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Effects of entomopathogenic fungi *Metarhizium anisopliae* in the control of *Melanacanthus scutellaris* (bean bug) and *Aedes aegypti* (mosquito) in Bauchi, Nigeria

[1]Deda Gayi Musa, [2*]Ahmad AbdulHameed, [3]Ahmad Jibrin Nayaya

[1,2,3]Department of Ecology,
Abubakar Tafawa Balewa University,
Bauchi,
Nigeria.

Email: dedagayikingsley@gmail.com

Abstract
There is an increase in the global interest for the use of biological control methods against insect pests. There is paucity of studies in the developing countries especially using indigenous fungal isolates as biocontrol agent. This study was undertaken to determine the pathogenicity and virulence of indigenous Metarhizium anisopliae against Aedes aegypti and Melanocanthus scutelari which are important pests causing human fever and destroying crops particularly bean. Metarhizium anisopliae was isolated locally from the soil and was tested against these insect pests in the laboratory using the following concentrations viz: 1×10^5 1×10^6, 1×10^7 and 1×10^8 conidia ml- for 14day. Both insects were highly sensitive to Metarhizium anisopliae with high percentage mortality. This study showed the potential of locally sourced Metarhizium anisopliae in the control of Aedes aegypti and Melanocanthus scutelaris

Keywords: *Aedes aegypti*, biological control, *Melanocanthus scutelari*, *Metarhizium anisopliae*, Insect pest

INTRODUCTION
Entomopathogenic fungi (EPF) are natural bioinsecticides of insect pests of agricultural importance. They infect and eventually kill insect pests within few days (Araújo and Hughes 2016; Yakubu *et al.*, 2022). This pathogenicity of Metarhizium helps reduce insect population and thus help in reducing crop loss (Sinha *et al.* 2016). Entomopathogenic fungi mode of actions shows some uniqueness from that of bacteria and viruses, it does not require ingestion, and rather they need contact action to invade their host directly through the cuticle.

Entomopathogenic Fungi are microorganisms that occur naturally within the environment. They have low toxic effect on mammals and general environment (Cox and Wilkin, 1996 Batta and Kavallieratos, 2018). Entomopathogenic fungi have evolved the potentials of developing on dead materials such as Cadavars, and so re-introducing more inoculum into the environment. With the normal practice of the conventional use of insecticides, entomopathogenic fungi have no long-term effect or persistence on the environment. (More *et al.*, 2000). Insects are a major cause of agricultural pest with others serving as vectors to

Author for Correspondence

parasite and to transmit different forms of diseases. Insect pathogens include entomopathogenic fungi, viruses, protozoa, bacteria and nematodes (Manzoukas *et al.,* 2019), may provide merits such as high efficacy and compatibility, with other form of integrated pest management (IPM) methods, therefore, they are highly considered to be one of the promising alternatives to chemical-based insect control (Rice and Cogburn, 1999; Schöller *et al.,* 2006; Shafighi *et al.,* 2014; Arora, 2015; Weinzierl and Higgins, 2019).

Recently, researchers have been exploring beneficial endophytic fungi with growth-promoting properties to find cost-effective and rapid approaches to enhance agricultural productivity and sustainability in areas that face environmental problems like salinity (Zhang *et al.,* 2016; Jaber, 2018; Dief *et al.,* 2021; Ali *et al.,* 2022; Anshu *et al.,* 2022; Chowdhury *et al.,* 2024). *Metarhizium anisopliae* (Metschn.) Sorokin and *Beauvaria bassiana* are entomopathogenic fungus that has been extensively studied in the field of biological control of insect pests (Chaudhary *et al.,* 2024; Peng *et al.,* 2022). Recent researches has indicated that *Metarhizium* spp. and *Beauveria* spp. exhibit a multifaceted survival strategy, relying not only on insect infestation for propagation but also demonstrating the ability to thrive as saprophytic and rhizosphere-competent fungi as well as establish themselves as plant endophytes (Chaudhary *et al.,* 2024). According to Kepler *et al.* (2015), this species has been included in a list of fungi that exhibit dual functionality, serving as both an entomopathogen and a promoter of plant growth. *Metarhizium* spp. and *Beauveria* spp. confer various advantages to a diverse range of host plants, such as alleviating stress conditions (Khan *et al.,* 2012; Chaudhary *et al.,* 2023) and enhancing plant growth (Liu *et al.,* 2022).

