Evaluation of Moringa oleifera Leaves and Stems Bark crude extract for controlling potato wilt caused by Fusarium species.

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Article

Keywords: Antifungal Activity, Crude Extract, Minimum Inhibitory Concentration, Potato Wilt

Posted Date: August 3rd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9721364/v1

Additional Declarations: No competing interests reported.

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List of abbreviations and acronyms

ABE Acetone Bark Extract
ALE Acetone Leaf Extract
EBE Ethanol Bark Extract
ELE Ethanol Leaf Extract
MIC Minimum Inhibitory Concentration
MP Medicinal Plant
NC Negative Control
PC Positive Control
ZOI Zone Of Inhibition

ABSTRACT

Fungal infections cause significant losses in many economic crops. Chemical control may be available to effectively and extensively reduce the effects of the most fungal disease but field application of these chemical fungicides may present a danger to the health of humans, animals, and the environment. This study was aimed to assess the antifungal activities of crude extracts of Moringa oleifera Lam. stems bark and leaves against Fusarium species. Four crude extracts (acetone and ethanol extract of stem bark and leaf) were tested for their effect on 10 Fusarium isolates at three different concentrations (40 mg/ml, 60 mg/ml, and 80 mg/ml) and at 100µg/ml (0.1 mg/ml) of positive control using agar-well diffusion method. All the quantitative data generated were analyzed by using SPSS statistical software for window version 20. At 40 mg/ml, concentration, zones of inhibition were recorded by; acetone crude extract of leaf: 11.00±1.00, 12.000±1.00 mm, at 60 mg/ml; 12.33±0.58, 15.33±0.58 mm, and at 80 mg/ml; 16.33±1.53 mm and acetone crude extract of stem bark: at 40 mg/ml; 9.00±1.00 mm, 6.67±1.155, at 60 mg/ml; 12.00±1.00, 14.67±0.58 and at 80 mg/ml; 15.67±1.53mm respectively; and by ethanol crude extract of leaf: at 40 mg/ml; 9.00±1.00,11.33±1.56 mm, at 60 mg/ml; 12.33±0.58 mm and at 80 mg/ml;16.00±1.00 mm and stem bark: at 40 mg/ml; 6.67±1.15 mm, 11.00±1.00, at 60 mg/ml;11.33±1.56, 12.00±1.00 mm, and 14.67±0.58 mm respectively at the respective concentration. From the recorded effect, acetone crude extract of the leaf at a concentration of 60 mg/ml and 80 mg/ml (p=0.073, and 0.493 respectively), and acetone crude extract of stem bark at a concentration of 80 mg/ml (p=0.201) and ethanol crude extract of the leaf at a concentration of 80 mg/ml (0.143) had non-significant growth effect on Fusarium species compared with the positive control (P> 0.05). Ethanol crude extract of stem bark at all the different concentrations had significant differences compared with the positive control (p=0.000, 0.000, 0.00, 0.00, and 0.012 respectively). Ethanol and

acetone extract of leaves have a minimum inhibitory concentration of 12.5 mg/ml and 6.25 mg/ml respectively and in the case of bark 25mg/ml and 12.5mg/ml respectively. The findings of this study revealed that each plant part possesses an antifungal effect on tested fungal strains varying with the type of solvent used and concentration, which might validate its traditional uses in treating potato wilts. Furthermore, extraction using the different solvent systems for each part of the plant may be useful to get more effective extracts.

***Key Words**: Antifungal Activity, Crude Extract, Minimum Inhibitory Concentration, Potato Wilt.*

1. INTRODUCTION

1.1. Background of the Study

Man is dependent on plants for almost every need and the destruction of crop plants due to infection by fungal pathogens has always been an area of prime concern. Researchers from all across the world are working to create strategies for managing plant diseases. The most common approach to disease control is chemical control. Synthetic fungicides are very effective because they inhibit pathogens by either damaging their cell membrane or its permeability or by preventing the pathogens' metabolic processes. (Bokshi *et al.,* 2003). The usage of synthetic fungicides has the drawback of being detrimental to soil, animal health, and human health. They enter the food chain and have a number of harmful consequences on the biosphere, contributing to significant declines in populations of beneficial soil organisms, soil acidification and compaction including thatch buildup, soil acidity and compaction, substantial drops in populations of beneficial soil organisms, and diminished disease resistance (Gurjar *et al.,* 2012). Farmers use a variety of conventional agricultural techniques to manage plant diseases. The mixture, which combines organic ingredients and plant extracts to prevent plant diseases, is an inexpensive, ecologically safe fungicide. Many ancient agricultural methods, such using organic materials (cow dung, oil cakes, etc.) to manage plant diseases, need to be validated. (Nene, 2013).

Therefore, in addition to well-known disease management techniques like crop rotation, the use of resistant cultivars, the planting of disease-free seeds, biological control, etc. for the control of fungal diseases, current thinking about plant and environmental protection suggests alternatives to pesticides and the use of other strategies (Deepa, and Kanika, 2014). According to Kagale et al. (2004), natural plant products represent a significant source of novel agrochemicals for the management of plant diseases. Primary and secondary metabolites produced by plants serve a variety of purposes, many of which have since been used by humans for their advantageous roles in a wide range of applications. The problem of increasing drug-resistant disease occurrences has brought attention to the antibacterial activity of plants and their metabolites. The ability of plants to overcome resistance of some microbes

has prompted research into their methods of action and the isolation of potent chemicals. Establishing the impact of the plant extracts in terms of their micro-static and micro-cidal activity as well as the range of organisms impacted is given special attention. This has enabled the exploitation of plants for the treatment of microbial infections and the development of new antimicrobial agents (Ncube *et al.,* 2008).

Phytochemicals, often known as natural products, are plant extracts that undergo biological screening using assays that are relevant to pharmacology. Because of its natural origin and fewer side effects, phyto-medicine is growing in popularity in both developed and developing nations. Natural products continue to be a significant source of novel pharmaceuticals, especially medicinal plants (Newman et al., 2003; Butler, 2004). Many nations have accepted plant-based medicines as legitimate treatments, and they are utilised all around the world (Balunas and Kinghorn, 2005).

Because antibiotics are so expensive in developing nations, using medicinal plants to treat infections is thought to be a safer and more cost-effective natural treatment option (Masika and Afolayan, 2002). Due to their strong pharmacological effects, minimal toxicity, and economic viability in comparison to manufactured medications, plants' medicinal qualities have also been studied in light of current scientific advancements worldwide. Approximately 80% of people in developing countries utilise traditional medicines made from medicinal plants (WHO 2002 reported in Igbinosa et al., 2009).

Herbal medicine is thought to be the main source of healthcare for 80–90% of Ethiopians. Traditionally, family illnesses have been treated at home with herbs, and sometimes traditional healers are sought (Elizabeth et al., 2014). Orthodox Christian traditional healers, also known as debteras in Amharic, may follow the Semitic Coptic medical system, the regional Arabic-Islamic medical system, or the religious traditions of Cushitic medicine. Within each system, there might be numerous differences in methodology. Herbal remedies are frequently combined with spiritual techniques, especially by the debteras (Getachew et al., 2001).

Moringa oleifera Lam is one of the plants. is the most widely used because of its many therapeutic applications and nutritional value, which have been valued for generations in many regions of both its native and introduced ranges. The Moringa plant is utilised for medical and other reasons in all of its parts (Anwar et al., 2007). In addition to its potential applications as a natural plant growth booster in agriculture, moringa is utilised as food and for human health. Moringa oleifera Lam. (Family: Moringaceae) has become increasingly significant in recent years because of its many applications and advantages for industry and agriculture (Ashfag et al., 2012). In agriculture and industry, the leaves of

this plant contain a bioactive substance that can be sprayed on crops indicating accelerated growth of young plants, which become more resistant to pests and diseases (Dalijit. *et al.,* 2013).

Studies on the antimicrobial properties of Moringa oleifera Lam stem bark. showed that methanolic extracts were the most effective solvents against some infections. Petroleum ether extract was shown to be inactive in the most recent study on the in vitro antibacterial and antifungal activity of Moringa oleifera Lam. stem bark against ten bacterial strains and six fungus strains. According to Devendra (2011), the various portions of the Moringa plant exhibit a wide range of bioactive components with varied polarities that may be useful in the development of new drugs (Devendra, 2011). Utilising plant extracts and biocontrol agents has been considered a practical way to manage plant diseases. Since plant-based fungicides are proven to have little effect on the environment, they seem to be one of the best options (Ramaiah and Garampalli, 2015). Potential candidates for the management of fungal illnesses include natural compounds, especially those derived from plants. One plant with significant potential in the pharmaceutical sector as a source of bioactive components for medication development is moringa. Moringa extracts have been shown to have a fungicidal impact on a number of soil-borne fungi, including Rhizoctonia, Pythium, and Fusarium (Moyo et al., 2012).

Nevertheless, to date, there is no research conducted on the antifungal activity of *Moringa oleifera* Lam. to treat plant disease caused by Fusarium spp in Ethiopia. Thus, the main aim of this study was to provide experimental evidence for the antifungal properties of *Moringa oleifera* Lam and to demonstrate the effectiveness of *Moringa oleifera* leaves and stems bark crude extract for preventing potato wilt caused by Fusarium species, considering the plant's therapeutic potential.

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2. MATERIALS AND METHODS

Description of the Study Area

The study was carried out at Ambo town and its surrounding in the West Shewa zone of Oromia Regional State. One of Ethiopia's nine regional states and ethnic divisions, Oromia Regional State makes up 34.3% of the nation's total land area. With more than 30 million residents, the state is the most populous in the nation. Approximately 12.2% of the region's total population is thought to live in metropolitan towns, with the other 87.8% living in rural areas. (CSA, 2008).

Ambo town is situated 60 kilometres northwest of Weliso town and 12 kilometres east of Guder town in the West Shoa Administrative Zone of Oromia Regional State. It is roughly 114 kilometres west of Addis Ababa on the main road leading to the Western region of Ethiopia. Ambo town, which occupies 8587 hectares of land, was founded in 1889. It is among Ethiopia's oldest towns. The lake that contains salt is the source of the name Ambo. The "Ambo Tsebel" hot spring has contributed to the growth of Ambo town. During the Haile Sellase era, the town's name was changed to Hagere Hiwot; it returned to its former name in 1974. The town was one of the most popular towns at the time since it had a master plan and municipal administration in 1931. since of its advantageous position, it has been the administrative, transportation, educational, and commercial hub of the West Shoa Zone. Abebe Doyo kebele borders Ambo town in the north; Uko Korke kebele borders it in the south; Kora Bala kebele borders it in the southwest; Ilamu Muja kebele of Ambo district borders it in the east; and Toke Kutaye district borders it in the west. There are six kebeles in the town. Three of these are pre-rural kebeles (Awaro in the east, Sankale Farisi in the west, and Oddo Liban Kisose in the north), and three are urban (01, 02, and 03). (Ambo Town Municipality, 2018). According to the 2014 census conducted by Ambo town municipality, the current population is estimated at 107,980; of which 52,912 were males and 55,068 were female inhabitants. Of the total population, 33,782 (29 percent) were youth in the age group 15-29 (Ambo Town Municipality Report, 2019).

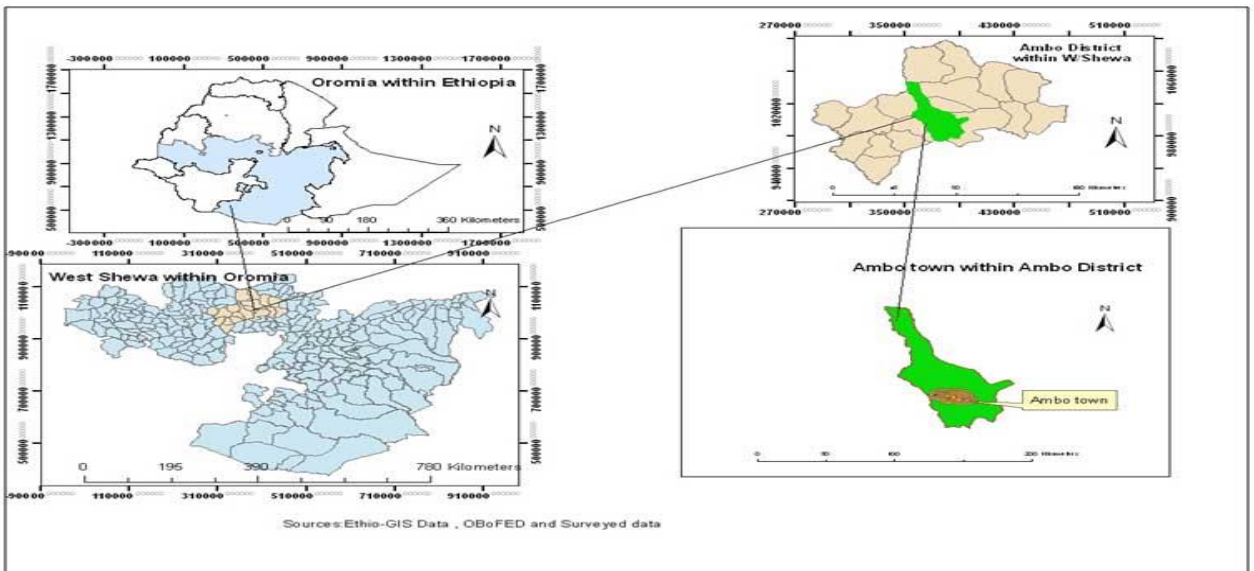

Figure.1: The map of study area, source: Ethio-GIS Data and surveyed data

The geographical (Astronomical) location of Ambo town is approximately between 8o 56'30" N - 8o 59'30" N latitude and between 37o47'30" E - 37o 55'15" E longitude. The average elevation of the town is 2090 meters above sea level and it varies from 2060 meters to 2140 meters above sea level. The town and its surrounding have a mean annual precipitation of 912 millimeters, which the highest rainfall concentration occurring from June to September and the mean annual temperature of the town is about 17.6 centigrade which is the characteristic of a warm temperature climate locally called Woina Dega (Badda Daree) (Ambo Town Municipality, 2018).

Materials and Chemical reagents

This research work has employed materials for grinding: mortar and pestle; for sieving: mesh (0.5 mm); for extraction and filtration: Gemmy Orbit shaker, Rota-evaporator, Whatmann NO 1 filter paper (110 mm), Measuring cylinder, balance and for antifungal susceptibility test; Petri dishes, phial, Para film, incubator, autoclave, electron balance, marker, flamer, spoon, Iron rod, fork, biological safety cabinet(hood), glove, newspaper, match, scissor, funnel, ruler, brush, micro-pipette, flask, cotton swab, cork-borer, test tube, and aluminum foil for the overall experimental work of this research work.

Chemicals reagents were used to carry out all the research work of this study. The non-polar and weak polar solvents of acetone, petroleum ether, and polar solvent of ethanol, The growth media PDA used in this research, and antibiotics used for inhibition of fungi . Distilled water is also used for washing materials, media preparation, and test solution preparation, and Copper sulfate is employed as a standard fungicide from Ambo university biology Laboratory.

Collection and Identification of Plant Materials.

Plant Sample Collection and processing

The plant sample of *Moringa oliefera* Lam. leaves and the stem bark was collected in a polyethylene bag on December 18/04/2012 E.C from Ambo University. The plant sample was identified with the help of a botanist (Jain and Rao1976). The collected leaves and stem bark of the plant were chopped with scissors into small pieces. Then the chopped plant material was shade dried on a newspaper independently up to Januar, 09/05/2009 E.C with a careful and continuous follow-up to avoid cross-contamination. The dried plant materials were then ground into a powdery substance by using a mortar

and pestle (Kebede et al. 2021). Then sieve it to get an appropriate size for extraction with the help of mesh (0.5 mm). Finally, the grounded powder was measured by electron balance, and a total weight of 823 g powder that constitutes a weight of 396 g of *Moringa oleifera* stem bark powder and 427 g of *Moringa oleifera* leaf powder was obtained. Then the measured plant materials were stored in clean dry glass containers of a size of 1000 ml and stored within a locker in the Biology Laboratory of Ambo University.

