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Unraveling the Potential of Household Electrolytes on Extricating Imidacloprid Residues from Tomatoes: Toxicity Assessment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Farmers that cultivate tomatoes use imidacloprid to combat sucking bugs even while the fruits are being harvested so that they can maintain a five- to six-time harvest. Decontaminating tomato fruits before eating is absolutely necessary due to the insecticide's slow dissipation rate and the fact that residues last in vegetables for 15–25 days. The quality of fruit has been compromised and cannot be used in salads, despite the fact that various heat procedures are effective in removing imidacloprid residue. Therefore, a study was conducted to assess the effectiveness of common electrolytes, namely NaCl and NaHCO3, and their combinations, on imidacloprid removal from tomato fruits. Fruits and washing solutions were extracted for imidacloprid residue and determined

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using the UFLC-PDA besides optimizing the concentration and period of decontamination or washing without dietary risk. Results indicates that the washing of the fruits with NaCl @ 1, 2 and 3% solutions for 10 minutes are efficient (98-100% removal) in decontaminating to below hazard quotient when sprayed with recommended 20 g ai/ha. Whereas, the $NaHCO_3$ or its combination with NaCl 1% for 10 minutes was found to be efficient (92-100%) in decontaminating the fruits when sprayed with 40 g ai/ha. Increasing the decontamination period beyond 10 minutes increased the residue load on fruits to above hazard quotient and was above 1.0 when NaCl was used. Study suggests the washing of tomato fruits with 2%$NaHCO_3$ or 1%NaCl+2% $NaHCO_3$ for 10 minutes is the efficient decontamination way and to ensure safety to the consumers.

Keywords: Tomato; imidacloprid residue; decontamination.

1. INTRODUCTION

Tomato (*Solanum lycopersicum*) is the largely consumed vegetable in the world and in Asia next to potato. India is the second-largest producer of tomatoes, produced 20.60 MT during 2022 against the world production of 189.1 MT [1] and currently contributes to 11% of the world’s total tomato production. Around the world, it is grown all year round, and people eat it raw or cooked. It is extensively grown both in the summer and the winter in India under a variety of ecosystems and primarily through sheltered farming. When grown via drip fertigation, tomatoes are fed heavily with important nutrients in order to maximize their potential production and earn more money. In order to manage insects and diseases during the growing season, pesticides and fungicides are used more frequently as a result of the crop's opulent growth, which also invites pest issues. Especially the sucking insects such as aphids and whitefly are the major pests infesting the tomato plants in India and reduce the fruit quality also [2]. To manage and protect the tomato crop, the imidacloprid, a nicotine-based systemic neurotoxin insecticide, applied frequently in India [3].

Imidacloprid,(NE)-N-[1-[(6-chloropyridin-3-yl)methyl]imidazolidin-2-ylidene]-nitramide is a patented chemical, manufactured by Bayer Cropscience (part of Bayer AG) and sold under trade names Kohinor, Admire, Advantage, Gaucho, Merit, Confidor, Hachikusan, Premise, Prothor, and Winner etc worldwide. It is a systemic insecticide and classified by the USEPA as both a toxic class II and a class III agent and the acute and chronic reference doses for imidacloprid infants (< 1 year) and children (1–12 years) set by the USEPA was 0.42 mg/kg per day and 0.057 mg/kg per day [4]. The imidacloprid residue concentrations (0.13 -0.46 mg/kg) in several fruits and vegetables were found to exceed the CODEX maximum residue limit [4]. The vegetables namely okra, bitter gourd, brinjal, tomato, onion, cauliflower, and chillies collected from different markets of southern Sindh were heavily contaminated with imidacloprid, and majority of samples violated the Japanese MRLs [5]. Kapoor et al. [6] observed that 15.20% of samples, including fruits, vegetables, and cereals, had imidacloprid and while about 22% fruits showed imidacloprid presence, 2% has above residue limits. Similarly, among the studied vegetables, 24% had imidacloprid and in 5.71% exceeds limits. About 21% of tested vegetables were contaminated with imidacloprid with less contamination in pumpkin [4]. Hence it is highly essential to decontaminate the fruits and vegetables before consumption for ensuring public health and environmental safety.

