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Inhibitory effect of *Irvingia* fruit waste extracts on some postharvest spoilage fungi

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ABSTRACT

The importance of fungi in plant food production and storage calls for urgent intervention on agro-allied practitioners. Although applied chemical pesticides are one of the effective and fast means for controlling fungal postharvest diseases, their continual use is increasing becoming worrisome. Attention, in recent times has been directed at exploring the potentials of botanicals (plant extracts) as alternatives to synthetic chemicals. Hence in this research the inhibitory effect of *Irvingia* fruit waste extract on three postharvest spoilage fungi (*Aspergillus, Fusarium* and *Mucor* species) was studied. Results showed that although ketoconazole (a fungicide) at 5mg/ml significantly ($P \leq 0.05$) inhibited all test fungi (mean percentage growth inhibition, 95.26%) more than *Irvingia* fruit waste extract, the latter at 0.4% and 1% concentrations also substantially inhibited all three test fungi (mean percentage growth inhibition being 53.26% and 72.45%, respectively). Findings further showed that the plant extracts were more active against *Aspergillus* and *Mucor* species compared to *Fusarium* species irrespective of concentration of plant extract. *Irvingia* fruit waste extracts would potentially serve as better alternatives or complementary to synthetic fungicides in the control of postharvest spoilage fungi.

Keywords Ketoconazole, *Irvingia gabonensis*, *Aspergillus, Fusarium, Mucor* species, fungicide, Postharvest spoilage fungi

1. INTRODUCTION

The significance of plant health and productivity has continually occupied a center stage in agricultural research; partly because of the ever increasing human population which has significantly heightened the demand for increase in food productivity, including those from plant source (Agrios, 2005; Ravan *et al,* 2005). Plant health and productivity are influenced by several factors, some of which include climate, genetic composition of the plant, soil composition and infection of plants by pathogens (Agrios, 2005; Ravan *et al,* 2005). Plant pathogens include bacteria, fungi, nematodes and viruses amongst others. Among these groups of pathogens, fungi accounts for most plant diseases. The importance of fungi in plant pathology and plant produce spoilage has placed a huge demand on Agro-allied practitioners for urgent intervention strategies (Duncan, 1999; Gurr *et al.*, 2011; Pennisi, 2010; Skamnioti and Gurr, 2009).

In particular, fungi are responsible for about 70% of the major crop diseases, resulting to huge economic losses year (Agrios, 2005). These plant pathogens cause extensive disease to seeds, seedlings, mature plants and aging plants, resulting in decreased growth and reproduction of plants. Furthermore, fungi are attributed to be one of the major causes of postharvest losses of agricultural produce. Microorganisms come in contact with naturally occurring foods such as fruits and vegetables whilst growing on the field, during harvest and handling (Bartz *et al.,* 2004; Beresford *et al,* 2006; Perrone *et al*, 2007). Some of such microorganisms persist on plant produce during storage where they cause postharvest spoilage and diseases. Some fungi whilst growing in certain food products produce toxic substances that result in food poisoning in man and animals when ingested (Pelczar et al., 1993; Amadi and Adeniyi, 2009). Species of postharvest spoilage fungi include *Aspergillus, Fusarium, Mucor, Botrytis, Penicillium* etc (Etebu, 2012, Amadi and Adeniyi, 2009; Beresford *et al,* 2006), and measures directed at controlling the growth of these fungi would invariably control postharvest spoilage occasioned by them.

Although applied chemical pesticides are one of the effective and fast means for reducing the loss of postharvest diseases, the use of synthetic chemicals are fast becoming undesirable and less popular because they are usually non-specific in their effects, killing beneficial organisms as well as pathogens. In addition, synthetic chemicals may have undesirable health, safety, and environmental hazards (Manczinger *et al*., 2002). At present, considerable efforts are directed at exploring the potentials of botanicals (plant extracts) as alternatives or

complementary to synthetic chemicals. Botanicals have the advantage of not only being readily available and affordable but are also non-phytotoxic and easily biodegradable (Akuesh *et al,* 2002; Okigbo and Nmeka, 2005; Okigbo and Omodamiro, 2006).

