

In Vitro Control of Postharvest Spoilage Fungi Affecting Sweet Potato (*Ipomoea batatas*) In Ebonyi State, South-Eastern Nigeria

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Abstract- Postharvest fungal spoilage is one of the major constraints to sweet potato production in Ebonyi State. The study investigated the *in vitro* control of the postharvest spoilage fungi affecting sweet potato in Ebonyi State with single and combined extracts of *Garcinia kola*, *Allium sativum*, *Zingiber officinale* and *Moringa oleifera* via Food Poison/Radial Growth technique. Single extracts were evaluated at 50 and 100 mg/ml concentrations of each plant extract while in the assay of combined plant extract, only 100mg/ml of each plant extract was mixed to provide extract combinations at equal mixing ratio of 2 (1:1) extracts. Extracts of *Zingiber officinale*, *Allium sativum*, *Moringa oleifera* and *Garcinia kola* when used individually at a lower concentration (50mg/ml), significantly inhibited the mycelial growth of all the phytopathogens *in vitro* when compared to the control but gave less than 50% inhibition which ranged from 5.44 - 29.56%. At a higher extract concentration (100mg/ml), the inhibition obtained was significantly ($P= 0.05$) higher than those of the control and lower concentrations but were still less than 50% except for garlic extract effect on *A. niger*. However, the use of two plant extract combinations (100mg/ml concentration each) showed mainly additive and synergistic extract/pathogen interactions that resulted in increased inhibition (above 50%) across most of the pathogens, though with a few antagonistic interactions. Combinations of *Allium sativum* and *Zingiber officinale* and *Allium sativum* and *Moringa oleifera* proved the best treatment combinations with no antagonism and the broadest spectrum antifungal activity.

Index Terms- Ebonyi State, In vitro control, Postharvest Spoilage Fungi, Sweetpotato,

I. INTRODUCTION

Sweet potato and other root and tuber crops are susceptible to fungal spoilage resulting in compromise on food quality and nutrition (deterioration in quality characteristics) (Alum *et al.*, 2019b) and great loss in storage roots, thus plaguing the availability of produce during off-season and causing a waste of farm inputs and scarce resources such as water. It also saps human effort and investments that can reduce farmers' incomes and increase consumers' expenses, thus adversely affecting the people's economic access to crop produce. Microbial spoilage

also compromises food (sweet potato) safety; posing a serious health concern.

Due to the negative economic importance of fungal pathogens, their control on sweet potato is needed. Several postharvest pathogen control methods are used and they include fungicide treatment, gamma irradiation and hydro-warming. However, these methods, though reported to have intermediate impacts in controlling spoilage and enhancing shelf life of sweet potato tubers (Ray and Ravi, 2005), have some drawbacks including not being readily available and affordable to farmers, being unfriendly to the environment, phytotoxic to man and with a great propensity to trigger resistance in the targeted pathogens (Okigbo and Nmeka, 2005). Given the drawbacks associated with the orthodox fungi and rot control approaches, focus has in recent times, shifted toward exploitation of plant extracts as novel fungicides in plant protection (Okigbo and Nmeka, 2005; Okigbo and Omodamiro, 2006). Many botanicals have been extensively researched on and proved to possess antimicrobial properties; hence myriads of reports have been documented stating the uses of plant extracts to control plantdiseases. Some plants tested for antimicrobial properties include *Chromolena odorata*, *Ocimum gratissimum*, *Moringa oleifera* and *Zingiber officinale* (Okigbo and Nmeka, 2005; Okigbo *et al.*, 2009a).

The development and application of potent and drawback-free decay control measures is critical to prevention of microbial spoilage and reduction of food losses to microbial attack. In spite of these recognitions, the control of fungi associated with sweet potato spoilage in Ebonyi State which in turn is notable for sweet potato production in South-eastern Nigeria seems not to have received attention. Moreover, where control has been attempted in other areas, attention has been focused on the use of single plant extracts which antimicrobial activities were always far less potent than those of synthetic chemicals employed as treatment checks. Furthermore, the antimicrobial activity of plant extracts that is observed in in-vitro conditions is quite different from its effect in complex food systems. In most cases antimicrobial activity is decreased due to interactions with food components. This could be a challenge in utilizing plant antimicrobials, as a higher concentration could result in unfavorable changes to the taste and aroma of food (Havelaar *et al.*, 2010). Combinations of antimicrobial plant extracts can lead to additive or synergistic effects on postharvest pathogens. Despite these recognitions, literature in Nigeria still lacks sufficient data on the potency of

combined plant extracts against microbial spoilage pathogens for use in sweet potato preservation. The potential benefits of reducing postharvest losses of sweet potato to mycodeterioration are large. It is critical to alleviation of poverty while reducing pressure on ecosystems, climate and water. It is also a strategy for contributing to food security enhancement and closing the food gap between food available today and food needed in 2050 to adequately feed the planet's projected 9.3 billion people.

The study was therefore aimed at investigating the *in vitro* antimicrobial activity of single and combined extracts of *Zingiber officinale*, *Garcinia kola*, *Allium sativum* and *Moringa oleifera* for the control of fungi responsible for sweet potato spoilage in Ebonyi State.

