



**INVESTIGATION OF ANTI MICROBIAL ACTIVITY OF
TRADITIONAL BOTANICAL PESTICIDES COLLECTED FROM
SIDAMA AREA, SOUTHERN ETHIOPIA**

MSc Thesis

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HAWASSA UNIVERSITY
College of Agriculture

November, 2022

HAWASSA, ETHIOPIA

**INVESTIGATION OF ANTI MICROBIAL ACTIVITY OF
TRADITIONAL BOTANICAL PESTICIDES IN
SIDAMA REGION, SOUTHERN ETHIOPIA**

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A Thesis Submitted to School of Plant and Horticultural Science
HAWASSA UNIVERSITY
College of Agriculture

In partial Fulfillment of the Requirements for the Degree of the Master of
Science in Agriculture (Specialization: Crop Protection)

November, 2022

HAWASSA, ETHIOPIA

COLLEGE OF AGRICULTURE

SCHOOL OF PLANT AND HORTICULTURAL SCIENCES

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DEDICATION

I proffer this thesis paper to my family members, especially my wife and my mother, for their committed partnership in the success of my thesis.

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ACKNOWLEDGEMENTS

First of all, I would like to thank the almighty God for his mercy and being source of my capability and strength to accomplish this thesis and in fact, throughout my life. Then, I would like to express my most profound thanks to my advisor; Dr Hirut Tsegaye for her patience, understanding, guidance and constructive comments. My heartfelt gratitude also goes to my teachers in Crop Protection department, especially to Dr. Elfinesh Shikur for being bridge to join this department eventhough she doesn't know what she has done for me. I really would like to thank Hawassa University crop protection laboratory and skillful workers there especially Kassa Gedebo for his patience, guidance and provision of necessary laboratory equipment and isolation and identification of pure culture of different microbial strains in the performance of the different laboratory procedures. Finally, I would like to thank my classmate Memhiru Mekiso for his financial and ideal support and my senior schoolmate Endale Girma unlimited help and Yikeber Simachew, teacher in Wachemo University, for his idea sharing in writing this thesis. At last, I thanks my neighbours Yosef Samuel and Tesfaye Garkebo including their family for WI-FI services.

Date of Submission:

ABBREVIATION AND ACCRONYMS

ANOVA	Analysis of Variance
BWE	Bacterial wilt of Enset
CLSI	Clinical and laboratory standards institute
CRD	Complete Randomized Design
DMSO	Dimethyl sulfoxide
EIAR	Ethiopian Institution of Agricultural Research
IPM	Integrated Pest Manegement
IZ	Inhibition zone
MBC	Minimum bactericidal concentrations
MDR	Multi drug resistace
MFC	Minimum fungicidal concentrations
MHA	Muller Hinton Agar
MICs	Minimum inhibitory concentrations
PDA	Potato dextrose agar
PPB	Plant pathogenic bacteria
PPF	Plant pathogenic fungi
RCBD	Randomized complete block design
WHO	World health organization

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“Investigation of Anti-microbial Activity of Traditional Botanical Pesticides in Sidama Region, Southern Ethiopia”

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Advisor: Hirut Tsegaye (PhD)

ABSTRACT

*Indigenous knowledge and systems of crop protection are at risk of extinction today because of the rapidly changing natural environments and cultural shifts at global scale. Many traditional pest control practices are disappearing because of introduction of foreign technologies like synthetic pesticides that assure short-term solutions to the problems. The aim of the present study was therefore, to identify the potential of botanical used by farmers in Sidama region, and investigate their antimicrobial activities against some plant pathogens. Information on traditional botanicals pesticides used for crop pests control was collected through observation, key informant interview, questionnaire, and group discussions. The antimicrobial activities of the common botanicals in the study area were investigated using filter paper disc and agar well diffusion method following standard procedure. The bioactive compounds from leaves of 16 botanicals were extracted with 95% ethanol, and the phytochemical tests were conducted. The result revealed that farmers in the study area have different ethnobotanical experience in their neighbourhood. Various plants belonging to different families were valued for pesticide activities. The botanically-derived pesticides were perceived to be affordable, effective, available, safe and dependable. However, estimating the proportion/ concentration of botanicals to be used for pest control where mentioned as a critical challenge. The ethanolic extract of *Cordia africana* (18.30mm) showed the maximum inhibition zone against *Fusarium vertecelloides* while the minimum antibacterial activity was observed by *Eucalyptus globules* extract (9.43mm) against *Ralstonia solanacearum* as compared with other botanicals included in the current work. Our investigation showed that the botanicals contain phenols, tannins, glycosides, flavonoids, saponins and steroids. The present work revealed that botanicals in the study area have potential to be used for crop pest control. In order to exploit this potential; standardized techniques of preparation, bio-safety and environmental guidelines are required.*

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Keywords: Inhibition zone, MICs, Antimicrobial activity, Phytochemicals screening

1. INTRODUCTION

1.1. Background of the Study

Subsistence farming is predominant in the rural areas of the developing world where it directly employs 50 - 70% of the population. Its' contribution to local and regional food security is crucial since they produce most of the staple food crops (Tefera, 2004). One of the major problems with agriculture nowadays is the demand for the production of more and more to provide food for the population which is in permanent augmentation. In realizing this, one of the stumbling blocks is the yield losses due to pests. It is estimated that field and storage pests destroy approximately 43% of potential production in developing Asian and African countries (Wang *et al.*, 2019).

In spite of the use of all available means of plant protection, about one-third of the yearly harvest of the world is destroyed by the pests. Losses at times are so severe to lead to famine in large areas in many countries of the world. So priority should be given to pre and post-harvest studies, particularly in humid tropical climates, where at least half of the food supply may be lost between harvest and consumption.

The increasing concern over the level of pesticide residues in food has encouraged researchers to look for alternatives to synthetic pesticides. Their indiscriminate use has led to the development of resistant strains of pests as well as different environmental and human health problems. In Ethiopia impact of pesticides to a given locality or on the environment has not been identified, assessed and compiled, there is no system for risk monitoring and communication ("The Place of Environmental Protection in the Growth and Transformation Plan of the Federal Democratic Republic of Ethiopia," 2013). Recently, in different parts of the world, attention has been paid

towards the exploitation of higher plant products as novel chemotherapeutics in plant protection. Because of non phytotoxicity, systemic, easy biodegradability and stimulatory nature of host metabolism, plant products possess the potential in pest management. The popularity of botanical pesticides is once again increasing and some plant products are being used globally as green pesticides. Botanical pesticides maintain biological diversity of predators (Amoabeng *et al.*, 2013) which makes their use in agriculture a sustainable pest management alternative to synthetic pesticides. It has been well argued that pesticidal plant products or botanical pesticides are an appropriate technology for resource-poor smallholder farmers. The scientific literatures documenting the bioactivity of plant derivatives to different pests continues to expand, yet only a handful of botanicals are currently used in agriculture.

In recent years there has been an attempt to replace the synthetic pesticides with less expensive, locally available, ecologically safe and socio-friendly options including botanicals (Nwokocha, *et al.*, 2016). Some of these plants include *Azadirachta indica* (neem) (Bankole and Adebajo, 1995) and *Cymbopogon citrates* (Nwokocha, *et al.*, 2016). The toxic effect of synthetic chemicals can be overcome, only by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Nwokocha, *et al.*, 2016). Extracts of many higher plants have been reported to exhibit antifungal properties under laboratory trails (Mohana *et al.*, 2008). Exploitation of plant metabolites in crop protection and prevention of biodeterioration caused by fungi appear to be promising.

Green plants represent a reservoir of effective chemotherapeutics and can provide valuable sources of natural pesticides (Bhoyar, 2021). Stem extracts of various plants are known to possess antimicrobial activity. Several workers have observed the antifungal activity of stem and its bark extracts (Rotimi *et al.*, 2015). Extracts of many higher plants have been reported to

exhibit antifungal and antibacterial properties under laboratory trials (Parekh *et al.*, 2006; Buwa and Staden, 2006; Mohana *et al.*, 2008). Many previous studies conducted in Ethiopia have shown the antimicrobial activities of many indigenous plants used in traditional medicine (Teferi and Hahn, 2003; Tesfaye *et al.*, 2006).

Plant species are estimated to be around 250–500 thousand (Cowan, 1999). However, only a small part of them was investigated for antimicrobial activity (Savoia, 2012; Petrosyan *et al.*, 2015). Plant extracts have great potential as antimicrobial compounds against micro-organisms (Nascimiento *et al.*, 2000). All plants containing active compounds are important. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. Botanicals (phytochemicals) are secondary metabolites that serve as means of the defense mechanism of the plants to withstand the continuous selection pressure from pests and other environmental factors. Many groups of phytochemicals such as steroids, alkaloids, terpenoids, phenolics and essential oils from more than 2000 plant species have been reported previously for their pesticidal activities.

The plants' secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules of neurotransmitters and thus have beneficial medicinal effects. Therefore, the random screening of plants for active chemicals is as important as the screening of botanically targeted species.

Furthermore, these smallholder farmers have been bypassed by agricultural modernization as new technologies were not made available to them on favorable terms, while some of which often do not suit their agro-ecological and socio-economic conditions. Pest management innovations are no exception. For instance, the promotion of synthetic pesticides in the control of pests through effective is expensive and has raised health and environmental concerns (Taluksder,

2006; Isman, 2007). The risks associated with the use of synthetic insecticides are even higher among small scale farmers because of lack of skills to obtain and handle pesticides appropriately (Saxena *et al.*, 1990). Thus, pests particularly insects, continue to ravage crops and without proper protection systems, farmers continue to lose most of their produce.

In addition, the experience of small holder farmers, who are typically resource poor, is that synthetic products are unaffordable, sporadically available, poorly labelled and packaged, adulterated and/or sold beyond their expiry date (Stevenson *et al.*, 2012). Considering the well documented health risks associated with synthetic pesticides, the need for alternatives is even more crucial (Wesseling *et al.*, 2001).

Phytochemical variation in pesticidal plants is a serious hurdle to their exploitation (Sarasan *et al.*, 2011) and it has been argued that they are generally not as effective as synthetic chemical pesticides, as they have relatively low, variable and sometimes unknown pesticidal activity. This leads to inconsistent efficacy, which is one of the many reasons why pesticidal plants have not been successfully commercialized. However, recent work indicates that pesticidal plants have lower impacts on beneficial insects compared to synthetic products and are cost beneficial when used as an alternative pesticide in vegetable production (Amoabeng *et al.*, 2013). The high diversity of African plant species with pesticidal properties and existing indigenous use of such plants by resource-poor farmers suggests that there is scope for developing a strong demand for more ecologically benign pathogen control.

Moreover, traditional farmers' perceptions of pest problems and indigenous control methods employed are not yet to be critically evaluated. The available information is mostly observational/ anecdotal and does not provide quantitative details about various socio-economic factors that influence the indigenous pest control practices (Altieri, 1993). As a result, the

development and extension of improved and adaptable pest management technology for small scale farmers in developing countries is being re-examined. Thus, traditional botanical pesticides which are environmentally friendly, cost effective and efficient needs to be recognized, valued, appreciated and it should be preserved, transferred, or adopted and adapted as integrated pest management elsewhere. Hence, the proposed study will be conducted to investigate the botanical pesticides in the study area and to evaluate the antimicrobial activities of selected botanicals.

1.2. Statement of the Problem

Pesticides are widely used to control/ prevent pests, weeds and other plant pathogens in an efferent to reduced and eliminate yield loss and maintain high product quality. Intensive use synthetic pesticides have led to the development of resistant strains of pests as well as different environmental and human health problems. Although the impact of these pesticides to a given locality or on the environment has not been clearly identified, assessed and compiled, there are indicators of the problems in different agricultural communities in Ethiopia. The toxic effect of synthetic chemicals can be overcome, by persistent search for new and safer pesticides accompanied by wide use of pest control methods, which are eco-friendly and effective (Mohana *et al.*, 2011). Reports indicate that in the last decades there have been attempts to replace the synthetic insecticides with less expensive, locally available, ecologically safe and socio-friendly options including botanicals. The attempts include exploitation of higher plant products as novel chemotherapeutants in plant protection. Because of non-phytotoxicity, easy biodegradability and stimulatory nature of host metabolism, plant products possess the potential in pest management. Commercial plant pesticide products Plant-based biopesticides are still just a promising, and in many cases unproven, alternative to synthetic pesticides even though for some pests, various crude or processed products have been commercialized (Khater 2012). By the beginning of the

year 2013, 400 active ingredients and over 1,250 biopesticide products had been registered and commercialized in the United States of America (U.S. Environmental Agency 2013).

The use of traditional approaches to protect agricultural crops from pests has been getting due attention. In Ethiopia, different botanicals have been used traditionally for plant pest control. To disseminate this knowledge and commercialize, there is a need to identify and investigate their efficacy. The proposed research is believed to help researchers and policy makers to exploit the potential of plant extracts in respect of plant pest management. Identification and investigation of the antimicrobial activities will contribute towards policy intervention framework for improving the use of botanical and reduce reliance on chemical pesticides that have been producing a potential health and environmental hazards. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Prusti *et al.*, 2008).

1.3. OBJECTIVES

1.3.1. General objective

- To contribute towards the development of safe, cheap and available methods of plant disease management in Ethiopia.

1.3.2. Specific objectives

- To assess the current information on the traditional botanical pesticides used for crop pest management in Sidama Region
- To determine the antimicrobial activities of selected botanicals against some plant pathogens under laboratory condition.
- To test the presence of major bioactive compounds of selected botanicals.

2. LITERATURE REVIEW

2.1. Plant diseases

Globally, enormous losses of the crops are caused by the plant diseases. Plant diseases are caused by pathogens (Singh, 2007). Plant pathogens are considered as plant pests. Pests are one of the serious problems faced by agricultural sector. Insect pests, plant pathogens and weeds can be collectively termed as pests. They represent a major constrain to crop production (Juroszek & Tiedemann, 2013). Agricultural production incurs substantial yearly losses because of plant diseases. Different kinds of organisms can cause plant diseases, including, bacteria, fungi, etc. Hence protection of plants from agriculture pest and pathogens is the preoccupation of agricultural scientist around the world (Agrios, 2005) and it is the unifying goal of plant pathology to control plant disease, and chemicals play a major role in accomplishing that goal in contemporary agricultural production (Epstein and Bassein, 2003; Ragsdale, 2000). In Ethiopia, few studies have been conducted and documented on method of traditional medicine preparations by using local medicinal plants, parts used and routes of applications (Kebu et al., 2004; Haile and Delenasaw, 2007; Ragunathan and Solomon, 2007).

2.1.1. Plant Pathogenic Bacteria

Higher plants contain potentially vast sources of nutrients for the myriad bacterial species in their environment, and most bacteria are small enough to pass through stomata and other natural openings in to the apoplast, the anteroom for these riches. It is the apoplastic colonizers that are the common pathogens that produce the rots, spots, wilts, cankers, and blights afflicting virtually all crop plants.

2.1.1.1 *Pseudomonas*

Pseudomonas syringae is a rod-shaped, Gram negative bacterium with polar flagella. As a plant pathogen, it can infect a wide range of species, and exists as over 50 different pathovars. It is unclear whether these pathovars represent a single species. *P. syringae* is a member of the *Pseudomonas* genus, and based on 16S rRNA analysis, it has been placed in the *P. syringae* group (Anzai *et al.*, 2000). It is named after the lilac tree (*Syringa vulgaris*), from which it was first isolated (Kreig *et al.*, 1984).

