

# Larvicidal and Synergic Effects of two Biopesticides (*Azadirachta indica* and *Metarhizium anisopliae*) against Larvae of *Culex quinquefasciatus* (Diptera, Culicidae) (Say, 1823)

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**Abstract:** Mosquitoes in general and *Culex quinquefasciatus* in particular have for a long time constituted a source of nuisance due to the diseases they transmit, stings and annoying buzzing. They are also a public health problem. This is why this study is aimed at finding a biopesticide that can fight effectively but also be an alternative to chemical residual pesticides in the environment. Concentrations of biopesticides used are ranged from  $2.5 \cdot 10^8$  to  $12.5 \cdot 10^8$  spores/ml with intervals of  $2.5 \cdot 10^8$  spores/ml for *Metarhizium anisopliae*. Concentrations ranging between  $8 \cdot 10^{-4}$  g/ml to  $40 \cdot 10^{-4}$  g/ml with intervals of  $8 \cdot 10^{-4}$  g/ml of *Azadirachta indica* (Suneem 1%) were used to fight against 100 three instars larvae of *Culex quinquefasciatus*. Concentrations of the mixture of *Azadirachta indica* (Suneem 1%) and *Metarhizium anisopliae* ranging from 0.02 ml +  $2 \cdot 10^8$  spores/ml to 0.02 ml +  $1 \cdot 10^8$  spores/ml were used to study synergic effects of these two biopesticides. One hundred (100) of three instars larvae of *Culex quinquefasciatus* are used as control and placed in the same jars with 500 ml of distilled water. *Metarhizium anisopliae* (green muscle) have caused 50% mortality of three instars larvae of *Culex quinquefasciatus* after 3 days. *Azadirachta indica* (Suneem 1%) have gaved a mortality more than 60% after 2 days. The mixture *Azadirachta indica* (Suneem 1%) and *Metarhizium anisopliae* caused a high mortality with a rate that exceeds 90% in 3 days. The mixture also prevented a larval moult thus stopping their growth and development. In summary, both *Metarhizium anisopliae* and *Azadirachta indica* (Suneem 1%) are effective against three instars larvae of *Culex quinquefasciatus*. Their mixture caused a synergic effect and thus increased their individual efficacy of two biopesticides.

**Keywords:** *Azadirachta indica*, *Metarhizium anisopliae*, *Culex quinquefasciatus*, mortality, efficacy

## I. Introduction

In Senegal and around the world, mosquitoes have always been considered a source of nuisance for humans, mainly because they can be vectors of disease. In fight against mosquitoes that cause many buzzing, sting and diseases such as yellow and dengue fevers and malaria, various methods based on chemicals products are adopted. These chemicals methods are most frequent to fight pests (Regnault-Roger and Hamraoui, 1997). However, due to their undesirable effects on environment and human health, the use of pesticides has been widely criticized in recent years (Lorito *et al.*, 1994). In response to the emergence of resistant species, some west african researchers studied double impregnation of mosquito net with two chemicals products

(pyrethroid and carbamate). The efficacy of this association was also demonstrated in a field trial in Côte d'Ivoire against mosquitoes of the genus *Anopheles* and *Culex* resistant to pyrethroids (Masson, 2003). This mixture involves a synergistic effect between these two insecticides, which gives it an advantage in control of mosquitoes. Thus, there is reduction of doses to be applied and better efficiency. Mixtures of chemical insecticides may be an effective strategy for resistance management, but are harmful to non-targets.

To develop alternatives for chemical methods, researchers now turn to biological insecticides such as neem (*Azadirachta indica* A. Juss) and fungi (*Aspergillus clavatus* and *Metarhizium anisopliae*).

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Taken separately or in mixture, these biological insecticides have shown better efficacy on mosquito larvae. Thus, the present study concerns a synergic effect between Suneem 1% (*Azadirachta indica*) and an entomopathogenic fungus (*Metarhizium Anisopliae*) on larvae of *Culex quinquefasciatus*. We have to show:

- larvicidal effects of Suneem1% on three mosquito larvae, in particular of *Culex quinquefasciatus*;
- larvicidal effects of the entomopathogenic fungus *Metarhizium anisopliae* on the same mosquito larvae;
- compatibility between Suneem 1% and the fungus *Metarhizium anisopliae*;
- synergic effects between the Suneem1% (*Azadirachta indica*) and the entomopathogenic fungus (*Metarhizium anisopliae*).

## II- MATERIALS AND METHODS

### 1- MATERIAL

#### 1.1 Biological material

##### - Suneem 1%

*Azadirachta indica* (Suneem 1%) was supplied by Senchim a Senegalese chemical industry (Dakar West Africa, Senegal). Suneem1% is biochemical product or biopesticides wich poison mosquitoes. It has larvicidal, adulticidal and anti-baiting properties in biological control.

