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The formulation and application of entomophatogenic fungi to infect mosquito

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Abstract

Entomophatogenic fungi is a potential bioinsecticide that hopely can handle the mosquito vector borne diseases and the insecticide resistant problems in all the world. Study of literature about entomophatogenic fungi include kinds of fungi, the quality of conidia, the carrier or formulation, method of application was held to get information and understanding about entomophatogenic fungi. *Beauveria bassiana, Metarhizium anisopliae* and *Aspergilus sp* were the popular entomophatogenic fungi that oil formulation have been known as the good formulation to infect entomophatogenic fungi to mosquito. Some method application were tried to infect the entomophatogenic fungi to mosquito and yield a different result but ovitrap could be a good consideration to used based on the capability to infect all stage of mosquito and can be modified appropriate by the bionomic of target species.

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Introduction

Entomophatogen fungi especially *Beauveria bassiana* and *Metarhizium anisopliae* is widely used by agriculture in Indonesia to fight the insect manifestation, but not yet for diseases vector control. Mosquito is the one of the most killer insect that responsible for spreading serious communicable diseases like dengue, malaria, lymphatic filarial, zika, chikungunya, etc that need an effective and efisien program to fight it. Fogging focus and Insectiside Residual Spraying become the main application to fight the mosquito vector control programme in Indonesia that the rapidly emerging insecticide resistance become the blocked obstruction to the programme. Some research finds insecticide resistance in many area, pyrethroid were detected resintant in many Southeast Asia country lie Cambodia, Indonesia, Laos, Singapore, Thailand, Timor Leste and Vietnam (Amelia-yap *et al*. 2018). Pyrethroid resistance also found in malaria vector *An. arabiensis* in Nouakchott Mauritania (Mint *et al*. 2018).

Besides of resistance, the long used of chemical insecticides can be poison for human and environment. A research report that organophosphate is responsible of 16,7% renal failure (Arefi *et al*. 2014), single exposure of DDT in the neonatal period leads to cognitive defects in adulthood, such as decreased habituation, a non-associative learning process, verbal, memory, quantitative, and perceptual performance skills (Abreu-Villaça and Levin 2018). Some chemical insectisides play an important role in our environment and daily life, they could be carcinogenic in laboratory animals and they could be implicated to lung, breast and colon cancers (Koca, Ustundag, and Yalcin 2016). The chemical insectiside persistence and bioaccumulation in the environment can cause the emerging evidence of adverse effects on wildlife off-target species (Abreu-Villaça and Levin 2018). The directly effect of malathion on some experimental animals, malathion showed negatively affected body weight, cerebral alterations and reproductive system on rats, alteration of protein, fat and energy metabolisms, and oxidative balance. While indirect effects, malathion residues found on animal products (honey, milk and meat) from livestock, bees, fishes and poultry and also observed in many vegetables such as onions, cucumbers, tomatoes and peppers (Koca, Ustundag, and Yalcin 2016).

Based on the negative effect on chemical insecticides, it needed to find some alternative to replace the use of chemical insectisides, and entomopathogen fungi that can be a potential choice. Entomophatogenic fungi, especially *M. anisopliae* is considered to be safe to vertebrates, humans and the environment (Zimmermann, 2007). Many factors determine the succes of entomopathogen fungi to infect mosquito such as the kinds and quality of entomophatogenic fungi, formulated or carrier and the method of application and the target species can also impact on the efficacy of fungal applications so the complete information about the entomophatogenic fungi was needed before applied the entomophatogenic fungi on the field that lead this study.

Material and methods

Search strategy

The search of the literature strategy are two readily available electronic databases to identify the international literature published: 1) PUBMED NCBI (National center for Biotecnology Informastion) and 2) google scholar database. English is the language or publication type restrictions. The following keywords and combinations were used: entomophatogenic fungi, *Baeuveria bassiana, Metarhizium anisopliae, Aspergilus*.

Selection criteria of study

The study selection process was performed by the first author. Potentially relevant studies were identified by screening the titles and the abstracts.

