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# *In Vitro* Evaluation of the Effect of Selected Plant Extracts on the Phytophthora Fungus Causing Disease in MD2 Variety of Pineapple in the Central Region of Ghana

**Mark Tutu Sarpong***

*Department of Crop Science, College of Agriculture and Natural Science, University of Cape Coast, Ghana*

***Corresponding Author:** Mark Tutu Sarpong, Department of Crop Science, College of Agriculture and Natural Science, University of Cape Coast, Ghana.

**Received:** October 18, 2016; **Published:** October 27, 2016

## Abstract

An *in vitro* study evaluation of the effect of selected plant extracts on the Phytophthora fungus causing the disease in MD2 variety of pineapple was carried out in the Central Region of Ghana. The plants extracts used were prepared from *Khaya senegalensis* (Desr.) A.Juss, *Azadirachta indica* (A.Juss), *Lantana camara* (L.) and *Momordica charantia* (L.). Compared to the mycelial growth inhibition over control at 1.25% concentration after 3 days, the highest inhibition of 90% was recorded by *Khaya senegalensis* (Desr.) A.Juss, *Lantana camara* (L) and Fosetyl -Al respectively while *Mormordica charantia* recorded 67.90 % of inhibition of the growth of mycelium *Phytophthora nicotianae var paradisica* (Dastur.). *Azadirachta indica* (A. Juss), recorded the least inhibition of 45%. The inhibition of mycelial growth of *Phytophthora nicotianae var parasitica* (Dastur.) over control at 1.25% concentration after 7 days of plating showed that at 90% inhibition Fosetyl -Al was the highest inhibitor of the mycelial growth of the *Phytopthora nicotianae var parasitica*. This was followed by: *Lantana camara* (L) with an inhibition of 55.2%, *Khaya senegalensis* (Desr.) A.Juss with an inhibition of 52.44% and *Mormordica charantia* with inhibition of 49.29%. It was noted that *Azadirachta indica* (A.Juss) with an inhibition of 36.95% was the least inhibitor of the growth of mycelial of the *Phytophthora nicotianae var paradisica* after the 7 days of plating The mycelial growth inhibition *Phytophthora nicotianae var parasitica* (Dastur.) over control at 2.5 % concentration after 3 days of plating showed that 90% inhibition was achieved in *Khaya senegalensis* (Desr.) A.Juss, *Lantana camara* (L) and Fosetyl -Al respectively followed by *Mormordica charantia* with an inhibition of 69.40% the plant extract with the least inhibition of 55 % of the mycelial growth of *Phytophthora nicotianae var parasitica* (Dastur.) was *Azadirachta indica* (A.Juss) The mycelial growth inhibition of *Phytophthora nicotianae var parasitica* (Dastur.) over the control at 2.5 % concentration after 7 days of plating showed that Fosetyl-Al was the highest inhibitor with 68.91% followed by *Lantana camara* (L) with 57.41%, *Khaya senegalensis* (Desr.) A.Juss with 55.46% and *Mormordica charantia* with 53.03% inhibition .The plant extract with the least inhibition of the mycelial growth of the *Phytophthora nicotianae var parasitica* (Dastur.) was *Azadirachta indica* (A.Juss) with inhibition of 40.68%.

***Keywords:*** *Khaya senegalensis; Azadirachta indica; Lantana camara; Phytophthora nicotianae var parasitica; Mormordica charantia*

## Introduction

Pineapple, *Ananas comosus* (L.Merr.) is the leading edible member of the family Bromeliaceae [1-3]. It is cultivated predominantly for its fruit that is consumed fresh or as canned fruit or juice. The edible portion constitutes about 60% of the fruit. The fruit has a sweet and sour taste and it contains about 80-85% water, 12-15% sugars of which, two-thirds is in the form of sucrose and the rest glucose and fructose; 19.9% carbohydrates; 0.1% fat, 0.4% protein; 10.6% ash; 0.5% crude fiber, 0.6% fruit acids (citric, mallic and nalic); vitamins: 3.6%

B1 (Thiamine), 1.2% B2 (Riboflavin) 1.6% A, 20% C, 1.1% Niacin, and minerals; 2% Calcium, 1% Phosphorus, 5% Iron, 3% Potassium and Sodium. One glass of juice (approximately 150 cc) contains an average of 75 calories [1,4].

