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Full Length Research Paper

In vitro evaluation of *Carica papaya* L. and *Lantana camara L.* extracts in the Control of Late blight of Potato caused by *Phytophthora infestans (*Mont.) De Bary

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ABSTRACT

***In vitro* study was conducted to evaluate the efficacy of two plant extracts of varying concentration in the control of late blight- causing fungal pathogen. Diseased leaves of Irish potato plants were collected from farms. The fungus was isolated from diseased potato leaf samples and identified using culture and morphological characteristics. The fungal species associated with the late blight of potato and subsequent rot was isolated and identified in this study as *Phytophthora infestans* Extracts of two plants namely: *Carica papaya* L. and *Lantana camara* L. were evaluated against *Phytophthora infestans* (Mont.) de Bary using three concentrations (20, 40 and 60g/l). Growth inhibition of the organisms varied with extract type and concentrations. *Carica papaya* extract was the most effective among the two extracts evaluated. It reduced the radial growth at 60g/l concentration to up to 75.29%. *Lantana camara* at 40g /l reduced the growth to the rate of 57.65%. There was significant difference in growth inhibition at ($p<0.05$) by the extract concentrations. The plant materials could serve as an alternative to synthetic fungicide in controlling late blight of potato and other rot inciting organisms.**

Keywords: Antifungal, *Phytophthora infestans*, *Carica papaya, Lantana camara*

INTRODUCTION

Solanum tuberosum popularly called Irish potato is a major food crop grown in more than 100 countries in the world, Plateau State Nigeria inclusive. According to FAO (2010), Irish potato is planted in 18.2 million hectares with total yield reached 314.1 million tons of tubers worldwide. Irish potato is consumed by more than one billion people in the world (FAO, 2008).It is a high quality vegetable food crop and used in preparing more than 100 types of recipes, potato fried chips, potato porridge, potato broth and so on. The protein content of Irish potato has a high biological value than cereals proteins.

Irish potato also known as king of the vegetables has emerged as fourth-most important food crop in India after rice, wheat and maize. The tuber is nutritionally a superior vegetable due to its edible energy protein. It has become an integral part of breakfast, lunch and dinner among the larger population. Being a short duration crop between 3-4 months, it produces more quantity of dry matter, edible energy and edible protein in less duration of time compared to cereals, like rice and wheat. Hence, irish potato is considered to be an important crop to achieve nutritional security, since it contributes largely to the food basket of the nation.

The Irish potato plants are subjected to attack by numerous diseases wherever the crop is planted. Fungal pathogen, *Phytophthora infestans* (Mont.) de Bary caused the epidemic late blight (LB) disease of potato as proposed by (Mont.) de bary. The timing of appearance and rate of disease progression help to determine the impact of the disease on the potato crop. The disease

occurs over a wide range of climatic conditions and depends in a large part, on the frequency of foliage wetting from rainfall, fog, dew, or irrigation, and on the nutritional status of foliage as well as cultivar. It has been reported that severe epidemics can reduce yields by up to 30% (Christ and Maczuga, 1989; Shtienberg *et al*., 1990). Potato production is challenged by diverse pathogens, the most damaging of which is the *oomycetes Phytophthora infestans*, the causal agent of late blight. Late blight epidemics can be destructive and sometimes cause total crop losses (Fry and Goodwin, 1997).

It has been estimated that the management of the disease cost 3.5 billion US dollars annually in developing countries alone (Rauscher *et al*., 2006). It results in foliage death and can cause extensive tuber rot in storage if the tubers become infected during the growing season. Control of late blight disease has been accomplished primarily by the application of chemical fungicides (Kim and Lee, 2003). In an attempt to modify this condition, some alternative methods of control were adopted. An experiment was conducted to assess the effect of curcumin polyphenol compound present in the rhizome of turmeric *Curcuma longa L.*). Tomato plants were treated with curcumin solution of 500 and 1000mg \ ml (which was dissolved in water and Dimethylsulfoxide (DMSO) respectively) and later inoculated with *P. infestans*. All treated plants survived and the level of late blight control achieved with curcumin was similar to that in plants treated with the fungicide chlorothalonil (Kim and Lee, 2003).). The primary reason for the application of synthetic chemicals on crops was to reduce crop loss due to plant pathogens to a level that is economically tolerable. This reduced the crop loss, thereby increasing crop production but with detrimental effects on the environmental quality and human health. According to Fry and Goodwin (1997), the high cost of fungicide applications, increasing awareness of health and environmental risks and worldwide pressures to minimize the use of chemical sprays, requires alternative control measures. Natural products isolated from plants appear to be one of the alternatives, as they are known to have minimal environmental impact and less danger to consumers, in contrast to synthetic pesticides (Varma and Dubey,1999). It is important to adopt disease control practices that will be affordable by the bulk of resource-poor farmers in our part of the world. Such plant protection strategy must be durable, compatible and integrable with the prevailing agricultural practices specific to our people.

