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Lethal Effects of the Mix (Suneem 1% and *Metarhizium anisopliae*) on Two Instars Larvae of *Culex quinquefasciatus* (*Diptera, Culicidae*) (Say, 1823) in their Natural Sites

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Abstract: ***Mosquitoes in general and Culex quinquefasciatus in particular have for a long time been a source of nuisance due to diseases they transmit, punctures and annoying buzzes. They are also a public health problem. That is why this study aim to find a biopesticide that can fight effectively but also be an alternative to persistent chemical pesticides in the environment. Thus, we decided to control the mosquito larvae of Culex in the wild with biopesticides suneem1% alone, Metarhizium anisopliae alone and their mixture. To arrive at this we determined the lodgings and determine their physicochemical conditions. We also evaluated larval densities before and after treatment with the ladle technique. This methodology allowed us to have results. The lodgings of Pikine, Kaffrine and Goudiry gave mortalities greater than 50% in 2 days. Suneem alone, Metarhizium anisopliae alone gave mortalities, but the formulation of these two biopesticides is more effective with high mortalities in 2 days. These control stage 3 larvae of Culex quinquefasciatus are used for these two treatments and placed under the same treatment conditions. The number of dead quinquefasciatus culex larvae increases in all treatment houses as a function of time. In sum, Metarhizium anisopliae and Suneem 1% are both effective against Culex quinquefasciatus stage 3 larvae. Their mixture caused a synergistic effect and thus increased their individual effectiveness.***

Keywords: Malaria, mosquitoes, Suneem1%, *Metarhizium anisopliae*

1. Introduction

In Senegal, as everywhere in the world, mosquitoes have always been considered a source of nuisance for humans, mainly because they can be vectors of diseases. As part of the fight against mosquitoes that cause nuisance (buzzing and bites) and diseases such as yellow fever, dengue, malaria, various control methods (mechanical, chemical and biological) are adopted. Chemical control with the use of insecticides is one of the most common methods of pest control (Regnault-Roger and Hamraoui, 1997). However, due to their undesirable side effects on the environment and human health, the use of pesticides has been widely criticized in recent years (Lorito et al., 1994). In the face of the appearance of resistant species, researchers from the Cheikh AntaDiop University in Dakar and the IRD (Research Institute for Development) have studied the effects of double impregnation of mosquito nets with two insecticides in the same way different action (a pyrethroid and a carbamate). The efficacy of this combination has also been demonstrated following a field trial in Côte d'Ivoire against mosquitoes of the Anopheles and Culex genus resistant to pyrethroids (Masson, 2003). This mixture involves a synergistic effect between these two insecticides, which gives it an advantage in the fight against mosquitoes. Thus, there is a reduction of the doses to be applied and a better efficiency. Chemical insecticide mixtures can be an effective strategy for managing resistance, but are harmful to non-targets. Insecticides are one of the most common methods used against mosquitoes. However, because of their undesirable side effects on the environment and human health, the use of conventional pesticides in this sense has been widely criticized in recent years (Lorito et al., 1994). In order to develop alternatives to chemical methods, microorganisms of various kinds, have just been studied and prospected for their biopesticide potential for insect pests such as mosquitoes (Chet et al., 1993). Of these biopesticides, endomopathogenic fungi (Aspergillus clavatus and *Metarhizium anisopliae*) and neem (Azadirachta indicaA.Juss) are currently considered to be the most important biological groups for mosquito control. Taken alone or in combination, these insecticides of biological origin have shown better efficacy on mosquito larvae. Taken alone or in combination, these insecticides of biological origin have shown better efficacy on mosquito larvae. In this context, the present study investigates the evolution of larval populations of *Culex quinquefasciatus* (Diptera, Culicidae) (Say, 1823) after synergistic and larvicidal effects of two biopesticides (Suneem 1% and *Metarhizium anisopliae*).

2. Material and Methods

1 Material

1.1 Biological material

- Suneem 1%

Suneem1% was supplied by Senchim a Senegalese chemical industry (Dakar, Senegal). Suneem1% is a biochemical product that can fight effectively against

mosquitoes. It has larvicidal, adulticidal and antiappealing properties in biological control.

