

## **In Vitro Control of Dry-Rot Disease Associated with *Solanum tuberosum* L. Using Varied Concentrations of Crude Extracts of *Daldinia cocentrica* (James BOLTON)**

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### **Abstract**

*Solanum tuberosum* L. (Irish Potato), popularly described as vegetable king, is a staple world's most productive crop. It is the source of nutrients such as crude protein, dietary fibres, anti-oxidants; such as vitamin C. Its availability and sustainability is threatened by post harvest deterioration such as dry-rot disease. This study was intended to in vitro control the disease, using varied concentrations (25%, 50% and 100%) of crude extract of *Daldinia concentrica* an endophytic fungus; known for its mycochemical actions against phytopathogenic fungal attacks on crops. The agar well food poison method of Martinus Berjerinck was used for the study. The results obtained from the study revealed that with 25% concentrations of the control agent; there was no impact of inhibition of the spread of the growth of the pathogen; however, 50% concentration showed an inhibition of 8mm and 100% concentration gave an inhibition of 12mm against the spread of the pathogen. This is an indication that the use of the control agent at higher concentrations can inhibit the growth of *Fusarium oxysporium*; the causal agent of potato dry-rot disease. It is likely that the control agent was able to inhibit the spread of the pathogen and inhibited the disease development; possibly due to the mycochemicals contained in the extract of the control agent.

**Keywords:** *P. solanum tuberosum*, *Daldinia concentrica*, Crude extracts, In Vitro, Agar well Poison, Potato Dry-rot Disease, *Fusarium oxysporium*.

### **Introduction**

*Solanum tuberosum* L. (Irish potato) is an annual swollen underground tuber crop that belongs to the family, solanaceae. It is commonly referred to as the "vegetable king." Being the most productive crop in the world, Irish potatoes are a substantial source of nutrients, including dietary fiber, antioxidants like vitamin C, and crude protein because of their low calorie content (Stevenson *et al.*, 2001). It is the fourth-largest yielding crop in the world, behind rice, wheat, and maize, and is a staple of most cuisines worldwide (FAOSTAT, 2008; Abbas *et al.*, 2011).

According to Irish Seed Sowers (2010), Robert Christie of Ballylaggart introduced *Solanum tuberosum* around 1882. However, the potato crop's supply, upkeep, and sustainability face significant challenges as a result of pathogenic attacks, wounds, and environmental factors, leading to decay when handled and stored. *Erwinia Chrysanthemi*, pectolytic bacteria, soft rot, dry rot illness brought on by *Fusarium* species, and other post-harvest deterioration,

such as black heart, which is caused by inadequate oxygen circulation in storage facilities is a major constraint in potato production (Tiwari *et al.*, 2020).

Potato dry-rot disease is a major threat to global potato production. It is a devastating fungal disease caused by *Fusarium*, world wide. The soil and seed-borne inoculum of *Fusarium* dry-rot is both seed and soil-borne, present in most growing areas. Its spread is associated with damage through the seed cuttings, gradings and harvesting. Wounds created during the handlings create entry route for the pathogen to enter and spread in the tissue (Aiko and Mehta, 2016).

The fungal infection of the crop accompanied by toxin development in the tubers is the causes of concern for consumer's health. This is because of the widespread dry-rot causing fungal species; *Fusarium graminearum*; reported to be a hemibiotroph, in which a variety of enzymes, toxins and secreted proteins may be involved in the pathogenesis of the hemibiotrophic fungi (Elsherbiny *et al.*, 2016). Sunken, wrinkled, dark to black tissue patches on tubers with decreased dry matter and shriveled flesh are symptoms of the potato dry-rot disease. *Fusarium graminearum*, the causative organism, causes infected tubers to dry out, but it can also induce damp rot, particularly when bacteria are also present (Aydm, 2019). Lesions initially show up as brown to black specks on the tuber's surface. Large hollow cavities may eventually grow from the lesions (Al-Mughrabi *et al.*, 2013; Tiwari *et al.*, 2020).

