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Toxicity Assessment of Varicosporium Alodeae and Articulospora Inflata on Anopheles Mosquito larvae in South West Nigeria

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Abstract: 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. Two entomopathogenic fungi namely *Varicosporium elodeae* and *Articulospora inflata* were assessed in *vitro* on different larval stages of *Anopheles* mosquitoes namely: second and fouth instars for their toxic activities. The treatments were conducted in a controlled environment for five days. High mortality was recorded in the groups treated with *V. elodeae* within 72 hours of post-treatment at LC_{50} (0.67 sfu/ml) at second instar while LC_{50} (1.876 sfu/ml) was observed for *A. inflata*. It was also revealed that the second instar was significantly different from fourth instar stages. Conclusively, *V. elodeae* and *A. inflata* could be a potential biopesticide for malaria vector control in Nigeria.
Keywords: Entomopathogenic fungi, malaria and biological control

Introduction

Malaria a life threaten disease, caused by protozoa in the genus *Plasmodium* is transmitted to people through the bites of infected female *Anopheles* mosquitoes. The insect bites between dusk and dawn. Transmission is more intense in places where the mosquito is relatively long-lived so that the parasite has time to complete its development inside the mosquito and where it prefers to bite humans rather than other animals[1]. This is the reason for occurrence of more than 85% of the world's malaria in Africa. In 2008, there were 247 million cases of malaria and nearly one million deaths. The disease occurs mostly among children living in Africa. It accounts for 20% of all childhood death. In Africa, a child dies of malaria attack every 45 seconds. This is due largely to level of immunity possessed by African children. Malaria can also result in miscarriage and low birth weight, especially during the first and second pregnancies. An estimation of 200,000 infants die annually as a result of malaria infection during pregnancy[1]. However in areas with less transmission and low immunity, all age groups are at risk.

Growing resistance of the etiologic agent to antimalarial medicines has spread very rapidly, undermining malaria control efforts. Idowu *et al.*[2] reported that about 50% of Nigeria population experienced at least one episode of malaria yearly. It has been reported especially in African and Asian

countries that chloroquine and sulfacoxine-pyrimethamine (SP) are no more effective as antimalarial drugs due to resistance of *Plasmodium* species[3]. Economically, malaria causes significant losses and can decrease gross domestic product (GDP) by as much as 1.3% in countries with high level of transmission (WHO, 2010).

Vector control is the primary public health intervention for reducing malaria transmission at the community level. It is the only intervention that can reduce malaria transmission from very high level to about zero. According to Lima *et al*.[4], the rapid increase of mosquito resistance to various chemical insecticides and the growing public concern over environmental pollution has resulted in the development of alternative methods for mosquito control, such as the use of biological agents. Biocontrol of malaria vector in Nigeria is a form of vector control that has not been adequately explored. Preliminary studies carried out showed that many microorganisms are associated with insects[5,6]. In furtherance of this work, this project was designed to assess the bioactivity of two fungi (*Varicosporium elodeae* and *Articulospora inflata*) isolated from insect cadavers (cockroach and housefly) on *Anopheles* mosquito larvae in south west Nigeria.

Materials And Methods

The isolation of entomopathogenic microrganisms was conducted at the Federal University of Technology, Akure (FUTA), Nigeria. Cockroaches and houseflies 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^{o}C for 72 h, after which the agar was observed for growth. The identification of fungi was carried out by comparison of their morphological characteristics with those described by Onions *et al.*[7], after examination under the microscope. The same procedure was repeated using houseflies.

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^{o}C. Conidal 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 loads of viable conidia were estimated as spore forming units, using a dilution plate count method.

The mosquito larvae were collected from stagnant waters. The A*nopheles arabiensis* larvae were reared in a meshed cage at 25^{o}C and 70% relative humidity under 14L:10D photoperiod with slight modifications according to Zhong *et al*.[8]. 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. Larvae were fed and sorted into second and fourth instars for bioassays.

Evaluation of Larvae Susceptibility to fungal isolates was carried out as follows. Larvae at each instar were divided into 25 larvae. Each of the mosquito stages was surface sterilised in separate petri dishes using 75% alcohol and rinsed with sterile water. They were treated with various concentrations of spore ranging from 1.3 to 6.5 Sfu/ml. Incubation was carried out for 5 days at 27^{o}C. Daily the number of dead larvae were counted. The LC_{25}, LC_{50} and LC_{75} were determined using probit analysis[9].