Full manuscript texts were assessed, all potentially relevant studies were evaluated against the predefined inclusion criteria. The study characteristics are research article, short communication, case report and systematic review or meta analytic used as criteria for eligibility. In order to include studies of comparable quality, we considered only data published in peer-reviewed journals. Thus data from unpublished literature, such as Ministry of Health or Justice reports, were not included.

Data extraction and analysis

Information about entomophatogenic fungi ability to infect mosquito. The coverage of this study and the information are showed by criterias : kinds of entomophatogenic fungi that infect mosquito, effect of entomophatogenic fungi infection to mosquito, the formulation and application method to infect mosquitos.

Result and discussion

Kinds of Entomophatogenic Fungi that Infect Mosquito

B. bassiana, *M. anisopliae, Isaria fumosorosea, I. farinosa, I. flavovirescens*, and *Lecanicillium spp*. were known as entomophatogenic fungi and potential biological control agents of mosquito especially *Ae. aegypti* (Darbro *et al*. 2011), *Coelomomyces stegomyiae var stegomyiae* can infected adult *Ae. albopictus* (Seye *et al*. 2018), *Beauveria brongniartii* and *Isaria javanica* were also can infect the mosquitos (Ramirez *et al*. 2018).

Many research have been held to identify and analys the effectiveness of entomopathogen fungi to fight mosquito vector and the effective method for application. One research find *B. bassiana* was significantly more virulent than *M. anisopliae*, (Howard, Koenraadt, Farenhorst, Knols, & Takken, 2010) but the other research stated that both fungus isolates are effective and persistent at low concentrations and short exposure times (Mnyone *et al*., 2009). The excessity of *M. anisopliae*, the 25th generation insects were find developed a transgenerationally primed resistance to *B. bassiana* but not to *M. anisopliae* (Dubovskiy *et al*., 2013) which dry conidia *M. anisopliae* were more effective than wet conidia (Ansari *et al*. 2011).

Aspergillus sp may be as productive and virulent against mosquito larvae as a well-the others well known entomophatogenic fungi (Thomas Bawin *et al*. 2016), where one research give a statement that *Aspergillus nomius* was as pathogenic as *B. bassiana* (Jaber *et al*. 2016). Some of *Aspergilus sp*, they are *Aspergilus ochraceus, Aspergilus kanagawaensis* and *Aspergilus sulphureus* were identified effective causing mortality of *Ae. fluviatilis* and *Cx. quinquefasciatus* larvae in at least 80%, while one of *Aspergilus flavus* strain only effective to against *Ae. fluviatilis.* (Aurea Maria Lage De Moraes, Gisela Lara Da Costa and Ricardo Lourenço De Oliveira 2001). *Aspergilus clavatus* was identified highly pathogenic against larvae of *Ae. aegypti, Cx. quinquefasciatus* and *An. gambiae* (Seye *et al*. 2018). The *Aspergilus terreus ethyl acetate* extract was identified as the potential larvicidal and pupicidal activity against three mosquito vectors *An. stephensi, Cx. quinquefasciatus*,and *Ae. aegypti* (Ragavendran and Natarajan 2015).

Based on literature the entomophatogenic fungi dose were used against mosquitoes ($2x10^{10}$ m^{-2}) (Ansari *et al*. 2011) although each of research used different dose and yield a different result too. The age did not tend to affect mosquito susceptibility to fungal infection although the older mosquitoes died relatively earlier than younger ones and non-blood-fed mosquitoes were more susceptible to fungus infection than blood-fed mosquitoes (Mnyone *et al*. 2011). *Cx. quinquefasciatus* larvae treated with *A. clavatus* still could pupate and produce infected adults but the infected can cause the mortality for its adults like the result of previous study that the mortality can be happen to adult *Ae. albopictus* infected by the fungus *Coelomomyces stegomyiae var stegomyiae* (Seye *et al*. 2018).

