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Efficacy of naturally *occurring antifungal agents* from five plants for the control of late blight *(Phytophthora infestans)* on Irish Potato

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Abstract

Fungicides are widely used in conventional agriculture to control plant disease. Prolonged usage often poses health problems. As modern society is becoming more health conscious, the use of botanicals is now emerging as one of the prime means to protect crops, their products and the environment from pesticides. The present study was undertaken to screen five selected plants *(Piliostigma thonningii; Maytenus senegalensis; Nauclea latifolia; Vernonia amygdalina* and *Cassia sieberiana)*; apply standard scientific procedures and to determine the toxicity and test their effectiveness for the control of *Phytophthora infestans* the causal organism of late blight of potato under field conditions. The experiment was arranged in completely randomized block design (CRBD). Botanicals were sprayed on well-established plants before disease occurrence and continued for 2 months at 3 days interval. Data was recorded on phytochemicals, chemomicroscopy of the various plant parts; disease severity and reduction. In the field trials, the greatest reduction of disease severity was achieved by *C. sieberiana* at 5% concentration and the least was obtained when potato plants were treated with *M. senegalensis* at 1% and 5% concentrations (46.1% and 45.2% respectively). The treatments were moderately effective, so these plants with antifungal properties could be utilized against the pathogens, at least to lessen the impact of the pathogen. These naturally occurring extracts or botanicals have a bright future in modern plant protection to replace conventional synthetic pesticides.

Key words: Efficacy; Naturally occurring; Antifungal; *Five plants*; Late blight

INTRODUCTION

The need to ensure security is often the prime factor governing a farmer's decisions, a fact which is often ignored. The main concern of developers and Scientists is to produce more, but what farmers seek, above all, is to live better by reducing the risk factors. The incidence and severity of pests have increased. All together, pests are responsible for the loss of a very significant proportion – usually estimated at around 35% of the world's crops. Their ravages starve the hungry of food and severely reduce the yield of cash crops (Deshi *et al*. 2014a; Chaube and Pundhir, 2005). Although pests are considered a major, if not the main constraint to increase crop productivity, such severe yield damages are seldom a permanent threat requiring continued and scheduled use of pesticides as insurance against income loss.

Late blight, caused by *Phytophthora infestans*, is an important and destructive disease on potato. It has historical significance as the cause of the Irish Potato Famine during the 1840s. This famine resulted in the death of more than one million people and the displacement of nearly two million more during a short, five-year period. This Irish Potato Famine is a devastating example of the epidemic potential of late blight. When late blight is present in a location or is introduced and environmental conditions are favourable, devastating crop loss can result if preventative control measures have not already been implemented.

The application of pesticides to combat the pests affecting the crop; has been increasing steadily. The use of pesticides has helped to increase production and productivity of crops. This cannot be disputed but basically the use of pesticides is another form of loss caused by diseases and other pests. In addition, they have caused recurrence, resurgence and resistance in the pests besides, causing extreme pollution of agro-ecosystem and the environment. Pesticides use usually comes along with a gamut of related problems. The effects of pesticides extend beyond agricultural systems. When this happens, the pesticide is often considered an environmental pollutant. Aside from this, most farmers do not have the economic means and knowledge or skill to control pests chemically.

Pest control is one of the key areas that affect profitability of farming. An important reserve for raising productivity and increasing the gross output of agricultural products is the elimination of losses of the harvest due to pest, plant disease and weeds. Most of the efforts in the past few years for the effective control of plant diseases have been focused on effective eradication or prevention through the development of synthetic chemical fungicides (Bajpai *et al*, 2004). However, increasing worry over the environmental load caused by the presently used synthetic fungicides has necessitated the search for fungicides of biological origin due to their numerous advantages over the synthetics (Parvu *et al*, 2010; Abubakar, 2009; Iherijika, 2002; Abdel-Hafez *et al*. 2014).

Bioactive compounds in plants can be defined as secondary plant metabolites eliciting pharmacological or toxicological effects. Secondary metabolites are produced within the plants besides the primary biosynthetic and metabolic routes for compounds associated with plant growth and development. Several of them are found to hold various types of important functions in the living plants such as protection, attraction or signalling (Bernhoft, 2010).

MATERIAL AND METHODS

Collection of Plant Materials

A range of plant materials were collected from their habitats at various locations within Central Zone of Plateau State, North Central, Nigeria during March 2013 – August, 2014. The identification of the plants were carried out with the aid of Internet resources and Medical Plants Handbook. Suspected Sample Validation was carried out in the Department of Plant Sciences and Technology, University of Jos, Nigeria where the voucher specimens was deposited in the herbarium of the department. All plants selected and used (table 1) were informed by discussion with various traditional healers.

