EFFECT OF PYROLYSIS PRODUCTS ON MANAGEMENT OF ASCOCHYTA BLIGHT (*Ascochyta rabiei*) AND PERFORMANCE OF CHICKPEA (*Cicer arietinum* L.)

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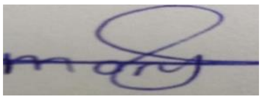
DECLARATION AND RECOMMENDATION

Declaration

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

Signature  Date: 15/09/2024

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Recommendation

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DEDICATION

I dedicate this thesis to my lovely daughter Precious Mwende. My brother, Fredrick Wanyonyi Simiyu for his love and financial support. My supportive parents Mrs Jesca and Mr John Simiyu who encouraged and inspired me throughout the academic journey.

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ABSTRACT

Chickpea is a drought-tolerant and nutritious legume with great potential to mitigate the impact of climate change on food and nutritional security. Its production is however limited by several fungal infections key among them is Ascochyta blight (AB) caused by *Ascochyta rabiei*. Efforts to manage AB have seen farmers resort to frequent use of synthetic fungicides which have negative impacts to human health and the environment. The objectives of this study were (i) to determine the antifungal effects of wood vinegar from selected feedstocks on *Ascochyta rabiei in vitro.* (ii) to determine the efficacy of biochar and wood vinegar on Ascochyta blight disease incidence and severity (iii) to determine the effects of biochar and wood vinegar on the performance of chickpea. Experiments were conducted in the laboratory, greenhouse and field. The laboratory experiment was conducted at Egerton University Biotechnology Laboratory to evaluate the antifungal activity of wood vinegar from maize cob, acacia trimmings, *Prosopis juliflora* and bean wastes on *Ascochyta rabiei in vitro* on potato dextrose agar (PDA) arranged in a completely randomized design (CRD). Greenhouse experiment was conducted in potted plants at Agronomy Research Field 7, Egerton University, and the field experiment at Agriculture Training Centre (ATC) Koibatek, Baringo County. The greenhouse and field experiments were arranged in a randomized complete block design (RCBD) and replicated three times. The potted plants were inoculated with *A. rabiei* at a spore concentration of $5x10^5$. Data collection from the *in vitro* experiment included mycelia growth inhibition. For greenhouse and field experiments, data was collected on crop emergence, plant height, days to 50% flowering, biomass, number of pods, yield, disease incidence and severity. Data was analysed using SAS version 9.4 using PROC GLM procedures. The laboratory experiment results showed that wood vinegar has significant antifungal effects against *Ascochyta rabiei.* Wood vinegar from maize cobs showed the highest inhibition at all concentrations tested with highest concentration of phenols at 4.56 mg/ml and low pH of 3.90. The results further revealed that AUDPC and PDI were less severe at 19.70% and 23.33% when biochar and wood vinegar from maize cob were applied in soil and foliar respectively. Wood vinegar and biochar from maize cobs increased the yield of chickpea at 3.35 t/ha This study demonstrated that pyrolysis products can be used to reduce Ascochyta blight incidence and severity and increase the performance of chickpea.

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LIST OF ABBREVIATIONS AND ACRONYMS

AB	Ascochyta Blight
ANOVA	Analysis of Variance
ASALS	Arid and semi-arid lands
ATC	Agricultural Training Centre
AUDPC	Area Under Disease Progress curve
Con	Concentration
CRD	Completely Randomized Design
DAE	Days After Crop Emergence
DAP	Di ammonium Phosphate
DSI	Disease Severity Index
FAO	Food and Agricultural Organization
F-C	Follin-Ciocateau
GLM	General linear model
HPR	Host Plant Resistance
ICRISAT	International crop research institute for semi-arid tropics
IDM	Integrated disease management
KCSAP	Kenya Climate Smart Agricultural project
LSD	Least significance difference
MIC	Minimum inhibitory concentration
MOA	Ministry of Agriculture
N	Nitrogen
NM	Nanometer
P	Phosphorous
PDA	Potato Dextrose Agar
PDI	Percent Disease Incidence
PROC	Procedure
RCBD	Randomized Complete Block design

CHAPTER ONE
INTRODUCTION

1.1 Background information

Chickpea (*Cicer arietinum* L.) also referred to as garbanzo beans, is a drought-tolerant and nutritious legume with great potential to mitigate the impact of climate change on food and nutritional security. It is a good source of human food providing proteins, vitamins, minerals, carbohydrates and fiber (Wang *et al*., 2021). The high content of proteins present in the grains plays a vital role in improving human diets (Jukanti *et al*., (2012). The crop is also known for improving soil health through nitrogen fixation in a cereal legume intercrop (Ananthi *et al*., 2017; Aziz *et al*., 2015), breaking the life cycle of some pathogens when it is grown during the off-season and addition of manure in the soils and improved soil structure in acidic soils (Khursheed *et al.,* 2022). Chickpea is grown on an estimated acreage of 12.3 hectares globally and ranked third after common beans (*Phaseolus vulgaris*) and *Pisum sativum* (field peas) (Vema *et al*., 2021). India is the leading producer of chickpea with 7.7 million tons which accounts for 68% of the world production ((FAOSTAT, 2012; ICRISAT, 2013), followed by Pakistan producing 0.1 million tons. Ethiopia leads in Africa with production of 400,000 tons which is 3.5% from an area of 231, 000 ha, followed by Tanzania with a production 67,000 tons from 115, 000 ha of land. In Kenya, 55,000 ha of land is under chickpea production with an annual production of about 15,000 to 18,500 tons (KARI, 2012). Ethiopia leads in production in Africa. In Kenya, annual estimations of chickpeas are 45 million tons and the area under production is 20-2200 ha (CRP, 2012). The major are major chickpeas producing areas in Kenya include parts of Eastern (Embu, Machakos, and Tharaka) and Rift Valley (Nakuru and Biomet) regions (ICRISAT, 2014).

In Kenya, the production of chickpeas has been showing a reduction trend both in the area under production and tonnage. Production in areas that practice chickpea farming has remained low as compared to the expected potential of 3600 kg/ha. Acceleration in yield reduction has been attributed to various biotic and abiotic factors. Bacteria, fungi, nematodes, temperatures, and humidity are major contributors to yield reduction in chickpea production. Ascochyta blight caused by *Ascochyta rabiei* (syn home rabies), also called by its teleomorph stage (*Didymella rabie*. Syn mycosphaerella) is a haploid heterothallic fungus and devastating disease of chickpea. It is among the major constraints that affect chickpea production all over the growing regions and it is associated with huge yield losses and low-quality grains (Aveskamp *et al*., 2014; Pande *et al*., 2011). The disease causes 10%-100% yield losses when the environmental conditions are conducive to disease development

(Sharma & Ghosh, 2016; Sing *et al*., 2022). The importance of Ascochyta blight continues to increase as chickpea acreage expands. The symptoms of damage are first noticed when blighted plants appear in the field. The necrotic lesions start from new leaves and stems then increase rapidly under wet and cool conditions joining with other spots on the leaves, buds, pods and petioles. Black spots that are less than 1 mm are seen in affected areas. In severe cases, the entire plant dries up. Upon harvesting, Ascochyta blighted seeds are shrunken and shrivelled as reported by Zhang *et al*. (2019).

Growing regions in Kenya sow chickpeas after the long rains in rotation with cereals crops like maize exposing the crop to terminal drought during the growing period. This has also been the trend in Asia and the Mediterranean regions n which the crop is usually sown during the spring periods allowing the crop to grow in summer periods. This has been a contributing factor to reduced yields and reduced biomass of the crop (Varshney *et al*., 2009). Sowing the crop in rainy seasons helps reduce terminal drought since there is a promotion of vegetative growth of the crop and hence an increase in the amount of yield. Unfortunately, farmers rarely achieve this scenario since the cool wet climate during the rainy season in the growing regions speeds up the development of Ascochyta blight (Kimurto *et al*., 2013). *Ascochyta rabiei* usually survives in the previous crop debris and in or on seeds. The disease-causing blight survives in form of pycnidia or mycelium or the teleomorph stage hence making it easy to spread through air born-spores. The asexual stage gives the pathogen prolonged periods of survival and unfortunately, no studies have been done on this state, especially in regions that are farming the crop in Kenya (Kimurto *et al*., 2013). To counter the effects of Ascochyta blight, various management strategies have been put in place (Fernandez *et al.,* 2010; Stoddardd *et al*., 2010). Among the most promising strategies include host plant resistant, application of foliar fungicides, crop rotation and adjusting the dates of sowing. The use of pyrolysis products from woody biomass to enhance performance and manage crop pests and diseases is an emerging and effective practical approach in sustainable agriculture.

Pyrolysis is one of the thermochemical technologies of converting biomass into energy and chemical products consisting of liquid, biochar and gas. Liquid pyroligneous acid obtained from condensation of pyrolyzed biomass contain several compounds that function as an antibacterial, antitermite, anti-fungal and antioxidant (Adfa *et al*., 2020). Biochar on the other hand can be integrated into traditional soil fertility strategies based on the use of composts, manures and anaerobic digesters to increase the agronomical value of the organic amendments (Rumpel & Chabbi, 2021). The use of pyrolysis products in crop production will support the agricultural sector in the "go organic" program in order to improve food security and conserve the environment. The proposed study was evaluated to determine the effects of various pyrolysis products on performance of chickpea and management of Ascochyta blight.

1.2 Statement of the problem

Chickpea production in Kenya and other regions in the tropics is highly limited by biotic and abiotic factors. Ascochyta blight is the most devastating foliage disease of chickpea and may cause 100% yield loss under wet and humid environmental conditions. The use of host plant resistance as a management strategy been rendered ineffective due to emergence of new strains of the pathogen. Furthermore, Ascochyta blight pathogen has the potential to remain in crop debris for a prolonged period of time due to its sexual stage (telemorph) resulting in frequent multiplication and spread of AB. Efforts to manage the disease and reduce crop losses have seen farmers resort to increased quantities and frequency of synthetic fungicide applications which have negative impacts on non-target organisms and the environment. Fungicide applications are also expensive and pose the risk of developing disease resistance pathogens. Thus, the use of natural pyrolysis products that include biochar and pyroligneous acid as an integrated disease management strategy provides an alternative to over-reliance on fungicides and Ineffective cultural measures and newly emerged pathotypes of AB.

1.3 Objectives

1.3.1 General Objective

To contribute to improved food and nutritional security through the use of pyrolysis products in the management of Ascochyta blight in chickpea

1.3.2 Specific objectives

i. To determine the antifungal effects of wood vinegar from selected feedstocks on *Ascochyta rabiei in vitro*

ii. To determine the efficacy of biochar and wood vinegar on Ascochyta blight disease incidence and severity

iii. To determine the effects of biochar and wood vinegar on the growth and yield of chickpea

1.4 Hypotheses

i. There are no significant antifungal effects of wood vinegar from selected feedstocks on *Ascochyta rabiei in vitro*

ii. Biochar and wood vinegar have no efficacy on Ascochyta blight disease incidence and severity
iii. Biochar and wood vinegar have no significant effects on the growth and yield of chickpea

1.5 Justification

To sustainably produce enough food for the expanding population, which is expected to reach 10 billion people by the year 2050, global agricultural production must rise at an average annual rate of 1.73%. In Kenya, annual agricultural production is expected to increase by 75% in order to meet the increasing population which is needs coming 2050 (FAOSTAT, 2017). Achieving this target has been hindered by biotic, abiotic, and climate change factors in the crop production sector and as such, there is a need to practice the cultivation of drought-tolerant crops.

Chickpea is a drought-tolerant and nutritious legume with great potential to mitigate the impact of climate change on food and nutritional security for an expanding world population. The crop is known for improving soil fertility, moisture conservation, and breaking the life cycle of pests and diseases when grown in fallow relay systems with other crops (Cheruiyot *et al*., 2001; Cheruiyot *et al*., 2003).

However, there has been a decline in the production of chickpea globally and this has been attributed to both biotic and abiotic factors. Among the most devastating biotic factors in production is the common *Ascochyta* blight which has lowered the global recommended tonnage production of (1.31 t ha^{-1}). A similar problem has been manifested in countries that produce the crop causing up to 100% yield losses (Sharma & Ghosh, 2016; Singh *et al*., 2012). In Kenya, losses ranging between 15-20% are experienced upon production and this is highly promoted by the cool wet climate speeds up the prevalence of the disease leading to yield loss, income loss and food insecurity (Kimurto *et al.*, 2013).

The over-reliance of fungicides to manage this disease has resulted in increased cost of production and also raised environmental and food safety concerns (Singh *et al.*, 2023). There is need for natural plant products that are cheap and safe alternatives. Recent research has focused on natural plant aqueous or organic soluble extracts for control of pests and diseases (Deshmukh *et al*., 2023). Burning of biomass residues such as maize cobs, invasive weeds species like *Prosopis juliflora*, bean wastes, and acacia trimmings as a mean of wastes disposal influences the soil biota, causes soil degradation loss of biodiversity, and associated human health risks (Ashokkumar *et al*., 2022). Therefore, it's critical to reduce the amount of plant biomass that is burned or wasted and to create sustainable, low-cost technologies to transform it into valuable bio products. One of these technologies is the pyrolysis process which entails conversion of woody biomass in oxygen limited environment (Solarte-Toro *et al.,* 2021). There are limited studies on the use of pyrolysis products from woody plants in the management of plant diseases. Deployment of pyrolysis products to manage AB in chickpea production system would be advantageous to the farmers arising from reduced cost of production which is incurred in the management of the disease by use of synthetic chemicals (Siah *et al.*, 2018). In addition, it will be an important component of the Integrated Disease Management which are programs aimed at reducing the occurrence, severity and incidence of Ascochyta blight in chickpea. The ultimate increase in production of chickpea will subsequently increase income, alleviate poverty and enhance development of food industry.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botany, Origin, and Importance of Chickpea

Chickpea is an annual herbaceous plant with branches at the base that are dispersed and spreading which makes the crop resemble a little bush. The majority of the plant's surface is coated in glandular or non-glandular hairs, but other genotypes lack hair. Cultivation of chickpea based on seed size and colour are categorized into macrosperma (Kabuli seeds) and microsperma (desi seeds) (Priyanka *et al*., 2018). Kabuli chickpea seeds are enormous (100-seed mass >25 g), cream-colored, round or ramhead seeds. The medium-to-tall plant has big leaflets, and white blooms, and doesn't have anthocyanins. Desi chickpea is small and angular-shaped and the color of the seed can be cream, black, brown, yellow, or green. Pod-1 contains 2-3 ovules, although only on average 1-2 seeds are formed. The plants have anthocyanin and are short with little leaflets and purple flowers.

Archeological evidence of chickpeas dates back 7500-6500 BC in the Middle East where Rochetti *et al*. (2024) reported on the occurrence of chickpeas in Bardur in Turkey. The crop probably originated in present-day south-eastern Turkey adjoining Syria where the annual three wild varieties of cicer have been traced back (Mathe & Khan, 2023). From this region, chickpeas spread to the eastern and the western parts, and the crop is grown during summer and spring and cool dry periods respectively. Initially, chickpea was taken to be a minor crop in the United. However, interest in the crop was gained when it was grown as an alternative to cereals when it was grown in spring in the Pacific Northwest and in the high plains where the rainfall was marginal. Chickpea has been found to be grown in almost 40 countries with Africa accounting for 5% of the total production while India leads in production.

Chickpeas has a wide range of uses among them boosting human nutrition. The highest nutritional makeup of any dry ingestible grain legume is found in chickpeas. Chickpea typically comprises 23% protein, 64% carbohydrates, 47% starch, 5% fat (mostly linoleic and oleic acids), 6% crude fiber, 6% soluble sugar, and 3% ash (Arooj *et al*., 2021; Fikre *et al*., 2020; Gupta *et al*., 2017; Kaur *et al*., 2021; Yadav *et al*., 2020). In terms of the mineral composition, phosphorous is the mineral component with the highest concentration (343 mg/100 g), followed by iron (7 mg/100 g), and zinc (3 mg/100 g) (Kiprop *et al*., 2016; Mudryj *et al*., 2014). The leaves of the chickpea can also be utilized as leafy vegetables particularly in Kenya. When cultivated in fallow systems with other crops, the crop is renowned for enhancing soil fertility, preserving moisture, and halting the spread of pests and diseases (Cheruiyot *et al*., 2001; Cheruiyot *et al*., 2002; Merga *et al*., 2019).

