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ARTICLE

Management of *Globisporangium ultimum* Infecting Groundnut and Bambara Groundnut Pods Using Diverse Methods

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ABSTRACT

Bambara groundnut and groundnut are leguminous crops of immense importance globally. However, these crops are exceedingly susceptible to *Globisporangium ultimum* groundnut pod rot disease which constitute foremost constraints to their production. The objective of proffering solutions to this pod rot disease was set and achieved using three diverse trials. Each experiment was conducted separately *in vitro* using completely randomized design and each treatment was replicated three times. Firstly, the trial conducted using synthetic chemicals; Team® (i.e. mancozeb + carbendazim) and Mancozeb® revealed that team inhibited mycelial growth of *G. ultimum* more than Mancozeb (at 50% and 100% concentrations) as from 24 hours after inoculation (HAI). Generally, mancozeb achieved 8%-100% inhibition, while team achieved 36%-100% inhibition. Secondly, the trial carried out utilizing plant extracts (African locust bean tree, mango, shea butter tree and pawpaw plant tissues) revealed that inhibition by plant extracts was lowest (8.0%) in shea butter (at 50% concentration) and highest (100% inhibition) at 24 HAI in African locust bean (*Parkia* sp.) (100% concentration), mango (100%) and shea butter (100%) . The best plant extracts were African locust bean tree (100% concentration), Pawpaw (100%), Mango (100%) and Shea butter tree (100%) followed by African locust bean (50%). Finally, the trial conducted using biocontrol agents (*Trichoderma* and *Cladosporium* spp.) revealed that these agents inhibited mycelial growth of *G. ultimum* by 12%-100%. All the biocontrol agents (*T. harzianum* NSBM, *T. virens* BGMZ2, *T. harzianum* AIM3, *Cladosporium cladiosporioides* AIGT, *C. cladiosporioides* AIPL and *T. viride* AIBK) were significantly different ($P \leq 0.05$) from the control at 96 HAI. Inhibition by bio-control agents generally ranged between 10%-90%. Thus groundnut pod rot disease complex can be effectively managed using these pesticides; nevertheless conducting of field based trials is being admonished.

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1. Introduction

Groundnut (*Arachis hypogaea* L.) is the thirteenth most important food crop and the fourth oil seed crop globally [1]. Nigeria produced 2.3 million metric tons (9.4%), China produced 4.6 million metric tons (18.8%) and India 6.9 million metric tons (27.9%) of groundnut, which means that Nigeria was the third largest global producer of groundnut in 2008 and Mali accounted for only 330, 000 tons [2,3]. Thus cultivating this crop in Nigeria can benefit both the local and international community immensely, considering the nutritional and economic value of groundnuts. Groundnut seeds are rich in vitamin E, niacin, falvin, calcium, phosphorus, magnesium, zinc, iron, riboflavin, thiamine and potassium [4]. Bambara groundnut (*Vigna subterranea* (L.) Verd.) is the third most important legume crop in Africa after groundnut (*A. hypogaea*) and cowpea (*Vigna unguiculata* (L.) Walp.) [5].

The amount of Bambara groundnut produced globally was 55,000 metric tons from 70,000 ha in 2000-2002, thus making Bambara groundnut the 11th most important world grain legume crop [6]. Recently the yield of Bambara groundnut in Africa was approximately 300,000 metric tons. Nigeria is the largest producer of Bambara groundnut (approximately 100,000 metric tons), followed by Burkina Faso 44, 712 metric tons, and Niger 30,000 metric tons as of 2015 [7]. As of 2017, Bambara groundnut was the third legume in Mali after groundnut and cowpea, and but it is said to be more resistant to drought, pests and diseases compared to groundnut and cowpea. Mali is not the first leading producer of this crop in Africa or globally [8].

In 2015, the total estimated production of 27,691 metric tons for Bambara groundnut as against 421,924 metric tons for groundnut was reported for Mali (Mali National Statistics) [2]. Unlike groundnut, which is cultivated in most subtropical and tropical regions, Bambara groundnut is mostly cultivated in West, Central and Southern Africa regions (i.e. mainly in Cote d'Ivoire, Zimbabwe, Nigeria, Togo, Cameroon and Mali) [9,10].

