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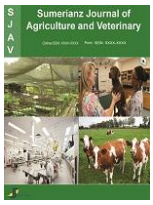

Original Article Open Access

Entomopathogenic Activity of *Metarhizium anisopliae* Against the Merchant Beetle, *Oryzaephilus mercator* (Fauvel): A Pest of Stored Date Palm Fruits

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Abstract

Date Palm faces serious challenges due to damage by insect pests, all of which reduce productivity. Attempts to control the insect pests have changed over time from conventional synthetic insecticides to natural control methods such as the use of biopesticides. The 50 adults of O. *mercator* were each inoculated with one of four different strains of *Metarhizium anisopliae* (Metsch) Sorok and *Beauveria bassiana* (F123, IC30, Biob and Bb). The set up was replicated four times. Mortality was recorded daily for 10 days in adults, mortality differed significantly ($P < 0.05$) within the days of exposure in adults. Biob was the most effective with adult mortality of 94.5% followed by F123, IC30 and Bb with 93%, 84% and 69% mortality respectively and the least mortality was recorded in the control with mortality rate of 40.5%. The dead adults *Oryzaephilus mercator* were placed in a controlled growth chamber to stimulate the development of fungal mycelia and confirm that the death was caused by infection of the M. anisopliae and B. bassiana isolates. The outgrowth of *Metarhizium anisopliae* and B. *bassiana* in all the treatments except control indicated that mortalities of *O. mercator* were actually caused by the isolates.

Keywords: *Entomopathogenicity; Oryzaephilus mercator*.

1. Introduction

Date palm (*Phoenix dactylifera*)**,** is a flowering plant in the palm family Arecaceae, cultivated for its edible sweet fruit [1]. *Phoenix dactylifera* grows 70–75 feet (21–23 m) in height, growing singly or forming a clump with several stems from a single root system. The leaves are 4–6 metres (13–20 ft) long, with spines on the petiole, and pinnate, with about 150 leaflets; the leaflets are 30 cm (12 in) long and 2 cm (0.79 in) wide.

Date palm products have been used for many years in feeding people as source of energy, nutrition, security, and as a healthy fruit. They are medically important plant.

Date palm leaves are used for Palm Sunday in the Christian religion. In North Africa, they are commonly used for making huts. Mature leaves are also made into mats, screens, baskets and fans. Processed leaves can be used for insulating board. Dried leaf petioles are a source of cellulose pulp, used for walking sticks, brooms, fishing floats and fuel. Date palm faces serious challenges ranging from diseases to damage by insect pests, all of which reduce productivity. The attempts to control insects have changed over time from chemicals to natural control methods, this is why the development of natural methods of insect control or biopesticides is preferred. This study focused on using biopesticide to adult instead of conventional synthetic insecticide which has been in use for years.

Merchant beetle (*Oryzaephilus mercator*) Coleopteran: Silvanidae are common pest species of date palm throughout the world [2]. They are opportunistic species, often travelling large distances associated with stored food stuffs and exploiting the opportunities provided by humans [3]. The insect feed on zUsing of entomopathogenic fungi in stored pest is now considered as one of the most promising alternatives to residual pesticides and fumigants. *Metarhizium anisopliae* is an entomopathogenic fungus with a worldwide distribution. It can be cultivated on both solid and liquid sterile media.

Chandler, *et al.* [4], pointed out that death by entomopathogenic fungi generally occurs in a period of 3 to 10 days after the infection, which is produced by water loss, nutrient deprivation, mechanical damage, and toxin action.

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Eziashi, *et al.* [5], pointed out that *M. anisopliae* and *B. bassiana* conidia (2.5 g of *Metarhizium* 2.5 x 10^{10} or 2.5g of *Beauveria* 10^{11}) concentration caused 100% mortality on adults *C. elaeidis*, on the sixth day. Addisu, *et al.* [6], stated that 1 x 109 *M. anisolpliae* caused more than 95% mortality for seven days on Macrotermes. The fungal-host relationship occurs through the adhesion and germination of conidia on the surface of the insect, followed by hyphae penetration through the cuticle. The process of host colonization initiates after penetration, with the penetrating hyphae becoming thicker and ramifying within the integument and the hemocoel of the insect, forming blastospores. The hyphae continue to grow and invade various internal organs after the death of the host and will subsequently emerge from the insect body and produce conidia that disseminate and infect other individuals [7]. The major drawbacks to the use of fungi for insect control are thought to be their poor stability in storage situations, and their high dependence, for efficacy, on climatic conditions in agricultural situations [8].