Figure 2: **Collection of *Moringa oleifera Lam.* leaf and stem bark.**

Isolation of the pathogen

Three plant samples and three soil samples were to isolate *Fusarium species* from different areas of West Shewa Zone Ilamu Jalina (Mexxii) Kebele which is 102 km west of Addis Ababa and 10 km east of Ambo town (Fig3) (Jain and Rao1976).. The leaf was cut into small pieces of 2-3 mm measured by ruler and cut scissor material and was collected in a polythene bag. Potato leaves were surface sterilized with Mercury chloride (HgCl2) for 3min rinsed in three changes of sterile distilled water washed repeatedly and placed In petri-plates containing PDA medium and incubated at 28°C (Mamta et al. (2013).

Figure 3: Collection of potato leaf infected by *Fusarium spp.*

An isolate of Fusarium was obtained from potato plants showing wilting symptoms. The lower leaves wilt, turn yellow, and then brown and eventually drop from the plant; the vascular system also turns brown (Garrett, 1970, Rich).

Preparation of the Extract

The extractions of the plant extract were performed according to the method in Geremew and Yalemtsehay, (2012) and Vongsak *et al*., (2013). Plant material was extracted using the maceration extraction method (using two different solvents, with increasing polarity: acetone and ethanol respectively). To perform extraction at 1:5 w/v, the powder (in weight) of both the leaf and stem bark were macerated separately in each of the two solvents (in volume). First, in a 1:5 w/v proportion, a weight of 300 g of each part of the grounded *Moringa oleifera* Lam. powder was dissolved in a solution of 1 and a half litter of acetone separately in a flask and shacked by hand until the solvent (acetone) and the solute(plant powder) mixed completely. Solvent (ethanol) has similar ratios and procedures as acetone. The dissolved solution was poured into a flask, packed with aluminum foil tied with plastic, and put on the shaker (Praveen and Jeyaprabha 2024). The mixture was then homogenized and allowed to stand for 24 hrs. After 24 hrs, the plant mixtures were then filtered by using a Whatmann filter paper No 1 which was giving filtrates and residues. The filtrate was then placed in a rotary evaporator at 40 oC to obtain crude extracts according to the methods in (Geremew and Yalemtsehay, 2012) and the solvents employed to dissolve the plant material were allowed to

evaporate. A residue of two claimed parts was shed-dried on aluminum foil and then macerated in the four (4) different crude extracts obtained from each part of *Moringa oleifera* by the two different solvents was placed in the ventilated hood until the solvents completely evaporated from the resultant plant extracts. Then the resultant crude extracts were measured by electric balance and placed in empty containers (phial) of which the weight was previously determined, then the measured crude extract was stored in the refrigerator at 40C until use as outlined in Praveen and Jeyaprabha (2024)

Extract Dilution to Prepare Working Concentrations

The crude acetone and ethanol extracts of *Moringa oleifera* Lam. leaves and stem bark was further diluted to make three different concentrations of working solution i.e. 40mg/ml, 60mg/ml, and 80mg/ml for the antifungal activity test. The density of suspensions inoculated into the plate for the susceptibility test was then determined and the working solution was three different concentrations; 40 mg/ml, 60 mg/ml, and 80 mg/ml, according to the methods in Neela *et al.,* (2014) and Ganie *et al.,* (2015). The first working solution was prepared by transferring 40 mg of each extract to the sterile test tube containing 1 ml of distilled water to give 40 mg/ml working concentrations. Similarly, the second and third working solutions were prepared by adding 60 mg and 80 mg of ethanol, petroleum ether, and acetone crude extracts of the plant to sterile test tubes with 1 ml of distilled water to make 60 mg/ml and 80 mg/ml of working concentrations (Ganie *et al.,* 2015).

Figure 4: Testing *Moringa oleifera* drug on *Fusarium* isolates.

Standard Fungal Pathogen

The test organism i.e. *Fusarium species* culture was obtained from the Ethiopian Institute of Agricultural Research in the Ambo plant protection research center.

252 **Standard Fungicides**

253 This study used copper sulfate as a standard fungicide and the test solution was prepared according to
254 the method (Nel *et al.,* 2007*,* Wakle, 2015). Copper sulphate was used as a positive control and
255 distilled water was used as a negative control.

256 **Antifungal Testing**

257 The effect of each plant crude extract on the mycelium growth of the tested fungus was determined
258 according to methods reported by Neela *et al.,*(2014) and Ganie *et al.,* (2015), and El-Mohamedy *et*
259 *al*., (2014).To determine the effect of the crude extracts on the mycelial growth of a test organism
260 (fungus), PDA was used as growth media and prepared according to the manufacturer's protocol (a
261 media prepared that 1000 ml of distilled water is required to dissolve 39 g PDA). Thirty-nine grams of
262 PDA was dissolved in 1000 ml of distilled water in a flask, and shacked by hand, then boiled on a hot
263 plate at 100 0C until it was completely mixed. Then sterilized by autoclaving at 121°C for 15 minutes
264 and left cooled to 50C in the ventilated biological safety cabinet (hood), dissolved antibiotic,
265 subsequently poured into sterile Petri dishes, and solidify at room temperature. Once the media poured
266 in the six Petri dishes get solidified, the plates were incorporated with five equally distant wells with
267 the help of a sterile cork-borer (6 mm diameter) for three different concentration of crude extracts and
268 their respective control separately in each Petri dishes dish for each plant part (Zisha et al. 2024;
269 Adnew et al. 2022). The seven-day-old fungal pathogen (*Fusarium species)* washed mycelia with
270 phosphate buffer and was placed at the center of the plate spread uniformly with a sterile cotton swab.
271 Following this, the wells were impregnated or filled with a 50 µl of each of the reconstituted plant
272 extracts which were from; 40 mg/ml, 60 mg/ml, and 80 mg/ml as well as the same volume from the
273 control solution from standard fungicides (copper sulfate) as a positive control and distilled water as a
274 negative control by micropipette (Ibrahim, and Abdul-Hafeez, 2023) The inoculated plate was
275 incubated at 28±2oC for 72 hrs. The inhibition zone of the colony diameter of the mycelial growth of
276 the inoculated fungi was measured by a ruler (in mm) after 3 days of inoculation with 3 replications
277 according to the methods in Neela *et al*., (2014).

278 **Determination of Minimum Inhibitory Concentration (MIC)**

279 The acetone, petroleum ether, and ethanol crude extract of *Moringa oleifera* Lam. leaves and stem
280 bark that showed significant antifungal activity at low concentrations in the agar well diffusion test
281 were selected for determining MIC. 100 mg of the dried acetone, petroleum ether, and ethanol crude
282 extracts were dissolved with 1 ml of distilled water in test tubes to obtain100 mg/ml (w/v) stock

solution and the solution was serially diluted as a start from 1:1,1:2, 1:4, 1:8, 1:16 and 1:32 to get 100 mg/ml,50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml and 3.125 mg/ml (v/v) concentrations of test solutions respectively. The growth media were first prepared and sterilized by autoclaving. The sterilized media were poured into sterilized Petri dishes and allowed to solidify (Al-Hatmi et al. 2017). Subsequently, by cutter material of *Fusarium species* was spread uniformly with a sterile cotton swab. After this 50µl from different concentrations of extract (100 mg/ml, 50 mg/ml, 25 mg/ml, 12.5 mg/ml, 6.25 mg/ml, and 3.125 mg/ml) were poured into each well. The plates were then incubated at 28oC for 72hrs. The lowest concentration which inhibited the growth of *Fusarium* was taken as the minimum inhibitory concentration (Jennifer. 2001; Ganie *et al*., 2015).

Data Analysis

All the experiments were carried out in triplicates. Zones of inhibition were analyzed using SPSS 20 statistical software package to compare means and statistical significance differences. Comparisons were made between the effect of the control group (standard drug (positive control), distilled water (negative control)) and the extracts (at different doses or concentrations) using two-way analysis of variance (ANOVA) (Tukey's HSD analysis). The zones of inhibition were expressed as mean ± SD by descriptive statistics of the triplicate test and statistical significance was calculated, 95% confidence interval was taken and P-values corresponding to $p < 0.05$ were considered statistically significant differences (Gomez and Gomez 1984).

3. RESULTS AND DISCUSSION

3.1. Extraction of *Moringa oleifera Stem bark and leaves*

In order to separate and characterise the desired chemical components from the plant materials, extraction is an essential initial step in the investigation of medicinal plants. Pre-washing, freeze-drying plant materials, grinding to create a homogeneous sample, which frequently improves the kinetics of analytic extraction, and enhancing the sample surface's contact with the solvent system were all part of the fundamental operation.

A material or an activity with desired qualities that is extracted from a plant's tissue, typically by treating it with a solvent, and used for a specific purpose is called a plant extract. Extraction was done using the procedure outlined in (Harborne, 1984). The results of this investigation included 22.39 g of acetone crude extract from Moringa oleifera leaves, 17.85 g of acetone crude extract from Moringa oleifera stem bark, 21.34 g of ethanol crude extract from Moringa oleifera leaves, and 29.74 g of ethanol crude extract from Moringa oleifera stem bark (**Fig 5**).

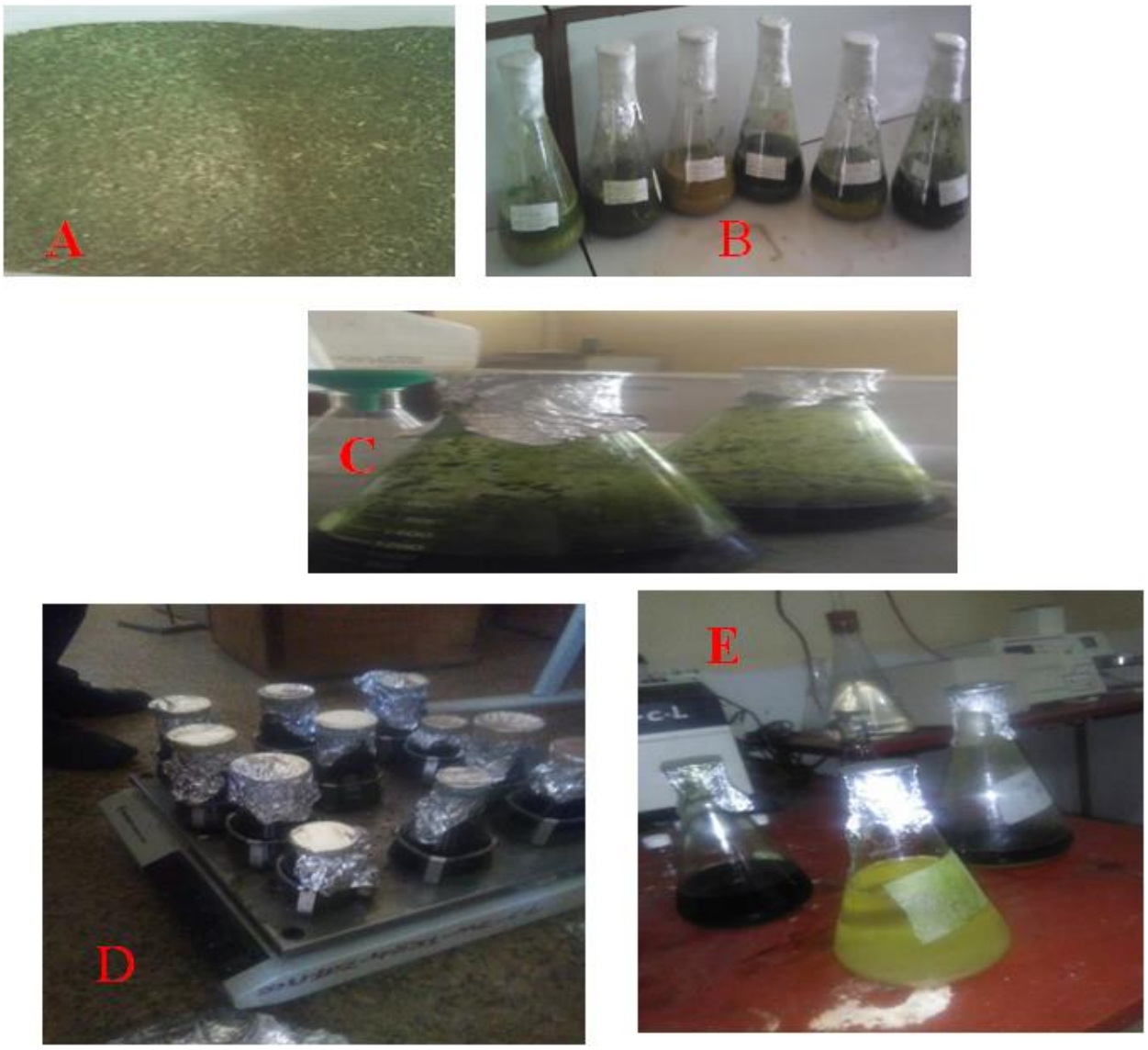

Figure 5: Powders of *Moringa oleifera* dissolved in solvent (acetone and ethanol), Shaker, Filtration of the crude extracts and Rotary evaporator

Isolation and Characterization of Fusarium *spp.*

Soil-borne Fusarium species are the cause of fusarium wilt. which, as free-living saprophytes, can endure in soil for extended periods of time without their host plants (Garrett, 1970). Soil samples taken from the study area were used to isolate the fungal pathogens. On the top of the solid media created for this purpose, a variety of distinct fungal strains emerged (Fig 6). For

327 additional characterisation and experimental investigation, only 10 isolates exhibiting the
328 traits of Fusarium species were chosen.

