Brazilian Journal of Biology
ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

https://doi.org/10.1590/1519-6984.227875
Original Article

The acetylcholinesterase as indicative of intoxication for pesticide in farmers of conventional and organic cultivation

H. H. F. Alves[a], *A. T. Silva*[b], *J. M. S. J. Pavão*[a], *T. J. Matos-Rocha*[a,c], *M. A. Souza*[a], *J. G. Costa*[a], *S. A. Fonseca*[a], *L. L. S. Pires*[a], *J. Faé*[b] *and A. F. Santos*[a,b]*

[a]Centro Universitário Cesmac, Maceió, AL, Brasil
[b]Universidade Estadual de Alagoas – UNEAL, Arapiraca, AL, Brasil
[c]Universidade Estadual de Ciências da Saúde de Alagoas – UNCISAL, Maceió, AL, Brasil
*e-mail: aldenirfeitosa@gmail.com

Received: August 25, 2019 – Accepted: February 9, 2020 – Distributed: August 31, 2021

Abstract

In this sense the objective of assessing the levels of pesticide poisoning in rural farmers of San Sebastian and take AL, using acetylcholinesterase enzymes Erythrocyte and plasma as biological indicator of intoxication. This is a prospective, transversal and descriptive variables analyzed were: year whose, sex, age group, education, location, condition, route of exposure. The data were acquired by collecting blood samples and socio demographic information of farmers. Was put as the determining factor the type of conventional and organic farming, and periods of drought and rainy. 56 volunteers were analyzed. The analyses were performed in the automatic biochemical Analyzer Cobas Integra 400 plus®. According to the results of the analyses, it was the largest number of individuals with reduced values of cholinesterase, specifically the Group of conventional farming, the period of greatest change index was in the rainy season, where the activity of AChE, expressive values presented in the city of São Sebastião, with 80% result of intoxicated, in the city of the foot – take, 21.73% over the same period. On analysis of the AChP, São Sebastião has obtained the highest number of contaminated with 18.75% and 30% respectively, in the District Take Foot stood between 10 and 21.73% of reduced levels of cholinesterase. This sets the organic system of cultivation, as the best alternative for prevention of future diseases, in addition to bringing quality of life for rural workers, as well as for consumers.

Keywords: cholinesterase inhibitors, agrochemicals, diagnosis.

A acetilcolinesterase como indicativo de intoxicação por pesticidas em agricultores de cultivo convencional e orgânico

Resumo

Nesse sentido objetivou-se avaliar os níveis de intoxicação por agrotóxicos em agricultores rurais de São Sebastião e Pé Leve - AL, utilizando as enzimas acetilcolinesterase Eritrocitária e Plasmática, como indicador biológico de intoxicação. Trata - se de um estudo prospectivo, transversal e descritivo cujas variáveis analisadas foram: ano, sexo, faixa etária, escolaridade, local, circunstância, via de exposição. Os dados foram adquiridos através da coleta das amostras de sangue e das informações sócio demográficas dos agricultores. Foi posto como fator determinante o tipo de cultivo convencional e orgânico, e os períodos de estiagem e chuvoso. Foram analisados 56 voluntários. As análises foram realizadas no analisador automático de bioquímica Cobas Integra® 400 plus. De acordo com os resultados das análises, percebeu-se maior número de indivíduos com valores diminuídos da colinesterase, especificamente no grupo de cultivo convencional, o período de maior índice de alteração foi na época chuvosa, onde a atividade da AChE, apresentou valores expressivos na cidade de São Sebastião, com resultado de 80% de intoxicados, na cidade do Pé – Leve, obteve-se 21,73% no mesmo período. Na análise da AChP, São Sebastião obteve maior número de contaminados com 18,75% e 30% respectivamente, já no Distrito Pé Leve ficou entre 10 e 21,73% de níveis diminuídos da colinesterase. Isso define o sistema orgânico de cultivo, como a melhor alternativa para prevenção de futuras patologias, além de trazer qualidade de vida para os trabalhadores rurais, como também para os consumidores.

Palavras-chave: inibidores da colinesterase, agroquímicos, diagnóstico.

1. Introduction

Currently, Brazil is one of the largest food producers in the world and, also, one of the countries that consumes the most agrochemicals in the production methods of various agricultural crops, developing technologies that meet the export demands (Carvalho et al., 2017). Pesticides are chemicals marketed in order to contribute to agricultural production processes, mainly on a large scale, still defined by the Ministry of the Environment, as agents of chemical, physical and Biological (Brasil, 2018).

Exposure to pesticides has set a serious public health problem. Rural workers lack the protection and care of their health and basic information about the risks inherent in the use of pesticides. The hegemonic productive model is immersed in several types of vulnerabilities, such as the institutional characterized by the almost absence of local technical assistance and inefficient surveillance, which ultimately allow the acquisition of pesticides without agronomic prescription and inappropriate use of these products, especially those related to low schooling, which, among other consequences, lead to a lack of understanding of the recommendations prescribed in the leaflets of these products (Preza and Augusto, 2012).

In human health, the effects may be different, depending on the product characteristics and exposure time, and may in the long term cause neurological or immunological disorders, as well as cancer, either by direct exposure to agrotoxicity or consumption of Contaminated food with large quantities of these chemicals (Della Vechia et al., 2016).

According to Carneiro (2015), the analysis of samples collected in all 26 Brazilian states conducted by the program for the analysis of pesticide residues in food (FOR) under Anvisa, reveals that one third of the food consumed daily By Brazilians is contaminated by pesticides.Essa contaminação está intimamente ligada à utilização de agrotóxicos acima da quantidade de suporte do ambiente, de modo a serem quimicamente transformados quando absorvidos pelas plantas e solo, sendo ingeridos pelas pessoas por meio dos alimentos e também transportados para os corpos d'água pelas chuvas (Ismael et al., 2015). Pesticides, in general, are classified as highly harmful to the environment and impact to a greater or lesser extent human health (Piovezan et al., 2013).

Part of the pesticides used has the ability to disperse in the environment, and another part can accumulate in the human organism, including in breast milk. The milk contaminated when consumed by newborns can cause health problems, because they are more vulnerable to exposure to chemical agents present in the environment, due to their physiological characteristics and to feed, almost exclusively with the Breast milk up to six months of age (Carneiro et al., 2012).