The aim of this study was to determine the effect of *metarhizium anisopliae* (biopesticide) on *Oryzaephilus mercator* adult.

2. Materials and Methods

The date palm fruits were introduced into sack bags and kept in Aluminum cages measuring 12 cm x 12 cm x 12 cm in Entomology Division laboratory NIFOR under room temperature. The cages legs were immersed in plastics filled with water to prevent natural enemies from entering into the cages to invade the date fruits kept in the cages.

The newly hatched adults and larvae were collected by cutting fresh date palm fruits into small pieces, peeling and shredding with the aid of razor blade. The collected adults and larvae were introduced into fresh date palm fruits from refrigerator at 4oc for 21 days in polythene bags to kill all hidden infestation because all the life stages are very sensitive to cold. 30 adults mixed sex of *Oryzaephilus mercator* were introduced into plastic containers measuring 4.5cm diameter by 2.5cm height and kept in Gallenkamp oven at 30°C. for 32 days for possible mass rearing into second filial generation for the work. Isolates of entomopathogenic fungi (*Meterhizium anisopliae*) were obtained from Pathologys Division of Nigerian Institute for Oil Palm Research (NIFOR) Benin.

The *metarhizium anisophae* and *Beauveria bassiana* isolates used were IC30, Bb, F123 and Biob stored in different plastic containers with tight lids and kept in a refrigerator at 4°C.

The plastic cages or containers used for the treatment were laden with whiteman paper cut to the size of the bottom of the plastic containers, 5.0g of each strain were weighed and 5.0g of quicker oat added into the container containing 50 adult beetles. The contents were gently shaken to ensure thorough admixture of adult beetles and the treatment powder, sprayed lightly with distilled water and covered with ventilated plastic lid.

The treatments were arranged in complete randomized block design and consist of 50 beetles (mixed sex) per replicate and the experiment was replicated four times. Four replicates of 50 adult beetles (200 adult *Oryzaephilus mercator*) were used for each treatment and the control. The plates of treated *Oryzaephilus mercator* were kept in the lab maintained at an average temperature of 28°C with range 26°C – 30°C and average ambient relative humidity range 92 – 96% relative humidity.

Observation of mortality was recorded on daily basis for ten days for adult. Dead adults from each treatment were kept in different containers for microscopic examination of the possible development of the hyphae and spore on the surface of the cadaver after incubated in humid chamber at 25°C to observe symptoms of mycosis. Microscopic preparations were carried out with the dead bodies to identify the fungus and check if it coincided with the initial isolate.

