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STUDY ABOUT THE EFFECT OF *BEAUVERIA BASSIANA* (VUILLEMIN IN 1912) ON THE AQUATIC STAGES OF *CULEX PIPIENS* (LINNÉ, 1758)

SONIA HAMID, FATMA HALOUANE, FATMA ZOHRA BISSAAD & FARIDA BENZINA

Laboratory of Valuation and Conservation of Biological Resources, Faculty of Science, University of M'hamed Bougara, Boumerdes, Algeria

ABSTRACT

The Culicidae are biting insects, the most harmful to people **(Himmi, 1991),** they are almost all bloodsuckers, and they are responsible of the spread of many important diseases such as malaria, yellow fever, and elephantiasis.

The Culicidae family is the most important; it includes three main kinds, pathogenic for humans: Anopheles, Aedes and Culex **(Feuillet & *al.*, 2006).**

Chemical insecticides are increasingly considered as the last means of fighting against the pest populations. Because they greatly increase production costs and that their excessive use can cause adverse effects on human, animal and environmental health.

In this context, the search for alternative methods of fighting takes all its importance, to replace their employment with reduced risk tools.

Entomopathogenic microorganisms occupy an important place among the alternative methods of fighting against pests insect. The fungus *Beauveria bassiana* is an entomopathogenic agent naturally present in the ecosystems. It offers a very interesting potential for controlling populations of mosquitoes **(Ziani, 2008)**

The objective of this study is to measure the impact of the use of insecticides preparations of *B. bassiana* that we have recently isolated, on populations of the *Culex pipiens* mosquito.

The found results reveal that the used strain showed a satisfactory efficiency against the eggs and different larval stages treated, comparatively with witnesses of the same age.

KEYWORDS: Entomopathogenic, *Beauveria bassiana, Culex pipiens,* Mosquitoes, Anopheles, Aedes and Chemical Insecticides

INTRODUCTION

Some groups of Diptera are responsible for most major diseases; this is the case of the Culicidae group that cause serious harm to humans and animals by their role of potential vectors of infectious diseases **(Rioux, 1958)**

In Algerian cities, *Culex pipiens L.* (Diptera: Culicidae) is the mosquito that presents the biggest interest by its large geographical distribution and its abundance which cause a serious harm. These insects are usually controlled by conventional insecticides for the most of them, chemical products that cause for long term secondary effects (effects on the no targeted organisms and resistance of the targeted species) **(Tabti and Abdellaoui-Hassaine, 2009).**

The Pesticides create a pollution that extends to phréatic table and to content of our food, they are harmful to health, wild fauna and biological balances. More, acquired resistance by some vectors tends to make pesticides ineffective **(Pintureau, 2009).**

Pathogenic fungi of insects occupy a particular place in invertebrates pathology and in research of organisms able to regulate the proliferation of harmful invertebrates, in vegetal and human or animal health **(Ferron *&al*., 1991 ; Lacey & *al.*, 1996).**

It is in this context that our work has been oriented, and dedicated to:

Check the influence of the isolated strain on the mortality of *Culex pipiens* eggs and larvae.

MATERIAL AND METHODS

Biological Material

Culex pipiens

The insects used in our study came from a breeding of mosquito species native of Algiers, identified by the team of the medical entomology of ecology laboratory of vectorial systems, and bred at the Pasteur Institute of Algeria (El Hamma appendix). Selected individuals are the eggs and the four larval stages.

Beauveria bassiana

This strain has been recently isolated (january 2012), from the soil of Boumerdes region, its identification was realised at botany department (Mycology service) of Agronomic National Institut (El Harrach), using the adhesive tape technical (figure 1 and 2).

