INHIBITORY EFFICACY OF SOME POTENTIAL LEAF EXTRACTS ON SOME ROOT PATHOGENS

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ABSTRACT

The efficacy of four potential leaf extracts of *Chromolaena odorata* (Stain weed), *Ocimum gratissimum* (Shrubby bacil) and *Cymbopogon giganteus* (Lemon grass) on some fungal root pathogens of *Aspergillus niger, Fusarium moniloforme, Rigidoporus lignosus*, and *Trichoderma* sp were investigated in invitro. The varied concentrations of the extracts at the rates of 5, 20, 60 and 100 g/l[1] impacted levels of inhibition on the root pathogens. At all levels of concentrations *Chromolaena odorata* consistently reduced and inhibited the growth of *Fusarium* most prominently ($P<0.01$), and with slight inhibitory effects on *Aspergillus* and *Trichoderma* ($P<0.05$) especially at the lowest concentration (5g/l[1]). However, effects of extracts at all concentrations failed to produce any discernable inhibition on *Rigidoporus.* Microscopic observation of the test pathogens are depicted for their structural characteristic. The present study reveals the inhibitory effects of the leaf extracts on root pathogens and the most efficacious extract are discussed for sustainable agriculture.

Key words: Plant extracts, root pathogens, inhibitory effects, varied concentrations of extracts

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INTRODUCTION

Chemical control of plant pathogens, parasites and pests essentially involves the use of synthetic pesticides that may likely lead to phytotoxicity, environmental pollution and pathogen

resistance (Yudelman *et al.*, 1998, Backman, 1997). Unsafe use of pesticides may result in deleterious effects on humans and animals. It is therefore imperative to develop naturally occurring fungicides, nematicides and pesticides which may be less toxic to man and animals but effective against pests, nematodes, fungi and other pathogens of various crops (Conway, 1995).

In recent years, much attention has been given to the use of non-chemical systems for seed treatment against seed borne pathogens. Plant extracts in this case have played significant role in inhibitory effects on plant pests and in the improvement of seed quality (Nwachukwu and Umechuruba, 2001). Plant extracts are products that are made out of plants in form of decoctions, infusions and powders (Adodo, 2004). Medical plants have been recognized for their characteristics and chemical attributes in nature.

Plant such as *Agerantum Conyziodes* commonly known as goat weed has a phytotoxicity that enables it to inhibit the growth of plants. This was discovered in wheat planted on soil previously infested with goat weed (Singh et al, 2003).

Chromolaena odorata L., known as Siam weed popularly called "Awolowo" plant in Nigeria, has the ability to suppress the growth of other plants growing with it (all elopathic properties), this is due to its ability to compete for conditions and the chemical components that are inhibitory to other plants around it (Apori *et al.*, 2000).

Leaf extracts of *Vernonia amygdalina* (bitter leaf), *Cymbopogon giganteus* (Lemon grass), bacil leaf and neem have inhibitory effects on fungal pathogens. Umuchuruba and Nwachukwu (1994 and 1999) reported that the four extracts significantly reduced incidence of *Aspergillus niger*, *Aspergillus flavus*, *Botryodiplodia theobromae*, and *Fusarium moniliforme* test when compared with their aqeous extracts.

Extracts of *Cymbopogon giganteus, Chromolaena odorata, Azadirachta indica* were employed on *Meloidogyne incognita* a causative agent of root knot of nematode of soybean as reported by Caveness (1967), Ogunfowora *et al*. (1983) who also indicated that nematodes did not initially constitute a serious problem on edible soybean in Nigeria. Akinyemi *et al.* (2005) have shown that ethanol and water extraction of *Ocimum gratissiumum* were effective against the growth of *Staphylococcus aureus.* In another study, leaf extrats of selected plants of *Chromolaena odorata* (Siam weed), *Ocimum gratissimum* (Shrubby bucil), and *Cymbopogon giganteus* (Lemon grass) were screed for their antifungal properties and used for the fungal pathogens associated with cassava root. The authors reported that *C. odorata* is a weed found in the tropic, in the family of Asteraceae, and the plant is known to grow rapidly and strongly scented perennial shrub or herb up to 3m tall, reproducing sexually from seeds and vegetatively from cut basal shoot. The stem is cylindrical, robust rather scrambling and dichotomously branched (Akobundu and Agyakwa, 1987). Freudenberg (1955) reported that the more physiological role of the lignin component is the permeation of polysaccharide wall and falling up of the intercellular organs. He claimed it is the high content of acidic detergent lignin that enhances the barriers of the cell wall of the host cell (North Corte, 1972).

