Residue of ^{14}C-Paclobutrazol in mango

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ABSTRACT

Paclobutrazol (PBZ) is a growth regulator used to control vegetative growth, stimulating the reproductive capacity of plants. The PBZ remains active in soil for a long time and its half-life varies with soil type and climatic conditions, which may severely affect the development of subsequent crops. Therefore, this study aimed to evaluate the residues and metabolites of ^{14}C-PBZ in pulp and peel mango Tommy Atkins. The tests were carried out with mangoes grown in pots of stainless steel and the application of ^{14}C-PBZ was held in the soil in the canopy projection. To evaluate the level of waste ^{14}C-PBZ held burning 200 mg of pulp and the organic oxidizer $^{14}CO_2$ evolution was detected by liquid scintillation spectrophotometer. The results of PBZ residues for pulp and peel were 1.65 and 0.37% after two years of application and 4.30 and 1.32% after one year of application, respectively. Analysis of the residue of ^{14}C-PBZ and its metabolites by high performance liquid chromatography (HPLC) coupled to a photodiode array detector and was not detected the presence of metabolites of the PBZ samples analyzed sleeves.

Keywords: – **High performance liquid chromatography, mango, paclobutrazol, translocation,** *Tommy Atkins*

I. INTRODUCTION

Paclobutrazol (PBZ), also known as "cultar", is an important agrochemicals in the culture of mango, being a regulator of growth inhibitor of gibberellin synthesis, making it possible to control the growth of trees, reducing the need for pruning and handling of the crop, with achieving a high degree of response in growth and flowering, factors essential for the development of orchards. This product is applied directly to soil and in Brazil there are few diagnostic environmental impact, becoming, therefore, require a detailed study on the behavior of paclobutrazol.

Soil usually is the last natural reservoir for pesticides and other agricultural inputs. From this compartment, these products can be released into the air, for water sources and to living organisms, especially for microorganisms. Pesticides and other inputs may still remain active in soil for long periods, interfering directly, or even preventing the development of new crops [1].

Use of radioisotope ^{14}C as tracer allow to calculate the waste in culture, information on the distribution of residues in plants and animals and in the soil profile, and show the efficiency of extraction procedures for various components of the waste. Therefore, analytical procedures more precise ensure a minimum of problems and maximum benefits in the use of chemicals, allowing the detection of minimal amounts of these waste products and metabolites, allowing a more accurate trial regarding the behavior of these substances the environment, and providing recommendations for the use and regulation of products. For analysis of fruit samples, and identification of ^{14}C-PBZ and its metabolites using the extraction method with solvents followed by dry combustion, where the $^{14}CO_2$ produced is captured in an alkaline solution and subsequently analyzed by liquid scintillation spectrometry (LSS) and high performance liquid chromatography (HPLC). Although, the aim of this study was to assess the presence of paclobutrazol residues in hoses fruits.

2. MATERIAL AND METHODS

2.1 Paclobutrazol

Paclobutrazol ([2RS, 3RS] -1 - [4-chlorophenyl] - 4,4-dimethyl-2-[1,2,4-triazol-1-yl] pentane-3-ol) belonging to the class of growth regulators the triazole chemical group, with hazard classification class III. Its solubility in water is 35 μg ml^{-1}. The radioactive isotope ^{14}C-paclobutrazol used in the experiments (Figure 1) presents specific activity = 3.577 mCi g^{-1} and radiochemical purity > 95 %.
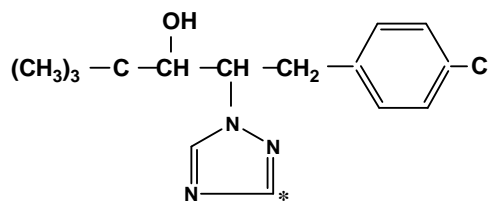

Figure 1. Chemical structure of paclobutrazol and * marking the location of ^{14}C

2.2 Aplication of paclobutrazol

To conduct the experiment with ^{14}C-paclobutrazol, was necessary that the plants were grown on stainless steel pots with soil, and the physical and chemical characteristics are: pH CaCl2 = 6.6, organic matter = 75 g dm^{-3}; Sand = 26 %; Silte = 23 % and Clay = 51 %.