The removal of pesticide residues from fruits and vegetables can be accomplished using a variety of techniques, such as washing with tap water, warm water and electrolytes, blanching, boiling, and other thermal and non-thermal procedures. The first step in reducing pesticide residues on a product's surface is washing [7]. The effectiveness of the washing procedure relies on the chemicals used, their mechanisms of action, their solubility in water, and when they are harvested [8]. Sonali sharma [9] reported that washing of fipronil and imidacloprid residues in chilli with microwave cooking reduced residues from 93%-100%. But however these methods may results in loss of vitamins etc. Suganthi et al. [10] stated that the decontamination techniques reduced imidacloprid residues in bitter gourd by 33-80% in 10 days and mentioned minimal risk to consumers, with RQ < 1 after 10 days of imidaclorpid application Naik et al. [11] studied the decontamination of imidacloprid from brinjal and okra involving boiling found that the removal up to 96.43% and 73.66% residues from brinjal and okra, respectively. Despite the fact that heat methods are used for decontaminating pesticides in many cases, there is a potential that some vegetables, notably tomato fruit, will lose quality. With this background the present study is essential to evaluate non thermal household electrolytes solution for optimizing the washing concentration and period is need of the hour to assessing the rate of imidacloprid residue removal from the tomato fruit and its toxicity assessment to the human being. Approximately 80% of tomatoes are consumed fresh, and the remaining 20% are used in the production of tomato paste, puree, ketchup, pickles, juices, and sauces [12].

2. MATERIALS AND METHODS

2.1 Chemical and Reagents

Certified Reference Standard material (CRM) of imidacloprid pestanal grade (>98%) was purchased from the Sigma-Aldrich was used for instruments calibration and recovery studies. Imidacloprid stock solution (1000 mg/L) was prepared by dissolving 0.1 g CRM in 100 ml of HPLC grade acetonitrile and from this, the working standards of 0.01 to 5.00 mg/L was prepared using the mobile phase for instrument calibration and the assessment of detection limits. High performance liquid chromatography (HPLC) grade acetonitrile of Merck and 0.2 micron filtered HPLC water from Millipore unit was used for the mobile phase. Other chemicals used in the study were of analytical grade from Hi-media / S.D. Fine Chemicals limited and Primary Secondary Amine (PSA) sorbent from Agilent technologies. The imidacloprid commercial formulation (Tatamida 17.8% SL), which had been diluted according to the procedures with high-quality water, was then applied to the tomato fruits.

2.2 Experiments Details

The experiments were laid out in a Factorial Completely Randomized Block Design consisting of 2 doses of imdacloprid viz., recommended – 20 g ai/ha (X) and double the recommended 40 g ai/ha (2X) doses as factor 1 and 9 washing treatments as factor 2 with three replications. Factor 2 includes washing with tap water alone, NaCl 1%, 2%, 3% solutions, $NaHCO_3$ 1%, 2% and 3% solutions and the combinations of NaCl + $NaHCO_3$ solutions. Three washing periods were the factor 3 viz. 5, 10 and 15 minutes.

Tomato fruits were collected from organic products outlet to conduct the recovery study and decontamination experiment by imposing treatments. The collected samples were washed with running tap water and then with distilled water to ensure the free of contamination before imposing the treatments. About 5-6 kg of tomato fruits were sprayed with the imidacloprid solutions (X and 2X dose) for complete wetting and then allowed for shade drying 1 hr. Later the imidacloprid treated fruits were portioned into 180-200 g and subjected washing using the prepared electrolyte solutions as detailed above. After washing treatment as per the stipulated washing periods of 5, 10 and 15 minutes, the fruits were removed and shade dried treatment and interval wise for 1 hr. Then the treatment wise washed fruits were subjected to imidacloprid residue analysis using UFLC-PDA.

2.3 Imidacloprid Residue Extraction and Determination

Imidacloprid residue from tomato fruits was extracted using QuEChERS (Quick Easy Cheap Effective Rugged Safe) method using acetonitrile and cleanup with anhydrous $MgSO_4$ and PSA [13]. Dried residue was re-dissolved in mobile phase and filtered with 0.22 µm nylon syringe filter for analysis in UFLC. Imidacloprid residue was analysed with Shimadzu Ultra Fast Liquid Chromatography (LC-20A) equipped with Quaternary Pump, Agilent C18 Column (4.6×150 mm, 5 µm), Auto Sampler and PDA detector. Imidacloprid was eluted with acetonitrile and water as mobile phase (80:20 v/v) using 1.0 ml flow rate and 10 µL injection volume at 270 nm.

2.4 Method Validation

The validity of the analytical approach used for the extraction and detection of imidacloprid was confirmed through the recovery studies. A known weight of tomato sample was fortified with known concentrations of imidacloprid standard ranged from 0.001 to 5.0 mg L^{-1}. After 1 hour of fortification, a spiked fruits was subjected to an extraction and clean up and then determined using UFLC. Quantification of residue concentration was accomplished by comparing the peak height response for samples with peak height of the standard. Precision standard deviation of replicate analysis of standard spiked at different concentrations was used to calculate the detection limit (DL) and quantification limit (QL).

