Entomopathogenicity Potentials of Molds Isolated from Soil and Plant Debris on Comparative Mortality of Bean Weevil (*Acanthoscelides Obtectus*)

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ABSTRACT

Entomopathogenic fungi, with their eco-friendly nature, have emerged as promising biocontrol agents for pest management. This study explores the entomopathogenic potential of various isolates, including *Aspergillus niger, Aspergillus flavus, Rhizomucor spp., Fusarium spp.,* and *Aspergillus terreus,* obtained from soil and plant debris. Confirming their entomopathogenic properties through morphological characteristics, the study aligns with previous research findings on *Fusarium spp.* and *Aspergillus sp.* isolation from diverse sources. In entomopathogenic assays against the Beans Weevil, all tested isolates exhibited efficacy, with *Rhizomucor* spp. demonstrating rapid and sustained mortality rates, reaching 100% within the initial day. *Fusarium spp*. displayed a cumulative impact, reaching full efficacy by the fourth day. *Aspergillus terreus* and *Aspergillus flavus* exhibited robust potential, achieving 100% mortality by the fourth day, while *Aspergillus niger* displayed slightly delayed onset but reached full efficacy by the third day. These results parallel findings on the effectiveness of *Aspergillus* species against various insects. Comparative analysis against conventional insecticides revealed entomopathogenic fungi's superior efficacy, with faster mortality rates than different concentrations of the insecticide. The water control group exhibited a gradual weevil mortality rate, emphasizing the natural course of mortality. The insecticide treatments, even at lower concentrations, consistently outperformed the water control group, highlighting the nuanced relationship between insecticide concentration and efficacy.

Key words: Entomopathogenicity, Fungi, Soil, Comparative mortality, Bean weevil

INTRODUCTION

The Bean Weevil, *Acanthoscelides obtectus* (Say) commonly known as bruchids is a significant pest of legumes, especially beans, (Rugumamu, 2014: Nta *et al.*,2019). They are light olive coloured and mottled with dark brown or grey reddish legs. They are widely distributed in almost all continents. The bean weevils are granivores, seen in stored products and typically infest various kinds of bean species particularly the species *Phaseolus vulgaris,* where they live for most of their lives inside the seed feeding on the seeds and leaving perfectly round holes in the beans. This reduces the food value as well as the germination potential of the seeds (Edet *et al.*, 2011), infestation of beans by *A. obtectus* begins in the field in drying beans pods and continues in the stored beans. They cause major losses in the field before harvest as well as in storage and has been classified as a primary pest with status 4, "destructive pest – a major pest rapidly capable of extensive damage"(Kljajić *et al.*, 2022). Seed losses can range from 7–40%, which means 1.59–9.12 million tons of damaged seeds each year in the world caused by bruchid. This equates to a loss of 1.59–9.12 million tons each year in the world (Mbogo *et al.*, 2009).

Chemical control is the most common practice of managing bean weevils, (Sağlam *et al.,* 2022), which is traditionally achieved with pyrethroids, organophosphates, and the fumigant phosphine (PH_3) and other synthetic insecticides (Lopes *et al.*, 2018). The increased use of chemical pesticide in the control of vector filthy insects has prompted some concern over the adverse effect of these chemical substances on living organisms and their environment. The harmful nature of these synthetic chemical pesticides leads to the inhibition of actions of enzymes and blocking of essential processes in organisms. There is increasing use of harmful organophosphates such as Actellic dust and Sniper (2, 2 dichlorovinyl-dimethyl phosphate) in the preservation of beans. Ideally, a pesticide must be lethal to the target species, but not to non-target species, including man, but unfortunately, this is not the case, hence the controversy and problem (Okafor *et al.,* 2019).

Entomopathogenic microorganisms such us fungi, bacteria, viruses, and protozoa are among the most promising alternatives to chemical insect control because of their high efficacy and compatibility (Mantzoukas *et al.*, 2022).

Numerous fungal microorganisms are pathogenic for many insect species and are able to control their natural populations by limiting their spread (Islam *et al.*, 2021). *Aspergillus niger, Aspergillus flavus, Rhizomucor spp, Fusaruim spp and Aspergillus terreus* are group of fungi, have been reported to efficiently control insects, demonstrating attributes such as high mortality rates, swift action, and abundant sporulation, which are valuable in insect control for agricultural purposes (Da Silva Santos *et al.*, 2020).

