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Spectra characterization, flavonoid profile, antioxidant activity and antifungal property of *Senecio bifrae* and its copper complex

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This study describes the synthesis, spectra characterization using UV-Visible and Infra-Red spectroscopic methods, flavonoid profile, antioxidant activity and antifungal property of *Senecio biafrae*-Cu complex and *Senecio biafrae*. UV-Visible spectra revealed bathochromic shifts for *Senecio biafrae*-Cu complex in comparison with *Senecio biafrae* which could be due to complexation. Differences between vibration spectra of *Senecio biafrae*-Cu complex and *Senecio biafrae* showed that carbonyl oxygen and hydroxyl groups were involved in complexation because of shifts in their positions. Kaempferol; a flavonol was found to be most abundant identified in both *Senecio biafrae* and its complex. *Senecio biafrae*-Cu complex had better antioxidant and antifungal activities against free radicals and pathogenic *Fusarium oxysporium*, *Aspergillus flavus* and *Fusarium solani* than *Senecio biafrae*. This study has shown that natural vegetables such as *S. biafrae* can also be used for complexation.

Key words: *Senecio biafrae*, flavonoid composition, antifungal property, inhibition, copper complex.

INTRODUCTION

In today's world, it is difficult to avoid exposure to heavy metals such as cadmium, mercury, lead, copper, chromium, nickel e.t.c. They enter into human body through food, water, air and skin. These heavy metals exert their toxic effects by producing free radicals which cause oxidative stress (Azeez et al., 2013). Copper (Cu) is essential for living but it has also been reported to have a major role in the production of the very reactive

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hydroxyl radical $HO^{\cdot}$ through the Fenton and Haber-Weiss reactions (Puig and Thiele, 2002; Panhwar et al., 2010; Riha et al., 2014). Free radicals such as hydroxyl radical generated by excessive exposure to copper leads to damage that affects lipids, protein/enzymes, carbohydrates, and DNA in cells and tissues (Bukhari et al., 2008). Each organism is imbued with mechanisms to safely excrete the excess Cu ions in the body but when this is hampered, chelation therapy can be used to remove excess Cu ions from vulnerable sites in critical organs (Malešev and Kunti, 2007; Dehghan and Khoshkam, 2012).

Chelation therapy is a promising method in treating pathological diseases arising from oxidative stress caused by excess or dysregulation of transition metals. This is achieved by suppressing the metal induced toxicity (Moridani et al., 2003). Chelating agents such as flavonoids have a natural way of complexing toxic metals and are easily excreted from the body. This plays a major role in limiting metal bioavailability and suppressing metal toxicity and is preferred to synthetic metal chelators because of toxicity (Moridani et al., 2003; Cao et al., 2015; Aaseth et al., 2015)

Flavonoids, a class of phenolic compounds have been shown to possess anti-inflammatory, antiviral, anticarcinogenic, antithrom-botic, antiallergic and hepatoprotective properties (Malesev and Kuntic, 2007). They are extensively distributed in plant-based foods. They are important natural antioxidants and free radical scavengers (Liu, 2003).

Flavonoid-metal complexes have been shown to possess higher antioxidant activity against free radicals and antimicrobial activity against pathogenic microorganisms such as *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* (Pereira et al., 2007; Dehghan and Khoshkam, 2012). Quercetin, morin, rutin kaemferol, naringin, catechin are some of flavonoids that have been used to chelate heavy metals such as Cu and they have proved to be better source of antioxidant (Pereira et al., 2007; Bukhari et al., 2008; Panhwar et al., 2010; Dehghan and Khoshkam, 2012). However, these flavonoids are eaten as part of food in vegetables or fruits but not isolated from them before they are consumed. Moreover, their concentrations in vegetables may differ from those concentrations reported for metal chelation. Hence, the use of vegetables such as *Senecio biafrae* which contain appreciable amounts of these flavonoid compounds (Muhammed et al., 2012). *S. biafrae* is one of the common vegetables consumed in Sierra Leone, Ghana, Benin Republic, Nigeria, Cameroon and Gabon. It is nutritive, rich in protein, ascorbic acid and polyphenols. It is also medicinal and has therapeutical properties. Its leaves contain various secondary metabolites such as dihydroisocoumarins, terpenoids, sesquiterpenes and amino acids (Dairo and Adanlawo 2007; Adefegha and Oboh, 2011).

