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Fungal Isolates from Banana Weevils (*Cosmopolites sordidus***) Cadaver as a Pest Control Option**

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ABSTRACT:

Synthetic pesticides are routinely used to control banana weevil Cosmopolites sordidus, a pest that causes 40 to 70% annual yield losses of Musa species. Biological control agents, including entomopathogenic fungi, are appropriate alternatives to synthetic pesticides. This study, evaluated Curvularia senegalensis, Fusarium verticillioides, Fusarium oxysporum species complex and Beauveria bassiana on eggs, instar stage two larvae and adult C. sordidus. Bioassays evaluated egg hatch inhibition, larvae, and adults mortality in response to 10^6 and 10^7 m⁻¹ conidia suspension applications of each strain. Plant protection potential in terms of rhizome injury reduction was evaluated by incorporating 10^7 ml⁻¹ conidia in soil potted plants at day 1 and 14 of the experiment and releasing weevils in a reciprocal manner at days 14 and 1, respectively. Both C. senegalensis and F. verticillioides at 10^7 ml⁻¹ conidia resulted in > 80 % egg hatch inhibition and larvae mortality, respectively, and >55% adult mortality. In contrast, to control, fungal treated plants had >50% peripheral and internal damage reduction. Additionally, a significant relationship between the dead weevils and rhizome injury accounted for 46 % tissue damage variance. In conclusion, all fungal strains, particularly Curvularia senegalensis, showed potentials as entomopathogen when applied on sorghum prior to weevil infestation.

Keywords: Biocontrol; entomopathogenic fungi; fungal virulence; rhizome damage

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INTRODUCTION

The banana weevil *Cosmopolites sordidus* Germar is one of the most serious pests within the Musaceae family, affecting plantain (*Musa x paradisiaca*), Abyssinian banana (*Ensete ventricosum*) and highland banana (*Musa* species) in tropics and sub-tropics. The organism of 10 to 16 mm length whose origin being Indo-Malaysia, causes annual yield losses between 40 to 70 % in banana and plantain growing regions of sub-Saharan Africa (Gold et al., 2003; Speijer et al., 2001). *C. sordidus* are characterised by four developmental stages, *viz* egg, larva, pupa and adult. *C. sordidus* eggs laid into notches created at the collar of the plant rhizome above the ground level hatch into larvae. Damages due to feeding larvae on the rhizome affect root development, nutrient and water uptake, plant stability during windy weather and death of plants during heavy damage (Gold and Messiaen, 2000). The adults are free living (*i.e.* not confined to host plant), negatively phototropic, thigmotactic, strongly hygrotropic, gregarious and displays death mimicry, and to a small extent shown to vector *Xanthomonas campestris* pv. *musacearum* in bananas (un published). *C. sordidus* exhibit restricted feeding habit, with adults feeding on dead plant material, while larvae feed and develop mainly on corms and pseudostem (Kiggundu, 2000). *C. sordidus* life cycle is between 2 to 4 years and adults can last for 180 days without food which undermines most conventional control methods.

Frequent applications of non-systemic insecticides in the soil around the plant mat are commonly used to

control adult weevils, but resistance among weevil populations is increasingly observed on organophosphates such as prothiofos, chlorpyrifos, pirimiphos-ethyl and ethoprophos) (Collins et al., 1991). Moreover, deposited eggs and developing larvae inside the plant tissue are not targeted. Integrated Pest Management (IPM) has long been advocated for, because its intervention goes beyond pest control, environmentally sound and suppresses the pests through the use of a wide variety of technological and management practices. Example of IPM program against *C. sordidus* include but not limited to the following; *Beauveria bassiana* and pseudostem traps (Nankinga, 1999), *B. bassiana* and aggregated pheromone traps (Tinzaara et al., 2007), Fallow and pheromone mass trapping (Rhino et al., 2010) among others. Consequently, integrating biological control options, based on applications of natural enemies such as entomopathogens (fungi, bacteria, and protozoa), predators or parasitoids might be appropriate alternatives to the use of chemical pesticides (Lazreg et al., 2007; Roy and Cottrell, 2008).

