

Evaluation of Biocontrol agents against *Lasiodiplodia theobromae* causing Inflorescence blight of Cashew in Nigeria

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Abstract: Biocontrol agents such as *Trichoderma viride* and *Aspergillus niger* hitherto neglected in the control of *Lasiodiplodia theobromae* causing inflorescence blight disease of cashew were evaluated in-vitro in the mycology laboratory, Plant Pathology Section, Cocoa Research Institute of Nigeria by dual culture technique; pathogen and the bio-agent at equal-distance, pathogen at periphery and pathogen at the centre respectively to determine their antagonistic effects and find out suitable management strategies. The results revealed that the selected bio-agents were able to inhibit the growth of the pathogen's mycelial significantly ($P = 0.05$) *A. niger* (31.87, 74.74, 80.12% respectively) and *T. viride* (25.38, 60.36, 90.48% respectively) and also reduce the mycelial extension of the pathogen significantly at the adopted dual culture techniques.

Keyword: antagonistic potential, bioagents, cashew, culture technique, Inflorescence blight

I. Introduction

Cashew (*Anacardium occidentale* L.) is a tree crop of considerable economic importance in Africa, Asia and Latin America. The tree produces a panicle inflorescence that bears male, female and hermaphrodite flowers [1]. Diseases and deterioration of environmental conditions often occur and result in serious economics losses [2].

Inflorescence blight disease caused by *Lasiodiplodia theobromae* is a major limiting factor affecting cashew nut production in Nigeria, causing 40-45% crop loss annually [3]; symptoms include withering of petals and other parts of the flower followed by a progressive dieback of the small peduncles from the tips and downward to the main floral shoots. The disease spreads through insects, which create wounds predisposing inflorescence axes to infection.

The pathogen, *L. theobromae* represents the asexual state of *Botryosphaeria rhodina* (Berk. & M.A. Curtis) Arx., with a worldwide distribution in tropical and subtropical regions and occurs on a very wide range of plants [4]. Hosts are mainly woody plants including fruit and tree crops. *Lasiodiplodia* species are common, especially in tropical and subtropical regions where they cause a variety of diseases [5].

Fungicides are still a primary means of controlling diseases; unfortunately some fungicides have lost their effectiveness because of development of resistance by plant pathogens [6]. Perceived negative effects of pesticides on agricultural land, water and soil pollution as well as other health problems have demanded that agricultural scientist pursue alternative controls that are more environmentally friendly, ecologically viable, medically safe and specific for controlling pathogens [7].

Therefore, biological control of plant pathogens has been considered as a potential control strategy in recent years and search for these biological agents is increasing [8].

Alternatively, antifungal agents produced by microorganisms may be use as biocontrol agent [9], as the materials based on microorganisms have properties such as: high specificity against target plant pathogens, easy degradability and low cost of mass production. Biological control offers an important alternative to synthetic chemicals.

Trichoderma is currently the most extensively researched biocontrol fungus and has been shown on a number of occasions to provide a protective effect against some fungi. There are however scarce information on its assessment as a bioagent potential to control inflorescence blight disease of cashew and hitherto for *A. niger* and even in the management of cashew diseases in Nigeria. Past research indicated that *Trichoderma* can parasitize fungal pathogens and produce antibiotics [10].

Research has been done on biological control potential of *Trichoderma* spp. against several pathogens attacking vegetables, fruits, field and industrial crops. *A. niger* has being scarcely used as a bioagent, however research findings has proof its status as a potential bioagent against some fungi.

Considering an array of chemical fungicides and botanicals trials as measures to control inflorescence blight disease of cashew in Nigeria of which appreciable level of successes have been reported, this preliminary investigation was to dive into the trend of global research in eco-friendly crop protection strategies against inflorescence blight disease of cashew as the level of use of antagonistic bio-agents on cashew diseases can not be substantiated in Nigeria.

The objective of this study was to initiate attempts to identify antagonistic bio-agents against *L. theobromae* the causal organism of cashew inflorescence blight disease in Nigeria.

II. Materials And Methods

Lasiodiplodia theobromae was isolated from inflorescences of cashew showing typical symptoms of inflorescence blight disease in Ibadan, Nigeria. It was microscopically identified on the basis of cultural and morphological characters. Pathogenicity of the isolate on cashew inflorescences was determined using the method of [11]. The isolate was maintained on acidified potato dextrose agar (PDA). Colony growth was observed and transferred to obtain pure culture.

Trichoderma viride and *Aspergillus niger* were tested *in-vitro* against *L. theobromae* in the laboratory of Plant Pathology Section, Cocoa Research Institute of Nigeria. The culture discs of 5mm in diameter of each of the test organisms and pathogen were cut aseptically from the colony of 6-day and 15-day old pure cultures respectively grown on PDA medium and kept at different positions according to the techniques employed for the study [12].