B.bassiana infection in adults mosquito starts when conidia enters through the spiracles and get germinated then invades the walls of the tracheae, and elease a toxin beauvericin, bassianin, bassianolide, beauverolides, and tenellin that kills the adults within 5 days. While *M. anisopliae*, conidia attaching the host cuticle, forming an appressorium, following by a penetration peg to enter the cuticle and entering the hemocoel, then hyphae formed that produces and releases toxins destruxins, swainsinone, and cytochalasin C that killing the mosquitos within 4-16 days (Narladkar, Shivpuje, and Harke 2015). The mortality time shows that entomopathogenic fungi are slow-killing agents, but it kill the mosquito before they are capable of transmitting the infective sporozoite stages (Mnyone *et al*. 2012). When the fungi infect, it recognize by the mosquito and show the immune elicitation as the reaction but can't eliminate the entomopathogenic fungal infection (Ramirez *et al*. 2018).

One research found that fungal infection can reduced the expression of resistance to insecticides that are permethrin and dichlorodiphenyltri-chloroethane (Farenhorst *et al*. 2009), while the combination of permethrin and *B. bassiana* treatments caused the blood feeding reduction of wild Cx. quinquefasciatus (Howard, Guessan, *et al*. 2010).

The Formulation

Some research used the different carrier to make entomophatogenic fungi formulation like tween 80 and distilled water, (Blanford *et al*. 2012), soy bean oil (Ramirez *et al*. 2018), sunflower oil (Albernaz, D.A.S., Tai, M.H.H. and Luz 2009), neem oil (Gomes *et al*. 2015), combination of water and emulsifiable vegetable oil (Graxol) at 10% of the oil (Sousa *et al*. 2013), combination of vegetable oil and isoparaffin formulations (Carolino *et al*. 2014), Enerpar oil and Shellsol oil (Mnyone *et al*. 2012), where ShellSol T is an effective carrier for entomopathogenic fungi formulated so the conidia or spore of *M. anisopliae* or *B. bassiana* were easy to mix and apply to the aplication surface (Bukhari, Takken, & Koenraadt, 2011) although a research claimed that 'dry' conidia kill mosquitoes faster than oil formulated ones (Ansari *et al*. 2011).

Peanut oil and Shellsol plus Ondina protected M. anisopliae conidia against the deleterious effects of a 6 h exposure to UV light significantly better than the other formulations tested. Emulsifiable oil fungal formulations such as water plus Emoleo®, water plus Codacide®, water plus Ashlade® and water plus Natur'l oil ® also provided significantly improved protection of conidia against UV light com- pared with the conventional water plus 0.05% Tween 80 formulation (Carolino *et al*. 2014). A new research found a combination formula that claimed a high stability of entomophatogenic fungi for long time with no viscosity, it was named by "IE # 4" are: a mixture of two oils of plant-origin (soybean oil: 28.50%, w/w and coconut oil: 19.50%, w/w), oil-soluble emulsifier (Tween 20: 2.0%, w/w), sterile distilled water (45.00%, w/w), glycerine (4.25%, w/w), and water-soluble emulsifier (Dehymuls k: 0.75%, w/w) (Batta 2016).

Oil formulations are known to immobile spore, improve spore survival and fungal efficacy against insects by facilitate the fungi adhesion to insect cuticle and reduce spore sensitivity to UV radiation (Bukhari, Takken, and Koenraadt 2011), but kinds of oils ussualy were used for entomophatogenic fungi formulation for research hard to find by ordinary people, whereas the entomophatogenic fungi are potential to autonomously used by people because the entomophatogenic fungi are available in the market now and there is no impossible thing that coconut oil, palm oil, olive oil or maybe honey can be a good carrier to infect entomophatogenic fungi to mosquito, like olive oil have sun protector factor (SPF) 7,549 and its good to protect the entomophatogenic fungi from the sun exposure (Kaur and Saraf 2010).

