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ORIGINAL ARTICLE

DETERMINATION OF IMIDACLOPRID PESTICIDE RESIDUES IN CUCUMBER AND EFFECT OF SOME FOOD PREPARATION METHODS IN REDUCING RESIDUES

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Abstract : Cucumber is widely cultivated in Iraq and the application of systemic and protective pesticides, the main choice of pests control particularly in greenhouse-grown systems. In this research imidacloprid pesticide was used with the recommended dose on the cucumber crop. Cucumber was harvested at 1h,1 ,3, 5, 7, 10, 14, 21days after the application to determine the residue of imidacloprid. The effect of peeling and washing with water (5^0C, 25^0C and 35^0C), sodium chloride solution, Sodium bicarbonate mixture with sodium chloride solution and liquid detergent was evaluated in reducing imidaclopride residues. Samples were extracted by the QuEChERS procedure and then analysed using High performance liquid chromatography (HPLC). One hour after pesticide application the maximum residue levels (MRLs) of imidacloprid were higher than that of Codex standard level. One day after pesticide application, the levels of imidacloprid were increased to reduce below MRLs in the seventh day after spraying. Food processes is used in this study. High percentage of the pesticide in the preliminary samples was reduced to 100% after one hour and the reduction rate after one day was 35-73%. The reduction rate in subsequent samples was low. Recovery test for target analytes was in the range between 103 and 113%. The washing and peeling procedures lead to the decrease of pesticide residues in greenhouse cucumbers. Among them, the peeling procedure has the greatest impact on residual reduction. The higher content of imidacloprid residues in flesh showed its penetration from the skin into the flesh. The results provided more understanding of imidacloprid distribution as well as the effective role of peeling in reducing residues in cucumber.

Key words : Imidacloprid, Pesticide, Residues, Cucumber, QuEChERS, HPLC.

1. Introduction

Fruits and vegetables are important components of the human diet since they provide essential nutrients that are required for most of the reactions occurring in the body. A high intake of fruits and vegetables (five or more servings per day) has been encouraged not only to prevent consequences due to vitamin deficiency but also to reduce the incidence of major diseases such as cancer, cardiovascular diseases and obesity. Like other crops, fruits and vegetables are attacked by pests and diseases during production and storage leading to damages that reduce the quality and the yield. In order to reduce the loss and maintain the quality of fruits and vegetables harvest, pesticides are used together with other pest management techniques during cropping to destroy pests and prevent diseases [Gilden *et al.* (2010)]. Cucumber plant *Cucumis sativus* is one of the most important vegetable crops in Iraq and world, this vegetable infested by several pests causing serious quantitative and qualitative damages. The use of pesticides in agriculture has helped to increase productivity and has thus contributed to steadily rising food production and led to increased yields in arable farming, but they can eventually pose a risk or threat to both animal and human life [Barbash (2006), PAN (2003)]. Residues of pesticides in raw foods could affect the ultimate consumers especially when freshly. Pesticide residues have been found in various fruits

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and vegetables; both raw and processed. One of the most common routes of pesticide exposure in consumers is via food consumption [Keikotlhaile *et al.* (2010)]. Imidacloprid 1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylideneamine,is an extensively used insecticide for crop protection in the worldwide from the last decade due to its low soil persistence and insecticidal activity at low application rate [Chao & Casida (1997)]. Imidacloprid is pesticides recommended for use on cucumber to control pests. Pesticide residues in agricultural crops can be reduced through post-harvest and pre-ingestion treatment. The reduction rates achieved during treatment vary according to the physical chemical properties of the pesticides, the shape and texture of the goods, and the methods used. Washing can lead to the removal of pesticides residues from agricultural commodities [Kwon *et al.* (2015)]. The removal of pesticides with the washing of raw agricultural commodities (RAC) may be performed not only through the dissolution of pesticide residues in the washing water or the rinsing with chemical baths (detergents, alkaline, acid, hypochlorite, metabisulfite salt, ozonated water etc.) [Holland *et al.* (1994)]. But also through the removal of dust or soil particles previously absorbed residues from the outer layer [Guardia Rubio *et al.* (2006)]. HPLC is now one of the most powerful tools in analytical chemistry. It has the ability to separate, identify, and quantify the compounds that are present in any sample that can be dissolved in HPLC, is the most accurate analytical methods widely used for the quantitative as well as qualitative analysis of pesticides [Rao *et al.* (2015)]. Present study determines residues level and reduce imidacloprid during use of household processes (washing water at different temperatures, solutions and detergent) in cucumber harvested after incubation in greenhouses.