To control *P. syringae* use of bactericidal compounds is common. However, this practice could cause serious damage to the environment and human health and also promotes the selection of pathogenic strains with increased tolerance to copper (Brent *et al.*, 1998). For these reasons, botanical pesticides deserve serious consideration in the framework of an integrated pest management strategy.

The antimicrobial effect of *Allium sativum* extract was confirmed in vivo tests on plants inoculated with suspension of *P. syringae* strains. *A. sativum* extract seemed to be the most effective treatment, when compared to the untreated control and the copper sulfate (Imen and Naima, 2018). In addition, Hassan and El-Meneisy (2014) demonstrated that garlic extract reduced the severity of the disease of bacterial Halo blight of bean caused by *P. syringae* pv. *phaseolicola*. In the same sense, Balestra *et al.* (2009) reported that *A. sativum* extract reduced the disease incidence and disease severity caused by *P. syringae* pv. *tomato*, *Xanthomonas vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*. It was reported that in vivo tests of aqueous extracts of *A. sativum* and *Ficus carica* fruits showed a reduction of the survival and the damages caused by *P. syringae* pv. *syringae*, and *Pseudomonas viridiflava* bacterial pathogens of kiwifruit (Balestra *et al.* 2008)

2.1.1.2 *Xanthomonas*

Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crop and are reported to have developed resistance to kanamycin, ampicillin, penicillin and streptomycin (Verma *et al.*, 1989; Bender *et al.*, 1990; Rodriguez *et al.*, 1997). Considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms (Hostettmann and Wolfender, 1997). Biopesticides has been suggested as an effective substitute for chemicals (Kapoor, 2001).

Xanthomonas campestris pv. *musacearum* (Xcm), is the causal agent of banana bacterial wilt disease. The disease was first reported in Ethiopia on enset, a family relative of bananas (Yirgou and Bradbury, 1968). It is currently found in all the enset growing regions and on wild enset plants (Brandt *et al.*, 1997). Bacterial wilt is a destructive disease in all enset growing areas of Ethiopia. The disease also attacks banana and other *Musa* spp (Viljoen, 2010). Banana bacterial wilt disease is currently restricted to Africa (Fenta and Karamura, 2012). Bacterial wilt attacks enset at any stage of growth, including full maturity (Brandt *et al.*, 1997). Once established in an area, the disease spreads rapidly and results in total yield loss (Weldemichael, 2008).

Bacterial diseases of plants, once established, are difficult to control owing to the lack of an effective chemical or other curative treatments (Biruma *et al.*, 2007). Study of Getahun and Masresha (2016) demonstrated that extracts of *Agarista salicifolia* and *Pycnostachys abyssinica* with high antibacterial activity in vitro against *X. campestris* pv. *musacearum* and it could be used for biopesticides control of Enset bacterial wilt.

2.1.1.3 *Ralstonia solanacearum*

Ralstonia solanacearum, the causal agent of bacterial wilt, is currently found on all continents and numerous islands located between the tropics of Cancer and Capricorn, causing disease on more than 200 plant species in over 50 families (Buddenhagen, 2009). According to Mansfield *et al.* (2012) *R. solanacearum* is considered as one of the world's most important/damaging phytopathogenic bacteria due to its lethality, broad geographic distribution and wide host range.

The use of chemicals has not been effective in the control of bacterial wilt of tomato because the pathogen is a soil borne and is systemic in its nature. The use of copper based bactericides and antibiotics seldom gave satisfactory control. Biopesticides because of their natural origin are biodegradable and they do not leave toxic residues or by-products to accumulate in the environment may give satisfactory control.

The water extract of two botanicals viz., *Ocimum gratissimum* and *Tylophora asthmatica* were effective in inhibiting the growth of *Ralstonia solanacearum*. *Ocimum gratissimum* extract showed highest inhibition zone of 28.66 mm (Kiran *et al.*, 2017). Saha *et al.* (2013) reported inhibitory effect of extracts of five *Ocimum* species against Gram-positive and Gram-negative bacteria and few plant pathogenic fungi.

2.1.2. Plant Pathogenic Fungi

Plant pathogenic fungi enter in to the plant either by making a hole on its skin (epidermis), or by growing in through the plant's breathing holes (stomata). Then they either poison and kill the plant cells before absorbing food from them, or simply steal nutrients from the living cells. The spores of some fungi come through the air and attack leaves, making dead spots or even killing the whole leaf. Some fungi live in the soil and enter roots. They can either block the water-

conducting cells or kill them, causing the plant to wilt. In many cases the plants is seriously damaged or may even die. So such pathogenic fungi can threaten numerous crop spp. Many fungal spp reported to cause disease on plants, and attempts were made to control using plant extracts.

2.1.2.1. Fusarium

Fusarium verticillioides (synonym, *Fusarium moniliforme* Sheldon; teleomorph, *Gibberella moniliformis* [synonym, *Gibberella fujikuroi* mating population A] is the most commonly reported fungal species infecting maize (Leslie, 1996). *Fusarium verticillioides* produces several toxins that have potential toxicity for humans and domesticated animals. The most significant of these toxins produced by *F. verticillioides* are the fumonisins (Desjardins *et al.* 1995). Since fumonisins can be detected in symptomatic and asymptomatic maize kernels, control of fumonisin contamination in maize has become a priority area in food safety research (Brown *et al.* 2001), and some guidelines for maximum fumonisin levels in human food and animal feeds have been issued (Food and Drug Administration, 2000)

There are many methods which are presently being used to control various plant pathogens including wilt pathogen such as physical, chemical, biological, cultural etc. Effective and efficient management of crop disease is generally achieved by the use of synthetic pesticides (Kiran *et al.*, 2006). Due to increased awareness about the risks use of pesticides, much attention is being focused on the alternative methods of pathogen control.

Joseph *et al.* (2008) reported that the Bioefficacy of plant extract to control *Fusarium solani* f. sp. *melongenae*. The *in vitro* efficacy of different plant extracts viz., *Azardiachta indica*, *Artemessia annua*, *Eucalyptus globulus*; *Ocimum sanctum* and *Rheum emodi* were tested to

control brinjal wilt pathogen. Different concentrations 5, 10, 15 and 20% of plant extracts was used in the study. All the plant extracts showed significant reduction in the growth of pathogen. Among the different extracts 20% of *Azardiachta indica* was found most effective followed by *Rheum emodi*, *Eucalyptus globulus*, *Artemessia annua* and *Ocimum sanctum*.

Adejumo (2012) studied that antifungall activity of plant extracts of *Piper. guineense*, *Garcinia kola*, *Aframomum. melegueta*, *Zingiber officinale*, *Cassia alata*, *Xylophia aethiopica* and Citrus limon and found good inhibitory effect against *F. verticillioides* in a decreasing order. Raju and Maridass (2011) also highlighted that the high level antibacterial and antifungal potential against Gram positive, Gram negative bacteria and moulds on seven Piper species used in indigenous system of medicine, might be due to the principal active constituents.

Velluti *et al.* (2004) reported the mycelial inhibition of *F. verticillioides* at 1000 mg/ml of lemongrass extract. Similarly, Study of Adejumo (2012) agrees with the reports that lemongrass was effective against *F. verticillioides* at 100 to 500 mg/ml. The antimicrobial activity of the oil is believed to be associated with the phytochemical components of lemongrass such as monoterpenes (Matasyoh *et al.*, 2007) which diffuse into and damage cell membrane structures.

Further studies on these effective botanicals should include bioassay-directed fractionation of the extracts which will lead to the isolation of the compounds that is showing considerable antifungal activity.

2.1.2.2. Aspergillus

Aspergillus flavus is an important pathogenic fungus affecting peanuts during storage (Amaike and Keller, 2011). In infected peanuts it can causes seed rot and reduce seed viability and germination (Kumar *et al.*, 2008). The pathogen can also produce aflatoxins, a group of fungal secondary metabolites, which are the most toxic carcinogens among known mycotoxins

(Krishnamurthy *et al.*, 2008). These metabolites are considered potent hepatocarcinogens in animals and may also be involved in primary liver cancer and other cancers in kidney, lung, and colon tissues in humans (Amaike and Keller, 2011).

To protect food and feedstuffs and to avoid aflatoxin contamination from *A. flavus*, there are several possible control strategies employed in peanut such as treating peanuts with fungicides, fumigants, or biocontrol agents (Kong *et al.*, 2010). Although the use of fungicides for controlling *A. flavus* is an effective measure, there are several negative effects of this method including generation of fungicide-resistant strains of *A. flavus*, and fungicide residues affecting food safety and as a source of environmental pollution (Droby, 2006). Therefore, development of effective biological controls is a priority with presumed lower risk to humans and the environment.

Rasooli *et al.* (2006) studied that the antifungal effects of essential oils from *Thymus eriocalyx* and *Thymus x-porlock* with special reference to the mechanism of inhibition of *Aspergillus niger* growth at ultrastructural level. *Thymus eriocalyx* and *Thymus x-porlock* oils inhibited *Aspergillus niger* at 125 ppm and 250 ppm with growth inhibition zone of 25 mm and 8 mm respectively. Fungicidal concentrations of *Thymus eriocalyx* and *Thymus x-porlock* oils were achieved at 250 ppm and 500 ppm with growth inhibition zones of 90 mm and 13 mm respectively.

2.1.2.3. Penicillium

Penicillium species are the main cause of deterioration and decomposition of a wide range of plant products after harvest, especially fruits, such as grapes (Gende *et al.*, 2008). These fungi are widespread, attacking various fruits, including grapes and especially during storage and often producing a variety of mycotoxins (Moslem *et al.*, 2011). Harmful mycotoxins and carcinogenic

compounds, such as citrinine, pat-ulin, penicillic acid and other secondary metabolites are produced by *Penicillium* spp. (Abramson *et al.*, 2009). Effective control of fruit diseases can also be achieved through many non-chemical control strategies (Sanzani *et al.*, 2010). One popular non chemical option for controlling plant diseases (Soylu *et al.*, 2005) is use of extracts and essential oils of herbaceous plants. Availability, low toxicity, and environmental friendliness make plant extracts attracted targets for investigation (Fawzi *et al.*, 2009 and Aqil *et al.*, 2010). Several plant extracts possess antifungal properties and can be used to suppress decomposing fungi (Ismail, 2008). Garlic is among the most promising natural plant materials with antifungal properties (Yassin *et al.*, 2013 and Znini *et al.*, 2011). Antifungal activity of plant extracts is noted against *Penicillium* spp. and other fungi, as well as reduced production of mycotoxins (Tas-keen *et al.*, 2011 and Minz *et al.*, 2012).

2.2. Indigenous knowledge of botanical for pest control

Traditionally, humanbeing are using natural products of animals, plants and microbial sources for thousands of years either in the pure forms or crude extracts (Parekh and Chanda, 2007). Traditional knowledge is the local knowledge that is unique to a given culture or society differing from the international knowledge generated by universities and research institutions. It is one of the tools used as cost effective survival strategy at the local level for decisions related with food security, human health, agriculture and household products protection and natural resources management (Grenier, 1998). These indigenous knowledge and techniques can be categorized as land preparation, soil fertility management, weed control, post harvest management and pest controlling strategies (Kuponiya and Bamigboye, 2009). From the different pest management strategies one is the use of traditional botanical pesticides (Pavela, 2007). Traditional botanical pest controlling methods are used to protect agricultural and household

products as natural, cost effective and easily accessible from nearby source. In addition, due to their biodegradability unlike synthetic chemical pesticides they are environmental friendly and hence favourable to the environmental sustainability and community wellbeing (Pavela, 2007).

Indigenous knowledge is transferred orally from generation to generation through herbalists and knowledgeable elders. The indigenous knowledge system in Ethiopia is not fully documented when compared to available multiethnic, cultural and flora diversity (Fisseha *et al.*, 2009; Mirutse *et al.*, 2009). Scientific documentation is to preserve valuable traditional knowledge for future generations (Martin, 1995 and Cotton, 1996). In Ethiopia, research and documentations on medicinal plants have started in recent times even though plants used traditionally as a source of medicine since time immemorial (Mesfin and Sebsebe, 1992). Many traditional remedies are remaining hidden due to migration of people, urbanization, influence of modern medicine and exotic cultures (Tafesse and Mekonnen, 2001). In Ethiopia, few studies have been conducted and documented on method of traditional medicine preparations by using local medicinal plants, parts used and routes of applications (Haile and Delenasaw, 2007; Ragunathan and Solomon, 2007).

Today many indigenous knowledge systems are at risk of extinction because of the rapidly changing natural environments and fast pacing economic, political, and cultural changes on a global scale (Grenier, 1998). Many traditional practices are disappearing because of the intrusion of foreign technologies like synthetic chemical pesticides that assure short-term solutions to problems (Grenier, 1998). Despite of killing pests effectively, these chemical pesticides are toxic to human beings and lead to severe environmental degradation if not properly used. Besides this, its fully integration into insect pest management systems is roughly disrupted as a result of the subsistence nature of production and high poverty levels. That is to mean high costs of synthetic

pesticides and associated toxicity risks discourage its integration into insect pest management systems (Mihale and Kishimba, 2004). As an alternative option, local community forced to rely on their indigenous knowledge and techniques in order to meet their daily needs which are most relevant to the rural poor and marginalized population. Farmers who use natural control methods obtain satisfactory yields and financially save on production inputs, and no longer forced to get into debt or sell off their livestock in order to repay loans for purchasing of pesticides. They were also able to control pests in a timely fashion using their own resources, rather than worry about how to access pesticides (Fantahun and Williamson, 2001). The use of plant extracts to control destructive insect pests or disease vectors is not new. Rotenone (*Derris* spp.), nicotine and pyrethrins have been used for a considerable time in small-scale subsistence and in commercial agriculture (Hillock and Bolin, 2004). For instance, the use of poisonous plants which have bio-pesticide properties is found in the Indian Rig Veda (2000 B.C.). More than 1,600 plant species have been reported to possess bio-pesticide properties at different parts of the world (Hoddy, 1991). Botanical pesticides are biologically active ingredients that derived from plants and used for the control pest. Currently the demand for environmental friendly pest management strategies is showing the tendency increase. Thus, the development and usage of alternative pesticides with minimal or non-ecological hazards like natural pesticides is highly recommended (Devlin and Zettel, 1999). It also used to maintain biological diversity and reduces environmental contamination and human health hazards. This becomes applicable when science-based approach is well developed to discover and determine their efficacy and attempts to either produce them on a commercial scale (Philogene and Lambert, 1993). Thus, traditional botanical pesticides which are environmental friendly, cost effective and efficient needs to be recognized, valued, appreciated and it should be preserved, transferred, or adopted and adapted as integrated pest

management elsewhere.

There has been a revival of interest in the plant products, as pesticides, for the control of field pests as well as stored product insect pests (Chodhary, 1990). Many plant products that are traditionally used by the farmers in developing countries appear to be promising (Jilani *et al.*, 1988). The neem derivatives have been reported to act as toxicant, antifeedant, repellent, oviposition deterrent, growth inhibitor, and sterilant (Saha, 2002). It is an age-old practice of traditional farmers to use a local plant in the storage of grain legumes (Teotia and Tiwari, 1980; Gill and Lewis, 1989). Various plant products have been tried recently with good degree of success as protectants against a number of stored product insect pests (Yadav and Bhatnagar, 1987; Ayyangar and Rao, 1989). Previous study indicated that *Bucida buceras*, *Breonadia salicina*, and *Xylothea kraussiana* extracts have been reported to have strong antifungal activity against *Aspergillus fumigatus* (Mahlo *et al.*, 2013).