Mosquito borne diseases are a major tropical health challenge, world-wide (Jemberie *et al*., 2018). Anthropogenic activities in tropical and subtropical countries play a significant role in increasing number of mosquito breeding sites (Chareonsook *et al*., 1996; Jamberie *et al*., 2018; Zuharal *et al*., 2021). Consequently, a high proportion of people suffer from viral transmission, including Japanese encephalitis, dengue fever and yellow fever (Heddini *et al.,* 2007; Nagi *et al*., 2011; Chakravarti *et al.*, 2012) as well as the transmission of other pathogens causing diseases such as malaria and filariasis, (Ghosh *et al.,* 2012; Kundu *et al.*, 2013). Chemical pesticides are extensively used to control adult mosquitoes as well as immature stages in their breeding sites (Jemberie *et al*., 2018). However, owing to the negative impacts they exhibit to the environment most especially non-target community of organisms and also the development of resistance by the target species of the insect, the need for alternative measures is paramount to replace those chemicals pesticides. As an alternative, the utilization of potential entomopathogenic fungi in pest control is considered an eco-friendly approach (Jemberie *et al*., 2018). Furthermore, the recent increase in the use of conventional chemical pesticides has not only contributed to an increase in food production, but also has resulted in adverse effects on the environment and non-target organisms. In view of these side effects, the necessity for sustainable crop production through eco-friendly pest management technique is being largely felt in the recent times (Sable *et al*., 2019). This present study is therefore aimed at providing knowledge base to the biocontrol of *Melanacanthus scutellaris* (bean bug) common name "*Kwarin Wake"* and *Aedes aegypti (*mosquito*)* common name "*Soro"* using locally sourced *Metarhizium anisoplae*.

Area of study

The research was conducted in Bauchi the capital city of Bauchi State North-eastern. The state occupies a total land area of 499,119km^2 (18,965 sq ml) representing about 5.3% of Nigerian's total land mass and is located between latitudes 90^{0}3' and 12^{0}3' north and longitudes 8^{0}50 and 11^0 east.

Collection of samples

Bean Bug
Insects were collected from agricultural farm just around August/September and was transferred to Abubakar Tafawa Balewa University Bauchi Laboratory. Bioassay was then immediately carried out on the transported bean bug.

Mosquito rearing and Maintenance.
The eggs of *Aedes* mosquito were collected and cultured at the Abubakar Tafawa Balewa University Bauchi. The eggs were then kept in plastic container to hatch (Jamberie *et al.,* 2018). Larvae were fed with brewer's yeast. Second and Third instars larvae were transferred to 20 x 14 cm square plastic bowls covered with gauze in which they pupated and emerged as adults (Gosh *et al.,* 2011). Adults on the other hand were transferred to 30 cm diameter plastic cages and provided with a 10% sucrose solution (Jamberie *et al.,* 2018).

Laboratory procedures
Entomopathogenic fungi were obtained from the soil using *Galleiria mallonela* as a bait from soil samples collected from Abubakar Tafawa Balewa University Bauchi (ATBU), Gwallameji (GWJ), Kagadama (KDM), New Lecture theater (NLT) according to (Ali-Shayeh *et al.,* 2002). The samples were baited separately for 7 days, moisture contents of positive soil samples to determined dehydration in a Pasteur oven at 105^0C for 24 h according to (Savim *et al.,* 2010). The cells were cultured on Potato dextrose agar (PDA) to obtain full growth on the petri dish. The viability of conidia for each isolate was determined by inoculating them onto PDA and assessing the germination after 24 h of incubation at 25^0C and under 16 h photoperiod. (Savim *et al.,* 2010).

Test of viability of *Metarhizium anisoplae* spore
The viable conidia of the different strains were determined by sub-culturing the conidial suspension. The spore was uniformly spread on the surface area of the PDA plates by using L-shaped glass rod. After inoculation, plates were then covered with sterilized cover slips, sealed with paraffin, and stored in a temperature-controlled incubator for 20-24 h (Yiang *et al.,* 2019). Percentage germination of spores was examined after the incubation period. A count was conducted on each plate using 16_X magnification under the microscope (Jamberie *et al.,* 2018).

Inoculations
Aedes mosquitos between 1-3 days old and adult Bean bug insect were used for the experiment. The mosquitoes and bug were introduced and exposed for different lengths of time to *Metarhizium anisoplae* conidia in 30 cm diameter plastic cages covered with white mosquito netting based on the protocol described by Scholte *et al*. (2003b). The treatments were replicated 3 times with 20 individuals per replicate. Each of the cages with the mosquitoes and bean bug had filter paper and containing a 10% sucrose solution. The control experiment had no conidia at all. Daily Mortality/survival in each cage plus the control was monitored and recorded (Mnyone *et al.,* 2009).

Determination of Mortality
Daily mortality of the inoculated insects and the control were recorded, and dead insects were removed from the cages and placed on glass petri dishes lined with Whatman filter paper moistened with distilled water to maintain high humidity (~90%) in the petri dishes and to encourage fungal growth. Each petri dish was sealed with parafilm and labeled with

date of death and number of cadavers recorded. The petri dishes were kept in the inoculation room and cadavers monitored for 2- 4 days for evidence of fungal growth on the surface of infected mosquito and Bean bug. The species of fungal growth were detected, confirmed morphologically using a dissecting microscope 400_X magnification (Mouatcho, 2010).

