Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
e-ISSN 1984-6428

| Vol 10 | | No. 5 | | April-June 2018 |

FULL PAPER

Synthesis and Biological Activity of Fe (III) Acetate for Microbial Control at Breeding Sites of *Aedes aegypti* (Diptera: Culicidae)

Jéssica Verger Nardeli[a], Andréa Haruko Arakaki[b], Tiago André Denck Colman[c], Isaías Cabrini[c,d], José Augusto Teixeira[a], Claudio Teodoro de Carvalho[c], Eduardo José de Arruda[c]*

[a]Institute of Chemistry, São Paulo State University - Araraquara, SP, Postal Code: 14800-060
[b]Institute of Chemistry, Federal University of Mato Grosso do Sul, Postal Code: 79074-460 Campo Grande, MS, Brazil.
[c]Department of Chemistry, Faculty of Exact Sciences and Technology - Federal University of Grande Dourados, Dourados-Itahum Highway, 12 km - P.O.B.: 533, Postal Code: 79804-970, Dourados, MS, Brazil.
[d]Department of Animal Biology, Institute of Biology, State University of Campinas, P.O.B. 6109, Monteiro Lobato Street, University City, Postal Code: 13083-862 Campinas, SP, Brazil.

Article history: Received: 08 February 2018; revised: 15 June 2018; accepted: 30 June 2018. Available online: 30 June 2018. DOI: http://dx.doi.org/10.17807/orbital.v10i5.1135

Abstract:

Unconventional approaches can be used control populations of disease-carrying insect to improve health, economic, social, and environmental standards. There is a need for prolonged reduction of vector populations, using low-toxicity and low-cost products in domestic settings. Metals such copper and iron have shown to be toxic to insect larvae, with a delay in reproduction, damage to the cells of the digestive system and the production of free radicals and oxidant species, resulting in tissue damage and death. To control insect breeding, we synthesized and characterized Fe (III) acetate via carbonate. Fe (III) acetate showed no toxic effect on mosquito larvae up to 1.000 mg L^{-1}, but was bactericidal for Gram-positive and Gram-negative bacteria, mainly due to the action of Fe (III) acetate.

Keywords: acetate; bactericidal; metallo-organic pesticides; mosquitoes; zika

1. Introduction

Mosquito-borne epidemics in Brazil are associated with several factors, including poor basic sanitation, droughts, mosquito adaptation to particular local conditions, lack of health education, and poor planning of mosquito control measures. Such preventive measures need to be undertaken throughout the year [1-3].

The mosquito *Ae. aegypti* has peridomiciliary habits with predominantly diurnal activity, especially in the twilight hours [4,5]. These insects are vectors for disease, often in epidemic form. The use of organophosphates and pyrethroids have become primary strategies for mosquito control and containment [1,2,4]. Insecticide resistance, in addition to increased availability of breeding sites, results in greater abundance of mosquitoes and consequent increased likelihood of epidemics of dengue, chikungunya, yellow fever and Zika [6]. The most rational way to control mosquitoes and to reduce the incidence of disease is to both slow the development of insecticide resistance, and to develop strategies to disrupt insect breeding.

There is a new class of multifunctional insecticides with several modes of activity, including digestive toxicity, effects on enzyme systems, central nervous system effects, and/or peripheral nervous system effects [7-9]. For example, an insecticide containing Cu (II) and/or Fe (III), a micronutrient present in soil, plants,

*Corresponding author. E-mail: ejarruda@gmail.com

microorganisms, and animals, has been shown to be toxic to *Ae. aegypti* [10].

Iron is a trace element that is essential for the maintenance of life, but that is present in soil in only low amounts. In plants, iron is involved in various metabolic activities and is a cofactor for various enzymes (catalase, peroxidase, cytochrome oxidase and xanthine oxidase), in addition to being essential to respiration, photosynthesis, and N_2 fixing [11,12].

In the mosquito gut, the peritrophic matrix (PM) consists of acellular compounds that surround food boluses. There are two types of peritrophic matrix: PM I, produced by the cells lining the midgut, and PM II, produced by cells of the cardia. Cardia cells form a group of specialized cells between the foregut and intestine. Adult mosquitoes produce PM I, while larvae produce PM II for defensive purposes and selection of nutrients [13].

The PM in mosquito larvae is responsible for separating ingested content from the intestinal epithelium. Marten et al. [14] estimated permeability of the caecal membrane that separates intestinal contents from the caecal gut lumen. Digestive enzymes secreted by the midgut epithelium through the PM reach the bolus, and the products of digestion cross the MP and are absorbed by epithelial cells [15].

Biting mosquitos feed on blood containing iron. The insect possesses mechanisms to prevent oxidative stress mediated by iron. It absorbs and/or quickly transports iron for excretion [16]. The majority of ingested iron is excreted within 72 hours in the form of heme [16]. Pascoa et al. [17] showed that, after digestion of hemoglobin, by *Ae. aegypti,* a large amount of heme is produced in the midgut of the mosquito.