The purposes of this study were to investigate the chelating property of *S. biafrae*; a source of different flavonoids and characterize *S. biafrae*- copper complex using UV-Visible and Infra Red spectroscopic methods; also, to investigate the antioxidant and antifungal activities of both *S. biafrae* and *S.biafrae*-copper complex.

MATERIALS AND METHODS

Collection of vegetable sample

S. biafrae leaves used for this study were purchased from farm around Olu-Ode market in Osogbo, Osun State, Nigeria. The vegetable was identified and authenticated by Mrs. F.M Tairu at National Horticultural Research Institute (NIHORT), Ibadan with voucher number NIH. 112427. The vegetables were lyophilized, ground, stored in foil paper and kept in a dessicator.

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), copper sulphate pentahydrate ($CuSO_4.5H_2O$), standard flavonoid compounds, methanol, sodium hydroxide, and aluminum chloride were used. All reagents were analar grade bought from Sigma Aldrich, Germany. Fungal culture, discs and agar were obtained from Microbiology unit, Fountain University, Osogbo. Deionized-distilled water was used all through the experiments.

Extraction

The method of Olajire and Azeez (2011) was used for this extraction. Fifty grams (50 g) of the powdered *S. biafrae* was soaked in 500 ml of 70% aqueous methanol and was shaken on orbital shaker for 4 h. The solution was filtered with Whatman No. 4 filter paper and the filtrate was evaporated at 40°C to dryness using a rotary evaporator.

Synthesis of *S. biafrae*-copper complex

1 g of *S. biafrae* extract was dissolved in 100 ml of methanol and stirred thoroughly until complete dissolution of the extract. 1 g of copper sulphate pentahydrate ($CuSO_4.5H_2O$) was added and the solution turned brownish-green upon stirring for 1hr 30 min. afterwards, the solution was filtered using Whatman No. 4 filter paper and the filtrate was evaporated at 40°C to dryness using rotary evaporator.

Instrumentation

UV–visible spectra of *S. biafrae* extract and *S. biafrae*-Cu complex were obtained using Jenway 6405 UV-Visible spectrophotometer and Infrared spectra were recorded using KBr pellets in the spectral range 4000 to 400 cm^{-1} on FTIR Spectrophotometer (Model 500, Buck Scientific Inc.).

Identification and quantification of flavonoid composition with gas chromatrography-flame ionization detector (GC-FID)

The procedures described by Whitehead et al. (1983) and Provan et al. (1994) were used for the extraction of flavonoids from *S.*

biafrae. Briefly, 50 mg of the sample was extracted with 5 ml of 1 M NaOH for 16 h on a shaker at ambient temperature. After this, the extract was centrifuged (5000 g). The residue was rinsed with deionized water, centrifuged again and the combined supernatants were placed in a disposable glass test tube which was heated at 90°C for 2 h to release conjugated flavonoids. The heated extract was cooled, titrated with 4 M HCl to pH < 2.0, diluted to 10 ml, with deionised water, and centrifuged to remove the precipitate. 15 ml of the supernatant obtained was passed through a conditioned Varian (Varian Assoc., Habor City, CA) Bond Elut PPL (3-ml size with 200 mg packing) solid- phase extraction tube at 5 ml min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under vacuum (-60 kPa) until the resin was thoroughly dried after which the flavonoids were eluted with 1 mL of ethyl vials. The PPL tubes were conditioned by first passing 2 mL of ethyl acetate followed by 2 ml of water (pH < 2.0).

The composition of flavonoid in *S. biafrae* extract and *S. biafrae*-Cu complex was analyzed using gas chromatography coupled with flame ionization detector (GC-FID). 1 µl of each solution was injected into GC (Hewlett-Packard Model 5890, USA) with FID which has HP-1 column (30 m × 0.25 µm × 0.25 mm id), nitrogen carrier gas, a detector section temperature of 320°C and a split ratio (20:1) mode inlet section (250°C). The column was initially at 60°C held for 5 min and increased at 15°C/ min for 15 min, maintained for 1 min and at 10°C/mi for 4 min held for 2 min. Flavonoids obtained were compared with their standards which were analyzed before the samples. Calibration curves of standard flavonoids analyzed were plotted and good correlation coefficients (r^2) between 0.9992 and 0.9998 were obtained.

