

How to Cite:

Mohammed, M. J., & Ali, S. T. (2022). Evaluation efficiency of *Metarhizium anisopliae* fungi in larva mortality *Culex pipiens*. *International Journal of Health Sciences*, 6(S1), 6539–6545.
<https://doi.org/10.53730/ijhs.v6nS1.6397>

Evaluation efficiency of *Metarhizium anisopliae* fungi in larva mortality *Culex pipiens*

Mohammed Jabber Mohammed

Department of Biology, College of Science, University of Thi-Qar, Iraq
Corresponding author email: mohammed86h55@gmail.com

Sadik Thajib Ali

Department of Biology, College of Science, University of Thi-Qar, Iraq

Abstract---This study has targeted the evaluation of the efficiency *metarhizium anisopliae* fungi in different concentrations of bio control four stages *Culex Pipiens* mosquitoes. This study has been showed different results by different concentrations and exposure periods for all stages larva *Culex Pipiens* that was sensitive to selective concentrations 2×10^5 , 2×10^7 and 2×10^9 spore/ml. The concentration 2×10^9 was recorded highest mortality in first stage reach (63.55), while lowest concentration While the rate of floating the same pours when concentration is 2×10^5 and reached (49.57) The rate of secondary and third, third and fourth phase has reached the top 2×10^9 (56.87, 48.55%, 44.80%), respectively, While the percharge rate itself is at least 2×10^5 (45.22%, 38.75%, 34.75%, 34.38%), respectively. With regard to exposure periods, the exposure period after (96) hours achieved a significant output at other exposure periods, where the rate of land phase The first (81.1%) is the rate of perhaps, while the exposure period (24) has given less rate for the same phase, amounting to 30.82%. The rate of destruction of the second, third and fourth and fourth pours was exposed (96) hours (79.17%, 65.27%, 60.83%), respectively, while the perpetrator rate for largely after a period of exposure (24.4%, 22.5%, 16.95 %) respectively.

Keywords---*Culex pipiens*, *Metarhizium anisopliae*, larvae, biological control.

Introduction

Culex pipiens is a major vector for many serious viral nurses, such as Sant louis , Elephant and West Nile Virus(*Aliota et al ., 2016*), (*Molaei et al., 2008*) which is infected with millions of people in the world (*CIRIMOTICH et al, 2011*), a recorded

about 700 million people or more people with Filariasis (WHO,2014) The researchers have methods development for its elimination, because of the damage caused by and its poor concerns of environmental pollution, researchers have examined other alternatives and most notably the vital control. Family and inexpensive and highly specialized pest direction (Bandani et al., 2000). *Metarhizium anisopliae* is one of the most used insects in bio-insects. This fungus affects their families after the external wall penetrates and reaches the cavity and growing the problem of ingest and is also produced toxins that kill the homes after 4-16 days of injury (Gayathri, 2010). The aim of study include study the impact of different concentrations of *Metarhizium anisopliae* on stages *Culex pipiens* and the relationship between the effect of the concentrations used and the time period for inciting percentage of mosquitoes.

Materials & Methods

Breeding *Culex pipiens* mosquitoes

Different non-adult roles of *Culex Pipiens* were collected from ponds, pools and margin in Maysan in October 2020 and to the December 2021 by using a net made of long arm tulle where insects are placed in glass bottles containing anasal water after eliminating chlorine with more than 48 hours and then quoted to the laboratory in the Faculty of Science / University of Dhi Qar. The contents of the glasses were emptied in plastic utensils and plastic ponds (70 x 40 x 15) cm containing chlorine-free water and then added to the mice-breeded mice and maize, rice and protein by (2) gram cloud for each nutrition basin larvae, the ponds covered with tulle cloth. The purpose of a permanent pure farm of the insect isolated virgins and put in a small plastic (500) ml with water with dimensions (1 x1x1) and put in the cage of education and envelope with tulle cloth and As well as putting into the cage of education Petri dishes containing a saturated cat on a diabetes by 10% to nourishes full after appearance (Al-Issa, 1999). The purpose of getting boats (Mohsen & Mehdi (1989)) where mosquitoes have been used on pigeon's blood after three days, putting a bath inside the cage then that he documented his lenders and his joined by the chest feathers to facilitate blood absorption. It is also placed inside the small plastic cage that contains the water of anasal to be a shop for the placement of mosquitoes after feeding on blood at about (3 -4) days. The egg boats were transferred to one liter pots containing the water. The resulting larvae flocks by adding mice fodder by 3 grams per basement, and to get rot in the cage water of and maintaining the vitality of larvae has been replaced by the cage water of all (4) days (AL-Sharook *et al* .,1991). Then we isolate the colony for two generations in the laboratory before the experiences. The insect was classification on it *Culex Pipiens* by Professor Kazem Saleh Al Hadalk / University of Basra / Faculty of Science.

