Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

OPEN ACCESS Asian Journal of Plant Sciences

ISSN 1682-3974
DOI: 10.3923/ajps.2016.35.41

Research Article
Phytochemical Constituents and Antifungal Properties of *Chromolaena odorata* L. and *Moringa oleifera* Lam on Fungal Rot of Cucumber (*Cucumis sativus* L.) Fruit

N.V. Chiejina and C.N. Onaebi

Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Enugu State, Nigeria

Abstract
This study investigated the phytochemical constituents and antifungal properties of *Chromolaena odorata* L. and *Moringa oleifera* Lam. against some pathogenic fungi of cucumber fruits. The fungi were *Geotrichum candidum* Link, *Mucor micheli* ex Staint-Amans, *Rhizopus stolonifer* Ehrenb. ex. Fr, *Aspergillus niger* Van Tiegh and *Fusarium oxysporum* Schlecht. The efficacy of the plant extracts varied with the plant pathogens and the various concentrations (20, 30, 40, 60, 80 and 100 mg mL^{-1}) tested. *In vivo* application of the extracts showed that *M. oleifera* had high fungitoxic effect that totally controlled the mycelial growth of *G. candidum*; with 100% inhibition at 100 mg mL^{-1}. *Moringa oleifera* extract also showed progressive retardations of the mycelial growth of *M. micheli* and *R. stolonifer*. Although *C. odorata* extract decreased the mycelial growth of the fungi with increase in concentrations, it was not as effective as *M. oleifera* since at 100 mg mL^{-1} it gave 64.97% inhibition of *G. candidum*. Phytochemical analysis of *C. odorata* revealed the presence of tannins, saponins, phenols, flavonoids, terpenoids, glycosides and alkaloids, while *M. oleifera* contained all the phytochemicals mentioned above except alkaloids. The presence of these active ingredients in the extracts may be responsible for the properties observed.

Key words: Phytochemicals, antifungal properties, *in vivo*, cucumber, pathogens, extracts

Received: November 18, 2015 **Accepted:** January 30, 2016 **Published:** March 15, 2016

Citation: N.V. Chiejina and C.N. Onaebi, 2016. Phytochemical constituents and antifungal properties of *Chromolaena odorata* L. and *Moringa oleifera* Lam on fungal rot of cucumber (*Cucumis sativus* L.) fruit. Asian J. Plant Sci., 15: 35-41.

Corresponding Author: C.N. Onaebi, Department of Plant Science and Biotechnology, University of Nigeria Nsukka, Enugu State, Nigeria
Tel: 08037292947

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Fruits, due to their high moisture content and nutrient composition are prone to pathogenic fungal attack which in addition to causing rots may also make them unfit for human consumption by producing mycotoxins (Moss, 2002). Postharvest diseases account for about 50% losses in fruits stored in poor storage conditions especially under high humidity. They pose a major problem to the agricultural industries (Agrios, 2005). Cucumber fruits are among the fruits susceptible to postharvest diseases caused by fungi under poor storage conditions. In Nigeria, fungi constitute the major limiting factor to the production of cucumber (NARI., 2004). Losses caused by fungal attack vary from 20-30% (Park *et al*., 2008). Since the end of the second world war, there has been a great boom in the use of fungicides for control of fungal diseases world wide. After the great justified alarm in the early 60s about the dangerous consequences to man and environment with respect to phytotoxicity, there came an urgent need for alternative method of plant disease control. This scenario necessitated the search for and the development of ecologically sustainable fungal control methods which are effective against the target species but cause minimal adversity for non-target species (Suleiman and Emua, 2009).