Determination of antioxidant activity

The method of Olajire and Azeez (2011) was used for the determination of antioxidant activity. 1, 0.8, 0.6, 0.4 and 0.2% solutions of *S. biafrae* extract and *S.o biafrae*-Cu complex were prepared. 1 ml of each concentration was measured into test tubes and 4 ml of 0.004 M DPPH was added to each tube in a dark room. The blank was prepared by measuring 1 ml of deionized-distilled water in a test tube and 4 ml of DPPH was added. Both blank and samples were allowed to stand for 30 min in dark room before reading at wavelength 517 nm. Percentage Inhibitory for both samples was calculated using this formula:

$$I\,\% = \frac{A_{blank} - A_{sample}}{A_{sample}}$$

Inhibitory concentration at which 50% of the free radicals were scavenged was extrapolated from the graph.

Determination of antifungal activity

The antifungal activity was determined using the poison food technique. Five-day old fungal cultures were punched aseptically with sterile cork borers of 4 mm diameter. The fungal discs were then put on the gelled agar plate. The agar plates were prepared by impregnating them with 100 ppm of ethanolic and aqueous solutions of *S. biafrae* extract and *S. biafrae*-Cu complex at a temperature of 45 to 50°C. The plates were then incubated at temperature of 25°C for fungi growth. The test fungus was then allowed to grow on poisoned plates with ethanolic and aqueous solutions of the extracts. Control tests were carried out simultaneously by putting fungal discs on a gelled agar plates containing 100 ppm of acetic acid as the positive control and 100 ppm of ethanol as the negative control. The effects of *Senecio biafrae* extract and *Senecio biafrae*-Cu on fungal growth were determined by measuring the diameter of the zone of inhibition obtained on poisoned plate and control plates. Percentage inhibition of mycelial growth using the method of Moslem and El-Kholie (2009) was calculated with the formula:

$$\%\ \text{Mycelial inhibition} = \frac{\text{Mycelial growth (control)} - \text{Mycelial growth (treatment)}}{\text{Mycelial growth (control)}}\ X\ 100$$

RESULTS

Flavonoid profile of *S. biafrae* and its Cu complex

Table 1 presents the flavonoid composition in *S. biafrae* extract and *S. biafrae*-Cu complex. In *S. biafrae* extract, the relative abundance of flavonoid compounds followed the trend; Kaempferol > quercetin > isorhamnetin > luteolin > apigenin while other were less than one. In *S. biafrae*-Cu complex, the trend was Kaempferol > isorhamnetin > quercetin > luteolin > apigenin while others were not detected. There were reductions of 56.71% in kaempferol, 83.94% in quercetin, 29.56% in isorhamnetin, 5.90% in luteolin and 45.07% in apigenin concentrations of *S. biafrae*-Cu complex compared with their concentrations in *S. biafrae* extract. This suggests that there was complexation between Cu (II) and the vegetable.

UV-visible spectra characterization of *S. biafrae* and its Cu complex

Figure 1 shows the characteristic peaks of flavonoids in *S. biafrae* extract and *S. biafrae*-Cu complex. In *S. biafrae* extract, absorption maxima (λ_{max}) were observed at 258 nm (Band II) and 375 nm (Band I). Bathochromic shifts in λ_{max} to 277 nm (band II) and 463 nm (band I) were observed in *S. biafrae*-Cu complex.

Vibrational spectra characterization of *S. biafrae* and its Cu complex

The coordination sites and binding properties in *S. biafrae* are presented in Table 2. The stretching vibration of C=O in *S. biafrae* occurred at 1658.05 cm^{-1} which was shifted to 1649.38 cm^{-1} in *S. biafrae*-Cu complex. The shift in O-H was from 3367 cm^{-1} in *S. biafrae* to 3426 cm^{-1} in *S. biafrae*-Cu complex. The bands at 1416.47, 1645 cm^{-1} and 1252.57 cm^{-1} are related to υ(C-OH), υ(C=C) and υ(C-O-C). The presence of υ (Cu-O) was observed at 408.04 cm^{-1} in *S. biafrae*-Cu complex but not in *S. biafrae*.