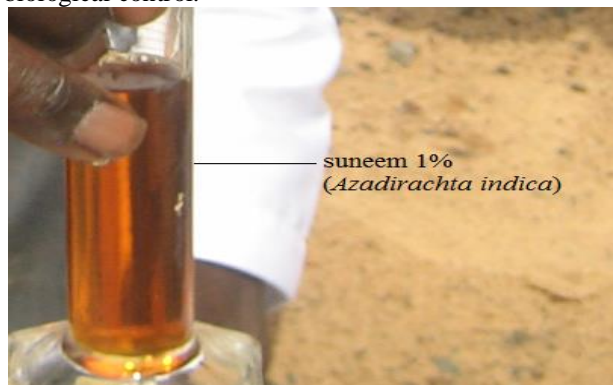


Figure 1: Biopesticide based on Suneem 1%

##### - *Metarhizium anisopliae*

It was synthesized supplied under the "green muscle label" by the Plant Protection Division (DPV) in Dakar, Senegal. Lyophilized spores form of strains were stored in bags according to laboratory conditions. Subsequently these strains were multiplied.

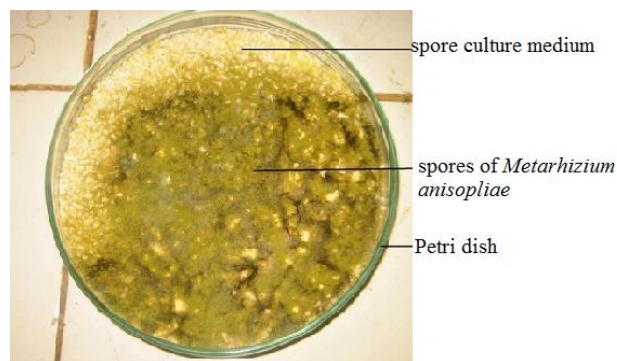


Figure 2: spores of *Metarhizium anisopliae*

#### 1.2 Animal material

The larvae mosquitoes of *Culex quinquefasciatus* were sampled in breeding sites located in suburbs of Dakar (Senegal, west Africa). Larvae were reared in wooden cages measuring 50 x 50 x 50 cm and under laboratory conditions (30 ° C, 52% relative humidity and day / night photoperiodicity of approximately 13/11 hours). These larvae are fed with bread powder.



Figure 3: Three instar larvae of mosquito : *Culex quinquefasciatus*

## 2- METHODS

### 2.1- Breeding sites surveyed

This study involved a sample of 16 breeding sites (ponds, marshes, valleys) selected according to different criteria : presence of mosquito larvae in a shelter, accessibility, perennality and non-treatment with insecticides. Two types were used: positive sites without vegetation and positive ones with vegetation.



Figure 4: Breeding site without vegetation



Figure 5: Breeding site with vegetation

## 2.2- Sampling Technique

Sampling of *Culex quinquefasciatus* larvae is done using the ladle method with a capacity of 500 milliliters. This technique consists of plunging the ladle into water and moving it with uniform movement while avoiding eddies.



Figure 6: Sampling method of *Culex quinquefasciatus* larvae by ladle

## 2.3 - *Culex quinquefasciatus* larvae treatment

### 2.3.1 - With Suneem1%

*Culex quinquefasciatus* larvae were treated directly in breeding sites. These latter were measured by a decameter to determine their extent. Volumes of water were obtained by calculation from these results. Larval densities before treatment are taken by ladle

method. Three shots of ladle of a volume of about 500 ml each made it possible to count the number of larvae and then to calculate the average densities for each breeding site. Thereafter, the breeding sites are treated with amounts of 25, 30, 35 and 40 ml.

### 2.3.2- With *Metarhizium anisopliae*

The larvae of *Culex quinquefasciatus* are treated directly in the breeding sites. After indicate water volumes of sites and larval densities before treatment by ladle method, larvae are treated with spores of *Metarhizium anisopliae*. Thus, amounts of 10 and 12.5 mg are applied in different breeding sites.

### 2.3.3 with mixture (*Metarhizium anisopliae* and Suneem 1%)

Larvae of *Culex quinquefasciatus* are treated directly in deposits with mixture of 50 ml of Suneem1% and 10 mg of spores of *Metarhizium anisopliae*.

## III-RESULTS

The results obtained are analyzed using the statistical software Rogui (R).