Bioassay

The virulence of *Metarhizium anisoplae* against the insects was determined using the protocol described by Scholte *et al.* (2003b). The insects were dusted with dry conidia of *M. anisopliae* and exposed for 14days. All the experiment were replicated three times with 20 insects each. Mortality rate was monitored daily.

The percent mortality was calculated by using following formula.

$$\text{Percentage mortality} = \frac{\text{Total no. of dead insect} \times 100}{\text{Total no. of inoculated insect}}$$

The data collected were analyzed in excel spread sheet and the differences were determined using error bars.

RESULTS

The percentage survival of *Melanacanthus scutellaris* infected with three spore concentrations of *M. anisopliae* obtained from different locations and that of the controls are presented in figure 1a, 1b, 1c, and 1d. The mean percentage survival was 5% at 14 days post-infection (dpi) at the highest concentration (109 spores/mL), 10% dpi at the intermediate concentration (107 spores/mL) and 15% dpi at the lowest concentration (105 spores/mL). In comparison, the mean percentage survival of controls without conidia (0.05% Triton x100) was 75% up to 14 days. The survival of *Melanacanthus scutellaris* exposed to *M. anisopliae* was significantly lower than the control at $p \leq 0.05$.

Plate 1. The Cadaver of *Aedes eagypti* covered with conidia of *Metarhizium anisopliae*.

Plate 2. The Cadaver of *Melanocanthus scutellaris* covered with conidia of *Metarhizium anisopliae.*

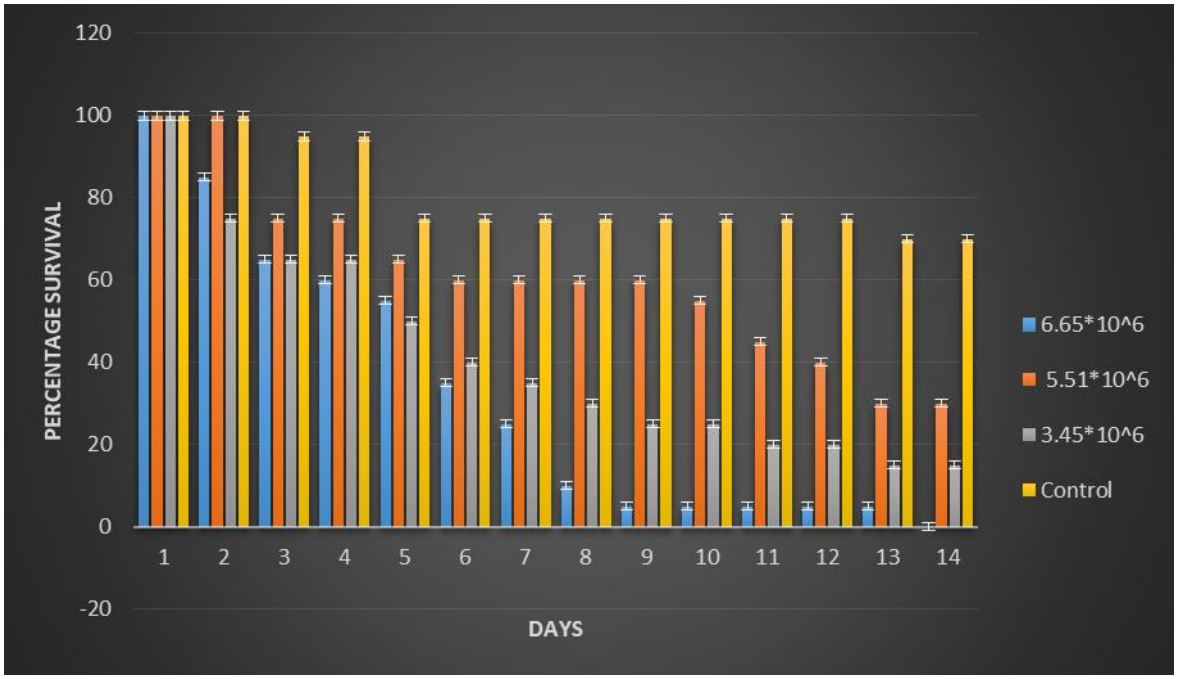

Fig1a. Percentage Survival of *Melanocanthus scutellaris* treated with *Metahrizium anisopliea* from New Lecture Theater, ATBU.

There was significant effect of concentration on the number of the adult *M. scutellaris* ($P \leq 0.05$). A less than 50% mortality was seen in day 6 from concentration $6.65 \times 10^{\wedge 6}$ and $3.45 \times 10^{\wedge 6}$ and same was recorded only after the 11 days from the date of bioassay in concentration $5.51 \times 10^{\wedge 6}$. Concentration $6.65 \times 10^{\wedge 6}$ showed complete mortality after 13 days, both concentration $3.45 \times 10^{\wedge 6}$ and $5.51 \times 10^{\wedge 6}$ recorded 20% survival after 14 days. The graph showed a significant and a steady mortality in all the concentrations of the isolate.

The time for 50% mortality (LT_{50}) values ranges from 5 – 12 days. The effect of the concentrations was significantly different from the actions of the isolates.

