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Evaluating Mosquito Biocontrol Effectiveness by Isolating and Characterizing Some Fungi Against *Culex Pipiens* and *Anopheles Stephensi* in Sothern Part of Iraq

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Abstract:

Objectives: Isolation *Aspergillus tamarii*, *Cladosporium herbarum*, and *Verticillium lecanii* fungi from naturally infected *Culex pipiens* and *Anopheles stephensi* insects were morphologically and molecularly identified.

Methods: In this study, populations of mosquitoes were cultured and examined to determine fungal infection and evaluated as potential agents against *C. pipiens* and *A. stephensi.*

Results: A variety of fungal isolates demonstrated differing degrees of pathogenicity 24 hours after treatment against *C. pipiens* and *A. stephensi* eggs, four-instar mosquito larvae, and adults. and as a biological control, it was found that the fungal suspension of each of the used fungi affected the life roles of the two mosquitoes. as it was more than the fungus suspension *A. tamarii, Cl. herbarum* on *V. lecanii*. The highest percentage of eggs mortality were (36.72, 48.97) %, (29.14, 42.25) %, and (24.45, 35.70) % of *C. pipiens* and *A. stephensi* when using the highest concentration of $1*10^5$ spore/ml, $3*10^5$ spore/ml and $2*10^5$ spore/ml of fungicide *A. tamarii, C. herbarum, V.lecanii* and respectively after 24 hours. The highest mortality rate was for the fourth larval stages, and the mosquito *A. stephensi* is more sensitive to type *C. pipiens* infection., as, in order (75.6, 67.19, 56.8) %, (74.18, 59.81, 50) %, and (65, 52,42.71) %.

Conclusions: Results highlight the significance of a mosquito's natural fungal opponent. All isolates had an impact on adults and larvae, although they were less successful against eggs. Both have the potential to develop, particularly against the larvae of the primary arbovirus, malaria, and lymphatic filariasis vectors, *A. stephensi*, and *C. pipiens*.

Keywords: *Entomopathogenic fungi; mosquito control; Culex pipiens; Anopheles stephensi.*

1 Introduction

Mosquitoes pose a serious risk to human health worldwide because they can spread infectious diseases such as arboviruses, malaria, and lymphatic filariasis., *A. stephensi* is a major vector of malaria in many countries of the world, including Iraq (Matthews,2011). where more than 250 million people, as well as more than a million deaths every year in the world, are infected with malaria (W.H.O, 2018.) while mosquitoes are *C. pipiens* a vector for various dangerous viral pathogens, including St. Louis virus (which causes encephalitis), West Nile, and dengue fever (W.H.O, 2014). It also transmits the nematode *Wuchereria bancrofti* which causes elephantiasis or the so-called Filariasis disease, which wastes the lives of millions of people. He scored more than 700 million people infected with filariasis, and about 103 million people in more than 80 countries face the risk of infection with this disease (Ishak *et al*., 2017) Perhaps chemical control was and is still the most effective in eliminating mosquitoes and reducing their damage in different regions of the world, where many manufactured pesticides have been used, but the damage caused by them was not little, as their use led to pollution of the air, water, and soil, Moreover, the target insects gained the ability to quickly adapt to toxic substances and to start developing immunity against them (Thongwat *et al*., 2018). Because of their selective specificity and environmental safety, entomopathogenic fungi represent an environmentally benign alternative that is gaining popularity in efforts to reduce the burden of vector-borne diseases (Bandani *et al*., 2000). Previous research has demonstrated the vast potential of microbial agents in the biocontrol of mosquitoes, so attention has turned to the use of fungal spore suspension in insect control to be a safe alternative to manufactured pesticides. This is because they are toxic, nutrient-inhibiting, or inhibitory substances in insects (Nielsen and Lewis 2012, Thakur *et al*., 2020) Among the eukaryotic organisms on Earth, fungi are the second biggest group, with an estimated 1.5 to 5.1 million species. (1-3) The fungal kingdom has important significance in human existence. (Hawksworth & Lücking, 2017; Pandian, 2023). and among these fungi species subordinate to the genera *Aspergillus*, *Verticillum, and Cladosporium*, are pathogenic to mosquitoes when the appropriate conditions are available for the development of spores of these fungi, (Kamalakannan *et al*., 2022; Pathan *et al*.,2021; Vivekanandhan *et al*,, 2018), *V. lecanii* is considered one of the deficient fungi as it is found in the soil and infects all soil insects, and it can infect mosquitoes (Sharma and Sharma, 2021). Previous studies have shown *A. tamarii* reported better larvicidal properties against mosquitoes, as for the fungus *C. herbarum* of the cystic fungi (Rana *et al*., 2020), and its effect on the whitefly *Bemisia* sp. and scale insects (Baskar *et al*., 2020). Fungal species identification is crucial in both basic (ecology, taxonomy) and applied (genomics) fields. Of the studies, 27% relied solely on molecular data, primarily from the internal transcribed spacer (ITS) region, for fungal identification (Tedersoo & Nilsson, 2016), while approximately 14% combined molecular and morphological data. This indicates that standardizing the taxonomic identification of fungi is a topic that could benefit from standardization, particularly in the application of bioactive (Raja *et al*., 2017). Because of the medicinal importance of the used mosquitos, and the fact that previous research that contributed to isolating local types of fungi and their use as a vital factor in the control is almost very few, and it did not previously address the fungus *A. tamarii*, *V. lecanii*, and *C. herbarum* Therefore, this study aimed to isolate three mentioned fungi from the of *Culex* spp. and *Anopheles* spp. that infected naturally for the first time in Iraq and evaluating their efficiency in controlling two types of mosquitoes; *C. pipiens* and *A. stephensi*.