Figure 1: Sunnem 1%: azadirachtin biopesticide

-Metarhizium anisopliae

It was awarded under the label "Green Muscle" by the Directorate of Plant Protection (DPV) of Dakar (Senegal). Lyophilized spores in the form of strains were stored in bags under laboratory conditions. Subsequently these strains were multiplied.

Figure 2: *Metarizhium anisopliae en* strain

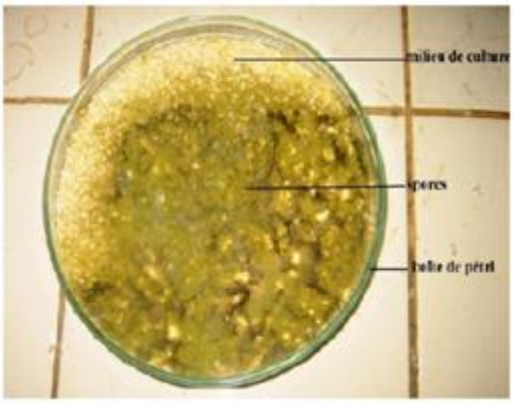


Figure 3: *M.anisopliae* en culture

-Animal material

The larvae of *Culex quinquefasciatus* tous stages were used as study material for this work. Larval densities were evaluated before any treatment. The larvae fed naturally and underwent the physicochemical conditions existing in their deposits.

Figure 4: Mosquito larva

2 Methods

2.1- Sampling houses

This study looked at a sample of 14 sites containing deposits consisting of basins, marshes and valleys selected according to different criteria: *relative* abundance of mosquito larvae, accessibility, durability and non-treatment with insecticides. These lodgings are structured in positive permanent lodgings in vegetation and positive permanent lodgings without vegetation.

Figure 5: Cottage without vegetation

Figure 6: Cottage with vegetation

2.2 Evaluation of larval densities before treatment

The evaluation of larval densities before treatment of *Culex quinquefasciatusa* larvae consisted of the use of the 500-ml ladle shot method. This technique consists of dipping the ladle in the water of the positive deposit then moving it with a uniform movement avoiding the eddies. This allowed *Cx quinquefasciatus* larvae to be collected and subsequently counted. This allows to reach the densities which are ratios of the number of larvae obtained to the volumes of waters of the ladles.£

Figure 7: Evaluation of pre-treatment larval densities of *Cx quinquefasciatus*

3. Treatment of *Culex quinquefasciatus* larvae

3.1 With Suneem1%

Culex quinquefasciatus larvae were treated directly in the breeding sites. These cottages were treated with amounts of 25% Sune, 25%, 35% and 40 ml. The cottages were measured thanks to a decametre to know their extent. The volumes of water were obtained by calculation from these results. To know the densities after treatment the method of the ladles was applied. In fact, three ladle shots with a volume of about 500 ml each made it possible to count the number of larvae and subsequently to calculate the average densities for each deposit.

3.2 With *Metarhizium anisopliae*

The larvae of *Culex quinquefasciatus* were treated directly in the breeding sites. Thus, quantities of *Metarhizium anisopliae* spores of 10 to 12.5 mg are applied in different breeding sites. After calculating the water volumes of the deposits, the larval densities were reevaluated by the same method of the ladles.

3.3 With the mixture Metarhizium anisopliae and Suneem 1%

The same methodology used with Suneem 1% and *Metarhizium anisopliae* was used again for mixing these two biopesticides. Only the concentrations used have changed. Indeed, a 40 ml concentration mixture of azardirachtin suneem1% was added to 10 mg of *Metarhizuim anisopliae* spores (40 ml of azardirachtin + 10 mg of *Metarhizuim anisopliae* spores)

4. Results

The results obtained are analyzed thanks to the logiciel statistique Rogui (R). II.1 Mortality from larvae of *Culex quinquefasciatus* traitées avecle mélange (Suneem1% et *Metarhizuim anisopliae*). Ces is a constituent of mortalities from larvae of *Culex quinquefasciatus* en fonction des doses du mélange et sont consignés dans le tableau 1.