2.2 Chickpea Production in Kenya

Small-scale farmers in Kenya's eastern region and the Rift Valley province, where better germplasm was introduced in the 1980s, primarily grow chickpeas. Recent studies have revealed that over the past 40 years, the crop has been grown locally in Kenya's coastal and eastern regions (Kimurto *et al*., 2014). Since then, the crop has spread throughout Kenya and is currently grown in a variety of agro ecological zones, including arid lowlands, medium altitudes, and dry highlands. Currently, Kenya produces between 40,000 and 55,000 tons of chickpeas in an area of 18,000 to 20,000 ha (Rubyogo *et al*., 2019). The national output is predicted to range between 540 and 1200 kg ha^{-1}. The last ten years have seen a decline in chickpea production, but in the last four years, interventions in the Rift Valley's Njoro, Koibatek, Bomet, and Naivasha have reported an increase in yield to 1500–3000 kg ha^{-1} (Ojiewo *et al*., 2020). About 70,000–100,000 tons of chickpeas are consumed annually thus, Kenya imports up to 100 000 tons of chickpea from Ethiopia, Sudan and Tanzania annually.

2.3 Constraints of Chickpea Production

Potential production is 1.31 t/ha globally which is less than the anticipated potential (FAOSTAT, 2014). Abiotic and biotic limits also contribute to production limitations. Abiotic restrictions such as drought, heat stress, and cold stress can lower yield losses by up to 50% in cases of drought and by 15-20% in cases of high and low temperatures (Gunes *et al*., 2008). Viruses, fungi, bacteria, and nematodes are a few examples of the biological limitations influencing chickpea production worldwide (Gurjar *et al*., 2011). A couple of pathogens mentioned cause diseases that reduce chickpea yields. Viral and bacterial infections also affect the crop at all stages of growth, although fungus is the main cause of the decline in chickpea production. The most dangerous fungi that affect chickpeas are Fusarium wilt (15% loss), Ascochyta blight (100% yield losse), grey mold (50%), and phytophthora root rot (70% yield loss).

2.4 Pyrolysis

Pyrolysis is a controlled heating process that produces biochar, volatile condensable compounds (such as wood vinegar, biochar and tar), and gaseous products like CH_4, H_2O, H_2, CO_2 and CO (Chen *et al*., 2021; Zaman *et al.*, 2017). These bio products have commercial value and serve and are important agricultural products. Pyrolysis could either be slow or fast depending on the decomposition temperature. Slow pyrolysis, also known as traditional pyrolysis, is a pyrolysis technology that originated in the early 1900s, when wood was pyrolyzed in industries for 24 hours to yield methanol, ethanol, acetic acid, and coal (Uddin *et al*., 2018). This technology employs continuous systems known as "charcoal" systems, which steadily heat the organic matter in an anaerobic atmosphere to temperatures greater than 400 °C, with minimum heating rates ranging from 5 to 7 °C/min and maximum heating rates ranging from 20 to 100 °C/min 9 (Chen *et al*., 2021). Maximum temperatures vary from 400 to 650 °C, with residence durations ranging from 5 to 30 minutes to several days, during which volatile organic molecules break and recombine to form char and other fractional liquids. Rapid pyrolysis is a pyrolysis process that increases the yield of high-quality liquid oil. This oil is an intermediate dense energy fuel that can be upgraded into hydrocarbons in petrol and diesel (Strahan *et al*., 2011). During fast pyrolysis, organic matter is thermally processed in the absence of oxygen at temperatures ranging from 600-650 °C at rates of up to 1000 °C per second. This causes the organic stuff to decay quickly, yielding large amount of vapours and aerosols with small amounts of gas and coal.

2.5 Biology and Epidemiology of Ascochyta rabiei

Ascochyta rabiei is a major constraint in chickpea production causing tremendous economic losses in farming regions (Singh *et al.,* 2022). Chickpea is the main host plant of the pathogen while other crops such as lentils, faba beans, and field peas have been reported to be affected by the pathogen. The pathogen thrives well in infected and contaminated seeds as well as infected volunteer chickpea crops (Abassy, 2024). During the off seasons, infected stubbles that are blown by wind act as mechanisms for short-distance dispersal. Spores produced by the wood pathogen are known to survive on machinery, skin, and clothing for a short period of time. Volunteer-infected chickpeas act as sources of inoculum that rapidly causes the spread of the disease when the environment is cool and wet. Infections occur when there are 3 hours of leaf wetness and temperature ranges of 5-30^{o}C (Shah, 2001). The fast development of the disease is seen when the temperature range is between 15^{o}C-30^{o}C with high relative humidity occurrences. Rapid infections are observed when the spores are moved further in the crop canopies by rain splash water.

2.6 Life Cycle of *Ascochyta rabiei*

Ascochyta rabiei exhibits sexual and asexual stages in its life cycle: asexual stages often referred as the anamorph and the sexual stage also called the teleomorph (Singh *et al*., 2022). The teleomorph stage occurs when two mating types are present on Ascochyta blight-infected crop or on crop debris (Owali *et al.,* 2019). Also there has to be high humid and low temperature 5^{o}C-10^{o}Cwhich facilitates oozing out of the spores allowing mating to occur. The successful mating leads to formation of the sexual fruiting bodies known as the psuedothesium which is technically present in the host tissues (Shah, 2001). The pseudothesium is black or brown in color and within it are the asci which contain 8 ascospores that are two-celledwhen the weather conditions are favorable and the pseudothesium is mature, it releases the ascospores in the air (Kerman, 2020).

Asexual reproduction is characterized by formation of fruiting bodies known as the pycnidia. The pycnidia contains an internal fertile layer known as the hymen and an opening known as the ostiole. The pycnidia produces the pycnidiopspores which appear as black dots produced on the tissues of the host. The pycnidiopspores are oval/lemon shaped and usually have a bend end on one side or in other cases, both ends can be bend (Abassy, 2024). On the artificial medial, the colonies are usually flat and submerged with a mycelium that is sparse. Pycnidiopspores are dispersed by air which later on lands and infects the chickpea plant. Upon landing, the pycnidiopspores develops an appressorium like structure on the surface of the hyphae that is used to puncture the epidermal layers of the plant and later the subepidermal layers (Oliver, 2024). At later stages, the pycnidia are formed which contain the pycnidiopspores that are dispersed by splash rain water or any other agents of dispersal. This is as illustrated in figure 1 below

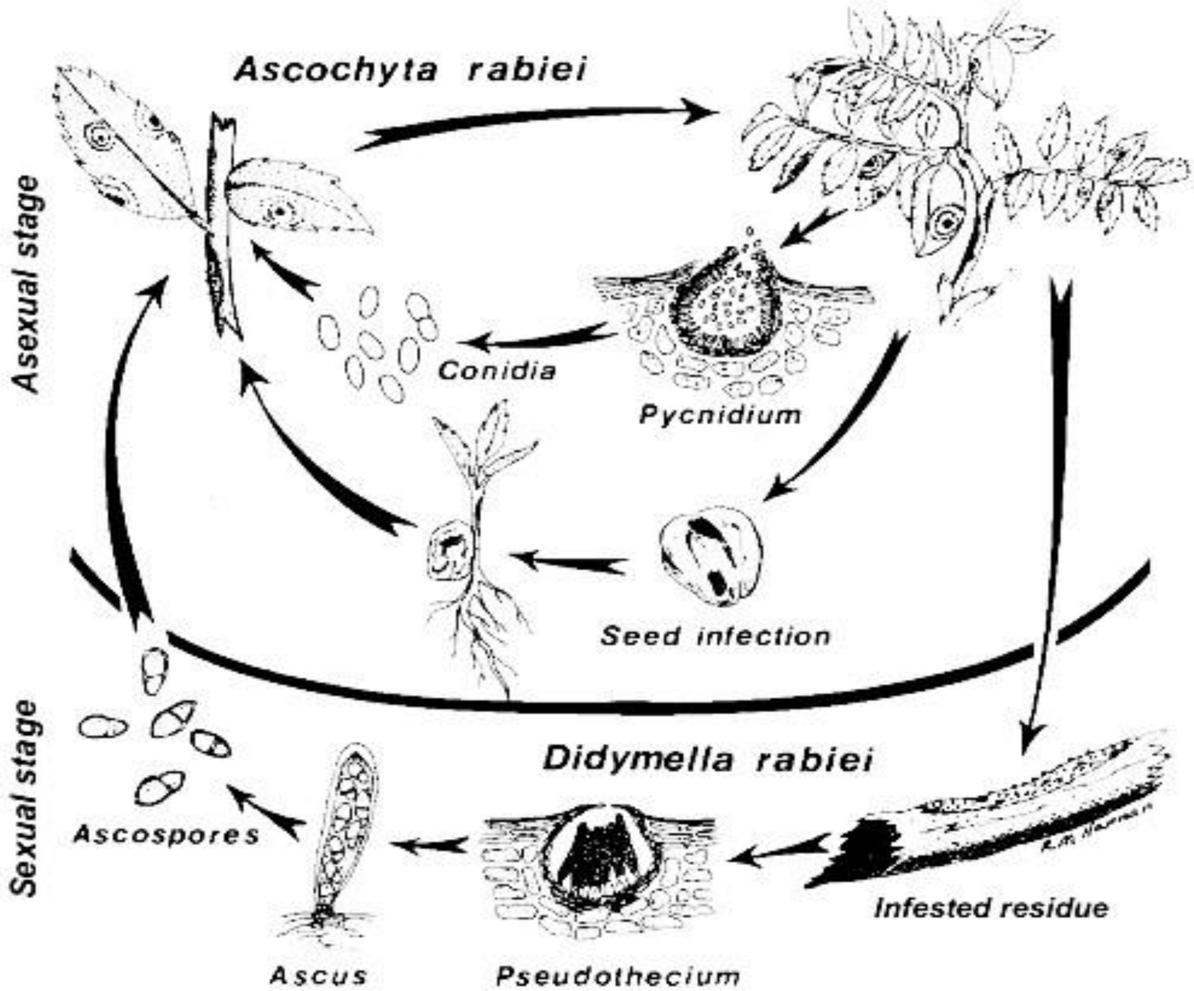

Figure 2. 1 : Life cycle of *Ascochyta rabiei* (Choudhary *et al*., 2022).

2.7 Symptoms of Ascochyta Blight

Ascochyta blight affects all the aerial wood parts of the crop: stem, leaves, petioles and the seeds when the environmental conditions are favourable. The disease is first noticed when the weather is humid and it manifests itself as creamy water soaked necrotic lesions on young/newer leaves of the crop (Nawaz, 2019). As the conditions continues to prevail, the necrotic lesions coalesce and form small circular necrotic lesions on leaves and pods which turn brown while some dark in color. With time, the oval lesions on leaves and pods develops concentric rings of pycnidia which are the fruiting bodies of the anamorph. (Davidson, 2012). On petioles and stems, the necrotic lesions becomes elongated also bearing the pycnidia/fruiting bodies in concentric rings. Necrotic lesions on stems appeared to be larger and this causes girdling of the lesions on the parts above the girdle. The incidence and severity of Ascochyta blight is more pronounced during the reproductive stage. This is as a result of the pycnidia, the asexual structures that harbored the pycnidiopspores (Koder *et al*., 2022; Liu *et al*., 2016).

Plate 2. 1: Dark oval lesion of Ascochyta blight in chickpea on pods. (Source, Author).

Plate 2. 2: Defoliated plant drying up due to increased severity of the disease at ATC Koibatek. (Source, Author).

2.8 Management of Ascochyta Blight

2.8.1 Biological Management of Ascochyta Blight

Biological management of crop pests and plant diseases has been emerging as the most viable and environmental approach to control crop pests and diseases (Ghazanfar *et al.*, 2018). These strategies are alternatives to using the use of chemicals and they show promising results and they are environmentally friendly even though their impact takes a long to be realized The methods involve the use of fungal and bacterial antagonists that are isolated from the crops rhizosphere as well as compost pits which have been used to manage crop pests and diseases. Various strains of fungi and bacteria such as Trichoderma, bacillus, and *Fusarium oxysporum* have been isolated and have demonstrated their efficacy in managing seed-borne and pathogenic fungi as well as enhancing nutrient acquisition and metabolization as reported (Ruano-Rosa *et al*., 2018).

Biological extracts from plants that have tested against *Ascochyta rabiei* in laboratory conditions and have recorded positive results on fungal growth inhibition on mycelial growth of the fungal colonies. Plant extracts such as fractions of n-hexane (dried leaves of *Syzgium camings* and *Chinopodium album* dissolved in n-hexane) has shown inbition properties against *A. rabiei* as reported by Sherazi *et al*. (2016). According to Javaid *et al.* (2020) fractions from n-butanol and ethyl acetate of *Tagetes erectus* have demonstrated inhibition properties in vitro bioassays. Fuithuremore, reports from (Ennouri *et al*., 2020; Erdogan & Keceli, 2021) shows that oils from peppermint (*Mentha piperita*) lemon gun that is scented

(*Corymbia citriodora*) and thymus vulgaris have demonstrated the same abilities of fungal growth inhibition of *Ascochyta rabiei* under in vitro experiments

2.8.2 Chemical Management of Ascochyta Blight of Chickpea

Management of Ascochyta blight by use of chemicals involves the application of fungicides. Even though this approach is harmful to the environment and humans. Pande *et al*. (2005) reported that it is the only approach that can be used to manage Ascochyta blight of chickpeas and achieve high profits since the resistant varieties are easily broken down by the emerging pathotypes of the pathogen which are virulent. Application of chemical fungicides can be achieved as seed dressers or as foliar fungicides. *Ascochyta rabiei* thrives in seeds, on infected crop residues and on volunteer chickpea and this have great potential to serve as sources of inoculum in new fields upon introduction (Moore *et al*., 2015). Infected seeds of chickpeas are usually quantified in terms of size. Small-seeded chickpea seeds are usually infected by Ascochyta blight as compared to large seeds. Infected crops usually produce tiny pods with tiny seeds as compared to healthy and vigorously growing crops. Various studies have shown great potential of seed dressing with fungicides as a way of reducing the severity and incidence of Ascochyta blight on emerging seedlings. Dressing of infected seeds with fungicides helps reduce the level of the inoculum though it cannot eradicate the inoculum completely. According to Schmitt *et al*. (2016) dressing of infected chickpea seeds with systemic fungicides such as stobilurins increases the resistance of young seedlings upon emergence. Furthermore, seed dressing with thiabendazole or a combination of other chemicals with thiabendazole helps prevent spread of Ascochyta blight through infected seeds (Singh *et al*., 2022).

Foliar applications of fungicides can either be systemic or protectants. Protectant fungicides are usually applied before infection of the disease occurs while systemic fungicides are usually applied after an infection has occurred or during periods when the conditions for AB build-up are favorable. Protectant fungicides such as chlorothanil when applied help delay the onset of the disease and this is as reported by Bretag *et al*. (2008). Systemic fungicides such as succinate dehydrogenase inhibitors, demethylation inhibitors, and quinone inhibitors are used to protect infected chickpeas against Ascochyta blight. When the crop is in the vegetative or podding phases, the application of proline and Endura is highly recommended if the conditions are favorable. According to Nganga *et al*. (2016) the application of tubeconazole or difeconazole was effective when the disease had occurred up to 3 days of infection upon overhead irrigation or after a splash of rain has occurred. The application of the same fungicide several times causes resistance. Therefore, there should be a well-scheduled plan for the application of the fungicides putting into consideration, the rates of application, time of application, and a rotation schedule of different fungicides (Fanning *et al*., 2022). The time of application of foliar fungicides is important. The most recommended time for foliar sprays is before flowering. According to Chongo *et al*. (2003) foliar applications before flowering were effective as compared to foliar applications made at reproductive stages.

2.8.3 Host Plant Resistance (HPR)

For the most part, resistance has held against the soilborne diseases. However, currently used cultivars have weak AB pathogen tolerance and are readily destroyed (Gan *et al*., 2006). The wood pathogen's sexual stage (telemorph), which greatly aids in the formation of new pathotypes or races. Integral to integrated pest control are cultivars that are tolerant of or resistant to a number of biotic or abiotic stressors. There are several reported variants having resistant characteristics. Evans, Dwelly, Sanford, and Mild's and other locally released genotypes like ICC7052 and ICC 4360 (Chandirasekaran *et al*., 2007; Gayacharan *et al*., 2020). Disease control has become easier thanks to the breeding of moderately resistant Kabuli or desi types. Chickpea leaf miner and Helicoverpa resistance have been identified, and work is being done to create cultivars that are resistant to these pests. It is still a remote possibility to manage Bruchus species and Sitona species through host plant resistance.

