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Analysis of residues from dithiocarbamate fungicides in vegetables produced in the region of Vargem Bonita, Federal District

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ABSTRACT

Dithiocarbamates are the most used fungicide and one of the most detected in food in Brazil. This study aims to analyze the levels of dithiocarbamate residues in vegetables produced in the region of Vargem Bonita, Federal District, and to compare the levels found with the maximum residue limits (MRLs) established by the Brazilian legislation. Eighty-four vegetable samples were obtained directly from 23 Vargem Bonita producers from June to November 2018. The analyzes were carried out using a spectrophotometric method, which determines the level of CS_2 produced by degrading the dithiocarbamate present in the sample. In total, 21 samples (25%) contained dithiocarbamate residues above the method's limit of quantification (LOQ, 0.05 mg/kg CS_2). Five of the 27 lettuce samples analyzed (18.5%) contained dithiocarbamates, at levels ranging from 0.06 to 0.50 mg/kg CS_2, below the MRL for this crop. Residues were also found in parsley, coriander and chicory samples (0.09 or 0.10 mg/kg CS_2), cultures for which there is no registered the use of dithiocarbamate in Brazil. Samples of arugula, chive, leek, and watercress, which also do not have registered use, tested positive; however, these crops are known to provide false positive results for CS_2, and the illegality of the residues cannot be confirmed. This study shows proper dithiocarbamate use in vegetables by Vargem Bonita farmers, except for parsley, coriander, and chicory, crops with a limited number of registered pesticides, which was probably the reason for the illegal dithiocarbamate use.

Keywords: Pesticides, maximum residue limit, spectrophotometry, CS_2.

RESUMO

Análise de resíduos de fungicidas ditiocarbamatos em hortaliças produzidas na região de Vargem Bonita, Distrito Federal

Os ditiocarbamatos são os fungicidas mais utilizados e mais detectados em alimentos no Brasil. Este estudo teve como objetivos analisar os níveis de resíduos de ditiocarbamatos em hortaliças produzidas na região de Vargem Bonita, Distrito Federal, e comparar os níveis encontrados com os limites máximos de resíduos (LMR) estabelecidos para esses alimentos. Foram coletadas 84 amostras de diferentes tipos de hortaliças diretamente de 23 produtores no período de junho a novembro de 2018. As análises foram realizadas utilizando o método espectrofotométrico, que determina o nível de CS_2 produzido pela degradação em meio ácido do ditiocarbamato presente na amostra. No total, 21 amostras (25%) continham resíduos de ditiocarbamatos acima do Limite de Quantificação do método (LOQ, 0,05 mg/kg CS_2). Cinco das 27 amostras de alface analisadas (18,5%) continham resíduos, com níveis de 0,06 a 0,50 mg/kg de CS_2, abaixo do LMR para essa cultura. Resíduos também foram encontrados em amostras de salsa, coentro e chicória, em níveis de 0,09 ou 0,10 mg/kg CS_2, culturas para as quais não existe registro de uso de ditiocarbamatos no Brasil. Amostras de rúcula, cebolinha, alho poró e agrião, que também não possuem registro para ditiocarbamatos, deram resultado positivo, porém essas culturas são conhecidas por fornecerem resultados falso positivos para CS_2, e a ilegalidade dos resíduos não pode ser confirmada. Esse estudo mostrou o uso adequado de ditiocarbamatos em hortaliças por agricultores da Vargem Bonita, com exceção de salsa, coentro e chicória, culturas com número limitado de pesticidas registrados, o que, provavelmente, tenha sido a razão do uso ilegal dos ditiocarbamatos.

Palavras-chave: Pesticidas, limite máximo de resíduos, espectrofotometria, CS_2.

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The rapid world population growth in the last century has increased significantly the demands for food production, requiring the introduction of new technologies in agriculture, including the intensive use of pesticides and fertilizers, mechanization, high-yield cultivars and irrigation (FAO, 2017). Pesticides increase agricultural productivity by protecting crops from pests and weeds (FAO, 2021). However, as these substances have a biocidal effect and do not affect only the target organisms, and if used incorrectly, there may be risks of negative effects on terrestrial and aquatic organisms and an impact on human health, both due to the consumption of water and food, and in the handling and application of these products (Spadotto *et al*., 2004).

