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Laboratory evaluation of entomopathogenic fungus *Metarhizium anisopliae* for the control of *Anopheles gambiae* s. L. Larvae in Bauchi Nigeria

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Abstract
The entomopathogenic fungus, *Metarhizium anisopliae* was cultured and isolated from soil samples which were obtained from the agricultural fields within Yelwa Campus of Abubakar Tafawa Balewa University Bauchi Nigeria. This fungus was used as biocontrol agent against the 4th instar larvae of *Anopheles gambiae* s. L. under ambient laboratory conditions. Five concentrations (1×10^5, 1×10^6, 1×10^7, 1×10^8 and 1×10^9 conidia/ml of the fungal isolate were prepared by adding the sterile 0.02% tween 80 in distilled water. Haemocytometer was used for counting the conidia. The results indicated an increase in the mean larval mortality from 4 to 96% as the concentration of the fungal isolate was increased from 1×10^5 to 1×10^9 Conidia/ml as the time of exposure was increased from 24 to 96 hours. This was reflected by the decrease in the values of LC_{50} from 5.75×10^8 to 7.10×10^5 while the values of LC_{90} decreased from 5.20×10^{11} to 9.77×10^7. This performance of the *M. anisopliae* in the biocontrol of mosquito larvae further encourages the use of the entomopathogenic fungi in an effort to reduce the spread of some vector-borne diseases in some parts of sub-Saharan regions of Africa.

Keywords: 4th instar larvae, *Anopheles gambiae, Metarhizium anisopliae*, biocontrol agent, soil sample

Introduction
Vector-borne diseases are an increasing cause of death and suffering worldwide. Of all the disease – spreading arthropods, mosquitoes are the greatest public health problem [1], causing physical, mental and health effects to man and other animals. As they are responsible for the transmission of various dreadful diseases such as malaria, filariasis, yellow fever and several types of encephalitis, World Health Organization, (WHO) declared mosquitoes as " public enemy number one" [2, 3, 4].
Anopheles species are the most important mosquito species as they are capable vector for malaria parasite, *Plasmodium* species. Approximately, half of the world's population is at the risk of malaria, particularly those living in lower income countries. It infects more than five hundred people per year and kills more than one million [5]. Control of disease – bearing vectors relies heavily on the extensive and intensive use of chemical insecticides. These chemicals are to certain extent quite successful in curving the vector – borne diseases. However, in view of some of the side effects of these chemical agents used in vector control, interest in environmentally friendly approaches and the use of biological control agents, have been revived. In line with these, the importance of entomophathogens has been highlighted as an environmentally – friendly pest/vector control method. According to Scholte [6] fungal diseases in insects are common, widespread and can decimate pest/vector populations in spectacular epizootics and virtually all insect orders are susceptible to fungal diseases.
Metarhizium species are known to be among the arthropod pathogens with broad geographic and host ranges) [7]. They are saprophytic in soil and parasitic on insects. *M. anisopliae*, in particular, naturally grows in soil and has been isolated from various arthropod cadaver and decaying materials. It causes disease known as green muscardine disease in various insects acting as a parasite. The potential use of this entomopathogenic fungus for controlling vectors of human and other animal diseases has been recently investigated with promising outcome. However, at present it has been produced as a commercial product and applied for control of different insect pests/vectors with no adverse effects to human or environment [6].
The present study was conducted to investigate the possibility of using *M. anisopliae* spore suspension to control *Anopheles gambiae* s. L. larvae under Sahel/Sudan savannah conditions.

Materials and Methods
Study Area
Abubakar Tafawa Balewa University Bauchi, Yelwa Campus is located at Longitude 9.792ºEast and Latitude 10.279º North.

Soil sample collection
Entomopathogenic fungus, *M. anisopliae* was isolated from the soil samples collected from an agricultural field where maize and cowpeas are cultivated as mixed crops within the Abubakar Tafawa Balewa University, Bauchi, Yelwa Campus. A two kilogrammes (2Kg) soil sample was collected from five (5) different sampling points and randomly mixed to obtain a homogenous sample which were kept in sterile polythene bags and transported to the laboratory for fungal culture.

