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Potential of *Beauveria bassiana* and *Metarhizium anisopliae* as Biological Management Agents of *Phytolyma fusca* (Hemiptera, Psylloidea)

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Abstract
Iroko gall bug *Phytolyma fusca* (Walker) is a major insect pest of *Milicia excelsa* militating against *Milicia excelsa* plantation establishment in West Africa. The efficacy of *Beauveria bassiana* (Bals.) Vuill. strain Bb S41 and *Metarhizium anisopliae* (Metsch.) Sorokin strains F123 and IC30 against adult *P. fusca* was evaluated at the pathology laboratory of Nigerian Institute of Oil Palm Research (NIFOR) Benin City, Nigeria using conidia suspension. Using Direct Contamination Assay method, 1g conidia of each fungal strain was applied to petri dishes containing ten newly emerged adult insects and no treatment was applied to control. Each treatment was replicated three times in a complete randomized design (CRD) and adult mortality was recorded daily for five days. All the treatments evaluated were effective against adult *P. fusca* under laboratory conditions. However, *M. anisopliae* strain IC30 was more effective than other strains giving 100% mortality of adult *P. fusca* at four days post-inoculation. *M. anisopliae* strain F123 recorded 100% mortality at five days post-inoculation; while *B. bassiana* strain Bb S41 recorded 90% mortality at five days post-inoculation and only 23% mortality was observed on the control experiment. The pathogenicity test results showed that only *B. bassiana* was pathogenic to adult *P. fusca* by forming mycosis on the cadaver of the insects. *Metarhizium anisopliae* and *B. bassiana* strains evaluated have shown great potential for biological control of *P. fusca,* hence, their use could be incorporated in the integrated pest management of *P. fusca* for sustainable establishment of *Milicia excelsa* plantation in the region.

Keywords: Milicia excelsa, entomopathogenic, mortality, iroko, pathogenicity

Introduction

Milicia excelsa (family; Moraceae) popularly called Iroko, is among the most economical hard wood species and among the most useful tree species found in indigenous rain forest of tropical Africa which is resistant to termites, timber borers; (organism causing decay and aquatic worms). *M. excelsa* has good working properties while the lumber and veneer are highly sort for construction works and decorations (Alder, 1989). There are no established iroko plantations as they grow in natural forest at a rate that is not sustainable and this is as a result of infestation of the plant by the gall forming insect; *Phytolyma species* which causes dieback of shoots after gall formation that results in the destruction of seedlings (Ofori *et al*., 2001). The phytolyma species attacking *M. excelsa* in Nigeria was recently confirmed to be *Phytolyma fusca* through morphormetric and molecular characterization study (Ugwu *et al*., 2019).

Phytolyma fusca (iroko gall bug) has seven life stages; eggs, five instars and adult (Ugwu and Omoloye, 2014), it usually attacks young leaves and form galls at the point followed by the rupturing of the adults which then lay numerous eggs on the young leaves, shoot and stem of the plant. The eggs then hatched into the first instar (nymph) which punctures the surface of the plant resulting in the formation of galls that engulfs the nymph. The gall burst at maturity and the adult bugs are released on the apical parts of the affected plant giving room for saprophytic fungal infection that finally causes dieback of terminal shoots. *P. fusca* infestation of *M. excelsa* results in stunted growth that affects the stem size and shape, reduction in regeneration rate and also affects the accumulation of biomass in the plant (Cobbinaah and Wagner, 1995; Agyeman *et al*., 2009) hence the need to control the iroko bug.

Entomopathogenic fungi (EPF) are excellent candidates

for use in biological control because they do not need to be ingested to cause infection rather they infect their hosts by contact and then direct penetration of the cuticle (Wright *et al.,* 2002). EPF are important in the regulation of insect populations in nature and numerous of the isolates of entomopathogenic fungi have been tested for their potential to control insect pests (Charnley, 2007). An attractive alternate method to chemical pesticides is these microbial biocontrol agents which are the natural enemies devastating the pest population with no hazardous effects on human health and the environment as reported by Khan *et al.* (2012). The authors, also reported that EPF has an important position among all the biocontrol agents because of its route of pathogenicity, broad host range and its ability to control both sap sucking pests such as mosquitoes and aphids as well as pests with chewing mouthparts.

