**ORIGINAL ARTICLE**

# Use of industrial waste microorganisms in biological vector control

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**ABSTRACT**

**Background and Objectives:** the use of microorganisms as biological control of disease vectors can be considered a less environmentally aggressive practice when compared to the chemicals used for this end. The present study evaluated the efficiency of fungal and bacterial cell suspensions, isolated from textile industrial waste, in the control of natural vectors *Aedes aegypti* and *Dermacentor nitens*, as a sustainable biological alternative for chemical control. **Methods:** seven fungal and six bacterial strains were evaluated. The isolates were grown in culture medium, nutrient broth and potato broth for bacteria and fungi, respectively. Aliquots of 2 mL of each microbial suspension were added directly to mosquito larvae and adult ticks. Changes in movement and paralysis of vectors at different exposure times, between zero and 20 minutes, and three and 24 hours were analyzed. **Results:** two bacteria and one fungus caused a slowdown in movement and/or increased ectoparasite movement after administration. Two bacterial isolates paralyzed the movements of one *Aedes aegypti* mosquito larva in its first stage of development, while one fungus caused increased larval movement at its most advanced stage of development. **Conclusion:** the microorganisms showed potential use as control of disease vectors. Subsequent activity tests of the possible secondary metabolites produced and the forms of administration of the microbial cultures will be performed. The results encourage further studies of optimization and characterization of cell extracts, which can be used as a sustainable tool in biological control.

**Keywords**: Biocontrol; Industrial Waste; *Aedes aegypti;* Tick; *Aedes aegypti.*

## INTRODUCTION

Control of vector-borne diseases presents a problem for humanity, being one of the main causes of morbidities in the population. Several factors can interfere with individuals' exposure to

these vectors, such as geographic, social and cultural distribution, educational levels of the population, and even the climatic characteristics of the region.[1] Mosquitoes of the genus *Aedes* (mainly *Aedes aegypti* and *Aedes albopictus*) and ticks of *Amblyomma cajennense, Amblyomma aurealatum, Amblyomma dubitatum,* and *Dermacentor nitens* species are among the vectors of greatest economic importance in Brazil.

*Aedes aegypti* is one of the species of greatest epidemiological importance in the transmission of severe arboviruses such as Dengue, Zika, Chikungunya and Yellow Fever in urban environments.[2] The arboviruses transmitted by *Aedes aegypti* have not only reached the Brazilian population, but have also shown gradual growth over the years. According to the epidemiological bulletin of the Ministry of Health, between 2018 and 2019, 596,381 cases of Dengue were confirmed, out of which 366 resulted in deaths, and there were 38,022 cases of Chikungunya which resulted in 15 deaths. Furthermore, there were 1,127,244 probable cases of dengue in Brazil, and in comparison to the same period in 2018, 170,628 probable cases were registered, an increase of 85% from 2018 to 2019. In the State of Paraná, 25 cases of Dengue (2 deaths) were confirmed in 2018, while in 2019, 351 cases were confirmed (21 deaths), showing a significant increase of 92.9% in cases of the disease in the period of one year.[3]

Tick species can transmit the same number or even more pathogens than any other group of arthropods, which feed on the blood of humans and animals.[4] Species of *Dermacentor nitens* (tropical horse tick) mainly affect horses and may occasionally parasitize other animals such as cattle and sheep.[5] These vectors carry the *Babesia caballi* bacteria, the causative agent of equine babesiosis. This disease causes, in horses, fever, anemia, jaundice, hepatomegaly and weight loss, leading to a decrease in animal performance, thus causing economic damage to breeders.[6,7]

Regarding public health, human infestations by species of *Dermacentor nitens* are uncommon, however, the presence of pathogenic bacteria such as *Rickettsia rickettsii* (cause of spotted fever) and *Borrelia burgdorferi* (cause of Lyme disease in humans) has already been reported in this tick species, with *B. burgdorferi* being found in species of *Dermacentor nitens* in the State of Paraná.[8]

The methodologies used in the control of mosquitoes that transmit these diseases of health and economic importance are generally chemical, through insecticides and larvicides, but they can also be mediated by mechanical control, where the main activities involve the protection, destruction or proper destination of breeding sites; reservoir drainage; as well as installation of screens on doors and windows. As a developing country with a predominantly tropical climate, the multiplication and propagation of insects in Brazil is favored by its conditions, and the

common methods of vector control show low sustainability, especially in the long run.[9] As well as in mosquito control, the most used method in tick combat for species that affect horses is the use of chemicals. However, due to the particularities of each tick species, attention must be paid to the different control methods and treatment periods.[7]