II. MATERIALS AND METHODS

Plant materials for antimicrobial evaluation

Plant materials used in this study were selected based on previous reports of their antimicrobial activities and include bulbs of *Allium sativum*, rhizomes of *Zingiber officinale*, seeds of *Garcinia kola* (bought from Ndoro local markets in Ikwuano LGA) and leaves of *Moringa oleifera* (collected from Ishiadu, Ibeku, Umuahia North LGA of Abia State). The plant materials were authenticated in the Department of Horticulture, NRCRI, Umudike

Preparation of Plant Extracts

After de-husking bulbs of *A. sativum* and peeling off the skin of *Z. officinale*, Bulbs of *A. sativum*, fruits of *G. kola*, leaves of *Moringa oleifera* and rhizome of *Z. officinale* were washed in clean water, air dried at room temperature for two weeks and 100 g of each weighed out separately and crushed in a blender.

Powdered plant materials were suspended separately in methanol (1 L) in conical flasks, thoroughly shaken, and allowed to stand for 24 hours at room temperature. The contents were first filtered through cheese cloth and then through Whatman filter paper No.4. The filtrates were concentrated in a water bath and stored in sterile beaker in the refrigerator until use. The concentrate (with a semisolid consistency) considered to be the 100% concentrate was diluted down to make up the required concentrations of 50mg/ml and 100mg/ml used for antifungal testing. Dilution was made with sterile distilled water before incorporation into PDA.

Test fungi

Postharvest fungal pathogens of sweet potato isolated in a previous study (Alum *et al.*, 2019a) were used as indicator organisms and include *Aspergillus flavus*, *Penicillium expansum*, *Aspergillus awamori*, *Aspergillus niger*, *Rhizopus oryzae*, *Fusarium solani* and *Botrydiploidae theobromae*.

Treatments evaluated: These included 50mg/ml and 100mg/ml of *Z. officinale* alone, 50mg/ml and 100mg/ml of *M. oleifera* alone, 50mg/ml and 100mg/ml of *G. kola* alone, 50mg/ml and 100mg/ml of *A. sativum* alone, Combination of 100mg/ml of *A. sativum* and 100mg/ml of *M. oleifera*, Combination of 100mg/ml of *A. sativum* and 100mg/ml of *Z. officinale*, Combination of 100mg/ml of *A. sativum* and 100mg/ml of *G. kola*, Combination of 100mg/ml of *M. oleifera* and 100mg/ml of *Z. officinale*, Combination of 100mg/ml of *M. oleifera* and

100mg/ml of *G. kola* and Combination of 100mg/ml of *Z. officinale* and 100mg/ml of *G. kola* including a negative control (sterile distilled water) and a positive control (mancozeb)

Antimicrobial Screening

The antifungal activities of the plant extracts were determined by Food Poison/Radial Growth Techniques described by Hanafey *et al.* (2012) with modifications.

Extracts were evaluated singly and in their combinations. For the purpose of assessing antifungal efficacy of single plant extracts, 50 and 100 mg/ml concentrations of each plant extract were assayed. For the purpose of combined plant extract evaluation, only 100mg/ml of each plant extract was mixed to provide extract combinations at equal mixing ratio of 2 (1:1) extracts.

A four equidistant section was created on each Petri dish by drawing two perpendicular lines at the reverse bottom of the plate, the point of intersection indicating the centre of the plate. An aliquot of 1 ml each of the extracts was separately introduced into the Petri dish containing 9ml PDA, carefully rotated to ensure even distribution of extract and allowed to set. A 4-mm cork borer was used to inoculate a disc of a 7day old pathogen culture on the medium containing extract just at the point of intersection of the two previously drawn lines at the bottom of the Petri dish in three replicates. Negative and positive control experiments were set up with the addition of water and mancozeb (Mancozeb 75% WP) respectively, each seeded in 1ml into 9ml PDA. Inhibitory effect of the extract was expressed as percentage inhibition and calculated using the formula:

$$\% \text{ Mycelial Inhibition} = \frac{(XC - YT) \times 100}{XC}$$

(1)

Where: XC = Average diameter of control

YT = Average diameter of fungal colony with treatment

Extract Pathogen Interactions Rating

Single extracts were rated for their inhibitory effects using the scale:

< 0% inhibition (not effective);

>0-20% inhibition (slightly effective);

>20-50% inhibition (moderately effective);

>50-<100% inhibition (effective);

100% inhibition (highly effective)

For Combined extracts, the synergism ratio for percentage inhibition was based on the Abbott formula as described in Burtram *et al.* (2015):

$$\text{Cexp} = (A + B + \dots + n) - (AB \dots n/100)$$

(2)

Where Cexp = expected efficacy of the mixture,

A and B and ...n = the control levels given by the individual single extracts from A to the last one (n) respectively making up the combination.

The synergy ratio (SR) between the observed (Cobs) and expected (Cexp) efficacies of the mixture was calculated as:

$$\text{SR} = \frac{\text{Cob}}{\text{Cexp}}$$

(3)

An SR >1.5 indicates a synergistic interaction between compounds;

0.5–1.5 indicates an additive interaction between compounds;

<0.5 indicates an antagonistic interaction between compounds.

