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In Vitro Effect of *Colocasia esculenta* (L.) Leaf Extracts on Mycelial Growth and Spore Germination of *Fusarium* species

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ABSTRACT

The use of biological agents to control pests and pathogens of agricultural crops are being currently explored globally because of their obvious selective advantage over the chemical alternatives. In this study, both aqueous and ethanolic extract of *Colocasia esculenta* leaf and corm peel extracts at concentrations 100 $mgml^{-1}$ were applied on actively growing mycelial cultures and spore suspensions of *Fusarium* sp. and monitored using standard microscopic and mycological techniques for 5 days. Both the aqueous and ethanolic corm peel extracts lack significant activities against the growth and spore germination of the test fungus. The percentage mycelia growth inhibitions of the leaf extracts were significant ($P<0.05$) and included 51.15% and 59.78% for aqueous and ethanolic extract respectively. The spore germination was also significantly inhibited ($P<0.05$) by both leaf extracts (71.05-86.09%). Findings from the study suggest that leaf extracts *C. esculenta* possess significant effects against mycelial growth and spore germination of *Fusarium* sp, and thus could be considered as a potential bioactive control agents against the test phytopathogens in plants

Keywords: Conidial germination, mycelial growth, phytopathogens, bioactive molecules.

INTRODUCTION

One of the major challenges of agricultural produce is high susceptibility to pests and microbial attacks, thereby affecting their economic and nutritional values. The identification and control of these pathogens both at pre- and post harvesting stages is crucial to reliable and sustainable food production. Most food crop spoilages are caused by fungi (Mbajiuka and Enya, 2014). Some of the effects of fungal attack on these crops include reduction of quality, changes in colour, tastes, smells, reduction in nutritional values, increase in free fatty acids (FFA) and reduction in germination ability (White and Jayas, 1993).

One of such common phytopathogenic fungi is *Fusarium* species (Gordon and Martyn, 1997). It is widespread in occurrence and attacks diverse agricultural crops. The fungi generally penetrates the vascular systems of the susceptible plants and impede water translocation thereby inducing leaves' stomata to close and wilt after initial signs of yellowing or browning of leaves before falling off completely (Gordon and Martyn, 1997). Several edible crops such as tomato, peas, water melon, and banana have been reported to be infected by *Fusarium* wilt diseases causing annual damage worth billions of dollars globally (Syneder and Hansen, 1989). These fungal species also produces a range of mycotoxic compounds that can negatively affect both livestock and humans that consume the infected crops.

Over the years, fungicide was introduced to control microbial crop diseases and that contributed greatly to increased food production and market values globally. Consequently, there was massive crop production and continuous application of synthetic fungicides to sustain the yield. As the years rolled by, it was discovered that regular application of these chemical agents, not only generated

resistant pathogens, but also was impacting negatively on the environment posing serious health risks (Komarek et al., 2010). Hence, the need to source for alternative fungicide agents with new formulations that combines effectiveness with eco-friendliness.

In recent years, fungicides from botanicals have been tested and found to possess great potential in controlling pathogens of humans and plants. Currently, several higher plants and their phytoconstituents have demonstrated remarkable successes in controlling plant diseases and also proven to be non-phytotoxic (Singh et al., 1990; Dubey, 1991). In Nigeria, plant extracts have been used to control fungal diseases of common crops, such as cowpea (Amadioha and Obi, 1998; Ogu and Owoeye, 2013), Banana (Okigbo and Emoghene, 2004) and yam (Okigbo and Nmeka, 2005; Ogu et al., 2011).

Colocasia esculenta (L.) Schott commonly called taro or cocoyam (Family: Araceae), is a perennial herbaceous plant, with large spherical corm (swollen underground storage stem) from which a few heart-shaped leaves emerge at tip on long petiole reaching 1-2m height (Onwueme, 1978). It is commonly grown for its edible starchy roots (corm) and leaves which served as food. Various extracts of the plant have been used in traditional medicines and scientifically proven to possess diverse phytocompounds with pharmacological properties against different human ailments (Prajapati et al., 2011). Considering the profound pharmacological properties of this plant, it is pertinent to investigate its fungicidal potentials against potential phytopathogenic fungi of common staple crops in developing countries.

Therefore, this work was carried out to assess the potential of cocoyam leaf extracts in controlling mycelial growth and spore germination of a common fungal pathogens (*Fusarium* species) isolated tomatoes plant.

