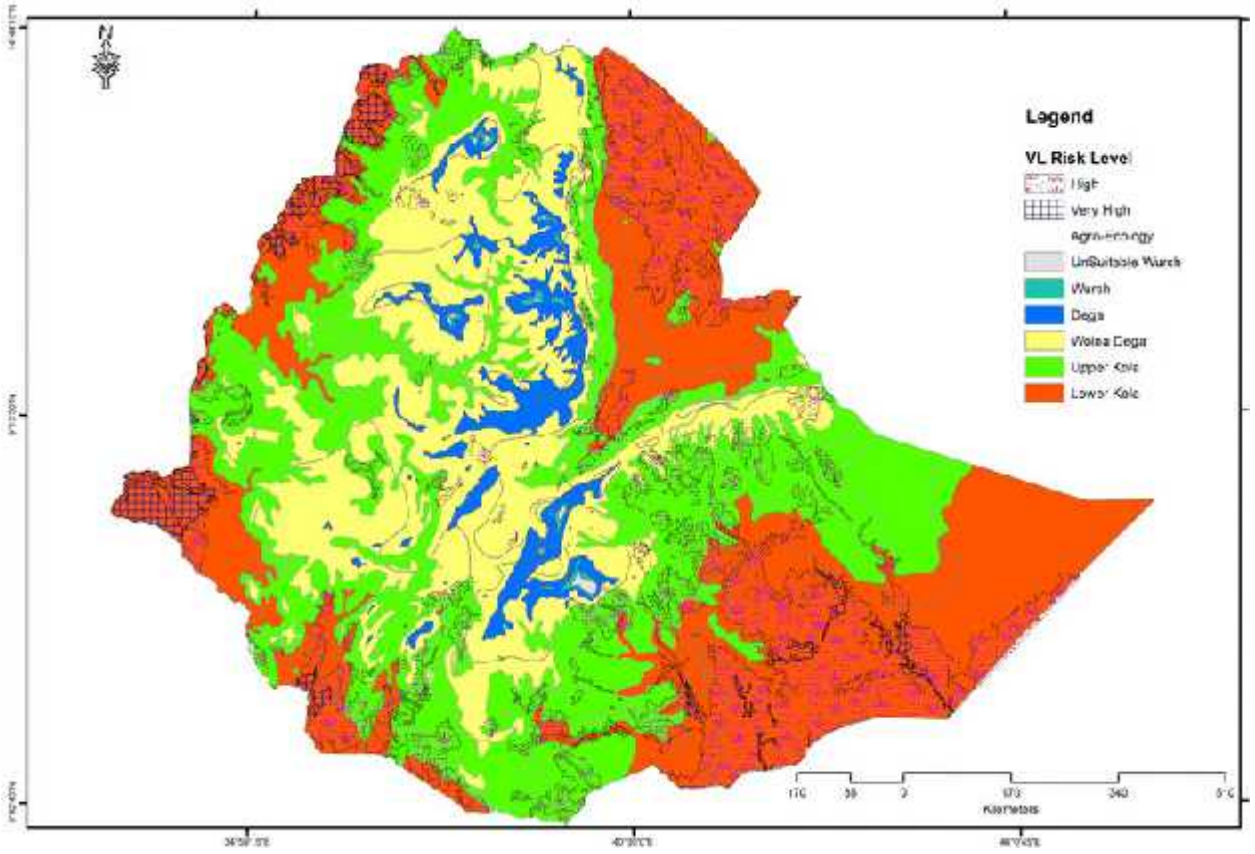
1.Addis Ababa 2.Afar 3.Amhara 4.Benishangul-Gumuz 5.Dire Dawa	6.Gambela 7.Harari 8.Oromia 9.Somali 10.SNNP: Southern Nations, Nationalities, & People's Region 11.Tigray

Appendix 3.1 General characteristics of trial sites from which anthracnose disease incidence and severity data had been collected in 2013

Trial Name	Latitude	Longitude	Altitude (m)	Region/Zone	AE Zone	Time
ADDIS ABABA						
AAU, 4kilo	9.037354	38.76779	2435.84	Addis Ababa	6	2013 -07-20
HADIYA						
Doisha	7.473552	38.4449	1900.00	Hadiya, SNNPR	1	2013 -09-20
Hossana	7.552825	37.85649	2309.44	Hadiya, SNNPR	2	2013 -08-01
Bonosha Mazoriya	8.042378	38.49944	1840.08	Hadiya SNNPR	3	2013 -08-08
East Badawacho	7.417574	38.19376	1774.78	Hadiya, SNNPR	1	2013 -08-17
Soro	8.142378	38.59944	1840.08	Hadiya SNNPR	3	2013 -08-18
GURAGE & SILTIE						
Sankura	7.353447	38.09112	1823.63	Silti, SNNPR	5	2013 -09-8
Sankura	7.353447	38.09112	1823.63	Silti, SNNPR	3	2013 -09-10
Worabe	7.737333	38.12154	1988.49	Silti, SNNPR	2	2013 -09-09
Alkeso	7.848471	38.18766	2096.36	Silti, SNNPR	4	2013 -09-18
Menaheria	7.918454	38.23706	2306.69	Silti, SNNPR	6	2013 -10-24
Qibet	7.949281	38.26793	2389.2	Gurage, SNNPR	3	2013 -09-21
Mareko-Guraghe Borders	8.024675	38.32799	2120.24	Mareko, SNNPR	3	2013 -10-22
Qoshe Fields	8.01513	38.53197	1872.82	Mareko, SNNPR	4	2013 -10-20
Butajira zuria	7.918454	38.23706	2306.69	Butajira, SNNPR	6	2013 -10-25
Meskan	7.949281	38.26793	2389.2	Butajira, SNNPR	3	2013 -10-23
Azernet-berbere	7.918454	38.23706	2306.69	Silti, SNNPR	6	2013 -10-24
WOLAITA						
Wolaita Sodo	6.852763	37.76414	1997.79	Wolaita, SNNPR	3	2013 -09-11
Humbo Tebella	6.703099	37.7751	1590.79	Wolaita, SNNPR	4	2013 -09-12
Mirab Abaya	6.652763	37.76414	1997.79	Gamogofa, SNNPR	2	2013 -09-13
Boditi (Damot Galle)	7.703099	37.7751	1590.79	Wolaita, SNNPR	4	2013 -09-14
Areka	7.852763	37.76414	1997.79	Wolaita, SNNPR	2	2013 -09-16
KAMBATA & ALABA						
Halaba Field A	7.317574	38.09376	1774.78	Alaba, SNNPR	6	2013 -11-07
Halaba Field B	7.389356	38.1042	1825.77	Alaba, SNNPR	3	2013 -11-08
Hadero	7.989356	38.4042	1825.77	Kambata, SNNPR	3	2013 -11-09
Mazoria	7.889356	38.3042	1815.00	Kambata, SNNPR	3	2013 -11-10
OROMIYA						
Wonji	8.4538411	39.280399	- -	Adama, Oromiya	2	2013 -10-20
Adama zuria	8.5263486	39.2583293	- -	Adama, Oromiya	2	2013 -10-20
Arsi Negelle	7.3610886	38.668713	- -	West Arsi, Oromiya	1	2013 -10-22
Nekemte	9.0893009	36.555386	- -	W. wollega Oromia	1	2013 -10-27
Gute	9.3208484	36.671451	- -	W. wollega Oromia	3	2013 -10-27
Ano	9.0928759	36.959483	- -	W. wollega Oromia	2	2013 -10-28

Bako	9.1248249	37.0588169	- -	W. wollega Oromia	2	2013 -10-29
AMHARA						
Bure	10.708145	37.0668651	- -	E. Gojam, Amhara	3	2013 -10-15
Finote-selam	10.697988	37.176773	- -	E. Gojam , Amhara	2	2013 -10-15

Appendix 3.2 Map of Agro-ecological zones (R1 to R6). Source: EFDRE, NMA



Appendix 3.3 Fungicides applied on Chili seedlings in different regions, zones, kebelles, and locations of Ethiopia (Survey Conducted in 2013)

Order No.	District and SD	Kebelle	Special location (Got)	Insecticide			Interval (days)*
				Fungicide Name	Active Ingredient	Concentrations	
1	Alaba	1	i	Mancozeb	Copper oxychloride	3.5g/l	3
2	Alaba	2	ii	Mancozeb	Copper oxychloride	0.5g/l	3
3	Alaba	3	iii	Mancozeb	Copper oxychloride	2.4g/l	5
4	Alaba	4	iv	Mancozeb	Copper oxychloride	0.65g/l	5
5	Alaba	5	v	Mancozeb	Copper oxychloride	dn	7
1	Humbo	1	i	Flint	Trifloxystrobin	dn	dn
2	Humbo	2	ii	Flint	Trifloxystrobin	5g/l	5
3	Humbo	3	iii	Flint	Trifloxystrobin	25 g/l	dn
4	Humbo	4	iv	Flint	Trifloxystrobin	1.5 g/l	7
5	Humbo	5	v	Flint	Trifloxystrobin	1.5 g/l	7
1	Mareko	1	i	Anvil	Hexaconazole	8.5g/l	7
2	Mareko	2	ii	Anvil	Hexaconazole	0.5g/l	dn
3	Mareko	3	iii	Volcano	Hexaconazole	dn	dn
4	Mareko	4	iv	Volcano	Hexaconazole	1.75ml/l	5
5	Mareko	5	v	Volcano	Hexaconazole	dn	7
1	Shashogo	1	i	Flint	Trifloxystrobin	dn	7
2	Shashogo	2	ii	Flint	Trifloxystrobin	dn	5
3	Shashogo	3	iii	Flint	Trifloxystrobin	dn	5
4	Shashogo	4	iv	Flint	Trifloxystrobin	dn	5
5	Shashogo	5	v	Flint	Trifloxystrobin	dn	7
1	Arsi negelle	1	i	Bayfidin	Triadimenol	dn	7
2	Arsi negelle	2	ii	Flint	Trifloxystrobin	dn	7
3	Arsi negelle	3	iii	Flint	Trifloxystrobin	dn	5
4	Arsi negelle	4	iv	volcano	Hexaconazole	dn	3
5	Arsi negelle	5	v	volcano	Hexaconazole	dn	7
	Mean						**13.7**
	SD						**5.25**

Dn= the respondent does not know

Appendix 3.4 Chili nursery pest and Disease Symptoms and Common Practices in Ethiopia, 2014. Low, Medium, Higher severity, very low severity damage (A, B, C and D)




Appendix 3.5: Survey Questionnaire

Questionnaire on Knowledge, Prevalence and Importance of Chili Anthracnose in Ethiopia

1. For the Nursery/FTC Personnel

1.1. Region……, 1.2. District……, 1.3. Administrative Post……

1.4. Location……., 1.5. Date…….

1.6. Interviewer……, 1.7. Respondent……

1.8. How many Chili seedlings (number) are in the nursery now? .....

1.9. For how long (number of days or months) the present seedlings have been maintained in the nursery? .....

1.10. How many (number) of the present seedlings have symptoms similar to those in the Photo ? ……

1.11. What is the ratio between diseased and healthy seedlings (that is X/Y: X = diseased and

Y = total number of seedlings)……..