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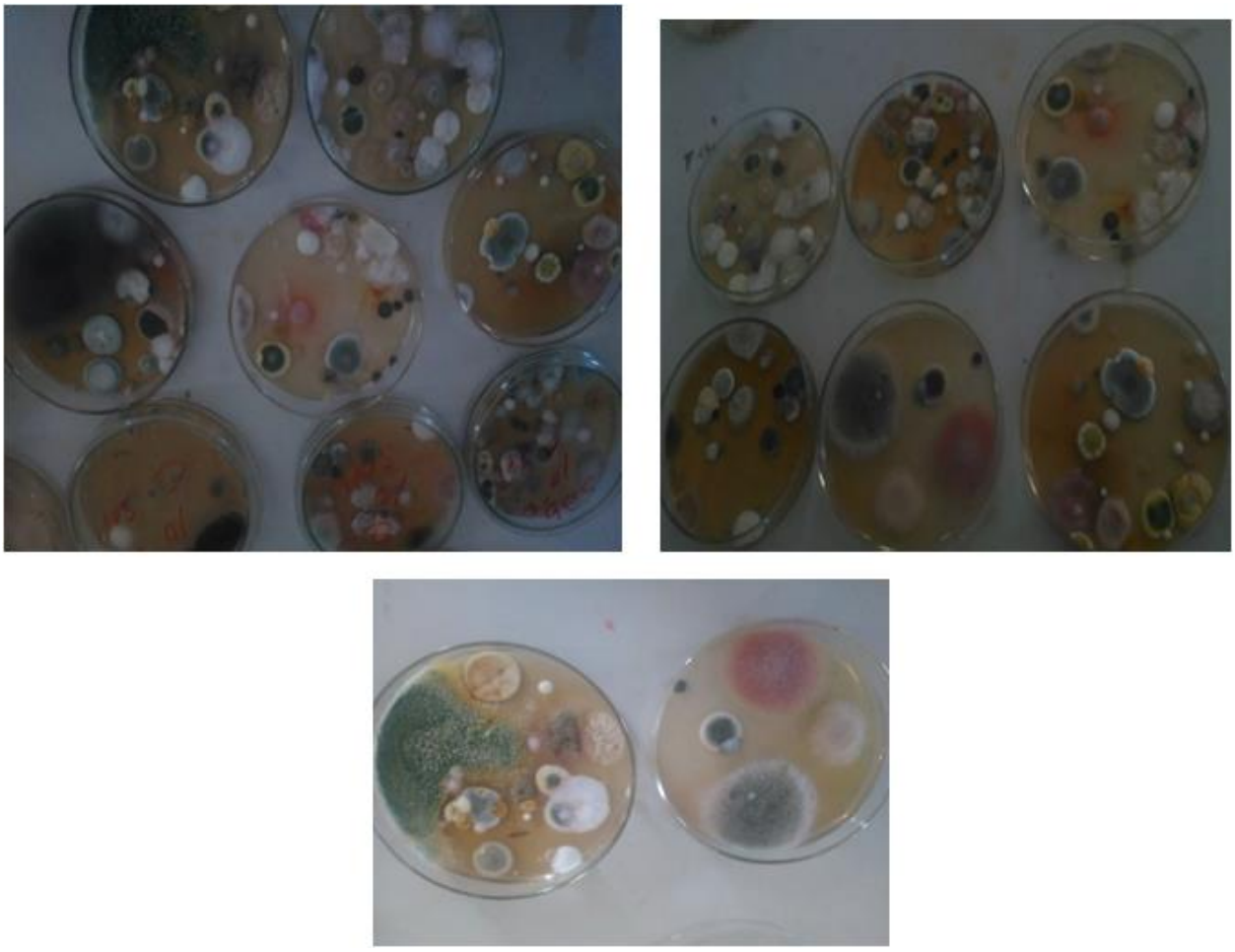

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Figure 6: Isolation and Purification of *Fusarium spp. By sub culturing*

Morphological characteri zation of the selected *Fusarium spp*

Morphological characteristics of Fusarium species include microconidia with an oval to kidney form, fragile, sickle-shaped macroconidia with thin walls. A single terminal chlamydospore and short monophialides create microconidia in false heads. Depending on the species, Fusarium colonies might have a cottony aerial mycelium and are typically pale or vividly coloured. They might be pale, yellow, brownish, pink, or reddish in colour (Fig. 7). Fusarium species' microscopic traits: Fusarium species are primarily identified by their unique characteristics, which include the sizes and forms of macro- and micro-conidia, the presence or absence of chlamydospores, colony appearances, pigmentations, and growth rates on agar media**.**

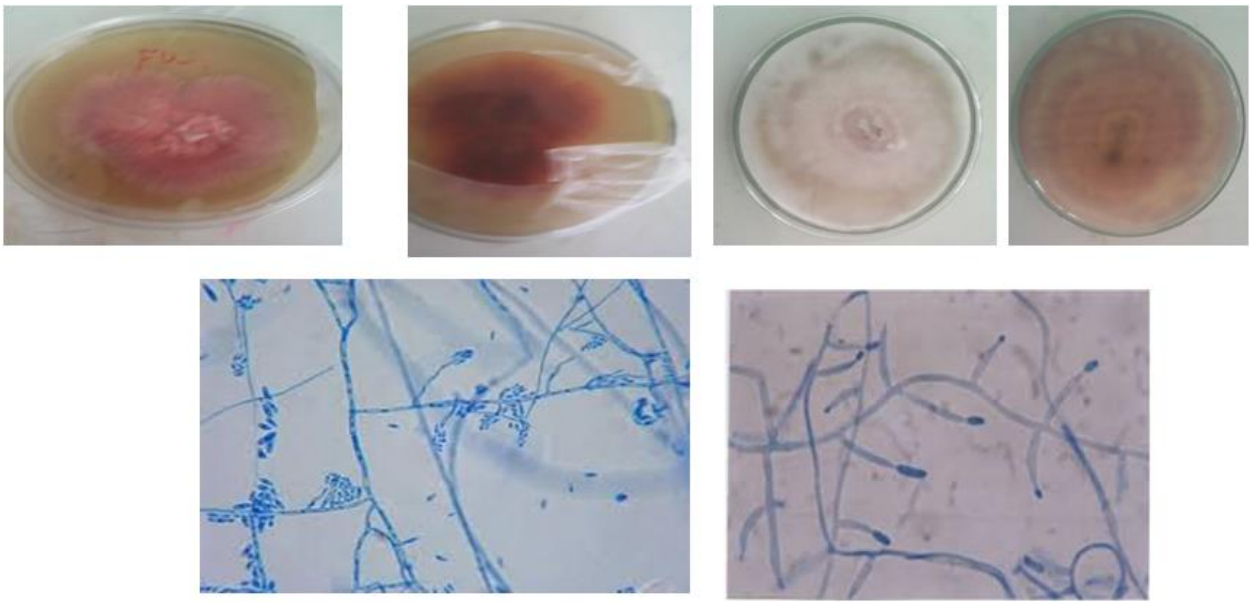
Fusarium species from spores which were morphologically various from different isolates obtained from the pathogens' pure cultures. Ocular and stage micrometres were used to measure spores at high power objectives (40x). On potato dextrose agar, Fusarium species develop, displaying both macro and microspores. An arrow points to three septate macrospores, whereas microspores are dispersed at random. For every isolate, microconidia, macroconidia, and chlamydospores were found**.**

Morphological Spores of *Fusarium* species of different isolates were from the pure culture of the pathogens. Spores were measured under high power objectives (40x) using ocular and stage micrometers. *Fusarium* species grow on potato dextrose agar showing the macro and

microspores. Three septate macrospores are indicated by an arrow, while microspores are spread randomly. The presence of microconidia, macroconidia, and chlamydospores was observed for all the isolates. A Fusarium species' macroconidia (spores) displaying the traits of foot cells (shown by an arrow). Conidiophore-bearing macrospore (arrow shown). The length of microconidia ranged from 5.80 to 8.05 µm. These findings fall within the range for Fusarium species reported by Ciampi et al. (2009). According to these authors, macroconidia are 27–55 mm long and 3–5 µm wide, with 3–5 septa. There could be millions of spores per millilitre in the spore suspension.

The larger conidia, known as macroconidia, have three to five cells and are progressively pointed and curled toward the ends. These spores are frequently observed in sporodochia-like groupings and on the surface of plants that have been killed by this infection. Chlamydospores are spherical, thick-walled spores with one or two cells that are formed either terminally or intercalary on older mycelium or in macroconidia. Surprisingly, they can infect other hosts for up to 30 years when dormant in the soil. Chlamydospores are thick-walled asexual spores produced by hyphae or conidia. They can be formed on dead host plant tissue in the final stages of the wilt disease (Marshall *et al*., 1981).

F_1 FRONT VIEW $F._1$ REVERSE VIEW$F._2$ FRONT VIEW$_2$ $F._2$ REVERSE VIEW



$F._1$ Morphological characteristics **$F._2$ Morphological characteristics**

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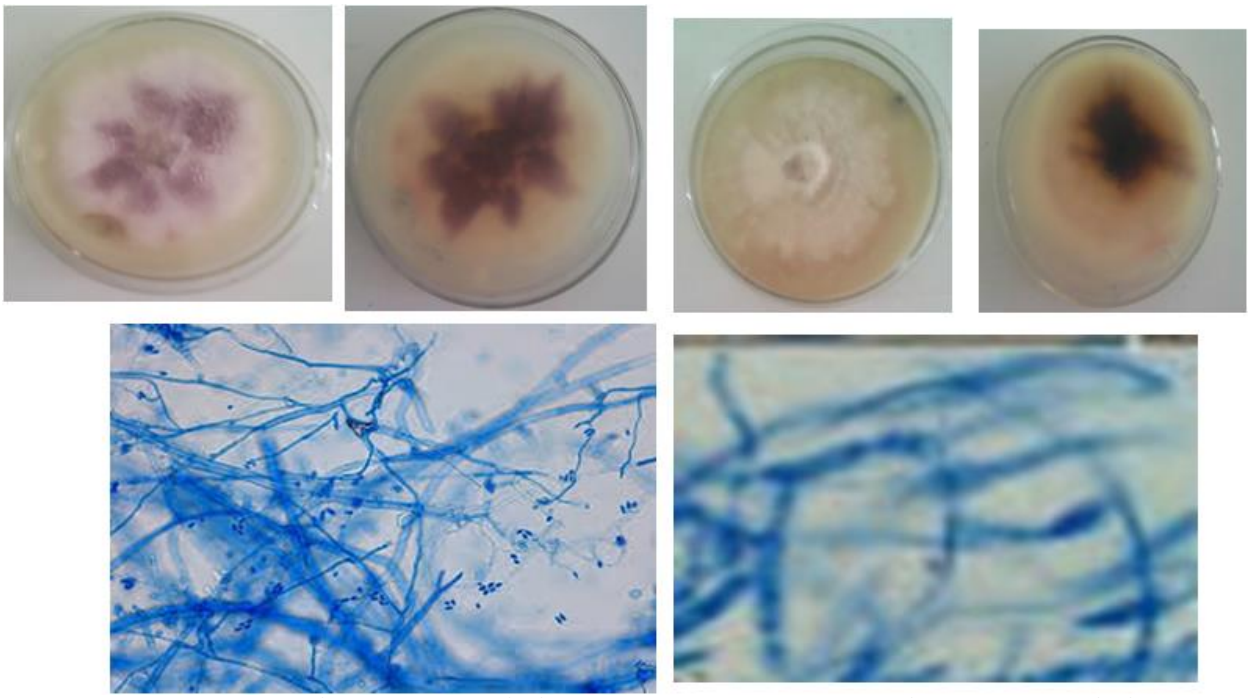
375 *F*. 3 FRONT VIEW *F*.3 REVERSE VIEW*F*. *F*. 4 FRONT VIEW *F*. 4 REVERSE VIEW



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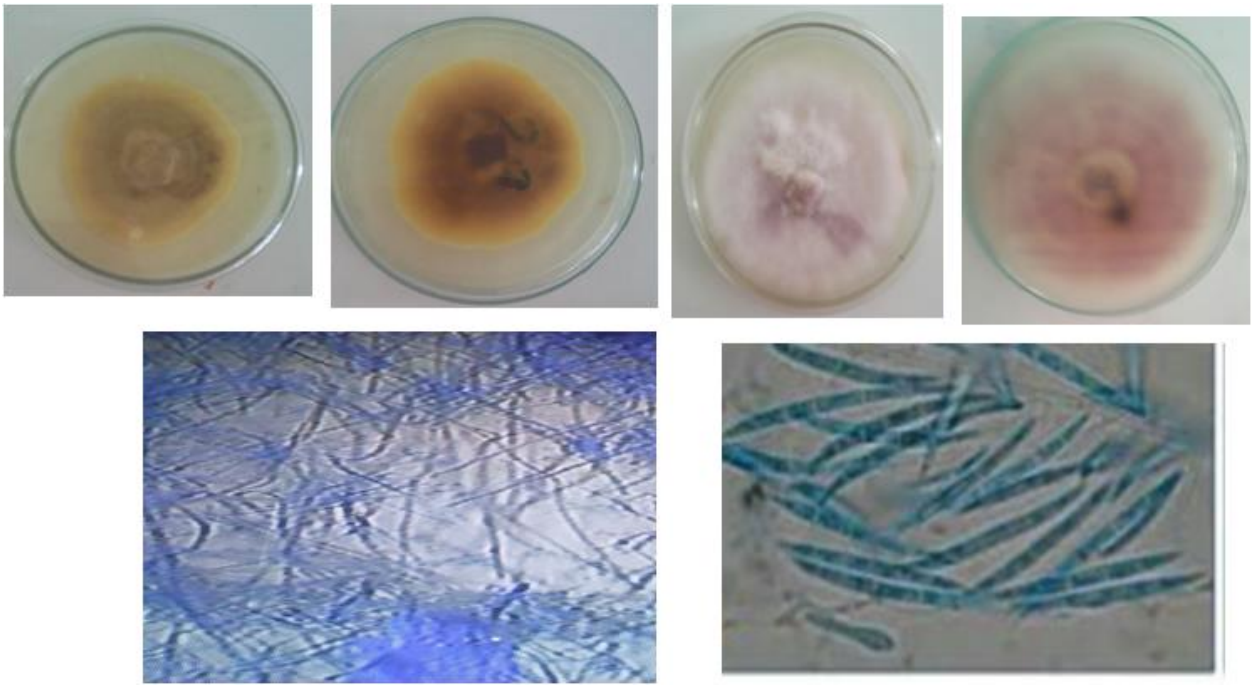
377 ***F*.$_3$ Morphological characteristics** ***F*.$_4$ Morphological characteristics**

378 *F*.5 FRONT VIEW *F*.5 REVERSE VIEW*F*. *F*. 6 FRONT VIEW *F*. 6 REVERSE VIEW



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380 ***F*.$_5$ Morphological characteristics** ***F*.$_6$ Morphological characteristics**

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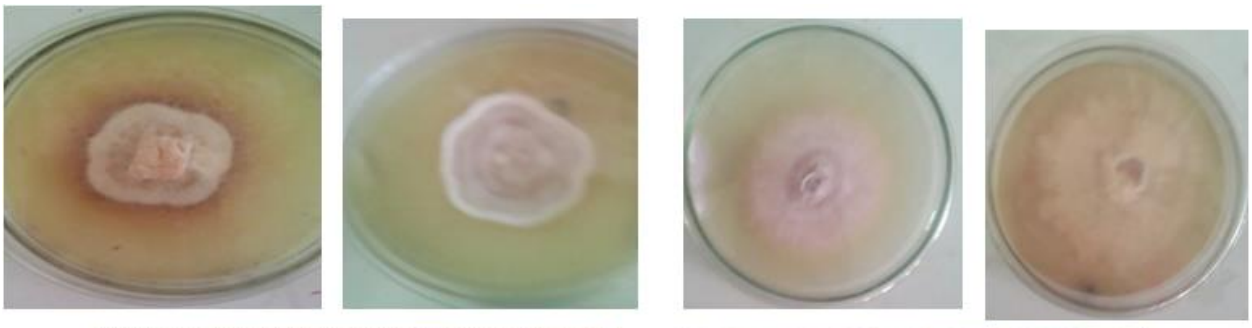
382 *F.*7 FRONT VIEW *F.*7 REVERSE VIEW *F.* 8 FRONT VIEW *F.* 8 REVERSE VIEW



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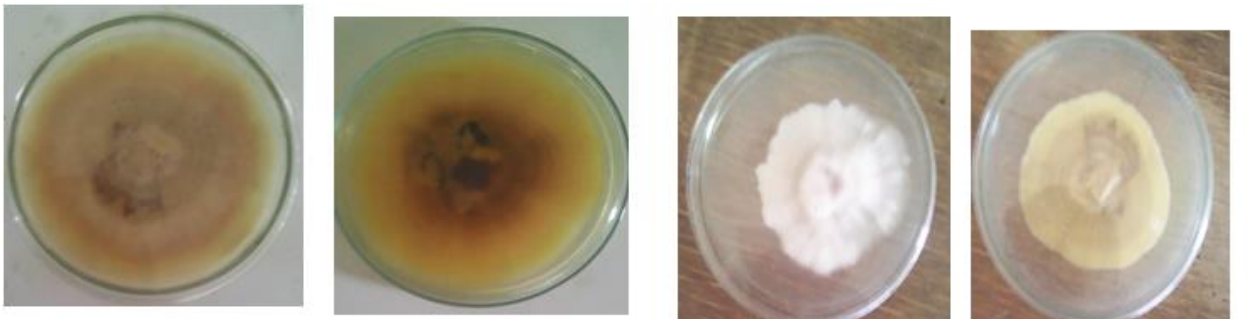
384 ***F.*$_7$ Morphological characteristics** ***F.*$_8$ Morphological characteristics**