Antioxidant activity *S. biafrae* and its Cu complex

Figure 2 shows the antioxidant activity of *S. biafrae* extract and *S. biafrae*-Cu complex. *S. biafrae*-Cu

Table 1. Flavonoid composition of *Senecio biafrae* and *Senecio biafrae*-Cu complex.

Flavonoid (mg/100 g)	*Senecio biafrae*	*Senecio biafrae*-Cu complex
Catechin	5.25e-6	ND
Rutin	2.04e-4	ND
Apigenin	3.75	2.06
Luteolin	4.41	4.15
Quercetin	63.31	10.17
Kaempferol	115.22	49.88
Myricetin	6.33e-4	ND
Naringenin	1.53e-3	ND
Epicatechin	2.79e-3	ND
Epicatechin-3-gallate	4.89e-5	ND
Gallocatechin	8.49-4	ND
Isoquercetin	0.21	ND
Kaempferol-3-arabinoside	4.27e-4	ND
Isorhamnetin	17.09	12.05
Orientin	3.82e-4	ND
Isoorientin	0.16	ND
Naringin	5.09e-4	ND
Daidzein	3.91e-4	ND
Morin	1.53e-3	ND
Quercitrin	7.25e-4	ND

ND, Not detected.

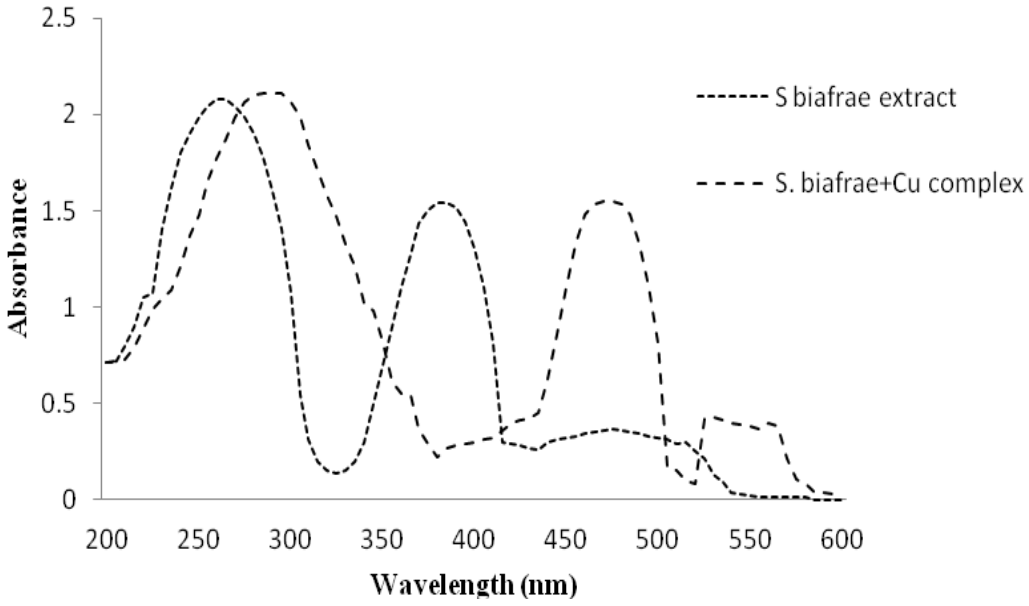

Figure 1. UV-Visible spectra of *Senecio biafrae* and *Senecio biafrae*-Cu extracts.

complex had 89.92% antioxidant activity while *S. biafrae* extract had 64.15%. Antioxidant activity of *S. biafrae*-Cu complex in this study is higher than what Dehghan and Khoshkam (2012) and Pereira et al. (2007) reported for Sn – quercetin and Cu - naringin complexes but lower than what Bukhari et al. (2008) reported Co – quercetin complex. Inhibitory concentration at which 50% of the radicals are scavenged (IC_{50}) for *S. biafrae*-Cu complex is

Table 2. Vibrational spectra bands (cm^{-1}) of *Senecio biafrae* and *Senecio biafrae*-Cu complex.

