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College of Graduate Studies

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Graduation Research Project

Title

Antifungal effects of Jatropha (*Jatropha curcas*) seeds and Argel (*Solenostemma argel* Del. Hayne) leaves aqueous extracts on the growth of the fungus (*Pestalotiopsis spp.)* in Guava *in vitro*.

تأثير المستخلص المائ لبذور الجاتروفا وأوراق الحرجل على نمو الفطر *(Pestalotiopsis spp.)* على الجوافة في المعمل

A dissertation submitted to Sudan University of Science & Technology in partial fulfillment of the Requirements for the Degree Master (M. Sc.) in Plant Protection

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الآية

قال تعالى:

(سُبْحَانَ الَّذِي خَلَقَ الْأَزْوَاجَ كُلَّهَا مِمَّا تُنبِتُ الْأَرْضُ وَمِنْ أَنفُسِهِمْ وَمِمَّا لَا يَعْلَمُونَ)

صدق الله العظيم

سورة يس الآية (36)

DEDICATION

TO MY LOVELY MOTHER

TO MY FATHER

TO MY SISTER AND BROTHERS

TO MY TEATCHERS

FINALLY TO MY FRIENDS ANDTHE SUDANESE PEOPLE

THIS WORK IS DEDICATED

ACKNOWLEMENT

First of all, I would like to express my thanks to almightily Allah, (the greatest), who helped me to complete this research. Secondly I would like to express my thanks and gratitude to my supervisor Dr. Ibrahim Saeed For his valuable advice throughout the whole period of research. My thankful also goes to my father and mother, my brothers and sister for their encouragement and moral support and to my friends Zakariya , Sufyan and Mobarak.

Abstracts

Guava (Psidium guajava) a vitamin C enrich fruit tree is grown abundantly throughout Sudan. In fact, it is an important fruit in many parts of the world where the climate is suitable for its production. The present investigation was undertaken under laboratory conditions of Plant Protection Department, College of Agricultural Studies, Sudan University of Science and Technology to study the effect of different concentration of aqueous extracts of jatropha seeds and argel leaves concentrations, on the growth of the fungus Pestalotiopsis sp., under laboratory conditions in order to formulate promising disease management approach. The fungus used in this was first confirmed as Pestalotiopsis sp. associated with canker scab based on its morphological and cultural characters. Three concentrations of the jatropha seeds extracts as well as that of argel leaves (25%, 50% and 100 %) were used in addition to control. The assessment of their inhibitory effect against the pathogen was expressed as fungal growth inhibition percentage. The results obtained showed that all concentrations of the two higher plants (25%, 50% and 100 %) reduced significantly ($P<0.05$) the fungal growth (56.2%, 75%, 75 %) respectively compared to untreated control. Moreover, the jatropha at 100% concentration demonstrated the highest inhibition of fungal growth (86%) followed in descending order by 50, 25% concentrations. Among Argel concentrations, the highest one (100%) reduced significantly the growth of the fungus (90%). Generally, the results showed that the antifungal activity increase with increase in extract concentration. Obviously, the test fungus differs in its response to the different concentrations but on the whole, growth inhibition increased with the concentration.

* The causal agents were identified as Pestalotiopsis spp. The identification was confirmed by morphological and culture characteristics, mainly the growth patterns and pigmentation.

ملخص البحث

الجوافة(*Psidium guajava*) والتى هى شجرة ذات ثمار غنية بفيتامين C مزروعة على نطاق واسع فى السودان. في الواقع انها شجرة مهمة في اجزاء كثيرة من العالم حيث المناخ المناسب لانتاجها.
تم اجراء هذا البحث في ظل الظروف المختبرية لقسم وقاية النبات، كلية الدراسات اللزراعية، جامعة السودان للعلوم والتكنلوجيا، لدراسة تاثير التركيزات المختلفة للمستخلص المائ لبذور الجاتروفا و اوراق نبات الحرجل على نمو فطريات (*Pestalotiopsis Spp*).

استخدمت ثلاثة تراكيز من المستخلصات بذور الجاتروفا بالاضافة لاوراق الحرجل (25، 50,100%) بالاضافة الى الشاهد. واعرب عن تقييم تاثيرها المثبط ضد الممرض كنسبة مئوية تثبيط نمو الفطريات. اظهرت النتائج التي تم الحصول عليها ان جميع تركيزات اثنين من النباتات العليا(25، 50، 100 %) خفضت بشكل كبير ($p<0.05$) نمو الفطريات 56.2 ، 75، 75 %) على التوالي مقارنة مع التحكم غير المعالجة. علاوة على ذلك اظهرت الجاتروفا بتركيز 100% اعلى تثبيط النمو الفطري(86%) تليها في تنازلي 50، 25 % تركيزات. بينت تركيزات الحرجل اعلى واحد 100% قلل بشكل كبير من نمو الفطريات (90%).

بشكل عام اظهرت النتائج ان النشاط المضاد للفطريات يزداد بزيادة تركيز المستخلص.

من الواضح ان فطر الاختبار يختلف في استجابته للتركيزات المختلفة ولكن بشكل عام يزيد تثبيط النمو مع التركيز.

*** وذلك من اجل الحصول على طرق ادارة واعدة .**

*** ولقد تم التعرف على الفطر على انه *pstalotiopsis spp* على حسب الصفات المزرعية والمورفلجية . انه الفطر المسبب للجرب في ثمار اشجار الجوافة.**

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CHAPTER ONE

CHAPTER ONE

INTRODUCTION

Guava (*Psidium guajava* L.) tree which is a native of Central America belongs to the family Myrtaceae. It is an important fruit in many parts of the world where the climate is suitable for its production. Besides being a rich source of vitamin C, the fruits have good amounts of vitamin A and B also. The fruits are eaten fresh and are commonly used for making jams, jellies, pastes and puddings (Sen. 1996 and Pandey. 2001).

In the Sudan, the guava is grown abundantly throughout the country. The fruit is a popular nutritious dessert; this besides being famous for its medicinal values as a prompt cure for digestive disorders and respiratory illness (Ali *et, al*., 2014). A number of old guava cultivars have been adopted by fruit growers. These are usually named after places of their intensive production like Shendi, Shambat, Sinja, Ganib, Sudani and Musaid. However, these are no longer distinct cultivars since the only method used for guava propagation is seed propagation which may not breed true to type. (Leipzig, 1996).

Despite the economic importance of this crop, its production is limited by some biotic factors in South Kordofan State (Abugebaha and Rashad areas) of Sudan and fungal diseases. Most of the guava fruits produced in these areas is associated with fruit canker cab. This disease was commonly found on the fruits right on the tree prior to ripening. The disease has made guava production in the region almost non-attractive to both farmers and in the home gardens. It has been reported that cankers cab has becomes a serious obstacle to guava cultivation; food values and

market price are falling and cause a great threat to expanding in Guava cultivation in that State (Booth key, 1977; Leipzig, 1996 and Ali *et, al*., 2014).