Data were subjected to Frequencies, Percentages and Analysis of Variance (ANOVA) using SPSS 20.0 Version and significant treatment means separated using Duncan's New Multiple Range Test.

III. RESULTS

The antifungal effects of four plant extracts viz: *Allium sativum*, *Moringa oleifera*, *Zingiber officinale* and *Garcinia kola* were evaluated singly and in their combinations against seven postharvest fungi of sweet potato and compared with the effect of mancozeb.

Antifungal activity of Single Plant Extracts against Postharvest Pathogens of Sweet potato

The antifungal activities of single plant extracts against postharvest pathogens of sweet potato are shown in Figure 1. All the extracts had activity against all the fungi, indicating and as well confirming earlier reports (Ijato, 2011) that all of the extracts possess anti-fungal properties. However, the inhibitory effect was found to vary with extract plant type, extract concentration, pathogen and extract constitution. Higher single plant extract concentrations exhibited stronger inhibition of all the test fungi than their lower concentrated counterparts. No plant extracts when used alone, irrespective of the concentration exhibited percentage mycelia growth inhibition that was up to 50% with the exception of *Allium sativum* effect on *A. niger*.

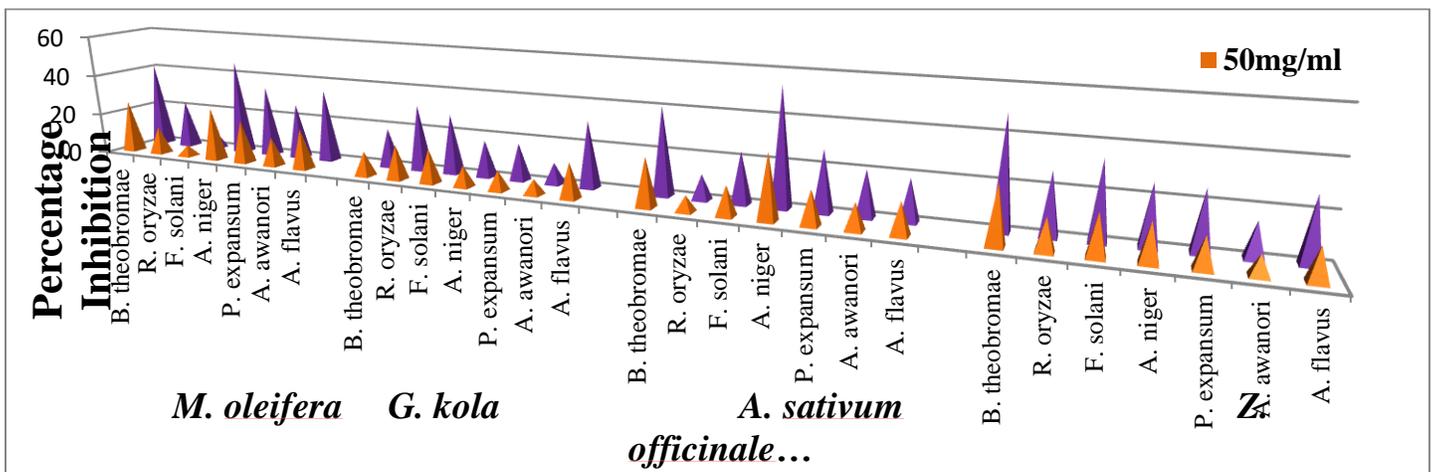


Figure 1: Percentage inhibition of mycelial growth of sweet potato postharvest fungi at different Concentrations of extracts of *Moringa oleifera*, *Garcinia kola*, *Allium sativum* and *Zingiber officinale*.

Comparative Evaluation of Single and Combined Plant Extracts against Postharvest Fungi of Sweet potato

The comparative effect of mancozeb, single and combined plant botanicals against the seven postharvest fungi isolated from symptomatic sweet potato roots from Ebonyi state is presented under this section.

Effect of Mancozeb on Sweet potato Spoilage Fungi

The inhibition percentage exhibited by Mancozeb on the test fungi is presented in figures 2 to 7. As posits in the figures, mancozeb exhibited a very high inhibition (all above 50% against all test fungi) that was significantly higher than those of plant extract and that include 91.41%, 96.01%, 93.88%, 100%, 100%, 100% and 85.91% respectively against *B. theobromae*, *R. oryzae*, *F. solani*, *A. niger*, *P. expansum*, *A. awamori* and *A. flavus*.

Effect of *Moringa oleifera* and *Zingiber officinale* Extract Combination on Sweet Potato Spoilage Fungi

The percentage (%) mycelial growth inhibition of garlic extract against food-associated fungi by poisoned food technique is presented in Figure 2. *Moringa oleifera* at the concentration of 1/10 showed significant but less than 50% inhibition of 41.44%, 22.92%, 8.84%, 46.54%, 35.15%, 34.28% and 26.81% against *B. theobromae*, *R. oryzae*, *F. solani*, *A. niger*, *A. flavus*, *P. expansum* and *A. awamori* respectively while individual use of *Z. officinale* at same concentration also exhibited less than 50% inhibition against all the tested fungi (*B. theobromae*, *R. oryzae*, *F. solani*, *A. niger*, *A. flavus*, *P. expansum* and *A. awamori* with percentage inhibitions of 49.09%, 27.50%, 34.01%, 26.42%, 27.56%, 26.04% and 15.22% respectively. However, when *Moringa oleifera* was combined with *Zingiber officinale*, results obtained showed additive interaction with only two fungi (*B. theobromae* and *P. expansum*) and antagonism against five fungi.