2. Materials and Methods

Chemicals, Reagents and Apparatus

Analytical standard imidacloprid (99.5% purity) and confidor (200 SL) systemic pesticide, rate of application: 50 ml / 100 L water from Bayer company (Germany), Acetonitrile (ACN) (liquid chromatography grade) Anhydrous magnesium sulphate ($MgSO_4$), and water ionized (HPLC grade) from ROMIL company (England), Sodium chloride (NaCl) (99.5% purty) were purchased from Sigma-Aldrich (Germany) and primary-secondary amine (PSA) was provided by Agilent Technology. HPLC (CTO-20AC) Shimadzu Corporation [Kyoto (Japan)].

Pesticide spraying and sampling

The field trial was performed in a greenhouse at a research station affiliated to the Agricultural Research Department, Ministry of Agriculture, Iraq in November 2017. The cucumber cultivar used was mawasim (the seeds from Agri seed company, USA). Pesticide was sprayed once using confidor (imidacloprid 20%sl). It was applied at the dosage recommended by regulation guidelines (50 ml/100 L). The samples were collected randomly from the lower, middle, and upper rows of bushes. About 2.5-3 kg of cucumber was collected after sprayed, and transported to the laboratory by a polyethylene plastic bag [Chavarri *et al.* (2004)]. Moreover, control samples (without any processing) were taken in each step to compare the results (Table 1).

Sample preparation and processing.

Each sample was divided into 8 parts according to the procedures required to reduce the residues of the pesticide imidacloprid after removing the impurities and soil.

Table 1: The dates for collecting cucumber fruits after imidacloprid spraying.

Samples	Date
Zero time (1 hour after sprayed)	7 /11/2017
1 day	8/11/2017
3 days	10/11/2017
5 days	12/11/2017
7days	14/11/2017
10 daye	17/11/2017
14 days	21/11/2017
21 days	28/11/2017

i) One of the parts before washing is crushed with a mixer and homogenized in a high speed laboratory homogenizer and then frozen at -20^{0}C pending analysis [Yang *et al.* (2012)].

ii) Another part from the eight parts was peeled with a special peel knife and the shell thickness was 0.3 mm [Leili *et al.* (2016)].

iii) Three parts were washed with tap water after the water temperature was controlled by an alcohol thermometer (5^{0}C, 25^{0}C and 35^{0}C) to wash each part in water at one specific temperature for 2 minutes and dried with a paper towel [Leili *et al.* (2016), Hassanzadeh *et al.* (2010), Elkins (1989)].

iv) Part of the eight parts were washed with 2% NaCl for 2 minutes with light rubbing by hand and then rinsed with water and dried with a paper towel [Shiboob (2012)].

v) The other part of the cucumber was also washed for 2 minutes with a solution of sodium carbonate and sodium chloride. Its concentration was 5%, mixed with two equal amounts of weight and diluted with water to form a concentration of 5%. After washing, it was washed with water and dried with a paper towel [Liang *et al.* (2012)].

vi) The last part of the cucumber was washed with a liquid detergent solution. The fruit was submerged for 2 minutes and then washed with water and blloted dry with a paper towel [Liang *et al.* (2012)].

All samples were crushed and homogenized and then frozen at -20^{0}C for pending analysis.

HPLC conditions

The HPLC (model Shimadzu LC-20AD Koyto, Japan) equipped with an UV detector at a wavelength of 230 nm and Waters column C18 25 mm × 4.6 mm was used to measure imidacloprid residue with the mobile phase of acetonitrile/water (10/90 v/v) with flow rate was 0.5 ml/min, Oven temperature was 40^0 C and last injection volume was 5µL.