The experiment consists of vessels with one hose plant, with two applications of 14C-PBZ, one after the other after pruning and harvesting the fruits of the season before. The application of paclobutrazol after the pruning of hose is used to enable the production season in addition to substantially increase the productivity [2].

Application of ^{14}C-PBZ was conducted according to Silva (2001) [3], via soil and projection of the crown. The application was in the afternoon, a day before the application received water and the vessels were removed 10 cm of soil layer on top, the projection of the crown and then made the application, followed by placing the soil removed. The vessels were kept in the care of good agricultural practice.

Implementation occurred in two periods, the first application of 14C-PBZ (application 1) were used four vessels, two of which received the application of the product and two were used as control. The second application (application 2) was performed nine months after application 1 in pots that had not received the treatment. Therefore, the experiment with plants that received the PBZ-14C in two different periods and the fruits analyzed in the same season of harvest it was verify the presence of waste of PBZ and metabolites.

The dose used (application 1) was 1.000 g ha^{-1}, which was prepared in a diluted solution volume of 750 mL of water/pot, containing 19.635 mg 14C-PBZ/vessel with activity of 80,000,000 dpm/pot, equivalent to 1.33 MBq. In application 2 was prepared in a diluted solution volume of 750 mL of water/pot containing 20.0 mg ^{14}C-PBZ/vessel with activity of 130,968,750 dpm/pot, equivalent to 2.18 MBq.

2.3 Evaluating the radioactivity of the fruits

Evaluation of fruits of hose plants is to determine levels of residues/metabolites of ^{14}C-PBZ in pulp sleeves, in order to verify the possibility of contamination/accumulation of PBZ in the soil for cultivation. This procedure was performed to harvest the fruit, then the removal of the shell and processed in a shake "Pique Walita", stored in glass bottle in frezzer. To determine the radioactivity was weighed 200 mg of samples in the gondola and subsequent firing porcelain in oxidized organic (900ºC for 3 min). The [14]CO_2 appears scintillator solution was captured in the carbo-sorbo and permafluor (2:1 v/v) and the radioactivity determined by LSS.

2.4 Paclobutrazol residues in mango fruits for analysis of HPLC/Flow

This work was developed in to 5 steps: (a) selection of growth regulator for mango samples, (b) optimization of chromatographic conditions, considering factors that supplies better resolution and less time of analysis, (c) development of method to analyse paclobutrazol in mango fruit, (d) determine paclobutrazol in mango samples by high performance liquid chromatography and (e) determine radioactivity in mango samples.

From the data of radioactivity was carried out to extract the pulp. The analysis for determination of residues of PBZ in chromatography, especially high performance liquid chromatography has been applied in studies of dissipation, especially in matrices such as soil and water.

For analysis we used standard analytical Paclobutrazol (Sigma) and the reagents and solvents: acetonitrile solution HPLC grade, ultrapure water (Pura-Q) (70:30), dichlorometane HPLC grade, anhydrous sodium sulphate PA and methanol HPLC grade.

Extraction procedure, adapted from Vaz et al. (2007) [4], followed up the steps as: Weigh 10 g of mango samples into a tube for centrifugal, add 30 mL of solution of acetonitrile: water (70:30); shake in Turrax for 2 min; centrifuge at 4000 rpm for 15 min, the supernatant transferred to round-bottomed flask, repeat the procedure in item 2 to 6, transfer the extract to the separation funnel, add 30 mL of dichloromethane, shake for ± 1 min, filter the organic phase in a filter paper containing ± 5 g of anhydrous sodium sulphate PA, repeat the procedure of item 9 to 11; evaporate the sample to dryness, resuspend in 10 mL of methanol in concentrator tube, inject 2 μL in high performance liquid chromatography coupled to a diode array detector and a Scitillation Flow Analyzer.