Table 1: Mortality (in%) of larvae of *Culex quinquefasciatus* traitées avec le mélange

days	Gîtes													
	KMF	MB	PKR10	KM U3	SY2	KMM	KML	PGR	DTK	TDS	YS	KF2S	KF2N	G
J1	47	49	52	54	46	48	51	53	50	54	49	48	53	50
J2	65	68	64	70	68	69	68	66	65	62	63	78	65	67
J3	93	97	91	96	96	95	94	90	93	90	91	96	98	94

Legend: KMF: «KeurMbayeFall», MB: «Mbao Baobab», PKR10: «Pikine Rue 10», KM U3: «KeurMassarUnité 3», KMM: «KeurMassar Marché», KML: «KeurMassar Lycée», PGR: «Pikine Guinaw Rail », DTK: « DjiddahThiaroyeKaw », TDS: « Thiaroye Darou Salam », YS: « Yembeul Sud », SY1: « Sante yalla1 », SY2: « Sante yalla2 »KF2S: « Kaffrine2 Sud », KF2N: « Kaffrine2 Nord », G: «Goudiry».

These results show a clear increase in the number of larvae of *Culex quinquefasciatus* mortes after treatment with the mixture. There are mortalities that reach and even exceed 50% in most of the deposits except some ("keurMbayeFall", "Mbao Baobab", "KeurMassar Marché" and "Sante Yallah 2") where they revolve around values 47% to 49%. %. The CL 50 was obtained very early after 24 hours of treatment under natural conditions. The CL 90 was obtained after 72 hours of treatment. Evidence that in the wild, the mixture (Suneem1% and *Metarhizium anisopliae*) seems to be the most effective and therefore the best in the fight against these *Culex quinquefasciatus* larvae.

1-Percentage mortality of culinary larvae treated with *Metarhizium anisopliae* The results of treatment with *Metarhizium anisopliae* are presented in the form of Percentages of *Culex* larvae mortality recorded in Table 2

Table 2: Percentages of mortality of culinary larvae treated with *Metarhizium anisopliae*

days	Sites													
	KMF	MB	PKR10	KMU3	SY2	KMM	KML	PGR	DTK	TDS	YS	KF2S	KF2N	G
J1	29	24	20	21	25	23	27	30	26	24	27	23	29	28
J2	40	39	43	47	49	38	36	51	49	47	48	49	52	50
J3	59	54	58	56	60	58	52	60	61	55	58	56	60	56
J4	96	93	95	91	97	90	91	96	94	93	95	92	94	95