2 Materials and Methods

2.1 Collection, Isolation, Identification, and Phylogenetic Analysis of Fungi associated with Mosquito

Naturally infected insects were obtained from Diwaniyah city, Al-Dagharai District (Iraq). In summary, external conidia from infected insects were placed onto potato dextrose agar (PDA) medium supplemented with 0.01% (w/w) chloramphenicol plate (PDA+) and 5% (w/w) sodium chloride. The plates were then incubated for seven days at 30 °C. To achieve pure culture isolation, the hyphal tip of the fungal colony was then moved to PDA plates. To identify the isolated fungi preliminary, the macro- and microscopic characteristics of the isolates were analyzed using the techniques of Samson Al-Rawei *et al*., (2018) and Hubka *et al*. (2016). The internal transcribed spacer (ITS) region of the rRNA gene was utilized for PCR amplification and for DNA extraction, sequencing, and BLAST, which were used to characterize fungi (Schoch e*t al*., 2012). The ITS universal primer set is positioned in front of the ITS 1, 5.8S, and. The nucleotide sequence of the ITS 1 primer is 5′ GTAACAAGGTTTCCGTAGGTG-3′ and that of the ITS 2 is 5′TTCTTTTCCTCCGCTTATTGATATGC-

3′ (AL-RIFAIE, 2023). A PCR program comprising an initial denaturation at 94 °C for 5 min was followed by 30 cycles of denaturing at 94 °C for 30 sec, elongations at, annealing of primer at 55 °C and 72 °C for 1 min 30 sec, and a final termination at 72 °C for 10 min was used in the GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) (Killelea *et al*.,2014). The primer pairs (ITS) and PCR products were delivered to the Macrogen DNA sequencing service in Korea after being refrigerated at 4°C. Direct sequencing was done on the PCR products in both directions. Using the Basic Local Alignment Search Tool (BLAST), nucleotide sequences were aligned and compared with the sequences of the other fungal isolates that were accessible in the NCBI database (Abdullah *et al*., 2019). Using MEGA 11, phylogenetic analysis of all fungal nucleotide sequences was compared.

2.2 Experimental conditions

All experiments were performed in rooms with climatic conditions similar to the outside environment.

Preparation of Spore Suspension: The isolates were cultivated for ten days at 37 °C on sterile substrates. Following the completion of the solid-state fermentation, the substrate (10 g) was gathered and overtaxed in a sterile test tube to suspend and loosen the spores. The mixture was then combined with 20 mL of 0.01% Tween 80. The contents of the dish were filtered through a fixed glass funnel containing a sterile piece of gauze with the addition of another 5 ml of distilled water to ensure the filtering of all fungal spores (Choi *et al*., 1999) To calculate the number of spores, take 1 ml of the filtrate and put on a slide count the modified white blood cells to count the spores: Improved Neubauer Haemocytometer to estimate the number of spores per unit volume, according to the number of spores in each of the four large squares in the corners of the slide to obtain the average number of spores in the box one (Bauer *et al*., 2002) and then multiply the result by 1*104 spore/ ml for the fungus *A. tamarii* (volume conversion factor) to obtain the number of spores in 1 ml of the fungal suspension. 3*105 spore/ ml for the fungus *C. herbarum* and 2*105 spore/ml for the fungus *V. lecanii*. To obtain a lower concentration, the following equation was applied (Lacey, 1997). Volume (ml) taken from the main suspension = desired concentration/concentration of the original suspension