Chromatographic conditions of HPLC equipment (model LC 1200, Agilent Technologies) was, flow 0.4 mL min^{-1}, mobile phase was methanol and water (90:10), wavelength 227 nm, retention time 6.42 min., column temperature: 50ºC, injection volume of 2.0 μL and C18 column (250 mm x 4.6 mm x 5 μm) The method validation [4] was obtained by means of parameters of selectivity, linearity, accuracy, robustness and recovery.

3. RESULTS

Validation of the method parameters were evaluated as selectivity, precision, recovery, limits of detection (LOD) and quantification (LOQ). The limits of detection (LOD) and quantification (LOQ) were 0.10 and 0.75 μg L^{-1}, respectively.

According to Lanças (2004) and Green (1996) [5,7], a simple way to verify the selectivity of the chromatographic method is to observe the presence of peaks in the retention time of analyte, injecting the blank with the same matrix to be analyzed. It should be noted the

absence of peaks near the retention time. Retention time (RT) of PBZ standard was 6.42 minutes.

To prove the selectivity of the method, extractions were made of fruit, without the addition of standard and injected in triplicate and is not observed interfering peaks near the retention time of default. Neither the matrix effect was observed. As the repeatability of the chromatographic method, standard samples were injected at the same concentration and evaluated, obtaining 0.017 as standard deviation and coefficient of variation (%) equal to 0.2654.

Linear range was between 0.75 – 3.0 μg L^{-1}, equation of a straight line was $y = 80{,}734x + 9{,}1331$ and the correlation coefficient obtained was 0,9942.
Assessing the recovery (%) of the method after the analysis of spiked (1.0, 1.5, 2.0 μg L^{-1}), obtained as a result the values of recovery samples, 86.58, 79.33 and 92.11% respectively (Table 1). In recovery, the values found are in the range of 70 to 120% for all products screened [6].

Table 1. Recovery and relative standard deviation for PBZ in mango samples (n=3)

Compound	Spike (μg L^{-1})	Recovery (%)[a]
	1.0	86.58
Paclobutrazol	1.5	79.33
	2.0	92.11

[a] = Average of 3 tests with 3 injections.

For quantification of the PBZ samples of pulp was used external standard method [8,9,10], using standard curve obtained under the same conditions of the sample. The calibration curve was established correlating standard versus peak area, in concentrations 0.75, 1.0, 1.5, 2.0, 3.0 μg L^{-1} (Figure 2). It was used as control sample of the fruit without fortification (addition of the standard). For the blank, was followed by the extraction procedure, using only the solvents. From the samples were not detect residues of PBZ within the method and conditions of study, as shown in the chromatogram of Figure 2 (RT = 6.42 min).
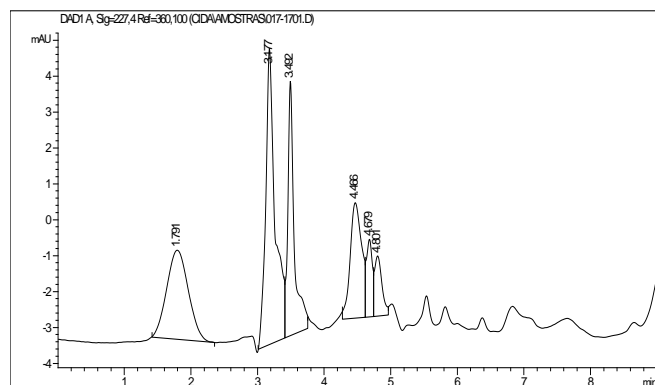

Figure 2. Chromatogram of analysis of PBZ residual in mango pulp.

4. DISCUSSION

Fruits collected showed a low percentage of radioactivity in the product applied by soil, an average of 1.65% to 4.30% for the pulp and to confirm that the analysis of ^{14}C-PBZ were measured by HPLC.

CONCLUSION

So, its concluded that as the translocation of Paclobutrazol at the plant air system, you can say that PBZ is not translocated to the fruit so that it can detect the presence of the original samples. The low level of radioactivity found in the fruit, through the combustion of the samples was confirmed by chromatography, which was not detected the presence of residues of ^{14}C-Paclobutrazol.

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