Isolation of *M. anisopliae* fungus from soil sample
Soil dilution method was adopted for the isolation of this entomopathogenic fungus. One gram (1gm) of homogenized soil sample was suspended in 9 ml of sterilized distilled water

mixed well and serially diluted. 1 ml of aliquots was transferred to sterile petriplates which was plated on DOA (dodine oatmeal agar) selective medium for screening entomopathogenic fungi (containing 200 ug/ml dodine and 50 ug/ml streptomycine), streptomycin was added to avoid bacterial growth. The petriplates were sealed with parafilm before incubation at 25ºC for 7 days. Pure culture of *M. anisopliae* were identified and re-isolated as described by Mohammadbeigi and Port [8].

Fungus Culture and Inoculum preparation

Isolates of the entomopathogenic fungus, *M. anisopliae* were cultured on potato dextrose agar (PDA) medium and incubated at 25ºC for 7 days. Fungal inoculum was prepared from by scrapping off the surface of the 7 days old culture with a sterilized glass rod and a homogenous conidial suspension was prepared in sterile distilled water by adding a few drops of the wetting agent Tween 80 (0.01%). The mixture was stirred with a magnetic stirrer for 10 minutes. Inoculums were prepared from 1 x 10^9 to 1 x 10^5 conidia/ml by direct counting on a haemocytometer.

Mosquito Rearing and Maintenance

The 4th instar larvae of *An. gambiae* s. l. were used for this bioassay. The larvae of *An. gambiae* s. l. were collected from the naturally infested ground pools within the ATBU, Bauchi, Yelwa campus during the rainy season and transported to the laboratory in plastic containers. In the laboratory, all the 4th instar larvae were separated for the bioassay while the remaining under develop larvae were fed on dog biscuits and yeast powder in the 3 : 1 ratio to reach the 4th instar larval stage as recommended by Gerberg [9].

Boassays

Thelarvicidal activity of *M. anisopliae* was tested against the 4th instar larvae of *Anopheles gambiae* s. L. under ambient laboratory conditions. The tests were conducted in glass beakers in accordance with WHO [2] procedure with slight modification where necessary. 1ml of each of the prepared conidia concentration (10^5, 10^6, 10^7, 10^8 and 10^9 conidia/ml) was measured separately and added to 250 ml beakers containing 49ml of sterile distilled water. This was replicated four times and a control treatment was set in which only sterile distilled water was used. Twenty five 4th instar larvae were introduced into the 250 ml beaker containing the sterile distilled water with the concentrations of the fungal isolate.

Analysis

Probit analysis was used to determine the efficacy of the fungal isolate by calculating the LC_{50} and LC_{90} after 24, 48, 72, and 96 hours [10]. The relationships between probit and log concentrations were established as probit equations and probit regression lines were drawn.

Results

The bio efficacy of soil inhabiting entomopathogenic fungus, *Metarhizium anisopliae* was assessed against the 4th instar larvae of *Anopheles gambiae* s. L. larvae under ambient laboratory conditions. The mean percentage mortality of the larvae at various fungus conidia concentrations of 10^5 to 10^9 at different exposure times of 24, 48, 72 and 96 hours is shown in table 1. From the results, the mean percentage mortality of the larvae varied from 4 to 96% with maximum mortality at the highest applied concentration of 10^9 conidia/ml after 96 hours of exposure. However, a general increase in the larval mortality was noticed as the exposure time and concentration of the fungal isolate were increased.

Table 1: Mean percentage mortality of 4th instar larvae of *Anopheles gambiae* s. L. exposed to different concentration of *Metarhizium anisopliae* isolate.

Concentration conidia/ml	Time (Hrs.)			
	24	**48**	**72**	**96**
Control	0	0	0	0
1 x 10^5	4	8	16	32
1 x 10^6	12	16	32	48
1 x 10^7	28	44	64	80
1 x 10^8	40	56	72	92
1 x 10^9	48	68	80	96

Table 2 shows the results of the efficacy of the *M. anisopliae* isolate against the 4th instar larvae of *An. gambiae* s. l. The values of the LC_{50} ranged between 5.75 x 10^8 to 7.100 x10^5 Conidia/ml as the time of exposure to the concentration of the fungus isolate increased from 24 – 96 hours. Similarly, for the LC_{90}, the values ranged between 5.20 x 10^{11} to 9.77 x 10^7 Conidia/ml with the increase for the time of exposure from 24 – 96 hours. The probit equations generated from the effects of the fungus isolate against the survival of the 4th instar larvae of the *An. gambiae* s. l. were used to drew the probit regression lines to show the relation between the mortality and the log concentration after the periods of 24, 48, 72 and 96 hours of exposure (Figs. 1-4).

