

Susceptibility of *Anopheles arabiensis* Mosquito Pupal Stage to Bioactivities of *Varicosporium elodeae* and *Articulospora inflata*

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One of the greatest challenges facing Africa in the fight against malaria is drug resistance. Resistance to chloroquine, the cheapest and most widely used antimalarial, is common throughout Africa. As a result of this trend, other control strategies are being considered. Biological control of malaria vector, an integral part of controlling malaria has rarely been exploited in Nigeria. Recent developments in this field show that certain fungi displayed activities against *Anopheles* mosquitoes. Investigation of bioactivities of two entomopathogenic fungi namely *Varicosporium elodeae* and *Articulospora inflata* were assessed *in vitro* on pupal stage of *Anopheles* mosquitoes in this research. The fungi were applied separately to the pupae. The treatments were conducted in a controlled environment for five days during which mortality of the pupae was checked. The result revealed that the two treatments were significantly different from each other. High mortality was recorded in the groups treated with *V. elodeae* within 24 hours of post-treatment. In contrast, death of the pupae started after 48 hours of treatment with *A. inflata*. *Varicosporium elodeae* appeared more potent than *A. inflata*; the lethal dose concentrations obtained for the former and latter fungi were LC₅₀ (2.64 sfu/ml) and LC₅₀ (5.486 sfu/ml) respectively. Conclusively, *V. elodeae* and *A. inflata* biopesticide can potentially be an important component of malaria control strategy in Nigeria.

Key words: Entomopathogenic Fungi, Malaria, Drug resistance and Bioactivity.

A recent upsurge of malaria in endemic disease area with explosive epidemics in many parts of Africa is probably caused by many factors, including rapidly spreading resistance to antimalaria drugs. Malaria in human is caused by a protozoan of the genus *Plasmodium* and the four subspecies, *falciparum*, *vivax*, *ovale* and *malariae*.

Plasmodium falciparum is responsible for the greatest illness and death in Africa. The disease is transmitted by the bite of female *Anopheles* mosquito of which *Anopheles gambiae* complex is the most efficient in the transmission of this disease in Africa (WHO, 2006). Malaria is an important social, economic and developmental problem affecting individuals, families, communities and countries. In the last decade, the prevalence of this disease has been escalating at an alarming rate, especially in Africa. An estimated 300 to 500 million cases each year cause 1.5 to 2.7 million deaths, more than 90% in children under 5 years of age in Africa (WHO, 2006); it is ranked third among major infectious disease threats in Africa after

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pneumococcal acute respiratory infections (3.5%) and tuberculosis (TB) (2.8%). Cases in Africa account for approximately 90% of malaria cases in the world (Thomas, 1998). There is a wide variation in the clinical manifestation of the disease, ranging from asymptomatic malaria, uncomplicated and with the most severe forms characterized by life-threatening complications; severe malaria anemia and cerebral malaria (Amodu *et al.*, 2005).

The control of malaria has been a preoccupation of man; various efforts have been put in place to reduce the scourge of this infection especially among pregnant women and children less than 5 years (Idowu *et al.*, 2008). In the past, emphasis was placed on the eradication of malaria; presently the main thrust is to control the disease. However, these strategies have not yielded sufficient and encouraging results considering the morbidity - mortality ratio. Vector control therefore remains one of the main elements of the malaria control strategy, particularly in areas of unstable and less intense transmission (Idowu *et al.*, 2008). According to WHO (2006), vector control remains the most generally effective measure to prevent malaria transmission and is therefore one of the four basic technical elements of the Global Malaria Control Strategy GMCS. Vector control represents an important part of the current global strategy for the control of major vector-borne diseases, and has a vital role in the prevention of malaria (WHO, 2006). The current study is to investigate the use of two entomopathogenic fungi *Varicosporium elodea* and *Articulospora inflata* as a control method against malaria vector in Nigeria, by infecting the pupal stage of *Anopheles* mosquito with their conidia.

MATERIAL AND METHODS

The isolation of entomopathogenic microorganisms was conducted in 2007 at the Federal University of Technology, Akure (FUTA), Nigeria. Cockroaches and housefly were collected into sterile containers from their natural breeding habitats (cupboards for cockroach and housefly around the refuse dumps) in FUTA. In the laboratory, adult cockroaches were placed inside a sterile petri dish containing 10mL of sterile water each (in triplicate). The petri dish was properly

shaken to ensure good washing away of particles that were on the cockroaches. One millimetre was taken from the wash water, serially diluted to 10^{-4} and 0.1ml of the 10^{-4} serial dilution was pour plated using potato dextrose agar. Incubation was done at 25°C for 72 h (fungi), after which the agar was observed for growth. Identification of the fungi was done by comparison of the observed morphological characteristics with those described by Onions *et al.* (1995), after examination under the microscope. The same procedure was repeated using housefly.

Fungal cultures were grown on Potato dextrose agar (200 g of potato, 20 g of glucose, 20 g of agar and 1000ml of distilled water) at 25°C. Conidial suspension was obtained by scraping conidia from 12 days old well sporulated cultures into an aqueous solution of 0.2 % Tween-80. The suspension was then filtered through muslin cloth to remove mycelium and the concentrations of viable conidia was estimated as spore forming units, using a dilution plate count method.