Hence this work attempts to investigate the potential use of postharvest *Irvingia gabonensis* (Aubry-lecomte ex o' Rorke) fruit waste extracts in the control of fungal postharvest diseases by studying its effect on three postharvest spoilage fungi. The findings of this work would potentially provide simple ways to minimize the growth of spoilage fungi on postharvest agricultural produce.

2. MATERIALS AND METHOD

The effect of postharvest *Irvingia* extract on mycelia growth of three test fungi (*Aspergilus* sp. *Fusarium* sp and *Mucor* sp), previously isolated from postharvest kernels of *Irvingia* fruits was studied. Fresh *Irvingia gabonensis* (Aubry-lecomte ex O' Rorke) fruits were harvested from a natural forest located in Amassoma (4°58′N 6°06′E) of Bayelsa State, Nigeria. The freshly edible part of the *Irvingia* pericarp was peeled, and pounded into paste under aseptic conditions. Sabouraud dextrose agar (SDA) was then prepared according to manufacturer's prescription, and supplemented with the resulting paste of *Irvingia* extract to arrive at 0.4 and 1% of extract respectively in separate Petri dishes.

Also, a third medium SDA was prepared and supplemented with Ketoconazole (a fungicide) to arrive at 0.5% (5mg/ml) of fungicide. Finally, a control medium consisting SDA only with no additional supplement was equally prepared according to manufacturer's prescription.

All four media (three test media and one control) were inoculated in triplicates with a 0.4cm diameter mycelia dish obtain from colony edge of 7day old pure cultures of each of the three test fungi (*Aspergillus, Fusarium* and *Mucor* species), and incubated at room temperature for 5days. The effectiveness of extract was recorded in terms of mycelia diameter and percentage growth inhibition. Percentage growth inhibition was calculated according to the formula adopted with slight modification from Whipps (1987)

$$\text{Percentage Growth Inhibition} = \frac{\text{DC - DS}}{\text{DC}} \times 100\%$$

Where, DC = Average diameter of fungal colony on control medium (SDA only); DS = Average diameter of fungal colony with SDA supplemented with *Irvingia* extracts or fungicide (ketoconazole).

All data were transformed according to Gomez and Gomez (1984) where appropriate and subjected to ANOVA using SPSS version 16.0 statistical software. Mean of transformed data were de-transformed (weighted), and Comparisons were made between the different media with respect to fungal mycelia growth and percentage growth inhibition.

3. RESULTS AND DISCUSSIONS

The different supplements integrated into Sabouraud dextrose agar (SDA) was observed to differentially influence the growth of the test spoilage fungi. SDA (control media) without any supplement significantly supported the growth of all three test fungi (*Aspergillus, Fusarium* and *Mucor* species) more than other SDA media wherein either *Irvingia* extract or fungicide was added (Fig. 1). After 5 days of incubation at ambient room temperature, the overall average colony diameter of test fungi on the control medium was 7.54cm (Fig. 1). The average colony diameter of the spoilage fungi on SDA amended with ketoconazole (5mg/ml) was only 0.54cm. This was significantly ($P \leq 0.05$) lower than the average colony diameter of the same test fungi on SDA separately amended with 0.4% (2.22cm) and 1% (3.58cm) of *Irvingia* fruit waste extract (Fig. 1).