Table 2: Efficacy of *Metarhizium anisopliae* isolate against the 4th instar larvae of *An. gambiae* s. l. after 24, 48, 72 and 96 hours of exposure.

Exposure Time (Hours)	Probit Equation	LC_{50} Conidia/ml	LC_{90} Conidia/ml
24	0.433X + 1.207	5.75 x 10^8	5.20 x 10^{11}
48	0.490X + 1.184	6.14 x 10^7	1.10 x 10^{10}
72	0.471X + 1.767	7.31 x 10^6	3.82 x 10^9
96	0.600X + 1.486	7.10 x 10^5	9.77 x 10^7

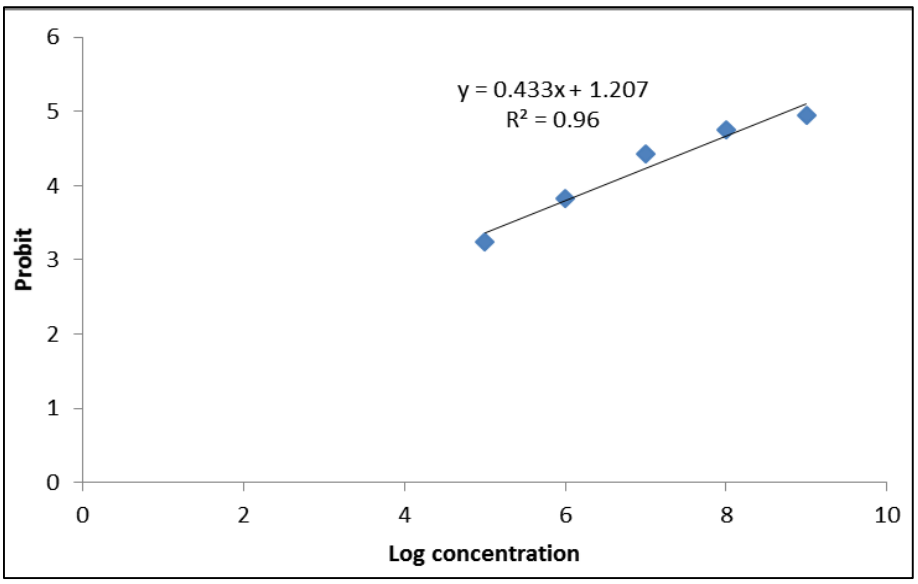

Fig 1: Probit regression line showing the relation between probit and log dose concentration of *Metarhizium anisopliae* against 4th instars larvae of *Anopheles gambiae* s. L. after 24 hours.

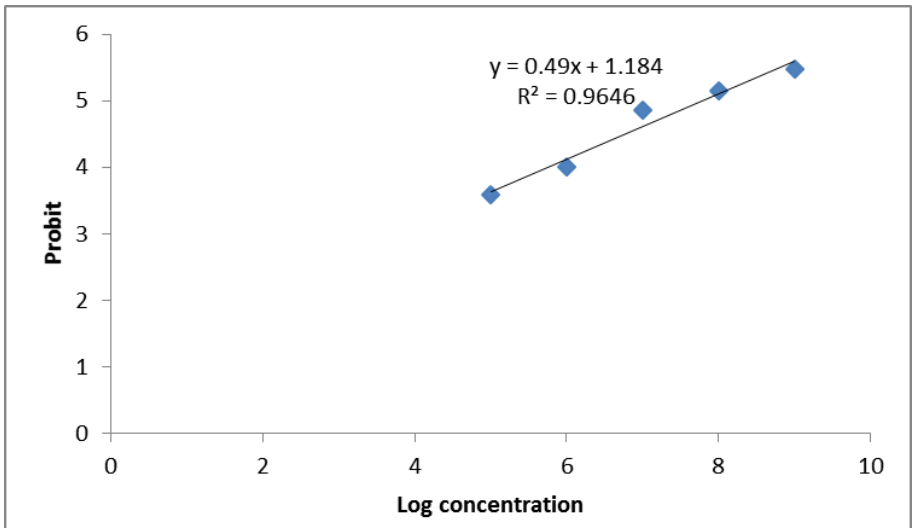

Fig 2: Probit regression line showing the relation between probit and log dose concentration of *Metarhizium anisopliae* against 4th instars larvae of *Anopheles gambiae* s. L. after 48 hours.

