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RESEARCH ARTICLE

MANAGEMENT OF HOST-PARASITE INTERACTIONS BETWEEN BAMBARA GROUNDNUT AND *Fusarium* WILT INDUCER

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ABSTRACT

Bambara groundnut (*Vigna subterranea* (L.) Verdc) is an excellent source of human and animal foodstuff and pharmaceutical resources but its production is hampered by fungi diseases. Synthetic pesticides, botanicals and bio-control sub-trials were conducted *in vitro* to control *Fusarium oxysporum* f.sp. voandzeia. The sub-trials were each set up using completely randomized design with three replications. Data were collected on radius of the growth and area covered by the colonies. Percentage inhibition of *F. oxysporium* (at 120 hours after inoculation (HAI)) using *Trichoderma* isolates ranged from 16 – 87%. Significantly (P ≤ 0.05) less area of medium was covered by the pathogen due to presence of *T. harzianum* NSBM, *T. harzianum* AIM16, *T. harzianum* AIM5, *T. harzianum* BGMP, *T. virens* BGMZ2, *T. harzianum* ZXMZ isolates and check compared to the control. Percentage inhibition of *F. oxysporium* (at 144 HAI) using Mancozeb and Copper(I)oxide+Metalaxyl ranged from 26 – 100% and both pesticides inhibited *Fusarium* significantly (P ≤ 0.05) more than the control. Percentage inhibition of *F. oxysporum* (at 144 HAI) using Eucalyptus, mahogany and cashew gums ranged from 5 – 100%. Eucalyptus 100% controlled *F. oxysporum* significantly more than all other treatments followed by the check, Eucalyptus 10% and Mahogany 100% (which were at par).

Keywords: biocontrol, pesticides, plant extracts, *Trichoderma***,** underutilized crops

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc. (syn. *Voandzeia subterranea* (L.) Thouars) a member of the plant family Fabaceae is an excellent source of flour, starch, fodder/hay, nuts/seeds and pharmaceutical resources in many communities especially in Africa and Asia (Indonesia, Thailand and Malaysia) although it is currently underutilized (Bamshaiye *et al*. 2011; Isadeha and Time 2018; Centre for Agriculture and Bioscience International (CABI) 2019; Khan *et al*. 2021).

Bambara groundnut was reported to be drought tolerant and relatively disease-free (Doku, 1996; Bamshaiye *et al*. 2011). Although Collinson (1997) pointed out that entire Bambara groundnut experiments were destroyed by diseases thus implying that Bambara groundnut is not as resistant to pests and diseases as previously purported. In fact, Goli *et al*. (1997) affirmed this view by reporting that when IITA Bambara groundnut accessions were screened for resistance to *Cercospora* leaf spot disease only 27% of the accessions were resistant to this disease.

Suleiman and Anyika (2016) further accentuated that fungi diseases cause serious deterioration of Bambara groundnut in humid settings. They said that the percentage

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incidence of fungal infections was between 40 -75% (for four landraces of cream white Bambara groundnut but the lowest incidence was reported on red Bambara groundnut landrace. Gwekwerere (1997) supported the view on the susceptibility of Bambara groundnuts by stating that during a survey in Zimbabwe (in 1989) the incidence of pests and diseases was high on Bambara groundnuts.

Presently, a dearth of knowledge exists on the control of fungi-induced diseases on Bambara groundnut. Suleiman and Anyika (2016) reported that different rates of aqueous leaf extracts of *Alternanthera brasiliana* generally retarded vegetative growth of the fungi isolates. Furthermore, they reported that the highest inhibition of fungi growth by this extract was obtained at a 40% concentration of *A. brasiliana*. Obagwu (2003) reported that when aqueous extracts of garlic either alone, or in combination with Benlate (benomyl 50% a.i., WP) were applied against brown blotch disease of Bambara groundnut (by *Colletotrichum capsici)* under field conditions, they controlled the fungus. Hence, he recommended a half dosage of benomyl (0.3 gL^{-1}) combined with 1% (w/v) garlic extract which was as effective as the full dosage of the chemical fungicide alone and gave complete control of the disease.

Lum *et al*. (2019) with the aid of some plant extracts successfully controlled *Fusarium oxysporum* f.sp. elaiedis; the causal agent of oil palm wilt *in vitro*. While Ndifon *et al*. (2015) with aid of numerous plant extracts (including eucalyptus and mahogany) successfully controlled *Fusarium oxysporum* f. sp. melongenae; the causal agent of African garden eggplant wilt. These related studies could be advanced for the control of *Fusarium* wilt of Bambara groundnut considering the importance of this wilt disease on Bambara groundnut in places like Kenya and even Nigeria.

