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RESEARCH ARTICLE

INHIBITORY EFFICACY OF MICROBIAL, BOTANICAL AND SYNTHETIC FUNGICIDES AGAINST *Athelia rolfsii* (*Sclerotium* STEM ROT) OF GROUNDNUT AND BAMBARA GROUNDNUT

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ABSTRACT

Solutions for groundnut stem rot by *Athelia rolfsii,* which accounts for major groundnut and Bambara groundnut yield losses, were investigated. Three in vitro trials were set up to assess the inhibition of the growth of *A. rolfsii* using microbial, botanical and synthetic fungicides. The radii of *A. rolfsii* were measured and inhibition of growth were calculated. The inhibition of *A. rolfsii* by *Trichoderma* and *Cladosporium* spp. at 72 hours after inoculation (HAI) ranged between 28-82%. Inhibition of *A. rolfsii* by mancozeb (100% concentration) was significantly more than the control and the other pesticide rates at 144 HAI. The inhibition by all the synthetic fungicides ranged between 10-90%. Eucalyptus gum, plum seeds, bark of African locust bean tree extracts inhibited growth of *A. rolfsii* throughout. All plant extracts controlled *A. rolfsii* with the efficacy ranging between 8-100% inhibition. At 144 HAI, Eucalyptus (100% concentration) controlled *A. rolfsii* significantly more than all the other treatments, followed by Eucalyptus (50%) and Parkia bark (100%). Parkia 100% controlled *A. rolfsii* significantly more than other treatments followed by plum 100% and orange seeds 100%. Thus *Sclerotium* stem rot can be effectively managed using the *Cladosporium cladosporioides*, *Trichoderma harzianum*, mancozeb®, team® and plant extracts above.

Keywords: *Arachis hypogaea*, mycoparasites, pesticides, plant extracts, *Sclerotium rolfsii*, *Vigna subterranea*

INTRODUCTION

Bambara groundnut (*Vigna subterranea* (L.) Verdc. in Fabaceae), which originated from sub-Sahara Africa (i.e. Nigeria-Cameroon), is classified as an underutilized leguminous crop, nonetheless it has the proven potential to contribute to improved food security (Tan *et al*. 2020; Ismaila *et al*. 2020). Bambara groundnut is mostly cultivated, in West Africa especially in Nigeria, for its seeds which are highly nutritious and can be considered as a wholesome food (Bamshaiye *et al*. 2011; Ismaila *et al*. 2020). Bambara groundnut is one of the most promising food legumes in Africa, due to its agronomic and nutritional potential but its production is being constrained by pests and diseases among which is groundnut stem rot (Kouassi and Zorobi, 2009).

Chen *et al*. (2014) and Wei *et al*. (2021) stated that groundnut (*Arachis hypogaea* L. in Fabaceae) is an important oil-seed crop that is widely cultivated especially in the subtropics and tropics. Pattee and Young (1982) and Kumar *et al*. (2020) pointed out that groundnut is a very important legume crop and its cultivation is mostly confined between latitudes 40°N and 40°S. Globally groundnut is cultivated on 25.5 million ha and yields 45.3 million tons of seeds (i.e. 1780 kg/ha). El -Sherbeny *et al*. (2020) said that groundnut is considered to be the crop of poor people since it is rich in oil, proteins, minerals, vitamins, medicinal compounds and also it serves as excellent feed for herbivores.

Production of groundnut is constrained by plant viruses, bacteria, fungi, insects like aphids, lack of resistance to drought, salinity and temperature (El-Sherbeny *et al*. 2020). In fact, groundnut production is mostly beleaguered by numerous economically important fungal diseases like tikka disease, rust, stem rot, collar rot which cause severe damage to the crop (Desai and Bagwan 2005).

