Ethiopian Journal of Environmental Studies & Management 14(4): 530 – 540, 2021.
ISSN:1998-0507 doi: https://ejesm.org/doi/v14i4.11
Submitted: June 01, 2021 Accepted: July 16, 2021

THE *In Vitro* INHIBITION OF *Macrophomina* AND *Lasiodiplodia* SPECIES USING THREE MEDICINAL PLANT EXTRACTS

OLADIPO, A.D.,[1] NWOGWUGWU, J.O.,[1] *OSUNLAJA, O.A.,[1] BATCHO, A.A.,[2] ALADE, O.D.[3] AND ABIMBOLA, B.O.[1]

[1]Pathology Section, Department of Forest Conservation and Protection, Forestry Research Institute of Nigeria, P.M.B 5054, Jericho, Ibadan, Oyo State, Nigeria
[2]Group of Molecular Physiology, Laboratory of Growth Regulators, Palacky University in Olomouc & Institute of Experimental Botany, ASCR, Slechtitelu 27 Czech Republic
[3]Federal University of Agriculture, Abeokuta, Nigeria
*Corresponding author: ayo.olugbenga@gmail.com

Abstract

This study was, undertaken to investigate the in vitro anti-fungal activities of ethanol extracts of three medicinal plants (Azadirachta indica, Chromolaena odorata and Zanthoxylum zanthoxyloides) against Macrophomina phaseolina and Lasiodiplodia theobromae isolated from Jatropha curcas. Antifungal bioassays using pour plate method were carried out by using leaf extracts of 10, 20, 30, 40 and 50% concentration in 100 ml of ethanol. In general, all the extracts significantly reduced the mycelia growth of the target fungi species, though there were significant differences (P<0.05) among different concentrations used. Increase in extracts concentration led to reduction in fungal mycelia growth therefore, the highest inhibition for each was at the highest concentration of 50%. C. odorata extracts exhibited the highest antifungal activity for M. phaseolina, while extracts of Z. Zanthoxyloides exhibited the highest antifungal activity for species of L. theobromae (100.00±0.0). This study therefore concludes that the ethanol leaf extracts of medicinal plants contain secondary metabolites with potentials to serve as bio-fungicides in the management of Macrophomina phaseolina and Lasiodiplodia theobromae, pathogens of Jatropha curcas and other forest tree species.

Key Words: *Antifungal, Jatropha curcas, Extracts, Secondary* metabolites

Introduction

Biological control strategy for plant disease management holds ample prospective for ensuring healthy species for agriculture and plantation farmers globally (Drenth and David, 2016). The use of synthetic chemicals for pest and disease control has been the primary method of controlling fungal diseases of crops both in developing and developed countries. But the use of the chemicals over time has led to pathogens resistivity (Adeniyi and Abiodun, 2015) and hence reducing productivity. These chemicals also have prohibitive cost, hazardous effects on the environment and ecosystem at large thereby posing threats to lives. There is therefore the need for increase on

emphasis of the importance of indigenous products in plant disease management which is eco-friendly, cheap and medically safe approach (El-Said, 2012).