1- Percentage mortality of culinary larvae treated with *Metarhizium anisopliae*

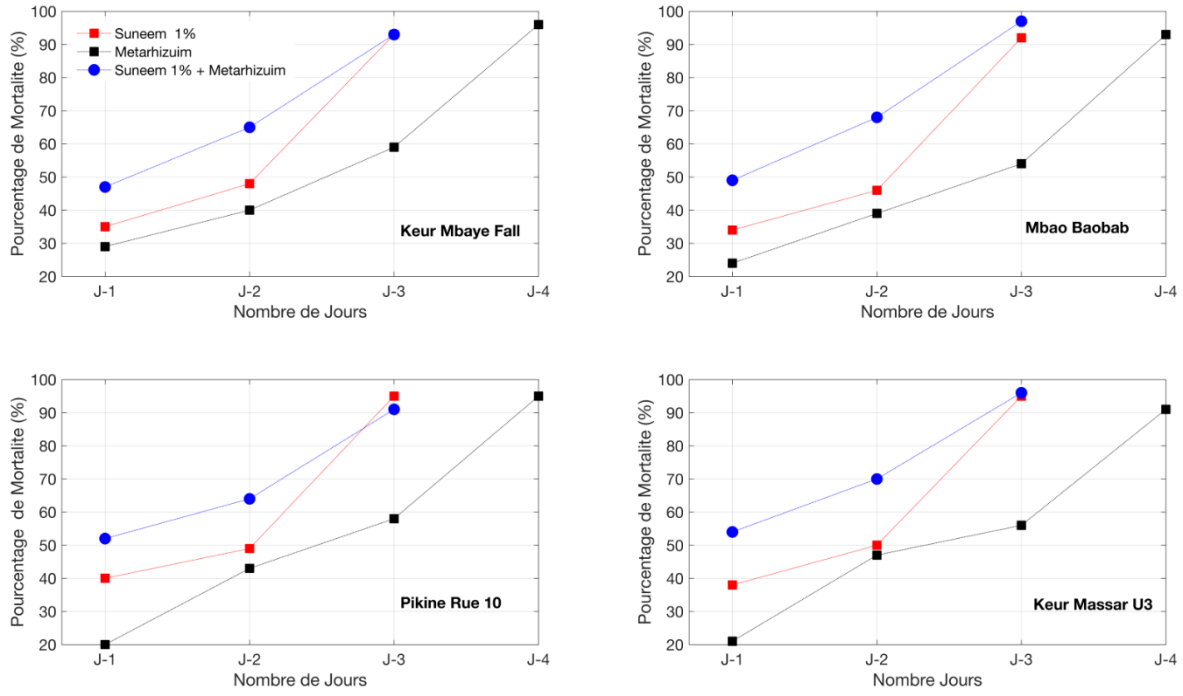
The results of treatment with *Metarhizium anisopliae* are presented in the form of Percentages of *Culex* larvae mortality recorded in Table 2

These results show a clear increase in the number of *Culex quinquefasciatus* larvae that died after treatment with *metarhizium anisopliae*. There are mortalities that reach and even exceed 50% in most of the deposits except some ("keurMbayeFall", "Mbao Baobab", "KeurMassar Marché" and "Sante Yallah 2") where they revolve around values 47% to 49%. %. The CL 50 was obtained very early after 48 hours of treatment under natural conditions. CL 90 was obtained after 96 hours of treatment. Evidence that in the wild, the mixture (Suneem1% and *metarhizium anisopliae*) seems to be the most effective and therefore the best in the fight against these *Culex quinquefasciatus* larvae. . Percentage mortality of *Culex* larvae treated with Suneem 1% The results of treatment with Suneem 1% are presented in the form of Percentages of mortality of *Culex* larvae recorded in Table 3.

Table 3: Percentages of mortality of *culex* larvae treated with Suneem 1%

days	Sites													
	KMF	MB	PKR10	KMU3	SY2	KMM	KML	PGR	DTK	TDS	YS	KF2S	KF2N	G
J1	35	34	40	38	36	33	34	45	42	40	35	36	39	37
J2	48	46	49	50	47	51	50	53	52	49	48	46	54	55
J3	93	92	95	95	93	96	93	92	95	97	95	94	93	97

These results show a clear increase in the number of *Culex quinquefasciatus* larvae that died after treatment with Suneem1%. There are mortalities that reach and even exceed 50% in most of the deposits except some ("keurMbayeFall", "Mbao Baobab", "KeurMassar Marché" and "Sante Yallah 2") where they revolve around values 47% to 49%. %. CL 50 was obtained very early after 24 hours of treatment under natural conditions. CL 90 was obtained after 72 hours of treatment. Evidence that in the wild, the mixture (Suneem1% and *Metarhizium anisopliae)* seems to be the most effective and therefore the best in the fight against these *Culex quinquefasciatus* larvae. II.2. comparative mortality of *Culex* larvae for the three biopesticides