Successes have been recorded in the use of extracts, *Chromolaena odorata* and or *Moringa oleifera* to control fungal pathogens (Okigbo *et al*., 2010; Devendra *et al*., 2011). Aman and Rai (2015) reported the antifungal activity of plant extracts against yellow sigatoka disease causing *Mycosphaerella musicola* in banana plantations. The antifungal activity of methanol extracts of *Leonotis nepetifola* L. and *Ocimum gratissimum* L. against asochyta blight (*Phoma exigua*) on french bean was also reported by Ochola *et al*. (2015). The biological activity of plant extracts are mainly as a result of the phytochemical constituents they contain. Leaf extracts of *C. odorata* and *M. oleifera* contain phytochemicals which offer an enormous potential as biocontrol agents of pathogens and a source of antimicrobial agents of therapeutic importance. It has been shown that *M. oleifera* leaf extract has antibiotic and antifungal properties against a wide range of pathogens like *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Aspergillus niger* and *Candida albicans* (Devendra *et al*., 2011). According to Ijato *et al*. (2010), leaf extract of *C. odorata* was effective in the control of fungal pathogens of diseased tomato infected with *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer* and *Geotrichum candidium*. Due to identifiable problems like chemical residues, phytotoxicity and pollution associated with chemical control strategies, alternative control methods are being explored. The aim of this research is to provide useful information on cheaper, affordable, natural and environment friendly bio-fungicide in the control of post-harvest fungal rot of cucumber fruit.

MATERIALS AND METHODS

The research was carried out from the month of August, 2010 to May, 2011 in Pathology Laboratory, Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Collection of plant materials: Diseased cucumber fruits were collected from Nsukka main market, Ogbete main market and Artisan market all in Enugu State. The leaves of *M. oleifera* and *C. odorata* were collected within the vicinity of University of Nigeria, Nsukka. Identification of the plants was confirmed by Mr. A. Ozioko of the Biodiversity and Conservation Centre, Nsukka.

Isolation of fungi from diseased cucumber fruits: The isolation method of Chiejina (2008) was used. Thin sections (2 mm diameter) were cut from periphery of diseased portions of cucumber fruits, sterilized in 0.1% mercuric chloride for 2-3 min and rinsed in three changes of sterile distilled water. The sections were plated into clean PDA plates and incubated at room temperature (27±2°C) for 6-7 days. Subcultures were made aseptically from the plates into similar clean PDA plates and were incubated under similar conditions until pure cultures were obtained. Small portions of the fungal cultures were teased and mounted in lactophenol in cotton blue on clean slides, covered with clean cover slips and viewed microscopically. Fungal identification was confirmed with the aid of books by Agrios (2005) and Ellis *et al*. (2007).

Pathogenicity test: The method of Okigbo and Ikediugwu (2000) was used. Cylindrical cores, 1 cm deep were removed from healthy cucumber fruits with sterile 5 mm diameter cork borer and 4 mm discs taken from the edge of a colony of test fungus were each placed downward into the holes in the fruits. The cores of the cucumber fruits were replaced after 2 mm pieces were cut off to compensate for the thickness of the agar inocula and the replaced core sealed with sterile petroleum jelly. Sterilized PDA used in place of the culture disc served as the control. On establishment of disease condition, inocula were taken from the infected fruits and cultured. The organisms were re-isolated and identified.

Plant extract preparation: The leaves of *M. oleifera* and *C. odorata* were dried at room temperature 27±2°C by spreading them on the floor for some days after which they were ground into fine powder.

Chiejina and Onaebichiemeka (2013) extraction method was used. Fifty grams each of the powdered plant material were soaked in 500 mL of absolute ethanol and allowed to stand for 2-3 days on a laboratory bench. The suspension was filtered using No. 1 Whatman filter paper. The filtrates were poured into plastic saucers and placed beneath a ceiling fan which evaporated the solvents leaving behind the crude extracts. The crude extracts were put into sterile bottles, labeled accordingly and stored in the refrigerator throughout the duration of the experiments.

The plant extracts were each dissolved in 50% concentration of dimethyl sulphoxide (DMSO; $(CH_3)_2SO$) in the ratio of 1:10 (1 g of crude extract dissolved in 10 mL of DMSO) to give a concentration of 100 mg mL^{-1}. Dilutions of 80, 60 , 40 and 20 mg mL^{-1} were made from the stock solution.