These species are generally considered soil borne fungi because of their abundance in the soil and their frequent association with plant roots as parasites or saprophytes. These species have usually been reported as plant pathogens causing serious diseases on many plant species (e.g., vascular wilts on a wide range of horticultural crops, crown rot and root rot diseases on many crops, head blight on cereal grain and other plant species) However, some of these species may act as weak to virulent entomopathogens and may live as saprophyte on dead insects (Batta, 2012).

An increasing number of studies demonstrating the potential of little known and explored *Fusarium species* as biological control agents of insects, *Fusarium avenaceum* was one of the entomopathogenic species of Fusarium that has been isolated from a number of insect species, and its pathogenicity to insects has been demonstrated in few species, such as greenhouse whitefly, *Trialeurodes vaporariorum* pruce budworm, *Choristoneura fumiferana*, and wheat stem sawfly, *Cephus cinctus*, However, no reports are yet available on the entomopathogenic activity of any *Fusarium species* against The Bean Weevil, *Acanthoscelides obtectus* This research study focuses on emphasizing the ecological and sustainable attributes of entomopathogenic fungi as effective alternatives for comparative Beans Weevil mortality and for a sustainable insect pest management.

MATERIALS AND METHODS

Sample Collection

Various samples were obtained from diverse sources, including soil, plant debris, and fruits was done for *Fusarium species* isolation. Selection criteria prioritized isolates with documented entomopathogenicity or recognized insecticidal properties. One hundred and fifty (150) adult Beans Weevils were collected from infested bean storage facilities to ensure a diverse representation of weight, life cycle developmental stage (age) and sex.

Isolation, Characterization and Identification of Entomopathogenic Fungi

Small portions of the fruit tissues harboring the spoilage organisms and connecting healthy tissue were surface disinfected by cleaning with cotton wool soaked in 0.1% silver nitrate solution. The excess disinfectant was then blotted out with sterile cotton wool. With the aid of a sterile knife and forceps the spoilt fruits portions were obtained and plated aseptically onto Sabouraud dextrose agar (SDA) in duplicates (replicate-plating). Incubations were done at room temperature until there were visible growths on the agar plates. The developing fungi were further purified by sub culturing onto freshly prepared SDA plates. Preliminary fungal characterization were done by studying the cultural characteristics and the slide culture technique for evaluating the fungal microscopic features with reference to the Manual of Fungal Atlases according to Frey *et al*. (1997); Barnett and Hunter, (2000); Watanabe, (2002) and Ellis *et al*. (2007).

Fungal Identification

This was done based on the description of the gross morphological appearance of fungal colonies on the SDA culture medium and the modified slide culture technique using lactophenol cotton blue stain for the microscopic evaluation under X10 and X40 magnification of the microscope.

Entomopathogenicity Assay

Selected number (15) of the weevil was introduced into petri-dishes containing pure culture of the fungal isolates and was incubated at room temperature for a 5 day period to monitor the kinetics of the death rate by entomopathogenicity. A control set-up was done using 10-folds serial dilution of a commercially sold pesticide (kill all) at concentration 0.1, 0.01, 0.001, 0.0001 and 0.00001 at the same incubation parameters.

Safety Considerations

Safety protocols for handling fungal cultures were strictly adhered to, including the use of personal protective equipment (PPE) and work within designated laboratory areas.

RESULTS AND DISCUSSION

Table 1. Assay of various fungi including *Fusarium spp* on efficacy as entomopathogen showing mortality rate of Weevil within the period of 5 days against the isolates

DAYS	*Aspergillus niger*	*Aspergillus flavus*	*Rhizomucor spp*	*Fusaruim spp*	*Aspergillus terreus*
1	3	4	15	3	7
2	15	12	15	11	15
3	15	15	15	12	15
4	15	15	15	15	15
5	15	15	15	15	15

Number of Weevil plated for the assay = 15

Table 2: Mortality Response of Beans Weevils to (control) Water and Various Concentrations of Conventional Insecticide over Time This table presents the mortality response of beans weevils subjected to different treatments, including water and various concentrations of a conventional insecticide, over a twelve-day period. The numerical values represent the number of deceased weevils at each respective concentration and time point.