The Application Method

The using of the trap to infected entomophatogenic fungi to mosquitos have to adapt with bionomic of the target mosquito. The resting target traps used for adult mosquitoes. One research with adult *Anopheles gambiae* as the sample tried to infect entomophatogenic fungi by mud panels, polyester netting and cotton cloth. The result, the risk of death for adult *Anopheles gambiae* exposed to conidia *M. anisopliae* on mud panels was higher than either polyester netting or cotton cloth, while mosquitoes exposed to *B. bassiana* on mud panels had a similar risk of death to mosquitoes exposed to this fungus on cotton cloth, in over-all, the poorest performance was consistently recorded for the polyester netting material (Mnyone *et al*. 2010), it is compatible with the bionomic of *Anopheles sp*, which like the pools or puddle that contact with soil directly. The other research found that polyester netting is a good candidate for operational use (Howard, Koenraadt, *et al*. 2010). The other research also found that fungal viability of *M. anisopliae* and *B. bassiana* significantly decreased when applied to the polyesther netting, but the effectiveness of the fungal treatment at killing mosquitoes did not significantly deteriorate (Farenhorst *et al*. 2010). Another research used black cotton cloth shown a good result to infect entomophatogenic fungi to mosquito (Carolino *et al*. 2014).

The application by delivery techniques that target host-seeking or house entering mosquitoes may be more effective than resting target techniques (Mnyone *et al*. 2011). The eave netting, eave curtains and cloth panels treated by *M. anisopliae* or *B. bassiana* failed to infect mosquitoes, but eave baffles and bed net strips shown the good result (Mnyone *et al*. 2012).

Aspergilus flavus have been tried to growth on a support as a biofilm in a liquid medium with the mosquito larvae as the goal target (T Bawin *et al*. 2014).

The culture filtrates of *Aspergilus niger* was identified have a lethal effect to adult *Cx. Quinquefasciatus, An. stephensi* and *Ae. aegypti*, although the mortality of exposured mosquitoes need process for a week or more (Singh and Prakash 2012). *Aspergillus clavatus* produced in a fungal biofilm bioreactor showed toxicity against *Cx. quinquefasciatus* larvae and adults The bioreactor was developed shows the chances to produce the fungal biomass simultaneously and can be used for industrial production (Seye *et al*. 2014). The combinatorial application of entomophatogenic fungi *A. flavus* combined with botanicals petroleum ether extract of *Cuscuta reflexa* against was found more effective to againts the *Anopheles stephensi* and *Cx. quinquefasciatus* larvae (Bhan, Mohan, and Srivastava 2013).

Many method can applied to infect entomophatogenic fungi to mosquito based on the stage and bionomic of target spesies, ovitrap maybe can consider as effective medium trap because especially for *Ae. aegypti*. The application of oil-based fungal formulations applied to ovitrap will be a more efficient to infect those eggs (Sousa *et al*. 2013), infect larvae inside eggs (Luz *et al*. 2008), but more ovitraps can infect all stage of mosquito (eggs, larvae, pupae and adults) because three stage in mosquito life cycle take place in aquatic condition that is can be mean ovitrap, and adults mosquitoes especially female mosquitoes should need ovitraps as breeding place.

Ovitrap and mosquito trap are the vector detection methods predicted dengue occurrence better than larval survey, and ovitrap presented longer duration periods than Mosquito trap (Melo, Scherrer, and Eiras 2012). The other advantage of ovitraps, it can be modified adapt to bionomic of the target spesies, for example ovitraps can be added by soil under the water similar as *Anopheles sp* breeding places.

Conclusion

Beauveria bassiana, Metarhizium anisopliae and *Aspergilus sp* were the popular entomophatogenic fungi that oil formulation have been known as the good formulation to infect entomophatogenic fungi to mosquito. Some method application were tried to infect the entomophatogenic fungi to mosquito like black cotton trap and polyester net but ovitrap could be a good consideration to used based on the capability to infect all stage of mosquito and can be modified appropriate by the bionomic of target species.

Recommendations

Entomophatogeni fungi can be used as vector control management in Indonesia, it is needed to co-working between health and agriculture institution of entomophatogenic fungi mass produce. All of the formulation is hard to find in Indonesia, so we need to looking for the new carrier of formulation that easy to find in Indonesia like olive oil or honey.

References

Abreu-Villaça, Yael, Edward D Levin. 2018. Developmental Neurotoxicity of Succeeding Generations of Insecticides. Human Subject System, February, 55–77. DOI:10.1016/j.envint. 2016.11.019.Developmental.