However, chemical control is often practiced to prevent diseases and protect crop plants against pathogens but one of the major problems with continuous use of chemicals is that resistance can be induced in target organisms in addition to contamination of the environment with toxic substances (Ali, 1996; Okigbo, 2006 and Carvalho, 2004). These observations make it necessary to explore more safe and reliable method. Recently, encouragement of botanical products to control pathogens in combination with proper cultural practices and use of resistant varieties was emphasized by many research workers (Mahr, 2008).

Obviously, no single approach for guava diseases control proved to be effective and without drawback. Therefore, integrated management strategies are the only solution to maintain plant health. These strategies should includes minimum use of chemicals for checking the pathogen population, encouragement of botanical products, modification of cultural practices and safe alternate antimicrobial compounds of higher plants.

This is stimulating to a series of research to combat this disease which account for huge losses in this crop that reach up to 80% or even more (Amusa, *et*, *al*,. 2006).

Based on the these information, this study was initiated with the objective of exploring the influence of different aqueous extracts of jatropha seeds and argel leaves concentrations, on the growth of the fungus *Pestalotiopsis sp.*, causal agent of canker cap in guava under laboratory conditions.

i- Isolation and identification of the fungus

ii- Investigate the antifungal activities of aqueous extracts of jatropha (*Jatropha curcas)* and Argel (*Solenostemma argel* Del. Hayne) on the growth of the fungus (*Pestalotiopsis Spp.*) the causal agent of canker cap in guava in vitro.

CHAPTER TWO

CHAPTER TWO

LITERATURE REVIW

2.1.Guava :

Guava (Psidium guajava L.) is grown in nearly every tropical and sub-tropical country in the world. Guava is very important fruit having 82% water, 0.7% protein, 11% carbohydrates and enough amounts of vitamins A, B, B2 and C plus some minerals (Bardi, 1975). However, diseases play a crucial role in limiting the yield of guava production. Scabby fruit canker, caused by Pestalotiopsis spp., is one of the most common fruit diseases in guava-growing areas and affects all developmental stages of guava fruit (Kwee & Chong, 1990). The genus Pesatlotiopsis was early descried by (Nag Rag, 1993). He cleared that the conidiomata of the genus was variable and ranging from acervuli to pycnidia. Conidiomata can be immersed to erumpent, unilocular to irregularly plurilocular with the locales occasionally incompletely divided and dehiscence by irregular splitting of the apical wall or overlaying host tissue. Conidiophores partly or entirely develop inside the conidiomata, and they can be reduced to conidiogenesis cells which are discrete or integrated, cylindrical, smooth, colorless and invested in mucus.

Scabby canker can drastically reduce fruit yield during the pre-harvest stage, and can also, lead to fruit losses during postharvest storage (Kaushik et al., 1972 and Kwee & Chong, 1990). Pestalotiopsis species are usually found in tropical and temperate ecosystems (Jeewon et al., 2004; Tejesvi et al., 2007 & 2009; Ding et al., 2009 and Liu et al., 2008 & 2009), and many cause plant disease in a variety of plants including canker lesions, shoot dieback, leaf spots, needle blight, tip blight, grey blight, scabby canker, severe chlorosis, fruit rots and leaf spots (Trapero et al., 2003 ; Sousa et al., 2004 and Espinoza et al., 2008). The genus Pestalotiopsis Steyaert is a heterogenous group of coelomycetous fungi consisting of 230 described species (Tejesvi et al., 2009) that are differentiated primarily on conidial characteristics such as size, septation, pigmentation, and presence or absence of appendages (Nag Rag 1993 and Sutton 1980). Some species have also, been identified based on their host occurrence (Kohlmeyer & Volkmonn-Kohlmeyer, 2001 and Chen et al., 2002). In the recent years, precise assessment of diversity and identification of fungi had a great impact on fungal taxonomy due to

rapid developments in molecular techniques (Phillips et al., 2007; Zhu et al., 2008 and Thongkantha et al., 2009). Fungal identification is more reliable when classical and molecular approaches are combined (Hyde & Soytong, 2008 and Than et al., 2008). Despite the broad application of random amplified polymorphic DNA (RAPD) based genetic markers for analysis of genetic diversity of fungal endophytes, little information is available on the species diversity of endophytes. RAPD analysis have been successfully used to identify strains (Pryor & Gilbertson, 2000 and Jana et al., 2003), characterize races (Malvick & Grau, 2001) and to analyzes virulence variability related to genetic polymorphisms (Kolmer & Liu, 2000 and Eman El-Argawy, 2012) in phytopathogenic fungi. RAPD can also, be used to detect genetic diversity in species of Pestalotiopsis (Tejesvi et al., 2007). At present at least 23 Pestalotiopsis species have been reported as endophytes some of which produce secondary metabolites with a great potential for anti-microbial to the control of plant diseases and anti-tumor medicinal application (Wei & Xu, 2004 ; Wei et al., 2005; Ding et al., 2009 ; Liu et al., 2009 ; Aly et al., 2010 and Xu et al., 2010).

Traditionally, the use of synthetic fungicides has been the preferred post-harvest treatment to control this microorganism (Aked et al., 2001). However, over time the reported use of fungicides has resulted in serious problems; the pathogens have developed resistance and residue levels have considerably increased (Mari et al., 2003). Chitosan is a naturally occurring polysaccharide derived from chitin that has exhibited potential to control several post-harvest plant diseases and to extend the shelf life of fruits and vegetables (Meng et al., 2008; Badawy & Rabea, 2009 and Eman El-Argawy, 2012). Several reports have shown that chitosan has antimicrobial activity and can interfere with spore germination and mycelial growth of phytopathogenic fungi (Rebea et al., 2003 and Muňoz et al., 2009). It was reported that chitosan confers protection against Botrytis cinerea in Vitis vinifera and controlled grey mould in cucmber plants (Romanazzi et al., 2006 and Nascimento et al., 2007).

2.2. Jatropha *(Jatropha curcas*):

Jatropha is a genus of approximately 175 succulent plant shrubs and trees (some are deciduous, like *Jatropha curcas* L).The name is derived from (Greek iatros=physician and trope =nutrition) Hence the common name physician .Jatropha is native to Mexico and Central America (Heller and Joachim, 1996 Morton, 1997; little, *et. al*, 1974).