Brazil is among the three largest

users of pesticides in the world since 2008. Data from the Food and Agriculture Organization of the United Nations (FAO) show that China was the largest user in 2018, with around 1.8 million tons, followed by United States and Brazil, with approximately 0.41 and 0.38 million tons, respectively (FAO, 2021). Dithiocarbamates are a group of non-systemic fungicides widely used in various crops worldwide. In Brazil, five compounds from this group are registered: mancozeb, metiram and propineb for foliar application, tiram for seed and soil treatment (potato only) and sodium metam for soil treatment (Anvisa, 2021). Mancozeb is the most used fungicide in Brazil (Ibama, 2020), being registered for 46 food crops in the country (Anvisa, 2021).

The toxicological relevance of dithiocarbamates is mainly due to its two plant and animal metabolites, ethylene thiourea (ETU), produced by ethylenebisditiocarbamates (such as mancozeb and metiram) and propylene thiourea (PTU), produced by propineb, which have been shown to be carcinogenic and mutagenic and causes thyroid hormone disruption in rats (Belpoggi & Maltoni, 2002; Axelstad *et al*., 2011). Despite the toxic potential of dithiocarbamates, studies assessing the risk of exposure to these fungicides in the Brazilian diet did not indicate a risk to user's health (Caldas *et al*., 2006; Jardim *et al.*, 2018).

To ensure safety against the risks caused by pesticides, it is necessary that they are used correctly, following the technical recommendations for use, or Good Agricultural Practices (GAP), which include the maximum dose and frequency of application, and the withdrawal period, which is the time between the last application and harvest. The use of the pesticides according to GAP ensures that the residues present in food are not above the maximum residue limit (MRL) and that the consumption of treated food is safe. Therefore, it is paramount that rural workers have knowledge of the correct use of pesticides to guarantee safe food for the population (Damalas & Eleftherohorinos, 2011; EU, 2021).

This study aims to verify if vegetables produced in the region of Vargem Bonita, Federal District, contain fungicide residues from the group of dithiocarbamates and, if confirmed, if the detected levels are within the MRLs established in the country.

MATERIAL AND METHODS

Sample collection

Vegetable samples were collected at Vargem Bonita's Rural Center, a rural community belonging to the Park Way administrative region, in the Federal District. This community is divided into agricultural production farms and residential lots. There are 67 farms, 56 of which effectively produce vegetables, mostly leafy vegetables, which supply the Federal District's Supply Center, restaurants, markets, and supermarkets in the region.

Eighty-four vegetable samples (Table 1) were collected in 23 farms, at four different times from June to November 2018, covering the regional dry and the rainy seasons, one sample of each crop from each property, one collection per property. One farm has an organic production stamp, the others are from conventional producers, who use pesticides for pest management. About 1 kg of each sample was collected, which is the amount recommended by the Codex Alimentarius Commission for pesticide analysis (CAC, 1999). The samples were immediately brought to the Laboratory of Toxicology, where they were stored in a freezer at <15°C for analysis.

Reagents and solutions

Sodium hydroxide (NaOH, 98% purity), diethanolamine (99% purity), and absolute ethyl alcohol were obtained from Dinâmica®. Copper acetate II monohydrate (98-102% purity) and tin chloride II ($SnCl_2$, 98-103% purity) were obtained from Vetec®. Hydrochloric acid (HCl, 32% PA) was purchased from Anidrol®.

The acid digestion solution was prepared by dissolving 31.25 g of $SnCl_2$ in 500 mL HCl, and making up to 2.5 L with distilled water. Complexing solution was prepared with 0.096 g of copper acetate II in 200 g of diethanolamine and the volume made up to 2 L with ethanol.

Determination of dithiocarbamate residues, expressed as CS_2

The samples were processed while still frozen, quickly cutting them into small pieces to avoid dithiocarbamate degradation and stored in a freezer until the moment of analysis (Mozzaquatro *et al*., 2019).