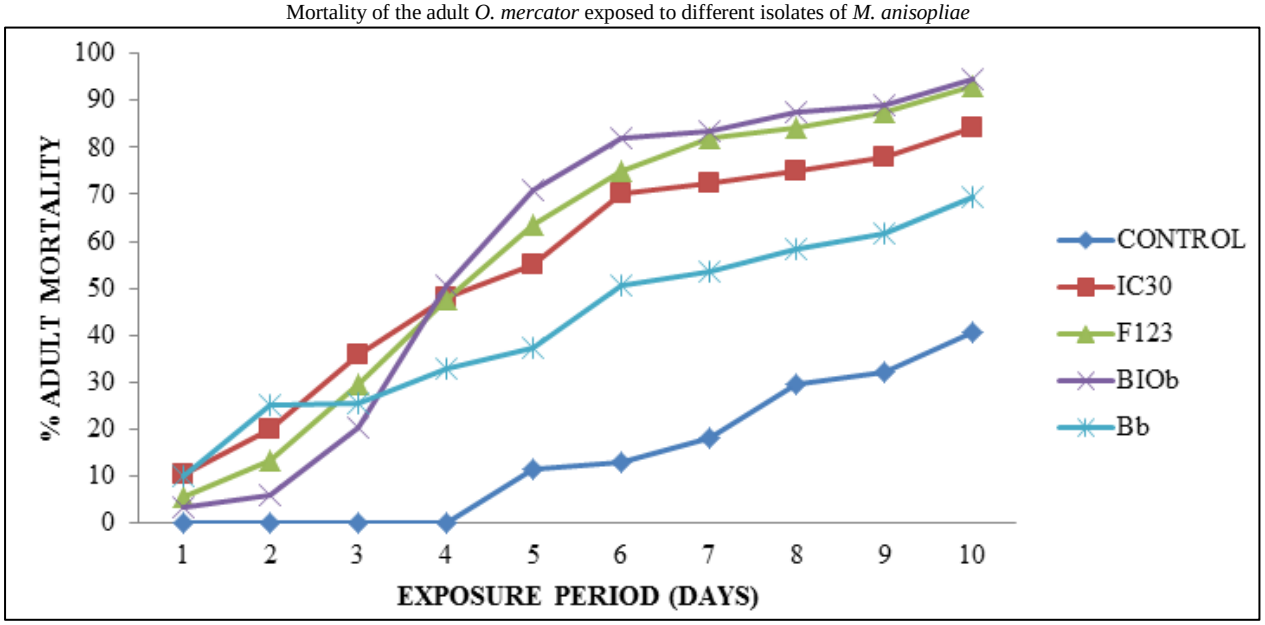
2.1. Statistical Analysis

Table-1. Mean cummulative percentage mortality (± SE) of *Oryzaphilus mercator* adults after exposure to *Meterhizium anisopliae*

		% Adult Beetle Mortality (Days post- treatment)											
Strain	Isolate conc. (conidia/ml)	1	2	3	4	5	6	7	8	9	10	F	sig
IC30	3 x 10^{-5}	10.5± 1.31^{A}	20± 3.03^{A}	36± 2.48^{B}	48 ± 2.35^{BC}	55 ± 2.33^{C}	70 ± 1.85^{D}	72.5± 1.65^{D}	75± 2.06^{D}	78± 2.20^{D}	84± 2.35^{D}	33.723	.000
F123	3 x 10^{-5}	5.5± 0.500^{A}	13.5 ± 2.25^{A}	29.5± 0.85^{B}	47.5 ± 3.35^{C}	63.5 ± 3.52^{D}	75 ± 2.38^{DE}	82± 1.29^{EF}	84± 1.15^{EF}	87.5± 1.65^{EF}	93± 1.50^{F}	58.695	.000
Bb	3 x 10^{-5}	10± 1.78^{A}	25± 6.51^{AB}	25.5± 6.75^{AB}	33 ± 6.44^{ABC}	37.5 ± 6.29^{ABCD}	50.5± 4.27^{BCD}	53.5± 4.15^{BCD}	58.5± 3.97^{CD}	61.5± 3.75^{CD}	69.5± 3.75^{D}	3.643	.004
Biob	3 x 10^{-5}	3.5± 0.63^{A}	6± 0.41^{A}	20.5 ± 3.57^{B}	50.5 ± 3.30^{C}	71 ± 1.85^{D}	82± 1.47^{DE}	83.5± 1.60^{EF}	87.5± 1.19^{EF}	89± 1.19^{EF}	94.5± 1.70^{G}	85.949	.000
CONT	0.00	0± 0.00^{A}	0± 0.00^{A}	0 ± 0.00^{A}	0 ± 0.00^{A}	11.5 ± 2.46^{AB}	13± 2.90^{AB}	18± 1.87^{ABC}	29.5± 4.61^{BC}	32± 5.82^{BC}	40.5± 9.42^{C}	3.557	.004

The same letters indicate non-significance differences while different letters in the same row indicate significant differences in regard to mean percentage mortality at p > 0.05 using student t-test (test), to test for standard error of mean (SEM)

Mortality of the adult *O. mercator* exposed to different isolates of *M. anisopliae*