2.8.4 Wood Vinegar and Biochar

Biochar is a solid material that is carbonated and it is created through the thermo-chemical conversion of biomass in an oxygen-restricted environment. (Karim *et al*., 2019; Rowan *et al*., 2022). Studies have revealed that biochar has positive impact on managing soil and crops, including buffering soil acidity (Xu *et al*., 2012), strengthening of the connections between soil and water (Novak *et al*., 2012), the capacity to hold and adsorb hazardous chemicals (Oleszczuk *et al*., 2012), the promotion of beneficial bacteria, and the inhibition of soil-borne diseases (Bonanomi *et al*., 2015; Jaiswal *et al*., 2019). Wood vinegar is a dark liquid produced when woody biomass is heated in an oxygen limited environment. Production of wood vinegar/ pyroligneous acid can be achieved through fast or slow pyrolysis. Slow pyrolysis is a conventional method and it is achieved when the temperatures are 400 ^oC while fast pyrolysis of wood vinegar is achieved when the lignocellulosic materials are rapidly heated at 500 ^oC (Aguirre *et al*., 2020). Contents of wood vinegar includes a family of organic and inorganic compounds such as lignin, cellulose, hemicellulose, ketones, starches, proteins, glycosides, alkaloids, and lipids (Balat *et al*., 2016; Zhai *et al*., 2015).

Pyroligneous acid has potentially been useful for plant growth promotion and disease control in agro-ecosystems (Oramahi & Yoshimura, 2013). All things considered, the research suggests that wood vinegar may be helpful in agro-ecosystems for promoting plant development and preventing diseases. Additionally, research indicated that wood vinegar can be a repellant to genuine flies, specifically insects of the order Diptera. Because of this, wood vinegar and biochar can be used in combination to suppress hazardous insects, soil-borne pathogens, and foliar diseases in crops.

2.8.5 Integrated Disease Management (IDM)

Disease management of Ascochyta blight can be achieved through integrated disease management strategies to ensure that the disease inoculums are kept below the economic threshold levels (Pande *et al*., 2009). The reports confirmed that the use of clean seeds and the burying of diseased crop residues reduced the levels of wood pathogen inoculum. Integration of cultural practices like crop rotation with nonhost crops and judicious application of foliar fungicides as well as the use of resistant cultivars helps reduce the yield losses caused by Ascochyta blight. The use of resistant cultivars is among the most economical, safe, and environmentally friendly approaches to managing the Ascochyta blight of chickpeas (Crutcher *et al*., 2022). Cultural and chemical approaches used alone cannot efficiently manage losses that are brought about by Ascochyta blight (Manjunatha *et al*., 2018). Avoiding planting chickpeas in the same wood paddock for around 3-4 years can be another practice to manage the disease. However, this approach is not practical since most farmers have very small pieces of land.

CHAPTER THREE

ANTIFUNGAL ACTIVITY OF WOOD VINEGAR FROM SELECTED FEEDSTOCKS ON (*Ascochyta rabiei*) *IN VITRO*

Abstract

A study to evaluate the antifungal activity of wood vinegar (pyroligenous acid) from maize cobs, acacia twigs, bean wastes, and an invasive tree species *Prosopis juliflora* against *Ascochyta rabiei* was conducted *in vitro* at Egerton University Njoro, Kenya. The physicochemical characteristics of the different wood vinegar were also determined. Antifungal effects of wood vinegar were evaluated at different concentrations (0.5, 1, 1.5, 2, 2.5, and 3% v/v) using a Petri dish bioassay arranged in a completely randomized design. A fungicide (Metalaxyl-M-40g/kg) and water were used as positive and negative controls, respectively. All the wood vinegars had a smoky odor with brown/yellow coloration and an average density of 1.06 g/cm^3. The wood vinegar from maize cobs showed a near acidic pH of 3.90 while bean wastes showed a near neutral pH of 7.16. Prosopis and acacia showed moderate acidity of 5.10 and 5.43, respectively. The highest concentration of phenols was recorded in wood vinegar from maize cobs (4.56 mg/ml) followed by acacia (3.52 mg/ml). The results from the antifungal assay showed wood vinegar treatment significantly ($P \leq 0.001$) reduced *A. rabiei* mycelia growth at all tested concentrations when compared to the untreated control. The minimum inhibition concentration was 0.5% v/v for all the tested wood vinegars. The percent mycelia growth inhibition generally increased with increasing concentration except for maize cob which showed 99.33% with complete inhibition at 0.5% v/v. Complete inhibition of the pathogen's growth (100%) for all the wood vinegars tested was achieved at 2.5% v/v concentration. This study showed that plant-based wood vinegar has antifungal activity against *A. rabiei*.

3.1 Introduction

Global production of chickpeas is limited by several fungal diseases key among them is Ascochyta blight (AB) caused by the pathogen; *Ascochyta rabiei*. This is a well-pronounced world pathogen that can cause yield losses of up to 100% under favorable weather on susceptible cultivars (Bahr *et al*., 2016; De Rossi *et al*., 2018; Sharma & Gosh, 2016; Tedesse *et al*., 2017). According to Kanouni *et al*. (2022) Ascochyta blight symptoms occur on aerial parts of the plant including leaves, stems, pods and flowers. Infected seeds are shrunken and shriveled and in severe cases, lesions having dark pycnidia are present (Kahraman & Ozkan, 2015; Zhang *et al*., 2019).

Limited cultivar resistance has led to over reliance on foliar applied fungicides in the management of the disease (Fanning *et al*., 2022). There is an increasing demand for natural pesticides that are not only efficient but safe to humans and friendly to the environment (Ngegba *et al*., 2020). Bio-pesticides, which are defined as pesticides derived from natural sources such as plants, bacteria, fungi, animals and some minerals, are potential alternatives pesticides and are gaining great research attention (Khursheed, *et al.,* 2022). Plant-derived biopesticides from many plant species are reported to be efficacious against several plant pathogenic microorganisms without imposing ill side effects (Shimira *et al*., 2021; Souto *et al.,* 2021). The antifungal effect of various plant derived products has been demonstrated by several researchers both *in-vitro* and *in-vivo* (Hassan *et al*., 2009; Kundu *et al.,* 2021; Wamani, 2020).

Wood vinegar (pyroligenous acid) is a highly condensed aqueous crude liquid produced through fast or slow pyrolysis of plant biomass materials in an oxygen-limited environment (Sangsuk *et al*., 2023). It has been widely used as natural pesticide based on old traditions and knowledge (Mhamdi, 2023; Trabelsi *et al*., 2021). Several studies have reported antifungal, antibacterial and insecticidal properties of wood vinegar (Arysad *et al*., 2020; Lee *et al*., 2011; Lee *et al*., 2022; Ma *et al*., 2011; Oramahi *et al.,* 2013). A study conducted by Urrutia *et al.* (2022) reported the insecticidal activities of wood vinegar against the Colorado potato beetle. Theapprat *et al*. (2015) reported that wood vinegar produced from *Dendrocalamus* asper (bamboo), *Eucalyptus camaldulensis*, *Azadirachta indica*, and *Leucaena leucocephala* exhibited antifungal properties against brown rot fungus and sapstain fungus. Termiticidal properties of wood vinegar investigated by Temiz *et al*. (2013) indicated that wood vinegar from giant cane has numerous components like aldehydes, ketones, phenols, and aldehydes which were effective against *Reticulitermes flavipes.* According to Shiny and Remadevi (2014) wood vinegar from shell oil can be used as a biodegradable termiticide and this greatly allows it to be used as a biopesticide.

The physico-chemistry and biological activity of wood vinegar are affected by many factors such as chemical composition of biomass pyrolysis system and refining method (Zhao *et al*., 2014). According to Zhao *et al.* (2014) the lignocellulosic components of agricultural biomass have different proportions and this contributes to differences in the composition of wood vinegar. It is on this premise that the current study undertook to determine the antifungal effects of wood vinegar from an invasive plant *Prosopis juliflora*, forest waste from acacia and agricultural waste from maize and beans on Ascochyta blight in chickpea *in vitro*. The study hypothesized differences in the efficacy of the wood vinegar from the different feedstocks. The characteristics of the wood vinegar from the selected feedstocks were also evaluated.

3.2 Materials and Methods

3.2.1 Preparation of Wood Vinegar from the Selected Feedstocks

Wood vinegar was produced from maize cobs, acacia, bean straws, and *Prosopis juliflora* locally known as Mathenge in Kenya. Maize cobs, bean straw and *Prosopis juliflora* were provided by the Perkerra National Irrigation Authority (NIA) at Marigat, Baringo County. Acacia trimmings were collected around Egerton University Njoro campus. All the materials were sun-dried to a moisture content of 12%. Wood vinegar from the different feedstocks was separately collected as condensed smoke produced through slow pyrolysis at 350^{o}C to 400^{o}C (Doti *et al.,* 2023) using a small vertical drum kiln developed by the Bioenergy Kenya Climate Smart Agriculture Project in the Department of Agricultural Engineering, Egerton University. The smoke condensate was allowed to settle at room temperature for 90 days, separating into layers of tar (at bottom), wood vinegar and water according to Zhai *et al*. (2015).

3.2.2 Physico-Chemical Properties of Wood Vinegar from the Selected Feedstocks

The odor of the wood vinegar was determined by raising the hand above the container and wafting the air towards the nose. Color was determined by visual observation of the liquid and comparisons made against a color chart. The pH values of wood vinegar were measured using a pH meter model (PHS-3CHS). The density was calculated using the formula (equation 1):

$$Density = \frac{\text{mass (kg)}}{\text{volume}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{Equation 1}$$

3.2.3 Preparation of Standard Garlic Acid and Folin–Ciocalteu Reagent

The garlic acid standards were prepared according to Shirazi *et al*. (2014) with some modifications. One gram (1 g) of garlic acid was weighed and dissolved in 100 ml of methanol resulting in a 1% solution of the garlic acid (10 mg/ml). Folin–Ciocalteu suspension was prepared by dissolving 10 g of sodium tungstate and 2.5 g of sodium molybdate in 70 ml of distilled water. This was followed by adding 5 ml of 85% phosphoric acid and 10 ml of concentrated hydrochloric acid. The resulting solution was refluxed for 10 hours before adding 15 g of lithium sulfate followed by 5 ml of distilled water and one drop of bromine water. The solution was refluxed again for 15 minutes and cooled to room temperature before use.

3.2.4 Determination of the Total Phenol Content in Wood Vinegar

The phenol concentration in wood vinegar was determined using Folin–Ciocalteu method according to Yang *et al*. (2016) with some modifications. An aliquot of wood vinegar (1 mL) was added to 1mL of 25% Folin–Ciocalteu suspension followed by 2 ml of 2% Na_2CO_3.The procedure was repeated with standard garlic solution whereby 1 mL aliquots (0.2, 0.4, 0.6, 0.8 mg/ml) were used. The solutions were thoroughly shaken and allowed to incubate for 30 minutes at room temperature. Methanol was used as a blank solution while distilled water was used to zero the spectrophotometer. Absorbance was measured at 760 nm using a UV–Vis spectrophotometer (Table 3.1). The measurement for each sample was done in triplicates. A standard garlic acid curve drawn from the different dilutions. The regression equation derived from the standard garlic acid curve (Fig. 3.1) was used to calculate the phenol concentrations of the selected wood vinegar

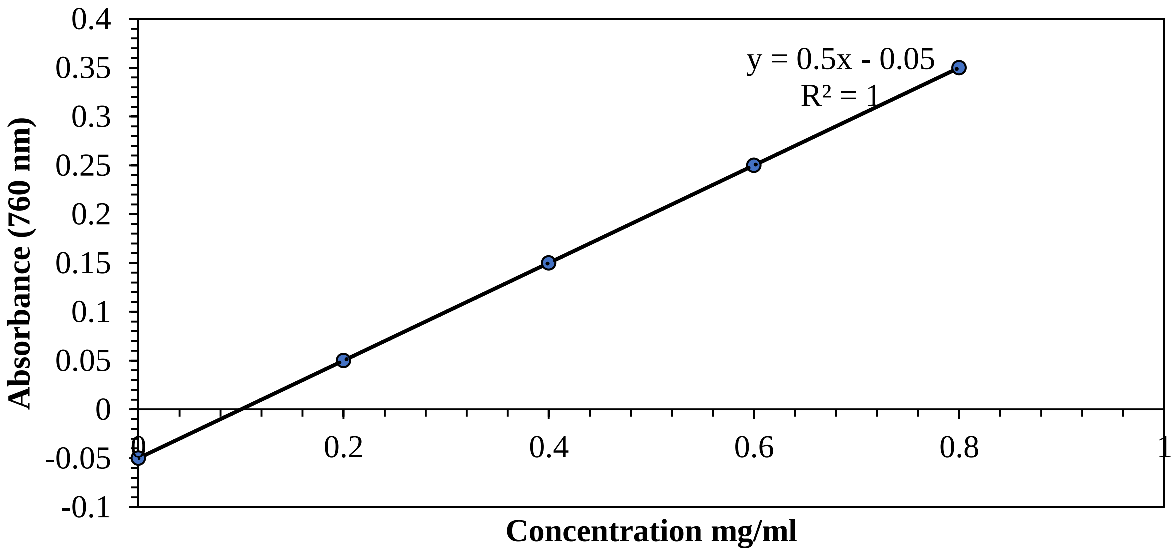

Figure 3. 1: Standard garlic acid curve

3.2.5 *Ascochyta rabiei* isolation and Culture Preparation

Chickpea plants exhibiting Ascochyta blight disease symptoms were collected from the field. Small pieces of diseased parts were cut off the plants using a sterile scalpel. The pieces were sterilized by dipping them in 2% sodium hypochlorite for 3 minutes, washing in 70% ethanol for 30 seconds and rinsing them five times in sterile distilled water. Potato Dextrose Agar (PDA) was prepared and added with doxycycline 300 g/L after sterilization and just before the media cools to prevent the growth of opportunistic pathogens. The mixture was dispensed into sterile petri dishes and left to set. The diseased materials were aseptically transferred onto the PDA plates and incubated at 24°C for 14 days. The plates were observed daily for fungal growth. Single spore technique was used to manage opportunistic pathogens and come up with a pure isolate of *A.rabiei.* Thereafter, the plates were flooded with sterile distilled water and *Ascochyta rabiei* spores were scrapped and harvested using a sterile spatula. The spore suspension was filtered through several layers of a Whatman paper to remove mycelia fragments. The pure culture of the resulting fungal growth was stained with lactophenol cotton blue solution and placed under a compound microscope magnification (×100) for identification based on morphological characteristics of *Ascochyta rabiei.*

3.2.6 Antifungal Assay.

Fungal growth inhibition was conducted according to Oramahi *et al*. (2018) using a Completely Randomized Design (CRD) with three replicates. The different wood vinegar (maize cobs, acacia, bean straws, and Prosopis) were tested at 0.5, 1.0, 1.5, 2, 2.5, and 3 % v/v concentrations. The fungicide Metalaxyl-M-40g/kg and distilled water were used as positive and negative checks, respectively. PDA media was separately mixed with the varying concentrations of the different wood vinegars except maize cobs. The mixture was autoclaved for 15 minutes at 121 °C and then poured into 90 mm diameter Petri dishes. In the case of the wood vinegar from maize cobs, the PDA was sterilized separately for 15 minutes at 121°C and mixed with wood concentrations of wood vinegar when the media was about to gel. After cooling, a 5mm plug mycelia from previously prepared pure culture was cut using a cock borer and centrally inoculated into the treatment plates. The plates were incubated at 25°C and monitored daily for the radial growth of fungi until maximum growth was observed (mycelia reached the edge) in the control plates. The presence of growth inhibition zones, an indication of antifungal activity, was observed. The minimum inhibitory concentration (MIC) for the different wood vinegars was also determined.

3.3 Data Collection

The odor color and density were determined as described under the physicochemical properties in the methodology. Total phenol concentration from acacia, *Prosopis juliflora*, bean straws and maize cobs was calculated according to the standard garlic acid curve equation (Fig. 3.1).

The radial growth (diameter) of the colonies from each Petri dish was measured and the percentage of fungal growth inhibition was calculated using Equation 2:

$I = \frac{C-T}{C} \times 100$…………………………………………………… Equation 2

where I = inhibition, as a percentage; C = colony diameter of mycelium from control Petri dishes (mm); and T = colony diameter of mycelium from the Petri dishes containing the wood vinegar (mm).

3.4 Data Analysis

All the data were analysed using SAS version 9.4 following *PROC GLM* procedures. Means were separated using Fischer's least significance difference at p=0.05. The following statistical model was used for the analysis

$Y_{ijkl} = \mu + F_i + C_j + CF_{ij} + \varepsilon_{ijk}$………………………………………………………3

where; Y_{ijkl} is observation of all the experimental plots; μ is the overall mean; F_i is the effect due to i^{th} feedstock, C_j is the effect due to J^{th} concentration, CF^{ij} is the effect due to the i^{th} feedstock and J^{th} concentration, ε_{ijk} random error component

3.5 Results

3.5.1 Physicochemical Properties of the Selected Wood Vinegar

The highest mean absorbance of the standard garlic acid (0.35) was recorded from the highest concentration of 0.8 mg/ml from maize cobs with the highest absorbance at 0.178 (Table 3.1). All the wood vinegars tested had a smoky odor with brown/yellow coloration. The wood vinegar from maize cobs showed a near acidic pH of 3.90 while bean straws had a near neutral pH of 7.16. Prosopis and acacia had a moderate acidity of 5.10 and 5.43, respectively. The average density of all the tested wood vinegar sources was 1.07 g/cm^3 while (Table 3.1).