The mosquito larvae were collected from stagnant waters. They were selected and differentiated using both physical and molecular characterisations. The *Anopheles arabiensis* pupae were reared in a meshed cage at 25°C and 70% relative humidity under 14L : 10D photoperiod with slight modifications according to Zhong *et al.* (2006). They were fed daily with Tetramin® fish food. This allowed them to reach maturity stage after which they were offered blood meal. Eggs laid on wet filter papers were transferred to water trays. Pupae were manually into containers containing sterile water.

The pupae were surface sterilised in separate petri dishes using 75% alcohol and rinsed with sterile water. There were three replicates and the control per treatment with 25 pupae in a container making up a replicate. One hundred pupae constituted a group. Susceptibility of the mosquito pupae to the fungal isolates was evaluated by subjecting the 25 pupae to fungi. Each group was inoculated with each of the varying conidia loads of 1.3 to 6.5 Sfu/ml. Incubation was carried out for 5 days at 27°C. Dead cadavers were removed daily and counted. The LC_{25} , LC_{50} and LC_{75} of the fungi were determined using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Application of the two pathogens, *V. elodeae* and *A. inflata* caused considerable mortality effect on the pupal stage of the *Anopheles* mosquito. Spore population and incubation time affected the percentage mortality

of the pupae (Fig. 1-2). There was increased mortality as the spore load increased. The digestion of mosquito was rapid when sufficient high spore number was used. *Varicosporium elodeae* produced higher number of dead pupa even within 1 day of post-infection than *A. inflata* in all the treated groups. This is supported by the

Table 1. Relative potency of *Varicosporium elodeae* and *Articulospora inflata* on the pupal stage of *Anopheles* mosquito larvae

No	Line name	LC ₂₅	Lower limit	Upper limit	1	2	Index	RR	Slope	LC ₅₀	LC ₇₅
1	<i>Varicosporium elodeae</i>	0.273	0.043	0.56	*	*	100	1	1.123	2.64	3.43
2	<i>Articulospora inflata</i>	0.542	0.095	0.837		*	52.127	2.865	0.926	5.486	14.654

Index compared with *Varicosporium elodeae*

Resistance Ratio (RR) compared with *Varicosporium elodeae*

lethal dose (LC₅₀) of 2.64 Sfu/ml for *V. elodeae* and 5.486 sfu/ml for *A. inflata* (Table 1). This means that *V. elodeae* was more effective and its spores infected their pupae better than *A. inflata*.

Higher mortality was noticed with increase in contact time of the fungi (Fig. 1 and 2). The percentage mortality was highest in treatments with *V. elodeae* with values significantly different from *A. inflata* at p ≤ 0.05. This disparity in activity

displayed by *V. elodeae* and *A. inflata* on the pupae at higher concentrations may be due to the fact that *V. elodeae* was able to digest the hard cuticle better the other fungus. Similar observation was reported by Manonmani *et al.* (2008). The result of the present work demonstrated that *Anopheles arabiensis* pupae are susceptible to *V. elodeae* and *A. inflata*, incubation time and fungal concentrations affected the level of mortality.

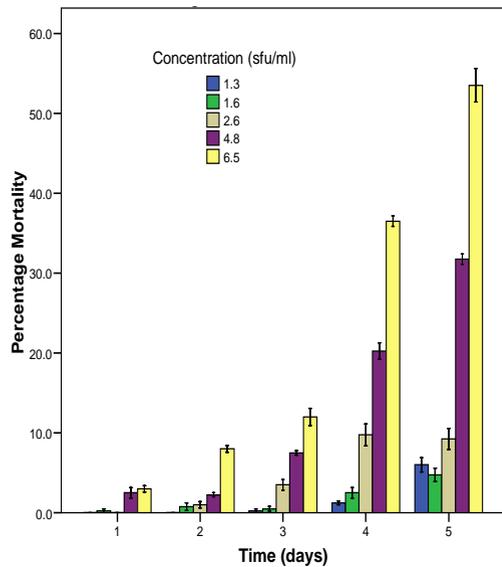


Fig. 1. Mortality effect of *Varicosporium elodeae* at different loads and incubation time on the pupal stage of *Anopheles* mosquito pupae

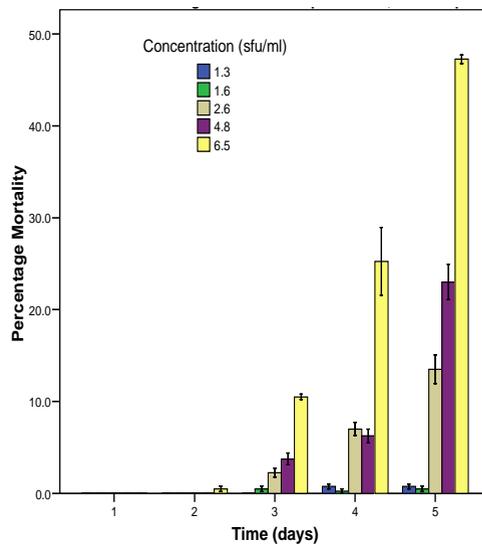


Fig. 2. Mortality effect of *Articulospora inflata* at different loads and incubation time on the pupal stage of *Anopheles* mosquito pupae

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