385 *F.9* FRONT VIEW *F.*9 REVERSE VIEW *F.* 10 FRONT VIEW *F.* 10 REVERSE VIE



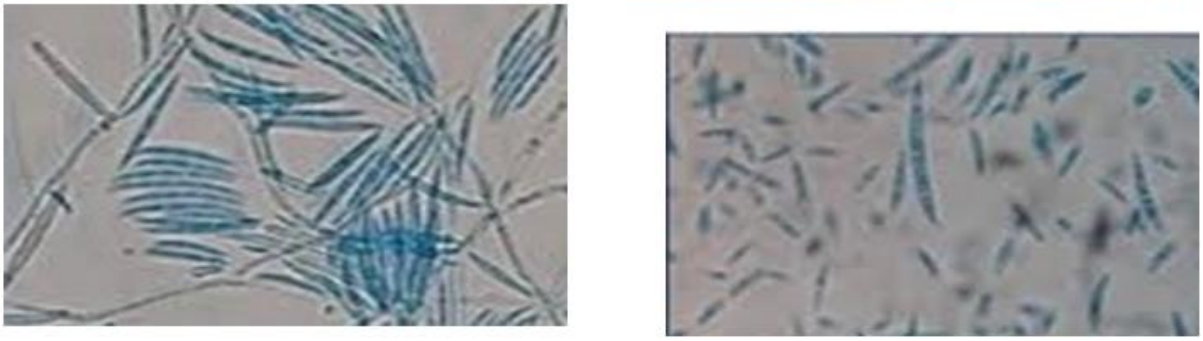


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Figure 7: Morphological Characteristics *Fusarium spp.*

Antifungal activity of the crude extracts

Four crude extracts (acetone and ethanol) were made and tested for antifungal properties against the pathogens under investigation. *Moringa olifera* Lam crude extracts' antifungal properties. Agar well diffusion was used in the lab to test extracts against Fusarium spp. at three different doses. The antifungal properties of several *Moringa olifera* Lam crude extracts. Tables 1 and 2 show information against the mycelium growth of Fusarium spp.

Antifungal activity of acetone crude extracts of *Moringa olifera* Lam. leaf against *Fusarium species.*

Acetone crude extracts of the leaves of *Moringa oleifera* Lam. were tested at three different concentrations i.e. 40 mg/ml, 60 mg/ml, and 80 mg/ml, for its' antifungal activity against the mycelium growth of *Fusarium species.* As indicated in Table 1, the zone of inhibition is directly proportional to the concentrations. Maximum inhibition was observed at 80 mg/ml of concentration which is a 16.33±1.53 mm zone of inhibition, whereas minimum inhibition was observed at 40 mg/ml on Isolates: *F*2, *F*4, *F*7, and *F*9 observed at 11.00±1.00 inhibition zone and Isolates: *F*1, *F*3, *F*5, *F*6, *F*8, and *F*10: 12.00±1.00 mm zone of inhibition. The intermediate concentration i.e. 60 mg/ml on Isolates: *F*2 has a 12.33±0.58 mm zone of inhibition and Isolates: on *F*1, *F*3, *F*4, *F*5, *F*6, *F*7, *F*8, *F*9, and *F*10 observed 15.33±0.58 comparison to positive control which showed 18.33±1.53 mm zone of inhibition. This indicates that positive control has the highest antifungal activity than crude extracts *Moringa oleifera* Lam. leave. The negative control used here, distilled water, showed no inhibition against tested fungal strains. Among the three different concentrations, 60 mg/ml and 80 mg/ml of the acetone crude extracts of *Moringa oleifera* Lam. leaves were highly effective against tested fungal strains, whereas at the concentration of 40 mg/ml, the acetone crude extracts of *Moringa oleifera* Lam. leaves were less effective in comparing with the positive control (Table 1)

Table1. Effects of acetone crude extract of *Moringa oleifera* Lam. leaf and stem bark against the mycelium growth of *Fusarium oxysporum* (mean ± SD, n = 3)

Plant part	Test materials	Control	Concentration (mm)	Test pathogen *(Fusarium spp.)*	Zone of inhibition (mm)=mea± SD, n=3	*P=value*
Leaf	Acetone crude extract	-	40 mg/ml	Isolates: F_2, F_4, F_7, and F_9	11.00±1.00	0.00
				Isolates: F_1, F_3, F_5, F_6, F_8 and F_{10}	12.000±1.00	0.000
			60 mg/ml	Isolates: F_2	12.33±0.58	0.00
				Isolates: F_1, F_3, F_4, F_5, F_6, F_7, F_8, F_9 and F_{10}	15.33±0.58	0.073
			80 mg/ml	All *Fusarium spp*		0.493
Bark	Acetone crude extract	-	40 mg/ml	. Isolates: F_1, F_3, F_4, F_7, F_8 and F_9	16.33±1.53 9.00±1.00	0.00
				Isolates: F_2, F_5, F_6 and F_{10}	6.67±1.155	0.00
			60 mg/ml	Isolates: F_2 and F_8	12.00±1.00	0.00
				Isolates: F_1, F_3, F_4, F_5, F_6, F_7, F_9 and F_{10}	14.67±0.58	0.012
			80 mg/ml	All *Fusarium spp.*	15.67±1.53	0.201

-	Positive control	Copper sulphate	100 µg/ml	All *Fusarium spp.*	18.33±1.53	
-	Negative control	Distilled water	1ml	All *Fusarium spp.*	0.00±00	0.00

SD= standard deviation; mm= millimeter; n = number of experimental replicates, P-value indicate statistical differences between the effect of each extract with the respective concentrations used; *P*-value < 0.05 is taken as significance difference (cut point of the significance value of this study).

The growth inhibitory activities of the acetone crude extract at a concentration of 80 mg/ml are significantly different from that of the negative control (distilled water) ($P < 0.05$) but not of the standard antifungal drugs ($P > 0.05$) (Table 1). This indicates that crude extract of *Moringa oleifera* Lam. showed antifungal effects which are not significantly different from the standard antifungal drugs (Table 1). This indicates that the extract has an inhibitory effect on tested fungal pathogens at a given concentration comparable to that of the standard antifungal drugs (copper sulfate). The reduced mycelial growths of the fungus were considered sensitive to the extract, and those plates with full mycelial growth were considered as resistant. The crude extract of *Moringa oleifera* Lam. at a concentration of 40 mg/ml and 60 mg/ml is significantly different from that of the standard drugs, the significant difference that exists between crude extract at a concentration of 40 mg/ml and 60 mg/ml ($P = 0.00$, and 0.012) and the negative control ($P < 0.05$) may suggest that they have some inhibitory effect against fungal strain tested in this study (Table 1).

Antifungal activity of acetone crude extracts of *Moringa oleifera* Lam. stem bark against *Fusarium* species.

As indicated in Table 1, acetone crude extract of *Moringa oleifera* Lam. Stem bark showed concentration dependent inhibition of mycelium growth of *Fusarium species.* The crude extracts at the concentration of: 40 mg/ml of *Moringa oleifera* Lam. Stem bark extract has produced 9.00±1.00 mm zone of inhibition on $F_{1,}$ $F_{3,}$ $F_{4,}$ $F_{7,}$ $F_{8\ and}$ F_{9} ; at concentration of 60 mg/ml the extract showed effectiveness on $F_{2\ and}$ F_{8} 12.00±1.00mm zone of inhibition and $F_{1,}$ $F_{3,}$ $F_{4,}$ $F_{5,}$ $F_{6,}$ $F_{7,}$ $F_{9\ and}$ F_{10} observed 14.67±0.58 mm zone of inhibition. At

concentration of 80 mg/ml of the extract all 10 of the isolates tested showed 15.67±1.53 mm zone of inhibition as comparison to positive control which was 18.33±1.53 mm zone of inhibition (Table 1).

The growth inhibitory activities of the acetone crude extract at a concentration of 80 mg/ml is significantly different from that of the negative control (distilled water) ($P < 0.05$) but not to the standard antifungal drugs ($P > 0.05$) (Table 1). This indicates that crude extract of *Moringa oleifera* Lam. Stem showed antifungal effects which are not significantly different from the standard antifungal drugs (Table 1). There is a significant difference between the crude extract of *Moringa oleifera* Lam. at the concentration of 80 mg/ml and the negative control (distilled water) but no significant difference between the crude extract at the concentration of 80 mg/ml(P=0.201) and the positive control (copper sulphate) ($P > 0.05$). this indicates that the extract has inhibitory effect on tested fungal pathogen at a given concentration in comparable to that of the standard antifungal drugs (copper sulphate). The reduced mycelial growths of the fungus were considered as sensitive to the extract, and those plates with full mycelial growth were considered as resistant. The crude extract of *Moringa oleifera* Lam. at a concentration of 40 mg/ml and 60 mg/ml are significantly different to that of the standard drugs, the significant difference that exists between crude extract at a concentration of 40 mg/ml and 60 mg/ml ($P = 0.00$, and 0.012) and the negative control ($P < 0.05$) may suggest that they have some inhibitory effect against fungal strain tested in this study (Table 1).

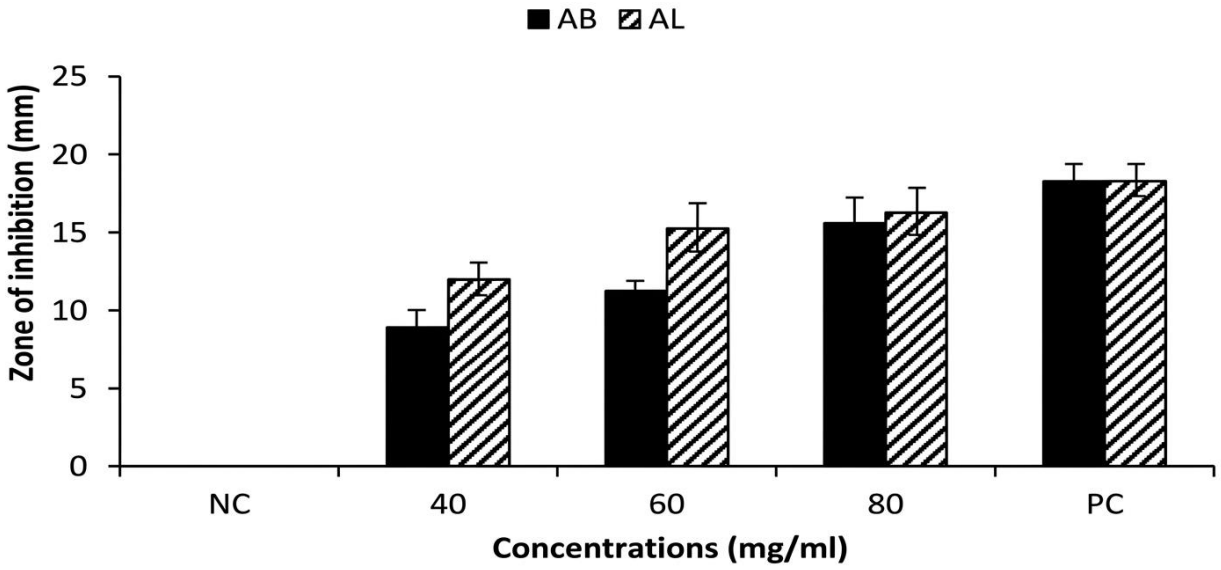

Figure 8: Effect of acetone crude extract of *Moringa oleifera* Lam. leaf and stem bark against *Fusarium species.*

Values represent the mean of triplicate and error bars denoted standard deviation from mean. Where, AL: Acetone Leaf Extract; AB: Acetone Bark Extract; PC: Positive Control; NC: Negative Control.

Antifungal activities of ethanol crude extracts of *Moringa oleifera* Lam. leaf against *Fusarium species.*

The effect of ethanol crude extracts of *Moringa oleifera* Lam leaves showed antifungal activities against the mycelium growth of tested fungal strains at different concentrations i.e. 40 mg/ml, 60 mg/ml and 80 mg/ml. The result presented in Table 2 showed that ethanol crude extracts at the concentrations of 80 mg/ml showed highest antifungal activity with mean inhibition value of 16.00±1.00 mm whereas at 40 mg/ml, ethanol extracts of leaf showed lowest antifungal activity with mean inhibition value of 11.00±1.00 mm. The intermediate concentration of 60 mg/ml, crude extracts has showed inhibition value of 12.33±0.58 mm of zone of inhibition in comparison to positive control which showed mean inhibition value of 18.33±1.53 mm zone of inhibition as indicated in (Table 2; Appendix 3).

Table 2. Effects of ethanol crude extract of *Moringa oleifera* Lam. leaf and stem bark against the mycelium growth of *Fusarium* (mean ± SD, n = 3)

Plant part	Test materials	Control	Concentration (mm)	Test pathogen *(Fusarium spp.)*	Zone of inhibition (mm)=mea± SD, n=3	*P=value*
Leaf	Ethanol crude extract	-	40 mg/ml	Isolates: F_1, F_8 and F_{10}	9.00±1.00	0.00
				Isolates: F_2, F_3, F_4, F_5, F_6, F_7, and F_9	11.33±1.56	0.00
			60 mg/ml	All *Fusarium spp.*	12.33±0.58	0.00
			80 mg/ml	All *Fusarium spp.*	16.00±1.00	0.143
Bark	Ethanol crude extract	-	40 mg/ml	Isolates: F_1 and F_4	11.00±1.00	0.00
				Isolates: F_2, F_3, F_5, F_6, F_7, F_8, F_9 and F_{10}	6.67±1.155	0.00
			60 mg/ml	Isolates: F_1, F_3, F_4, F_5, F_7, F_9 and F_{10}	11.33±1.56	0.00

				Isolates: F_2 , $F_{6 \text{ and }} F_8$	12.000±1.00	0.000
			80 mg/ml	All *Fusarium spp.*	14.67±0.58	0.012
-	Positive control	Copper sulphate	100 µg/ml	All *Fusarium spp.*	18.33±1.53	
-	Negative control	Distilled water	1ml	All *Fusarium spp.*	0.00±00	0.00

SD= standard deviation; mm= millimeter; n = number of experimental replicates, P-value indicate statistical differences between the effect of each extract with the respective concentrations used; *P*-value < 0.05 is taken as significance difference (cut point of the significance value of this study)

The growth inhibitory effect of the ethanol crude extract of *Moringa oleifera* Lam. leaves at 40 mg/ml and 60 mg/ml were significantly different from the negative control, distilled water (*P* = 0.00). There is significance difference between the ethanol crude extracts of *Moringa oleifera* Lam. leaves at 80 mg/ml (*P*=0.143) with negative control (*P*<0.05) but did not showed significance differences with positive control (copper sulphate) (*P*>0.05) at the concentration of 80mg/ml (*P*=0.143). This indicates that the crude extracts at the concentration of 80 mg/ml has anti-fungal activity against *Fusarium* species comparable with positive control (copper sulphate). So that the plant extract (environmentally-friendly) can be used to treat *Fusarium* species potato wilt by replacing the standard fungicidal chemicals.

Antifungal activities of ethanol crude extracts of *Moringa oleifera* Lam. stem bark against *Fusarium species*.