Figure 1: Macroscopic Appearance of *Beauveria bassiana* (Original, 2012)

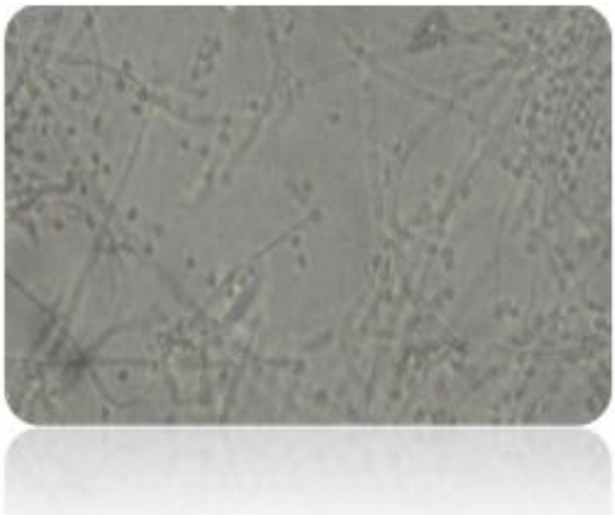


Figure 2: Hyphae and Spores of *Beauveria bassiana* x40 (Original, 2012)

TREATMENT TECHNICAL

Preparation of the Entomopathogenic Solution

From the colonies aged from 8 to 15 days, samples are taken and put into flask containing 500 ml of sterile distilled water that is hermetically shut to prevent contamination.

To allow a maximum release of spores, flask is shaked for 30 minutes, by adding 2-3 drops of tween 80 reagent, period after which the spores concentration is evaluated using a hématimétric cell (Malassez cell).

Used Dose

In order to realize a preliminary test allowing to assess the toxicity of the local strain *Beauveria bassiana* that we have recently isolated, we have choosen a high dose $0.33x10^7$ spores / ml. We have opted for this dose according to the

findings of many researchers in microbiological fighting, Meikle *& al.* (2007), Wang *& al.* (2002), Fan *& al.* (2012) and Halouane (2008), who have obtained satisfactory results in treating various types of insects with a fungal suspension of *B. bassiana* (10^7spores/ml).

The selected dose is applied on eggs and four larval stages of *Culex pipiens.*

Type of Treatment

To administrate the fungal solution to our insect, we have selected the submerging method, which consists to submerge the individuals in the fungal solution in reason of ten individuals of each larval stage (with three repetitions of each larval stage during 5 to 15 minutes and then they are put back into their natural habitation (lake water).

For the eggs, we have proceeded to submerge a tray of eggs into the entomopathogen solution.

Control individuals trays and larvae are distributed in the same manner and they have the same number that treated individuals, except that they have been submerged into sterile distilled water, and then they are put back in their natural habitation (lake water).

- The cumulative mortalities and symptomatology are daily checked.

Variance Analysis

In aim to confirm the treatment efficiency on the larval stages, we relied on the variance analysis with two criterias for classification of cumulative and corrected mortality rates after 96h.

Probit Analysis

In aim to estimate the LT50 for the dose of 0.33x 10^7spores/ml (LT50 = lethal time at which we obtain 50% of mortality), we realized the transformation of corrected mortality percentages into probit **(Bliss in Cavelier, 1976)** and the transformation of time into decimal logarithm **(Cavelier, 1976).**

These transformations have allowed us to establish equations of the regression lines

« probit- logarithm» of type:

$$y= ax+b$$

y= corrected mortalities Probit

x= logarithm of time

a = the slope

RESULTS

Effect of *Beauveria bassiana* on Eggs of *Culex pipiens*

The sensitivity of the eggs of *Culex pipiens* to *Beauveria bassiana* seems important which no hatching have been highlighted throughout the treatment period.

After 96h (3^{th} day) the tray of eggs sinks into the water reflecting their death.

Effect of *Beauveria bassiana* on the Mortality of Larvae of *Culex pipiens*

The corrected mortalities percentage observed after 5 days of treatment with *Beauveria bassiana* are represented in figure n°3.