Indeed, the health status of Bambara groundnuts in Africa has not been resolved. It has been reported that Bambara groundnut suffers from infections caused by all manner of pests and pathogens (fungi, viruses, nematodes, bruchid insects, etc.). Kola (2005) reported that dark-coloured Bambara groundnut seeds (Sb4-4A and Sb8-1) with the exception of one dark-coloured cultivar (Swazi Vs A), were less infected by fungi especially *Aspergillus, Fusarium* and *Chaetomium* spp.; compared to the light-coloured accessions (i.e. As7 and Sbas1-8).

Isadeha and Time (2018) reported that the Bambara groundnut is cultivated all over Nigeria, with the exception of the swampy areas. Nafula *et al*. (2021) observed that Bambara groundnut yield was low mainly because of biotic stresses. Moreover, they affirmed that *Fusarium oxysporum* Schlecht f.sp. voandzeia was one of the most destructive fungi associated with Bambara groundnut in Kenya. The study showed that disease incidence (76 - 79%), disease severity (43 - 46%) and area under disease progress curves varied contingent on the Bambara groundnut landrace cultivated.

Nigeria is the largest global producer of Bambara groundnut well ahead of other global leading producers mostly found in West Africa (Khan *et al*. 2021). Due to the high demand for Bambara groundnut, its cultivation has expanded from the drier to the humid parts of Nigeria that are often disposed to disease outbreaks due to the higher relative humidity in these areas. It was pointed out that these infections may result in low percentage germination of seeds, low seedling vigor, reduction of growth and yield; increased seed and seedling diseases (Singh 2011); a reduction of carbohydrate, protein and oil content of seeds (Bhattacharya and Raha 2002); and contamination of grains when mycotoxins are produced (Barros 2011).

This portends a situation whereby the cultivation of this crop may be completely hampered by pests and diseases resulting in the disappearance of the many dishes that are relished internationally. The erstwhile noteworthy disappearance of groundnut pyramids in Nigeria has been widely documented as being the outcome of the aphid -borne virus disease epidemic in the country and Bambara groundnut may follow likewise.

Based on the economic and socio-economic importance of the Bambara groundnut to Africa and Nigeria in particular, it was deemed necessary to carry out this study with the objective of accessing the management of *Fusarium* pathogens isolated from Bambara groundnuts in south-eastern Nigeria. This study may help one to accept or reject the hypothesis that Bambara groundnut seed infections can be controlled with mostly locally sourced agents.

MATERIALS AND METHODS

This research was carried out at the Faculty of Agriculture Laboratories in Alex Ekwueme Federal University Ndufu-Alike in Ebonyi State. The University is located in Ndufu-Alike (6.069^0N by 8.199^0E) about 21 km from Abakaliki, the State capital. Ebonyi State is in the derived savanna zone of Nigeria with a humid tropical climate. The State inhabitants practice cultivation of many crops among which is the Bambara groundnut.

Isolation and identification of the fungi associated with Bambara groundnut seeds

The Bambara groundnut seeds (lines 1-7) assessed in this trial were obtained from a breeder at the University of Nigeria, Nsukka, Enugu State. Other seeds utilized were obtained from local farmers in Ebonyi State, owing to the non-availability of improved varieties. The fungi were isolated using potato dextrose agar (PDA) medium which was autoclaved at 120ºC and 15 psi for 15 minutes according to the manufacturer's instructions and incubated at room temperature.

The isolated fungi were sub-cultured to obtain pure cultures which were used to identify the fungi with the aid of literature on fungi morphology and identification keys (Barnett and Hunter 1972). The fungi isolates were stored in the freezer for use later in determining their pathogenicity and possible management techniques.

Sub-trial 1: Biocontrol of *F. oxysporium* with *Trichoderma* isolates

The *F. oxysporum* f.sp. voandzeia isolate utilized in this sub-trial was obtained from the Bambara groundnut seeds (line 5) from Nsukka. While the *Trichoderma* isolates were obtained from the mushrooms, crops (Bambara groundnut, guava, maize and beans) and farmland soils from West Cameroon and southeastern Nigeria. The method of isolation of these fungi is discussed above. The experiment was laid out in Petri dishes using a completely randomized design and each treatment was replicated three times. The treatment set consisted of seven *Trichoderma* isolates (six *Trichoderma harzianum* and one *Trichoderma virens* isolates) and a chemical pesticide check. The check was inoculated with the *Fusarium* isolate alone. The agar medium was inoculated with a 2-mm disc of the pathogen or biological control agent placed at the edge of the plate.