Pest suppressive soils often contain entomopathogens, which prevent insect pests from reaching a critical threshold despite favorable environmental conditions (Vegaa et al., 2009). Soil fungi are possibly the most adaptable infectious biocontrol agents as they are self-propagating, grow on a wide range of hosts and infect different insect developmental stages (Ali-shtayeh et al., 2003). And because of a wide host range, fungi like *Curvularia* have species that may exists as endophytes epiphytes, saprophytes and as pathogen (Manamgoda et al., 2015), but yet to be reported as an entomopathogen. Nonetheless *Fusarium* and *Beauveria* are reported as endophytes as well as entomopathogens (Ochieno, 2010). In this study, three fungal isolates from *Cosmopolites sordidus* cadavers were identified in Ghana and evaluated along with the known *Beauveria bassiana* isolate IMI372439: *Curvularia senegalensis, Fusarium verticillioides* and *Fusarium oxysporum* species complex. The objective of this study was to test in laboratory assays the susceptibility of different developmental stages of *C. sordidus* to fungal contamination and to investigate in pot experiments whether soils inoculated with various fungal conidia result in lower survival rates of eggs, larvae and adults for reducing plantain rhizome damage.

II. MATERIALS AND METHODS

2.1. Fungal strain isolation and identification

Fungal isolates from the species C. senegalensis (Cs), F. verticillioides (Fv) and Fusarium oxysporum species complex (FOSC) were obtained from banana weevil cadaver collected from the plantain fields at the Crops Research Institute (CRI) in Kumasi, Ghana (6° 41' 0" North - 1° 37' 0" West). Fungal strains were determined following the morphological identification key described by (Mathur and Kongsdal, 2003). For confirming their identity, fungal cultures were sent to the Leibniz Institut Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany), where strains were verified by sequencing rDNA internal transcribed spacer (ITS) and rDNA large subunit (LSU) fragments (Schoch et al., 2012) using standard fungal primers (https://sites.duke.edu/vilgalyslab/r PCR; >ITS1F (forward) CTTGGTCATTTAGAGGAAGTAA and >LR5 (reverse) dna primers for fung) for TCCTGAGGGAAACTTCG, and sequencing; >ITS4 TCCTCCGCTTATTGATATGC and >LR4 ACCAGAGTTTCCTCTGG (both reverse). Obtained rDNA-ITS/ LSU sequences were confirmed by databases Westerdijk Fungal Biodiversity Institute (http://www.wi.knaw.nl/), NCBI BLAST (https://www.ncbi.nlm.nih.gov/) and MycoBank (http://www.mycobank.org/). The fungal isolate B. bassiana (Bb; IMI number 372439) from the culture collection of the Centre for Agriculture and Biosciences International (CABI) was selected as a positive standard because of its virulence to C. sordidus (Nankinga and Moore, 2000; Prabhavathi and Ghosh, 2014). All fungal isolates were cultured on fresh potato dextrose agar (PDA) at 27 ±2 °C in the darkroom (Kaushal and Singh, 2010).

2.2. Inoculum production

For inoculum production, 14-day-old sporulation cultures were flooded with sterile water, containing 0.05 % Tween 20, and conidia were gently dislodged using a sterile L-shaped glass rod. The conidia suspension was calculated by counting conidia with a Neubauer haemocytometer (BS.74B, Weber, England) under a bright field microscope (Leica Microsystems DM500, Wetzlar, Germany). The conidia suspension was diluted as appropriate with sterile water, containing 0.05 % Tween 20, and used for inoculation experiments and as a starter kit for mass production on a

sorghum (*Sorghum vulgare*) carrier as recommended by Sahayaraj and Namasivayam, 2008. Conidia produced on a carrier are more tolerant to the adverse environmental condition than conidia produced in liquid media (Mahassneh and Ali-shtayeh, 1999). The carrier was prepared by washing and soaking whole grain sorghum in water overnight. Excess water was then drained and grains were dried to attain on average 50 % moisture content. The sorghum was placed in flasks and autoclaved at 121 °C and 1034.21 hPa for 30 min. After cooling, conidia suspension at 109 ml-1 was added under a laminar flow hood. Flasks were incubated at 27 ±2 °C for 15 days and shaken daily to evenly distribute the inoculum, to avoid clumping and to guarantee uniform fungal development throughout the carrier substrate. After incubation, subsamples were suspended in sterile water with 0.05 % Tween 20 and subsequently filtered with a double-layered cheese cloth. The conidia filtrate was used to determine the conidia density per gram of colonized sorghum using the haemocytometer.