3. Results

Pathogenicity tests revealed that *M. anisopliae* and *B. bassiana* were pathogenic to adults *O. mercator*. Death was observed on treated *O. mercator* using *M. anisopliae* from six to ten days after inoculation with different days of sporulation shown white mycelia growth. Treated and dead adults of *O. mercator* after three days of inoculation were observed with no external and visual sporulation of *M. anisopliae* and five days later they were observed with external and visual sporulation of white mycelia growth of *M. anisolpiae* (Plate 1A, B). The *O. mercator* adults treated with *M. anisopliae* also showed external and visual sporulation of white mycelia growth. Infection started from the *O. mercator* mandible through the thorax and abdomen. Both the *O. mercator* and its feed were covered with green colour called Green Muscardine disease and white colour called White Muscardine disease (Plate 2 A,B). Mortality of adults *O. Mercator*, as shown in table 1, started 24 hours after inoculation with isolate IC30, F123, Bb, and Bioblast except the control treatment without mortality. The treatment mean biopesticide differ significantly from one another at ($p < 0.05$) with treatment Biob having the highest mortality rate follow by treatment F123, IC30 and Bb respectively. Though there was no significant difference among FI23 and IC30 but these treatment effect were however different significantly ($p< 0.05$) from the control which had the lowest mortality rate. The mortality curve shown that immediately after inoculation into fresh medium, the population remains temporarily unchanged up to fourth days (lag phase) in control. The lag phase is also experienced in Biob between day 1 and day 2 while in Bb between day 2 and day 3, Other treatments show exponential phase of mortality (log phase) from day one to the 6th day of the study. The work indicates that bioactive formulation of all the entomopathagenic fungi used in this study showed mortality of adult *O. mercator* within a day of exposure. Highest percentage mortality was recorded within 10 days and the efficacy level with highest mortality was recorded in Biob followed by F123, IC30, Bb with percentage mortality of 94.5, 93.0, 84.0, and 69.5 respectively, the least percentage mortality of 40.5 was recorded in the control.

Pathogenicity of *Metarhizium anisopliae* and *Beauveria bassian*

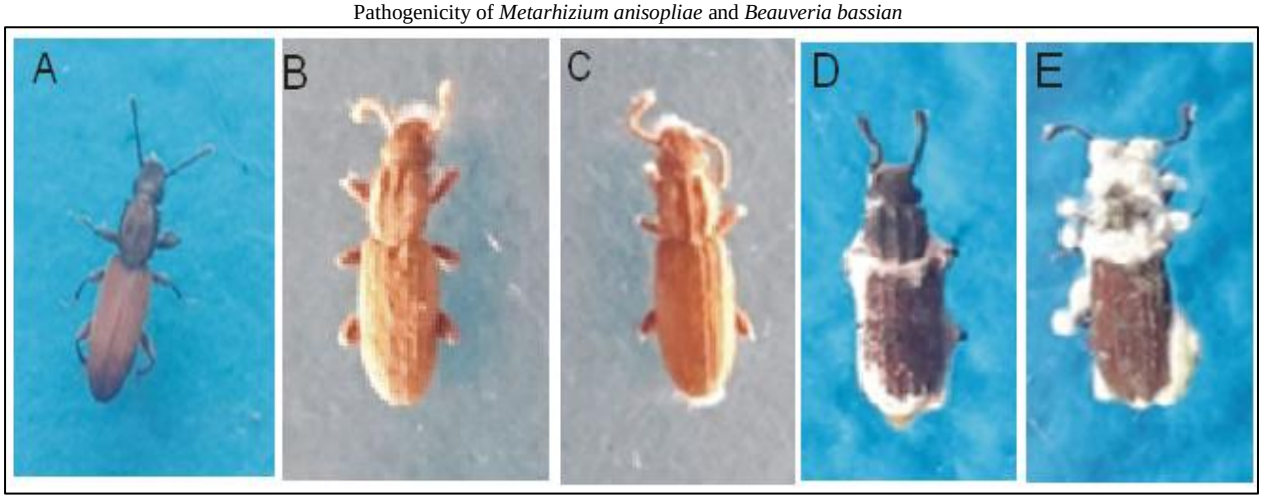


Plate 1. (A,B,C,D,E) Light Photographs of Adults *Oryzaephilus mercator* treated with *Metarhizium anisopliae*