Extraction

The QuEChERS method (quick, easy, cheap, effective, rugged, and safe) was used for the extraction of imidacloprid from the cucumber samples (AOAC 2007.01). 10.0 g of homogenized sample was placed into a 50 ml centrifuge tube with 10 ml of ACN. Then at vortex for 5 minutes ensuring that the solvent interacted well with the entire sample. Then, 4.0 g of anhydrous $MgSO_4$, 1.5gm Na - acetate and 1.0 g of NaCl was added repeating the shaking process for 1 minute to prevent coagulation of $MgSO_4$ and put in centrifuge 3500 rpm /5 min, the upper layer was cleaned by dispersive solid-phase extraction with 0.5 g of primary secondary amine and 150 mg of anhydrous $MgSO_4$. The mixture was then shaken for 1 minute and centrifuged for 5 minutes at 3500 rpm. Clean the extract with a 0.45µm filter and, then 5µl of this solution were injected into HPLC.

Recovery and calibration studies

Recovery experiments were carried out by spiking cucumber at different levels to establish the reliability and validity of analytical method adopted. Calibration curves and cucumber fortified samples were prepared by using working standard solutions. Cucumber samples fortified at 0.1 and 0.5 mg kg^{-1} were processed as previously described and analyzed by HPLC to evaluate the accuracy and the precision of the analytical procedure. Recovery tests were replicated three times for each fortification level. The limit of detection (LOD mg kg^{-1}) of each analyte was determined as the lowest concentration giving a response three times the standard deviation of the baseline noise defined based on the analysis of three control samples. The limit of quantification (LOQ mg kg^{-1}) was determined as the lowest concentration of a given compound giving a response that could be quantified with relative standard deviation lower than 15%. [Hassanzadeh *et al.* (2012), Xia & Ma (2006)].

3. Results and Discussion

Method's Performance

The analytical method was developed so as to provide a rapid, accurate, and efficient means of determining imidacloprid residues in cucumbers. Mean recovery value obtained for imidacloprid was 103% and 113% with relative standard deviation (RSD) values below 15% in the fortification range from 0.5 to 0.1 mg kg^{-1}. Calibration for quantification was carried out by use of external standard calibration curves; calibration curves were linear with correlation coefficients being better than 0.998 for both analytes (Fig.1). Retention time of imidacloprid under these conditions was observed to be 6.859 minutes. The LOD and LOQ for both analytes in the cucumbers fruits were 0.001 and 0.027 mg kg^{-1}, respectively, ensuring LOQ values

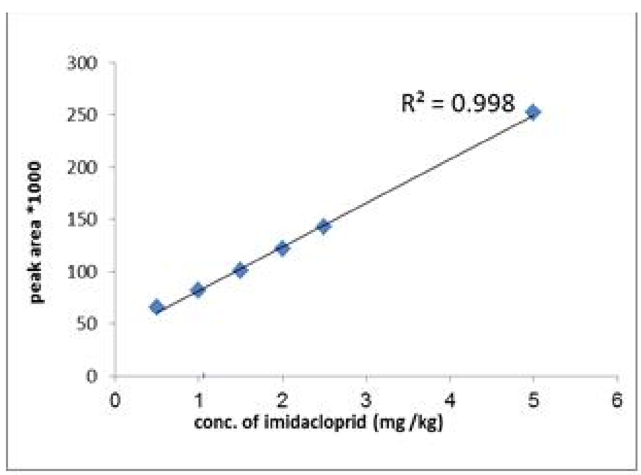

Fig. 1: HPLC chromatogram of standard solution and calibration curve of imidacloprid

significantly lower than the MRLs established by the Codex. Sample chromatogram of retention time for imidacloprid at samples were analyzed in HPLC is shown in Fig. 2.