Table 3. 1: Absorbance, Density and pH of Wood Vinegar from Selected Feedstock

Wood vinegar	Absorbance (760 nm)	pH	Density (g/cm^3)
Maize cobs	0.178	3.90	1.08
Acacia	0.126	5.43	1.06
Prosopis	0.113	5.10	1.06
Bean straw	0.112	7.16	1.06
Methanol	-0.05		

3.5.2 Phenol Concentration

The results showed significant differences in the concentration of phenols in the wood vinegars tested. The highest concentration of phenols 4.56 mg/ml was recorded in the vinegar from maize cobs, followed by acacia (3.52) which was not significantly different from bean straw and Prosopis.

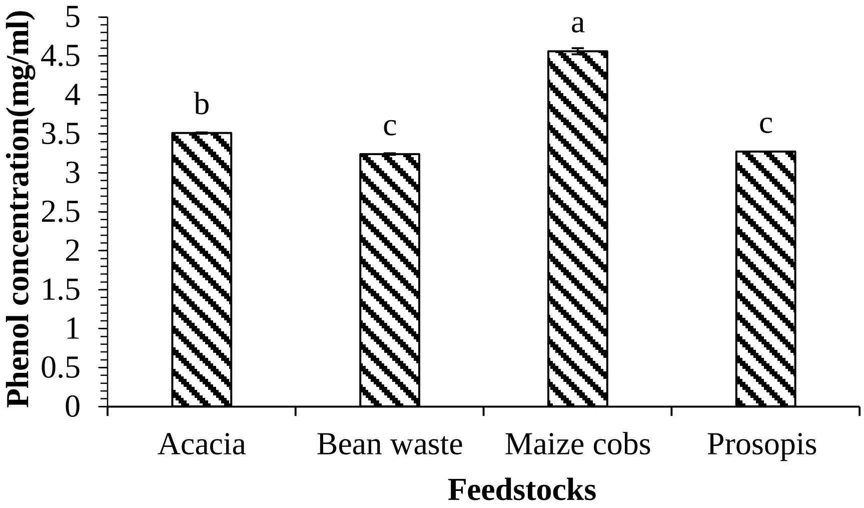

Figure 3. 2: The mean concentration of phenols from selected wood vinegar.

3.5.3 Morphological Characteristics of *A. rabiei*

Under microscopic observation, *Ascochyta rabiei* was circular in shape and brown/ dark in color. The pycnidium was spherical and loosely held on the mycelium. The conidiophores were light brown in color and submerged on PDA. The surface of the pathogen was shiny and rough and some sparse mycelium could be seen on the aerial parts. Following these features, the pathogen was identified as *A.rabiei* (Baite *et al*., 2016; Crociara *et al*., 2022) as shown in plate Plate 3.1 A, B and C below.

A B C

Plate 3. 1: A, B and C are the representation of front view, pigmentation in agar and Ascospores of *Ascochyta rabiei* respectively as observed under the microscope at x100.

3.5.4 Antifungal Effects of Wood Vinegar from Selected Feedstocks

Results showed that feedstock, concentration, and the interaction between feedstock and concentration were highly significant on fungal growth inhibition at $P \leq 0.001$ (Appendix 1).

Wood vinegar from all the feedstocks differed significantly and they exhibited a decline in the growth of the fungus with wood vinegar from maize cobs and acacia showing maximum results on fungal growth inhibition with means of 99.82% and 86.65% respectively (Table 3.2).

Table 3. 2: Performance of the Metalaxyl-M and Wood Vinegar from Selected Feedstocks on Growth Inhibition of Ascochyta rabiei In Vitro.

Feedstock	**Percent fungal Growth inhibition**
Acacia	86.65^{b}
Bean straws	67.97^{e}
Maize cobs	99.82^{a}
Metalaxyl-M-40g/kg	75.31^{c}
Prosopis juliflora	73.73^{d}
Negative control	0.00^{f}
Lsd	0.58

Means followed by the same letters along the column are not significantly different according to Fischer's least significance difference at $P \leq 0.05$.

The results further showed that wood vinegar treatment, significantly ($P \leq 0.001$) reduced *A. rabiei* mycelia growth at all tested concentrations compared to the untreated control (Table 3.3). The minimum inhibition concentration was 0.5% for all the tested wood vinegar. The percentage of mycelia growth inhibition generally increased with increasing concentration with maize cob showing complete inhibition even at low concentrations. Complete inhibition of the pathogen's growth (100%) was achieved at a concentration of 2.5% v/v for all the wood vinegars. Antifungal activity of the different wood vinegars was indicated by the presence of clear growth inhibition zones in the inoculated plates. Plate 3.2 shows plates of radial fungal inhibition zones for the different vinegars at six concentrations with water and Metalaxyl-M 40g/kg fungicide with a standard application rate of 50 g/L being used as controls.

Table 3. 3: Mycelial growth inhibition of *Ascochyta rabiei* upon exposure on semi-volatile organic compounds of wood vinegar from acacia, bean waste, maize cobs, fungicide and Prosopis at different concentrations. Values are the means of three replicates ± SD.

Feedstock / Con	0	0.5	1	1.5	2	2.5	3
Maize cobs		100.00±0.00	99.57±0.43	100.00±0.00	100.00±0.00	99.33±0.33	100.00±0.00
Acacia		58.81±0.22	70.41±0.3	98.67±0.67	100.00±0.00	100.00±0.00	100.00±0.00
Bean straws		11.31±0.39	46.99±0.03	65.11±0.39	84.41±1.34	100.00±0.00	100.00±0.00
Prosopis juliflora		11.77±0.59	30.61±0.65	100±0.00	100.00±0.00	100.00±0.00	100.00±0.00
metalaxyl-M-40g/kg		24.08±0.61	38.81±1.86	89±0.76	100.76±0.00	100.00±0.00	100.00±0.00
Water	0.00±0.00						

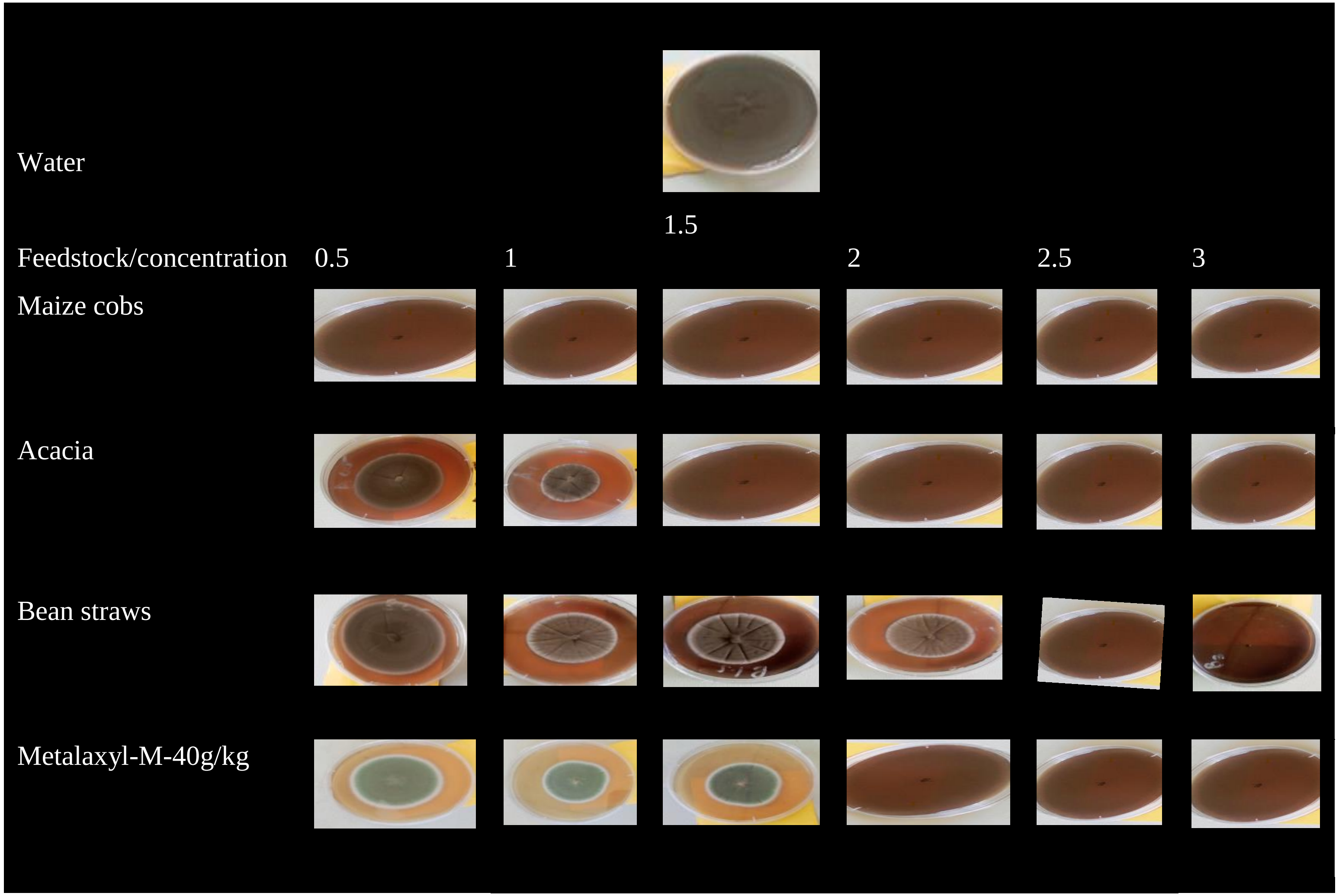

Plate 3. 2: Radial fungal growth inhibition zones for *Ascochyta rabiei* following in vitro assay of wood vinegar and fungicide at different concentrations.

3.6 Discussion

All the wood vinegar tested appeared to be of good quality in terms of physical properties; a smoky odor with brown/yellow coloration and an average specific density of 1.07 $g.mL^{-1}$. Theapparat *et al*. (2018) described the qualities of good wood vinegar as having a smoky odor, no visible suspended matter and transparent liquid with a brown / yellow coloration, pH xvalue of about 3 and a specific density of 1.010-1.050 $g.mL^{-1}$. The near ideal pH of 3.90 was only observed in wood vinegar from maize cobs followed by acacia with 5.10. The organic and inorganic components of the acid contribute to the yellow color as well as the smoky odor of the acid as further confirmed by Mathew *et al*. (2015).

Results from the current study also showed the presence of varying concentrations of phenols in the wood vinegar tested. The highest concentration of phenols 4.56 mg/ml was obtained from maize cobs followed by acacia with 3.52 mg/ml. Previous studies have reported that the physical and chemical properties of wood vinegar are influenced by several factors key among them being the type of feedstock carbonized (Ghodake *et al.*, 2021). Wood vinegar from biomass varies in their chemical structure due to differences in the approximate amount of lignin, cellulose and, hemicellulose (Lu *et al*., 2020). Differences in the proportion of these constituents not only depend on the plant species but are also influenced by other factors such as habitat, age and part of the plant among others (Grewal *et al*., 2018). Variations in the composition of feedstock used for the preparation of wood vinegar are therefore the most likely reason for the different properties of the product.

Most research has found wood vinegar possesses high contents of phenols. The phenolic compounds are biopolymers that form the major components of cell walls in woody plant bark. According to Bouket *et al.* (2022) wood vinegar contains phenolic compounds that are either syringol or catechol type. The Boresults are further confirmed by Liu *et al*. (2021) who reported that wood vinegar contains around 70% of phenolic compounds. These components of wood vinegar have demonstrated excellent antifungal activity against different fungal pathogens (Cadenillas *et al*., 2022; Silva *et al*., 2020; Simonetti *et al.,* 2019; Yilar *et al*., 2019) which is consistent with the findings of the current study. It is therefore a prompt to carry out further research to test the components of wood vinegar individually and their specific antifungal activities. However, several authors have indicated that it is not possible to find out the mode of action of wood vinegar due to the diversity of volatile organic components. The plant-based bioactive compounds including alcohols, alkaloids, phenols, tannins, and terpenes present in wood vinegar have been found to delay sporulation and mycelial development as well as inhibit germ tube elongation (Mansour *et al.*, 2023; Ngegba *et al*., 2022).

In the current study, wood vinegar from maize cobs caused complete inhibition of Ascochyta rabiei mycelial growth in vitro even at low concentrations of 0.5% V/V. Complete inhibition of fungal growth for acacia, bean straw and Prosopis was achieved at the concentration of 2.5% V/V in-vitro. The stronger antifungal activity of wood vinegar from maize cobs can be attributed to the higher phenol content and acetic acid indicated by the near-ideal pH of 3.90. High contents of organic acids and phenols may correlate with strong antimicrobial activity (Deng *et al.*, 2023; Shiny *et al.*, 2024). A similar study by Bouket *et al.* (2022) reported differences in fungal growth inhibition of Pythium mycelia by wood vinegar from Almond, pomate, pine, pomegranate and walnut.

3.7 Conclusions

This study demonstrated that the tested wood vinegars possess antifungal activity against *Ascochyta rabiei* pathogen well exemplified by their ability to completely inhibit mycelial growth at the concentration of 2.5% v/v. The higher potential of wood vinegar from maize which showed complete fungal growth inhibition at 0.5% v/v, is due to the higher proportion of phenols and organic acids indicated by the lower pH. Farmers can produce wood vinegar from biomass waste such as branches trimmed from trees and crop residues making it a clean energy material with low cost and high benefits. The low cost of production can also be attributed to savings from the use of synthetic pesticides. Further research is however recommended to establish specific compounds in wood vinegar that are responsible for fungal growth inhibition of *Ascochyta rabiei* and their stability under field conditions.

CHAPTER FOUR

EFFICACY OF BIOCHAR AND WOOD VINEGAR ON MANAGEMENT OF ASCOCHYTA BLIGHT (Ascochyta rabiei) AND GROWTH OF CHICKPEA (*Cicer arientinum* L.)

Abstract

Field and greenhouse experiments were conducted to determine the efficacy of pyrolysis products (biochar and wood vinegar) from maize cobs and acacia twigs on the management of Ascochyta blight and the performance of chickpea. Greenhouse experiment was conducted in field 7 at Egerton university, Njoro while field experiments were conducted at ATC Koibatek during July-October 2022 and April-August 2023 growing seasons. The treatments were arranged in a factorial randomized complete design (RCBD) both in greenhouse and ATC Koibatek experimental fields. Data was collected the following parameters: Disease incidence, disease severity scoring at seedling, vegetative, flowering and podding growth stages. Data on performance parameters included yield, plant height, crop biomass and 50% flowering. The data obtained was subjected to analysis of variance following PROC GLM procedures using SAS version 9.4 and mean separation was done using Fischer's LSD test at $P \leq 0.05$. In the greenhouse experiment, there was an increase in the number of pods with 81.00 pods/plant, biomass (59.14g), plant height (57 cm), and a reduction of days to 50% flowering (56.67%) from a combination of maize cobs biochar and wood vinegar from maize cobs (T7). Furthermore, the same treatment (T7) showed a reduction in disease incidence and severity by 23.33% and 19.70%, respectively. The correlation analysis indicated a negative correlation between disease incidence/severity and the morphological aspects of chickpea. In the field experiment, treatment T7 showed an increase in number of pod/plants, biomass, plant height, plant count and 50% flowering with means of 54.33 cm, 0.22 kg/ha, 60.50 plant/plot and 50.00% days to flowering respectively. Furthermore, T7 recorded higher yields in season 1 and 2 with means of 3.57 t/ha and 3.40 t/ha. Treatment 7 showed reduction in disease incidence and severity with means of 24.75% and 11.33% respectively. This research, therefore, identified a combination of maize cob biochar and its respective vinegar in enhancing the performance of chickpeas and management of Ascochyta blight.

4.1 Introduction

Ascochyta rabiei is the most devastating fungal wood pathogen that causes Ascochyta blight and leads to great negative effects in chickpeas (Deokar *et al.*, 2019). The disease begins in the distal plant section and may cause wilting before the diseased plant eventually dies (Mahmood *et al.,* 2019). The diseased plant shows lesions ranging in colour from dark brown to black that can be found on seeds, branches, stems, leaflets, and stalks. Temperatures between 5 and 30°C, with an ideal temperature of 20°C and a relative humidity of more than 80%, are favorable for fungus virulence. The pathogen remains viable for 13 years in infected seeds and up to 4 years in the soil on crop residue (Naseer *et al.,* 2022). It can be effectively managed through chemical practices such as seed and foliar applications of synthetic fungicides (Namriboi *et al.,* 2018). However, the use of natural plant-based remedies against this infection is being investigated due to the negative effects of fungicides on the ecosystem (Shuping & Eloff, 2017). According to numerous studies (Akhtar *et al*., 2020; Bottger *et al*., 2018; Javaid *et al*., 2020), many plants are abundant in secondary metabolites that have inhibitory effects on fungal pathogens. Additionally, using plant-derived extract in the management of crop pests and diseases is risk-free, environmentally safe, and less expensive to use than fungicides (Palanichamy *et al*., 2018).