A. Shows healthy and untreated adult *Oryzaephilus mercator* after ten days of incubation with no external and visual symptom at x40 magnification

B. Shows treated and death adult *Oryzaephilus mercator* after incubation with external and visual sporulation of white mycelia growth of *M. anisopliae* conidia. Infection started from the O. mercator mandible at x40 magnification.
C. Shows treated and death adult *Oryzaephilus mercator* after incubation with external and visual sporulation of white mycelia growth of *M. anisopliae* conidia. Infection started from the O. mercator mandible and anus (openings) at x40 magnification.
D. Shows treated and death adult *Oryzaephilus mercator* after incubation with external and visual sporulation of white mycelia growth of *M. anisopliae* conidia. Infection started from the mandible, thorax and down to the abdomen at x40 magnification.

Plate 2A: Shows treated and dead adult Oryzaephilus Mercator after 10 days of incubation with external and visual sporulation of white and green mycelia growth of *M. anisolpiae* conidia. Both the *O. mercator* and its feed were covered with green colour called Green Muscardine disease at x40 magnification

Plate-2B. Shows treated and dead adult *Oryzaephilus mercator* after 10 days of incubation with external and visual sporulation of white mycelia growth of *B. bassiana* conidia. Both the *O. mercator* and its feed were covered with white colour called White Muscardine disease at x40 magnification

4. Discussion

Entomopathogen infection can be accomplished in six steps that include; - adhesion of the fungal conidia to insect cuticle, - germination of conidia, - penetration of host cuticle, - defeating the host defense responses, - vegetative growth inside the insect body and - post mortal sporulation [9]. Each step is influenced by a range of integrated intrinsic and external factors, which ultimately determine the pathogenicity. [10] stated that adhesion is achieved through the secretion of mucilage. Invariably, if the entomopathogen fail to adhere to the insect body, that entomopathogen considered avirulent. After adhesion, the next factor for the virulence of a strain is the enzymes that hydrolyze the epidermis of the insect. The most important enzymes secreted by entomopathogenic fungi are lipases, proteases and chitinases, which are produced sequentially, reflecting the order of the substrates they encounter. Glinski and Buczek [11], point out that the hard, waterproof cuticle, biochemical activity of the juice of the middle intestine, and its peritrophic membranes, along with the tracheal system form a physiological mechanism and barriers that effectively protect the cavity of the body against invasion by fungi. The same result was obtained in this

study when mortality caused by different treatments in adult. Higher mortality was recorded in adult of different treatments as the days of exposure increase, this means that the enzyme that degrade the exoskeleton increases as the day increases hence making it possible for the fungi to infest *O. mercator*. Along with different degrading enzymes (such as lipase, protease, chitinase) which account for the virulence of different entomopathogenic fungi Fan, *et al.* [12], certain secondary metabolites of these fungi also possess insecticidal activities and contribute to the pathogenesis of the fungal strains [13]. Some metabolites may also act as the defensive tool by protecting the fungi from certain hostile factors such as competitive micro-organism [14].

The difference in the mortality of different strain of *M. anisopliae* used in this study may be as a result of difference in the degree of temperature tolerance by different strains. This is in line with the report of Dimbi, *et al.* [15], responses of *M. anisopliae* and *B. bassiana* isolates germination was seen at 25 and 30^0C where 30^0C was best for the radial growth of the colony. Other factors also contributed to the difference in the mortality of the different isolates used in this study other than temperature because the work of [16] and Milner, *et al.* [17], showed that the strains of *M. anisopliae* had better adaptability to tolerate high temperatures for germination. Wang and St. Leger [18], reported that the molecular mechanisms involved in the capacity of *M. anisopliae* to adhere to both insects and root. A study by Wang [19] examining genetic expression demonstrated that *M. anisopliae* could act as both a pathogen (growing on the cuticle and in the hemolymph of insect hosts) and a saprophyte in the rhizosphere (growing on the bean root exudates) therefore the use of *M. anisopliae* can serve both as control when the insects has infested the dates and also as preventive measure to reduce infestation. Wang and St. Leger [18], reported that the molecular mechanisms involved in the capacity of *M. anisopliae* to adhere to both insects and root. A study by Wang [19] examining genetic expression demonstrated that *M. anisopliae* could act as both a pathogen (growing on the cuticle and in the hemolymph of insect hosts) and a saprophyte in the rhizosphere (growing on the bean root exudates) therefore the use of *M. anisopliae* can serve both as control when the insects has infested the dates and also as preventive measure to reduce infestation This is in line with the report of Dimbi, *et al.* [15], that the ability of entomopathogen to tolerate different temperature