Jatropha curcas L. (physic nut) is a perennial, monoecious shrub, belonging to the family Euphorbiaceae (Govaerts *et al.,* 2000). The plant is a native of Central America but is now widely distributed in the tropics and subtropics of Africa and Asia (Foidl *et al.,* 1996; Kumar *et al*., 2013). Its distribution was spread by the Portuguese traders due to its several properties which include rapid growth, hardiness, easy propagation and widely ranging usefulness (Dhilon *et al.,* 2008). *J. curcas* is a crop of significant economic importance. The seeds are being used in the production of biodiesel which is an environmentally friendly energy source (Qian *et al*., 2010; Can, 2014; Kamel *et al.,* 2016; Guan *et al*., 2017). All parts of the plant are used in traditional medicine and as raw material for pharmaceutical and cosmetic industries as well as in the control of land degradation (Paramathma *et al*., 2006; Martinez-Herrera *et al.*, 2008). Mampane *et al*. (1987) reported on the anti-cancerous properties of '*Jatrophine'*, an alkaloid contained in the latex of *J. curcas.* The latex is also used as a topical application for skin diseases, rheumatism and for sores on domestic livestock (Gübitz *et al.,* 1999). The oil from its grains and extracts obtained from its leaves have proven to be highly toxic for many microorganisms, insects and animals, hence a potential pesticides which is mainly due to its phorbol ester content (Nwosu and Okafor, 1995; Solsoloy and Solsoloy, 1997; Achten *et al.*, 2008). However, there are many fungal pathogens which are a threat to large scale production of *Jatropha* (Rao *et al*., 2011; Nwogwugwu 2015). The plant has been reported to be cultivated in Ekiti state (Aransiola *et al*., 2012), Delta state (Okpeke *et al*., 2015) of Nigeria. These fungal pathogens have become serious problem for farmers/researchers all over the world and 56.9% yield losses have been reported due to attack by different diseases and insect pests on its leaves and fruits (Singh *et al*. 2007). Several researchers have reported the antimicrobial and pesticidal efficacy of Neem (*Azadirachta indica* A. Juss. (Choudhary *et al*., 2017), *Chromolaena odorata* (L.) R. M. King and H. Robinson (Okey, 2015) and *Zanthoxylum zanthoxyloides* (L.) Waterman (Paulin *et al*., 2018) on some pathogenic fungi. Plants are known to be store houses of biochemicals that contribute to suppressing of phytopathogens. These biochemicals contain Nitrogen compounds and Phenolics which function as a defense and chemical signals molecule against pathogens (Shoaib *et al*., 2018). Kumar *et al*. (2013) reported that the symptoms of *Lasiodiplodia theobromae* are leaves becoming yellowing at the initial stage, drooping and shedding of leaves, which later leads to disease symptoms spreading on the stem and roots. The infected stem will also show necrotic lesions on branches as scars which become brownish and sunken. Infected roots also exhibit discolouration and browning of vascular tissues. While the symptoms of collar rot of *Jatropha curcas* caused by *Macrophomina phaseolina* may include yellowing of leaf, wilting, leaf fall, blackening, rotting of the roots and collars (Singh *et al.,* 2018). The present study was, therefore, undertaken to investigate the *in vitro* anti-fungal activities of *Azadirachta indica, Chromolaena odorata* and *Zanthoxylum zanthoxyloides* extracts against

Macrophomina phaseolina and *Lasiodiplodia theobromae*, causal pathogens of collar and fruit rot of *Jatropha curcas.*

Materials and Methods

Study Area

The experiment was carried out at the Plant Pathology Laboratory of Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo state located on longitude 03˚51′20″ E to 03˚53′43″ E and latitude 07˚23′18″ N to 07˚23′43″ N, belonging to the derived savannah agroecological zone. The mean annual rainfall over the study area is about 1500 mm while mean monthly temperature is about 27°C (Ayeni *et al*., 2020).

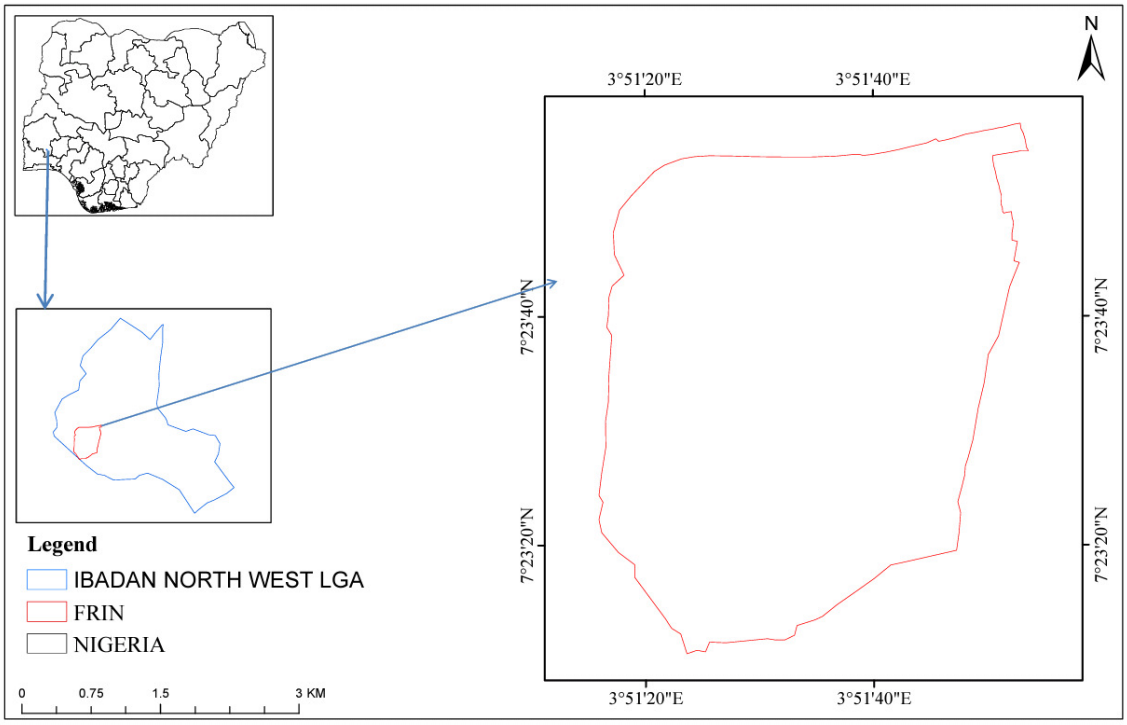

Fig. 1: Scaled map of study area

Sources of Materials Used

Fresh leaves of *Azadirachta indica, Chromolaena odorata* and *Zanthoxylum zanthoxyloides* were collected from the premises of FRIN, Ibadan, Oyo state (Plate 1). After thorough washing with distilled water, leaves were air-dried at room temperature for 7 days, ground with a warring blender (Binatone Blender produced in United Kingdom) into fine powder and stored in clean dry bottles.

