The Potency of Oilpalm-Pressed Fibre (OPPF) Against Fungal Pathogens Associated with Various Handling Wounds on Yam (*Dioscorearotundata* Poir)

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ABSTRACT

Yam (Dioscorea rotundata Poir) is a staple food in Nigeria and Africa, providing about 200cal per capita. Its production is limited by many factors. Of utmost concern, is the various handling wounds on the yam tubers that create entry points for pathogenic rot fungi. This study intended to use oil palm – pressed fibre (OPPF) as a biocide to treat the wounded tubers. The completely randomised design was adopted and treatments replicate to obtain mean percentages of weight loss. There was no fungal contamination recorded and no weight loss of the tubers. However, from days 8 and >8, six fungal pathogens were isolated and these included; Aspergillus, spp, Penicillium spp, Botrydioplodia theobromae, Fusarium moniliforme, Sclerotium rolfsii, and Rhizopus stolonifer. Similarly, the untreated (control) wounded samples also harboured same six fungal species of those recorded above, few days after incubation and weight losses of 2.09%, 3.70%, 4.19%, 6.66%, 11.97%, and 13.9% were recorded from the untreated samples. The presence of the fungal rot pathogens on the various wounded surfaces affected the viability of the tubers and caused weight losses. Highest % weight losses of 64.69% was recorded for B. theobromae, Penicillium spp and Sclerotium rolfsii, while lowest weight loss was recorded for Aspergillus spp at 45.5% and 49.87 on days 20 and >20 respectively. The results showed that treatment with OPPF controlled the fungal contamination on the wounded surfaces, especially on short term. Based on the findings of this study, we recommend OPPF as a biocide to treat various handling wounds of yam tubers and the subsequent preservation from pathogenic fungal rots.

Key words: *Yam, Handling wounds, OPPF, Biocide, Control, Weight loss, Pathogenic rot fungi*

INTRODUCTION

Yam, (*Dioscorea rotundata* Poir) is a monocoteledon, classified as a tuber crop. It belongs to the family Discoreaceae. Yam is an important carbohydrate and staple food crop in Nigeria and African diet which provides about 200 calories per capita daily (Iloba, 1996).

Nigeria is reported as the world's largest producer of yam; accounting for half of global production. In 2008, the Food and Agricultural Organization (FAO), reported that Nigeria produced 18.3 million metric tonnes of yam from 15 million hectares; representing 73.8% of total yam produced in Africa (Ogali *et al.*, 1991). According to the figures in 2008, it is also reported that yam production has doubled since 1985; with Nigeria producing 35.17 million metric tonnes with value equivalent to US $5.64 (FAO, 2011).

However, the production of yam in Nigeria is substantially short and cannot meet its growing demands at present use. Yam production is not without limitations, a times its limitations could be occasioned by its tedious manual labour in cultivation; coupled with the attendant problem of pests and diseases that could affect its growth, yield and storage (Ecobar-Sanchez *et al.*, 2016).
Of utmost importance of the limitations to yam production is that, it is often associated with various handling wounds, ranging from cultivation – nurturing, harvest to storage. And no matter how small yam wounds may be, they could provide points of entry for fungal wound pathogens (Passam *et al.*, 1976b; Okigbo and Emila, 2010). The disease causing agents resulting from the handling wounds of yam tubers reduce the quantity and quality of the produce and make them unappealing to consumers and lead to consequent loss to the farmers (Amusa *et al.*, 2003). Although Passam *et al.* (1976b) had reported that suberization of cut tuber surfaces can be induced under laboratory conditions and at high temperature (35^{o}C) and relative humidity of about 85 – 90%. This however depends on the equilibrium moisture content which is important to primary processing and storage of farm crops (Ezeike, 1981). If the wounded yams are well exposed early after wounding, self-healing of the wounds may set in due to the physiological process involved in the formation of suberin and periderm over the wounds (Biggs, 1985). This is possible with concentration of some enzymes such as polyphenol, oxidase during the healing process (Christopher *et al.*, 1989).

Control of diseases of tuber crops has received extensive attentions of research, though many were tended towards the use of field inventories to eradicate or reduce the effect of decay of yam tubers caused by rot-causing fungal pathogens, especially in post-harvest. However, efficient fungicides may be, they are not desirable, owing to their residual actions and general hazards on consumers (Ogundana and Dennis, 1981).