Ocimum gratissimum L (family: Labiatae) is an annual herb, strongly scented, about 2-3m tall. The leaves are edible but their seeds are poisonous. It is cultivated extensively worldwide as potted herb and bedding plant. The plant contains essential oils and oleoresins, with the oil containing high concentration of linacoal and methychavicol (extragole) in the ratio of 2 or 3:1, while other constituents founds in low concentrations include 1, 8 – cineole, eugenol, alpha-terpinol, beta-caryophyllene, geraniol, sabinene, alpha-phellandrene, gamma-terpene, myrcene, limonene, ocimene and paracyme, are said to have storng antibacterial and antifungal activities (Mcmaton, 1988).

The present study investigates the potential use of some plant extracts namely *Ocimun gratissiun, Chromolnenu odorata, Cymbopogon giganteus* to control some root pathogens of *Aspergillus* sp, *Fusarium* sp, *Trichoderma* sp and *Rigidoporus* sp.

MATERIALS AND METHODS

Fresh leaves of potential inhibitory plants of C*hromolaena odorata* (Siam weed – 'Awolowo' plant – local name in Nigeria), *Ocimum gratissiumum* (shrubby bucil), *Cymbopogon giganteus* (Lemon grass) were sourced from a garden at the University of Benin, Benin City, Nigeria.

The test root fungal pathogens for the study included, *Fusarium* sp, *Aspergillus* sp, *Rigidoporus* sp, *and Trichoderma* sp were isolated from pure culture prep from disease sections of cassava (*Manihot esculenta* Crantz) root tuber obtained from an intercropped cassava/rubber (*Hevea brasiliensis* Muell. Arg.) from a field plot at Rubber Research Institute of Nigeria (RRIN) main station, Iyanomo, Benin City, Edo State.

Isolation of test pathogens from inoculation procedures was carried out inside a laminar air flow chamber in the plant protection laboratory of RRIN which was sterilized with UV light for about one hour.

Samples of pieces of diseased cassava tubers obtained randomly from the rhizosphere of the field plot were bulked and washed in three discharges of tap water. The sample pieces were then disinfected in 10% sodium hypochlorite solution. The cut pieces were then plated in 20ml of potato dextrose agar (PDA). The medium (PDA_) was prepared according to standard methods (Tuite, 1969). Pilated samples were incubated for five days under diffused laboratory light and temperature at 27^0C. To prevent bacterial growth on PDA cultures, streptpmycin and penicillin antibiotics were added to the PDA at the rate of 0.2ml f stock solution per 20ml PDA (Ayanru, 1982).

The composition of the stock solution was made up of 0.1g of streptonmycin and 10^6 international units (I.U) of penicillin added to 20ml of sterile distilled water.

Test root pathogens were isolated from pure cultures and identified microscopically using microscopy methods according to Barnette and Hunter (1972).

Leaves of test plants of *Chromolaena Odorata, Ocimum gratissiumum* and *Cymbopogon giganteus* were extracted in 70% ethanol for two minutes, rinsed in distilled water and sun dried for two weeks. The dried leaf samples were then grounded thoroughly using an electric blender. Four levels of concentrations of the extracts were prepared at $5g/l^{-1}$, $20g/l^{-1}$, $60g/l^{-1}$, and $100g/l^{-1}$ by adding 5g, 20g, 60g, and 100g of grounded samples separately into one litres of water. The leaf sample content in each 1000ml flasks were vigorously stirred and allowed to stand for 24 hours. The extracts were filtered using Whatsman filter paper (9cm). The filtrates collected were sterilized at 15 p.s.i at 121^0c. The various concentrations of extracts obtained were each dispensed into the PDA medium by adding 2ml of each extract concentration using a sterile injectable syringe. The mixture was then homogenized by gently swirling the plates and allowed to solidify.

A 5 cm mycelia disc from each pure culture of *Aspergillus, Fusarium, Rigidoporus and Trichoderma* species was inoculated at the centre of the medium and observed for inhibitory effects of the amended leaf extracts.

RESULTS

Observed inhibitory effects of the various concentrations of leaf extracts (5, 20, 60 and 100g/l^{-1}) were obtained. Figures 1 showed that at 5g/l^{-1} extracts of *Chromolaena odorata, Ocimum gratissiumum and Cymbopogon giganteus* impacted varied levels of inhibitory effects on *Fusarium moniloforme, Aspergillus niger, Rigidoporus lignosus, and Trichoderma* sp. *Chromolaena odorata* produced the greatest effect on *Fusarium* ($P<0.01$), while it slightly inhibited the growth of *Aspergillus*, and *Trichoderma* ($P<0.05$). *Rigidoporus* remained unhibited by the extract. Extracts of *Ocimum gratissimum* and *Cymbopogon giganteus* at 5g/l^{-1} had lesser inhibitory effects on *Aspergillus, Fusarium* and *Trichoderma* but more on *Fusarium* ($P<0.05$) while no inhibition on *Rigidoporus* was observed. At the 20g/l^{-1} concentration, extracts of the test plants had greater inhibitory effects on *Fusarium*, and slight effect on *Aspergillus* when compared to effects on other pathogens (Fig 2). However, *Chromolaena odorata* highly reduced growth of *Fusarium,* and no inhibitory effects on *Trichoderma* and *Rigidoporus* were recorded (fig 2). Increased concentrations at 60g/l^{-1} and 100g/l^{-1} had the greatest inhibition on *Fusarium* while *Aspergillus*, *Trichoderma* and *Rigidoporus* remained unhibited at these concentrations (figs 3 and 4).