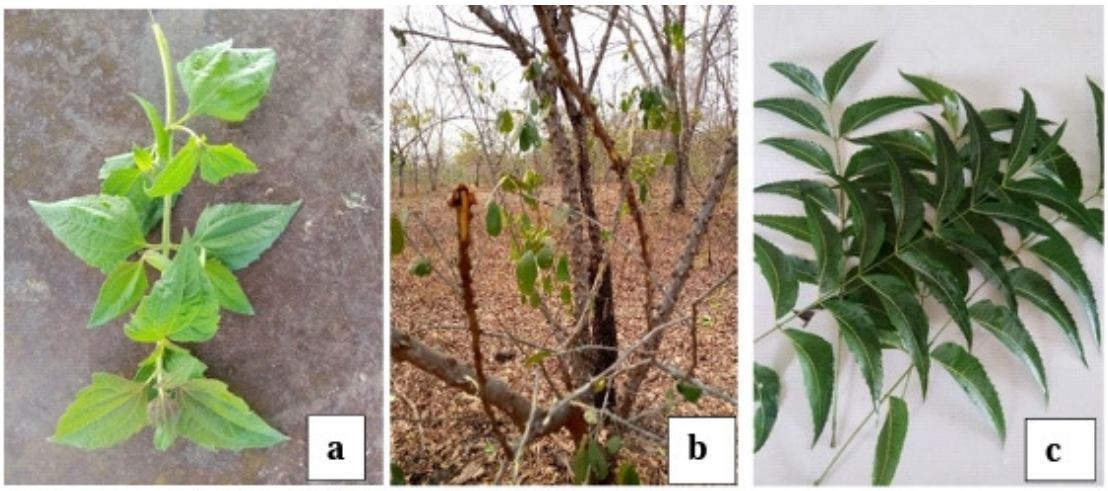

Plate 1: Plants used for the leaf extracts; (a) *Chromilaena odorata* (b) *Zanthoxylum zanthoxyloides* (c) *Azadirachta indica*

Preparation of Extracts

The extracts (*A. indica, C. odorata* and *Z. zanthoxyloides*) were prepared by weighing 10, 20, 30, 40 and 50 g of powdered leaf materials of the three plant species and soaked in 100 ml of absolute ethanol (Sigma-Aldrich produced in USA) to obtain 10, 20, 30, 40 and 50% concentration each respectively. Materials were left for 72 hours at room temperature. After that extracts were filtered through 4 layers of sterile muslin cloth and centrifuged (Heamatocrit, Hawskley produced in USA) at 3,500 rpm for 20 minutes. The supernatants solution was collected and stored in sterile containers (Savaliya *et al*., 2015).

Preparation of Culture Medium

Potato Dextrose Agar (PDA) medium was prepared by dispensing 39 g of PDA powder in 100mL distilled water in a conical flask, plugged with cotton wool, properly wrapped, and sterilized at 121°C for 15minutes. The medium was allowed to cool, then acidified by addition of lactic acid. The cooled agar was dispensed aseptically into sterile glass petri-dishes inside the inoculating chamber and allowed to solidify.

Isolation and Identification of Pathogens

Jatropha plants showing symptoms of collar and fruit rot were collected from the study area. The diseased portions of collar and fruit were cut into 0.5 cm pieces and surface sterilized by 1% sodium hypochlorite solution for 1 min and then thoroughly rinsed with sterilized distilled water. These pieces were placed on potato dextrose agar (PDA) plates aseptically and incubated at 25±2°C for 7 days. The fungal colonies were sub-cultured on freshly prepared PDA plates to obtain pure cultures of the pathogens by isolating single spores as described by Slippers *et al.* (2004). For *Macrophomina phaseolina* the colour of fungal colony was grey which darkens with age and characteristic black coloured oblong microsclerotia were observed (plate 2). On the bases of these characteristics, the isolated fungus was identified as *M. phaseolina* as reported by Wylie (1993) and Watanabe (2002). Fungal colonies of *Lasiodiplodia theobromae* on PDA were also white, with abundant fluffy aerial mycelia which

turned greyish sepia to greyish black, reverse observed to be fuscous black to black (plate 2) (Celiker and Michailides, 2012).