Based on the fore-goings, many researchers have advocated for the use of plant materials with bioactive potentials against pathogenic fungi that cause rot and decay in yam. Some have used materials such as woodash, palm oil, palm kernel oil, lime-juice, vegetable oils, neem ash etc; but the use of oil palm-pressed fibre (OPPF) has not been reported sufficiently. Although, it has been reported that oil palm fibres contain some phytochemicals with vital biological activities. Adams (2001) reported that OPPF contains about 0.6 – 39kg of phenolic compounds; 4.0 – 62kg of sterols, tools and essential oils. Kumar *et al.* (2008) reported that plant essential oil contain a powerful disinfectant known as thymol which is antifungal. They reported that thymol's antifungal potency arises from its phenolic structure. Thymol is reported to bean effective organic fungicide (Ahmed *et al.*, 2010). Thymol belong to the class of naturally occurring essential oil known as biocide, shown to be an effective organic fungicide such as cavacrol. It is evidenced that when thymol is applied, it has the tendency to eliminate all pathogenic fungi invasion (Karaman *et al.*, 2001).
Numpaque *et al.* (2011) suggested that the antifungal potentials of thymol is caused by its ability to alter the fungal morphology and cause hyphal aggregations, resulting in reduced hyphal diametres and walls.

Segvic *et al.* (2007) also reported that thymol is lipophilic, enabling it to interact with the fungal cell membrane; thereby altering its permeability by permitting loss of macromolecules. Zambonelli *et al.* (2004) also added that the essential oil in OPPF is correlated with thymol content, hence some fungal molds are sensitive to it.

There are no reported extensive works on the control of rot-causing fungal pathogen on yam hence, the dearth of information on it regarding the control of the extracellular pectolytic enzymes produced at various wound sites by the pathogens.

This study is therefore intended to use OPPF as biocide to disinfect the wounded yam surfaces and allow the organic fungicidal potency of OPPF to interface with the fungal pathogenic rot agent that may have their ways into the yam tissues through the various handling wounds and destabilize their morphology and integrity of their hyphal wall, thereby controlling their morphology and integrity of their hyphal wall, thereby controlling their impacts on the viability of the yam tubers.

MATERIALS AND METHODS

Freshly harvested yam tubers and oil palm fruits used in this study were procured from Nkpolu-Oroworukwo, market in Port Harcourt Local Government Area of Rivers State Nigeria. The tubers were brought to the department of Plant Science and Biotechnology of Rivers State University Laboratory where the studies were carried out. The tubers were identified as *Dioscorea rotundata* prior by a taxonomist with the University, Dr. M. G. Ajuru. The palm fruits were processed in the same laboratory to obtain the oil palm-pressed fibre (OPPF).

Various types of handling wounds on the yam tubers were identified and simulated according to the methods of Elenwo and Yubedee (1999).

SIMULATION OF HANDLING WOUNDS ON YAM TUBERS

(a) Scar Wounds: This represents the type of wound produced when kitchen knife or any sharp object are used to scrape off soil adhering to the tubers after harvest. This practice is common with local subsistence farmers. To simulate this type of wound, an area of about 1cm x 2cm x 0.5 deep is peeled off the tuber surface, using sterile knife or scapel.

(b) Sharp Wounds: These types of wounds are incurred during harvesting, when farmers use digging pegs, sharp knives and or any other sharp objects to dig out the tubers from the mounds. The wounded surfaces were surface sterilized by wiping the surfaces with 5% ethanol. But to simulate sharp wounds, a distance of about 2cm was marked off the sharp end. The tuber to be wounded was then pierced up to the 2cm mark at the tip of the peg or digger. Sharp wounds can also be inflicted when tubers are cut into pieces for cooking or planting, and the remaining portion kept back to the kitchen. The remnant portion kept back to the kitchen ought to be preserved as long as it last, from fungal rot-causing pathogens. Yam tubers are wounded at the head, middle and the tail region respectively (Elenwo, 2009).

(c) Crush Wounds: These types of wounds are usually incurred when tubers are tipped from basins, baskets or even lorries, during transportation and offloading from the farm into the store and to the market. To simulate crush wounds in this study, tubers were allowed to drop from a height of about 1m unto a hard floor. The surface with which the tuber hit on the floor may or may not become open while bruised, but it is soft to touch with some tissues lacerated. These could become entry points by rot-causing pathogenic fungi (Okigbo and Emeka, 2010)

(d) Natural Wounds: Natural wounds are incurred when tubers are detached from the parent stalks, especially during weeding, adjustment of the vines to remove weeds could cause tuber to detach from the parent stalks. When that occurs, natural wound is created and the tuber may become exposed to pathogenic rot-causing fungi in the field before harvest. For this study, natural wounds were not simulated. However, a tuber can incure one or more of the identified types of handling wounds at a time.
The wounded yam tubers were incubated on laboratory benches and monitored.