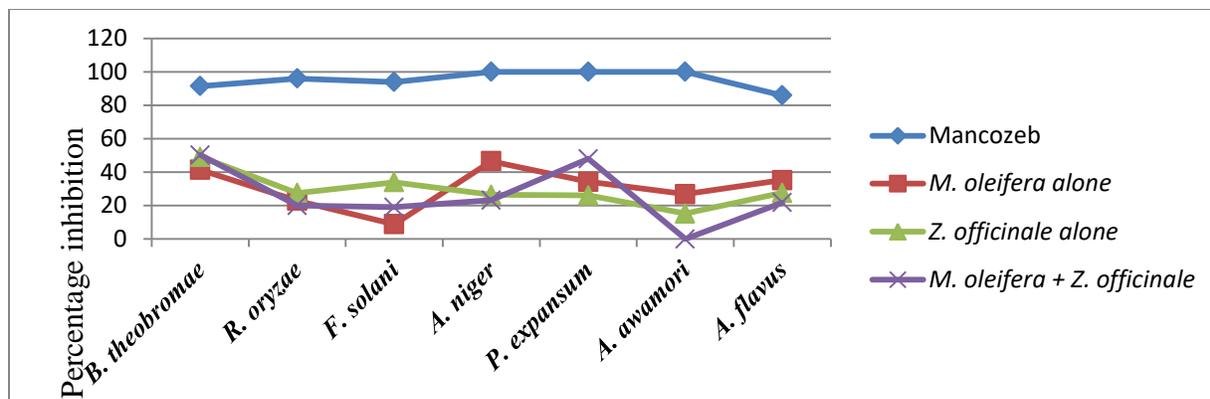


Figure 2: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Moringa oleifera* and *Z. officinale* against postharvest fungi of sweet potato

Effect of *Garcinia kola* and *Moringa oleifera* Extract Combination on Sweet potato Spoilage Fungi

The percentage (%) mycelial growth inhibition of single and combined extracts of *Garcinia kola* and *Moringa oleifera* against food-associated fungi by poisoned food technique is presented in Figure 26. At same concentration, *Garcinia kola* showed percentage inhibitions that were significantly different from the control but less than 50% inhibition and ranged from 10.15% to 31.67%. However, the combination of *Garcinia kola* extract with *Moringa oleifera* extract led to an additive effect (observed value of 47.927% as against expected value of 51.39% with synergy ratio of 0.93 on only one fungi (*Penicillium expansum*) while antagonistic effect was observed in other fungi.

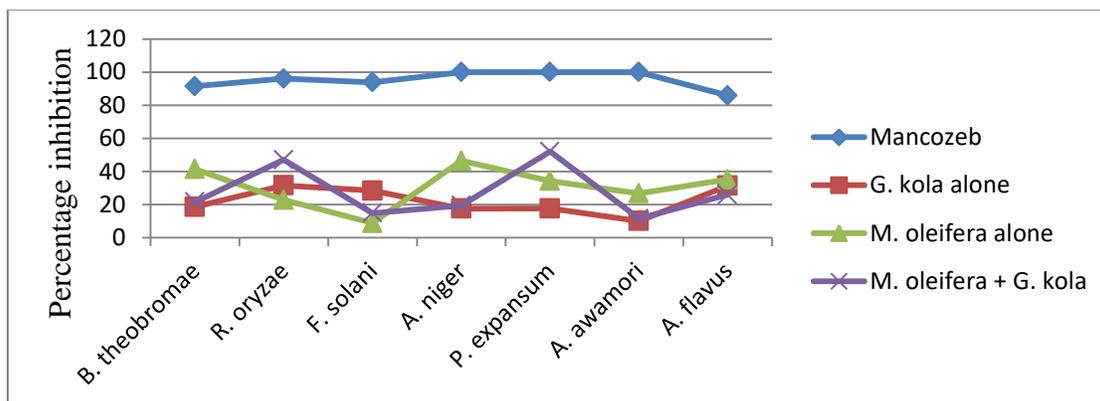


Figure 3: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Garcinia kola* and *Moringa oleifera* against postharvest fungi of sweet potato

Effect of *Moringa oleifera* and *Allium sativum* Extract Combination on Sweet potato Spoilage Fungi

The percentage inhibition of *Moringa oleifera* when used alone at the concentration of 1/10 is given in Section 5.2.3 above. At same concentration, *Allium sativum* showed maximum percentage inhibitions of 54.09% on *A. niger* and others below-50% inhibitions against other fungi (*B. theobromae*, *R. oryzae*, *F. solani*, *A. flavus*, *P. expansum* and *A. awamori*) with respective inhibition of 40.99%, 11.67%, 28.81%, 19.05%, 28.13% and 21.02%) that were significantly different from the control but less than 50% inhibition. When *Moringa oleifera* was combined with *Allium sativum* (Figure 27), a synergistic effect was observed on two fungi (*R. oryzae* and *P. expansum* with observed values of 55.42% and 100% against expected values of 31.91% and 52.77% respectively) and additive effects were observed on the other five tested fungi (*B. theobromae*, *F. solani*, *A. niger*, *A. flavus* and *A. awamori*) with respective observed percentage inhibition values of 75.22%, 36.74%, 83.65%, 52.56% and 60.15% as against respective expected values of 65.45%, 30.55%, 75.46%, 47.54% and 42.21%.

