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Investigating the Effect of Washing, Saturating and Cooking Procedures on the Residual Amounts of Butachlor and Diazinon Vegetable Pesticides in White Rice

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ABSTRACT

Objective: The usage of pesticides has increased during the recent years. One the other hand, presence of harmful pesticides residue in rice and other food has caused a great concern among the consumers. Therefore, it is important to develop some pragmatic procedures to increase the food safety along with the reduction in consumption of pesticide residues. **Methods:** A gas chromatograph with electron capture detector was used to determine of residue of butachlor herbicide and diazinon insecticide in washed white rice, steeped white rice, cooked white rice to abkesh cooking method. The method consist of: 1- preparation calibration standards, 2- draw calibration curve, 3- Samples preparation 4- liquid phase extraction, 5- clean up, 6- analysis with GC electron capture. **Results:** The average percent of loss of the butachlor herbicide in washed white rice, steeped white rice, abkesh white rice were 69.99% , 81.00% , 90.73% and 66.22% , 72.67% , 88.90% for diazinon insecticide, respectively. The results obtained from effect of processes on the residual contents of butachlor herbicide in white rice indicated that the highest and lowest residual contents of pesticides in white rice were related to control treatment and abkesh cooking treatment, respectively. **Conclusions:** The results of present study proved that rice processing treatments such as washing, steeping, and cooking lead to a significant reduction of pesticides' residue. Therefore, more process treatments, caused more reduction pesticides residue in white rice treatments.

1.INTRODUCTION

Nowadays we know that the agricultural poisons have not influence merely on the targets but after entering into ecosystem they can have side effects on other creatures (Cavas, 2011). Accumulation and duration of these pollutants in agricultural products and in aquatic environments are serious threats for living creatures and can directly influence on human through food chain. Most of these chemical compounds are mutant and lead to cancer or growth reduction (Kumar et al., 2010). However there may be very low densities of these agricultural poisons in the environment not leading to quick and observable damages, they can cause damages at the level of death for living creatures (Dong Nguyen et al., 2008). After wheat, rice is one of the most important grains. Comparing with wheat, its industrial value is less

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but its nutritive consumptions are much more important so that the rice is the main food of about 50% of earth population and having less protein, however, its protein quality is higher than wheat's (Jahn et al., 2000). Butachlor is a selective and pre-planting pesticide to fight with one-year grasses, some of wide sheets and some of aquatic weeds used in direct and seedling and oat. Diazinon is an organic phosphorus insecticide have tactile, respiratory and digestive effects on mites through disturbing choline stabilizer. It is used to fight with pests in vegetables, tobacco, grass, planted vegetables, farms and ecological vegetables and plants. In Iran, it is used widely against stem worm (Gorjan et al., 2009).One percent of pesticides poured on the plant is absorbed. Two to five percent of the toxins used in the soil are absorbed to the root. They show that a great part of consumed pesticides contact with non-target cases. In Iran some toxins are used in rice farms that can kill salmon 0.57 in a million. Concentration of Butachlor at the beginning of spraying in the farm is about 3 parts in a million which is three times lethality for salmon. It should be noted that the lethality of these toxins is more than very dangerous toxins like parathion (Mousavi, 2010).