Then the result is multiplied by the volume of the suspension to be prepared, and this is how the concentrations were prepared: Fungal spore suspension *A. tamarii* ($1*10^5$, $1*10^4$, $1*10^3$, $1*10^2$). Fungal spore suspension *C. herbarum* ($3*10^5$, $3*10^4$, $3*10^3$, $3*10^2$) and Fungal spore suspension *V. lecanii* ($2*10^5$, $2*10^4$, $2*10^3$, $2*10^2$). Pathogenicity to the mortality rate of two mosquito species *C. Pipiens* and *A. stephensi*: The method of Altre *et al*. (1999), was employed to investigate the pathogenicity assays. In a nutshell, third-instar *C. pipiens* and *A. stephensi* larvae were taken from a lab colony, reared on blood or individual eggs at 100 eggs per replicate of type *A. stephensi* was used with a soft brush and placed separately in a 250 ml plastic container containing 100 ml of each concentration of suspended fungi from the tested fungi, as well as spraying the eggs on the surface at the same concentration in which it was placed by a hand sprinkler at an amount of 5 ml for each replicate from a height of 15 cm., and maintained in laboratory conditions of 15:9 h light: dark and 30 °C until used. Only sterile distilled water is sprayed on the control treatment to guarantee that every egg is exposed to the fungal solution. The eggs were observed until they hatched and the mortality rate was computed when the pots were placed in the incubator at a temperature of 25 ± 2 °C (Ali and Haitham, 2017).

2.3 The effect on the mortality rates of the fourth larval stage

Each fungus from the tested fungi for the two types of mosquitoes (separately) was taken and distributed into four containers, each containing 100 ml of each concentration of the suspension concentrations. The fifth contains sterile distilled water (control treatment). Then the treated larvae were transferred with a soft brush to 250 ml glass containers containing sterile distilled water to which the larvae food was added by 10 mg. The vessels were placed in the incubator at a degree of 25 ± 2 m and a light period (D) / L) 10/14 h, then the mortality rate was calculated within 24 hours of treatment (Mamai *et al*., 2019).

2.4 The effect on the adult mortality rates

A sufficient number of pupae of each species were taken from the stock culture, and they were placed individually into 10 ml tubes and closed the tubes with a piece of cotton until they were transformed into adults, then I prepared glass balls of 1-liter capacity in each of them with a piece of cotton saturated with a sugary solution 10 % was placed in a small dish, spraying each baker with 5 ml of each concentration of fungal suspensions with a hand sprinkler from a height of approximately 15 cm, while the control treatment was sprayed with sterile distilled water, after which 10 adults were transferred by an aspirator from each of the newly emerging males and females of the two species .to the treatment bikers, this experiment was repeated three times for each concentration and the same for the control treatment, the treatments were incubated under the same conditions, the mortality rate was calculated daily for a period of24 h. (Leisnham *et al*., 2014; W. H. O, 2006).

2.5 Data analysis

The results were analyzed statistically using SPSS software using the Completely Randomized Design (CRD) with two factors and a single-factor experiments. The percentages of data were analyzed after the Acrsine transformation and the averages were compared using the Revised least significant difference (RLSD) at a significant level. (P = 0.05) (Al-Rawei *et al*.,2000), percentages of depreciation were calculated and corrected according to the Abbott Formula (Abbott, W. 1925): % Corrected Loss = Mortality % in treatments - Mortality % in control x 100 ÷100 - Mortality % in control.

3 Results

The identification of more than a hundred mosquito's specimens from five provinces (Dywanyah, Kut, Babylon, Najaf, and Karbalah) in the central of lraq, indicated that they were *C. pipiens* and *A. stephensi* according to Keys (Al-Rawei *et al*.,2000), and Three fungal species were identified from naturally infected insects and accounted for 12.3% of the total quality-filtered reads: A. tamarii, *C. herbarum* and *V. lecanii*. where the anatomy of *A. tamarii* colonies on SDA expanding quickly medium, white mycelium, fluffy; Reverse uncolor, sometimes pinkish; nonetheless, conidia have thick, rough walls and are roughly pyriform to globose, with a conspicuously olive brown conidial color; In tiny heads, phialides are biseriate, whereas in large heads, they are uniseriate (De Hoog, and Guarro, 1995; Käärik *et al*., 2012). Colonies produced by *C. herbarum* can be They are flat, green, or brown, with a diameter of 3-7 mm. The conidia (5.5-13 * 3.8-6 µm) also exisare branched and have swollen ends, with smooth or rough walls. The spores are lemon-shaped, conical, or oval, and are usually single-celled and carried terminally on a short, neck-like structure (Prasil, and de Hoog, 1988). The developing colony of the fungus *V. lecanii* is characterized by the radial shape of cottony eggs and appears to be zero due to its production of pigments. The conidiophores are transparent, smooth-walled, and carry near their end secondary radial branches, and metulaue, and each one bears phialid structures carrying several conidia at its end (AlAVO, 2015). This fungus produces three types of the conidia are oval or lemon-shaped, namely Macroconidia, Intermediatconidia, and Microconidia (Figure 1).