Among the fungus that infest most grown crops, including Irish potatoes, *Fusarium* species are the most devastating. Nonetheless, Stevenson *et al.* (2001) reported that optimal storage conditions along with prudent and appropriate cultural practices play a critical role in determining the frequency and severity of any storage rot, including potato dry-rot disease. They also said that handling potatoes carefully will lower the risk of infections; yet, since some conditions that lead to dry rot are inevitable, various approaches have also been tried to monitor the occurrence of the disease. According to Daami-remadi (2012), to combat potato dry-rot disease, researchers have experimented with the use of nanotechnology, hostile microorganisms, and chemical techniques to combat potato dry rot disease. Nevertheless, because of their environmental unfriendliness and lingering effects, chemical methods are undesirable regardless of how effective they may be.

Recently, the utilization of phyto and mycochemicals derived from fungus and plants has received attention. Since these plants and fungus, which are mostly aromatic and mycotic in nature, have been shown to be efficient against phyto-pathogenic fungi, they are abundant sources of phytochemicals, mycochemicals, and bioactive substances (Bajanowski *et al.*, 2013; Heltoft *et al.*, 2015). A variety of higher plants, mostly aromatic and medicinal plants, as well as several fungus polypores has been reported to be rich sources of bioactive chemical compounds. According to Roche *et al.* (2004), the bioactive substances are secondary metabolites that have strong anti-phytopathogenic fungal effects on plant diseases. These bioactive substances containing secondary chemicals that have been broken down by fungal endophytic polypores and plants can be acquired as extracts. The chemical compounds found in the extract are kept in the cell vacuoles of the plant so that when these tissues are extracted, they show promise as an antifungal agent for sick crops (Mahmoud *et al.*, 2010). These secondary metabolites are grouped based on their biosynthesis and origins. They include:

- (i) Terpenoids, (nitrogen-containing compounds);
- (ii) Phenyl propanols (Phenolic compounds) and

(iii) Cyanohydrin etcetera (Croteau *et al.*, 2000).

Examples are replete in literature on bioactive compounds among plant and fungal materials as extracts which can produce intended effect on plant pathogenic fungi (De-lange *et al.*, 1976; Crotaras *et al.*, 2004 and Gillizer *et al.*, 2012). These bioactive chemicals or compounds may include: alkaloids, malicin, nimaazals, tanins, flavonoids, coumarins, monoterpene, polypeptides, glycosides, saponins, and the phenolics. These compounds have been reported to exhibit antifungal potentials against some phytopathogens such as; *Botrytis cinerea*, *Fusarium* species, *Calocedrus, macrolepis*. They also inhibit mycelial growth of *F. solani*, *Pestalopsis funerea*, *Colletotrichum gloesporioides* and *Ganoderma australe* (Adrian, 1976 and 1977; Armah *et al.*, 1999). It is also reported that some of them such as T – Muurolol and  $\alpha$  cadinol could be used as natural bio-alternative control against broad spectrum of plant pathogenic fungi (Martinez, 2012; Romanazzi *et al.*, 2012; Chan *et al.*, 2013; Maria Fernanda *et al.*, 2019).

The use of aromatic higher plant extracts as an alternative to pesticides has become more popular as a result of researchers' focus shifting to the use of environmentally acceptable sources to control phytopathogenic fungus on crops. The antifungal properties of fungal endophytic polypores against phytopathogens are also well-known; yet, little research has been done with sources of mycochemicals against phytopathogens

Therefore, it is on this premise that this study is intended to use crude extract of an endophytic polypore; *Daldinia concentrica* to control in vitro, the potato dry-rot disease as indicated in this study.

## Materials and Methods

### Study Area

This study was carried out in the laboratories of Plant Science and Biotechnology, as well as Food Science and Technology, in the Faculty of Science and Agricultural Science respectively; both of which are of Rivers State University, Port Harcourt, which lies in the coordinates of  $4^{\circ} 20' 5''$  S  $1^{\circ} N$  (Lat) and  $6^{\circ} 20'$  and  $7^{\circ} 35'$  E (Long); bounded on the South by the atlantic ocean; to the North by Imo and Abia States; to the East by Akwa Ibom and to the West by Bayelsa State.