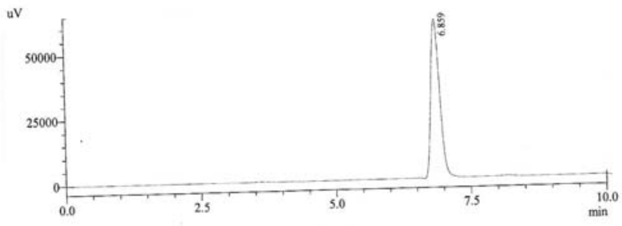

Fig. 2: Retention time of imidacloprid

Imidacloprid Residues

The obtained results of this study show that imidaclprid residues on cucumber fruits are comparable to those reported in earlier studies. The initial residues of imidacloprid for the investigated unwashed cucumber fruits were 1.179 mg/Kg. These figures were decreased to 0.931 after 7 days of spraying. Which is less than MRL according to the recommendations of the Codex Alimentarius Commission (2004) of the World Health Organization and the Food and Agriculture Organization, where the maximum limit of residues of imidacloprid pesticide in the fruits of cucumber is 1 mg/kg, which can be harvested after the seventh day of spraying [Nasr *et al*. (2014)]. Data in Table 3 indicate the amount of imidacloprid residues on and in cucumber fruits taken after different time intervals from the last foliage applications. It is noted that the pesticide completely disappeared in the sample of the tenth day and the following samples, since no traces of the pesticide were detected. The decrease of residues was not significantly ascribed to the growth diluting effect but caused by degradation of the pesticide itself [Nasr *et al*. (2014)]. Half-life (t/2) for degradation of imidacloprid in cucumber was observed to be 6.45 days. Imidacloprid is a systemic pesticide with physical/chemical properties that allow residues to move into treated plants and then throughout the plant via xylem transport and translamilar between leaf surfaces movement.

Following the residues of the imidacloprid pesticide in the fruits of the cucumber harvested at intervals specified in this study, we note that the remaining pesticide was raised in cucumber to reach its peak in the harvested sample. After three days of spraying, the remaining residues were 5.41 mg/kg. It can be explained that the phloem transmits sugars that are represented in green leaves to other parts of the plant. In certain circumstances, sugar can move in the bark from its storage places to the growing places.

It is the same path normally taken by pesticides in the phloem, as the leaves of small plants do not export sugar including the pesticides that reach these leaves and remain in the bark. Small leaves begin to export sugar, they usually move up and towards the top of the plant. Therefore, the orientation of the pesticides from these leaves to the roots is unlikely because the roots usually get their food from the leaves advanced in the near age and given that the fruits and seeds are the centers that gather sugar in which it condenses, during the life of the plant and during the formation of fruits and seeds transmitted to pesticides and gather them [Ahmed (2003)].

Table 3: Residues of the imidacloprid pesticide in the harvested samples

Time after application(day)	Residues (mg/kg)
Zero time (1 hour after sprayed)	1.179
1	1.942
3	5.410
5	2.890
7	0.931
10	0.000
14	0.000
21	0.000

The concentration of the imidacloprid pesticide used when spraying was 100 mg/kg. But, the different levels of initial deposits of both tested pesticides on fruits of cucumber are mainly due to many factors; the ratio of surface to mass area and character of treated surface, smooth or rough and waxy or non-waxy [Abo El-Ghar & Ramadan (1962)]. Systemic and non-systemic character of both compounds, high wax content of fruit surface and hydrophilic balance of investigated pesticides controlled the penetrability of applied agrochemicals into fruit tissues [Cabras *et al.* (1998)]. Degradation and dissipation residues of imidacloprid from cucumber fruits happened because the initial deposits and residues at different intervals of these pesticides are influenced by different factors: evaporation of the surface residue which is dependent on temperature condition, biological dilution which is dependent on the increase mass of fruits, chemical or biochemical decomposition, metabolism and photolysis. Great interest to note that the same factors were studied by several investigators. Christensen (2004) reported that the decline of pesticides may due to biological, chemical or physical processes, or if still in the field, due to dilution by growth of the crop. Plant growth particularly for fruits is also responsible to a great extent for decreasing the pesticide residue concentrations due to growth dilution effects [Walgenbach *at al*. (1991)]. In addition, the rapid dissipation of originally applied pesticide are dependent on a variety of environmental factors such as sunlight and temperature [Lichtenstien (1972)]. However, high temperature is reported to the major factor in reducing the pesticides from plant surface [Awad *et al*. (1967)]. Light plays an important role in the behavior of pesticide in the environment [Zeep and Cline (1977)]. In this respect, several investigations have been carried out to examine the residual behavior of these pesticides on treated plants. Wagner (2016) reported that imidacloprid is rapidly moved through plant tissues after application, and can be presented in detectable concentrations in tissues such as leaves, vascular fluids, and pollen. The disappearance of the imidacloprid pesticide permanently and not to monitor the remaining samples in the sample drawn after 10 days of spraying came in line with the results found by Hassanzadah as the imidacloprid pesticide was not monitored in cucumber fruits after 13 days of spraying, and pointed out that its dissipation could mainly be attributed to degradation by chemical and physical properties and less by growth dilution

