In vitro inhibitory effect of leaf and seed extracts of *Moringa oleifera* on growth of *Fusarium oxysporum* associated with leaf spots of *Zanthoxylum zanthoxyloides*

[*]Onyekachi Francis Akanu Ibiam[1], Felicia W. Nmom[2], Ezeibe Chidi Nwaru[3], Inemesit Ndarake Bassey[4], Smart Chima Nwosu[5], Dennis Chidozie Udechukwu[1] and Tobechukwu Ebele Okeke[1]

[1]Department of Biology, Faculty of Biological Sciences, Alex Ekwueme Federal University, Ndufu-Alike Ikwo, Ebonyi State, Nigeria
[2]Department of Plant Science and Biotechnology, Faculty of Biological Sciences, Rivers University, Nkpolu-Orworukwo, Rivers State of Nigeria
[3]Department of Plant Science and Biotechnology, Abia State University Uturu, Abia State Nigeria
[4]Department of Botany and Ecological Studies University of Uyo, Akwa Ibom State, Nigeria
[5]Department of Public Health, School of Public and Allied Health, Babcock University, Ilesan, Ogun State, Nigeria
[*]Corresponding author's email: drakanuibiamjr@yahoo.com

Abstract

Fusarium oxysporum was found associated with the leaf spot disease of *Zanthxylum zanthoxyloides.* The goal of this study was to employ *Moringa oleifera* aqueous leaf and seed extracts to inhibit the development of *F. oxysporum in vitro* at doses of 100, 200, 300, 400, and 500 mg mL^{-1}. Tests for the presence of certain secondary metabolites were also carried out. Naturally occurring substances such as alkaloids, flavonoids, saponins, phenols, steroids, terpenoids and cyanoglycosides were identified in the samples. The extracts from the two parts of the plant were effective against the growth of *F. oxysporum*. The highest inhibitory effects of leaf extract occurred at 400 and 500 mg mL^{-1}, with a mean diametric measurement value of 0.57 cm and the least at 100 mg mL^{-1} with a value of 1.28 cm. Similarly, the highest effect of seed extract was at 500 mg L^{-1} with a value of 0.76 cm and the least at 100 mg mL^{-1} with 1.34 cm fungal growth as compared to1.92 cm fungal diameter recorded in the control treatment. This work shows that the test fungus could be controlled effectively using concentrations of 400 and 500 mg mL^{-1} leaf and seed extracts of *M. oleifera*.

Keywords: *Fusarium oxysporium*, Inhibitory, Leaves, *Moringa oleifera*, *Zanthoxylum zanthoxyloides.*

Introduction

Zanthoxylum zanthoxyloides is a tree grown in Nigeria, and is also grown in West African countries, and used in traditional medicines to treat a number of diseases such as malaria, as reported by Goodman *et al.* (2019). In order to combat chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum*, he employed dichloromethane extracts of the root-bark, stem-bark, and a methanol extract of the stem-bark, with inhibitory concentrations of 50% at values between 1 and 10 g mL^{-1}. Ascaris suum, a parasitic nematode of swine, and a close relative of human nematode Ascaris lumbricoid, was controlled by ethanolic extract of Z. zathoxyloides in vitro, due to the anthelmintic properties of the plant as reported by Williams *et al.* (2016). Boye *et al.* (2012) and Ogunrinade *et al.* (2021) reported the anti-inflammatory and gastro-protective effects of *Z. zanthoxyloide*. Some of its medicinal bioactivities included anti-trypanosomal (Dofuor *et al.,* 2019), anti-malarial (Enechi *et al.,* 2019), antihelminthic (Olounladé *et al.*, 2012), and antisickling (Tine *et al.*, 2017; Emeke *et al.,* 2019). Flavonoids, tannins, coumarins, fluoroquinoline, oxylipin, aromatic compounds, acridone alkaloids, fluoride, and zantholic acid, among others, were observed, according to Dofuor *et al.* (2020) in *Z. zanthoxyloides*, and shown to be of great medicinal value against foliar diseases caused by bacteria and fungi.