This study was designed to evaluate the efficacy of *Carica papaya* L. and *Lantana camara* L. extracts in the inhibition of the radial growth of the pathogen and to provide some of the information required in the use of different concentrations of the extracts as an alternative to synthetic fungicides for the effective control of late blight disease of irish potato.

METHODOLOGY

Isolation of *P. infestans* (Mont.) de Bary

Irish potato farms in Jos North Area of Plateau State Nigeria were surveyed. Leaves of the potato plant showing symptoms of late blight in the field were collected from the three locations. Three farms were sampled per location. Five samples were collected from each farm. The leaves were collected and preserved in an airtight sterilized polythene bags. The leaf samples were taken to the Plant Science and Technology Laboratory for isolation of the pathogen. The three methods used for the isolation of *Phytophthora infestans* (Mont.) de Bary were Direct screening of the diseased leaves to identify the symptoms of late blight disease on the potato leaf, Blotter method and Agar plate method. The Blotter Method, as described by Aneja (2007) was carried by using potato leaves containing both the diseased and healthy looking tissues that were cut across lesions of 5 to 10 mm square. The cut surfaces were surfaced sterilized by dipping in 70% ethanol for 15 seconds. Treated leaves were washed in three changes of sterile distilled water and blotted dry on clean, sterile paper towels to remove sterilant. The pieces were aseptically transferred to sterilized Petri dish lined with moist Whatman filter paper and incubated at 25^0c for 3-5 days, with continual sprinkling of sterile distilled water on the Whatman filter paper, to keep it moist. When growth was established, the hyphae were transferred directly to nutrient medium. With the aid of inoculating needle, colonies produced from the germinating spores on the Whatman filter paper were aseptically transferred to Wheat Dextrose Agar (WDA) (Johnson and Booth, 1983), an improvised selective medium for the growth of *Phytophthora infestans*.

The fungal isolate obtained from infected leaves was inoculated into healthy tubers of Irish potato to determine whether the organism could induce similar symptoms on re-inoculation. Inoculation of tubers and storage of inoculated tubers were carried out in the Laboratory using the same fungus isolated from the diseased leaves. Fresh and healthy Irish potato tubers were washed in running tap water to remove soil and other debris on the tuber surface. The whole tubers were surface-sterilized in 70% ethanol, after which they were rinsed with sterile distilled water. Each tuber was bored to a depth of 1cm, using a flame- sterilized cork borer at middle of the tuber, 3 mm mycelial discs of the test pathogen were placed at the bottom of the hole and covered with the tuber piece earlier removed and then sealed with Vaseline to prevent extraneous infection. The control set-up consisted of tubers that were similarly bored into but inoculated with sterile potato dextrose agar (PDA) discs of 3mm, and covered with Vaseline. All inoculated tubers were enclosed in polyethylene bags moistened with sterile cotton wool soaked with sterile distilled water to maintain a high relative humidity and incubated at 28 ± 2^0C for 7 days. At the end of the incubation period, the tubers were cut open at the point of inoculation and observed for disease development.

Preparation of the plant extracts from Carica papaya and Lantana camara

Leaves of the plant samples were collected and identified as *C.papaya* and *L.camara* leaves were washed with sterile distilled water to remove dust and air-dried at room temperature (28±2°C) for fourteen days, to reduce the moisture. These dried leaves were ground using pistil and mortar into powder and stored in air-tight containers.

To obtain aqueous solution of these powdered plants extract, three concentrations (20, 40 and 60g) were prepared, by dissolving the powder in 1litre of distilled water respectively, vigorously agitated and left for 24 hours to stand before filtration. The mixture was then filtered using sterile muslin cloth (Oloyede *et al*., 2012).

The filtrates were used as plant extract in the experiment. The synthetic fungicide (Z force) was prepared according to the manufacturer's instruction by dissolving 0.125g per litre of water.

Assessment of plant extracts against *P. infestans* (Mont.) de Bary

The antifungal properties of the extracts were tested using the radial growth method as described by Banso *et al*. (1999). PDA medium was prepared by autoclaving at 121°C and cooled to 45°C. Afterwards, the extracts were poured into separate flasks, plugged with cotton and heated for about 10 minutes to avoid contamination (Madari and Singh, 2005).