Bands	*S. biafrae*	*S. biafrae*-Cu
υ (O-H)	3367.00 s	3426.00 s
υ (C=O)	1658.05 s	1649.38 s
υ (C-H)	2947.66 s	
υ (C-OH)	1416.47 m	1402 m
υ (C-O)	1028.48 s	
υ (C=C)	1645.00 s	1631.00 s
υ (C-O-C)	1252.57 s	1212.1 m
υ (Cu-O)		408.04 s

s, Strong; m, medium.

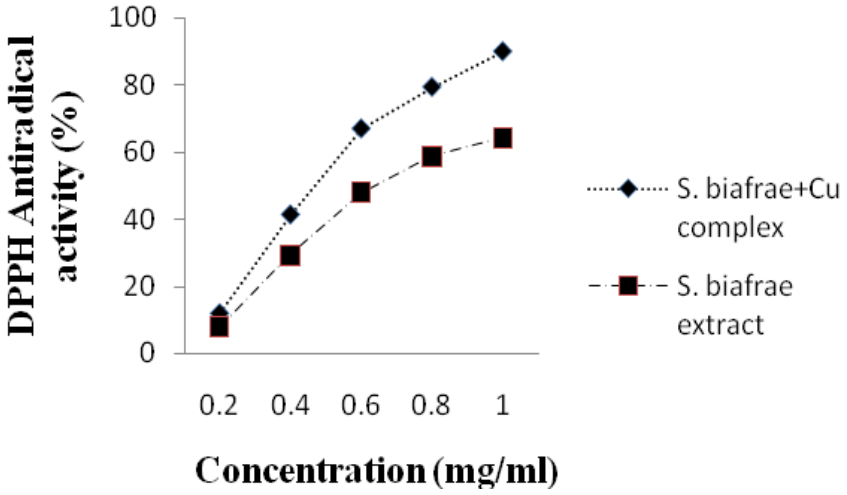

Figure 2. Antiradical efficiency of *S. biafrae* and *S. biafrae*-Cu extracts.

Table 3. Antifungal activity (% mycelia inhibition) of *S. biafrae* and *S. biafrae*-Cu complex.

S/N	Organism	Aq. extract of *S. biafrae*	Aq. extract of *S. biafrae*-Cu	Eth. extract of *S. biafrae*	Eth. extract of .*S biafrae*-Cu
1	*Fusarium oxysporium*	14.3	28.6	62.9	57.1
2	*Aspergillus flavus*	50.0	45.0	55	62.3
3	*Fusarium solani*	17.7	20.6	32.4	35.3

Aq., Aqueous; Eth., ethanolic.

0.45 mg/ml and for *S. biafrae* extract is 0.68 mg/ml.

Antifungal activity of *S. biafrae* and its Cu complex

Table 3 presents the results of antifungal activity of both aqueous and ethanolic *S. biafrae* extract and *S. biafrae*-Cu complex against *Fusarium oxsporium*, *Aspergillus flavus* and *Fusarium solani.* These results are represented as percentage mycelia inhibition. Aqueous *S. biafrae*-Cu complex had better antifungal activity against *F. oxysporium* and *F. solani* than aqueous *S. biafrae* extract while reverse was obtained for *A. flavus*. Ethanolic *S. biafrae*-Cu complex had higher antifungal activity against *A. flavus* and *F. solani* than ethanolic *S. biafrae* extract while otherwise was obtained for *F. oxysporium*. Very high antifungal activity was observed against *F. oxxysporium* and *A. flavus* with ethanolic

extract while average activity was observed against *A. flavus* with aqueous extract. Percentage mycelial inhibition was highest against *F. oxysporium* followed by *A. flavus* and *F. solani*.

DISCUSSION

Vegetables are one of the dietary sources of flavonoids (Erlund, 2004; Olajire and Azeez, 2011). Flavonoids have anticancer, antifungal anti-inflammatory and contribute significantly to antioxidant activities of vegetables (Tapas et al., 2008; Ghasemnezhad et al., 2011). Flavonol; a class in flavonoids dominated the abundance of flavonoid contents in both *S. biafrae* and *S. biafrae*-Cu complex with kaempferol and quercetin. Therefore, it could be inferred that the activity of flavonoids in both the extract and complex was dictated by flavonols. The contents of quercetin and kaempferol show that both *S. biafrae* and its complex have anti-inflammatory, antibacterial, antioxidant and are effective metal chelators (Malesev and Kuntic, 2007; Pereira et al., 2007; Bukhari et al., 2008; Panhwar et al., 2010; Dehghan and Khoshkam, 2012).