Zhou et al. [18], utilizing mass spectrometry (ICP-MS), quantified radioisotopic labeling of iron and blood constituents during the first gonotrophic cycle in *Ae. aegypti*. Excess Cu (II) and Fe (III) disrupted homeostasis and triggered degradation reactions of organic substances (nucleic acids, proteins, and lipids) via free radicals, reactive oxygen species, and lipid peroxidation [19].

Oxidative stress can be exploited as a strategy for population control of insect vectors. The target insect's metabolism can be induced to produce radicals and oxidizing species *in situ*. Oliver and Brooke [20] demonstrated the effects of oxidative stress on the longevity, blood feeding, and insecticide resistance in *Anopheles arabiensis* and *Anopheles funestus*.

Iron is an essential and beneficial metal. Plants have developed strategies to minimize iron deficiency and to increase iron absorption from the soil. In some monocotyledonous and dicotyledonous plants, the reduction of Fe (III) to Fe (II) happens in acidic conditions in the rhizosphere, caused by the extrusion of protons by H^+-ATPases through the plasma membrane. The reduction of Fe (III) to Fe (II) is promoted by iron chelate reductase. After reduction, Fe (II) is transferred by specific membrane transporters to the interior of cells [21]. Iron deficiency is the most prevalent human nutritional deficiency worldwide, especially affecting young children and pregnant women. The deficiency persists over decades, causing negative effects and potentially irreversible developmental delays [22]. In Brazil, a recent systematic review found the median prevalence of anemia was 53%, mainly in children under 2 years of age [23].

Iron is found in macromolecule complexes with proteins, including enzymes, cytochrome, myoglobin and hemoglobin. The best dietary source of iron, because of its greater proportion as heme group, is meat, especially red meat and organ meats (liver, kidney, and heart). During lactation in women, there is a reduction in physiological iron content reaching concentrations of 0.35 mgL^{-1} at about the fifth month. At this stage, about 70% of the iron requirements could be met by complementary foods [24]. Lozoff et al. [25] emphasized that iron deficiency with or without anemia cause serious consequences in the development of the fetus, resulting in hindered motor and neurophysiological development, and affecting cognitive and emotional development of children, adolescents and pregnant women [26--28]. There is controversy regarding the role of iron deficiency in immune responses. Some investigators argue that iron deficiency predisposes individuals to infections, while others suggest that iron protects against microorganisms. The incidence of malaria, for example, is significantly lower in iron-deficient children [29, 30].

Bacteria colonize various organs in mosquitoes, including the midgut, and to a lesser

extent, the salivary glands and reproductive organs [31, 32]. The bacterium *Asaia spp.* was detected in salivary glands and reproductive systems of various mosquitoes, including *Ae. aegypti*, *Anopheles gambiae* and *Anopheles stephensi* [33]. The endosymbiont *Wolbachia* was also detected in the head, muscles, Malpighian tubules, ovaries, and testes of *Culex pipiens* and *Aedes albopictus* [34]. Strikingly, *Wolbachia* was also found in *A. albopictus* hemolymph, which is generally assumed to be bacteria-free [34].

As mosquito-associated bacteria rely on some of the nutrients from the insect meal for growth, the nutrient composition of food sources may directly impact the diversity of bacteria present [32]. Zouache et al. [35] showed that around half of the bacterial diversity in field populations of *Ae. albopictus* was explained by the sex of the mosquito, with greater diversity observed in females. Interestingly, the genera *Pseudomonas*, *Serratia* and *Enterobacter* are frequently found in females of several mosquito species [36-38].

Bacteria selectively attract mosquito females seeking oviposition sites. Among others, *Bacillus cereus* and *Pseudomonas aeruginosa* were noted as being effective attractants. It was concluded that *Ae. aegypti* displays discriminatory behavior in selecting individual bacterial species for oviposition [39]. It was demonstrated that the composition of skin microbiota affects the degree of attractiveness of humans to mosquito species [40]. For example, *Corynebacterium minutissimum* produces volatile compounds including lactic acid and butyl butyrate that attract *An. gambiae* [41]. Moreover, bacteria from breeding sites and water-soluble compounds secreted by those bacteria can stimulate the hatching of *Ae. aegypti* eggs [42] and attractiveness to breeding sites. The microbiological control is an important part of the vector control due to the attractiveness of the females to the breeding sites due to signaling by volatile compounds of the microbiota

This study describes a synthesis of Fe (III) acetate salts and use of the reaction product for analysis of insecticidal properties in *Ae. aegypti* larvae, and bactericidal activity against *Gram-negative* and *Gram-positive* bacteria.

2. Results and Discussion

2.1. Characterization

2.1.1. Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR)

Fe (III) acetate is a coordination compound more commonly known as basic iron acetate (III). With the formula of $Fe(CH_3COO)_3$, it is a salt with low solubility in water, but is soluble in ethanol [43].

The proposed synthetic route allowed us to obtain Fe (III) acetate for evaluation of bactericidal and insecticidal activity. We performed characterization for FTIR for different synthetic routes of Fe (III) acetate.