Table 1. Test Plants used for naturally occurring antifungal agents and assays

Name	Family	Plant part used
Pliliostigma thoninningii (Shum.)	Fabaceae	Flower, fruit, leaf, bark
Maytenus senegalensis	Calatraceae	Leaf, stem, bark, root
Nauclea latifolia	Rubiceae	Leaf, stem, root
Vernonia amygdalina Del.	Asteraceae	Leaf, stem, root, seeds
Cassia sieberiana	Fabaceae	

Preparation of Plant Extracts

Fresh plant parts were collected, weighed and shade-dried. They were ground to fine powder using sterilized pestle and mortar. The powder was stored in air tight bottles. The technique described by Odey *et al*. (2012) was used with slight modifications. Each residue was used to prepare solutions of different concentrations for test of antifungal activities.

Laboratory Procedures

The fractions of various plant powder extracts were subjected to preliminary phytochemical screening to identify the secondary metabolites present. The methods of analysis employed were those described by Trease and Evans (2004); Harbone (1998) and Soforowa (1993). Using conventional protocols for detecting the presence of alkaloids, tannins, carbohydrates, saponins, plavonoids, steroids, anthraquinones and cardiac glycosides. Chemomicroscopy were also conducted.

Mycological Studies:

Chemical and reagents: All the chemicals and solvents used in this study were standard and of analytical grade.

Isolation and pathogenicity tests of causal pathogen: Sis fungal isolates were isolated from naturally infected potato leaves and tubers showing blight symptoms. Pathogenicity tests of *Phytophthora infestans* isolates were carried out. The inoculums were prepared by culturing each of the tested isolates on PDA medium at 27^0C for 15 days. Then 10ml of sterile distilled water was added to each plate and colonies were carefully scraped with sterile needle. The resulting conidial suspension from each isolation was adjusted to 5 x 10^6 spores/ml and used on 20 potato plants using an atomizer. After inoculation, plants were covered with polyethylene bags for 48 hours; bags were removed and plants were kept under greenhouse conditions. Two weeks after inoculation, disease severity was recorded in each treatment following the score chart proposed by Hadian (2012).

Field testing of plant extracts against late blight disease: Field trials were conducted for two consecutive growing seasons (2013 and 2014) at the Teaching and Research Farm of the Department of Agricultural Science Education, Federal College of Education, Pankshin outskirt of the city of Jos, Plateau State, Nigeria. The field shows late blight incidence during 2012 cropping season (plates 1 and 2). Hence experiment is carried out at the natural inoculums potential of the soil. Field preparations were done mechanically during the growing seasons. The experiments were conducted in 2 x 2 m^2 plots with replications.

Data Analysis and Presentation: Statistical analysis was performed using SPSS version 11.0 Computer Package and the pooled mean values were separated on the basis of least significant difference (LSD) at the 0.05 probability level. Experiment carried out in a single factor, completely randomized block designs (CRBD).

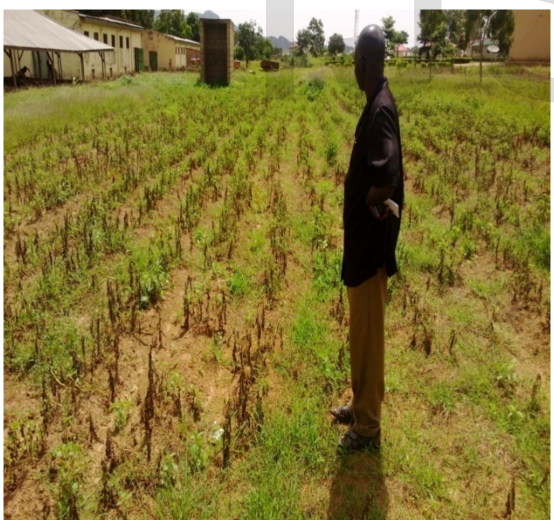

Plate 1: Potato field showing late blight

Plate 2: Late blight outbreak in neighbouring

Table 2: Phytochemical profiles of the various studied plants (showing the presence of secondary metabolites).