Jatropha Tree

2.2.1. Classification of jatropha:

Kingdom: planta

Division: Magnoliophyta

Class: Magnoliopsida

Order: Malighiales

Family: Euphorbiaceae

Subfamily: Cortonoideae

Tribe: Jatropha

Genus: *Jatropha*

Species: *curcas*

2.2.2. Description:

Jatropha or phyicnut can grow to a height of about 3 to 5 meters .if the condition, are favorable they can grow to height of about 8 to 10 meter .with spreading branches and stubby twinge with smooth grey bark and they emit white water latex when it is chopped (James A ducke.1983).normally five root are formed seed, one tap root and 4 lateral root and 4 lateral roots Leaves deciduous, board and usually simple alternate but apically crowded, ovate, acute to acuminate, basally cordate, deeply palmate 3 to 5 lobed, green or pale green in color.

Flower: Several too many in greenish cymes, yellowish, bell shaped are formed terminally on branches.

Fruits: Small capsule –like round fruit about 2.5-4 cm in diameter there green and fleshy when immature.

Seed: 2 or 3 black seed each about 2 cm long (Morton, 1977, little *et al*, 1974).

2.2.3 Varieties:

Cape Verde variety: This is small seeds (weight of 1.000grains is about 682g.length of seed is about 16.8mm). This variety is found almost in all countries of the world, except Central America (Becker & Makkar, 1998).

Nicaragua variety: This variety is different from the cape Verde variety by larger leafs ,which have a more rounded form and by larger seeds(weight of 1.000 grains is about 878g)and length of seed is about 20.3 mm). The yield of the trees seems to be the same because there are less on fruits on a tree than with the Cape Verde variety (Becker & Makkar, 1998).

Nontoxic Mexican variety: Weight of grains is between 524g and 901g). Birgit Veracruz, are very appreciated by the population as food. However this Non-toxic variety of Jatropha could be a potential source of oil for Human consumption, and the seed cake can be a good protein for human as well as for live stocks (Becker & Makkar, 1998).

2.2.4 Distribution:

Though native to America the species is almost pan tropical now widely planted as a medicinal plant which soon tends to establish itself? It is listed, e.g. as awed in Brazil, Fiji, Honduras, India, Jamaica, Panama, PuertoRico, and Salvador, the plant was spread as evaluable hedge plant to Africa and Asia by Portuguese traders. (Holm, *et. al*, 1979). In Sudan; Jatropha is found in many areas such as Khartoum state, in central Sudan kassala state, in east and kordofan state, in the west .it is also dominant in the southern states especially in baher eljebel and baher elgazal state where the farmer used it as hedges to protect house gardens. Jatropha project was in kotum, north Darfur, with participation of the German development a Service (list and Horhammer, 1969-1979, Henning, 2001).

2.2.5 Ecological requirement:

Jatropha will have its best result when it is planted in rainy season .so it would be easy to prepare seedling during dry season and them ready before the rain season. Jatropha plant grows on wide range of climate and soils and can be established marginal/poor soil. In fact, *Jatropha acurcus* grows almost anywhere, even on gravelly, sandy and saline soils It can

also thrive on the poorest stony soil and grow in the crevices of rocks (James, 1983). Jatrohais found in tropical subtropical zones and also in regions that has low temperature has capacity to with stand little forest .they can live with the minimum water content and can live for several month without water by shedding its leave s to reduce the transpiration loss.(Duke and Wain, 1981).

2.2.6 Cultivation:

Growth readily, from cutting or seeds. Cuttings strike root so easily that the plant can be used as energy producing living fences post. (Morton, 1977, Little, *et. al*, 1974).

2.2.7 Yield and economics:

According to (Gaydou, *et. al,* 1982)seed yield approach 6-7MT/HA with 37%oil.They calculated that such yields could produce the equivalent of 2,100-2,800liters fuel oil/ha in Madagascar, they have 10,000ha of purging nut, each producing 2,400oil/ha for potential production of 24,000,000liters. The plant yields more than four times as much fuel per hectare as soybean, as and more than ten times that of maize (Michael, 2006).

2.2.8 Chemistry:

Par100g,the seed is reported to contain 6.6g H2O,18,9g protein38,0g fat,33.5g total carbohydrate,15,5gfiber and 4.5g ash(Duke and Atchley, 1983). Leaves, which show anti-leukemic activity, contain a-amyrinsitosterol, stigmastterol, andcompesterol, 7-keto-6sitosterol, stigmast-5ene-3-6, 7-a-adiol (Morton, 1981). Leaves contain isovitexin, saccharose, raffinose, stachyose, glucose, fructose, galactose, protein and oil. Oleic and linoleic-acid (List & Horhammer, 1969-1979). Curcasin, arachidic, linoleic -, myristic-, oleic-, palmitic-, and steric-acids are also reported (Perry, 1980).

2.2.9 Seeds and its toxicity:

The seed of physic nut are a good source of oil; which can be used as a diesel substitute .however; the seed of J.curcas are in general toxic to humans and animal .curuin; ataxic protein is olated from the seed s; was found to inhibit protein synthesis in invite or studies. The high concentration of phoebe esters present in Jatropha seed has been identified as the main toxic agent response bled for Jatropha toxicity (Adolfat, *et.al*; .1984; .Makkae, *et.al*; 1997).

Several cases of J.curcas nut poisoning in human after accidental consumption of the seed have been reported with symptoms of giddiness; vomiting and in the extreme condition even death have been recorded (Backer and Makker; 1998).

Ionizing radiation treatment could serve possible additional processing method for in activation or removal of certain anti nutritional factors such as phoebe esters ;phytates saponins and lections (siddhueaju *et.al*;.2002). It is not possible to destroy phorbolesaters by heat treatment because they are heat stable and can with stand roasting temperature as high as 160c for 30min. however ;it is possible to reduce its concentration

in the meal by chemical treatments this treatment is promising ; but in economic term it is expensive to produce Jatropha meal from it (Areqheore, *et.al*; 2003).(Martinez-Herrera, *et.al*, 2006).

2.2.10 Uses of Jatropha:

As hedge: Jatropha is an excellent hedging plant for protection ofagricuturalfield against damaged by livestock as unsalable to cattle and goats.

As food: The physic nut seed is eaten in certain region of Mexico once it has been boiled and roasted (Delgado and Parado; 1989).

As pesticide: The oil and aqueous extract from oil have a potential as an insecticide. From instance it has been used in the control of pulse; potato corn (Kasushik and Kumar; 2004). Methanol extracts of Jatropha seed (which contains biodegradable toxins) are being tested in Germany for control of bilharzias-carrying water snails.

As an energy source: The oil from Jatropha is regarded as a potential fuel substitute. Air new Zealand Houston based continental airlines have run testes in jan.2009; further demonstrating the viability of Jatropha oil as jet fuel. Japan air also conducted test flights in Jan .2009 as well.