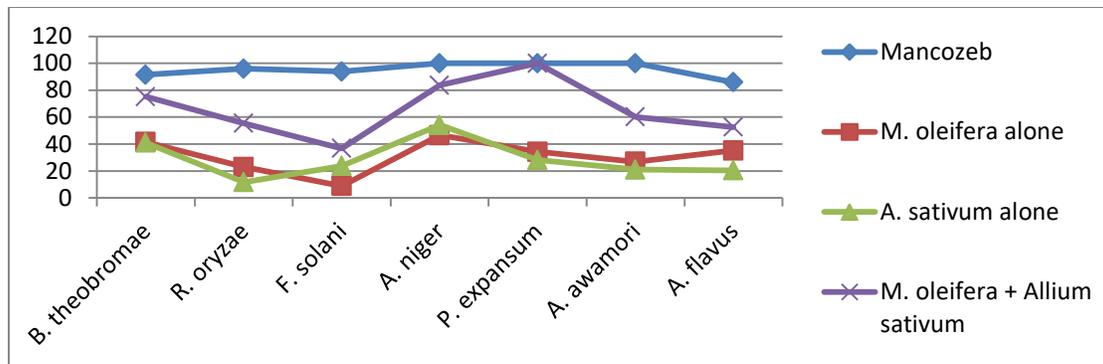


Figure 4: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Moringa oleifera* and *Allium sativum* against postharvest fungi of sweet potato

Effect of *Zingiber officinale* and *Allium sativum* Extract Combination on Sweet potato Spoilage Fungi

Zingiber officinale extract had pathogen dependent inhibition that was significantly different from the control but not above 50% inhibition when used alone at a concentration of 1/10. They included inhibitions of 49.09%, 27.50%, 34.01%, 26.42%, 27.56%, 26.04% and 15.22% respectively against *B. theobromae*, *R. oryzae*, *F. solani*, *A. niger*, *A. flavus*, *P. expansum* and *A. awamori*. As shown in Figure 28, the extract of *Zingiber officinale* exerted a synergistic effect against four fungi viz: , *F. solani*, , *A. flavus*, *P. expansum* and *A. awamori* (with respective observed percentage inhibition values of 88.44%, 76.92%, 69.18% and 78.11% as against respective expected values of 49.72%, 41.36%, 33.06% and 45.84%) and additive effect against the other fungi: *B. theobromae*, *R. oryzae* and *A. niger* (with respective observed percentage inhibition values of 51.25%, 94.17% and 98.74% as against expected values of 35.97%, 71.99% and 66.22%) when it was combined with *Allium sativum*.

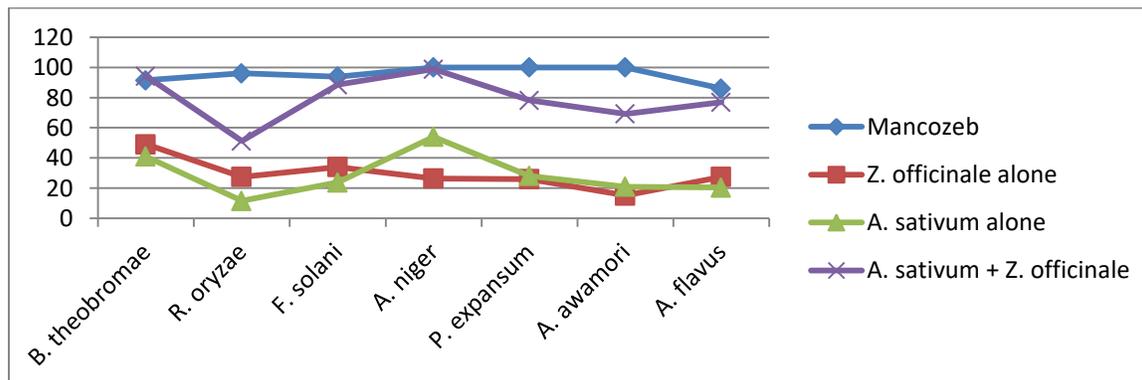


Figure 5: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Zingiber officinale* and *Allium sativum* against postharvest fungi of sweet potato

Effect of *Garcinia kola* and *Allium sativum* Extract Combination on Sweet potato Spoilage Fungi

Garcinia kola showed percentage inhibitions that were statistically significantly different from the control but less than 50% inhibition and includes 18.64%, 31.67%, 28.47%, 17.61%, 31.41%, 17.71% and 10.15% against *B. theobromae*, *R. oryzae*, *F. solani*, *A. niger*, *A. flavus*, *P. expansum* and *A. awamori* respectively. However, the combination of *Garcinia kola* extract with that of *Allium sativum* (Figure 29) led to an antagonistic effect on *R. oryzae* (observed value of 19.17% as against expected value of 39.64%) and an additive effect was observed in the other tested fungi (*B. theobromae*, *F. solani*, *A. niger*, *A. flavus*, *P. expansum* and *A. awamori*) with respective observed percentage inhibition values of 58.55%, 50.34%, 80.50%, 35.77%, 52.09% and 30.43% as against respective expected values of 55.27%, 45.5%, 62.17%, 44.48%, 40.86%, and 29.05%); with the effect on *A. flavus* trending towards antagonism (Synergy ratio = 0.80).