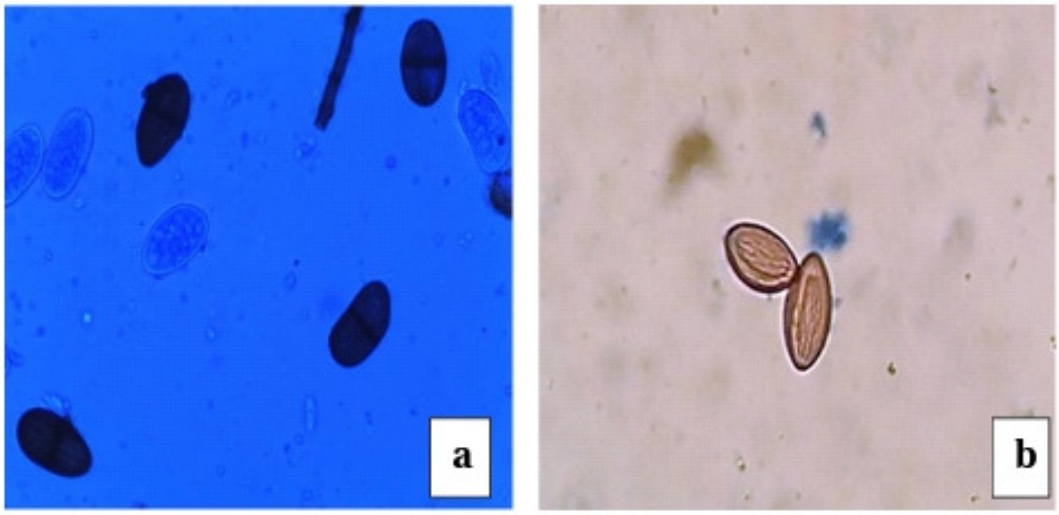

Plate 2: Photomicrograph showing mature and hyaline unicellular comidia of *Lasiodiplodia theobromae* (a) and *Macrophomina phaseolina* (b) isolated from infected fruit collar rot of *J. curcas*

***In vitro* Anti-fungal Assay**

The inhibitory effect of the three plant extracts were assessed using the food poisoned technique (Adeniyi and Abiodun, 2015); 2 ml of each extract was aseptically added to 20ml of sterilized and cooled PDA in a petri dish, which has been gently agitated and allowed to solidify. Mycelial discs of *Macrophomina phaseolina* and *Lasiodiplodia theobromae* were prepared using a sterilized 5 mm diameter cork borer from the tips of 7 days old fungal culture and placed in an inverted position on the extract-amended PDA. Each treatment was replicated three times. Petri plates without extract but with 2 ml of sterile distilled water (SDW) served as negative control while 2 ml of Benlate solution (0.5 g in 100 ml of sterilized distilled water) served as positive control. Plates were incubated at room temperature for 7 days. The radial mycelia growth was recorded at 7 days after inoculation (DAI), when the upper surface of the control treatment was fully covered with the mycelial of the pathogens. The growth was measured using a ruler and recorded in centimetres (cm). The percentage mycelia growth inhibition was calculated using the formula described by Choudhary *et al.,* (2017) thus:

$$I = \frac{C-T}{C} \times 100 \text{ ---------- (1)}$$

Where, I= Percent Inhibition

C= Colony diameter in Control plate

T= Colony diameter in Treated plate

Data Collection and Statistical analysis

The effectiveness of extracts was recorded in terms of percentage inhibition according to equation 1. The data were subjected to analysis of variance (ANOVA) followed by Duncan's Multiple Range Test to separate the treatment means at $P < 0.05$ on Statistical Package for Social Sciences (SPSS) version 11.0 (SPSS, 2001).

Results and Discussion

Antifungal Activity of Chromolaena odorata Extract

Among the three test plant species, extracts of *C. odorata* were found most effective against *M. phaseolina.* Concentration of 50% of the extracts exhibited highest inhibition for both pathogens, resulting in total reduction for *M. phaseolina* and 73% reduction for *L. theobromae*. At concentration lower than 50%, they were found to be comparatively less active as shown in table 1. This corroborates the findings of Okey (2015) who reported that leaf extracts from *C. odorata* were found to have inhibitory effects on *L. theobromae* and *M. phaseolina*. This inhibition they observed may be attributed to the high levels of fungi toxic substances such as flavonoids, tannins and saponins found in the extract and that increase in concentration had a corresponding increase in percentage inhibition of growth and sporulation of the pathogens. Ngane *et al.* (2006) also reported that extract of *C. odorata* leaves and some of its fractions examined have antifungal properties by dilution methods on solid and liquid media, using yeasts and filamentous fungi. The authors reported that both extracts and fractions can inhibit *in vitro* growth of *Cryptococcus neoformans*, *Microsporum gypseum, Trichophyton mentagrophytes* and *Trichophyton rubrum*. The chemical analysis of the extract and fractions also revealed the presence of biologically active constituents such as coumarins, flavonoids, phenols, tannins and sterols which have been attributed to have fungicidal activity.