Table 1: Relative potency of *Varicosporium elodeae* and *Articulospora inflata* on the second instar of *Anopheles* mosquito larvae

No	Line name	LC_{25}	Lower limit	Upper limit	1	2	Index	RR	Slope	LC_{50}	LC_{75}
1	*Varicosporium elodeae*	0.178	0.027	0.4	*	*	100	1	1.123	0.67	2.73
2	*Articulospora inflata*	0.361	0.065	0.707		*	49.307	2.028	0.926	1.876	10.324

Legend:
Index compared with *Varicosporium elodeae*
RR: Resistance ratio compared with *Varicosporium elodeae*

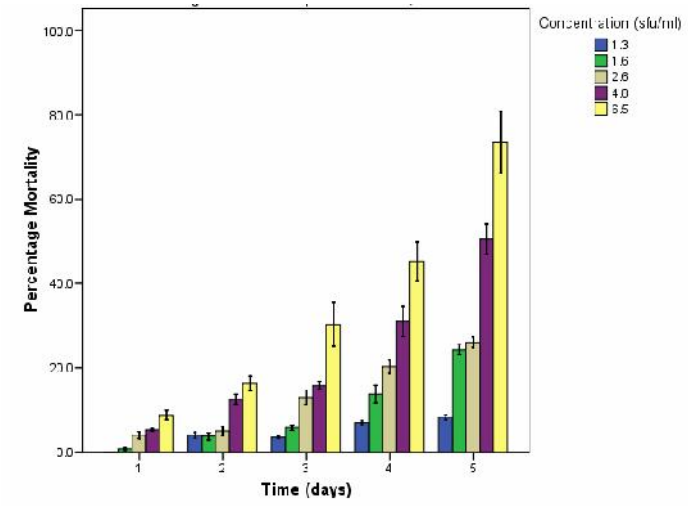

Figure 1: Effect of infection at different concentrations and incubation time of *Varicosporium elodeae* with the 2nd instar of *Anopheles* mosquito larvae.

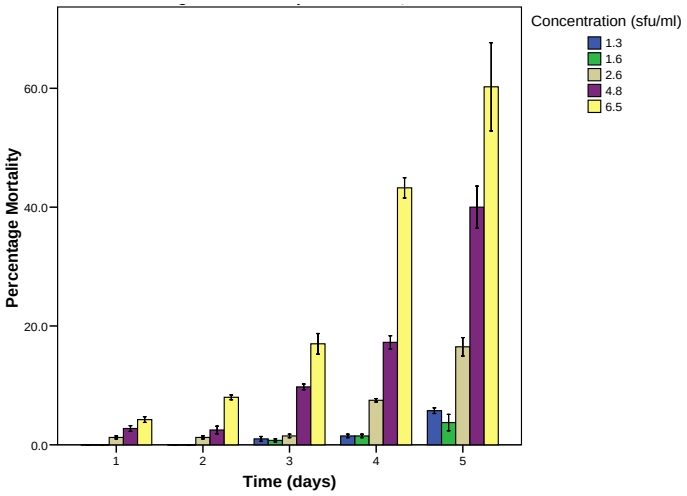

Figure 2: Effect of infection at different concentrations and incubation time of *Varicosporium elodeae* with the 4th instar of *Anopheles* mosquito larvae

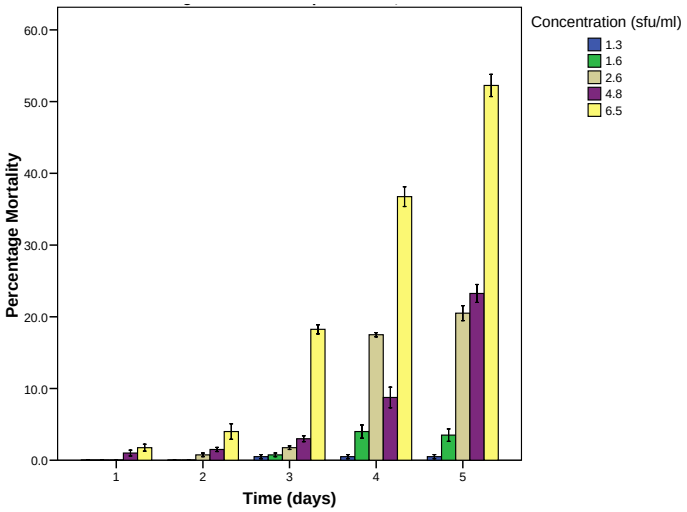

Figure 3: Effect of infection at different concentrations and incubation time of *Articulospora inflata* with the 2nd instar of *Anopheles* mosquito larvae

