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Full Length Research Paper

Adulticidal effect of fungal pathogen, *Metarhizium anisopliae* on malarial vector *Anopheles stephensi* (Diptera: Culicidae)

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The entomopathogenic fungus, *Metarhizium anisopliae* is being considered as a biocontrol agent for the adult mosquito of *Anopheles stephensi* (Malarial vector). In the present experiment was carried out in the laboratory of 30-50 male and female adult mosquitoes were exposed to *M. anisopliae* (exposed to 1 x 10^6 conidia/ml of oil or water suspension). In our results, there was 96% and 94% adult mortality was observed in oil and water formulated conidia of *M. anisopliae*. Similarly, adult emergency rate was also decreased with increasing concentration (1x10^8 conidia/ml). Finally, we conclude that the fungal spores or cells developed within insect cuticle which is suppress the cellular defence system and also fungal grow on the legs and wings to arrest the mosquito movement.

Key words: Entomopathogenic fungi, *Metarhizium anisopliae*, *Anopheles stephensi.*

INTRODUCTION

Malaria rank amongst the world's most prevalent tropical infectious diseases. An estimated 300 - 500 million people are infected with malaria annually, resulting in 1.5 to 3 million deaths (WHO, 2000). The impact of persistence of entomopathogenic fungi on insects and on filage has not been extensively studied. Conidia of hyphomycetous fungi strongly adhere to insect cuticle, and the attachment of conidia to cuticles is through to involve non-specific adhesion mechanisms mediated by the hydrophobicity of the cell wall (Bouclas et al., 1988, 1991). Considerable research is now focusing on the formulation of conidia in oils, but the influence of these formulations on the rain fastness of conidia is unknown (Poprawski et al., 1997). Survival of entomopathogenic fungi requires a delicate balance of interaction between the fungus, host and the environment. In general, the life cycle of the entomopathogenic fungi involves an infective spore stage, which germinates on the cuticle of the host, forming a germ tube that penetrate the cuticle and invades the hemocoel of the insect host (Hajek and Leger, 1994).

The fungus multiplies within the insect and kills it; death is due to toxin production by the fungus or multiplication to inhabit the entire insect. Under favorable environmental condition, the fungus grows out of the cadaver, and forms conidiophores or analogous structure and sporulates. Alternatively, many species form some type of resting stages capable of forming or releasing a type of spore. Spores need new hosts, so the fungus needs a strategy for dissemination. Therefore, the important point is that the environment and host are crucial to the survival and reproduction of the fungus. Insect pathogens have a long history of recognition despite the relatively recent understanding of microbial infections. Descriptions of entomopathogenic fungi can be found in drawings from several centuries ago (Simson et al., 1988). Reviews dealing with entomopathogenic fungi and their development as microbial control agents are available (Ferron, 1985; Butt et al., 2001; Upadhyay, 2003). The available literature on entomopathogenic fungi for mosquito control, however, is rather scattered and void of recent reviews (Roberts, 1974; Ferron et al., 1991). Hence in the present investigation, an attempt has been made to evaluate the effect of the fungal pathogen, *Metarhizium anisopliae*, on the adulticidal effect on malarial vector, *Anopheles stephensi*.

MATERIALS AND METHODS

The mosquito larvae was maintained in our laboratory at 28 ± 2ºC,

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Table 1. Adult *Anopheles stephensi* exposed to dry conidia (1 x 10^6 conidia/ml) of the entomopathogenic fungus *Metarhizium anisopliae* for different time duration.

Species	No. of mosquitoes released	Exposure time	Mortality (%)	LT_{50} ± SE
				Control Treated
A. stephensi	100	8 days	94	18.00±1.00
		72 h	76	3.24±0.23
		48 h	67	11.68±1.16
		24 h	54	3.29±0.59
				8.86±1.31
				3.38±0.27
				7.65±1.30
				3.75±0.29

75 - 85% RH, under 14 L: 10 D photoperiod cycles. The larvae were fed with dog biscuits and yeast at 3:1 ratio. The feeding was continued till larva transform into the pupal stage. The mosquito larvae were collected in a container from the drinking water tank and rain water pool near Bharathiar University, Coimbatore, Tamil Nadu, India. The mosquito larva was kept in to the laboratory at room temperature (28 ± 2ºC).

Bioassay 1

Dry conidia of *M. anisopliae* were supplied by T- Stanes research and development Pvt. Limited, Coimbatore. Conidia were suspended in distilled water and germination percentages were determined on potato dextrose agar (PDA). Conidia were suspended in either sterile distilled water or oil formulation. The concentrations of conidia were estimated using a heamocytometer and the number of viable conidia/ml was calcula-ted as a percentage germination x the concentration of conidia, as determined from the hemocytometer count. The conidia concentrations were adjusted to 2 x 10^8 viable conidia/ml.

Conidia inoculation in water or oil containing 10^2, 10^4, 10^6 and 10^8 conidia was pipetted out. The 3rd and 4th instars larvae were kept into the different conidial concentration and the mortality was also calculated. The number of death larva was counted at different time interval.

Bioassay 2

For each of three replicate, 100 adult mosquitoes of *A. Stephensi* (malaria vector), aged 1 - 2 days at the start of the experiment, were placed in a cage covered with muslin cloth mosquito netting. The mosquitoes were offered 10% glucose solution absorbed into white laboratory filter paper placed in a glass vial. This suspension dusted with 100 mg dry conidia and placed over the filter paper. Mosquitoes landing on the suspension to consume glucose would thus be exposed to conidia through tarsal contact or head and thorax region when feeding through the holes in the suspension. The suspensions remain in the cage until the end of the bioassay (Minimum of 8 days).