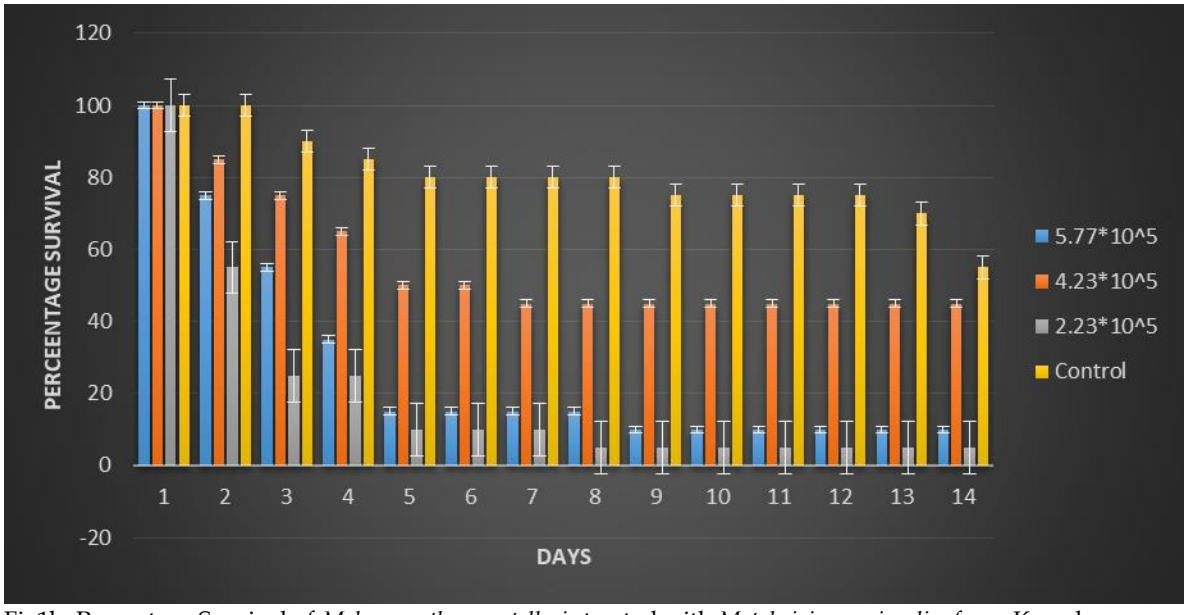

Fig1b. Percentage Survival of *Melanocanthus scutellaris* treated with *Metahrizium anisopliea* from Kagadama.

A 50% mortality was seen in day 2&3 from concentration 5.77 × 10^5 and 2.23 × 10^5 respectively. However, same was recorded after 5 days of the bioassay from concentration 4.23 × 10^5. Concentration 5.77 × 10^5 showed a 95% mortality after 9 days, concentration 2.23 × 10^5 showed 95% survival after 8 days, and just 50% survival 4.23 × 10^5 recorded after 14 days. The time for 50% mortality (LT_{50}) values ranges from 2 – 4 days. The effect of the concentrations was significantly different.

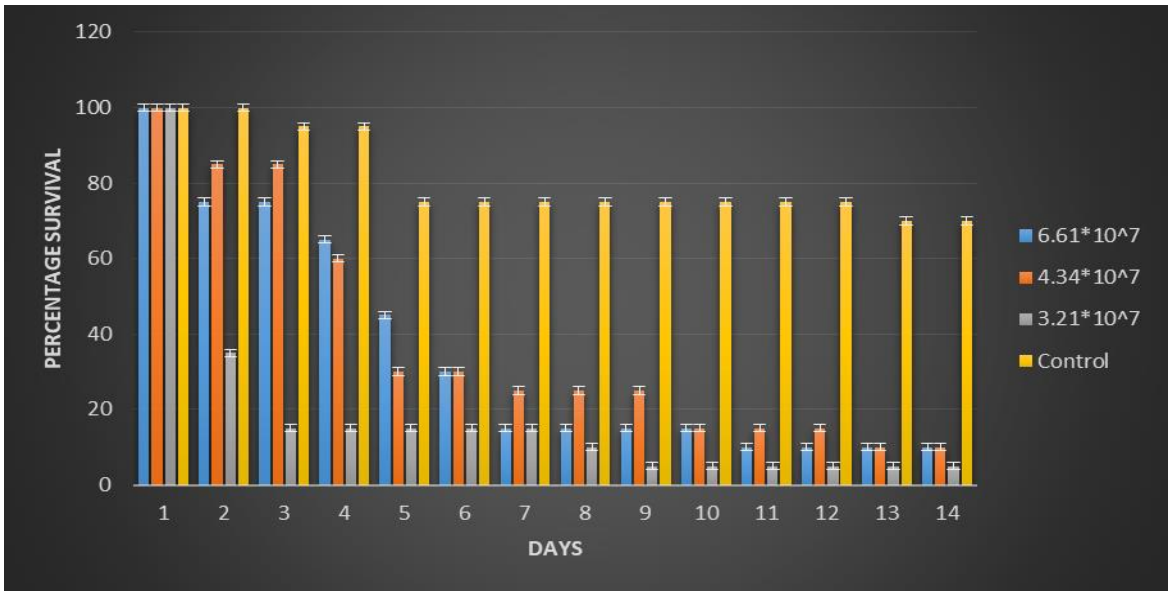

Fig1c. Percentage Survival of *Melanocanthus scutellaris* treated with *Metahrizium anisopliea* from Gwallameji.