Biological control methods use predators or pathogens with the potential to reduce the vector population.[10] Among the available pathogen alternatives, bacteria, fungi or parasites that may release toxins are used, as in the case of *Bacillus thuringiensis*, which has a larvicidal action.[9] This bacterial isolate, although achieving effective action in reducing the number of immature *Aedes* in treated containers in the short term, lacks effectively proven action in reducing dengue morbidity in the long term.[9,11] Studies in the literature have shown great potential in vector control using microorganisms. The efficacy of bacteria of the species *Xenorhabdus indica, X. stockiae* and *Photorhabdus hainanensis* in the control of *Aedes aegypti* mosquitoes was proven through the death of larvae due to exposure to bacteria.[12] In the same context, researchers evaluated the effect of the fungus *Metarhizium anisopliae* on larvae of the mosquito *Aedes aegypti* in its second larval stage (L2), with mortality ranging from 10 to 100% .[13]

In the biological control used against ticks, strains of entomopathogenic fungi can be evaluated for their ability to interrupt the life cycle of various insects and arachnids. The pathogenicity of *Beauveria bassiana* fungi against mites of the species *Tetranychus urticae* was shown by fourteen authors.[15] In a similar way, positive results of fungi of the species *Beauveria bassiana* and *Metarhizium anisopliae* have already been demonstrated in the control of ticks of the species *Dermacentor nitens*.[14]

In this sense, the search for new compounds to use as biocontrol is necessary. Liquid waste from textile industry treatment plants can be considered a promising environment in the search for these compounds, since it has unique characteristics, such as a large amount of suspended solids, high demand for oxygen and organic compounds, generating an environment of high temperature that is conducive to the proliferation of a large microbial load, adapted to these extreme conditions.[16,17] Thus, the present study aimed to evaluate the biotechnological potential of cell suspensions of fungi and bacteria isolated from industrial textile waste, regarding their ability to be used as sustainable biological tools in the control of vectors of health importance.

## METHODS

Ticks of the species *Dermacentor nitens* were kindly provided by veterinarian Cléber Cardeal (UDC) from private property horses. Eggs from the laying of *Aedes aegypti* were supplied by

the Zoonosis Control Center (CCZ) of Foz do Iguaçu, collected in strategic *ovitrap*[18] arranged by the municipality, which are produced with Eucatex wooden reeds to which the eggs are attached.

Microorganisms preserved in 20% glycerol at -20ºC, previously isolated from textile industry residue, were reactivated in Petri dishes with the culture media: a) bacteria reactivation: adapted agar nutrient (NA), composed of 2 g $L^{-1}$ of meat peptone, 3 g $L^{-1}$ of peptone and 15 g $L^{-1}$ of bacteriological agar; b) reactivation of fungi: potato dextrose agar (BDA) composed of 5 g $L^{-1}$ of glucose, 200 g of previously boiled cut potatoes and 15 g $L^{-1}$ of agar. The same broth culture media were used for microbial growth in 50 mL flasks to perform activity tests against vectors. The plates were incubated in a bacteriological oven at 37ºC (bacteria for 2 days) and at 28ºC (fungi for 7 days). The liquid media were incubated at the same temperatures, at 150 rpm in a shaker.

In order to assess the effects of microbial cultures on larvae in the first stage of development (L1), egg hatching was carried out in a monitored manner to synchronize the tests. For this, Eucatex straws containing the mosquito eggs were placed in 500 mL bottles containing water, so that the straw was upright and the eggs were completely immersed in water. After 3 to 4 hours, the eggs hatched and it was possible to visualize, by naked eye, small larvae moving in the water and accumulating in the bottom of the bottles (Figure 1). After the larvae were released, animal feed bran was added to the same bottles, in order to provide nutrition to the larvae during the test periods, eliminating the death bias due to lack of food. In order to avoid evasion of mosquitoes that could occasionally evolve from larvae to adult stages, a mosquito containment system composed of a plastic box with a screen on the lid was developed.

**Figure 1.** Hatching of *Aedes aegypti* eggs by immersing an Eucatex reed containing laying in water. The larvae accumulate in the bottom of the beaker

The experiments of cell culture exposure to vectors were performed according to Vitta *et al*., 2018[12], with modifications. The vectors were transferred to glass Petri dishes separately, with four *Dermacentor nitens* ticks (Figure 2) and five *Aedes aegypti* larvae in the first stage (L1) (about 1 mL of the water where the eggs hatched was added the Petri dish). Two mL of each microbial suspension was added over the vectors. Changes in the movement of vectors under the following exposure times were analyzed: zero minutes, 20 minutes, 3 hours, and 24 hours. An additional test was carried out, exposing *Aedes aegypti* larvae at a more advanced stage (L4). Petri dishes containing the same number of vectors and distilled water were used as controls. The experiments were carried out in duplicates.