DAYS	Water	0.1	0.01	0.001	0.0001	0.00001
1	6	14	14	3	0	2
2	6	15	15	4	5	4
3	8	15	15	6	10	7
4	8	15	15	13	10	7
5	11	15	15	15	10	8
6	13	15	15	15	10	10
7	13	15	15	15	13	13
8	14	15	15	15	14	14
9	14	15	15	15	14	14

10	14	15	15	15	14	14
11	15	15	15	15	15	14
12	15	15	15	15	15	15

Table 3: Colonial and Microscopic Identifications of the Various Fungi Isolates.

Colony morphology	**Microscopy**	**Identity**
Colonies were compact with white or yellow basal felt covered by a dense layer of dark-brown to black conidial heads.	Conidial heads are large (up to 3 mm by 15 to 20 µm in diameter), globose, dark brown, becoming radiate and tending to split into several loose columns with age. Conidiophore stipes are smooth-walled, hyaline or turning dark towards the vesicle. Conidial heads are biseriate with the phialides borne on brown, often septate metulae. Conidia are globose to subglobose (3.5-5 µm in diameter), dark brown to black and rough-walled	*Aspergillus niger*
Colonies are granular, flat, often with radial grooves, yellow at first but quickly becoming bright to dark yellow-green with age.	Conidial heads are typically radiate, later splitting to form loose columns (mostly 300-400 µm in diameter), biseriate but having some heads with phialides borne directly on the vesicle (uniseriate). Conidiophore stipes are hyaline and coarsely roughened, often more noticeable near the vesicle. Conidia are globose to subglobose (3-6 µm in diameter), pale green and conspicuously echinulate. Some strains produce brownish sclerotia.	*Aspergillus flavus*
Colonies are typically suede-like and cinnamon-buff to sand brown in colour with a yellow to deep dirty brown reverse.	Conidial heads are compact, columnar (up to 500 x 30-50 µm in diameter) and biseriate. Conidiophore stipes are hyaline and smooth-walled. Conidia are globose to ellipsoidal (1.52.5 µm in diameter), hyaline to slightly yellow and smooth-walled.	*Aspergillus terreus*
Colonies had rapid growth, 4.5cm in 4 days. Aerial mycelium was white to cream with orange-yellow pigmentation. Colour on the reverse side was dark-yellow. Colonies were incubated at 30^{o}C for 5 days.	Conidiophores were hyaline, simple, bearing spore masses at the apexes. As tall as the length of macroconidia by a few times. Conidia were hyaline and phialosporous. 3- 5 macroconidia were present, which were fusiform, cylindrical, moderately curved with an indinstinctly pedicellate foot cell and a short blunt apical cell, 28-42 x 4-6 µm. Microconidia were abundant, which were cylindrical to oval, one to two-celled borne on lateral phialides, 8-16 x 2-4.5µm. Chlamydospores were hyaline, globose, smooth to rough-walled, borne singly and in pairs on short lateral branches, 6- 10 µm.	*Fusarium solani*
Colonies are typically suede-like and cinnamon-buff to sand brown in	Sporangiophores are hyaline and mostly sympodially branched with long branches erect and shorter branches becoming circinate (recurved). Sporangia are spherical,	*Mucor* *circinelloides*

colour with a yellow to deep dirty brown reverse.	varying from 20-80 µm in diameter, with small sporangia often having a persistent sporangial wall. Columellae are spherical to ellipsoidal and are up to 50 µm in diameter. Sporangiospores are hyaline, smooth-walled, ellipsoidal, and 4.5-7 x 3.5- 5 µm in size. Chlamydospores are generally absent. Zygospores are only produced in crosses of compatible mating types and are reddishbrown to dark-brown, spherical with stellate spines, up to 100 µm in diameter and have equal to slightly unequal suspensor cells.	

Discussion

Entomopathogenic fungi are microorganisms that can infect and kill insects at different stages of their life-cycle. They have gained attention as potential biocontrol agents in pest management due to their environmentally more friendly nature. The effectiveness of entomopathogenic fungi depends on various factors, including the fungal species, application methods, environmental conditions, and the target pest species.