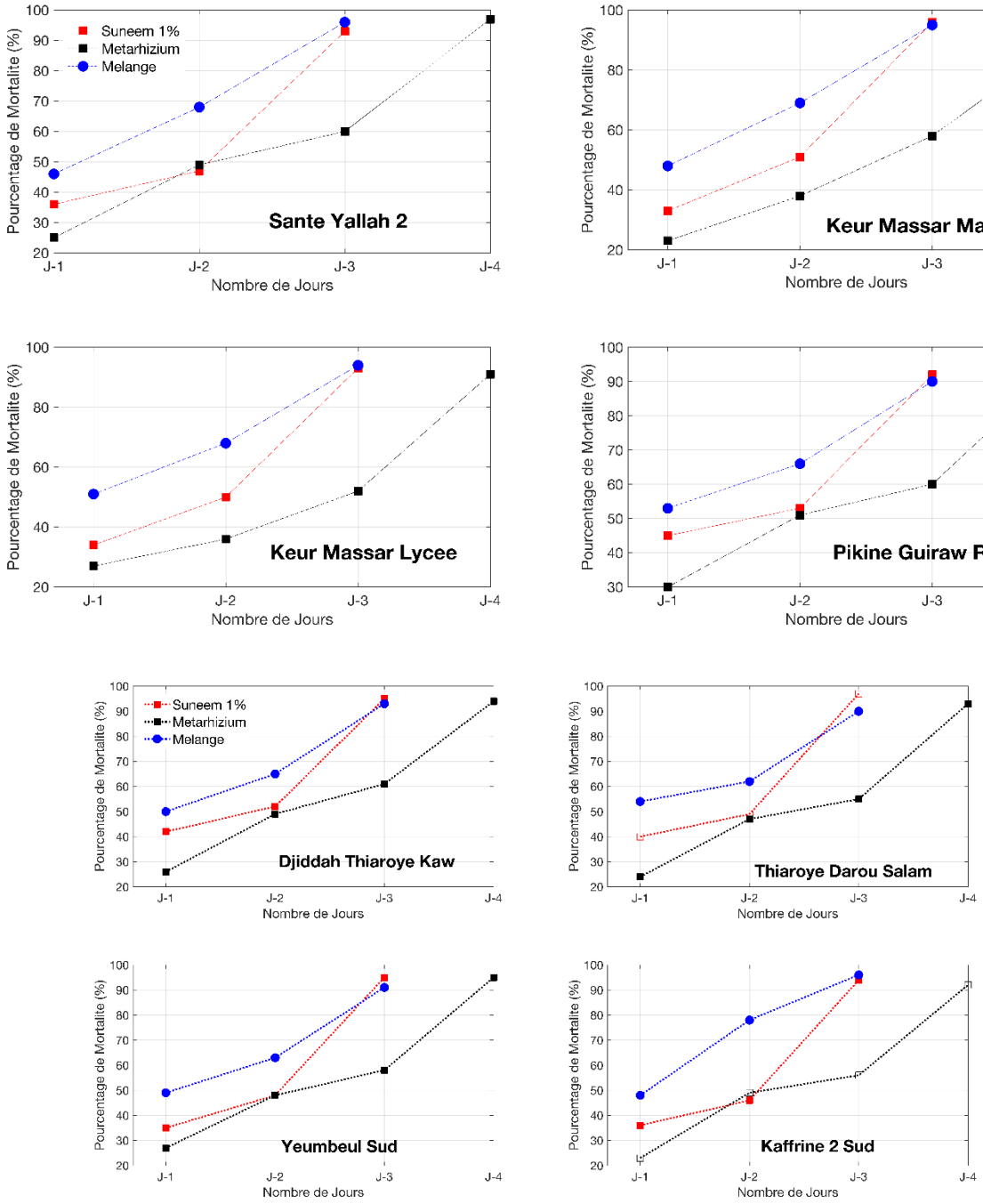


Figure 8: Comparative figures of Culex larval mortalities for the three biopesticides

These graphs illustrate cumulatively three marked parameters that are:

Percent mortality of *Culex quinquefasciatus* larvae;

- The toxicity of the three biopesticides;
-Their effectiveness.

These parameters are analyzed in a comparative way. Thus, Figure 8 shows, as a function of time and natural conditions, that the number of dead larvae of *Culex quinquefasciatus* is more significant for the mixture, followed by Suneem 1% and *Metarhizium anisopliae* and this in all the treatment deposits.