Fungal Culture: The cultures of *Botrydioplodia theobromae, Aspergillus* species *Sclerotium rolfsii, Fusarium chrysogenum, Rhizopus stolonifer* and *Penicillium* species which were used in this study were isolated from the cultured wounded yam tubers from harvesting, transportations, sales and storage respectively. These fungi had been reported by (Elenwo and Yubedee, 1999 and Elenwo, 2009).

The cultures were maintained on Potato Dextrose Agar (PDA) medium until when they were required for use.

Isolation of the Pathogens: The infected tissues of the variously wounded yam tubers cultured on open laboratory benches were picked at the periphery of the wounds overgrown with fungi with the aid of a sterile forceps and inoculated unto PDA and incubated at 27 ± 2^0C for 5 days and monitored closely daily. The cultures were severally subcultured for proper identification

Identification of Fungi Pathogens of Wounded Yam Tubers: Identification of fungal pathogens associated with various handling wounds on yam tubers was done based on the description of the growth characteristics of the gross morphological appearance of the fungal colonies on the PDA culture medium and the slide culture technique for microscopic evaluation with reference to the manual of fungal Atlas (Sarah *et al.*, 2016; Barnett and Hunter).

Pathogenicity Test: Apparently healthy, fresh yam tubers with simulated various handling wounds were washed under running tap water and surface sterilized with 70% alcohol. Mycelial broth of the isolated fungi were prepared using the method of Shirun and Golam (2012). Using a sterile cork borer, holes of about 1cm were made at 3 position each of the wounded portions of the tubers and 5mm disk from a 5 day old culture of the isolated fungal/wound, using a sterile cork borer were inserted into the holes and covered with sterile transparent white polyethene bag for 48 hours and kept on the laboratory benches at a temperature of 28 ± 2^0C. The experiment was monitored daily as symptoms began to manifest.

Treatment of Wounded Yam Surfaces with OPPF
Two kilogram (2kg) of palm fruits were procured from Nkpolu-Oroworukwo market, brought to the Rivers State University, Plant Science and Biotechnology Laboratory and cooked to soft. The fruits were violated in a mortar, using pistil to separate the pericarp from the kernel. The removed pericarp bearing oil palm was diluted with 500ml of tap water to obtain the pressed fibre. The oil palm – pressed fibre still contained some traces

of the oil palm mixed with water. The pressed out fibre was used to disinfect the wounded surfaces of the yam tubers. These were incubated on the open laboratory benches at room temperature (between 28 – 30^{0}C). The initial weight of the tubers were taken and they were reweighed at intervals of four days (4 days) each in line with the method of Ogunde and Orbevba (2009) and calculated as:

$$\% \text{ weight loss} = \frac{\text{Weight Loss}}{\text{Initial Weight}} \text{X} \frac{100}{1}$$

A close constantobservations were made for any fungal growth. The manifested fungi were recorded based on mycelial forms under the microscope.

Control Test: Different types of the wounded yam surfaces were incubated in same manner as the treated set of experiment; but no kind of treatment was applied to them.

RESULTS AND DISCUSSION

Table I: Fungi Contamination on Wounded Yam Surfaces, Days 1 – 4 After Treatment with OPPF

Types of Wound	Treatment	Fungi Species Isolated
Scar	OPPF	–
Crush	OPPF	–
Sharp	OPPF	–
Control	Nil	Very few colonies of *Aspergillus* spp *Sclerotium rolfsii, R. stolonifer, B. theobromae*and *Fusarium chrysogenum*

Table 2: Fungal Contamination of Wounded Yam Surfaces >4 Days After Treatment With OPPF.

Types of Wound	Treatment	Fungi Species Isolated
Scar	OPPF	*Penicillium* species, *S.rolfsiiAaro*and *A. niger*
Sharp	OPPF	*A. niger, S. rolfsii, Penicillium* species and *B. theobromae*
Crush	OPPF	*Aspergillus* species, *Penicillium* species, *S. rolfsii, Fusarium chrysogenum, R. Stolonifer* and *B. theobromae*
Untreated	Nil	*Aspergillus* species, *Penicillium* species, *Rhizopus stolinifer Botrydioplodia theobromae, Sclerotium rolfsii* and *Fusarium*

Table 3: Effect of Oilpalm – Pressed Fibre Treatment on Percentage Weight Loss of Wounded Yam