Table 1: Percentage inhibition by *Chromolaena odorata* extract

Concentration (%)	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
10	51.67±2.35^{b}	64.11±2.35^{b}
20	66.68±2.35^{b}	79.08±2.35^{c}
30	77.68 ±2.96^{c}	86.42 ±2.35^{d}
40	86.28±2.35^{c}	91.73 ±2.35^{d}
50	100.00±0.00^{d}	95.47 ±3.53^{d}
SDW(-ve control)	0.00±0.00^{a}	0.00±0.00^{a}
Benlate (+ve control)	100.00 ±0.00^{d}	100.00±0.00^{e}

Means followed by same letter(s) in the same row are not significantly different ($p \leq 0.05$)
SDW – Sterile Distilled Water

Antifungal Activity of Zanthoxylum zanthoxyloides Extract

All the concentrations of *Z. zanthoxyloides* extracts significantly reduced biomass of the two pathogens assessed. Increase in concentration also resulted in increase in the percentage inhibition of both pathogens. However, extracts of *Zanthoxylum zanthoxyloides* were found most effective against *Lasidioplodia* spp. Concentration of 50% of the extracts exhibited highest for both pathogens resulting in total reduction for *Lasidioplodia* spp biomass and 84% reduction for *Macrophomina* spp (Table 2). Essential oil of *Z. zanthoxyloides* harvested in western Burkina Faso has been reported to have an inhibitory action on the five phytopathogenic fungi (*C. graminicola*, *C. lunata*, *F. moniliforme*, *F. verticillioides* and *M. phaseolina*) (Paulin *et al*., 2018). It has been reported that the

extracts of this plant contain aporphines: tembetarine, berberine, magnoflorine; furoquinolines: 8-methoxydictamine, skimmianine, 3-dimethylallyl- 4-methoxy-2- quinolone; the benzophenanthridine: fagaronine, dihydroavicine, chelerythrine and canthine -6-one (Adesina, 2005; Bowden, 1990) and that these are responsible for its reported antimicrobial action (Adesina, 2005; Odebiyi and Sofowora, 1973).

The *Zanthoxylum* genus, which includes more than 549 species (Doring, 2017) distributed in the tropics and temperate regions, can also be an important source of biomolecules for the control of pathogenic fungi. This has been reported to be an advantage because several species of this genus whose organs are used to extract the essential oil are also used in food. (Abdou-Bouba, 2009).

Table 2: Percentage inhibition by *Zanthoxylum zanthoxyloides* extract

Concentration (%)	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
10	52.38±2.35^{b}	54.22±3.53^{b}
20	66.24±2.35^{c}	56.44±2.35^{b}
30	70.69±3.53^{d}	57.12±4.71^{b}
40	71.35 ±2.35^{d}	78.29±3.53^{c}
50	84.53 ±2.04^{e}	100.00±0.0^{d}
SDW (-ve control)	0.00±0.00^{a}	0.00±0.00^{a}
Benlate (+ve control)	100.00 ±0.00^{f}	100.00 ±0.00^{d}

Means followed by same letter(s) in the same row are not significantly different ($p \leq 0.05$)
SDW – Sterile Distilled Water

Antifungal Activity of Azadirachta indica Extract

Extracts of *A. indica* also significantly showed decline biomass of the two pathogens assessed. Increase in concentration also resulted in increase in the percentage inhibition of both pathogens. Concentration of 50% of the extracts exhibited the highest inhibition for both pathogens resulting in 73% reduction for *L. theobromae* biomass and 71% reduction for *M. phaseolina* as shown in Table 3. This corroborates the findings of Nwogwugwu and Batcho (2019) who reported higher inhibition potentials of *A. indica* in the disease suppression of *Sclerotium rolfsii* at higher extract concentrations. However, the inhibition of *M. phaseolina* observed in this study is contrary to the findings of Oluma and Elaigwe (2006) who observed that extracts of *A. indica* had no inhibitory effect on the mycelia growth of *M. phaseolina* though Javaid and Rehman (2011) reported up to 85% reduction in biomass of *M. phaseolina* by aqueous extracts of *A. indica.* Similarly, de Rezende Ramos *et al*. (2007) have reported 35% growth reduction of mycelia of *Phytophthora* on neem (*A. indica*) leaf extract media. Choudhary *et al.* (2017) reported that ethanol leaf extract was more effective than the water extracts of neem and also reported that other workers have evaluated the efficacy of various extracts of neem leaf on seed borne fungi *Aspergillus* and *Rhizopus* and results confirmed that growth of both the fungal species was significantly inhibited and controlled with both ethanol and water extract. Furthermore, ethanol extract of neem leaf was most effective as compared

to aqueous extract for retarding the growth of both fungal species (Choudhary *et al.,* 2017). Many chemicals and biological active compounds have been identified in the leaf extract of *A. indica* (e.g phytol, octadecatrienoic acid, methyl ester, hexadecanoic acid, methyl ester, etc.) (Hossain *et al*., 2013) which may be the reason for its fungicidal effect.