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In 2021, CAB International reported the presence of *Athelia rolfsii* (Curzi) C. C. Tu & Kimbr. which causes groundnut stem rot/ *Sclerotium* stem rot in Nigeria. It is reported to be able to infect more than 500 host plant species (Ferreira and Boley 1992; Mullen 2001; Kwon 2010, Priya *et al*. 2013; El-Nagar *et al*. 2013; Ünal *et al*.; 2019; Bowen et al. 2021).

Athelia rolfsii (in the family Atheliaceae in class Agaricomycetes) has numerous synonyms like *Botryobasidium rolfsii* (Saccardo) Venkat.*; Corticium centrifugum* (Lév.) Bresad.; *Corticium rolfsii* Curzi; *Hypochnus centrifugus* (Lév.) Tul.; *Pellicularia rolfsii* (Curzi) E. West; *Sclerotium rolfsii* Sacc.; *Sclerotium rolfsii* var. rolfsii Saccardo) and causes groundnut stem rot; root and stem wilt of groundnut; southern blight; southern stem rot, *Sclerotium* stem rot or white mould (Ünal *et al*. 2019; CABI 2021). Cavalcanti *et al*. (2018) reported that *A. rolfsii* is widespread and causes direct damage to crops which induces secondary metabolite production in plants.

Kumar and Thirumalaisamy (2016) emphasized that these diseases reduce the pod yield of groundnut and also quality of groundnut haulm. Grahame (2014) reported that *Sclerotium* stem rot causes up to 10-25% reduction in groundnut pod yields worldwide including more than 45 countries in Africa. These diseases cause severe seedling mortality which result in patchy crop stands and reduction in yield which range between 25-50%. Liamngee *et al*. (2015) reported that globally losses of 10-20 million US dollars have been associated with *A. rolfsii* and yield losses range from 1–60%, despite considerable research on control of this pathogen.

A. rolfsii cannot be controlled by a single method, such as the use of fungicides or cultural rotation with resistant plants (de Sousa and Blum 2013). Mullen (2001) reported that applying fungicides to soil may require large quantities of synthetic pesticides which may not be practicable in many situations especially for resource poor farmers like those in Africa. Mullen (2001) further lamented that methyl bromide which previously successfully controlled southern blight has been banned. Moreover, it has been pointed out that in field studies, the required quantity of biological agents may be very high and not practicable in most farming operations. Many cultural practices like fertilization ad lib and continuous cropping have led to accumulation of toxic substances and increased disease epidemics in peanut (Jadon 2018; Htoon *et al*. 2019).

Cavalcanti *et al.* (2018) reported that *A. rolfsii* has been controlled by various researchers on tomato, groundnut, garlic, black pepper, yams, common bean among other crops using seven *Trichoderma* spp. (*Trichoderma virens*, *T. koningii, T. Viride, T. harzianum, T. asperellum, T. Longibrachiatum* and *T. reesei*); four *Bacillus* spp. (*Bacillus subtilis*, *B. amyloliquefaciens*, *B. Cereus*), four *Streptomyces* spp. (*Streptomyces globisporus, S. flavotricini, S. Pactum, S. Senoensis*), three *Pseudomonas* spp. (*Pseudomonas putida, P. fluorescens, P. aeruginosa*) and Rhizobacteria sp. Ferreira and Boley (1992) reported that the most commonly used biocontrol agents include *T. harzianum*, *T. viride, B. subtilis, Penicillium* spp., and *Gliocladium virens*. While Ünal *et al*. (2019) reported that Bacillus *cereus* strain 44bac and *Stenotrophomonas rhizophila* strain 88bfp were more effective than the other bacteria strains (by 91 and 90.1% efficacy respectively) in controlling southern blight.