A total of 146 billion MT of agricultural and forestry residues are produced globally each year, most of which are dumped in landfills or burned to ashes. Use of techniques like pyrolysis can be used to transform biomass into gaseous, liquid, and solid fuels (biochar) which are valuable commercially and provide crucial organic resources for use in agriculture. Numerous studies have shown the potential ability of liquid wood vinegar to have antibacterial, antifungal, and antioxidant properties. Application of wood vinegar has shown to be successful against plant diseases in addition to plant growth. These studies also show wood vinegar's ability to control bacteria and fungi that cause plant diseases (Mathew *et al*., 2015).

The pyrolysis process yields biochar which may be included in traditional fertilization techniques, particularly in systems of organically managed food production, it may have significant implications for the development of a circular economy in agriculture. Numerous studies have demonstrated the agronomic advantages of applying biochar to soil fertility, crop nutrition, and productivity (Manirakiza & Seker, 2020; Sanchez *et al*., 2019). Since biochar and pyroligneous products have been demonstrated to promote crop productivity and suppress crop pests and diseases, minimal research has been done on biochar from maize cobs, bean wastes, acacia trimmings, and *Prosopis juliflora*. This study, therefore, aimed at determining the effects of biochar soil applied and wood vinegar foliar spray in managing Ascochyta blight in chickpea and effects of biochar and wood vinegar on the performance of chickpea.

4.2 Materials and Methods

4.2.1. Field Experimental Site Description

The field experiments were conducted at the Agricultural training center ATC Koibatek, Baringo County following the short rain season of July-October 2022 and long rain season April-August 2023. ATC-Koibatek lies at an altitude of 1890 meters above sea level, (latitude 1^{o} 35' S and longitude 36^{o} 66' E) and it is located in the midlands of the upper agroecological zone. The typical annual temperature ranges from 18.2-24.3^{o}C, and the average annual precipitation is 767 mm. The soils are deep, sandy loam, vitric andosols with moderate to high soil fertility (Jaetzold & Schmidt, 2012). The temperatures and rainfall figures throughout the study period are shown in Table 4.1 below.

Table 4. 1: Rainfall and Temperatures Recorded over the Growing Seasons.

Season	Mean monthly temperature ^{o}C	Mean monthly rainfall (mm)
1 (July-October 2021)	30.81	19.48
2 (April- August 2022)	16.80	54.84

4.3. Greenhouse Assay

A susceptible chickpea variety Chania desi 2 (92944) was used to determine the efficacy of wood vinegar on Ascochyta blight incidence and severity, a potted plant experiment was conducted in the greenhouse at Field 7, Egerton University Njoro. Planting pots measuring 15 cm depth and 13 cm wide were filled with autoclaved fine sand and arranged in a factorial randomized block design (RCBD) replicated three times. The experiment included four soil and foliar treatments. Soil applied treatments included separately mixing sand with biochar from acacia and maize cobs, and DAP in pots before planting while the untreated served as the negative control. Foliar applied treatments included selected wood vinegar from *in vitro* experiments (acacia and maize cobs) 3% v/v, a fungicide (Metalaxyl-M-40 g/kg) and untreated (negative control). Biochar was applied at a ratio of 50% biochar and 20% sterilized fine sand and 30% sterilized loam soil. DAP was applied at 2 g per pot while foliar application of the fungicide. Chickpea Seeds were sown in the pots (5 seeds per pot) and after germination, a pure culture of an aggressive isolate of *A. rabiei* on chickpea dextrose broth was used for inoculation. The fungal growth was removed from the flasks and adjusted to 5×10^{5} conidia/mL using a hemocytometer. A calibrated hand sprayer was used to apply the inoculum on the crops that were two weeks old. Wood vinegar and Metalaxyl-M-40 g/kg foliar spray was done at the four chickpea growth stages; Seedling

(21 days after crop emergence, DAE), vegetative (44 days DAE), 50% flowering (60 DAE) and 50% podding. The treatments used are in Table 4.2below

Table 4. 2: Experimental Treatments.

T1	Negative control
T2	DAP/Fungicide (combination of di-ammonium phosphate and a fungicide)
T3	Acid 1 (wood vinegar from maize cobs)
T4	Biochar 1 (biochar from maize cobs)
T5	Acid 2 (wood vinegar from acacia)
T6	Biochar 2 (biochar from acacia)
T7	Acid 1/Biochar 1 (combination of wood vinegar and biochar from maize cobs respectively)
T8	Acid 2/Biochar 2(combination of wood vinegar and biochar from acacia respectively)
T9	Acid 1/Biochar 2 (combination of wood vinegar from maize cobs and biochar from acacia
T10	Acid 2/Biochar 1- (combination of wood vinegar from acacia and biochar from maize cobs)
T11	DAP (di-ammonium phosphate)
T12	Fungicide
T13	DAP/Acid 1 (combination of di-ammonium phosphate and wood vinegar from maize cobs)
T14	DAP/Acid 2 (combination of di-ammonium phosphate and wood vinegar from acacia)
T15	Biochar1/Fungicide (combination of biochar from maize cobs and a fungicide)
T16	Biochar 2/ Fungicide (combination of biochar from acacia and fungicide)

4.4 Data Collection

Data on the following parameters were collected;

a) Percent Disease Incidence

Percentage disease incidence (PDI) was measured based on the number of diseased plants per pot. The percent PDI was calculated as the ratio of the diseased plant to the total number of plants multiplied by 100%. The percent disease incidence (PDI) was calculated as described below

$PDI = \frac{\text{no of diseased plants}}{\text{total number of plant}} \times$ 100%…………………………………………. ……….Equation 4

b) Disease Severity

Scoring of disease severity was done using a 1-9 rating scale as described by Pande *et al*. (2011) where 1 indicates no symptoms, 2 indicates minor lesions that are prominent on the apical stems, 3 indicates lesions that are between 5 and 10 mm in size and a clear drooping of apical stems, 4 indicates lesions that are evident on all plant wood parts and 5 indicates lesions on all plant parts, defoliation has begun, branch dryness and cracking, mild to moderate; 6 is the same as five in terms of lesions, defoliation, broken, and dry branches; 7 is the same as five in terms of lesions, defoliation, broken, and dry branches; 8 is the same as 7 in terms of symptoms, but up to fifty percent of the plants are killed; and 9 is the same as seven in terms of symptoms, but up to all the plants are dry (100%) of the plants killed. Disease severity index was calculated according to Monaim (2011) with the formula given below:

$DSI = \frac{\sum d}{d\ max \times n} \times$………………………………………………………………………. Equation 5

Where: d is the disease rating of each plant

d max is the maximum disease rating and n is the total number of plants examined in each plot. The disease severity index was used to calculate the area under disease progressive curve AUDPC using the given equation

$AUDPC = \sum_{i=1}^{n=1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$…………………………………… ……………...Equation 6

in this case; n was the total number of observations, t was time of each reading in days, y_i was the cumulative disease severity expressed as a proportion at the i^{th} observation, y_{i+1} was disease severity on assessment date $(i + 1)$, t_i was the time (days after planting) at the i^{th} observation and t_{i+1} is the second assessment date of two consecutive assessment

c) Chickpea Growth Data

To determine the effects of biochar and wood vinegar on growth of chickpea, the following data were be recorded; number of days to 50% flowering (recorded as the days from crop emergence up to when the 50% of the crops had flowered, number of pods per plant was counted from three plants and averaged. Plant height was measured by a tape from the base of plant to tip at end upon maturity of the crop, Crop biomass (dry) for three plants was measured by a weighing balance from the laboratory after oven drying for 24 hours.

4.5 Data Analysis

All the data were analysed using SAS version 9.4 following *PROC GLM* procedures. Means were separated using Fischer's least significance difference at $P \leq 0.05$. Pearson correlation matrix was employed to check the relationship between disease incidence, AUDPC and performance aspects of chickpea.

Analysis for incidence and severity was conducted using the following statistical model

$Y_{ijkl} = \mu + S_i + R_j + T_k + S_l + TS_{kl} + \varepsilon_{ijkl}$…………………………………………

Equation 7

where; Y_{ijkl} is observation of all the experimental plots; μ is overall mean; S^i is effect due to i^{th} stage; R_j was the effect due to the j^{th} replicate, T_k was the effect due to k^{th} treatment, TS_{kl} was the effect due to the k^{th} treatment and l^{th} stage, ε_{ijkl} random error component

Analysis for the morphological and area under disease progress curve (AUDPC) of chickpea at the greenhouse conditions was done using the following statistical model

$Y_{ijk} = \mu + R_i + T_j + \varepsilon_{ijk}$…………………………………… ……………………Equation 8

Where where; Y_{ijkl} is observation of all the experimental units; μ is the overall mean; R_i is the effect due to i^{th} replicate; T_j is the effect due to the f the j^{th} treatments, ε_{ijkl} random error component.

4.2.2 Field Assay

The field assay which was a validation of the greenhouse experiment was conducted during July-October 2022 and April-August 2023 growing seasons at ATC Koibatek. The chickpea variety used was similar to the one used in the greenhouse. A two-factor experiments were conducted on plots measuring 2 m × 2 m arranged in a factorial randomized complete block design (RCBD) with three replications. Treatments and combinations are as illustrated in Table 4.2 above. Biochar was applied at 2 kgs per plot and well mixed with the soil in the holes before planting. Two chickpea seeds were placed in the hole with a planting depth of 5-8 cm and spaced at 50 ×10 cm giving a plant population of 400000 plants per ha. Natural diseases infection was allowed to take place in the field experiment. Weed management was achieved by manual weeding as well as rogueing. Insect pest management was achieved by application of (Escort) following manufactures recommendations

4.2.3 Data Collection

Data collected on disease incidence, disease severity and growth parameters were similar as described in section 4.4 above.

Grain yield was obtained by weighing dry grains at 12 % moisture from each experimental unit in the laboratory using an electric weighing balance. Stand count was recorded as total number of crops germinated at 50% germination in each plot.

4.2.4 Data Analysis

All the data were analysed using SAS version 9.4 following *PROC GLM* procedures. Means were separated using Fischer's least significance difference at $P \leq 0.05$.

In the field experiments, the following statistical model were used to perform the analysis for disease incidence, disease severity, area under disease progress curve and morphological aspects, respectively.

$Y_{ijkl} = \mu + R_i + S_j + T_k + ST_{jk} + S_l + SS_{jl} + TS_{kl} + STS_{jkl} + \varepsilon_{ijkl}$……….…………Equation 10

where; Y_{ijkl} is observation of all the experimental plots; μ is overall mean; S_i is effect due to i^{th} season; R_j was the effect due to the j^{th} replicate, T_k was the effect due to k^{th} treatment, ST_{jk} was the effect due to the j^{th} season and k^{th} treatment, S_l was the effect due to l^{th} stage, SS_{jl} was the effect due interaction between j^{th} season and l^{th} stage, TS_{kl} was the effect due to interaction between the k^{th} treatment l^{th} stage, STS_{jkl} was the effect due to interaction between the j^{th} season, k^{th} treatment and l^{th} stage, ε_{ijkl} random error component.

$Y_{ijkl} = \mu + S_i + R_j + T_k + ST_{ik} + \varepsilon_{ijkl}$……………………………………………………...11

where; Y_{ijkl} is observation of all the experimental plots; μ is overall mean; S_i is effect due to i^{th} season; R_j was the effect due to the j^{th} replicate, T_k was the effect due to k^{th} treatment, ST_{ik} was the effect due to the i^{th} season and k^{th} treatment, ε_{ijkl} random error component

4.6 Results

4.6.1 Effect of Biochar and Wood Vinegar on Percent Disease Incident (PDI) In the Greenhouse Experiment.

The analysis of variance from the greenhouse experiment showed that treatments, growth stage and the interaction between treatments and growth stage were highly significant at (*$P \leq 0.001$*). Potted plants treated with biochar and later sprayed with wood vinegar showed significant differences in disease incidence compared to the control (T1). Soil treatment with biochar and foliar application of wood vinegar had a significant reduction on disease incidence. The highest percentage disease incidence in the greenhouse experiment was recorded from T1 with a mean percent disease incidence of 65.00% while the lowest disease incidence was recorded from T7 at 23.33% (Figure 4.1).

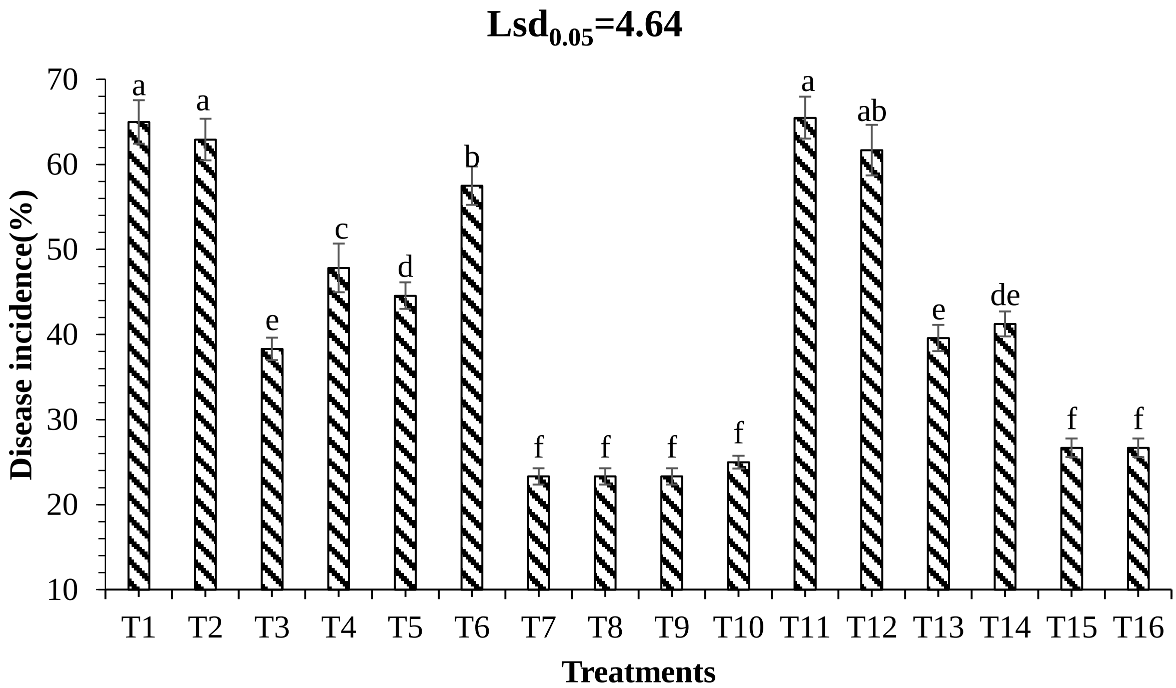

Figure 4. 1: Percent disease incidence in the greenhouse. Bars with similar letters are not significantly different according to Fischer's least significance difference at $P \leq 0.05$.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.2 Effect of Biochar and Wood Vinegar Treatments on Percent Disease Incidence (PDI) at different Chickpea Growth Stages under Greenhouse experiment.

In the greenhouse, the interactive effect of treatments and crop growth stages differed significantly from the controls at $P \leq 0.001$. The highest PDI for all the treatments were recorded at podding with DAP (T11) recording 100% mean PDI which did not differ significantly from the negative control (T1). The lowest Ascochyta blight incidence was recorded from cobs biochar and cobs vinegar (T 7) with mean PDI of 20% flowering, 40% podding, 13.00% seedling and 20.00% vegetative (Table 4.3).

Table 4. 3: Effect of Biochar and Wood Vinegar Treatments on Percent Disease Incidence at different Chickpea Growth Stages.