Constituents	Study Plants/Parts																	
	P. thonningii				***M. senegalensis***				***N. latifolia***			***V. amygdalina***			***C. sieberiana***			
	Flower	**Fruit**	**Leaf**	**Bark**	**Leaf**	**Stem**	**Bark**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Seed**
Alkaloids	-	+++	++	+	-	++	-	+	-	+++	++	-	+	-	+	+	+	+
Carbohydrates	+	++	++	+++	-	-	-	-	+	++	+	+	+	+	+	+++	+++	+
Cardiac glycosides	+++	++	++	+	++	-	-	++	+	+	+++	+	+	+	+	+++	+++	+
Anthraquinones	+	-	+	++	+	+	+	+++	-	++	+	-	-	-	-	++	++	-
Flavonoids	+++	++	+++	++	-	-	-	-	++	+++	++	+	+	+	+	+++	+++	-
Reducing sugar																+++	+++	
Saponins	++	-	+	+	++	+++	+++	++	-	+	+++	+	+	+	+	+	+++	-
Steroids	+++	+	+	+	+	++	+	++	+	+	+	+	-	-	+	-	+++	-
Tannins	++	+	++	+++	++	+++	+++	++	-	++	+	+	+	+	+	+++	+++	-
Triterpene												+	+	+	+	+	+	+

Key: + = Low concentration; ++ = moderate; +++ = High concentration; - = not detected.

Table 3: Chemo-microscopy of various plant parts showing the presence of some primary metabolites.

	Study Plants/Parts																	
	P. thonningii				*M. senegalensis*				*N. latifolia*			*V. amygdalina*			*C. sieberiana*			
Constituents	**Flower**	**Fruit**	**Leaf**	**Bark**	**Leaf**	**Stem**	**Bark**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Leaf**	**Stem**	**Root**	**Seed**
Calcium oxalate	+	++	+	+++	+++	+	-	-	+	++	+++	+	+	+	+	+	+	-
Lignin	+	+++	++	++	++	+	+	-	+	+++	+	+	+	+	+	+	+	-
Proteins	+++	++	+	+	+	-	-	+	+	+++	+							
Oil and Fats	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+
Starch	+	++	+	+	++	++	+	+++	+	++	+	+	+	+	+	+	+	-
Tannins					++	+	-	-	-	++	+							

Key: + = Low concentration; ++ = moderate; +++ = High concentration; - = not detected.

Table 4: Influence of five plant extracts on late blight disease of potato under green house and field conditions.

Treatment	Concentration (%)	Green house conditions		Field Conditions	
		Disease Severity (%)	Reduction (%)	Disease Severity (%)	Reduction (%)
P. thonningii	1	28.7[b]	47.0	37.9[c]	36.0
	5	26.4[b]	51.2	36.2[c]	38.9
M. senegalensis	1	35.2[a]	34.9	46.1[a]	22.1
	5	30.3[b]	44.0	45.2[a]	23.6
N. latifolia	1	27.8[b]	48.6	41.3[b]	30.2
	5	24.3[bc]	55.1	38.2[c]	35.5
V. amygdalina	1	19.4[cb]	64.1	28.4[d]	52.0
	5	17.2[cb]	68.2	27.1[d]	54.2
C. sieberiana	1	20.8[bc]	61.6	27.3[d]	53.9
	5	15.3[d]	71.7	25.19	57.6
Infected control		54.1*	0.0	59.2*	0.0

Values in the column followed by different letters indicate significant differences among treatments according to the least significant difference (P = 0.05)

RESULTS

The potential of natural products has been recognized since antiquity. Table 1 showed the plants selected for use in the present study. All the plants selected and used were informed by a discussion with various traditional healers. Products research of the past decades have clearly demonstrated that natural products represent an unparalleled source of molecular diversity for drug discovery and development that fight disease. Results from table 2 are various plant powdered extract subjected to preliminary phytochemical screening, these help to show and identify the secondary metabolites present on each plant extract. The phytochemicals detected are: alkaloids, anthraquinones, carbohydrates, cardiac glycosides, flavonoids, reducing sugar, saponins, steroids, tannins and triterpene.

A total of eighteen (18) plant extracts from the five (5) plants were tested for the presence or absence of ten (10) secondary metabolites (18 x 10 = 180). One hundred and twenty were found present while sixty (60) were absent amongst the plant extracts. Cardiac glycosides and tannins (16 each) had highest occurrence, followed by saponins (15); carbohydrates and steroids, (14 each); flavonoids (13). Alkaloid and anthraquinones had 12 and 11 respectively while triterpene and reducing sugar had the least (7 and 2 respectively).