1.11.1. On transplanted seedlings ……..…….

1.11.2. On non-transplanted seedlings……..……...

1.12. Are the seedlings watered? (Yes/no). How often (number of times per day or per Week)……

1.13. Are the plots sprayed with fungicides before flowering?

Yes…….. No……..……

1.14. If yes, how? .....……

1.15. What is the effect of spraying? …….

How is the spraying effect measured? ……..

1.16. Is there any of the years when you have no seedlings in the nursery?……

Which one (from …… to ……

1.17. Are the mother sprayed with chemicals? ……

What is the chemical…….…….. and

Rate of application ……. And

Frequency?……

Aimed to control (pest and disease names) ……..

1.18. Are the seedlings in the nursery sprayed with any chemical?……...……..

Which one (name)?……. , At which frequency?…….. and which rate of application? ……..

1.19. What is the actual damage of the symptoms observed on seedlings? (Signal the correct answers).

1.19.1. They only defoliate the seedlings……..…….…..….

1.19.2. They do kill the seedlings…….

1.19.3. They cause no problem to the seedlings……..……..…

1.20. When (period of months) are the symptoms observed? …….…….

1.21. When do (month) the appearance of new symptoms end?.....

1.22. Do nearby adult plants (up to 300 m) show similar symptoms or not?……

2. For the farmers and 'other' personnel

2.1. Region …… . 2.2. District……., 2.3. Administrative Post……..

2.4. Location……., 2.5. Date……..

2.6. Interviewer……., 2.7. Respondent……

2.8. When preparing from mature plants, have you ever come across symptoms similar to those illustrated in the photos above?……..

2.9. Do such symptoms affect the quality of the chili fruit?……..…..…

2.10. If yes to 2.9, then how do you assess the damage?……....

2.11. What do you do to minimize the damage?……

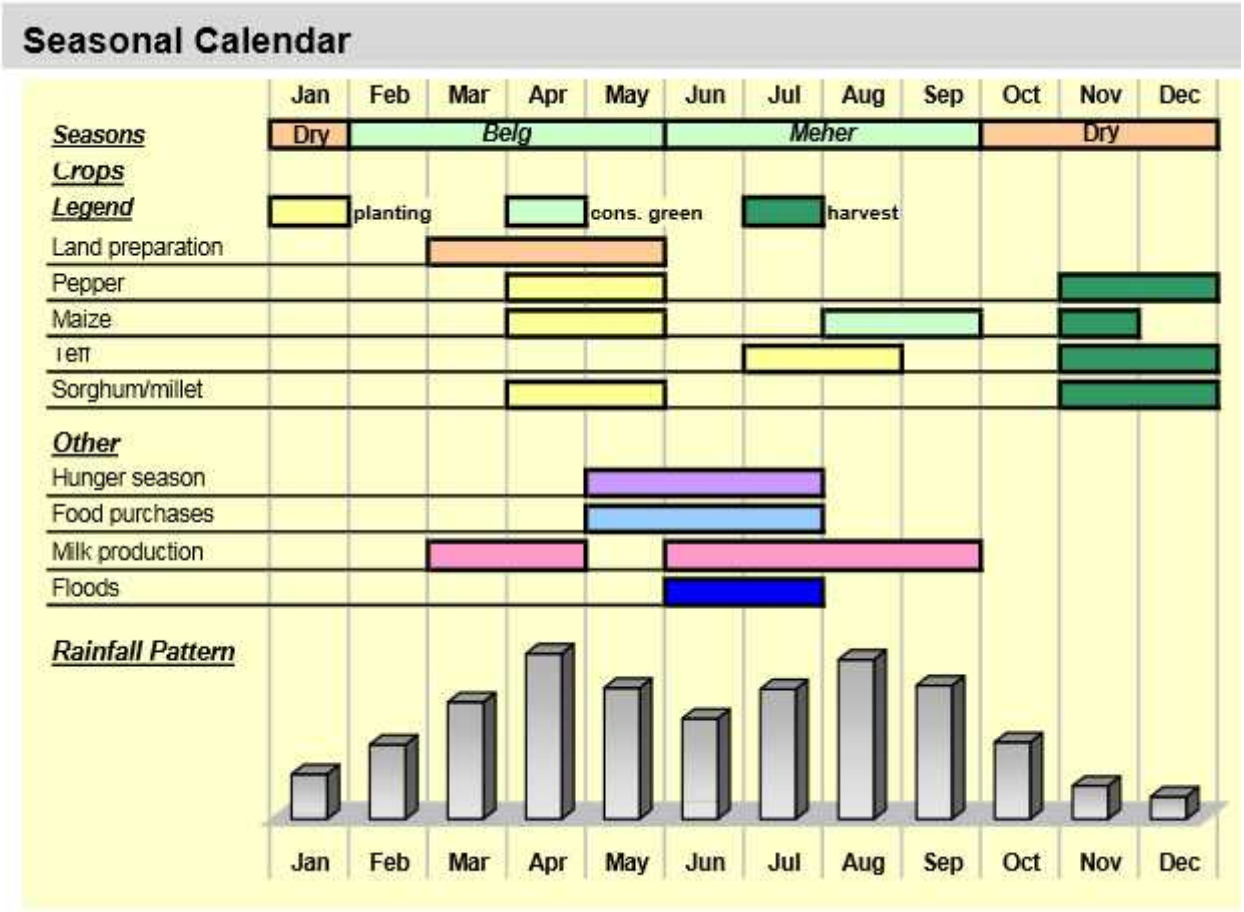
2.12. Are the matured plants sprayed in order to control these symptoms? ……

2.13. If yes, what is the chemical, the rate and the frequency of application? ……

2.14. Describe how the seedlings are uprooted from the nursery, packed and transported to the nursery ….…….……

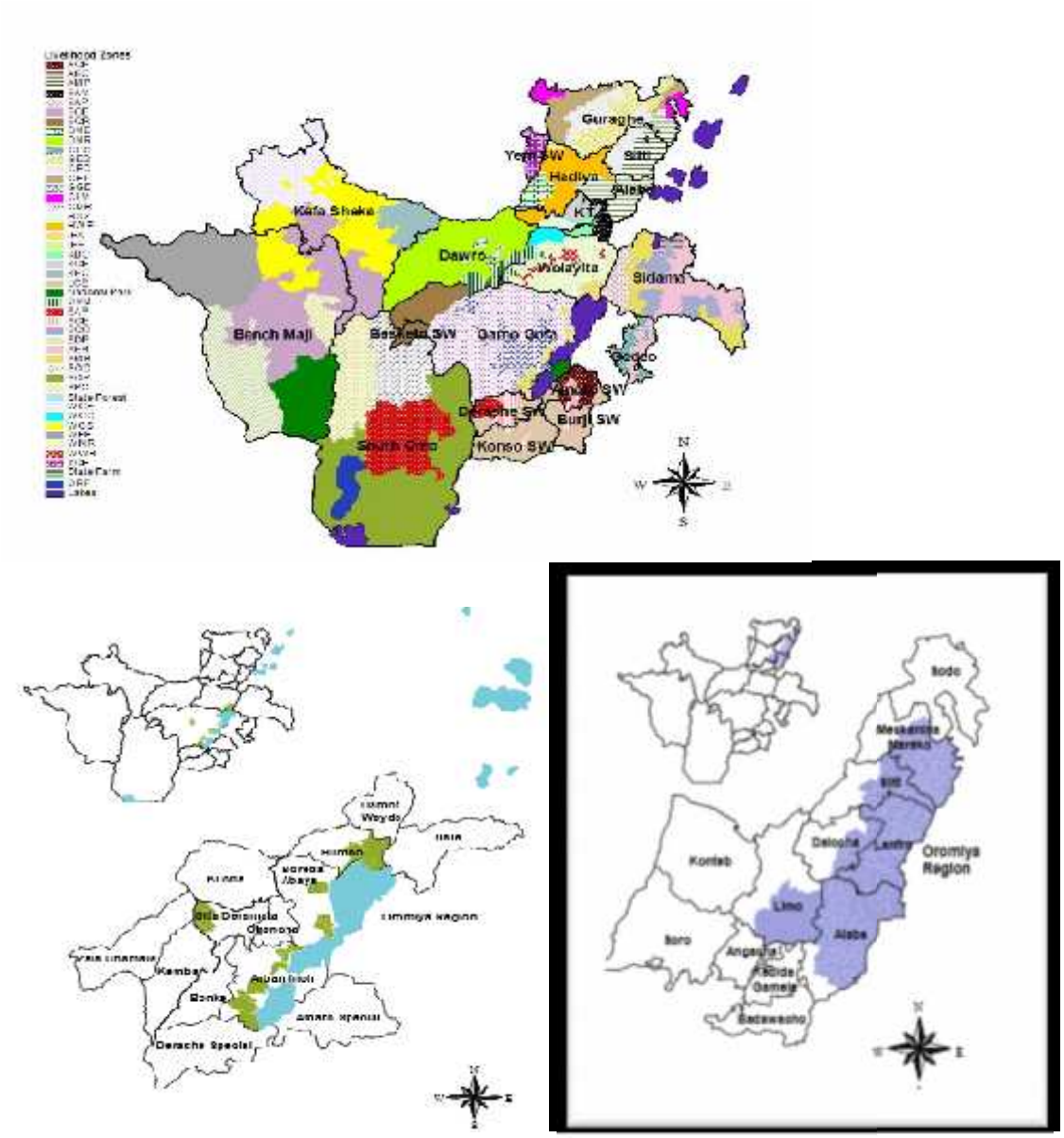
2.15. What basis (criteria) do you use to group the Region, e.g. by size, color, cultivar, quality, Origin, etc ……...……

Appendix 3A: Calendar of Events on Pepper Production in Ethiopia



Source of rainfall data: National Meteorological Service Agency (NMSA) Data Archives (long-term average).