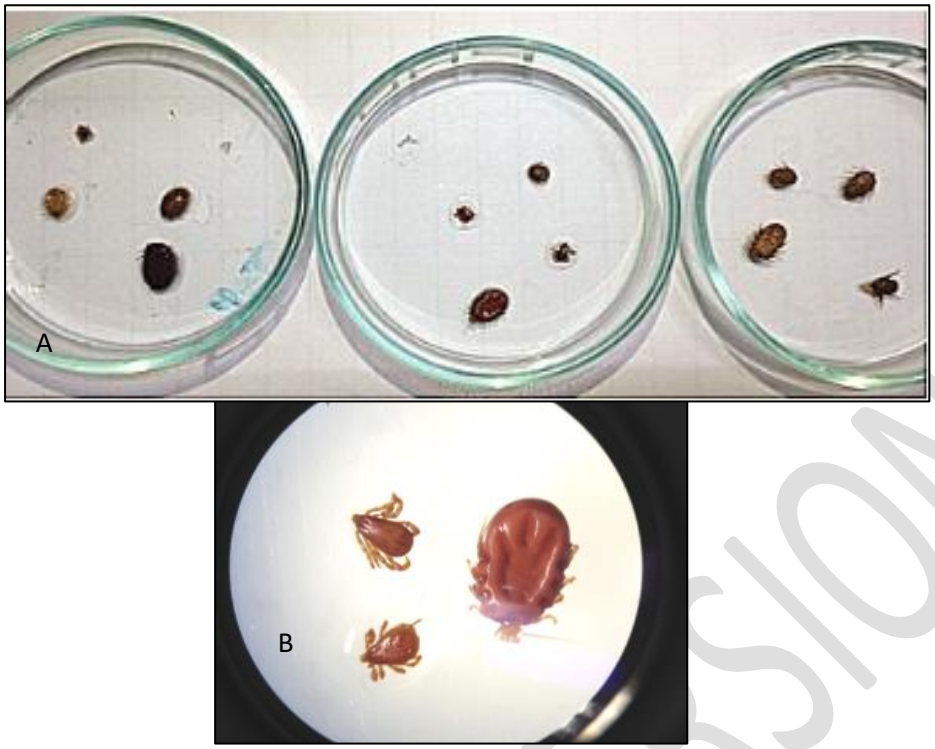


**Figure 2.** A) ticks in Petri dishes before adding the microbial suspension; B) ticks chosen for testing. Stereomicroscope visualized images

## RESULTS

The results found after evaluating the biocidal activity using microbial isolates against ticks of the species *Dermacentor nitens* are shown in Table 1.

**Table 1 -** Results of *Dermacentor nitens* tick exposure tests to microbial cultures

| Microbial culture | 0 minutes | 20 minutes | 3 hours | 24 houres |
|---|---|---|---|---|
| **Bacteria** | | | | |
| ITB 04 | I | I | VIII | VIII |
| ITB 05 | II | V | VIII | VIII |
| ITB 01 | IV | VI | VIII | VIII |
| ITB 12 | VIII | VIII | VIII | VIII |
| ITB 16 | VIII | VIII | VIII | VIII |
| **Fungi** | | | | |
| ITF 17 | VIII | VIII | VIII | VIII |
| ITF 04 | III | VII | VIII | VIII |
| ITF 02 | III | VI | VIII | VIII |

| | | | | |
|---|---|---|---|---|
| ITF 03 | III * | V | VIII | VIII |

[I] Without changes. Ticks continued with the same movement pattern when compared to control;
[II] Deceleration of tick movements when administering the culture;
[III] Brief increase in tick movement when in contact with the culture;
[IV] Notable increase in the movement of ticks when in contact with the culture;
[V] Ticks showed less movement than in the previous time and less than the control;
[VI] Marked decrease in movements. Ticks practically became immobile when compared to the previous time, and to control;
[VII] The ticks remained with the same intensity of movement as the previous time, being similar to the control;
[VIII] All ticks remained immobile;
* the cultured fungus cells visibly adhered to tick feet.