***Metarhizium anisoplia* colony development**

The isolation used *Metarhizium anisopliae* obtained from the Ministry of Science and Technology / Agricultural Research Department, then deals to the incubator with a temperature $27^{\circ} \pm 2$ m and a relative moisture degree of 80 ± 5 for 14 days (Hoe et al.2009).

Prepare the fungal suspension *Metarhizium anisopliae*

The suspension prepared by added (5) ml from distilled water sterilized to fungal farm for age 14 days on the central Potato dextrose agar in plastic Petri dishes with added (0.2) ml of 80 tween with concentration (0.01), after that, the spores harvested by the L-steam glass rod, after which the contents of the dish were nominated by a glass suppression installed with a piece of sterile gauze with addition (5) ml other from the sterile distilled water to nominate all fungal craps, then collect the suspend composed of 10) put in flask as stock solution. To calculate the number of spores fungal suspension used haemocytometer transfer (1) ml of the basis solution to (99) ml (from the sterile distilled water to ease the basis solution, and put (0.1) ml on chamber slide according to (Aube and Gagnon, 1969).

Effect the fungal suspension *Metarhizium anisopliae* on mortality mosquitoes

Take (40) portrayed from all phases of the left-wing, which is insulated by isolating the pipelines that preceded in the pipes of education until insert and access to the required phase, as was dependent on the size of the gauge, which is increasingly weakened, Of the mushrooms and then distributed four three of them containing (100) ml of each concentration of the veterinary commentator concentrations, and the fourth have only sterilized water) treatment (for two minutes and then transferred treatment only by brush (250) ml (10) mg / mL, the container pots have been placed on therapy therapies thereafter at the incubator (25 ± 2 m and light period 14:10) / L) Clock and then calculated the percentage of destruction after (96, 72, 48, 24) hours (Soni and prakash, 2011).

Statistical Analysis

All results are analyzed by full random design multi-factors Complete Randomized Factorial Design (C.R.D) and tested among the averages using the lowest differences of Least Significant Differences (L.S.D) and at the probability (0.05).

Result and Discussion

The figures (1, 2, 3, 4) showed effect of different concentrations of *M. Anisopliae* is in the four stages mortality of cx.pipiens, which differed results with different exposure of time, when centering 2×10^9 recorded highest mortality with the rate of 63.55%, while the lowest rate of the same phase was (49.57%) and when concentration 2×10^5 , the rate of secondary and fourth and fourth phase has reached the top 2×10^9 (56.87%, 48.55%, 44.80%), while the rate of fluctuations itself is at least 2×10^5 (45.22%, 38.75%, 34.38%), respectively. As for exposure periods, the period after the exposure (96) has achieved a significant output Where the rate of oil phase (81.1%) is the rate of perhaps, while the exposure period (24) has given less rate for the same phase, with 30.82%. The rate of destruction of the second, third and fourth and fourth pours was exposed (96) hours (79.17%, 65.27%, 60.83%), respectively, while the perpetrator rate for largely after a period of exposure (24.4%, 22.5%, 16.95 %) respectively.

In terms of interference between each of the species used and exposure periods, the focus of 2×10^9 has achieved the highest mortality after a period of exposure (96 hours and when the first hurdle has reached 86.67% murder), and the lowest killing ratio achieved when concentration 2×10^5 and after a period of exposure (24 hours and was at the fourth stage, which amounted to killing (13.33%). The increase in property ratios increases the focus is attributed to the increase in the number of staples and then increased proportion and number of developing states when the host is attacked and folds for insect (immunotherapy) because the immune system of the insect (for larvae) can defend the body when the crowds are only credited Increased focus (high concentrations). The immune system is less efficient and loses (Scholete et al., 2003). The results of the statistical analysis showed significant differences in paired killings among the four larva floors at a moral level (0.05) to treat control.