Pineapple production contributes immensely to the economy of Ghana in 2004 a total of 71,805 metric tons of pineapple exported from the country earned the country US $ 22,069 000. This amount increased to 46,694 metric tons an average earning of US $ 13,430 000 in 2006. In 2009 Ghana earned US $ 10,628,000 by exporting 41,567 metric tons [5]. Pineapple generates an estimated rural income of GH¢ 6 million to 2500 households in the rural communities [6].

The large commercial farms in the villages have provided employment to the inhabitants both in the urban and rural areas of the country. This has helped in minimizing the rural urban drift and also served a boost for cottage industrialization [7].

Commercial pineapple production in Ghana was initially based on the cultivation of the smooth cayenne variety. The MD2 variety later became the standard for the international market. This variety is now the standard for the international market. This variety is sweeter, grows to a uniform size, and ripens evenly than the other varieties. Currently the MD2 represents 70%-75% of the European Union market and its price is about two times more than the smooth cayenne [8].

Consequently, Ghanaian farmers set out to rapidly to expand the production of MD2.The government therefore mad a budgetary allocation of US $2 million [9]. Furthermore, the World Bank in 2005 allocated US $2 million through MoFA to support smallholder farmers in the country to have access to the 'MD2' planting materials in order to ensure continuous production [9]. Ghana in 2004 had 11% share of the export market from developing countries to the European Union [8].

The MD2 variety is now the standard for the international market, because it is sweeter, grows to a uniform size, and ripens evenly than the other varieties

Currently the MD2 represents 70%-75% of the European Union market and its price is about two times more than the smooth cayenne [8].

Unfortunately, however, the MD2 variety has been found to be more susceptible to the Phytophthora rot disease than the smooth cayenne. This rot has affected the production of pineapple to the extent that Ghana is not able to meet her quota of MD2 on the world market [3,8,10].

The susceptibility of the variety to the attack of Phytophthora varies with losses ranging from 0% to 100% within the first 60 days of planting. Once the disease infects any plant that plant is lost completely in terms of producing quality fruit. The Phytophthora rot attacks all stages of the pineapple. The rising demand for organically produced pineapples is making it more important for the use of botanicals for the control of the Phytophthora rot because it is prohibited to use any chemicals in organic pineapple production [11].

The use of botanicals in the production of pineapples is not popular among farmers in the study area.

The devastating nature of the Phytophthora rot and the need for organic pineapples make it very essential that a study into the management Phytophthora rot by use of plant extracts in the MD2 variety in the Central Region of Ghana is intensively carried out in order to make available cost effective and alternative management measure for the disease so as to save the pineapple industry in Ghana.

### Characteristics of Phytophthora nicotianae var. parasitica (Dastur) and Phytophthora cinnamomi (Rands)

*Phytophthora cinnamomi* (Rands) thrives in cool to warm conditions, but is relatively inactive in very acidic soils with pH less than 4. It occurs at pH 5 or higher and is severe at pH 7 and above. *P. cinnamomi* it requires a temperature range of 25 to 36°C for growth. Little rot

occur below 25˚C. It thrives in any poorly drained soil especially in dust and is therefore often seen alongside roads. The survival spores of *P. cinnamomi* (Rands) may persist up to six years the soil [12].

*Phytophthora nicotianae var. parasitica* (Dastur) thrives in warm to hot conditions. It is relatively inactive in soils below pH 4.5 but not above pH 8. It does not thrive in infertile, light, sandy soils and does not like anaerobic conditions. It survives in dry conditions but not in dust. Optimum soil temperature of 19 to 25˚C is best for infection. No infection occurs below 15˚C, infection is slow at 30˚C and nil at 33˚C, infection is through the soil [12].

*Phytophthora nicotianae var. parasitica* (Dastur) can infect and kill plants in dry, warm to hot conditions. It is less dependent on free water than Phytophthora cinnamomi (Rands) for the production of spores and for plant infection. Infection is through leaf axils [12].

## Transmission of Phytophthora diseases

The soil is an important reservoir of inoculum for all diseases caused by *Phytophthora species* especially *Phytophthora nicotianae var. parasitica* (Dastur) and *Phytophthora cinnamomi* (Rands). Chlamydospores germinate in the soil or on decaying vegetation in the presence of an appropriate stimulus, such as root exudates, to produce zoospores. The zoospores then move towards and infect the roots or move to the surface in soil water where rain splash disperses them into stems or leaves of healthy plants [13].