Medicinal use: All parts of Jatropha (seeds & leaves & bark) have been used in traditional medicine and for veterinary purposes for a long time (Dalziel, 1955;Duke, 1988).The oil has a strong purgative action is also widely used for skin diseases and to soothe pain such as that caused by rheumatism. The oil is used as cathartic purgative and for the treatment of skin ailments (Duke; 1988).latex used to dress sores an against Plasmodium falciparum; p.vivax; provable and p.malariae. (Perry, 1980).The seeds used to treat arthritis; gout and jaundice. Leaves regarded as anti-parasitic; rubefacinot for paralysis. Rheumatism; also applied to scabies to hard tumors (Hart well; 1967-1971).

2.3 Hargel *(Solenostemma argel*):

Hargel (*Solenostemma argel*) is a plant or plant part valued for its medicinal, aromatic or savory qualities. Herb plants produce and contain a variety of chemical substances (Shayoub, 2003). Herbs had been used all cultures throughout history. The primitive man observed and appreciated the great diversity of plants available to him. The plants provided food, clothing, shelter, and medicine (Shayoub, 2003).

Certainly herbs used in some infections, cough, cold, stomach upset, indigestion, catarrh, constipation and so on (WHO, 2002; Mcntyres 2003). Herbal medicine has provided the world's population with safe, effective and low cost natural substances (medicine) for centuries (Shayoub, 2003).

The plant hargel is a member of the family Asclepiadaceae that comprises numerous medicinal plants, like Calotropis procera, Marsdenia

obyssinicna and Huernia mecrocarpa, known for their cardiac activity. Hargel grows naturally in the northern parts of the Sudan and extends from Berber to Abu-Hamad, especially the Rubatab area. It is also widely distributed throughout North Africa (Egypt, Libya and Algeria) and Saudi Arabia (Ahmed, 2004). hargel is used in the folk medicine as a effective fought remedy ;infusion of leaves for gastrointestinal, cramps, as laxative (Filipescu *et al* 1985); stomachache; anticolic, antisyphilitic if used for prolonged period of 40 to 80 days (Boulos, L., 1983) and as anti inflammatory (Jobeen, *et al*., 1984). Hargel has antimicrobial effect to some bacteria and fungi. (Abd Elhady, *et al* 1994a, b) and has antiviral activity to new castle disease virus.

Hargel leaves are used in indigenous medicine for the treatment of some diseases such as the disease of liver and kidney. It is an effective remedy for bronchitis and is used to treat neuralgia. It is used as incense in the treatment of measles and sometimes crushed and used as remedy for healing wounds. The leaves are infused to treat gastro-intestinal cramps and stomach colic (Abd Elhady, *et al*., 1994c) Sulieman *et al.,* (2009) reported that harjal aqueous extracts have antimicrobial activity against two fungi (*Aspergillus niger* and *Pennicilium italicum*) and two Gram negative bacteria (*Escerichia coli* and *Salmonella typhi*).

2.3.1 Morphological description:

A perennial, 60 cm high, with several vigorous stems. The leaves are, oval, leathery and covered with fine hairs. The numerous flowers have white petals, and a strong smell. Their inflorescences are giving the plant attractive look. The fruits are thick, 5 cm. long and 1.5-2 cm wide, green with violet lines; they contain pubescent seeds.

The plant has a long flowering period from March to June (El-kamali, 2001).

2.3.2 Regional:

Algeria, Libya and Egypt. No particular threat is reported for Algeria, but in Egypt the plant is vulnerable because of its intensive overuse. The largest population of Solenostemma argel grows in the upper part of the Wadi Allaqi conservation area and from 1989 on has been protected by Egyptian law.

The plant is cultivated on a farm in the downstream part of Wadi Allaqi to increase its population and promote the cultivation of this economically important plant. Hargel is Tropical plant that spreads across the central Sahara to the Sinai and the southeastern (Arabian) desert.

2.3.3 Ecology:

The plant grows in extremely dry conditions with a yearly rainfall of around 50-100 mm. It grows on the gravelly soils and on the stony and pebbly soils (El-kamali, 2001).

CHAPTER THREE

CHAPTER THREE

MATERIALS AND METHODS

3.1: Location of the study:

This study was conducted under laboratory conditions at Plant pathology Department, College of Agricultural Studies "Shambat", Sudan University of Science and Technology within February, 2019 to investigate the antifungal activity of crude aqueous extracts of some higher plants Jatropha and Argel against canker cap fungus (*pestalotiopsis spp.)* of guava.

3.2. Collection of infected guava fruits:

Random samples of fruits, showing typical symptoms of canker cab, were collected from guava trees at Abugebaha and Rashad areas orchards of South Kordofan State. Collected samples were kept in plastic bag and brought to laboratory for isolation and identification of the fungus.

Method of collecting infected guava fruits from different orchards at Abugebaha and Rashad areas

3.3. Collection of botanical samples:

Samples of Argel (*Solenostemma argel* L.) leaves were obtained from retailer's stores at Khartoum North local market. The leaves were freed from foreign materials like stones, sand and dust, before being kept in the Lab., for further investigation. At the time of experiment the leaves were dried, and milled using laboratory mill into fine powder. Similarly, Jatropha seeds were obtained from centre of forestry research at Suba. Collected seeds were crushed to obtain powder. Aqueous extract of the powder (50g/500ml) was used to prepare different concentrations (25, 50 and 100% extract) from each of the two powders.

Jatropha seeds

3.4. Isolation, and identification of the fungus:

Previously collected samples of infected guava fruits showing typical symptoms of the disease were cut into small pieces approximately 0.5 to 1.0 cm, washed thoroughly with the tap water, surface sterilized with Clorox (NaOCl) (1% concentration) for 1 minute, rinsed three times in sterilized distilled water and dried on sterilized filter paper. The sterilized pieces were then plated at the rate of 4 sections per plate, one at the centre

and 3 ones circled, on sterilized glass Petri-dishes containing potato dextrose agar medium (PDA). The inoculated Petri dishes were incubated at 25^0C for 5 days. Growing fungus was further sub-cultured on PDA medium for further purification of the fungus. Purified growing fungus was examined under compound microscopic based on the method of (Booth key, 1977) to confirm that the fungus is (*Pestalotiopsis sp.)*

Fungus identification by growth habit character and spores using microscopic examination to confirm that the fungus is Pestalotiopsis *sp.* was supplemented by other identification aids such as Burgess *et al.*, (1994); Giha (1996); (Sutton and Dyko, 1989); Abbasher *et al.*, 2013. Standard books and research papers were also consulted during the examination of this fungus (Elliott, and Edmonds, 2004). The purified isolates were maintained on PDA medium for further studies.

3.5. Preparation of crude aqueous extracts:

Aqueous extracts of each of the plant materials were prepared as recommended by Okigbo (2006). The obtained fine powder form Argel leaves and Jatropha seeds were weighed (25, 50 and 100 gm) and placed in 100 ml conical flask each and completed to 100 ml sterilized distilled water to obtain the three concentrations and it was placed in a shaker for 4 hrs. The extracts were filtered overnight to obtain 25 % 50% and 100% concentrations.