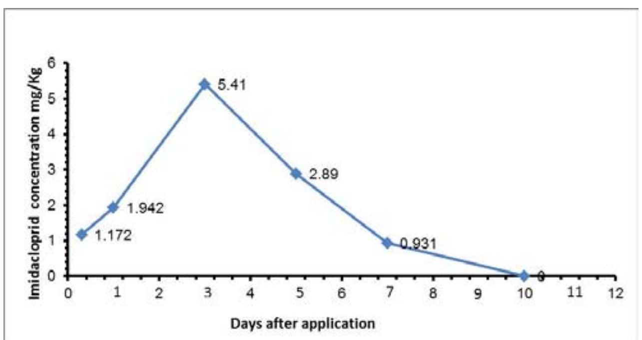

Fig. 3: Dissipation of imidacloprid from cucumber following its application

effects when the cucumber were almost mature [Hassanzadeh *et al*. (2012)] (Fig.3).

The effect of peeling and washing with water and solutions on the residues of imidacloprid

The food preparation process in this experiment had an effect in reducing the residues of the imidacloprid pesticide. However, this reduction does not continue because the pesticide of imidacloprid is a systemic pesticide which, over time is applied to the plant tissues and fruits. It is difficult to remove or reduce a large percentage of the herbicides. These manufacturing operations are carried out to minimize some of these residues. Peeling which, is the removal of the crust or the covers of the fruit has noted significantly reduced or reduced the pesticide has reached 100%, as well as the washing of water at different temperatures and solutions used in the experiment. However, this proportion of the reduction decreased as we departed from the date of spraying for residues observed in subsequent samples and the reason is as we mentioned earlier the nature of the systemic pesticide and this is what we see in Table 4.

The preparatory processes used in this study to reduce the residues of imidacloprid contributed to the reduction of the pesticide significantly in the samples withdrawn after one hour and one day after spraying with a reduction of 73% -100%. The peeling in reducing the residues of imidacloprid in primary deposits is the most food preparation. The effectiveness of the removal of pesticide residues from the fruit, which works to remove the crust of all layers of wax and other classes. (Fig.4) and these were identical with what was found by Lilei *at al*. (2016) but in the subsequent samples there was no effect of the process of peeling in the reduction of pesticide. Con Imidacloprid pesticides Which move into the plant over time. The systemic action of a pesticide residue in this case is not always correlated with decreased reduction of pesticide

Table 4: Imidacloprid Residues after Preparation of Processes (mg kg^{-1})

Samples after applicatio n	No proess	Peeling	Washing with water at temperture			Wash with solutions		
			5^0C	25^0C	35^0C	2% NaCl	5% NaCl + Na bicarbonate	Detergent
Zero time	1.179	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1 day	1.942	0.87	1.521	1.207	1.004	1.002	1.000	1.211
3 days	5.410	3.55	4.820	4.105	3.799	4.103	4.183	4.282
5 days	2.890	2.211	2.762	2.527	2.400	2.411	2.406	2.400
7 days	0.931	0.898	0.928	0.917	0.900	0.900	0.912	0.911