Sub-trial 2: Effects of synthetic pesticides on *F. oxysporum* associated with Bambara groundnut

The experiment was carried out using Petri dishes. It was laid out in the laboratory using a completely randomized design (CRD) with 5 treatments and eacb treatment was replicated three times. The treatment set included control, Mancozeb 100%, Mancozeb 50%, C.O.M. (i.e. Tandem™) 100% and C.O.M. 50%. Mancozeb (with a.i mancozeb WP recommended at 2 000 g/ha, it is a commercial contact fungicide) was utilized at the rate of 5 g/1 000 ml distilled water for 100% concentration, while C.O.M. (with a.i wettable powder of copper (I) oxide (60%) + Metalaxyl (12%) recommended at 800 g/ha, it is a systemic fungicide) was utilized at rate of 4 g/1 000 ml distilled water for 100% concentration. Each treatment consisted of three levels (0.0, 50 and 100%).

Sub-trial 3: Effects of plant extracts on *F. oxysporum* f.sp. voandzeia with Bambara groundnut

The experiment was carried out using Petri dishes. It was laid out in the laboratory using a completely randomized design (CRD) with 8 treatments and each treatment was replicated three times. The treatment set included control, synthetic chemical check (Mancozeb a.i at the rate of 5 g/1 000 ml distilled water for 100% concentration), eucalyptus

(*Eucalyptus globulous*), mahogany (*Kharya senegalensis*) and cashew (*Anacardium officinale*) resins/gums (3 g resins/45 ml agar for 100% concentration). There was no special preparation for the gums except for using boiling water to ensure the dissolution of the gums. Each treatment consisted of three levels (0.0, 50 and 100%).

Data collection

The radius of the fungus colony was measured using a transparent ruler at 24 hours intervals starting from day 1 through day 8. The measurement of the area covered by the colony was recorded using the disk improvised from a plastic Petri dish with eight equal sectors obtained by ruling lines through its center with a fine tip permanent marker pen. The percentage inhibition of the pathogen was calculated using equation 1.

$$PI = ((C - T)/C) \times 100\% \qquad \text{...... Eqn 1}$$

Where,

PI= Percent inhibition of growth of the fungus

C= Perpendicular radius of fungus colony in control plate

T= Perpendicular radius of the fungus colony in treated plate

Data analysis

The percentage data collected were arc-sine transformed. The data were subjected to analysis of variance (ANOVA) and the means were separated using Student Newmann Keul's (SNK) test (as obtainable with Genstat® statistical package; Discovery 2nd Edition). Descriptive statistics were used to illustrate the trends in the growth of the pathogen and its management.

RESULTS AND DISCUSSION

Sub-trial 1: Biocontrol of *F. oxysporium* with *Trichoderma* isolates

The percentage inhibition of *F. oxysporium* using *Trichoderma* isolates is presented in Fig. 1. It was observed that the amount of control of *F. oxysporum* f.sp. voandzeia by all isolates of *Trichoderma* except for *T. harzianum* AIM12B was comparable with that produced by the synthetic check (i.e mancozeb 100%). Generally, the percentage inhibition ranged from 27 – 87% but when *T. harzianum* AIM 12B was included the inhibition ranged from 16 – 87%.

The 120 hours after inoculation data (HAI) on bio-control of this pathogen were subjected to analysis of variance and the means were separated using SNK ($P \leq 0.05$). It was observed that the synthetic check produced significantly the highest control of *F. oxysporum* f.sp. voandzeia followed by isolate