Fig. 1: *Colocasia esculenta* (L.) plant (Heuze et al., 2015)

MATERIALS AND METHODS

Collection and Processing of *Colocasia esculenta*

Matured *Colocasia esculenta* plants were manually uprooted from nearby farm lands in Ugbowo, Benin-City, Edo State Nigeria. They were thoroughly washed with distilled water and then separated into leaves. Each sample was dried in the oven at 70^0-80^0C for 15 minutes and at room temperature of 28 ± 2^0C for 72 hours before extraction. The final weight was obtained after grinding in a mortar with a pestle. The powdered material obtained were then extracted using aqueous and ethanol

Preparation of aqueous and ethanolic *Colocasia esculenta* Leaf extracts

The powdered plant materials were separately weighed (100g) and macerated in 500ml distilled water and 95% ethanol for 48hours. The macerated solutions were filtered separately through a clean cheese cloth before evaporating the extract to dryness over a steam bath. The extract was weighed and stored for further analysis.

Isolation of Fungal Pathogens from tomatoes

Samples of spoilt tomatoes were sourced from Oba Market in Benin City, Nigeria, and aseptically cultured on sulphate streptomycin fortified-Potato Dextrose Agar (Hi-media) using microbiological techniques and incubated at room temperature (28 ± 2^0C) for maximum time of 5-7 days. The mycelia colonies showing

white, compacted, surface and white underside characteristics of *Fusarium* species as described earlier (Alexopoulus and Mims, 1979; Roca *et al*., 2005) were subcultured and confirmed for further analysis

Determination of Effect of extract on the mycelia growth of fungal isolates

Extract at 100 $mgml^{-1}$ of the leaf was prepared using 20% Dimethyl Sulfoxide (DMSO), from which 2ml was withdrawn and dispensed aseptically into sterile Petri-dishes before pouring 18 ml sterile molten streptomycin-fortified PDA. The plates were swirled gently clockwise and anti-clockwise and allowed to set. With a sterile cock borer (6mm), mycelial discs were aseptically cut from fresh culture of test fungal pathogens and gently layered upside down at the centre of the set extract-antibiotic-PDA Petri-plates. Positive and negative control contained $10mgml^{-1}$ carbendazim and DMSO. The experimental set up was done in completely randomized design in triplicates. The effect of extracts on the radial growth of the fungus was measured at intervals of 24 hours. Colonies showing regular colonies were measured in two directions at right angles to each other on the reverse side of the plates, while the irregular colonies were measured along the longest and shortest directions and the average used as the colonial growth (Islam et al., 2003). The effect of the extracts on mycelial growth was expressed as percentage mycelia inhibition using the formula below (Ogu et al., 2011)

$$\text{Inhibition of myceliail growth } (\%) = \frac{MGC - MGT}{MGC} \times 100 \ldots \text{(i)}$$

MGC= Mycelial growth in control plates,
MGT= Mycelial growth in treated plates

Determination of Effect of extract on spore germination

Potato Dextrose broth was prepared according to specifications from which 4.5ml was pipette aseptically into sterile three test-tubes containing 0.5ml each of the various plant extracts. Fresh plates of each fungal culture were flooded with sterile distilled water to harvest the spores. The purified spores were adjusted to 10^5 spores/ml and used for the assay (Nandhini and Anjana, 2015). An aliquot (100µl) of the spore suspension was added into each test tubes, mixed properly and incubated at ambient temperature on a rotary shaker for 24hrs. The positive and negative control set up same amount of carbendazim (10mg/ml) and sterile distilled water respectively. The contents were thoroughly mixed and thereafter, with a sterile Pasteur pipette, a drop was placed on clean grease-free microscope slide and fixed with lactophenol cotton blue stain before microscopic examination for spore germination. The spore was considered germinated if the length of the germ tube was at least half the length of the conidium and was counted out of 100 randomly selected conidia in three replicate slides (Nandhini and Anjana, 2015). The spore germination expressed in percentage was calculated using the following formula

$$\text{Spore Germination}(\%) = \frac{\text{Number of Germinated spores}}{\text{Total number of spores}} \times 100 \ldots \text{(ii)}$$

Statistical Analysis

Statistical analysis of the data was done using Analysis of Variance on the replicated completely randomized experimental design. The significant means at $P<0.05$ were separated using the Duncan's Multiple Range Tests (SAS, 2002)