Ferreira and Boley (1992) reported that when plant residues (compost, oat, or straw) were added to the soil they reduced disease incidence. They argued that the addition of soil amendments may increase populations of antagonistic soil microorganisms. In fact, Bulluck and Ristaino (2002) later on reported that the use of organic amendments, cotton gin trash and swine manure effectively controlled southern blight disease through improved colonization of soil by antagonistic *Trichoderma* spp. Along with species of *Trichoderma*, other biological agents, such as *G. virens, B. subtilis, and Penicillium spp., antagonized S. rolfsii. T. koningii* also reduced the number of sclerotia (Latunde-Data, 1993). *Streptomyces philanthi* was

effective in reducing the infection by *S. rolfsii* (Boukaew *et al*. 2011).

Shew (n.d.) reported that many fungicides have excellent activity against southern stem rot. Teaching Research Extension Service (TRES) (no date) reported that chemical control of southern blight is possible with Vitavax®, Terraclor® (PCNB), Tilt® or Folicur®. When these chemicals were combined with EPTC (a herbicide) in autoclaved soil, *S. rolfsii* activity was diminished, even though EPTC (S-ethyl dipropylthiocarbamate: a herbicide) alone stimulated growth of the pathogen. In natural soil, the effectiveness of *T. viride* was reduced in the presence of EPTC. T. viride in combination with pentachloronitrobenzene (PCNB) has been shown to provide good disease control and better yields in artificially inoculated field plots (tomato) than in non-inoculated, untreated field plots. *T. viride* without PCNB provided statistically similar disease control although the yield was lower. PCNB alone was less effective than PCNB combined with *T. viride* or *T. viride* alone.

G. virens have been shown to rapidly degrade *S. rolfsii* strain SR-1 in soil. *G. virens* colonized *S. rolfsii* strain SR-3 but the sclerotia can germinate under good conditions. Formalin and chlorobromopropene are among the most promising fumigants for treatment of seed beds or fields for valuable crops. Mullen (2001) reported that the soil fungicide, (PCNB), azoxystrobin and a soil insecticide (chlorpyrifos) significantly controlled southern blight.

The addition of organic amendments such as compost, oat or corn straw, or cotton gin trash to soil sometimes reduces *Sclerotium* stem rot incidence and development. Furfuraldeyde, an organic (sugar derivative) amendment, changed the soil microflora, leading to a decrease of *S. rolfsii* severity. Also, neem oil and pine bark extracts or pine bark powder caused reduced growth of *S. rolfsii*. Biological control of *S. rolfsii* has been achieved to some degree with bacteria (*B. subtilis*), actinomycetes, a mycorrhizal fungus, or certain *Trichoderma* species (Mullen 2001).

Cladosporium species are often isolated from different surfaces and organic matter. Some of them may colonize leaf lesions formerly caused by plant pathogenic fungi as secondary invaders (Ellis 1976; Bensch *et al*. 2012). Many species(> 7000 of *Cladosporium* are plant pathogens i.e., they are cause leaf spots and other lesions (Dugan *et al*. 2004; Schubert 2005; Rivas and Thomas 2005, Deshmukh and Rai 2005; Bensch *et al*. 2012) or they occur as hyperparasites on other fungi (Assante *et al*. 2004; Heuchert *et al*. 2005; Bensch *et al*. 2012; Zhan *et al*. 2014; Torres *et al*. 2017). The major problem with groundnut stem rot is that it affects the economic part of the crop thus directly affecting the farmer's income.

Literature search revealed a dearth of knowledge about the control of groundnut / *Sclerotium* stem rot in the humid tropics especially in Nigeria. Thus the objective of this research was to assess the inhibitory efficacy of biocontrol, botanical and synthetic pesticides for the control of *A. rolfsii* isolated from groundnut and Bambara groundnut. The trial was restricted to biocontrol, botanical and synthetic fungicides application against *A. rolfsii* in vitro

MATERIALS AND METHODS

Site of the study

This research was carried out at the Faculty of Agriculture Laboratories in Alex Ekwueme Federal University, Ndufu-Alike in Ikwo Local Government Area (at 6.0690N by 8.1990E), about 21 kilometers from Abakaliki; Ebonyi State capital. Ebonyi State is in the derived savanna zone of Nigeria with a humid tropical climate. The cultivation of groundnut and Bambara groundnut in Ebonyi State is a fruitful venture though the humid environment seems to be encouraging more than a fair share of pathogenic fungi infections on these two groundnut crops.