Appendix 3B : Pepper livelihood zones in SNNP, Ethiopia



Appendix 3C : Pepper livelihood zones in SNNP, Ethiopia

CODE	NAME OF LIVELIHOOD ZONE	GEOGRAPHY AREA AND AGR-ECOLOGY
GLM	Gurage lowland maize and teff LZ	North-east lowland
AMP	Alaba-marako lowland pepper LZ	North-east lowland
GET	Gurage-siltie Enset and Teff LZ	North-east midland/lowland
GEC	Gurage-siltie Midland Enset and chat LZ	North-east midland
GEB	Gurage-siltie Highland Enset and Barley LZ	North-east highland
YCE	Yem cereal and Enset LZ	North-east midland/highland
HWE	Hadiya-Kembata Cereal and Enset LZ-Hadiya SZ	North-east midland/highland
KCE	Hadiya-Kembata Cereal and Enset LZ-Kembata SZ	North-east midland/highland
BAM	Badawacho-Alaba Maize LZ	North-east midland
KBC	Kedida-Badawacho coffee LZ	North-east midland
HGZ	Hadero Ginger LZ	North-east lowland/midland
WCG	Wolaita Ginger and coffee LZ	Central midland/lowland
WMR	Wolaita Maize and Root crop LZ	Central midland/lowland
DMR	Dawro-konta maize and root crop LZ	Central midland/lowland
GMR	Gamo-Goffa Maize and root crop LZ	Central midland/lowland
BCRB	Basketo-melo coffee and root crop LZ	Central midland/highland
WWB	Wolaita Barley and wheat LZ	Central midland/highland
GGEB	Gamo Gofa Enset and Barley LZ	Central midland/highland
OVM	Omo valley maize and sorghum LZ	Central lowland
IBA	Chamo-Abaya Irrigated Banana LZ	Central lowland
BAP	Bilate Basin Agro-pastoral LZ	Eastern lowland
SMB	Sidama maize belt LZ	Eastern lowland/midland
AEC	Awassa Chat and Enset LZ	Eastern lowland
SCO	Sidama Coffee LZ	Eastern lowland
GCO	Gedeo Coffee LZ	Eastern lowland
SEB	Sidama-Gedeo Highland Enset and Barley LZ	Eastern highland
LCE	Southern special woredas lowland cereal LZ	Southern lowland
SCE	Southern cereal, Enset and Root Crop LZ	Southern midland/lowland
ACE	Amaro Coffee and Enset LZ	Southern midland/lowland
SOP	South Omo Pastoral LZ	South-west rangeland
SPO	Salamago Pastoral LZ	South-west rangeland
SDP	Surma Agro- Pastoral LZ	South-west low/midland
SAP	Southern Ago- Pastoral LZ	South-west lowland
SOC	South Omo crop LZ	South-west high/midland
WCE	Sheka cereal and Enset LZ	Western midland/highland
KEC	Keffa cereal and Enset LZ	Western midland/highland
BCE	Bench-keffa cereal and Enset LZ	Western midland/highland
ECS	Western Coffee and species LZ-Eastern sub-zone	western midland
WCS	Western Coffee and species LZ-western sub-zone	western midland
WFP	Western forest products LZ	western low/midland

Appendix 3D: Characteristics of Alaba-Mareko Lowland Pepper LZ

This relatively food secure zone has a valuable cash crop industry that attracts migrant laborers from other zones. The population is relatively sparse and land-holdings are large enough to allow even poor households to grow nearly 60% of their food needs as well as gaining more than 60% of their cash earnings from sale of pepper, as do middle and better-off households. In addition, teff and other crops are sold. Livestock production, especially cattle, is important for middle and better-off households, where sales amount to some 20% of annual cash earnings. Even poor households make around one-tenth of their income from selling butter. There is no irrigated production in recent years; but floods from neighboring highlands are also frequent problem, although at the same time as causing damage they deposit fertile silt.

Appendix 3E:- Anthracnose incidence means on Chili seedlings per regions, zones, kebelles, and locations of Ethiopia (Survey Conducted in 2013)

Order No.	Regions(Zones)	District and SD	Special location (Got)	Incidence on Seedlings (%)		
				Transplanted	Planted	Mean
1	SNNP, Alaba sp. wor	Alaba	A	36	100	68
2	SNNP, Alaba sp. wor	Alaba	B	42	3	22.5
3	SNNP, Alaba sp. wor	Alaba	C	28	14	21
4	SNNP, Alaba sp. wor	Alaba	D	34	11	22.5
5	SNNP, Alaba sp. wor	Alaba	E	56	21	38.5
		Mean		**21.78**	**29.8**	**34.5**
		SD		**10.64**	**16.56**	**16.56**
1	SNNP, Wolaita	Humbo	A	22	4	13
2	SNNP, Wolaita	Humbo	B	23	19	21
3	SNNP, Wolaita	Humbo	C	15	5	10
4	SNNP, Wolaita	Humbo	D	8	6	7
5	SNNP, Wolaita	Humbo	E	9	3	6
		Mean		**8.56**	**7.4**	**11.4**
		SD		**7.02**	**6.58**	**6.03**
1	SNNP, Guraghe	Mareko sp. dis.	A	12	5	8.5
2	SNNP, Guraghe	Mareko sp. dis.	B	2	9	5.5
3	SNNP, Guraghe	Mareko sp. dis.	C	41	6	23.5
4	SNNP, Guraghe	Mareko sp. dis.	D	25	4	14.5
5	SNNP, Guraghe	Mareko sp. dis.	E	36	8	22
		Mean		**23.2**	**3.6**	**3.6**
		SD		**16.3**	**2.07**	**2.07**
1	SNNP, Hadiya	Shashogo	A	12	6	9
2	SNNP, Hadiya	Shashogo	B	2	8	5
3	SNNP, Hadiya	Shashogo	C	41	7	24
4	SNNP, Hadiya	Shashogo	D	25	9	17
5	SNNP, Hadiya	Shashogo	E	36	8	22
		Mean		**23.2**	**4.23**	**4.23**
		SD		**16.3**	**1.14**	**1.14**
1	Oromiya, Lome	Arsi negelle	A	2	2	2
2	Oromiya, Lome	Arsi negelle	B	4	5	4.5
3	Oromiya, Lome	Arsi negelle	C	1	6	3.5
4	Oromiya, Lome	Arsi negelle	D	2	2	2
5	Oromiya, Lome	Arsi negelle	E	1	7	4
		Mean		**2**	**4.4**	**3.2**
		SD		**1.23**	**2.3**	**1.15**
1	Oromiya, East Shoa	Adama	A	3	2	2.5

2	Oromiya, East Shoa	Adama	B	2	5	3.5
3	Oromiya, East Shoa	Adama	C	1	8	4.5
4	Oromiya, East Shoa	Adama	D	5	9	7
5	Oromiya, East Shoa	Adama	E	4	6	5
		Mean		**3**	**6**	**4.5**
		SD		**1.58**	**2.7**	**1.7**
		Overall Mean		**13.6**	**9.24**	**10.24**
		Overall SD		**6.7**	**5.8**	**6.06**

NA=No plants available; SD= Standard deviation

Appendix 3.F Anthracnose incidence means on Chili seedlings in different Agro-ecological zones, Seasons and locations in some selected areas of Ethiopia

Agro-Ecological Zone	Order No.	District and SD	Kebelle	Special location (*Got*)	Mean Incidence on Seedlings (%)		
					On-season	Off-season	Overall
Upper Kolla	1	Alaba	Keb.1	1	93	10	51.5
Upper Kolla	2	Alaba	Keb.2	2	52	13	32.5
Upper Kolla	3	Alaba	Keb 3	3	32	16	24
Upper Kolla	4	Alaba	Keb.4	4	42	17	29.5
Upper Kolla	5	Alaba	Keb. 5	5	57	25	41
		Mean			**55.2**	**16.2**	**35.7**
		SD			**23.21**	**5.63**	**10.76**
Upper kolla	1	Humbo	Keb.1	1	27	14	20.5
Upper Kolla	2	Humbo	Keb.2	2	33	19	26
Upper Kolla	3	Humbo	Keb 3	3	35	15	25
Upper Kolla	4	Humbo	Keb.4	4	28	16	22
Upper Kolla	5	Humbo	Keb. 5	5	19	13	16
		Mean			**28.4**	**15.4**	**21.9**
		SD			**6.23**	**2.31**	**3.97**
Woyina Dega	1	Maraqo	Keb.1	1	52	25	38.5
Woyina Dega	2	Maraqo	Keb.2	2	62	29	45.5
Woyina Dega	3	Maraqo	Keb 3	3	51	16	33.5
Woyina Dega	4	Maraqo	Keb.4	4	55	14	34.5
Woyina Dega	5	Maraqo	Keb. 5	5	66	18	42
		Mean			**57.2**	**20.4**	**38.8**
		SD			**6.53**	**6.35**	**5.04**
Upper Kolla	1	Shashogo	Keb.1	1	72	16	44
Upper Kolla	2	Shashogo	Keb.2	2	56	18	37
Upper Kolla	3	Shashogo	Keb 3	3	67	17	42
Upper Kolla	4	Shashogo	Keb.4	4	75	19	47
Upper Kolla	5	Shashogo	Keb. 5	5	56	18	37
		Mean			**65.2**	**17.6**	**41.4**
		SD			**8.871**	**1.141**	**4.39**
Upper Kolla	1	Arsi negelle	Keb.1	1	72	22	47
Upper Kolla	2	Arsi negelle	Keb.2	2	54	50.5	52.25
Upper Kolla	3	Arsi negelle	Keb 3	3	71	66	68.5
Upper Kolla	4	Arsi negelle	Keb.4	4	82	12	47
Upper Kolla	5	Arsi negelle	Keb. 5	5	81	27	54
		Mean			**72**	**35.5**	**53.75**