2.3. Banana weevil collection and rearing

Adult weevils were trapped according to Ogenga-Latigo and Bakyalire (1993) from infested plantations nearby the CRI. Weevils were maintained on weekly cut plantain rhizomes inside 10 L plastic buckets that were closed with perforated lids for aeration. Buckets were kept in the laboratory at ambient temperature. This procedure ensured an adequate supply of eggs, larvae and adult weevils for experiments.

2.4. Laboratory bioassay

Based on preliminary experiments, conidia suspensions of 106 and 107 ml-1 were selected for infection studies using different C. sordidus developmental stages. Twenty brown spotted weevil eggs (3-day-old), twenty larvae of instar stage two (5-day-old) and twenty unsexed adult weevils were dipped into the two inoculums of each fungus, respectively, while controls were treated with distilled water with 0.05 % Tween 20. The whole experiment was replicated three times. Treated adult weevils were put in 250 ml flasks, corked with cotton plug, while eggs and larvae were separately kept on petri dishes with moistened filter paper. Larvae and adult weevils were supplied with freshly cut rhizome pieces and all specimens were incubated at 27 ±2 °C in the darkroom for up to 10 days. The numbers of unhatched eggs, dead larvae and dead adult weevils were counted daily from the 4th day of incubation and percentage values were calculated as proposed by (Zeng et al., 2010). To confirm the infectivity of fungal isolates (Bos, 1981), about 5 specimens with visual signs of mycosis from each replicate were surface sterilized with 70% ethanol (1 min) and 1 % sodium hypochlorite (2 min) and subsequently rinsed with sterile water. After blotting off excess water, specimens were placed on moistened filter paper in petri dishes that were sealed with parafilm. Incubation was at 27 ±2 °C in the darkroom for up to two weeks until mycosis developed and confirming microscopically (Amscope B490B-3M-digital microscope-US) that mortality was due to respective fungal infection. Microscopically re-isolated conidia from weevil eggs, larvae and adults that died as result of respective fugal treatments, were further used to infect different weevil growth stages in attempt of confirming Koch's postulate.

2.5. Plant material

Sword suckers of the plantain cultivar 'Apantu' were grown in 10 L plastic pots filled with sterile peat soil. Potted plants were placed 60 cm from each other under an outdoor shade structure that transmitted approximately 60 % of incident light. Plants were weekly fertilized with 5 g nutrient mixture of 23 % N, 10 % P2O5 and 10 % K2O for three months prior to treatment application and, in absence of rainfall, sufficiently watered. Ninety plants with uniform growth and with at least six fully expanded green leaves were selected for the experiment.

2.6. Experimental design of pot experiments

Experimental plants were assigned in a randomized complete block design to five blocks and eighteen treatments. Within each block, one plant was subjected to one of the following treatments: inoculation with four fungal strains (*Cs, Fv, Bb, FOSC*), each with conidia applications of 20 ml of 10^7 ml⁻¹ suspension (SUS) and 20 to 25 g of sorghum carrier (SC) with conidia equivalent of 10^7 g⁻¹ at the commencement (1d) of the experiment and 14 days later (14 d). In addition, there were two control plants per block without fungal inoculation (UTC), one for each fungal

application time. Both conidia application types were incorporated in the soil just below the plant collar. Sexed weevils (Rukazambuga et al., 1998), four female and four male adults, were introduced to each pot at either 2 weeks after fungal inoculation at 1 d or 2 weeks prior fungal inoculation at 14 d, ensuring that weevils were exposed to each fungus for 6 weeks until the end of the experiment at 8 weeks. Each pot was wrapped with insect netting to prevent the escape of the weevils.

2.7. Soil analysis

Soil samples at approximately 5 cm depth of each pot were taken fortnightly from 2 to 8 weeks after the commencement of the pot experiment to determine fungal density as number of spores per gram of soil, following the method described by (Smith and Skipper, 1979) and using the haemocytometer. Additional soil samples were taken at the commencement, half-way point and termination of the experiment, respectively, and analyzed for pH, organic carbon (Rousk et al., 2009), extractable phosphorus as it affects the growth and abundance of most fungi (Kleinman et al., 2002) and soil texture classes (Kovács et al., 2004) at the CRI.