Albernaz DAS, Tai MHH, Luz C. 2009. Enhanced Ovicidal Activity of an Oil Formulation of the Fungus *Metarhizium anisopliae* on the Mosquito *Aedes aegypti*. Medical and Veterinary Entomology **23,** 141–147.

Amelia-yap, Zheng Hua, Chee Dhang Chen, Mohd Sofian-azirun, Van Lun Low. 2018. Pyrethroid Resistance in the Dengue Vector *Aedes aegypti* in Southeast Asia: Present Situation and Prospects for Management. Parasites & Vectors **11(332),** 1–17. DOI:10.1186/s13071-018-2899-0.

Ansari Minshad Ali, Edward C Pope, Simon Carpenter, Ernst-jan Scholte, Tariq M Butt. 2011. Entomopathogenic Fungus as a Biological Control for an Important Vector of Livestock Disease: The *Culicoides* Biting Midge. PLoS ONE **6(1),**1–8. DOI:10.1371/journal.pone.0016108.

Arefi Mohammad, Fakhroddin Taghaddosinejad, Peyman Salamaty, Davood Soroosh, Hami Ashraf, Mohsen Ebrahimi. 2014. Renal Failure Prevalence in Poisoned Patients. Nephro Urology Monthly **6(2),** 10–14. DOI: 10.5812/numonthly.11910.

Aurea Maria Lage De Moraes, Gisela Lara Da Costa, Mariangela Ziccardi De Camargo Barcellos, Pedrina Cunha De Oliveira Ricardo Lourenço De Oliveira. 2001. The Entomopathogenic Potential of *Aspergillus* spp. in Mosquitoes Vectors of Tropical Diseases. Journal Basic Microbiology **41(1),** 45–49.

Batta Yacoub A. 2016. MethodsX Invert Emulsion : Method of Preparation and Application as Proper Formulation of Entomopathogenic Fungi. MethodsX **3,** 119–27. DOI: 10.1016/j. mex.2016.02.001.

Bawin T, Boukraa S, Seye F, Raharimalala FN, Zimmer J, Delvigne F, Francis F. 2014. Biofilms from Entomopathogenic Fungi in Mosquito Control. 19th National Symposium on Applied Biological Sciences 2014.

Bawin Thomas, Fawrou Seye, Slimane Boukraa, Jean-Yves Zimmer, Fara Nantenaina Raharimalala, Quentin Zune. 2016. Production of Two Entomopathogenic *Aspergillus* Species and Insecticidal Activity against the Mosquito *Culex quinquefasciatus* Compared to *Metarhizium anisopliae*. Biocontrol Science and Technology **26(5),** 617–29. DOI:10.1080/09583157.2015.1134767.

Bhan Sweta, Lalit Mohan, Srivastava CN. 2013. Combinatorial Potentiality of *Aspergillus flavus* and *Cuscuta reflexa* against Mosquito Vectors. Advances in Bioresearch **4(4),** 99–105.

Blanford Simon, Nina E Jenkins, Andrew F Read, Matthew B Thomas. 2012. Evaluating the Lethal and Pre-Lethal Effects of a Range of Fungi against Adult *Anopheles stephensi* Mosquitoes. Malaria Journal **11(1)** DOI:10.1186 /1475-2875-11-365.

Bukhari Tullu, Willem Takken, Constantianus JM Koenraadt. 2011. Development of *Metarhizium anisopliae* and *Beauveria bassiana* Formulations for Control of Malaria Mosquito Larvae. Parasites & Vectors **4(1),** DOI:10.1186/1756 -3305-4-23.

Carolino Aline T, Adriano R Paula, Carlos P Silva, Tariq M Butt, Richard I Samuels. 2014. Monitoring Persistence of The Entomopathogenic Fungus *Metarhizium anisopliae* Under Simulated Field Conditions with The Aim of Controlling Adult *Aedes aegypti* (Diptera : *Culicidae*). Parasites & Vectors **7(1),** 1–7. DOI:10.1186/1756-3305-7-198.

Darbro Jonathan M, Robert I Graham, Brian H Kay, Peter A Ryan, Matthew B Thomas. 2011. Evaluation of Entomopathogenic Fungi as Potential Biological Control Agents of the Dengue Mosquito, *Aedes aegypti* (Diptera: *Culicidae*). Biocontrol Science and Technology **21(9),** 1027–47. DOI:10.1080/09583157.2011.597913.