High soil exchangeable calcium and magnesium cation concentration are positively correlated with disease severity [14]. In addition, high nitrogenous fertilizer, salinity and phosphorus enhance the disease. High water tables and excess irrigation provide suitable conditions for increased zoospore inoculum levels and subsequent root infections [14].

The use of water from newly contaminated ponds and reservoirs for irrigation, especially in overhead sprinkler and drip systems, transmits the disease although the effect may be seasonal [15].

Mud on farm tools, laborers' shoes, vehicle wheels, or animals may carry infected soil over long distance. Wind disposal of chlamydospores in dry period also occurs and wind borne spread by sporangia of up to 245 m has been recorded [16].

## Epidemiology of Phytophthora rot

Phytophthora rot tends to be serious in the wet season. High soil pH above 6 promotes disease development. The pathogen can be transmitted through suckers, and if pineapple is grown for two seasons, disease incidence and severity is likely to be very high. Excessive use of nitrogen-based fertilizers further increases susceptibility of pineapple to the disease due to the increased uptake of water from the soil matrix [14]. The pathogen can survive in the soil or in plant debris as chlamydospores for many years [17]. The development of Phytophthora rot disease depends greatly on the effect of humidity and temperature on the different stages of the life cycle of the fungus. Sporangia germinate almost entirely by means of zoospores at temperature up to 12˚C or 15˚C, while above 15˚C sporangia may germinate directly by producing a germ tube. Temperatures above 30˚C check the growth of the fungus in the field but do not kill it and the fungus start to grow again when temperature is favourable [18].

### Cultural Control of Phytophthora

This is the purposeful manipulation of the environment in which the pineapple is growing and it include such practices like planting and cultivation of the soil in order to make it undesirable for pests so as to reduce the damage caused by pests and diseases [18].

Cultural control measures in the control of Phytophthora in pineapple production include checking of high soil moisture levels and improving aeration by increasing drainage, and paying attention to mineral nutrition.

Movement of soil and water from diseased fields into healthy ones should be avoided. The fungus readily moves from field to field in moist soil, on tools, vehicles, bins, ladders, shoes, domestic and wild animals. Shovels, soil augers and trowels are to be dipped in 70% ethanol or rubbing alcohol before reuse. Severely affected plants should be removed [19-21].

Mother plots or sucker plots which are the plots with the plants left after the harvesting of the fruits and allowed to produce suckers as the planting material for the next season [8]. These plots are treated with fungicide to enhance the production healthy planting materials. The use of inoculum-free irrigation water is highly recommended. The removal of diseased plants, planting in well-drained sites and the avoidance of infected soil all help to reduce spread, but have limited use, as *Phytophthora nicotianae var*. *parasitica* (Dastur) is often imported in infected plants, soil or irrigation water [13].

Rotation with non-solanaceous hosts may also aid control [20,21].

**Chemical Control of Phytophthora**

The disease can be reduced by the removal of infected or dead plants after treating with fungicide. Pathogen spreading through irrigation channels can be reduced by introducing a sack of copper sulphate crystals which release copper ions slowly with the passage of water, but more than twice during the irrigation period [18].

In the 1970s and 1980s, systemic fungicides with specific activity against species of Phytophthora and related fungi revolutionized control of the rot diseases. The first of these compounds were metalaxyl (Ridomil) and fosetyl-Al (Alliette) [22-24]. The phosphonates, including fosetyl-Al and its active breakdown product phosphorous acid and potassium phosphate, have been effective when applied as foliar sprays and soil application [22-24]. Foliar and soil applications of Alliette or phosphorous is reported to control the Phytophthora rot [25]. In South Africa and Australia salts of phosphonic (phosphorous) acid, particularly potassium phosphonate (potassium phosphate) have been registered for foliar sprays [23].

A recommended treatment for healthy and lightly infected plants in South Africa, New Zealand and Australia, is a foliar application of 0.8 - 1% buffered phosphorous acid 4-6 times annually [25].

In South Africa they first developed stem injection of avocado with fosetyl-Al and phosphonates to successfully control root rot caused by *Phytophthora cinnamomi* (Rands) in avocado, and its adaption in pineapple production has given good results in several pineapple producing countries [26].

Soil fumigation with methyl bromide or aerated steaming of soil is effective in nursery, but this may allow a rapid build-up of a pathogen, if introduced subsequently, due to the absence of competitors. Compounds such as metalaxyl have good mobility in soils and may be used as a prophylactic treatment, when applied as a soil drench [18].