In addition, the current results showed the reverse relationship between the age of the larva and the percentage of depreciation. 86.67, 85.00, 70.00, 66.68% respectively, and the reason for this reverse relationship is that the first larvae is an immune system is incomplete and the body's wall in the first larvae is slim and tight making it easily breached by mushrooms The main way to enter it by is a direct breakthrough for the body wall through the bacterial tube that consists two days later from contacting the body wall and gets an injury when the moisture and temperatures are available (Senthamizhlselvan, 2010). That the reason for the disparity of murder in these fungi may return to those fungi on the secretion of fungal design and analysts affecting the vital effectiveness of living and vital objects leading to its destruction (Ibrahim, 1998).

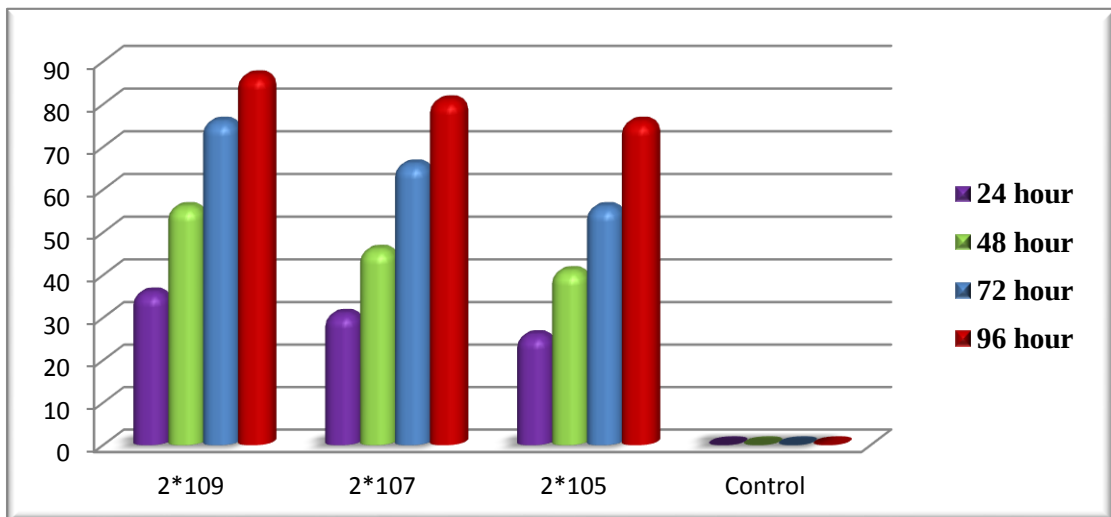


Figure (1) The effect of different concentrations of *Metarhizium anisoplia* in the ratios mortality of the first stage of *Culex pipiens*

Value LSD concentrations = 0.74

Value LSD exposure periods = 0.85

Value LSD Overlapping = 2.96

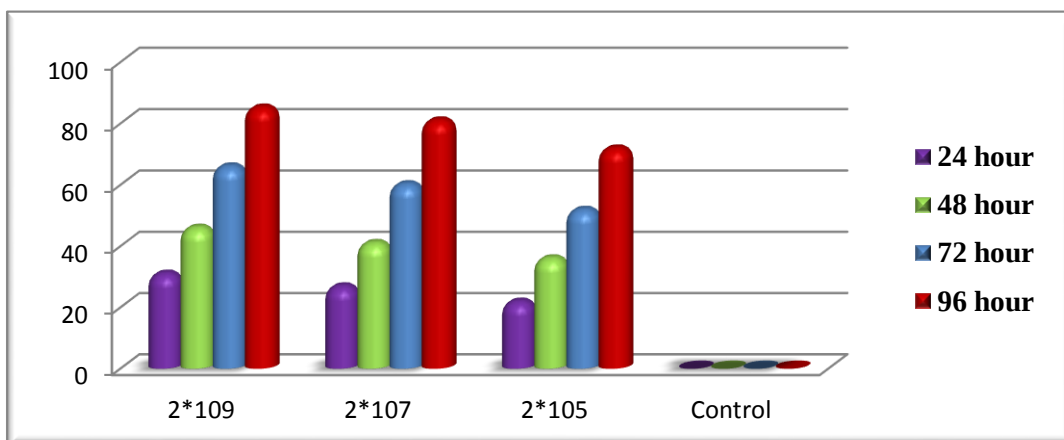


Figure 2. The effect of different concentrations of *Metarhizium anisoplia* in the ratios mortality of the second stage of *Culex pipiens*