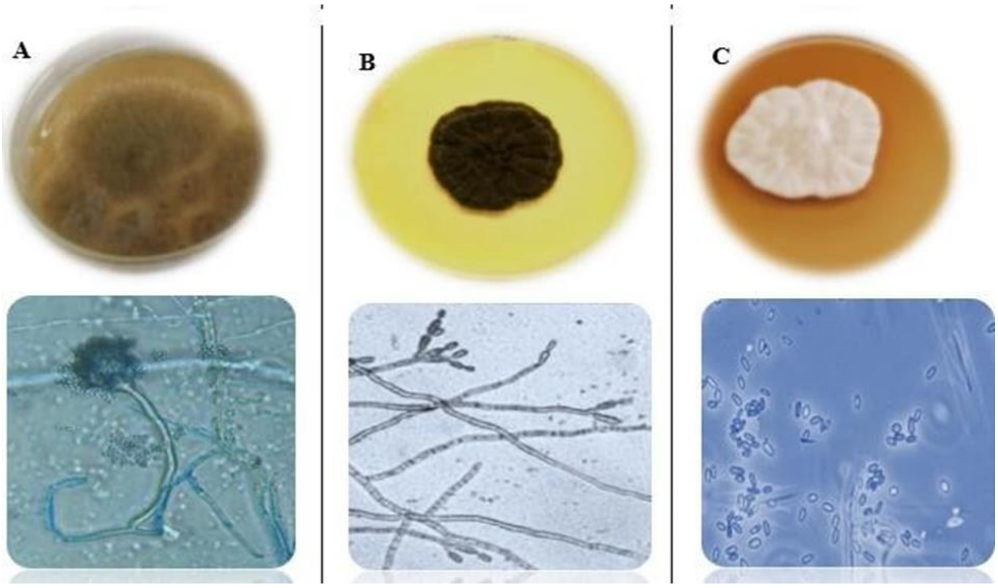


Figure 1: Morphological features of Cladosporium and cladosporioides cultured on SDA medium. Light microscopy of conidiophores, ramoconidia, and conidia (a-r x40). A. *A. tamarii*, B. *C. herbarum*, C. *V. lecanii*.

The results showed that the sequence of nitrogenous bases of study isolates, their number was 569 PB, the result was identical percentage (90%) with many global and local isolates found in the NCBI gene bank (Figure 2) which confirms and supports the validity of the phenotypic and microscopic diagnosis of this fungus (Kozel and Wickes, 2014; Abdullah *et al*., 2019). The fungal isolates' phylogenetic tree demonstrates It is assumed that certain species are more closely related than others. For instance, it was discovered that *A. tamari* shared a closer ancestry with *A. aflatoxiformans* and pseudomonas. Similar nonentity isolates are known to survive, according to the evolutionary tree of the fungi used in this study. The species underwent a small amount of elaboration to assist assure their survival (Goettel & Glare, 2010).