Treatments	Growth stages			
	Flowering	Podding	Seedling	Vegetative
	% disease incidence (PDI)			
T1	80.00±0.00	100.00±0.00	20.00±0.00	60.00±0.00
T2	100.00±0.00	50±0.00	25.00±0.00	40.00±0.00
T3	40.00±0.00	60.00±0.00	20.00±0.00	33.33±6.67
T4	60.67±0.00	90.00±0.00	0.00±0.00	40.67±0.00
T5	46.67±3.33	46.67±5.00	70.00±1.67	23.33±7.26
T6	70.00±0.00	90.00±0.00	20.00±0.00	50.00±0.00
T7	20.00±0.00	40.00±6.67	13..00±0.00	20.00±0.00
T8	20.00±6.67	40.00±0.00	13.33±6.67	20.00±0.00
T9	20.00±0.00	40.00±0.00	13.33±6.67	20.00±0.00
T10	20.00±0.00	40.00±0.00	20.00±0.00	20.00±0.00
T11	75.00±0.00	100.00±0.00	25.00±0.00	50.00±0.00
T12	76.67±5.33	33±1.67	23.33±7.26	38.33±3.33
T13	43.33±3.33	65.00±5.00	21.67±1.67	21.67±5.00
T14	43.33±5.00	65.00±1.67	21.67±5.00	35.00±0.00
T15	26.67±0.00	40.00±6.67	13.33±6.67	26.67±1.67
T16	20.00±6.67	46.67±0.00	20.00±0.00	20.00±0.00

Figures in a column whose SE values do not overlap are significantly different at $P \leq 0.05$.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.3 Effect of Biochar and Wood Vinegar on Disease Severity and Area Under Disease Progress Curve (AUDPC).

The analysis of variance from the greenhouse experiment showed that treatments were highly AUDPC at *$P \leq 0.01$)* on and incidence *(*Appendix 2).

Disease severity differed depending on the efficacy of the applied treatment on potted plants (Plate 4.1).

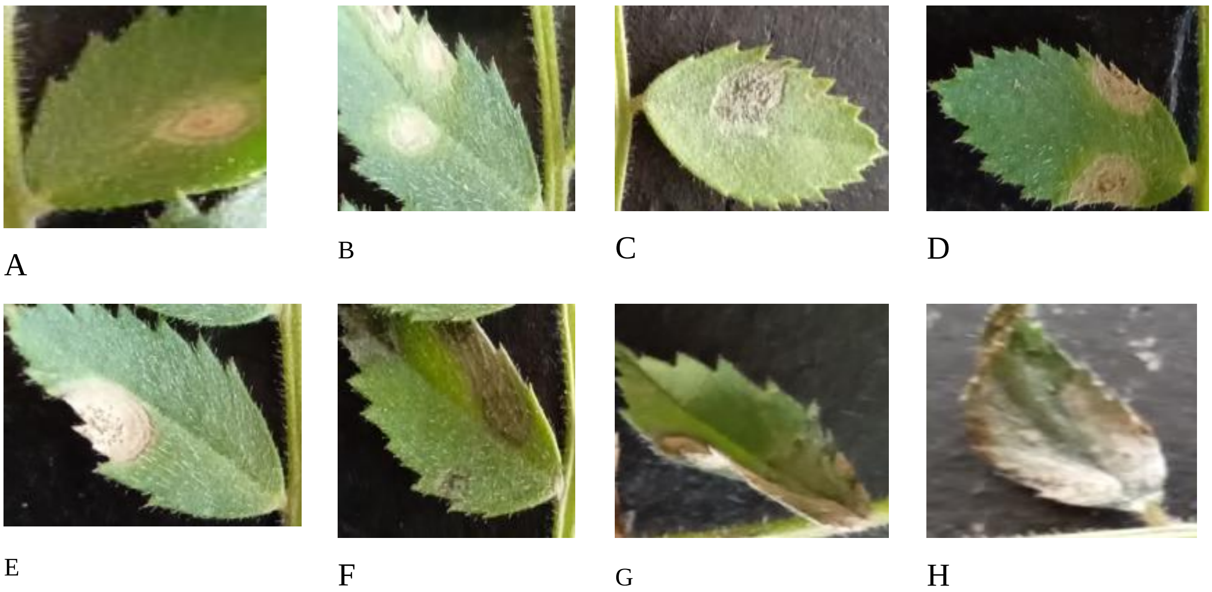

Plate 4. 1: Ascochyta blight disease severity for different biochar and wood vinegars: A-biochar and wood vinegar from maize cobs, B-biochar and wood vinegar from acacia, C-biochar from acacia and wood vinegar from cobs, D-biochar from cobs and wood vinegar from acacia, E-fungicide and biochar from maize cobs, F-fungicide and biochar from acacia, G-DAP and fungicide, H-negative control.

The area under disease progress curve *(*AUDPC) significantly differed with the treatment combinations. The lowest (19.70%) was recorded in T7 (biochar and wood vinegar from maize cobs) with and the highest in T1 (Negative control) recording a mean of 68.19% (Figure 4.1a). sole application of DAP (T11) and fungicide (T12) showed moderate reduction in AUDPC with mean percent of 40.44% and 42.26% respectively and were significantly different from the controls (Figure 4.2).

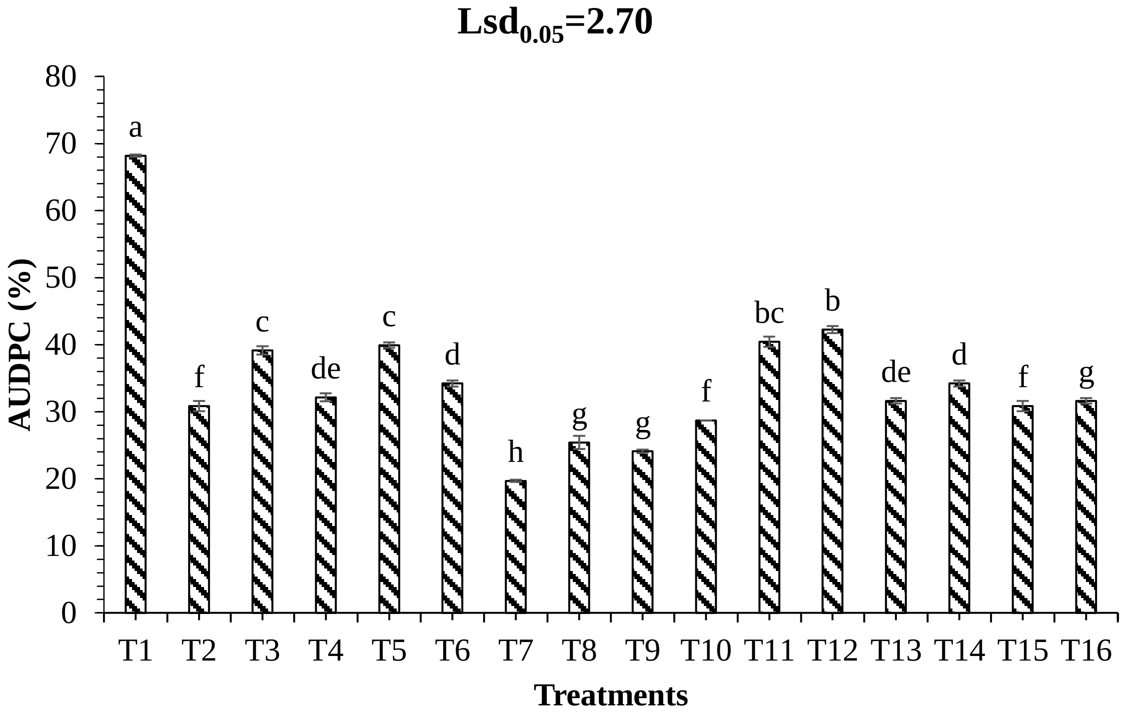

Figure 4. 2: Area under disease progress curve in the greenhouse. Bars with similar letters are not significantly different according to Fischer's least significance difference at P≤0.05.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.4 Effect of Biochar and Wood Vinegar on Percent Disease Incident (PDI) in the Field Experiment.

The analysis of variance showed significant differences in mean PDI depending on the efficacy of the treatment applied treatment (Plate 4.2)

Plate 4. 2: Treatment variations in Aschochyta blight PDI in the field. (Source, author).

The lowest percent disease incidence was recorded in plots treated T7 which had slightly a lower mean of 14.51% (1.86) while the highest percent disease incidence of 53.59% (6.47) was recorded in the untreated control (T1). The percent disease incidence for T2 (DAP + Fungicide), was as high as 42.11% and was not significantly different from foliar application of the fungicide. (Figure 4.3).

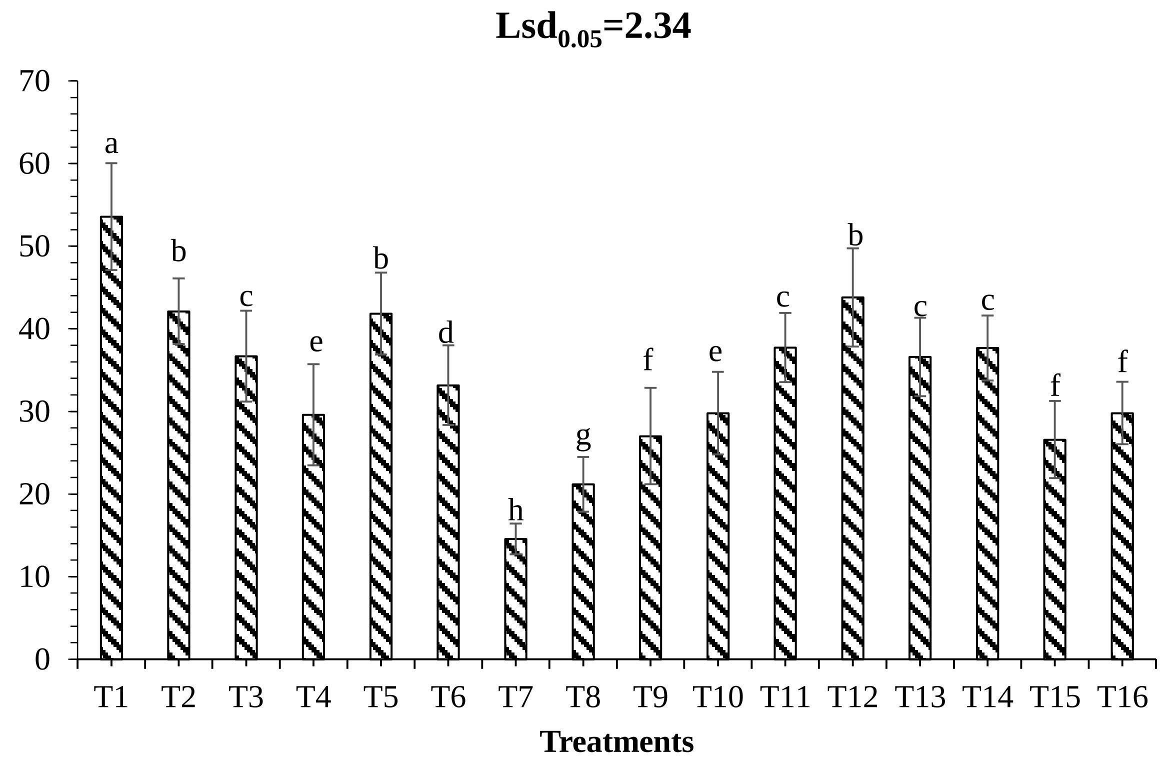

Figure 4. 3: Percent disease incidence in the field. Bars with similar letters are not significantly different according to Fischer's least significance difference at P≤0.05.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.5: Effect of Biochar and Wood Vinegar Treatments on Percent Disease Incidence for the Two Seasons.

Interaction between season and treatments on percent disease incidence revealed that disease incidence was more pronounced in season 2 than in season 1 during the planting period. As was evident from the two seasons, the highest percent disease incidence was recorded from the negative control (T1) with mean percent disease incidence reducing from 48.78% and 58.40% in season 1 and season 2 respectively. The lowest mean percent disease incidence was recorded from T7 with a mean reduction of 13.30% and 15.73% in season 1 and season 2 respectively. The response to the application of foliar fungicide and soil amendments varied greatly while others did not. Biochar and wood vinegar from maize cobs indicated to be the most efficacious in reducing disease incidence of Ascochyta blight under field experiment in the two seasons (Table 4.4).

Table 4.4: Effect of Biochar and Wood Vinegar Treatments on Percent Disease Incidence during Season one and two.

Treatments	PDI	
	Season 1	Season 2
T1	48.78±9.34	58.40±9.14
T2	37.56±7.49	46.67±9.10
T3	33.65±6.62	39.69±7.67
T4	26.9±5.64	32.18±6.35
T5	38.74±8.13	44.93±8.95
T6	31.60±7.00	34.74±6.68
T7	13.30±2.61	15.73±2.71
T8	18.32±4.20	24.09±5.15
T9	23.77±5.22	30.25±5.99
T10	24.39±5.19	32.20±7.66
T11	35.69±8.28	39.78±7.58
T12	40.75±8.59	46.84±8.99
T13	36.14±7.38	37.05±6.94
T14	36.03±7.03	39.33±6.88

T15	23.14±4.96	29.98±5.71
T16	23.38±5.16	27.12±6.21

Figures in a column whose SE values don't overlap are significantly different at $P \leq 0.05$.

Key

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.6: Effect of Biochar and Wood Vinegar on Percent Incidence at different Chickpea crop growth Stages under Field Conditions.

The effect of the application of selected treatments indicated a significant effect on percent disease incidence (PDI) at different growth stages of chickpea in season 1 and season 2 (Table 4.5). Foliar and soil application of wood vinegar and biochar showed significant differences at all growth stages of the crop when compared to the control in the two seasons. No significant differences were observed amongst the treatments at the seedling stage apart from the control experiments in season 1. Variations in response in the application of biochar and wood vinegar started showing up from vegetative, flowering and podding stages of the crop in season 1. In season 2, variation in response to the application of biochar and wood vinegar was evident in some of the pyrolysis products as early as seedling stage. Soil application of T7 recorded the lowest PDI in the two seasons with means of (1.68%, 10.79%, 16.36% and 24.35%) at seedling, vegetative, flowering and podding respectively in season 1 and (4.42%, 10.39%, 23.06% and 25.05%) at seedling, flowering, vegetative and podding respectively in the second season. Percent disease incidence was high at podding in all the treatments in the first and the second season.

Table 4. 5: Effect of Biochar and Wood Vinegar on Percent Disease Incidence at Different Chickpea Growth Stages during Season 1 and 2.

	Season (1)				Season (2)			
	Stage				**Stage**			
Treatments	seedling	Vegetative	Flowering	Podding	seedling	Vegetative	Flowering	podding
T1	8.04±1.01	33.15±0.93	64.20±1.57	89.74±2.21	16.93±1.55	44.01±2.33	77.40±2.41	95.26±1.40
T2	1.63±0.05	28.86±2.54	54.39±0.34	65.35±4.11	3.33±0.00	36.11±2.22	63.89±1.11	83.33±0.96
T3	1.59±0.00	28.93±3.53	42.86±0.92	61.24±1.53	3.38±0.10	31.53±0.78	52.30±1.08	71.56±3.52
T4	1.64±0.08	20.22±1.66	33.34±2.10	52.54±0.89	3.24±0.05	25.410±1.42	38.91±0.40	61.16±1.28
T5	1.65±1.45	28.19±5.22	51.98±2.61	73.20±0.07	3.39±0.06	33.91±0.58	25.41±1.45	83.09±1.73
T6	1.70±0.08	24.89±0.79	34.23±5.22	65.58±0.53	3.24±0.05	28.62±1.03	43.33±296	63.73±1.22
T7	1.68±0.07	10.79±2.39	16.36±2.11	24.35±1.90	4.42±2.09	10.39±0.46	23.06±2.18	25.05±0.79
T8	1.69±2.22	8.93±0.34	28.67±1.12	33.98±0.07	3.35±0.02	12.84±1.08	35.24±4.44	44.73±3.06

Values recorded are means ± SE from three replicates. Values in a column whose SE values don't overlap are significantly different at $P \leq 0.05$

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

Table 4.5: Continued…

	Season (1)				Season (2)			
	Stage				**Stage**			
Treatments	seedling	Vegetative	Flowering	Podding	seedling	Vegetative	Flowering	podding
T9	1.63±0.05	16.37±1.37	30.05±3.07	47.01±5.13	3.39±0.10	23.82±1.68	38.08±4.27	55.69±5.62
T10	1.64±0.03	20.29±3.27	26.33±0.66	49.32±0.90	2.42±0.68	25.80±2.91	44.36±6.67	68.22±8.34
T11	1.61±0.03	23.65±0.16	40.87±1.43	76.62±0.54	3.58±0.11	32.30±2.12	52.61±2.7	70.64±3.45
T12	2.16±0.51	28.84±1.96	53.29±1.54	78.72±0.31	3.43±0.44	37.12±1.79	64.58±0.99	82.24±1.23
T13	1.71±0.11	29.70±3.55	44.56±1.45	68.58±2.71	3.39±0.06	31.41±3.86	49.61±3.81	63.80±3.15
T14	1.74±0.09	31.8±0.15	44.57±0.72	65.99±1.98	3.37±0.07	37.69±1.54	52.90±2.51	63.38±1.81
T15	1.62±0.03	15.43±0.58	29.78±0.38	45.73±1.18	3.41±0.04	23.31±0.82	38.66±0.00	54.54±1.15
T16	1.62±0.02	15.69±0.70	28.10±0.23	48.11±1.44	3.35±0.11	22.72±1.60	26.50±11.62	52.91±2.34

Values recorded are means ± SE from three replicates. Values in a column whose SE values don’t overlap are significantly different at $P \leq 0.05$.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar, T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.7 Effect of Treatments on Disease Severity at different Crop Growth Stages in the Field.