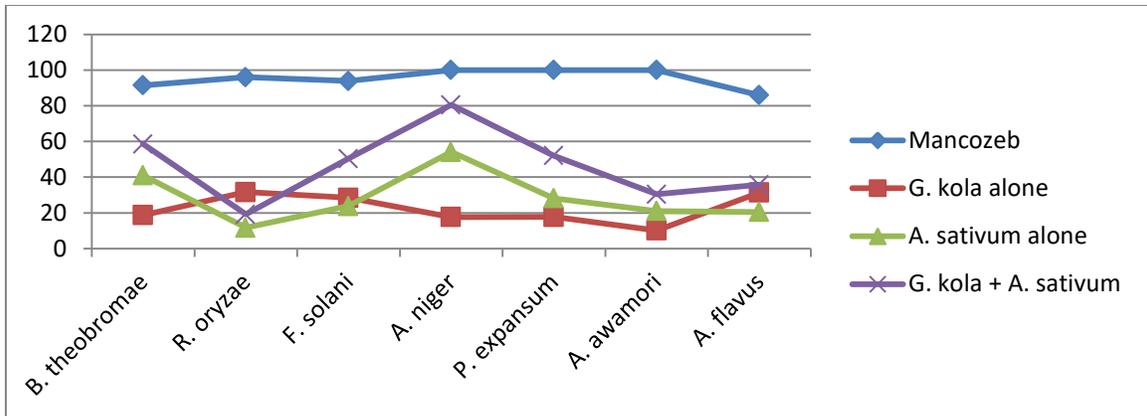


Figure 6: Comparative percentage mycelial growth inhibition of Mancozeb, Single and Combined extracts of *Garcinia kola* and *Allium sativum* against postharvest fungi of sweet potato

Effect of *Garcinia kola* and *Zingiber officinale* extract combination on sweet potato spoilage Fungi

On application as single extracts, neither *Garcinia kola* nor *Zingiber officinale* could exhibit up to 50% mycelia growth inhibition. However, the combination of *Garcinia kola* with *Zingiber officinale* (Figure 30) had a synergistic effect in *R. oryzae* (76.12% observed value against 50.46% expected value) an additive effect in *B. theobromae*, *F. solani*, *A. niger* and *A. flavus* (with respective observed percentage inhibition values of 75.13%, 71.43%, 39.62% and 75.00% as against respective expected values of 58.61%, 52.8%, 39.38%, and 50.30%) and an antagonistic effect in two fungi (*A. awamori* and *P. expansum* with observed percentage inhibitions of 10.87% and 18.75% against expected values of 23.83% and 39.14% respectively).

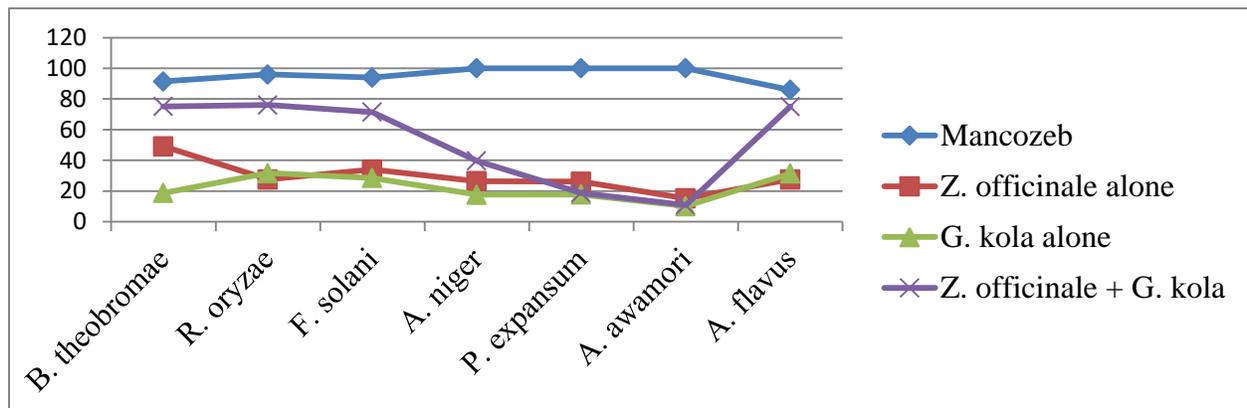


Figure 7: Comparative percentage mycelial growth inhibition of Single and Combined extracts of *Garcinia kola* and *Zingiber officinale* against postharvest fungi of sweet potato

