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RESEARCH ARTICLE

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# Efficacy of Some Plant Extracts on Growth and Germination of *Rhizopus stolonifer* and *Fusarium oxysporum* Isolated from Rotten Irish Potato Tubers

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**ABSTRACT**

*The antifungal activity of leaf extracts of Azadirachta indica,Ricinus comunis and Mangifera indica on growth and germination of Rhizopus stolonifer and Fusarium oxysporum associated with storage rot of Irish potato tubers were investigated. Results showed that all the plant extracts showed significant ($p<0.05$) mycelial growth and spore germination inhibition of the test fungi. The inhibition in growth and spore germination increases with corresponding increase in the extract concentration. The extract of A. indica proved highly effective in inhibiting the growth and spore germination of R. stolonifer. Similar results were also obtained in growth and spore germination of F. oxysporum. The highest inhibition in growth and spore germination was observed in A. indica at highest concentration and the least was recorded in M. indica. It would be recommended that these plant extracts should be use by farmers as alternative to synthetic chemicals.*

**Keywords:** Leaf extract, *Rhizopus stolonifer*, mycelial growth, spore germination.

## INTRODUCTION

Irish potato (*Solanum tuberosum* L. ) belongs to the family *Solanaceae*[6]. Irish potato was introduced into Nigeria in later part of the 19th century by the Europeans, notably tin miners in Jos Plateau [4]. Irish potato is ranked fourth in importance after rice, wheat and maize.It has a high nutritive value and is grown for food, livestock feed and for industrial purposes [9].

Postharvest loss of root and tuber crops has been a serious problem to farmers, as more than 40% of their harvest may be lost to decay [11]. Irish potato like other tropical food crops, is attacked by many pathogens which includefungi, bacteria and viruses. Fungal rots constitute a major problem of irish potato production [12].It was identified that *Rhizopus oryzae, Fusariumredolens, Butryodiplodia theobromae*, *Fusariumoxysporum* and *Penicillium species* were responsible for post harvest rot of Irish potato tubers in southwestern Nigeria.

Synthetic chemicals have been used to control post harvest rot of many root and tuber crops. The continuous use of these chemicals develop resistance in the pathogens and cause a number of environmental and health problem[7]. The use of the plant extracts in control of plant disease is now universally accepted. These plant extracts have been reported to be safe, non-toxic and effective against plant pathogens [14]. There are several local plant species whose their extracts proved effective in controlling post harvest rot of tuber crops. [10] reported that extracts of *A. indica*

and *Afronum melegueta* inhibited the growth of *A. niger, B. theobromae, F. solami* and *P. italicum* isolated from rotted cassava. [1] observed that leaf extract of *Alchornea cordifolia, Anona muricata, allium sativum, Gacinia kola and Zingiber officinale* suppressed the growth of *A. flavus, A. niger, B. theobromae, F.solani, F. oxysporum* and *R. stolonifer.*

The aim of this paper is to determine the efficacy of leaf extracts of *A. indica, R. communis* and *M. indica* in inhibiting the growth and spore germination of *R. stolonifer* and *F.oxysporum.* -

## MATERIALS AND METHODS

### Sample Collection

The fungal isolates; *Rhizpopus stolonifer* and *Fusarium oxyporum* used in this study were obtained from stock cultures of the fungal isolates associated with storage rot of irish potato tubers from the mycology laboratory of Usmanu Danfodiyo University, Sokoto. Fresh leaves of *Azadrichtaindica, Ricinus communis* and *Mangifera indica* were collected from fadama plots in Gwadabawa, Gwadabawa local government, Sokoto state.

### Preparation of Plant Extracts

This was carried out using the method described by [2]. Fresh leaves of *Azadirachta indica, Ricinus communis* and *Mangifera indica* were washed throughly with tap water and then with sterile distilled water. The leaves were sun-dried for 5 days. The dried samples were separately grinded in laboratory using blender. The grounded samples were sieved to obtained powdered samples for extraction. Then 25g, 50g, 75g and 100g of each powdered samples were mixed with 100ml ethonal solution in the bottle to produce 25%, 50%, 75% and 100% concentrations. The extracts were sieved through muslin cloth and stored in sterile conical flasks until use.