Damage to the leaves and other parts of the plants by fungal diseases, which could reduce food and medicinal values, are becoming of great economic importance to man (Khan and Javaid, 2020, 2021, 2022). It has become pertinent to seek the isolation and identification of the fungi causing diseases in economically important plant species and using plant extracts for the management of these fungi to afford man the maximum beneficial use of

plants (Banaras *et al.*, 2020; Javed *et al.*, 2021). In recent years, many studies have been carried out to control disease caused by *Macrophomina phaseolina, Sclerotium rolfsii, Fusarium oxysporum* and other fungi using crude plant extracts (Khan and Javaid, 2013; Ferdosi *et al.*, 2022), as well as using dry biomass of plants as soil amendment (Javaid and Khan, 2016; Ali *et al.*, 2020; Jabeen *et al.*, 2021).

All portions of the *Moringa* tree are edible and have been used by people for a very long time, and *Moringa* has played a significant role in resolving the majority of the nutritional and general illness control issues that the globe faces (Fahey, 2005). Fungi like *Trichophyton*, *Epidermophyton*, *Microsporum*, *Trichosporon*, *Geotricum* and *Candida* could be controlled by extracts of *Moringa* (Agu, 2008). According to Dwivedi and Enespa (2012), the plant's entire body, including the roots, flowers, bark, stem, leaves, and seeds, have antimicrobial qualities. A number of antioxidants, including ascorbic acid, fatty acids, and phenolic acids are present in this plant (Alhakmani *et al*., 2013; Vongsak *et al*., 2014). Numerous substances, including quercetin, zeatin, -sitosterol, kaempferol, and caffeoylquinic acid, have also been reported by Sharma and Pliwal (2013). Myricetin, rutin, chlorogenic acid, glycosides niaziminin and niazin have been reported by Mbikay (2012). The plant was also said to possess antibacterial and antifungal properties (Arora *et al*., 2014; Arora and Onsare, 2014; Belay and Sisay, 2014). No literature exists on the use of *Moringa* aqueous leaf and seed extracts for the management of foliar fungal diseases of *Z. zanthoxyloide*s; hence, this work was aimed at isolation and identification of the fungi associated with foliar diseases of *Z. zanthoxyloides*, and the use of aqueous extracts of the leaves and seeds of *M. oleifera* for the management of these fungi *in vitro.*

Materials and Methods

Sample collection

Fresh leaves of infected *Z. zanthoxyloides* were collected from a farm at Mgbabor village, off Presco Campus, Ebonyi State University, Abakaliki. Ebonyi State of Nigeria. Seeds and leaves of *M. oleifera* were obtained from dealers at Eke-Aba market, Abakaliki Metropolis, Nigeria. The research was carried out at the Department of Applied Biology Laboratory Ebonyi State University, Abakaliki, Nigeria.

Isolation of fungi

Standard method of isolation was used. In a 500 mL conical flask, 20 g of potato dextrose agar (PDA) was mixed with 50 mL of distilled water and blended by stirring. Its volume was raised up to 500 mL in 1 L flask. Aluminum foil was used to close the flask's mouth. The medium was autoclaved for 15 minutes at 121 ºC and 103 kPa pressure to sterilize it, together with some 90 cm Ostwald Petri dishes covered in aluminum foil. One gram of chloramphemicol (to inhibit bacterial growth) was added into the medium and stirred with magnetic stirrer. After flaming the flask's mouth, it was then quickly transferred onto sterile Petri dishes. They were allowed to gel. 4% sodium hypochlorite was used to make sterile 4mm portions of infected *Z. zanthoxyloides* leaves, followed by a number of rinses in sterile distilled water. They were inoculated onto the media in the Petri dishes using sterile forceps. Four pieces per Petri dish, were placed at evenly spaced spots, and incubated for 48 h at a temperature of 252 ºC in the incubator. To obtain pure cultures, subcultures of the fungal isolates were made. Their habit character and spore features were used to identify them, following the manual by Barnett and Hunter (2000).