### Source and Collection of Samples

The sampling areas for this study were Ikwerre (Kelga) and Port Harcourt (Phalga) Local Government Areas of Rivers State. The potato tubers infected with dry-rot disease were procured from Nkpolu-Oroworukwo, Mile 3 market, Port Harcourt, while the treatment agent; *Daldinia concentrica* was obtained from the wild in a home garden, particularly Omuanwa in Kelga of Rivers State; where the vegetation is similar to that of Niger Delta within the main forest belt. The sample, *Solanum tuberosum* was identified and confirmed by a plant taxonomist; Dr. M. G. Ajuru, while the treatment agent; *Daldinia concentrica* was identified by a plant mycologist; Dr. F. W. Nmom.

### Sample Description and Preparation

The potato tubers (*S. tuberosum*) were identified based on their morphological features and as infected based on the symptoms manifested as dry-rot disease. The *D. concentrica* was also identified based on their hard rounded dark-brown balls which older ones were black as charcoal; often best described as cramp ball or king Alfred's cake.

The potato tubers were procured fresh and taken to the laboratory for microscopic work; while *D. concentrica* were sun-dried at  $37^{\circ}C$  for 3 days and later grounded to powder form.

The powdered form was further sun-dried at 37<sup>o</sup>c for 48 hours to maintain consistency without forming clumps. The powdered treatment agent was taken for mycochemical analysis.

### **Media Preparation**

The media used for this study was sabouraud Dextrose Agar (SDA) and was prepared according to the manufacturer's prescription.

### **Isolation of Fungal Pathogen**

The sample was washed under running tap water and disinfected with 10% sodium hypochlorite solution. Sterile knife was used to cut 10mm of the sample and placed on prepared Sabouraud Dextrose Agar (SDA) plates. The inoculated plates were incubated at room temperature for 2 – 7 days and they were observed for growth colonies from which were subcultured to obtain pure cultures

### **Macroscopic and Microscopic Examination of Fungal Isoaltes**

Microscopic examinations and cultural morphology were used to identify the isolates. After 48 hours of growth at 30°C, morphological investigations were conducted on the media plates used to isolate the fungal isolates. Based on these studies, colonies of the individual fungal isolates were identified based on their size, shape, pigmentation, elevation, and texture. The wet mount technique was applied, following Cheeseburgh's (2005) instructions. Using a sterile wire loop, a tiny amount of the isolate was removed and put on a grease-free, clean microscope slide. Next, a drop of lactophenol blue was added, spread, and covered with cover slips. This was inspected under a microscope (x10) to identify the hyphae and (x40) to look for other distinguishing characteristics.

### **Extraction of Crude Extract of *Daldinia concentrica* Used for In Vitro Test**

2g of powdered *D. concentrica* was weighed into 5ml of 20% ethanol and stored for 24 hours. The crude extract of *D. concentrica* was diluted in two-folds into concentrations of 50% and 25% and stored in sterile bottles.

### **In Vitro Test with *D. concentrica* for Antifungal Activities**

In vitro test for the determination of antifungal activities of *D. concentrica* on the fungal isolate from *Solanum tuberosum* (Irish Potato) was done using conventional Kirby-Bayer Agar well diffusion method according to National Committee on laboratory standard (NCLS) recommendation (2002). This was carried out with 100%, 50% and 25% concentrations of the crude extract of *D. concentrica*; while Ketoconazole (200mg) was used as control antifungal drugs. Using 0.5 MacFarland standard prepared as a comparator, a turbid suspension of *Fusarium oxysporium* was created in sterile distilled water. The Muller Hinton Agar media surface was evenly streaked with a sterile swab stick dipped in the fungal suspension, then pressed against the test tube walls to allow any excess to drip off. A 6mm sterile cork-borer was then used to bore wells in the media. Each concentration of the *D. concentrica* extract was added using a sterile pipette, and the mixture was then left to absorb. After a 24-hour incubation period at room temperature, the plates were examined for potential zones of inhibition..