Jatropha seeds powder

3.6. Test procedure:

Food poison techenic was used in this study (Rao and Srivastava, 1994) to evaluate the effect of each concentration on linear fungal growth. Initially, fresh fungal growth was prepared from previously maintained culture. The Potato Dextrose Agar (PDA) medium was prepared and dispensed in 100 ml in conical flasks (250 ml), then amended with the required concentration from jatropha and Argel extracts before being solidified, agitated and poured into sterilized glass Petri dishes. Three plates, containing 30 ml of PDA, were assigned for each concentration and left to solidify. The other three plates with PDA medium were served as control.

Each solidified medium was then inoculated centrally by a fungal growth disc cut by a sterile cork-borer (No.5) from an edge of an actively growing culture of the fungus where opposite poles were marked at the back of each plate and incubated at 25°C in incubator and radial growth of pathogen was measured at 24 h intervals.

The Petri dishes of each concentration were arranged in a complete block design in incubator and incubated at 25 C^0 for 5 days. The growth of the fungus was measured and calculated successively after 3, 4 and 5 days after inoculation. The effect of each extract concentration on linear fungal growth was calculated as percentage of reduction in diameter of fungal growth (R) where: -

$$R = \frac{dc - dt}{dc} \times 100$$

Where R = Percentage reduction of the growth, dc= diameter of controlled growth and dc = diameter of treated growth

3.7. Experimental design:

The experiment was arranged in a Complete Randomized block Design.

3.8. Statistical analyses:

The obtained data was statistically analyzed according to analysis of variance (ANOVA); -Duncan's Multiple Range Test was used for mean separation.

CHAPTER FOUR

CHAPTER FOUR

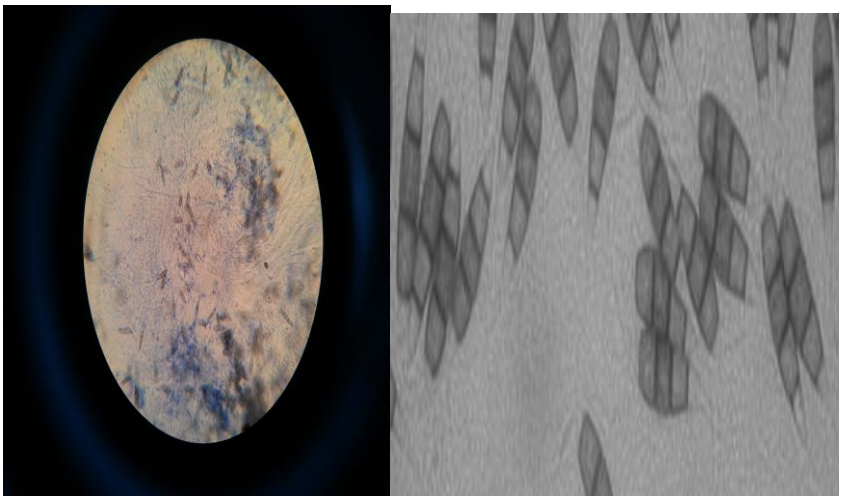
RESULTS

This study was conducted under laboratory conditions of plant pathology, College of Agricultural Studies, Sudan University of science and Technology during February, 2019 to investigate the inhibitory effect of jatropha seeds and argel leaves aqueous extracts against the test fungus.

4.1. Identification of the fungus:

Isolation and identification of the fungus *Pestalotiopsis spp.* Was performed depending on the cultural characteristic shapes as described by Giha (1996); Sutton and Dyko, (1989) and Abbasher *et al.,* (2013).

Plate, 1 presents typical shape of spore and conidia of the fungus *Pestalotiopsis spp.*



Plate, 1: *Pestalotiopsis* fungus in Petri dish and spores under microscope.

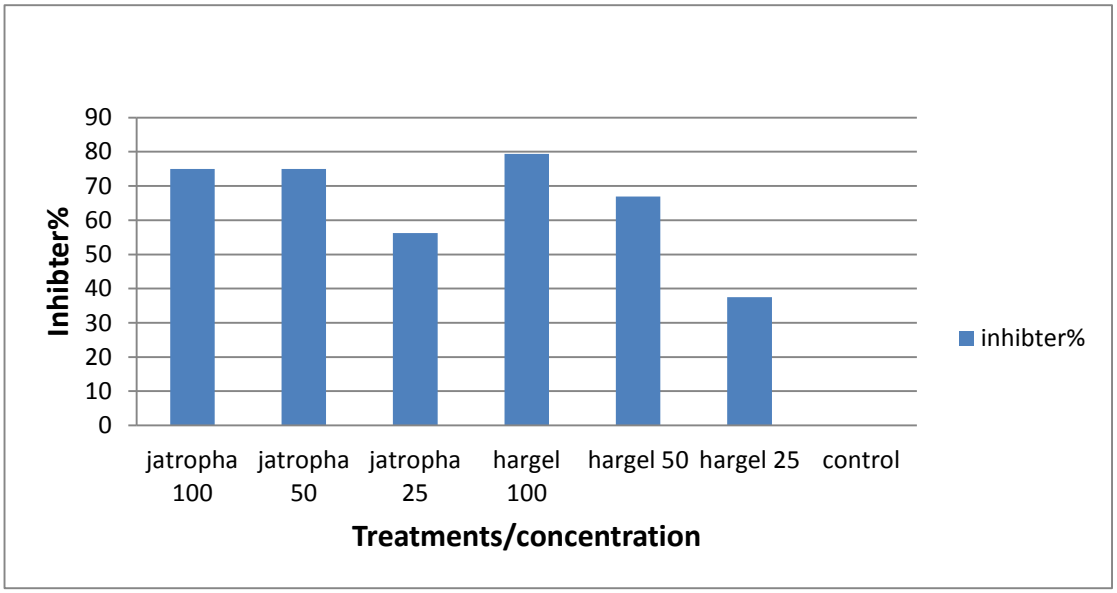
4.1.1. Classification of fungus:

Kingdom: Fung
Division: Ascomycota
Class: Sordariomycetes
Subclass: Xylariomycetidae
Order: Xylariales
Family: Sporocadaceae
Genus: *Pestalotiopsis*
Species: adusta, arachidis, asiatica, …

4.2. Table, 1and figure, 1: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth *of* *Pestalotiopsis sp.* after three days after inoculation

Treat	Conc	R1	R2	R3	Total	Mean	Mean%	Inhibition %
Jatropha 100%		3	4	5	12	4	25 (7.1)**e**	75
Jatropha 50%		7	0	5	12	4	25 (5.0)**g**	75
Jatropha 25%		7	7	7	21	7	43.8 (6.2)**f**	56.2
Argel 100%		3	4	3	10	3.3	20.6 (8.4)**d**	79.4
Argel 50%		5	7	4	16	5.3	33.1 (10.5)**c**	66.9
Argel 25%		8	10	12	30	10	62.5 (29.1)**b**	37.5
Control		15	20	13	48	16	100 (53.1)**a**	0
SE±							0.74	
C.V.%							9.3	
LSD							0.8405	

