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Research Article

Application of Plant-Base Fungicides to Control Aflatoxigenic Fungi Producing Mycotoxins in Stored Cowpea Seeds

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Abstract

The inhibition of aflatoxin production in stored grains and pulses was achieved by the use of chemicals. However, these chemicals constitute serious health hazards to humans and animals. It is neither environmentally friendly nor safe for the sustenance of the ecosystem. Therefore, the current study was designed to investigate the use of plant extracts in the eradication of seed- and air-borne aflatoxigenic species of fungi in line with the recommendations of World Health Organization [WHO]. Aflatoxin detoxification and mortality of aflatoxigenic fungal strains by plant-based fungicides were examined using rigorous laboratory procedures. The strains of Aspergillus flavus, A. Parasiticus and A. Fumigatus were totally killed by 1000mg/mL cons. of the phyto-fungicides (100% death recorded). Also, 100% aflatoxin detoxification was recorded by the application of 750 and 100mg/mL concentration of the plant-based fungicides used for this experiment. The use of plant extracts for control of fungal pests is safe and less expensive than synthetic pesticides. More so, they are environmentally friendly and constitute no health risk to humans and animals. This will help reduce pollution from chemical sources and also contribute to a sustainable development of the ecosystem.

Keywords: Aflatoxin production; Phyto-fungicides; Detoxification and mortality of aflatoxigenic fungal strains; Synthetic pesticides; Sustenance of the ecosystem

1. Introduction

Aflatoxin is a regular contaminant of stored cowpea seeds in Nigeria and around the globe, constituting health hazard to humans and animals, serious danger to some beneficial microbes within the environment and other endangered species (plants and animals inclusive). The inhibition of aflatoxin production in stored grain and pulses was achieved over time from antiquity till modern age by the use of chemicals, basically preservatives and pesticides [1]. Pesticides (especially fungicides and insecticides) inhibit the transmission of spores of mycotoxigenic fungi, reduce fungal growth and minimize insect infestation of crops [2]. Chemical control of plant disease has been of immense benefit in the production of viable seeds for consumption and cultivation, however, the use of chemicals constitute serious danger to human and animal health, because they are carcinogenic [3], and neither environmentally friendly nor safe [4]. Biologically active compounds have been proven to be abundant in medicinal plants [5]. Some essential compounds from plants possess antimicrobial, fungicidal and insecticidal activities [3]. The use of some phytochemicals as food preservatives (unlike synthetic fungicides) leave no toxic residue on treated produce, require no pre-harvest interval during application or dosage limitation and contain many bioactive metabolites which makes pathogens' development of resistance to them less likely [6]. Several workers have affirmed their efficacy for managing field and storage disease of produce [7, 8, 4]. Fandohan *et al.* [9] reported significant inhibitory effects of essential oil from seeds of the neem tree (*Azadirachta indica*) on the growth of *Fusarium verticillioides* and fumonisin contamination in maize. Also, Suleiman *et al*. (2008) reported the effect of aqueous leaf extracts on *Fusarium* species isolated from cowpea. The leaves of *Ageratum conyzoides* was used as preservatives in cowpea and maize storage bins to repel insects and disallow storage fungi in traditional homesteads of Benin Republic [10]. Adegoke and Odesola [11] investigated the effects of essential oil of lemon grass in the control of seed mycoflora of cowpea and maize in storage. Many methods have been adopted to control aflatoxin in cowpea and other crops, these methods can be grouped into two which are; preventive approach and curative approach. Preventive approach aims at preventing the growth of aflatoxigenic fungi before harvest (pre-harvest) and after harvest (post-harvest). On the other hand curative approach involves the de-activation of already existing aflatoxin in cowpea and other crops. Generally, the best control of fungal toxins is usually the eradication of the fungi that produce them. Therefore, the current study was designed to investigate the use of plant extracts in the control of seed- and air-borne aflatoxigenic species of fungi below the disease threshold level in line with the recommendations of World Health Organization [WHO]. The outcome of this research will be pertinent to cowpea farmers and merchants as it will help to protect/sustain life and ensure the circulation of disease free and non- toxic cowpea seeds for consumption worldwide and to better ameliorate the problem of environmental pollution.