Spectrophotometric analysis was carried out using the vertical system developed by Caldas *et al.* (2001). In this system, the CS_2 released after an acid hydrolysis step, under heat for 45 minutes, of the dithiocarbamate present in the sample (150 g) with 175 mL of digestion solution. The CS_2 is carried by a nitrogen flow, passing through a 10% NaOH solution (20 mL) (Trap 1), which retains the acid vapors from the acid digestion. The CS_2 then forms a yellow complex when reacted with 15 mL of complexing solution (Trap 2), which was transferred quantitatively to a calibrated volumetric flask of 25 mL and the volume completed with ethanol. The intensity of the complexed yellow solution was measured on a Shimadzu® UV-1650 PC spectrophotometer at 435 nm against an analytical curve of CS_2, and the result expressed as mg CS_2/kg. The method was previously validated at the Laboratory of Toxicology for the vegetables included in this study, with a LOQ (Limit of Quantification) of 0.05 mg CS_2/kg, being accredited by Brazilian National Institute of Metrology, Standardization and Industrial Quality (Instituto Nacional de Metrologia, Qualidade e Tecnologia Industrial, INMETRO) by ISO 17025 (CRL-0447).

RESULTS AND DISCUSSION

Table 1 shows the results of the 84 samples analyzed, in CS_2, and the MRLs for each crop (Anvisa, 2021), allowing to compare whether the levels of residues are in accordance with current legislation.

As the most produced vegetable in the Vargem Bonita region, lettuce was the crop with the highest number of collected samples (27 samples of different varieties). Also, lettuce was the crop with the highest number of positive samples (5 samples; 18.5%), with levels between 0.06 and 0.50 mg/kg CS_2, lower than the MRL of 3 mg/kg. This large range of concentration is expected as different lettuce varieties produced in different farms were analyzed. In the 2017/2018 Pesticide Residues in Food Program (Programa de Resíduos de Pesticidas em Alimentos, PARA), conducted by the Brazilian National Regulatory Health Agency, 286 lettuce samples were collected throughout Brazil, 40 (14%) contained dithiocarbamate residues, of which 4 had residues above the MRL (Anvisa, 2020).

Table 1. Levels of dithiocarbamates, as CS_2, in the samples collected in 2018 at the Vargem Bonita's region of the Federal District, Brazil and the maximum residue limit (MRL) for each crop. Brasília, UnB, 2018.

Sample	**mg CS_2/kg (n)**	**MRL, mg CS_2/kg**
Arugula[b]	0.40; 1.1	NR
Bean pod	<0.05	0.3
Beet root	<0.05 (3)	0.3
Carrot	<0.05 (2)[a]	0,1
Chayote	<0.05	NR
Chicory	0.09	NR
Chili, *de cheiro*	<0.05 (2)	NR
Chili, *dedo-de-moça*	<0.05	NR
Coriander	<0.05 (4); 0.10	NR
Chive[c]	0.05, 0.07; 0.08; 0.10 (2); 0.20	NR
Eggplant	<0.05	0.5
Ginger	<0.05 (2)	NR
Leek[c]	0.09; 0.20 (3)	NR
Lettuce, *americana*	<0.05 (6); 0.20; 0.50	3
Lettuce, frise	<0.05 (8)[a]; 0.06; 0.20	3
Lettuce, *lisa*	<0.05 (2)	3
Lettuce, *mimosa*	<0.05	3
Lettuce, red *mimosa*	<0.05 (4)	3
Lettuce, red	0.07	3
Lettuce, romain	<0.05	3
Parsley	<0.05 (5); 0.09	NR
Pumpkin, *baianinha*	<0.05	1
Pumpkin, *moranga*	<0.05	1
Scarlet eggplant	<0.05	NR
Spinach	<0.05 (3)	NR
Sweet peper	<0.05 (3)	3
Sweet potato	<0.05	0.1
Tomato, cherry	<0.05	0.1
Watercress[b]	1.4	NR
Yam	<0.05	NR
Zuchinni, *Itália*	<0.05 (5)[a]	1
Zuchinni, *menina*	<0.05 (2)[a]	1

n= numer of samples, one when not specified; NR= not registered for the crop, Anvisa (2020); [a]one sample from organic farmer; [b]may give false positive results (*Brassicaceae* sp.); [c]may give false positive results (*Amaryllidaceae* sp.).

The only authorized dithiocarbamate for lettuce is metiram, however the method of analysis used in the study does not allow the identification of the compound applied in the field, since all are degraded to CS_2 in the analytical method. Moreover, it is not possible to know if the samples contain unauthorized dithiocarbamates. Although there are specific methods for the determination of dithiocarbamates in foods (Crnogorac *et al.*, 2008; López-Fernández *et al.*, 2012), the indirect method of analysis in this study is used by monitoring programs carried out in Brazil and in other countries, being the basis for the establishment of the MRLs (Anvisa, 2020; Efsa, 2021; Dawe, 2020). It is important to point out that none of the specific methods can discriminate all the dithiocarbamate compounds, including mancozeb from metiram.