Cultivation of Bambara groundnut however, is currently being invigorated in the Americas and Asia due to its health benefits and the fact that Bambara groundnut does well in those areas [9]. The cultivation of groundnut and Bambara groundnut in Africa especially in Nigeria is a potentially fruitful venture but currently heavy rainfall and high relative humidity have been a source of concern for farmers of these two groundnut crops, as far as disease outbreaks including pod rots are concerned.

Bambara groundnut and groundnut plants are concurrently susceptible to soil-borne root pathogens and foliar diseases which constitute major constraints to production of this legume [11,12]. These infections result in low yields and quality of the produce. Combined yield losses due to incidence of diseases in groundnut can be as high as 50% [13].

The soil-borne diseases infecting groundnuts are mainly caused by fungi and nematodes as well as some spore-forming bacteria [14]. 'Compared to groundnut and cowpea, Bambara groundnut is more resistant to drought, pests and diseases' it appears that this statement is highly misleading and is an over-generalization. Bambara groundnuts are mostly attacked by fungi, root knot nematodes, viruses, insects and mites [15-24]. May be Bambara groundnut in the semi-arid and arid ecological regions is rather tolerant to pests and diseases which is expected [8] but it is quite susceptible to pests and diseases in the semi-humid and humid ecological regions.

Pod rot disease complex essentially could be caused by pathogenic infections and factors like excessive soil moisture, wide variations in soil moisture, calcium deficiency, insect and nematode feeding, as well as irrigation with poor quality (salty) water [14]. Groundnut pod rot or groundnut pod rot/root rot/wilts are the two major classes of groundnut pod rot disease complex.

Pod rots consist mainly of two kinds as follows: *Pythium* pod rot caused by *Pythium* spp. (characterized by greasy-appearing, brown to black lesions on a softened pod) and *Rhizoctonia* pod rot by *Rhizoctonia solani* (characterized by dry, brown to dark brown lesion on a firm pod). However, groundnut pod rot is also jointly caused by *Fusarium solani, Fusarium oxysporum, Macrophomina phaseolina, Rhizoctonia solani, Sclerotium rolfsii* and *Aspergillus niger*. The groundnut pod rot/root rot/wilt disease is induced by *Rhizoctonia solani* (whose teleomorph stage is *Thanatephorus cucumeris*) [25].

Pod rot by *R. solani* and *Pythium* spp. is often more problematic in furrow-irrigated than pivot irrigated fields. Regrettably, often times the most important and obvious pathogen (observed as the causal agent of the disease), may be tackled as the cause of disease, while further investigation into other pathogens (that may be present in the disease situation), is often omitted. Thus research conducted so far on some of the economically important soil-borne and foliar diseases of groundnut is inadequate. Of course this makes sense since disease causal organisms may take on more or less importance in different ecological zones and cultural practices [26].

Soil-borne diseases of groundnut such as *Rhizoctonia* limb and pod rot, Pythium pod rot, *Cylindrocladium* black rot and *Sclerotinia* blight are among the most difficult diseases to manage. *R. solani* is most devastating on

mature plants leading to decay of pegs, pods and stems [27]. Terbuconazole and propiconazole have dual soil-borne and foliar activity for managing groundnut diseases. Management of pod rot disease complex is feasible with use of cereal-cereal-groundnut crop rotation and seed treatment with thiram, or application of soil amendments (like gypsum, rice hull, fish meal) as well as avoiding excessive irrigation before harvesting among other measures [25].

T. harzianum isolate grew over *Bipolaris oryzae* and the antifungal metabolites of *T. harzianum* completely inhibited growth of pathogen thereby preventing mycelial growth of B. oryzae in vitro [26]. *Trichoderma* isolates *in vivo* after colonizing plant roots often kill several major root rot fungi (i.e. *Pythium, Rhizoctonia*, and *Fusarium*) thus enabling roots to grow faster by overcoming microbial stresses that normally inhibit plant growth [27]. Generally the mechanisms employed by *Trichoderma* isolates include organic matter decomposition, mycoparasitism, cellulose degradation and phosphate solubilizing activity [28]. Competition for nutrients by biocontrol agents is a very popular mechanism engaged by some effective control agents.

Trichoderma species (*T. viride* and *T. harzianum*) inhibited five seed borne phytopathogens (i.e. *Curvularia lunata, F. oxysporum, Alternaria alternata, Colletotrichum gloeosporioides* and *R. solani*) [29]. *Trichoderma* isolates inhibited mycelial growth of *A. alternata* (14.6% - 26.8%), *Penicillium* sp. (9.37% - 34.4%), *A. niger* (31.7% - 42.5%) [30]. Two isolates of *Trichoderma* spp. (*T. harzianum* and *Trichoderma asperellum*) significantly reduced *F. oxysporum* disease severity (20.0% - 44.0%) and increased the dry weight of the crop (23.0% - 52.0%) and *T. virens* treatments performed significantly better than the control [31] in Iran.