As noted in Table 2 the fungal growth inhibition was concentration dependent. The highest anti-fungal activity was recorded at 80 mg/ml to which all the10 *Fusarium* isolates: showed 14.67±0.58 mm zone of inhibition and the intermediate concentration 60 mg/ml has produced 12.00±1.00 mm zone of inhibition on *F*2, *F*6 and *F*8 and 11.33±1.56 mm zone of inhibition

on *F*1, *F*3, *F*4, *F*5, *F*7, *F*9 and *F*10. On the other hand ethanol crude extracts of *Moringa oleifera* Lam. stem bark at the concentration of 40mg/ml show the lowest inhibition of mycelium growth of *Fusarium species*.

The result indicates that ethanol stem bark extracts at the concentration of 40 mg/ml (P = 0.00) have the lowest inhibitory effect on the tested pathogen. Although the antifungal activities of stem bark extracts at a concentration of 60 mg/ml and 80 mg/ml (P = 0.00 and 0.012 respectively) against the tested fungal species were found to be significantly different from positive controls (P>0.05), in the most cause's a concentration at 80 mg/ml showed higher antifungal activities against *Fusarium species.* When compared with the chemical fungicides copper sulfate (positive controls), all ethanol crude extracts of *Moringa oleifera* Lam. stem bark showed a significantly lower zone of inhibition than the positive control (copper sulfate) (Table 2).

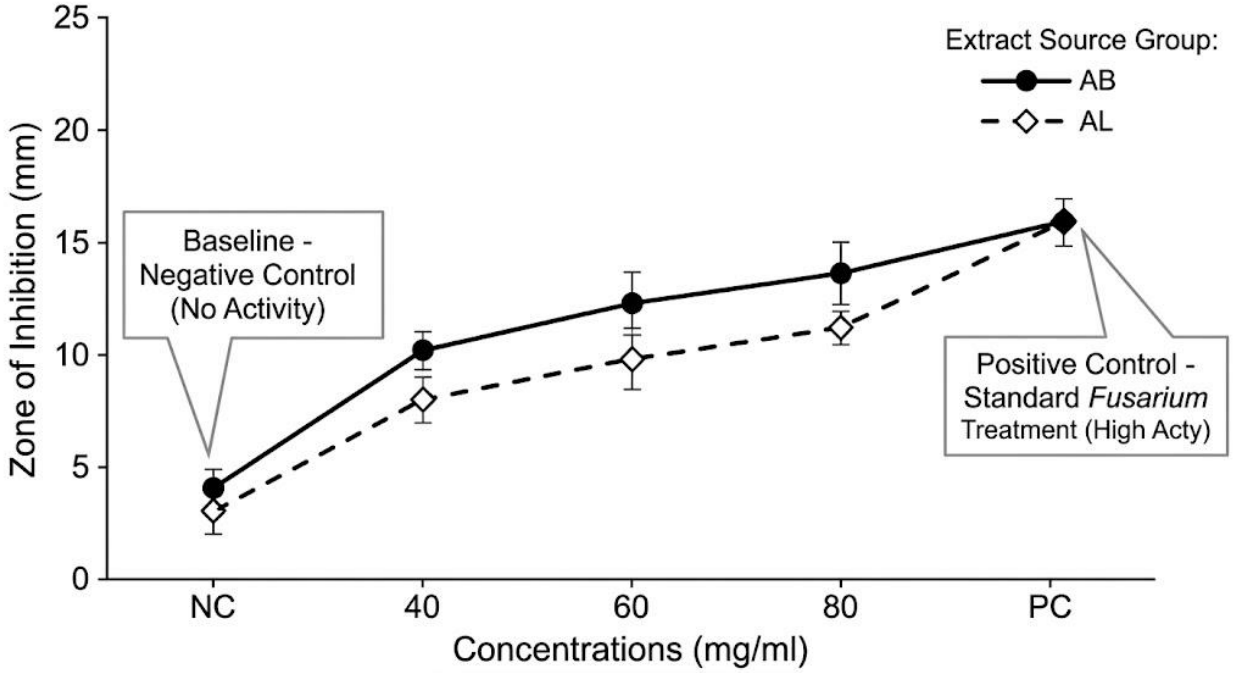

Figure 9: Effect of ethanol crude extract of *Moringa oleifera* Lam. leaf and stem bark against *Fusarium species*.

Values represent the mean of triplicate and error bars denoted standard deviation from mean. Where, EL: Ethanol Leaf Extract; EB: ethanol Bark Extract; PC: Positive Control; NC: Negative Control.

Determination minimum inhibitory concentration of the crude extracts

The minimum inhibitory concentration (MIC) assay was employed to evaluate the effectiveness of the crude extracts that showed antimicrobial activities in the previous tests. The extracts of *Moringa oleifera* Lam. leaves and stem barks were subjected to the concentration range from 100 mg/ml of 50% up to the lowest concentration of 3.125 mg/ml. The MIC results of all the plant part are shown on (Table 3, 4, 5, and 6).

Table 3: Minimum inhibitory concentrations (MIC) of acetone crude extract *Moringa olifera* Lam. Leaf against test organism (*Fusarium species*)

Test organism	*Moringa oleifera* Lam. acetone leaf extract (mg/ml)					
	100	50	25	12.5	6.25	3.125
Fusarium species	++++	+++	+++	+	+	-

- = none effective, + = weak effect, +++ = strong effect

As shown from table 3 the MIC value of acetone crude extract of *Moringa oleifera* Lam. leaf against on *Fusarium* isolates is 6.25 mg/ml.

Table 4: Minimum inhibitory concentrations (MIC) of acetone crude extract *Moringa olifera* Lam. stem bark against test organism (*Fusarium species*).

Test organism	*Moringa oleifera* Lam.acetone stem bark extract (mg/ml)					
	100	50	25	12.5	6.25	3.125
Fusarium species	+++	+++	++	+	-	-

551 - = none effective, + = weak effect, +++ = strong effect

552 As shown from table 4 the MIC value of acetone bark extract of *Moringa oleifera* Lam.
553 Against on *Fusarium* isolates is 12.5 mg/ml. This indicates that acetone bark extracts against
554 the test pathogens confirm the low activity of the extract from that particular plant part at low
555 concentrations.

556 **Table 5: Minimum inhibitory concentrations (MIC) of ethanol crude extract *Moringa***
557 ***oleifera* Lam. stem bark against test organism (*Fusarium specie).***

Test organism	*Moringa oleifera* Lam. ethanol leaf extract (mg/ml)					
	100	50	25	12.5	6.25	3.125
Fusarium species	++++	+++	++	+	-	-

558 - = none effective, + = weak effect, +++ = strong effect

559 As shown from table 5 the MIC value of ethanol leaf extract of *M. oleifera* Lam. against on
560 *Fusarium* isolates is 12.5 mg/ml. The MIC value of ethanol leaf extract of *Moringa oleifera*
561 Lam. against *Fusarium* isolates is 12.5 mg/ml. As denoted in the above tables, the acetone
562 crude extract of the leaves of *Moringa oleifera* Lam. exhibited the lowest MIC at 6.25 mg/ml
563 against *Fusarium species* (Table 3).Whereas, the MIC value produced by ethanol crude
564 extract of *Moringa oleifera* Lam. Leaf was . 12.5 mg/ml against *Fusarium species* (table 5).

565 **Table 6: Minimum inhibitory concentrations (MIC) of ethanol crude extract *Moringa***
566 ***oleifera* Lam. stem bark against test organism (*Fusarium species)***

Test organism	*M. oleifera* Lam. ethanol stem bark extract (mg/ml)					
	100	50	25	12.5	6.25	3.125
Fusarium species	++++	+++	+	-	-	-

567 - = none effective, + = weak effect, +++ = strong effect

568 As shown from table 6 the MIC value of ethanol stem bark extract of *Moringa oleifera* Lam.
569 against on *Fusarium* isolates is 25 mg/ml. As denoted in the above tables, the acetone crude
570 extract of the leaves of *Moringa oleifera* Lam. exhibited the lowest MIC at 6.25 mg/ml
571 against *Fusarium species* (Table 3). Whereas, the MIC value produced by ethanol crude

572 extract of *Moringa oleifera* Lam. stem bark was recorded at highest concentration i.e. 25
573 mg/ml against *Fusarium species* (Table 6).

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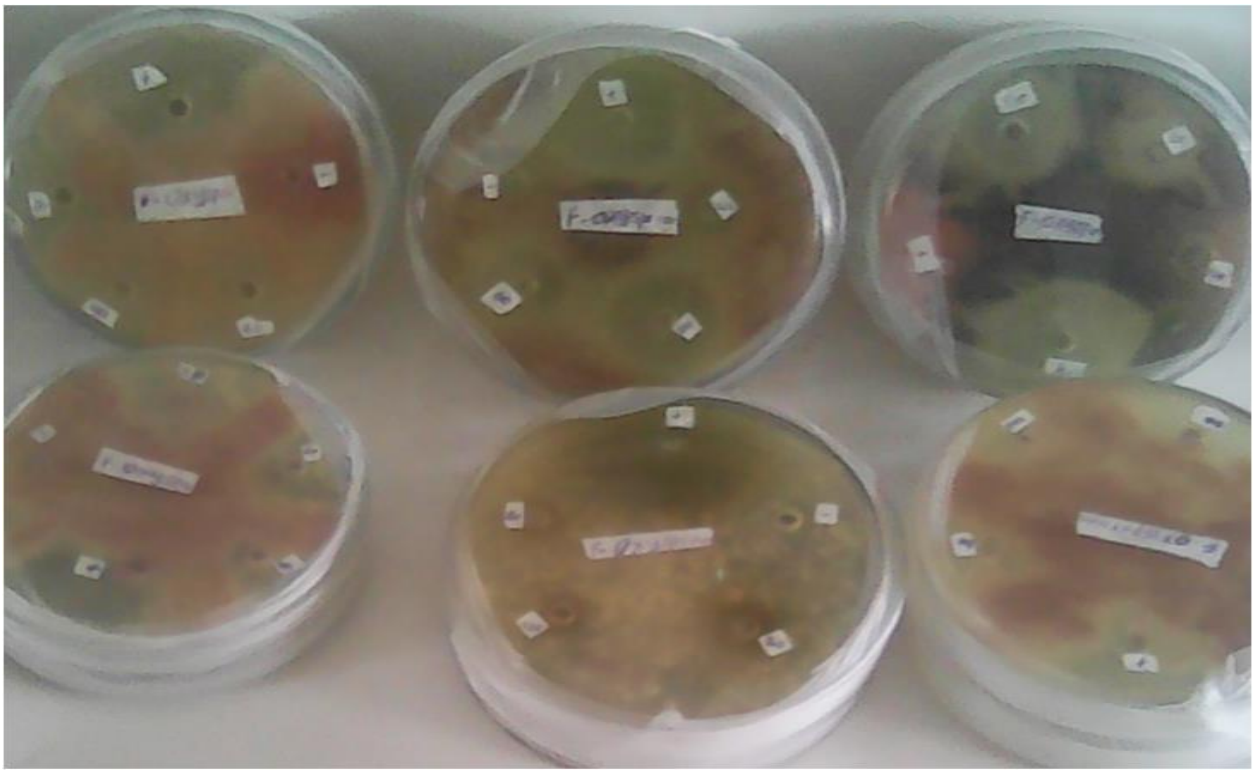
575 **Antifungal susceptibility tested results.**



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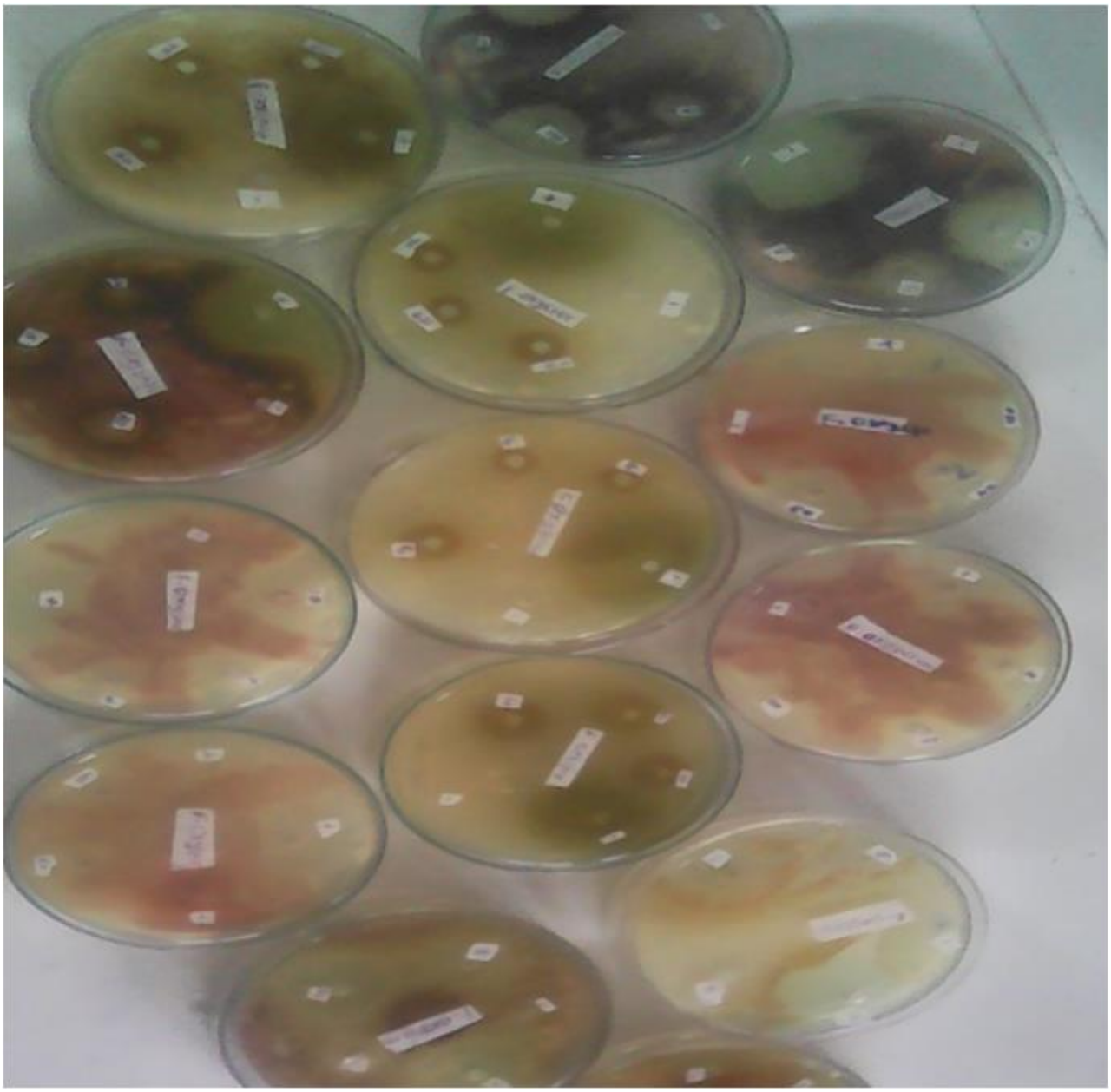