Table 3: Percentage inhibition by *Azadirachta indica* extract

Concentration (%)	*Macrophomina phaseolina*	*Lasiodiplodia theobromae*
10	0.00±0.00[a]	2.45±2.35[a]
20	45.78±2.35 [b]	21.61±2.35[b]
30	61.46 ±2.35 [c]	49.54 ±2.35 [c]
40	69.85±2.35[c]	58.19±1.27 [c]
50	71.34±2.35[c]	73.16±2.35[d]
SDW (-ve control)	0.00±0.00[a]	0.00±0.00[a]
Benlate (+ve control)	85.88±0.09 [d]	89.41±0.00[e]

Means followed by same letter(s) in the same row are not significantly different ($p \leq 0.05$)
SDW – Sterile Distilled Water.

Conclusion

From this study it can be concluded that ethanol extracts of allelopathic trees (*Azadirachta indica, Chromolaena odorata*) possess antifungal properties which can effectively be used to manage *Macrophomina phaseolina* and *Lasidioplodia theobromae*. Further studies are however required to isolate and identify these effective antifungal ingredients from these extracts and pot trials be carried out to further establish the inhibition potentials of these extracts. The antifungal ingredients can also be a potential substitute in preparation of inorganic fungicides since they are environmentally friendly.

References

Abdou-Bouba, A. (2009). Contribution to the study of the development of a functional food based on spices from Cameroon: Physico-chemical and functional characterization. PhD thesis, University of N'Gaoundéré, N'Gaoundéré, Cameroon. pp 228

Achten, W.M.J., Verchot, L., Franken, Y.J., Mathijs, E., Singh, V.P., Aerts, R. and Muys, B. (2008). Jatropha bio-diesel production and use. *Biomass & Bioenergy*, 32(12): 1063-1084.

Adeniyi, D.O. and Abiodun, J. (2015). *In vitro* evaluation of plant extracts against *Lasiodiplodia theobromae* causing cashew inflorescence blight. *African Journal of Biotechnology*, 14(13): 1139-1142.

Adesina S. K. (2005). The Nigerian Zanthoxylum; Chemical and Biological values. *African Journal of Traditional Complementary and Alternative Medicine*, 2(3): 282 – 301.

Aransiola, E.F., Daramola, M.O., Ojumu, T.V., Aremu, M.O., Layokun, S.T. and Solomon, O.B. (2012). Nigerian Jatropha Curcas Oil Seeds: Prospect for Biodiesel Production in Nigeria. *International Journal of Renewable Energy Research*, 2(2): 317-325.

Ayeni, O.D., Adediran, A., Ofordu, C.S., Mba, N.C., Amoo-Onidundu, O.N., Arabomen, O. and Okumodi, B.O.

(2020). *Journal of Meteorology and Climate Science,* 18(1): 89 – 98.

Bowden G.H.W. (1990). Effects of fluoride on the microbial ecology of dental plague. *Journal of Dental Research*. 69: 653-659.

Can, O. (2014). Performance and exhaust emissions of a diesel engine fuelled with a waste cooking oil biodiesel mixture. *Energy Conversion and Management Journal.* 87 : 676–686.

Çeliker, N.M. and Michailides, T.J. (2012). First report of *Lasiodiplodia theobromae* causing canker and shoot blight of fig in Turkey. *New Disease Reports*, 25:12. [doi:10.5197/j.2044-0588.2012.025.012]

Choudhary, S., Dwivedi, A.K. and Singh, P. (2017). Antifungal activities of neem leaf extracts on the growth of Macrophomina phaseolina in vitro and its chemical characterization. *Journal of Environmental Science, Toxicology and Food Technology*, 11(4): 44-48.

de Rezende Ramos, A., Falcao, L.L., Barbosa, G.S., Marcellino, L.H and Gander, E.S. (2007). Neem (*Azadirachta indica* Juss) components: Candidates for the control of *Crinipellis perniciosa* and *Phytophthora* sp. *Microbiological Research,* 162: 238-43.