### Mycochemical Determination and Analysis of *D. concentrica*

Mature fruit bodies of the fungus, *D. concentrica* in this study were analyzed for the presence and quantity of mycoactive chemicals such as Alkaloids, Tannins, Phenols, Saponins, Terpenoids, Oxalate and Hydrogen Cyanide; using standard methods as described by Obadoni and Ochuko (2001).

#### Procedure for analysis

##### Test for Alkaloids

The method described by Harbourne (1973) was used to determine the presence and (quantity) of alkaloids in the sample; *D. Concentrica*.

##### Procedure for Analysis:

One gramme (1g) of the sample (w) was weighed into a volumetric flask; 40ml of 10% acetic acid in ethanol was added and shaken. This was allowed to stand for 4 hours and then filtered. The filtrate was evaporated to about a quarter of its original volume. To it was added 3 drops of Conc. NH<sub>4</sub>OH solution in order to precipitate the alkaloids. The former precipitate was filtered through a weighed filter paper, labeled as (w1).

The filter paper was placed and allowed to dry at 60°C for 30-60 mins until it was constant. The filter paper was weighed again and recorded as (w2). Percentage alkaloids was determined using the formula:

$$\% \text{ Alkaloids} = \frac{W_2 - W_1}{W}$$

Where:

W<sub>2</sub>= Weight of filter paper + residue

W<sub>1</sub>= Weight of empty filter paper

W= Sample weight

##### Test for Tannin

Tannin was determined using the method of Jaff (2013).

##### Procedure:

10ml of distilled water was added to a flask containing 1g ground sample, and the mixture was shaken. The stand was centrifuged for 15 minutes at 2500rpm after being left at room temperature for 30 minutes. A 10ml volumetric flask was filled with 1ml of the supernatant, 1ml of folin-ceocalteu reagent, 1ml of saturated Na<sub>2</sub>CO<sub>3</sub> solution, and 10 ml of distilled water to dilute the mixture. The experiment was incubated at room temperature for thirty minutes. Centrifugation was not used in this process, which yielded standard tannic acid standards of 20, 40, 60, 80, 100, and 120 mg/L from a 500 ppm stock (i.e., 50 mg of tannic acid standard dissolved in 100ml of distilled water).

The absorbance of the above tannic acid concentration was read at a wavelength of 725nm and calibration curve for the tannic acid standard was drawn which stood as absorbance against concentration. To obtain the tannic acid concentration of the sample; the absorbance of the sample down the concentration axis was traced and extrapolated. To calculate the tannic acid content (mg/kg) the formula below was used:

Tannic acid content (mg/kg) = concentration obtained in mg/lx

$$\text{Tannic acid content (mg/kg)} = \frac{\text{Conc. in mg/d} \times \text{Vol. of Sample} \times \text{DF}}{\text{Sample Weight (1000g)}} \times \frac{100}{1}$$

Where DF= Dilution factor, but if not diluted, then DF=1

##### Test for Flavonoid

The test for flavonoids adopted was as reported by Sofowore (1993) and Harbourne (1973).

**Procedure:** 0.30g of the sample was weighed into a beaker and extracted with 30cm<sup>3</sup> of distilled water for 2 hours and then filtered with Whatman filter paper, 125mm to 10cm<sup>3</sup> of the aqueous filtrate of the sample was added 5cm<sup>3</sup> of 1.0M of diluted ammonia solution, followed by the addition of 5cm<sup>3</sup> of conc. H<sub>2</sub>SO<sub>4</sub>. Appearance of yellow colouration which disappeared on standing confirmed the presence of flavonoids in the sample. To calculate the content of flavonoid in the sample, the formula of Ezeonu (2016) was adopted as shown:

$$\%(\text{w/w}) = \frac{\text{RE} \times \text{D} \times 10^2}{\text{Weight of Sample}} \times \frac{100}{1}$$

RE= Tutin equivalent (ug/ml)

V= total volume of sample (ml)

D= Dilution factor

W= Sample wt.

#### **Test for Total Phenol Using Tannic Acid**

The method of Furkmen *et al.* (2006) was used to determine total phenol from the sample.