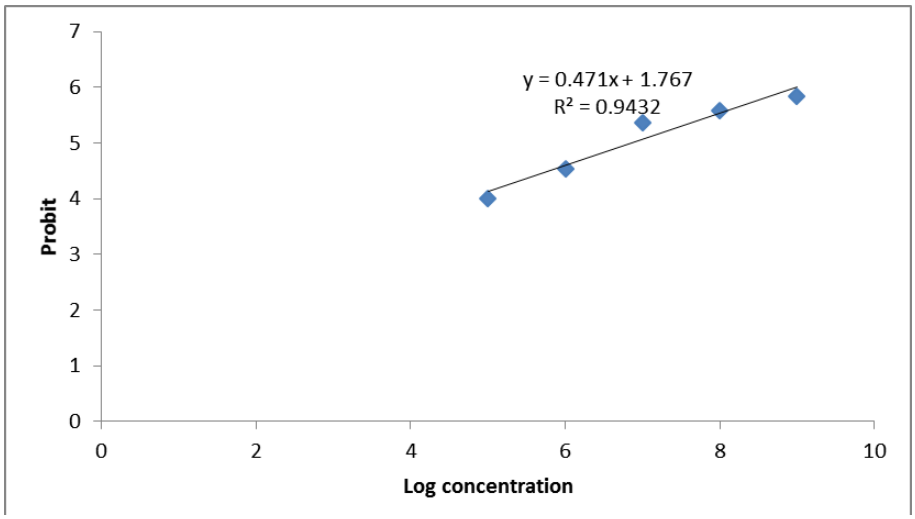

Fig 3: Probit regression line showing the relation between probit and log dose concentration of *Metarhizium anisopliae* against 4th instars larvae of *Anopheles gambiae* s. L. after 72 hours.

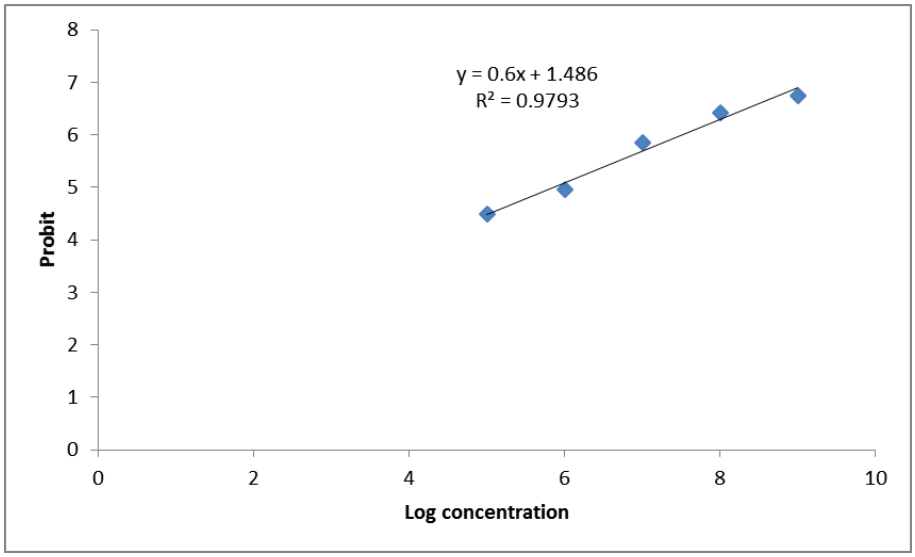

Fig 4: Probit regression line showing the relation between probit and log dose concentration of *Metarhizium anisopliae* against 4th instars larvae of *Anopheles gambiae* s. L. after 96 hours.

