IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 9 Ver. I (Sep. 2015), PP 60-63
www.iosrjournals.org

Biological and physiological effects of *Metarhizium anisopliae* on *Culex quinquefasciatus.*

Ali M. Al-Dahbi[1], Maki Hamed Abed-Ali[2]
[1]*Assist Lecturer, Faculty of dentistry, Dijlah University College. ali.maki@duc.edu.iq*
[2]*PhD. Department of protection-college of Agriculture, Baghdad University*

Abstract: *The results of studying the effects of M. anisopiliae spores on mosquito, C. quinquefasciatus showed a biological effects represented by immature mortality. The mortality increased proportionally with the concentrations of fungal spores, which reached (at high concentration 2×10^{11} spores / ml), to 86.6, 56.6% in first and late instar larvae, respectively. An important to mention that cumulative death rate was significantly associated with the time, which reached to 56% at 7 day after treatment.*

In addition, M. anisopiliae had a long period permanence in aquatic habitats; in which the residual effects stay 30 days in aquatic habitats after treatment at laboratory conditions. Interestingly, the long period exposure of fungal spores (30 minutes) to high heating (100 C^o) does not affect the spore's ability to kill mosquito.

Furthermore, the study revealed that the fungal spores preferentially infects mosquito's head and syphon.

I. Introduction

For many years, we have enjoyed the benefits of using pesticides and is still the most efficient substance available and the substance of choice in the mosquito-control programs, such as Ultra-Low Volume (ULV) spraying,and contact pesticideswhich targeting adult mosquito'ssites. Thestrategiesthat targetthe immature stages at their breedingsites (i.e. aquaticenvironments) should be containing safe substances. Therefore,Biopesticide which include; insectgrowth regulators(IGR), plant extracts and microbial pest control agents (MPCA) such as Bacillus thuringiensisand Bacillus sphricus have been usedagainst immature stages of mosquitoes. It was found that the most common problem sarising from the use of pesticides (especially with mosquito control)include, the development of resistanceas well as the harmful side effects on non-target organisms (people, animals, soil, water, etc.), which in turn leads to the emergence of scary mosquitoes(1; 2; 3). Ithas been noted that fungi like thefast-killing viruses in which have the ability of changing and varying in host specialization with species and within the isolates of the same species. It is worth mentioning that the Hyphomyceteshavea broad host range compared to Entomophthorales, which are usually havehigh host specialization (4).Insect fungal pathogens have beenexploredas agents of biological control in many researches since the seventies, and there are examples of commercial products are available despite being limited.

However, the use of fungi like other microbial factors has become known to be use din way ssimilar to conventional insecticides (i.e.,without the need to secondary recycling in the environment).For instance, Beauveria bassiana (MycotrolO)is provided to seed sprouts in the indoor nursery before they are planted in the field,this practiceis very effectivein the fight againstDiamond-BackMoth (DBM). AtUnited States in the openfieldpractices, B. bassiana, was substantially reducethe abundance of DBMlarvaandwhenoverlappedwithBacillus thuringiensis(Bt) had foughtthreepestsfrom Lepidoptera (5). And this technique reduced the number of Bt uses and thus contributed to resistance management

It has been foundthatfromthoseinsectpathogens;Lagenidium giganteum, Metarhizium anisopliae, Tolypocladium sp, Culicinomyces and Bacillus sp have a highefficiencyinintegrated mosquitoes managementalsofound thatsuch thingsinterestingfor beingcharacterized cheap and high virulence especiallywhenisolated frominfectedinsects(6, 7, 8, 9).

This study was conductedfor the aim of usingmicrobial control agent (M. anisopliae) againstimmature stagesofC. quinquefasciatus mosquito in its breeding sitesto find outthe efficiency ofM. anisopliae,by testingdifferent concentrationsfromdrysporesof the fungus, to find outdurationthatthefungusremainseffective,the impact ofsome environmental factorsin efficiency of thefungus,method ofimpactandthe reason forthe death oftreated mosquito.