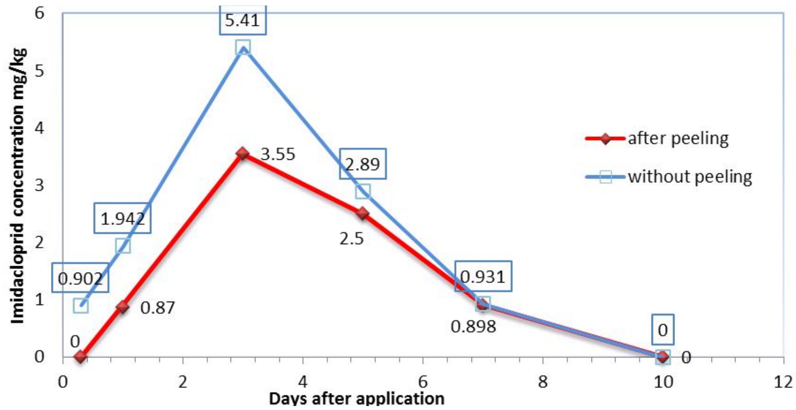

Fig. 4: Peeling effect in the reduction of imidacloprid residues

residues through peeling. Thus, although, residues of the systemic organophosphorus phorate were only reduced by 50% through peeling of potatoes [JMPR (1992)] and similarly disyston residues in potatoes were only reduced by 35% after peeling [Holland *et al.* (1994)], quinalphos residues on apples and procymidone residues on tomatoes were reduced by =73% [Cengiz *et al.* (2007)].

Furthermore, the reported data of the reduction of dichlorvos and diazinon on cucumber by 57.2% and 67.3% respectively [Cengiz *at al.* (2006)].

Washing is the most common form of processing which is a preliminary step in both household and commercial preparation. Loosely held residues of several pesticides are removed with reasonable efficiency by varied types of washing processes [Street (1969)]. The efficiency of washing depends on the location of the pesticide on the surface of the plant or within the tissue. The greater the washing time the greater the time of the reduction of the pesticide, the greater the solubility of the pesticide in water depending on the polarity of the pesticide and its tendency to link to the wax layer and the temperature of the wash water. Warm water is more effective than cold water and when we observe the results obtained from the use of water washing has had an effect in the disposal of residues imidacloprid despite the difference in temperature of the water washed in the samples, although there was a slight difference between them and the reduction that Imidacloprid of water-soluble pesticides. Fig. 5 shows the effect of water washings at 35^{0}C higher in reducing pesticide residues in primary deposits while the effect of washing was 1^{0}C and 5°C but less in the same deposits. However, the reduction rate was lower in subsequent samples due to the nature of the regular pesticide, its residue can't be disposed of as long as the spray is removed even if the washing time is increased [Al-Nawa *et al.* (2006)]. Al-Nawa and others tested the washing process with the tap water of the cucumber treated with the systemic methomyl pesticide. Note that the pesticide is 60% Day of spraying. In another experiment, the effect of washing with tap water was reduction in imidacloprid 42% [Leili *et al.* (2016)]. Washing with water at 35^{0}C in this experiment had a greater effect than tap water on the imidacloprid pesticide as studies showed that warm water washing has the potential to reduce pesticide residues. Washing with tap water was more than 60% in some vegetables [Randhawa *et al.* (2007)], but cold water washing in this experiment had less effect than washing at a higher temperature. In another study, however, the effects of cold water washing for 2 minutes had an effect in reducing the pesticide residue of beans to 45%. Was washed with cold water for two minutes for the fruits of the tomatoes in the removal of 30-77% of ethylene bis-dithiocarbamates (EBDC) fungicides [Holland *et al.* (1994)].

Sodium chloride (NaCl) solution is largely used to decontaminate the pesticide residues from different fruits and vegetables. Studies have proved effective in removing pesticide residues from fruits and vegetables [Holland *et al.* (1994)]. This was observed in this study as it had an effect in reducing the imidaclopriide if it was reduced in the first sample after one hour of spraying 100% and decreased the reduction rate in the subsequent samples as the reduction rate did not exceed 10% in the sample taken after 7 days of spraying (Fig. 6). Shiboob found that washing in 1% sodium chloride solution for 1 minute reduced the Dimethoate pesticide in the option to 83%. [Shiboob (2012)]. It was found that washing with sodium chloride solution 10% gave a greater loss 3-4 times the residues of pesticides for fruits washed with water washed with water [Abou-Arab (1999)]. did not differ significantly from ordinary washing with tap water but their dipping in 2% sodium chloride solution for 10 minutes removed higher amounts of triazophos and acephate residues [Phani-Kumar *et al.* (2000)]. Although washing with tap water has proved to be a significantly effective, simple, common and convenient method for removing pesticide contaminants from food surfaces. However, it was found that the solutions were more effective in removing pesticide residues. The sodium chloride solution and 5% sodium bicarbonate had a role in reducing the imidacloprid of the cucumber and the detergent solution also had a role in reducing the pesticide (Fig. 6).