IV. DISCUSSION

The outcomes from the single extract antifungal evaluations made it clear that all the tested plant extracts when used individually had activity against all the tested fungi, indicating and as well confirming earlier reports (Ijato, 2011) that all of the extracts possess anti-fungal properties. Ethanolic extracts of garlic was revealed by results of an investigation by Akinmusire *et al.* (2014) to inhibit the growth of *Aspergillus ustus*; *Aspergillus niger* and *Penicillium* species. The results of the present study also confirmed earlier observations of Tagoe *et al.* (2011), who had reported that ethanol extract of garlic is active against *Aspergillus flavus*, *Aspergillus niger* and *Cladosporium herbarum*. The antimicrobial activity of the Moringa extract was in keeping with the previous studies done by Busani *et al.* (2012) who reported on the antibacterial properties of *M. oleifera* seed and leaf. The inhibitory effects of aqueous extracts of moringa leaf on mycelia growth of *B. theobromae*, *A. niger*, *F. solani* and *R. stolonifer* and their attendant rots on sweetpotato had earlier been reported by Alum *et al.* (2014).

The inhibitory effects of the extracts tested in the present study was shown by results to vary with extract plant type, extract concentration, pathogen and extract constitution. On the basis of pathogen sensitivity, all the tested fungi were more or less sensitive to the four plant extracts, though sensitivity was generally low with the exception of *A. niger*. *R. oryzae* (Figure 1) was most sensitive to *Garcinia kola* and showed least susceptibility towards *A. sativum* at both concentrations. *B. theobromae* was most susceptible to *Z. officinale* and least sensitive to *Garcinia kola* extract. *F. solani* in turn was most and

least susceptible to *Z. officinale* and *M. oleifera* respectively. *A. niger* was the most susceptible of all the tested fungi was most sensitive to *A. sativum* followed by *M. oleifera* and least sensitive to *G. kola*. *A. flavus* was most sensitive to *M. oleifera* and least susceptible to *A. sativum*. *A. awamori* and *P. expansum* was most sensitive to *M. oleifera* and least sensitive to *G. kola*.

With respect to extract concentration, results on the study, using individual extracts alone showed that at a low extract concentration (50mg/ml), percentage inhibition was significantly better than the control but generally low (below 30% i.e inhibition from 5.44 to 29.56%) across the tested fungi. However, increasing the extract concentrations to 100mg/ml caused Significantly higher percentage inhibition against all the test fungi than their lower concentrated counterparts. This finding is in agreement with findings of several researchers (Amionye and Ataga, 2007; Alhussaen *et al.*, 2011). Alhussaen *et al.* (2011) obtained results that indicated that garlic extract had a concentration dependent activity against *Pythium ultimum* isolated from tomato seedlings. Alhussaen *et al.*, (2011) in their study, reported that undiluted garlic extract showed a high control activity with no growth as compared to the biotic control without the extract whereas diluted garlic extracts 10% and 5% reduced the fungal growth to 15.5% and 41% respectively.

Increasing concentrations of these extracts implied an increase in the active ingredients of the solutions which acts on the fungi thereby affecting their physiological processes and consequently lowering the growth of the fungi. That notwithstanding, none, apart from garlic extract against only *A. niger* exhibited percentage mycelia growth inhibition that was up to 50%. This is in keeping with findings of several researchers, for instance, results obtained by Okigbo and Nmeke (2005) in their

investigation of hot water extracts of *Z. officinale* seeds via radial growth technique for antimicrobial activity against *Fusarium* species., *A. flavus* and *A. niger* showed that *Z. officinale* exhibited 33.3%, 31.5%, and 18.2% mycelial growth inhibition respectively. Possible explanations for the generally low percentage inhibition recorded in this study may be due to the fact that the concentrations were low.

However, with the concomitant increases in percentage inhibition attendant with increase in extract concentration observed in this work and corroborated by other works, chances are that further increase in single extract concentration could produce correspondingly increase in percentage inhibition of phytopathogen but that may trigger unfavorable outcome on the organoleptic properties of food produce or worse still trigger emergence of resistance as was the case with overuse of chemical fungicides. From the forgoing and based on a survey report harnessed from some of Ebonyi sweet potato farmers who reported to have in time past used neem leaf extracts on a postharvest context but had to stop as they found the extracts impacting on the organoleptic characteristics of their food, there is need for development of a more potent control agent that will possess high activity at low dose rate. Moreover, the result of the antifungal action of the individually used plant extracts showed that none of the extracts had broad spectrum activity against the test fungi. The outcomes of susceptibility experiment depicted that whereas extract of garlic showed the highest inhibitory effect at both concentrations (29.56 and 54.09%) against *A. niger*, it gave inhibitions as low as 7.28% and 11.67% against *R. oryzae*. *Allium sativum* exhibiting the highest percentage inhibition amongst *Zingiber officinale* and other extracts in the present studies is in keeping with findings by Iram *et al.* (2012) and Skrinjar and Nemet (2009). The antimicrobial activity of garlic is believed to be due to the effect of allicin, the main ingredient in garlic, generated by the phosphopyridoxal enzyme allinase and ajoene. The second highest percentage inhibition (26.12% and 49.09%) was exhibited by *Zingiber officinale* extract against *B. theobromae* but the same plant extract could exhibit only 8.7% and 15.22% inhibitions against *A. awamori*. Similarly, *Moringa oleifera* which exhibited the third highest inhibition percentage (25.79% and 46.54%) shown against *A. niger* barely inhibited (5.44% and 8.84%) *F. solani*. Given the fact that none of the single extracts exhibited broad spectrum antimicrobial activity and considering also the broad spectrum of pathogens involved in the post harvest food spoilage complex; for any alternative control methods to be efficient, such alternative control methods should not be too specific.