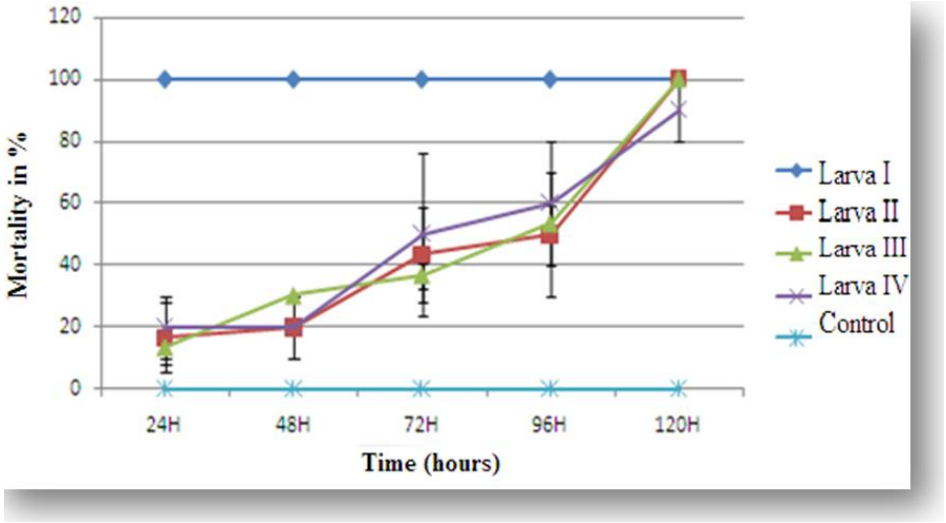


Figure 3: Mortality Rates for the Different Larval Stages (L1, L2, L3 and L4) of *Culex pipiens* Witnesses and Treated for the Dose $0.33x10^7$ Spores /ml of *B Beauveria bassiana*

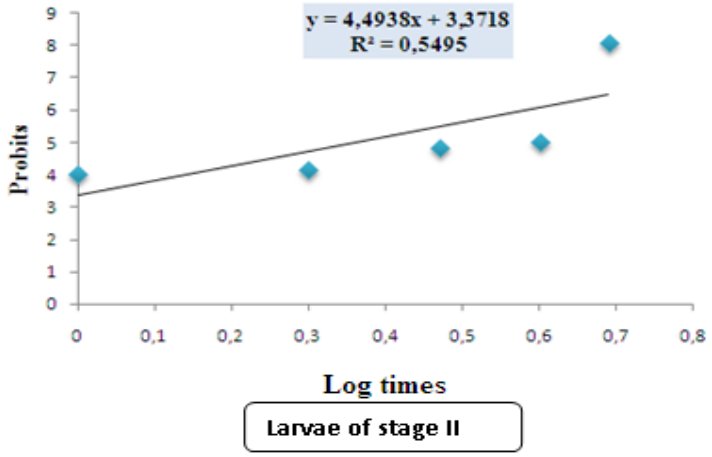
According to the figure n°3, it was noticed that the larval mortality evolves in the time and varies significantly according to the different larval stages treated, The results of the treatment effect revealed a highly significant difference between the mortality rates registered for the different larval stages of *Culex pipiens* and the control individuals during the treatment ($pr<0.05$). Indeed the highest mortality was recorded in the larvae of the first stage or we recorded 100% mortality from the first day of treatment.

For larval stages II, III and IV, we can note that the recorded mortalities during treatment start to appear from the first day by reporting 16.66% for the second larval stage, 13.33% and 20% for the third and fourth stages respectively. These mortalities are in the range until the fourth day or we recorded a mortality of 50%, 53.33% and 60% for stages II, III and IV respectively. Total mortality of 100% was obtained during the fifth day of treatment for the three larval stages.

According to these results, we can note that the first larval stage have an increased sensitivity to the fungal treatment comparatively to others stages.

LT50: calculates of LT50 (lethal times: causing 50% of mortality) of *Beauveria bassiana* tested at the high dose (10^7), informs us about the importance of the fungus effect along the time.

The values of LT50 correspond to the dose ($0.33x10^7$spores/ml) and every larval stage, are directly estimated from the equations of regression lines (figure n°4) and represented in the histogram (figure 5).