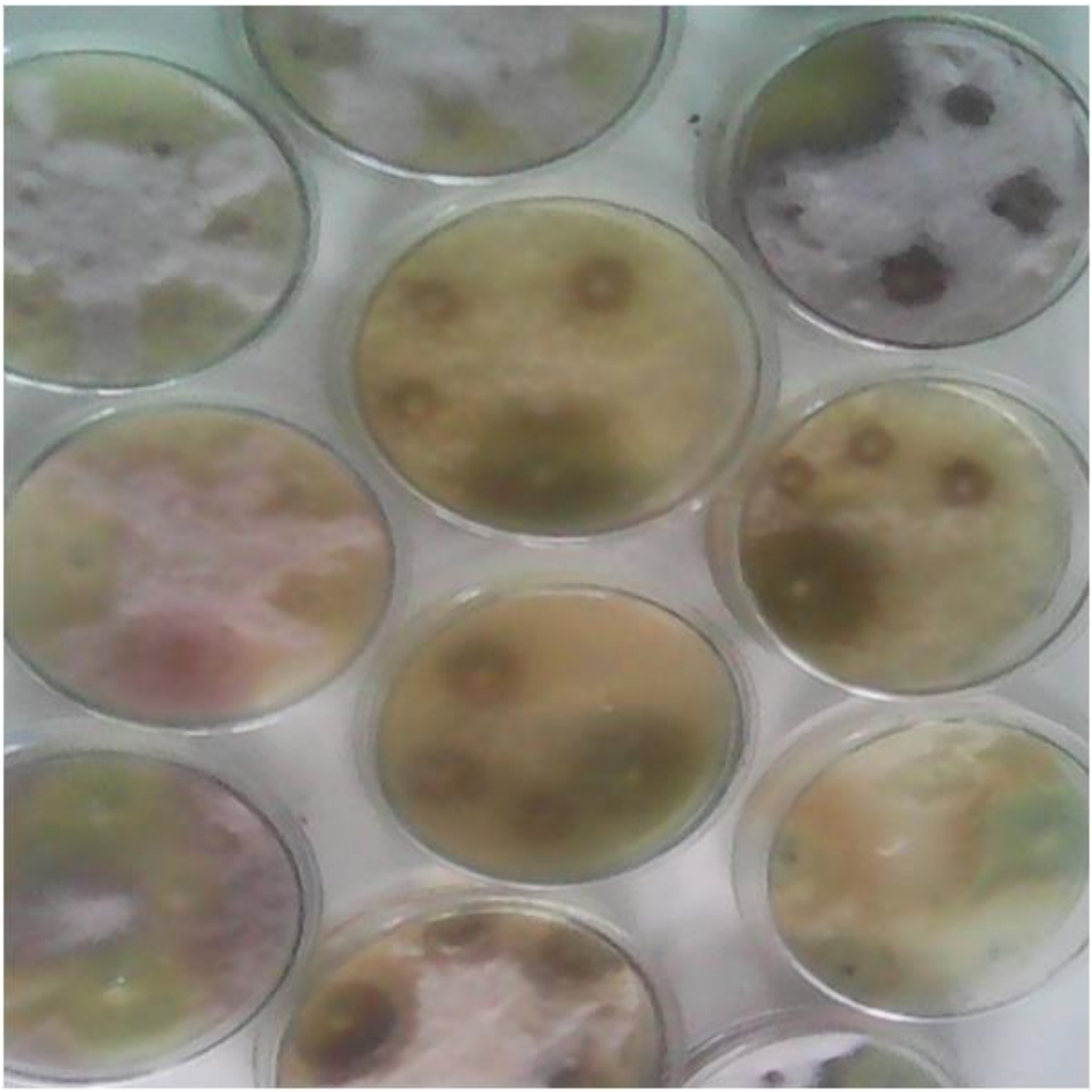

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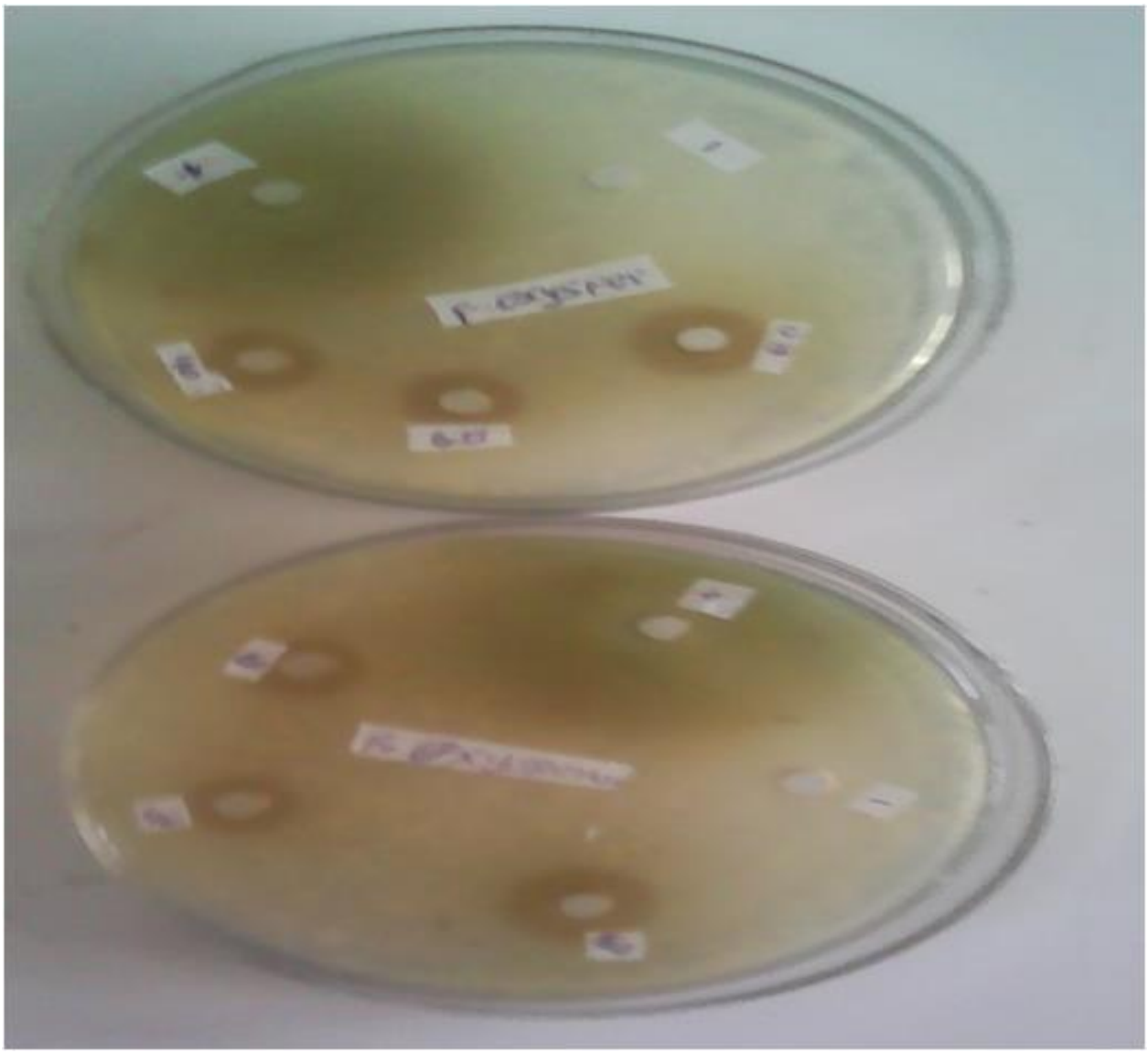

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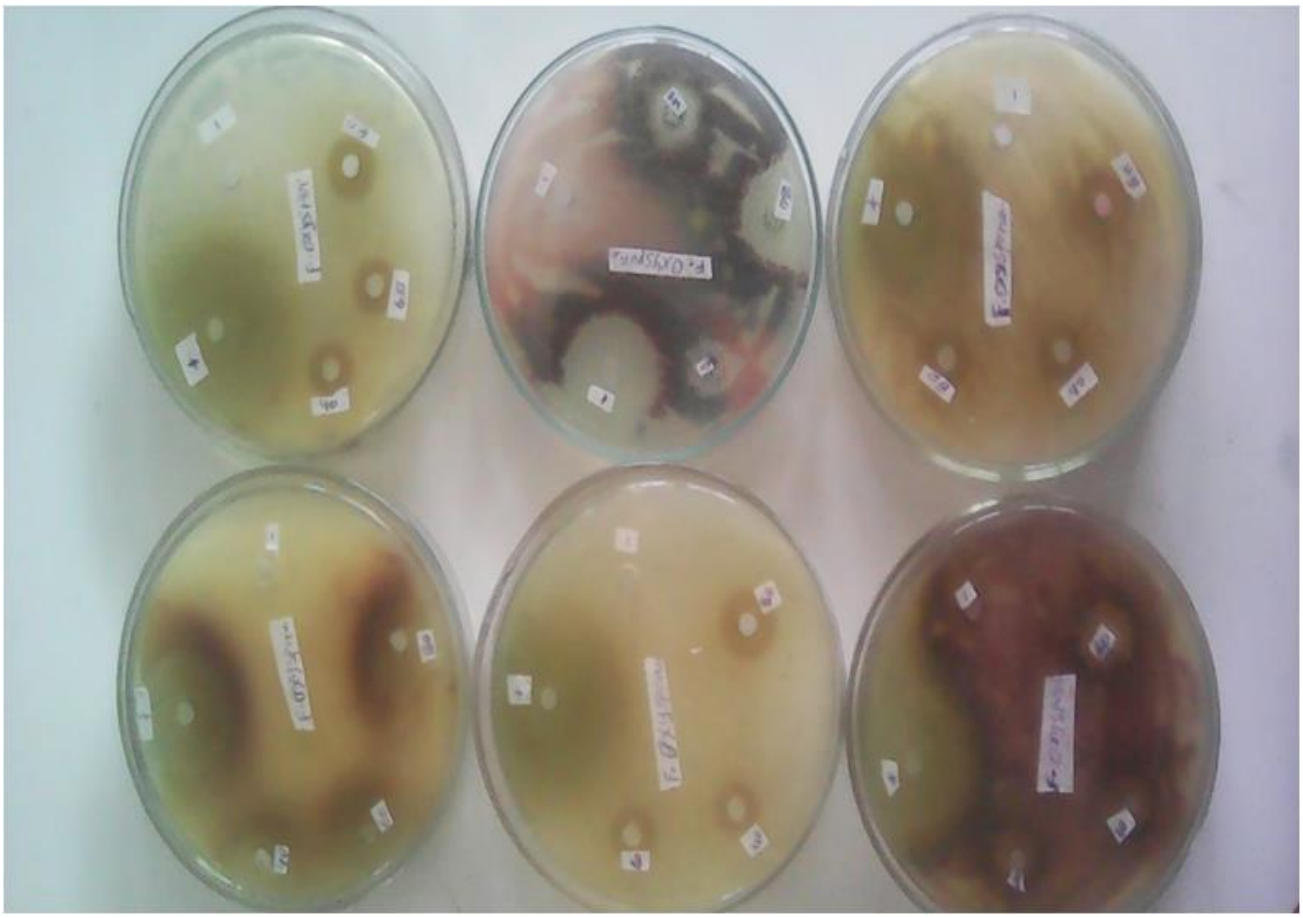


Figure 10: **Antifungal susceptibility test results.**

The results of the present study are discussed below.

Different phytochemical compounds would be more or less soluble in different types of solvents. The findings of Ncube et al. (2008), who reported methods for evaluating the antibacterial activities of naturally occurring substances derived from plants, lend credence to this. This suggests that the target chemical composition of the crude extracts meant to be prepared for antibacterial activity is influenced by the solvent selection. Secondary metabolites function as antimicrobial substances, inhibiting or eliminating the infections through several ways. Nevertheless, the mechanism underlying the plant extract's inhibition of mycelium growth was left for further investigation. *Moringa olifera* Lam. leaf and stem bark crude extract exhibited concentration-dependent antifungal activity. High concentrations often shown superior antifungal effectiveness against *Fusarium* species mycelium growth. The study's findings unequivocally demonstrate that plant parts, such as leaves and stem bark, had antifungal efficacy against the pathogens under research at all tested concentration. The tested

606 *Moringa olifera* Lam's antifungal properties increased as the concentration rose. The study's
607 findings demonstrated that the better antifungal efficacy of acetone crude extracts of *Moringa*
608 *olifera* Lam. leaves against Fusarium species at a concentration of 80 mg/ml. Nevertheless,
609 the effect was not greater than the positive control. The findings demonstrated that using plant
610 extracts as natural fungicides can reduce the need for synthetic fungicides for controlling plant
611 pathogenic fungi This finding was consistent with the works of Moyo *et al*., (2012) who
612 report about antimicrobial activities of *Moringa oleifera* Lam. leaf extracts, and Talreia,
613 (2010) who reports crude extract of flavonoids of *Moringa oleifera* Lam. against bacterial and
614 fungal pathogen. Their report shows that the increase in concentration of the crude extracts,
615 resulted in the increase of zone of inhibition. The results of this finding were also in
616 conformity with El-Mohamedy *et al*., (2014) who, reported the antifungal activity of *Moringa*
617 *oleifera* Lam. extracts as natural fungicides against some plant pathogenic fungi *In-vitro.* The
618 effectiveness of the antifungal activities of the plant also varied according to the examined
619 plant parts. In this study, the crude extracts of *Moringa olifera* Lam. leaf and stem bark
620 showed a significant growth inhibition against the tested fungal species. The result of this
621 study showed that the acetone crude extract of leaves was more potent than ethanol crude
622 extracts. The difference in activity could be related to the different secondary metabolites
623 presented and extracted from each part of the plant. Whereas the ethanol crude extracts of the
624 plant illustrated less antifungal activity compared to acetone extracts and positive control
625 (copper sulfate).

626 The antifungal activities of the ethanol and acetone extracts of the two parts of the plant
627 against the test pathogens varied. The findings of this study revealed that the plant parts
628 extracted by acetone provided more consistent antifungal activities compared with those
629 extracted using ethanol. This result agreed with the findings of Neela *et al.,* (2014) who
630 reported antifungal activities of selected medicinal plant extract against *Fusarium oxysporum.*
631 The result of this study also showed that greater antifungal activities were recorded by leaf
632 extracts and this suggests that more of the bioactive ingredients are lodged in these parts. This
633 agreed with the work of Abdulkadir *et al.,* (2015) who report the antifungal activity of
634 *Moringa oleifera* extracts as a natural fungicide against some plant pathogenic fungi *In-vitro*.
635 Moyo *et al*., (2012) reported antimicrobial activities of *Moringa oleifera* lam leaf extracts.

The works mentioned so far may serve as supportive evidence for the presence of antifungal properties in *Moringa olifera* Lam. leaves and stem bark reported in the present study.

The antifungal activity of acetone crude extract of *Moringa olifera* Lam. leaves against *Fusarium* isolates was tested at various concentrations i.e. 40 mg/ml, 60 mg/ml, and 80 mg/ml. These three concentrations showed inhibitory activity on the mycelium growth of *Fusarium* isolates at 40 mg/ml concentration mean inhibition value was observed at 12.000±1.00 and 11.00±1.00 mm, at 60 mg/ml; 15.33±0.58 mm and 12.33±0.58 at60 mg/ml 16.33±1.53 mm inhibition zone respectively. Acetone crude extracts of stem bark of the plant at the respective concentration were observed to have little antimicrobial activities against the tested fungal species with diameters of zones of inhibition of at 40 mg/ml; 9.00±1.00mm and 6.67±1.155, at 60 mg/ml 12.00±1.00 and 14.67±0.58mm and 80 mg/ml; 15.67±1.53mm. Ethanol crude extracts of stem bark of the plant at the respective concentration showed diameters of zones of inhibition of 6.67±1.15 mm, 12.00±1.00 mm, and 14.67±0.58 mm and the leaf extract showed 11.00±1.00 mm, 12.33±0.58 mm, 16.00±1.00 mm zone of inhibition. The standard antibiotics used in this experiment showed the highest antifungal activities and the diameter of the zone of inhibition was recorded (18.33±1.53 mm). In the present study, the antifungal properties were found to be best from leaves extract of the *Moringa olifera* Lam. than that of stem bark.