Generally, all extracts at increasing concentrations consistently inhibited and reduced the growth of *Fusarium* sp; and *Chromolaena* odorata had the greatest inhibition on *Fusarium* (figs. 1, 2, 3 and 4). However, all extracts at the varied concentrations failed to produce any discernable inhibitory effects on the growth of *Rigidoporus.*

Observed microscopic characteristics of test pathogens are illustrated in plates 1, 2, 3 and 4.Cultural appearance of *Aspergillus niger* showed as a black mould, which consisted of mycelium of interwoven mass of hyphae that branched freely and spread through the medium. Microscopic observation (plate 1) revealed hyaline hyphae septate much branched. conidiphore tended upright, simply terminating in a globose that bears phialides (phialospores) at the apex.

Cultural characteristics of *Fasarium moniliforme* consists of a much branched and cottony appearance often tinge of pink purple or yellow. Microscopic revelation (plate 2) showed a sickle or typical canoe-shaped conidia borne on conidiophores on verticillate arrangement on short hyphal branches.

Trichoderma viride in culture appear as bright green due to clusters of conidia at the tips of the aerial hyphae. Microscopic examination (plate 3) showed hyaline conidiophores that are much branched but not verticillate. Phialides are singly or in groups – conidia (phialospores). In culture it is easily identified by its rapid growth and green patches.

Rigidoporus Lignosus appeared as a whitish cottony mould consisting of mass of hyphae, interwoven and branch extensively. It spreads very rapidly in culture. Microscopic observation (plate 4) showed copiously branched hyphae, hyaline in nature with conidia (phialospores) borne singly or in clusters on terminal ends of hyphae.