Organophosphate and carbamates represent the main class of insecticides involved in poisoning cases. These compounds inactivate plasma and erythrocyte acetylcholinesterase enzymes, causing elevation in acetylcholine levels, leading to an acute cholinergic syndrome, with the emergence of muscarinic, nicotinic and nervous system signs and symptoms Central (CNS), being these manifestations dependent on the dose and the route of exposure involved in the occurrence (Yankovskaya et al., 2018).

It is believed that acute intoxication by acetylcholinesterase inhibiting insecticides is responsible for more deaths than any other class of chemicals. The intoxication resulting from occupational exposures in the workplace, particularly in farmers, is generally less severe (King and Aaron, 2015).

A valuable indicator of the relationship between exposure to pesticides and health problems is the determination of erythrocyte and plasma cholinesterase. The inhibition of these enzymes by organophosphorated compounds leads to the accumulation of acetylcholine, and the organism starts to present a series of undesirable manifestations (Pinto, 2017).

Anticholesterasic Agents act as inhibitors of the enzyme acetylcholinesterase, accumulating acetylcholine in cholinergic receptors, causing the post-synaptic cholinergic transmission does not cease at the appropriate time, thus leading to hyperstimulation (Araújo et al., 2016).

Cholesterases (ChEs) are enzymes hydrolases belonging to the Serinoesterases group, with specificity by choline esters, but are from a family that differs from serine-proteases, presenting more identity with other esterases such as Carboxylesterases, microsomal esterase of rabbits, esterase-6 of Drosophyla and proteins with adhesion properties, among other properties, which probably lost the catalytic function throughout the evolution such as Neuroliguins, Neurotactins, Gliotactins and Thyroglobulin (Pezzementi and Chatonnet, 2010).

Cholesterases constitute a group of enzymes that have in common the property of hydrolysing esters, differentiating each other in relation to the substrate. The human organism has two types of cholesterases: acetylcholinesterase (Specific cholinesterase, true or erythrocyte) and pseudocholineriasis (plasmatic, nonspecific or serum cholestarase) (Shmitz, 2003).

Cholinesterase activity may be increased in situations of hypercholesterolemia, obesity with triglycerides, hyperthyroidism, parkinsonism and when using benzodiazepines, androgens, antibiotics and insulin. It is diminished during pregnancy, in Hypocholesterolemia, in the states of malnutrition, in hepatic cirrhosis, in tuberculosis, rheumatoid arthritis, acute infections and during the use of Phenothionamines (Shmitz, 2003). Other drugs also lead to decreased enzymatic activity: sulfates, fluorides, citrate, fenotiazins, codeine and others (Andrade Filho et al., 2001).

The diagnosis of acetylcholinesterase inhibitor poisoning is typically clinical and is based on anamnesis with history of exposure and physical examination. The simultaneous presence of muscarinic and niconic symptoms strongly suggests exposure to these compounds and, to the suspicion, immediate empirical treatment is necessary (King and Aaron, 2015).

Within this context, the objective of this study is to evaluate the levels of pesticide poisoning in rural farmers

of São Sebastião and Pé Leve-AL, using erythrocyte and plasma acetylcholinesterase enzymes, as indicators Biological intoxication.

2. Material and Methods

The present work is part of the research evaluation of the action of pesticides in humans and the environment: a focus on the APL of Horticulture, approved by the Research Ethics Committee of CESMAC under CAAE: 57804316.5.0000.0039 and Opinion Number: 1.766.735.

2.1. Type of study

This is an experimental analytical study, classified as quantitative regarding the approach. All selected individuals were asked to sign the Informed consent form (ICF). Subsequently, a form was applied to each farmer, with questions aimed at obtaining information on sociodemographic characteristics.

2.2. Data collection sites and biological samples

Samples of blood collection were carried out at the farmers' homes and at the headquarters of the associations of rural farmers in São Sebastião and the District Pe Leve-AL. Together with the sociodemographic information of each volunteer of the research.

2.3. Sample

The sample consisted of farmers of small farms of conventional and organic cultivation. It was understood as a small property that is characterized as family farming.

2.3.1. Size and sampling

It was used for the sample calculation, the number of farmers accredited in the farmers' associations of the municipalities selected by the research. For the sample calculation, the following criteria were adopted: 5% confidence limit and confidence interval of 95%. The hypothetical frequency of the result factor in the population (p) was 50%, a criterion adopted when the real frequency is not known. The calculation was made through the OpenEpi® program available online.

2.4. Procedures

2.4.1. Determining the socio-demographic profile of the farmer

In the present study, the questionnaire allowed the characterization of the socio-demographic profile of the study population, their practices and attitudes towards the use of pesticides and the health conditions of this population. To achieve the purpose to which the instrument is proposed, the questionnaire was structured containing the following variables: Personal data: Name, age, address, marital status and schooling.

2.4.2. Collection of blood material

Biological samples were obtained by venous puncture of the peripheral blood (15mL) in vacuum tubes. The environment of the implementation of this procedure was the facilities of the health units. At the time of collection, two tubes were used, one with ethylene diamine tetra sceptical acid anticoagulant (EDTA) and the other without. After clot formation, the tubes without anticoagulant were centrifuged at 2000 rpm to obtain the serum that was separated in tubes of Dry tests. The transport of all samples was in isothermal boxes with Gelox for the research partner Laboratories. The laboratories were stored between 2-8 °C until they were subjected to the analyses.

2.4.2.1. Determination of erythrocyte cholinesterase levels

The blood samples were analyzed in a Test Mate Che cholinesterase (model 400) equipment, which enables rapid test for erythrocyte cholinesterase. The Test-mate ChE Cholinesterase Test System reagents are based on the Ellman method. Acetylthiocholine (AcTC) and Butyrilthiocholine (BuTC) are hydrolysed, respectively, by Erythrocyte cholinesterase (AChE), producing carboxylic acid and thiocholine that react with Ellman's reagent (DTNB-dithiyrobenzoic acid), producing a Yellow staining that is measured spectrophotometrically at 450nm. The rate of color formation is proportional to the amount of cholinesterase.

The AChE reagent has a specificity above 95% due to the addition of a specific plasma cholinesterase inhibitor (PChE), the As1397 (10-(Alfadietilaminopropionil)-Fenotiaziana). The Test-mate ChE Photometric analyzer is calibrated in its manufacture and no additional calibration is required.