1ml of stock solution from each concentration of the two plants was added to PDA in the separate Petri dishes and mixed. In the control no extract was incorporated into the agar plate, while 1 ml synthetic fungicide was added to separate agar plate. After the medium solidified, the plates containing the medium were incubated overnight. A 3mm cork borer was used to remove fungal discs from 7 days old culture of *P. infestans* and were placed in the centre of each Petri plate, point inoculation with the aid of an inoculating needle. The position of the disc was marked on the base of the dish with a marker pen and two orthogonal axes passing through the centre of the disc were marked to be used as references for recording growth. Each treatment was replicated three times with ten plates per replication.

Plates were incubated at temperature of 28 ± 2^0c for 7 days. Radial growth along each line was recorded at exactly 24 hr intervals as the mean growth along two directions on the perpendicular lines drawn on the reverse side of the plates using transparent measuring rule. The inhibitory activity of each treatment was expressed as the percent growth inhibition as compared to the control (0 %) using the following formula:

$$\text{Growth inhibition } \% = \frac{Dc-DT}{DC} \times 100$$

Where, DC = Diameter of control and DT = diameter of fungal colony with treatment.

The data on effect of the treatments on the growth of pathogens was analyzed by analysis of variance (ANOVA), and treatment means were compared by Fishers least significant difference test (LSD) at P = 0.05.

RESULTS AND DISCUSSION

The occurrence of the fungus as shown on table 1 was found to be high at location 2 (ECWA Staff), followed by Location 1(Rukuba road), with Location 3 showing the least occurrence of late blight disease on the farms.

Table 1: Occurrence of *Phytophthora infestans* in different locations

Fungus	Location		
	L1	L2	L3
Phytophthora infestans	++	+++	+

Key:
L1 Location -Rukuba road
L2 Location -Ecwa Staff
L3 Location -Utan
\+ Mild occurrence
++ Moderate occurrence
+++ High occurrence

Plate 1: Blighted potato leaves

Plate 2: Blighted potato leaves

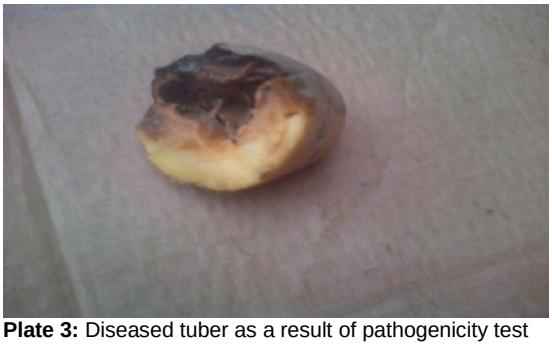

Plate 3: Diseased tuber as a result of pathogenicity test

Plate 4: Healthy un-inoculated tuber

Plate 5: Plate view of *Phytophthora infestans*

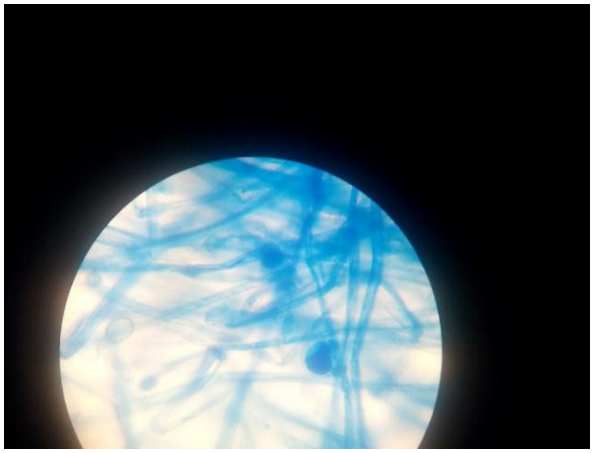

Plate 6: Micro graph of *Phytophthora infestans*

Table 2: Effect of *Carica papaya* and *Lantana camara* extract on radial growth (cm) of *P. infestans* after 7 days.

* Intervals	I	II	III	Total	Mean
Extracts/ concentration					
Lantana camara 20%	2.9	3.4	3.9	10.2	3.4
Lantana camara 40%	3.6	3.2	4.0	10.8	3.6
Lantana camara 60%	3.2	5.2	4.2	12.7	4.2
Carica papaya 20%	2.4	3.2	1.6	7.2	2.4
*Carica papaya*40%	3.4	4.1	4.8	12.4	4.1
Carica papaya 60%	3.0	1.2	2.1	6.3	2.1
Z force (Fungicide)	0.50	0.3	0.7	1.5	0.5
Control	8.5	8.5	8.5	25.5	8.5

*I,II,III are sampling intervals. Each is a Mean of 10 replicates

From the Anova, there is significant difference between the various plant extract concentration used.