### 1- Larval densities with Suneem 1%

The results of treatment with *Azadirachia indica* (Suneem 1%) are presented in form of larval densities recorded in Table 1

Gites Products	Parameters	KMF	MB	PKR10	KM U3	PGR	DTK	TDS	YS	KF	G
Suneem 1%	DLAvT	134	99	98	154	109	96	75	95	89	89
	DLApT	47	32	35	46	37	34	28	35	36	29
	TM	65	68	64	70	66	65	62	63	60	67

**Table 1:** Front larval densities and after treatment with *Azadirachia indica* (Suneem 1%).

Legend: KFM: Keur Mbaye Fall, MB: Mbaob Baobab, PKR10: Pikine Street 10, KM U3: Keur Massar U3, PGR: Pikine Guinaw Rail, DTK: "Djiddah Thiaroye Kaw", TDS: "Thiaroye Darou Salam", YS: "Yembeul Sud", KF: "Kaffrine", G: "Goudiry", DLAvT: Larval densities before treatment, DLApT: Larval densities after treatment, TM : Mortality rates.

Larval densities of *Culex quinquefasciatus* decrease after treatment with *Azadirachia indica* (Suneem 1%). Average mortality rate which around 70% and higher.

**Table 2:** Larval densities before and after treatment with *Metarhizium anisopliae*

Gites Products	Parameters	KMF	MB	PKR10	KM U3	PGR	DTK	TDS	YS	KF	G
<i>Metarhizium anisopliae</i>	DLAvT	107	82	90	141	87	92	69	84	85	92
	DLApT	44	38	38	62	35	36	31	35	37	40
	TM	59	54	58	56	60	61	55	58	56	56

Legend: KMF: "Keur Mbaye Fall", MB: "Mbaob Baobab", PKR10: "Pikine Street 10", KM U3: "Keur Massar U3", PGR: "Pikine Guinaw Rail", DTK: "Djiddah Thiaroye Kaw" TDS: "Thiaroye Darou Salam", YS: "Yembeul Sud", KF: "Kaffrine", G: "Goudiry", DLAvT: Larval densities before treatment, DLApT: Larval densities after treatment, TM: Mortality rates.

*Metarhizium anisopliae*. This reduction is close to 50% mortality. In natural environment, *Metarhizium anisopliae* is effective against *Culex quinquefasciatus* larvae.

**3- Larval densities with mixture (*Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*)**  
The results of treatment with mixture (*Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*) are presented in the form of larval densities recorded in Table 3.

**Table 3:** Larval densities before and after treatment with mixture (*Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*)

Gites Products	Parameters	KMF	MB	PKR10	KM U3	PGR	DTK	TDS	YS	KF	G
Melange	DLAvT	164	103	115	162	113	109	98	112	107	98
	DLApT	11	08	06	08	09	05	03	06	06	03
	TM	93	92	95	95	92	95	97	95	94	97

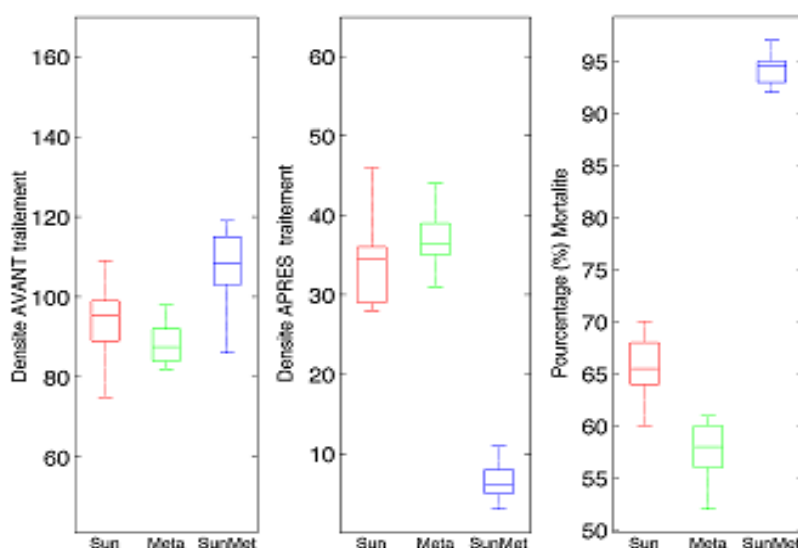
**Legend :** KFM: "Keur Mbaye Fall", MB: "Mbao Baobab", PKR10: "Pikine Street 10", KM U3: "Keur Massar U3", PGR: "Pikine Guinaw Rail", DTK: "Djiddah Thiaroye Kaw" TDS: "Thiaroye Darou Salam", YS: "Yembeul Sud", KF: "Kaffrine", G: "Goudiry", DLA<sub>v</sub>T: Larval densities before treatment, DLA<sub>p</sub>T: Larval densities after treatment, TM: Mortality rates.

These results show that larvae of *Culex quinquefasciatus* underwent heavy mortalities following treatment with mixture (*Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*). These

mortalities revolve around 95%. Therefore mixture (*Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*) is effective against the larvae of *Culex quinquefasciatus*.