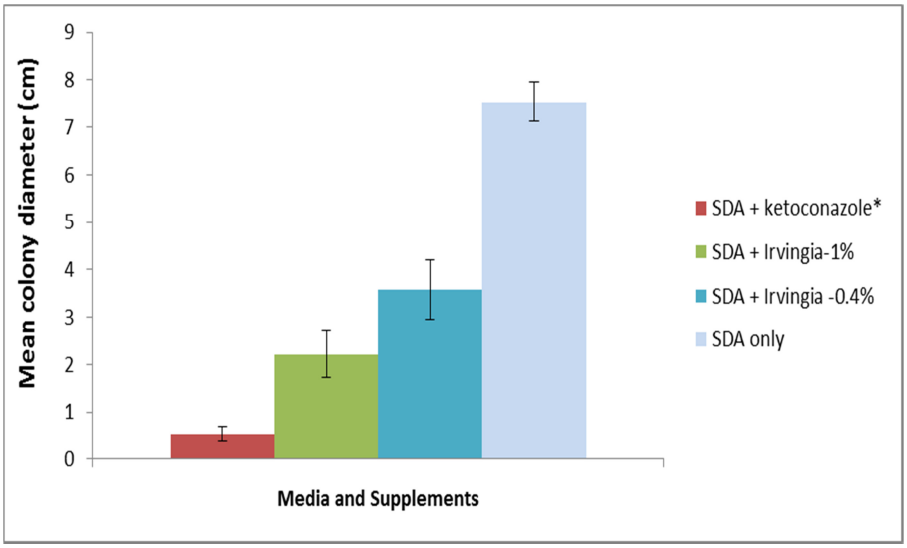

Fig. 1: Overall effect of *Irvingia* fruit waste extracts and ketoconazole (fungicide) on postharvest spoilage fungi

*Concentration of ketoconazole (fungicide) was 5mg/ml

Sabouraud dextrose agar (SDA), without any supplement has often been used as the standard medium for the primary isolation of fungi and is still widely used (Brun, *et al.* 2001; Sandven and Lassen, 1999; Silva, *et al.,* 2004; Scognamiglio, *et al.*, 2010). In this work, SDA alone with no additional supplement was used as control medium of growth, and all three test spoilage fungi grew luxuriantly in it. In particular, average colony diameter of *Aspergillus, Fusarium* and *Mucor* species on SDA (control medium) after 5 days of incubation at room temperature were 6.28cm, 7.67cm and 8.67cm, respectively (Fig. 2), being significantly greater in comparison to colony diameter on SDA supplemented with either Ketoconazole or *Irvingia* fruit waste extract (Fig. 2). The fungi used in this work are well known postharvest spoilage organisms; known to cause extensive damage in fruits and vegetables (Etebu, 2012, 2013; Sweeney and Dobson 1998; Kabak *et al* 2006). Suffice to reiterate that all the postharvest spoilage test fungi used in this work were isolated from commercial *Irvingia* kernels bought from a local market. An earlier work by Etebu and Bawo (2012) had shown that members of these genera of fungi are common spoilage of commercial *Irvingia* kernels sold in different markets of Bayelsa State in Nigeria.

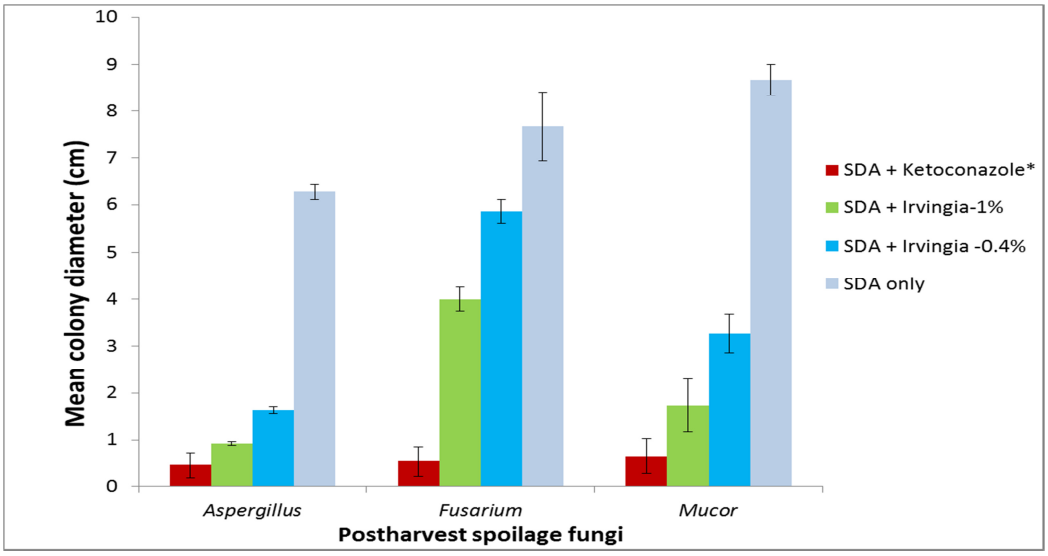

Fig. 2: Comparative growth response of various postharvest spoilage fungi to Sabouraud dextrose agar separately supplemented with *Irvingia* fruit waste extracts or ketoconazole (fungicide)