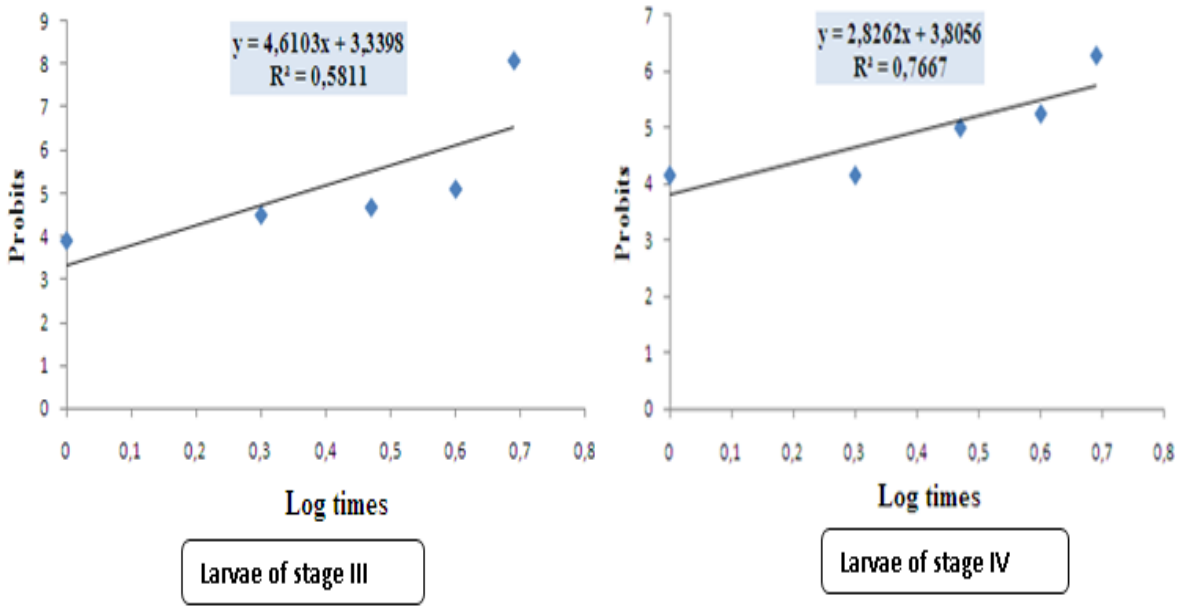


Figure 4: Efficiency of the Dose 0.33x10^{7}sp/ml in the Time against the Larval Stages of *Culex pipiens*

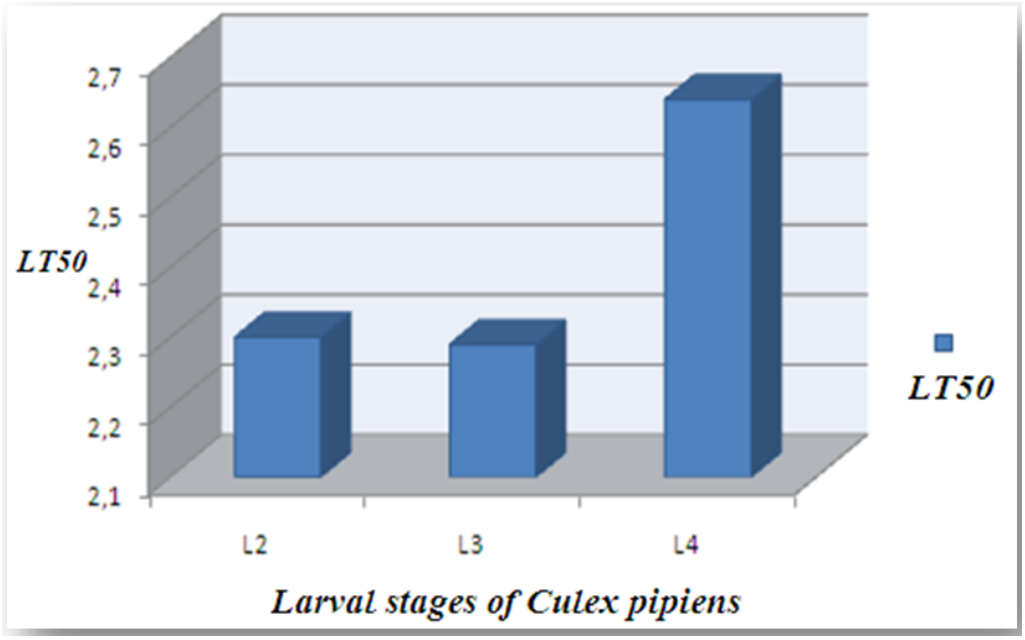


Figure 5: LT_{50} Obtained after Treatment of *Culex pipiens* Larvae with *B. Beauveria bassiana* at a Concentration of 0.33x10^7 sp/ml

The calculated lethal times, vary slightly of a larval stage to another. The most elevated time is noted during the treatment of the fourth larval stage with *B. bassiana* (0.33x10^{7}sp/ml) → 2.64 day. The obtained lethal time 50 during the treatment of larval stage II and III with the same dose are respectively 2.30 days and 2.29 days this may show that these two larval stages present a similar sensitivity to the treatment with *B **Beauveri**.bassiana*.