#### **Procedure:**

1.00g of ground sample was weighed into a conical flask and 10ml of ethanol was added and plugged with aluminum foil. This was vigorously shaken and left to stand for 30 mins for proper extraction after which the set up was filtered to obtain clear supernatant which was used for total phenolics assay. In order to get the total phenolics; 1ml solution of the supernatant was pipetted into a test tube and 0.5ml 2N folinciocalleu reagent and 1.5ml of 7% Na<sub>2</sub>CO<sub>3</sub> solution were added. This was made up to 10ml with distilled water and vigorously shaken and allowed to stand for 90 mins. The absorbance was read at 765nm. The following concentrations of tannic acid standard 20, 40, 60, 80, 100 and 120mg/L were prepared. The absorbance of the above tannic acid concentration was read off and calibration curve for the tannic acid standard was drawn for absorbance against concentration. And to obtain the concentration of the sample, the absorbance of the sample down the concentration axis was traced and extrapolated and then calculated as:

$$\text{Phenol content mg/kg} = \frac{\text{Conc. Obtained m/g} \times \text{Vol. Sample} \times \text{DF}}{\text{Sample Weight}}$$

Where:

DF= Dilution factor and if not diluted, it is read as DF=1.

#### **Test for Saponin**

The method reported by Obadoni and Ochuko (2001) was used to determine total saponin of the sample.

#### **Procedure:**

2.5ml of 20% aqueous ethanol were added to a conical flask containing 20g(WO) of the ground sample. The material was heated to 50°C with stirring for four hours in a hot water bath before being filtered. The apparatus was once again extracted, this time combining the extract with 40ml of 20% ethanol. The extract was allowed to evaporate in a water bath at 90°C, reducing its volume to 20ml. After transferring the concentration into a 250 ml separating funnel, 10.25ml of 5% aqueous NaCl was used twice to wash the mixed butanol layer. The remaining solution was collected and weighed into a petri dish recorded as W1.

The petri dish was dried in an oven at about 90°C. The petri dish was reweighed and recorded as W<sub>2</sub>. The total saponin was calculated as:

$$\% \text{ Saponin Content} = \frac{W_2 - W_1}{W_0} \times \frac{100}{1}$$

Where:

W<sub>2</sub> = Reweighed weight of petri dish

W<sub>1</sub> = Initial weight of petri dish

W<sub>0</sub> = sample weight.

### Test for Terpenoid

The Methodology used to determine terpenoid is as reported by Ejikeme *et al.* (2014).

#### Procedure:

0.30g ground sample was weighed into a conical flask and extracted with 30cm<sup>3</sup> and the component extracted for 2 hours. A mixture of 2cm<sup>3</sup> chloroform and conc. 3cm<sup>3</sup> H<sub>2</sub>SO<sub>4</sub> were carefully added to 5cm<sup>3</sup> of the extract to form a layer. The presence of a reddish brown colouration at the interface was an indication and positive results for the presence of Terpenoids. Percentage Terpenoids was calculated as:

$$\% \text{ Terpenoids} = \frac{\text{Weight of Terpenoids}}{\text{Sample of Weight}} \times \frac{100}{1}$$

### Test for Oxalate

The methods of Munro (2000) was adopted to determine Oxalate in *Daldinia concentrica*.

#### Procedure:

1g of the ground sample was weighed into a conical flask and 75ml of 3NH<sub>2</sub>SO<sub>4</sub> was added. This was allowed to stand for 30 mins, after which it was filtered using a Whatman no.1 filter paper. 25ml of the filtrate was pipetted into a beaker and 2 drops of methyl red indicator was added. The set up was allowed to boil in a hot titration against 0.05m of KMnO<sub>4</sub> was made until a faint pink color persisted for at least 30 seconds. The content of Oxalate in the sample was determined by taking 1ml of 0.05m KMnO<sub>4</sub> as equivalent to 2.2mg Oxalate represented as;

$$\text{Oxalate content (mg/100g)} = \frac{\text{Titre Value} \times 2.2 \times \text{DF}}{\text{Weight}} \times \frac{100}{1}$$

Where:

2.2mg= mass equivalent Oxalate value of 1m of 0.05m of KMnO<sub>4</sub> solution.