Treatments	Initial Weight (g)	Percentage Weight Loss in Days					
		4	8	12	16	20	>20
Treated samples	650	NWL	NWL	1.69	2.46	2.76	3.23
Untreated samples	810	2.09	3.70	4.19	6.66	4.97	13.95

Legend = NWL = No Weight Loss

Table 4: Effect of Fungal Pathogens on the Percentage Weight Loss of Untreated Wounded Yam Tubers

Pathogen	Initial Weight (f)	% Weight Loss in Days					
		4	**8**	**12**	**16**	**20**	**>20**
Penicillium	810	2.09	3.70	11.85	26.29	52.59	64.69
***Aspergillus* sp**	780	16.66	30.38	38.07	45.25	45.38	49.87
Sclerotium rolfsii	680	20.58	34.70	35.0	40.88	51.76	59.70
Rhizopus stolonifer	650	21.49	34.90	39.32	51.98	52.59	57.31
Botrydioplodia theobromae	520	19.23	31.53	36.53	41.15	59.23	59.80
Fusarium chrysogenum	786	16.61	40.21	36.03	47.03	56.72	58.53

The results of this study are presented on Tables 1 – 4. The results showed that from days 1 – 4 after treatment with OPPF, there was no fungal growth observed on the wounded surfaces of the yam tubers except on the untreated (control) as indicated on table I. However, 8 days after the treatment, six major fungal species were identified from the various wounded surfaces. Identifications were made microscopically based on their mycelial growth characteristics as confirmed with the illustrations of fungal Atlas by Barnett and Hunter (1972) and Sarah *et al.* (2016). The fungal species identified included: *Aspergillus* species, *Penicillium* species, *Botrydioplodia theobromae*, *Rhizopus stolonifer*, *Sclerotium rolfsii* and *Fusarium chrysogenum*. The lacerated crush wounded surface seemed secured to habour more of the isolated fungi as much as the untreated wounded surfaces.

The results of the effect of the oil palm – pressed fibre (OPPF) on the weight loss of the wounded yam tubers is presented on Table 3. The results showed a varying percentage of losses compared to the untreated (control). There was no significant weight loss recorded for the treated samples for days 4 and 8; however, 1.69%, 2.46%, 2.76% and 3.2% weight losses were recorded for days 12, 16, 20 and >20 respectively. Notwithstanding, the untreated samples recorded higher percentage weight losses for the period of examination compared to the treated samples.

Percentage losses of 2.09%, 3.70%, 4.19% 6.66%, 11.97% and 13.95% were recorded for days 4, 8, 12, 16, 20 and <20 respectively from the untreated samples
The results presented in Table 4 revealed different levels of pathogenicity across the tested organisms. The results showed that *R. stolonifer* had higher values of weight loss represented as 21.49%, 34.90%, 39.32% and 51.91% for days 4, 8, 12 and 16 respectively. Highest percentage losses at 64.69%, 59.70 and 59.80 on days 20 and >20 were recorded for *Botrydioplodia theobromae*, *Penicillium* spp and *Sclerotium rolfsii* respectively. Lowest losses were also recorded for *Aspergillus* spp at 45.38% and 49.87 on days 20 and >20 respectively.

The results obtained from this study indicated that from days 1 – 4 after treatment with OPPF, there was no fungal growth noticed until after 8 days of treatment. This implies that wounded surfaces were coherent and intact, though the crush wound with lacerated tissues were later contaminated with six fungal species. The results also indicated that the treated yam tubers did not loose significant weight until many days when the treatment effect may have worn out of the yam tissues.

The result indicated that suberization may have been induced over the cut or wounded surfaces which seem to agree with the report of Passam *et al.* (1976b) who reported that suberization of cut tuber surfaces is induced under laboratoryconditions of high temperature 35^0C and relative humidity (85 – 90%); a clear description of the conditions and environment where this study was carried out. It also relates to the report of Ezeike (1981) who reported on the concept of equilibrium moisture content as being importantly related to primary processing and storage of farm crops. This implies that loss of moisture may have contributed to weight loss of the wounded yam tubers. It is also possible that self-healing may have also contributed to the coherent and intact surfaces of the treated wounded tubers. This is in agreement with the subjections of Biggs (1985) who reported that wound healing in tubers involves some physiological processes; of which the wounded surfaces can induce the formation of Suberinand Periderm based on the integrity and moisture content of the starch layer which forms at the surface of the wound.