As Liang *et al.* (2012) reported, the highest loss of trichlorfon and dimethoate from cucumber was caused by washing in 5% sodium carbonate solution, while 5% sodium bicarbonate solution caused the highest reductions in dichlorvos, fenitrothion and chlorpyrifos [Liang *et al.* (2012)] Solutions of NaOH, potassium dichromate and soap are used as decontaminating agents. Dipping of fruits in NaOH solution removed 50 to 60 percent surface residues of pyrethroids compared to 40 to 50 percent removal by hydrolytic degradation with NaOH and a detergent solution removed 50 to 60 percent residues [Ahmed *et al.* (2011)]. Washing of the detergent with several concentrates has also contributed to reducing pesticide residues in fruits and vegetables (Fig. 6).

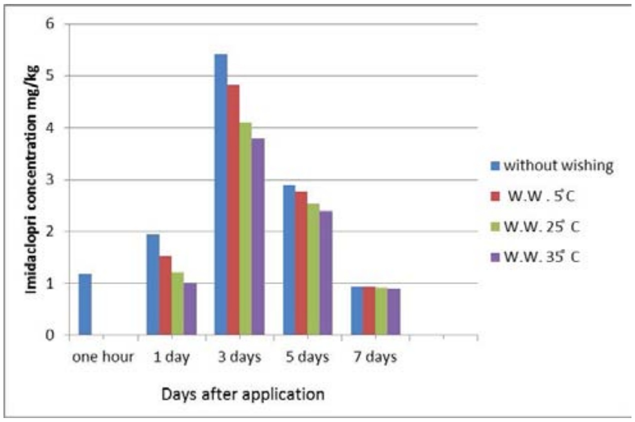

Fig. 5: Effect of washing with water at different temperatures in reducing residues imidacloprid

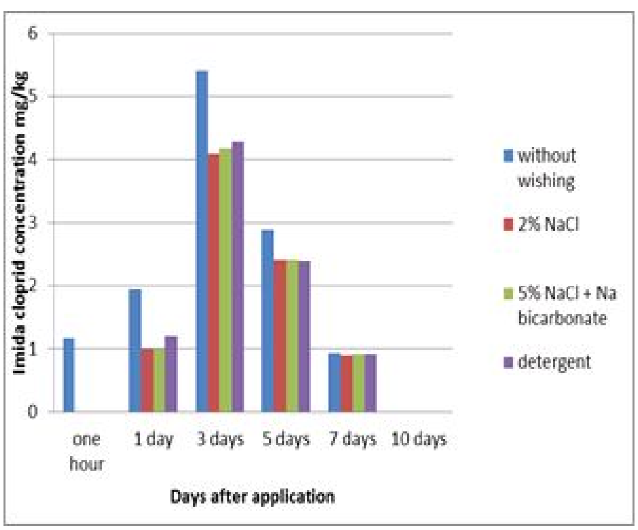

Fig. 6: Showing the effect of wishing with solutions on the reduction of imidacloprid residues

4. Conclusion

The results obtained from this study showed that imidacloprid residues remain in cucumber and pepper, especially after a few days of pesticide spraying. During the study period, we observed that imidacloprid residues begin to rise in cucumber fruits after an hour of spraying. And until the third day of spraying to decrease thereafter to reach the maximum allowed on the seventh day (because of the movement of the pesticide within the plant, especially the quantities of pesticide spread on the surfaces of new leaves developing). The safety period exceeded 7 days as the result differed with the company's recommendation as the safety period was set three days. Supervisors should control the harvesting of products and prevent farmers from selling them before the 10th day of spraying, due to the presence of pesticide residues.

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