Table 3: Comparison of radial growth of control and concentration of extracts from *C. papaya* and *L.camara*

Treatment	Mean radial growth	Difference	L.S.D	Probability rating
Control	8.5 – 8.5	0	1.121	
Z force	8.5 – 0.5	8	1.121	$p < 0.05$
L. camara 20%	8.5- 3.40	5.1	1.121	$p < 0.05$
L. camara 40%	8.5- 3.60	4.9	1.121	$p < 0.05$
L. camara 60%	8.5- 4.25	4.25	1.121	$p < 0.05$
C. papaya 20%	8.5- 2.4	6.1	1.121	$p < 0.05$
C. papaya 40%	8.5- 4.15	4.35	1.121	$p < 0.05$
C. papaya 60%	8.5- 2.1	6.4	1.121	$p < 0.05$

The mean radial growth in the control experiment differs significantly ($p<0.05$) from the growth obtained from the concentrations used in the extracts of the two plants. There was a significant difference among the extracts concentration after seven days and the test of mean showed difference in inhibition as compared to the control.

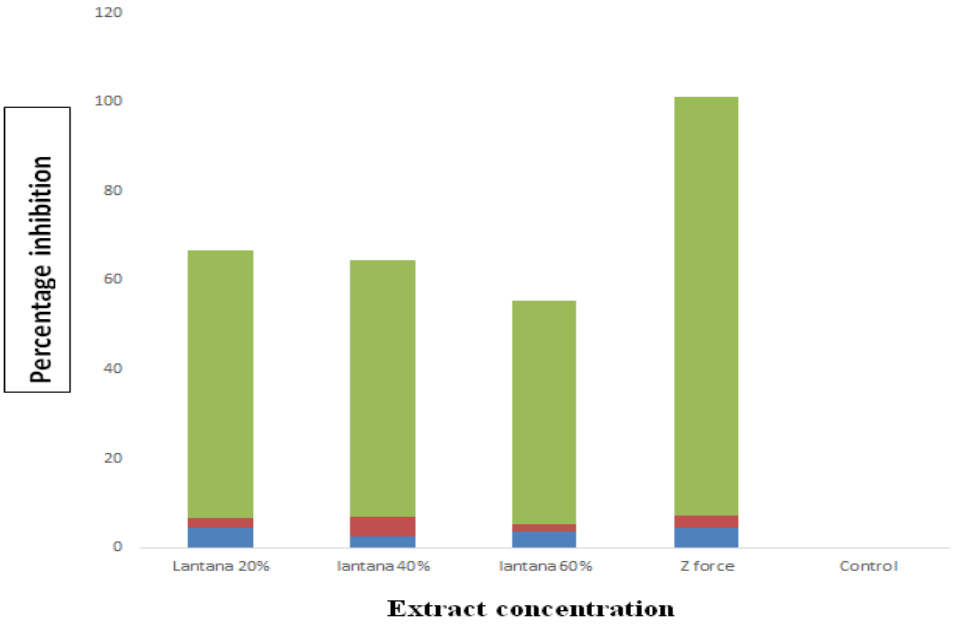

Fig. 1: Percentage inhibition of *Lantana camara* extract at varying concentration

From the chart above it shows that the plant extract reduced the radial growth of the organism when compared with the control which contains no plant extract. The synthetic fungicide recorded the highest inhibition rate of 94%.