Bioassay 3

The coconut oil was added to the (10% oil- formulated for the suspension). Different concentration of conidia/ml. In the bioassay, 1 ml of various oil-formulations was pipette evenly over the filter paper. This paper was gently placed in the cage. Mosquitoes were offered 10% glucose solution placing over the cage. The dead mosquitoes were removed from the vial after three days. Then the dead mosquitoes were examined into the optical microscope for conidial penetration.

Statistical analysis

Experimental test of percentage mortality observed was corrected by Abbott's formula (Abbott, 1925). The lethal concentration of 50% and 95% (LT_{50} and LT_{95}) were used to measure differences between test samples.

RESULTS

The fungal cells developing within the insects may possess an outer coat, which is neutral to circulating heamocytes or they are effectively masked by host proteins or by producing immuno-modulating substances which suppress the cellular defence mechanism, the fungal cells may be tolerant to the humoral and cellular defence system of the insects. The *M. anisopliae* showed to be pathogenicity of larvae of *A. stephensi*, of the mosquito larvae when exposed to 1 x 10^6 dry conidia. The mortality was also calculated at different time interval. The hundred mosquito larvae were exposed into the dry conidia on the 8th day at 72 h, 48 h and 24 h time interval. The mortality was observed about 96% at the 8th day exposure of *M. anisopliae* and the 72 and 48 h of expo-sure the mortality was about 76 and 67%, respectively (Table 1). In this experiment where mosquito larvae were expos-ed to dry conidia, the fungal sporulation was observed in 95% of the insect. Effect of *M. anisopliae* on the growth and development of *A. stephensi* is given in the Table 2. Larval, pupal and adult emergency was steadily reduced after the treatment of different conidial concentration. The total emergency was also significantly reduced after the treatment of *M. anisopliae* at higher dose level. *M. anisopliae* exposed to adult mosquito of *A. Stephensi*, when mosquito were exposed to 10% oil formulation and conidia concentration was expressed in 1 x 10^6 at different time interval. The 100 number of adult mosquito of *A. Stephensi* was exposed in to the conidial formulation, the mortality was observed in different time

Table 2. Effect of different concentration of *Metarhizium anisopliae* on the growth and development of *Anopheles Stephensi*.

Concentration (conidia/ml)	Mean duration of each Instars (days)			Total no. of days	Total emergency (%)
	Larvae	Pupae	Adult		
1x 10^2	6.0±1.0	2.0±2.2	2.0±0.8	15.0±0.8	80
1x 10^4	5.0±1.0	1.5±1.4	2.0±1.4	13.5±1.4	71
1x10^6	4.0±1.2	1.0±1.2	1.5±0.6	11.5±0.4	60
1x10^8	4.0±1.8	0.5± 1.0	1.0±0.6	6.0±1.2	40

Table 3. Adult *Anopheles stephensi* exposed to different doses of coconut oil formulated *Metarhizium anisopliae* (conidia/ml of 10% oil-formulation).

Concentration (conidia/ml)	No. of Mosquitoes released	Exposure time	Mortality (%)	LT_{50} ± SE
1x10^6	100	8 Days	96	5.85 ± 0.26
1x 10^6	100	72 h	70	6.65±0.43
1x10^6	100	48 h	49	5.08±1.61
1x 10^6	100	24 h	40	3.75±0.29

duration, in which 96% of mortality was observed in after 8 days and the 70% of mortality was observed at 72 h interval (Table 3). The mosquito survival in the untreated group was not significantly different from that observed for mosquitoes exposed to the lowest dose. The LT_{50} and SE were significantly different from the survival of adult mosquitoes.

DISCUSSION

Biological control at the larval stages of development of mosquitoes is one of the techniques which afford a cheap, easy to use and environmental friendly method of mosquito control. Natural insecticides are phytotoxic and do not accumulate chemical residue in the flora, fauna and soil. In laboratory study, the adult mosquito *A. stephensi* were susceptible to *M. anisopliae*.

Many biological control agents have been evaluated against larval stages of mosquitoes, of which the most successful ones comprise bacteria such as *Bacillus thuringiensis israelensis* and *Bacillus sphericus* (Fillinger et al., 2003). Entomopathogenic fungi are considered excellent candidates for biopesticides due to their safety, relatively limited host range, ease of production and suitability of large scale production (Ferron, 1981). Current intra-domiciliary vector control depends on the application of residual insecticides. Although biological control agents have been developed against aquatic mosquito stages, none are available for adults.

Epidemiology models on malaria show that adult and larval mosquito survival is the most sensitive component of vector capacity (Miller, 1973). For the successful conidial attachment and in the end, killing of a mosquito, a threshold number of conidia per unit surface area are required. In our lethal dose response experiment the lowest dose resulting in a significant effect on mosquito survival was 1 x 10^8 conidia/ml. In order to achieve the highest possible impact of the fungus on the mosquito population, it is desirable that the other pathways besides the primary mode of contamination are utilized. The results of this study show that laboratory condition is more significant to the field (Scholte et al., 2003). The results from the current study shows that the daily survival rates of *M. anisopliae* infected adult as well as larval mosquitoes at any given moment in the mosquito life span, is lower than non-infected mosquitoes, and that their life span is reduced, provided that the conidia dose is high enough. Prospects for developing this adult and larvae mosquito control strategy are promising and may in due course be developed into a mosquito control tool. Hence, the present investigation has been made to evaluate the adulticidal effect of *M. anisopliae* to evaluate good biocontrol agent of *A. stephensi*. Finally, we discuss about fungal pathogen interacting with adult mosquito as an attempt to control the mosquito in the laboratory level.

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