RESULTS AND DISCUSSION

The exploitation of bioactive phytochemicals in control of phytopathogens cannot be over emphasis in the light of the global yearning for sustainable agricultural development. In this study, it was observed that the aqueous and ethanolic leaf extracts of *C.esculenta* displayed significant ($P<0.05$) antifungal activities against the mycelial growth of *Fusarium* sp. The percentage mycelial growth inhibition elicited by the aqueous and ethanolic leaf extracts were 51.15% and 59.78% respectively. The corm peel extracts

however did not show any inhibitory effect against the test fungi. This finding is an indication that the leaf of the plant possesses significant bioactive phytochemicals when compared with the corm peels. According to Damilola *et al.* (2013) the major bioactive ingredient present in the leaf of *C. Esculenta* plants were saponins, tannins, flavonoids, alkaloids and total phenols. The antifungal action observed from the leaf extracts could be attributed to these phytochemicals. Previous studies had reported that leaf extracts of this plant possess promising antibacterial and antifungal activities against common human pathogens (Kubde et al., 2010; Singh et al., 2011; Nogodula et al., 2012; Nakade et al., 2013; Chakraborty et al., 2015). The differences in the activities could be attributed to the differences in the extracting solvents. This finding is in concordance with the reports of earlier researchers that extracting solvent affect the amount of extractable bioactive molecules (Ogu et al., 2011; Ogu et al., 2012). The poor activities of the corm peels in this study suggest that either the extracting solvents used couldn't extract the available stored biomolecules or the peels lack sufficient bioactive molecules.

Table 1: Effect of *C. Esculenta* leaf extracts (100 mgml^{-1}) on mycelial growth of *Fusarium* sp. after 5days of incubation

Extract	Mean Diameter of Mycelia (mm ± SE)	Percentage Inhibition (%)
Control	68.25 ± 0.56	00.00
Leaf Aqueous	33.34 ± 2.01	51.15*
Leaf Ethanol	27.45 ± 1.21	59.78*
Peel Aqueous	67.99 ± 3.12	00.04
Peel Ethanol	67.01 ± 1.58	01.81

n=3; *P<0.05

The effect of the extracts on the germination of spores of the test fungi followed a similar pattern as the effects of mycelia growth inhibition. The percentage inhibition of the spores ranged from 71.05-86.09 % for the leaf extracts and 19.54 - 26.03 % for corm peel extracts. Also a better inhibitory effect was exhibited by the ethanolic leaf extracts, though not significantly (P<0.05) different from the aqueous leaf extracts. The effects of corm peel extracts on the spore germination were better than effect against radial mycelial growth of the test fungi. The peel extracts could possibly possess some levels of antifungal activities against the spore germination of the test fungi. This further buttresses and reinforces the antifungal potency and potentials exploitable from cocoyam plant tissues as documented earlier (Prajapati*et al*., 2011). The findings in this study is in line with the reports of others on the antifungal potentials of leaf extracts against several food borne pathogens such as *Fusarium oxysporum, Botryodiplodia theobromae, Aspergillus niger, Aspergillus flavus, Verticillium theobromae* and *Penicillium oxalicum* of common food crops (Okigbo and Emoghene, 2004; Igeleke and Ayanru, 2007; Ogu and Owoeye, 2013; Siddique et al., 2014; Nweke, 2015). Hence, the ethanolic and aqueous extracts of *C. esculenta,* like other medicinal botanicals, possess antifungal activities against potential food borne pathogens.

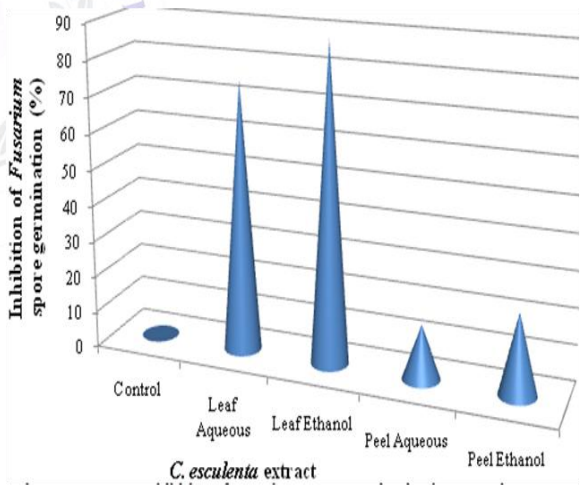

Fig-2: Effect of *C. esculenta* extracts on *Fusarium* spore germination after 24hrs

CONCLUSION

The findings from this study have shown that aqueous and ethanolic leaf extracts of *C. Esculenta* contain antifungal phytochemicals that can effectively inhibit the mycelial growth and spore germination of *Fusarium species* under in vitro experimental conditions. It also revealed that better antifungal activity was observed with the ethanolic leaf extract fraction,

though not significantly different from the aqueous fractions. Hence, leaf extract of *C. esculenta* could be suggested to hold a very promise potential as a biological fungicide to control the test phytopathogens in diseased plants. Purification and further in vivo test of the bioactive molecules are essential.

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