The measurement of cholinesterase activity is calculated by the photometric analyzer through the equation:

$$\text{Blood U/mL} = \frac{(A/\text{min})(\text{assay volume mL})}{(\varepsilon, \text{mM}^{-1}\,\text{cm}^{-1})(\text{“light path”}\ \text{cm})(\text{blood mL})}$$

The determination of cholinesterase activity is then refined later, through the following adjustments listed to obtain the final cholinesterase value:

- Reagent white adjustment-a small non-specific fraction (about 15%) is subtracted from the value of the dosed cholinesterase activity;
- Temperature adjustment-using the temperature sensor in the analyzer, both measurements of the cholinesterase activity and the "Blank" activity of the reagent are normalized at 25 °C;
- Hemoglobin adjustment – for AChE, hemoglobin normalization occurs according to sample volume and iron content; Therefore, the AChE indexes are more accurately expressed in U/g Hgb.

As recommended in the operating manual, a capillary was filled with 10µL of blood, then placed in a test tube, and agitated vigorously for 15 seconds. The test tube was gently turned to the vertical, until the capillary adhered to the side of the vial until the ideal time for reading. After the test key was triggered, it was observed for ten seconds to the reading message, followed by another message for removal of the tube, continuing again in the repetition of the test by tightening the testing key. After the dissolution of

the reagent with three drops of distilled water, it was added to the test tube, suffering a gentle agitation for 5 seconds. The capillary was aligned and the test tube inserted into the analyzer, and the testing key was pressed, continuing the assay. Were taken as reference values the expected values determined from normal samples from donors of blood bank (men and women), between 20 and 60 years of age (Table 1).

The decrease of cholinesterase to < 50% of normal indicates possible pesticide poisoning requiring removal of exposure and/or treatment with anticholinergic agents such as atropine and Pralidoxime. Suspected cases of poisoning can be confirmed by monitoring cholinesterase for a subsequent increase and level of equilibrium in the activity after 1-3 months of exposure. If the reference line values are obtained, a decrease in cholinesterase to < 70% of the reference line values can be interpreted as possible pesticide poisoning.

2.4.2.2. Determination of plasma cholinesterase levels (AChP)

The analyses were performed in the Automatic Biochemistry Analyzer COBAS INTEGRA® 400 Plus. This equipment has as main features: be fully automated, with capacity to perform up to 400 tests/hour; has random access to exams; Integration of 4 measuring principles; Methodologies: End point, fixed time and kinetics; Working with mono or bi reagents.

Determination of plasmatic cholinesterase enzymatic activity in plasma samples by continuous colorimetric kinetic methodology. It is based on a kinetic test by the method with Butyrilthiocholine. The color decrease can be measured in wavelength between 405 and 415 nm. The results will be expressed in international units per liter: U/L. Reference range for men and women aged 40 years or more: 5320-12920 U/L. Reference range for women aged 16 to 39 years, not pregnant, who are not taking contraceptives Hormonal: 4260-11250 U/L. Reference range for women aged 18 to 41 years, pregnant or taking hormonal contraceptives: 3650-9120 U/L.

3. Results and Discussion

3.1. Sociodemographic variables

Analyzing the sociodemographic profile of the interviewees, in relation to gender (Table 2), it was observed that the majority of the evaluated farmers belong to the male sex 92.68%, in the Dist. Pé Leve-AL, and 86.6% in São Sebastião-AL. These findings demonstrate our sociocultural reality, where males are involved in agricultural activities, and women are responsible for performing domestic work (Vikkey et al., 2017). Corroborating the data obtained in our study, Ubessi et al. (2015), when evaluating the socio-motdemotrgáfic profile of 434 rural workers in the northwest region of Rio Grande do Sul, there was a predominio male with 91.6%. Additionally, Preza and Augusto (2012) also found male predominance (96.5%) among the 29 farmers in northeastern Brazil.

It was found that the most prevalent age group between the two cities was between >40 and ≤60 (60%) (Table 2)

Table 1. Reference values for erythrocyte cholinesterase (AchE).

	N	Average	Standard deviation	Track
AChE, U/mL	40	3.68	0.47	2.77-5.57
AChE, U/g Hgb	40	27.1	2.9	21.9-37.3

N = Number of individuals.

Table 2. Sociodemographic characteristics of farmers of São Sebastião - AL and Pé Leve – AL.

Sociodemographic Variables		São Sebastião-AL		Distrito Pé Leve - AL	
		N	FR (%)	N	FR (%)
Age (Years)	≤ 20	0	0	2	4.87
	20 < I ≤ 40	2	13.33	17	41.46
	40 < I ≤ 60	9	60	17	41.46
	IS ≤ 60	4	26.66	5	12.19
Sex	Male	13	86,66	38	92.68
	Female	2	13.33	3	7.31
Marital status	Married	12	80	34	82.92
	Single	2	13.33	5	12.19
	Divorced	0	0	1	2.43
	Widowed	1	6.66	1	2.43
Education	Incomplete fundamental	10	66.66	19	46.34
	Complete fundamental	1	6.66	6	14.63
	Can't read	3	20	6	14.63
	Complete Higher Education	1	6.66	2	4.87
	Incomplete high school	-	-	4	9.75
	Full High School	-	-	4	9.75

N = Number of individuals; FR = Relative frequency.

. In the study conducted by Vasconcelos et al. (2014), by characterizing the profile of 112 rural workers who use pesticides in coffee plantations in Minas Gerais, with respect to the age group, 45 (40.18%) aged between 31 and 50 years and 40 years (35.71%) between 18 and 30 years. Similar mean age was also reported by Cotton et al. (2018), in a survey with farmers in a community in Australia, noted that most of the participants in their research were male with an average between 45 and 64 years of age. Vasconcelos et al. (2014) mention that the age factor in rural workers can cause a serious health problem, as young people and the elderly are more susceptible to illness due to exposure to chemical agents.

As for the marital status of farmers (Table 3), the majority are married (as), 82.92%, in the District Pé Leve-AL and 80% in the city of São Sebastião-AL. The workforce was characterized as predominantly familiar, because the other family members also participate in the crop cultivation process. Corroborating this study, Onyeisi et al. (2017), seeking to analyze enzymatic changes by pesticides in farmers in the municipality of Valença do Piauí, observed that the majority (73.9%), those involved were married.