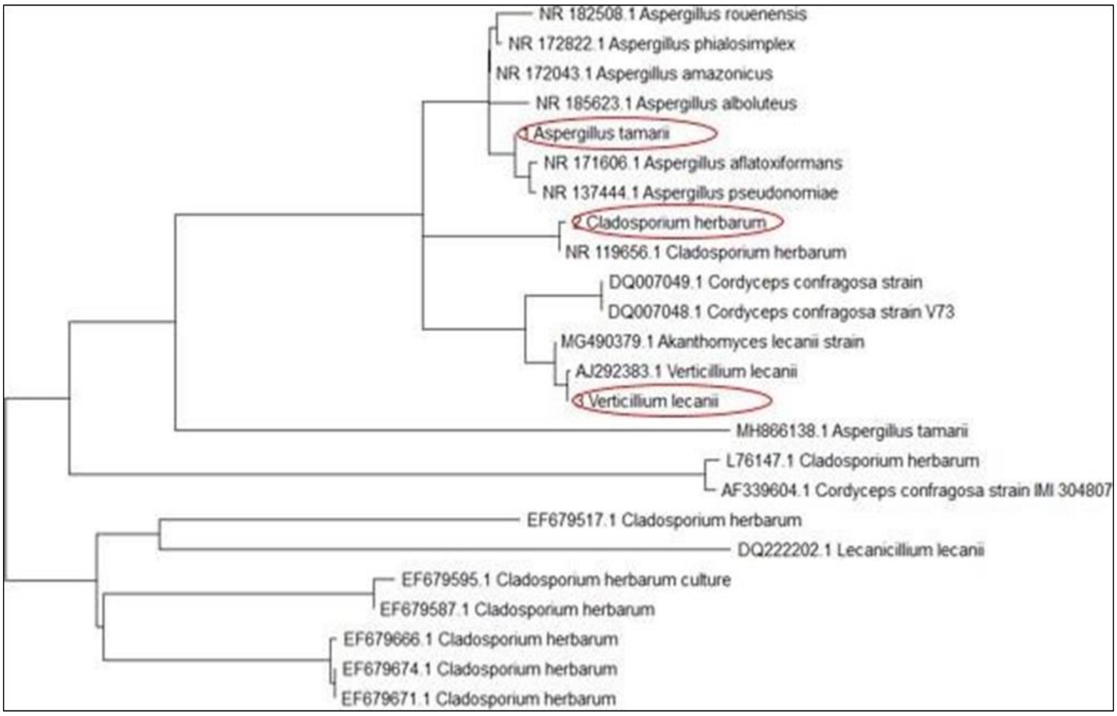


Figure 2: Phylogenetic tree of partial ITS gene sequences by maximum likelihood. Note: Sequences from this study are shown in the rectangle red.

3.1 The effect of fungicide suspensions *A. tamarii, C. herbarum* and *V. lecanii* in the mortality of two mosquitoes *C. pipiens* and *A. stephensi*:

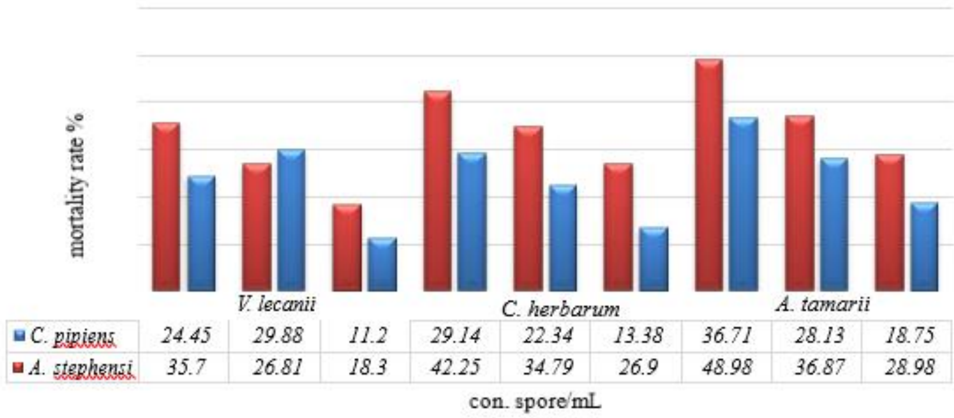
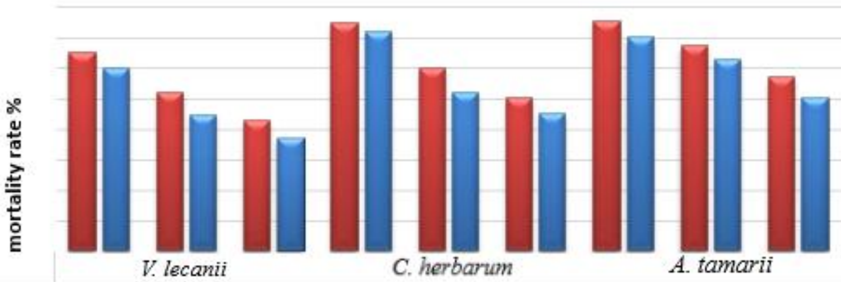


Figure 3: Effects of using different concentrations of fungal spore suspensions *A. tamarii, C. herbarum* and *V. lecanii* on eggs mortality of two mosquitoes species *C.pipiens* and *A. stephensi* L.S.D value below 0.05 significance level for interference = 0.458

(Figure 3) Results of the effect of different concentrations of *A. tamarii, C. herbarum* and *V. lecanii*, respectively, in the egg decimation percentages of two *C. pipiens* and *A. stephensi* were observed that there was a direct relationship between the concentration of the fungal suspension and the percentage of hatching as the mortality rate increased with the increase in concentration and the statistical analysis showed that there are significant differences between the concentrations of the fungal suspensions and that the type of mosquito *A. stephensi* is more sensitive to the fungal suspension compared to the second type in all stages.

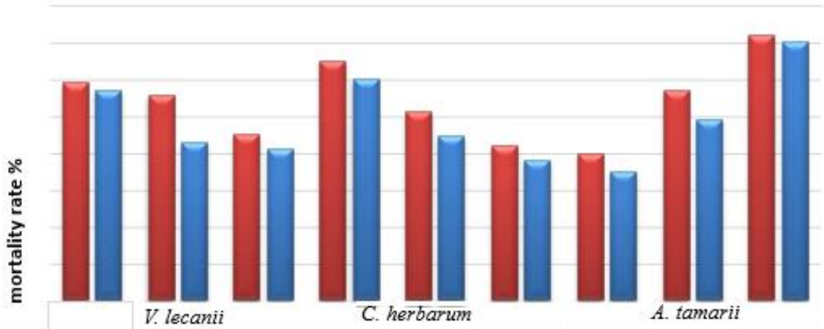


C. pipiens	60	44.8	37.03	72.15	51.98	45	70	62.84	50
A. stephensi	65	52	42.71	74.81	59.81	50	75.6	67.19	56.8

con. spore/mL

Figure 4: The effect of using different concentrations of fungi on the fourth larval stage of *C. pipiens* and *A. stephensi* L.S.D below 0.05 significance level for interference = 0.156