Concentration 3.21 × 10^7 showed a 50% mortality after day 2 followed by concentration 4.34 × 10^7 recording a 50% mortality in between day 4 - 5. More so, concentration 6.61 × 10^7 recorded a 50% mortality after 5 days. Concentration 5.77 × 10^5 showed a 95% mortality after day 9, concentration 2.23 × 10^5 showed 95% survival after day 8. Concentration 4.23 × 10^5 recorded 50% survival after day 14. The time for 50% mortality (LT_{50}) values was seen on day 3. The effect of the concentrations was significantly different.

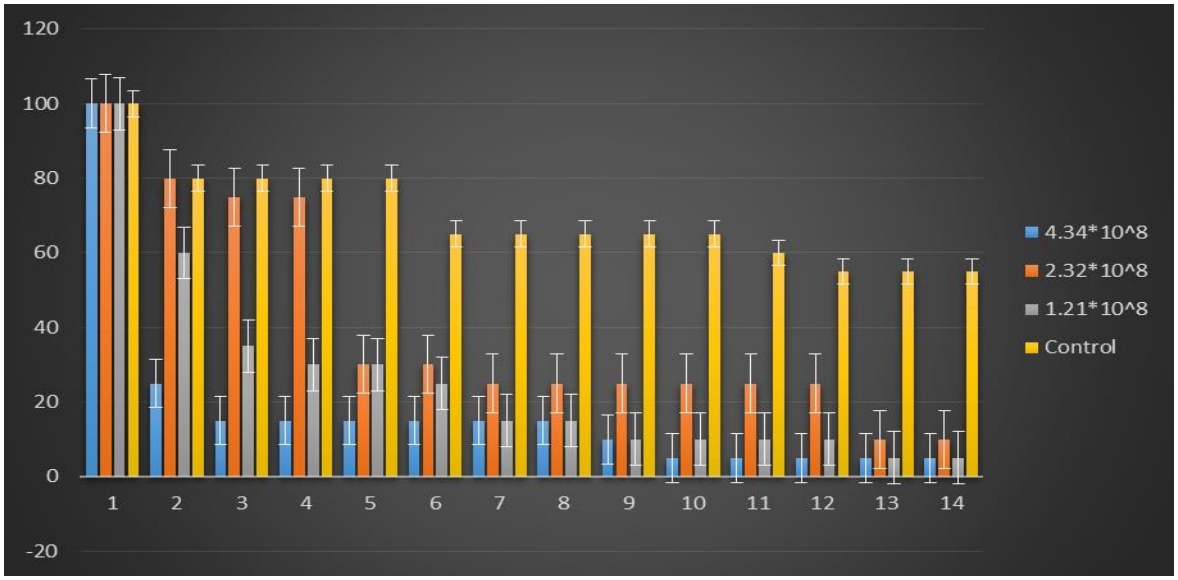

Fig1d. Percentage Survival of *Melanocanthus scutellaris* treated with *Metarhizium anisopliea* from Abubakar Tafawa Balewa University.

A 50% mortality was observed in day 2&3 from concentration 4.34 × 10^8 and 1.21 × 10^8 respectively. However, same result was recorded after 5 days of the bioassay from concentration 2.32 × 10^8. Concentration 4.34 × 10^8 showed a 95% mortality after 3days, concentration 1.21 × 10^8 showed 95% survival after day 7. Concentration 2.32 × 10^8 recorded 95% survival after day 13. The time for 50% mortality (LT_{50}) values ranges from 2, 3 and 5 days. The effect of the concentrations was significantly different.