Dhilon, R.S., Hooda, M.S. and Handa, A. (2008). Scope of Jatropha cultivation in wastelands of India, in Exotics in Indian forestry, edited by Chauhan, S. K., Gill, S.S., Chauhan, R. and Sharma, S. C. (Agrotech Publishing Academy, Udaipur, India). 308-319.

Doring, M. (2017). GBIF Type Specimen Names. GBIF Secretariat. Checklist dataset https://doi.org/10.15468/sl9pyf accessed via GBIF.org on 2021-06-14.

Drenth, A. and David, I. G. (2016). Fungal and oomycete diseases of tropical tree fruit crops. *Annual Review of Phytopathology,* 54: 373–395.

El-Said, R.M.F. (2012). Control of root rot of chickpea caused by *Sclerotium rolfsii* by different agents and gamma radiation. M.Sc. thesis, Faculty of Science in Microbiology (Mycology), Al-Azhar University.

Foidl, N., Foidl, G., Sanchez, M., Mittelbach, M. and Hackel, S. (1996). *Jatropha curcas* L. as a plant–source for the production of biofuel in Nicaragua. *Bioresource Technology,* 58(1): 77-82.

Govaerts, R., Frodin, D.G. and Smith, A.R. (2000). World checklist and bibliography of Euphorbiaceae (with Pandaceae). Royal Botanical Gardens, Kew.

Guan, Q., Li, Y., Chen, Y., Shi, Y., Gu, J., Li, B., Miao, R., Chen, Q. and Ning, P. (2017). Sulfonated multi-walled carbon nanotubes for biodiesel production through triglycerides transesterification. *Royal Society of Chemistry Advances*, 7(12): 7250–7258. DOI:10.1039/C6RA28067F

Gübitz, G. M; Mittlebach, M. and Trabi, M. (1999). Exploitation of the tropical oil seed plant *Jatropha curcas.* L. *Bioresource Technology,* 67: 73-82.

Hossain, M.A., Al-Toubi, W.A., Weli, A.M., Al-Riyami, Q.A. and Al-Sabahi, J.N. (2013). Identification and characterization of chemical compounds in different crude extracts from leaves of Omani neem. *Journal of Taibah University of Sciences*, 7:181–188.

Javaid, A. and Rehman, H. A. (2011). Antifungal activity of leaf extracts of

some medicinal trees against *Macrophomina phaseolina*. *Journal of Medicinal Plants Research*, 5(13): 2868-2872.

Kamel, D.A., Farag, H.A., Amin, N.K. and Fouad, Y.O. (2016). Biodiesel synthesis from non-edible oils by transesterification using the activated carbon as heterogeneous catalyst. *International Journal Environmental Science Technology,* 14: 785–794.

Kumar, R., Kumari, A., Sinha, A. and Maurya, S. (2013). New report of *Lasiodiplodia theobromae* causing *Jatropha* decline in Eastern Plateau and Hill region of India. *Archives of Phytopathology and Plant Protection,* DOI: 10.1080/03235408.2013.840100

Mampane, K.J., Jouberty, P.J. and Hay, I.T. (1987). *Jatropha curcas*: Uses as a traditional Tswana medicine and its role as a cause of acute poisoning. *Phytotherapy Resource,* 1: 50-51.

Martinez-Herrera, J., Siddhuraju, P., Francis, G., Davila-Ortiz, G. and Becker, K. (2008). Chemical composition, toxic/antimetabolic constituents, and effects of different treatments on their levels, in four provenances of *Jatropha curcas* L. from Mexico. *Food Chemistry*, 96(1): 80-89.

Ngane, A.N., Etame, R.E., Ndifor, F., Biyiti, L., Amvam Zollo, P.H. and Bouchet, P. (2006). Antifungal Activity of *Chromolaena odorata* (L.) King & Robinson (Asteraceae) of Cameroon. *Chemotherapy*, 52: 103-106.

Nwogwugwu, J.O. (2015). Response of *Jatropha curcas* L. to Fungal Pathogens in Southwestern Nigeria. PhD Thesis submitted to University of Ibadan, Ibadan, Nigeria.

Nwogwugwu, J.O. and Batcho, A.A. (2019). Antifungal potentials of some botanicals on *Sclerotium rolfsii schum.* the causal pathogen of damping-off of *Gmelina arborea* Roxb. in Ibadan, Southwestern Nigeria. *Journal of Plant Diseases and Protection*, 126(6): 543-551. http://doi.org/10.1007/s41348-019-00267-z.

Nwosu, M.O. and Okafor, J.I. (1995). Preliminary studies of the antifungal activities of some medicinal plants against *Basidiobolus* and some other pathogenic fungi, *Mycoses,* 38:191-195.