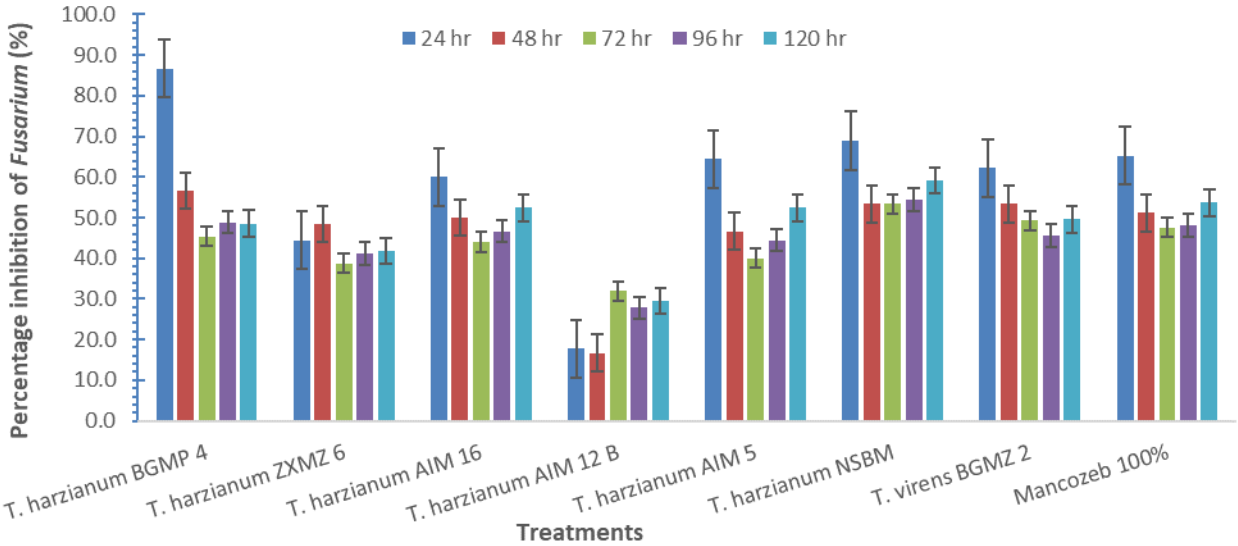

Figure 1: Percentage inhibition of growth of *F. oxysporum* using *Trichoderma* isolates in south eastern Nigeria

T. harzianum NSBM. However isolate *T. harzianum* NSBM was at par with isolates *T. harzianum* AIM16, *T. harzianum* AIM5, *T. harzianum* BGMP and *T. virens* BGMZ2. Isolate *T. virens* BGMZ2 was significantly at par with isolate *T. harzianum* ZXMZ. In terms of control of *Fusarium* sp., the least statistically effective isolate was *T. harzianum* AIM 12B which was significantly different from all other isolates and check but significantly inhibited *F. oxysporum* f.sp. voandzeia more than the control.

Analysis of variance and means separation using SNK ($P \leq 0.05$) (based on 120 HAI data) was carried out for the area covered by the colony of *F. oxysporum* f.sp. voandzeia under the influence of *Trichoderma* isolates or check. It revealed that significantly less area of the medium was covered by the pathogen due to the presence of *T. harzianum* NSBM, *T. harzianum* AIM16, *T. harzianum* AIM5, *T. harzianum* BGMP, *T. virens* BGMZ2, *T. harzianum* ZXMZ and *check* (which were significantly different from isolate *T. harzianum* AIM *12B* and control).

Sub-trial 2: Effects of synthetic pesticides on *F. oxysporum* associated with Bambara groundnut

The percentage inhibition of *F. oxysporum* (based on 144 HAI data) using synthetic pesticides is presented in Fig. 2. It was observed that the amount of inhibition of *F. oxysporium* f.sp. voandzeia by all the synthetic pesticides ranged from 26 – 100%. The 144 hours of data on chemical control of this pathogen was subjected to analysis of variance and the means were separated using SNK ($P \leq 0.05$). It was observed that C.O.M. 100% controlled *F. oxysporium* f.sp. voandzeia significantly more than all the other treatments followed by Mancozeb 100%. Mancozeb 100% controlled the pathogen significantly more than C.O.M. 50% and Mancozeb 50% (which were at par). However all chemical treatments controlled *Fusarium* significantly more than the control.