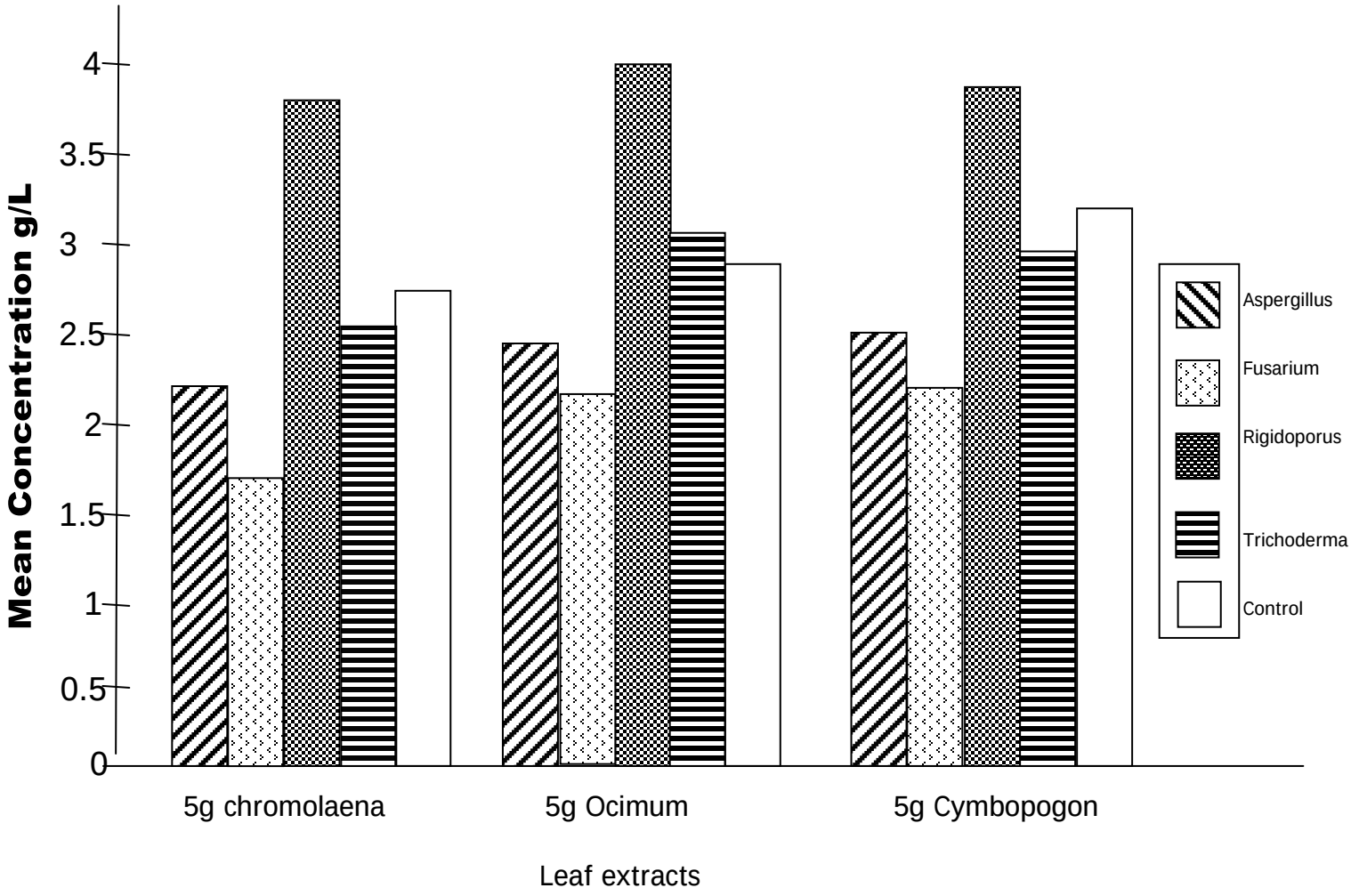

Fig.1. Mean inhibition of leaf extracts (5g/L) on test organisms.

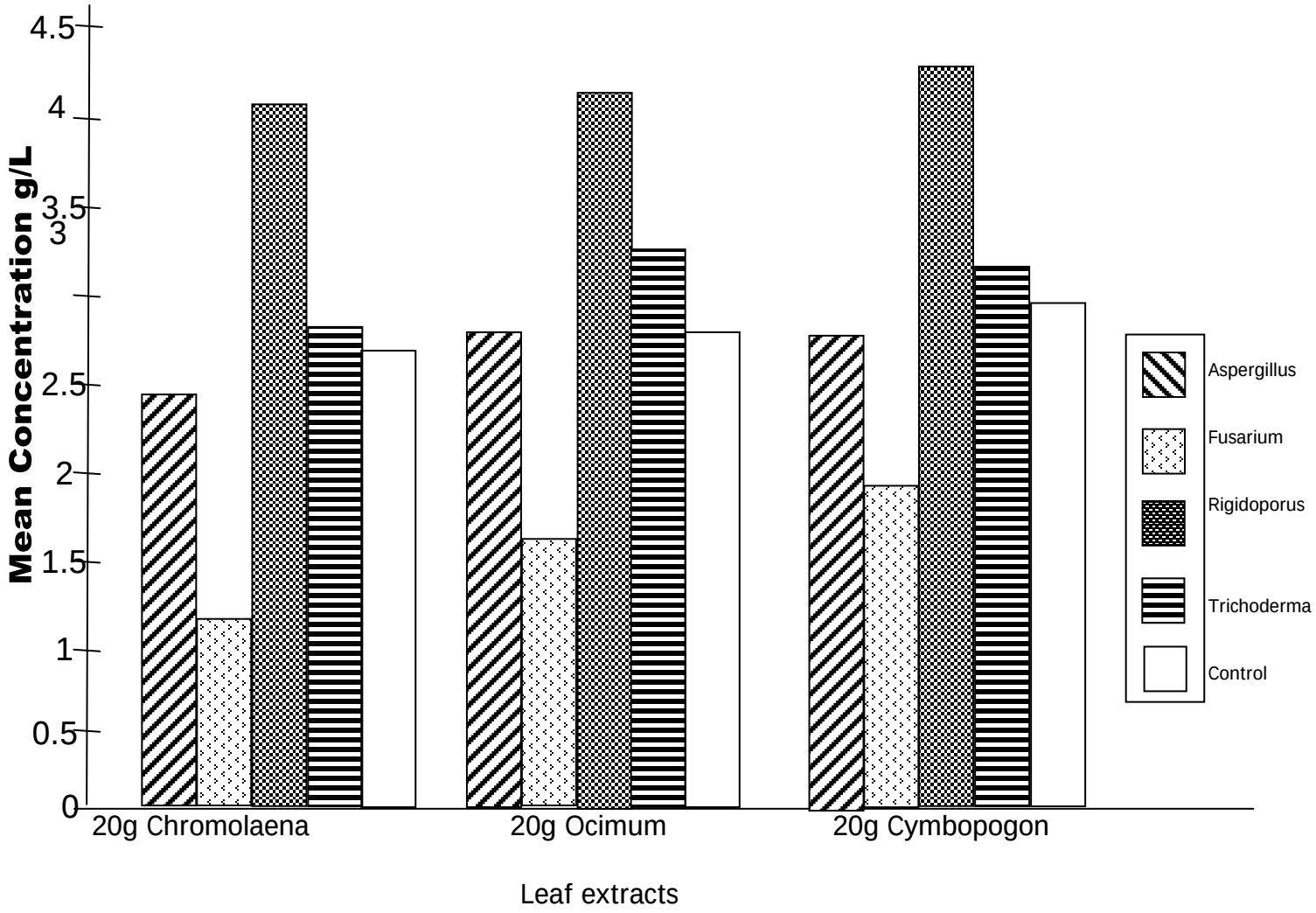

Fig. 2. Mean inhibition of leaf extracts (20g/L) on test organisms.