Farenhorst Marit, Bart GJ Knols, Matthew B Thomas, Annabel FV Howard, Willem Takken, Raphael N Guessan. 2010. Synergy in Efficacy of Fungal Entomopathogens and Permethrin against West African Insecticide-Resistant *Anopheles gambiae* Mosquitoes. PLoS ONE, **5(8),** 1–10. DOI:10.1371/journal.pone.0012081.

Farenhorst Marit, Joel C Mouatcho, Christophe K Kikankie, Basil D Brooke, Richard H Hunt, Matthew B Thomas, Lizette L Koekemoer, Bart G J Knols, and Maureen Coetzee. 2009. Fungal Infection Counters Insecticide Resistance in African Malaria Mosquitoes. Proceeding of the National Academy of Sciences **106(41),** 17443–47. DOI:10.1073/pnas.0908530106.

Gomes Simone A, Adriano R Paula, Anderson Ribeiro, Catia OP Moraes, Jonathan WAB Santos, Carlos P Silva, Richard I Samuels. 2015. Neem Oil Increases The Efficiency of The Entomopathogenic Fungus *Metarhizium anisopliae* for The Control of *Aedes aegypti* (Diptera : *Culicidae*) Larvae. Parasites & Vectors **8(669),** 1–8. DOI:10.1186/s13071-015-1280-9.

Howard Annabel FV, Raphael N Guessan, Constantianus JM Koenraadt, Alex Asidi, Marit Farenhorst, Martin Akogbéto, Matthew B Thomas, Bart GJ Knols, Willem Takken. 2010. The Entomopathogenic Fungus *Beauveria bassiana* Reduces Instantaneous Blood Feeding in Wild Multi-Insecticide-Resistant *Culex quinquefasciatus* Mosquitoes in Benin, West Africa. Parasites & Vectors **3(87),** 1–11.

Howard Annabel FV, Constantianus JM Koenraadt, Marit Farenhorst, Bart GJ Knols, Willem Takken. 2010. Pyrethroid Resistance in *Anopheles gambiae* Leads to Increased Susceptibility to the Entomopathogenic Fungi *Metarhizium anisopliae* and *Beauveria bassiana.* Malaria Journal **9(168),** 1–9. DOI:10.1186/1475-2875-9-168.

Jaber Sana, Alex Mercier, Khouzama Knio, Sylvain Brun, Zakaria Kambris. 2016. Isolation of Fungi from Dead Arthropods and Identification of a New Mosquito Natural Pathogen. Parasites & Vectors **9(491),** 1–10. DOI:10.1186/s13071-016-1763-3.

Kaur Chanchal Deep, Swarnlata Saraf. 2010. In Vitro Sun Protector Factor Determination Herbals Oils Used in Cosmetics. Pharfacognosy Research **2(1),** 22–25.
DOI: 10.4103/ 0974-8490.60586.

Koca Yakup Onur, Ahmet Onder Ustundag, Melis Yalcin. 2016. The Paradox of Malathion Used for Human Life. Agronomy **LIX,** 515–20.

Luz C, Tai MHH, Santos AH, Silva HHG. 2008. Impact of Moisture on Survival of *Aedes aegypti* Eggs and Ovicidal Activity of *Metarhizium anisopliae* Under Laboratory Conditions. Memorias Instituto Oswaldo Cruz **103(2),** 214–15.

Melo Diogo Portella Ornelas De, Luciano Rios Scherrer, Eduardo Alvaro Eiras. 2012. Dengue Fever Occurrence and Vector Detection by Larval Survey, Ovitrap and Mosquitotrap: A Space-Time Clusters Analysis. PLoS One **7(7),** 1–14. DOI:10.1371/journal.pone.0042125.

Mint Aichetou, Mohamed Lemine, Mohamed Aly, Ould Lemrabott, El Hadji, Amadou Niang, Leonardo K Basco, *et al*. 2018. Pyrethroid Resistance in the Major Malaria Vector *Anopheles arabiensis* in Nouakchott, Mauritania. Parasites & Vectors **11(344),** 1–8.
DOI:10.1186/s 13071-018-2923-4.