The effect of food processing on pesticide residues in rice has been the focus of a few recently published articles. Chen et al. showed the amount of organo-chlorin pesticide in rice, human fat and fish using two-dimension gas chromatography. The results showed that the residual concentration level of the pesticide in rice was less than human fat and fish. So, the residual of this pesticide in the foods should be measured to consider its potential hazards (Chen et al., 2007). In another study, Funk et al. investigated the residual level of pesticides in cooked rice and noodle. Noodle and rice were cooked in chicken soup with Lindane, Dildrin and DDT compounds. The results showed that a small amount of each pesticide has been transferred to the cooking rice and noodle. However, a portion of the pesticides were residual in the rice and the noodle. The amount reduced while cooking which can be caused by distillation, thermal decomposition or both (Funk et al., 2009). Kaushik et al. investigated food processing methods to reduce the residual pesticides. The results showed that processing operations like thermal, freezing, grounding, peeling, malting and fermentation operation reduced the pesticides significantly (Kaushik et al., 2009). Wang et al. probed the daily consumption and danger assessment for human due to organo-phosphorus toxins based on Cambodia food data. Dichloro diphenyl trichloroethane (DDT) and (HCHs) hexachlorocyclohexanes in Cambodia foods were significantly more than developing or developed countries verifying the probability of cancer for the Cambodian (Wang et al., 2011). Pareja et al. searched the analysis methods of the residual pesticides in rice. They considered extracting and cleaning ways, e.g., liquid-liquid extraction, solid-phase extraction, liquid under-pressure extraction, jelly chromatography and liquid- hypercritical extraction with QuEChERS methods (quick, easy, cheap, effective, strong and safe) which currently are widely used. Traditionally, determining the residual amount of pesticides in rice with gas chromatography is done through the mass spectrometer. The application of a new group of pesticides has led the lab to use liquid chromatography with mass spectrometer (Pareja et al., 2011).Yang et al. considered the synergistic effect of washing and cooking on removing the pesticides in different foods. Most of the residual amounts of the determined pesticides was lower than the highest amount of the residual level. The results showed that a mixture of washing and cooking remove the residual pesticides effectively (Yang et al., 2012).

2. MATERIALS AND METHODS

2.1. Samples

It is important to note that the samples of white rice are free from investigated vegetable pesticides. First 1 Kg of raw white rice is transferred to lab and the laboratory samples are weighed at the amount of 15 g. The population of the study are T_1 treatment is raw white rice which is spiked with investigated vegetable pecticides. T_2 is the raw rice with pesticides which is washed 5-6 times to be cleaned fully. T_3 is the raw rice with pesticides which is washed 5-6 times to be cleaned thoroughly then saturated in a dish containing 5-6 times more water than rice weight at 25 °C for 30 minutes and finally the water is emptied. T_4 is sample 3 cooked by abkesh method. In this method, the rice is washed 5-6 times to be cleaned; then, it is saturated in a container having 5-6 times more water than the rice weight. In the next phase the water is emptied and the rice is boiled for 12 minutes in the water (5-6 times more than the raw rice weight) and the water is emptied and cooking continues for other 10 minutes (Sengupta et al., 2006; Rewthong et al., 2011).

2.2. Preparing working standards

First the original standard Butachlor and Diazinon toxins were made so that 0.01 g (10 mg) of the toxins was weighed and then, separately in a 10 ml volumetric flask with stone, it was reached to the mass of 10 ml. Every ml of this solution contains 1 mg toxin. Due to high concentration of the original standard of Diazinon and Butachlor, an interface standard was prepared. To this end, specific densities of the toxin were taken from the original standard by micro syringe. First, a solution with 100 PPM was made up of the two toxins. Then, based on the order of working standard, 10 mg/ml and 5 µg/ml, were made for the most concentrated solvent of Diazinon and Butachlor with stone. Then other working standards were prepared with 50/50 ratio and finally, Butachlor with concentrations of 10 µg/ml , 5 µg/ml , 2.5 µg/ml and 1,25 µg/ml and Diazinon with concentration of 5 µg/ml , 2.5 µg/ml and 1,25 and 0.62 µg/ml were injected to gas chromatograph machine to draw the standard curve(Anonymous, 2001). The first figure shows the chromatogram curve of the most concentrated standard solution of Butachlor pesticide and Diazinon insecticide.

2.3. The conditions of gas chromatograph machine

Nitrogen gas was used as the carrier gas. The primary temperature of the column was 180 kept in 2 minutes, the so-called retention time. This act is done to separate the solvent peak fully from other peaks. Then with a slope 5 °C/min in the temperature increased continued to 240 °C. Electron Capture Detector (Ecd) with 280°C temperature setting was used. Injector temperature is 250°C. The model and the type of the used column is BPI, Capillary with 0.15 mm ×50 m and 0.5 internal diameter.

2.4. Drawing the calibration curve

Drawing the calibration curve is one of the quality control factors which is mostly used for determining the accuracy of analysis machines. First, different concentrations of the standards of the toxins are injected to gas chromatograph; then, using the concentration and the area of the standard peaks of the toxins, the calibration curve is drawn and the regression line is resulted. This equation is used to calculate the residual amounts of toxins in white rice.