2. Methodology

2.1 Fungal pathogens

The fungal pathogens of cowpea seeds used for this experiment were obtained from the Department of Botany, Faculty of Science, University of Ibadan, Ibadan, Oyo State, Nigeria.

2.2 Preparation of plant-based fungicides

The procedure for preparation of plant extract as described by Okigbo and Ogbonnaya [12] was adopted with little modifications. Freshly collected leaves of *Bryophyllum pinnatum* and *Petiveria alliacea* was weighed, decontaminated and pretreated using 70% ethanol (to remove germs), rinsed in deionized water (to

remove alcohol residue), pulverized and trickled using standard laboratory equipment.

The concentrations used were 500 mg/mL, 750 mg/mL and 1000 mg/mL. All extract was stored in the refrigerator at 4^0C in air tight bottles.

2.3 Aflatoxin detoxification and fungi mortality experiment

The plant-based fungicides (1mL) and Potato Dextrose Broth (9mL) were homogenized in sterile McCartney bottles in a laminar airflow chamber. Radial mycelia plug of 5mm diameter (7day old culture of each pathogen) was aseptically inoculated into each bottles in replicates of three (3). This was done for each of the extracts and at different concentrations as well as the control setup for the experiment (The control was setup in McCartney bottles containing PDB and the individual pathogens only). The inoculants were characterized according to the treatment applied, treatment concentration, and the pathogen's identity. They were labeled appropriately and incubated at 25 ± 2°C for 7days. At the end of the experiment, the fungi mycelia were harvested and weighed to determine the fresh weight (M_1), oven dried for 3 hours at 80°C and weighed again to determine the dry weight (M_2). The mycelia weight for the control experiment was recorded too (M_o). The formula below was used to determine the percentage weight loss of the pathogen:

Fungi Mortality Rate (%) = $\frac{M_o - M_2}{M_o} x\ 100$

Where,

M_o = The dry mycelia weight of the control experiment (Untreated pathogens)

M_2 = The dry mycelia weight of the treated pathogen(s)

2.4 Experimental layout for bio-treatment

A completely randomized design was used for the experimental set up for bio-treatment.

2.5 Data analysis

Data collected was organized and analyzed using Costat 6.451 statistical software and the homogeneity of means was determined using Duncan Multiple Range Test (DMRT). Data was represented as means and standard deviation.

3. Results

3.1 Fungal pathogens of stored cowpea seeds used for the experiment

- *Aspergillus flavus*
- *Aspergillus parasiticus*
- *Aspergillus fumigatus*

Note: All the pathogens used for this experiment were confirmed to be highly aflatoxigenic.

3.2 Fungi mortality rate (FMR) experiment

It was observed that 1000mg/mL of each treatment applied had 100% mortality effect on all the aflatoxigenic pathogens of cowpea seeds investigated in this research i.e. The strains of *Aspergillus flavus* were totally killed by 1000mg/mL concentration of *Petiveria alliacea*, while similar effects was recorded for *A. parasiticus* and *A. fumigatus* with 100% destruction recorded by applying 1000mg/mL concentration of *Bryophyllum pinnatum* (Table 1). Also, 750mg/mL of Plant-based fungicide obtained from *Petiveria alliacea* totally killed the strains of *Aspergillus flavus* only (100% mycelia mortality). Other concentrations of the treatment had appreciable phyto-fungitoxic effects on the aflatoxigenic pathogens too (Table 1).

3.3 Aflatoxin detoxification

100% aflatoxin detoxification was recorded by the application of 750 and 100mg/mL concentration of the plant-based fungicides used for this experiment (Table 2). Application of 500mg/mL concentration of each treatment was unable to detoxify or totally inhibit aflatoxin production (Table 2).