		SD			**11.24**	**22.14**	**8.82**
Upper Kolla	1	Adama	Keb.1	1	73	22	47.5
Upper Kolla	2	Adama	Keb.2	2	82	25	53.5
Upper Kolla	3	Adama	Keb 3	3	61	28	44.5
Upper Kolla	4	Adama	Keb.4	4	55	29	42
Upper Kolla	5	Adama	Keb. 5	5	64	36	50
		Mean			**67**	**28**	**47.5**
		SD			**10.61**	**5.24**	**4.51**
		Overall Mean (%)			**57.5**	**22.18**	**39.84**
		Overall SD			**6.27**	**7.63**	**2.83**

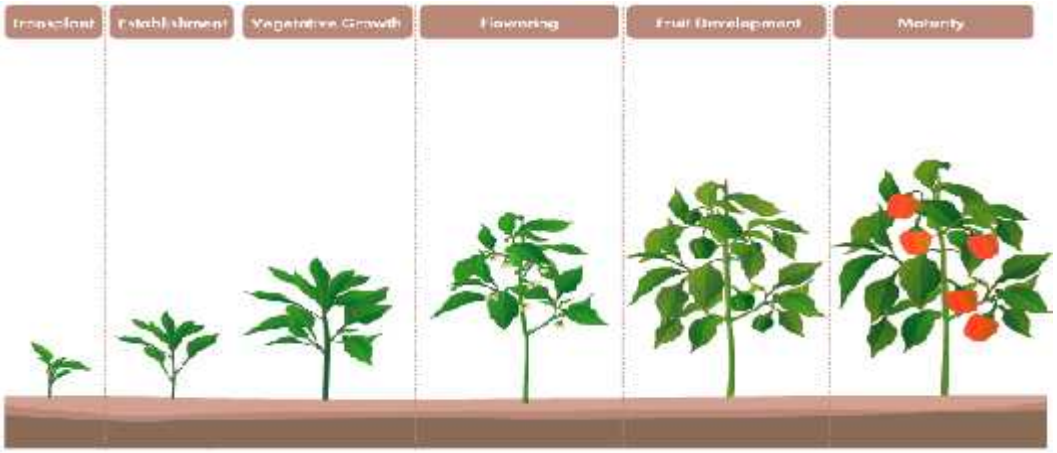
SD= Standard deviation

Appendix 3G Prevalence Incidence and Severity of Chili seedlings in 132 farms in different regions, zones, kebelles, and locations of Ethiopia (Survey Conducted in 2013)

S. No.	District	No. of Fields Assessed	Mean Percent of Anthracnose disease:		
			Prevalence	Incidence	Severity
1	Humbo	4	70	37	70
2	Wolaita sodo Zuria	6	60	75	50
3	Mirab Abaya	8	100	50	50
4	Boditi (Damot Galle)	4	50	70	70
5	Areka	5	56	50	30
	Mean of Wolaita		67.2	56.4	54
1	Hossana zuria (Lemo)	6	100	70	70
2	Doisha	7	100	50	50
3	Shashogo	6	50	30	30
4	East Badawacho	4	75	40	40
5	Soro	5	50	50	50
	Mean of Hadiya		75	48	48
1	Mareko-Guraghe Borders	8	60	30	30
2	Qosha zuria	6	50	60	50
3	Butajira zuria	6	70	40	70
4	Meskan	4	70	75	70
5	Azernet-berbere	5	40	40	40
	Mean of Guraghe and siltie		58	49	52
1	Alaba	6	100	35	80
2	Sankura	7	80	70	75
3	Worabie zuria	8	80	75	70
4	Hadero	4	70	75	60
5	Mazoria	5	70	80	75
	Mean of KA		80	67	72
1	Adama-Wonji	4	40	40	14
2	Mojo-Ziway-Meki	4	30	30	13
3	Arsi negelle	3	40	10	14
4	Amhara-Bure	4	70	70	17
5	Amhara- Finoteselam	3	40	14	14
	Mean of Oromiya & Amh.		**44**	**32.8**	**14.4**
	Standard deviation		**14.32**	**12.54**	**20.96**

Appendix 4A: Pepper phenological guide (including visual key)

Appendix 4B: Pepper phonological stage



Appendix 4C: Initial phenological stages

Third Phase

The shoot has grown to 10 cm, the four most external leaves are 10-12 cm long, the next two are 6-8 cm in length while the internal leaves, which almost wrap the flower bud, are 2-3 cm long. The inflorescence length from the base bract (i.e. the ultimate small leaf not yet fully developed), is approximately 2-3 cm long. The reddish- brown color of the leaves is becoming attenuated and a yellow orange shade is beginning to appear at the bottom of the leaves.

Fourth Phase

The earliest four leaves progressively lose their typical reddish brown color of the earlier stages and become pale green. The four younger leaves, formed later, still retain their native color, toning progressively in color from the petiole. The toning at this stage covers the first two to three veins. The four proximal leaves have reached their maximum size (between 13 - 17 cm long by 8-10 cm wide), whilst the four distal leaves are still growing. The inflorescence starts to open, the length from the inflorescence bract is approximately 8-10 cm, and the branches are 3-4 cm long and usually four to five in number.

Fifth Phase

The rachis of the inflorescence grows longer reaching an average size of 12-14 cm from the bract. All the leaves are fully developed and the first four leaves have toned to a more or less intense green color, depending on the type of the pepper fruit tree. The leaf area becomes thicker and coriaceous. The coloring of the four following leaves covers about 40-50% of the leaf area from the petiole, the inflorescence has opened further and the laterals (5 or 6) are 6-8 cm long.

Sixth phase

All the leaves are green and of a definitive size. The rachis of the inflorescence has grown longer to a length of 14-17 cm. The panicle has laterals fully spread and some are in anthesis.

Seventh phase

Approximately 50% of the flowers are open and the panicle is fully developed. The coriaceous leaves of the shoots are dark green in color.

Appendix 4D: photos of Intermediate disease stages on pepper leaves and fruits





Appendix 5.1: Standard diagrams showing leaves and Fruits affected with anthracnose disease

Key: infection levels. 0 = no infection; 1 = [0- 10%] leaves surface covered with actively sporulating mycelium, indicated by dark shading; 2 = [10 -25%]; 3 = [25 -50%]; 4 = [50-75%]; 5=>[75-99%]; 6=>99%. (Source: Survey, 2015).

Appendix 5 . 2 : Standard photos 1-5, showing pepper leaves and Fruits Affected with Anthracnose (*Colletotrichum capsici* (syd.) Buter and Bisby)

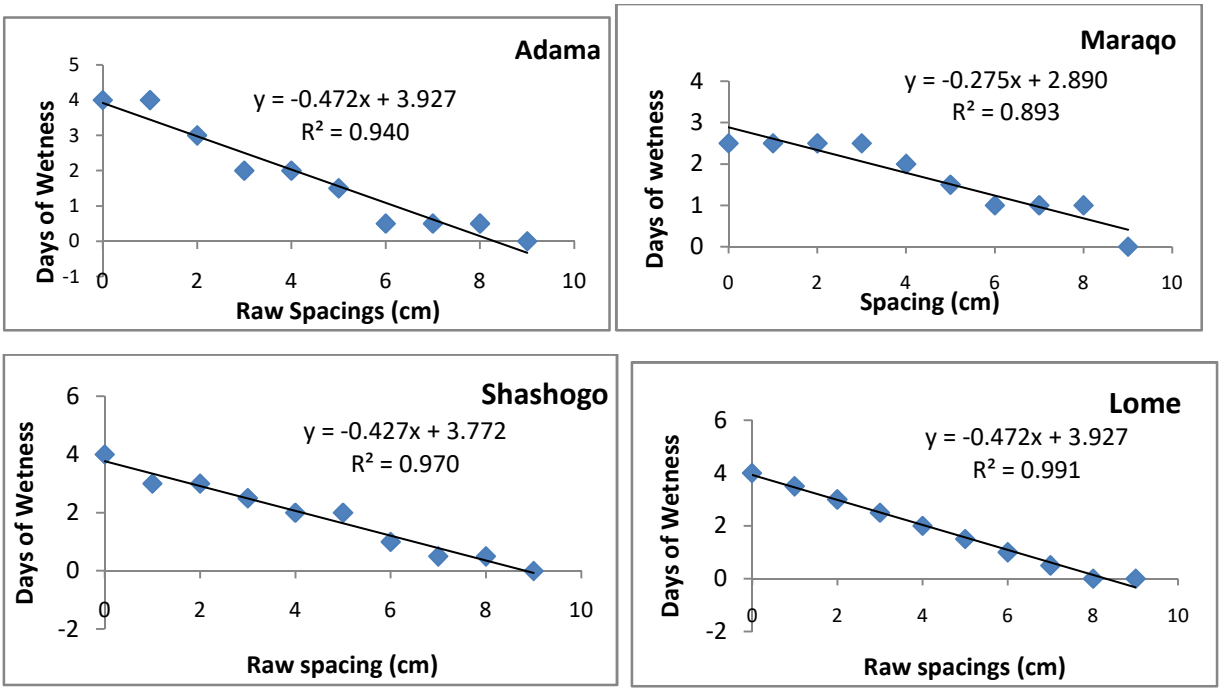
Key: Anthracnose development stages: 1 = [0- 5%] leaf surface with necrosis, 2 = [5 -10%], 3 = [10 -25%], 4 = [25 -75%], 5= [75-99%], 6= >99-100%. (Survey, 2015)

Appendix 5.3. List of Isolates of *Trichoderma* Spp Obtained from AAU, their Designations and Formulation (Recommended By: Tesfaye, 2015 (Unpublished);

Iso. name	Major gr. of the isolate	Formulation	Iso. name	Major gr. of the isolate	Formulation
Tri 1	37	Cotton seed	Tri 11	Th	Straw
Tri 2	37	Straw	Tri 12	Th	Coffee husk
Tri 3	37	Coffee husk	Tri 13	Th	Straw+husk
Tri 4	69	Cotton seed	Tri 14	47	Cotton seed
Tri 5	69	Straw	Tri 15	47	Straw
Tri 6	69	Coffee husk	Tri 16	47	Coffee husk
Tri 7	V	Cotton seed	Tri 17	Ad	Cotton seed
Tri 8	V	Straw	Tri 18	Ad	Straw
Tri 9	V	Coffee husk	Tri 19	Ad	Coffee husk
Tri 10	Th	Cotton seed	Tri 20	Ad	Straw+husk