The minimum inhibitory concentration (MIC) value of acetone and ethanol crude extracts of *Moringa olifera* Lam. leaves and stem barks against the tested Fusarium isolates ranged from 3.125 mg/ml to 200 mg/ml. The minimum inhibitory concentration (MIC) assay was employed to evaluate the effectiveness of the crude extracts that showed significant antimicrobial activities in the previous tests. Among the crude extract of *Moringa olifera* Lam. acetone extracts of the leaf had the lowest MIC value 6.25 mg/ml. on the other hand, the highest MIC value was exhibited by ethanol extracts of *Moringa olifera* Lam. stem bark against the mycelium growth of *Fusarium* isolate*s* with the minimum inhibitory concentration (MIC) value of 25 mg/ml. The relatively low minimum inhibitory concentration values recorded for the leaf acetone extracts of *Moringa olifera* Lam. against the test pathogens confirm the high activity of the extract at low concentrations. Extracts with lower MIC scores are very effective antimicrobial agents. In this study, the crude extract of *Moringa olifera*

Lam. leaves has the least activity against the tested pathogen. This result can be supported by Abdulkadir *et al.,* (2015) that had reported similar results for the antimicrobial activities of ethanolic extracts of *Moringa oleifera* Lam. on some pathogens. All the above reports were also estimated to confirm the observed inhibitory effect of *Moringa olifera* Lam. extracts in this study. In general, the antimicrobial effect of *Moringa olifera* Lam. leaves involved a comparison of the acetone crude extract with the commercially available standard antifungal drug by comparing the mycelia growth inhibition zones. The result noted that the commercially available standard antifungal drug showed a larger inhibitory effect than the *Moringa olifera* Lam. leaf acetone extract. As a result, acetone crude extracts of Moringa olifera Lam. leaves were found to be the most effective, and it may be a promising material for managing diseases caused by this fungus. The extract's broad spectrum of activity was demonstrated by its activity against the tested fungal strain; as a result, it can be used to obtain antibiotic components for drug development that can be employed to control potato fungal wilt disease.

4. CONCLUSION AND RECOMMENDATIONS

4.1. Conclusions

For a very long time, wilts, particularly those caused by fungal pathogens (Fusarium species), have posed a serious threat to potato yield. People are attempting to solve these problems through both conventional and scientifically validated approaches. There have been reports of issues with using synthetic treat to treat fungal wilts, primarily because the fungus has evolved resistance. Chemical fungicides are hazardous to humans and other animals, toxic to non-target organisms, and damage the environment**.** Besides, due to affordability and accessibility of these drugs, people opt to rely on traditionally available substances around them. In this regard higher plants take the major part of the sources. Based on the results of this study it could bring further evidence that the *Moringa olifera* Lam. plants extracts such as leaves and stem bark, have the potential of becoming powerful and safe alternative means of disease control instead of the harmful synthetic fungicides.

- The use of crude extracts of this plant could alleviate the problems of potato wilt disease by inhibiting the mycelium growth of *Fusarium species*.

- Therefore, the findings of the present study revealed that *Moringa olifera* Lam. exhibited antimicrobial effect by the crude extracts against the fungal strain (*Fusarium species*).
- The antimicrobial effect of the crude extract of each solvent (acetone and ethanol) was found to be concentration dependent or the nature of the plant part phytochemical content against the tested pathogens.
- In the present study, crude extracts of *Moringa olifera* Lam. leaves and stem bark showed antimicrobial effect on the selected fungal strains in which those extracted with weak polar solvents (acetone) having high effects while those extracted with polar solvent (ethanol) lower effect.

5.2. Recommendation

The present research work attempted to fill the gap of knowledge about the antifungal activity of *Moringa olifera* Lam. Based on the conclusion of the results of the experiments conducted in the present study, the following points are recommended:

1. Since the present study focused only on the antifungal activities of crude extract of *Moringa olifera* Lam.leaf and stem bark, similar studies need to be done on other parts of the plant.
2. In order to widen the knowledge of antimicrobial effect of this plant, similar researches have to be carried out on other pathogenic microbes other than (*Fusarium species*) used in the present study.
3. Only crude extracts from the plant were tested for its effect on mycelium growth of *Fusarium species*. Hence, further study has to be conducted in order to isolate the biochemical compounds, purify the antifungal constituents from *Moringa olifera* Lam.extracts and test active principles for such activity.
4. Finally, great effort and attention should be given to conserve this plant through informing and educating people about its medicinal values.

Data availability confirmation statement

All the data were presented in the manuscript.

Declaration of interest statement

There is no competing interest pertaining to this article.

Funding: No fund was received

REFERENCES

Abdulkadir, I., Nasir, I., Sofowora, A., Yahaya, F., and Ahmad, A. (2015). Phytochemical screening and antimicrobial activities of ethanolic extracts of *moringa oleifera* lam on isolate of some pathogens. *Journal of Applied Pharmacy,***7** (4): 1-7.

Adane Abraham (2009). Agricultural biotechnology research and development in Ethiopia.*African Journal of Biotechnology*, **8**(25): 7196-7204.

Adnew A., Shifa H., Mohammed A. (2022). Antifungal activity of plant extracts against postharvest mould fungi associated with coffee (Coffea arabica L.) in Bale Zone. Ethipion Journal of Agricultural Science,12:107–124

Aktaruzzaman, M., Sheng, J., Jae, H., Young, H and Byung, S. (2014). First report of potato stem-end rot caused by *Fusarium oxysporum* in Korea.*Journal of Mycobiology,* **42**(2): 206– 209.

Al-Hatmi A M. S., Curfs-Breuker I, Sybren de Hoog G. Meis J F. and Verweij E. 2017 Antifungal Susceptibility Testing of Fusarium: A Practical Approach J. Fungi, 3, 19; doi:10.3390/jof3020019

Anwar, F., Latif, S., Ashraf, M., and Gilani, A. (2007).*Moringa oleifera* a food plant with multiple medicinal uses.*Phytochemistry Research*, **21**: 17-25.

Ashfaq, M., Basra, S., and Ashfaq, U. (2012). Moringa: A miracle plant of agro-forestry. *Journal of Agriculture Science,* **8**: 115-122.

Ayed, F. (2005). Fusrium wilt of potato: cultivars behavior and chemicals and biological control. Master of Science on plant and environmental protection. Horticulture of chott nmrium, Tunisia, p: 85.

Ayed, F., Daami, R., Jabnoun, K., and El-mahjoub, M. (2006). Potato vascular wilt in Tunsia; incidence and biocontrol by Trichderma spp. *Journal of Plant Pathology,***5**(1): 92-98.

Balunas, M., and Kinghorn, A. (2005). Drug discovery from medicinal plants.*Journal of Life Science,* **78**: 431-441.

Bawa, I. (2016). Management strategies of *Fusarium* wilt disease of tomato incited by *Fusarium*

oxysporum f. sp. lycopersici (Sacc.). International Journal of Advanced Academic Research, **2**(5): 1-11.

Berdy, J. (2005).Bioactive microbial metabolites.*Journal of Antibiotcs,* **58**(1): 1–26.

Berhe Tadesse, Minaleshewa Atlabachew and Kebede Nigussie Mekonnen.(2015). Concentration levels of selected essential and toxic metals in potato (*Solanum tuberosum* L.) of West Gojjam, Amhara Region, Ethiopia.*Springerplus.***4**: 514.

Bokshi, A., Morris, S., and Deverall, B. (2003). Effect of benzothiadizol and acetylsalicylic acid on fusarium diseases of potato.*Plant Pathology,* 52: 22– 27.

Butler, M. (2004).The role of natural product chemistry in drug discovery.*Journal of Natural Products,***67** (12): 2141– 2153.

Choueiri, E., El-Zammar, S., Jreijiri, F., Mnayer, D., Massaad, R., Saad, A., Hanna, L. and Varveri,
C. (2004).Phytosanitary status of potato in the Bekaa valley in Lebanon. *Journal of Agriculture,* **34**: 117-121.

Daami, M., Jabnoun, H., Ayed, F., and El-Mahjoub, M. (2011).Comparative aggressiveness on *Verticillium dahliae*, *Verticillium albo-atrum* and *Verticillium tricorpus* on potato as measured by their effects on wilt severity, plant growth and subsequent yield loss. *Journal of Plant Science and Biotechnology*, **5**: 1-8.

Daljit, S., Jemimah, O., and Harpreet, K. (2013). Bioprospecting of *Moringa (Moringaceae):* microbiological perspective.*Journal of Pharmacology and Phytochemistry,***1**(6): 193-215.

Deepa, H., and Kanika, S. (2014).A Review: Plant extracts a new source of antimicrobial potential.
*Research Journal of Pharmaceutical, Biological and Chemical Sciences,***5**(3): 597-628.

Devendra, B., Srinivas, N., Prasad, V., and Swarna, P. (2011). Antimicrobial activities of *Moringa oleifera* (Lam.) leaf extract, against selected bacterial and fungal strains. *International Journal of Pharmacology and Biological Sciences,* **2**(3): 234-242.

Dwivedi, S and Enespa, A. (2012).Effectiveness of extract of some medicinal plants againstsoil-borne fusaria causing diseases on lycopersicon esculantum and solanum melongena plants.*International Journal of Pharmacology and Bio Sciences,* **3**(4): 1171 – 1180**.**

Elizabeth Avigdor, Hans Wohlmuth , Zemede Asfaw and Tesfaye Awas (2014). The current status of knowledge of herbal medicine and medicinal plants in Fiche, Ethiopia.*Journal of*

Ethno-biology and Ethno-medicine, **10**: 38.

El–Mohamedy, R. Aboelfetoh, M., and Abdalla, A. (2014). Evaluation of antifungal activity of *Moringa oleifera* extracts as natural fungicide against some plant pathogenic fungi *In-vitro.Journal of Agricultural Technology,* **10**(4): 963-982

Ephrem, G. (2015).Disease management practice on potato (*solanum tuberosum*l.) in Ethiopia.*World Journal of Agricultural Research,* **3**(1): 34-42.

Farkhondeh, O., Masoud, Z., and Alireza, M. (2013).Biological control of *Fusarium* wilt of potato (*Fusariumoxysporum* f. sp. *tuberosi*) by *Trichoderma* isolates under fieldcondition and their effect on yield. *Journal of Crop Protection.***2** (4): 435-442.

Ganie, S., Mudasser, Z., Surender, S., Surender, S., Ranjana, S., and Sujata, G.(2015).Antifungal efficacy of *Moringa oleifera* (Lam.).*American Journal of Phyto-medicine and Clinical Therapeutics,***3**(01): 028-033.

Garima, M., Pradeep, S., Ramesh, V., Sunil, K., Saurabh, Jha, K., and Khosa, L. (2011). Traditional uses, phytochemistry and pharmacological properties of*Moringa oleifera* plant. *An overview Scholars Research Library,***3**(2): 141-164.

Garrett, S. D. 1970. Pathogenic root - infection fungi. Cambridge University Press, New York

Geremew Tafesse and Yalemtsehay Mekonenn (2012).Anti-diarrheal activity of leaf extract of *Juniperusprocera* and its effect on intestinal motility in albino mice. *Journal of Medical and Biomedical Sciences*, **1**(4): 7-12.

Getachew Addis, Dawit Abebe , Kelbesa Urga (2001). A survey of traditional medicinal plants in Shirka District, Arsi Zone, Ethiopia.*Ethiopian Journal of Pharmacology,***19**: 30–47.

Gildemacher, P., Kagnongo, W., Ortiz, O., Tesfaye, Agajie., Woldegiorgis Gebremedhin., Wagoire, W., Kakuhenzira, R., Kinyae, P., Nyongesa, M., and Struik, P. (2009). Improving potato production in Kenya, Uganda and Ethiopia.*Potato Research*, **52**: 173-205.

Gomez AK, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. Paperback John Wiley and Sons New York, Chichester, pp 680

Gurjar, M., Ali, S., Akhtar, M., Singh, K. (2012). Efficacy of plant extract in plant disease control. *Journal of Agricultural Science*, **3**: 425-433.

Gurib, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects Medicine*, **27**: 1 - 93.

817 Hafizi, R., Salleh, B., and Latiffah, Z. (2013).Morphological and molecular characterization
818 of *Fusarium solani* and *Fusarium oxysporum* associated with crown disease of oil palm.
819 *Journal of Microbiology,* **44**(3): 959–968.
820 Hammer, K., Carson, C., and Riley, T. (1999). Antimicrobial activity of essential oils and other
821 plant extracts. *Journal of Applied Microbiology,***86**(6): 985.
822 Harborne JB, (1984). Phytochemical methods, 2nd edition, Chapman and Hall publications,
823 London, New York, pp. 288.
824 Hegde, H. (2015). An overview of *Moringa* production in Ethiopia.*International Journal of*
825 *Science and Research,* **4**(4): 6-14.
826 *Ibrahim, O.H.M., Abdul-Hafeez, E.Y. (2023). The Acetone Extract of Albizialebbeck Stem*
827 *Bark and its In Vitro Cytotoxic and Antimicrobial Activities. Horticulturae, 9, 385.*
828 Igbinosa, O., Igbinosa, E., and Aiyegoro, O. (2009). Antimicrobial activity and phytochemical
829 screening of stem bark extracts from *Jatrophacurcas (Linn). African Journal of*
830 *Pharmacology*, **3**(2): 058-062.
831 *Jain SK and Rao RR. (1976). A handbook of field and herbarium methods. Today and*
832 *Tomorrow's Printers and Publishers, New Delhi, India*
833 Jennifer, M. (2001).Determination of minimum inhibitory concentration.*Journal of antimicrobial*
834 *chemotherapy,* **48**: 5-16.
835 Kagale, S., Marimuthu, T., Thayumanavan, B., Nandakumar, R., and Samiyappan, R.
836 (2004).Antimicrobial activity and systemic resistance of plant.*Physiology and Molecular*
837 *Plant Pathology,* **65**: 91–100.
838 Kebede T., Gadisa E. and Tufa A. (2021). Antimicrobial activities evaluation and
839 phytochemical screening of some selected medicinal plants: a possible alternative in the
840 treatment of multidrug-resistant microbes. PLoS One, 16(3).
841 Kerkeni, A., Daami, R., Khedher, M. (2013). In vivo evaluation of compost extracts for the
842 control of the potato *Fusarium* wiltcaused by *Fusarium oxysporum f. sp. African* Journal *of*
843 *Plant Science and Biotechnology*, **7**: 36-41
844 Khoury, C., Bjorkman, A., Dempewolf, H., Ramirez, J., Guarino, L., and Jarvis, A.
845 (2014). Increasing homogeneity in global food supplies and the implications for food
846 security. *Processing National Academy of Science*, **111**: 4001–4006.
847 Lopez, V. (2011). Are traditional medicinal plants and ethnobotany still valuable approaches in

pharmaceutical research? *Ethnobotany in pharmaceutical research,* **10** (1): 3 – 10.

Lutaladio, N., and Castaldi, L. (2009). Potato: the hidden treasure. *Journal of Food,***22**: 491–493.

Luis, S., Rubio, C., Gonzalez, D., Gutierrez, J., Revert, C., and Hardisson, A. (2011). Comparative study of the mineral composition of several varieties of potatoes (*Solanum tuberosum* L.) from different countries cultivated in Canary Islands (Spain). International *Journal of Food Science Technology,***46**: 774–780.

Mamta, J., Rashmi, Srivastava, A., Sharma, k., and Anil P. (2013). Isolation and characterization of Fusarium oxysporum, a wilt causing fungus, for its pathogenic and non-pathogenic nature in tomato *(Solanum lycopersicum*). *Journal of Applied and Natural Science.* 5(1): 108-117.