#### 4- Comparison of larval densities for the two biopesticides used (*Azadirachia indica* (Suneem 1%) , *Metarhizium anisopliae*) and their mixture

The evolution of these larval densities as a function of the two biopesticides taken separately and their mixture is presented in diagram. 7 corresponding to pads:



**Figure 7:** Comparative larval densities of the three biopesticides used (Suneem1%, *Metarhizium anisopliae* and the mixture (*Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*). **Legend :** Sun: "Suneem1%"; Meta: "*Metarhizium anisopliae*"; SunMeta: "Suneem + *Metarhizium anisopliae*"

It is found that the larval densities after treatment for each biopesticide are much lower than densities of the mixture. The mortality rates are very strong for the mixture, followed by *Azadirachia indica* (Suneem 1%) and *Metarhizium anisopliae*. Ultimately, the mixture appears to be much more effective than Suneem1% and *Metarhizium anisopliae*.

#### IV- DISCUSSION

The use of natural products such as *Azadirachia indica* (Suneem 1%) , *Metarhizium anisopliae* and the mixture of the two biopesticides in the control of mosquito larvae in general and those of *Culex quinquefasciatus* in particular is more than current. In Australia, Rae (2004) carried out treatments on larvae of *Chortoicetes terminifera* (the locusts of Australia)

with Green Guard®, formulated from a strain of *Metarhizium anisopliae*.

Results on the application of *Azadirachia indica* (Suneem 1%) to larvae of *Culex quinquefasciatus* confirm the work of Attri and Prasad (1980) which showed the efficacy of neem oil on Culicidae larvae and Scott and Kaushik (2000) on the efficacy of Margosan-O® on *Culex quinquefasciatus*, those of Sèye et al. (2004) with formulated neem oil (LD50 at 3 mg / l in 48 hours) and those of Ndione et al. (2007). Lepage et al., (1992) used several strains of entomopathogenic fungi (*Metarhizium anisopliae*, *Cordyceps militaris* and *Tolyposcladium cylindrosporum*) to control a few biting diptera. They concluded that all the strains tested, *Metarhizium anisopliae*, seem to be the most interesting for the

biological control of larvae of biting Diptera. Saint-Louis et al., (2001) confirmed that *Metarhizium anisopliae* is a promoter fungus for biological control due to its efficacy at all stages of an insect development and could be an excellent candidate as an alternative measure to synthetic pesticides. In the context of biological control or integrated pest management. These results are similar to those obtained by Alves et al. (2002), which recorded an early mortality of larvae of *Culex quinquefasciatus* treated with *Metarhizium anisopliae* from the first day of treatment.

The work of Ravallec *et al.* (2003) demonstrated that *Metarhizium anisopliae* had a distinct effect on *Aedes albopictus* larvae. These results for the applications of an entomopathogenic fungus such as *Metarhizium anisopliae* on insect larvae confirm the Work of Touré (2006) which showed that the spores of *Metarhizium anisopliae* caused the mortality of the Senegalese locust with a LD50 of 4 days and a TL100 of 7 days for a dose of  $4.3 \cdot 10^7$  spores / ml. Our study fits right Line with these various published articles. Indeed, the results of our study allowed us to note a considerable decrease in the range of 50%, 70% and 95% respectively for treatments with *Metarhizium anisopliae*, *Azadirachta indica* (Suneem1%) and the mixture of both. These results confirm and even complement the work of the authors cited above. The adoption of this innovative method of mixing effective biopesticides, thus creating a synergistic effect in the control of the larvae of *Culex quinquefasciatus*, has several advantages in particular resulting in a decrease in mosquito larvae on the one hand and related diseases and other diseases preserve an already fragile ecosystem. Indeed, our natural treatments showed average values of temperature, PH and relative humidity respectively of the order of 30 ° C, 10 and 60%. The work of Blanford & Klass (2004) Concluded that temperatures below 38 ° C during the day and above 20 ° C overnight are considered favorable in the control of mosquitoes.

In addition to the stimulating factor that is the physicochemical conditions of natural deposits, the time factor or duration of treatment appears to be determinant in the results. The residence of the larvae in the treated zone for at least 48 hours, gives a better efficiency to the treatment. Mortality and sporulation after 6 h and 24 h of exposure are lower than those after 48 h.

### Conclusion

In summary, it seems relevant to remember that *Azadirachta indica* (Suneem 1%) and *Metarhizium*

*anisopliae* are very effective against larvae of *Culex quinquefasciatus* in particular, and against mosquito larvae in general. But this efficacy appears much more important when these two biopesticides are mixed. In perspective it opens up to the scientific community a use of the entomopathogens (bacteria or fungi), of deregulators of growth in a much more effective biological control.

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