II. Materials and methods

Collection and diagnosis of mosquitoes

Mosquitoes larvae of C. quinquefasciatus wascollected andbred from identified isolates in themedical and veterinaryInsectsLaboratoryin the College ofAgriculture/ Baghdad university which diagnosedinDepartment of Plant Protection laboratoriesusingdiagnostickeys (10)and specialistsin the classification ofinsects.

Fungi:

The fungal strain that used in this study were commercially available ofMetarhizium anisopliae Strain F52 (®Met52)

The effects of different concentrations of the fungus in the larval stage of M. quinquefaschiatus

Bioassays wereused to determine the effects of different concentrations of M. anisopliae in early and late instar of C. quinquefasciatus larvae, these were done by exposing tenlarval isolatesto 5ml of fungus concentration at different concentrations (2×10^{11}, 2×10^{9}, 2×10^{7} Spore/ ml). Each fungus concentration (5ml) was poured in sterile petri dishes(60 mm × 15 mm)with 45 ml tap water.The tap water was leaved 24 hours to get rid of chlorine. For a period of week, the dishes were monitoring every day to isolate and account the dead larvae, taking into account to check the water level in each petri dishes to avoid losing water due to evaporation. The larvawere examined under an optical microscope. It should be mention that only water were used in control group.Each treatment and control groups included three replicates

Duration of fungal effectivity in the aquatic environment:

The purpose of this bioassay to determine if there was a difference in the effect of the fungus persistence period in the aquatic environment after treatment to kill larval stages. It has been assumed that no significant difference in death between different periods of persistence,that means the once treatment will be sufficient to combat instead of re-treatment. As the continuation and long existence of an effective fungus in the larval environment is crucial to not hesitate to field application.The bioassay was done in three periods, at1, 15 and 30 day after treatment.Tenlarvae of first instar used in this treatmenttreated with fungal concentration of10^{11} Spore / ml of water. The larvae exposed to fungal spore as mentioned above andonly water usedin control treatment. Each treatment with three replicates. The death was accounteddaily for 7 days after larval exposed to fungus. The experiment conducted in temperature and relative humidity of laboratory.

Effect of high wet-bulb temperatureonthe efficiency of fungal spore

To determine the effect of wet-bulb temperature on spore efficiency in aquatic environment, tubes containing spores with screw caps weretransferred to water bath (Chem-Index/ USA), Three different temperature were used to treat fungal spore (30ºC, 50ºC, 100 ºC). We assumed that the relative humidity is 100%.

III. Physiological effects:

Mode of action and cause of death:

To determine the mode of toxic action (i.e., whether the cause of death involves the direct toxic effects of fungi or to another reasons);the bioassay was conducted by treating tenearly instar larvae with 10^{11} spore/ ml of fungus. The dead larvae then washed with ethyl alcohol to eliminate the remaining spores and microbes on their surface; afterwards they incubated at 37ºC for seven days to allow the fungus to grow.Then, the larvae were examined under a microscope to observe the fungal growth (i.e., to determine if the death was due to fungal growth blocked the spiracles of larval syphon or by mycotoxins).

Statistical analysis

Experiments designed according to the complete randomization and use Least Significant Difference (LSD)to compare means by using the statistical program SPSS.

IV. Result and discussion

Table 1: Dead percentage of different instar of C.quinquefasciatus after exposed to different concentration of M. anisopliae

Concentration (spore/ ml)	Early instar	Late instar
cotrol	0	0
2×10^{7}	26.6	15
2×10^{9}	50	30
2×10^{11}	86.6	56.6
LSD	12.153	12.144

The table (1) revealed, that the death rates in early and late larval instars of C.quinquefasciatus significantly increased with concentration, reached to 26.6% and 15% in early and late instar respectively at concentration 2×10^{7}, while it reached to 86.6% and 56.6% inearly and late instar at 2×10^{11}, respectively. Although,it can be difficult to compare the present results with other results of various researches, however, the present study is in accordance with the report of Daoust et al. 1982 (11) and Roberts 1982 (6), whichindicate that death rates of C. pipiens to different concentrations of M. anisopliae was increased or decreased depending on fungal concentration.