Various workers have screened large number of plants for their fungitoxic properties. Manoharachary and Gourinath (1991) found that aqueous leaf extract of *Eucalyptus lonceolatus* was inhibitory for the germination and growth of *Curvularia lunata*, *Cylindrocarpon lichenicola* and *Fusarium solani*. Singh and Prasada (1993) found that leaf extract of *Azadirachta indica* and *Ocimum sanctum* inhibited the growth of *Fusarium oxysporum*. Mostly the aqueous extract of plants has been used to evaluate their fungitoxic properties (Thapliyal *et al.*, 2000 and Algesaboopathi and Balu, 2002). Shafique *et al.*, (2007) from experiment recommended that aqueous extracts of allelopathic trees especially those of *Azadirachta indica* and *Mangifera indica* can be used to treat the wheat grains for 10 minutes before sowing or storage to reduce the fungal incidence. Similarly, Meena *et al.*, (2010) tested leaf extract of ten medicinal plants against *Alternaria cucumerina*. They reported that, leaf extract of *Azadirachta indica*, *Calotropis*

gigantia and *Aloe barbadensis* were found to be effective in controlling spread of *Alternaria cucumerina*. Aqueous extract of *Eucalyptus angophoroides* showed retardation of growth of storage fungi which reveals that it is most fungitoxic and can be used in biopesticides formulations.

2.2.1. Antimicrobial Activities of *Allium sativum*

Garlic (*Allium sativum*), is one of the edible plants which has generated a lot of interest as a medicinal panacea. Previous studies reported the insecticidal, fungicidal, acaricidal, nematocidal, and bactericidal properties of garlic (Lalla *et al.*, 2013). A wide range of microorganisms including gram-positive and gram-negative bacteria (Whitemore and Naidu 2000), fungi, protozoa, and viruses have been shown to be sensitive to crushed garlic preparations (Koch and Lawson 1996).

Previous studies reported that *A. sativum* exhibited activity against many pathogenic bacteria and fungi at different rates (Pyun and Shin 2006, and Saravanan *et al.* 2010). The effectiveness of garlic extract against a range of plant pathogenic organisms was shown by growth inhibition of the plant pathogenic bacteria *Agrobacterium tumefaciens*, *Erwinia carotovora*, *Pseudomonas syringae* pv. *maculicola*, *P. syringae* pv. *phaseolicola*, *P. syringae* pv. *tomato*, and *Xanthomonas campestris* pv. *campestris* (Curtis *et al.* 2004). Triki *et al.* (2008) showed that the antimicrobial property of the garlic extract is due to an active molecule which is allicin. This molecule accumulates naturally at ambient temperatures during garlic bulbs storage (Amagase *et al.*, 2001). Allicin crosses the cell membrane easily and undergoes thiol-disulfide exchange reactions with free thiol groups in proteins. It is thought that these properties are the basis of its antimicrobial action (Miron *et al.* 2000). The action of biological active ingredient of allicin which exhibits its antimicrobial activity mainly by immediate and total inhibition of RNA

synthesis, although DNA and protein syntheses are also partially inhibited, suggesting that RNA is the primary target of allicin action (Deresse, 2010).

2.2.2. Antimicrobial Activities of Neem

Neem (*Azadirachta indica* Juss) is a fast growing tree that is native to the Indian subcontinent and which is now distributed throughout Southeast Asia, East and Sub-Saharan Africa, and parts of Central America (Hora, 1981). Unlike most of the pesticides available in the market, neem extracts are apparently nonpoisonous to man and animals and non-polluting to the environment. In crop protection, the seed can be used in the forms of neem oil, neem kernel powder and neem press cake. In the store, it can be used in the form of powder or oil (Teshome, 1991). Grainge and Ahmed (1985) reported that the effective range of neem is insecticidal, repellent, anti-feedant, growth inhibiting, fungicidal and nematocidal. Neem is effective against numerous pests and it has been shown that it can control over 100 species of insects, mites and nematodes. The pulp of neem completely prevents the development of resting forms of *Rhizoctonia solani* (Singh *et al*, 1980). Adane and Abraham (1995) reported the gradual action of botanical plants such as leaf powder of neem and *Persian lilac* against *Sitophilus zeamais* and they recorded the highest mortality due to the application of the botanicals at 28 days after the treatment application. *Azadirachta indica* and *Polyalthia longifolia* showed their antifungal activity against *Macrophomina phaseolina*, *Rhizopus stolonifer* and *Penicillium digitatum* (Rajendra and Ashok, 2011).

2.2.3. Antimicrobial Activities of *Justicia schimperiana*

Justicia schimperiana is common shrub growing in moist mountain forest, usually near streams and rivers, in evergreen scrub on hill slopes, forest clearings, different plantations, waste ground or planted. It belongs to the family of *Acanthaceae*. It is a leafy shrub with much branched easily breakable stems, simple and opposite, long oval and tip pointed leaves and white or yellow white flowers. It is up to 4 m high, with slightly unpleasant smell (Hedberg *et al.*, 2006).

The presence of compounds with diverse chemical class including alkaloids, lignans, flavonoids and terpenoids was reported (Corrêa and Alcântara, 2012). Other chemical class of chemicals like essential oils, vitamins, fatty acids and salicylic acid, steroids also reported to reside in the *Justicia* (Corrêa and Alcântara, 2012).

Murthy *et al.* (1993) screened the antibacterial activity of different parts (leaves, stem, bark and root) of *Justicia schimperiana* and found it to be active against the tested bacterial species (*S. aureus*, *E. coli*, *B. cereus*, *P. mirabilis*, *K. pneumonia* and *P. aeruginosa*). Moreover, Kothai and Thirunalasundri (2011) reported antibacterial effect of aqueous and ethanol root extracts against both clinical and standard strains of *S. pyogenes*. By contrast, Tamirat *et al.* (2015) reported the absence of detectable zone of inhibition of root extract of *Justicia schimperiana* against *S. pneumoniae*, *S. pyogenes* and *S. typhi*.

2.2.4. Antimicrobial activities of *Eucalyptus globulus*

Eucalyptus globulus belongs to the family of Myrtaceae which is indigenous to Australia. *Eucalyptus globulus* Labill has been planted as the main tree species in the central highlands of Ethiopia since 1895. *Eucalyptus* leaves are rich sources of essential oils, flavonoids or tanins, which are responsible for their antibacterial, larvicidal, fumigant, antioxidant activities and

anthelmintic properties (Topiar *et al.*, 2015).

According to Sabramania *et al.* (1994), laboratory trials conducted with six plant species against *Callosobruchus chinensis* infesting red gram (pigeon peas) showed that extracts of *Eucalyptus globulus* were the most promising. Moreover, Rajendra and Ashok (2011) reported that, aqueous extract of *Eucalyptus angophoroides* found to be fungitoxic for the growth of *Alternaria dianthicola*, *Curvularia pellescens*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum*.

2.2.5. Antimicrobial activities of *Vernonia amygdalina*

Vernonia amygdalina, a species in the family *asteraceae*, is a tropical shrub with height of 1-3mm, petiole leaf of about 6mm in diameter (Ibrahim *et al.*, 2009). *Vernonia amygdalina* commonly called bitter leaf in English, “oriwo” in Edo, “ewuro” in Yoruba, “shikawa” in Hausa, and “olubu” in Igbo (Oboh and Masodje, 2009). The leaves are consumed as vegetable and condiments, after macerating and washing thoroughly to remove the bitterness. The bitter *V. amygdalina* is due to the presence of alkaloids, saponins, tannins and glycosides. Antihelmitic, antimalarial, antitumorigenic, hypoglycemic and hypolipidaemic properties of *Vernonia amygdalina* have been reported (Izevbigie *et al.*, 2004).

2.2.6. Antimicrobial activities of *Ruta chalepensis*

Ruta is a genus of *Rutaceae* family and features primarily shrubby plants that are native to the Mediterranean region and usually grow on rocky slopes. The three most diffused species are *Ruta chalepensis* L., *Ruta graveolens* L., and *Ruta montana* L. (Pollio *et al.*, 2008). It is used for culinary purposes to flavor foods and as an aperitif (Pieroni, 2000). This plant is commonly used

as a traditional medicinal plant. It is protective against various disorders such as rheumatism, fever, mental disorders, dropsy, neuralgia, menstrual problems, convulsions, and other bleeding and nervous disorders (Pollio *et al.*, 2008). With regard to the phytochemical composition of *Ruta chalepensis* extracts, Aguilar-Santamaria and Tortoriello (1995) have reported that its leaves and young stems contain alkaloids, flavonoids, phenols, amino acids, furocoumarins, and saponins. However, data on the bioactivities of *R. chalepensis* are scarce. Almog *et al.* (2011) found that the ethanol extract of the plant reduced the oxidative stress in experimental animal models by modulating the activities of various antioxidant enzymes.

Moreover, Fakhfakh *et al.* (2012) reported interesting in vitro anti-oxidant activities of the essential oil and solvent extracts of wild *R. chalepensis*. Studies on the antimicrobial activity of *R. chalepensis* are limited; however, Al-Bakri and Afifi (2007) found that dimethyl sulfoxide extracts of *R. chalepensis* possess promising antibacterial activity. Several researchers suggested the relevance of identifying the metabolic differences between wild and cultivated specimens to assess their quality during the domestication process (2009). In some instances, the wild forms of plants may demonstrate different phytonutrient profiles from the cultivated ones. Several studies have stated that most wild species preserve higher biological capacity and richer secondary metabolites, compared with the cultivated species (Economal *et al.*, 1999; Luseba *et al.*, 2011)

2.2.7. Antimicrobial activities of *Ocimum lamiifolium*

Ocimum lamiifolium is an indigenous plant in Ethiopia, locally called “dama-kassie” (in Amharic) and is one of the most common traditional medicinal plants used in Ethiopia (Mirutse *et al.*, 2010). *Ocimum lamiifolium* extracts are also known to have antibacterial, antifungal, insect

repellent (Ermiyas Dagne, 2009), and antiinflammatory activities in humans (Kashyap *et al.*, 2011). Destaw and Yalemtehay (2015) reported that the crude extract of *Ocimum lamiifolium* (Ol) used to inhibit the growth of some selected pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Shigella boydii* and *Staphylococcus aureus*.

2.2.8. Antimicrobial activities of *Calpurnia aurea*

Calpurnia aurea is a plant within the family of Fabaceae. It is widely distributed in Ethiopia with a local name “digita” in Amharic and used in traditional medicine to treat diverse medical conditions in humans and animals (Zorloni, 2007). Abdella *et al.* (2013) reported that all parts of the plant are used for different human and animal diseases. Tadege *et al.* (2005) have also reported that different parts of *Calpurina aurea* are used to treat different diseases such as diarrhea, stomach-ache, bowel, and bladder disorders.

2.2.9. Antimicrobial activities of *Cordia Africana*

Cordia is a pantropical genus of flowering plants that belongs to Boraginaceae family. (Alemayehu *et al.*, 2016), from which *Cordia africana* (Lam.) is one of the common forest tree species in the family. *Cordia africana* was found to be a very good source of total phenol antioxidants (Tewolde-Berhan *et al.*, 2015). The ethnomedicinal use of *C. africana* in treatment of various diseases has been validated in several experimental studies. Ethanol extracts of the plant exhibited in vivo antiinflammatory (Tijjani *et al.*, 2015) and antioxidant (Ganesan *et al.*, 2015 and Alhadi *et al.*, 2015) and antinociceptive activities (Tijjani *et al.*, 2015). Another study on the leaf and stem bark extracts of *C. Africana* also showed that the plant possesses anti-inflammatory, antibacterial, and antioxidant activities (Isa *et al.*, 2016). Several studies reported

that phytochemical analysis of the extracts of *C. africana* is a rich source of essential secondary metabolites (Alhadi *et al.*, 2015 and Asrie *et al.*, 2016). Various compounds like flavonoids, triterpenes, tannins, alkaloids and fatty acids possessing wide range of bioactivities were isolated from different plant parts of *Cordia* species (Thirupathi *et al.*, 2008).

2.3. Prospects and Limitation of Biopesticides

Biopesticides are competitive subclass of pesticides that are naturally occurring organisms or compounds that suppress the growth and proliferation of pests' population by diverse mechanisms of action, excluding those that interfere with pests' nervous systems (Tijjani *et al.*, 2016; Marrone, 2019; Nuruzzaman *et al.*, 2019; Wattimena and Latumahina, 2021). They are categorized into three groups: microbial biopesticides, biochemical biopesticides, and botanical biopesticides (Liu *et al.*, 2019; Ram and Singh, 2021).

In the nearest future, biopesticides could replace synthetic pesticides without significantly affecting productivity and yield, if their potentials are fully maximized (Mishra *et al.*, 2020). The limitation confronting the full adoption of biopesticides are the high cost of refined commercial products, inability to meet global market demand, differing standard method of preparations and guidelines, dose determination of active ingredients, the susceptibility of biopesticides to several environmental factors, ephemeral stability, and slow action among others. While expecting these limitations to be addressed through research breakthroughs in the coming years, farmers, especially in rural areas, can take advantage of a crude plant extract in protecting plants for improved yield (Stevenson *et al.*, 2017). The effectiveness of the application of pesticides, including biopesticides, is maximal when incorporated in IPM (Quarles, 2013). An IPM is a pest-control option, equivalent to an effective multi-faceted approach involving the combination of

cultural practices and other suitable control tactics into one management program to achieve a long-lasting reduction of pest population and associated problems (Sadof *et al.*, 2021). Positive externalities that go along with biopesticides besides significant pest population reduction are social acceptability, economic viability, and environmental stewardship, the three domain concept of sustainable development.

2.4. Advantages of Biopesticides

Biopesticides exert their inhibitory effects through multiple modes of actions such as growth regulators, gut disruptors, metabolic poison, neuromuscular toxins, and non-specific multi-site inhibitors (Sparks and Nauen, 2015; Dar *et al.*, 2021). These multiple modes of action against targeted pests obliterate the chances of developing resistance as is common with chemical pesticides. The intensive use of conventional pesticides in industrial-scale farming over a long period, especially in the Green Revolution era, also created challenges such as pesticide-related pollution, post-harvest chemical consumption through bioaccumulation, biodiversity losses, and insurgence of secondary pests and elimination of natural/beneficial enemies. These negative consequences are not associated with the use of biopesticides. Thus, prohibitive restrictions are continually imposed on synthetic pesticides to reduce their numbers with time. For instance, there has been a reduction to 250 active ingredients of conventional pesticides in 2009 as opposed to more than 1,000 in 2001 while entrance of new conventional pesticides into the market reduced from 70 in 2000 to 28 in 2012 (McDougall, 2013). The direct result of the declining number of classical pesticides is the increased demand for biopesticides for some beneficial reasons. These benefits include, but are not exclusive to, altering the course of pest resistance, low toxicity properties, complementary input to synthetic pesticides,

eco friendliness, specificity (thus have little or no negative impact on non-target organisms and humans), biodegradability, and little or no problem of post-harvest contamination, stability against abiotic stress (Deravel *et al.*, 2014; Kalpana and Anil, 2021), and compatibility in integrated pest management (IPM).