Treatment	Location	Aflatoxigenic Pathogen	Fungi Mortality Rate (%)		
			500mg/mL	750mg/mL	1000mg/mL
Petiveria alliacea	Sasa	*A. flavus*	96	98	100
	Bodija	*A. flavus*	17	100	83
	Oja-Oba	*A. flavus*	33	67	83
	Sasa	*A. parasiticus*	22	78	89
	Bodija	*A. parasiticus*	25	75	75
	Oja-Oba	*A. parasiticus*	14	71	71
	Sasa	*A. fumigatus*	-17	67	83
	Bodija	*A. fumigatus*	0	86	86
Bryophyllum pinnatum	Sasa	*A. flavus*	96	96	96
	Bodija	*A. flavus*	17	17	83
	Oja-Oba	*A. flavus*	33	17	67
	Sasa	*A. parasiticus*	33	44	89
	Bodija	*A. parasiticus*	25	38	88
	Oja-Oba	*A. parasiticus*	43	57	100
	Sasa	*A. fumigatus*	33	17	100
	Bodija	*A. fumigatus*	14	29	14

Table 1: The mortality rate of the treated fungal pathogens of store cowpea seeds.

Treatment	Location	Aflatoxigenic Pathogen	Aflatoxin Detection		
			500mg/mL	750mg/mL	1000mg/mL
Petiveria alliacea	Sasa	*A. flavus*	-	-	-
	Bodija	*A. flavus*	+	-	-
	Oja-Oba	*A. flavus*	+	-	-
	Sasa	*A. parasiticus*	+	-	-
	Bodija	*A. parasiticus*	+	-	-
	Oja-Oba	*A. parasiticus*	+	-	-
	Sasa	*A. fumigatus*	+	-	-
	Bodija	*A. fumigatus*	+	-	-
Bryophyllum pinnatum	Sasa	*A. flavus*	-	-	-
	Bodija	*A. flavus*	+	+	-
	Oja-Oba	*A. flavus*	+	+	-
	Sasa	*A. parasiticus*	+	+	-
	Bodija	*A. parasiticus*	+	+	-
	Oja-Oba	*A. parasiticus*	+	-	-
	Sasa	*A. fumigatus*	+	+	-

	Bodija	*A. fumigatus*	+	+	+

Table 2: Aflatoxin detection in stored cowpea seeds after treatment.

3.4 Fresh weight loss

It was observed that application of 1000mg/ml of *Petiveria alliacea* decreased the fresh mycelia weights of *Aspergillus flavus* isolated from cowpea samples collected from Sasa [0.01g], Bodija [0.02g] and Oja-Oba [0.05g] markets, respectively, *A. parasiticus* (Isolated from Oja-Oba [0.05g], and Sasa [0.05g] market samples, respectively), and *A. fumigatus* (Isolated from Bodija [0.09g], and Sasa [0.07g] market samples, respectively). Also, *Bryophyllum pinnatum* was able to effectively reduce the fresh mycelia weight of *A. flavus* (isolated from Sasa [0.13g], Bodija [0.04g] and Oja-Oba [0.14g] markets, respectively), *A. parasiticus* (from Oja-Oba [0.03g], Sasa [0.04g], and Bodija [0.04g] markets, respectively), and finally, *A. fumigatus* (obtained from Sasa [0.03] only) compared to the untreated pathogens used as control for this experiment i.e. *A. flavus* (Sasa [0.14g], Oja-Oba [0.22g] and Bodija [0.21g], respectively), *A. parasiticus* (Oja-Oba [0.28g], Sasa [0.32g], and Bodija [0.29g], respectively), and *A. fumigatus* (Bodija [0.31g] and Sasa [0.29g], respectively) as recorded in Table 3. There was no significant difference in the fresh weight composition of the aflatoxigenic pathogens with 500 and 700mg/mL phyto-fungicides and the control set up for the experiment ($P \leq 0.05$) as shown in Table 3.