## Plant Extracts for the Control of Phytophthora Rot

Extracts from plants like *Carica papaya* (L.), *Amorphophallus companulatus* (Surana), *Azadirachta indica* (A.Juss.), *Gnidia glauca* (Fresen.), *Pongamia pinnata* (L), *Strychnous nux vomica* (L), *Allium cepa* (L), *Lantana camara* (L) and others have all proven to control the Phytophthora rot to some extent [27].

***Azadirachta indica* (A. Juss)**

*Azadirachta indica* (A. Juss) (Neem) belongs to the in the mahogany family Meliaceae.

*Azadirachta indica* (A. Juss) kernel has been found to be amongst the most prominent in reducing the disease situation in pineapple. Azadirachtin which is one of the 70 limonoids which have antifungal property occurs in all parts of the *Azadirachta indica* (A. Juss) [28].

***Lantana camara (L)***

The effectiveness of lantana extracts as biocides which include antifungal activity have been reported [29].

***Momordica charantia (L)***

*Momordica charantia* (L) also called bitter melon or bitter gourd has been used in trials against *Fusarium oxysporum* f. sp. *Melonis* (Schlecht), *Macrophomina phaseolina* (Tassi) Goid and *Alternaria solani* (Sorauer) and was found to have exhibited various degrees of inhibition of the three pathogens. The variability in antifungal activity exhibited by *Momordica charantia* extracts has proven that there are a variety of antifungal compounds with different characteristics and probably different modes of action [30].

***Khaya senegalensis* (Desr.) A.Juss**

*Khaya senegalensis* (Desr.) A.Juss (African mahogany) like neem belongs to the family Meliaceae. It has been proven to contain anti-fungal compounds like seneganolide A, 2-acetoxyseneganolide A and methyl 6-hydroxyangolensate which are effective against Botrytis cinerea (De Bary) Whetzel the fungus that causes grey rot in strawberry, grapes and tomatoes [31]. The effect of *K.seneghalensis* against *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.) has been reported by van der Puije [32].

## Methodology

Pineapple leaves showing signs of rot from the heart were randomly picked from plants. An average of three leaves was picked from each the plants and they were each put in separate sample bags. The leaves were carefully examined and leaves with suspected live fungus in the lead margins selected.

The lead margins of the leaves were carefully cut with a knife into approximately 1 mm x 1 mm pieces. Two pieces were picked from each of the 6 leaves with forceps and surface sterilized in a 1% NaOCl (Sodium hypochlorite) for two minutes (2 mins.) The 12 pieces were rinsed two times in sterile distilled water, and then blotted dry with sterile filter paper [33]. The pieces were then placed on the carrot agar [34] in 9 cm petri dishes. There were six replications.

The plates were incubated at 25°C for three to seven days. Sub cultures of cultures that contained more than one fungal colony, were made by successive transfer of mycelia portions onto fresh carrot agar in order to obtain pure cultures [34].