The entomopathogens; *Metarhizium anisopliae* and *Beauveria bassiana* are entomopathogenic fungi that are present in soils throughout the world (Zimmermann, 1993) and have been used extensively as biocontrol agent against many insect pest of plants (Alean *et al*., 2004, Jaramillo *et al*., 2005, Amnuaykanjanasin *et al*., 2013). *Metarhizium* species also known as green muscardine fungus have long been recognized for their biological control potential against arthropod (Bischoff *et al*., 2009). Infections of arthropods by *Metarhizium* species are easily recognized a few days after death, when the fungus grows out of the arthropod integument and forms reproductive structures. Initially only fungal hyphae that appear white is seen, but as conidia form and mature they often take on a characteristic olive green colour however, depending on the species and strain of *Metarhizium*, spores can range in colour from white to yellow to brown and green (Tanada and Kaya, 1993). The insect disease caused by *B. bassiana* is called white muscardine disease. When the microscopic spores of the fungus come into contact with the body of an insect host, they germinate, penetrate the cuticle, and grow inside, killing the insect within a matter of days. Afterwards, a white mold emerges from the cadaver and produces new spores (Barbarin *et al*., 2012).

Furthermore, EPF has a unique mode of infection in which insect is infected through the cuticle. *B. bassiana* and *M. anisopliae* penetrate the proteineous cuticular barrier of insects (Ranga, 2011) and proliferate throughout the host thereby resulting in death of infected insect. The cuticle penetration is as a result of combined action of mechanical force and the enzymatic action of those enzymes secreted by the fungus. Such enzymes include proteases, chitinases, lipases and lipoxygenases which break down the cuticle, offering nutrients to the fungus (Lubeck *et al*., 2008; Boldo *et al.,* 2009 and Mustafa and Kaur, 2009). The pathogenecity of these entomopathogens against the pupa, larva and adult *Coelaemenodera elaeidis* in oil palm has been reported by Eziashi, (2017). According to Mwamburi, (2021). *B. bassiana* and *M. anisopliae* were effective against fall armyworm (*Spodoptera frugiperda*). Similarly, the biocidal ability of *B. bassiana* with selected botanicals against *Bactrocera dorsalis* (oriental fruit fly) was reported by Ugwu and Nwaokolo, (2020). This study therefore evaluated the potential of one strain of *B. bassiana* and two strains of *M. anisopliae* against *P. fusca* under laboratory conditions.

Materials and Methods

Collection of adult Phytolyma fusca

Matured galls were collected from infested young *Melicia excelsa* plant growing on the students *Gmelina arborea* demonstration plot of Federal College of Forestry Ibadan, Oyo state Nigeria.The Federal College of Forestry Ibadan is located at latitude 7° and 9° N longitude 3° and 58° E of Greenwich Meridian Time (GMT) with annual rainfall of 1300 to 1500 mm and relative humidity of 80 to 85% average (FCF, 2021). The galls were kept in a plastic cage and transported to the Nigerian Institute of Oil Palm Research (NIFOR) for bioassay. The mature galls ruptures to release adult *P. fusca* which was used for the bioassay (Plate 1A)

Collection of fungal isolates and bioassay

The stock culture of *Beaveria bassiana* strain Bb S41 and *Metahizium anisopliae stains* F123 and IC30 were collected from Nigerian Institute for Oil Palm Research (NIFOR) Benin City, Nigeria. The bioassay of the fungal isolates against adult *P. fusca w*as also carried out at the pathology laboratory of (NIFOR) using spore powder of the fungal strains.

Mortality Test

The mortality test was conducted on newly emerged adult *P. fusca* from the ruptured *M. excelsa* galls following Eziashi *et al*. (2017) method of Direct Contamination assay, one gram (1g) conidia of each strain was applied to the center of plastic Petri dishes containing sterilized moistened Whatman filter paper (No: 1) using a sterilized spatula. Ten newly emerged adult insects were added to the treated Petri dishes and no treatment was applied on control. The plates were covered with muslin clothes to prevent the escape of the insects and also to give room for aeration before covering them with their lids. Each treatment was replicated three times in a complete randomized design (CRD) including control. Adult mortality was recorded daily for five days.