*Concentration of ketoconazole (fungicide) was 5mg/ml

Table 1: Comparative effect of *Irvingia* fruit waste extracts and ketoconazole (fungicide) on inhibition of various postharvest spoilage fungi

	Mean Arcsine transformed % Inhibition				Mean De-transformed % Inhibition			
Extract	*Aspergillus*	*Fusarium*	*Mucor*	Mean	*Aspergillus*	*Fusarium*	*Mucor*	Mean
Irvingia-0.4%	59.35	29.04	52.21	**46.87**[a]	74.01	23.56	62.45	**53.26**
Irvingia -1%	67.33	43.76	63.94	**58.34**[b]	85.14	47.84	80.7	**72.45**
Ketoconozole	77.50	77.65	77.11	**77.42**[c]	95.32	95.43	95.02	**95.26**
Mean	**68.06**	**50.15**	**64.42**	**60.88**	**86.04**	**58.94**	**81.357**	**76.32**

Although the test fungi used in this work have been implicated with postharvest spoilage of *Irvingia* fruits (Etebu, 2012, 2013), it is noteworthy to emphasize that these earlier works succinctly showed that *Irvingia* fruits had no pathological symptoms when the fruits were still green and unripe (Etebu, 2012). The implication of this feat is that fungi could not colonize the fruit at that stage or could not grow since disease severity of postharvest *Irvingia* fruits have been shown to be directly proportional to fungal population responsible for disease (Etebu, 2013).

Some species of the genera of test fungi used in this work, particularly *Aspergillus* and *Fusarium*, have been reported to produce secondary metabolites generally termed mycotoxins, and these metabolites are toxic to humans and animals who ingest food contaminated by them (Amadi and Adeniyi, 2009; Jestoi, 2008). *Irvingia* kernels are widely used in Nigeria and other African countries, majorly for soup thickening purposes (Matos *et al*., 2009). Although, there is yet no report of illness arising from consumption of *Irvingia* kernels, mycotoxins have been shown to occur among *Irvingia* kernels whilst in storage (Adebayo-Tayo *et al.,* 2006), making their consumption a potential risk. It is therefore very pertinent to seek ways to control the contamination of these fungi.

Interestingly, findings from this work showed that all three media supplements inhibited the three test fungi in varying degrees (Table 1). On the overall ketoconazole (fungicide) inhibited the growth of fungi as much as by 95.26%. Its percentage growth inhibition of test fungi was significantly ($P \leq 0.05$) higher than those grown on SDA supplemented with *Irvingia* extracts. Ketoconazole is a broad-spectrum antifungal drug (Como and Dismukes, 1994; Jones, 1987); a weak dibasic compound, and insoluble in water except in very low pH (Daneshmend and Warnock 1988). This apparent insolubility probably explains why its fungal inhibitory effect in this study was not 100%. This notwithstanding, ketoconazole could still be regarded to have greatly inhibited the growth of all three test fungi. Sangoyomi (2004), relying on Whipps formula to calculate percentage growth inhibition rated antagonists of rot fungi as follows: 0% inhibition (not effective), > 0 - 20% inhibition (slightly effective), > 20 - 50% inhibition (moderately effective), > 50 - <100% inhibition (effective), 100% inhibition (highly effective). Going by this scale, the inhibitory effect of ketoconazole would well be said to be very effective against all three test fungi.