Effect of plant extracts on fungal mycelial growth: A modified method of Uzuegbu and Okoro (1999) was used. Healthy cucumber fruits were surface sterilized with 95% ethanol and rinsed in several changes of sterile distilled water. The fruits were perforated using a sterile cork borer (3 mm diameter) and the cores removed with a sterile forceps. Test plant extract (0.5 mL) was pipetted into each perforated area and allowed to percolate for a few minutes. Mycelial discs cut from each of the test fungus were inoculated into the holes and the holes were plugged with the cores. Cucumber fruits inoculated with clean mycelial discs without plant extracts served as the control. The inoculation sites were sealed with vaseline (jelly) to prevent contamination. The diameters of the inoculated sites were measured after five days. Fungi toxicity was recorded in terms of percentage colony inhibition and calculated according to the equation:

$$\text{Growth inhibition (\%)} = \frac{DC - DT}{DC} \times 100 \quad (1)$$

Where:
DC = Average diameter of control
DT = Average diameter of fungal colony with treatment

Phytochemical analysis: Qualitative detection of the presence of saponins, tannins, alkaloids, flavonoids, phenols, glycosides and terpenoids was carried out on the extracts as follows:

Test for saponins: The ability of saponins to produce frothing in aqueous solutions was used as a screening test for its presence. Five millilitres of ethanolic extract of each of the test plants were shaken with distilled water in a test tube, frothing which persisted on warming was taken as evidence for the presence of saponins (Sofowora, 1993).

Test for tannins: Five millilitres of each of the extracts were diluted with 20 mL distilled water and 3-4 drops of 10% ferric chroride solution added. A blue-black green precipitate indicates the presence of tannins (Trease and Evans, 2005).

Test for alkaloids: Two millilitres of each of the extracts were each measured into a test tube and a few drops of picric acid solution were added. An orange coloration indicates the presence of alkaloids (Trease and Evans, 2005).

Test for flavonoids: Four millilitres of each of the plant extracts were heated with 10 mL of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 mL of the filtrate was shaken with 1 mL of dilute ammonia solution. A yellow coloration indicates the presence of flavonoids (Sofowora, 1993).

Test for phenols: Equal volumes of plant extract and ferric chloride solutions were mixed together and the presence of phenols is indicated by a deep bluish green colour (Sofowora, 1993).

Test for terpenoids: Five millilitres of each extract were mixed with 2 mL chloroform and 3 mL concentrated H_2SO_4 added so as to form a layer. A reddish-brown precipitate at the interface indicates the presence of terpenoids (Harborne, 1998).

Test for glycosides: Twenty five millilitres of sulphuric acid was added to 5 mL of each extract in a test tube and boiled for 15 min, cooled and neutralized with 10% NaOH. Five millilitres of Fehlings solution was added. The presence of glycosides was indicated by brick red precipitate (Koruthu *et al*., 2011).

RESULTS

Identification of pathogens: Five fungal isolates were obtained from diseased cucumber fruits and identified as *Geotrichum candidum*, *Mucor micheli, Rhizopus stolonifer*, *Aspergillus niger* and *Fusarium oxysporum* as was confirmed by the pathogenicity test carried out.

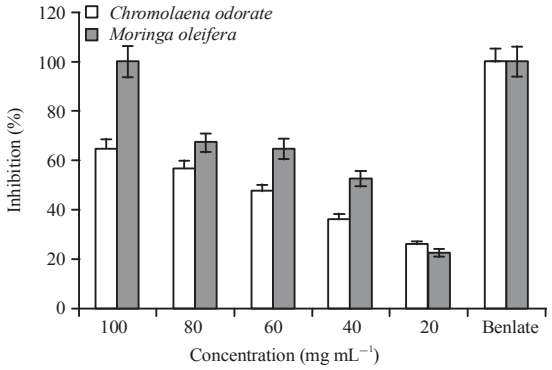

Fig. 1: Effects of ethanolic plant extracts on *in vivo* treatment of *G. candidum*

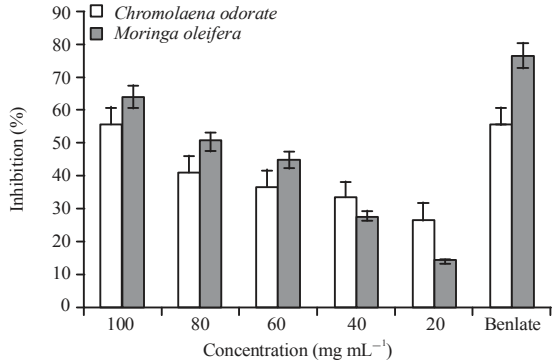

Fig. 2: Effects of ethanolic plant extracts on *in vivo* treatment of *M. micheli*