Discussion

The use of conventional synthetic insecticides in vector control is facing a threat due to the emergence of insecticide resistance in insect vectors, in addition to the other problems such as negative effects on non-target organisms including humans and the environment, leaving toxic residues in food products and are not easily biodegradable. As a result of these and other side effects of the synthetic insecticides, either counter measures or development of newer vector control strategies are needed. The use of bio-control agents is now under consideration as a suitable alternative to the use of synthetic insecticides [11]. These biological control agents like bacteria, protozoa, nematodes, viruses and most fungi were found to exert considerable control of insect vector populations [6].

Among the entomopathogenic fungi, *Metarhizium* species are the most common ones with a worldwide distribution that survive in diverse habitats including fresh water, soil surfaces and aerospaces [12]. Many laboratory studies have shown the potential of *M. anisopliae* as a mosquito control agent from the different parts of the world [13, 14, 15, 16, 17a, b, 18, 19, 20, 21, 22].

As demonstrated in the earlier trials, the present evaluation of the bioefficacy of the fungus, *M. anisopliae* against the 4th instar larvae of the malaria vector, *An. gambiae* s. l. showed a very promising outcomes. From these results, the control performance of this entomopathogenic fungus was found to increase as the concentration of the fungus isolate and time of exposure were increased (Table 1). This is indicated by the decreased in the values of LC_{50} from 5.750×10^8 to 7.100×10^5 Conidia/ml across the time of exposure from 24 to 96 hour (Table 2). Similar results were reported by Scholt [23] and Blandford [24]. This could be attributed to the facts that the death of the insects might results from a combination of factors such as mechanical damage resulting from tissues invasion, depletion of nutrient resources and released of some other toxins as a result of larval invasion by the fungus [25].

The results obtained from this trial further encourages the use of entomopathogenic fungi to control mosquito vectors responsible for the spread of many mosquito borne diseases in dry areas of Sub-Saharan Africa. However, the nature of the breeding habits of some medically important vector species such as *An. gambiae* complex may prove to be difficult to use these fungi as biocontrol agents against their larvae. The larval habitats of these mosquito species include a variety of transient, mainly sunlit, rain water pools, burrow- pits, drains, hoof prints around pond and water bodies. Most of these sites are transient, and in some areas breeding is highly seasonal, following the rainfall pattern of that specific area. It may be very unlikely that under normal field conditions, larvae of *Anopheles* mosquitoes are in contact with any of the entomopathogenic fungi to be applied as a biocontrol agent. Entomopathogenic fungi were found to control the larvae of other mosquito species such as *Culex* and *Aedes* effectively than in the case of *Anopheles* larvae but controlled all the adults from the species of *Anopheles*, *Aedes* and *Culex* successfully without any difficulties [6].

Conclusion

In conclusion, the results obtained from this study confirmed that the locally isolated entomopathogenis fungus, *M. anisopliae* has the potential to reduce larval populations of An. gambiae s, l. under laboratory conditions. This can lead to the indirect reduction of the adult mosquitoes that transmit malaria and other important mosquito-borne diseases in our communities.

References

1. World Health Organization. Malaria Control Today-Current WHO recommendations Working Document Roll Back Malaria Department, WHO. 2005, 75.
2. World Health Organization. Report of the WHO informal consultation on the Evaluation and testing of insecticides, CTD/WHOPES/IC/96.1. Control of Tropical Diseases Division. World Health Organization, Geneva. 1996.
3. Sachs J, Malaney P. The economic and social burden of malaria. *Nature.* 2002; 416(6881):581.
4. Boutayeb A. The double burden of communicable and non-communicable Disease in developing countries.