2.5. Sample preparation

2.5.1. Contaminating the rice with vegetable pesticide

1 cc of the mixture of the most concentrated standard was used to contaminate every 15 g of white rice. Then, regarding the mentioned treatments, the related processes were done on 15 g of white rice for every laboratory samples.

2.6. Liquid Phase Extraction

First, 15 g of the prepared white rice was transferred to a lid covered glass container, then 50 cc of Petroleum Ether solvent (40-60 °C boiling point) was added and mixed with a shaker (speed: 120 RPM) for 30 minutes. After that, the top liquid of the glass container was transferred to a beaker and again 50 cc of Petroleum Ether solvent was added to the rice in the covered container and mixed for 30 minutes by a shaker .Next, the top liquid in the glass container was added to the previous beaker and all the stages were repeated once. Totally, 150 cc of Petroleum Ether solvent was used and the time for shaking in extraction phase was 90 minutes. In the next phase, the top liquid was filtered by 25 g of Sodium Sulfate Anhydrous in a 250 ml beaker through filter paper. The refined liquid was evaporated to the volume of 5 ml (Anonymous, 2001).

2.7. Clean up

First the column was filled with organic solvent (N-Hexane) and then 1 cm of Sodium Sulfate Anhydrous (to absorb the residual water in the sample), 5 cm of activated silicagel special for column (60 mesh) were added to be deposited. Next, 0.5 cm of activated florezyl was used to remove the fat. Again, 1 cm of sodium sulfate Anhydrous was added to the cleanup column and the refined liquid in extraction phase evaporated to 5 ml was transferred slowly by the column's wall not to disturb the layers in the column and the Teflon valve was opened to empty the organic solvent to 1 cm residual in the column. Finally, 150 cc of hexane was added to the content of the cleanup column slowly by the column's wall. To add the refined liquid related to extraction phase to the column and adding N-Hexane in the last stage to the cleanup column, the Teflon tap was a little open to implement the cleanup act. The liquid of this phase was collected in the beaker and then was dried by the water bath (50-60 °C). Then it was solved in 2 cc of stone and injected to gas chromatograph machine (Anonymous, 2001).

2.8. Data analysis method

According to the mentioned treatments, to analyze the data of the experiment, the fully random repeated in three times is used. In this research, 4 treatments are done and regarding a three-time repetition, there will be 12 experimental units. Dunken multi-range test is done through SPSS Software to compare the means.

3. RESULT

Based on the results of mean comparison of the residual amounts of Butachlor and Diazinon pesticides in samples of white rice it was shown that washing, saturating and cooking processes reduced significantly the residual amounts of both pesticides in the rice samples in relation to control treatment. There was a meaningful difference between washing and other processes regarding the residual amounts of Butachlor but there wasn't a meaningful difference between saturation and cooking processes. Abkesh cooking method, saturating and then washing in order showed more reduction of Butachlor in white rice. There wasn't a meaningful difference between washing and saturation processes regarding the residual amounts of Diazinon and also there wasn't a meaningful difference between saturation and cooking processes. However, there was a meaningful difference between washing and abkesh cooking method. The processes of cooking, saturation and washing showed the highest level of reduction in residual toxin in white rice samples, respectively.

The figures 2, 3, 4, and 5 show the chromatogram curve of the control treatment rice, the washed rice, the saturated rice and the cooked rice.

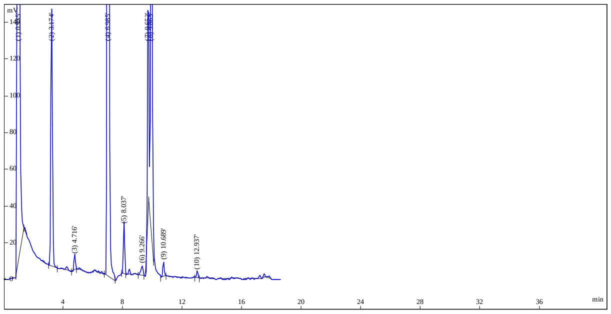


Figure 1. A representative chromatogram obtained for the two pesticides (diazinon, Rt=3.174; butachlor, Rt= 6.985)