Azadirachta indica, Ocimum basilicum and *Crotalaria juncea* effectively inhibited *Aspergillus, Fusarium* and *Rhizoctonia* spp. Whereas *Acacia nilotica, Eucalyptus camaldulensis* and *Prosopis juliflora* showed least potential mycelial inhibition capacity against *Aspergillus flavus, A. niger, F. solani, M. phaseolina* and *R. solani* [32]. *Rosmarinus officinalis* oil and methanolic extracts effectively controlled many bacteria species including: *Staphylococcus aureus, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus feacalis, Escherichia coli, Staphylococcus epidermidis, Bacillus subtilis* and *Candida albicans* [33].

From the foregoing gen, it can be ascertained that both groundnut and Bambara groundnut producers could benefit from intervention aimed at preventing loss of economic yield attributed to pod rot disease complex globally. Pod yield loss may affect the crop both in the field and even in store as this disease complex may predispose the pods to subsequent damage in store by other pathogens and pests. The low income that farmers obtain from discolored and unhealthy pods is a direct justification for this intervention, because the huge risks in agricultural production can only be reduced through access to quality knowledge which is scarce as far as pod rot disease complex is concerned. This research was conceived with the main objective of tendering some management solutions to this disease complex.

2. Materials and Methods

2.1 Site of the Study

This research was carried out at the Faculty of Agriculture Laboratories in Alex Ekwueme Federal University, Ndufu-Alike at Abakaliki (at 6.0690N by 8.1990E). Abakaliki is the Ebonyi State capital and it is situated 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.

2.2 Isolation and Identification of the Fungi Utilized

Infected groundnut and Bambara groundnut pods utilized for this research were obtained from the University Research and Teaching Farm. The *Cladosporium cladosporioides* (Fresenius) de Vries isolates and *Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish. 2010 (syn. *Pythium ultimum* Trow, 1901), isolates were acquired from these infected pods. While the *Trichoderma* isolates were obtained from Bambara groundnut seeds, mushrooms, crop seeds and farmland soils collected from south eastern Nigeria and West Cameroons.

The fungi (*C. cladosporioides* isolates, *Trichoderma* spp. and *G. ultimum*) were isolated using dehydrated commercial potato dextrose agar (PDA) medium which was autoclaved at 120ºC and 15 psi for 15 minutes according to the manufacturer's (Lifesave Biotech) instructions. 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 [34].

2.3 Trial 1: Biocontrol of *Globisporangium ultimum* 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 *T. harzianum* isolate NSBM, *T. virens* isolate BGMZ2, *T. harzianum* isolate AIM3, *T. viride* isolate AIBK, *Cladosporium cladiosporioides* isolate AIPL, *C. cladosporioides* isolate AIGT and a control. The control was inoculated with the *G. ultimum* 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.

2.4 Trial 2: Effects of Synthetic Pesticides on *Globisporangium ultimum 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, and each treatment was replicated three times. The treatment set included control, mancozeb 100% concentration, mancozeb 50%, Team 100% and Team 50%. Mancozeb® (usually applied at a rate of 2000 g/ha 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 (0.0, 50 and 100% concentrations) and they were applied into the Petri dishes according to the layout. The *in vitro* rates were actually drawn (after obtaining the standard recommended field fungicide quantities) at 0, 50 or 100 µL per petri dish as required.

2.5 Trial 3: Effects of Plant Extracts on *Globisporangium ultimum*

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*), bark of mango (*Mangifera indica*), bark of shea tree (*Vitellaria paradoxa* (formerly *Butyrospermum parkii*), and pawpaw seeds (*Carica papaya*). *Parkia* sp., mango and shea plant tissues utilized were each weighed at 333.3 g tissues per L of distilled water to make 100% concentration while sun dried pawpaw seeds were utilized at the rate of 166.7 g/L to give 100% concentration. Each treatment consisted technically of three levels (0.0, 50 and 100% concentrations) and they were applied into the Petri dishes according to the layout.