Another important aspect evaluated was the level of education among farmers. It was seen that the low level of education was predominant, and 14.63% stated that they had attended only between the 1st and 4th grade of elementary school, 66.6% did not even finish elementary school, and of those who stated that they could not read 20% was in São Sebastião-AL and 14.63% in Pé Leve-AL (Table 2). Studies conducted with rural workers in other regions of Brazil, corroborate the data of this research where Rambo (2016), in the state of Mato Grosso, found that 78.54% of the interviewees did not complete elementary school, Souza (2008) identified that 71.5% do not have the complete fundamental, compared with the studies by Sangalli et al. (2014), the percentages are lower 67%, PE and 43.9%, MS, respectively.

The level of education is a relevant aspect, because as described in the study Santos et al. (2015), when analyzing the correlation between the level of education and the occurrence of poisoning in farmers in the municipality of Garanhuns-PE, there was a higher incidence of pesticide poisoning in individuals with incomplete elementary school, followed by 25% of illiterate individuals, in this sense, according to the authors, the level of education was a determining factor in the occurrence of intoxications.

Another important aspect is that the high number of producers with low schooling can possibly compromise on the farmer's understanding of the product's package leaflet instructions, not only in terms of its form of application, but also in relation to dangerousness. This information is made available only on the product label, which favors the exposure of workers to pesticides. Exposures that tend to increase given the lack of knowledge about instructions on how to use defensive, say Damalas and Eleftherohorinos (2011).

For Barroso and Wolff (2009), the fact that he is literate does not mean that the worker understands the information contained in the labels and/or leaflets of pesticides, especially because the packaging has a lot of technical information. The low level of education of the interviewees demonstrates the vulnerability of these individuals regarding the perception of risk and the understanding of the information contained in the labels of pesticide packaging (Preza et al., 2011).

Although in the rural environment studied, low schooling was found, the high rate of illiteracy and low schooling in Brazilian agriculture contributes, directly, to the increase in the number of poisoning with pesticides. This fact that determines a series of visual communication policies (such as the use of illustrations, figures, colored bands, etc.) in products and reports directed to this audience. These elements are found on product labels sold for this agricultural purpose, in theory to inform those people who do not have reading/writing skills (Damalas and Eleftherohorinos, 2011; Preza et al., 2011).

3.2. Laboratory analyses

The Brazilian law states that all agricultural workers must be submitted to periodic medical examinations with cholinesterase measurements, however, these are not provided by public health services. The exposure assessment and health care of family farmers in Brazil are limited by the informal organization of these workers and their distribution in approximately 4.3 million small properties (Brasil, 2018), the constant and prolonged exposure to low doses of multiple pesticides, the distance to health services, the shortage of laboratories with available analytical capacity,

Table 3. Plasma acetylcholinesterase activity (AChP) in conventional and organic farming farmers, Alagoas, 2017 and 2018.

	AChP activity in Farmer (U/L)					
	1ª collection			**2ª collection**		
	Given value MIN-MAX	**Reduced N (FR%)**	**Normal N (FR%)**	**Given value MIN-MAX**	**Reduced N (FR%)**	**Normal N (FR%)**
São Sebastião	3.188,2-8982,1	3 (18.75)	13 (81.25)	4.406,2-6.990	3 (30)	7 (70)
Pé Leve	2.927,4-11.823,4	4 (10)	36 (90)	3.542,3-8.796,3	5 (21.73)	18 (78.26)
Conventional*	2.927,4-11.823,4	7 (12.5)	49 (87.7)	3.542,3-8.796,3	8 (24.24)	25 (75.75)
Organic	-	-	-	5.598-11.250	0 (0)	23 (100)

N = Number of individuals; FR = Relative frequency. *Summation of the data of the conventional and organic cultivation villages.

and the absence of an integrated intoxication reporting system (Buralli et al., 2018; Carneiro, 2015).

To present the results of plasma and erythrocyte activity values, levels and relative frequency were organized into tables, taking into account the types of conventional and organic cultivation in two periods of the year. It was found from the analysis of data from the 1st collection (Table 3), that farmers from both cities under study showed a considerable decrease in activity of (AChP), where the number of enzymatic reduction was higher in the city of São Sebastião-AL, with 18.75%, this percentage was higher compared to the Pé-leve District-AL, which obtained 10% of intoxicated individuals in the same period; may indicate acute or chronic intooxytion, it is observed that these results were found in farmers of the conventional cultivation system, which may be directly linked to the use of pesticides.

According to Araújo et al. (2007), in the conventional production system, exposure to agrochemicals is not restricted only to direct contact with the products during their preparation and spraying, because farmers usually participate in all stages of the from transport, storage and utlization to the disposal of the packaging of the products.

In the study conducted by Araújo et al. (2007) when evaluating in an agricultural community located in Nova Friburgo, (RJ), to know the clinical and laboratory aspects of multiple exposure to pesticides in a representative sample of 102 farmers, of both sexes, with regard to the results, we can infer that the levels of cholinesterase reduction found were high (20%), which is related to the fact that 44% of the workers examined reported recent exposures, reporting that the use of pesticides had been within seven days before the date of collection of blood samples for toxicological examination.

In the research, it was seen that, despite pesticide contamination, the percentage of farmers with low AChP activity (Table 3) was lower than individuals who presented normal values of the same enzyme. However, the producers of the organic cultivation system did not show a decrease in colinetherapeutic activity in any of the collected collections.

In the studies by Nganchamung et al. (2017), Mesquita Filho and Pereira (2011), Faria et al. (2009) and Stroparo and Braguini (2011) demonstrated a significant decrease in plasma cholinesterase levels, in most of the individuals studied, although the evaluation was composed mostly of men, the results corroborate the findings of this study Study. In the study conducted by Goethel et al. (2013), the smallest part of individuals are male, however, a significant number of these presented decreased levels of cholinesterase, which can be compared with the other studies mentioned above, which corroborate the results of the present study.