SYMPTOMATOLOGY

Our observations show decrease movements of infected individuals of *Culex pipiens*, they feed little and they have difficulties in moulting; furthermore, their life duration is reduced. After death, the insects are hard and mummified, they develop conidia on the body, it's muscardine's development, it is white to *Beauveria bassiana* (the color of their spores) (figures 6, 7 and 9).

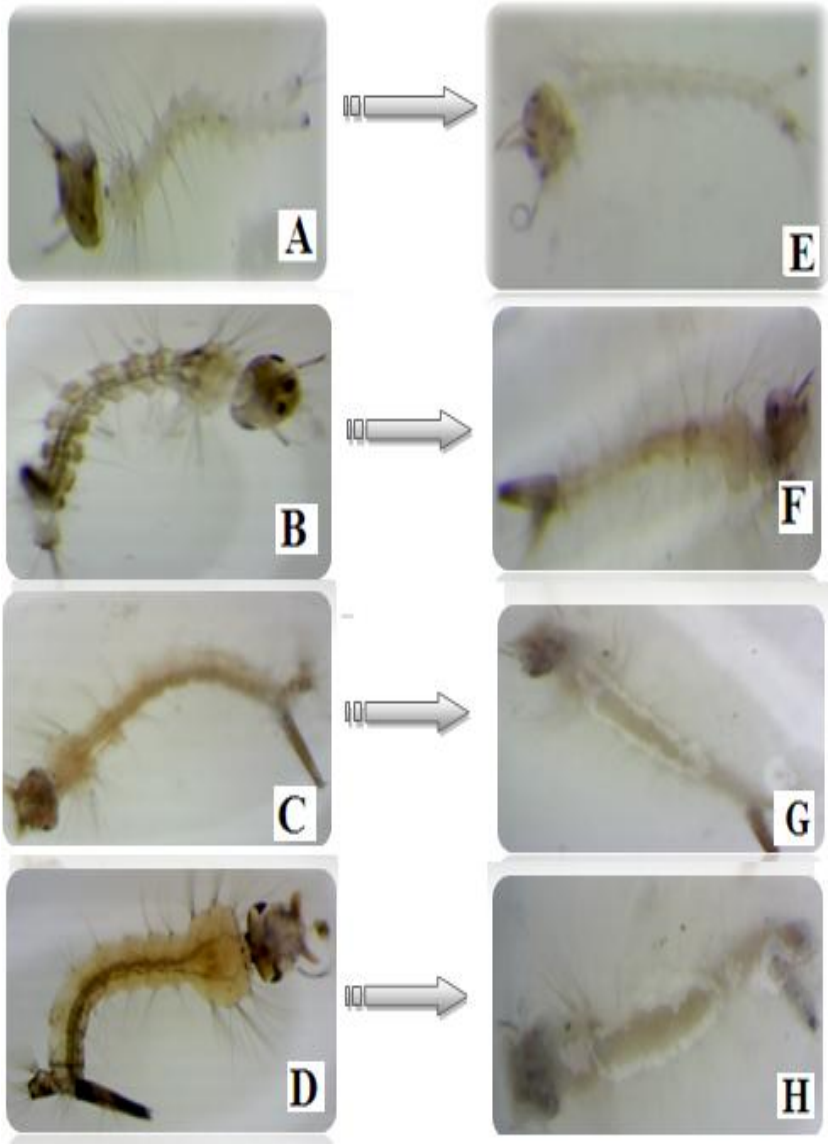


Figure 6: Larval Stages I, II, III and IV of *Culex pipiens* Witnesses (A, B, C and D) and Treated (E, F, G and H) x40 (Original, 2012)

During treatment, we have noted for the larval stage IV of the *Culex pipiens,* the passage of some larvae to pupal stage whose emergence of nymphs was blocked leading to the individual's mortality (figure n° 7).