2.5. Bioactive Components of Plants

Plants are the richest source of bioactive compounds. Bioactive metabolites are classified into primary or secondary metabolites, depending on their functional role (Sharma *et al.*, 2019). Plants and fungi produce thousands of secondary metabolites according to the physiological stage, tissue localization (floral and non-floral leaves, fruits, or bark), environmental conditions, and other biotic or abiotic stress. These compounds may be involved in the primary physiological function of the cell; they may participate in the control of cell growth and cell development, acting as plant growth substances, i.e., plant hormones. Nevertheless, their main function is ecological, especially with regards to the plant defense against herbivores, bacteria, and fungi (Mithöfer and Maffei, 2017).

2.5.1. Phenolic Compounds

The antioxidants found in plants are mainly phenolic compounds (phenolic acid and flavonoids). Phenolic compounds are aromatic hydroxylated compounds, possessing one or more aromatic rings with one or more hydroxyl groups, being commonly found in vegetables, fruits and many food sources. Phenolics form a significant portion of our diet, and some of which are among the most potent and therapeutically useful bioactive substances (Apak *et al.*, 2007).

Structure activity relationships of two phenol derivatives (cinnamaldehyde and eugenol) were

studied on two phytopathogenic fungi, namely, *Rhizoctonia solani* and *Fusarium oxysporum*. Phenol antifungal activity was shown to depend on the chemical structure. In particular, aldehydes, acid groups, conjugated double bonds, and the length of CH chain outside the ring have increased the antifungal activity (Xie *et al.*, 2017). While aldehydes and acid groups may be more prone to react with amino acid residues of proteins through hydrogen bonds and induce conformational modification because of the proton release ability, the length of the CH chain increases hydrophobicity, a major determinant of phenol capability to enter the plasma membrane (Ben Arfa *et al.*, 2006; Dambolena *et al.*, 2011).

2.5.2. Flavonoids

Flavonoids represent a large group of phenolic compounds with antioxidant activity that occur naturally in fruits, vegetables, grains, barks, roots, stems, flowers, and derived products like tea and wine. These compounds have been linked to reduce the risk of major chronic diseases (Liu, 2004).

2.5.3. Tannins

Plant tannins are polyphenols that are widely found in terrestrial plants and in some marine plants (phloroglucinol). As natural polyphenolic compounds, tannins display antibacterial effects. Research has investigated the effects of different levels of tannins from sweet chestnut tannin and they found that in 28-day-old chickens, 1,000 mg/kg tannins reduced the numbers of *E. coli* and other harmful bacteria in the small intestine (Biagia *et al.*, 2010).

It has been reported that the amount of hydroxyl groups in tannins and the release of hydrogen peroxide are important indicators to evaluate the antibacterial properties, which are positively

correlated with antioxidant properties. For instance, trihydroxy b-cycloflavonol (gallic catechin) has stronger effects on *Streptococcus*, *Clostridium*, and *Staphylococcus* than dihydroxy b-cyclocatechin (Anita *et al.*, 2014). Because tannins come from a wide range of sources and have diverse antibacterial effects, screening and identifying tannins that are effective and specific to target microorganisms will be the work of ongoing research.

2.5.4. Glycoside

Glycosylation of metabolites in plants serves multiple purposes. Fan *et al.* (2015) isolated several glycosides from water spinach (*Ipomoea aquatica*). These compounds were analyzed against six different cancer cell lines and compounds 80-82 showed marked in vitro cytotoxicity. Jia *et al.* (2015) isolated six glycosides from *Dregea sinensis* and showed cytotoxicity towards human leukemia cells (HL-60).

2.5.5. Saponins

The physiochemical and biological properties of saponins have led to a number of traditional and industrial applications. They have traditionally been used as natural detergents. The combination of a hydrophobic aglycone backbone and hydrophilic sugar molecules confers foaming and emulsifying properties of saponins (Moses *et al.*, 2014). The name ‘saponin’ is derived from the Latin word ‘sapo,’ meaning soap, as a soapy lather forms when plants containing saponins are agitated in water. The chemical structure of saponins may be transformed during storage or processing. Therefore, the selection of methods appropriate to storage of plant material is a key part of each efficient technology (Hossain *et al.*, 2010).

2.5.6. Steroids

In the last decade, important advances have been made in elucidating the metabolism (biosynthesis and catabolism) and signaling pathways of sterols and brassinosteroids (BRs) as well as their importance for plant growth and development, in both model plants and crops (Schaller, 2004; Choe, 2006; Clouse, 2011; Williams, 2011). Both sterols and brassinosteroids (BRs) control several traits of agronomic importance, such as plant growth, photosynthesis, architecture, and flowering time.

When applied exogenously, BRs induce resistance against a broad range of diseases, notably against fungal and bacterial pathogens in tobacco and in rice. In rice, treatment with BL protected the plant against rice blast and bacterial rice blight diseases, whereas in tobacco, it enhanced resistance against the bacterial pathogen *Pseudomonas syringae* and the fungal pathogen *Odium* species (Nakashita *et al.*, 2003). Another important aspect of the plant protective action of BRs is their capacity to improve virus resistance. Treatment of tobacco plants with BL reduced infection by the Tobacco mosaic virus (Nakashita *et al.*, 2003). Similarly, potato (*Solanum tuberosum*) cuttings cultured in a medium containing brassinosteroids (BL), epibrassinolide (EBL), or 28-homobrassinolide were more resistant to viral infection through all developmental stages (Khripach *et al.*, 2000). BR analogs also possess biological effects on insects. More particularly, brassinosteroids (BRs) can influence ecdysteroid-dependent steps of insect development (Zullo and Adam, 2002).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted in six districts (Awassa Zuria, Chuko, Malga, Wondo Genet, Yirgalem and Dalle) of Sidama Region, Southern Ethiopia. The Region lay in 6°29'N of latitude and 38°31'E of longitude. The antimicrobial activities of selected plant extracts were checked in Hawassa University, College of Agriculture, crop protection laboratory.

3.2. Survey of Botanical Pesticides Traditionally Used By Farmers

With discussion of administrative officials key informants (those who have knowledge of study area) and farmers were selected and help us to identify botanicals which have been used traditionally by the farmers to control major pests of the area. Part/s of the plant used, method of preparation and application were also identified. Plants reported to be pesticides were collected and on the spot identification of individual plant species carried out with the help of expertise, pictorial aids and literature materials. For plant specimens whose species identification in the field becomes difficult specimens were prepared and preserved for further species identification in National Herbarium Museum of Addis Ababa University.

3.3. Identification of Pathogens and Preparation of Plant Extracts

3.3.1. Plant Pathogen

Selected botanicals were evaluated against some plant pathogenic fungi and bacteria. These pathogens: *Fusarium vertecelioides*, *Aspergillus flavus*, *Penicillium notatum* identified from Maize (*Zea mays*) and *Xanthomonas campestris* (*X.c.pv.m*), *Ralstonia solanacearum* and *Pseudomonas syringae*, were isolated from Enset (*Ensete ventricosum*) were involved in this study using the well and disc methods. The plant pathogenic fungi and bacteria were isolated,

identified in the Crop Protection laboratory and their pure cultures were preserved for further analysis.

3.3.2. Preparation of plant extracts

Sixteen Selected botanical which are widely used by the farmers in the management of plant pathogens were brought to the lab and stored in the appropriate condition. The selected plant materials were washed thoroughly 2-3 times with running water and once with sterile distilled water and then air-dried and powdered with the help of blender. Twenty five gram (25g) of the grind selected plant materials were soaked each in 125 ml ethanol for 24 hours (Idris *et al.* 2009). The extracts were filtered through a sieve to remove debris. The filtrates were then filtered through filter paper. The final filtrates were evaporated in a water bath at 40°C to get the crude extracts. The crude extracts were stored at 4°C until required. This was used for phytochemical and antimicrobial analysis. Table.1 showed the names of selected botanicals.

Table 1 Botanicals, their local names and area of collection

Botanical name	Local names(Amharic)	Family names	Area of Collection
<i>Alium sativum</i>	Nech shinkurt	<i>Alliaceae</i>	Yirgalem
<i>Artemisia afra</i>	Chikugn	<i>Asteraceae</i>	Yirgalem
<i>Azadirachta indica</i>	Yekinin zaf (Neem)	<i>Meliaceae</i>	Awassa Zuria
<i>Brucea antidysenterica</i>	Abalo	<i>Simaroubaceae</i>	Chuko
<i>Calpurnia aurea</i>	Digita	<i>Fabaceae</i>	Chuko
<i>Cordia Africana</i>	Wanza	<i>Boraginaceae</i>	Malga
<i>Croton macrostachyus</i>	Bisana	<i>Euphorbiaceae</i>	Dalle
<i>Eucalyptus globules</i>	Nech Bahir-zaf	<i>Myrtaceae</i>	Malga
<i>Justicia shimperiana</i>	Sensel	<i>Acanthaceae</i>	Wondo Genet
<i>Moringa stenopetala</i>	Shiferaw	<i>Moringaceae</i>	Malga
<i>Nicotiana tabacum</i>	Tinbaho	<i>Solanaceae</i>	Chuko
<i>Ocimum basilicum</i>	Besobila	<i>Lamiaceae</i>	Dalle
<i>Ocimum lamifolium</i>	Damekesie	<i>Lamiaceae</i>	Chuko
<i>Rosmarinus officinalis</i>	Siga metibesha	<i>Labiatae</i>	Dalle
<i>Ruta chalepensis</i>	Tenadam	<i>Rutaceae</i>	Yirgalem
<i>Vernonia amygdalina</i>	Grawa	<i>Asteraceae</i>	Awassa Zuria

3.4. Determination of extraction yield

The percentage yield of each extract was obtained using the formula (Anokwuru et al., 2011).

$$\text{Percentage yield} = \frac{(\text{Weight of crude extract}) \times 100}{\text{Weight of sample}}$$

Weight of sample

3.5. Preparation of Solution

Each sample extract was mixed with dimethylsulfoxide (DMSO) and 100 percent (100mg/ml) was used to test both antibacterial and antifungal activities.

3.6. Antimicrobial Activities test of Ethanolic Extracts

Antimicrobial activity was evaluated by measuring the zone of inhibition against the test microorganisms (Cushnie & Lamb, 2005). In this study, all the tests assays were done in triplet for each treatment and control groups. The results were presented as Mean $\pm$ SE with the help of Microsoft Excel 2007 version.

3.6.1. Antibacterial Activity Test

Antibacterial activities of the ethanolic extract of the selected plants were determined by Well and disc method (Stoke and Ridgway, 1980). Selected plant pathogenic bacteria (*Xanthomonas campestris* (X.c.pv.m), *Ralstonia solanacearum* and *Pseudomonas syringea*) were inoculated in to recommended culture media. The Inoculants were incubated at 37 ± 1 °C for 24 h. The culture suspensions were then prepared and adjusted by comparing against 0.4-0.5 Mc Farland turbidity standard tubes. The recommended broth media (20 ml each) was prepared and poured into different sterilized petri dish (10×90 mm diameter) after injecting 100 µl of the respective

bacterial culture suspension. The media were distributed in Petri dishes homogenously.

The ethanolic extracts obtained from the dried selected plants were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 100mg/ml (Tepe *et al.*, 2005). A well of 6 mm in diameter was bored at the centre of each inoculated and solidified media with help of sterilized cork borer. Then 100 µl of the plant extracts were filled into the wells directly.

At the end of the incubation period (24 h), the inhibition zone (diameter) formed on each media were measured. Inhibitory activity of the DMSO was also tested as negative control. Studies were performed in triple time and the results were expressed as average values. Standard antibiotics; Tetracycline (30 µg) and penicillin (10 µg) was used as the reference discs.

3.6.2. Antifungal Activity Test

The plant pathogenic fungal cultures (*Fusarium vertecelioides*, *Aspergillus flavus* and *Penicillium notatum*) were incubated at room temperature for 48 h in liquid Potato Dextrose media. The culture suspensions were prepared and adjusted by comparing against 0.4-0.5 McFarland turbidity standard tubes. Potato Dextrose agar media (20 ml) were poured into sterilized Petri dishes (10x90 mm diameter) after inoculation with the fungal cultures (100 µl) and distributed homogeneously and allowed to solidify. With the help of sterilized cork borer a well of 6 mm in diameter was bored at the centre of the media in the plate. The plant extracts of 100 µl was filled into the wells of agar plates directly. The plates were incubated at 28 ± 1 °C for 3-4 days. Fungicides (Mancozeb) were used as the reference. After the incubation period, zone of inhibition was measured, tabulated and compared with reference.

3.7. Determination of Minimum Inhibitory Concentration (MIC)

The MICs of the extract for each test microorganism was regarded as the agar plate with the lowest concentrations without growth (Oboh *et al.*, 2007 and Vamanu, 2012). The standard agar dilution protocol with double dilution was used. From a stock solution, a double dilution method (to bring 100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25mg/ml, 3.125mg/ml and 1.56mg/ml) were performed by using sterile digital micropipette (CLSI, 2012). The standard concentration of the extract used was 100mg/ml. Dimethylsulfoxide (DMSO) was used as the diluent. All testes were done in triplicates.

3.8. Phytochemical Constituent Test

Each plant extract was tested for the presence of various secondary metabolites (phytochemical) such as tannins, glycosides, phenolics, flavonoids, saponins and steroids. The analysis was employed for the presence of various secondary metabolites (phytochemical constituent) as described by (Kokate, 2000).

The extracts were subjected to phytochemical tests for plant secondary metabolites such as tannins, glycosides, phenolics, saponins, flavonods, and steroids following the standard laboratory techniques (Trease and Evans, 2004).

3.8.1. Test for Tannins

Few mL of each plant extract solutions were taken in different test tubes followed by the addition of aqueous basic lead acetate formation of reddish brown bulky precipitate indicates the presence of tannins.

3.8.2. Test for Glycosides

When α -naphthanol and concentrated sulphuric acid was added to each plant extract solutions, reddish violet ring at junction of two layers indicates the presence of glycosides.

3.8.3. Test for Phenolics

When 5% glacial acetic acid and 5% sodium nitrite were added to each plant extract solutions, an appearance of a muddy niger brown color indicates the presence of phenols.

3.8.4. Test for Flavonoids

When aqueous basic lead acetate was added to each plant extract solutions, production of reddish brown precipitate is an indication of presence of flavonoids.

3.8.5. Test for Saponins

Five milliliter of each plant extract was shaken vigorously to obtain a stable persistent froth indicates the presence of saponins.

3.8.6. Test for Steroids

Few drops of concentrated sulphuric acid were added to the plant extract solution in chloroform, a red color appearance at the lower layer indicates the presence of steroids.

3.9. Data Analysis

For laboratory works, one way analysis of variance was carried out by using the general linear model procedure and treatment means were separated by using least significant difference (LSD) test at 5% significance level using SAS software package (SAS, 2010). Comparison between means of data for inhibition zones were done to determine significance differences among different plant extracts and microbes. During analysis Turkey's Honestly Significantly Different

at $p < 0.05$ after a one way analysis of variance (ANOVA) was used to determine zone of inhibition against crude extract.

4. RESULTS AND DISCUSSION

4.1. Survey of Botanical Pesticides Traditionally Used By Farmers

The survey work was started on January 10/2012 E.C to have information about crop diseases and treatment methods in selected six woredas of Sidama Region, southern Ethiopian. This survey information helped the researcher to select effective botanicals of the study area. For each botanical from each woreda people put their method of treatment of microbial diseases of crops. Some people in selected woredas are model farmers and experienced local healers of crop diseases. Even they did not know what the causative agent of the crop disease was, but their trials were successful in their field.

Field observation revealed that ethnobotanical knowledge on botanical pesticides is freely shared among the respondents. Also respondents were enthusiastic in utilizing them in their farms. These respondents transcend the varying socioeconomic classifications used in this study.