Mnyone Ladslaus L, Matthew J Kirby, Dickson W Lwetoijera, Monica W Mpingwa, Emmanuel T Simfukwe, Bart GJ Knols, Willem Takken, Tanya L Russell. 2010. Tools for Delivering Entomopathogenic Fungi to Malaria Mosquitoes: Effects of Delivery Surfaces on Fungal Efficacy and Persistence. Malaria Journal **9(246),** 1–7.
DOI:10.1186/1475-2875-9-246.

Mnyone Ladslaus L, Matthew J Kirby, Monica W Mpingwa, Dickson W Lwetoijera, Bart GJ Knols, Willem Takken, Constantianus JM Koenraadt, Tanya L Russell. 2011. Infection of *Anopheles gambiae* Mosquitoes with Entomopathogenic Fungi: Effect of Host Age and Blood-Feeding Status. Parasitology Research **108,** 317–22.
DOI:10.1007/s00436-010-2064-y.

Mnyone Ladslaus L, Issa N Lyimo, Dickson W Lwetoijera, Monica W Mpingwa, Nuru Nchimbi, Penelope A Hancock, Tanya L Russell, Matthew J Kirby, Willem Takken, and Constantianus JM Koenraadt. 2012. Exploiting the Behaviour of Wild Malaria Vectors to Achieve High Infection with Fungal Biocontrol Agents. Malaria Journal **11(1),** 87.
DOI:10.1186/ 1475-2875-11-87.

Narladkar BW, Shivpuje PR, Harke PC. 2015. Fungal Biological Control Agents for Integrated Management of *Culicoides spp*. (Diptera : *Ceratopogonidae*) of Live
tock. Veterinary World **8 (February),** 156–63. DOI:10.14202/vetworld. 2015.156-163.

Ragavendran, Chinnasamy, Devarajan Natarajan. 2015. Insecticidal Potency of *Aspergillus terreus* against Larvae and Pupae of Three Mosquito Species *AnophelessStephensi, Culex quinquefasciatus*, and *Aedes aegypti*. Environment Science Pollution Research **22,** 17224–37.
DOI:10.1007/s11356-015-4961-1.

Ramirez L, Christopher A Dunlap, Ephantus J Muturi, Ana BF Barletta, Alejandro P Rooney. 2018. Entomopathogenic Fungal Infection Leads to Temporospatial Modulation of the Mosquito Immune System. Neglected Tropical Diseases, April 1–24. DOI:10.1371/journal.pntd.0006433 April.

Seye Fawrou, Thomas Bawin, Slimane Boukraa, Jean-yves Zimmer, Mady Ndiaye, Frank Delvigne, Frédéric Francis. 2014. Pathogenicity of *Aspergillus clavatus* Produced in a Fungal Biofilm Bioreactor toward *Culex quinquefasciatus* (Diptera : *Culicidae*). Journal Pesticide Science **39(3),** 127–32. DOI:10.1584/jpestics.D14-006.

Seye Fawrou, Oumar Faye, Mady Ndiaye, Ebrima Njie, José Marie Afoutou. 2018. Pathogenicity of the Fungus, *Aspergillus clavatus*, Isolated from the *Locust, Oedaleus Senegalensis*, against Larvae of the Mosquitoes *Aedes aegypti*, *Anopheles gambiae* and *Culex quinquefasciatus.* Journal of Insect Science **9(1),** 1–7.

Singh Gavendra, Soam Prakash. 2012. Lethal Effects of *Aspergillus niger* against Mosquitoes Vector of Filaria, Malaria, and Dengue : A Liquid Mycoadulticide. The Scientific World Journal **2012,** 1–5. DOI:10.1100/2012/603984.

Sousa NA, Lobo LS, Rodrigues J, Luz C. 2013. New Insights on The Effectiveness of *Metarhizium anisopliae* Formulation and Application Against *Aedes aegypti* Eggs. The Society for Applied Microbiology **57,** 193–99. DOI:10.1111/lam.12097.