Phyto-chemical screening

Leaf and seed extracts of *M. oleifera* in aqueous forms, were processed in accordance with the description of Uzoekwe and Mohammed (2015). Crushed leaf and seed samples (150 g) of the plant were soaked separately in 1.7 L for 72 h and filtered, using a muslin cloth (2 mm pore size), concentrated with a rotary evaporator at 50 ºC and stored in air tight containers in a refrigerator, until subsequent use.

Test for flavonoids

Extract of each sample (1 mL) in test tubes were mixed with 5 mL of dilute ammonia. Each mixture received an addition of concentrated sulfuric acid, which produced a yellow tint that showed the presence of flavonoids.

Test for tannins

Two different test tubes containing 1 mL extract of each sample were heated for 5 min to boil. Two drops of 15% ferric chloride were added to each of the extracts in the tubes, which gave blue-black coloration indicating the presence of tannins.

Test for cyano-glycosides

In two different test tubes, extract of the two samples were mixed with glacial acetic acid. A drop of 15% ferric chloride and 1 mg mL^{-1} of concentrated sulfuric acid were added to the mixture of the respective samples and glacial acetic acid. The presence of cyano-glycoside was demonstrated by a brown coloring that developed at the contact.

Test for saponins

Five milliliters of distilled water were mixed each with 1 mL of each of the sample extracts in two different test tubes. The mixture after shaking vigorously, was observed for frothing, which confirmed the presence of saponins.

Test for steroids

A mixture of concentrated sulfuric acid and acetic acid was added to each of the sample extracts in two separate test tubes. The combination turned blue-green from violet, indicating the presence of steroids.

Test for terpenoids

Salkwoski's test was used to prove the presence of terpenoids. Each of the sample extracts (1 mL) in two test tubes were mixed respectively with 2 mL of chloroform and 3 mL of concentrated sulfuric acid. The appearance of a reddish-brown coloration at the interface proved the presence of terpenoids.

Test for alkaloids

To demonstrate the presence of alkaloids in the sample extracts, Wagner's test was employed. In two distinct test tubes, one milliliter of each sample extract was combined with three drops of Wagner's reagent (2 grams of iodine and 6 grams of potassium iodide, dissolved in 100 milliliters of distilled water). The presence of alkaloids was confirmed by a reddish-brown color.

Preparation of plant extracts

The method of Nweke and Ibiam (2012) was modified by using mature leaves and seeds of the test plant for the preparation of the extracts for the work. They were gathered, washed several times using sterile, deionized water, chopped into tiny pieces, and left to be dried by the air for seven days to a constant weight. They were blended into powder in a sterile Waring blender, and sieved through a sterile cheese cloth (2 mm pore size). Cold aqueous extracts of the powdered two sample parts of the plant, were prepared individually, by separately adding 1, 2, 3, 4 and 5 g of each of the sample parts into 250 mL conical flasks to prepare different concentrations of the extracts, in the ratio of 1:1, 2:1, 3:1, 4:1, and 5:1 w/v of extract and water . Aqueous extract concentrations of 100, 200, 300, 400, and 500 mg mL^{-1} were obtained from the resultant solutions of the extracts, respectively. They were left over the next night to enable the extraction of the plant parts' chemical components, after being properly mixed for 5 minutes on the magnetic stirrer. To keep the prepared extracts fresh until needed, they were placed in the refrigerator.