DF= Dilution factor

W= sample weight in gram.

### Test for Hydrogen Cyanide

The method of Railes (1992) was used to determine Hydrogen cyanide in the test sample.

#### Procedure:

5g of the ground sample was weighed into a conical flask and 50ml distilled water was added. This was allowed to stand overnight and then filtered. 2ml of the filtrate was pipetted into a test tube and 4ml of alkaline picrate was added and the tube corked. It was incubated in a water bath for 5 mins at 80°C. A change in color from yellow to reddish brown after incubation indicated the presence of Hydrogen cyanide.

After detection was made, the absorbance was read at 510nm and a blank containing 2ml distilled water which contained 4ml alkaline picrate solution was made; then cyanide

standard curve with 1, 2, 3, 4, 6, 8 and 10mg/L cyanide standard was prepared. The set up was extrapolated and the prepared graph from an existing graph was traced down the absorbance of the sample and concentration axis, so as to obtain concentration of the sample in mg/L. The calculation was made as:

$$\text{Mg/kg HCN} = \frac{\text{Conc. in mgl} \times \text{Vol. of Sample} \times \text{DF, if present}}{\text{Sample Weight}} \times \frac{100}{1}$$

### Test for Phytate

Phytate was determined in the test sample using the methods of Russell (1980).

#### Procedure:

2g of the ground sample was weighed into a 250ml conical flask and 100ml of 2% conc. HCl added. This was allowed to stand for 3 hours and filtered. 50ml of the filtrate was pipetted into a 250ml beaker. A 107ml of distilled water was added to improve acidity; while 10ml of 0.3% ammonium thiocyanate (NH<sub>4</sub>SCN) was added as indicator. Standard iron iii chloride (FeCl<sub>3</sub>) solution which contained 0.00195 Fe/mg was titrated until a brownish yellow color appeared and persisted for 5 mins to indicate the presence of phytate in the sample.

Calculation was made as:

$$\text{Phytic acid g/kg} = \frac{0.00195 \times \text{Volume of FeCl}_3 \text{ Consumed} \times \text{DF}}{\text{Sample Weight}} \times \frac{100}{1}$$

### Results and Discussion

**Table 1: Mean Percentage of Mycochemical Contents in *Daldina concentrica***

<u>Parameter</u>	<u>Composition</u>
Tannin (µg/100g)	94.945
Oxalate (µg/100g)	2.675
Hydrogen & Cyanide (µg/100g)	12.03
Flavonoid(mg/100g)	9.355
Alkaloid (%)	34.165
Phytate (%)	10.444
Saponin (%)	6.425
Terpenoid (%)	14.425



**Table 2: Macroscopic and Microscopic Characterization of the Fungus Isolated from the Potato Tubers.**

Isolate Code	Macroscopic Characteristics	Microscopic Characteristics	Probable Organism
1	White fluffy growth with yellow patches on the reverse	Septate hyphae with canoe-like shape of conidia	<i>Fusarium oxysporium</i>

**Table 3: Antifungal Susceptibility Test of *D. concentrica* on *Fusarium oxysporium***

S/N	Sample Concentrations	Zone of Inhibition (mm)
1	<i>Dablinia concentrica</i> (25%)	0mm
2	<i>D. concentrica</i> (50%)	8mm
3	<i>D. concentrica</i> (100%)	12mm

The results of this study are presented in Tables 1 – 3. The results on Table 1 revealed that some mycochemicals are present in the control agent, and they include: phenols, tannins, flavonoids, alkaloids, saponins, terpenoids, as well as oxalate, phytate and hydrogen cyanide. The results also indicated the mean percentage mycochemical contents in the control agent of the study, which showed that tannins (94.945) had the highest values, followed by alkaloids (34.165), while saponin (6.425) and oxalate (2.675) were of the lowest contents; although oxalate was of less importance to the essence of the study which bothers on antifungal properties. The results on Table 2 showed that the causal organism of the potato dry-rot disease is *Fusarium oxysporium* after microscopic study which revealed the organism as white fluffy growth with yellow patches on the reverse. Microscopically, it showed presence of septate hyphae with canoe-like shaped conidia, the actual description of *F. oxysporium*. The results presented on Table 3 showed the in vitro control of the fusarium dry-rot with crude extract of *Daldonia concentrica*. The results of the in vitro control indicated that at 25% concentration of the extract; there was 0mm inhibition; however, at 50% concentration of the control agent, there was 8mm inhibition and at 100% concentration; there was 12mm inhibition of the spread of the pathogen.