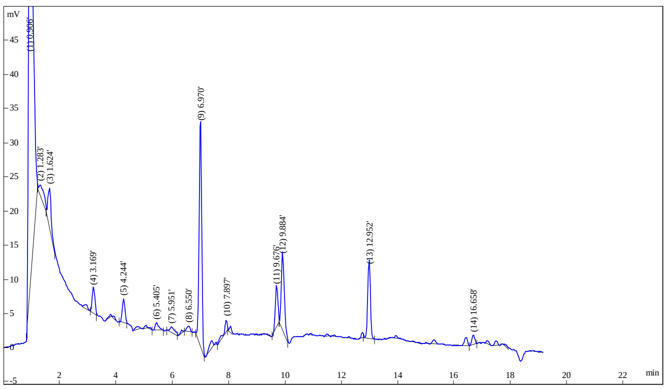


Figure 2. Chromatogram obtained from two pesticides (diazinon, Rt=3.169; butachlor, Rt= 6.970) in control sample

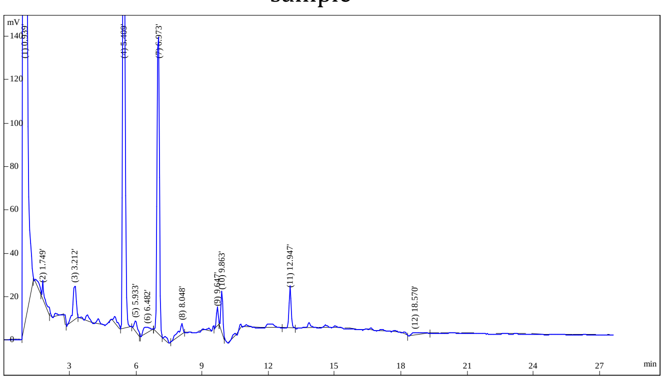


Figure 3. Chromatogram obtained from two pesticides (diazinon, Rt=3.166; butachlor, Rt= 6.929) after washing procedure

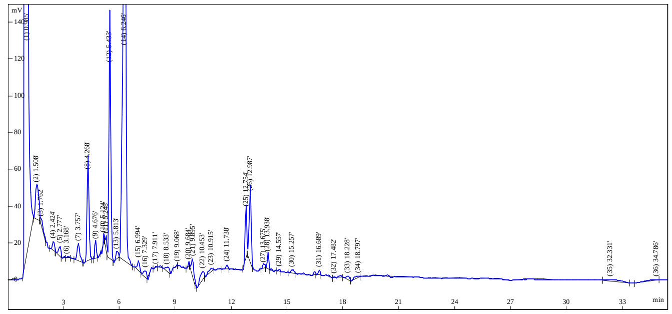


Figure 4. Chromatogram obtained from two pesticides (diazinon, Rt=3.212; butachlor, Rt= 6.973) after saturating procedure

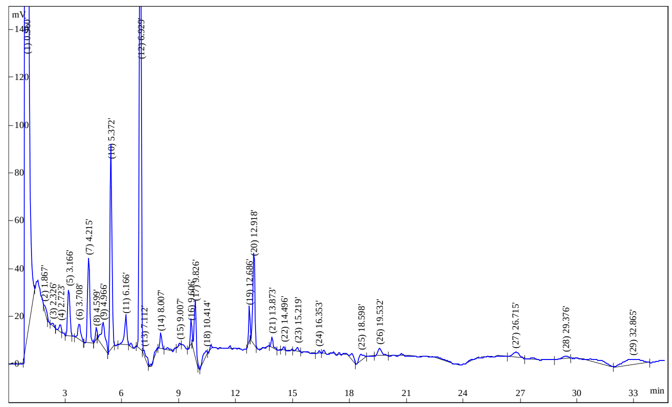
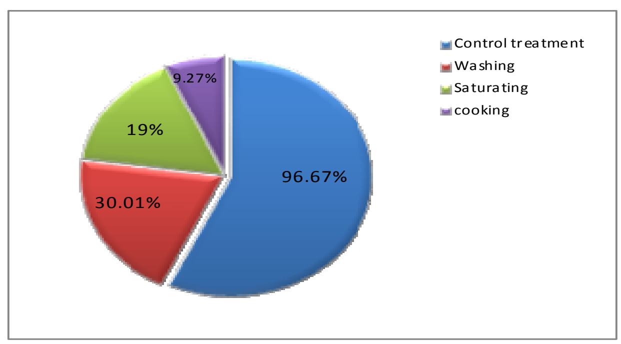