Fig, 1:



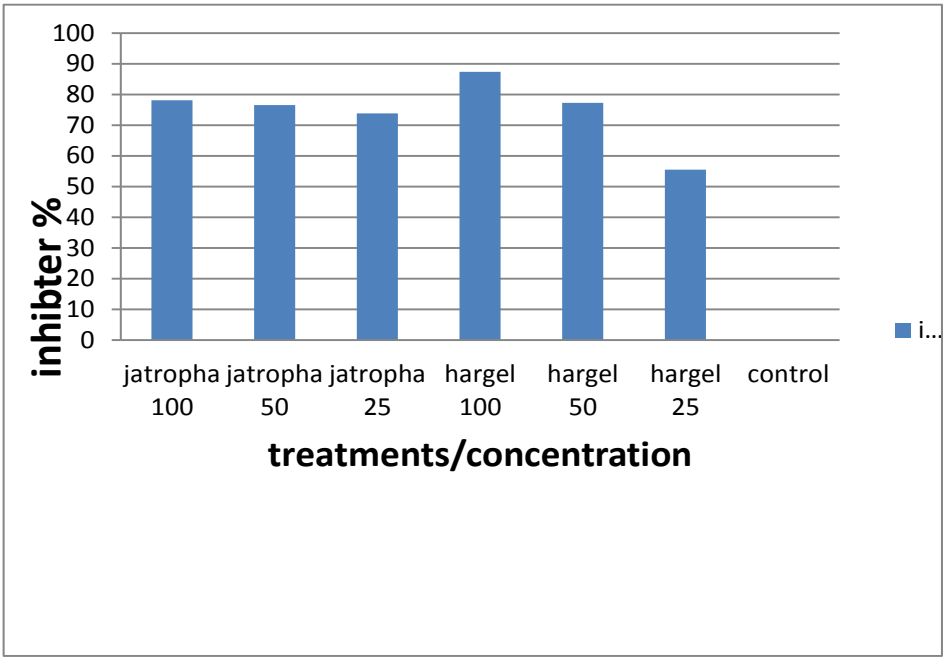
Figure, 1 and table, 1: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth *of* *Pestalotiopsis sp.* after three days after inoculation

Three days after inoculation, jatropha (seeds) extract concentrations as well as that of the argel were invariably exhibited inhibitory effects against the fungal growth. However, the highest concentration of the jatropha extract (100%, 50%) and argel (100%, 50%) gave the highest inhibition zones percent (75, 75, 79.4 and 66.6 %) respectively. However, the inhibitory effect from all concentrations tested was significantly (P s 0.05)different from control (Table,1 and Fig,1). Followed by jatropha 25 and argel 25 which gave the lowest inhibition zone percentage (56.2 and 37.5) respectively.

4.3 Table, 2 and figure, 2: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth *of* *Pestalotiopsis sp.* after four days from inoculation

Treat conc	R1	R2	R3	Total	Mean	Mean%	Inhibitory %
Jatropha 100%	8	10	6	24	8	21.9 (5.0)**f**	78.1
Jatropha 50%	13	6	7	26	8.6	23.5 (6.2)**e**	76.5
Jatropha 25%	11	10	8	29	9.6	26.2 (8.4)**d**	73.8
Argael 100%	5	5	4	14	4.6	12.6 (10.5)**c**	87.4
Argel 50%	7	10	8	25	8.3	22.7 (29.0)**b**	77.3
Argel 25%	12	10	27	49	16.3	44.5 (5.2)**f**	55.5
Control	42	38	30	110	36.6	100 (53.1)**a**	0
SE±						0.80	
C.V.%						8.7	
LSD						0.7211	

Fig, 2



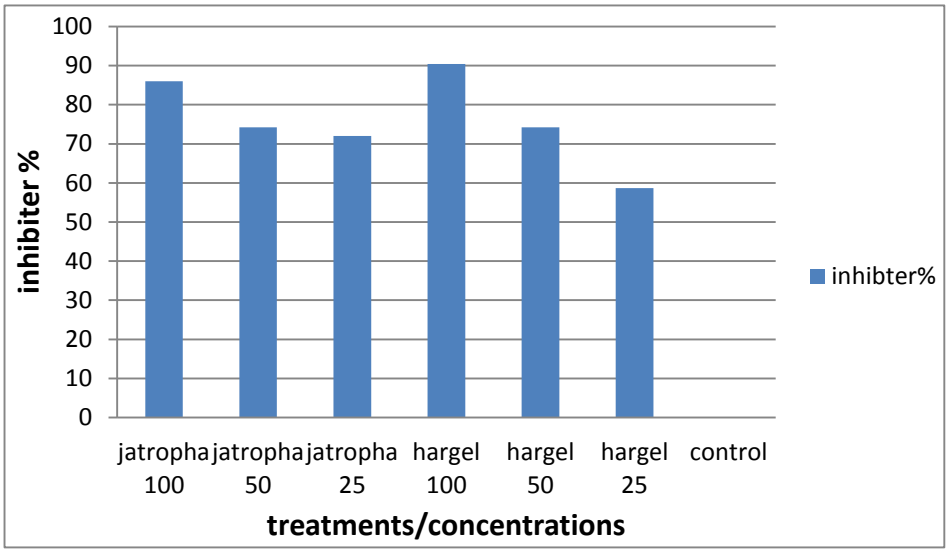
Figure, 2 and table, 2: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth *of Pestalotiopsis sp.* after four days from inoculation

In day four after inoculation, all concentration of the jatropha extract (100%, 50, 25%) and argel (100%, 50 %) gave the highest inhibition zones percent (78.1, 76.5 ,87.4, 77.3, and 73.8 %) respectively compared to control. This inhibitory effect from all concentrations tested was significantly ($P<0.05$) different from control. However, argel 25 was lowest inhibition zone percentage (55.5). (Table, 2 and Fig, 2).