Appedndix 5.4. *Colletotrichum capsici* and *T. harzianum* isolate Tri3 on PDA plate (***Upper left:*** Isolates showing high inhibitions of Mycelial growth of *Colletotrichum capsici* in presence of Trichoderma isolates; ***Upper right:*** Isolates showing low inhibition of mycelial growth of *Colletotrichum capsici;* ***Lower left:*** Isolates showing moderate inhibition of mycelial growth of *Colletotrichum capsici;* ***Lower right:*** Control)

Appendix 6.1. Regression between Planting density and Days of wetness, in nurseries at Adama, Maraqo, Shashogo and Lome in 2013



Appendix 7: Publications 1 - Abstract

International Journal of Sciences: Basic and Applied Research (IJSBAR)

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(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Evaluation of Extracts of Some Noxious Plants against Coffee Berry Disease (*Colletotrichum kahawae L.*)

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Abstract

Aqueous, alcoholic and dry extracts of five different noxious plants, namely, *Senna occidentalis, Melia azendrachta, Parthenium hysterophorus, Calotropis procera* and *Argemone mexicana* were tested in *in vitro* condition with the purpose of evaluating their inhibiting effects against *C. kahawae.* Data were collected on radial growth and percent inhibition. ANOVA had indicated that the aqueous and aqueous extracts of *Melia azendrachta* gave highest zones of inhibition followed *by Senna occidentalis* with 76.02%, 59.95%, respectively. Alcoholic extracts *Calotropis procera* and *Senna occidentalis* gave the highest zones of inhibition with 72.75% and 63.62%, respectively. *Senna occidentalis* had superior inhibition effect in both aqueous and ethanol extracts when applied 24 hours after inoculation with 83.37% and 80.67%. However, the aqueous extracts of all the botanicals tested were superior to *Clotropis procera* when applied 12 hours before inoculation of the fungus. Except ethanol extract of *Senna occidentalis*, the other botanicals were not statistically different from the control. This indicated that there is relationship between the time of application and efficacy of the antifungal compounds from these botanicals. However, the aqueous extracts of the seeds of these plants gave a better inhibitory effect than the leaf parts. Dry extracts of *Senna occidentalis* followed by *Argemone mexicana* gave better inhibitory effect against *C. kahawae* with 76.37% and 70.55%, respectively. Moreover, the extracts of *Calotropis procera* and *Melia azendrachta* had been with a promisingly higher inhibitory effect in this study. Therefore, in vitro evaluation and testing of these botanical plant extracts have antifungal compounds which reduced the mycelia growth of *Colletotrichum kahawae.*

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Appendix 7.2: Publications 2 - Abstract

International Journal of Sciences: Basic and Applied Research (IJSBAR)

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(Print & Online)

Prevalence, Incidence and Severity of Anthracnose (*Colletotrichum* Spp) in Main Chili Growing Areas of Ethiopia

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Abstract

Chili anthracnose is the key constraint that hampers chili production in Ethiopia. But scientific information on the magnitude of the problem was not sufficient. Thus, this survey was aimed to assess the prevalence of chili anthracnose, weaknesses in management and formulate appropriate recommendations. 132 purposively selected chili fields, 106 farms and 26 nurseries throughout the chili livelihoods of Ethiopia had been surveyed. To evaluate the perceptions of farmers, semi-structured questionnaire had been used administered among 132 farmers. Data on incidence, severity and prevalence, and their variation across different locations, seasons and agro-ecological zones, had also been collected. The obtained data had been analysed through descriptive statistics using IBM SPSS 20.0. The highest and lowest disease spread was observed in Alaba and Shashogo with cumulative incidence of 41.88% and 19.81%, respectively. From the chili farms, the highest incidence was found in Arsi negelle followed by Alaba with the value of 31.66% and 28.66%, whereas the lowest incidence in farms was found in Humbo and Mirago with 13.63% and 14.89%. Nurseries with a highest incidence had been observed in Humbo and Alaba with values of 13.5% and 13.02%, respectively. The disease incidence was low, 4.13% and 1.28%, in Shashogo and Arsi negelle.

* Corresponding author

Appendix 7.3: Publications 3 - Abstract

SJIF IMPACT FACTOR (2015): 5.79 CRDEEP Journals
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Full Length Research Paper

Morpho-pathological Variability of Chili Anthracnose (*Colletotrichum capsici* (Syd))Bisby and Butler) in Southern Nations Nationalities Peoples and Oromiya Region, Ethiopia

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Abstract
Chili had immense dietary and economic importance for Ethiopians. Ironically, it suffers profound losses in yield due to anthracnose caused by Colletotrichum capsici. But scientific information on variability of the Colletotrichum spp in the country is insufficient. Thus, the present study was undertaken to determine the morpho-pathological variability to design better management practices of Colletotrichum capsici to avoid losses. Morpho-pathological variations were studied in twenty isolates of C. capsici were collected from main chili growing farms located in southern and oromiya regions. The isolates were cultured and identified. Variations among isolates in terms of Colony size, color, shape, marginal pattern and characteristics had been observed. Nine differential virulence patterns were depicted amongst 20 locally acclaimed chili genotypes. Cultural tests had categorized the isolates into ten different groups. Disease incidence was also. Colonies varied in their cultural attributes ranging from cottony to fluffy, mostly suppressed with regular to irregular margins. Color of colonies ranged between cottony white to dark grey. Growth rate of isolates was between 22.0-69.5mm. Morphological studies of isolates revealed variations in their color, size, shape, acervuli production, setae size and shape, conidia. Average conidial size varied from 18.00-33.3 µm and average setae size varied from 77.2-181.2 µm. In conclusion, the existence of variations among Colletotrichum capsici isolates strengthened the present knowledge on the identification and understanding pathogen which has immense contribution for ultimate anthracnose disease management.

Key Words: Chili anthracnose, Colletotrichum capsici, Morphological variability, Pathological variability

Introduction

Chili (*Capsicum frutescence* L.) is an important cash crop grown worldwide (Makari *et al.*, 2009; Bosland and Votava, 2000)and in Ethiopia (Seloshi, *et al.*, 2014; EEPA, 2003). It is prone to number of fungal bacterial and viral diseases (Devi and Prakasam, 2014). Which significantly affect its production and quality? However, huge losses to the crop are incurred mostly by fungal diseases. Of these diseases, dieback and fruit rot has assumed the status of major disease in some important chili growing countries(Makari *et al.*, 2009; Sharma *et al.*,2005). Anthracnose causes extensive pre and post-harvest damage to chili fruits causing anthracnose lesions (Mehrotra and Aggarwal, 2003). Even small anthracnose lesions on chili fruits reduced their marketable value (Masoodi *et al.*, 2013). *Colletotrichum capsici* is most adhesive that adhere to the plant surface and remain latent until such physiological changes occur in the fruit and cause economic losses to the farmers due to low fruit quality and is marketability(Canoon *et al.*, 2011; Noirwung *et al.*, 2012). Many post-harvest diseases of fruit exhibit the phenomenon of quiescence in which symptoms do not develop until the fruit ripens (Masoodi *et al.*, 2013;Agrios, 2005).*Colletotrichum* species have the most adhesive discs that adhere to plant surfaces and remain latent until physiological changes occur in fruits (Mehrotra and Aggarwal, 2003).

Chili anthracnose usually develops under high humid conditions when rain occurs after the fruits have started to ripen with reported losses of up to 84% (Rajapakse *et al.*, 2007; Tamaru *et al.*, 2003; Tamaru, 2004). Economic losses caused by the disease are mainly attributed to lower quality and marketability. Variability of the progeny exhibits a characteristic that is different from those present in the ancestral individuals or descent individuals, this individual is called a variant (Agrios, 2005).

Sharma *et al.* (2005) studied the pathogenic variability in *C. capsici* studied and found 15 pathotypes of *Colletotrichum capcisi* that existed from 30 isolates studied in India and proposed 15 pathotypes of *C. capsici* existed among 37 isolates from different chili

Appendix 7.4: Publications 4 - Abstract

International Journal of Sciences: Basic and Applied Research (IJSBAR)

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(Print & Online)

In Vitro Evaluation of Fungicides, Plant Extracts and Antagonists (*Trichoderma* Spp) on Chili Anthracnose (*Colletotrichum capsici* (Syd.))

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Abstract

Chili anthracnose caused by *Colletotrichum capsici* is one of the most devastating diseases that deter chili production in Southern Ethiopia. This study was conducted in 2014 with the aim to judiciously manage *Colletotrichum capsici* causing anthracnose of Chili. Six fungicides, namely, Ranomyl, Tilt-250 EC, Vitavax-200, Royal 50 WP, Dithane M 45 and Ridomil at concentration of 150, 250 and 300 ppm; and leaf extracts of garlic, ginger, onion and neem at three different concentrations (15%, 10% and 5%) were evaluated against the radial growth and mycelial dry weight of *Colletotrichum capsici*. Concurrently, 20 Trichoderma isolates formulated with residues of different crops were also tested against *Colletotrichum capsici* by using dual culture technique. In all these three experiments the treatments were arranged in CRD and data was analyzed through ANOVA. All the treatments were statistically different from the untreated check. From the six fungicides, all test concentrations of Tilt-250 EC significantly inhibited the mycelial growth of *Colletotrichum capsici*. Vitavax-200 and Royal 50WP gave 55.33-77.33 and 50.67-83.67, and 20.33-69.33mm and 20.67-73.67g, radial growth and mycelial dry weight of the test pathogen, respectively. Dithane M-45 and Capsaicil were found to be significantly inferior to the rest of the fungicides.

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Appendix 7.5: Publications (under process) 5

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Ref. No. EJBS/1817/323/16
Date 12 August 2016

Mr. Serawit Handiso
Dept. of Microbial, Cellular and Molecular Biology
Addis Ababa University
PO Box 1176
Addis Ababa

Subject: Reception of manuscript

Dear Mr. Serawit,

This is to kindly acknowledge the receipt of your manuscript entitled "Implications of seedling age and density on development of chili (*Capsicum frutescence* L.) Anthracnose (*Colletotrichum capsici*) leaf symptoms". Thank you very much for sending your manuscript to our journal. We will inform you about the status of your manuscript in the near future.