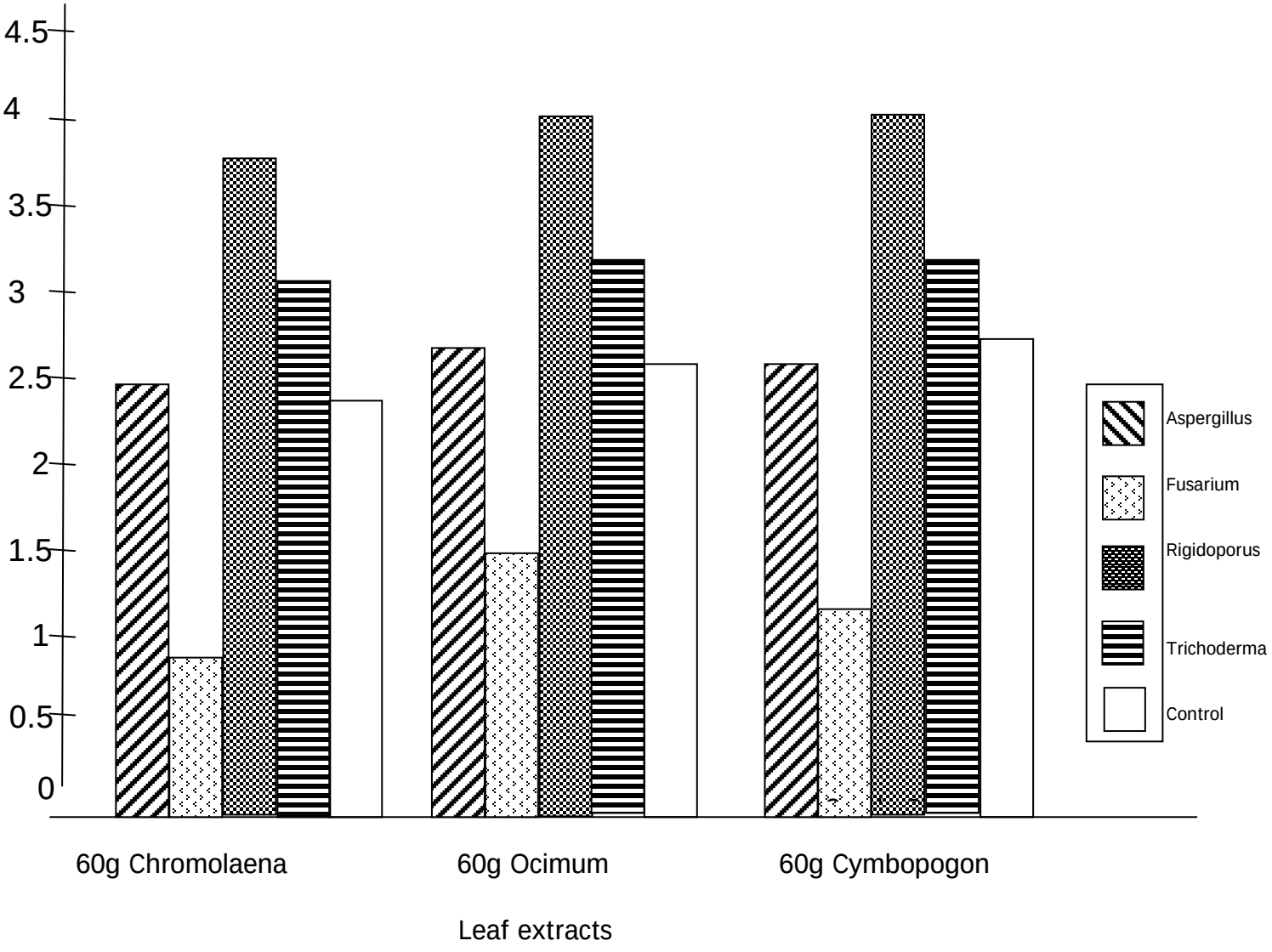

Fig. 3. Mean inhibition of extracts (60g/L) on test organisms.