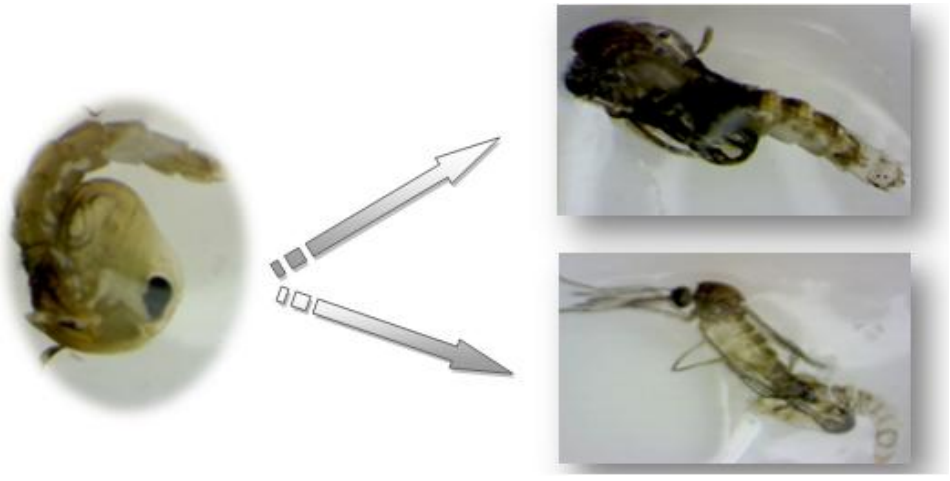


Figure 7: Blocked Emergence at Two Levels x40 (Original, 2012)

Some nymphs gave a deformed imagos with atrophied wing (figure 8).

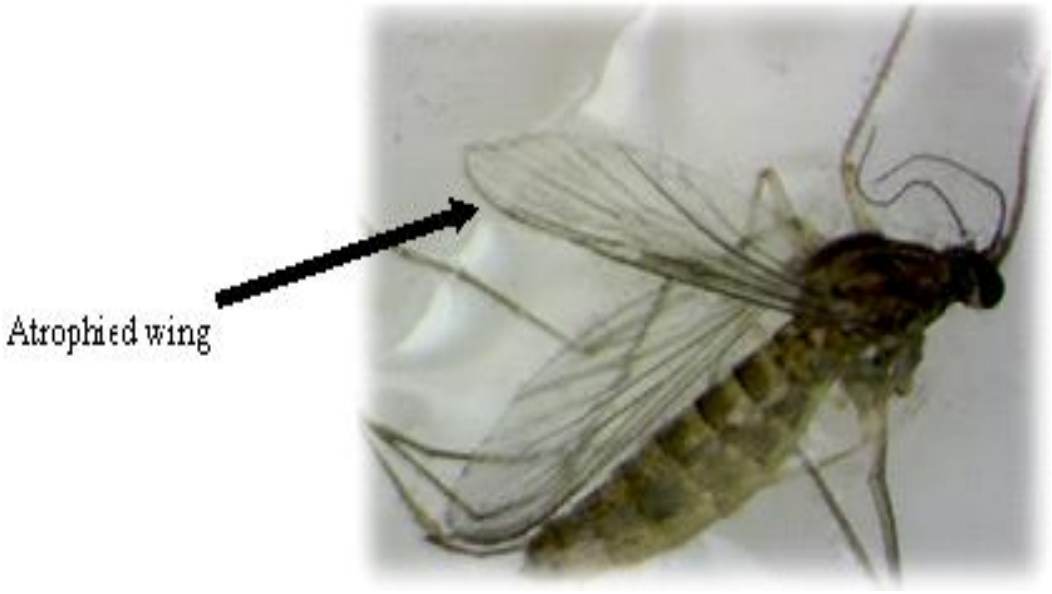


Figure 8: Deformed Adults of *Culex pipiens* with an Atrophied Wing x40 (Original, 2012)

We have found a strong proliferation of the fungus on the cadavers of deformed adults (figure 9).

Figure 9: Proliferation of the Fungus on the Deformed Adults of *Culex pipiens* x40 (Original, 2012)

In parallel, during the same period, we haven't found mortality in control individuals treated with sterile distilled water.

The tray of eggs of *Culex pipiens* treated with *Beauveria bassiana* presents after 24 h of treatment, a beginning of fungus formation on the surface without hatching (figure « B ») .

The fungus grows after 48 hours until covering all the treated tray of eggs.