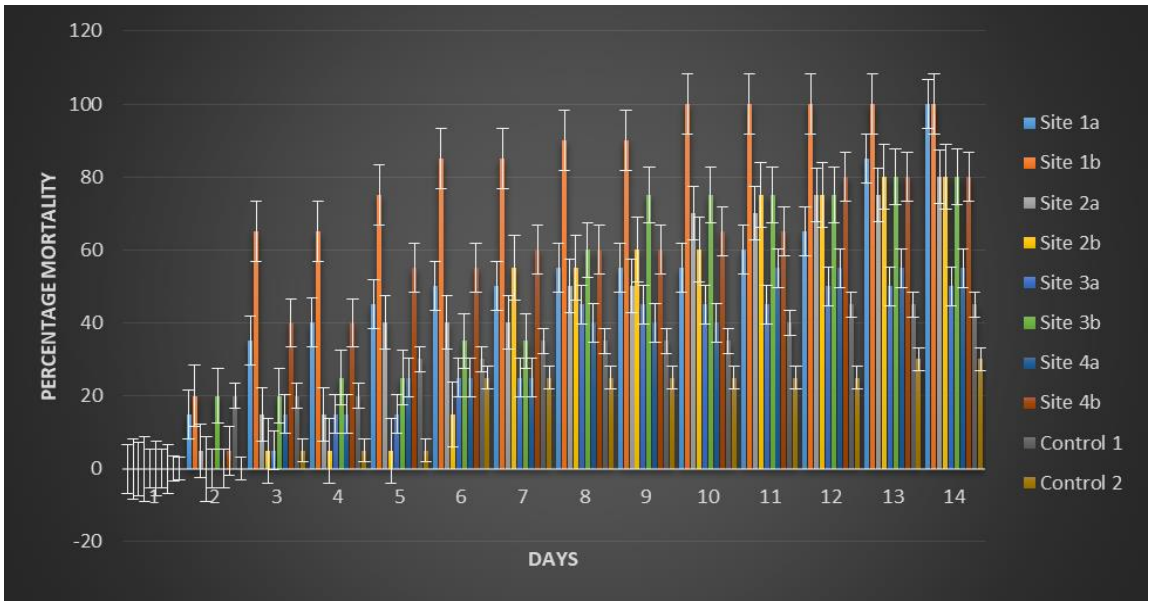

Fig2. Percentage Survival of adult Aedes Mosquito treated with *Metarhium anisopliea*

The effect of *M. anisopliae* against *Aedes aegypti* is shown in Fig 2. The virulence of *M. anisopliae* towards *Aedes aegypti* was higher and significantly different from the control treatment ($P < 0.05$).

DISCUSSION

The effects of *M. anisopliae* has been extensively studied as a control fungus of insect pests of agricultural importance as a highly effective entomopathogenic fungus but recent studies have reported that this fungus exhibits endophytic characteristics, allowing it to establish colonization within plant roots (Chowdhury *et el*., 2024). Furthermore, these studies have also demonstrated the fungus's ability to enhance nutrient acquisition and promote overall plant growth (Liu *et al.,* 2017; Shaalan *et al.,* 2021; González-Pérez *et al.,* 2022). Furthermore, the result of the research is in consonance with the work of Zuharah *et al.,* (2021) who showed the high virulence of *Metarhizium anisopliae* on *Ae. albopictus* and *Ae. aegypti* mosquito larvae at a conidia concentration of 1×10^6/ml within 7 days of the treatment period. The fungus displayed high larvicidal activity against laboratory and field strain of *Aedes. eagypti* larvae with LC_{50} values (9.6 × 103 /ml, 1.3 × 103 /ml) and LC_{95} values (1.2 × 10^6/ml, 5.5 × 105 /ml) respectively.

Different strains vary in their host specificity, virulence factors such as toxins and cuticle degradation enzyme complex (CDE) production (Petlamul & Prasertsan 2012). Most of the times fungal isolates perform well in laboratory bioassays, by exhibiting higher mortality rates within 1-2 weeks, although their virulence may be significantly variable according to the host specificity, origin, and culture history of individual isolates, this has also resonated from the result of this research on *Melanocanthus scuttelaris*. Even though no work has been recorded on the bean insect tested against EMF. (Cherry *et al.,* 2005).

Prior to field use, laboratory screening is vital step to identify the potential against insect pests (Cherry *et al.*, 2005), this has clearly been taken care of by these studies. The two steps (screening and virulence) to select potential isolates in our study have been used effectively by many other workers (Moino *et al.*, 1998). Within fungal taxa, a single isolate can exhibit restricted range towards their host (Inglis *et al.,* 2001) and isolates recovered from the host, exhibit high virulence towards that pest than the isolates recovered from soil or non-related species. Moreover, their laboratory screening is a vital step to identify the virulent strain prior field application (Cherry *et al.,* 2005).

This study showed the great potentials of locally sourced *M. anisopliae* as a myco-insecticide biocontrol agent of *Aedes. aegypti* and *Melanocanthus scuttelaris*

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REFERENCES

Ali, S.; Zhen, H.; Ren, S. (2010). Production of cuticle degrading enzymes by *Isaria fumosorosea* and their evaluation as a biocontrol agent against diamondback moth. *J. Pest Sci*. 83, 361–370.

Agriculture & Agri-Food Canada (AAFC). (2012). Directory of Biopesticides in Organization for Economic Cooperation and Development Countries. http://www4.agr.gc.ca/AAFCAAC/display afficher. Do? id= 1315941819381&lang=eng.

Ansari, M.A., Butt, T.M. (2011). Effects of successive subculturing on stability, virulence, conidial yield, germination, and shelf-life of entomopathogenic fungi. *J. Appl. Microbiol*. 110 (6), 1460–1469.