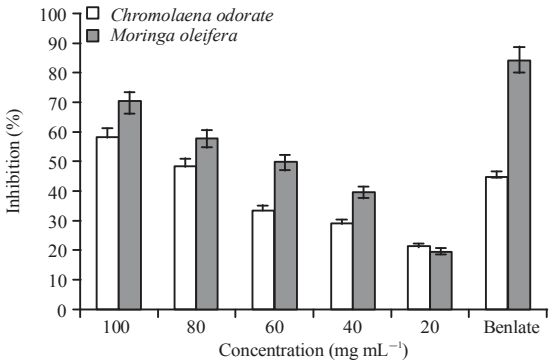

Fig. 3: Effects of ethanolic plant extracts on *in vivo* treatment of *R. stolonifer*

Effects of plant extracts on radial growth of pathogens: Ethanolic extracts of *Chromolaena odorata* and *Moringa oleifera* where found to be fungitoxic on the mycelial growth of the rot fungi. The disease development of *Geotrichum candidum* was inhibited by the plant extracts and benlate solution. The inhibitory effects of the plant extracts increased as the concentrations increased. *Moringa oleifera* extracts exhibited higher inhibitory effects than *C. odorata* at all the concentrations except at the lowest concentration of 20 mg mL^{-1} (Fig. 1). Figure 2 shows the fungitoxic activities of the plant extracts on mycelial growth of *Mucor micheli*. The fungi toxic effects of *M. oleifera* extract were more potent than those of *C. odorata* at higher concentrations than at the lower. Results on the effects of ethanolic plant extracts on *in vivo* treatment of *Rhizopus stolonifer* are shown in Fig. 3. The results revealed that both plant extracts produced significant ($p<0.05$) levels of inhibition of mycelial growth of *R. stolonifer* at the various concentrations. Figure 4 shows the results of the effects of the plant extracts on disease development of *A. niger* in cucumber fruits. Ethanolic extracts of *M. oleifera* and *C. odorata* at 100 mg mL^{-1} concentration gave 100 and 66.6% inhibition, respectively. *Moringa oleifera* extracts were the most effective in arresting disease development and this compared very well with benlate at higher concentrations. The effects of the ethanolic leaf extracts and benlate solution on the mycelial growth of *Fusarium oxysporum* was significantly different ($p<0.05$) for all the concentrations. The two extracts at 100 mg mL^{-1} and benlate solution at 20 mg mL^{-1} gave 100% inhibition. The least effect (14.0%) was produced by *M. oleifera* extract at 20 mg mL^{-1} (Fig. 5). Once more, *M. oleifera* extract was more potent than *C. odorata* extract except at the lowest concentrations tested.

Phytochemical analysis: The phytochemical screening of *Chromolaena odorata* revealed the presence of saponins, tannins and terpenoids in moderate concentrations while alkaloids, flavonoids, glycosides and phenols were in low concentrations. The phytochemical screening of *Moringa oleifera* revealed the presence of flavonoids in high concentration, terpenoids and glycosides in moderate concentration. Tannins, saponins and phenols were in low concentrations while alkaloids were absent (Table 1).

DISCUSSION

The organisms associated with the rot of cucumber fruit in the present study were *Geotrichum candidum, Mucor micheli, Rhizopus stolonifer*, *Aspergillus niger* and *Fusarium oxysporum*. These organisms have been associated with post harvest rot of fruits (Onyeke and Ugwoke, 2011; Chiejina and Onaebichiemeka, 2013). Roting may probably start in the soil and progress in storage. This may happen when infected fruits do not show perceptible external symptoms (Okigbo and Ogbonna, 2006). Some of these pathogens like *R. stolonifer* and *M. micheli* produce