(Figure 4) shows the effect of using concentrations of fungi spore suspensions under investigation on the *tamarii*, B. *C. herbarum*, C. *V. lecanii* percentage of mortality of the fourth larval stages of two mosquitoes *C. pipiens* and *A. stephensi* was superior to the fungus suspension *A.tamarii* and *C. herbarum*, in all concentrations in *V. lecanii* had a mortality rate when using the fungi suspension, while the mortality was absent in the control treatment, which indicates the existence of a direct relationship between concentration and mortality rates.



C. pipiens	57	42.98	41	60	44.71	38	35	49.13	70
A. stephensi	59	55.6	45	65	51.01	42	40	56.87	72

con. spore/mL

Figure 5: The effect of overlapping different concentrations of fungi A. tamarii, C. herbarum and V. lecanii in the percentages of mortality of adults in the two C. pipiens and A. stephensi L.S.D below 0.05 significance level for interference = 0.769

(Figure 5) indicates the effect of different concentrations in adult mortality of *C. pipiens* and *A. stephensi* percentage were (70,72) % when using highest concentration of $3*10^5$ spore / ml of the fungus suspension *A. tamarii* while lowest concentration $1*10^2$ spore/ml, (35, 40)%, and (60, 65)% in highest concentration of fungus suspension *C. herbarum* a while the lowest mortality was at the lowest concentration $3*10^2$ spore/ml, (38, 42)%, and as for the fungus suspension *V. lecanii*, the highest mortality was at the highest concentration $2*10^5$ spore/ml (57, 55) %, for both two types of mosquitoes, respectively, in the same period. The mortality in the control treatment was non-existent. Moreover, we conclude from the above figure that the relationship between the concentrations of innate suspensions and each of the percentages of mortality and the duration of exposure was positive, and the statistical analysis demonstrated the presence of significant differences in the percentages of adult mortality. For the concentrations used, the statistical analysis also showed that there are significant differences according to concentration *A. stephensi* was more sensitive to infection with fungal suspensions compared with *C. pipiens*. This means that when used, all mushrooms contribute equally to reducing the spread of malaria. It's critical to have a formula that distributes the spores while keeping them on the surface for optimal concentration. This not only simplifies application but also lowers the quantity of spores needed. Using organic dry formulations is crucial for controlling Anopheles sp. because of the way spores enter the larval body (Daoust *et al*., 1982). The permanence of fungal spores must also be increased, which calls for this preparation. UV radiation, humidity, and temperature can all affect fungal spores. Because high relative humidity encourages spore germination, releasing spores above the water's surface may have unfavorable effects (Gunnarsson *et al*., 2011). To measure the virulence of the fungi, the values of LC_{50} and LC_{90} were calculated, which represent the basic value in test methods.

Table (1): Shows the LC_{50} and LC_{90} values for the bioassay of suspensions *A.tarmaii* , *C. herbarum* and *V.lecanii* in the eggs and fourth larva stage and adults of my *C. pipiens* and *A. stephensi* mosquito

Fungi	**Mosquitospecies**	Insect' sstages	LC50 value (LCL–UCL)	LC90 value (LCL–UCL)	X2	P - value	Regression equation
A.tamarii	***C.pipins***	Egg	3.06×10^{6} (1.964×10^{6}-7.414×10^{7})	1.153×10^{7} (7.273×10^{6}-7.512×10^{6})	3.500	0.174	Y=-0.47+1.53E-7*X
		L4	1.109×10^{7} (9.427×10^{6}-1.319×10^{7})	2.507×10^{7} (1.714×10^{7}-3.213×10^{7})	0.726	0.696	Y=-1.03+9.5E-8*X
		Adult	1.074×10^{7} (8.940×10^{6}-1.275×10^{7})	2.574×10^{7} (1.472×10^{7}-4.26×10^{7})	1.315	0.518	Y=-0.93+9.07E-8*X
	A.stephensi	Egg	2.778×10^{6} (1.231×10^{6}-5.622×10^{6})	1.588×10^{7} (9.631×10^{6}-5.986×10^{7})	5.989	0.050	Y=-0.28+9.96E-7*X
		L4	1.078×10^{7} (8.314×10^{6}-1.371×10^{7})	2.584×10^{7} (1.96×10^{7}-3.484×10^{7})	0.644	0.752	Y=-0.92+8.75E-8*X
		Adult	7.596×10^{6} (5.194×10^{6}-9.006×10^{6})	1.895×10^{7} (1.375×10^{7}-1.25×10^{7})	0.210	0.900	Y=-0.86+1.14E-7*x
C.herbarum	***C.pipins***	Egg	4.874×10^{6} (1.983×10^{6}-7.524×10^{7})	1.490×10^{7} (8.53×10^{6}-4.965×10^{7})	3.811	0.149	Y=-0.63+1.31E-7*X
		L4	1.475×10^{7} (9.324×10^{6}-1.389×10^{7})	2.517×10^{7} (2.034×10^{7}-3.10×10^{7})	0.084	0.959	Y=-1.12+9.63E-8*X
		Adult	4.18×10^{5} (2.986×10^{6}-	8.558×10^{6} (6.50×10^{6}-	1.	0.452	Y=-0.07+1.57E-