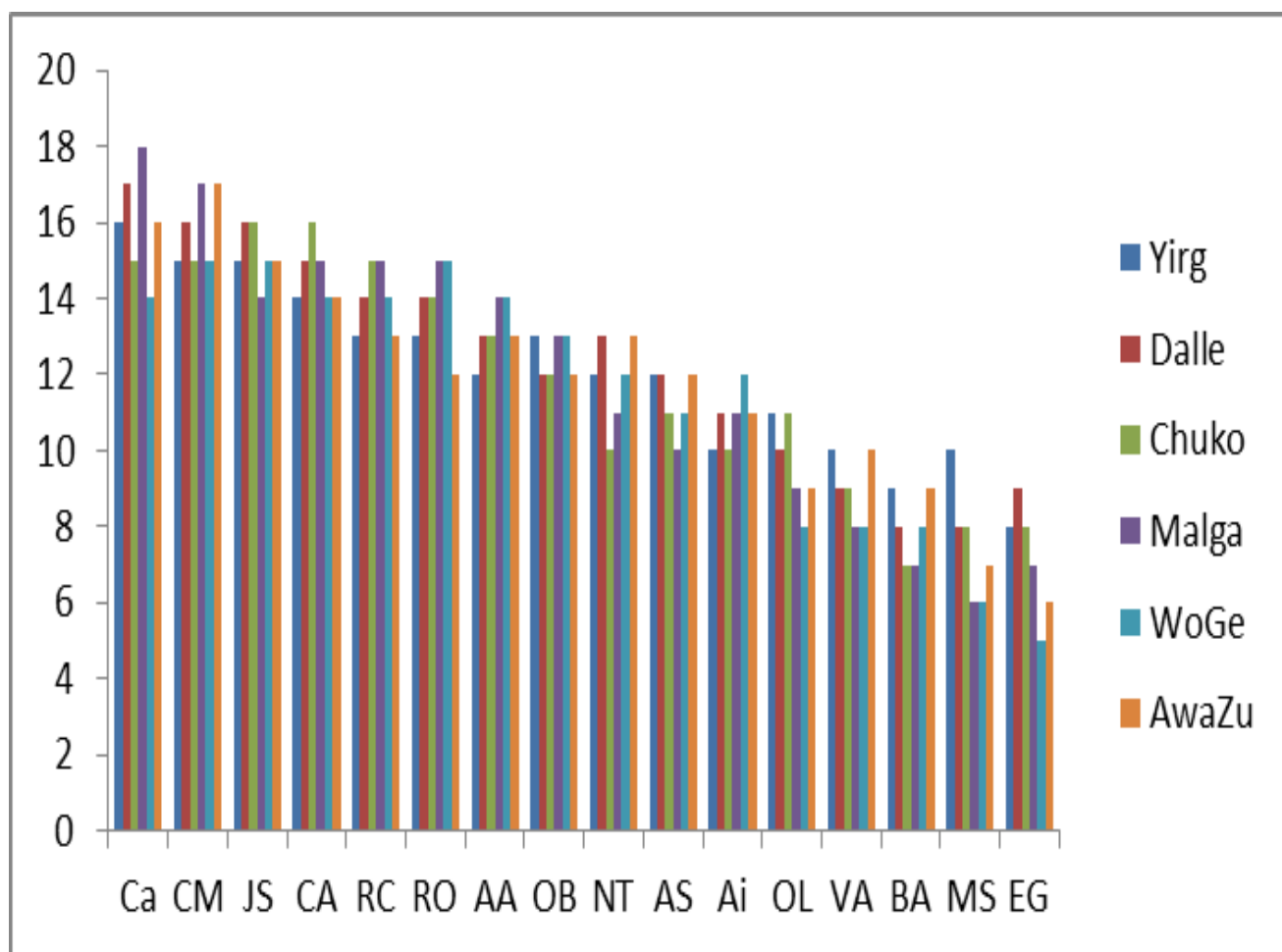


Figure:1. Graphical representation of survey on botanicals in Sidama Area.

The abbreviations stands for *Cordia Africana*, *Croton macrostachyus*, *Justicia shimperiana*, *Calpurnia aurea*, *Ruta chalepensis*, *Rosmarinus officinalis*, *Artemisia afra*, *Ocimum basilicum*, *Nicotiana tabacum*, *Alium sativum*, *Azadirachta indica*, *Ocimum lamifolium*, *Vernonia amygdalina*, *Brucea antidysenterica*, *Moringa stenopetala* and *Eucalyptus globules* respectively.

4.2. The use of botanicals in the study area

A total of 120 respondents were selected based on the level indigenous knowledge and techniques for crop pest managements. All respondents' mentioned that their household or agricultural products were dominantly affected by one or more factors connected with climate, pest and other problems related to human activities. More than 65.5% of respondents' household and agricultural products were affected by both climate and pest whereas for 34.5% of

respondents pest alone were considered as major factor for the damage or loss of crops. In the same way, the studies made in Eastern Ethiopia showed that pest problems were mentioned by tremendous number of the farmers as a major constraint upon their production that followed by drought and others (WFP, 2009). A report by Tadele (2004), showed that elements of climate, the rainfalls amount and temporal distribution is the most determinant for fluctuations in crop production of Ethiopia.

To minimize and prevent the damage by the crop pest, all respondents reported as they try all their best to control the pest by different methods or techniques. However, few respondents did not know any means used to control some crop pest. In general, the respondents use either traditional, modern or both methods for their crop pest managements. The majority (75.2%) of respondents prefer to use both traditional and modern methods simultaneously. However, the respondents who prefer traditional and modern methods alone were 10.3% and 14.5%, respectively. The variation in the preference of the methods might be associated to level of income and, level of education, social background of respondents and the type of crop pest needed to be controlled. For more than 72% of respondents the reasons of preference for cultural methods were associated to its low cost (Table 2).

Field observation revealed that most of the farmers were resource-poor and operated at subsistence level. They lacked the resources to purchase chemical pesticides. Even when such resources were sourced from diverse means, the chemicals are only available in towns hence extra cost is incurred on transportation to purchase them. Also, the existence of adulterated chemicals has been noticed in the study area. Quite often, difficulties abound in the identification of genuine chemicals. Similarly, proliferation of chemicals abounds with directives on utilization written in diverse foreign languages. Thus considerable mistakes, such as the use of over-dose or

under-dose, were being made by farmers. All these made the use of botanical pesticides the farmers' preference.

The other reasons for preference of traditional method mentioned were due to its easily applicability and accessibility, cultural norm or custom, its low negative impact or environmental friendly and adopted unknowingly from ancestors (Table 2).

As a traditional botanical based pest controlling method, they use the leaves, stems, fruits, seeds, roots or the combination of plant parts to kill or repel the pests. The dominantly used plant species as organic pesticides or repellents were 'Neem tree' (*Azadirachta indica*), 'Abalo' (*Brucea antidysentrica*), 'Digita' (*Calpurnia auria*), 'Bisana' (*Croton macrostachyus*), 'Nech Bahirzaf' (*Eucalyptus globulus Labill.*), garlic (*Allium sativum*), 'Shiferaw' (*Moringa stenopetala*), 'Tinbaho' (*Nicotiana tabacum*), 'Damakesie' (*Ocimum Lamiifolium*), 'Girawa' (*Vernonia amygdalina*), etc. (Table.3). The names in the quotation mark indicates local name whereas the names in the bracket shows scientific or botanical name.

Table 2. Respondents' response on reasons of preference for pest controlling methods

Item	Alternatives	Percentage
Reason of preference for traditional method	Easy use and cheap	72.3
	Easily applicability and accessibility	10
	Cultural norm or custom	7.6
	Low negative impact	5
	Adopted unknowingly from ancestor	5.1
Total		100

4.3. The botanical formulations and efficacy of their control among farmers in Sidama region

In bid to mitigate the damaging effect of pests on crops, farmers used variety of means, ranging from planting some indigenous plants that harbor birds/insect predators which feed on the dominant crop pests, using specific plant part, whole plant or animal product ash. Plants used in the field included *Azadirachta indica* (Neem plant), *Brucea antidysentrica* (Abalo), *Calpurnia auria* (Digita), *Vernonia amygdalina Del.* (Girawa), *Croton macrostachyus Del.* (Bisana), *Cycas*. Neem leaves, *Moringa stenopetala* (Shiferaw), *Nicotiana tobaccum* (Tobacco) and *Ruta chalepensis* L. (Tenadam). These plants were grinded and mix with water for the preparation of botanical formulations to apply (spray) on the crop field to prevent and/or control crop pests. Table 3 presents inventory of different botanical pesticide products formulated by farmers.

The preparations of the extracts of the plants used require no technology. Water, obtained freely from the river, is often used for the extraction (Table 3). Thus, the use of the botanically-derived

pesticides was perceived to be cheap, easily prepared and readily available. Pyrethrum flower powder used as natural insecticide for years in traditional farming system has no toxicity to human being and animals and it possesses ecological benefits that have led to increasing worldwide production (Ban *et al.*, 2010). Anjarwalla *et al.* (2016) revealed that farmers in Ghana similarly expressed preference for the control of pests using botanical pesticides. Chemical pesticides were perceived to be costly, toxic and dangerous to the health. Estimates by WHO revealed that about 200 000 people are killed annually as a result of direct pesticide poisoning (CAPE, 2015).

The most prominent among the materials used in the preparation of some of the identified botanical formulations are *Azadirachta indica*, *Croton macrostachyus*, and tobacco leaves. In addition to the aforementioned materials, wood ash is also a common botanical product used among farmers. It was ascertained from our survey that 41.8% of the farmers were believed that indigenous control methods were as effective as synthetic pesticides. A very small proportion (7.2%) had their indigenous control method not effective. The remaining proportion did not provide the estimated rating although they were using plant botanicals formulations for the control of field pests mainly insect pests.

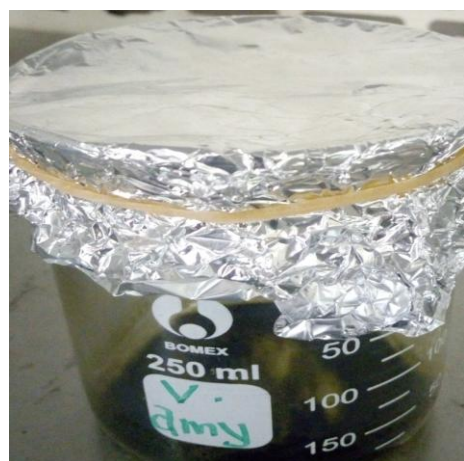
Table 3. Plant products used by farmers to prepare the botanical pesticide formulation

S/N	Scientific name of the plant	Mode of preparation/ formulation
1	<i>Croton macrostachyus Del.</i> , <i>Calpurnia auria</i> , <i>Moringa oleifera</i>	The leaves of these plants are grinded, soaked in water for four hours or more, after which the mixture is filtered and sprayed to control most crop diseases
2	<i>Azadirachta indica</i> , <i>Eucalyptus globulus</i> , and <i>Nicotiana tobaccum</i>	The leaves of Neem, Eucalyptus and tobacco are parboiled and soaked for some time. After this, the mixture is filtered and kept for between 2 and 4 days, before being used. The solution produces repugnant odor that repells insect pest.
3	<i>Azadirachta indica</i> , <i>Vernonia amygdalina Del.</i> and <i>Ruta chalepensis L.</i>	The leaves of these plant species are soaked in water and boiled for some time. Thereafter, the mixture is filtered and kept for 5 days. After the time, the filtrate is sprayed on the field.
4	Tobacco leaves/powder with clove and neem leaves	The leaves of neem leaves and tobacco together with clove are sundried, grinded separately soaked in water in an air tight container. After 3 days the mixture is filtered and sprayed to crops.
5	<i>Artemisia afra</i> <i>Croton macrostachyus Del.</i> , and <i>Calpurnia auria leaves</i>	The leaves of these plant species are macerated and soaked in water for some time. Thereafter, the filtrate is sprayed on the field to control crop diseases.
6	Wood ash	The ash is put in a cloth bag, tied to the end of plant stock and by the beating the bag, the ash sprinkled on the crops.
7	<i>Nicotiana tabacum</i>	Extracts from <i>Nicotiana tabacum</i> is mixed with wood ash and spread on the crop field to trap insect pest attacking crops.

4.4. Extraction yield

Ethanollic extraction method was employed to isolate the bioactive components from botanical plants (Fig. 2). The extraction yield is a measure of the solvent efficiency to extract specific components from the original material. percentage yield of crude extract was shown in table 4.

Vernonia amygdalina extract



All crude extracts



Figure:2. Ethanollic extracts of selected botanicals

In this study, the highest yield of crude extract 2.77g (11.08%) was obtained from leave of *V. amygdalina* which followed by *C. macrostachyus* 2.06g (8.84%). However, the lowest yield 1.7g (6.8%) was obtained from *E. globulus* (Table: 4). This result showed similar yield that reported by Amare *et al.* (2013) from *Ocimum lamiifolium* leaf (9.4%) crude extract. Regarding *Caplurnia aurea* leaf the extraction yield (7.6%) was obtained and this result showed less yield that reported by Melese *et al.* (2019) on 80% ethanol (20.2%). Koffi *et al.* (2010) showed that ethanol extract were found to be more effective than aqueous extracts of the same plant in general, as a result of higher solubility of organic compounds in ethanol.

A comparable result (9.4%) was reported by Amare *et al.* (2013) with 80% ethanol extract of

Calpurnia aurea leaf. In the case of *Ocimum lamiifolium* leaf the yield (21.5%) extracted from 80% ethanol (Melese *et al.*, 2019).

In this study, one way ANOVA showed that significant variation 2.14 was observed between *V.amygdalina* and *C.macrostachyus* in terms of percentage yield of crude extracts. There is variation observed between *C.amygdalina* and *J.schimperiana* ($p=0.064$) and *C.aafricana* and *J.schimperiana* ($p=0.72$) at 0.05 confidence interval. Different yield of crude extracts were obtained from leave of selected botanicals.

Table 4. Percentage yield of crude extract (Mean Sample Weight=25g)

Plants	Weight of extract(g)	Yield (%)
<i>Alium sativum</i>	1.86	7.44
<i>Artemisia afra</i>	1.92	7.68
<i>Azadirachta indica</i>	1.75	7
<i>Brucea antidysenterica</i>	1.99	7.96
<i>Calpurnia aurea</i>	1.90	7.6
<i>Cordia Africana</i>	2.04	9.6
<i>Croton macrostachyus</i>	2.06	8.24
<i>Eucalyptus globules</i>	1.7	6.8
<i>Justicia shimperiana</i>	2.01	8.04
<i>Moringa stenopetala</i>	1.8	7.2
<i>Nicotiana tabacum</i>	1.95	7.8
<i>Ocimum basilicum</i>	1.89	7.56
<i>Ocimum lamifolium</i>	1.89	7.56
<i>Rosmarinus officinalis</i>	1.8	7.2
<i>Ruta chalepensis</i>	1.8	7.2
<i>Vernonia amygdalina</i>	2.77	11.08

4.4. Phytochemical screening

In the present study the qualitative analysis of the phytochemical constituents test in the ethanolic extract were carried by selected botanicals from the study area. Phytochemical constituents are responsible for antimicrobial activity of plant species.

Phytochemical analysis indicated the presence of alkaloids, saponins, polyphenols, terpenoids and flavonoids in most of crude extract of selected botanicals (Table 5). Among the tested phytochemicals, glycoside and steroids were absent in the ethanolic crude extracts of *Eucalyptus globules*, *Cordia africana* and *Ocimum basilicum*.