### Effect of Plant Extracts on Growth of the Isolates

Effect of the plant extracts on the growth of *R. stolonifer* and *F. oxysprum* was investigated using the food poisoning technique [13]. One milliliter (ml) of each extract at varying degrees (25%, 50%, 75%, 100%) was poured into each Petri dishes and 9ml of the PDA was added to each Petri dishes which gave PDA- extract mixture with corresponding 2.5%, 5.0%, 7.5% and 10% extract concentrations. The plates were gently rotate to ensure even dispersion of the extracts. The agar-extract mixture was allowed to solidify. Each plate is inoculated at centre with 4mm diameter agar containing 7 days old culture of each isolate. Each treatment consists of 3 replications.The control experiment was without addition of extract but distilled water was used. All the inoculated plates were incubated at 28 +/- 2 $^0$C for 5 days. At the end of incubation period growth and inhibition of the extract was calculated according to the method described by [17].

$$\text{Percentage inhibition} = \frac{R1 - R2}{R2} \times 100$$

Where
R1 = The farthest radial distance of pathogen in control plate
R2 = The farthest radial distance of pathogen in extract incorporated agar plates.

### Effect of Plant Extracts on Spore Germination of the Isolates

The effect of different concentrations of the leaf extracts of *A. indica, R. communis* and *Mangifera indica* on spore germination of *R stolonifer* and *F. oxysporum*. One millliitre of each extract at varying concentrations (25%, 50%, 75% and 100%) was poured into 50ml conical flasks and 9ml of the PDB was added to each conical flask which gave PDB-extract mixture with corresponding 2.5%, 5.0%, 7.5% and 10% extract concentration. These concentrations were used to study spore germination of the isolates.

Spore suspensions of *R. stolonifer* and *F. oxysporum* containing 20-30 spores per microscopic field was prepared from 7 days old cultures of isolates. One drop of spore suspension was put in a glass slides containing a drop of different concentrations of plant extract. Hence slides were kept in moist chamber prepared by putting two folds of filter paper in both side of the Petri plates were incubated at 28 $^0$C for 24hrs [16]. The percentage spore germination was recorded using formula given by [5].

$$\text{Percent spore germination} = \frac{\text{Number of spore germinated}}{\text{Total number of spores}} \times 100$$

**Statistical Analysis**
The data obtained was subjected to one-way analysis of variance (Anova).Mean seperations were carried out using Least Significant Differences (LSD) at $p<0.05$

## RESULTS

**Effect of Plant Extracts on Growth of the Isolates**
Different concentrations (2.5%, 5.0%, 75% and 10%) of the extracts of *A. indica, R. communis* and *M. indica* were evaluated against *R. stolonifer* and *F. oxysporum.* The results of antifungal screening of ethanolic extracts of the three plant species are presented in table 1 and 2.The maximum inhibition of *R. stolonifer* growth was found at highest concentration of 10% (table 1). It was followed by the concentration of 7.5%, 5 and 2.5% respectively. The extract of *A.indica* at highest (10%) was the most effective inhibiting the growth *R. stolonifer* followed by the extracts of *R. communis* and *M.indica* respectively. See table 1

**Table 1: Effect of plant extract on growth of *R. stolonifer***

| | Inhibition of mycelia growth (%) | | | |
|---|---|---|---|---|
| Plant extract | 0.5 | 2.5 | 5.0 | 10 |
| *A. indica* | $56.2^{b}$ | $62.3^{b}$ | $67.5^{b}$ | $80.2^{a}$ |
| *R. communis* | $25.2^{C}$ | $30.5^{C}$ | $52.0^{b}$ | $65.2^{b}$ |
| *M. indica* | $20.3^{C}$ | $28.6^{C}$ | $46.1^{C}$ | $58.0^{b}$ |
| Control | $0.0^{d}$ | 0.0 | 0.0 | 0.0 |

*$^{a,b,c}$ mean in a columm with different superscripts are significantly different ($p<0.05$)*
*values are means ± standard error of 3 replications*

The percentage inhibition of mycelial growth of *F. oxysporum* also varied with the type of leaf extracts and extract concentration. *Azadirachta indica* leaf extract gave the highest inhibition while *M. indica* gave the lowest. All the leaf extracts of the plant species gave the highest percentage inhibition of mycelial growth at the highest concentration (10%) followed by 7.5%, 5.0% and 2.5% respectively. The results are presented in table 2.