The Fourier transform infrared (FTIR) spectra of the synthesized and recrystallized Fe (III) acetates are presented in **Figure 1 (a, b, c)**.

Figure 1(a) Fe (III) acetate obtained from carbonate without recrystallization (crude Fe (III) acetate), **Figure 1(b)** Fe (III) acetate obtained via carbonate and recrystallized and **Figure 1(c)** Fe (III) acetate obtained by another synthetic route from the metallic iron powder [44] for comparisons. The assignments of the Fe (III) acetate bands observed in the infrared spectrum are summarized in **Table 1**. The difference between the ν_a (COO^-) and ν_s (COO^-) modes is 108 cm^{-1} [44] and 123 cm^{-1}, which is slightly larger than the ionic value (137 cm^{-1}). Therefore, the formation of a bridging trinuclear iron complex might be suggested from the infrared data [44]. Through comparison between the synthetic routes **(Figure 1)**, **(a)** without recrystallization and **(b)** with recrystallization was observed to decrease in intensity of characteristic -OH bands in the presence of water. The biological tests were carried out with the compound of Fe (III) acetate, the FTIR is shown in **Figure 1a**.

We performed a test for the obtain Fe (III) acetate by mixing with iron oxide and acetic acid. The FTIR analyzes of the product suggest the formation of Fe (III) acetate.

3.1.2. Simultaneous Thermogravimetry and Differential Thermal Analysis (TG-DTA)

TG-DTA curves of the Fe (III) acetate synthesized are shown in **Figure 2**. The thermal decomposition occurs in four consecutives steps between 26-670°C, with total mass loss of

57.35%.

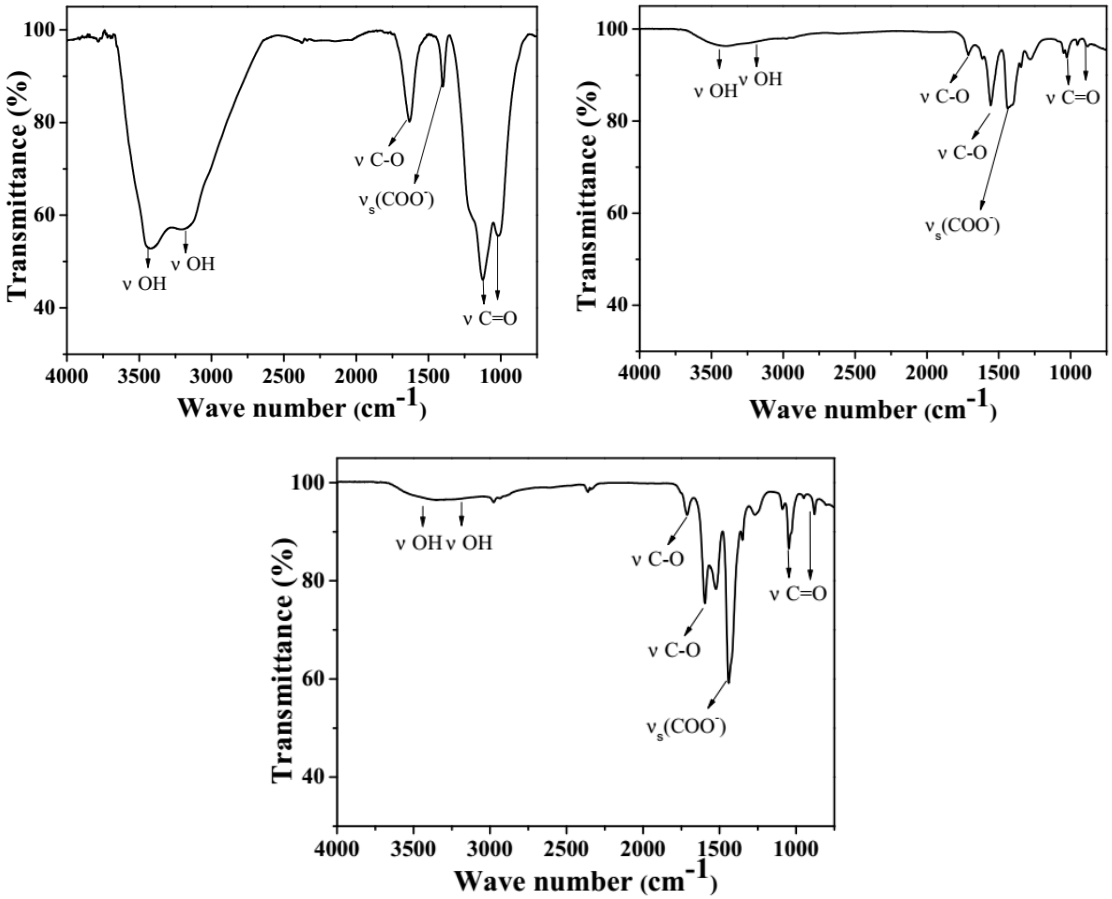

Figure 1. FTIR-ATR spectra for Fe (III) acetate: **(a)** Fe (III) acetate synthesized without recrystallization (crude Fe (III) acetate) **(b)** recrystallized Fe (III) acetate **(c)** recrystallized Fe (III) acetate produced from powder metal Fe^0 [44].