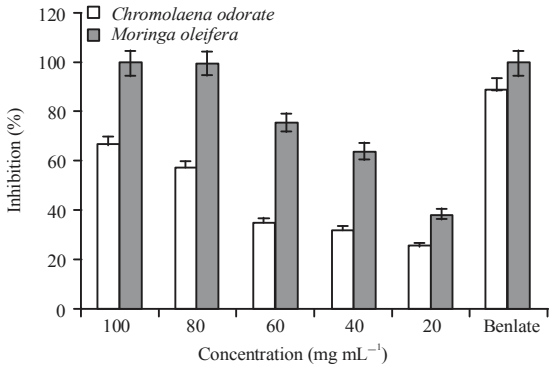

Fig. 4: Effects of ethanolic plant extracts on *in vivo* treatment of *Aspergillus niger*

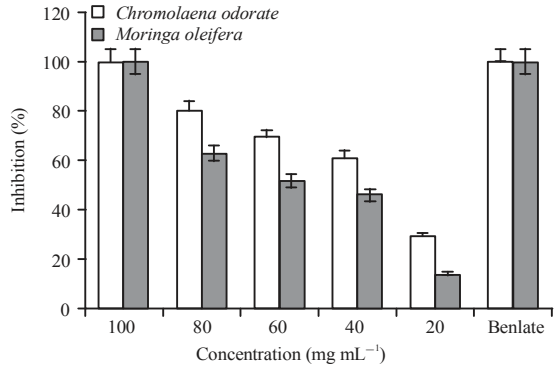

Fig. 5: Effects of ethanolic plant extracts on *in vivo* treatment of *F. oxysporum*

Table 1: Phytochemical screening of *C. odorata* and *M. oleifera* extracts

Compounds	*Chromolaena odorata*	*Moringa oleifera*
Saponins	++	+
Tannins	++	+
Alkaloids	+	-
Flavonoids	+	+++
Phenols	+	+
Terpenoids	++	++
Glycosides	+	++

+++: Present in high concentration, ++: Present in moderate concentration, +: Present in low concentration, -: Absent

numerous air-borne sessile spores that can easily land on the fruits while on display in the market. This is in line with the view of Chiejina (2008) on salad vegetables.

The results indicated that the tested plant extracts, *Moringa oleifera* and *Chromolaena odorata*, caused a significant reduction in the radial growth of the pathogens. This shows that they have fungitoxic potentials. The observed fungitoxicity of the extracts comfirms the report of Ijato *et al*. (2010) who reported fungitoxic activity of *Azadirachta indica* and *Chromolaena odorata* against *A. niger, F. oxysporum, R. stolonifer* and *G. candidum.* The radial growth and spore germination of *Fusarium oxysporum* was reported to be inhibited by extracts of *C. odorata* (Okoi and Udo, 2010). Dwivedi and Sangeeta (2015) also reported the inhibition of the radial growth of *Fusarium oxysporum* f. sp. ciceri by some medicinal plant extracts in which *M. oleifera* was among. The inhibitory effect gradually increased with increase in the concentration of the extracts. This observation supports the report of Daouk *et al*. (1995) that the reduction in microbial population depends on the concentration of the extracts and that high concentrations can completely inhibit the growth of microorganisms. Suleiman and Emua (2009) also observed toxicity of plant extracts at higher concentrations. *Moringa oleifera* had higher fungitoxic effect than *C. odorata. Moringa oleifera* extracts completely controlled the mycelial growth of *G. candidum*; with 100% inhibition at 100 mg mL^{-1}. *Moringa oleifera* extract also showed progressive retardations of the mycelial growth of *M. micheli* and *R. stolonifer*. Jabeen *et al*. (2008) and Oluduro (2012) obtained fungal inhibitory activities with extracts of *M. oleifera* as was observed in this study confirming the antifungal potency of the extracts. Extracts from *M. oleifera* at 100 mg mL^{-1} concentration was the closest to benlate at 20 mg mL^{-1} which is the standard fungicide for rot control in cucumber. The disparity in the concentration of leaf extracts and benlate was that the extract was in crude form while benlate was refined. Therefore, leaf extracts required higher concentration to work efficiently.