Araújo JPM, Hughes DP. (2016). Diversity of entomopathogenic fungi. Which groups conquered the insect body? Adv Genet 94:1–39. https://doi.org/ 10.1016.

Barredo, E. & De Gennaro, M. (2020). Not just from blood: Mosquito nutrient acquisition from nectar sources. *Trends Parasitol*. 36, 473–484.

Batta, Y.A. (2013). Efficacy of endophytic and applied Metarhizium anisopliae (Metch.) Sorokin (Ascomycota: Hypocreales) against larvae of Plutella xylostella L. (Yponomeutidae: Lepidoptera) infesting Brassica napus plants. *Crop Prot*., 44, 128–134

Blanford, S. Shi, W. Riann, C. Marden, J.H. Koekemoer, L.L. Brooke, B.D. Coetzee, M. Read, A.F. & Thomas, M.B. (2011). "Lethal and pre-lethal effects of a fungal biopesticide contribute to substantial and rapid vector control". *PLoS ONE* 6: e23591.

Bidochka, M.J., Kamp, A.M., Lavender, T. M., Dekoning, J., De Croos, J. N. A. (2001). Habitat association in two genetic groups of the insect pathogenic fungus Metarhizium anisopliae: uncovering cryptic species? Appl. Environ. *Microbiol*. 67, 1335-1342

Chakravarti, A. Arora, R. & Luxemburger, C. (2011). "Fifty years of dengue in India". Transactions of the Royal Society of Tropical Medicine and Hygiene 106: 273-285.

Cox, D.R. (1972). Regression models and life tables. *J Royal Statist Soc*, B: 187-220.

Dubovskiy, I.M. Whitten, M.M. Yaroslavtseva, O.N. Greig, C. Kryukov, V.Y. Grizanova, E.V. Mukherjee, K. Vilcinskas, A. Glupov, V.V. Butt, T.M. (2013). Can insects develop resistance to insect pathogenic fungi? *PLoS ONE* 8 (4), e60248.

EPA (2003) Environmental Protection Agency. http://www.epa.gov/oppbppd1/biopesticide.

FAO (2021). The Impact of Disasters and Crises on Agriculture and Food Security. https://doi.org/10.4060/cb3673en

Jaber, L.R.; Vidal, S. (2010). Fungal endophyte negative effects on herbivory are enhanced on intact plants and maintained in a subsequent generation. Ecol. *Entomol*, 35, 25–36.

Jemberie, W. Hill, S. Rännbäck L.M. Ondiaka, S. Raja, N. Ignell, R. (2018). Evaluation of entomopathogenic fungi Metarhizium anisopliae against dengue virus mosquitoes Aedes aegypti (Diptera: Culicidae) *ENTOMON* 43(1): 07-18.

Jiang, W. Peng, Y. Ye, J. Wen, Y. Liu, G. Xie, J. (2019). Effects of the Entomopathogenic Fungus Metarhizium anisopliae on the Mortality and Immune Response of Locusta migratoria. Insects 11, 36; doi: 103390/insects11010036

Joel Claude Mouatcho (2010). The Use of Entomopathogenic Fungi against Anopheles Funestus Giles (Diptera: Culicidae). PhD Thesis pp162.

Kepler, R.M.; Luangsa-Ard, J.J.; Hywel-Jones, N.L.; Quandt, C.A.; Sung, G.H.; Rehner, S.A.; Aime, M.C.; Henkel, T.W.; Sanjuan, T.; Zare, R.; et al. (2017). A phylogenetically-based nomenclature for Cordycipitaceae (Hypocreales). *IMA Fungus*, 8, 335–353.

Liu, N. (2015). Insecticide resistance in mosquitoes: Impact, mechanisms, and research directions. Annu. Rev. *Entomol*. 60, 537–559. https://doi.org/10.1146/annureviento-010814-020828.

Mantzoukas, S. Eliopoulos, P.A. (2020). Endophytic entomopathogenic fungi: A valuable biological control tool against plant pests. *Appl. Sci*, 10, 360

Mantzoukas, S. Pettas, I.; Lagogiannis, I. (2020). Stored product pests as models for trapping entomopathogenic fungi from olive tree orchards in Western Greece. J. *Stored Prod. Res*, 87, 101584.

Mantzoukas, S. Pettas, I. Lagogiannis, I. Mpekiri, M. (2019). Insecticidal action of Several Isolate of Entomopathogenic Fungi against The Granary Weevil *Sitophilus granaries.* Journal of Agriculture 9 (10), 222.

Mnyone, L.L., Kirby, M.J., Lwetoijera, D.W., Mpingwa, M.W., Simfukwe, E.T., Knols, B.G., Takken, W., Russell, T.L. (2010). Tools for delivering entomopathogenic fungi to malaria mosquitoes: effects of delivery surfaces on fungal efficacy and persistence. Malar. J. 9, 246. https://doi.org/10.1186/1475-2875-9-246.