4.4. Table, 3 and fig, 3: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth *of* *Pestalotiopsis sp.* after five days after inoculation

Treat conc	R1	R2	R3	Total	Mean	Mean%	Inhibiter %
Jatropha 100%	5	6	8	19	6.3	14 (6.2)**e**	86
Jatropha 50%	13	12	10	35	11.6	25.8 (8.4)**d**	74.2
Jatropha 25%	15	12	11	38	12.6	28 (10.5)**c**	72
Hargel 100%	5	4	4	13	4.3	9.6 (29.0)**b**	90.4
Hargel 50%	10	12	13	35	11.6	25.8 (5.8)**ef**	74.2
Hargel 25%	12	12	32	56	18.6	41.3 (5.2)**f**	58.7
Control	45	45	45	135	45	100 (53.1)**a**	0
SE±						0.85	
C.V.%						8.72	
LSD						0.6871	

Fig, 3



Figure, 3 and figure, 3: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth *of Pestalotiopsis sp.* after five days from inoculation

In day five after inoculation, the concentration of the jatropha extract (100%, 50, 25%) and argel (100%, 50 %) continued to give the highest inhibition zones percent (86 , 74.2 ,72, 90.4, and 74.2 %) respectively compared to control. This inhibitory effect from all concentrations tested

was significantly different from control. The Argel 25 remained the lowest inhibition zone percentage (58.7). (Table, 3 and Fig,3).

4.5. Statistical analyses

The obtained data was statistically analyzed according to analysis of variance (ANOVA); -LSD Range Test was used for mean separation

- Data in parentheses were transformed using square root transformation ($\sqrt{X + 0.5}$) before analysis.

4.6. Table, 4: Effect of aqueous extracts of jatropha seeds and argel leaves on radial mycelial growth of *Pestalotiopsis sp.* after three, four and five days from inoculation

Treatments	Conc	Mean after 3 days%	Mean after 4 days%	Mean after 5 days%
Jatropha	100	7.1 e	5.0 f	6.2 e
	50	5.0 g	6.2 e	8.4 d
	25	6.2 f	8.4 d	10.5c
Argel	100	8.4 d	10.5 c	29.0 b
	50	10.5 c	29.0 b	5.8 ef
	25	29.0 b	5.2 f	5.2 f
Control		53.1 a	53.1 a	53.1 a
SE±		0.74	0.80	0.85
C.V. %		9.3	8.7	8.72
LSD		0.8405	0.7211	0.6871

CHAPTER FIVE

CHAPTER FIVE

DISCUSSION

5.1. Discussion:

Obviously, the non rational uses of synthetic pesticides have caused serious problems to human and animal health in addition to their negative impact on environment. These problems include contamination of the biosphere, toxicity to man, animal and beneficial insects and other non target organisms.

This have drawn the attention of the researchers and paved the way to them to adopt new pest management strategies based on safe alternate products of low environmental persistence, highly specific, cheep, available and biodegradable (Sanixa, *et, al.*, 198). This gradual rise in resistance of fungal pathogens for synthetic chemicals highlights the need to find alternative safe sources Erdogrul, 2002).This was further highlighted by Agrafotis (2002) who reported that the development of new different antimicrobial agents more safe is very important step. In this context, the search for an eco-friendly way of managing this fungus in tomato which offers an alternative to fungicides is highly demanding.

Historically, numerous phytochemicals have been isolated from different plants which are now being prescribed by medical practitioners all around the world (Newman, *et al.*, 2000). In fact, higher plants are extremely abundant with biologically active secondary metabolites. Over 80% of all known Alkaloids, Terpenioid, Phenols and other secondary metabolite were produced by higher plants (Siddig, 1993). Many plant extracts or products have proven to be as potent as many conventional synthetic pesticides and are effective at very low concentrations. On the other hand

botanical insecticides possess great advantages over synthetic pesticides in being more environmentally friendly and accepted by the majority of the farmers, governmental organizations and decision makers (Kelang, 2001).

The present work was conducted in order to study the inhibitory effects of jatropha seeds and harjal crude aqueous extracts on the growth of the fungus *Pestalotiopsis sp.* in guava. The results revealed that the Jatropha seeds and harjal crude aqueous extracts consistently exhibited an inhibitory effect on fungal growth with significantly higher inhibition zones percent. Similar studies which explored the effect of extracts of many higher plants have been reported to exhibit antibacterial, antifungal and insecticidal properties under laboratory trials (Bullerman, 1974; Abdel-Rahim *et al.*, 1989; Al-Jali *et. al.*, 1997; Satish *et. al*., 1999; Okigbo and Ogbonnaya, 2006; Shariff *et. al*., 2006; Ergene *et. al.*, 2006; Kiran and Raveesha, 2006; Mohana and Raveesha, 2006).

Lai (2004) said that many herbs and spices included Harjal can yield medicinal compounds. Antibacterial activity of spices and other plants are well documented (Alicia, 1981). Vlietinek *et al.* (1995) screened about 100 medicinal plants, used by traditional healers to treat infections in Rwanda, for their antibacterial, antifungal and antiviral properties. Their study showed that 45% were active.

In Sudan many studies were carried out for testing the antimicrobial activity of some medicinal plants. Ahmed (2004) tested the extracts of 10 Sudanese medicinal plants against microbial organism. He found a marked antimicrobial effect.

The results obtained in this study also showed that all extract of *J. curcas* plant seeds which screened against test fungi, expressed an antifungal effect compared to control. This antimicrobial effect of Jatropha parts extract was also demonstrated by Aiyelaagbe *et. al.,* (2000). In their study

the antimicrobial activity of extracts of Jatropha sp. was investigated and they proved that all the extracts exhibited some broad spectrum antimicrobial activity especially at higher concentration.

However, Devappa, e.t al., (2010) reported that a number of Jatropha species contain a cocktail of toxic and antimicrobial compounds. In these, Phorbol esters and curcin appear to be the foremost toxic phytochemical present in organic solvent extracts and aqueous extracts, respectively.

Moreover, the current study showed that the screened concentrations of the two plants differ in their reactions to test fungi. Likewise the test fungi responded differently to the different concentrations of extracts. This variability in response which expressed by test fungi to different Jatropha extracts was also reported by Aiyelaagbe (2001). In that investigation, he explained that the majority of the studies involving *Jatropha* plant parts or extracts demonstrated their inhibitory effects on infectious or harmful microorganisms at variable degree.

In conclusion, the current study shown that the plants *Jatropha curcas and Argel* are potential source of antimicrobial agents and its activity against various microbes may be sufficient to perform further studies for isolation and identification of the bioactive ingredient (s) for large scale utilization..

5.2. Conclusions:

The present study indicated that the crude aqueous extracts of jatropha seeds and *harjal* have antimicrobial activity against the tested organism. The study also revealed that the highest inhibitory effects of the two plants extracts were found when using the concentrated extract.

From the present work it could be recommended that: the argel plant extracts can be used as antifungal agent. And it can be used in the food industry to flavor food and to compact contamination by micro-organisms.

5.3. Recommendation:

It was recommended to use natural antifungal such as jatropha and hargel which inhibits the fungal growth (*Pestalotiopsis Spp*) especially at concentration 25%, 50%, 100%.