Antifungal bioassays

Two milliliters of each of the sample extracts was inoculated into 18 mL of PDA dispensed into Petri dishes according to the concentrations. No extracts were added into the control which had only the PDA. The whole set-up was allowed to solidify. The text fungus was inoculated at the center of the media in the Petri dishes for the treatments and control. Each concentration was replicated three times, including the control, giving a total of 18 Petri dishes per extract sample used. Diametric measurements of the fungal growth were taken on daily basis for 7 days, to determine the extent of inhibition of the vegetative growth of the fungal pathogen by the extracts. To assess the test fungus's capacity to withstand the effects of the extracts and resume further growth, period of the control experiment was extended by extra three days.

Statistical analysis

The results were analyzed statistically for the data generated from the *in vitro* inhibitory studies, using the leaves and seeds of *M. oleifera*, and the statistical package for social sciences (SPSS) was used. Means and standard deviations, as well as the analysis of variance (ANOVA) at $P \leq 0.5$, and Duncan multiple range were used to test the significance of performance values of the concentrations of the two parts of the plant, within and between the concentrations of the two parts, and the period of the experiment.

Results and Discussion

Fusarium oxysporum was isolated from infected leaves of *Z. zanthoxyloides* (Fig. 1). Earlier, Uzochukwu (2015) also isolated this fungus from the infected leaves of the plant. Phytochemical analysis of aqueous seed and leaf extracts of *M. oleifera* revealed the presence of saponins, cyanogenic glycosides, terpenoids, alkaloids, flavonoids, phenols, steroids, and tannins (Table 1). A similar qualitative phytochemical test carried out using the methanolic and ethanolic leaf and seed extracts of the plant by Ahmadu *et al.* (2021), confirmed the presence of phytochemicals compounds like phenolic compounds, alkaloids, anthaquinones, steroids, tannins, terpenoids and saponins in the *M. oleifera* leaf extract. Oniha *et al.* (2021) also obtained coumarins, terpenoids, triterpenoids, anthocyanins, beta-cyanins, cardiac glycosides, phenols, saponins, and tannins from the aqueous leaf extracts of the plant. The result of phyto-screening by the above researchers confirm the result of the phyto-screening obtained in this work with respect to the presence of corresponding phytochemicals observed.

Tables 2 and 3 show the values of the *in vitro* inhibitory effect of the aqueous extracts of *M. oleifera* leaves and seeds respectively, on the vegetative growth of *F. oxysporum*. There was a significant difference between the test groups and the control ($P \leq 0.05$). There was a progressive inhibitory effect of the aqueous leaf extracts on the vegetative growth of the fungus from day 1 to day 10. With respect to duration of exposure, there was a significant increase in inhibitory effect of *M. oleifera* leaf extract on the growth of *F. oxysporum* from day 1 to 10, across all concentrations. The highest effect was observed at extract concentration of 400 and 500

mg mL^{-1}, with a mean diametric measurement of 0.57 cm each, followed by 300 mg mL^{-1} with 0.63 cm, 200 mg mL^{-1} with 0.64 cm, and least effect at 100 mg mL^{-1} with 1.28 cm diameter. The fungal growth increased from day 1 until it became constant from the eighth day at 100 and 200 mg mL^{-1}, and from the day 7to the tenth day, from 300 to 500 mg mL^{-1}. The above results differed from the control, which had the highest diametric value of 1.92 cm. The growth values increased from day one until ninth day, when it became constant (Table 2).

Significant differences were existed between the test groups and the control ($P \leq 0.05$). The highest effect was observed at extract concentration of 500 mg mL^{-1} with a value of 0.76 cm followed by 400, 300, 200 and the least at 100 mg mL^{-1} with mean diametric measurements of 0.79, 0.86, 0.98 and 1.34 cm, respectively, while the control was 1.92 cm. The fungal growth increased from day one until it became constant from the ninth to the tenth day at 100 and 300 mg mL^{-1}, and also in control; and from the eighth to the tenth day for 400 and 500 mg mL^{-1}. With respect to duration of exposure, there was a progressive significant increase in inhibitory effect of *M. oleifera* seed extract on the growth of the fungus from day 1 to day 10, across all groups (Table 3).