Sub-trial 3: Effects of plant extracts on *F. oxysporum* f. sp. voandzeia with Bambara groundnut

The percentage inhibition of *F. oxysporum* f.sp. voandzeia (based on 144 hours after inoculation data) using poison food technique with plant extracts is presented in Figure 3. It was observed that the amount of inhibition of *F. oxysporum* by all the botanical pesticides ranged from 5 – 100%. The best plant extract based on percentage inhibition of pathogen

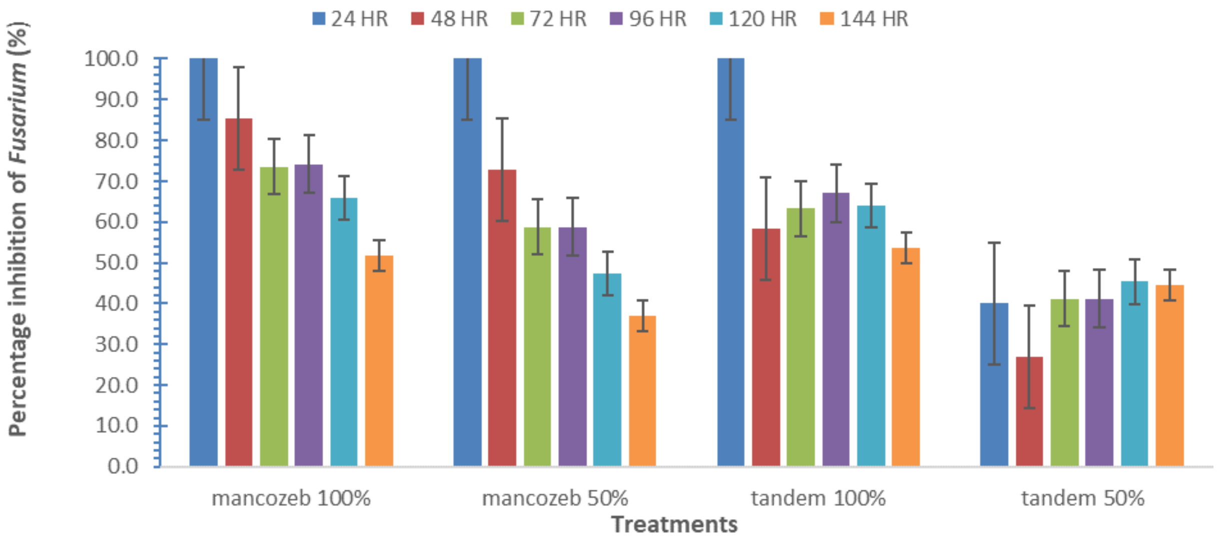

Figure 2: The percentage inhibition of *F. oxysporium* from Bambara groundnut *in vitro* in south eastern Nigeria
tandem is C.O.M. (Copper(I)Oxide+Metalaxyl)

growth was Eucalyptus 100%. For brevity performance of the lower concentrations of each extract was left out since they tended to perform similar to the 100% concentration.

The 144 hours after inoculation data on control of this pathogen by the extracts from plant resins were subjected to analysis of variance and the means separated using SNK ($P \leq 0.05$). It was observed that Eucalyptus 100% controlled *F. oxysporum* f.sp. voandzeia significantly more than all the other treatments followed by the check, Eucalyptus 10% and Mahogany 100% (which were at par). However, pathogen control by Mahogany 100% was also at par with Mahogany 10% and Cashew 100%. Cashew 10% was the least effective treatment based on the mean ranking although it inhibited the pathogen significantly more than the control.

The findings on the application of chemical fungicides to control fungi on Bambara groundnut confirmed the findings of Obagwu (2003) on the use of benlate to control *Colletotrichum capsici* on Bambara groundnut. *Fusarium* wilt disease of tomato (Lycopersicon esculentum L.) was inhibited by different chemicals in the laboratory and glasshouse. The synthetic fungicides (Nativo®, (at 1000 and 500 ppm), Topsin-M (1000 ppm), Curzate, Melody Duo (10 ppm) and Carbendazim were the most effective chemicals compared to Cabriotopc, Prevailc and Antracol (Gul *et al*. 2021). These findings by Gul *et al*. (2021) confirm the findings of this study that chemical pesticides have variable inhibition rates on *Fusarium* species hence the need to investigate the effects of each one of them separately.

These findings on the use of plant resin extracts were corroborated by the findings of Suleiman and Anyika (2016), Ndifon *et al*. (2015) and Lum *et al*. (2019) who effectively controlled pathogenic fungi including *Fusarium* spp. with the aid of plant extracts. Mycelia growth of *Fusarium verticilloides* was inhibited by betel (Piper betle) leaf, clove leaf and galangal (i.e. Thai ginger or Siamese ginger) plant extracts in Parkistan. In the field, the application of the *Bacillus subtilis,* either alone or in combination with plant extracts, was able to suppress *Fusarium verticilloides* infection (Suriani *et al*. 2021). It can be clearly observed that both bacteria and fungal agents exist in nature that can be used to control fungal pathogens like *Fusarium* spp.