Isolation and identification of the fungi utilized in the trials

Infected groundnut and Bambara groundnut stems utilized for this research were obtained from the University Research and Teaching Farm. While the *Trichoderma* isolates were obtained from Bambara groundnut seeds, mushrooms, crop seeds and farmland soils collected from eastern Nigeria and West Cameroons. The *Cladosporium cladosporioides* (Fresenius) de Vries isolate was obtained from diseased groundnut stems.

The fungi (*C. cladosporioides*, *Trichoderma* spp. and *S. rolfsii* /*A. rolfsii*) 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. The isolated *C. cladosporioides*, *Trichoderma* and *S. rolfsii* were sub-cultured to obtain pure cultures which were used to identify the fungi with the aid of literature on fungi morphology (Barnett and Hunter 1972).

Trial 1: Biocontrol of *A. rolfsii* using *C. cladosporioides* and *T. harzianum* isolates

The experiment was laid out in petri dishes using completely randomized design and each treatment was replicated three times. The treatment set consisted of 4 *Trichoderma harzianum* isolates (*T. harzianum* AIBN, *T. harzianum* BGMP, *T. harzianum* ZXMZ, *T. harzianum* BGMZ3), *Cladosporium cladosporioides* and a control. The control was inoculated with the *S. rolfsii* isolate alone. The agar medium was inoculated with 2-mm disc of the pathogen or biological control agents placed at the edge of the plate according to the layout. This dual culture trial was inoculated by placing the different cultures at the edge of the petri dish. The data were collected as shown below.

Trial 2: Effects of synthetic pesticides on *S. rolfsii* in vitro

The experiment was carried out using petri dishes. It was laid out in the laboratory using a completely randomized design (CRD) with 5 treatments, each treatment replicated three times. The treatment set included control, mancozeb 100% concentration, mancozeb 50%, team 100% and team 50%. Mancozeb® (applied at a rate of 2000 g/ha - it is a contact fungicide) while team® (recommended at a rate of 800 g/ha is a wettable powder of carbendazim (12%) + mancozeb (63%) and it is a systemic and contact fungicide) were utilized to compose the treatments. Each treatment consisted of three levels (control 0.0, 50 and 100% concentrations) and they were applied into the Petri dishes according to the layout. The data were collected as shown below.

Trial 3: Effects of plant extracts on S. rolfsii

The experiment was carried out using petri dishes. It was laid out in the laboratory using a completely randomized design (CRD) with 10 treatments, with each treatment replicated three times. The treatment set included control, bark of African-locust bean tree (*Parkia biglobosa*), resin from Eucalyptus tree (*Eucalyptus globolus*), seeds of orange (*Citrus sinensis*), seeds of plum (Syzygium *cumini*) and team® (100% concentration). All plant tissues utilized were each weighed at 333.3 g tissues per L of distilled water) to make 100% concentration. Each treatment consisted technically of three levels (control 0.0, 50 and 100% concentrations) and they were applied into the petri dishes according to the layout. The data were collected as shown below.

Data collection and analysis used for all the sub-trials

The radius of the fungus colony was measured using a transparent ruler at 24 hour intervals starting from day 1 (24 hours after inoculation (HAI)) through day 8. The percentage inhibition of the pathogen was calculated using equation 1.

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

Where
PI = % 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
*perpendicular refers to 'right angle' in this context. Other radii could be taken especially if inhibition from the source or bio control organism varies and is not uniform at all

fronts. This radius was decided on based on experience.