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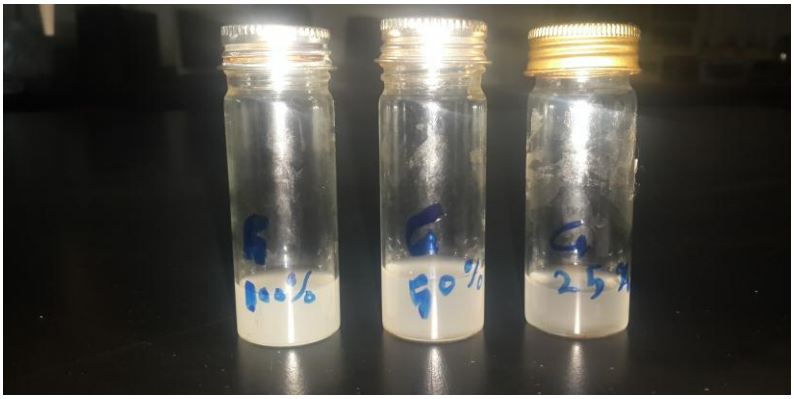
APPENDIX

APPENDIX

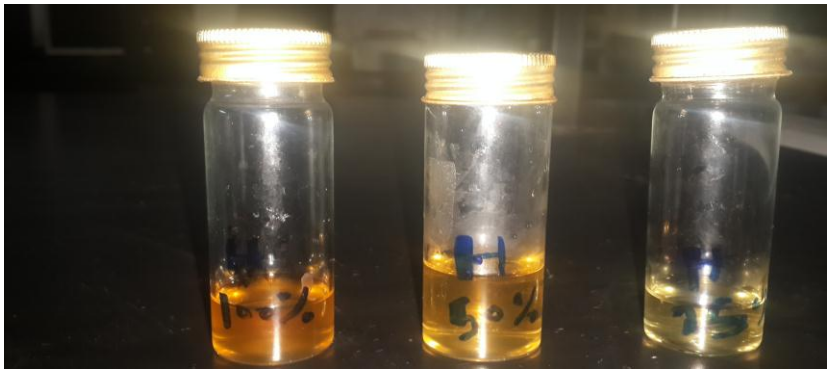
Materials, tools and equipments used in this study:

- Gloves
- Camera
- Marker pen
- Electric blender
- Petri-dishes
- Sensitive balance
- Incubator
- Needle
- Flame burner
- Laminar flow cabinet
- Compound microscope
- Autoclave
- Slides
- Aluminum foul
- Water path
- Potato dextrose agar(PDA)
- Filter papers
- Medical cotton

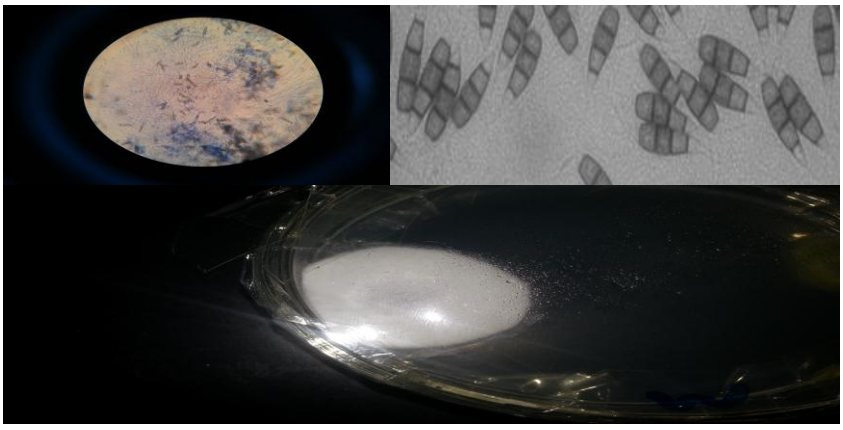
Tools and equipments used in the experiment



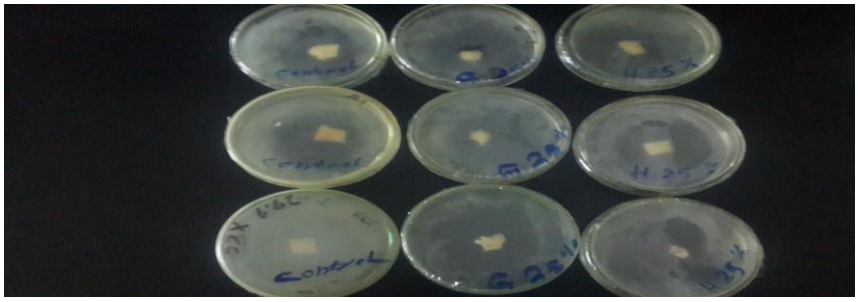
Jatropha aqueous extracts concentrations (100%, 50% and 25%)



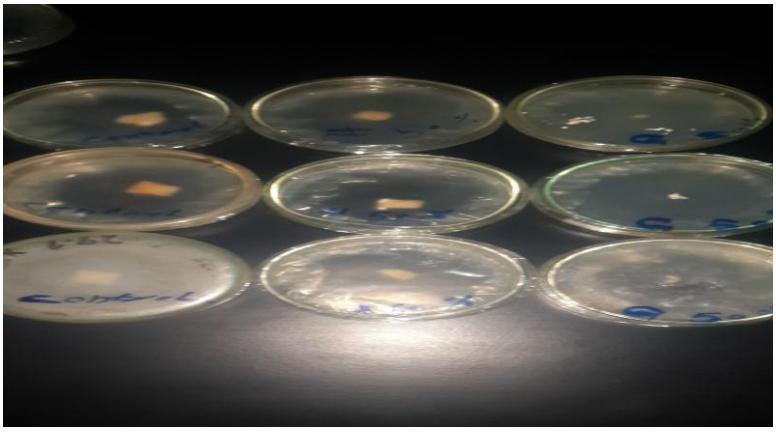
Hargel aqueous extracts concentrations (100%, 50% and 25%



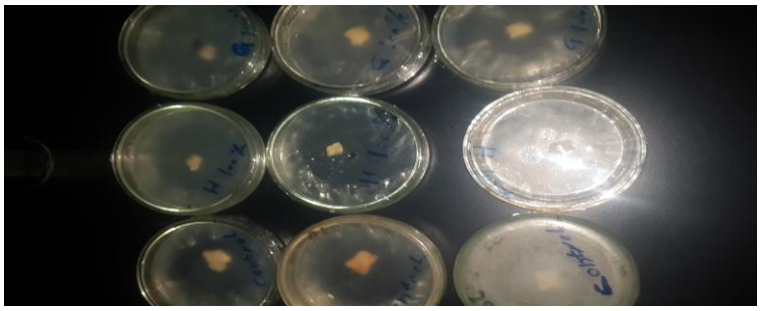
Fungus in Petri dish and spores under microscope.



Jatropha 25%, hargel 25% and control in PDA



Jatropha 50%, Argel 50% and control in PDA



Jatropha 100%, Argel 100% and control in PDA