The inhibitory effects of plant extracts on mycelial growth of plant pathogenic fungi have been claimed to lie in their phytochemical constituents which include alkaloids, tannins, flavonoids, phenols, saponins and terpenoids (Anyasor *et al*., 2011). The phytochemical analysis carried out on the test plants showed the presence of alkaloids, tannins, flavonoids, phenols, saponins and terpenoids in *C. odorata* while *M. oleifera* contained all the phytochemicals stated above except alkaloids. Anyasor *et al*. (2011) were able to show the presence of terpenoids, tannins, saponins, anthraquinones, phenols and cardiac glycoside in extracts of *C. odorata* and Bamishaiye *et al*. (2011) also noted the presence of alkaloids, tannins, phenolics, saponins, flavonoids and steroids in extracts of *M. oleifera*. Majority of these phytochemicals were equally identified in *C. odorata* and *M. oleifera* extracts in this study. The fungicidal properties of the extracts hindered the mycelial development of the fungi by probably affecting their metabolism which may have resulted in their inability to use the required substrates efficiently. This agrees with the study of Chiejina and Ukeh (2012) on phytochemical analysis of methanolic extracts of *Aframomum melegueta* and *Zingiber officinale*. The presence of these identified phytochemicals supports their use as antimicrobial agents. The fact that plant

extracts from *M. oleifera* and *C. odorata* were used to control the rot of cucumber fruit makes them a possible substitute for synthetic fungicide. This approach to plant disease management is economically viable and poses little environmental risk. Moreover, the plants are available to farmers in Nigeria who do not have ready access to synthetic fungicides.

ACKNOWLEDGEMENT

The authors extend their appreciation to Dr. C.C. Onyeke of the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka for his valuable contributions in the course of this research.

REFERENCES

Agrios, G.N., 2005. Plant Pathology. 5th Edn., Academic Press, New York, USA., ISBN-13: 9780120445653, Pages: 922.

Aman, M. and V.R. Rai, 2015. Antifungal activity of fungicides and plant extracts against yellow sigatoka disease causing *Mycosphaerella musicola*. Curr. Res. Environ. Applied Mycol., 5: 277-284.

Anyasor, G.N., D.A. Aina, M. Olushola and A.F. Aniyikaye, 2011. Phytochemical constituent, proximate analysis, antioxidant, antibacterial and wound healing properties of leaf extracts of *Chromolaena odorata*. Ann. Biol. Res., 2: 441-451.

Bamishaiye, E.I., F.F. Olayemi, E.F. Awagu and O.M. Bamshaiye, 2011. Proximate and phytochemical composition of *Moringa oleifera* leaves at three stages of maturation. Adv. J. Food Sci. Technol., 3: 233-237.

Chiejina, N.V., 2008. Mycoflora of some salad vegetables. Bio-Research, 6: 392-395.

Chiejina, N.V. and J.A. Ukeh, 2012. Antimicrobial properties and phytochemical analysis of methanolic extracts of *Aframomum melegueta* and *Zingiber officinale* on fungal diseases of tomato fruit. J. Nat. Sci. Res., 2: 10-15.

Chiejina, N.V. and C.N. Onaebichiemeka, 2013. *In vitro* fungicidal activity of two plant extracts against five phytopathogenic fungi of cucumber (*Cucumis sativus* L.) fruit. Int. J. Applied Nat. Sci., 2: 61-68.

Daouk, R.K., S.M. Dagher and E.J. Sattout, 1995. Antifungal activity of the essential oil of *Origanum syriacum* L. J. Food Prot., 58: 1147-1149.

Devendra, B.N., N. Srinivas, V.S.S.L.P. Talluri and P.S. Latha, 2011. Antimicrobial activity of *Moringa oleifera* Lam., leaf extract, against selected bacterial and fungal strains. Int. J. Pharma Bio Sci., 2: B13-B18.

Dwivedi, S.K. and Sangeeta, 2015. Efficacy of some medicinal plant extract against *Fusarium oxysporum* f. sp. *ciceri* causing chickpea wilt. Asian J. Crop Sci., 7: 138-146.

Ellis, D.H., S. Davis, H. Alexiou, R. Handke and R. Bartley, 2007. Descriptions of Medical Fungi. 2nd Edn., Adelaide Medical Centre for Women & Children, Australia, ISBN-13: 9780959851267, Pages: 198.