Since, the first collection was performed during the dry season, it was noticed that the amount of intoxicated was not high, that is, most farmers presented normal levels of plasma acetylcholinesterase in relation to the rainy season. Mattiazzi (2017), points out that the fact that the results are shown within normal limits can be justified by the low use of pesticides cholinesterase inhibitors by workers, or also, as also mentioned by Murakami et al. (2017), because blood samples were collected in a period in which this type of pesticide was not used.

For this age of drought, (Table 4), it was found that most individuals had normal Levels of AChP, highlighting the Pé-leve District with 90% of individuals with normal enzymatic activity, this result differs with that of Faria et al. (1999), where the majority of rural fruit-producing workers in the municipalities of Antônio Prado and Ipê-RS, presented reduced levels of AChP during the dry season, characterizing intoxication between October and January, the same authors explain that in this period due to the high temperatures, the body absorbs more toxins released by pesticides and also because applications are more intense at this time of year. The severity of these intoxications was considered mild-moderate in 80% of cases and as severe in 20% of the occurrences.

According to the 2nd collection (Table 3), performed at the rainy season, it was found that the percentage of intoxicated increased considerably in the city of São Sebastião and Pé-Leve District, to 30% and 21.73%, respectively, this result demonstrates that in relation to the dry season, there was a considerable reduction in AChP levels in the rainy season.

Thus, it was possible to observe that in the conventional cultivation system for the total number of samples collected in both cities, during the rainy season, the number of intoxicated increased, this may be related, to the fact that it is in this period the most intense use of pesticides, with the greater probability of contamination.

Table 4. Relative frequency of erythrocyte acetylcholinesterase in farmers of conventional and organic cultivation, Alagoas, 2017 and 2018.

	AChE U/Ml activity in farmer					
	1ª collection			**2ª collection**		
	Given value MIN-MAX	**Reduced N (FR%)**	**Normal N (FR%)**	**Given value MIN-MAX**	**Reduced N (FR%)**	**Normal N (FR%)**
São Sebastião	2.02-4.75	1 (6.25)	15 (93.75)	0.77-3.63	8 (80)	2 (20)
Pé Leve	3.43-4.91	-	40 (100)	0.24-4.40	5 (21.73)	18 (78.26)
Conventional*	2.02-4.91	1 (1.78)	55 (98.21)	0.24-4.40	13 (39.39)	20 (60.60)
Organic	-	-	-	2.79-4.46	0 (0)	23 (100)

N = Number of individuals; FR = Relative frequency. *Summation of the data of the conventional and organic cultivation villages.

According to Gomes and Barizon (2014), in Brazil the information on the presence of pesticides in water and soil is limited, evidencing a gap in information on the real environmental condition in our country in the face of excessive exposure to pesticides. A study conducted in two municipalities by the Federal University of Mato Grosso and Osvaldo Cruz Foundation on water contamination in the state of Mato Grosso, one of the main grain producers, found the presence of chemicals in rainwater and in the air, it demonstrated that the most worrying is that more than 50% of these residues are present in the rainwater, showing their volatilization and precipitation also for urban areas (Moreira et al., 2012).

According to the evaluation of erythrocyte acetylcholinesterase (AChE) where the test with it is more reliable, because this remains in the body for longer, that is, the determination of AChE activity is more valuable in case of suspected prolonged exposure, since erythrocyte concentrations take longer to decrease than serum AChP concentrations, and require more time for normalization after exposure to agrochemicals (Henry, 2008; Ribeiro and Mella, 2007). It was found in the analysis of the 1st collection made in conventional cultivation (Table 4), that only the city of São Sebastião presented intoxicated individuals in the dry season, with a percentage of 6.25% of the population of farmers studied with reduced levels of AChE .

According to the analysis of enzymatic activity, in blood samples of individuals of the Organic System, it was found that in the city of São Sebastião-AL there were no changes in the levels of erythrocyte acetylcholinesterase. Emphasizing that in the Pé-Leve district, also in evaluations made with samples collected in a dry and rainy period, no volunteers in the research showed a reduction in The EA.

Regarding the evaluation of erythrocyte acetylcholinesterase levels, in the 2nd collection, in rainy season, it was noted that according to (Table 4), there was a sudden increase in the number of intoxicated in the City of São Sebastião, from 6.25% to 80%, proving the action of the pesticides on the activity of the Enzyme AChE, reducing it significantly and characterizing them as anticolinetherapeutic agents. Results similar to those of this research were found by Dhananjayan et al. (2012), where working with enzymatic activity of rural workers, found a significant decrease in erythrocyte cholinesterase levels in cases of exposures to pesticides, observing that the lower the level of enzyme activity, the higher the degree of intoxications

Attention is drawn to the Pé-Leve District of which in the first collection at the time of drought there were no cases of intoxication, however in the analysis performed in the 2nd collection, there was a jump in the percentage of individuals with reduced levels of The EA, from 0 to 21.73%, the as the number of individuals with normal enzymatic activity decreases the number of infected increases, proving the malesman action of pesticides for such enzymes.

In studies conducted by Quandt et al. (2010), in farmers in North Carolina, there were – reductions in ache activity levels, with decreases >15% of an individual's highest value, identifying and considering a reduction in activity significant colineteric. A recent study by Cotton et al. (2018) showed that 14.9% of the 41 farmers involved there was a significant reduction in AChE from exposure to insecticides. Vikkey et al. (2017), evaluating levels of poisoning in farmers in the Central Republic of Benin, there was a significant decrease in AChE of 60.61% of the population studies. Nganchamung et al. (2017). It obtained a reduced value of The EA of 50.0%, of the 90 farmers in its research, corroborating that of this research. Results similar to those of this research in relation to the reduction of AChE were obtained by Magauzi et al. (2011) in Zimbabwe (24.1%) and Vikkey et al. (2016) in Benin (26.82%). As it was presumed, the producers of the organic system who had blood samples collected and analyzed did not present negative changes in relation to TheE levels in the body. In the specific case of the State of Alagoas, Sousa et al. (2012) they mention that there is only one cooperative of certified organic farmers, representing about 2% of the crops developed in the state. For the authors, the adoption of alternative controls can be a viable strategy for the replacement of pesticides, common in horticulture. And consequently decrease the probability of poisoning by synthetic agrochemicals, used in agriculture.