In this study, *Aspergillus niger, Aspergillus flavus, Rhizobium spp, Fusaruim spp* and *Aspergillus terreus* were isolated and characterized from soil, plant debris within Nnamdi Azikiwe University, Awka and screened for their entomopathogenic potentials against specific number of the Beans weevil (*Acanthoscelides obtectus*). The isolates were confirmed based on morphological and biochemical characteristics of the various isolates. The findings in this study are in accordance with Abrar *et al*. (2023) which isolated entomopathogenic *Fusarium spp* from soil and leaf samples in Manga Forest Pakistan. Yan *et al*. (2022) in in their study isolated *Aspergillus sp*. From plant debris Dong *et al*. (2023) also isolated *Fusarium spp*. From peach plant debris in China.

Table 1. Shows result the entomopathogenic assay of the various fungal isolates against beans weevil. The study reveals that all tested isolates, including *Aspergillus niger, Aspergillus flavus, Rhizomucor spp., Fusarium spp.*, and *Aspergillus terreus,* exhibit entomopathogenic efficacy against Beans Weevils. *Rhizomucor spp.,* exhibited rapid and sustained mortality rates, reaching 100% within the initial day (all 15 weevils died on the first day of exposure). *Fusarium spp*., consistent with its recognized entomopathogenic nature, displayed a gradual yet cumulative impact on Beans Weevils. Mortality rates steadily increased, reaching 100% by the fourth day. The observed pattern aligns with expectations, substantiating *Fusarium spp.'s* potential as a biocontrol agent.

Both *Aspergillus terreus* and *Aspergillus flavus* demonstrated robust entomopathogenic potential, achieving 100% mortality by the fourth day.

These *Aspergillus species* exhibited a rapid and sustained impact on Beans Weevils, indicating their efficacy in pest management strategies. *Aspergillus niger,* while effective, displayed a slightly delayed onset, with notable mortality observed by the second day and reaching 100% by the third day. This is consistent with the findings of Abrar *et al.* (2023) which showed that both fungi isolate (*Fusarium spp.)* were effective against *A. aegypti* population. Numerous studies have reported that *Aspergillus* species have high pathogenicity against different insects, indicating that the fungus has promising biological control potential in pest management (Ibarra-Cortes *et al.*, 2018; Zhang *et al.*, 2015; Lin *et al.*, 2021; Karthi *et al.*, 2018). Assessing the mortality patterns over time *Rhizomucor spp.* stands out as the most efficient entomopathogenic organism in achieving rapid and sustained mortality rates (at 100% mortality rate on the first).

Table 2 represents the results for the comparative control groups for this study. The table provides a comprehensive overview of the mortality response of beans weevils to varying insecticide concentration treatments, using the control (water) over a twelve-day period. The water-insecticide dependent control exhibited a gradual weevil mortality rate indicative by reduced mobility of the bean weevil. Conversely, the conventional insecticide at Concentration variations yielded diverse outcomes. The highest concentration (0.1) demonstrated indicated a rapid mortality rate, achieving complete weevil mortality by day 11. Similarly, the 0.01 concentration exhibited comparable effectiveness, reaching full weevil mortality by the same day. The 0.001 concentration, while displaying a delayed effect, eventually achieved complete weevil mortality by day 12. In contrast, the lower concentrations (0.0001 and 0.00001) exhibited a more gradual impact, with maximal mortality observed by day 15. This concentration dependent response highlights the nuanced relationship between insecticide concentration and efficacy.

Comparative Mortality analysis, between the control groups against the entomopathogenic fungi for entompathogenic efficacy against beans suggest Entomopathogenic fungi to possess a competitive efficacy against Beans weevils as compared to the carcinogenic conventional insecticide. This observation aligns with Douro *et al*. (2013) comparative study, which reported the efficacy of entomopathogenic fungi surpassing conventional insecticides in the management of cotton pests.

CONCLUSION

This study sheds light on the entomopathogenic potential of *Aspergillus niger, Aspergillus flavus, Rhizobium spp., Fusarium spp.,* and *Aspergillus terreus* against Beans Weevils. The findings underscore the efficacy of these fungi in exerting entomopathogenic activity, with Rhizobium spp. demonstrating exceptional efficiency by achieving rapid and sustained mortality rates. Comparative analysis with conventional insecticides reveals the superiority of entomopathogenic fungi, as they exhibit faster mortality rates than all concentrations of the insecticides. The study reaffirms the ecological and sustainable attributes of entomopathogenic fungi as viable alternatives for pest management.

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