Three parsley, coriander and chicory samples were also positive for dithiocarbamates, however these crops have no registered use of these fungicides and are, therefore, irregular in terms of Brazilian legislation. These samples were collected in the same farms where the CS_2 positive lettuce samples were collected, a crop for which the use of dithiocarbamates is legal. This indicates that farmers do not always consider the authorization to use the pesticide product in a crop, but its availability in the property. Parsley, coriander, and chicory are included in the group of smaller crops according to INC 1/2014 of the Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento, MAPA), as they have few products available for pest control, which can leave farmers with no options. However, it is not possible to say whether producers were aware of the illegality of this use. The use of pesticides in crops for which there is no authorization was identified in the PARA and the Programa Nacional de Controle de Resíduos e Contaminantes (PNCRC) conducted by MAPA. Data reported by the two programs for the period 2001 to 2010 showed that 72%

of the irregularities referred to the use of unauthorized pesticides for the crop (Jardim & Caldas, 2012). Similar results were found in the PARA 2017/2018, which analyzed 4616 samples of 14 foods (Anvisa, 2020).

Dithiocarbamates are not systemic, they are contact fungicides, that is, they do not translate through the plant (Fanjul-Bolado *et al*., 2020). For this reason, residues are unlikely to be found in root or tuber vegetables such as beets and carrots. Indeed, no dithiocarbamate residues were identified in any sample of this group of vegetables (Table 1).

Thirteen analysed samples were vegetables from the families *Brassicaceae* and *Amaryllidaceae*, which can generate false positive results in the analyzes due to phytogenic generation of CS_2 if the plant naturally contains compounds with sulfur or carbon-sulfur bonds (Perz *et al*., 2000; Ma'mun, 2021). In this study, all samples from the families *Brassicaceae* (watercress and arugula) and *Amaryllidaceae* (chives and leeks) showed positive results for CS_2 (0.05-1.4 mg CS_2/kg). Although these cultures do not have an established MRL, it is not possible to state that the CS_2 found was due to dithiocarbamate application.

Very few studies have analyzed dithiocarbamates in vegetables produced in Brazil, probably because these compounds need a specific method and are not included in the multiresidues methods for pesticide analysis (Anvisa, 2020; Mozzaquatro *et al.*, 2022). Vivian *et al.* (2006) identified dithiocarbamate residues in 17.6% of the 68 vegetable samples collected at an agroecological fair in the city of Santa Maria, State of Rio Grande do Sul, but none showed values above the MRL. Carvalho & Barbosa (2013) analyzed dithiocarbamate residues in 136 tomato samples produced in Minas Gerais between 2006 and 2008. About 66% had dithiocarbamate residues, but no sample had residues above the MRL. Monitoring data from PARA and PNCRC showed that 16.2% of the 30,786 samples of 30 foods collected from 2005 to 2015 in all Brazilian states contained dithiocarbamate residues (Jardim *et al.*, 2018). Tomatoes and peppers were the vegetables with the highest percentage of positive samples (30-34%), and 14.6% of lettuce samples contained these residues.

Several countries conduct programs to monitor pesticide residues in food. Australia data for the 2017-2018 period show that only 4 apple samples out of the 248 analyzed were positive for dithiocarbamates, none above the MRL (3 mg/kg CS_s), but 7 out of 85 pear samples were positive (8.4%), 4 of which above the MRL (Dawe, 2020). Dithiocarbamates were among the most frequently pesticides found in the European Union's monitoring program in 2019, with 34% of the more than 13,000 food samples containing residues above the MRL (Efsa, 2021).

In conclusion, 25% of the 84 vegetable samples analyzed collected from Vargem Bonita producers were positive for dithiocarbamates, as CS_2, from which five were lettuce samples, but all with levels below the MRL established by ANVISA. Three chicory, parsley, and cilantro samples were positive, but these crops have no dithiocarbamate registered use, which indicates the irregular use of the product, probably due to the lack of other legal phytosanitary options in the country. These results indicate the need of a constant monitoring of these fungicides by government agencies to mitigate any risk arising from the incorrect use of dithiocarbamate products.

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