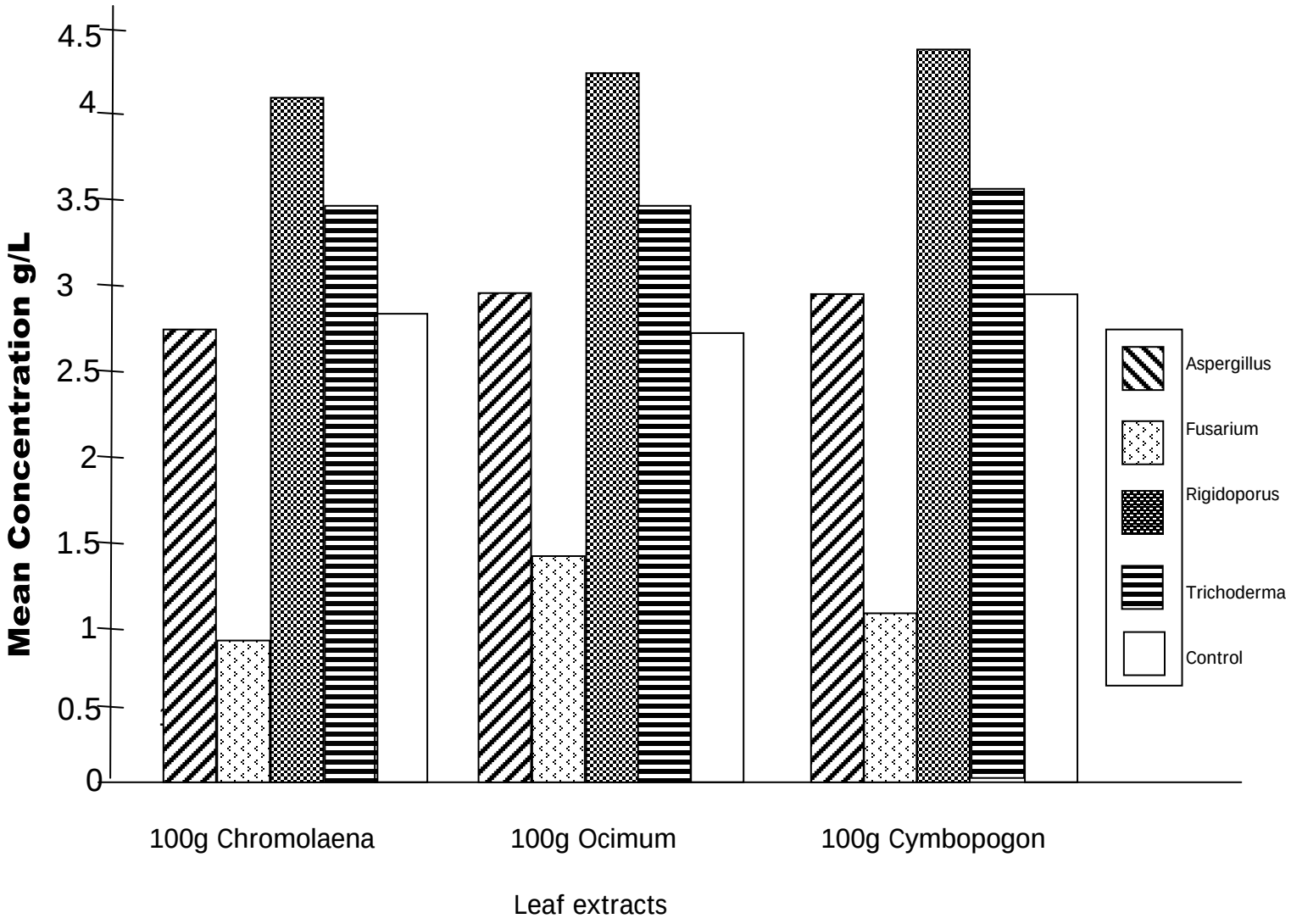

Fig. 4. Mean inhibition of extracts (100g/L) on test organisms.