2.8. Chlorophyll content

Chlorophyll content at the base, center and apex of the second youngest fully opened leaf of each plant was measured fortnightly using a chlorophyll meter (SPAD[™]-502, Minolta, Japan). The average indexed chlorophyll content of each measured plant was used for treatment comparison and the larger the index the greater the chlorophyll loss (Deol et al., 1997).

2.9. Rhizome damage

At the end of the experiment, weevil mortality (Henderson and TILTON, 1955) and the percent rhizome damage as caused by feeding larvae were determined. The latter included exposing peripheral and internal feeding tunnels and counting the total number of grids with damaged and intact rhizome tissue, respectively, by using a mesh wire (grid size 1 cm²) (Ogenga-Latigo and Bakyalire, 1993). Single grids were thereby visually evaluated by applying scores of one third, two third and one, according to the degree of damage level. Assessment of peripheral damage included wrapping the mesh wire around the pared rhizome, whereas assessment of internal damage consisted of two transverse cuts at 2 and 5 cm below the collar, respectively, and overlaying the mesh wire on both tissue surfaces to count and calculate the mean cross section damage. Percent total rhizome damage was then determined from peripheral and internal damage caused by *C. sordidus* (Ntonifor et al., 2006).

2.10. Statistical analysis

The data were analyzed using a generalized linear mixed model (GLIMMIX) and General Linear Model (GLM) procedure of SAS 9.4 (SAS Institute, Inc. Cary, NC, USA) (Gbur et al., 2012). The variables were percentage egg inhibition, larvae or adult weevil mortality, soil fungal spore density, SPAD index value and percentage of rhizome damage. GLIMMIX procedure assumed equal variance and was specified with binomial distribution for dependent variables and LS-means statement for the fixed effects and unbalanced data. GLM procedure considered multiple comparison for the p-value and confidence limits for the LS-mean differences. Results were graphically displayed using SigmaPlot 12.3 (Systat Software Inc, San Jose, CA, USA).

III. RESULTS

3.1. Identification of fungi

The rDNA sequences of Cs (Index 1) GenBank accession number MT476857.1, matched Cochliobolus geniculatus NCBI GenBank accession JN943416.1,Westerdijk Fungal Biodiversity Institute isolate number CBS149.71, the NCBI GenBank accession number HG779001 and the MycoBank number MB296254 with a possible synonym of C. geniculate (Tracy and Earle) Boedijn (MycoBank 265873) (Madrid et al., 2014). The rDNA sequences of Fv (GenBank MT476859) (Index 2) also matched (100%) the CBS576.78 while FOSC rDNA sequences (GenBank MT476858) (Index 3)

matched (99%) a number of forma specialis that form the complex (clades) such as Fusarium oxysporum f. sp. vasinfectum, f. sp. apii, f. sp. rhois, f. sp. nicotianae etc. However, FOS f. sp. cubense (Laurence et al., 2014) which causes vascular wilt disease in banana was not observed.

3.2. Laboratory bioassays

All All fungal strains inhibited significantly egg hatching of *C. sordidus* when compared to the control treatment (Fig. 1a). Unlike for *Bb*, the two conidia suspension used for each fungal treatment did inhibit differentially egg hatching. The percentage of egg inhibition was over 80% for both *Cs* and *Fv* and below 50% for *Bb* and *FOSC* (Fig. 1a). All fungal strains induced significantly greater mortality of *C. sordidus* instar stage 2 larvae compared to the untreated control (Fig. 1b). At both conidia suspensions, high percentages of larvae mortality of above 75% were recorded for *Cs* and *Fv*. In contrast, larvae mortality was below 40% for *Bb* and *FOSC*, except for *Bb* applied at 10^7 ml^{-1} conidia suspension with a treatment effect that was similar to that of *Cs* and *Fv*, respectively (Fig. 1b). Adult weevil mortality was between 35 to 65% across all fungal treatments and zero for the control (Fig. 1c). For *Cs* and *Fv*, weevil mortality was significantly greater in the higher conidia suspension, an effect that was not seen for *Bb* and *FOSC* (Figure. 1c)..

3.3. Re-infection and re-isolation of the fungi

To confirm the infectivity of all fungal isolates, re-incubation of surface sterilized fungal inhibited eggs as well as dead larvae and adult weevils, respectively, resulted in death of re-infected weevil growth stages and considerable mycelia growth on surface sterilized cadavers of the respective fungal strain (Figure. 2.).