**Table 2: Effect of plant extract on growth of *F. oxysporum***

| | Inhibition of mycelia growth (%) | | | |
|---|---|---|---|---|
| Plant extract | 0.5 | 2.5 | 5.0 | 10 |
| *A. indica* | 45.1 | 56.2 | 67.4 | 86.4 |
| *R. communis* | 28.3 | 48.2 | 52.5 | 69.9 |
| *M. indica* | 32.2 | 43.2 | 56.1 | 57.0 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 |

*$^{a,b,c}$ mean in a columm with different superscripts are significantly different ($p<0.05$)*
*values are means ± standard error of 3 replications*

**Effect of Plant Extracts on Spore Germinationof theIsolates**
The effects of the leaf extracts of *A. indica*, *R.communis* and *M. indica* on spore germination are presented in table 3 and table 4. All leaf extracts inhibited spore of the isolates. The results showed that different concentration of the plant extracts caused significant inhibition in the spore germination *R. stolonifer*. However, the maximum inhibition in spore germination was found in the highest concentration (10%). It was followed by 7.5%, 5.0%, and 2.5% concentration of the plant extracts. The extract of *Azadirchta indica* at highest concentration was found most effective in inhibiting the spore germination of *R. stolonifer* followed by highest concentration of *R. communis* and *M. indica* respectively. The results are presented in table 3.

**Table 3: Effect of plant extracts on spore germination of *R. stolonifer***

| | | Spore germination (%) | | |
|---|---|---|---|---|
| Plant extract | 2.5 | 5.0 | 7.5 | 10 |
| *A. indica* | 32.2 | 24.5 | 16.3 | 12.3 |
| *R. communis* | 38.6 | 28.5 | 18.5 | 16.2 |
| *M. indica* | 46.8 | 30.2 | 24.8 | 22.5 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 |

*$^{a,b,c}$ mean in a columm with different superscripts are significantly different ($p<0.05$)*
*values are means ± standard error of 3 replications*

The results on the effect of the leaf extracts of the plant species on spore germination of *F. oxysporum* are presented in table 4. The leaf extracts of the three plant species inhibit spore germination of the fungus. The maximum inhibition in spore germination was found at highest concentration(10%). It was followed by 7.5%, 5.0 and 2.5%. concentrations of plant extracts. The extract of *A. indica* at highest concentration was found most effective in inhibiting spore germination of *F.oxysporum*, it was followed by *R. comunis* and *M. indica* respectively.

**Table 4: Effect of plant extract on spore germination of *F. oxysporum***

| | Concentration/Spore germination (%) | | | |
|---|---|---|---|---|
| Plant extract | 2.5 | 5.0 | 7.5 | 10 |
| *A. indica* | 32.6 | 21.3 | 12.5 | 9.9 |
| *R. communis* | 52.9 | 40.3 | 23.8 | 19.6 |
| *M. indica* | 56.5 | 32.3 | 26.4 | 22.5 |
| Control | 0.0 | 0.0 | 0.0 | 0.0 |

*$^{a,b,c}$ mean in a columm with different superscripts are significantly different ($p<0.05$)*
*values are means ± standard error of 3 replications*

## DISCUSSION

This study revealed that the leaf extracts of *A. indica, R.communis* and *M. indica* and their concentration had considerable effect on growth and germination of *R. stolonifer* and *F. oxysporum*. It is clear from the results, that concentration of the leaf extracts of the three plant species showed maximum inhibition in growth and spore germination of isolates as compared to the control. At highest concentration all the leaf extracts proved highly effective ininhibiting growth and germination of the fungal isolates,this is follwed by lower concentrations ; 7.5%, 5.0% and 2.5% respectively. The results also indicate that the extract of *A. indica* was highly effective as compared with the extracts of *R. comunis* and *M*. indica. This is similar to the findings of [10] who reported that *A. indica* is more active in inhibiting the growth of *A. niger, B. theobromae, F. oxysporum* and *P. oxalicum* associated with postharvest rot of cassava tubers. [15] observed that extract of *A. indica* and *Chromolalena odonata* inhhibited thegrowth of *A. niger, F. oxysporum, R. stolonifer* and *Geotrichum candidum*. The work of [2] indicated that extracts of *Allium sativum* and *A. indica* inhibitthe growth of *Sclerotia rolfsii, B. theobromae, R. solani* and *A. niger*. The ability of the leaf extract of *A. indica*, *R. comunis*, and *M. indica* to inhibit growth and spore germination of *R. stolonifer* and *F. oxysporum* could be due to presence of fungitoxic compounds in the extracts of the three plant species [2].

## CONCLUSION

It was revealed from the study that the leaf extracts of *A.indica* caused inhibition in growth and spore germination of *R. stolonifer* and *F. oxysporum*. This demonstrated fungitoxic potential of the leaf extracts of these three plant specis against the two pathogenic fungi. Therefore the use of the leaf extracts of these plants provide better alternative to synthetic chemicals which are expensive and pose potential danger to the farmers, marketers, consumers and environment.The leaf extracts can be use as biopesticides for the control of postharvest rot of irish potato tubers.

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