Table 2 the percentage of early larval instar death of mosquito in aquatic environment

concentration	Periods (day after treatment)		
	1 day	15 day	20 day
0	6.6	2.6	13.6
2×10^{11}	56.6	60	60
t-test	8.66	8	8

Theearly larval instar groups were placed in petri-dishes (for 7 days) treated with $2\times10^{11}$of M. anisopliae spores (the more effective concentration on mosquito larvae) at different intervals (1, 10 ,20 day).It has been shown that no significant difference ($P<0.01$) in death ratio among larvae for all treatments in aquatic environment (table 2).Whereas it showed significant differences over the control group, this indicatethat one-time treatment with fungal spores was sufficient to achieve a significant larval death as a compared with control which, in turn, it support the hypothesis that our studybased upon; spraying fields for once is enough to achieve effective and long-term control in aquatic environments.

Table 3 Percentage cumulative mortality of larvae of the early stages of C. quinquefasciatusafter 7 days of exposure to M.anisopliae

Concentration Spore/ml	**Time (day)**				**LSD**
	2	**4**	**6**	**7**	
control	0	0	0	0	-
2×10^{7}	0	8	20	26.6	1.23
2×10^{9}	0	22	40	50	2.31
2×10^{11}	0	32	52	56.6	3.1
LSD	**-**	**1.86**	**2.32**	**3.4**	

From the result that obtained from table 3, we conclude that percentage cumulative mortality significantly increases in direct proportion to the passage of time, which reached 57% at the 7^{th} day after treatment. It is worth mentioning that death did not occur in the first 2 days after treatment.Allen et al (1995) (12)stated that fungal growth presented two phases: first, a lag phase (3 days) and then a rapid growth phase (> 3 days), where after 5 days fungi become more abundant. This lag may be due to the slower colonization speed when starting from spores vs. vegetative mycelium .This result is agree well withthat obtained by Clark et al.(1968) (13), which stated that the fungal the development of spores take about 2-3 days post treatment to kill the larvae treated with M. anisopliae.

Table (4) Impact ofwet-bulb temperature onM. anisopliae

Temperature	**Death%**
30	56.6
50	50
100	36.6
LSD	22.08

It was shown that a significant differences among death rates according to wet-bulb temperature. Ranged from 56.6% to 36.6 % at 30 $C^{o}$and 100 C^{o} , respectively (table 4).This result seems to be in general agreement with the results obtained by Zimmermann (1982) (14), when he referred, that M. anisopliae spores did not kill by the high temperature with presence of high relative humidity. On another hand, results varied with Rangel et al (2005) (15) when they showed that fungus spores was killed with high dry heat. This study indicates spores of M. anisopliae resistance to high wet temperature (relative humidity about 100%) for along two hours, that's means M.anisopliae can be used to control immature stage of C. quinquefasciatus in high temperature habitats. The difference betweenresults may be due to different methodologies of research, amount of spores exposed to heat, fungal isolates and methods of fungal heating. If we take into account,Zimmermann (1982)(14) heated the spores by boiling water bath while the other used oven.

Interestingly, our resultsfound that fungal infection confining on syphon (perispiracular valves) andmouthparts, which form a typical mycelial growth, making the primary cause of the death, is due to larval suffocation, however the fungal growth was observed more in Syphon than the mouthparts. This result is consistent with Meranpuri and Khachoatourians (1991) (16), which revealed that that spores germination consisted the entire larva's body and is focused on the basis on the back of the body and head.

In addition, it has been found that the fungus remains constant in perispiracular valves even after washing with alcoholbecause of the folds in the lobes. In similar study, but with Beauvaria bassiani were found that germ tube penetrated perispiracular valves. The mycelium growth inside the siphon and after four day, the siphon appeared full of with mycelium (12). Researcher did not refer to exact cause of death because of mechanical damage occurred before death. Finally, cause of death may be due to mechanical damage because of block respiratory radical and tracheal system that cause suffocation or to produce toxicant inside treated larvae.