Trans. R. Soc. Trop. Med. Hygiene. 2006; 100(3):191-199.
5. World Health Organization. Handbook for Integrated Vector Management. Geneva. WHO Press, 2012. WHO/HTM/NTD/VEM/2012.3
6. Scholte EJ, Samson BGJ RA, Takken W. Entomopathogenic fungi for mosquito control: A Review. Journal of Insect Science. 2004; 4 (19):24-36.
7. Elham AS, Shigidi MT, Hassan SM. *Metarhizium anisopliae* as a Biological Control agent against *Hyalomma anatolicum* (Acari: Ixodidae). Pakistan Journal of Biological Sciences. 2013; 16(24):1943-1949.
8. Mohammadbeigi A, Port G. Efficacy of *Beaueria bassiana* and *Metarhizium anisopliae* Against *Uvarovistia zebra* (Orthoptera: Tettigoniidae) via contact and ingestion. Int. J. Agric. Crop Science. 2013; 5(2):138-146.
9. Gerberg E, Barnard D, Ward R. Manual for Mosquito Rearing and Experimental Techniques. Revised edition. Lake Charles, LA, American Mosquito Control Association, Inc., *Bulletin*, 5.
10. Finney DJ. Probit analysis, 3rdedn. Cambridge University Press, London, 1971.
11. Dhaliwal GS, Koul O. Biopesticide and Pest Management: Conventional and Biotechnological Approaches. Kalyni Publishers, New Delhi, 2007, 455.
12. Hajek AE, Ledger RJ. Interaction between fungal pathogen and insect hosts. Annual Review of Entomology. 1994; 39:293-322.
13. Roberts DW. Some effects of *Metarhizium anisopliae* and its toxins on mosquito larvae. In: Van der Laan, editor. Insect Pathology and Microbial Control. Amsterdam: North-Holland Publishing Company, 243-246.
14. Roberts DW. *Coelomomyces*, *Entomophthora*, *Beauveria*, and *Metarhizium* as parasites of mosquitoes. Miscellaneous Publications of the Entomological Society of America. 1970; 7:140-155.
15. Roberts DW. Fungal infections of mosquitoes. In: Aubin A; Belloncik S; Bourassa JP; LaCouriere F; Pellissier M, editors. Mosquito Control. 1974, 143-193.
16. Ramosker WA. An examination of the long-term epizootic potential of various artificially introduced mosquito larval pathogen. *Mosquito News.* 1982; 42:603-607.
17. Daoust RA, Roberts DW. Studies on the prolonged storage of *Metarhizium anisopliae* conidia: effect of temperature and relative humidity on conidial viabilityand virulence against mosquitoes. Journal of Invertebrate Pathology, 1983a; 41:161-170.
18. Daoust RA, Roberts DW. Studies on the prolonged storage of *Metarhizium anisopliae* conidia: effect of growth substrate on conidial survival and virulence against mosquitoes. Journal of Invertebrate Pathology. 1983b; 41:161-170.
19. Agudelo-Silva F, Wassink H. Infectivity of a Venezuelan strain of *Metarhizium anisopliae* to *Aedes aegypti* larvae. Journal of Invertebrate Pathology. 1984; 43:435-436.
20. Sandhu SS, Rajak RC, Sharma M. Bioactivity of *Beauveria bassiana* and *Metarhizium anisopliae* as pathogens of *Culex tritaeniorhynchus* and *Aedes aegypti*: effect of instar, dosages and time. Indian J, Microbiology. 1993; 33:191-194.
21. Alves SB, Alves LFA, Lopes RBA, Pereira RM, Viera SA. Potential of some *Metarhizium anisopliae* isolates for control of *Culexquinque fasciatus* (Diptera: Culicidae). Journal of Applied Entomology. 2002; 126:504-509.
22. Scholte EJ, Ng'habi K, Kihonda J, Takken W, Paaijmans K, Abdula S *et al*. Entomopathogegic fungus for control of adult African Malaria mosquitoes. Science. 2005; 308:1641-1642.
23. Amora SSA, Bevilqua CML, Feijo FMC, Pereira RHMA, Alves ND, Freire FMA *et al*. The effects of the fungus *Metarhizium anisopliae* var. *acridum* on different stages of *Lutzomyia longipalpis* (Diptera: Psychodidae). *Acta Tropica*, 2010; 113(3):214-220.
24. Scholte EJ, Njiru BN, Smallegang RC, Takken W, Knols BGJ. Interaction of malaria (*Anopheles gambiae* s. s.) and filariasis (*Culex quinquefasciatus*) vectors with the entomopathogenic fungus Metarhizium anisopliae. Malaria Journal. 2003; 2:29.
25. Blanford S, Chan BHK, Jenkins N, Sim D, Turner RJ, Read AF, Thomas MB. Fungal pathogen reduces potential for malaria transmission. *Science.* 2005; 308:1638-1641.
26. Rajasekhar P, Kalidas P. Mechanisms involved in the entomopathogenesis of *Beauveria bassiana*: A Review. Asian Journal of Environmental Science, 2010; 5(1):65-74.