3.5 Dry weight loss

It was observed that at 1000mg/mL conc., the dry mycelia weights of all the aflatoxigenic pathogens were significantly affected by the antifungal properties of *Bryophyllum pinnatum* and *Petiveria alliacea* (Table 4). 750mg/mL formulation of the plant extracts of *Petiveria alliacea* inhibited the production of mycelia mass of *Aspergillus flavus* isolated from cowpea samples from Sasa [0.01g], Bodija [0.00g] and Oja-Oba [0.02g], respectively, *A. parasiticus* (from Sasa [0.02g], Bodija [0.02g] and Oja-Oba [0.02g] respectively), and *A. fumigatus* (from Sasa [0.02g], and Bodija [0.01g], respectively). The bio-treatment application of *Bryophyllum pinnatum* was only effective in the control of *A. flavus* isolated from Sasa market only (0.02g). 500mg/mL formulation of the plant extracts of *Bryophyllum pinnatum* and *Petiveria alliacea* effectively reduced the dry matter of *Aspergillus flavus* (0.02g each from Sasa market) only, compared to the control experimental set up i.e. *A. flavus* (Sasa [0.50g], Bodija [0.06g] and Oja-Oba [0.06g] respectively), *A. parasiticus* (Sasa [0.09g], Bodija [0.08g] and Oja-Oba [0.07g] respectively), and *A. fumigatus* (Sasa [0.06g], and Bodija [0.07g] respectively) as recorded in Table 4.

Treatment	**Location**	**Aflatoxigenic Pathogens**	**Fresh Mycelia Weight (g)**		
			500mg/mL	**750mg/mL**	**1000mg/mL**
Petiveria alliacea	Sasa	*A. flavus*	0.14 ± 0.12^{b}	0.09 ± 0.07^{cd}	0.01 ± 0.01^{h}
	Bodija	*A. flavus*	0.24 ± 0.10^{ab}	0.04 ± 0.03^{d}	0.02 ± 0.00^{h}
	Oja-Oba	*A. flavus*	0.21 ± 0.15^{b}	0.22 ± 0.02^{bcd}	0.05 ± 0.06^{fgh}
	Sasa	*A. parasiticus*	0.29 ± 0.03^{ab}	0.17 ± 0.05^{bcd}	0.05 ± 0.06^{fgh}
	Bodija	*A. parasiticus*	0.27 ± 0.04^{ab}	0.15 ± 0.04^{bcd}	0.15 ± 0.06^{cde}