Table 1. Spectroscopic data of Fe (III) acetate and identification of the wavenumbers (cm^{-1}); assignments of the Fe (III) acetate bands observed in the FTIR spectra (cm^{-1}).

Acetate anion	Fe (III) acetate; cm^{-1} [44]	Fe (III) acetate; cm^{-1}	Assignments [45, 46]
-	-	3423	ν (OH)
-	-	3166	ν (OH)
-	-	1626	ν (C=O)
1553	1523	1523	νa (COO^-)
1416	1415	1400	νs (COO^-)
1347	1345	1345	δ (CH_3)
-	1047	1123	ρoop (CH_3)/ν C=O
1020	1025	1027	ρip (CH_3)/ν C=O
928	973	-	ν (C–C)
-	658	-	δ (COO^-)
-	581	-	ρoop (COO^-)
-	-	466	σ (COO^-)

ν= stretching; δ= deformation; ρ= rocking; oop= out-of-plane; ip, in plane; s, symmetric; asym = asymmetric; δ = angular deflection; ν = stretching; sym = symmetric.

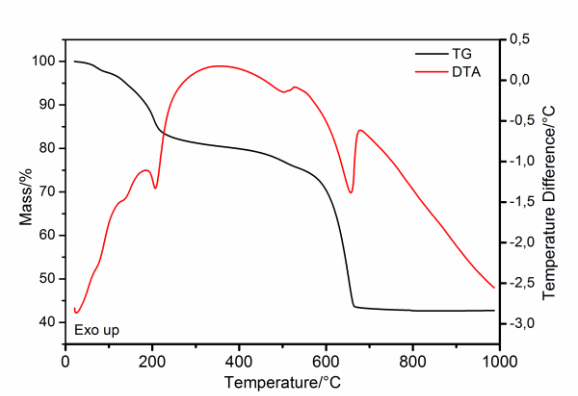

Figure 2. TG-DTA curves of the Fe (III) acetate synthesized.

The first two steps of thermal decomposition were attributed to the dehydration, which is in accordance whit the FTIR-ATR data. The dehydration step occurs between 25 – 250°C, with mass loss 18.01%, associated with two endothermic events in the DTA curve between 120–153°C and the peak at 206°C corresponding to release of the three hydration water molecules of the Fe (III) acetate trihydrated ($\Delta m_{theoretical}$ = 18.83%).

The anhydrous compound is stable up to 420°C and above this temperature, in the third and fourth steps, thermal decomposition occurs between 420-535°C and 535-670°C, respectively, associated with endothermic peaks at 498 and 657°C in the DTA curve. These steps of mass loss were attributed to thermal decomposition of the acetate with release, probably, CO_2 as the main gaseous product. The final residue formed after thermal decomposition of Fe (III) acetate was a mixture of Fe_2O_3 and Fe_3O_4 characterized by their magnetic properties.

In order to support our experimental data, we find a recent study, Souza et al. [47] performed the synthesis of Fe (III) acetate hydrate, and showed that hydrated structure could be $[Fe_3O(CH_3COO)_6 (H_2O)_3]CH_3COO$. The analysis of the material by thermogravimetry (TG) up to 600ºC leaded the compound to the stable final residue of hematite, Fe_2O_3 [45]. The difference observed in the residue obtained by Souza et al. [47] and the residue observed in this study probably should be associated the experimental conditions, which were not the same.

Compounds containing Fe (II) and Fe (III) ions with low solubility can be used as bioactive agents to exert microbicide and/or insecticidal activity to control the breeding of *Ae. aegypti*. Thus, may be interesting to work with this type of material because in Brazilian soils, there are varying amounts of oxides and Fe (II) and Fe (III) hydroxides that may aid microorganisms to control the food chain by determining which species are involved in the attraction of females to breeding sites [11, 12].

Furthermore, a study performed by Ladeira et al. [48] demonstrated the chemical properties and reactivity of a chemical species such as metal oxidation state, degree of protonation of an acid or metal interaction with substances or species present in the aqueous medium can be changed or modified depending on the environmental conditions. The Fe (III) ion behavior in an aqueous medium is very interesting because different chemical equilibria are established from the pKa values: 2.2, 3.5 and 9.6, since the behavior of Fe (III) can be assessed by the equilibrium established between the metal ion and the aqueous medium. Fe (III) at pH close to 2 forms the metal complex $[Fe(H_2O)_6]^{3+}$ with 6 (six) water molecules. In aqueous media, the complex $[Fe(H_2O)_6]^{3+}$ undergoes hydrolysis to form $[Fe(OH) (H_2O)_5]^{2+}$, according to **Equation 4** [51].

$$[Fe(H_2O)_6]^{3+}_{(aq)} + H_2O_{(aq)} \rightarrow [Fe(OH)(H_2O)_5]^{2+}_{(aq)} + H_3O^+_{(aq)} \qquad pKa = 2.2 \qquad (4)$$

The pKa value of the hydrolysis reaction of $[Fe(H_2O)_6]^{3+}_{(aq)}$ in **Equation 5** shows that at pH 2.2, establishing an equilibrium 50% of Fe (III) as $[Fe(OH)(H_2O)_5]^{2+}$, which can undergo sequential deprotonation as shown in **Equations 5**, **6** and **7** by the variation of pH [48].