Odebiyi, O.O. and Sofowora, E.A. (1973). Antimicrobial alkaloids from a Nigerian chewing stick (*Fagara zanthoxyloides*). *Planta Medical Journal,* 36: 204-207.

Okey, E.N. (2015). The Effect of *Chromolaena odorata* leaf extract on fungi associated with cassava (*Manihot esculenta*) (CRANTZ) tuber rot. *Journal of Agriculture and Veterinary Science,* 8(10): 63-68.

Okpeke, M.Y., Okorodudu, B.O. and Onyeagocha, S.U.O. (2015). Assessment of Farmers' Awareness of The Economic Importance of Physic Nut (*Jatropha curcas*) in Ndokwa East Local Government Area, Delta State, Nigeria. *Global Journal of Agricultural Research*, 3(4): 34-49

Oluma H.O.A. and Elaigwe, M. (2006). Antifungal activity of extracts of some medicinal plants against *Macrophomina phaseolina*. *Nigeria Journal of Botany.* 19(1):121–8.

Paramathma, M., Venkatachalam, P., Sampathrajan, A., Vairavan, K., Jude Sudhagar, R., Parthiban, K.T., Subramanian, P. and Kulanthaisamy,

P. (2006). Cultivation of Jatropha and biodiesel production. Sri Sakthi Promotional Litho Process. 40 pp.

Paulin, O., Ouattara, L., Bonzi, S., Yameogo, J.T. and Somda, I. (2018). Evaluation of antifungal activity and phytotoxicity of the essential oil of *Zanthoxylum zanthoxyloides* fruits. *Agricultural Science Research Journal,* 8(4): 92–99.

Qian, J., Shi, H. and Yun, Z. (2010). Preparation of biodiesel from *Jatropha curcas* L. oil produced two-phase solvent extraction. *Bioresource Technology,* 101(18): 7036 – 7042. doi: 10.1016/j.biortech.2010.04.018.

Rao, C.S., Kumari, P., Wani, S.P. and Marimuthu, S. (2011). Occurrence of black rot in *Jatropha curcas* L. plantations in India caused by *Botryosphaeria dothidea*. *Current Science*, 100: 1547-1579.

Savaliya, V.A., Bhaliya, C.M., Marviyaand, P.B. and Akbari, L.F. (2015). Evaluation of phytoextracts against *Macrophomina phaseolina* (Tassi) Goid causing root rot of sesame. *Journal of Biopesticides*, 8(2): 116-119.

Shoaib, A., Munir, M., Javid, A., Awan, A.Z. and Rafiq, M. (2018). Antimycotic potential of *Trichoderma* sp. And leaf biomass of *A. indica* against the charcoal rot of pathogen, *M. phaseolina* (Tassi) Goid in Cowpea. *Egyptian Journal of Biological Pest Control,* 28: 26 doi.org/10.1186/s41938-018-0031-6

Singh, A., Patel, P., Agrawal, P. K. (2018). Management of Collar Rot Disease Caused by *Macrophomina phaseolina* in *Jatropha curcas* – Green Diesel Plant. *Journal of Plant Pathology and Microbiology,* 9(1): 430 - 433. doi: 10.4172/2157-7471.1000430.

Singh, R.A., Munish, K. and Haider, E. (2007). Synergistic cropping of summer groundnuts with *Jatropha curcas*. A new two-tier cropping system for Uttah Pradesh. *International Crops Research Institute for the Semi-Arid Tropics Journal,* 5(1): 1-2.

Slippers, B., Burgess, T., Wingfield, B.D., Crous, P.W., Coutinho, T.A. and Wingfield, M.J. (2004). Development of SSR markers for *Botryosphaeria* spp. with *Fusicoccum* anamorphs. *Molecular Ecology Notes,* 4: 675-677.

Solsoloy, A.D. and Solsoloy, T.S. (1997). Pesticidal efficacy of formulated *Jatropha curcas* oil on pests of selected field crops. In Biofuels and Industrial Productions from *Jatropha curcas,* edited by G M. Gubitz, M. Mittelbach and M. Trabi (Graz University of Technology, Austria). pp. 216-226.

Statistical Package for Social Sciences (SPSS). (2001). SPSS for Windows, version 11.0. Statistical Package for the Social Sciences, SPSS Inc., Chicago, USA.

Watanabe, T. (2002). Identification of Fungi from: Pictorial Atlas of Soil and Seed Fungi (2nd ed.) Morphologies of Cultured Fungi and Key to Species CRC Press, Washington DC. https://www.routledgehandbooks.com/doi/10.1201/EBK1439804193-c3

Wyllie, T.D. (1993). Charcoal rot. In Sinclair JB, Backman PA (eds). Compendium of Soybean diseases (3rd ed.), APS Press, St. Paul, MN. Pp. 30-33.