There were significant differences at $P \leq 0.001$ in Ascochyta blight severity levels among the tested treatments in the two seasons (Table 4.6). The negative control T1 showed the highest disease severity at podding with a severity of 8.83 and was significantly different from the rest of the treatments. Susceptibility in this treatment was high and the performance as well as the yield of the crop was low. In the treatment, AB symptoms were observed on all parts of the plant and defoliation ranged between 10%-80%. The lowest disease severity was recorded from T7 with a mean severity of 3.50 at podding which was significantly lower than the rest of the treatments. In this treatment (T7), the disease severity ranged between 10%-30% throughout the growth stages which was slightly lower as compared to the rest of the treatments. The highest mean severity of 5.71 was recorded from the negative controls experiment while the lowest mean severity of 1.96 was recorded from maize cob biochar and cobs vinegar and was significantly different from other treatments Table 15. However, T8 (acacia vinegar+acacia biochar), T9 (cobs vinegar +acacia biochar) T10 (Acacia vinegar+cobs biochar), T15 (Cobs biochar+Fungicide) and T16 (Acacia biochar+Fungicide) showed moderately lowest severity when compared to the negative control.

Table 4. 6: Effect of Treatments on Disease Severity at different Chickpea Growth Stages in the Field.

Treatments	Seedling	Vegetative	Flowering	Podding	Mean severity
T1	1.67±0.21	5.33±0.92	7.00±0.00	8.83±0.17	5.71
T2	1.00±0.00	1.50±0.22	1.88±0.17	5.50±0.50	2.42
T3	1.00±0.18	2.67±0.21	3.83±0.31	6.67±0.33	4.21
T4	1.00±0.00	1.67±0.21	3.50±0.22	5.50±0.67	4.00
T5	1.17±0.17	2.67±0.33	4.17±0.40	7.00±0.26	2.58
T6	1.17±0.17	1.50±0.22	4.17±0.40	5.50±0.67	2.88
T7	1.00±0.00	1.50±0.22	2.00±0.26	3.50±0.99	1.96
T8	1.00±0.00	1.33±0.21	2.17±0.40	3.33±0.33	2.00
T9	1.00±0.00	1.17±0.17	2.33±0.42	4.50±0.99	2.25
T10	1.00±0.00	1.50±0.22	2.67±0.21	4.50±0.67	2.29
T11	1.17±0.00	3.67±0.71	5.67±0.21	6.50±0.22	2.92
T12	1.00±0.18	3.00±0.77	5.00±0.45	6.83±0.31	3.75
T13	1.00±0.00	1.50±0.22	3.00±0.00	4.83±0.60	3.08
T14	1.00±0.00	1.67±0.21	3.33±0.33	5.50±0.67	2.00
T15	1.00±0.00	1.33±0.21	2.50±0.56	4.33±0.71	2.46
T16	1.00±0.00	1.33±0.21	2.50±0.56	5.00±0.73	2.46
Lsd	2.23	1.08	0.82	1.13	0.53

Figures in a column whose SE values don't overlap are significantly different at $P \leq 0.05$.

Key

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.8 Effect of Biochar and Wood Vinegar on Area Under Disease Progress Curve (AUDPC) in the Field.

The highest area under disease progress curve was recorded in Treatment 1 (negative control) had with a mean percent AUDPC of 35.17% and was significantly different from other treatments. The lowest AUDPC was recorded from T7 with a mean percent disease incidence of 11.33% (Fig 4.4).

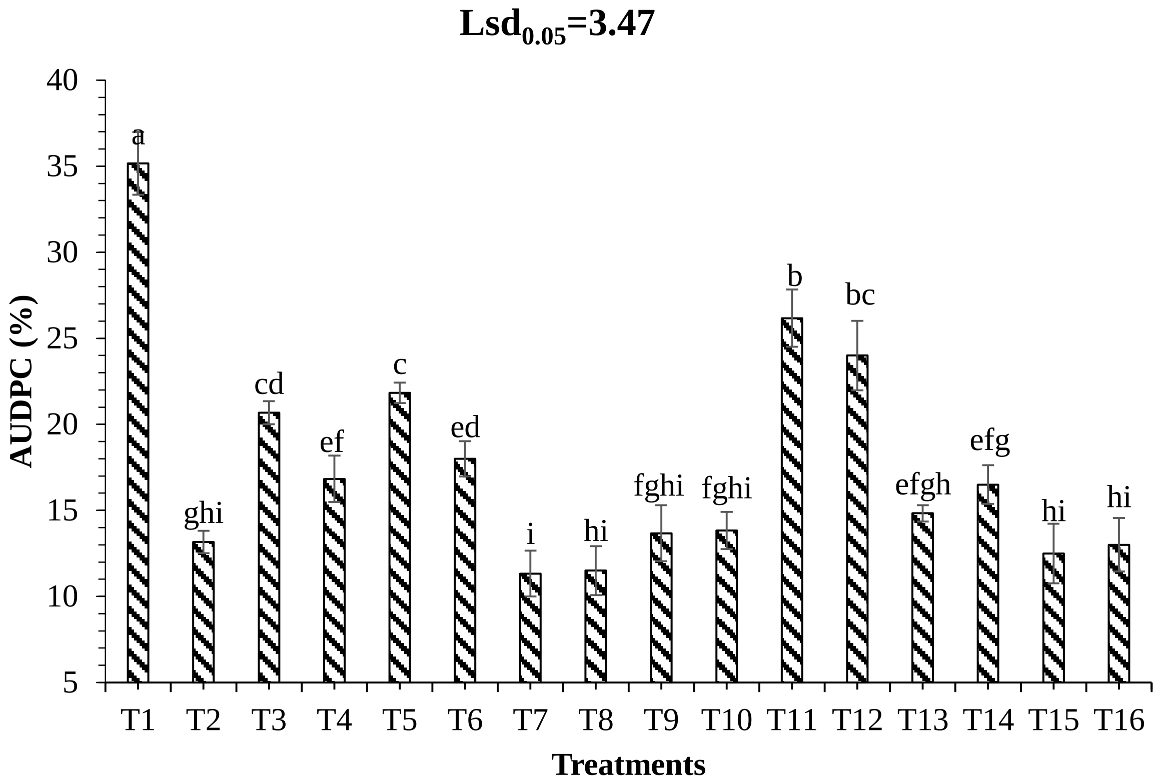

Figure 4. 4: Area under disease progress in the field. Bars with similar letters are not significantly different according to Fischer's least significance difference at $P \leq 0.05$.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.9: Effect of Biochar and Wood Vinegar on Performance of Chickpea in the Greenhouse Experiment.

In the greenhouse experiment, the analysis of variance revealed that treatments were significant on the performance of chickpea at $P \leq 0.001$ (Appendix 2). The treatments had a significant effect on plant height, crop biomass, number of pods per plant and days to 50% flowering as compared to the rest of the treatments and the normal farmer's practice (T2) (Table 4.7). The plants that were sown in T7 showed the highest plant height with means of 57.33 cm, while T1 (negative control) had the lowest height at 15 cm (Table 4.7). The highest biomass was also recorded from plants sown in pots treated with T7 at 55.64 kg while T1 had 3.31 kg. There was an increase in the number of pods at 81.00 for T7 as compared to the negative control at 3.67. Days to 50% flowering (DTF) also differed from the treatments. The highest mean at 50% flowering was recorded from T7 with 56.67% and negative control had 6.67% (Table 4.7).

Table 4. 7: Effect of Treatments on Growth Parameters of Chickpea in the Greenhouse Experiment.

Treatments	Plant height (cm)	Biomass (kg)	No. pods/ plant	Days to 50% flowering
T1	15.00 ± 0.58^{i}	3.31 ± 0.58^{l}	3.67 ± 0.88^{fgh}	6.67 ± 1.67^{e}
T2	43.33 ± 0.88^{d}	15.98 ± 0.88^{h}	32.67 ± 7.88^{cde}	46.67 ± 0.00^{bc}
T3	31.00 ± 1.00^{f}	16.30 ± 1.00^{h}	12.00 ± 0.58^{ghi}	20.00 ± 0.00^{d}
T4	51.00 ± 1.15^{c}	29.92 ± 1.15^{f}	29.00 ± 0.58^{def}	50.00 ± 0.00^{abc}
T5	30.00 ± 0.58^{fg}	11.50 ± 0.58^{j}	10.00 ± 0.58^{ghi}	20.00 ± 0.00^{d}
T6	50.33 ± 0.33^{c}	24.12 ± 0.33^{g}	20.67 ± 6.17^{efg}	50.00 ± 3.33^{abc}
T7	57.33 ± 0.58^{a}	55.64 ± 0.58^{a}	81.00 ± 1.00^{a}	56.67 ± 1.00^{a}
T8	56.33 ± 0.88^{ab}	55.64 ± 0.54^{e}	58.67 ± 0.88^{b}	57.67 ± 0.88^{a}
Lsd	2.21	1.42	12.24	8.02

Means followed by the same letters along the column are not significantly different according to Fischer's least significance difference at $P \leq 0.05$.

Table 4.7: Continued…

Treatments	Plant height (cm)	Biomass (kg)	No. pods/ plant	Days to 50% flowering
T9	$56.33±0.33^{ab}$	$50.82±0.33^{b}$	$58.00±6.10^{b}$	$56.67±0.00^{a}$
T10	$55.00±0.58^{ab}$	$49.40±0.88^{b}$	$44.00±2.08^{c}$	$56.67±0.0^{a}$
T11	$25.67±1.76^{h}$	$12.96±1.76^{i}$	$14.33±0.33^{fgh}$	$16.67±3.33^{d}$
T12	$28.00±0.58^{fg}$	$7.98±0.58^{k}$	$7.67±0.67^{fgh}$	$13.33±3.33^{ed}$
T13	$40.67±0.88^{e}$	$15.59±0.88^{h}$	$20.33±1.45^{fg}$	$43.33±3.33^{c}$
T14	$39.33±0.33^{e}$	$11.49±0.33^{j}$	$17.00±0.58^{fgh}$	$46.67±3.33^{bc}$
T15	$54.33±0.88^{b}$	$42.53±0.58^{c}$	$40.33±6.36^{cd}$	$50.00±0.00^{abc}$
T16	$52.00±0.58^{c}$	$38.58±0.88^{d}$	$34.67±3.38^{cd}$	$53.33±3.33^{ab}$
Lsd	2.21	1.42	12.24	8.02

Means followed by the same letters along the column are not significantly different according to Fischer's least significance difference at $P \leq 0.05$.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

4.6.10: Effect of Biochar and Wood Vinegar on Growth and Yield of Chickpea in the Field Experiments.

The treatments also showed a significant effect on yield and yield-related parameters. There was an increase in yield and number of pods per plant upon application of cobs biochar and cobs wood vinegar (T7) with a mean of 3.57 t/ha and 3.40 t/ha for the for season one and two respectively. which was significantly different from other treatments. The lowest yield was obtained from the negative control experiment with a mean of 0.27 t/ha and 0.21t/ha for season 1 and 2 respectively. (Figure 4.5 and Fig 4.6 respectively). Similarly, T7 showed promising results in increasing the number of pods per plant. Plots with this treatment showed increased pods per plant with a mean of 108.67% and was significantly different from other treatments (Table 4.8). Agronomic parameters such as plant height, biomass, plant count and 50% flowering showed an increase in plots applied with biochar and wood vinegar. Application of T7 recorded high biomass with a mean of 0.22 kg/ha and was statistically different from other treatments. Similarly, T7 showed a pronounced increase in plant count per plot, plant height and 50% flowering with means of 55.50 plants per plot, 54.33 cm and 50% respectively and was significantly different from other treatments. Negative control (T1) recorded lower means in biomass, plant count, plant height and 50% flowering with means of 0.01kg/ha, 51.50 PC, 17.17cm and 20% flowering respectively and was statistically different all the parameters with other treatments (Table 4.8).

Table 4. 8: Effect of Treatments on Biomass, Stand Count, Plant Height And 50% Flowering of Chickpea in the Field (July-October 2022, April-August 2023).

Treatments	Biomass kg/ha	Plant count/ plot	Plant height(cm)	50% flowering	Number of pods/plants
T1	$0.01±0.00^{d}$	$51.50±6.11^{cde}$	$17.17±0.95^{d}$	$20.00±9.49^{c}$	3.33^{g}
T2	$0.03±0.00^{d}$	$53.00±5.62^{bde}$	$42.17±1.05^{b}$	$40.00±0.00^{b}$	62.50^{c}
T3	$0.05±0.02^{dc}$	$58.83±3.12^{abc}$	$28.67±1.56^{c}$	$20.00±0.00^{c}$	26.67^{e}
T4	$0.02±0.00^{d}$	$51.50±1.28^{a}$	$49.83±0.40^{a}$	$50.00±0.00^{a}$	34.17^{de}
T5	$0.01±0.00^{d}$	$53.83±6.09^{abcd}$	$36.50±4.27^{b}$	$21.67±1.67^{c}$	28.50^{e}
T6	$0.03±0.00^{d}$	$60.33±1.38^{ab}$	$50.50±0.67^{a}$	$50.00±0.00^{a}$	31.00^{de}
T7	$0.22±0.16^{a}$	$60.50±2.66^{abcde}$	$54.33±0.49^{a}$	$50.00±0.00^{a}$	108.67^{a}
T8	$0.16±0.02^{ab}$	$50.33±6.04^{de}$	$53.50±1.15^{a}$	$50.00±0.00^{a}$	103.00^{a}
T9	$0.11±0.01^{abcd}$	$60.00±1.13^{ab}$	$54.00±1.10^{a}$	$50.00±0.00^{a}$	79.83^{b}
T10	$0.10±0.01^{bcd}$	$58.67±1.56^{abc}$	$51.33±1.09^{a}$	$50.00±0.00^{a}$	82.50^{b}
T11	$0.01±0.00^{d}$	$56.67±2.75^{abcd}$	$41.33±5.51^{b}$	$20.83±0.83^{c}$	17.00^{f}
T12	$0.01±0.00^{d}$	$53.33±4.58^{abcde}$	$29.00±1.63^{c}$	$13.33±2.11^{d}$	7.33^{g}
T13	$0.02±0.00^{d}$	$56.00±1.69^{abcde}$	$39.67±3.29^{b}$	$38.33±1.67^{b}$	38.67^{d}
T14	$0.02±0.00^{d}$	$48.00±4.62^{e}$	$37.83±0.95^{b}$	$38.33±1.67^{b}$	35.67^{de}
T15	$0.07±0.02^{bdc}$	$56.83±2.57^{abcd}$	$52.50±1.06^{a}$	$50.00±0.00^{a}$	86.67^{b}
T16	$0.15±0.02^{abc}$	$60.83±0.87^{ab}$	$51.83±1.01^{a}$	$50.00±0.00^{a}$	83.33^{b}
Lsd	0.11	8.17	6.78	5.93	9.49

Means followed by the same letters along the column are not significantly different according to Fischer's least significance difference at $P≤0.05$.

T1- Negative control, T2- DAP+fungicide, T3- Cobs vinegar, T4-Cobs biochar,T5-Acacia vinegar, T6-Acacia biochar, T7-Cobs vinegar+cobs biochar, T8-Acacia vinegar+acacia biochar, T9- Cobs vinegar+acacia biochar, T10-Acacia vinegar+cobs biochar, T11-DAP, T12-Fungicide, T13-DAP+cobs vinegar, T14-DAP +Acacia vinegar, T15 Cobs biochar+Fungicide, T16 Acacia biochar+Fungicide.

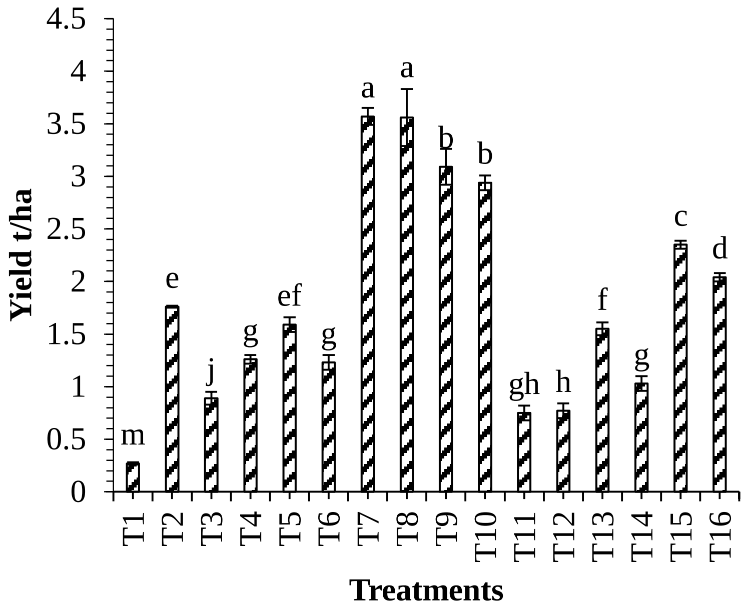

Figure 4. 5: Yield t/h for the first season. Bars with similar letters are not significantly different according to Fischer's least significance difference at P≤0.05.