Sincerely,

Seyoum Mengistou (Prof.)
Editor-in-Chief

Appendix 7.6: Publications (under process) 6

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Ethiopian Journal of Biological Sciences

Ref. No. EJBS/1818/324/16
Date 12 August 2016

Mr. Serawit Handiso
Dept. of Microbial, Cellular and Molecular Biology
Addis Ababa University
PO Box 1176
Addis Ababa

Subject: Reception of manuscript

Dear Mr. Serawit,

This is to kindly acknowledge the receipt of your manuscript entitled "Performance evaluation of some chili pepper (*Capsicum frutescence* L.) genotypes for anthracnose (*Colletotrichum capsici* (zyd)) resistance in SNNP region of Ethiopia". Thank you very much for sending your manuscript to our journal. We will inform you about the status of your manuscript in the near future.

Sincerely,

Seyoum Mengistou (Prof.)
Editor-in-Chief

Appendix 7.7: Publications 7 - Abstract

American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
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The Nexus between Incidence and Severity of Chili Anthracnose (*Colletotrichum capsici* (Syd.) Bisby and Butler) on Chili in SNNPR, Ethiopia

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Abstract

The incidence-severity relationship for chili anthracnose, caused by *Colletotrichum* spp., was studied on ten released Chili/pepper genotypes to determine the feasibility of using disease incidence to estimate indirectly disease severity in order to establish the potential damage caused by this disease in southern region, Ethiopia. Data were statistically analyzed by regression. Anthracnose leaf incidence was consistently associated with leaf severity and their relationships can be estimated using the linear function across locations, crop seasons, and genotypes. Thus, the use of easily assessed incidence data had been recommended for determination of severity as well as epidemic comparisons, genotype and seasonal evaluation in chili anthracnose management. This study will pave the way for chili producing farmers for cheaper and efficient approach tailoring it for determination of economic threshold level and launch opportune management practices.

Keywords: Anthracnose; Chili; Incidence; i-s-Relationship; Severity.

1. Introduction

Chili anthracnose, caused by *Colletotrichum capsici*(Syd.), is one of the most important chili disease in pepper growing regions of Ethiopia [1], which manifests its symptoms in both leaves and young fruits [2].

* Corresponding author.

Appendix 7.8: Publications 8 – Abstract

SJIF IMPACT FACTOR (2015): 5.42 CRDEEP Journals
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International Journal of Basic and Applied Sciences. Vol. 6 No. 3, 2017. Pp. 77-82
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Full length Research Paper

Determinants for Frequency of Fungicide Applications against Chili Anthracnose (*Colletotrichum capsici* (Syd.) in Southern Ethiopia

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Abstract
Chili anthracnose is posing a severe menace to chili production in southern Ethiopia. Study on fortnightly treatments of the fungicide Ridomil starting at different chili phenological stages was carried out so as to devise a strategy for appropriate frequency and timing for spraying against anthracnose diseases on chili. Two factor factorial arrangements with completely randomized block design had been employed. Variety, viz., MaraqoFana and OdaHaro, was assigned as factor A while fungicide frequency was assigned as factor B with four levels, viz., flushing, flowering, fruiting and control. The treatments were replicated four times. Data on incidences of anthracnose on leaves severity on fruits were collected. The financial impact and profitability of frequent spraying was computed. The economic implication of high frequency application of Ridomil in the current context of fungicide use on chili in Ethiopia was evaluated. A maximum of 7 applications starting from flushing successfully prevented the disease development and significantly reduced the incidence of leaf anthracnose. The best economic profit was obtainedwith7 applications of Ridomil for chili with potential yield of at least 29 kilograms per season per plot. In light of this research, small scale chili farmers will be able to lessen pesticide treadmill thereby escalating ecological safety and profitability.

Key Terms: Chili Anthracnose, Financial Impacts, Frequency, Fungicide, Ridomil

Introduction

Chili (*Capsicum frutescence* L.) is a crop of increasing importance in the economies of sub-Saharan Africa (Nsabiyera *et al.*, 2012). However, what is being produced has been below the potential that this area is capable of producing (Nsabiyera*et al.*, 2012; Lemma*et al.*, 2008). For instance, over the past many years, hot chili production in Ethiopia has stagnated at 0.4 tonnes per ha, with yields remaining lower than the average global production of 28 metric tons (Lemma *et al.*, 2008). Moreover, the quality of the produce realized does not meet the stringent standards of the international markets, where most countries face fierce competition from major producing countries such as India and China (Thampi, 2003). The poor quality of the produce is largely attributed to biotic and abiotic stresses in the field and the poor quality cultivars grown by farmers (Tumime *et al.*,2010). Particularly, attack by different pest infestations or infectious can cause significant losses in chili production (Ochoa-Alejo and Ramirez-Malagon, 2001). The most common diseases of most chili peppers are phytopathogenic fungi, bacteria, and viruses. By and large, from 50% (Pakdeevaraporn *et al.*,2005) to 100% loss had been was observed due to chili anthracnose (Melanie and Sally, 2004); causing severe defoliation of plants, resulting in reduced yield and loss of quality of harvested fruit when severe damage occurs on enlarging fruits(Melanie and Sally, 2004).

Control and/or prevention of these diseases and their vector populations are usually through use of chemical sprays on diseased plants and use of various cultural practices. In developing countries such as Uganda and Ethiopia, farmers largely use pesticides for disease and pest control on hot chili (Karungi*et al.*, 2010). Ever-increasing loads of disease management via chemicals accompanied by rising cost make pesticides non-affordable for small scale farmers(Lemma *et al.*, 2008).Application of pesticides arbitrarily over time has also resulted in a buildup of resistance among target pests and pathogens (Flint, 1999). Pests and diseases are best managed using host resistance, which is a cheap option for farmers (Dawiller*et al.*, 2007).

However, in cases where disease-resistant cultivars are not available, fungicide control would be the best control strategy. In some countries like Brazil, fungicides such as copper oxychloride, copper hydroxide, zinc + manganese, carbamate, captafol, benomyl, dithianon, anilazine, bitertanol, tridimenol and triforine, are used to control the disease (Freire*et al.*, 2002). However, the choice of fungicides depends on various factors such as biological efficiency, crop sensitivity (Usciquate, 2013), economic feasibility(Freire*et al.*, 2002), environmental aspects (Sijaona, *et al.*, 2001), previous exposure to a certain fungicide or group (Siddiqui*et al.*, 2001)and local regulations for registration (Arnau, 2000). In general, there is no sufficient research information vis-à-vis the frequency of fungicides application in Ethiopia. Thus this paper was initiated with the aim to determine the efficacy of various fungicides rate and frequency against chili anthracnose in south western regions of the country.

Appendix 7.9: Publications 9 - Abstract

American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
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Integrated Management of Anthracnose (Colletotrichum capsici (Syd.)): Implications to Disease Reactions, Quality and Growth Parameters of Three Genotypes of Chili

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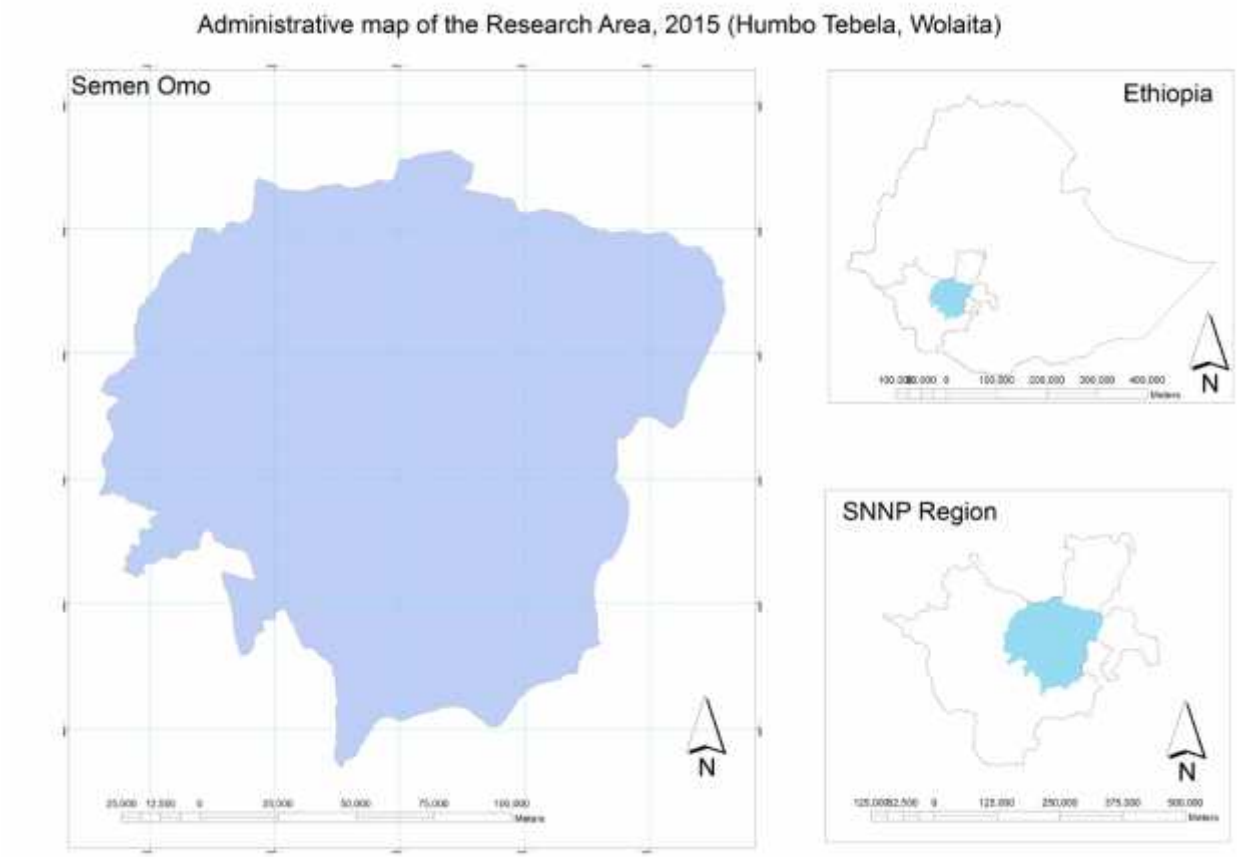
Abstract

Anthracnose (*Colletotrichum* spp) is one of the most important disease that decimate chili production in Ethiopia. The efficacy of three Trichodamus isolates viz., AAU-37, AAU-Th and AAU-69 with aqueous leaf extracts Onion, Garlic, Neem and *Cassia* spp were harmoniously applied on Oda Haro, Marako Fana and Melka zala pepper varieties, with the aim to manage chili anthracnose in rainy season of 2013. The treatments were arranged in RCBD replicated thrice. Data on disease reaction, quality and growth characteristics of chili had been collected. Analysis of data was carried out using ANOVA. The lowest plant infection (17.8%), leaf infection per plant (15.2%), percent diseased leaf area(15.2%)and infected fruits per plot (17.4%) was observed on combined application of isolates *Trichoderma* spp, plant extracts and Ridomil in Marago fana variety. Regarding the growth parameters, viz. the highest Mean Percent establishment (81.67), mean days to 50% flowering (65.33), mean days to 50% maturity (82) days to first harvest (106.3) in was observed in T16, T16, T4, and T8, respectively. From the quality parameters, the highest mean number of branches per stem (9), mean canopy diameter (24.8), mean number of flowers per plant (9.6) and mean plant height (61.4) in T10, T15, T6 and T7, respectively. Both negative and positive control showed higher incidence and severity as compared to single and combined application of isolates *Trichoderma* spp, plant extracts and Ridomil. Therefore, integrated use of *Trichoderma* spp and plant extracts can be recommended. Conventional fungicides will be replaced by antagonists and botanicals. This will improving crop quality and growth maximize profitability of chili and ultimate sustenance.