For experts the solution for agriculture would be to merge organic methods with modern technologies used in traditional plantations. Some of the points emphasized are: crop rotation, natural pest management, agricultural diversification and livestock, improvements in soil condition from composting use, green and animal fertilization (Ciclo Vivo, 2016). Researchers ensure that organic farming is able to meet all the food needs in the world, regardless of climate change. They also justify this statement: "[…] organic farms have the potential to produce high yields as a result of the higher water retention capacity in soils grown without pesticides" (Ciclo Vivo, 2016).

Stotz (2012), in a survey of family farmers from Sumidouro - RJ, where most producers and family members are in São José de Ubá at 146 km, considers the conventional agriculture system positive in economic terms, considering as an option necessary and irrevocable life, despite the health risks itself. In this sense, Melo et al. (2012), show that organic agriculture can be the best form of production that guarantees health and environmental protection. However, this type of production has some obstacles, such as the scarcity of financial resources applied and the lack of specific knowledge that makes it impossible to practice this modality.

4. Conclusions

It is inferred in this study that the vast majority of the interviewed farmers do not have complete elementary education, in addition, most of them are of the genus Maculine with ages between 40 and 60 years, which make them even more vunerable to contamination by Agrotoxicity due to the immune system is more prone to risk.

Thus, this study allowed collecting biological data on the risks of pesticide poisoning and analyzing these data according to the recommendations of the World Health Organization. In our study, it was possible to low schooling among farmers, which in some ways can influence the understanding of the leaflets and labels of pesticides, thus compromising handling, transportation, packaging and disposal of these products. We demonstrated high inhibition of AChE in the farmers who participated in this study. Routine monitoring of AChE may allow early recognition of frequent and continuous exposure to pesticides, since non-use of personal protective equipment is closely linked to the level of intoxication.

It is concluded that exposure to pesticides in conventional cultivation the chance of pesticide poisoning is higher, and that the organic system of cultivation, remains the best alternative for the prevention of future pathologies, besides bringing quality of life to the Rural workers, as well as for consumers, because caring for the environment is taking care of human health.

References

ANDRADE FILHO, A., CAMPOLINA, D. and DIAS, M.B., 2001. *Toxicologia na prática clínica*. 1. ed. Belo Horizonte: Folium.

ARAÚJO, A.J., LIMA, J.S., MOREIRA, J.C., JACOB, S.C., SOARES, M.O., MONTEIRO, M.C.M., AMARAL, A.M., KUBOTA, A., MEYER, A., COSENZA, C.A.N., NEVES, C. and MARKOWITZ, S., 2007. Exposição múltipla a agrotóxicos e efeitos à saúde: estudo transversal em amostra de 102 trabalhadores rurais, Nova Friburgo, RJ. *Ciência & Saúde Coletiva*, vol. 12, no. 1, pp. 115-130. http://dx.doi.org/10.1590/S1413-81232007000100015. PMid:17680063.

ARAÚJO, C.R.M., SANTOS, V.L.A. and GONSALVES, A.A., 2016. Acetilcolinesterase – AchE: Uma Ensima de Interesse Farmacológico. *Revista Vitual de Química*, vol. 8, no. 6, pp. 1818-1834.

BARROSO, L.B. and WOLFF, D.B., 2009. Riscos e segurança do aplicador de agrotóxicos no Rio Grande do Sul. *Disciplinarum Scientia*, vol. 10, no. 1, pp. 27-52.

BRASIL. Ministério do Meio Ambiente, 2018 [viewed 13 January 2019]. *Agrotóxicos* [online]. Available from: http://www.mma.gov.br/seguranca-quimica/agrotoxicos

BURALLI, R.J., RIBEIRO, H., MAUAD, T., AMATO-LOURENÇO, L.F., SALGE, J.M., DIAZ-QUIJANO, F.A., LEÃO, R.S., MARQUES, R.C., SILVA, D.S. and GUIMARÃES, J.R.D., 2018. Respiratory condition of family farmers exposed to pesticides in the State of Rio de Janeiro, Brazil. *International Journal of Environmental Research and Public Health*, vol. 15, no. 6, pp. 1203. http://dx.doi.org/10.3390/ijerph15061203. PMid:29890615.

CARNEIRO, F.F., 2015. *Dossiê ABRASCO: um alerta sobre os impactos dos agrotóxicos na saúde*. São Paulo: Expressão Popular.

CARNEIRO, F.F., PIGNATI, W., RIGOTTO, R.M., AUGUSTO, L.G.S., RIZZOLO, A., FARIA, N.M.X., ALEXANDRE, V.P., FRIEDRICH, K. and MELLO, M.S.C., eds., 2012. *Dossiê Abrasco: um alerta sobre os impactos dos agrotóxicos na saúde*. Rio de Janeiro: Abrasco.

CARVALHO, M.M.X., NODARI, E.S. and NODARI, R.O., 2017. "Defensives" or "pesticides"? A history of the use and perception of pesticides in the state of Santa Catarina, Brazil, 1950-2002. *História, Ciências, Saúde-Manguinhos*, vol. 24, no. 1, pp. 75-91. http://dx.doi.org/10.1590/s0104-59702017000100002. PMid:28380166.

CICLO VIVO, 2016 [viewed 13 January 2019]. *Estudo mostra que agricultura orgânica pode alimentar o mundo inteiro* [online]. Available from: http://ciclovivo.com.br/noticia/estudo-mostra-que-agricultura-organica-pode-alimentar-o-mundo-inteiro/

COTTON, J., EDWARDS, J., RAHMAN, M.A. and BRUMBY, S., 2018. Cholinesterase research outreach project (CROP): point of care cholinesterase measurement in an Australian agricultural community. *Environmental Health*, vol. 17, no. 31, pp. 1-11. http://dx.doi.org/10.1186/s12940-018-0374-1. PMid:29606131.

DAMALAS, C. and ELEFTHEROHORINOS, I.G., 2011. Pesticide exposure, safety issues and risk assessment indicators. *International Journal of Environmental Research and Public Health*, vol. 8, no. 5, pp. 1402-1419. http://dx.doi.org/10.3390/ijerph8051402. PMid:21655127.

DELLA VECHIA, J.F., CRUZ, C., SILVA, A.F., CERVEIRAJR, W.R. and GARLICH, N., 2016. Macrophyte bioassay applications for monitoring pesticides in the aquatic environment. *Planta Daninha*, vol. 34, no. 3, pp. 587-603. http://dx.doi.org/10.1590/s0100-83582016340300021.