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

RESULTS AND DISCUSSION

The inhibition of *A. rolfsii* by all the isolates of *Trichoderma* and *Cladosporium* spp. was significantly more than the control (Fig. 1). The inhibition by all the isolates of *Trichoderma* and *Cladosporium* spp. ranged between 28-82% but the inhibition seemed to decline with time. The inhibition of *A. rolfsii* by bio control agents utilized started off higher at 24 hours after inoculation (HAI) then declined steeply at 72 HAI when the trial was terminated. The inhibition at 72 HAI was still high enough compared to some of the works reviewed herein. The inhibition of *A. rolfsii* by mancozeb 100% was significantly more than the control and the other rates of the chemicals (Fig. 2). The inhibition by all the synthetic pesticides ranged between 10-90% but the inhibition declined with time. The inhibition of *A. rolfsii* by synthetic fungicides utilized started off higher at 24 HAI then declined gently till 144 HAI when the trial was terminated. The inhibition at 144 HAI was still high enough for mancozeb compared to team.

All the plant extracts were able to inhibit the growth of *A. rolfsii* throughout the period of the trial (Fig. 3). Eucalyptus gum (100% concentration) was the best plant extract

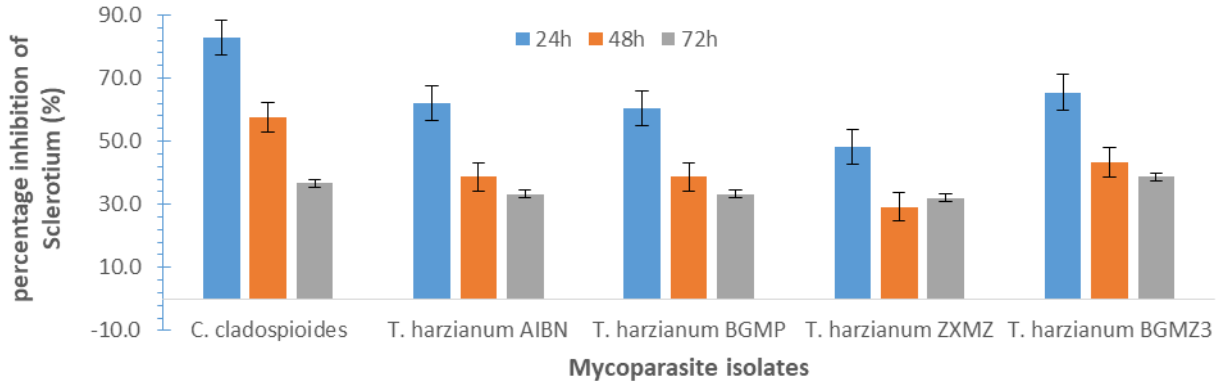

Figure 1. The effects of *Trichoderma* and *Cladosporium* isolates on *A. rolfsii in vitro*
NB: error bars were calculated based on standard error