Figure 5. Chromatogram obtained from two pesticides (diazinon, Rt=3.168; butachlor, Rt= 6.994) after the cooking procedure



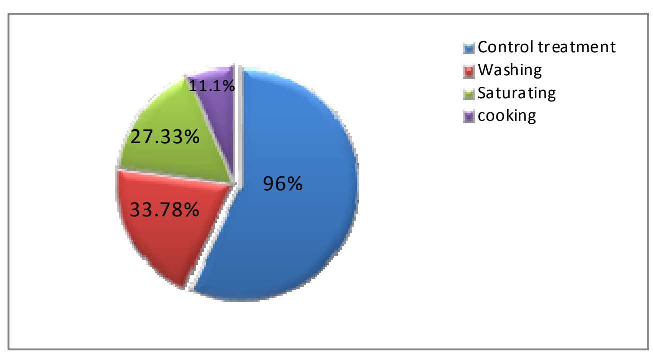


Figure 6. The results on the effect of studied procedures on the residual amount of Diazinon pesticide in white rice

4. DISCUSSION

The surface remain of the toxins are caused by simple washing process but systemic remain of pesticides keep on inside the texture. There are evidences for different types of agricultural products and pesticides showing that the remain of pesticides reduce in line with the time of washing process caused solubility and the tendency of internal cuticular waxes or deeper layers (Ahmed et al., 2011). The reason of the reduction of the residual amount

of the pesticides in washing process is their polarization degree of and solubility in the water which reduces the toxins to specific amounts depending on time and washing process. Regarding Butachlor which is a systemic pesticide penetrating to the plant tissue, increasing the volume of the water and washing time can reduce it more. It should be noted that the solubility and the tendency of cuticular waxes movement interpret this reduction. On the other hand, the reason of more reduction in residual pesticides of white rice samples in saturating process comparing to washing process is the rational between time and washing process where increasing time and volume of the water reduces more the amounts of the residual toxins comparing to washing process. Parameters such as hydrolization level, evaporation pressure, solubility in water and molecular weight has effects on reduction of pesticides after the cooking processes (Shoeibi et al., 2011). The stages and the conditions used in cooking are numerous. The details of time, temperature, humidity reduction rate, opening or closing of cooking system have high effects on the residual levels of pesticides. Hot washing and blanching act have more influence in relation to cold washing. The decomposition and evaporation of the residual level of pesticides increase with more cooking temperature (Ahmed et al., 2011).

RECOVERY

The recovering of the method is done to approve the accuracy, exactness and repeatability of the method. Regarding that the present study is on the samples of the raw white rice and the cooked white rice, the recycling on them is repeated three times based on the experiment method. The recycling was estimated based on calibration curve. LOQS, Retention Time, concentration and contamination level of the rice related to two pesticides for recovering test and recovering percentage are shown in table 1.

Table 1:
Specifications of LOQs ,Concentration of pesticides for recovery test , level contamination of rice with Pesticides for recovery test, recovery(%)

Average recovery (%)		contamination Level of rice with Pesticides for recovery test		Concentration of pesticides for recovery test		LOQ (µg/kg) (ppb)	pesticides
Raw rice	**Cooked rice**	**Raw rice**	**Cooked rice**	**Raw rice**	**Cooked rice**		
84.33	94.35	0.8, 1.6, 2.4	1.65, 3.3, 4.95, 6.6	8	50	40	Butachlor
82.58	104.5	1.25, 2.5, 3.75	1.65, 3.3, 4.95, 6.6	12.5	50	40	Diazinon

CONCLUSION

The results of the study showed that washing, saturating and cooking processes had meaningful difference in comparison with control treatment. The results of the residual levels of Butachlor and Diazinon pesticides in the samples of white rice showed that the control treatment had the highest residual amount of the pesticides and the sample of cooked rice in abkesh method had the lowest amount of the residual toxins. In effect, the increase of the processes phases caused more reduction of the residual pesticides in white rice samples.

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