References

[1]. Regis, L.; Silva-Filha, M. H. N. L.; de Oliveira, C. M. F.; Rios, E. M.; da Silva, S. B.; Furtado, A. F., 1995: Integrated control measures against Culex quinquefasciatus, the vector of filariosis in Recife. Memo´ rias Instituto Oswaldo Cruz 90, 115–119.

[2]. Shaker, A. H., Areeg, H. S. AL-Dhahir and Wissal A. H. 2010. An assessment for some extracts activity of an algae Char sp. On mosquitoes 4th larval instar of Culex quinquefasciatus. Mesan journal of academic science. 9 (17) 170-184

[3]. Abed-Ali M.H., 2013. Biological effects of Chitin synthesis inhibitor Applaud (buprofezin) on Culex quinquefasciatus in polluted water. Iraqi journal of sciens. 24 (4) 842-846.

[4]. Pell JK, J Eilenberg, AE Hajek and DS Steinkraus. 2001. Biology, ecology and pest management potential of Entomophthorales. In: Fungi as biocontrol agents: progress, problems and potential (eds TM Butt, C Jackson & N Magan). CABI International, pp. 71-154.

[5]. Vandenberg JD, AM Shelton, WT Wilsey and M Ramos. 1998. Assessment of Beauveria bassiana sprays for control of diamondback moth (Lepidoptera: Plutellidae) on crucifers. Journal of Economic Entomology 91, 624-630.

[7]. Roberts, D. W., 1970: Coelomomyces, Entomophthora, Beauveria and Metarhizium as parasites of mosquitoes. Misc. Publi. Entomol. Soc. Am. 7, 140–155.

[8]. Lacey, L. M.; Lacey, L. A.; Roberts, D. W., 1988: Route of invasion and histopathology of Metarhizium anisopliae in Culex quinquefasciatus. J. Invertebr. Pathol. 52, 108–118.

[9]. De Barjac, H.; Sutherland, D. J., 1990: Bacterial Control of Mosquito and Black Flies. New Brunswick: Rutgers University Press, 349 pp.

[10]. Sandhu, S. S.; Rajak, R. C.; Sharma, M., 1993: Bioactivity of Beauveria bassiana and Metarhizium anisopliae as pathogens of Culex tritaeniorhynchus and Aedes aegypti: effect of instar, dosages and time. Indian J. Microbiol. 33, 191–194.

[11]. Gillies MT, Meillon B. 1968 The Anophelinae of Africa South of the Sahara (Ethiopian zoogeographical region).2nd edition., 343.

[12]. Daoust, R. A.; Ward, M. G.; Roberts, D. W., 1982: Effect of formulation on the virulence of Metarhizium anisopliae conidia against mosquito larvae. J. Invertebr. Pathol. 40, 228–236.

[13]. Allen, E.B., Allen, M.F., Helm, D.J., Trappe, J.M., Molina, R., Rincon, E., 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. In: Collins, H.P., Robertson, G.P., Klug,

[16]. M.J. (Eds.), The significance and regulation of soil biodiversity. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 47±62.

[19]. Clark TB, Kellen WR, Fukuda T, Lindegren JE, 1968. Field and laboratory studies on the pathogenicity of the fungus Beauveria to three genera of mosquitoes. Journal of Invertebrate Pathology: 11:1-7.

[20]. Zimmermann G, 1982. Effect of high temperatures and artificial sunlight on the viability of conidia of Metarhizium anisopliae. Journal of invertebrate pathology; 40:36-40.

[21]. Rangel DEN, Braga GUL, Anderson AJ, Roberts DW, 2005.Variability in conidial thermotolerance of Metarhizium anisopliae isolates from different geographic origins. Journal of invertebrate pathology; 88:116-125

[22]. Miranpuri GS and Khachoatourians GG, 1991. Infections sites of the entomopathogenic fungus Beauveria bassiana in the larvae of the mostquito Aedes aegypti. Entomol. Exp. Appl.; 59:19-27.