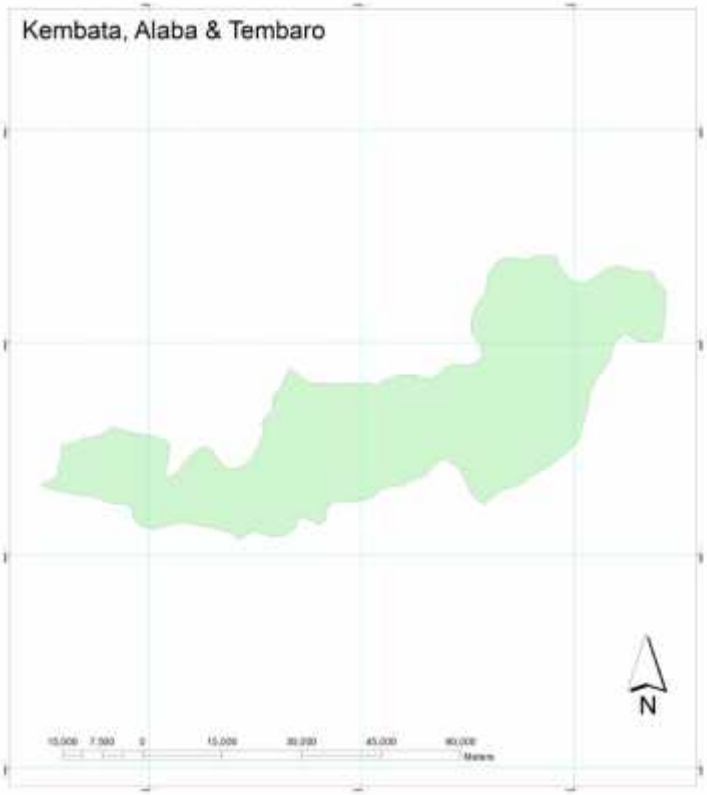
Key Terms: Anthracnose; infection; Quality; Growth Parameters; Integrated Disease Management; Plant Extracts.

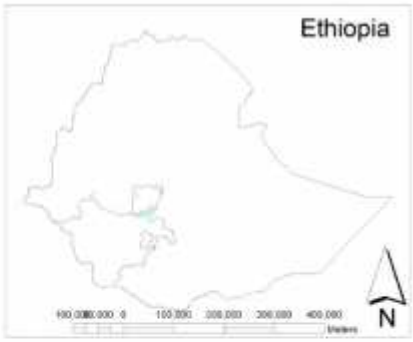
* Corresponding author.

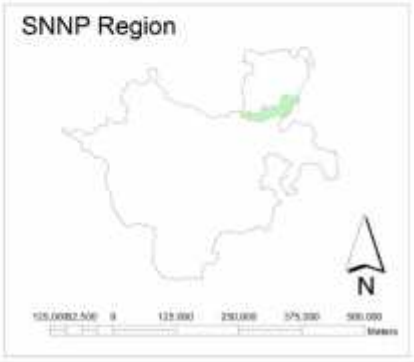
Appendix 8: Administrative Maps of the Research Area



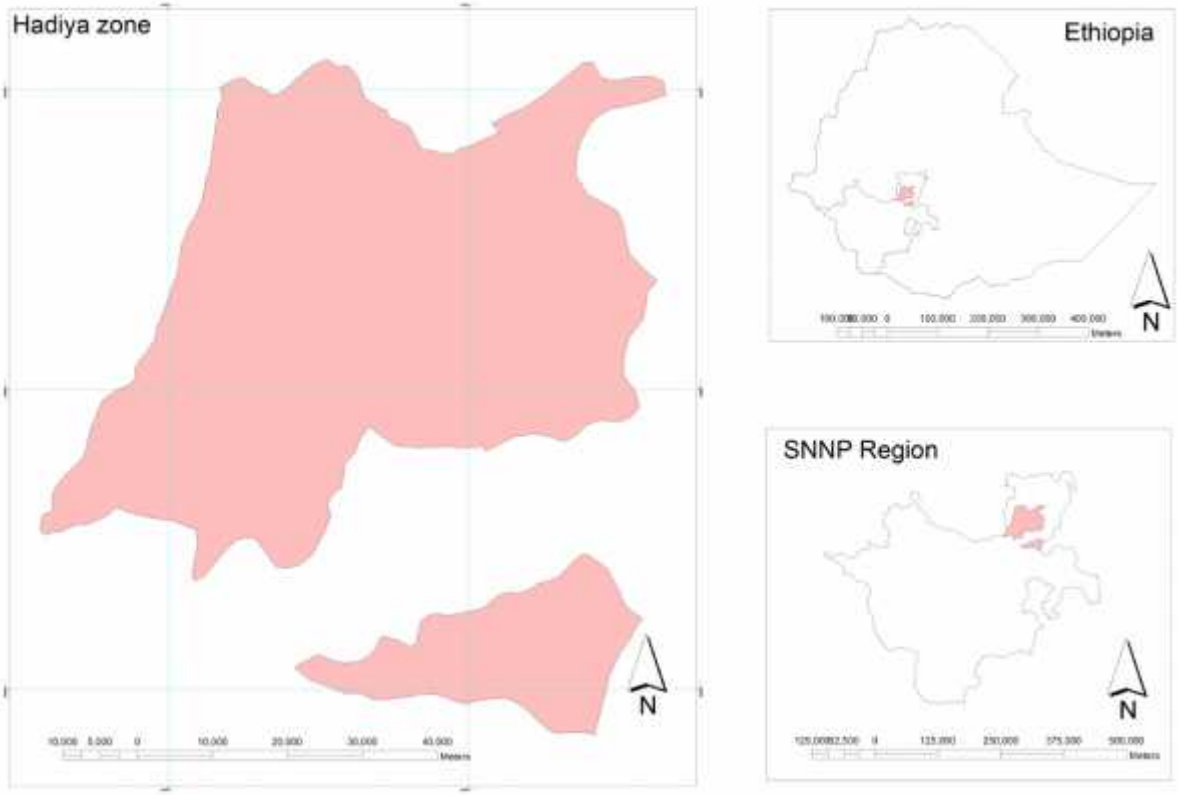
Administrative map of the Research Area, 2015 (Kembata Alaba and Tembaro)