Marshall, E. M., Alois, AB, and Beckham, C. H. 1981. Fungal Wilt diseases of plants, Academic press, New York

Masika, P and Afolayan, A. (2002). Antimicrobial activity of some plants used for treatment. *International Journal of Antimicrobial Agents*, **26**(5): 129-134. Mamta, J., Rashmi, Srivastava, A., Sharma, k., and Anil P. (2013). Isolation and characterization of Fusarium oxysporum, a wilt causing fungus, for its pathogenic and non pathogenic nature in tomato *(Solanum lycopersicum*). *Journal of Applied and Natural Science.* 5(1): 108-117.

Mengistu Ketema., Degefu Kebede., Nigussie Dechssa., and Feyisa Hundessa (2016). Determinants of adoption of potato production technology package by smallholder farmers: evidences from eastern Ethiopia. *Review of Agricultural and Applied Economics,* **19**(2): 61–68.

Morton, J. (1991). The horseradish tree, *Moringa (Moringaceae)*: a boon to arid lands. *Journal of Botany*, **45**: 318-333.

Moyo, B., Masika, P., and Muchenje, V. (2012). Antimicrobial activities of *Moringa oleifera* (Lam.) leaf extracts. *African Journal of Biotechnology*, **11**(11): 2797-2802.

Ncube, N., Afolayan, A., and Okoh, A. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends *African Journal of Biotechnology*, **7**(12): 1797-1806.

Neela, F., Sonia, I., and Shamsi, S. (2014). Antifungal activity of selected medicinal plant extract on *Fusarium oxysporum schlechtthe* causal agent of *Fusarium* wilt disease in tomato. *American Journal of Plant Sciences,* **5**: 2665-2671.

879 Nel, B., Steinberg, C., Labuschagne, N., and Viljoe, A. (2007). Evaluation of fungicides and
880 sterilants for potential application in the management of *Fusarium* wilt of banana.*Journal*
881 *of Crop Protection*, **26**: 697-705.
882 Nene, Y. (2013).Asian Agricultural history foundation.*Progressive report,***7**: 185-201.
883 Newman, D., Crag, G., and Snader, K. (2000).The influence of natural products upon drug
884 discovery.*Natural Product Reports*, **17**: 215-234.
885 Nyunza, G., and Mwakije, A.(2012). Analysis of round Potato marketing in Tanzania, the case of
886 Rungwe district Tanzania. *International Journal of Business and Social Sciences*, **3**(23):
887 86-96.
888 Olawale, E., Isaac, K., and Labode, P. (2015). Trends and policy correlates of agricultural
889 production and agricultural production sustainability outcomes in Ghana and Nigeria.
890 *Journal of Sustainable Development*, **8**(9): 71-78.
891 Olson, M. (1999).Plant family Moringaceae.*Journal of Botany,***4**: 84-92.
892 Osorio, G., Felix, E., Enciso, R., Carolina, G., Noe, P., Lukas, A., and Luz, B. (2016).
893 Association analysis for disease resistance to *Fusarium oxysporum* in cape gooseberry
894 (*Physalis peruviana L*). *Genomics*, **17**: 248-264.
895 Padayachee, B., and Baijnath, H. (2012).An overview of the medicinal importance of
896 Moringaceae.*Journal of Medicinal Plants Research,***6**(48): 5831-5839.
897 Paliwal, R., Sharma, V., and Pracheta, A. (2011). A review on horse radish tree *Moringa*
898 *oleifera*: A multipurpose tree with high economic and commercial importance. *Asian*
899 *Journal of Biotechnology,***3**: 317-328.
900 Praveen T and Jeyaprabha J (2024) Antifungal potential of the spices and medicinal plant
901 extracts on the fungal growth of Fusarium oxysporum International Journal of Advanced
902 Biochemistry Research; SP-8(11): 19-22
903 Ramachandran, C., Peter, K., and Gopalakrishnan, P. (1980). Drumstick (*Moringa oleifera*): a
904 multipurpose Indian vegetable. *Botany*, **34**(3): 276-83.
905 Ramaiah, A., and Garampalli, K. (2015). In vitro antifungal activity of some plant extracts
906 against *Fusarium oxysporum f. sp. lycopersici*. *Plant Science Journal,* **5**(1): 22-27.
907 Saremi, H., and Amiri, M. (2010). Exploration of potato cultivar resistant to the major fungal
908 pathogen on potato wilting disease in Iran.*Journal of Food and Agricultural*
909 *Environment*, **8**: 821- 826.

Sasidharan, S., Chen, Y., Saravanan, D., Sundram, K., and Yoga, L. (2011).Extraction, isolation And characterization of bioactive compounds from plants' extracts. *African Journal of Traditional, Complement and Alternative Medicine.* **8**(1): 1–10.

Semagn Asredie, Donald, H., Walter, D., Keith, P., David, W., Fentahun Mengistu, and Steffen, S., (2015).Potato variety diversity, determinants and implications for potato breeding strategy in Ethiopia.*Journal of Potato Research*, **92**: 551-558.

Spooner, D., and Salas, A., (2006).Handbook of potato production, improvement, and Postharvest management in chapter one about structure, biosystematics and genetic resources. The Haworth Press, Pp, 1-39.

Spooner, D., Nunez, J., Trujillo, G., Rosario, M., Guzman, F., and Ghislain, M. (2007). Extensive simple sequence repeat genotyping of potato landraces supports a major reevaluation of their gene pool structure and classification. *Proceedings of the National Academy of Sciences*, **104**(49): 19398-19403.

Talreja, T. (2010).Screening of crude extract of flavonoids of *Moringa oleifera* against bacterial And fungal pathogens.*Journal of Phytology,* **2**(11): 31-35.

Tewodros Ayalew. (2014). Analysis of seed potato(*Solanum tuberosum* L.) systems with special focus in Ethiopia.*Journalof Agricultural Research,* **8**: 122–135.

Trabelsi, B., Abdallah, R., Ammar, N., Kthiri, Z., Hamada, W., and Remadi, M. (2016).Bio-suppression of *Fusarium* wilt disease in potato using nonpathogenic potato-associated fungi. *Journal of Plant Pathology and Microbiology,* **7**(4): 1-8.

Ugonna, C., Jolaoso, M., and Onwualu, A. (2013). A technical appraisal of potato value chain in Nigeria, *Global Journal of Arable Crop Production,* **1**(1): 059-069.

Vongsak, B., Sithisarn, P., Mangmool, S., Thongpraditchote, S., and Wongkrajang, Y. (2013). Maximizing total phenolics, total flavonoids contents and antioxidant activity of *Moringa oleifera* leaf extract by the appropriate extraction method. *Crops Production*, **44**: 566-571.

Wakle, G. (2015).Effect of copper oxychloride against development of tuber rot. *Centre for Information of Biotechnology,* **4** (3): 34-35.

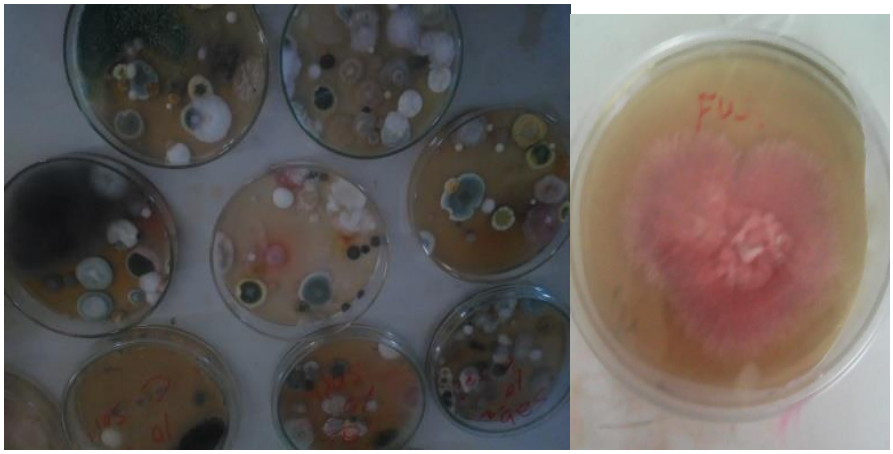
Wassu Mohamed. (2014). Genetic Variability in potato (*Solanum tuberosum* L.) genotypes for late blight (*Phytophthorainfestan*s (Mont.) *de Bary*) resistance and yield at Haramaya, Eastern Ethiopia. *East African Journal of Sciences,* **8**(1): 13-28.

WHO (2002). Traditional medicine: growing needs and potential. WHOpolicy perspectives on medicines. World Health Organization,Geneva Pp. 1-6.

Zisha M, Dhar1D W and Manzoor U 2024)Fungicidal potential of selected plant extracts against Fusarium oxysporum African Journal of Biologicl sciences 6(8)

7. Appendices

Appendix 1: Plant Collection (*Moringa oleifera leaf,* stem bark and Potato wilt infected by *Fusarium* spp).



951

952

953 **Appendix 2**: Laboratory activities.

Pairwise Comparisons						
Dependent Variable: Zone of inhibition (mm)						
(I) Concentration (mg/ml)	(J) Concentration (mg/ml)	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
40mg/ml	60mg/ml	-3.333[*,b,c]	.894	.039	-6.537	-.130
	80mg/ml	-4.333[*,b,c]	.894	.007	-7.537	-1.130
	100ug/ml	-6.333[*,b,c]	.894	.000	-9.537	-3.130
	1ml	12.000[*,b,c]	.894	.000	8.797	15.203

60mg/ml	40mg/ml	3.333*,b,c	.894	.039	.130	6.537
	80mg/ml	-1.000b,c	.894	1.000	-4.203	2.203
	100ug/ml	-3.000b,c	.894	.073	-6.203	.203
	1ml	15.333*,b,c	.894	.000	12.130	18.537
80mg/ml	40mg/ml	4.333*,b,c	.894	.007	1.130	7.537
	60mg/ml	1.000b,c	.894	1.000	-2.203	4.203
	100ug/ml	-2.000b,c	.894	.493	-5.203	1.203
	1ml	16.333*,b,c	.894	.000	13.130	19.537
100ug/ml	40mg/ml	6.333*,b,c	.894	.000	3.130	9.537
	60mg/ml	3.000b,c	.894	.073	-.203	6.203
	80mg/ml	2.000b,c	.894	.493	-1.203	5.203
	1ml	18.333*,b,c	.894	.000	15.130	21.537
1ml	40mg/ml	-12.000*,b,c	.894	.000	-15.203	-8.797
	60mg/ml	-15.333*,b,c	.894	.000	-18.537	-12.130
	80mg/ml	-16.333*,b,c	.894	.000	-19.537	-13.130
	100ug/ml	-18.333*,b,c	.894	.000	-21.537	-15.130

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

b. An estimate of the modified population marginal mean (I).

c. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Bonferroni.

954 **Appendix 3. ANOVA values for the comparison of the antifungal effect of acetone**
955 **crude extract of *Moringa oleifera* Lam. leaf at different concentrations on the test**
956 **pathogens with the standard drug (copper sulphate)**

957

958 **Appendix 4. ANOVA values for the comparison of the antifungal effect of acetone**
959 **crude extract of *Moringa oleifera* Lam. stem bark at different concentrations on the test**
960 **pathogens with the standard drug (copper sulphate)**

961

Pairwise Comparisons						
Dependent Variable: Zone of inhibition (mm)						
(I) Concentration (mg/ml)	(J) Concentration (mg/ml)	Mean Difference (I-J)	Std. Error	Sig.[d]	95% Confidence Interval for Difference[d]	
					Lower Bound	Upper Bound
40mg/ml	60mg/ml	-2.333[a,b]	.966	.364	-5.793	1.127
	80mg/ml	-6.667[a,b,*]	.966	.000	-10.127	-3.207
	100ug/ml	-9.333[a,b,*]	.966	.000	-12.793	-5.873
	1ml	9.000[a,b,*]	.966	.000	5.540	12.460
60mg/ml	40mg/ml	2.333[a,b]	.966	.364	-1.127	5.793
	80mg/ml	-4.333[a,b,*]	.966	.012	-7.793	-.873
	100ug/ml	-7.000[a,b,*]	.966	.000	-10.460	-3.540
	1ml	11.333[a,b,*]	.966	.000	7.873	14.793
80mg/ml	40mg/ml	6.667[a,b,*]	.966	.000	3.207	10.127
	60mg/ml	4.333[a,b,*]	.966	.012	.873	7.793
	100ug/ml	-2.667[a,b]	.966	.201	-6.127	.793
	1ml	15.667[a,b,*]	.966	.000	12.207	19.127
100ug/ml	40mg/ml	9.333[a,b,*]	.966	.000	5.873	12.793
	60mg/ml	7.000[a,b,*]	.966	.000	3.540	10.460
	80mg/ml	2.667[a,b]	.966	.201	-.793	6.127
	1ml	18.333[a,b,*]	.966	.000	14.873	21.793
1ml	40mg/ml	-9.000[a,b,*]	.966	.000	-12.460	-5.540
	60mg/ml	-11.333[a,b,*]	.966	.000	-14.793	-7.873
	80mg/ml	-15.667[a,b,*]	.966	.000	-19.127	-12.207
	100ug/ml	-18.333[a,b,*]	.966	.000	-21.793	-14.873

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Bonferroni.

962 **Appendix 5. ANOVA values for the comparison of the antifungal effect of ethanol**
963 **crude extract of *Moringa oleifera* Lam. leaf at different concentrations on the test**
964 **pathogens with the standard drug (copper sulphate)**

965

Pairwise Comparisons					
Dependent Variable: zone of inhibition					
(I) Concentration	(J) Concentration	Mean	Std. Error	Sig.[d]	95% Confidence Interval for

		Difference (I-J)			Difference[d]	
					Lower Bound	Upper Bound
40mg/ml	60mg/ml	-1.333[a,b]	.789	1.000	-4.158	1.492
	80mg/ml	-5.000[a,b,*]	.789	.001	-7.825	-2.175
	100ug/ml	-7.333[a,b,*]	.789	.000	-10.158	-4.508
	1ml	11.000[a,b,*]	.789	.000	8.175	13.825
60mg/ml	40mg/ml	1.333[a,b]	.789	1.000	-1.492	4.158
	80mg/ml	-3.667[a,b,*]	.789	.009	-6.492	-.842
	100ug/ml	-6.000[a,b,*]	.789	.000	-8.825	-3.175
	1ml	12.333[a,b,*]	.789	.000	9.508	15.158
80mg/ml	40mg/ml	5.000[a,b,*]	.789	.001	2.175	7.825
	60mg/ml	3.667[a,b,*]	.789	.009	.842	6.492
	100ug/ml	-2.333[a,b]	.789	.143	-5.158	.492
	1ml	16.000[a,b,*]	.789	.000	13.175	18.825
100ug/ml	40mg/ml	7.333[a,b,*]	.789	.000	4.508	10.158
	60mg/ml	6.000[a,b,*]	.789	.000	3.175	8.825
	80mg/ml	2.333[a,b]	.789	.143	-.492	5.158
	1ml	18.333[a,b,*]	.789	.000	15.508	21.158
1ml	40mg/ml	-11.000[a,b,*]	.789	.000	-13.825	-8.175
	60mg/ml	-12.333[a,b,*]	.789	.000	-15.158	-9.508
	80mg/ml	-16.000[a,b,*]	.789	.000	-18.825	-13.175
	100ug/ml	-18.333[a,b,*]	.789	.000	-21.158	-15.508

Based on estimated marginal means

*. The mean difference is significant at the .05 level.

a. An estimate of the modified population marginal mean (I).

b. An estimate of the modified population marginal mean (J).

d. Adjustment for multiple comparisons: Bonferroni.

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