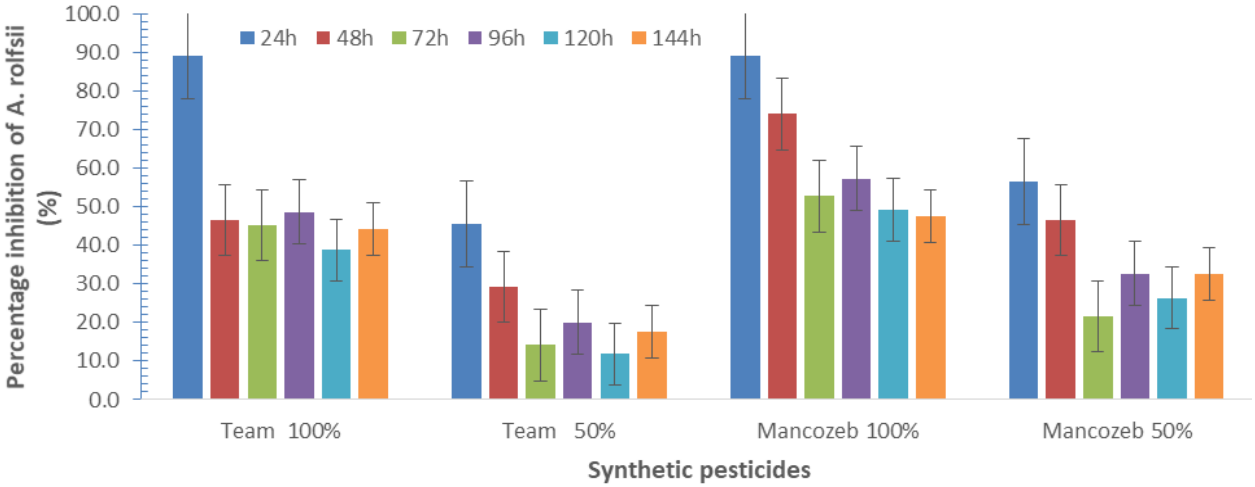

Figure 2. The effects of synthetic pesticides on *A. rolfsii in vitro*
NB: error bars were calculated based on standard error

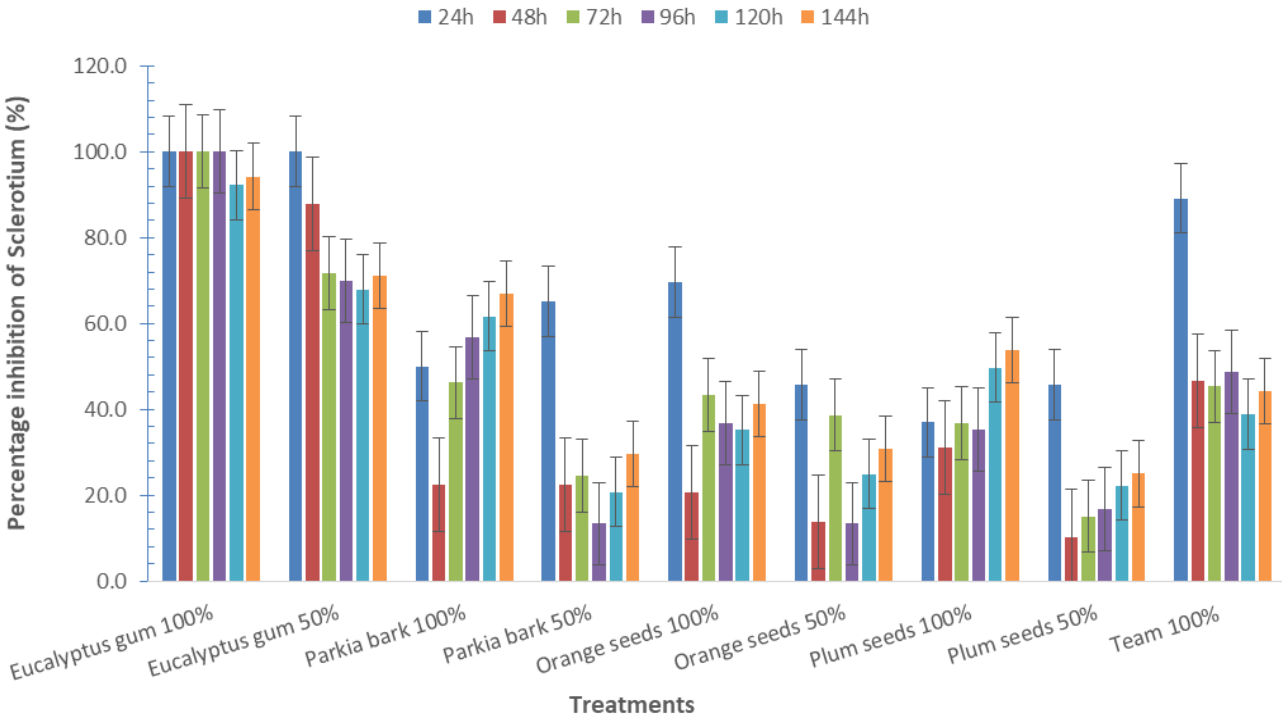

Figure 3. The effects of plant extracts on *A. rolfsii in vitro*
NB: error bars were calculated based on standard error