Similarly *Irvingia* fruit waste extract, also significantly inhibited all three test spoilage fungi (Table 1). Specifically, average percentage growth inhibition occasioned by *Irvingia* fruit waste extract at 0.4% and 1% concentrations in SDA were 53.26% and 72.45%, respectively (Table 1). As shown earlier, a substance is considered to be an effective antagonist when it is able to retard or inhibit the growth of a given test fungus by a percentage margin of over 50% (Sangoyomi, 2004). The findings of this work showed that *Irvingia* fruit waste

extract indeed has fungal inhibitory potentials. Studies on the control of plant pathogens have been centered on the application of botanicals (plant extracts). They are considered as better alternatives or complementary to synthetic fungicides because they are not only readily available and affordable but are both non-phytotoxic and easily biodegradable (Akuesh et al, 2002; Okigbo and Nmeka, 2005; Okigbo and Omodamiro, 2006).

Results further showed that percentage inhibition of fungi was dependent on the concentration of *Irvingia* extract supplement. Whilst 0.4% of *Irvingia* fruit waste extract supplement inhibited the growth of the test fungi by 53.26% the overall mean percentage growth inhibition caused by 1% of the same *Irvingia* extract was as much as 72.45%, being significantly ($P \leq 0.05$) higher than the former (Table 1). Besides, although all supplements (fungicide or *Irvingia* extract) in this work inhibited the growth of all test fungi, results showed a significant ($P \leq 0.05$) interaction between supplement and fungi, indicating that the degree of growth inhibitory effect a given supplement had on the test fungi varied from fungus to fungus. A close look at Table 1 further revealed that ketoconazole inhibited all test fungi *Aspergillus, Fusarium* and *Mucor* species at about the same degree 95.32%, 95.43% and 95.02% respectively. This was definitely not the case with the *Irvingia* extracts. The growth inhibitory effect of the *Irvingia* supplement on the test fungi clearly showed a given pattern, *Aspergillus* > *Mucor* >*Fusarium* irrespective of the extract concentration. More specifically, the mean percentage growth inhibitory effect of 0.4% of *Irvingia* supplement on *Aspergillus, Mucor* and *Fusarium* was 74.01%, 62.45% and 23.56, respectively. In the same manner, the mean percentage growth inhibitory effect of 1% of *Irvingia* supplement on *Aspergillus, Mucor* and *Fusarium* was 85.14%, 80.70% and 47.84% respectively (Table 1). This result further substantiates the fact that concentration plays a significant role in growth inhibition of fungi irrespective of fungal type used in this study.

The antimicrobial activity of botanicals has been attributed, in part, to phytochemicals which they possess (Okigbo and Ajalie, 2005; Okwu and Joshia, 2006). Recent studies have shown that extract of the mesocarp of postharvest *Irvingia gabonensis*, which are often thrown away as wastes by locals of the Niger Delta region of Nigeria, contain phytochemicals such as alkaloids, flavonoids, saponins, tannins and glucosides (Etebu, 2012; 2013). More emphatically, *Irvingia* fruit wastes used in this present study were fresh and still green. The pioneering work of Etebu (2012) showed that *Irvingia* fruits, at this stage, possess a relatively high amount of alkaloids and flavonoids which decreased with time after harvest. Alkaloids are a group of phytochemicals occurring in plants, and are intended to defend the plant against herbivore, fungi, bacteria, viruses, and even other competing plants (Ashihara *et al* 2008; Wink, 1988). Although the specific alkaloid(s) present in *Irvingia* fruits are yet unknown, it does appear that the relative abundance of alkaloids in mature, fresh and green *Irvingia* fruits was probably reseponsible for the inhibitory effect of *Irvingia* fruit waste extract on the postharvest spoilage fungi observed in this work. *Irvingia* fruit waste extracts would therefore potentially serve as better alternatives or complementary to synthetic fungicides.

4. CONCLUSION

The urgent need for healthy and environmentally friendly sources of fungicides in the control of fungi implicated in the reduction or spoilage of plant produce cannot be overemphasized Findings from this study showed that extracts of *Irvingia* fruits which are often treated as waste and thrown indiscriminately, substantially inhibited all three postharvest spoilage fungi at considerably low concentrations of 0.4% and 1%, respectively. Consequently, *Irvingia* fruit waste extracts would potentially serve as better alternatives or complementary to synthetic fungicides.

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