The tick control experiments against the tested microbial suspensions showed positive results (with the exception of isolates ITB 12, ITB 16 and ITF 17), as the ticks remained immobile throughout the experiment. The best results observed were the cell extracts of two bacteria (ITB 01 and ITB 05) because shortly after the application of the microbial suspension, there was an abrupt change in the movement of the ticks. The ITB 01 extract promoted a notable increase in movement and the extract ITB 05 promoted a slowdown in the movements of ticks. Thereafter, there was complete paralysis of the movements of all ticks. The extracts ITF 02 (*Penicillium* sp.), ITF 03 and ITF 04 also showed relevant results, with a brief increase in movement soon after the application of the suspension. On the other hand, throughout observation of the ITF 03 suspension, it was noted that cells of the fungus adhered to the feet of the ticks. For the other isolates, the results were considered negative. The ticks submitted to the control tests with distilled water did not show any change in movement until the time "20 minutes", and after the time "3 hours" there was a high decrease in movements, progressively decreasing until the time of 24 hours. After 24 hours, the majority (90%) of ticks stopped moving, and those that survived showed a drastic reduction in movement.

The results of the exposure tests for *Aedes aegypti* larvae in the first stage of development (L1) and late stage (L4) against microbial cultures are shown in Table 2. The decrease or increase in larval movement was compared to the control plate containing distilled water, which was analyzed as a comparison for each time.

**Table 2 -** Results of exposure tests of *Aedes aegypti* larvae to microbial cultures

| ***Aedes*** | ***aegypti* (early stage of development) – L1** | | | |
|---|---|---|---|---|
| **Microbial culture** | **0 minutes** | **20 minutes** | **3 hours** | **24 hours** |
| **Bacteria** | | | | |
| ITB 12 | I | II | III | IV |
| ITB 12 | I | III | III | III |
| ITB 15 | I | V | VI | VI |
| ITB 15 | I | II | III | V |
| ITB 16 | I | VII | VII | II |

| | | | | |
|---|---|---|---|---|
| ITB 16 | I | III | III | II |
| **Fungi** | | | | |
| ITF 17 | VIII | IX | III | IX |
| ITF 17 | I | IX | III | II |
| ***Aedes aegypti* (3 days since egg hatching) – L4** | | | | |
| **Microbial culture** | **0 minutes** | **20 minutes** | **3 hours** | **24 hours** |
| **Bacteria** | | | | |
| ITB 01 | I | II | III | III |
| ITB 01 | I | II | III | III |
| **Fungi** | | | | |
| ITF 02 | I | II | III | III |
| ITF 02 | I | II | III | III |
| ITF 03 | I | II | III | III |
| ITF 03 | I | II | III | III |
| ITF 34 | VIII | X | III | II |
| ITF 34 | VIII | X | III | II |

[I] No changes. The larvae continued to move similarly to the control;
[II] The larvae showed decreased movement, being slightly less mobile than in the previous time and in the control;
[III] The larvae maintained the movement intensity of the previous time;
[IV] 01 larva presented paralysis of movements. The others maintained the same movement intensity as the previous time;
[V] 01 larva became practically immobile. The others maintained the same movement presented in the previous time;
[VI] 01 larva presented movement paralysis. The others showed a brief decrease in movement, being less mobile than in the previous time and slightly less than the control;
[VII] 02 larvae significantly decreased their movements compared to the control. The others maintained the same movement presented in the previous time;
[VIII] The larvae showed increased movement intensity, compared to the control;
[IX] The larvae decreased the intensity of movement when compared to the previous time, being similar to the control;
[X] The larvae maintained the movement intensity of the previous time, being greater than the control at this time.

The larvicidal activity experiments evaluated against the tested microbial suspensions were positive, considering that all evaluated isolates presented activities against the larvae of *Aedes aegypti*. The best results were observed with the suspensions of isolates ITB 12 and ITF 15, which caused paralysis of movement of at least one larva (20%) compared to the control (water), after 20 minutes of testing. The suspension of the ITB 16 isolate significantly reduced the movements of two larvae (40%) after 20 minutes and 3 hours of evaluation. The suspension of the ITF 17 isolate, on the other hand, increased the movement speed of the larvae, observed immediately after the administration of the microbial suspension.

Microbial suspensions were tested against *Aedes aegypti* larvae in a more advanced stage (after 3 days since egg hatching), possibly in L4 stage of larval development, with more developed morphological characteristics. At this stage, structures of the head, chest and abdomen are more visible and it is possible to notice the siphon responsible for breathing on the water surface.[20]

Results showed an increase in the movement intensity of the larvae, right after addition of the microbial suspension of the ITF isolate 34 (Table 2).