UV-Visible spectroscopic method was used to characterize the *S. biafrae* and its complex because each coloured sample has a characteristic absorption. In metal – flavonoid complexes, bathochromic shifts are usually observed in UV-Visible spectra because the complexes are often coloured (Malesev and Kuntic, 2007). The bathochromic shifts observed in the λ_{max} of the brownish - green *S. biafrae*-Cu complex confirm its formation (Bukhari et al., 2008; Dehghan and Khoshkam, 2012). These bathochromic shifts were caused by increased conjugative effects of *Senecio biafrae*-Cu complex (Malesev and Kuntic, 2007). Band I absorption in *S. biafrae* extract is characteristic of flavonol absorption (352 to 385 nm) suggesting that flavonols dominated the flavonoid compounds in the extract (Malesev and Kuntic, 2007).

To further confirm the formation of complex, functional groups used for binding were analyzed using Infra-Red spectroscopic method. The shifts observed in the positions of C=O, OH, υ(C-OH), υ(C=C) and υ(C-O-C) were due to complexation. These shifts suggest there was complexation in *S. biafrae* involving the carbonyl oxygen and hydroxyl group of the flavonoids in the vegetable (Bukhari et al., 2008; Panhwar et al., 2010).

The presence of υ (Cu-O) in *Senecio biafrae*-Cu complex indicates the formation of metal complex whereas it was absent in *S. biafrae* (Panhwar et al., 2010; Dehghan and Khoshkam, 2012).

Antioxidant activity of *S. biafrae* and its complex was determined in terms of their electron donating abilities using DPPH method. This is a suitable parameter to establish free radical scavenging and health status of these samples (Stoilova et al., 2007; Bukhari et al., 2008; Edziri et al., 2011). Higher antioxidant activity and lower IC_{50} of *Senecio biafrae*-Cu complex compared to *S. biafrae* values shows that it possesses higher potency against free radicals than *S. biafrae* because the lower the IC_{50} the more potent the extract is (Olajire and Azeez, 2011). Higher antioxidant activity of the *Senecio biafrae*-Cu complex suggests that Cu (II) has significantly modified the chemical composition of the flavonoids in the vegetables (Bukhari et al., 2008). This could also be due to the decrease in oxidation potential of flavonoid present in the vegetable by Cu (II) complexation thus, making it readily oxidized, more reactive and effective than free *S. biafrae* (Bukhari et al., 2008; Panhwar et al., 2010). This complex possessed better antioxidant activity that Sn – quercetin and Cu – naringin complexes (Dehghan and Khoshkam 2012; Pereira et al., 2007).

Metal complexes have been reported to possess higher antifungal activity than free ligand such as flavonoids and are used to treat infectious diseases (Al-Amiery et al., 2012). Higher antifungal activity of *Senecio biafrae*-Cu complex against pathogenic *F. oxsporium*, *A. flavus* and *F. solani* which have been implicated in the production of toxins could be connected with antifungal agent such as Kaempferol and quercetin present in it (Cho et al., 2008; Gupta et al., 2009; Dahham et al., 2010). The results of different solvent activities affecting the antifungal potency of extract as obtained in the result of this study is in agreement with results of Dahham et al. (2010) who reported that methanolic extract of *Punica granatum* L. was more potent than aqueous extract of the same plant. This shows that ethanolic extract is better utilized as fungicide than aqueous extract. Since fungi cause diseases and the synthetic fungicides use for their control cause environmental and health hazards (Kanwal et al., 2010) ethanolic extract of *S. biafrae*-Cu complex could be employed to kill off fungi.

Conclusion

This study has reported differences in UV-Visible and Infra-Red spectra of *S. biafrae* and its complex. Shifts were observed in the λ_{max} and vibrational frequencies of *S. biafrae* compared with *S. biafrae*-Cu complex. *S. biafrae*-Cu complex had better potency against free radicals and pathogenic *F. oxsporium*, *A. flavus* and *F. solani* than *S. biafrae*.

Conflict of Interest

The authors have not declared any conflict of interest.

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