Moreover, the degree of fungal induced mycoses tended to increase for all strains from 6 to 12 days of incubation. The microscopic characterization of conidia (Figure. 3) from mycotic re-infected weevil eggs, larvae and adults, respectively, confirmed that mortality of C. sordidus was due to the infection of the respective fungal strain (Meyer et al., 2008; Muerrle et al., 2006) of pathogen re-isolation.

3.4. Pot experiment

Soil characteristics. The soil used for the experiment was a sandy loam in a moderately acidic pH range (5.5 to 6.0), a concentration of extractable P ranging between 116.4 to 489.5 ppm and an organic carbon content of about 1.6%. Soil properties represent the three samples taken at the start, halfway through and at the end of the experiment.

Soil spore density. The number of fungal spores per gram of soil increased continuously for all fungal strains throughout the experimental period of 8 weeks (Figure. 4). However, conidia of each fungus was more abundant in SC at all sampling times than what was observed in SUS. Irrespective of fungal strain, the SC-1d treatment had with 4 to 7 billion spores per gram soil the highest spore density at the end of the 8-week experimental period. All other fungal treatments reached a final spore density of below 3.5 billion spores per gram soil, except SC-14d and SUS-14d for Cs.

3.5. Chlorophyll SPAD values

In general, neither the fungal strain nor the fungal application time in relation to the introduction of weevils had any effect on the SPAD index of young 'Apantu' leaves. In contrast, the method of conidia application significantly affected the SPAD index for example at week 6 and 8 of sampling. 'Apantu' plants treated with SUS tended to have greener leaves than those treated with SC (Figure. 5).

3.6. Rhizome damage

'Apantu' plants treated with either of the four fungal strains had only half of the rhizome injury compared to the untreated controls that had more than 40 % tissue damage (Fig. 6a). Plants treated with *Cs* and *Bb* were about 50 % less damaged by *C. sordidus* than those treated with *Fv* and and *FOSC* (Fig. 6b). While the method of fungal application did not significantly affect the rhizome damage caused by feeding larvae, plants treated 2 weeks before weevils were introduced had less damage than those treated 2 weeks after weevil exposure (Fig. 6a). *C. sordidus* caused less peripheral than internal damage (Fig. 6b; Fig. 7).

Fungal mycelia growth, observed in the larvae feeding tunnels (Figure. 7), likely caused larvae mortality and thus resulted into reduced rhizome damage in treated plants.

There was a significant ($p \le 0.001$) relationship between the number of dead weevils and rhizome injury, accounting for about 46 % of the variance in tissue damage (Figure. 8).

IV. DISCUSSION

4.1. Identification of fungi

Fungal taxonomy is complicated due to considerable morphological and physiological variation and the limited number of morphological markers, but renders important for a range of reasons such as for example the development of bio-pesticides. The isolates were initially identified up to genus level by microscopic evaluation of colony color and conidia morphology and consequently classified to *Dothideomycetes* (*Cs*) and *Sordariomycetes* (*Fv* and *F. oxysporum*). Both genera have a wide host range and geographical distribution (Manamgoda et al., 2015, 2012). To identify the isolates to species level, molecular data were used such as the rDNA internal transcribed spacer (ITS) and rDNA large subunit (LSU) sequence. The rDNA ITS/LSU sequences were then compared to sequences in various databases with a 99-100 % match. The rDNA ITS is commonly used, because it is regarded as a DNA barcoding marker for the identification of fungi to species level (Raja et al., 2017). Since the rDNA ITS could not separate *Fusarium oxysporum* species complex into its members (clades), multi-locus sequence typing (MLST) approach is proposed. *Cs* and *Fv* are classified as biosecurity level (BSL) 1, while *FOSC* members to BSL 1+ (Hoog et al., 2019), both not considered dangerous organism according to the German Committee on Biological Agents.