Carbohydrates, flavonoids, reducing sugars and triterpene were not detected in all the *Maytenus senegalensis* parts used in this study. Reducing sugars and triterpene were only detected in *Vernonia amygdalina* and *Cassia sieberiana* extracts. This study also reveals the non presence of anthraquinones in *V. amygdalina*. Some of these secondary metabolites appear in certain parts of the same plant with differential concentrations (table 2).

Table 3 is the result of chemo-microscopy of a total of eighteen (18) plant parts showing the presence of some primary metabolites (calcium oxalate, lignin, proteins, oil and fats, starch and tannins). Calcium oxalate was detected in 15 parts; starch was detected in all extracts except in the seeds of *Cassia sieberiana*. Lignin was next to starch $^{16}/_{18}$. Protein presence was only detected in three of the study plants (table 3) and tannins occurring in only two (*M. senegalensis* and *Nauclea latifolia*) while oil and fats occurred moderately in the fruit of *Piliostigma thonningii* and as traces in *N. latifolia* and *C. sieberiana*.

The different concentration of the five plant extracts significantly reduced the late blight disease (Table 4). The most effective treatments with plant extracts under greenhouse conditions were *C. sieberiana* at 1% and 5% concentration, followed by *V. amygdalina* at 1%

and 5% concentration. The least reduction of disease index was achieved by *M. senegalensis* at both 1% and 5% concentrations (34.9 and 44 respectively). Other treatments with plant extracts were moderately effective.

All treatments significantly reduced the late blight disease under field conditions (Table 4). The greatest reduction of disease severity was achieved by *C. sieberiana* at 5% concentration and the least reduction was obtained when potato plants were treated with *M. senegalensis* at 1% and 5% concentrations (46.1% and 45.2% respectively). The other treatments were moderately effective.

DISCUSSION

Natural compounds of plant origin are biodegradable, often of low toxicity and pose low danger to the environment if used in small amounts (Keita *et al*. 2001; Okosun and Adedire, 2010). Plants are capable of synthesizing an overwhelming variety of small organic molecules called secondary metabolites. This agrees with the finding of the present study in which ten (10) of such were detected (Table 2). Other workers (Al-Samarai *et al:*, 2005) had reported that the biosynthesis of several secondary metabolites is constitutive.

Laboratory studies of extracts of plant species have revealed powerful fungitoxicities in relation to many fungal pathogens (Hay and Waterman, 1993). Such bioactive chemicals either exhibit germination, growth and multiplication of pathogens or lethal to the pathogens. Several workers (Bhatm, 2001; Letessier *et al.*, 2001; Parven and Kumar, 2002; Agrios, 1997; Kamalakannan *et al*., 2001; Meena and Muthsamy, 2002) had shown that many plants extracts control many anamorphic fungal plant pathogens and also effective against other Ascomycota and Basidiomycota leaf pathogens. They assessed the potential biological activity of extracts from a wide range of plants on various micro organisms.

The high blight reduction observed by *C. sieberiana* followed by *V. amygdalina*, then *P. thonningii* in this study showed that they have high fungicidal properties with *C. sieberiana* recording the highest. This is in line with Kim *et al*. (2004); Abubakar (2009); Abdel-Hafez *et al*. (2014); Cohen *et al*. (2003), Al-Samarai *et al* (2012) who previously had discovered the effectiveness of antifungal activity of some aqueous plant extracts.

Organic, plant based pesticides that rely on plants' natural defenses against pathogens may not only be effective and inexpensive for protecting crops, but also safer and more environmentally friendly. The greenhouse and field experiments indicated that the foliar sprays of potato plants with plant extract resulted in a significant reduction in late blight infection. These results are similar to previous works on the role of plant extracts in fungal disease control. Several authors including Curtis *et al*. (2004); Krebs *et al*. (2006); Qaesem and Abu-Blan (1996) Ro”hner *et al*. (2004) reported that plant extracts from non-host plant species caused reduction of the late blight disease and suppressed the mycelia growth of *Phytophthora infestants*.

Many higher plants have been reported to produce economically important organic compounds, representing large reservoir of chemical structures with biological activity (Ncube *et al*. 2008). Biologically active compounds from plants will often have activity against organisms with which the producing plant does not have to cope. Recently, in different parts of the world, attention has been paid towards exploitation of higher plant products as novel chemotherapeutics for plant protection because they are mostly non phytotoxic and easily biodegradable (Isman, 2006). Such products of higher plant origin may be exploited as eco-chemical and biorational approach in integrated plant protection program (Hadian, 2012).