Comparative observation of the mean diametric inhibitory effect of the plant's leaf and seed, showed that the mean diametric values with the same letters indicated no significance, whereas those with different letters indicated significant difference in the performance of the extracts, both within the concentrations, and between, the period of the experiment and control (Table 2 and 3).

This performance of the leaf extracts over that of the seeds could be due to the fact that leaf extract contained more efficacious phytochemicals than its seed extract counterpart. This is confirmed by the report of Ahmadu *et al*. (2021), who stated that crude extracts' antifungal bioassay results, showed that methanol leaf extract (99%) inhibited mycelial development more effectively. Oniha *et al.* (2021) used the aqueous leaf extracts of this plant to inhibit the growth of the following fungal species of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* and *Aspergillus fumigatus* at MIC value of 15.625 mg mL^{-1}, though our results were obtained at a higher concentration.

Extracts of leaves and seeds of *M. oleifera* have been effective in treating certain plant and animal fungal diseases, including *Trichophyton, Epidermophyton, Microsporium, Trichosporon, Geotricum,* and *candida* (Agu, 2008). Ayirezang *et al.* (2020), reported that the minimum inhibitory concentration of *M. oleifera* leaves and seeds extracts at 25 mg mL^{-1} may serve as natural antifungal for controlling growth of food spoilage fungi. Tesfay *et al*. (2021) revealed that *M. oleifera* extracts have potentials in reducing *Botrytis cinerea* and *Colletotrichum gloeosporoides*, the principal post-harvest disease fungus that cause poor quality and decreased shelf-life of blueberries. Their findings lend more evidence to the possibility that *M. oleifera* has antifungal characteristics. With varied efficiency, minimum inhibitory doses of the leaves' crude extracts were established as being 75 mg mL^{-1} for *Aspergillus flavus* and 100 mg mL^{-1} for *Rhizopus stolonifer* (Suraka *et al.*, 2021).

Conclusion

This study was about to investigate the use of crude extracts of the leaves and seeds of *M. oleifera* in the control of *F. oxysporum* associated with foliar disease of *Z. zanthoxyloides* leaves. The study reveals that leaf and seed extracts of the plant were effective in decreasing the vegetative growth of the fungus. Increasing concentrations of the extracts showed significant decreases in fungal growth. However, further studies are required to confirm the efficacy of the extracts in the treatment of foliar fungal diseases of *Z. zanthoxyloides in vivo*.

Table 1: Qualitative determination of the presence the phytochemicals (saponins, cyogenic glycosides, phenols, volatile oils, terpenoids, alkaloids, flavonoids and tannins) in the acqeuos extracts of the leaves and seeds of *M. oleifera*

Phyochemical	Lea Extract	Seed Extracts
Saponins	+	+
Alkaloids	+	+
Flavonoids	+	+
Flavonoids	+	+
Phenols	+	+
Steroids	+	+
Terpenoids	+	+
Cyogenic glycosides	+	+

+ sign represent present.

Table 2: Diametric measurement of the growth of *Fusarium oxysporum* inhibited by the aqueous extract of *Moringa oleifera* leaves.

Days	Extract concentration (mg mL^{-1})					
	Control	**100**	**200**	**300**	**400**	**500**
1	1.15±0.26b	1.12±0.26b	0.51±0.07b	0.49±0.05b	0.49±0.05b	0.48±0.02b
2	1.23±0.25a	1.14±0.29b	0.53±0.09c	0.51±0.03c	0.50±0.06c	0.50±0.06c
3	1.24±0.26b	1.14±0.29b	0.55±0.10e	0.53±0.04cd	0.52±0.07de	0.52±0.07de
4	1.25±0.20b	1.15±.31b	0.57±0.11d	0.54±0.05c	0.53±0.08d	0.53±0.08d
5	1 .25±0.25a	1.23±0.26b	0.58±0.12de	0.56±0.10cd	0.54±0.09d	0.54±0.09d
6	1.28±0.10b	1.23±0.25a	0.60±0.13cd	0.60±0.11bc	0.54±0.09c	0.54±0.09c
7	1.28±0.07b	1.25±0.20b	0.60±0.13c	0.63±0.12b	0.57±0.10bc	0.57±0.10bc
8	1.59±0.29a	1.28±0.10b	0.64±0.19b	0.63±0.12b	0.57±0.10b	0.57±0.10b
9	1.92±0.30a	1.28±0.07b	0.64±0.19d	0.63±0.13b	0.57±0.10cd	0.57±0.10cd
10	1.92±0.30a	1.28±0.26b	0.64±0.20d	0.63±0.13b	0.57±0.11cd	0.57±0.11cd