4.2. Treatment effects on eggs, larvae and adult weevils

Entomopathogenic isolates from a host organism are typically of great virulence that leads to some level of damage (Liu et al., 2002). Of the three fungal strains isolated from weevil cadaver, Cs and Fv were more virulent against the different life stages of C. sordidus than Bb and FOSC (Figs.1, 2). Such variation in the virulence among fungal isolates has often been reported (Alshareef and Robson, 2014; Valero-jiménez et al., 2014) and can be compensated for to some extent with increasing conidia concentration as has been demonstrated in the current study (Fig. 1c). The pathogen's application time in relation to the developmental stage of the pest will also affect the degree of disease severity. For example, the physiochemical properties of the egg, larvae or adult cuticle (Grizanova et al., 2019; Inglis et al., 2001) may affect conidia adhesion and viability. The observed rapid germination and hyphal growth of Fv spores was also reported on Melodogyne graminicola, a namatode that damages rice roots (Le et al., 2016) and Tropidacris collaris, a grasshopper pest that consumes all plant material (Pelizza et al., 2011), a fungal characteristic that may be related to a relatively high infectivity rate. After spore attachment and germination on the host surface, the fungus penetrates through the insect cuticle, joints or creases where the insect's protective covering is thinner, with either germ tubes or an appressorium formation to infect the weevil host (St Leger and Wang, 2010). The application of adhesive fungal suspensions may lead to an improved pathogen virulence towards insect pests with hard to penetrate cuticles. In agreement with (Njau et al., 2011), the mortality of adult weevils was generally lower than that of eggs and larvae, a variation that could be attributable to differences in the composition and thickness of the cuticle for the various stages of the weevil life cycle. After the death of the weevil, the fungus grew out through the exoskeleton and began to produce spores.

4.3. Soil properties and spore density

The major soil properties that affect optimum fungal germination and multiplication are pH, available phosphorus and organic carbon, which were all within the recommended range (Miranda and Harris, 1994; Rath et al., 1992; Tawaraya et al., 1996). However, fungi with a slow growth pattern on infected cadaver such as *Cs* rely on insect carbohydrates rather than soil carbon (Vegaa et al., 2009). The observed differences in soil spore density among fungal treatments are likely more dependent on timing and method of fungal application rather than on soil properties. For example, the number of spores per gram of soil was much greater when conidia was applied on

sorghum (Fig. 4) that presumably supplied carbon and nutrient required for fungal multiplication (Zahmatkesh et al., 2017). However, the number of spores in the soil did not correlate well with fungal infectivity as for example *Fv* with the greatest soil spore density (Fig. 4) was not most effective againist weevil damage (Fig. 6b).

4.4. Treatment effects on potted plants

Treatment effects on potted plants. The weevil *C. sordidus* affects Musa and Ensete species with stunted growth, leaf yellowing, loss of roots and eventually premature death (Okolle et al., 2009); however, plants have few or no symptoms when treated with appropriate infectious biological control agents. Entomopathogenic endophytic fungi not only target the various life stages of *C. sordidus* but also grow into tunnels (galleries) within the corm (Fig. 7) where they can infect feeding pathogens. Despite mycelia growth in larvae feeding tunnels, fusarium wilt disease and corm discoloration symptoms were not observed. Optimum temperature for most entomopathogens to grow, infect and cause disease to the insect pest host is between 15-30°C (Ansari et al., 2014; Inglis et al., 2001). The results here indicate that applications of fungal strains prior to weevil infestation is a preferred option for managing crop damage.

V. CONCLUSIONS

In this study we were able to ascertain that isolates of *Cs*, *Fv* and *FOSC* are pathogenic to *C. sordidus* both in laboratory and pot experiments. All strains, but in particular *Cs*, show promising potentials as entomopathogenic fungi when applied on sorghum to plantain plants prior to weevil infestation. They can then self-propagate and survive in moderately acidic soils with adequate levels of organic carbon to infect and cause mortality to *C. sordidus*. However, further work is needed to test their biological potential on field plants and whether their efficacy is sufficient enough to provide crop protection against weevil damage. Entomopathogenic fungi are effective pest control options to include in IPM for *C. sordidus* while reducing the reliance of chemical pesticides and their associated risk of pesticide resistance and contribute to sustainable management practices for *Musa* spp.