The findings of this study revealed that *Fusarium oxysporium* is the causal agent of dry-rot disease of potato crop. It could be that these potato tubers were wildly wounded through handlings that may have led to the collapse of the tubers when infected. This seem to be in tandem with the report of Aiko and Mehta (2016) and as supported by Tiwari *et al.* (2020); who suggested that wounds created during handling processes may create entry route for the fusarial pathogen to enter and spread in the tissues of the crop. They emphasized that if the disease can affect the plant in the field, then the damage could occur during handling and storage. The study also revealed that the dry-rot disease is accompanied by shrinking and shrivelling with black to brown rots on the surface tissue; expressing its etiology along the lesion development on the outer surface of the tubers. This finding agrees with the submission of Kolaei *et al.* (2013) and Latus-Zietkiewicz *et al.* (1987) who reported that potato tubers are the major plant parts directly affected by the dry-rot disease which is accompanied with shrinking and shrivelling on outer surface. According to them, the black

or brown rot which the pathogen produces in the crop occur internally while the wounds act as entry route for the pathogen where it causes rotting of the internal tissues; while the indirect effect of the infection can be observed as necrotic lesions on the damaged primary and secondary potato plant even while in the field. It is on this premise that the findings of this study align with their reports.

Another finding of this study is that the control agent; *Daldinia concentrica* was effective in the inhibition of the spread of the pathogen, especially at higher concentrations. This could be possible because the extracts of the control agent contained some secondary metabolites which are antifungals in action; hence, it is a fungal endophyte with mycotic substances. This finding is clearly in line with the submissions of Rocha *et al.* (2004) and supported by Mahmoud *et al.* (2010) who reported that some higher plants and fungal polypores are rich sources of phytochemical and mycoactive chemical compounds; some of which are polyporous fungi which are endophytic in their habit. These mycoactive compounds of endophytic fungi occur as secondary metabolites in a wide range of variety and mushrooms. The chemicals are also obtained as mushroom extracts and essential oils containing secondary compounds metabolized by plants and fungal endophytic polypore. The chemicals or compounds are stored in the fungal cell vacuoles, such that when the materials are used in the form of extract, they have the potentials for antifungals and biofungicides.

The findings also agree with the reports of Armah (1999); Maria Fernanda (2019), Martinez (2012). Romanazzi (2012) and Chang (2013) altogether suggested that mycoactive compounds or chemicals exercised antifungal potentials against *Botrytis cinerea*, *Fusarium* and macrolepis species and inhibited mycelial growth of *Folani*, *Pestalopsis funera*, *Colletotrichum gleosporoides* and Ganoderma substrate.

The findings of this study clearly align with the reports of Adrian (1976 and 1977); which implies that some of the mycoactive chemical compounds such as 1-muurolol and  $\alpha$ -cadinol could be used as natural bio-alternative control against a broad spectrum of plant pathogenic fungi in the stead of elaborate use of field inventories which are not ecofriendly.

### **Conclusion**

The findings of this study has shown that *Fusarium oxysporium* is the causal agent of the dry-rot disease of potato tubers. It also indicated that the pathogen affects the internal tissues of the crop which manifest physically as sunken, shrinkled and shrivelled on the epidermis of the potato tubers. Additionally, the study also revealed that the crude extracts of *Daldinia concentrica*; a fungal endophytic polypore at higher concentrations (50 – 100%) is effective in controlling the spread of the causative pathogen in vitro; owing to its components of mycoactive chemical compounds, potent against phytopathogenic fungi. We hope to create another platform testing for invivo study using same control agent in the nearest future.

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