	Oja-Oba	*A. parasiticus*	0.28 ± 0.03^{ab}	0.09 ± 0.11^{cd}	0.14 ± 0.07^{cdef}
	Sasa	*A. fumigatus*	0.25 ± 0.11^{ab}	0.19 ± 0.01^{bcd}	0.07 ± 0.09^{efgh}
	Bodija	*A. fumigatus*	0.28 ± 0.04^{ab}	0.10 ± 0.13^{bcd}	0.09 ± 0.06^{efgh}
Bryophyllum pinnatum	Sasa	*A. flavus*	0.08 ± 0.07^{b}	0.10 ± 0.12^{bcd}	0.13 ± 0.07^{defg}
	Bodija	*A. flavus*	0.20 ± 0.10^{b}	0.52 ± 0.73^{ab}	0.04 ± 0.04^{gh}
	Oja-Oba	*A. flavus*	0.26 ± 0.06^{ab}	0.86 ± 0.51^{a}	0.14 ± 0.11^{cdef}
	Sasa	*A. parasiticus*	0.47 ± 0.49^{a}	0.53 ± 0.51^{ab}	0.04 ± 0.03^{gh}
	Bodija	*A. parasiticus*	0.14 ± 0.00^{b}	0.18 ± 0.10^{bcd}	0.04 ± 0.03^{gh}
	Oja-Oba	*A. parasiticus*	0.15 ± 0.11^{b}	0.17 ± 0.13^{bcd}	0.03 ± 0.03^{h}
	Sasa	*A. fumigatus*	0.21 ± 0.16^{b}	0.30 ± 0.01^{bcd}	0.03 ± 0.04^{h}
	Bodija	*A. fumigatus*	0.22 ± 0.02^{b}	0.20 ± 0.00^{bcd}	0.30 ± 0.07^{ab}
Control	Sasa	*A. flavus*	0.14 ± 0.00^{b}	0.14 ± 0.00^{bcd}	0.14 ± 0.00^{cdef}
	Bodija	*A. flavus*	0.21 ± 0.00^{b}	0.21 ± 0.00^{bcd}	0.21 ± 0.00^{bcd}
	Oja-Oba	*A. flavus*	0.22 ± 0.00^{b}	0.22 ± 0.00^{bcd}	0.22 ± 0.00^{bc}
	Sasa	*A. parasiticus*	0.32 ± 0.00^{ab}	0.32 ± 0.00^{bcd}	0.32 ± 0.00^{a}
	Bodija	*A. parasiticus*	0.29 ± 0.00^{ab}	0.29 ± 0.00^{bcd}	0.29 ± 0.00^{ab}
	Oja-Oba	*A. parasiticus*	0.28 ± 0.00^{ab}	0.28 ± 0.00^{bcd}	0.28 ± 0.00^{ab}
	Sasa	*A. fumigatus*	0.29 ± 0.00^{ab}	0.29 ± 0.00^{bcd}	0.29 ± 0.00^{ab}
	Bodija	*A. fumigatus*	0.31 ± 0.00^{ab}	0.31 ± 0.00^{bcd}	0.31 ± 0.00^{a}

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

Table 3: Fresh weight measurement of aflatoxigenic pathogens treated with phyto-fungicides.

Treatment	**Location**	**Aflatoxigenic Pathogen**	**Dry Mycelia Weight (g)**		
			500mg/mL	**750mg/mL**	**1000mg/mL**
Petiveria Alliacea	Sasa	*A. flavus*	0.02 ± 0.03^{d}	0.01 ± 0.01^{ef}	0.00 ± 0.01^{c}
	Bodija	*A. flavus*	0.05 ± 0.03^{bcd}	0.00 ± 0.01^{f}	0.01 ± 0.01^{c}
	Oja-Oba	*A. flavus*	0.04 ± 0.03^{bcd}	0.02 ± 0.01^{def}	0.01 ± 0.01^{c}
	Sasa	*A. parasiticus*	0.07 ± 0.01^{bcd}	0.02 ± 0.01^{def}	0.01 ± 0.00^{c}
	Bodija	*A. parasiticus*	0.06 ± 0.02^{bcd}	0.02 ± 0.01^{def}	0.02 ± 0.01^{c}
	Oja-Oba	*A. parasiticus*	0.06 ± 0.02^{bcd}	0.02 ± 0.01^{def}	0.02 ± 0.01^{c}
	Sasa	*A. fumigatus*	0.07 ± 0.02^{bcd}	0.02 ± 0.01^{def}	0.01 ± 0.01^{c}
	Bodija	*A. fumigatus*	0.07 ± 0.01^{bcd}	0.01 ± 0.02^{ef}	0.01 ± 0.01^{c}
Bryophyllum Pinnatum	Sasa	*A. flavus*	0.02 ± 0.02^{d}	0.02 ± 0.03^{def}	0.02 ± 0.01^{c}
	Bodija	*A. flavus*	0.05 ± 0.02^{bcd}	0.05 ± 0.04^{bcde}	0.01 ± 0.01^{c}
	Oja-Oba	*A. flavus*	0.04 ± 0.00^{bcd}	0.05 ± 0.01^{bcde}	0.02 ± 0.01^{c}