Medicinal plants remain a rich source of novel therapeutic agents. Many plant species are still unevaluated chemically or biologically. Several studies regarding the action of plant extracts against some phytopathogenic fungi have been performed (Al-Samarai *et al*. 2012; Kim *et al*. 2004; Abubakar, 2009; Parvu *et al*. 2010). The quality and quantity of the biologically active compounds from plant extracts significantly depend on the species, the plant organ and harvest time (Abubakar, 2009; Gujar *et al*. 2012; Al-S amarai *et al*. 2012; Iherijika, 2002; Orji *et al*, 2003).

CONCLUSION

Plant based antimicrobials represent a rewarding and vast untapped resources and have enormous potential for developing antimicrobial agents. The natural product compounds detected from the five studied plants have some form of biological activity, those known as active principles and such could evolve to become a discovery “lead.” A continued exploration of medicinal plants are needed today so that those plants that have shown promising antifungal activity can be investigated further.

REFERENCES

Abdel-Hafez, S. I. I.; Abo – Elyousr K. A. M.; and Abdel-Rahsim I. R. (2014). Effectiveness of plant extracts to control purpose blotech and *Stemphylium* blight disease of Onion (*Allium cepa* L.) in Assiut, Egypt. *Phytopathology and Plant Protection.* 47(3):377-387.

Abubakar, El-MM. (2009). Efficacy of crude extracts of garlic (*Allium sativum* Linn.) against nosocomial *Escherichia coli.*, *Staphylococcus aureas*, *Stretococcus pneumonia* and *Psuedomonas aeruginosa*. *J. Med. Plants Res*. 3:179-185.

Agrios, G. (1997). *Plant Pathology*, 4th edn., Academic Press, New York.

Al-Samarai G., Singh H., and Syarhabil M. (2012). Evaluating eco-friendly botanicals (natural plant extracts) as alternatives to synthetic fungicides. *Annals of Agricultural and Environmental Medicine*. 19(4): 673-676.

Bajpai V., Shin S. Y., Kim M. J., Kim H. R. and Kang S. C. (2004). Antifungal activity of bioconcerted oil extracts of linoleic acid and fractionated dilutions against phytopathogens. *Rhizoctonia solani* and *Botrytis cinera*. *Agricultural Chemistry and Biotechnology*. 47:199-204.

Bernhoft A. (2010). A brief review on bioactive compounds in plants. In: Bioactive compounds in plants – benefits and risks for man and animals. Bernhoft, A. (ed.), Oslo: the Norvegian Academic of Science and Letters.

Bhatm, N. (2001). In vitro evaluation of some leaf extracts against *Fusarium* spp. Causing yellows of ginger in Sikkim. *Plant Disease Research* 16(2): 259-262.

Chaube H. S. and Pundhir, V. S. (2005). *Crop diseases and their management*. Prentice-Hall of India Private Ltd, New Delhi. 703 pp.

Cohen, Y. A.; Bader B. and Ben-Daniel, Y. (2003). Fungicidal preparations from *Inula viscose*. Available from http://www.infodienst-m/r.Bwl/la/ivwo/ecofruvit.

Curtis H., Noll U., St'ormann J., and Slusarenko A. J. (2004). Broad-spectrum activity of the volatile phytoanticipinallicin in extracts of garlic (*Allium sativum* L.) against plant pathogenic bacteria, fungi and Oomycetes. *Physiological and Molecular Plant Pathology*. 65:79-89.

Deshi S. N.; Wonang D. L. and Dafur B. S. (2014a). Control of Rots and Spoilage of Agricultural Products: A Review. *Academic Journal of Interdisciplinary Sciences 3(7):38-43.*

Edeoga H. O.; Okwu D. E., and Mbaebie B. O. (2005). Phytochemical Constituents of some Nigerian Medicinal Plants. *Afri. J. Biotechnol*. 4:685-688.

Gujar M. S., Ali S., Akhtar M., and Singh K. M. (2012). Efficacy of plant extracts in plant disease Management. *Agric. Sci*. 3(3): 425-433.

Hadian S. (2012). Antifungal Activity of some Plant Extracts against some Pathogenic Fungil in Iran. *Asian J. Exp. Bio. Sci*. 3(4): 718.

Harbone J. B. (1998). *Phytochemical Methods. A guide to Modern techniques of Plant analysis*. 3rd edition. Chapman and Hall Pub. London, UK.