DHANANJAYAN, V., RAVICHANDRAN, B., ANITHA, N. and RAJMOHAN, H.R., 2012. Assessment of acetylcholinesterase and butyrylcholinesterase activities in blood plasma of agriculture workers. *Indian Journal of Occupational and Environmental Medicine*, vol. 16, no. 3, pp. 127-130. http://dx.doi.org/10.4103/0019-5278.111755. PMid:23776322.

FARIA, N.M.X., FACCHINI, L.A., FASSA, A.G. and TOMASI, E., 1999. Estudo transversal sobre saúde mental de agricultores da Serra Gaúcha (Brasil). *Revista Brasileira de Saúde Pública*, vol. 33, no. 4, pp. 391-400. http://dx.doi.org/10.1590/S0034-89101999000400011.

FARIA, N.M.X., ROSA, J.A.R. and FACCHINI, L.A., 2009. Intoxicações por agrotóxicos entre trabalhadores rurais de fruticultura, Bento Gonçalves, RS. *Revista de Saúde Pública*, vol. 43, no. 2, pp. 335-344. http://dx.doi.org/10.1590/S0034-89102009005000014. PMid:19225687.

GOETHEL, G., NASCIMENTO, F., DANI, C., MASCARENHAS, M., SEBBEN, V. and FUNCHAL, C., 2013. Avaliação de parâmetros bioquímicos e toxicológicos de fumicultores do município de Venâncio Aires/RS. *Revista Brasileira de Pesquisa Saúde*, vol. 15, no. 1, pp. 105-112. http://dx.doi.org/10.21722/rbps.v15i1.5586.

GOMES, M.A.F. and BARIZON, R.R.M., 2014. *Panorama da contaminação ambiental por agrotóxicos e nitrato de origem agrícola no Brasil: cenário 1992/ 2011*. Jaguariúna: Embrapa Meio Ambiente, 35 p.

HENRY, J.B., 2008. *Diagnósticos clínicos e tratamento por métodos laboratoriais*. 20. ed. São Paulo: Manole.

ISMAEL, L.L., ROCHA, E.M.R., LINS FILHO, L.A. and LIMA, R.P.A., 2015. Resíduos de agrotóxicos em alimentos: preocupação ambiental e de saúde para população paraibana. *Revista Verde*, vol. 10, no. 3, pp. 24-29. http://dx.doi.org/10.18378/rvads.v10i3.3459.

KING, A.M. and AARON, C.K., 2015. Organophosphate and Carbamate Poisoning. *Emergency Medicine Clinics of North America*, vol. 33, no. 1, pp. 133-151. http://dx.doi.org/10.1016/j.emc.2014.09.010. PMid:25455666.

MAGAUZI, R., MABAERA, B., RUSAKANIKO, S. and COULIBALY, B.M., 2011. Os efeitos na saúde de agrotóxicos

entre trabalhadores rurais em fazendas comerciais de Kwekwe District, Zimbabwe. *The Pan African Medical Journal*, vol. 9, pp. 26-32. http://dx.doi.org/10.4000/vertigo.6432. PMid:22145061.

MATTIAZZI, A.L., 2017. *Exposição a agrotóxicos e alterações auditivas em trabalhadores rurais*. Cerro Largo: Universidade Federal da Fronteira Sul, 97 p. Dissertação de Mestrado em Desenvolvimento de Politicas Públicas.

MELO, K.C., SOUSA, R.E.M., ANASTASSIOY, C.A., GÓES, K.O.C. and SILVA, A.A., 2012. Análise socioeconômica e ambiental dos produtores familiares do município de Juína/MT e suas dificuldades. In: *Anais do XXI Encontro Nacional de Geografia Agrária*, 2012, Uberlândia. Uberlândia: UFU.

MESQUITA FILHO, M. and PEREIRA, R.C., 2011. Manejo, uso de equipamentos de proteção individual (epi) e intoxicação por agrotóxicos entre os trabalhadores da lavoura do morango do sul de Minas Gerais. *Revista Espaço para a Saúde*, vol. 13, no. 1, pp. 23-34.

MOREIRA, J.C., PERES, F., SIMÕES, A.C., PIGNATI, W.A., DORES, E.C., VIEIRA, S.N., STRÜSSMANN, C. and MOTT, T., 2012. Contaminação de águas superficiais e de chuva por agrotóxicos em uma região do estado do Mato Grosso. *Ciência & Saúde Coletiva*, vol. 17, no. 6, pp. 1557-1568. http://dx.doi.org/10.1590/S1413-81232012000600019. PMid:22699646.

MURAKAMI, Y., PINTO, N.F., ALBUQUERQUE, G.S.C., PERNA, P.O. and LACERDA, A., 2017. Intoxicação crônica por agrotóxicos em fumicultores. *Saúde em Debate*, vol. 41, no. 113, pp. 563-576. http://dx.doi.org/10.1590/0103-1104201711317.

NGANCHAMUNG, T., ROBSON, M.G. and SIRIWONG, W., 2017. Associação entre sangue atividade da cholinesterase, efeitos organofosforados resíduos de pesticidas sobre mãos e saúde entre os agricultores do pimentão em Ubon Ratchathani Province, Nordeste da Tailândia. *Roczniki Panstwowego Zakladu Higieny*, vol. 68, no. 2, pp. 175-183. PMid:28646835.

ONYEISI, O.J., COSTA, C.P.T., SANTOS, S.F.L., COSTA, R., AMARAL, M.P.F. and CHAVES, S.V.T., 2017. Apoptose em Trabalhadores Expostos ao Agrotóxico Organofosforado. *Revista Interdisciplinar*, vol. 10, no. 2, pp. 83-88.

PEZZEMENTI, L. and CHATONNET, A., 2010. Evolution of cholinesterases in the animal kingdom. *Chemico-Biological Interactions*, vol. 187, no. 1-3, pp. 27-33. http://dx.doi.org/10.1016/j.cbi.2010.03.043. PMid:20359467.

PINTO, G. D. A., 2017. *Capacidade de degradação do agrotóxico clorpirifós por bactérias láticas*. Rio de Janeiro: Universidade Federal do Estado do Rio de Janeiro, 62 p. Dissertação de Mestrado em Alimentos e Nutrição.