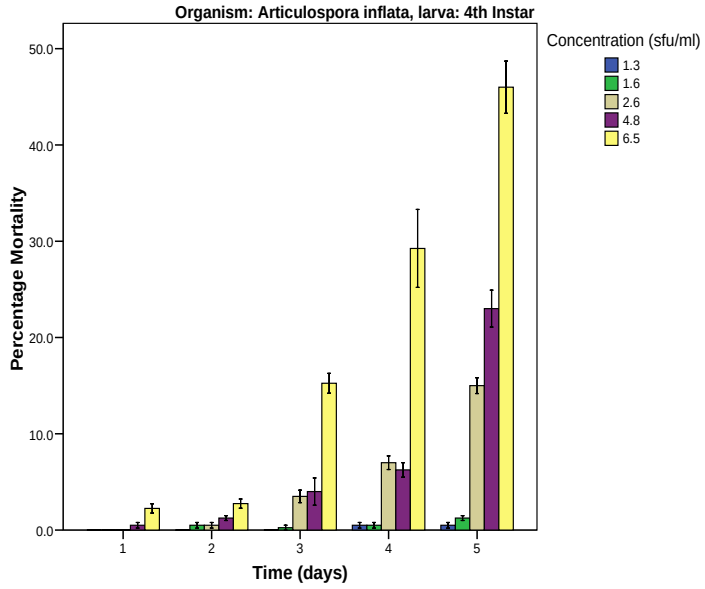

Figure 4: Effect of infection at different concentrations and incubation time of *Articulospora inflata* with the 4th instar of *Anopheles* mosquito larvae.

Results And Discussion

Spore population and incubation time were seen to affect the percentage mortality (Fig 1- 4). There was increased mortality as the spore load increased. The digestion of mosquito was rapid when sufficient high spore number was used. *Varicosporium elodeae* exhibited higher larvicidal activity than *Articulospora inflata* in all the treated groups. This observation was noticed after day 3 of infesting the larvae with the organisms. This might be as a result of the fact that fungal spores infect their host by germination of mycellia which penetrate the host exoskeleton. There might have been toxin production. Similar data was published by Scholte[10].

Incubation time displayed direct relationship with the mortality of the larvae. As the contact time increased, there was corresponding increase in mortality (Fig. 1-4). The percentage mortality was highest in the 2nd instar treatment with values significantly different from 4th instar stages at $p \leq 0.05$ for *V. elodeae* (Fig.1 and 2). In contrast, the observed percentage mortality was not significantly different between the second and the fourth instar at $p \leq 0.05$ for *A. inflata* (Fig. 3 and 4). This difference in larval mortality expressed by *V. elodeae* and *A. inflata* on the fourth larvae at higher concentrations of the spores may be as a result of the age and the hardiness of the larvae cuticle. Similar result was reported by various scientists including Manonmani *et al.*[11]. This indicates that the fungal mycelia could penetrate the softer cuticle of the larvae in the 2nd instar faster and deeper within a shorter contact time than the 4th instar of the larvae. *Varicosporium elodeae* appeared more potent than *A. inflata* based on the LC_{50} of 0.67 Sfu/ml and 1.876 Sfu/ml for the two fungi respectively. Microorganisms with high degradative ability are good biocontrol agent[12].

The result of the present work demonstrated that *Anopheles arabiensis* larvae are susceptible to *V. elodae* and *A. inflata*. Since these fungi were sourced from insects it then means that insects are good and cheap source of microorganisms capable of eradicating mosquito. Therefore, we conclude that the application of *V. elodeae* and *A. inflata* on malaria vector larvae could significantly reduce the parasite transmission thereby leading to reduction of malaria risk. Biological control methods have potential as a new strategy for malaria control in Nigeria.

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