In summary, for the application of the synthesized product, it is important to understand these equilibria as they allow us to understand the type and activity of Fe (III) compounds in the breeding environment of insect vectors. Fe (II) and Fe (III) ions are present in soil, dust, and waste, where they can accumulate, solubilize and exert toxic activity, thereby helping control

microbes (bacteria, fungi, protozoa and other microorganisms) that are present in the breeding sites of insect vectors [48].

$$[Fe(OH)(H_2O)_5]^{2+}_{(aq)} + H_2O_{(aq)} \longrightarrow [Fe(OH)_2(H_2O)_4]^{+}_{(aq)} + H_3O^{+}_{(aq)} \quad pKa = 3.5 \quad (5)$$

$$[Fe(OH)_2(H_2O)_4]^{+}_{(aq)} \longrightarrow [Fe(OH)_3(H_2O)_2]_{(aq)} + H_3O^{+}_{(aq)} \quad pKa = 6.3 \quad (6)$$

$$[Fe(OH)_3(H_2O)_2]_{(aq)} \longrightarrow [Fe(OH)_4]^{-}_{(aq)} + H_3O^{+}_{(aq)} \quad pKa = 9.6 \quad (7)$$

These considerations must be made because the pH of natural waters, as well as the waters at mosquito breeding sites are in range of 5 to 8. In this situation, the predominant species in the middle are $[Fe(OH)_2(H_2O)_4]^+$ and $[Fe(OH)_3(H_2O)_2]$, with the latter reacting and precipitating as $Fe(OH)_3$. Understanding the chemical speciation is important, because $Fe(OH)_3$ precipitates with humus in the rivers, lakes or breeding sites. Oxidation of organic material on the Fe (III) reduces the metal ion to Fe (II), generating distinct chemical species according to **Equation 8** [48].

$$[Fe(H_2O)_6]^{2+}_{(aq)} + H_2O_{(aq)} \longrightarrow [Fe(OH)\,(H_2O)_5]^{+}_{(aq)} + H_3O^{+}_{(aq)} \quad pKa = 9.4 \quad (8)$$

The pKa of the hydrolysis reaction in **Equation 8** shows that within the pH range of natural waters, but below pH 9.6, the Fe (II) ion is soluble in water of lakes, rivers or breeding sites. In the middle of the water body or near the oxygen-rich surface, it is again oxidized to Fe (III) according to **Equation 9**. Thus, the redox cycle can be restarted with Fe (III) continuously in an oxidizing environment [48]. The bottom of the breeding sites of *Ae. aegypti* can be considered reservoirs of Fe (II) ions and humus, the latter being part of the food of the larvae. The larvae must rise to the surface to breathe, which regions are rich in Fe (III). At the bottom of the water at breeding sites, insect uptake accumulated humus together with Fe (II), which can accumulate in the insect's body and subsequently can be oxidized to Fe (III) by metabolism or surface regions. The Fe (III) ions possess (bio) activity against microorganisms (bacteria, fungi, protozoa and others), and lower the activity to larvae of *Ae. aegypti*.

$$2\,[Fe(H_2O)_6]^{2+}_{(aq)} + \tfrac{1}{2}\,O_{2(g)} + 2\,H^{+}_{(aq)} \longrightarrow 2\,[Fe(H_2O)_6]^{3+}_{(aq)} + H_2O_{(l)} \quad \Delta E^{o} = 0{,}46V \quad (9)$$

Toxicity bioassays, according to the methodology described by Nardeli et al. [9] with 3rd instar larvae (L3) of *Ae. aegypti*, showed no toxicity of the reaction product Fe (III) acetate until 1,000 mgL^{-1} (ppm) concentration. Bactericidal activity was then performed for *Gram-negative* and *Gram-positive* bacteria.

2.2. Applications

2.2.1. Antibiogram

The antibiogram can indicate the resistance of bacteria to antimicrobial agents. This information may contribute to develop a control strategy for *Ae. aegypti*, the imposition of conditions unfavorable to the aquatic insect breeding, especially for intervention in the food chain. The L3 instar requires microorganisms, micronutrients and humus (organic matter decomposition) that are essential for their development and volatile signaling for females during oviposition. However, one can propose the Fe (III) acetate as active bactericidal agents for interference in the food chain of *Ae. aegypti*. The experiments were performed with Fe (III) acetate in concentrations of 10^{-1} mol L^{-1}, 10^{-2} mol L^{-1}, 10^{-3} mol L^{-1} and 10^{-4} mol L^{-1} with reference to the molar mass of Fe (III)

acetate. Due to the balance established in the pH of the solution the different forms resulting from the hydrolysis of the ion Fe (III) acetate will be the insect's layout to be absorbed and exert toxic activity.