Culture discs of test organism and the pathogen were placed opposite each other at 4cm apart in the petriplate containing 20ml PDA and the antagonistic properties of the test bioagents were exhibited (dual culture technique). Pathogen at the periphery technique had the culture disc of the pathogen placed aseptically 4cm away radially from at four corners keeping one disc of the test organism at the centre in the petriplate. The third technique (pathogen at the centre) had the culture disc of the pathogen placed in the centre and four similar discs of the test organism were placed 4cm away from the pathogen at the periphery in the petriplate. The culture discs of the pathogen were kept at respective places of pathogen in each technique without bioagents served as control [12].

All treatments were incubated at room temperature (28±2°C) and the radial growth of the pathogen was measured after 6 days. There were three replications of the treatments, the percent growth inhibitions (PGI) was calculated as follows:

$$PGI = \frac{dc - dt}{dc} \times 100$$

where: Diameter of mycelial colony in control (dc),
Diameter of mycelial colony in treatments (dt),
Percent growth inhibition (PGI)

III. Results And Discussion

The antagonists tested *in-vitro* was significantly superior over control in the three techniques employed against *L. theobromae*. In the dual culture technique, *T. viride* and *A. niger* differ significantly with the control, however there was no significant difference between the test organisms. The percent growth inhibition was higher in *A. niger* (31.87%) while that of *T. viride* was 25.38%, with 52.80mm colony diameter of the pathogen in the former while the latter was 57.80% (table 1).

The findings were in harmony with those obtained by [13] which reported *T. viride* as an active bioagents reducing the radial growth of *Fusarium solani* by 5.2cm and reduced growth percentage by 57.8%. *T. viride* inhibited significantly highest growth of *Colletotrichum gloeosporioides* (15.33mm) and had percent growth inhibition of 60.69% [12]. [6] reported *T. harzianum*, and *T. viride* exhibited great zone of inhibition in dual culture *in-vitro* test against *L. theobromae* isolated from banana fruits.

A. niger also had the higher percent growth inhibition of 74.74% and reduced colony diameter of pathogen to 19.20mm which differ significantly from *T. viride* and the control with 30.13mm and 76.0mm respectively, and *T. viride* had 60.36% growth inhibition (TABLE 1).

This observation shows *A. niger* as more active bioagents strain against *L. theobromae* in pathogen at periphery technique. This differ from the earlier finding of [12], which reported *T. viride* as a more active bioagents with higher percent growth inhibition over *A. niger* in the pathogen at periphery test against *C. gloeosporioides* causing anthracnose of Indian bean. [14] in his early findings reported the antagonistic effect of *T. viride*, *T. harzianum* and *A. niger* against *C. gloeosporioides*.

IV. TABLE

Table 1: Efficacy of antagonist bioagents against *L. theobromae* in-vitro

Test organism	Colony diameter of Pathogen (mm)*	Percent growth inhibition (%)
Pathogen and BCA at equidistant Technique		
<i>Trichoderma viride</i>	57.83a**	25.38
<i>Aspergillus niger</i>	52.80a	31.87
Control	77.50b	0
LSD	7.31	
Pathogen at Periphery Technique		
<i>Trichoderma viride</i>	30.13b	60.36
<i>Aspergillus niger</i>	19.20a	74.74
Control	76.0c	0
LSD	3.12	
Pathogen at Centre Technique		
<i>Trichoderma viride</i>	8.0a	90.48
<i>Aspergillus niger</i>	16.7b	80.12
Control	84.0c	0
LSD	1.37	

*Each value is the mean of three replicates

**Means followed by the same letter in the same column are not significantly different according to LSD (5%)

[8] also reported *T. viride* showed maximum inhibition of 71.41% over control in *Rhizoctonia solani* (from tomato), and best antagonist inhibiting 67.91 and 66.21% over control in *Sclerotium rolfsii* (collar rot of tomato) and *Sclerotinia sclerotiorum* (web blight of beans) in dual culture test respectively. In the pathogen at the centre technique, *T. viride* treatment had 8.0mm pathogen diameter which differ significantly with *A. niger* and control, and had 90.48% growth inhibition. *A. niger* also differ significantly from the control reducing pathogen colony to 16.7mm (table 1).

A. niger, *T. viride*, *T. harzianum*, *T. longibrachyatum*, *B. subtilis* and *P. fluorescence* were strong and potent antagonists against *C. gloeosporioide* [15]. It was observed from the study that the antagonists tested by three different techniques were effective against *L. theobromae* and may be of helpful use as potential bioagents.

V. Conclusion

Depending on the technique adopted, each of *T. viride* and *A. niger* proved to be highly antagonistic. However, *A. niger* had a higher antagonistic bioagent potential against *L. theobromae* on cashew inflorescence in two of the three techniques employed in this study. Further researches could unfold exploiting this potential of *A. niger* on many pathogens and managing its adverse effects on crops.

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