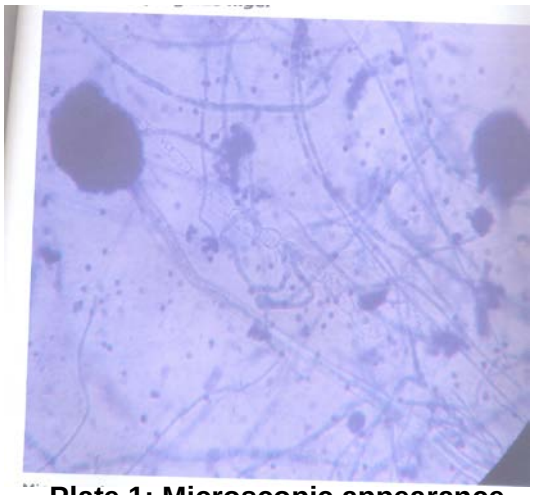

Plate 1: Microscopic appearance of *Aspergillus niger*

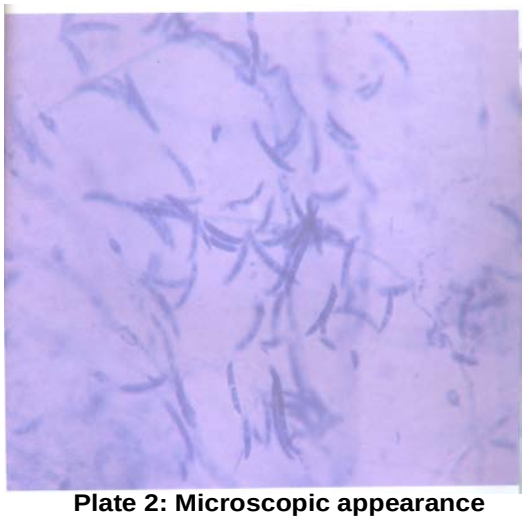

Plate 2: Microscopic appearance of *Fusarium moniliforme*

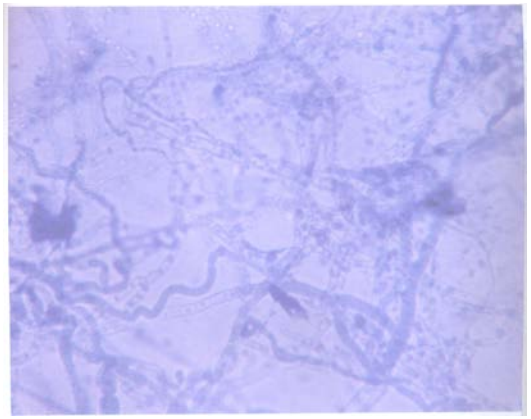

Plate 3: Microscopic Appearance of *Trichoderma* sp

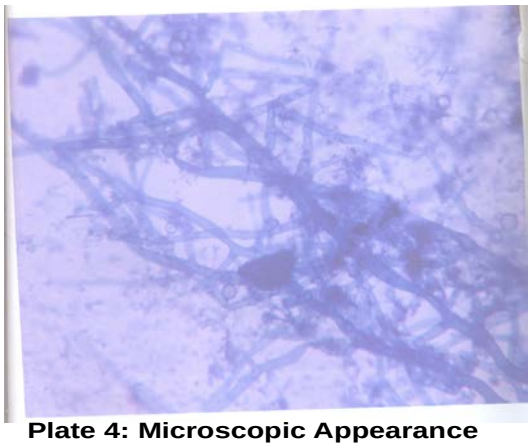

Plate 4: Microscopic Appearance of *Rigidoporus lignosus*

DISCUSSION

The inhibitory efficacy of the different plant extracts investigated showed that the extracts at varied concentrations of 5, 20, 60 and 100 g/l^{-1} reduced growth of *Aspergillus*, *Fusarium* and *Trichoderma* while *Rigidoporus* was unaffected. The inhibitory effects of the leaf extracts may be attributed to the water soluble chemicals present in the extracts. Dey and Harborne (1989), Weinderfield and Roder (1991), Singh *et al*. (2003). Reported such chemicals to either dissolve the cytoplasm or render it inactive and these chemicals – phenol, lignin, terpene and flavanoids in the leaf extracts are capable of penetrating the microbial walls thus

complicate microbial metabolic process as feeding deterrent. In falvanoids, carbon 2 and 3 are hydrogenated, consisting of double bonds, and this property enhances extract inhibitory action due to interaction with proteins and enzymes of pathogens (Herborne, 1980).

The biosynthesis of a chemical compound, sequiterperne found in plants provides a defense mechanism; since related compounds, ipomearoneand ipomeone exhibit potent antifungal properly against pathogenic fungi (Dey and Herborne, 1989) Nidiry (1999) found leaf extract of tomatos reduces mycelia growth of *Colletotrichum gloeosporioides* on spore germination. Findings in the present study on the inhibitory effect of test leaf extracts is confirmed by the works of Umuchuruba and Nwachukwu (1994 and 1999) where leaf extracts of *Cymbopogon giganteus, Ocimum gratissimum, Vernonia amygdalina* (bitter leaf) significantly reduced the incidence of *Aspergillus niger, Aspergillus flavus, Botryodipiodia theobromal*, and *Fusarium moniliforme.* Aside effects on pathogenic fungi, leaf extracts are known to reduced incidence of root-not nematodes and bacterial studies by Caveness (1967), Ogunfowora *et al.* (1983) indicated the use of *Cymbopogon giganteus, Chromolaena odorata, Azadirachla indica* affected the development of *Meloidogyne incognita* of soybean. Similarly, inhibitory effect of *Ocimum gratissiumum* significantly reduced the growth of Staphylococcus aureus (Akinyemi *et al.,* 2005).