Pathogenicity Test

To ascertain that insect mortality was caused by the entomopathogens, a pathogenicity test was carried out. Dead insects from each plate were transferred to the center of separate plates lined with sterile moistened Whatman No 1 filter paper and left at room temperature for possible colonization.

Statistical Data Analysis

The data obtained for five days were subjected to Analysis of Variance (ANOVA). Significant means were separated by Tukey's Honestly Significant Difference at 5% level of significance using ASSISTAT statistical software 7.6 beta.

Results and Discussion

The lethal effect of fungal strains on adult Phytolyma fusca

All the fungal strains evaluated were toxic to adult *P. fusca* and caused mortality at varied rates after inoculation (Table 1). The IC30 strain of *M. anisopliae* outperformed the other strains from the first day to the fourth day after inoculation by causing higher mortality of adult *P. fusca*. *Phytolyma fusca* treated with *M. anisopliae* recorded the highest mortality rates from day one to day four with mean values of 3.33, 6.67, 10 .00 and 10.00 respectively. *M. anisopliae* (F123) and *B. bassiana* strain (BbS41) recorded the highest mortality on the 5th day with a mean value of 10.00. The Bb S41 strain recorded the least mortality among the isolates from day one to day three with mean mortality rates of 1.33, 3.00 and 4.67 respectively. The control trial recorded the least mortality among the treatments from day one to day four. The mortality observed on day four (1.00) and day five (1.33) for control could be attributed to nutritional stress. Furthermore, there were significant differences ($P< 0.05$; $P<0.01$) among the treatments on the mean mortality rates of *P. fusca* recorded on days two, three, four and five. However, there were no significant differences ($P>0.05$) in the mean mortality rate of *P. fusca* among the treatments on day one. The total percentage mortality of *P. fusca* after five days of inoculation ranged from 7.76% - 100% mortality (Figure1.). The two *Metahizium anisopliae* strains (F.123, and IC30) recorded 100% mortality at five days post inoculation while *B. bassiana* strain (Bb S41) recorded 90% *P. fusca* mortality at five days post-inoculation. Only about 8% mortality was observed in the control experiment. *M. anisopliae* strain IC30 recorded 100% mortality at four days post inoculation indicating that is the most active isolate against *P. fusca*. among others. This result agrees with the report of Eziashi, *et al.* (2017) that hundred percent mortality of adult *Coelaemenodera elaeidis* of oil palm treated with 2.5 g (2.5×10^{11}) of *M. anisopliae* recorded hundred percent mortality on the 4th post-incubation day while *C. elaeidis* treated with 2.5 g (2.5×10^{10}) of *B. bassiana* recorded 100% mortality on the 6th post-incubation day. The results of our study also corroborate the earlier study by Erler and Ozgur Ates (2015) who reported that *B. bassiana* strain PPRI 5339 and *M. anisopliae* strain F52, were effective against *Polyphylla fullo* (June beetle) larvae. Mwamburi, (2021) also reported the effectiveness of *B. bassiana* and *M. anisopliae* on fall armyworms. Similarly, Ugwu and Nwaokolo (2020) reported that *B. bassiana* was effective against *Bactrocera dorsalis* pupating larvae causing about 83% mortality. Moreover, Munoz (2000) also reported mortality levels between 20 and 98.7% when 16 strains of *B. bassiana* were evaluated against *C. capitata* adults. In addition, Swiergiel *et al.* (2016) submitted that the commercial strain *B. bassiana* isolate was lethal to apple sawflies under laboratory conditions.