The medicinal potential of most plants majorly depends on the presence of active phytochemical compounds called secondary metabolites (Das *et al.*, 2010). The phytochemical screening test of the 80% ethanol extract of most selected botanicals revealed the presence of the following secondary metabolites: alkaloid, tannins, saponin, flavonoids, polyphenols, and terpenoids. Even though, the mechanism of the antimicrobial activity of the crude extract is not known, there is possible speculation which is based on the secondary metabolites present in the extracts.

The antimicrobial activity of most extracts of selected botanicals could be contributed by alkaloids they possess. Alkaloids are reported to have antimicrobial activity against different pathogenic microorganisms (Singh *et al.*, 2011). Possible mechanism of action of extracts having alkaloids could be disruption of cell membranes or inhibition of the protein synthesis of bacteria (Sathyabama & Kingsley, 2013). Tannins are the other compounds that have been found in extracts of the most selected botanicals and reported in different studies to have antimicrobial activities (Wink, 2005). The antimicrobial mechanism of tannins might be because of its membrane damaging effects and inhibition of metabolic pathways of bacteria like oxidative

phosphorylation which could lead to death of the microorganism (Funatogawa *et al.*, 2004; Scalbert, 1991). The phenolic compounds are among secondary metabolites found in the plant extracts and reported to have antimicrobial activity (Thippeswamy *et al.*, 2013). The mechanisms thought to be responsible for plant extracts with phenolic compounds could be; phenolic toxicity to microorganisms including enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Mason & Wasserman, 1987). Saponins are among secondary metabolites found in the crude extract of some selected botanicals extract. There are reports on antimicrobial activity of saponins (Soetan *et al.*, 2006; Mattana *et al.*, 2010). Saponins could contribute for antimicrobial activity of current plant extracts. The antimicrobial mechanism of saponins is not well understood. Flavonoids are hydroxylated aromatic substances which are known to be synthesized by plants in response to microbial infection (Dixon *et al.*, 1983). So, it not surprising that they have been found in-vitro to be effective antimicrobial substances against a wide array of microorganisms (Borris, 1996).

Terpenoids are other phytochemicals found in the selected botanical extracts which have antimicrobial activity. Terpenoids were reported to have antimicrobial activity against bacteria (Habtemariam *et al.*, 1993), fungus (Ayafor *et al.*, 1994), virus (Hasegawa *et al.*, 1994) and protozoa (Ghoshal *et al.*, 1996). The mechanism of action of terpenoids is not fully understood but, it is speculated to involve membrane disruption by the lipophilic compounds (Brehm1Stecher and Johnson, 2003). Generally, there are different speculations in antimicrobial mechanism of medicinal plant extracts, based on the phytochemical they contain. Even though the mechanism are not well understood, the extracts of the study plants have antimicrobial property with different degrees of activity against tested bacterial and fungal species; which

confirm the potential of the extracts of leaves of selected botanicals for isolation of effective compounds in the treatment of infections caused by the tested bacteria and fungi. This effect of extracts of the leaf of botanicals might be attributed to either the individual class of compounds present in the crude or to the synergistic effect that each class of compound exert to give the observed antimicrobial activity findings.

The crude extract of *Justicia schimperiana* was positive for qualitative phytochemical screening test of alkaloid, tannins, saponin, flavonoids, phenols and terpenoids. There are reports on antibacterial activity of alkaloids against gram positive and negative bacteria (Singh & Verma, 2011). The remaining secondary metabolites (saponin, tannins, flavonoids, polyphenols and terpenoids) found in the crude extract of *Justicia schimperiana* also reported to have antibacterial activity against pathogenic bacteria species (Jasmine *et al.*, 2007; Wink, 2015). So, the antibacterial activity of the crude extract of the leaf of *Justicia schimperiana* might be because of synergistic or additive effect of these secondary metabolites (Akharaiyi, 2011).

Table: 5. Phytochemical analysis for ethanolic leaf extracts.

N ^o	Selected botanicals		Phytochemicals				
	(Test plants)	Tannin	Glycoside	Phenolics	Flavanoids	Saponins	Steroids
1	<i>Allium sativum</i>	+	+	+	+	+	+
2	<i>Artemisia afra</i>	+	+	—	+	—	+
3	<i>Azadirachta indica</i>	+	+	+	+	+	+
4	<i>B. antidysenterica</i>	+	+	+	+	+	+
5	<i>Calpurnia aurea</i>	+	+	+	+	+	—
6	<i>Cordia africana</i>	+	—	+	+	+	—
7	<i>C. macrostachyus</i>	+	—	+	+	+	+
8	<i>Eucalyptus globules</i>	+	—	+	+	+	—
9	<i>Justicia shimperiana</i>	+	+	+	+	+	+
10	<i>Moringa stenopetala</i>	+	+	+	+	+	+
11	<i>Nicotiana tabacum</i>	+	+	+	+	+	—
12	<i>Ocimum basilicum</i>	+	+	+	+	+	+
13	<i>O.lamiifolium</i>	+	+	+	+	+	+
14	<i>Ruta chalepensis</i>	+	+	+	+	+	+
15	<i>Rosmarin officinalis</i>	+	+	+	+	+	+
16	<i>V.amygdalina</i>	—	+	+	+	+	+

Key: + = present; - = absent



Figure:3. Color change results in phytochemical tests

4.5. Antifungal and Antibacterial Activities of Extracts

Antifungal & antibacterial activities of the sixteen selected plants extracts were evaluated against some plant pathogenic fungi (*Fusarium vertecelliodes*, *Aspergillus flavus* and *Penicillium verrucosum*) and bacteria (*Xanthomonas campestris* (*X.c.pv.m*), *Ralstonia solanacearum*, and *Pseudomonas syringea*). The antimicrobial activities were confirmed by the appearance of clear zone around the plant extracts that inhibit the growth of bacteria/ fungi on the media.

4.5.1. Antibacterial Activity

The antibacterial activities of each of the crude ethanol extracts of the plants were determined using filter paper disc and agar well diffusion methods of Stoke and Ridgway (1980). All leave extracts possessed potential antibacterial activity against *Xanthomonas campestris* *pv.m*, *Ralstonia.solanacearum* and *Pseudomonus syringea* (Table 6). The antibacterial efficacy of the ethanol extracts of the plant against selected bacteria showed varied levels of inhibition. The extracts showed good activity against the test bacteria ranging from 17.18–6.17 mm. The activities of the sixteen extracts of the plant were compared with standard drug tetracycline.

Among the ethanolic leaf extracts of sixteen botanicals, *C.africana* exhibited the highest inhibition zone (17.18 ± 0.03 mm) against *X.campestris* by well diffusion method whereas; *C.macrostachyus* was the second with inhibition zone of 16.85 ± 0.05 mm followed by *J.schimperiana* with inhibition zone (16.06 ± 0.05 mm) and the lowest botanical was *E.globulus* with its inhibition zone (7.00 ± 0.01 mm) by well diffusion method (table,6).

The study showed that ethanol extract of *Calpurnia aurea*, *Croton macrostachyus* and *Jastica shimperiana*, exhibited 15.82, 15.43 and 15.06 mm zone of inhibition respectively, against *R.solanacearum* by well diffusion method. Whereas in the case of well method the values were decreased to 14.87, 12.49 and 12.43 mm by ethanol extract of *Calpurnia aurea*, *Croton macrostachyus* and *Jastica shimperiana* used respectively against *R.solanacearum*. The above figures are by standard (100g /ml).

Pseudomonas syringae was observed to be susceptible to all of the plant extracts, used, the zones of inhibitions were 16.34, 15.97 and 15.55 mm by well diffusion method, and 15.24, 13.3 and 12.97 mm by filter paper disc method of ethanolic extract of *Calpurnia aurea*, *Croton macrostachyus* and *Jastica shimperiana*, respectively. Generally, the selected botanicals showed their effectiveness on fungi than in bacterial species.

The observations were supported by earlier reports (Kiran *et al.*, 2017) that the water extract of two botanicals viz., *Ocimum gratissimum* and *Tylophora asthmatica* were effective in inhibiting the growth of *Ralstonia solanacearum*. *Ocimum gratissimum* extract showed highest inhibition zone of 28.66 mm. In addition, Getahun and Masresha (2016) found that extracts of *Agarista salicifolia* and *Pycnostachys abyssinica* with high antibacterial activity in vitro against *X. campestris* pv. *musacearum* and it could be used for biopesticides control of Enset bacterial wilt.

This study demonstrated a comparable result with the findings obtained by Shemsu *et al.* (2013), which recorded inhibition zone of the methanol extract of *Calpurnia aurea* leaf to be 14 mm against *E. coli*, followed by 11mm against *S. aurea*, 9 mm against *S. typhimurium* and 9 mm against *P. aeruginosa*. In agreement to the current study, Binyam *et al.* (2014) reported high antibacterial activity (13.3 mm) against *E. coli* and (8.6 mm) against *S. typhimurium* with methanol extract of leaf of *Calpurnia aurea*.

Ocimum lamiifolium leaf showed antibacterial activity against all tested bacteria; on *E. coli* (14.7±2.39 mm) followed by (13.2±1.49 mm) against *S. aureus* (11.84±0.74 mm) against *S. typhimurium* and (10.63±0.54 mm) against *P.aeruginosa*. In contrast, methanol extract of *Ocimum lamiifolium* didn't show any zone of inhibition against *P.aeruginosa* (Teklit Gebregiorgis Amabye and Said Mussa, 2015) but in this study *Pseudomonas spp* had zone of inhibition of 9.94±0.03 mm. This variation might be due to extraction solvent. Similarly, ethanol crude extract of *Ocimum lamiifolium* showed antibacterial activity against *E. coli* (13.5 mm) and against *S. aureus* (12 mm) and also water crude extract of *Ocimum lamiifolium* against *E. coli* (15.5 mm), *S. aureus* (13.5 mm) and *P. aeruginosa* (13 mm) (Amare *et al.*, 2013).

During this study, it was also observed that well diffusion method was the best for evaluating antibacterial activities of the selected botanicals during this study. On the other hand, standard antibiotic tetracycline (30µg/disc) showed more significant antibacterial activity against all tested bacteria showing the larger zone of inhibition in every case. Most plates showed the zone of inhibition by the plant extracts against the plant pathogenic bacteria, while pure culture of the pathogens is given in agar plates.

Table: 6. Size of inhibition zone (mm) by ethanolic extract of selected botanical against the sample plant pathogenic bacteria (well and disc method)

	<i>Xanthomonas campestris</i>		<i>R.solanacearum</i>		<i>Pseudomonas syringe</i>	
SB	Well(mm)	Disc(mm)	Well(mm)	Disc(mm)	Well(mm)	Disc(mm)
Ca	17.18±0.03 ^a	14.85±0.05 ^a	15.82±0.05 ^a	12.49±0.05 ^a	16.34±0.08 ^a	13.34±0.28 ^a
Cm	16.85±0.05 ^a	14.18±0.97 ^{ba}	15.43±0.05 ^b	12.43±0.33 ^b	15.97±0.05 ^{ba}	12.97±0.03 ^b
Js	16.06±0.03 ^b	14.06±0.01 ^{bc}	15.06±0.03 ^c	12.06±0.32 ^{cb}	15.55±0.02 ^{bac}	12.35±0.05 ^{cbd}
CA	15.49±0.01 ^c	13.01±0.00 ^{bc}	14.47±0.02 ^d	11.80±0.65 ^{cbd}	15.14±0.00 ^b	12.22±0.01 ^{cb}
Rc	15.01±0.00 ^d	12.49±0.34 ^{ecd}	14.02±0.01 ^e	11.02±0.00 ^{ced}	15.01±0.06 ^c	12.14±0.01 ^{cbd}
Ro	14.51±0.00 ^e	11.84±0.00 ^{efd}	13.50±0.01 ^f	10.50±0.01 ^{fed}	14.01±0.00 ^d	11.01±0.00 ^{ced}
Aa	14.01±0.00 ^f	11.01±0.34 ^{efd}	13.02±0.01 ^g	10.35±0.01 ^{feg}	13.50±0.01 ^{ed}	10.50±0.58 ^{fed}
Ob	13.82±0.03 ^f	10.82±0.35 ^{efg}	12.44±0.01 ^h	9.44±0.37 ^{thg}	13.02±0.01 ^e	9.39±0.01 ^{thg}
Nt	13.02±0.01 ^g	10.35±0.02 ^{htg}	12.00±0.01 ⁱ	9.00±0.05 ^{ihg}	12.06±0.04 ^f	9.02±0.30 ^{teg}
As	12.39±0.00 ^h	9.39±0.35 ^{hi}	11.09±0.03 ^j	8.42±0.30 ^{ihj}	11.56±0.05 ^{gf}	8.56±0.36 ^{hg}
Ai	11.06±0.02 ⁱ	8.60±0.33 ^{hig}	10.32±0.04 ^k	7.99±0.32 ^{ij}	11.05±0.03 ^g	8.38±0.33 ^{hg}
Ol	10.27±0.03 ^j	8.39±0.01 ^{ji}	9.86±0.02 ^l	7.19±0.90 ^{kj}	9.94±0.03 ^h	7.61±0.01 ^{ihg}
Va	9.44±0.01 ^k	7.44±0.02 ^j	9.00±0.01 ^m	6.00±0.34 ^k	9.12±0.03 ^{ih}	7.46±0.36 ^{ih}
Ba	8.92±0.02 ^l	5.92±0.1 ^j	8.28±0.02 ⁿ	5.94±0.04 ^k	8.50±0.01 ^{ij}	6.17±0.03 ^{ij}
Ms	8.41±0.01 ^m	6.41±0.56 ^j	7.85±0.03 ^o	5.85±0.04 ^k	7.89±0.04 ^j	5.89±0.36 ^{ij}
Eg	7.00±0.01 ⁿ	6.00±0.03 ^{jk}	6.17±0.02 ^p	3.50±0.00 ^l	6.45±0.04 ^k	4.45±0.01 ^j
Cont	0.00±0.00 ^o	0.00±0.00 ^k	0.00±0.00 ^q	0.00±0.00 ^m	0.00±0.00 ^l	0.00±0.00 ^k

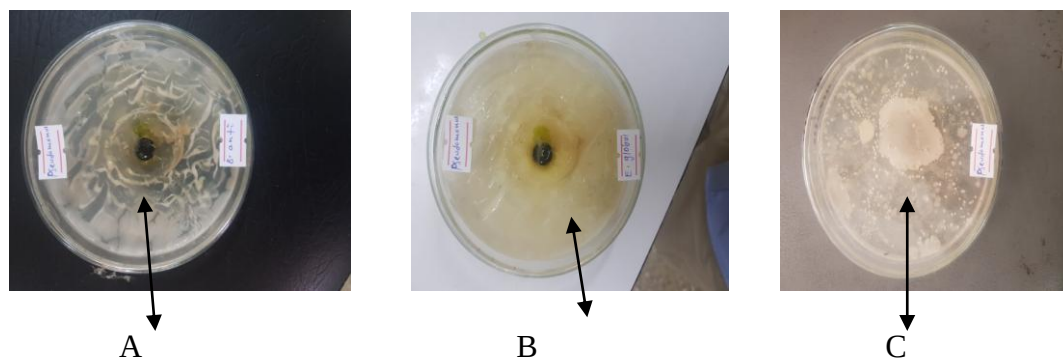


Figure: 4. Zone of inhibition by botanicals against plant pathogenic bacteria; (a) *C.africana* against *Xanthomonas campestris* (b) *J.schiperiana* against *Ralstonia solanacearum*, and (c) *C.macrostachyus* against *Pseudomonas syringae* (well method)

4.5.2. Antifungal Activity Test

In this study, *C.africana* showed the highest IZ (18.30 ± 0.01 mm) against *F.verteceloides* however *E.globulus* exhibited the lowest IZ (8.40 ± 0.01 mm) against *P. verrucosum*. Significant antifungal activity of *Calpurnia aurea* was observed against all plant pathogenic fungi. Among the phytopathogenic fungi, *F.verteceloides* (18.3 and 15.3 mm) was highly susceptible followed by *A.flavus* (18.27 and 15.34 mm) and *P.verrucosum* (17.04 and 15.7 mm) both in well and disc diffusion methods, respectively. The observed results were highly significant at 5 and 1% levels of significance.