From the forgoing, the need to seek for control agents with high biological activity against the fungi at low rate application and a broad spectrum activity became obvious. Based on expositions by Sharom *et al.* (2014) that identifying synergistic combinations of the natural plant compounds could result in control strategies with high biological activity (enhanced antimicrobial activity), low dose rate application and a low risk of pathogen-resistance development and the paucity of information on the activity of combined plant extracts against sweet potato postharvest fungi, the present study comparatively evaluated the plant extracts (*M. oleifera*, *G. kola*, *A. sativum* and *Z. officinale*) in their two by two combinations and mancozeb used as a positive control.

Mancozeb exhibited appreciable inhibition that was above 50% against all the test fungi. The result of the present study agrees with several reports on the antifungal activity of mancozeb. Suleiman and Sule (2016) had earlier reported an inhibition as high as 92.22% against *P. expansum*. Dar *et al.* (2013) in like manner reported after their investigation that that mancozeb proved to be the best for the growth inhibition of *F. solani* and *F. oxysporum*. Chirag (2014) reported 100% inhibition of *F. solani* by mancozeb at different concentrations including 1000, 1500, 2000 and 2500ppm. This study was not able to verify the mechanism of action of mancozeb but according to reports in literature, Mancozeb inhibits enzyme activity in fungi by forming a complex with metal-containing enzymes including those involved in the production of adenosine triphosphate (ATP).

As aforementioned, single extracts of the four plant species exhibited weak percentage inhibition (below 50%) against all the test fungi except for *Allium sativum* extract and *A. niger* interaction. However, the results of evaluation of the two-by-two combination (50mg/ml concentration each) of plant extracts showed three different kinds of interactions viz: synergism, additivity and antagonism; with additive interaction predominating, followed by synergistic interactions, both of which resulted in increased inhibition (above 50%) across majority of the pathogens. The observed synergistic as well as additive interactions provided increased antimicrobial activity using lower concentration when used together while antagonist interaction provided decreased antimicrobial activity. Of the plant extract combinations, *A. sativum* and *Z. officinale* was found most effective followed by *A. sativum* and *M. oleifera*; both extract combinations proving the most potent with broadened spectra of activities and inhibition values slightly comparable to those of Mancozeb. While *Zingiber officinale* and *Allium sativum* exerted a synergistic effect against four fungi viz: *F. solani*, *A. flavus*, *P. expansum* and *A. awamori* and additive effect against the other fungi (*B. theobromae*, *R. oryzae* and *A. niger*), *Moringa oleifera* and *Allium sativum* extract combination gave synergistic effects against two fungi and additive effects on the other five fungi, with no record of antagonism. The cause of increased efficacy is unknown, although possession of the different modes of action by the active phytochemical of the constituent plant botanicals might be a factor resulting to additive and synergistic effects.

On the contrary, *Moringa oleifera* plus *Zingiber officinale* and *Garcinia kola* plus *Moringa oleifera* extract combinations exhibited the poorest antifungal interaction; giving antagonistic interaction against five of the seven test fungi and additive effect but below 50% inhibition on the other two fungi. *Moringa oleifera* and *Allium sativum* gave synergistic effects against two fungi and additive effects on the other five fungi. *Garcinia kola* and *Allium sativum* led to an antagonistic effect on *R. oryzae* and an additive effect was observed in the other tested fungi (in spite of which inhibition against two fungi (*P. expansum* and *A. awamori*) were less than 50%. *Garcinia kola* and *Zingiber officinale* exhibited a synergistic effect in *R. oryzae*, additive effect in *B. theobromae*, *F. solani*, *A. niger* and *A. flavus* and an antagonistic effect in two fungi (*A. awamori* and *P. expansum*). The reason for the reduced fungi sensitivity to the combinations that exhibited antagonism that could be indicative of competitive

inhibition resulting from constituents in the plant extracts competing for the mode of action sites of the active ingredients.

Conclusion

In conclusion, the study showed that none of the single extracts exhibited broad spectrum antimicrobial activity, thus suggesting that where a broad spectrum of pathogens are involved in the post harvest food spoilage complex; none of the plant extracts if used alone can be an efficient alternative to synthetic fungicides. The study as well showed that plants antimicrobial agent's combination can modify the antimicrobial activity and that while some treatment combinations perform better than their individual counterparts when used alone, not all extract combinations are effective despite the potency of their individual components. This thus suggested that empirical formulation and application of plant botanical combinations as protectants without prior verification may not yield good outcomes. The plant extract combinations that exhibited synergism and additivity should be developed to enhance antimicrobial potentiation while antagonistic extract combinations should be avoided due to emerging resistance microorganisms. Combinations of *A. sativum* extracts with either *Z. officinale* or *M. oleifera* extract hold promising prospect for use in the development of novel biofungicide for management of postharvest fungi of sweet potatoes.

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