The charts in **Figure 3** show the diameter of the inhibition zones (no bacterial growth) obtained in antibiograms performed with Fe (III) acetate, $Fe(CH_3COO)_3$, tested with several *Gram-positive* and *Gram-negative* bacteria, i.e., *S. aureus* ATCC-25923 (+), *Escherichia coli* ATCC-25922 (-), *L. monocytogenes* ATCC-7644 (+) and *S. typhimurium* ATCC-14028 (-).

The experimental results showed that the Fe (III) acetate in successive dilutions and even low concentrations have bactericidal activity.

Despite the acetate remaining active in dilutions of the order of 10x times used, the activity is concentration-dependent. This suggests that the low solubility of the acetate and saturation of the environment at breeding sites can be exploited to maintain the toxic activity of insecticides for long time.

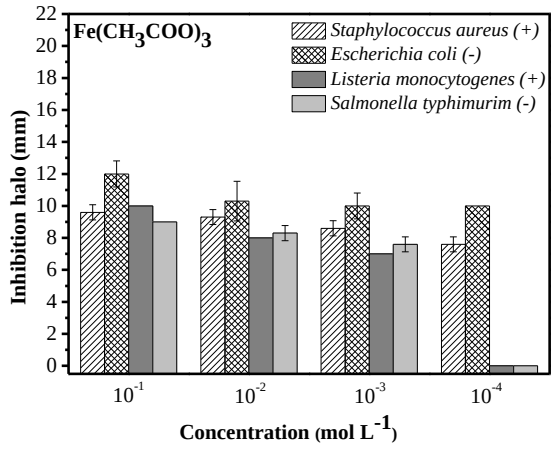

Figure 3. Antibiogram results for *Gram-positive* and *Gram-negative* bacteria with Fe (III) acetate.

2.2.1.1. Statistical analysis

The results demonstrate that the toxic effect of Fe (III) acetate is concentration-dependent, causing high mortality especially of *E. coli*. The statistical analysis for some of the comparisons was not possible because of the similarity of the results between repetitions. ANOVA showed a significant difference ($p < 0.05$), confirmed by Tukey test for (Table **2**).

1) comparison between all species of bacteria at each concentration. It is observed that for these analyzes comparisons was no significant difference in all comparisons, except in 10^{-4}, confirming that Fe (III) acetate acts differently in each species of bacteria;

2) comparing the concentrations for each species of bacteria. In these comparisons, it was observed that in general, *E. coli* and *L. monocytogenes* suffered minor toxic effect from Fe (III) acetate;

3) comparing the *Gram-positive S. aureus* and *L. monocytogenes* at each concentration. In this case, only at 10^{-1} mol L^{-1} concentration was there no significant difference, which shows that the action of Fe (III) acetate is not related to permeability of the cell wall, but to the concentration, as there in no difference in permeabilization Fe (III) acetate between species bacteria.

4) comparing the *Gram-negative E. coli* and *S. typhimurium.* In this case, there was no significant difference between 10^{-2} mol L^{-1} and 10^{-4} mol L^{-1}. As for Gram-positive bacteria, permeability of the Fe (III) acetate is different among species.

Figure 4 illustrates the concentration of commercial antibiotics used as standards. The antibiotic streptomycin exerts activity against *Gram-negative*, and penicillin against *Gram-positive* bacteria. It may be noted that acetates had greater inhibition compared to antibiotics in terms of the inhibition halo.

The size of inhibition halos between Fe (III) acetate and antibiotics are similar, but antibiotics are more toxic due to lower concentrations that were used in this study.

The simplicity of synthesis of the compounds, as well as their recycling and low toxicity properties, allow their domestic use to control the larvae in their breeding habitat, encouraging research in this area to develop alternatives with low toxicity and production cost. Such recyclable products can be used for population control of

mosquito vectors despite their adaptability and competency to survive in extreme conditions [6].

Table 2. Results of the statistical analyzes for Fe (III) toxicity comparisons in bacteria.

Comparation	Concentrations			
1	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	F = 15.000	F = 4.444	F = 12.266	F = 969.00
	p = 0.0016	*p* = 0.0406	*p* = 0.0028	*p* = < 0.0001
2	Species of Bacteria			
	E. coli	*L. monocytogenes*	*S. typhimurium*	*S. aureus*
	-	-	F = 317.8333	F = 7.000
			p = < 0.0001	*p* = 0.0129
3	*Gram-positive* (*S. aureus* and *L. monocytogenes*)			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	F = 1.000	F = 16.0000	F = 25.0000	F = 49.0000
	p = 0.624	*p* = 0.0172	*p* = 0.0088	*p* = 0.0034
4	*Gram-negative* (*E. coli* and *S. typhimurium*)			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
	F = 27.0000	F = 4.5000	F = 12.2500	-
	p = 0.0079	*p* = 0.1007	*p* = 0.0257	

F ratio = value that shows if there is difference between the comparative averages; *p* = lower level of significance with which the null hypothesis would be rejected