Ethanol Extracts of *Croton macrostachyus* also, prevented the growth of all tested plant pathogenic fungi. *Croton macrostachyus* showed the best *in vitro* antifungal activities (18.03 mm) against *F.verteceloides* which was followed by inhibition zone of 17.57 and 16.45 mm in diameter against *A.flavus* and *P.verrucosum*, respectively by well method. The antifungal inhibition zone of 17.13, 16.83 and 15.94mm were recorded against *F.verteceloides*, *A.flavus* and *P.verrucosum*, respectively when *Jastica shimperiana* applied by well methods. The minimum inhibition zone (7.16 mm) by *E.globulus* extract was obtained against *A.flavus* by paper disc diffusion method.

Mahlo *et al.* (2016) reported that three plant pathogenic fungi (*Penicillium janthinellum*, *Trichoderma harzianum* and *Fusarium oxysporum*) had significant sensitivity to the plant extracts (average MIC values of 0.28 mg/ml). *Fusarium oxysporum* was reasonably sensitive.

Previous study indicated that botanicals such as; *Bucida buceras*, *Breonadia salicina*, and *Xylothea kraussiana* have been reported to have strong antifungal activity against *Aspergillus fumigatus* (Mahlo *et al.*, 2013). *Aspergillus niger* was reported to be resistant to Dichloromethane, aqueous and methanolic extracts of 14 plants used traditionally in Paraguay (Portillo *et al.*, 2001).

The report by Rajendra and Ashok (2011) revealed that, *Azadirachta indica* and *Polyalthia longifolia* showed its antifungal activity against *Macrophomina phaseolina*, *Rhizopus stolonifer* and *Penicillium digitatum*. They also reported that, aqueous extract of *Eucalyptus angophoroides* found to be fungitoxic for the growth of *Alternaria dianthicola*, *Curvularia pellescens*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Rhizopus stolonifer*, *Penicillium digitatum* and *Penicillium chrysogenum*. Moreover, Sabramania *et al.* (1994), laboratory trials conducted with six plant species against *Callosobruchus chinensis* infesting red gram (pigeon peas) showed that extracts of *Eucalyptus globulus* were the most promising.

In fungal case, this study showed more effectiveness than the bacterial case. For instance, *Fusarium vertecellioides* become more susceptible to *Cordia Africana* by inhibition zone 18.30 ± 0.01 and the lowest inhibition zones 9.43 ± 0.04 was recorded using *E.globulus*.

Table: 7. Size of inhibition zone (mm) by ethanolic extract of selected botanical against the sample plant pathogenic fungi (well and disc method)

	<i>F.vertecelioides</i>		<i>A.flavus</i>		<i>P.verrucosum</i>	
SB	Well(mm)	Disc(mm)	Well(mm)	Disc(mm)	Well(mm)	Disc(mm)
Ca	18.30±0.01 ^a	15.30±0.13 ^a	18.27±0.02 ^a	15.27±0.36 ^a	17.04±0.04 ^a	13.46±0.09 ^a
Cm	18.03±0.06 ^a	15.21±0.06 ^{ba}	17.57±0.04 ^b	14.23±0.04 ^b	16.45±0.06 ^b	13.45±0.06 ^b
Js	17.13±0.04 ^b	15.03±0.04 ^{bc}	16.83±0.04 ^c	13.83±0.06 ^c	15.94±0.03 ^c	12.94±0.03 ^{cb}
CA	16.48±0.02 ^c	14.13±0.02 ^{dc}	16.22±0.06 ^{dc}	13.50±0.01 ^c	15.45±0.01 ^d	12.45±0.01 ^{cb}
Rc	16.02±0.01 ^d	13.48±0.01 ^{de}	15.93±0.01 ^{de}	13.22±1.00 ^d	14.98±0.01 ^e	13.64±0.88 ^{cd}
Ro	15.49±0.00 ^e	13.02±0.00 ^{fe}	15.50±0.01 ^{fe}	12.93±0.33 ^e	14.50±0.01 ^f	11.83±0.32 ^{de}
Aa	14.99±0.01 ^f	12.49±0.01 ^{fg}	14.96±0.00 ^{fg}	12.30±0.33 ^e	14.00±0.00 ^g	10.34±0.66 ^{de}
Ob	14.48±0.01 ^f	11.10±0.01 ^{hg}	14.46±0.02 ^g	12.00±0.56 ^f	13.47±0.04 ^h	10.47±0.04 ^{ef}
Nt	14.17±0.07 ^g	11.48±0.07 ^{hg}	13.66±0.02 ^h	11.79±0.29 ^g	13.04±0.01 ⁱ	10.04±0.01 ^{ef}
As	13.82±0.02 ^h	11.17±0.02 ^{hi}	13.43±0.04 ^h	10.76±0.05 ^g	12.50±0.02 ^j	9.50±0.02 ^{ef}
Ai	12.96±0.02 ⁱ	10.82±0.02 ^{ji}	12.56±0.05 ⁱ	10.56±0.35 ^h	11.49±0.01 ^k	9.49±0.01 ^{fg}
Ol	12.29±0.03 ^j	9.96±0.03 ^j	11.76±0.02 ^j	9.09±0.50 ^h	10.79±0.02 ^l	8.51±0.02 ^{fg}
Va	12.08±0.01 ^k	9.31±0.12 ^{jk}	11.31±0.02 ^j	8.37±0.02 ⁱ	10.51±0.01 ^m	7.79±0.12 ^{ig}
Ba	11.29±0.03 ^k	9.29±0.03 ^k	10.37±0.02 ^k	7.98±0.33 ^j	10.05±0.02 ⁿ	7.39±0.35 ^{hi}
Ms	10.76±0.02 ^l	8.16±0.58 ^{kl}	9.83±0.05 ^k	7.16±0.28 ^k	9.18±0.01 ^o	7.18±0.01 ^{hi}
Eg	9.43±0.04 ^m	7.09±0.29 ^l	8.78±0.05 ^l	6.45±0.02 ^k	8.40±0.01 ^p	6.40±0.01 ⁱ
Con	0.00±0.00 ^o	0.00±0.00 ^m	0.00±0.00 ^m	0.00±0.00 ^{kl}	0.00±0.00 ^q	0.00±0.00 ^j

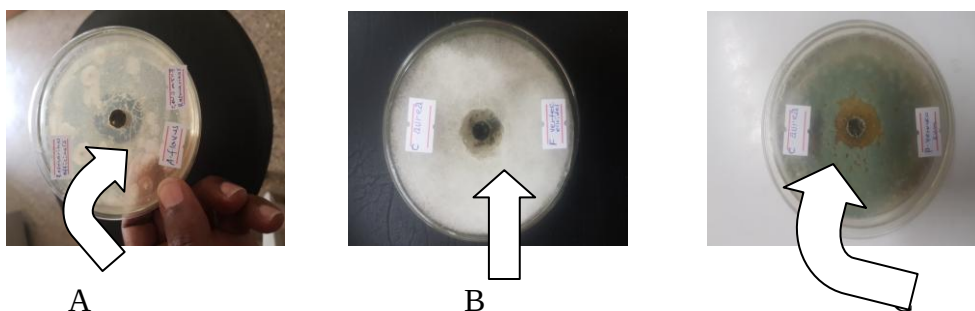


Figure 5 Zone of inhibition by botanicals against plant pathogenic fungi; (a) *J.schimperiana* against *Aspergillus flavus* (b) *C.africana* against *Fusarium vertecellioides*, and (c) *C.machrostachyus* against *Penicillium verrucosum* (well method)

5. Conclusion and Recommendation

5.1. Conclusion

In the present work information on traditional botanical pesticides used for the control of crop pests were gathered in Sidama region from six randomly selected representative districts. In most cases, a specific part of the plant will be grinded and macerated with water for easy of application. It was ascertained that 41.8 % of the farmers believed that indigenous crop pest control methods are as effective as synthetic pesticides. The dried leaf parts of botanicals were extracted with ethanol. The highest crude extract was obtained from leave of *Vernonia amygdalina* (11.2%) followed by *Croton macrostachyus* (8.2%). However, the lowest yield (6.8%) was obtained from *Eucalyptus globulus*. The present investigation revealed that the ethanolic extracts the botanical pesticides exhibited inhibitory effects on the growth of *Aspergillus niger*, *Fusarium vertecelloides*, and *Penicillium verrucosum* and bacteria (*Xanthomonas campestris* (X.c.pv.m), *Ralstonia solanacearum*, and *Pseudomonas syringea*). Ethanolic extract of *Cordia africana* (Wanza) showed the maximum inhibition (18.30 mm) against *Fusarium vertecellioides* while the minimum botanical activity on bacteria observed by *Eucalyptus globulus* is(9.43mm) against *Ralstonia solanacearum* as compared with other botanicals included in the current work. Our result showed that most selected botanicals contain phenols, tannins, glycosides, flavonoids, saponins and steroids. The critical challenge in using botanicals for crop pest control in the study area was estimation of the proportion of the ingredients used in plant based formulations.

5.2 Recommendation

Based on this study the following recommendations were made:

1. Awareness creation activities should be done with stakeholders (farmers, Developmental agents, NGO, etc.) on the advantage of using botanicals.
2. Non target effects of the botanicals at different rates and frequencies should be studied.
3. Other parts of the effective botanicals should be tested for efficacy.
4. Standardized techniques of preparation, bio-safety and environmental guideline are required for effective utilization of potential botanicals in crop pest control method
5. Further phytochemical and biological activity studies should also be conducted on this plant to isolate the specific antimicrobial activity in it.

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7. Appendix

7.1 Appendix 1

ANOVA: 1. Xanthomonas campestris

SV	DF	SS	MS	F-V	P-V	CV
Treatment	16	547.9	34.24	1168.55	<.0001	1.31
Error	34	0.99	0.029			
Total	50	548.88				

ANOVA: 2. Pseudomonas syringea

SV	DF	SS	MS	F-V	P-V	CV
Trt	16	531.5	33.21	258.25	<.0001	2.86
Error	34	4.37	0.12			
Total	50	535.87				

ANOVA: 3. Ralstonia solanacearum

SV	DF	SS	MS	F-V	P-V	CV
Trt	16	497.51	31.09	10558.1	<.0001	0.44
Error	34	0.1	0.002			
Total	50	497.61				

ANOVA: 4. Aspergillus flavus

SV	DF	SS	MS	F-V	P-V	CV
Trt	16	426.62	26.66	340.99	<.0001	1.98
Error	34	2.65	0.078			
Total	50	429.28				

ANOVA: 5. Fusarium verteceliodes

SV	DF	SS	MS	F-V	P-V	CV
Trt	16	356.12	22.25	1250.98	<.0001	0.92
Error	34	0.60	0.017			
Total	50	356.72				

ANOVA: 6. Pennicilium verrucosum

SV	DF	SS	MS	F-V	P-V	CV
Trt	16	416.58	26.0367564	712.11	<.0001	1.43
Error	34	1.24	0.0365627			
Total	50	417.83				

7.2 Appendix-2

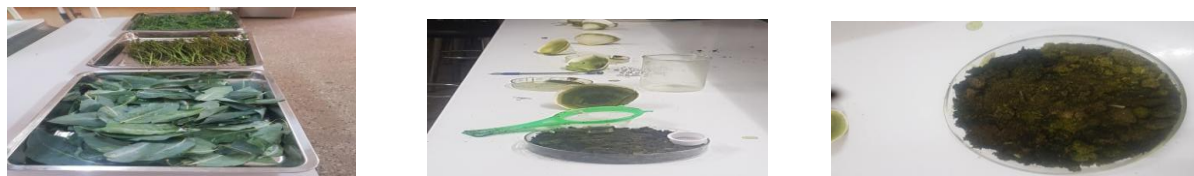


Figure 6 Plant materials and preparation process of extracts



Figure 7 Water bath to get crude extract & measuring extracts by sensitive balance



Figure 8 Xanthomonas colony and inhibition zones of botanicals against bacteria



Figure 9 Inhibition zones on plates made by fungus

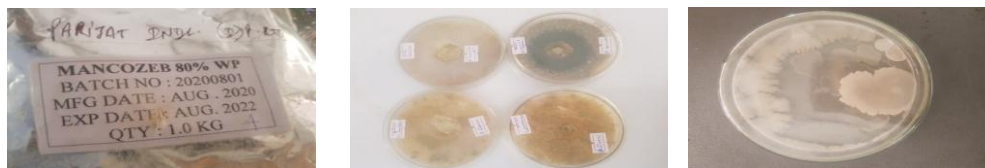
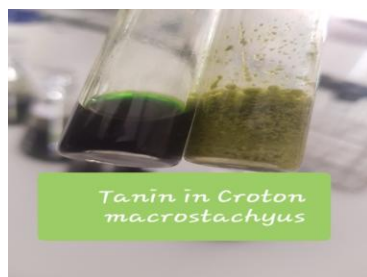


Figure 10 Mancozeb as +^{ve} control



A



B



C

Figure 11 Phytochemical test

As shown, “A” represents method of adding chemicals “B” represents presence of phytochemical tannin and “C” shows persistent froth after shake to test saponin.