Furthermore, *M. anisopliae and B. bassiana* strains have been successfully used to control different insect pests under field conditions (Puterka 1999; Lababidi 2002; Lacey *et al.*, 2001, 2011). The efficacy of *M. anisopliae* and *B. bassiana* strains could have been influenced by the prevailing environmental conditions during the study. Environmental factors like temperature, humidity and sunlight play intense role on field persistence of entomopathogenic fungi (EPF) especially at the commencement of their growth, sporulation, and infection to the cuticle of host insect pests and this period lasts 3 to 8 h for many EPF (Vidal and Fargues 2007).

Pathogenicity of the Beauveria bassiana and Metahizium anisopliae to adult P. fusca

The results of the pathogenicity showed white mycelia growth (mycosis) on the insect cadaver after 3-5 days of incubation indicating that the strains were pathogenic to *P. fusca* (Plate 2a-2b). Only *B. bassiana (*Bb S41) strain exhibited pathogenic effects on *P. fusca* while the *M. anisopliae* strains and control did not show any pathogenicity to the *P. fusca* cadaver. The mycosis started on the body of *P. fusca* on the 3rd day after inoculation and progresses till the 5th day. On the fifth day full mycosis was observed on the cadaver of the *P. fusca.* The pathogenicity of entomopathogens (*B. bassiana)* in the insect could be attributed to their ability to penetrate the insect body through mechanical pressure and the action of a mixture of enzymes (chitinases, proteases and lipases) which dissolves the insect cuticle (Lubeck *et al*., 2008; Boldo *et al.,* 2009 and Mustafa and Kaur, 2009).

Conclusion

Based on the results of this study, it was concluded that all the three entomopathogenic fungi isolates evaluated were all effective against adult *P. fusca* under laboratory conditions. *M. anisopliae* strain IC30 proved more toxic to adult *P. fusca* than F 123 and Bb S41. Only *B. bassiana (*Bb S41) strain exhibited pathogenic effects on *P. fusca* by forming mycosis on the cadaver of the *P. fusca* from three to five days post inoculation. The use of the evaluated *M. anisopliae* and *B. bassiana* strains could provide viable alternative biocontrol for *P. fusca* and could be incorporated in the integrated pest management of *P. fusca* for sustainable establishment of *M. excelsa* plantations in the region.

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1A

1B

Plates 1A: Rupturing Milicia excelsa gall and 1B; newly emerged adult P. fusca

Table 1: Sequential mean mortality rate of adult *Phytolyma fusca* treated with *B. bassiana* and *M. anisopliae* (Days)

Treatment	1	2	3	4	5
B. bassiana					
Bb S41	1.33ab	3.00ab	4.67b	8.00ab	10.00a
M. anisopliae					
F 123	1.67ab	6.00a	6.33ab	6.00b	10.00a
IC30	3..33a	6..67a	10.00a	10.00a	0.00c
Control	0.00b	0.00b	0.00c	1.00c	1.33b
Significant level	Ns	*	**	**	**

Means on the same column having different letters are significantly different (P≤0.05) according to Tukey's Honestly Significant Difference (HSD)

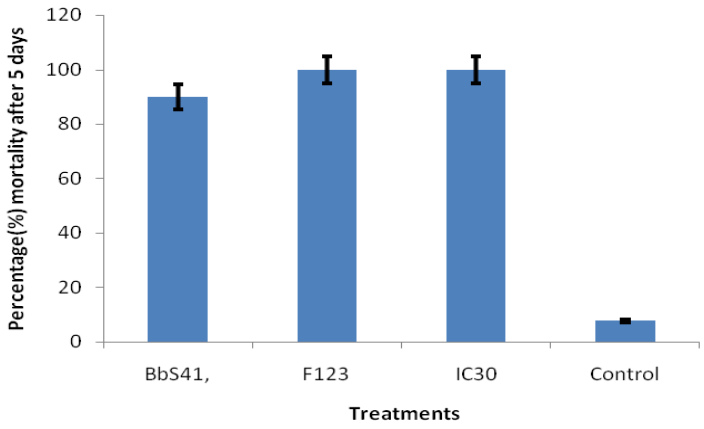

Figure 1. The total percentage mortality of *P. fusca* after 5 days

Plate 2a: 3days post death

Plate 2b: 4days post death

Plate 2c: 5days post death

Plate 2d: 6days post death (full mycosis)