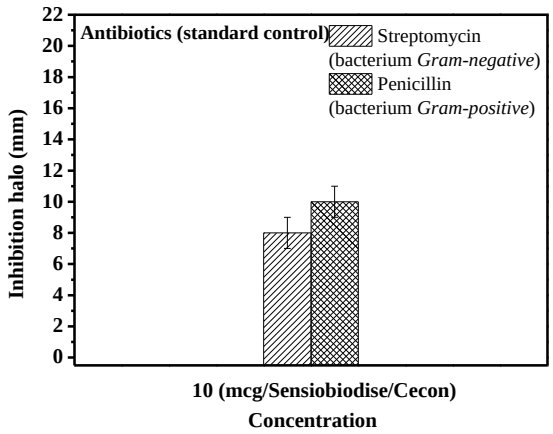

Figure 4. Concentration of antibiotics [44] (standards) against inhibition halo (mm) with standard deviation ± 1.0.

3. Material and Methods

3.1. Synthesis

Fe (III) acetate is product available and has the following characteristics: CAS: 2140-52-5; molecular weight (MW): 232.98 g moL^{-1}; molecular formula: $C_6H_9FeO_6$; purity: 95%; boiling point: 117.10ºC at 760 mmHg. The methodology for the synthesis of Fe (III) [49] for the synthesis of carbonate salts ($Fe_2(CO_3)_3$) using aqueous solutions of their salts was as follows: iron sulfate nonahydrate ($Fe_2(SO_4)_3.9H_2O$) was dripped into a saturated solution of sodium hydrogen carbonate, $NaHCO_3$. The precipitate, ($Fe_2(CO_3)_3$), was filtered with qualitative Whatman 40 filter paper and washed thoroughly with distilled water for removal of the sulfate ion, SO_4^{-2}. The carbonate iron was centrifuged for 30 minutes (Centrifugal Excelsa II FANEM) at 2000 rpm. The supernatant was removed, and precipitate was washed with ice-cold distilled water again to avoid losses due to solubility and ensure the removal of sulfate ions and/or interfering ions. After sedimentation of the material, the supernatant was removed with a Pasteur pipette and a qualitative test was performed for sulfate ion in an aqueous solution of $BaCl_2$.

Fe (III) acetate was synthesized by adding stoichiometric quantities of acetic acid (CH_3COOH) to the respective carbonate. The aqueous suspension was heated slowly up to near ebullition until total neutralization of the carbonate. The resulting solutions was maintained in an ice bath for recrystallization from acetate, and filtered. The reaction system was stirred until complete elimination of carbon dioxide. Subsequently, the mixture was filtered and the solvent was evaporated. 7.21g of dark red crystals were obtained, with yield 70.55%. Crude Fe (III) acetate crystals were purified by recrystallization. 1 g of crude Fe (III) acetate crystals was placed into 250 mL beaker, and 100 mL 96.5% ethanol was added. The mixture was heated to 70ºC and mixed on a magnetic stirrer. Most Fe (III) acetate dissolved. The solution was then filtered through fine paper filter. The resulting clear dark red solution was poured into an

evaporating dish, and air-dried. 0.23 g of dark red crystals were obtained (yield 22%). The product was then packed in a glass bottle and maintained in a vacuum desiccator containing anhydrous silica. Fe (III) acetate ($Fe(CH_3COO)_3$) is insoluble in water, but soluble in ethanol, and is brownish-red.

The chemical reactions to obtain acetate Fe (III) acetate via carbonate are shown in **Equations 1, 2** and **3**.

$$Fe_2(SO_4)_{3(aq)} + 6\ NaHCO_{3(aq)} \rightarrow Fe_2(CO_3)_{3(aq)} + 3\ Na_2SO_{4(aq)} + 3\ H_2O_{(l)} + 3\ CO_{2(g)} \quad (1)$$

$$Fe_2(CO_3)_{3(s)} + 6\ CH_3COOH_{(aq)} \rightarrow 2\ Fe(CH_3COO)_{3(aq)} + 3\ H_2CO_{3(aq)} \quad (2)$$

$$Fe(CH_3COO)_{3(aq)} \rightarrow Fe(CH_3COO)_{3(s)} \quad (3)$$

The compound of crude Fe (III) acetate, hydrated $Fe(CH_3COO)_3$, and recrystallized Fe (III) acetates were characterized by FTIR analysis.

3.2. Characterization

3.2.1. Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR)

Fe (III) acetate was chemically characterized by means of Fourier Transform Infrared Attenuated Total Reflectance (FTIR-ATR) using a Nikolet IS5 spectrophotometer with an Attenuated Total Reflectance attachment (scans = 32, energy scanning from 600 to 4000 cm^{-1}, resolution = 2 cm^{-1}).

2.2.2. Simultaneous Thermogravimetry-Differential Thermal Analysis (TG-DTA)

Simultaneous TG-DTA curves were obtained with a thermal analysis system, model SDT 2960 from TA Instruments. The purge gas was dry air, with a flow rate of 100 mL min^{-1}. A heating rate of 10°C min^{-1} was used with samples weighing about 10 mg. An alumina crucible was used for recording the TG-DTA curves.