Values are expressed as mean±SD. Mean values in the same row with the same superscripts are not significantly different, while those with different superscripts are significantly different at $P \leq 0.05$

Table 3: Measurements of the growth (cm) of *Fusarium oxysporum* inhibited by the aqueous extract of *Moringa oleifera* seeds.

Days	Extract concentration (mg mL^{-1})					
	Control	**100**	**200**	**300**	**400**	**500**
1	1.15±0.26b	1.00± 0.02a	0.80 ± 0.06a	0.75±0.10a	0.73±0.05a	0.51±0.07bc
2	1.23±0.25a	1.21± 0.03a	0.82± 0.08a	0.77±0.11ab	0.76±0.07bc	0.57±0.08b
3	1.24±0.26b	1.23± 0.06a	0.84± 0.10a	0.78±0.12a	0.77± 0.09b	0.57±0.08bc
4	1.25±0.20b	1.25± 0.07a	0.89± 0.14a	0.79±0.13ab	0.78±0.10c	0.57±0.08bc
5	1 .25±0.25a	1.27± 0.13a	0.90± 0.17a	0.80±0.14ab	0.78±0.11c	0.57±0.08bc
6	1.28±0.10b	1.28± 0.15a	0.93± 0.19a	0.82±0.15ab	0.78±0.12c	0.58±0.08bc
7	1.28±0.07b	1.30± 0.21b	0.95± 0.23b	0.84±0.16b	0.78±0.13c	70.73±0.03a
8	1.59±0.29a	1.32± 0.22b	0.97± 0.24b	0.85±0.17b	0.79±0.14c	0.76±0.04b
9	1.92±0.30a	1.34± 0.23b	0.98± 0.26b	0.86±0.18b	0.79±0.14c	0.76±0.05b
10	1.92±0.30a	1.34± 0.23b	0.98± 0.26b	0.86±0.18b	0.79±0.14c	0.76±0.06b

Values are expressed as Mean±SD. Mean values in the same row with the same superscripts are not significantly different, while those with different superscripts are significantly different at $P \leq 0.05$.

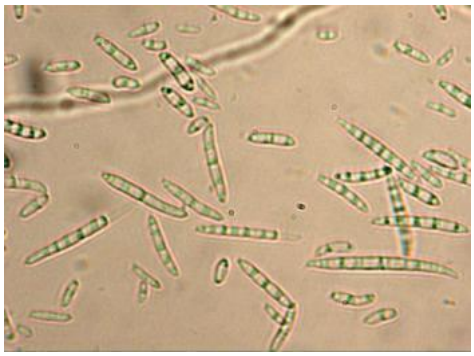

Fig. 1: *Fusarium oxysporum.*

References

Agu MC, 2008. Effects of organic manure types on root-gall nematode disease and African yam bean yield. *Plant Sci. Res.*,**1**: 17-19.

Alhakmani F, Kumar S, Khan SA, 2013. Estimation of total phenolic content, *in vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera. Asian Pacific J. Trop. Biomed.*, **3**: 623-627.

Ali A, Javaid A, Shoaib A, Khan IH, 2020. Effect of soil amendment with *Chenopodium album* dry biomass and two *Trichoderma* species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. *Egypt. J. Biol. Pest Control*, **30**: 102.