			6.782×10^{6})	1.135×10^{7})	588		7*X
	A. stephensi	Egg	4.484×10^{6} (1.964×10^{6}-5.642×10^{6})	1.850×10^{7} (1.106×10^{7}-6.170×10^{7})	3.227	0.199	Y=-0.41+9.31E-7*X
		L4	1.465×10^{7} (9.321×10^{6}-2.391×10^{7})	3.744×10^{7} (2.41×10^{7}-4.322×10^{7})	0.535	0.765	Y=-1.13+6.72E-8*X
		Adult	9.750×10^{6} (5.550×10^{6}-1.165×10^{7})	2.287×10^{7} (1.689×10^{7}-3.09×10^{7})	1.199	0.549	Y=-0.96+1.03E-7*X
V.lecanii	***C.pipins***	Egg	6.094×10^{6} (3.754×10^{6}-2.202×10^{7})	1.539×10^{7} (9.138×10^{6}-5.977×10^{7})	2.401	0.301	Y=-0.85+1.41E-7*X
		L4	1.436×10^{7} (1.216×10^{7}-1.812×10^{7})	3.384×10^{7} (2.46×10^{7}-5.098×10^{7})	0.283	0.868	Y=-0.95+6.7E-8*X
		Adult	2.001×10^{7} (1.141×10^{7}-2.217×10^{7})	4.367×10^{7} (3.167×10^{7}-5.84×10^{7})	0.891	0.648	Y=-1.1+5.86E-7*X
	A. stephensi	Egg	5.566×10^{6} (2.633×10^{6}-5.782×10^{6})	1.585×10^{7} (9.742×10^{6}-5.997×10^{7})	2.099	0.350	Y=-0.7+1.27E-7*X
		L4	1.116×10^{7} (1.7233141-2.918×10^{7})	5.208×10^{7} (3.705×10^{7}-7.25×10^{7})	0.094	0.954	Y=-0.88+4.18E-8*X
		Adult	1.076×10^{7} (8.801×10^{6}-1.206×10^{7})	2.752×10^{7} (1.32×10^{7}-3.342×10^{7})	0.356	0.837	Y=-0.83+7.77E-7*X

(LC_{50}) concentration that kills 50% of population, (LC_{90}) concentration that kills 90% of population, (LCL) lower confidence limit, (UCL) upper confidence limit, (χ^2) chi-squared. Three replicates were used in each treatment, n = (375). No mortality was recorded in the negative control group.

Tables (1) show the LC_{50} and LC_{90} values that the mosquito species *A. stephensi* is more sensitive to the fungal suspension compared to the eggs of the second type, as it reached less than these values after 24 hours of treatment, and these values increase directly as the insect stage progresses.

The P-value was also calculated (which is a measure to clarify the extent to which we have proof or evidence to reject the hypothesis The null and we take the alternative hypothesis in the test that we have), and the regression equation, which is a statistical equation that expresses the relationship between two variables and is used to estimate past values and predict future values and helps in describing the degree of the relationship between the variables. In the laboratory, the three fungi have shown potential as larval control agents. To check its suitability in the field, experiments must be carried out under natural conditions, in which, in addition to the persistence and effectiveness of the spores, the influence of the fungal species on its non-target effects must be observed.