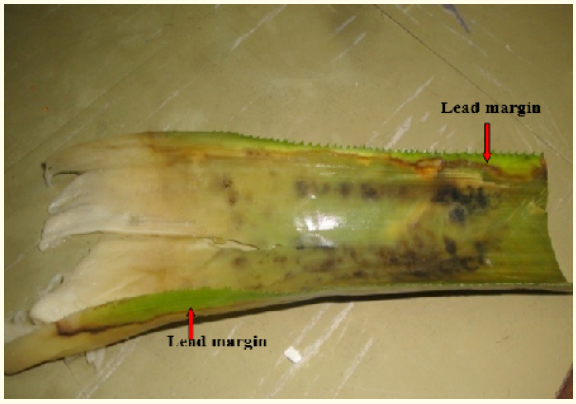


***Plate 1:*** *A Sample of pineapple leaf used for the plating showing the lead margins.*

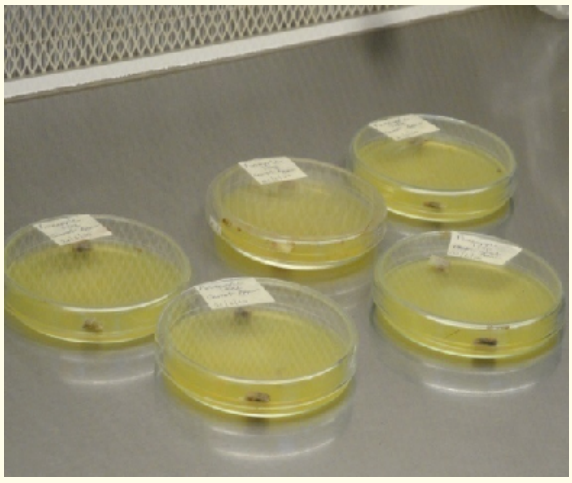

***Plate 2:*** *Plated pineapple leaf pieces on carrot agar medium.*

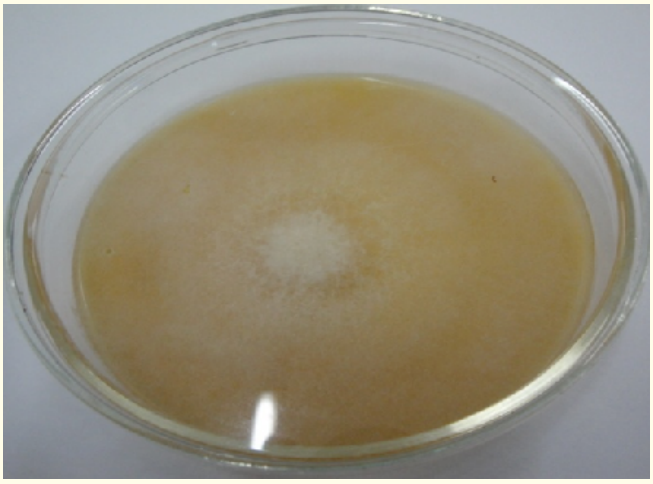

***Plate 3:*** *Pure culture of the isolated Phytophthora fungus.*

Identification of Phytophthora fungi was done with the help of as electronic microscope and based on literature by NCSU [14].

Further confirmation of the isolated fungus was carried out at the pathology laboratory of the Cocoa Research Institute of Ghana –Tafo in the Eastern Region of Ghana.

## Mass culture of pathogen

Multiplication of the *Phytophthora nicotianae var*. *parasitica* that was obtained was done by transferring of portions of the mycelia from the Phytophthora from the pure culture onto 10 other petri dishes containing fresh carrot agar and incubated at 25°C. The petri dishes were kept at a 25°C in a dark room for use in the evaluation and transmission experiments [34].

### General mycological methods

Throughout the laboratory experiments general mycological methods were used.

All the nutrient media used for the laboratory work were sterilized by autoclaving for 15 minutes at 12˚C and a pressure of 15 pounds per square inch (Psi) or 103.4 Kpa before use [35].

Also, all the glassware, knives, cork-borers, forceps and needles were sterilized by heating at 140oC for 3 hours in an electric oven [35].

During the sub culturing of the pure culture the forceps, needles that were used were flamed to red hot and air cooled before use.

The culture room was sterilized with 10% dettol solution and allowed to settle for 15 minutes.

Hands and internal surfaces, including the work surfaces, walls and any objects inside the laminar flow hood were disinfected with 70 percent ethanol [35].

### Pathogenic isolate

The pathogenic fungus used for this study was an isolate of *Phytophthora nicotianae var*. *paradisica* (Dastur.) obtained from the pathology laboratory of the Cocoa Research Institute, Tafo. A stock culture of the isolate was kept in a refrigerator at a temperature of 6˚C for use in subsequent experiment.

### Culture medium

Carrot agar was used for the isolation and laboratory evaluation of the plant extracts. The medium was prepared by cutting, weighing and boiling 200g of fresh carrots in distilled water for 45 minutes. The boiled carrots were ground in an electric blender, sieved and topped to 1 litre with distilled water. Twenty grams (20g) of plain agar was added to the sieved carrot extract and heated in a water bath. The mixture was then autoclaved for 15 minutes at 121˚C and a pressure of 15 pounds per square inch (Psi) or 103.4 Kpa.