Monroe, A. N. A. Williams, S. Ogoma, C. Karema, and F. Okumu, (2022). Reflections on the 2021 World Malaria Report and the Future of Malaria Control, Springer, Berlin, Germany.

Moore D, Bridge PD, Higgins PM, Bateman RP, Prior C. (1993). Ultra-violet radiation damage to Metarhizium flavoviride conidia and the protection given by vegetable and mineral oils and chemical sunscreens. *Annals of Applied Biology*, 122:605-616.

Okumu, F. Gyapong, M. Casamitjana, N. Castro, M.C. Itoe, M.A. Okonofua, F. Tanner, M. (2022.). "What Africa can do to accelerate and sustain progress against malaria," *PLOS Global Public Health*, vol.2, no.6, Article ID e0000262.

Peng, G.; Wang, Z.; Yin, Y.; Zeng, D.; Xia, Y. (2008). Field trials of Metarhizium anisopliae var. acridum (Ascomycota: Hypocreales) against oriental migratory locusts, Locusta migratoria manilensis (Meyen) in Northern China. *Crop Prot*, 27, 1244–1250.

Rhodes, V. L, Matthew, B. Kristin, T. (2018). The interplay between dose and immune system activation determines fungal infection outcome in the African malaria.mosquito, *Anopheles gambiae*. *Developmental & Comparative Immunology* 85,125-133.

Sable S. B, Deore P.B, Bangar V.R and Kurhade K.C. (2019). Effect of Different Spore. Concentration of Entomopathogenic Fungi Isolates against Mealy Bug *Maconellicocus hirsutus*. International Journal of Current Microbiology and Applied Sciences 8(3): 2166-2175. *Developmental and Comparative immunology* Vol. 15.125-133.

Sevim, A.; Demir, I.; Demirba ˘G, Z. (2010). Molecular characterization and virulence of Beauveria spp. from the pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). Mycopathologia, 170, 269–277

Scholte EJ, Takken W, Knols BGJ. (2003). Pathogenicity of six east African entomopathogenic fungi to adult Anopheles gambiae s.s. (Diptera: Culicidae) mosquitoes. Proc Exp Appl *Entomol* NEV, Amsterdam 14: 25-29.

Scholte, E.J., Takken, W., Knols, B.G. (2007). Infection of adult *Aedes aegypti* and Ae. *albopictus* mosquitoes with the entomopathogenic fungus Metarhizium anisopliae. *Acta. Trop.* 102, 151–158. https://doi.org/10.1016/j.actatropica.2007.04.011.

Sinha KK, Choudhary AK, Kumari P. (2016). Entomopathogenic fungi. *Ecofriend* Pest Manag Food Secur. https://doi.org/10.1016/B978-0-12-803265-7. 00015-4.

Shah, P.A.; Pell, J.K. (2003). Entomopathogenic fungi as biological control agents. Appl. *Microbiol. Biotechnol*., 61, 413–423.

W. H. O, (2017). "Global vector control response 2017-2030," Tropical Medicine and Infectious Disease, vol. 7, no. 7, p125.

Wei, G.; Lai, Y.; Wang, G.; Chen, H.; Li, F.; Wang, S. (2017). Insect pathogenic fungus interacts with the gut microbiota to accelerate mosquito mortality. *Proc. Natl. Acad. Sci*. USA, 114, 5994–5999

Yakubu, M.N. Adamu, F.M. Barde, A.A. Muhammad, M. Tahir, F. (2018). Effect of the entomopathogenic fungus Metarhizium anisopliae against Mosquitos (Aedes aegypti) in vitro conditions. *Int J Innov Approach Sci Res*. https://oi.org/10.29329/ijiasr.2018\.140.2.

Yakubu, N. M, Ladan, M.A, Deba, F.A, Safiya, U.S. Abdulhameed, A. Tahir, F. (2022). Biodiversity and virulence characterization of entomopathogenic fungi isolated from soils in different regions of Nigeria. *Egyptian Journal of Biological Pest Control*. 32:93 https://doi.org/10.1186/s41938-022-00593-9

Zhang, D. Gao, F. Jakovlic, I. Zou, H. Zhang, J. Li, W. X. Wang, G.T. (2020). "PhyloSuite: an integrated and scalable desktop platform for streamlined molecular sequence data management and evolutionary phylogenetics studies," *Molecular Ecology Resources*, vol. 20, no. 1, pp. 348–355.

Zhang, J., W. Huang, C. Yuan, Y. Lu, B. Yang, C. Y. Wang and E. Ling. (2017). Prophenol oxidase-mediated ex vivo immunity to delay fungal infection after insect ecdysis. *Front. Immunol*. 8:1–14.

Zhang, P.F. You, Y.W. Song, Y. Wang, Y.Z. Zhang, L. (2015). First record of Aspergillus oryzae (Eurotiales: richocomaceae) as an entomopathogenic fungus of the locust, *Locusta migratoria* (Orthoptera: Acrididae). *Biocontrol Sci. Technol.* 25, 1285–1298.