4 Discussion

Resistance of mosquito vectors to pesticides is frequently regarded as a serious danger to the recent advancements in the management of malaria. Nonetheless, research evaluating the effects of treatments and pesticide resistance has revealed a patchy application of epidemiological and entomological criteria. Firstly, the efficacy of insecticides may be underestimated as the assessment test does not account for mosquito susceptibility to infection. Moreover, relationships between vector competency and pesticide resistance have unexpected implications for interventions. This study intended to highlight the role of isolated fungi as an alternative control measure against mosquitoes, the reason for the ability of these fungi to penetrate the eggshell is due to the complementarity of the enzymatic and mechanical activities they have, as they can secrete the enzymes protease, chitinase, and lipase, in addition to the power of mechanical action (Liu *et al*., 2007; Frey, 2019). The increase in mortality rates by increasing the concentration is due to the increase in the number of spores, and then the increase in the percentage of spores developing when attacking the host and weakening the immune system of the insect, in addition to the immune system of the larvae can defend the body at concentrations Sessile However, when the concentration increases, the device may lose mechanically block the pathways that allow them to enter through the mouth or siphon. The linked spores harm the larval tissues when they germinate because of their vegetative (ONG'WEN, 2018). The latent immune system needs to address a wide range of offenses in this situation. Resistance to the control agent is less likely to develop the more diverse the modes of action are (Mulla *et al*., 2003). The lack of a lasting effect is a major drawback, even though exposure for just one day is sufficient to result in a considerable death rate. By looking for tolerant isolates and formulations, this can be resolved (Rutjens, 2023). According to observations made by Lord *et al*. (1987), Wilson *et al*. (1990), and Sandhu et al. (1993), infection can also move from larvae to adults. This study documents the transmission of infection from the larvae of A. stephensi to the pupae and adults of *A. tamarii*, *C. herbarum*, and *V. lecanii*. (Šebesta *et al*., 2022). non-target effects *V. lecanii* is a suspended fungus that contains enzymes that help the fungus penetrate the shell of insect eggs and cause the death of the embryos inside it (Damialis *et al*., 2015), while another study (Aboelhadid *et al*., 2018), confirmed that exposing Rhipicephalus annulatus eggs to *V. lecanii* spores at a concentration of $1*10^1$ its efficiency (Bossen *et al*., 2023). Insects infected with fungi may live 3 to 5 days as a result of spores germination and penetration of fungal hyphae through the respiratory orifices, which causes suffocation of larvae as a result of closing the respiratory openings, as well as the growth of the fungus in the middle channel of the larvae and depletion of nutrients and after 72 hours the fatty tissue is destroyed and thus the percentage of larvae mortality may reach 100% after 96 hours, and some treated larvae die during molting as they fail to molt and remain attached to the molted layers (Goettel, and Glare,2010; Sarowar *et al*., 2013). The additional Toxins released by the fungus and its larvae induce blood poisoning when spores are consumed. The larvae may have rejected the spore mass as food because of the enormous clump size and density of the mass, which inhibited spore attachment. As a result, high concentrations did not provide better temporal and spatial coverage, which might have increased the death rate. To maximize the benefits of natural control, fungal spores should be used instead of uprooted endotoxins (Bossen *et al*., 2023) While most spores adhere to the inside of the larval body, some

spore/ml did not significantly affect the hatching rate of these eggs. A study (Abdullah *et al*., 2009) indicated that exposing the eggs of *C. pipiens* of *B. bassiana* spores at a concentration of $3*10^5$ spore/ml led to all of them being destroyed, as for study used *C. quinquefasciatus* and *A. pulcharhimus* (Al-Karawi and Hanaa, 2012), showing that the treatment of eggs *C. quinquefasciatus* and *A. pulcharhimus* pylori spores of *L. lundbergii* led to their mortality by 56% and 59.33%, respectively, at a concentration of $3 *10^7$ spore/ml. (Sissani *et al*., 2014) indicated. The hatching ratio of *C. pipiens* exposed to *M. anisopliae* spores at a concentration of $3 *10^3$ spore/ ml decreased to 40%, and ALmshkur and Saeed (2014) also noted a decrease in the incidence of mosquito eggs *C. quinquefasciatus* increased by 59% at a concentration of $2 *10^6$ spore/ml when exposed to *C.keratinophilum* spores, However, these tests were performed under lab conditions, which is almost impossible to occur under field conditions because the fungal spores themselves are sensitive to environmental factors (Souza *et al*., 2023). Therefore, it is important to test the off-target effects of these fungi in a more realistic setting. In humans, this is a clinical case.

5 Conclusions

This study has demonstrated that the fungus species employed affects the mosquito mortality rate at all phases. It's interesting to note that all fungal species can destroy mosquitoes. Nevertheless, there was no correlation observed between the mortality rate and either elevating the concentration of fungal spores or decreasing the duration of spore exposure. Spore clumping made it difficult to achieve uniform coverage over time and space, which led to inconsistent outcomes. It's critical to have an effective delivery system and a spreadable formulation for the spores to spread across the water's surface to stop fungus from injuring unintentional targets. For best results, a formulation of this kind needs to be created.

Recommendations:

To ensure effective and safe mosquito control, it is important to develop appropriate delivery methods and formulations that allow for even dispersion of fungal spores across the water's surface.

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