## Results

Table 1 presents the effects of the plant extracts on the mycelial growth of *Phytophthora nicotianae var*. *parasitica* (Dastur.).

| Treatments | 3 days at 1.25% conc. | 7 days at 1.25% conc. | 3 days at 2.5% conc. | 7 days at 2.5% conc. |
|---|---|---|---|---|
| Control | 0.00 | 0.00 | 0.00 | 0.00 |
| ***Khaya senegalensis*** | 90.00 | 52.44 | 90.00 | 55.46 |
| ***Azadirachta indica*** | 45.00 | 36.95 | 55.00 | 40.68 |
| ***Mormordica charantia*** | 67.90 | 49.29 | 69.40 | 53.03 |
| ***Lantana camara*** | 90.00 | 55.20 | 90.00 | 57.41 |
| **Fosetyl-Al** | 90.00 | 90.00 | 90.00 | 68.91 |
| **S.E.D** | 6.47 | 1.109 | 6.29 | 1.844 |
| **$L.S.D_{0.05}$** | 14.69 | 4.16 | 13.71 | 4.018 |

***Table 1:*** *Mean mycelial diameter inhibition of Phytophthora nicotianae var parasitica (Dastur) over control after treatment.*

From Table 1, it can be seen that the mycelial growth inhibition over control at 1.25% concentration after 3 days, the highest inhibition of 90% was recorded by *Khaya senegalensis* (Desr.) A.Juss, *Lantana camara* (L) and Fosetyl -Al respectively while *Mormordica charantia* recorded 67.90 % of inhibition of the growth of mycelium *Phytophthora nicotianae var*. *paradisica* (Dastur.). *Azadirachta indica* (A. Juss), recorded the least inhibition of 45%.

The inhibition of mycelial growth of *Phytophthora nicotianae var. parasitica* (Dastur.) over control at 1.25% concentration after 7 days of plating showed that at 90% inhibition Fosetyl -Al was the highest inhibitor of the mycelial growth of the *Phytopthora nicotianae var. parasitica*. This was followed by: *Lantana camara* (L) with an inhibition of 55.2%, *Khaya senegalensis* (Desr.) A.Juss with an inhibition of 52.44% and *Mormordica charantia* with inhibition of 49.29%. It was noted that *Azadirachta indica* (A.Juss) with an inhibition of 36.95% was the least inhibitor of the growth of mycelial of the *Phytophthora nicotianae var. paradisica* after the 7 days of plating (Table 1).

The mycelial growth inhibition *Phytophthora nicotianae var*. *parasitica* (Dastur.) over control at 2.5 % concentration after 3 days of plating showed that 90% inhibition was achieved in *Khaya senegalensis* (Desr.) A.Juss, *Lantana camara* (L) and Fosetyl -Al respectively followed by *Mormordica charantia* with an inhibition of 69.40% the plant extract with the least inhibition of 55 % of the mycelial growth of *Phytophthora nicotianae var*. *parasitica* (Dastur.) was *Azadirachta indica* (A.Juss) (Table 1).

The mycelial growth inhibition of *Phytophthora nicotianae var*. *parasitica* (Dastur.) over the control at 2.5 % concentration after 7 days of plating showed that Fosetyl-Al was the highest inhibitor with 68.91% followed by *Lantana camara* (L) with 57.41%, *Khaya senegalensis* (Desr.) A.Juss with 55.46% and *Mormordica charantia* with 53.03% inhibition .The plant extract with the least inhibition of the mycelial growth of the *Phytophthora nicotianae var*. *parasitica* (Dastur.) was *Azadirachta indica* (A.Juss) with inhibition of 40.68% (Table 1).

## Discussion

The reduction in mycelial growth of *Phytophthora nicotianae var*. *parasitica* (Dastur) in the laboratory by both extracts of *Lantana camara* (L) and *Khaya senegalensis* (Desr.) A.Juss and the reduction of infection in the field by *Khaya senegalensis* and *Azadirachta indica* (A.Juss) are indicative of the antifungal properties of these botanicals. These findings confirm results of earlier studies by (Anon, 2011) who noted the antimicrobial and fungicidal properties of *Lantana camara* [36]. Abdelgaleli., *et al*. [31] evaluating extracts of *Khaya senegalensis* has isolated seneganolide A, 2-hydroxyseneganolide A and 2-acetoxyseneganolide which have antifungal properties. Naik., *et al.* [37] have observed antifungal compounds in neem extracts.

From the *in vitro* evaluations an appropriate combination of the applications of the extracts of *Lantana camara*, *Khaya senegalensis* and *Azadirachta indica* could probably effectively minimize the problem of Phytophthora rot in MD2 and hence increase the production of organic pineapple in Ghana.

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**Volume 4 Issue 1 October 2016**