followed by Eucalyptus gum 50% although all the different plant extracts (at 100% concentration) were comparable with the check (100% concentration). All the plant extracts controlled *A. rolfsii* with the efficacy ranging between 8.0-100% inhibition. It was observed that inhibition by Eucalyptus gum 100% stayed high throughout the 144 hours of the trial while Parkia (50 and 100%) and Plum seeds 100% exhibited increase in inhibition with time compared to other treatments.

All the isolates of *T. harzianum* controlled *A. rolfsii* significantly ($P \leq 0.05$) more than the control, 72 HAI. The synthetic pesticides (mancozeb 50% and 100% and team 100% concentration) controlled *A. rolf*sii significantly ($P \leq 0.05$) more than team 50% and control. However, team 50% was significantly better than the control at 144 HAI.

At 144 HAI, Eucalyptus gum (100% concentration) controlled *A. rolfsii* significantly more than all the other treatments, followed by Eucalyptus gum 50% and Parkia bark 100%. Parkia 100% controlled *A. rolfsii* significantly more than the other treatments followed by plum seeds 100% and orange seeds 100%. Finally, Parkia bark 50%, plum seeds 50% and orange seeds 50% were only significantly better than the control at reducing the growth of *A. rolfsii*.

The management of *A. rolfsii* was effectively carried out using different methods of disease control but none of the methods gave persistent full control which concurred with the statement by de Sousa and Blum (2013) that *A. rolfsii* cannot be controlled by a single method, such as the use of fungicides or cultural rotation with resistant plants. The efficacy of *Trichoderma* isolates in inhibiting the growth of *A. rolfsii* corroborated the findings of Ferreira and Boley (1992) and Cavalcanti *et al*. (2018) who used isolates of *Trichoderma* spp. to inhibit pathogenic fungi on various crops including groundnuts. The use of *Cladosporium* sp. as bio control agent was fairly new and it proved quite effective thus corroborating the findings that some *Cladosporium* species occur as hyperparasites on other fungi (Bensch *et al*. 2012; Torres *et*

al. 2017; Assante *et al*. 2004; Zhan *et al*. 2014). In fact, *Cladosporium cladosporioides* and *Cladosporium pseudocladosporioides* were reported to be particularly mycoparasitic on *Puccinia* rust agent (Torres *et al*. 2017) which specifically affirmed the results of this trial.

It seems use of chemical pesticides will be with us for a little longer seeing that it was one of the effective methods of managing the pathogen. Actually, Teaching Research Extension Service (TRES) (no date) reported that chemical control of southern blight is possible with vitavax®, terraclor®, tilt® or folicur® which validated the findings of this trial. Meanwhile Ndifon *et al*. (2015) reported that Eucalyptus gum and other plant extracts were effective against *F. oxysporum* which affirmed the findings of this trial on efficacy of plant extracts against *A. rolfsii*. Fontem *et al*. (2014) and Lum *et al*. (2019) reported that application of plant extracts from weeds and trees effectively controlled *F. oxysporum* which also corroborated the findings of this trial on use of chemicals.

CONCLUSION

Athelia rolfsii is one of the major diseases of groundnut and bambara groundnut worldwide. Its management has been a major challenge globally hence this research was carried out. Three in vitro trials that were carried out showed that *Trichoderma* and *Cladosporium* isolates, mancozeb®, team® and plant extracts (Eucalyptus gum, Parkia bark, orange seeds and plum seeds) could effectively control *A. rolfsii* effectively. The higher concentrations of the plant extracts were more effective and were recommended while more research continues on the use of these different methods of managing *A. rolfsii.* 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 *Athelia* spp. in vivo.

AUTHOR CONTRIBUTION

NEM conceived the topic, carried research and wrote the paper.

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