Arora DS, Onsare JG, Kuar H, 2013. Bioprospecting of *Moringa* (Moringaceae): microbiological perspective. *J. Pharmacog. Phytochem.*, **1**: 193-215.

Arora DS, Onsare JG, 2014. *In vitro* anti-microbial evaluation and phytoconstituents of *Moringa oleifera* pod husks. *Ind. Crops Prod.,* **52**: 125-135.

Ayirezang FA, Azumah B, Achio S, 2020. Effects of *Moringa oleifera* leaves and seeds extracts against food spoilage fungi. *Adv. Microbiol*., **10**: 27-38.

Banaras S, Javaid A, Khan IH, 2020. Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina*. *Int. J. Agric. Biol.,* **24**: 1376-1382.

Barnett HL, Hunter BH, 2000. Illustrated Genera of imperfect fungi 4th Edition. Mac. Graw Hill. pp. 219.

Belay K, nd Sisay M, 2014. Phytochemical constituents and physicochemical properties of medicinal plant (*Moringa Oleifera*) around bule hora. *Chem. and Mat. Res.***, 6**: 61-72.

Boye A, Koffuor GA, Boampong JN, 2012. Gastroprotective effect and safety assessment of *Zanthoxylum zanthoxyloides* root bark extract. *Am. J. Pharmacol. Toxicol.,* **7**: 73-80.

Dofuor AK, Djameh GI, Ayertey F, 2019. Antitrypanosomal effects of *Zanthoxylum zanthoxyloides* extracts on African trypanosomes. *Evid. Based Complement. Altern. Med.* **2019**: 1730452.

Dofuor AK, Ayertey F, Bolah P, 2020. Isolation and antitrypanosomal characterization of furoquinoline and oxylipin from *Zanthoxylum zanthoxyloides. Biomolecules,* **10**: 1670.

Dwivedi SK, Enespa A, 2012. Effectiveness of extract of some medical plants against soil borne fusaria causing diseases on *Lycopersicon esculantum* and *Solanum melongena*. *Intern. J. Pharma. Biol. Sci.,* **3**: 1171-1180.

Emeke U, Obontu TJ, Olushola I, Akinyelen A, 2019. Salivary fluoride retention: a comparative analysis between fluoride containing chewing sticks and a non-herbal fluoridated toothpaste. *J. Contemp. Dent. Pract.,* **20**: 370-376.

Enechi OC, Amah CC, Okagu IU, 2019. Methanol extracts of *Fagara zanthoxyloides* leaves possess antimalarial effects and normalizes haematological and biochemical status of plasmodium berghei-passaged mice. *Pharmac. Biol.,* **57**: 577-585.

Fahey SD, 2005. *Moringa oleifera*. A review of the medical evidence for its nutritional, therapeutic and prophylactic properties, Part1. Retrieved from *http://www.TFLJournal.org.*

Ferdosi MFH, Ahmed H, Khan IH, Javaid A, 2022. Fungicidal potential of flower extract of *Cassia fistula* against *Macrophomina phaseolina* and *Sclerotium rolfsii. J. Anim. Plant Sci.,* **32**: 1028-1034.

Goodman CD, Hoang AT, Diallo M, McFadden GI, Wangensteen H, 2019. Anti-plasmodial effects of *Zanthoxylum zanthoxyloides. Planta,* **85**: 1073-1079.

Jabeen N, Javaid A, Shoaib A, Khan IH, 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel. J. Plant Pathol.,* **103**: 901-913.

Javaid A, Khan IH, 2016. Management of collar rot disease of chickpea by extracts and soil amendment with dry leaf biomass of *Melia azedarach* L. *Philipp. Agric. Sci.,* **99**: 150-155.

Javed S, Mahmood Z, Khan KM, Sarker SD, Javaid A, Khan IH, Shoaib A, 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. *Sci. Rep.,* **11**: 8417.