Test extracts at increasing concentrations significantly inhibited the radial growth of pathogens except *Rigidoporus lignosus.* However, the highest concentration of $100g/l^{-1}$ rapidly reduced the growth rates of *Fusarium,* and extracts of *Chromolaena odorata* exerted the greatest inhibitory effect on *Fusarium* when compared to other inhibitory actions of other test extracts. The growth rates of *Asporgillus* were consistently inhibited at the lowest concentration ($5g/l^{-1}$) than at the highest concentration ($100g/l^{-1}$) (fig.13 and 16). Comparative effects of extracts showed that *Chromolaena odorata* proved t be effective extract while *Ocimum gratissiumus* had the least inhibitory effect. Results also showed that extracts of *C. Odorata* and *C. giganteus* exerted more inhibition on *Trichoderma* compared to effects of other extracts. Extracts at various concentrations failed to inhibit the growth of *Rigidopurus lignosus. Rigidoporus lignosus* is a highly destructive fungus of natural rubber (*Hevea brasiliensis)*. Over half of a plantation can be destroyed with time (Begho, 1995).

REFERENCES

Adodo, A. 2004. *Nature Power*. 3rd Edition. Generation Press Limited, Lagos, nigeria. 290pp.

Akinyemi, K.O, Oladipo, O., Okwara, C.E., Ibe, C.C. and Fasure, K.A. 2005. Screening of crude extract of six medicinal plants in South West Nigeria. Unorthodox Medicine for antimethicillin resistant *Staphylococcus aureus* activity. Complimentary and Alternative Medicine 5:6

Akobundu, I.O, and Agyakwa, C.W.A. 1987. Handbook of West African Weed. International Institute of Tropical Agriculture, Ibadan. 512 pp

Apori, S.O, Long, R.J, Castrol, F.B and Orskov, E.R. 2000. Chemical Composition and nutritive value of leaves and stems of tropical weeds, *Chromolaena odorata.* Forage science 55 (5): 77-81

Ayanru, D.K.G. 1982. The control of damping off of cotton seedlings by Fungicidal seed treatment. *Niger. Agric. J.* 17/18**:**63-72.

Backman, P.A. 1997, Pesticide input now and into the 21st Century. In: B.K. English, R.L. White and L.H. Chuang (eds) Crop and Livestock technologies Symposiu. Iowa State University Press, Iowa 125-128

Begho, E.R. 1995. Incidence and methods of controlling nursery and field diseases in rubber husbandary. In: O.B.C. Uraih and E.R. Begho. Hevea Plantation Establishment. Proceedings of the training workshop on Hevea Plantation establishment held at RRIN, Iyanomo, Benin City, Nigeria 2nd – 4th Akwete sub-station RRIN. 44-55.

Caveness, F.E. 1967, End of tour progress report on Nematology, Ministry of Agriculture and Natural Resources, Research Division, Ibadan, Nigeria 119 pp.

Conway, G. 1995. The depletion of Natural resources. The impact of food. A 2020 vision for food, *Agriculture* and the environment. International food policy Research Institute, Washington, D.C. 125pp.

Dey, P.M and Harborne, J.B. 1989. methods in Plant Biochmistry. Plant phenolics. Academic Press Ltd, London 1:552

Freudenberg, K. 1995. Lignin. In:K. Peach and M.V. Tracey (eds). Modern Methods of Plants. Springer, Berlin 516pp.

Harborne, J.B. 1980. Encyclopedia of physiology (eds: A. Pirson and M.H. Zimmerman. Springer, Berlin 329 – 402.

Mcmaton, C. 1988. White Lotus Aromatics. Newsletter. Newsletter Archive 5pp

Nidiry, E.S.J 1999. Antifungal activity of tomato seed extracts. Filoterapia 70 (2): 181 – 183

North Corte, D.H. 1972, Annual Review of plant physiology 23:113-132.

Barnett, H.L and Hunter, B.B. 1972. Illustrated genera of imperfect fungi

Nwachukwu, E.O. and Umuchuruba, C.I. 2001. Antifungal activities of some leaf extracts on seed borne fungi of Africa yam bean seeds. Seed germination and seedling Emergence. Journal of Applied Science and Environment Management 5:29-32

Ogunfowora, A.O, Oyekan, P.O. and Afolabi, N.O. 1983. Association of Nematodes with premature senescences of Soybean plants in South-Western Nigeria, *Proceedings of Third National Meetings of Nigerian Soybean. Scientist 3:103-107*

Singh, P.H, Batish, D.R., Kaul, S. and kohili, R.K. 2003. Phytotoxic interference of Agerantum conyzoide with wheat (Triticum aestivum L.) *Journal of Agronomy and Crop Science 185 (5): 34-346.*

Umuchuruba, C.I. and Nwachukwu, E.O. 1994. Efficacy of certain fungicides against seed borne fungi of African yam bean seeds. *International Journal of Pest Management 4 (2):126-131*

Wiendenfield, H. and Roderm E. 1991. Pyrozidine alkaloids from Agenrantum conyzoides. Phytochemistry 25:2625-2627

Tuite, J. (1969). Plant Pathological Methods. Burgess Publishing Co. Minn. USA . 239 pp.

Yudelman, M., Ratta, A. and Nygard, D. 1998. Pest management and food Production: looking to the future, food, agriculture and the environment. *Discussion paper 25, International Food Policy Research Institution*, United States of America 50pp.