2.6 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 7. The percentage inhibition of the pathogen was calculated using Equation (1).

$$PI = ((C - T) / C) \times 100\% \quad (1)$$

where,

PI = Percentage 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' because other radii could be obtained especially the longest radius away from the source / front of inhibition.

The data for the last interval in each trial were subjected to analysis of variance (ANOVA) and the means separated using Student Newmann Keul's (SNK) method (as obtainable with Genstat® Discovery, Second Edition statistical package). Descriptive statistics were used to illustrate the trends in growth of the pathogen and its management as time passed.

3. Results and Discussion

Passage of time trend: The effects of synthetic pesticides against *G. ultimum in vitro* revealed that team (mancozeb + carbendazim) inhibited mycelial growth of *G. ultimum* more than mancozeb at all levels, 24 hours after inoculation (HAI) (Figure 1). In fact Mancozeb inhibited *G. ultimum* by 8%-100% while team inhibited it by 36%-100%. It was observed that the higher rates of all the plant extracts inhibited *G. ultimum* more than the control (Figure 2). The inhibition by plant extracts ranged from 8.0% (at 120 HAI) in shea butter (at 50% concentration) to 100% inhibition at 24 HAI in *Parkia* sp. 100%, mango 100% and shea butter 100% plant extract concentrations. The results showed that all the biocontrol agents inhibited mycelial growth of *G. ultimum* in vitro (Figure 3). The control was highly variable and isolate dependent. It varied from 100% inhibition by *Trichoderma* isolates at 24 HAI to 12% inhibition at 48 HAI. The percentage inhibition somehow improved after 48 HAI in some treatments.

ANOVA and means separation for the last day of data collection for each trial: The results revealed no significant difference ($P \leq 0.05$) between all mancozeb rates and control at 120 HAI. However, there was a significant difference ($P \leq 0.05$) between all the rates of team and all other treatments. There was a significant difference ($P \leq 0.05$) between the plant extracts and control at 120 HAI. The best plant extract were bark of African locust bean tree (*Parkia* sp.) 100% concentration,

Pawpaw 100%, Mango 100% and Shea butter tree 100% followed by Parkia sp. 50%, Pawpaw 50%, Mango 50% and Shea butter tree 50% compared to the control. All the biocontrol agents (*T. harzianum* NSBM, *T. virens* BGMZ2, *T. harzianum* AIM3, *Cladosporium cladiosporioides* AIGT, *C. cladiosporioides* AIPL and *T. viride* AIBK) were significantly different ($P \leq 0.05$) from the control at 96 HAI. The control by bio-control agents generally ranged between 10%-90%. The means separations were sufficient for the objective of this work and so no need to belabour the issue of multiplying entities in an unprofessional manner by going further to rank more means for all the intervals. Based on the patterns obtained in the charts and need to avoid long windy articles that lead to complicated confusion the final means were first ranked and gave acceptable significant differences, the author deemed this sufficient rather than proceeding to rank means obtained during earlier data collection intervals. This could be seen as duplication of presentation of results since the charts and ANOVA have already given sufficient leads on what to expect. The length of time that control application is effective is one of the key requirements for pest management. And if the final day of data collection shows significant difference between the treated plots and negative control then this should be sufficient evidence to say that the control can be utilised up to that number of days. The control is not shown in the figures presented but it was zero based on the formula used for the study. The error bars were based on standard error using IBM SPSS.