## DISCUSSION

Reports in the literature have demonstrated the potential of using microorganisms such as bacteria and fungi as agents that produce compounds that interfere in the life cycle of mosquitoes, including *Aedes aegypti*,[19], as well as microbial use against tick species of epidemiological importance such as the carriers of spotted fever.[20] However, reports with the use of microbial agents isolated from industrial waste with potential use against ticks of economic importance for horse breeding to recreational and/or sports activities, such as those of the species *Dermacentor nitens*, are very rare. Furthermore, the use of microorganisms recovered from samples of industrial textile waste, which may be adapted to relatively stressful conditions, as biological tools for the control of disease vectors (*Aedes aegypti* and *Dermacentor nitens*) is still unprecedented.

The effect of fungal cultures on ticks may have been provided by the adhesion and development of hyphae of fungi in the integument, similarly to the performance of entomopathogenic fungi.[21] In this work, the authors reported that the fungus invaded the host by diffuse colonization, not showing a preference for specific host tissues, with rupture of the intestinal wall and leakage of content to the hemocoel. The host's death was detected between 4 to 5 days of infection. In our study, three bacteria (ITB 01, 04 and 05) and three fungi (ITF 02, 03 and 04), were able to totally paralyze the four ticks after 3 hours of exposure to microbial suspensions, demonstrating the potential of these microorganisms for the control of vectors such as ticks, among them, those of the species *Dermacentor nitens*.

Tests with isolates ITB 12, ITB 16 and ITF 17 were carried out with a batch of ticks that showed reduced movement speed from the start, which possibly influenced the results. Thus, for these microbial isolates, the results were disregarded, since the arachnids were possibly stressed due to the collection and packaging processes before the time of the test.

Laboratory results indicate that bacteria can present pathogenic tools against ticks. However, their mode of action and their value as potential biocontrol agents have yet to be determined.[22] Therefore, our results encourage further studies with the use of environmental bacteria and their metabolites in the control of ticks of epidemiological and economic importance, especially after the promising results achieved with the bacterial strains, which showed tick-control action after 3 hours of contact of the vectors.

The tick-control action may have occurred either through the contact of the compounds produced by the microorganisms and secreted in the medium, or by the direct action of microbial cells in the metabolism of ticks. Thus, new experiments are needed to determine whether the microbial isolates are producers of secondary metabolites with tick-control action or whether the microbial cells themselves produce an effect against these arachnids, and then define what is the best strategy for using these microorganisms as candidate agents for biological control against tick species.

In this work, it was demonstrated that there are sustainable alternatives for the biological control of vectors that are responsible for diseases of great epidemiological impact and that affect thousands of people in Brazil, such as Dengue, Zika, Chikungunya and Yellow Fever. The larvicidal action of microbial suspensions on larvae of the mosquito *Aedes aegypti* was observed by the reduction of movement speed of the vectors when they come in contact with the suspensions, which possibly contain compounds of toxic action for the vectors. This toxicity can be caused mainly by the oral route in immature stages of the larvae, a mechanism also reported in 2018[12], which was termed as oral toxicity by ingestion, in this case performed by the ingestion of microbial cells by larvae of the mosquito *Aedes aegypti* and not by contact of a chemical compound. Another possible action would be the interference with normal chemical reactions of the nervous system, disabling the normal movement of the vector, representing a mechanism of action similar to compounds of the classes of organophosphates, carbamates and pyrethroids.[11] This hypothesis could explain the reason why some tested microbial suspensions caused an increase in the movement of the vector after contact, suggesting that there was a chemical imbalance in its nervous system due to the increase in electrical discharges.[23] Thus, it is possible to infer that the compound contained in the microbial suspension may delay larval development, preventing them from becoming active vectors.

Performed screenings showed the potential of microbial cell extracts for biological control of vectors of epidemiological interest. Additional studies should be carried out in order to define activity temperature, effectiveness in the control of ectoparasites in other stages of the cell cycle of larvae and ticks, and ways of administering microbial extracts that are more efficient in combating the studied vectors. Thus, the microorganisms evaluated in the present study proved to be potential candidates for use, in the near future, as biological tools in the control of vectors. The results found in our work show that microorganisms derived from industrial waste have great potential to be used as agents to combat vectors of health importance, including mosquitoes and ticks, as a sustainable alternative to the use of chemical compounds.

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**Authors' Contributions**

RAF, NLC and JRO contributed to the design, analysis, writing and revision of the article;

CC, SRG, JAR and ASL contributed to the design of the article.

MRZP contributed to the planning and design of the article, analysis, writing and final review of the article.

All authors have approved the final version to be published and are responsible for all aspects of the work, including ensuring its accuracy and integrity.