Khan IH, Javaid A, 2013. Antifungal activity of *Melia azedarach* L. fruit extract against *Sclerotium rolfsii*, the cause of collar rot disease of chickpea. *Mycopath,* **11**: 9-13.

Khan IH, Javaid A, 2020. First report of *Curvularia lunata* causing postharvest fruit rot of banana in Pakistan. *Int. J. Agric. Biol.,* **24**: 1621-1624.

Khan IH, Javaid A, 2021. Molecular characterization of *Penicillium expansum* associated with blue mold disease of apple in Pakistan. *Pak. J. Bot.,* **53**: 2299-2303.

Khan IH, Javaid A, 2022. *Penicillium echinulatum* causing blue mold on tomato in Pakistan. *J. Plant Pathol.,* **104**: 1143.

Mbikay M, 2012. Therapeutic potential of *Moringa oleifera* leaves in chronic hyperglycemia and dyslipidemia. a review. *Front. Pharmacol.,* **3**: 24.

Ogunrinade FA, Guetchueng ST, Katola FO, 2021. *Zanthoxylum zanthoxyloides* inhibits lipopolysaccharide- and synthetic hemozoin-induced neuroinflammation in BV-2 microglia: roles of NF-κB transcription factor and NLRP3 inflammasome activation. *J. Pharm. Pharmacol.,* **73**: 118-134.

Nweke C, Ibiam OFA, 2012. Effect of extracts from leaves, bark and roots of *Azadiratch indica* L on the vegetative growth of *Colletotrichum gloeosporoides* fruit rot pathogen of soursop (*Annona muricata* L) fruits. *Agric. Biol. J. North Am.,* **3**: 481-485.

Olounladé EVB, Azando MS, Hounzangbé-Adoté, 2012. *In vitro* anthelmintic activity of the essential oils of *Zanthoxylum zanthoxyloides* and *Newbouldia laevis* against *Strongyloides ratti*. *Parasitol. Res.,* **110**: 1427-1433.

Sharma V, Paliwal R, 2013. Isolation and characterization of saponins from *Moringa oleifera* (*Moringaceae*) pods. *Int. J. Pharm. Pharmaceut. Sci.,* **5**: 179-183.

Suraka B, Ibrahim D, Suleman KU, Usman SI, 2021.

Antifungal activity of *Moringa oleifera* leaves crude extract against *Aspergillus flavus* and *Rhizopus stolonifer*. *Dutse J. Pure Appl. Sci.*, **7**: 9-17

Tesfay NA, Mbili SZ, Mditshwa NCA, Magwaza LS, 2021. Antimicrobial properties of *Moringa oleifera* extracts on blueberry post-harvest fungal diseases. *Acta Hort.*, **1306**: 329-334

Tine Y, Renucci F, Costa J, Wélé A, Paolini J, 2017. A method for LC-MS/MS profiling of coumarins in *Zanthoxylum zanthoxyloides* (Lam.) B. Zepernich and timler extracts and essential oils. *Molecules*, **22**: 174.

Uzocukwu A, 2015.Effect of fungi associated with the leaf psot of *Zanthoxylum zanthoxyloides* on the proximate and anti-nutrient composition of the leaves. B. Sc. Project. Ebonyi State University, Abakaliki. Nigeria.

Uzoekwe NJ, Mohammed JJ, 2015 Phytochemical, Proximate and Mineral contents of leaves and back of *Ficus capensis. J. Appl. Sci. Environ. Manage*., **19**: 633-637.

Vongsak B, Sithisarn P, Gritsanapan W, 2014. Simultaneous HPLC quantitative analysis of active compounds in leaves of *Moringa oleifera* Lam. *J. Chromat. Sci.*, **52**: 641-645.

Williams R, Soelberg J, Jäger AK, 2016. Anthelmintic properties of traditional African and Caribbean medicinal plants: identification of extracts with potent activity against Ascaris suum *in vitro*. *Parasite*, **23**: 24.