0.76 = Value LSD concentrations
 exposure periods = 0.83 Value LSD
 Value LSD Overlapping = 2.93

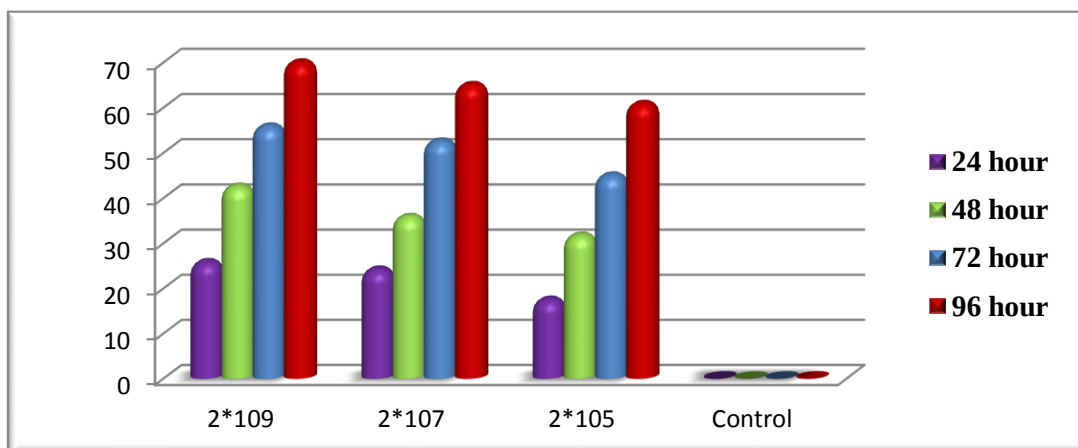


Figure 3. The effect of different concentrations of *Metarhizium anisoplia* in the ratios mortality of the third stage of *Culex pipiens*

Value LSD concentrations = 0.78
 Value LSD exposure periods = 0.83
 Value LSD Overlapping = 2.94

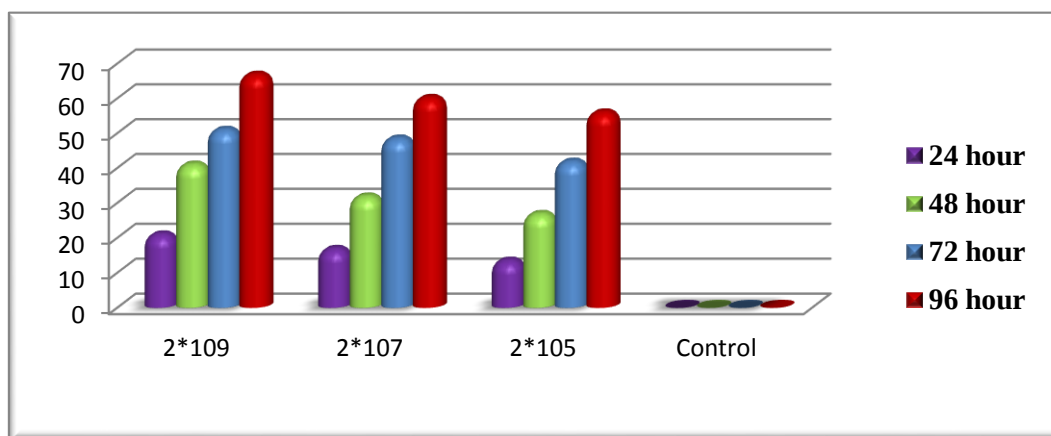


Figure (4) The effect of different concentrations of *Metarhizium anisoplia* in the ratios mortality of the fourth stage of *Culex pipiens*

Value LSD concentrations = 0.76

Value LSD exposure periods = 0.89

Value LSD Overlapping = 2.91

Conclusion

The concentrations of *Metarhizium anisoplia* showed to effect in mortality all stages larvae of *Culex Pipiens* . The first and second stage was more mortality than third and fourth in larvae stages, with regard to exposure periods, the exposure period after (96) has achieved a significant superiority at other expenses.