A few strains of *Pseudomonas flourscence* and *Bacillus subtilis* can control the growth of

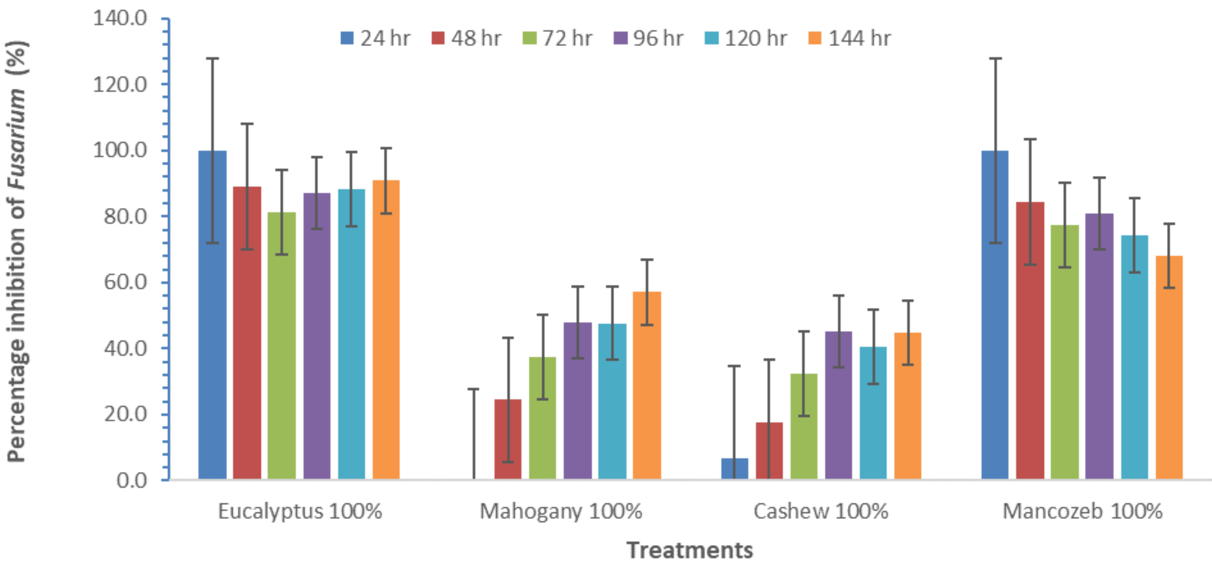

Figure 3: Percentage inhibition of growth of *F. oxysporum* due to application of plant extracts *in vitro*

Fusarium oxosporium in vitro (Bari *et al.* 2019) which affirmed the findings of this study that biocontrol agents can be used to inhibit the growth of *Fusarium oxysporium*. Ndifon (2022) reported that *Athelia rolfsii* was effectively controlled using *Trichoderma* isolates, synthetic fungicides and plant extracts which corroborates the findings of this study.

This study showed that the *Eucalyptus* resin and *Trichoderma* isolates were able to provide percentage inhibitions that were close to those of the synthetic mancozeb (100% concentration). This is quite encouraging and should be investigated more. The synthetic chemicals were applied at very low rates since the ultimate aim of researching their use nowadays is to provide more evidence for an integrated disease management project.

CONCLUSIONS

Fusarium wilt is one of the major diseases of Bambara groundnut. Its management has not been researched hence this study was carried out. Three in vitro trials that were carried out showed that *Trichoderma harzianum* and *T. virens* isolates, Mancozeb®, Tandem® (i.e. Copper (I) Oxide + Metalaxyl) and plant resin extracts (Eucalyptus, mahogany and cashew) could effectively control the growth of *Fusarium oxysporum*. The higher concentrations of the plant extracts were more effective and were recommended while more research is ongoing on the use of these different methods of managing the pathogen. Based on the findings of this research, it is recommended that more work be carried out on plant extracts, bio-control agents and synthetic fungicides against *Fusarium* wilt of Bambara groundnut *in vivo*.

AUTHOR CONTRIBUTION

NEM conceived topic, coordinated research, carried out research, collected data, wrote the paper. NEM, EA, CGO and AFC edited the paper and supplied resources. NEM and EA analyzed data

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