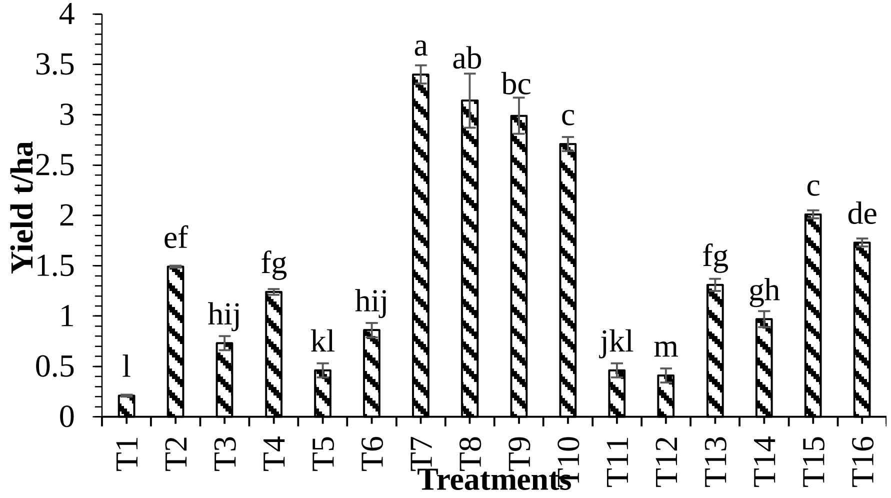

Figure 4. 6: Yield in t/ha for the second season. Bars with similar letters are not significantly different according to Fischer's least significance difference at P≤0.05.

4.6.11: Correlation Analysis for Morphological Aspects, Disease Incidence and Area Under Disease Progress Curve in the Greenhouse.

Pearson's correlation (r) was used to assess the relationship between performance parameters of chickpea, disease incidence, and severity due to response from biochar and wood vinegar applications. The study revealed that biomass had a significantly strong positive correlation with plant height at $P \leq 0.001$ r (0.88). similarly, the number of pods had a significantly strong positive correlation with plant height and biomass at $P \leq 0.001$ and $P \leq 0.01$ with r (0.82) and r (0.90) respectively. Days to 50% flowering exhibited a significant strong positive correlation with plant height, biomass and number of pods at $P \leq 0.001$ r (0.96), r (0.78) and r (0.78), respectively. The area under the disease progress curve exhibited a significantly strong negative correlation with plant height, biomass and the number of pods and DTF at $P \leq 0.001$ with r (-0.88), r (-0.75), r (-0.78) respectively. Percent disease incidence had a strong negative association with plant height and the number of pods and a moderate negative association with DTF with r (-.074), r (-0.72) and r (-0.68) respectively. Similarly, PDI exhibited a significant strong negative association biomass at $P \leq 0.001$ with r (-0.82). However, PDI showed a moderate positive association with AUDPC at $P \leq 0.01$ and r (0.69) (Table 4.9).

Table 4. 9: Correlation for Morphological Aspects, Disease Incidence and AUDPC.

	Plant height	Biomass	Number of pods	Days to 50% flowering	AUDPC
Biomass	0.88***				
Number of pods	0.82***	0.90**			
Days to 50% flowering	0.96***	0.78**	0.78***		
AUDPC	-0.88***	-0.75***	-0.78***	-0.85***	
Incidence %	-0.74**	-0.82***	-0.72**	-0.68**	0.69**

*** significant at 0.001, **Significant at 0.01, * Significant at 0.05.

4.7 Discussion

From this study, it was found that the efficacy of wood vinegar in reducing disease incidence and severity of Ascochyta blight was highly effective under greenhouse as well as field conditions. The antifungal effect of wood vinegar in suppressing Ascochyta blight could be attributed to the presence of phenols, acetic acid and organic compounds such as creosole. Similarly, a study conducted by Saberi *et al*. (2013) demonstrated reduced severity of root and crown of cucumber upon application of wood vinegar with the maximum concentration showing reduced severity. Similarly, wood vinegar foliar applied helped reduce disease incidences of dirty panicle and brown rot in paddy rice (Chuaboon *et al*., 2016). Furthermore, El-Fawy *et al*. (2023) reported the ability of wood vinegar from guavas in reducing disease severity of black dot disease in potatoes.

The stage of crop growth of chickpea also influenced percent disease incidence (PDI). PDI was low at the seedling stage and progressively increased through the growth period. This is due to the fact that as the disease progresses, the lesions elongate on stems which causes stem girdling breaking and dying of the plant. Similarly, the spreading of the disease continues to the rest of the crops throughout the growth period. PDI and severity were high in the second season as compared to the first season. The high levels of humidity as well as low temperatures in the second season favored the development and rapid spread of Ascochyta blight. These results are further confirmed by Kimurto *et al*. (2013) who found out that the susceptibility of AB on chickpea was favored by cool and wet conditions. A study conducted by Nganga *et al*. (2016) further confirmed that ascochyta blight is rapidly spread under low humidity and temperatures.

Disease severity and incidence were reduced in the seedling, vegetative, flowering and podding stages of the crop. A combination of biochar soil applied and wood vinegar foliar applied showed a significant reduction in disease incidence and severity at all the growth stage of the crop in the the two seasons and in the greenhouse experiment. A study conducted by El-Gamal *et al*. (2022) confirmed that foliar application of natural products from plant suppressed the severity of stem rust after foliar application at different stages.

The addition of sole wood vinegar showed a significant increase in the growth aspects of chickpea when compared to the negative control. Moreover, the addition of sole biochar obviously increased the growth parameters of chickpea over the control. However, a combination of biochar and wood vinegar showed the greatest promotion effects on the growth aspects and yield of chickpea. This can be attributed to an improvement in the physical properties such as improved soil PH, increased cation exchange capacity, and improved soil structure enhancing translocation of nitrogen (N) and P from the soil to the crop (Zheng *et al*., 2013). Additionally, this result shows that a combination of wood vinegar and biochar greatly contributed to increase in yield of the crop. In addition to enhancing the physical properties of soil, wood vinegar acts as a catalyst for physiological and biochemical processes in plants such as cell growth, photosynthesis, and nutrient absorption, increasing the glossy appearance of the plant thus increasing yields. These results are consistent with several researchers after experimentation on several crops. Pan *et al*. (2017) found out that the sole application of biochar and wood vinegar as well as the combination of the two products significantly promoted the growth of cucumber. Likewise, the best results of yield and growth of watermelon were recorded after the application of 10% wood vinegar and local formulation (Zulkarami *et al*., 2011). Consistently, an increase in tomato fruit was demonstrated with wood vinegar application on tomatoes as reported by Ofoe *et al*. (2022). Another study conducted by Essa *et al*. (2023) showed that foliar spray of wood vinegar and algae helped improve the yield and quality of several varieties of faba beans with the highest concentration of 4ml giving the best results.

The presence of esters such as methyl acetate and methyl formate accelerates development and plant growth. Moreover, N which is known to promote plant growth is highly found in wood vinegar and this could be attributed to an increase in biomass on plants treated with wood vinegar (Luo *et al*., 2019). From this study, it is evident that there is need to apply biochar and wood vinegar in foliar management of Ascochyta blight in chickpea.

4.8 Conclusion

The greenhouse and field experiment revealed that a combination of biochar and wood vinegar helps improve the growth aspects of the plant, increases the yield production and suppress disease development in the crop. This is attributed to the multiple benefits in translocation in plants, improving soil physical and chemical properties, enhancing nutrient supply in crops due to soil amendment after soil and foliar applications of biochar and vinegar as well as the chemical components present in wood vinegar. The effects of biochar and wood vinegar to enhance plant growth and suppression of diseases and the mechanisms under which the impacts are brought about needs to be studied.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATION

5.1 General Discussions

Management of crop diseases using chemical fungicides negatively impacts on the environment and human health. Therefore, use of plant derived products to manage crop diseases is an approach sustainable and eco-friendly (Shuping & Eloff, 2017). The mycelial growth of *Ascochyta rabiei* was highly influenced by the concentration of wood vinegar. The concentration of wood vinegar at 2.5% v/v and 3%v/v showed maximum mycelial growth inhibition in all the feedstocks. This is in accordance with Firouzbehi *et al*. (2021) where wood vinegar at different concentrations showed differences on inhibition of rot fungi when used as a preservative.

In this study, the results indicated that biochar and wood vinegar help improve crop growth, reduce disease incidence and severity of Ascochyta blight and increases the yield in chickpea. The application of these products as soil and foliar whether as sole or combined treatments helps amend the soil thus improving the growth aspects of the crop. Disease pressure was more intense during podding than early crop growth stages. This indicates that the application of wood vinegar and for foliar management of AB should be done throughout the crop growth stages to reduce development of the disease on the crop. Also, Ascochyta blight was much pronounced in pots and experimental plots with zero application of biochar and wood vinegar. The unsprayed pots and plots had so much defoliation and the susceptibility of the crops to the diseases was high. This indicates that foliar application of wood vinegar is key in reducing the severity of the disease. Foliar application of the chemical fungicide also showed effectiveness in management of Ascochyta blight *in vitro* and *in vivo* AB. This is a clear indication that as much as we advocate for natural products in management of crop diseases, they cannot be fully eliminated but can be combined with several strategies to be ecofriendly (Ruano-Rosa *et al*., 2018). Combination of 50% biochar and 3%v/v wood vinegar from maize cobs and acacia showed the highest performance and reduced severity of AB than other treatments in the greenhouse which was a similar case to the field experiment when a combination of 2kgs of biochar and 3%v/v of wood vinegar was applied. This shows that when crop diseases are well managed, the quality and quantity of the produce are maximumly produced and hence increased income for farmers (Jackson *et al*., 2020).

5.2 Conclusions

i. Wood vinegar from maize cobs exhibited maximum mycelial growth inhibition at 2.5%v/v and 3% v/v and this is a clear justification of employing the products in integrated disease management of Ascochyta blight in chickpea.
ii. Application biochar and wood vinegar at recommended rates at in the greenhouse and field experiment throughout the four crop growth stages improved the performance and yield of chickpea as well reducing the severity and PDI of Ascochyta blight.
iii. Numerous natural products in wood vinegar reduces the development of *Ascochyta rabiei*. This reduces the spread of Ascochyta blight and thus suppression of the disease

5.3 Recommendations

From the study that was conducted, the following recommendations are drawn

i. Wood vinegar from different feedstocks should be investigated to determine the antifungal effects of different disease-causing microorganisms *in vitro.*
ii. Farmers should adopt biochar and wood vinegar in management of Ascochyta blight in chickpea under greenhouse and field conditions
iii. Biochar and wood vinegar application should be employed by farmers in chickpea production programs to help promote crop growth and increased yield.
iv. Further studies should be conducted to explore the actual active ingredients /secondary metabolites in wood vinegar responsible for inhibition/suppression of AB.

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APPENDICES

Appendix A: Analysis of variance for concentration and feedstock on fungal growth inhibition.

Source of variation	Df	Fungal growth inhibition
Feedstock	4	2882.02***
Concentration	5	10011.47***
Feedstock × Concentration	20	893.19***
Error	58	0.77
R^2		0.99
CV		1.09

*** significant at 0.001, CV coefficient of variation, R^2 coefficient of determination

Appendix B: Analysis of variance for the effect of treatments on plant height, biomass **number** of pods and days to 50% flowering under greenhouse

Source of variation	df	Plant height(cm)	Biomass(kg)	No. pods/plant	Days to 50% flowering	AUDPC
Replicates	2	6.27**	2.56**	14.25	94.27**	0.02
Treatments	15	532.76***	906.62***	1409.93***	970.97***	0.81**
R2		0.99	0.99	0.93	0.96	0.57
CV%		3.09	3.21	24.26	11.97	12.09

*** significant at 0.001, **Significant at 0.01, CV coefficient of variation, R^2 coefficient of determination.

Appendix C: Analysis of variance for percent disease incidence under greenhouse

Source of variation	df	Incidence
Replicates	2	72.01
Treatments	15	3102.81***
Stage	3	21343.93***
Treatments*Stage	45	419.03***

R^2	0.97
CV	13.73

*** significant at 0.001, CV coefficient of variation, R^2 coefficient of determination.

Appendix D: Analysis of variance for yield and performance parameters of chickpea for the two seasons (July-October 2022, April-August 2023).

Source of variation	df	Yield t/hectare	Biomass kg/ha	Number of pods per plant	Plant count per plot	Plant height(cm)	50%flowering
Season	1	1.40***	0.02	709.5937***	2081.34***	7.59	94.01
Replicates	2	0.29	0.01	133.7917	57.07	13.57	30.47
Treatments	15	5.92***	0.02***	7093.7104***	96.82*	740.96***	1189.15***
Season*treatment	15	0.06	0.01	71.4604	99.85*	3.37	87.343***
CV%		21.12	152.40	15.88	12.65	13.61	13.43
R^2		0.92	0.48	0.96	0.62	0.84	0.92

* Significant at 0.05, ** significant at 0.01, *** significant at 0.001, CV-coefficient of variation, R^2 coefficient of determination.

Appendix E: Analysis of variance (ANOVA) for disease incidence on chickpeas in the field.

Source of variation	df	Incidence
Replicates	2	95.08***
Season	1	3021.35***
Treatment	15	2233.19***
season*treatment	15	43.15***
Stage	3	60697.91***
season*stage	3	167.77***
Treatment*stage	45	313.41***
season*treatment*stage	45	27.67**
Error	254	4311.44
Cv %		0.98
R2		12.27

Significant at $P \leq 0.01$, * Significant at $P \leq 0.001$, CV coefficient of variation, R^2 coefficient of determination.

Appendix F: Analysis of variance (ANOVA) for disease severity at different growth stages of chickpea and area under disease progress curve at the field

Source of variation	df	seedling	vegetative	flowering	Podding	AUDPC	Mean severity
Season	1	0.09	0.01	4.59***	17.51***	70.04***	2.34***
Replicates	2	0.07	7.70	1.01	44.042	35.66	2.22
Treatments	15	0.18***	7.78***	12.41***	12.10***	253.09***	5.97***
Season*treatments	15	0.07	0.52	1.19***	0.73	8.46	0.17
CV%		18.79	45.27	20.37	17.90	17.02	15.04
R^2		0.61	0.71	0.87	0.83	0.87	0.88

*** significant at 0.001, CV-coefficient of variation, R^2 coefficient of determination.

Appendix G: Abstract for publication from objective one

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Antifungal effect of wood vinegar from selected feedstocks on *Ascochyta rabiei in vitro*

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A study to evaluate the antifungal activity of wood vinegar (pyrogenous acid) from maize cobs, acacia twigs, bean straw and an invasive tree species *Prosopis juliflora* against *Ascochyta rabiei* was conducted *in vitro* and at Egerton University Njoro, Kenya. The physicochemical characteristics of the different wood vinegar were also determined. Antifungal effects of wood vinegar were evaluated at different concentrations (0.5, 1, 1.5, 2, 2.5 and 3% v/v) using a Petri dish bioassay arranged in a completely randomized design. A fungicide (Metalaxy-M-40 g/kg) and water were used as positive and negative controls, respectively. All the wood vinegars had a smoky odor with brown/yellow coloration and an average density of 1.06 g/cm^3. The wood vinegar from maize cobs showed a near acidic pH of 3.90 while bean straws showed a near neutral pH of 7.16. Prosopis and acacia showed moderate acidity of 5.10 and 5.43, respectively. The highest concentration of phenols was recorded in wood vinegar from maize cobs (4.56 mg/ml) followed by acacia (3.52 mg/ml). The results from the antifungal assay showed wood vinegar treatment significantly ($P \leq 0.001$) reduced *A. rabiei* mycelia growth at all tested concentrations when compared to the untreated control. The minimum inhibition concentration was 0.5% v/v for all the tested wood vinegars. The percent mycelia growth inhibition generally increased with increasing concentration except for maize cob which showed 99.33% and complete inhibition at 0.5 and 1.5 % v/v concentrations, respectively. Complete inhibition of the pathogen's growth (100%) for all the wood vinegars tested was achieved at 2.5% v/v concentration. Plant-based wood vinegar has antifungal activity against *A. rabiei.*

Key words: Antifungal, *Ascochyta rabiei,* polyphenols, pyrogenous acid, wood vinegar.

Appendix H: Research permit from Nacosti

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