	Sasa	*A. parasiticus*	0.06 ± 0.01^{bcd}	0.05 ± 0.01^{bcde}	0.01 ± 0.01^{c}
	Bodija	*A. parasiticus*	0.06 ± 0.01^{bcd}	0.05 ± 0.02^{bcde}	0.01 ± 0.01^{c}
	Oja-Oba	*A. parasiticus*	0.04 ± 0.03^{bcd}	0.03 ± 0.02^{cdef}	0.00 ± 0.01^{c}
	Sasa	*A. fumigatus*	0.04 ± 0.02^{bcd}	0.05 ± 0.01^{bcde}	0.00 ± 0.01^{c}
	Bodija	*A. fumigatus*	0.06 ± 0.01^{bcd}	0.05 ± 0.01^{bcde}	0.06 ± 0.01^{b}
Control	Sasa	*A. flavus*	0.50 ± 0.10^{bcd}	0.50 ± 0.10^{a}	0.50 ± 0.10^{a}
	Bodija	*A. flavus*	0.06 ± 0.01^{bcd}	0.06 ± 0.01^{bcd}	0.06 ± 0.01^{b}
	Oja-Oba	*A. flavus*	0.06 ± 0.01^{bcd}	0.06 ± 0.01^{bcd}	0.06 ± 0.01^{b}
	Sasa	*A. parasiticus*	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}	0.09 ± 0.01^{b}
	Bodija	*A. parasiticus*	0.08 ± 0.01^{bc}	0.08 ± 0.01^{bc}	0.08 ± 0.01^{b}
	Oja-Oba	*A. parasiticus*	0.07 ± 0.01^{bcd}	0.07 ± 0.01^{bc}	0.07 ± 0.01^{b}
	Sasa	*A. fumigatus*	0.06 ± 0.01^{bcd}	0.06 ± 0.01^{bcd}	0.06 ± 0.01^{b}
	Bodija	*A. fumigatus*	0.07 ± 0.01^{bcd}	0.07 ± 0.01^{bc}	0.07 ± 0.01^{b}

Means with the same alphabets down the column are not significantly different at P≤0.05 using Duncan Multiple Range Test (DMRT) for separation of statistically significant means. Data collected were represented as "Means ± SD" only

Table 4: Dry weight measurement of aflatoxigenic pathogens treated with phyto-fungicides.

4. Discussion

The research conducted showed that *Petiveria alliacea* and *Bryophyllum pinnatum* were very effective in detoxification and inhibition of aflatoxin production in stored cowpea seeds collected from local markets within Ibadan, Oyo State, Nigeria. Also, these phyto-fungicides showed a tremendous capacity for elimination of aflatoxigenic fungal pathogens of stored cowpea seeds too. The success achieved by the application of these plants-based fungicides can be attributed to the presence of high levels of antifungal substances present in the plant samples. A similar report was given by Silva *et al*., [13] who noted the ability of *P. alliacea* to inhibit the growth of aflatoxigenic fungal species and also *Colletotrichum gloeosporioides*. The dry mycelia weight of most of the isolated aflatoxigenic species was significantly reduced by the antifungal activity of *P. alliacea* and *B. pinnatum*. This was in connection with the research ideology of Alabi *et al*., [14] who initially investigated the fungitoxic and phytotoxic effects of aqueous extract of *B. pinnatum* on some fungal pathogens that induced wilting in cowpea grown in Ago-Iwoye, Ogun State, Nigeria. They also reported that the plant extracts significantly reduced the Disease Infection Rate (DIR) in treated plants.

5. Conclusion

The use of plant extracts for control of fungal pests is safe and less expensive than synthetic pesticides. There are no side effects to high dosages of botanical extracts applied for the treatment or management of plant diseases. Production of plant-based fungicides are very easy to setup, cheap, available and affordable, and there are no skills or precautions needed for application. More so, they are environmentally friendly and constitute no health risk to humans and animals. This will help reduce pollution from chemical sources and also contribute to a sustainable development of the ecosystem.

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