5. Discussion

In this study, the control of larvae of *Culex quinquefasciatus* with respectively 1%, *Metarhizium anisopliae* and the mixture (Suneem 1%, *Metarhizium anisopliae*) gave respectively a percentage of larval mortality of 50% after 24 hours and 90% after 72 hours of treatment with larvae. Suneem 1% of 50 ml.The results concerning the application of Suneem 1% against the larvae of *Culex quinquefasciatus* confirm the work of Attri and Prasad (1980) who showed the effectiveness of neem oil on larvae of CulicidaeDL 50 and LD 90 and in terms of mortality and those of Scott and Kaushik (2000) on the efficacy of Margosan-O® on the *Culex* of *quinquefasciatus*, those of Sèye et al. (2004) with neem oil formulated (LD50 at 3 mg / l in 48h) and those of

Ndioneet al. (2007) .These same mortality rates of 50% after 24h and 90% after 96h of treatment with quantities of *Metarhizium anisopliae* spores of 12.5 mg were registered.

These findings confirmed the work of Rae (2004) in Australia on the treatment of larvae of Chortoicetes terminifera (Australian locusts) with Green Guard®, formulated from a strain of *Metarhizuim anisopliae*. Lepage et al (1992) used several strains of entomopathogenic fungi (*Metarhizium anisopliae*, Cordycepsmilitaris and Tolypocladium cylindrosporum) to control some biting Diptera, They concluded that of all the strains tested, *Metarhizium anisopliais* the most interesting for the biological control of larvae of biting dipterans. St. Louis et al. (2001) confirmed that *Metarhizium anisopliae* is a promoter fungus for biocontrol by sonefficiency on all stages of insect development and could be an excellent candidate as an alternative measure to synthetic pesticides under biological control or integrated control. These results are similar to those obtained by Alves et al. (2002) who experienced early mortality of *Culex quinquefasciatlas* larvae by *Metarhizium anisopliae* from day 1 of treatment. The works of Ravallecet al. (2003) demonstrated that *Metarhizium anisoplia* had a distinct effect on Aedesalbopictus larvae. These results concerning the applications of an entomopathogenic fungus such as *Metarhizium anisopliae* on insect larvae confirm the work of Touré (2006) who showed that the spores of *Metarhizium anisopliae* resulted in the mortality of the Senegalese locust with a TL4 of 4 days and TL100. of 7 days for a dose of 4.3.107spores / ml. In the same way, the 50% and 90% mortality rates were recorded with the larvae treated with the mixture (a mixture) of 40ml concentration of azardirachtin (Suneem1%) added to 10mg of *Metarhizuim anisopliae* spores (40ml of azardirachtin + 10mg spores of *Metarhizuim anisopliae*). These results confirm the work of Seye Fawrou (2012).

Our study fits in perfectly with these various published articles. In fact, the results of our study have allowed us to note a considerable decrease of around 50%, 70% and 95% respectively for the treatments with *Metarhizium anisopliae*, Suneem1% and the mixture of both. In terms of efficiency the mixture appears more effective followed by Suneem1% and *Metarhizium anisopliae*; this could be explained by a summation of the active principles of these two biopesticides. These results confirm and even complete 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 *Culex quinquefasciatus* larvae, has several advantages, notably resulting in a reduction of mosquito larvae, on the one hand, and related diseases, and on the other, preserving an already fragile ecosystem. Indeed, our natural treatment sites have shown 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 during the night are considered favorable in the fight against 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 determining in the results. The larvae stay in the treated area for at least 48 h, provides better treatment efficacy. Mortality and sporulation rate after 6 hours and 24 hours of exposure are lower than those after 48 hours exposure.

6. Conclusion

In sum, it seems relevant to remember that Sunnem 1% and *Metharizium anisopliae* are very effective against larvae of *Culex quinqueafasciatus* in particular and against mosquito larvae in general. But this effectiveness appears much more important when these two biopesticides are mixed. Moreover, in perspective it opens to the scientific community a use of entomopathogens (bacteria or mushrooms), of deregulators of growth which could be effective in a biological fight against mosquitoes.

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