PIOVEZAN, G., PRADO, J., JORGENSEN, K.O., GOMES, S., ROZIN, L. and ROMANO, R.G., 2013 [viewed 22 January 2019]. *Impacto sociológico da fumicultura em agricultores* [online]. Curitiba: UFPR. Available from: http://www.humanas.ufpr.br/portal/sociologiasaude/files/2013/12/impactosociológico-da-fumicultura.pdf

PREZA, D.L.C. and AUGUSTO, L.G.S., 2012. Vulnerabilidade de trabalhadores rurais frente ao uso de agrotóxicos na produção de hortaliças em região so Nordeste do Brasil. *Revista Brasileira de Saúde Ocupacional*, vol. 37, no. 125, pp. 89-98. http://dx.doi.org/10.1590/S0303-76572012000100012.

PREZA, D.L.C., NOGUEIRA, T.F. and AUGUSTO, L.G.S., 2011. Práticas na comercialização e na indicação de agrotóxicos em região produtora de hortaliças no Estado da Bahia. *Magistra*, vol. 23, no. 4, pp. 168-174.

QUANDT, S.A., CHEN, H., GRZYWACZ, J.G., VALLEJOS, Q.M., GALVAN, L. and ARCURY, T.A., 2010. Cholinesterase depression and its association with pesticide exposure across the agricultural season among Latino farmworkers in North Carolina. *Environmental Health Perspectives*, vol. 118, no. 5, pp. 635-639. http://dx.doi.org/10.1289/ehp.0901492. PMid:20085857.

RAMBO, J.R., 2016. *Produzir e vender? O caso do Programa de Aquisição de Alimentos de agricultores familiares assentados da reforma agrária em Tangará da Serra-MT*. Ilha Solteira: Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista, 180 p. Tese de Doutorado em Agronomia.

RIBEIRO, A. C. C. and MELLA, E. A. C., 2007. Intoxicação ocupacional por organofosforados: a importância da dosagem de colinesterase. *Iniciação Científica Cesumar*, vol. 9, no. 2, pp. 2007.

SANGALLI, A.R., SCHLINDWEIN, M.M. and CAMILO, L.R., 2014. Produção e geração de renda na agricultura familiar: um diagnóstico do assentamento rural Lagoa Grande em Dourados, Mato Grosso do Sul. *Ciência e Natura*, vol. 36, no. 2, pp. 180-192. http://dx.doi.org/10.5902/2179460X11064.

SANTOS, L.A.O., MUNIZ, L.S., ANDRADE, H.M.L.S. and ANDRADE, L.P., 2015. Impacto das Intoxicações causadas por agrotóxicos no município de Garanhuns-PE. *Cadernos de Agroecologia*, vol. 10, no. 3.

SHMITZ, M.K., 2003. *Intoxicação por agrotóxicos inibidores da colinesterase*. Florianópolis: Universidade Federal de Santa Catarina, 41 p. Trabalho de Conclusão de Curso em Medicina.

SOUSA, M.F., SILVA, L.V., BRITO, M.D. and FURTADO, D.C.M., 2012. Tipos de controle alternativo de pragas e doenças nos cultivos orgânicos no Estado de Alagoas, Brasil. *Revista Brasileira de Agroecologia*, vol. 7, no. 1, pp. 132-138.

SOUZA, L.C., 2008. *Análise de situações de vida e trabalho de mulheres da área rural de Nova Friburgo-RJ*. Rio de Janeiro: Escola Nacional de Saúde Pública Sergio Arouca, Fundação Oswaldo Cruz, 39 f. Dissertação de mestrado em Saúde Pública.

STOTZ, E.N., 2012. Os limites da agricultura convencional e as razões de sua persistência: estudo do caso de Sumidouro, RJ. *Revista Brasileira de Saúde Ocupacional*, vol. 37, no. 125, pp. 114-126. http://dx.doi.org/10.1590/S0303-76572012000100014.

STROPARO, L.F.E. and BRAGUINI, W.L., 2011. Avaliação da exposição à organofosforados entre produtores de tabaco de uma localidade do município de Irati – Paraná. *Publicatio UEPG: Ciências Exatas e da Terra, Ciências Agrárias e Engenharias*, vol. 17, no. 1, pp. 39-50. http://dx.doi.org/10.5212/Publ.Exatas.v.17i1.0004.

UBESSI, L.D., UBESSI, C., KIRCHNER, R.M., JARDIM, V.M.R. and STUMM, E.M.F., 2015. Uso de equipamentos de proteção por agricultores que utilizam agrotóxicos na relação com problemas de saúde. *Revista de Enfermagem UFPE on line*, vol. 9, no. 4, pp. 7230-7238.

VASCONCELOS, M.V., FREITAS, C.F. and SILVEIRA, C.A., 2014. Caracterização do uso de agrotóxicos entre trabalhadores rurais. *Revista Saúde*, vol. 40, no. 2, pp. 87-96. http://dx.doi.org/10.5902/2236583411934.

VIKKEY, H.A., FIDEL, D., PAZOU ELISABETH, Y., HILAIRE, H., HERVÉ, L., BADIROU, A., ALAIN, K., PARFAIT, H., FABIEN, G. and BENJAMIN, F., 2017. Risk factors of pesticide poisoning and pesticide users' cholinesterase levels in cotton production areas: Glazoué and Savè Townships, in

Central Republic of Benin. *Insights Saúde Ambiental*, vol. 11, pp. 1-10. http://dx.doi.org/10.1177/1178630217704659. PMid:28469452.

VIKKEY, H.A., IBRAHIM, M.C., HERVÉ, L., FIDÈL, D., FABIEN, G., PAUL, A. and FAYOMI, B., 2016. Evaluation des indicateurs bilaterais ologiques d'exposition pesticidas aux organophosphorés et la fonction hépatique des agriculteurs de la Commune de Gogounou au nord-est du Bénin. *J Biol Soe Clin Bénin*, vol. 24, pp. 9-14.

YANKOVSKAYA, E., TATTAR, A. and FISHEL, M., 2018. Quality estimation with force de cored attention and cross lingual embeddings. *Procedings of Third Cinfereced on Machine Translations*, vol. 2, pp. 816-821. http://dx.doi.org/10.18653/v1/W18-64093.