Appendix **Table 8** Antimicrobial activities of botanicals by concentration 6.125%

Trt	<i>Xcpm</i>	<i>Ps.</i>	<i>Rs</i>	<i>Af</i>	<i>Fv</i>	<i>Pv</i>
Ca	0.47±0.00a	0.43±0.00a	0.40±0.00a	0.50±0.00a	0.50±0.00a	0.45±0.00a
Cm	0.40±0.00a	0.37±0.00b	0.34±0.00ab	0.48±0.00b	0.43±0.00b	0.42±0.00b
Js	0.34±0.00ab	0.36±0.00c	0.33±0.00b	0.43±0.00c	0.40±0.00c	0.39±0.00c
CA	0.33±0.00b	0.35±0.00d	0.33±0.00b	0.41±0.00c	0.40±0.00c	0.38±0.00c
Rc	0.33±0.00b	0.34±0.00e	0.33±0.00b	0.35±0.00d	0.36±0.00d	0.34±0.00d
Ro	0.31±0.00b	0.34±0.00f	0.32±0.00b	0.31±0.00e	0.32±0.00e	0.31±0.00e
Aa	0.30±0.00b	0.29±0.00g	0.30±0.00c	0.30±0.00e	0.31±0.00e	0.30±0.00e
Ob	0.29±0.00bc	0.28±0.00g	0.28±0.00d	0.29±0.00e	0.30±0.00e	0.29±0.00e
Nt	0.29±0.00c	0.26±0.00h	0.28±0.00d	0.29±0.00e	0.30±0.00e	0.29±0.00e
As	0.25±0.00c	0.26±0.00h	0.28±0.00d	0.27±0.00f	0.29±0.00f	0.28±0.00f
Ai	0.25±0.00c	0.25±0.00i	0.28±0.00d	0.24±0.00g	0.26±0.00g	0.26±0.00g
Ol	0.24±0.08d	0.23±0.00j	0.21±0.07e	0.24±0.00g	0.25±0.00h	0.26±0.00g
Va	0.16±0.00e	0.20±0.08k	0.18±0.00f	0.24±0.07g	0.16±0.00i	0.23±0.00h
Ba	0.14±0.00f	0.16±0.00kl	0.15±0.00g	0.15±0.00h	0.12±0.00j	0.14±0.00i
Ms	0.13±0.06f	0.13±0.06kl	0.12±0.00h	0.07±0.07i	0.06±0.00k	0.05±0.00j
Eg	0.05±0.05g	0.07±0.00m	0.06±0.00i	0.06±0.00j	0.00±0.08kl	0.00±0.00k
Cont	0.00±0.00h	0.00±0.00n	0.00±0.00j	0.00±0.00k	0.00±0.00l	0.00±0.00

Appendix **Table 9** Antimicrobial activities of botanicals by concentration 12.25%

Trt	<i>Xcpm</i>	<i>Ps.</i>	<i>Rs</i>	<i>Af</i>	<i>Fv</i>	<i>Pv</i>
Ca	2.10±0.00 ^a	1.82±0.00 ^a	1.78±0.00 ^a	2.29±0.01 ^a	2.19±0.00 ^a	1.89±0.00 ^a
Cm	1.84±0.00 ^{ba}	1.74±0.00 ^{ba}	1.73±0.00 ^a	2.21±0.00 ^{ba}	2.02±0.00 ^{ba}	1.73±0.00 ^{abc}
Js	1.66±0.00 ^{ba}	1.69±0.00 ^{ba}	1.55±0.00 ^a	1.94±0.00 ^{bac}	1.92±0.00 ^{ba}	1.62±0.00 ^{bdc}
CA	1.55±0.00 ^{ba}	1.48±0.00 ^{ba}	1.50±0.00 ^a	1.93±0.01 ^{bdac}	1.89±0.00 ^{ba}	1.53±0.00 ^{edc}
Rc	1.52±0.00 ^{ba}	1.42±0.00 ^{ba}	1.49±0.00 ^a	1.89±0.00 ^{bac}	1.56±0.00 ^{ba}	1.37±0.01 ^{ba}
Ro	1.44±0.00 ^{ba}	1.33±0.00 ^{ba}	1.39±0.00 ^a	1.56±0.00 ^{bdac}	1.52±0.00 ^{ba}	1.24±0.00 ^{edf}
Aa	1.43±0.00 ^{ba}	1.25±0.00 ^{ba}	1.31±0.00 ^a	1.55±0.00 ^{bdac}	1.41±0.00 ^{ba}	1.19±0.01 ^{egf}
Ob	1.32±0.00 ^{ba}	1.21±0.00 ^{ba}	1.25±0.00 ^a	1.40±0.00 ^{bdac}	1.27±0.00 ^{ba}	1.18±0.00 ^{egf}
Nt	1.32±0.00 ^{ba}	1.12±0.00 ^{ba}	1.21±0.00 ^{ba}	1.36±0.01 ^{bdc}	1.20±0.00 ^b	1.18±0.00 ^{hgf}
As	1.24±0.36 ^{ba}	1.11±0.27 ^{ba}	1.12±0.33 ^a	1.31±0.53 ^{bdac}	1.17±0.49 ^{ba}	0.99±0.26 ^{egf}
Ai	1.13±0.01 ^{bc}	1.11±0.01 ^{bac}	0.99±0.00 ^{ba}	1.29±0.00 ^{bdc}	1.16±0.00 ^{bc}	0.90±0.00 ^{hg}
Ol	1.13±0.33 ^{ba}	0.97±0.32 ^{ba}	0.99±0.32 ^a	1.19±0.33 ^{bdac}	1.13±0.00 ^{bc}	0.84±0.00 ^{hi}
Va	1.05±0.32 ^{ba}	0.96±0.32 ^{bac}	0.95±0.32 ^{ba}	1.12±0.33 ^{bdac}	1.06±0.33 ^{ba}	0.78±0.00 ^{hij}
Ba	1.01±0.32 ^{ba}	0.94±0.32 ^{bac}	0.95±0.32 ^{ba}	1.09±0.02 ^{dec}	1.00±0.32 ^{ba}	0.55±0.00 ^{ij}
Ms	0.93±0.32 ^{bc}	0.85±0.32 ^{bac}	0.93±0.32 ^{ba}	0.97±0.33 ^{dec}	0.99±0.33 ^b	0.54±0.00 ^{ij}
Eg	0.83±0.32 ^{bc}	0.75±0.32 ^{bc}	0.82±0.32 ^{ba}	0.81±0.32 ^{de}	0.98±0.32 ^{bc}	0.48±0.01 ^j
Cont	0.00±0.00 ^c	0.00±0.00 ^c	0.00±0.00 ^b	0.00±0.00 ^e	0.00±0.00 ^c	0.00±0.00 ^k

Appendix Table 10 Antimicrobial activities of botanicals by 25% concentration

Trt	<i>Xcpm</i>	<i>Ps.</i>	<i>Rs</i>	<i>Af</i>	<i>Fv</i>	<i>Pv</i>
Ca	4.42±0.00 ^a	4.01±0.00 ^a	3.56±0.00 ^a	5.34±0.00 ^a	4.57±0.00 ^a	4.02±0.00 ^a
Cm	4.01±0.00 ^b	3.56±0.00 ^b	3.31±0.00 ^b	4.52±0.00 ^b	4.29±0.00 ^b	3.55±0.00 ^b
Js	3.66±0.00 ^c	3.20±0.00 ^c	3.01±0.00 ^c	4.07±0.00 ^c	3.77±0.00 ^c	3.19±0.00 ^c
CA	3.40±0.00 ^d	3.01±0.00 ^d	2.80±0.00 ^d	3.98±0.00 ^{dc}	3.47±0.00 ^d	3.01±0.00 ^d
Rc	3.19±0.00 ^e	2.82±0.00 ^e	2.61±0.00 ^e	3.79±0.00 ^d	3.27±0.00 ^e	2.80±0.00 ^e
Ro	3.00±0.00 ^f	2.60±0.00 ^f	2.40±0.00 ^f	3.46±0.00 ^e	3.04±0.00 ^f	2.58±0.00 ^f
Aa	2.73±0.03 ^g	2.20±0.00 ^g	2.37±0.03 ^g	3.21±0.03 ^f	2.80±0.00 ^g	2.33±0.00 ^g
Ob	2.60±0.00 ^h	2.00±0.00 ^h	2.09±0.00 ^h	3.03±0.00 ^{gf}	2.58±0.00 ^h	2.11±0.00 ^h
Nt	2.37±0.00 ⁱ	1.92±0.00 ⁱ	2.00±0.00 ⁱ	2.87±0.00 ^h	2.40±0.00 ⁱ	1.93±0.00 ⁱ
As	2.19±0.00 ^j	1.80±0.00 ^j	1.93±0.00 ^j	2.60±0.00 ^{ih}	2.18±0.00 ^j	1.72±0.00 ^j
Ai	2.00±0.00 ^k	1.56±0.00 ^k	1.64±0.00 ^k	2.41±0.00 ^{ij}	2.00±0.00 ^k	1.55±0.00 ^k
Ol	1.79±0.00 ^l	1.39±0.00 ^l	1.54±0.00 ^l	2.19±0.00 ^{kj}	1.91±0.00 ^k	1.40±0.00 ^l
Va	1.60±0.00 ^m	1.20±0.00 ^m	1.31±0.00 ^m	2.00±0.00 ^{kj}	1.70±0.00 ^{lm}	1.19±0.00 ^m
Ba	1.39±0.00 ⁿ	1.00±0.00 ⁿ	1.08±0.00 ⁿ	1.80±0.00 ^k	1.50±0.00 ⁿ	1.01±0.00 ⁿ
Ms	1.21±0.00 ^o	0.91±0.00 ^o	1.00±0.00 ^o	1.50±0.00 ^l	1.31±0.00 ^o	0.89±0.00 ^o
Eg	1.03±0.00 ^p	0.72±0.00 ^p	0.85±0.00 ^p	1.34±0.00 ^l	1.11±0.00 ^p	0.86±0.00 ^o
Cont	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^m	0.00±0.00 ^q	0.00±0.00 ^p

Appendix Table 11 Antimicrobial activities of botanicals by concentration 50%

Trt	Xcm	Ps	Rs	Af	Fv	Pv
Ca	9.74±0.01 ^a	9.50±0.01 ^a	9.50±0.00 ^a	11.62±0.01 ^a	11.05±0.00 ^a	11.62±0.01 ^a
Cm	9.30±0.00 ^b	9.08±0.01 ^b	9.01±0.00 ^b	11.18±0.00 ^b	10.71±0.00 ^b	11.18±0.00 ^b
Js	8.87±0.01 ^c	8.57±0.00 ^c	8.57±0.00 ^c	10.82±0.00 ^c	10.14±0.00 ^c	10.82±0.00 ^c
CA	8.38±0.00 ^d	8.11±0.00 ^d	8.50±0.0 ^d	10.35±0.00 ^d	9.86±0.00 ^d	10.35±0.00 ^d
Rc	7.95±0.00 ^e	7.65±0.01 ^e	8.12±0.00 ^e	9.87±0.00 ^e	9.43±0.00 ^e	9.87±0.00 ^e
Ro	7.51±0.00 ^f	7.16±0.00 ^f	8.00±0.01 ^f	9.44±0.00 ^f	8.99±0.00 ^f	9.44±0.00 ^f
Aa	7.01±0.00 ^g	6.71±0.00 ^g	7.67±0.00 ^g	8.97±0.00 ^g	8.48±0.00 ^g	8.97±0.00 ^g
Ob	6.51±0.00 ^h	6.22±0.00 ^h	7.49±0.00 ^h	8.46±0.00 ^h	8.01±0.00 ^h	8.46±0.00 ^h
Nt	6.02±0.00 ⁱ	5.87±0.01 ⁱ	7.23±0.00 ⁱ	8.01±0.00 ⁱ	7.48±0.00 ⁱ	8.01±0.00 ⁱ
As	5.54±0.00 ^j	5.38±0.00 ^j	7.00±0.00 ^j	7.51±0.00 ^j	7.01±0.00 ^j	7.51±0.00 ^j
Ai	5.07±0.00 ^k	4.92±0.00 ^k	6.88±0.00 ^k	7.01±0.00 ^k	6.47±0.00 ^k	7.01±0.00 ^k
Ol	4.56±0.00 ^l	4.45±0.00 ^l	6.49±0.00 ^l	6.51±0.00 ^l	5.97±0.00 ^l	6.51±0.01 ^l
Va	4.07±0.00 ^m	3.91±0.00 ^m	6.00±0.00 ^m	6.00±0.00 ^m	5.46±0.00 ^m	6.00±0.00 ^m
Ba	3.56±0.00 ⁿ	3.44±0.00 ⁿ	5.50±0.00 ⁿ	5.52±0.02 ⁿ	5.01±0.00 ⁿ	5.52±0.00 ⁿ
Ms	3.03±0.00 ^o	3.00±0.00 ^o	5.01±0.00 ^o	5.01±0.00 ^o	4.49±0.00 ^o	5.01±0.00 ^o
Eg	2.55±0.00 ^p	2.49±0.00 ^p	4.50±0.01 ^p	4.50±0.00 ^p	4.00±0.01 ^p	4.50±0.00 ^p
Con	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q

Appendix Table 12 Antimicrobial activities of botanicals by concentration 75%

Trt	Xcm	Ps	Rs	Af	Fv	Pv
Ca	14.21±0.01 ^a	13.08±0.02 ^a	12.63±0.34 ^a	15.01±0.01 ^a	15.49±0.00 ^a	14.00±0.00 ^a
Cm	13.50±0.00 ^b	12.72±0.01 ^b	12.01±0.01 ^b	14.30±0.01 ^b	14.96±0.00 ^b	13.49±0.00 ^b
Js	13.01±0.00 ^c	12.20±0.01 ^c	11.42±0.00 ^c	13.51±0.00 ^c	14.12±0.00 ^c	13.01±0.00 ^c
CA	12.50±0.00 ^d	11.98±0.00 ^d	11.01±0.00 ^d	13.03±0.00 ^d	13.49±0.00 ^d	12.51±0.00 ^d
Rc	11.97±0.00 ^e	11.50±0.00 ^e	10.50±0.00 ^e	12.62±0.00 ^e	13.06±0.00 ^e	12.02±0.01 ^e
Ro	11.49±0.00 ^f	11.10±0.00 ^f	10.01±0.00 ^f	12.15±0.00 ^f	12.68±0.01 ^f	11.48±0.00 ^f
Aa	11.04±0.00 ^g	10.65±0.00 ^g	9.51±0.00 ^g	11.71±0.00 ^g	12.22±0.00 ^g	11.05±0.00 ^g
Ob	10.56±0.01 ^h	10.12±0.00 ^h	9.08±0.00 ^h	11.24±0.00 ^h	11.94±0.01 ^h	10.55±0.00 ^h
Nt	10.55±0.00 ⁱ	9.55±0.00 ⁱ	8.61±0.00 ⁱ	10.78±0.00 ⁱ	11.50±0.00 ⁱ	10.08±0.00 ⁱ
As	10.15±0.00 ^j	9.12±0.00 ^j	8.13±0.00 ^j	10.24±0.01 ^j	11.01±0.00 ^j	9.61±0.00 ^j
Ai	10.00±0.00 ^k	8.65±0.01 ^k	7.75±0.00 ^k	9.85±0.01 ^k	10.76±0.00 ^k	9.11±0.00 ^k
Ol	9.80±0.00 ^l	8.18±0.00 ^l	7.22±0.00 ^l	9.29±0.00 ^l	10.31±0.01 ^l	8.75±0.00 ^l
Va	9.20±0.00 ^m	7.75±0.00 ^m	6.86±0.01 ^m	8.75±0.00 ^m	9.83±0.01 ^m	8.21±0.01 ^m
Ba	8.75±0.00 ⁿ	7.21±0.00 ⁿ	6.31±0.00 ⁿ	8.30±0.01 ⁿ	9.29±0.00 ⁿ	7.90±0.00 ⁿ
Ms	8.25±0.00 ^o	7.00±0.00 ^o	5.85±0.00 ^o	7.89±0.00 ^o	8.87±0.00 ^o	7.45±0.00 ^o
Eg	7.76±0.01 ^p	6.50±0.00 ^p	5.49±0.00 ^p	7.29±0.01 ^p	8.59±0.01 ^p	7.05±0.01 ^p
Cont	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q	0.00±0.00 ^q

BIOGRAPHY OF THE AUTHOR

The author was born in Kecha Kebele, Soro woreda, Hadiya Zone, Southern Regional State in 1980, EC. He attended his primary education at Kecha Primary School and completed his secondary school education at Gimbichu Secondary School. He joined Adama Science and Technology University, biology department in 2006 and graduated with bachelor of degree in biology. After completion of his BSc study, he had worked at Hadiya Zone of, Soro Woreda, Jajura High School and Preparatory School and he is serving there till now. Finally, he joined Hawassa University College of Agriculture, School of plant and horticultural Science in 2019 to pursue his Master of Science study in Plant pathology program.