The sinking of the eggs in water, testifying their death after 96 hours (figure 10 « C »)

No mortality was recorded for the tray of eggs control treated with distilled water where we have noticed a hatching after three days of treatment (figure n° 10 « A »).

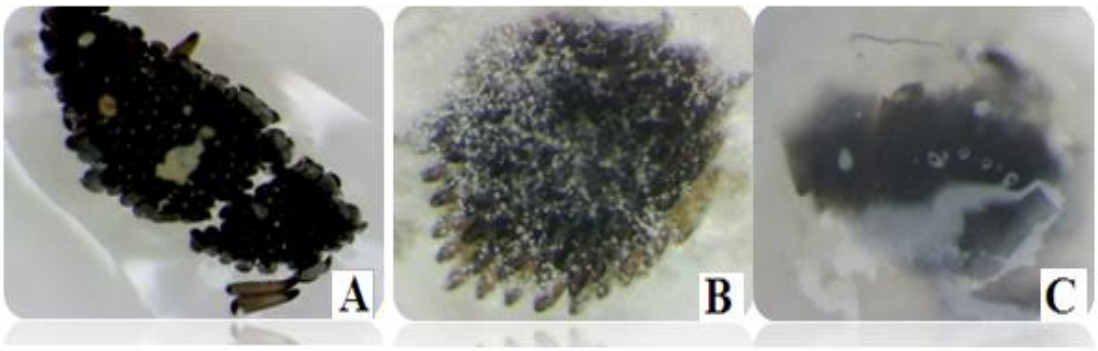


Figure 10: Tray of Eggs of *Culex pipiens,* Witness (A) and Treated (B and C) x40 (Original, 2012)

In fact, same symptoms have been observed by many authors, like Leger *and al.* (1988), McCoy *and al.* (1988) and Boutelis *and al.* (2010).

DISCUSSIONS

According to the variance analysis, the results of our biological tests show a highly significant difference between the mortality rates registered for the different larval stages and control individuals during the treatment.

For larvae of first stage, the difference is very highly significant or we recorded a total mortality rate (100%) from the first day of treatment demonstrating the sensitivity of the larvae (stage I) of *Culex pipiens* to a fungal treatment, as it was already confirmed by Riba *and al.* (1984), by treating the larvae of *Culex pipiens* with a fungal suspension of *Metarhizium anisopliae* in a concentration of 10^5 spores / ml, these larvae are particularly sensitive to the fungus which more than 70% of them die from the first day before the fungus germination, in opposite, in the same conditions, no mortality has been recorded for the two others species *Aedes aegypti*, *Anopheles stephensi*, this can be caused by the sensitivity to the fungus treatment which may be different from a mosquito species to another.

The corrected mortality rates for the II, III and IV stages are in general, close values situated between 16% and 20% for the first day, 20% and 30% for the second day and between 50% and 60% for the fourth day to reach 100% for the fifth day of treatment. Similarly, Millet *and al* (2007) has demonstrated that no correlation has been found between the age of larva and the time passed until its death, by treating the larvae of *Paysandisia archon*, by using a concentration of $7x10^7$sp/ml of *B. bassiana.*

This same author notes that the mortality of the larvae reaches 100% from the 14^{th} day of treatment. After that, the dead larvae are covered with mycosis. In another side, our experimentation has registered to the different larval stages of *Culex pipiens* a cumulative mortality of 100% after only five days.

Of same, we have noted a mortality of 16.66%, 13.33% and 20% respectively for the larval stages II, III and IV for the first day of treatment, 20%, 30% and 20% for the second day, 43, 33%, 36.66% and 50% for the third day and 50%, 53.33% and 60% for the fourth day.

If we compared these results with those obtained by Millet and al (2007) who has mentioned a mortality less than 10% for the first, second and third day, 12% for the fourth day and in final a mortality of 30% for the fifth day. We can conclude that the entomopathogenic *B.bassiana* has probably showed an interesting efficiency against the *Culex pipiens* larvae than the *P. Archon* larvae.

Many others searchers interested to the study of biological activity of entomopathogenic against different orders of insects.

Against the acarids, the entomopathogenic fungus *B. bassiana* in a concentration of 10^7sp/ml has registered a mortality of 100% in a period of 5 to 8 days in adults of *Varroa destructor* (Meikle *and al*, 2007).