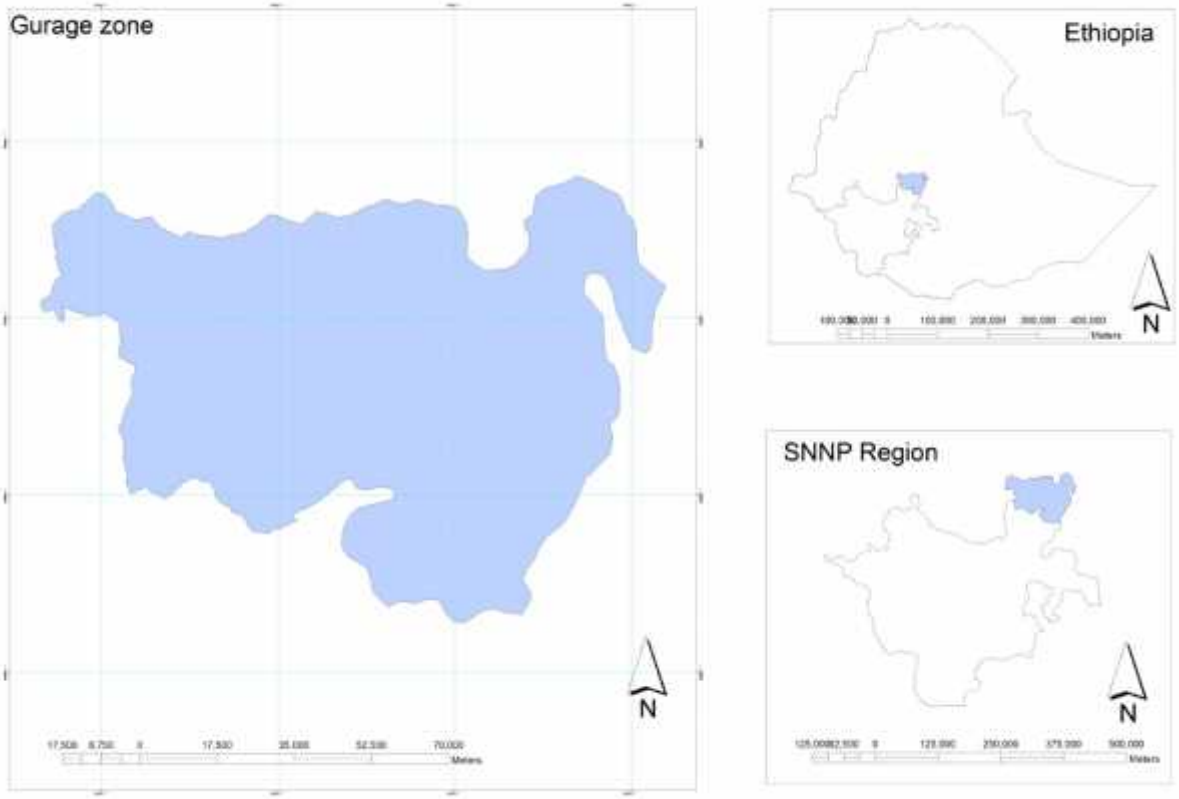




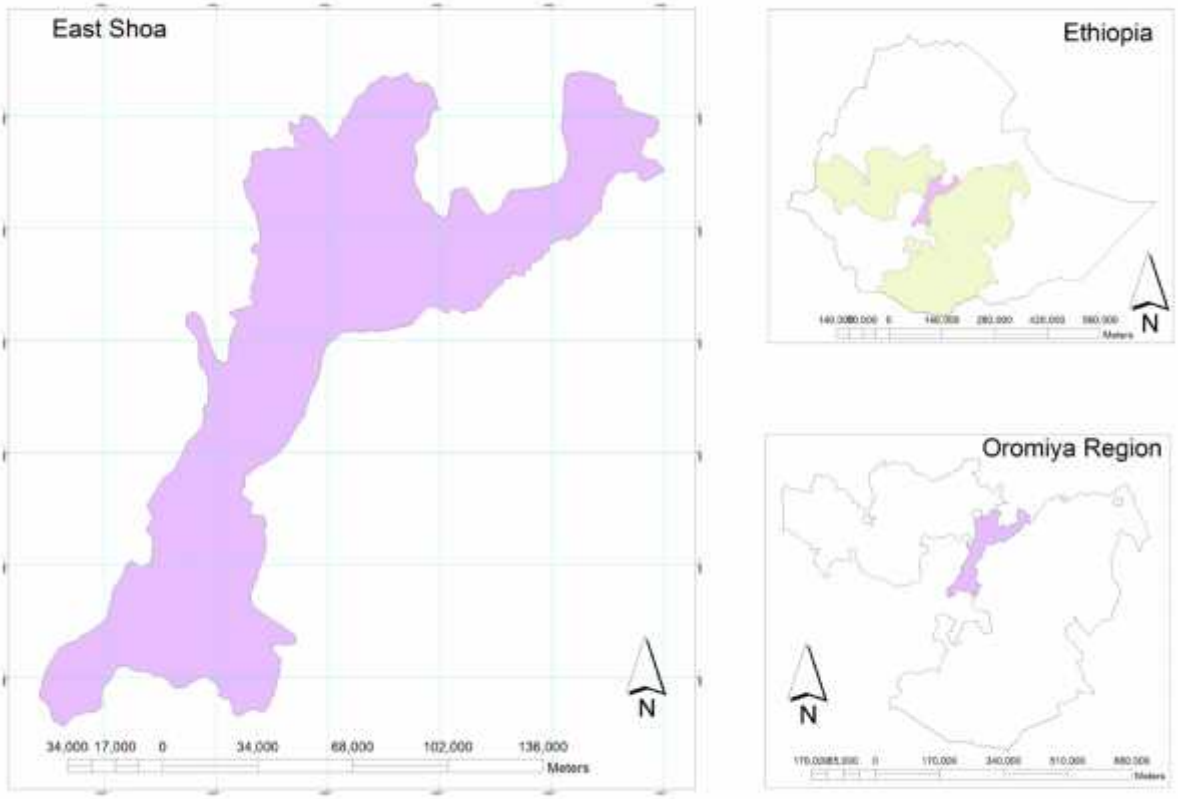
Administrative map of the Research Area, 2015 (Hadiya Zone)

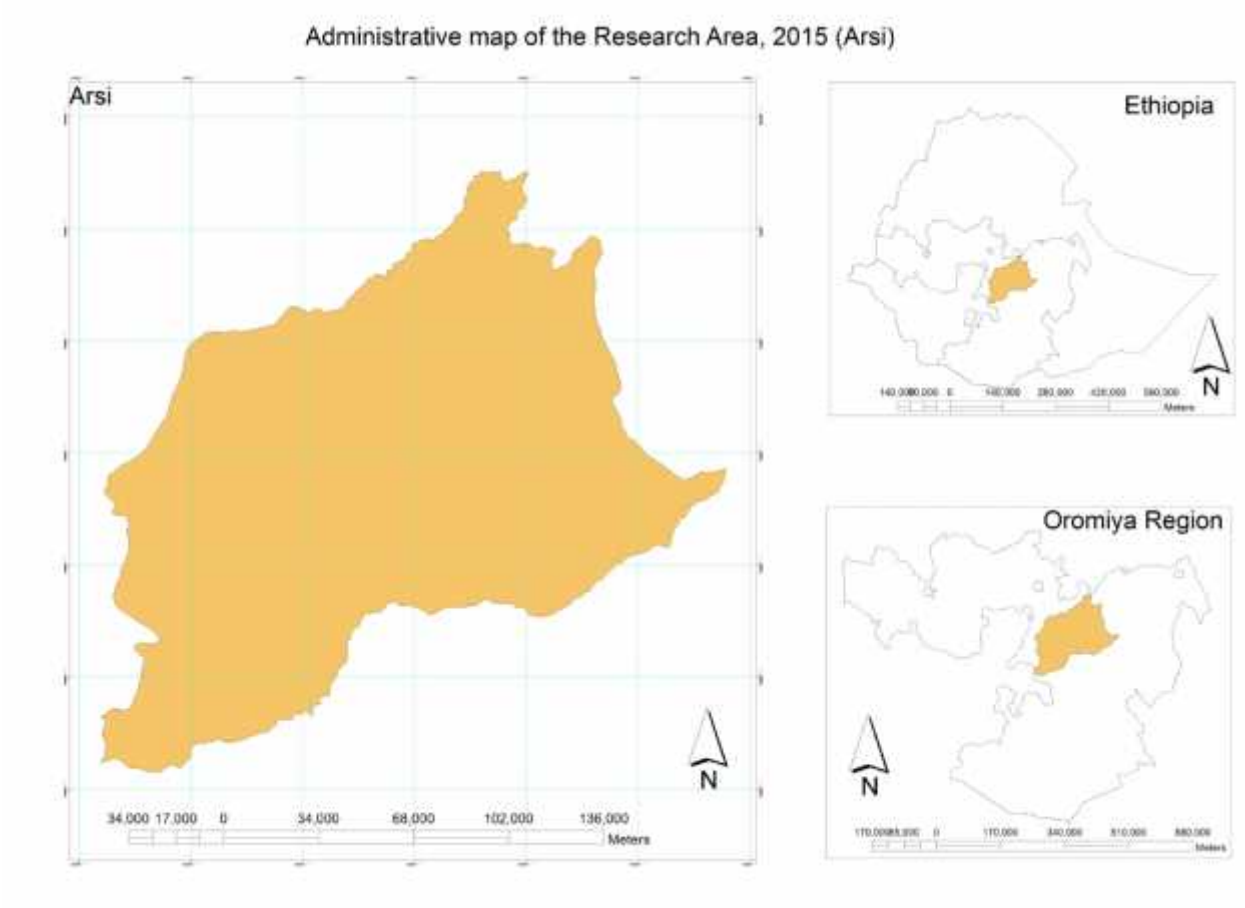


Administrative map of the Research Area, 2015 (Gurage Zone)

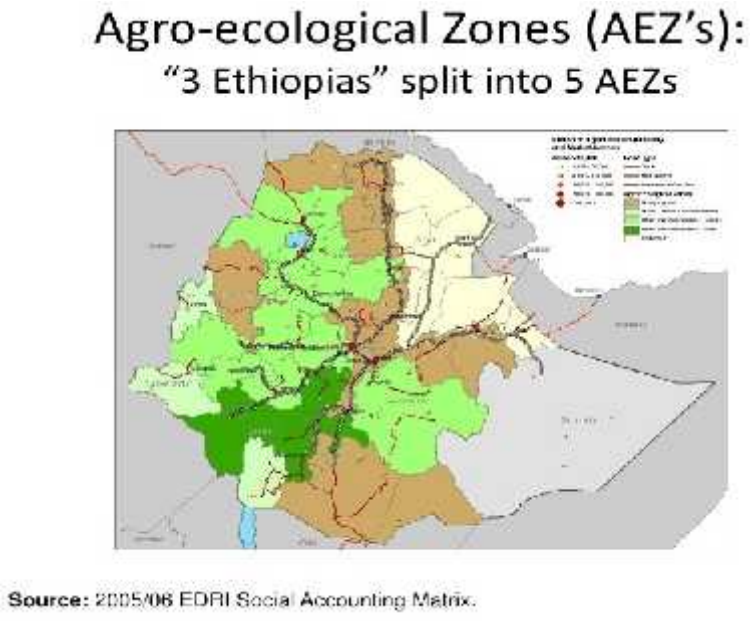


Administrative map of the Research Area, 2015 (East Shoa)





Appendix 9: Agro-ecological map of Pepper Cultivation in Ethiopia (Source: NMA)



Source: 2005/06 EDRI Social Accounting Matrix.

Appendix 10: Chili pepper *(Capsicum frutescence*)(Crop Variety Release, 2009)

10a.1 Varieties under production

10a.1.1 Variety: Melka Shote (PBC 223)
10a.1.1.1 Year of release: 2006
10a.1.1.2 Breeder/Maintainer: MARC/EIAR

10a.2.2 Variety: Melka Awaze (PBC 600)
10a.2.2.1 Year of release: 2006
10a.2.2.2 Breeder/Maintainer: MARC/EIAR

10a.2.3 Variety Oda Haro
10a.2.3.1 Year of release: 2005
10a.2.3.1 Breeder/Maintainer: BARC/ OARI

10a.2.4 Variety: Melka Zala (PBC 972)
10a.2.4.1 Year of release: 2004
10a.2.4.2 Breeder/Maintainer: MARC/EIAR

10b. Sweet/Hot Pepper (*Capsicum annum)*

10b.2 Varieties under production

10b.2.1 Variety: Melka Dima (Papri King)
10b.2.1.1 Year of release: 2004
10b.2.1.2 Breeder/Maintainer: MARC/EIAR

10b.2.2.Variety: Melka Eshet (Papri Queen)
10b.2.2.1 Year of release: 2004
10b.2.2.2 Breeder/Maintainer: MARC/EIAR