It could also mean that, while the healing of the wounded surfaces were taking place, some enzymes may have been concentrated, in line with the suggestions of Christopher *et al.* (1989) who suggested that during wound healing of tubers, activities of certain enzymes such as polyphenol oxidase occur in high concentrations in the proximal half of the tuber than in the distal portions. They also suggested that increase in lipoxygenase activity at the wounded site could become a localized phenomenon. This is most likely what would have taken place in this study of managing handling yam wounded yam tubers.

The use of oil palm – pressed fibre (OPPF) as a control strategy on wounded yam surfaces is expected to be effective against fungal contamination due to the report of Jude *et al.* (2020) who submitted that yam tubers contain some bioactive compound, such as phenols, flavonoids, saponin, diosgenin, diosgenin, diocorin and hydrogen cyanide. These on their own part are anti-fungals, coupled with the bioactive potentials of the OPPF. And it therefore seemed that there was a sort of interactions between both sets of bioactive compounds both in the tubers and OPPF.

The potency of OPPF against fungal contamination is not in doubt, hence Paul-Victor *et al.* (2017) had already reported that plant fibres and their composites have underlying principles of wound healing in plants. They reported that the natural fibres are relevant to product quality and provide inspiration for biomimetric healing; even in synthetic materials.

The reasons why treated wounded yam surfaces could not be contaminated for as long as 4 days are many. One of the reason could be because, the treated wounded yam surface may have responded to quick healing; in line with the report of Passam *et al.* (1976b), when they reported that when yam tuber is cut or wounded, their tissues respond to healing by an almost immediate increase in metabolic activity; the time at which their respiration rate is also high: resulting in induction or increase in the activity of certain enzymes, notably amylase and invertase which activity is highest in the layers of the cells adjacent to the wound.

It is also possible that the bioactive chemicals in OPPF synergized with the physiological processes of the wounded tuber surfaces. Infact, Adams (2001) had earlier reported that OPPF has some phytochemicals with vital biological activities, being found to contain about 0.6 – 39kg of phenolic compounds; about 4.0 – 62kg.

The OPPF may have as well acted as disinfectant over the surfaces of the handling wounds; in line with the report of Kumar *et al.* (2008) who suggested that plant essential oil contains a powerful disinfectant, known as **thymol** which is also anti-microbial on fungal and bacteria. Their suggestion agrees with the findings of this study that the thymol's antifungal potency is because of its phenolic structure and as an effective fungicide as supported by Ahmed *et al.* (2010).

The findings of this study on the effect of the treatment of the tuber wound surfaces with OPPF is a clear indication and alignment with the report of Karaman *et al.* (2001), who reported that thymol contained in the essential oil is a known biocide for effective fungicide such as cavacrol, evidenced that when thymol is applied, it could eliminate pathogens.

The preservation of the shelf-life of the wounded yam tubers by the OPPF treatment may have also resulted from the mechanism of actions of the antifungal compounds in the OPPF, which agrees with the report of Numpaque *et al.* (2011); that the antifungal nature of thymol is due to its ability to alter the fungal hyphal morphology and cause hyphal aggregation, resulting to reduced hyphal diameter and lyses of the fungal hyphal cells.

Little wonder, why the wounded surfaces were coherent and intact, especially the sharp – wound sort. It could be that the active antifungal compound in the biocide; thymol may have interacted with the fungal cell membrane, in tandium with the report of Segvic *et al.* (2007) that thymol is lipophytic, enabling it to interact with the cell membrane of the fungal cells; thereby altering the cell membrane permeability by permitting the loss of macromolecules. It also agrees with the suggestions of Zambonelli *et al.* (2004) who supported this view and added that the activity of the essential oil in the OPPF is correlated with their thymol content; hence some fungal molds are sensitive to it.

As for the crush wounded surfaces that had the highest fungal load, it is also clear the wounded surfaces which tissues were lacerated were more ready entry points for the isolated fungi which also agrees with the opinion that fungi can penetrate their host through wounds.

CONCLUSION

This findings of this study has established that oil palm-pressed fibre (OPPF) is an effective disinfectant and an organic fungicide against fungal contamination of handling wounds of yam tubers. The study therefore exerts that the effectiveness of the OPPF was due to the bioactive compounds contained in the biocide, which may have interacted with the suberin on the wound surfaces to bring about the healing of the wound and the consequent control of the rot fungal pathogens that could have led to the rot of the tubers.

Based on the findings of this study, we therefore recommend OPPF as an organic disinfectant and biocide against handling wounds on yam tubers and subsequently prolong the shelf-life of the tubers in storage.

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