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Figure legends:

Figure 1 Effect of fungal treatments (*Cs* - *Curvularia senegalensis*, *Fv* - *Fusarium verticillioides*, *Bb* - *Beauveria bassiana*, *FOSC* - *Fusarium oxysporum* species complex) with 10^6 and 10^7 ml⁻¹ conidia suspensions, respectively, compared to an untreated control (UTC) on mean percent inhibition of egg hatching (a), larvae (instar stage 2) mortality (b) and adult weevil mortality (c) of *C. sordidus*. Vertical bars indicate standard error of the means (3 replicates, each with n=20) and fungal strains at the two conidia suspension with no significant difference (p ≤ 0.05) are indicated by same letters.

Figure 2 Re-growth of *C. senegalensis, F. verticillioides, B. bassiana* and *Fusarium oxysporum* species complex (*FOSC*) on surface sterilized, previously with the same fungal strains inhibited eggs as well as dead larvae and adult weevils, respectively, after 6 and 12 days of re-incubation.

Figure 3 Re-isolated conidia of *C. senegalensis* (a), *F. verticillioides* (b), *B. bassiana* (c) and *Fusarium oxysporum* species complex (*FOSC*) (d) from weevil (*C. sordidus*) cadavers. Conidia magnification is 100 X.

Figure 4 Mean number of spores per gram soil of at (a) 2-weeks, (b) 4-weeks, (c) 6-weeks and (d) 8-weeks post inoculation with *C. senegalensis* (*Cs*), *F. verticillioides* (*Fv*), *B. bassiana* (*Bb*) and *Fusarium oxysporum* species complex (*FOSC*) applied at 20 ml of 10^7 ml^{-1} suspension (SUS) or 20 to 25 g of sorghum carrier (SC) with conidia equivalent of 10^7 g^{-1} at the beginning (1d; weevils introduced 2 weeks later) of the experiment or 14 days later (14 d; weevils introduced 2 weeks earlier). Vertical bars indicate standard error of the means (n = 5) and treatment effects at each sampling time with no significant difference (p ≤ 0.05) are indicated by same letters respectively.

Figure 5 Mean SPAD index of the second youngest fully opened leaf (average of records at the base, centre and apex) of the plantain cultivar 'Apantu' at (a) 2-weeks, (b) 4-weeks, (c) 6-weeks and (d) 8-weeks of exposure to weevils (*C. sordidus*). Weevil damage was not controlled (UTC) or controlled by the fungal strains *C. senegalensis* (*Cs*), *F. verticillioides* (*Fv*), *B. bassiana* (*Bb*)and *Fusarium oxysporum* species complex (*FOSC*) applied at 20 ml of 10^7 ml⁻¹ suspension (SUS) or 20 to 25 g of sorghum carrier (SC) with conidia equivalent of 10^7 g⁻¹ at the beginning (1d; weevils introduced 2 weeks later) of the experiment or 14 days later (14 d; weevils introduced 2 weeks earlier), respectively. Vertical bars indicate standard error of the means (n=5) and treatment effects at each sampling time with no significant difference (p ≤ 0.05) are indicated by same letters.

Figure 6 Mean percent rhizome damage of plantain, cultivar 'Apantu', after 6 weeks of exposure to weevils (*C. sordidus*): a) fungal application effects, (b) overall fungal effect on total, peripheral and internal damage. Weevil damage was not controlled (UTC) or controlled by the fungal strains *C. senegalensis* (*Cs*), *F. verticillioides* (*Fv*), *B. bassiana* (*Bb*) and *Fusarium oxysporum* species complex (*FOSC*) applied at 20 ml of 10^7 ml⁻¹ suspension (SUS) or 20 to 25 g of sorghum carrier (SC) at the beginning (1d; weevils introduced 2 weeks later) of the experiment or 14 days later (14 d; weevils introduced 2 weeks earlier), respectively. Vertical bars indicate standard error of the means (n=5) and treatments with no significant difference (p ≤ 0.05) to untreated controls are indicated by same letters.

Figure 7 Weevil (*C. sordidus*) damage on 'Apantu' rhizomes: Pared rhizome with peripheral larvae tunneling (a); mycelia growth in larvae feeding tunnels marked with red circles (b).

Figure 8 Relationship between total number of dead weevil (n=90) and rhizome damage. Equation of the linear relationship is y = -4.1x + 45.3. $R^2 = 0.46$. Confidence limits are indicated by the dash lines.



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Indexes:

Index 1

> MT476857 [Curvularia senegalensis]

Index 2

> MT476859 [Fusarium verticillioides]

Index 3

>MT476858 [Fusarium oxysporum species complex]