Hay R. K. M., and Waterman P. G. (1993). *Volatile oil crops: Their Biology, Biochemistry and Production*. Edited by R. K. M. Hay and P. G. Waterman. Essex: Longman Scientific and Technical.

Ihejirika G. O. (2002). Fungicidal Effects of Selected Medicinal Plants on Post-Harvest Rots Biodeterioration of Banana. *African Journal of Science and Technology*. 3(1 & 2): 68:70.

Isman M. B. (2006). Botanical insecticides, deterients and repellents in modern agriculture and an increasingly regulated world. *Annual Review of Entomology*. 51:45-66.

Kamalakannan A.; Shanmugam V.; Surendran M., and Rinivasan R. S. (2001). Antifungal properties of plant extracts against *Pyricularia grisea*, the rice blast pathogen. *Indian Phytopathology*. 54(a): 490-492.

Keita S. M.; Vincent C.; Schmit J. P.; and Belanger A. (2001). Efficacy of essential oil of *Ocimum basilium* L., and *O. gratissium* L. applied as an insecticidal fumigant and powder to control *Callosobruchus maculates* (F.) (Coleoptera: Bruchidae). *Journal Stored Product Research*. 37:339-349.

Kim Y.; Lee C.; Kim H.; and Lee H. (2004). Anthraquinones isolated from *Cassia tora* (Leguminosae) seed show an antifungal property against phytopathogenic fungi. *Journal of Agricultural and Food Chemistry* 51:1578-1581.

Krebs H., Dorn B., and Forrer H. R. (2006). Control of late blight of potato with medicinal plant suspensions. *Agrarforschung*. 13:16-21.

Letessier M. P.; Svoboda K. P. and Walters D. R. (2001). Antifungal activity of the essential oil of hyssop (*Hyssopus officinalis*). *Journal of Phytophathology*. 149 (11-12): 673.

Meena B. and Muthusamy M. (2002). Fungitoxic properties of plant extracts against *Sclerotium rolfsii* in jasmine. *Journal of Ornamental Horticulture* Newseries. 5(1): 82-83.

Ncube, N. S.; Afolayan A. J. and Okoh A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. *African Journal of Biotechnology*. 7(12): 1797-1806.

Odey M. O.; Iwara L. A.; Udiba U. U.; Johnson J. T.; Inekwe U. V.; Asenye M. E. and Victor O. (2012). Preparation of Plant Extracts from Indigenous Medicinal Plants. *International Journal of Science and Technology*. 1(12): 688-692.

Okosun O. O.; and Adedire C. O. (2010). Potency of Cowpea Seed Bruchid; *Callosobruchus maculates* (Fabricius) [Coleoptera: Bruchidae]; of African Nutmeg Seed (*Monodora myristica* (Gaentn.) Dual) Extracted with Different Solvents. *Nigerian Journal of Entomology*. 27:89-95.

Orji E. C.; Oguezue D. C., and Orji M. U. (2003). Evaluation of Anti-microbial Activities of Leaf extracts of *Morinda lucida* and *Erythrina* senegalensis for medical Uses. International Journal of Gender and Health Studies: 1(1):79-83.

Parveen S. and Kumar V. R. (2002). Effect of extracts of some medicinal plants on the growth of *Alternaria trichina. Journal of Phytological Research*. 13(2):195-196.

Parvu M.; Barbu-Tudoram L.; Rosca-Casian O.; Vlase L., and Tripon S. C. (2010). Ultrastructural Changes in *Fusarium oxysporum* F. Sp. Tulipae hyphae treated invitro with *Allium fistulosum* plants extracts. *Annals of RSCB*. 2:62-72.

Qasem J. R., and Abu-Blan H. A. (1996). Fungicidal activity of some common weed extracts against different plant pathogenic fungi. *Journal of Phytopathology*. 144:157-161.

Ro"hner E.; Carabet A.; and Buchenaves A. (2004). Effectiveness of plant extracts of Paeniasuffrticosa and Hederalelix against diseases caused by *Phytophthora infestans* in tomato and *Pseudoperonospora cubensis* in cucumber. *Journal of Plant Disease and Protection*. Iii(i): 83-95.

Soforawa A. (1993). *Medicinal Plants and Traditional Medicine*. WHO, Document No. 30:69 pp.

Trease G. E. and Evans W. C. (2004). Pharmacognosy.(4^{th} Ed.). USA: W.B. Sounders.