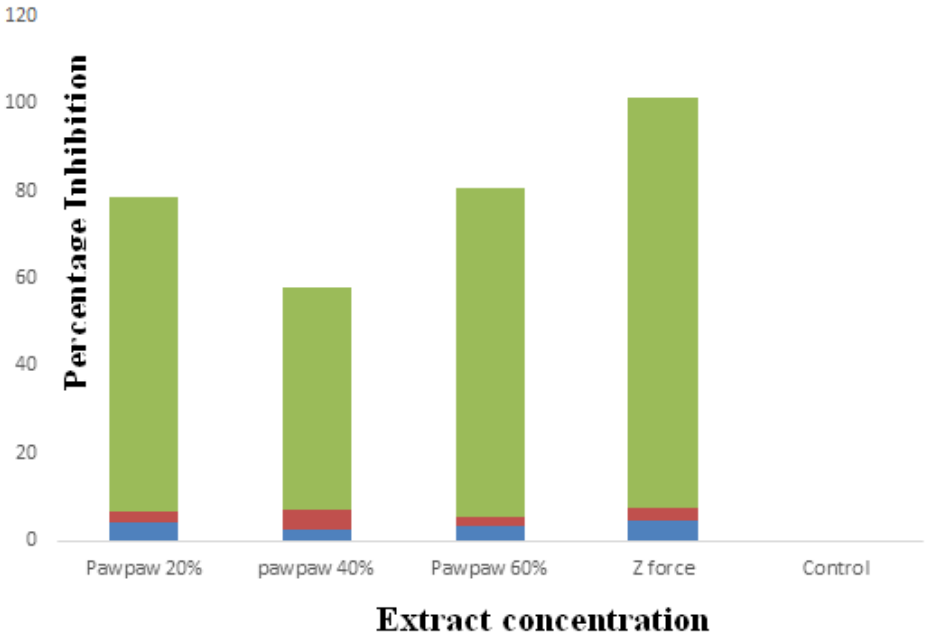

Fig. 2: Percentage inhibition of *Carica papaya* (pawpaw) extract at varying concentration

This chart presents the effect of aqueous extract of plant material against the radial growth of *P. infestans*. This result shows considerable amount of growth inhibition by *Carica papaya* at 60% concentration, with the synthetic fungicide recording the highest inhibition rate.

The pathogen associated with the diseased condition in this finding was *P.infestans*. The effects of *C. papaya* and *L. camara* extracts at various concentrations on the growth of *Phytophthora infestans* are shown in table 2. The various concentration both extracts reduce the radial growth significantly. The degree of control by these

extracts varied and was highly significant ($p<0.05$) at 20 and 40% for *L. camara* and highly significant at 20 and 60% for *C. papaya* as shown on table 3.

The results showed that the two extracts significantly ($p< 0.05$) reduced the radial growth of the pathogen with *C. papaya* giving higher reduction of 75.29% radial growth at 60% concentration (fig.2).There was a significant difference among the extracts concentration after seven days and the test of mean showed difference in inhibition as compared to the control. The extracts of *Lantana camara, Carica papaya*, are of great use in agriculture, public health, medicine, cosmetics and many more. Investigation on the antifungal properties of *C. papaya* and *L. camara*, on the growth of isolates of *P. infestans* showed that crude extracts of these plants possess some inhibitory components which caused significant reduction in radial growth of the fungus *in vitro.* According to Oloyede *et al. (*2012), the presence of lantadene in *Lantana camara,* purpin and acetogenins in *Carica papaya* indicates that these plants can serve as antimicrobial agents.

The result from this study agrees with the results of Amadioha *(*1998)*,* Owolade and Osikanlu (1999) who reported the efficacy of extracts from *C. papaya, A. ciliata, C. odirata*, among other extracts in reducing the mycelial growth of fungi as *Erysiphe cichoracearum, Collectrotrichum capsid* and *Protomycopsis phaseoli*, and compared favourably with the chemical fungicides Benlate and Ridomil. In addition, Tijjani *et al.* (2010) reported a significant inhibitory property of Neem (A*. indica*) and Moringa (*Moringa oleifera*) extracts on the mycelia growth of *Rhizopus stolonifer*. Akpa *et al*. (1991) also reported a significant inhibitory property of Neem (*A. indica*) extracts on mycelia growth of *Collectotrichum graminicola* just as found the extracts of *Ocimum gratissimum* to reduce the radial growth. This study has also confirmed and established that the antifungal activites of these crude extracts can be used or applied as post-harvest tuber treatment against tuber rot in potato caused by *P.infestans*. The inhibition of the growth of *Phytophthora infestans* by *Lantana camara* and *Carica papaya* is similar to earlier findings by Udo *et al.* (2001) who reported the high potency of extracts of garlic on the inhibition of growth and sporulation of fungal pathogens on *Ipomoea batatas* and *Diocorea* spp.

Compared to synthetic chemicals, extracts of plant origin offer the benefits of pre and post-infection fungi toxicity and are not known to leave toxic effect on produce. This therefore makes them an input of choice particularly in organic farming and in low- input conventional farming systems. They are eco-compliant; being less likely to harm farm-friendly bees, butterflies and earthworms. Application of plant-derived pesticides not only in field interventions, but especially in storage will spell improvement in sustainable food production and food security programmes of developing countries of the World with nearly 1 billion severely hungry people. Use of plant extracts to fight rots and spoilage diseases decimating stored agricultural products in Sub-saharan African countries like Nigeria, undoubtedly reflects the least–cost method of arresting phyto-fungal diseases in the country.

CONCLUSION

In conclusion, this study had shown that the extracts from leaves of *Carica papaya* and *Lantana camara* have the potential to control late blight of potato, especially foliar and tuber diseases caused by *P.infestans.*

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