3.3. Applications

3.3.1. Antibiogram

The analysis of the biological activity was performed by inhibition zone tests with antibiogram, using Fe (III) acetate in concentrations of 10^{-1} mol L^{-1}, 10^{-2} mol L^{-1}, 10^{-3} mol L^{-1}, and 10^{-4} mol L^{-1} against the following bacteria: *Escherichia coli* ATCC-25922 (*Gram-negative*), *Staphylococcus aureus* ATCC-25923 (*Gram-positive*), *Salmonella typhimurium* ATCC-14028 (*Gram-negative*), and *Listeria monocytogenes* ATCC-7644 (*Gram-positive*). The antibiotics penicillin and streptomycin were used as standards for comparison, using standard *Gram-positive* and *Gram-negative* bacteria. The antibiograms allowed us to assess the potential bactericidal compounds according to their concentrations used. One can thus evaluate the inhibitory action of Fe (III) acetate for bacteria.

Toxicity assessment of Fe (III) acetate was made using methodology described in Nardeli et al. [9]. The antibiograms were prepared by disk diffusion testing of following the methodology of Kirby-Bauer (Drew et al.) [50]. The test was performed by spreading a 100 mL (stage 3) aliquot of the bacterial culture with sterile Drigalski handle in a Petri dish containing medium Mueller-Hinton agar. In the center of the Petri dish was placed a disk of filter paper impregnated with the antibiotic solution. The bacteria were separately cultivated in Mueller-Hinton broth for 24 hours at 37°C in BOD chamber. The inoculation was carried out with the use of inoculum with optical density of 1.0 at 600 nm wavelength.

The Petri dishes were prepared with Mueller-Hinton agar culture medium for subsequent inoculation. The solidified culture medium was inoculated with 100 µL broth and the microorganisms were subsequently placed on autoclaved filter paper disks of 6 mm, treated with various concentrations of 10^{-1} mol L^{-1} to 10^{-4} mol L^{-1} Fe (III) acetate. The impregnated paper discs were placed on the clamp surface of the medium in the center of the Petri dish. The Petri dish was incubated inverted at 28°C for 48 hours, to make

reading the results. The formation of inhibition zone on the surface of the culture medium around the impregnated disc indicates the absence of bacterial growth, revealing the inhibitory action of the antibiotic on the strain of bacteria. Analyzes were performed in quadruplicate for each concentration studied.

Species distribution diagram for Fe (III) acetate can be displayed in the concentration range of 10^{-3} mol L^{-1} to 10^{-5} mol L^{-1} (pH 0-10) for different species that coexist in an aqueous medium and are pH-dependent: $[Fe^{3+}]$, $[Fe(OH)^{2+}]$, $[Fe(OH)_2^{+}]$, $[Fe(OH)_3^{2-}]$, $[Fe(OH)_4^{3-}]$, $[Fe(OH)_5^{2-}]$, $[Fe(OH)_6^{3-}]$ [51].

3.3.1.1. Statistical analysis

Comparative analyzes were performed by ANOVA and Tukey test (significance level α = 0.05), using the BioEstat 5.3 Software. The dimensions of the inhibition zones between species of *Gram-positive/Gram-negative* versus concentration were used for comparisons.

4. Conclusions

Synthesis of hydrated Fe (III) acetate was carried out by way of carbonate, obtaining a mixture of Fe salts with yield approximately 70.6%. Fe (III) acetate had no larvicidal activity up to 1.000 mgL^{-1} (ppm), possibly due to the low solubility of the compounds and metal species in equilibrium. The results of antibiograms showed the potential of the Fe (III) acetate to inhibit the growth of *Gram-positive* and *Gram-negative* bacteria compared with penicillin and streptomycin. The permeability of the Fe (III) acetate through the cellular membrane varies depending on the bacterial species. There was no concentration-dependent specificity for *Gram-positive* or *Gram-negative* bacteria. This suggests that antibiotic activity reduces bacteria concentration, thereby imposing an unfavorable environment for insect reproduction by reduced attraction to breeding sites, and by influencing the survival of *Ae. aegypti* larvae. Fe (III) acetate is possibly composed of acetate and/or hydroxides, that have no larvicidal activity, but that have bactericidal activity. Fe (III) acetate and/or hydroxide ions can act as a reservoir and/or a carrier, triggering Fe (III) to be taken up by cells, causing oxidative stress and thus microorganisms control at mosquito breeding sites. Fe (III) ions are metal species with bactericidal activity, but low insecticidal activity for *Ae. aegypti*. The presence of decaying organic material (humus) and viability of occurrence of redox reactions of Fe (II) to Fe (III) suggest that iron salts are suitable compounds for the development of devices (*eg*, red ceramic), for *Ae. aegypti* control in household and gardening spaces. Nevertheless, the availability of Fe (III) should be improved.

Acknowledgments

CNPq, CAPES/PROCAD, FUNDECT, UNESP/Araraquara, UNICAMP, UFMS, UFGD.

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