According to Wang *and al* (2002), a suspension of *B.bassiana* with a concentration of 5.10^7sp/ml showed a mortality of 100% in adults of *Coptotermes formosanus* after about 4 to 8 days.

In the same case, Fan *and al* (2012), has registered a mortality of 90% after about 144 hours (5 à 7 days), by treating *Galleria mellonella* with a fungal suspension concentrated at 5.10^7 sp/ml of *B.bassiana*.

Besides, Halouane (2008) noted a beginning of mortality of *Schistocerca gregaria* in the third day after the treatment with a suspension of *B. bassiana* (0.164x 10^7spores/ml) to reach 100% in the 7^{th} day.

The results obtained by the authors quoted above, seem the same at the point of view of cumulated mortality (total of 100%), knowing that they used a fungal suspension of *B bassiana* (10^7sp/ml) against adults of different species of insects.

In fact, Ravallek *and al* (1986), has demonstrated that the cumulative mortality during the treatment of *Toxorhynchites amboinensis* by a spores suspension of *M. Anisopliae* in a concentration of 10^7sp/ml, corresponds to 40% at the fifth day of treatment while a cumulative mortality is observed in the 19^{th} days.

If we want to deduct the $LT_{50,}$ according to our experimentation, we note that for the second larval stage, LT50= 2.30 days, for the third larval stage, LT50= 2.29 days and for the fourth larval stage, LT50= 2.64 days.

According to Riba *and al* (1984), the LT50 relative to a treatment on larvae of *Aedes aegypti* with a strain of *M. anisopliae* is 1.7 days at a concentration of 10^6sp/ml. This difference in the LT50 value would be caused in one side by the efficiency of the entomopathogenic strain, the sensitivity of mosquitoes to the fungal treatment that can be differentiated from a mosquito kind to another. In another side, by the selected dose of the fungal suspension.

In same, Millet *and al* (2007), has noted an LT50 of the treated larvae of *P. archon* by *B. bassiana* (concentration of 10^7sp/ml) of 5 to 6 days.

The same author has mentioned that the mortality of corn borer remained weak during the test, with no more of 30%, so, he noted that the *B bassiana* isolate is more pathogenic against the larvae of *P. Archon* than against the corn borer, which is its original host.

The toxicity of the entomopathogenic *B. bassiana* against eggs of *Culex pipiens* seems more accented than against its larvae, that cause the death of the tray of eggs treated from the third day of treatment without no hatching.

According to Millet *and al* (2007), 42% of treated eggs of *p. archon* with *B. bassiana* have developed the mycoses and don't hatch while six larvae issued from 17 hatched eggs have dead which gives a larval survival of 24%.

Using of the entomopathogenic fungus against mosquitoes eggs allow not only to limit their hatching but also to induce a diminution of larval viability that are infected when they go out of the egg. It's a good result because it makes possible a treatment from the egg stage in order to multiply the action chances (Millet *and al*, 2007).

CONCLUSIONS

The use of entomopathogenic microorganisms as agents of biological fighting against harmful insects, knew a remarkable rise during the last decades, due to major environmental problems as the ecosystems pollution and mainly the resistance of several insects against chemical insecticides. The necessity and acuity to find efficient alternatives of fighting

against a harmful insects others than chemical fighting, prompted us to explore new avenues with the entomopathogenic micro-fungus *Beauveria bassiana.* Many studies have demonstrated the insecticide potential of *B. bassiana*. It may be used against different harmful insects belonging to several orders **(Greathead *and al.*, 1994; Todorova *and al.*, J996; Todorova *and al.*, 2002a; Kouassi *and al.*, 2002, Tadela and Pringle, 2003; Cornia *and al.*, 2004 *and* Sabbahi *and al*, 2008aa)**.

The preliminary knowledge of etiological relationships between entomopathogenic funguses and mosquitoes are sufficiently advanced to be continued by tests in natural conditions in order to obtain a better knowledge of epidemiological components of this parasitic complex **(Riba *and al*, 1984).**

We have showed through these tests a very good efficiency of *B bassiana* strain that is recently isolated, on the eggs and the different larval stages of mosquito *Culex pipiens*.

These results portend the possibility of the realization of new tests in natural conditions. That must be conducted on a larger scale (on the field), in order to confirm the results presented in this study.

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