Profile not only varies between the strain but the themal tolerance between the isolate is also significant. Other factors also contributed to the difference in the mortality of the different isolates used in this study other than temperature because the work of [16] and Milner, *et al.* [17], showed that the strains of *M. anisopliae* had better adaptability to tolerate high temperatures for germination. Wang and St. Leger [18], reported that the molecular mechanisms involved in the capacity of *M. anisopliae* to adhere to both insects and root. A study by Wang [19], examining genetic expression demonstrated that *M. anisopliae* could act as both a pathogen (growing on the cuticle and in the hemolymph of insect hosts) and a saprophyte in the rhizosphere (growing on the bean root exudates) therefore the use of *M. anisopliae* can serve both as control when the insects has infested the dates and also as preventive measure to reduce infestation. Pathogenicity of entomopathogenic fungi *Beauveria bassiana*, *Beauveria bronaniartii* and *Metarhizium anisopliae* to adult *O. elegans.* The study which are detrimental to both man and environment. This study considers different isolates of *M. anisopliae* such as IC30, Biob, F123 and Bb. From the result of the study, IC30 achieved 100% mortality of larvae on the 3rd day of exposure while other treatments achieved 100% larval mortality on the 4th day of exposure. This means that IC30 is the most effective for the control of larval stage of *O. mercator*. When the same treatment was applied to the adults, none of the treatments achieved 100% adult mortality but highest mortality was recorded in Biob followed F123, IC30 and Bb respectively after 10 days of exposure. It was observed that Biob which has the highest mortality after 10days has the least between day 1 to day 3. This may be as a result of low production of the enzyme that will enhance the penetration of the entomopathagen on the insects. It was also observed that IC30 which was 2nd to the least effective recorded the highest adult mortality within the first 3 days of exposure. This implied that IC30 has higher tendency of early enzyme secretion when exposure. This is in line with report of [15] which stated that endoprotease enzymes are the most important facilitating factors for penetration of entomopathogenic fungi into the cuticle have been separate in different species. The out-growth of fungi on the cadaver of the insect as shown in plate 2 was an indication that mortality of the adult is as a result of entomopathogenic effect of *the isolates.* This is in line with the report of Alves [7], that the fungal-host relationship occurs through the adhesion and germination of conidia on the surface of the insect, followed by hyphae penetration through the cuticle and invade various internal organs. The major drawbacks to the use of fungi for insect control are thought to be their poor stability in storage situations, and their high dependence on climatic conditions in agricultural situations [8]. This problem was overcome in this study by applying it fresh after manufacture or stored under refrigeration for future use. This study showed that the isolates are very effective in control of adult *O. mercator.* Similar observation was made by Masoud [20], on the study of the pathogenicity of entomopathogenic fungi *Beauveria bassiana*, *Beauveria brongniartii* and *Metarhizium anisopliae* to adult *O. elegans*.

5. Conclusion

The findings show that *M. anisopilae* strains have potential for adoption as an Integrated Pest Management (IPM) tool. The beautiful thing about this isolates is that, they don't kill immediately rather slow in action as the fungus grows when it comes in contact with openings of the anterior and posterior regions such as the mouth and anus. As observed in this study, isolates of *M. anisopliae* and *Beauveria bassiana* are suitable to infect the *oryzerphilus mercator* in laboratory condition and has the potential to be developed as a microbial agent for controlling the pest.

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