Harborne, A.J., 1998. Phytochemical Methods a Guide to Modern Techniques of Plant Analysis. 3rd Edn., Springer, New York, USA., ISBN-13: 9780412572609, Pages: 302.

Ijato, J.Y., S.D. Oyeyemi, J.A. Ijadunola and J.A. Ademuyiwa, 2010. Allelopathic effect of leaf extract of *Azardirachta indica* and *Chromolaena odorata* against post harvest and transit rot of tomato (*Lycopersicum esculentum* L). J. Am. Sci., 6: 1595-1599.

Jabeen, R., M. Shahid, A. Jamil and M. Ashraf, 2008. Microscopic evaluation of the antimicrobial activity of seed extracts of *Moringa oleifera*. Pak. J. Bot., 40: 1349-1358.

Koruthu, D.P., N.K. Manivarnan, A. Gopinath and R. Abraham, 2011. Antibacterial evaluation, reducing power assay and phytochemical screening of *Moringa oleifera* leaf extracts: Effect of solvent polarity. Int. J. Pharmaceut. Sci. Res., 2: 2991-2995.

Moss, M.O., 2002. Mycotoxin review-1. *Aspergillus* and *Penicillium*. Mycologist, 16: 116-119.

NARI., 2004. Post-harvest handling technical series. Technical Bulletin, No. 28, Cucumber Post Harvest Care and Market Preparation, National Agricultural Research Institute, pp: 13.

Ochola, S.O., J.O. Ogendo, I.N. Wagara, J.O. Ogweno, J.G. Nyaanga and K.O. Ogayo, 2015. Antifungal activity of methanol extracts of *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. against ascochyta blight (*Phoma exigua*) on French bean. Asian J. Plant Pathol., 9: 27-32.

Okigbo, R.N. and F.E.O. Ikediugwu, 2000. Studies on biological control of postharvest rot in yams (*Dioscorea* spp.) using *Trichoderma viride*. J. Phytopathol., 148: 351-353.

Okigbo, R.N. and U.O. Ogbonna, 2006. Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on postharvest yam (*Dioscorea* spp.) rot. Afr. J. Biotechnol., 5: 727-731.

Okigbo, R.N., C.A. Agbata and C.E. Echezona, 2010. Effects of leaf extracts of *Azadirachta indica* and *Chromolaena odorata* on post harvest spoilage fungi of yams in storage. Curr. Res. J. Biol. Sci., 2: 9-12.

Okoi, A.I. and S.E. Udo, 2010. Effect of leaf extracts of three plant species on mycelial growth of *Fusarium oxysporum*. Niger. J. Mycol., 3: 101-109.

Oluduro, A.O., 2012. Evaluation of antimicrobial properties and nutritional potentials of *Moringa oleifera* Lam. leaf in South-Western Nigeria. Malaysian J. Microbiol., 8: 59-67.

Onyeke, C.C. and K.I. Ugwuoke, 2011. Effects of botanical extracts on the mycelial growth of seed-borne fungi of the African yam bean, *Sphenostylis stenocarpa* (Hochst ex a. Rich) Harms. Niger. J. Biotechnol., 22: 1-7.

Park, I.K., J. Kim, Y.S. Lee and S.C. Shin, 2008. *In vivo* fungicidal activity of medicinal plant extracts against six phytopathogenic fungi. Int. J. Pest Manage., 54: 63-68.

Sofowora, A., 1993. Medicinal Plants and Traditional Medicine in Africa. 2nd Edn., Spectrum Books Ltd., Ibadan, Nigeria, ISBN-13: 9782462195, Pages: 289.

Suleiman, M.N. and S.A. Emua, 2009. Efficacy of four plant extracts in the control of root rot disease of cowpea (*Vigna unguiculata* [L.] Walp). Afr. J. Biotechnol., 8: 3806-3808.

Trease, G.F. and W.C. Evans, 2005. Pharmacognosy. 5th Edn., Elsevier, New Delhi, India, Pages: 585..

Uzuegbu, J.O. and O.K. Okoro, 1999. Protection of mechanically injured yellow yam (*Dioscorea cayenensis*) tubers from fungal rot using pastes of some local herbs. J. Sustain. Agric. Environ., 1: 262-266.