References

- Aliota, M. T., Peinado, S. A., Osorio, J. E., & Bartholomay, L. C. (2016). *Culex pipiens* and *Aedes triseriatus* mosquito susceptibility to Zika virus. *Emerging infectious diseases*, 22(10), 1857.
- Al-Issa, Abbas. (1999). Effect of growth organizers Methoprene (Altosid) and Lufenuron (Match) on the life of *Culex quinquefasciatus* and *Culex Molestus*. Master thesis - Department of Plant Protection / Faculty of Agriculture - University of Baghdad.
- Al-Sharook, Z., Balan, K., Jiang, Y., & Rembold, H. (1991). Insect growth inhibitors from two tropical Meliaceae: Effect of crude seed extracts on mosquito larvae 1. *Journal of applied Entomology*, 111(1-5), 425-430.
- Aube, C., & Gagnon, C. (1969). Effect of carbon and nitrogen nutrition on growth and sporulation of *Trichoderma viride* Pers. ex Fries. *Canadian Journal of Microbiology*, 15(7), 703-706.
- Bandani, A. R., Khambay, B. P. S., Faull, J. L., Newton, R., Deadman, M., & Butt, T. M. (2000). Production of efrapeptins by *Tolypocladium* species and evaluation of their insecticidal and antimicrobial properties. *Mycological Research*, 104(5), 537-544.
- Cirimotich, C. M., Dong, Y., Clayton, A. M., Sandiford, S. L., Souza-Neto, J. A., Mulenga, M., & Dimopoulos, G. (2011). Natural microbe-mediated

- refractoriness to Plasmodium infection in Anopheles gambiae. *Science*, 332(6031), 855-858.
- Gayathri, G., Balasubramanian, C., Moorthi, P. V., & Kubendran, T. (2010). Larvicidal potential of Beauveria bassiana (Balsamo) Vuillemin and Paecilomyces fumosoroseus (Wize,) Brown and Smith on Culex quinquefasciatus (Say). *Journal of Biopesticides*, 3(1), 147.
- Hoe, P. K., Choon-Fah, J. B., Jugah, K., & Rajan, A. (2009). Evaluation of Metarhizium anisopliae var. anisopliae (Deuteromycotina: Hyphomycete) isolates and their effects on subterranean termite Coptotermes curvignathus (Isoptera: Rhinotermitidae). In *Am. J. Agric. Biol. Sci.*
- Ibraheim, Ismail Khalil; Jubouri and the center of Mohammed snow. (1998). Fungal toxins are raised and raised. Agricultural Research Center. 243 pages.
- Mohsen, Z. H., Ouda, N. A., Mehdi, N. S., Zaiya, H. H., & Al-Chalabi, B. M. (1989). Toxicity of various larvicides and formulation against larvae and pupae of Culex quinquefasciatus and other non-target arthropods. In *Proc. Sci. Conf. Baghdad* (pp. 7-11).
- Molaei, G., Andreadis, T. G., Armstrong, P. M., & Diuk-Wasser, M. (2008). Host-feeding patterns of potential mosquito vectors in Connecticut, USA: molecular analysis of bloodmeals from 23 species of Aedes, Anopheles, Culex, Coquillettia, Psorophora, and Uranotaenia. *Journal of medical entomology*, 45(6), 1143-1151.
- Scholte, E. J., Takken, W., & Knols, B. G. J. (2003). Pathogenicity of six East African entomopathogenic fungi to adult Anopheles gambiae ss (Diptera: Culicidae) mosquitoes. In *Proc Exp Appl Entomol NEV, Amsterdam* (Vol. 14, pp. 25-29).
- Senthamizhlselvan, P., Sujeetha, J. A. R., & Jeyalakshmi, C. (2010). Growth, sporulation and biomass production of native entomopathogenic fungal isolates on a suitable medium. *Journal of Biopesticides*, 3(2), 466.
- Soni, N., & Prakash, S. (2011). Aspergillus niger metabolites efficacies against the mosquito larval (Culex quinquefasciatus, Anopheles stephensi and Aedes aegypti) population after column chromatography. *Am J Microbiol Res*, 2, 15-20.
- World health Organization. (2014). Management of insecticide resistance among vectors of public health importance: report of the ninth meeting of the Global Collaboration for Development of Pesticides for Public Health (GCDPP), 9-10 September 2014.