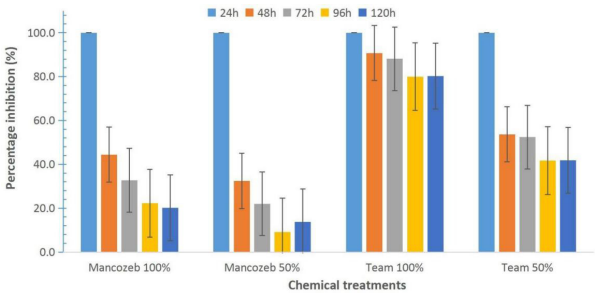

Figure 1. Effects of synthetic pesticides against *G. ultimum*

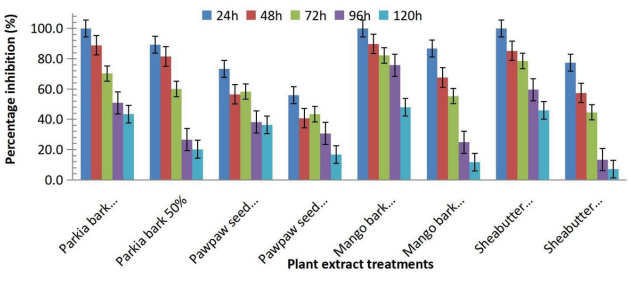

Figure 2. Effects of plant extracts against *G. ultimum*

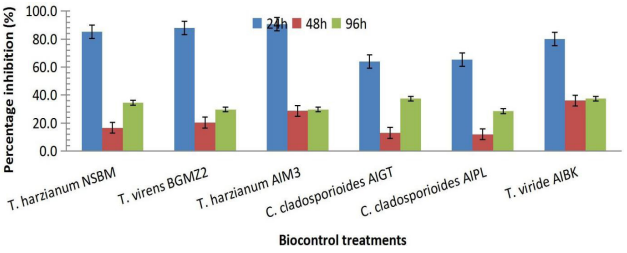

Figure 3. Effects of bio-control agents against *G. ultimum*

The plant extracts showed great potentials of controlling pathogenic fungi which concurred with the results of Ndifon and Lum [35] who successfully utilized aqueous extracts from five plants for management of *Aspergillus niger* [36]. Similarly, utilization of aqueous extract of some plants including *Eucalyptus globulus* significantly reduced mycelial growth of *Aspergillus* species [37]. Turmeric and garlic extracts were effective in inhibiting radial growth of *Aspergillus niger* isolated from Bambara groundnut seeds [38]. These findings also affirmed the findings that plant extracts could be effective against fungi pathogens of Bambara groundnut.

In the current trial, it was observed that synthetic fungicides generally control fungi more than plant extracts which affirmed the results of Ndifon and Lum [24,35] that Mancozeb and Tandem (metalaxyl + Copper II oxide) produced higher inhibition of the fungus compared to the plant extracts. Also Mancozeb was effective in inhibiting radial growth of *Aspergillus niger* isolated from Bambara groundnut seeds [38]. Team (i.e. mancozeb+carbendazim) inhibited mycelial growth of *G. ultimum* more than Mancozeb alone which agreed with the findings that Tandem inhibited the growth of *Aspergillus* sp. more than Mancozeb alone [36].

Trichoderma harzianum isolated from groundnut and Bambara groundnut soils effectively controlled *Rhizoctonia solani;* a causal agent of leaf blight of Bambara groundnut *in vitro* [39]. These findings corroborated the findings of the current trial using isolates of *Trichoderma* species. No case of *Cladosporium* species being applied against pathogens of groundnut and Bambara groundnut was encountered, but the isolates of this fungus were quite effective against *G. ultimum*. Thus the search for biocontrol agents should not be limited to those currently being assessed. *T. harzianum* isolates inhibited *Macrophomina phaseolina* (20.2% - 58.7%) in dual culture trials [40]. Three *Trichoderma* species (*T. harzianum, T. atroviride* and *T. longibrachiatum*) showed high mycelial growth inhibition potential against two isolates of *Sclerotinia sclerotiorum*, while *T. atroviride* reduced the growth of the pathogen by 85.0% - 93.0% [41]. These findings agreed with the findings on the current

biocontrol trial using *Trichoderma* and *Cladosporium* spp. In the discussion section herein it was necessary to utilize more of other crops and control agents because very little work has been carried out on most of the pathogens of groundnut and Bambara groundnut. This statement affirms the statement that for most of Africa including South Africa no registered chemicals exist for the control and management of the diseases of Bambara groundnut [15].

4. Conclusions

Groundnut and Bambara groundnut have a great potential to supply mankind with protein and oil but their production is being hindered by many diseases among which is *Globisporangium ultimum* pod rot disease. Three trials were set up to proffer solutions to the threat being posed by pod rot disease. African locust bean bark, pawpaw seeds, shea butter bark, and mango bark may hold the key to controlling the disease. These plant materials are cheap, available and may pose no threat to man and the environment. The synthetic fungicides (Team and Mancozeb) were able to control the fungus at very low rates which could be good for integrated management practices. Finally the biocontrol of this fungus using *Trichoderma* and *Cladosporium* spp. shows that the feasibility of these agents being able to maintain the pathogen below economic injury level exists.

Author Contributions

N.E.M.conceived the topic, designed the study, carried out the trial, analyzed the data, wrote the manuscript and edited it.

Conflict of Interests

The author declares no conflict of interests.

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