2.5 Decontamination Efficiency and Toxicity Assessment

The efficiency of the imposed treatments on removing the imidacloprid residue from the fruits was evaluated using the reduction rate) and processing factors as described in literature [8].

$$Decontamination\ rate\ \% = (1 - PF) * 100$$

$$Processing\ factor\ (PF) = \frac{WTr}{NCr}$$

Where,

WTr- Residue in treatment (mg/kg);
NCr- Residue in control (No treatment) mg/kg.

The dietary intake and chronic intake risk assessment of the imidacloprid was calculated to assess the chronic consumer health risk (hazard quotient, HQ) based on the ratio of estimated daily intake (EDI) and acceptable daily intake (ADI) was fixed as 0.01 mg/kg which is the instrument detection limit as well as the MRL for imidacloprid in most of the vegetables. The average adult weight was estimated to be 60 kg, and the tomato consumption in India is assumed to be 50 g/day for calculating the EDI and ADI. The formula is as follows,

$$EDI = Median\ residue\ x \frac{food\ consumption}{body\ weight}$$

$$HQ = \frac{EDI}{ADI} x\ 100$$

If the HQ is > 1.0, it is considered as not safe to consume and undetected sample is calculated using LOD (0.01 mg/kg).

2.6 Statistical Analysis

The data collected during the course of investigation was statistically analyzed using 'R' software. Graphical analysis was carried out using the MS-Excel software 2013 version. The Pearson correlation analysis was carried out between variables and constant parameters at a significance level of P= 5.

3. RESULTS AND DISCUSSION

3.1 Method Validation and Recovery Studies

The suitability of the instrument conditions and extraction method for detecting and determining the imidacloprid by the UFLC-PDA was assessed imposing recovery and calibration studies. Imidacloprid was eluted at 2.72±0.2 minutes (Fig. 1) under the described instrument conditions. Instrument detection limit was assessed through external standard calibration in the working range of 0.01 to 2.5 mg/L imidacloprid standards. Calibration graph was constructed using the concentration against the area was found be to be linear with the r2 value of 0.994 (Fig. 2) and instrument detection limit was found to be 0.01 mg/L. Recovery experiment conducted by fortifying the imidacloprid concentration from 0.01-1.00 mg/L showed >98% recovery with the quantification limit of 0.05 mg/L.

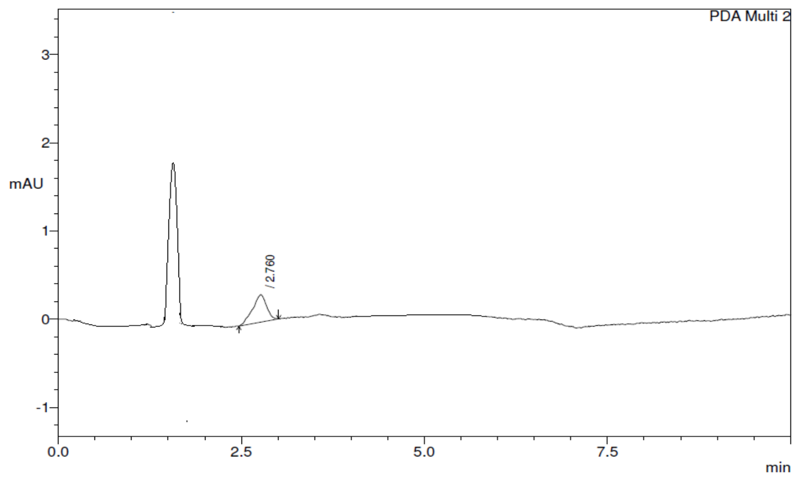

Fig. 1. Chromatogram of imidacloprid standard (0.01 mg/L) detected in UFLC-DAD

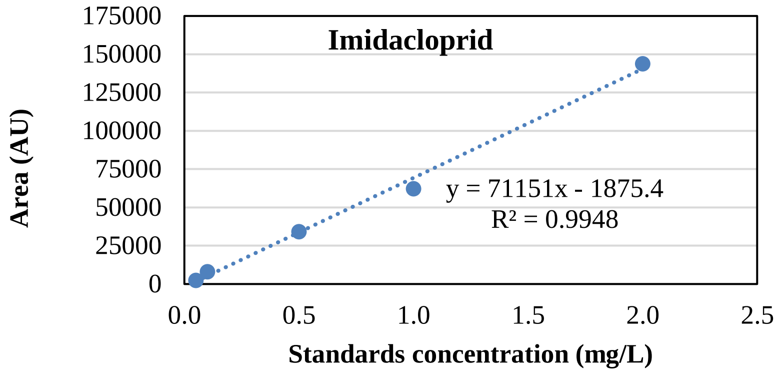

Fig. 2. Calibration graph of imidacloprid standards determined with UFLC-DAD

3.2 Efficacy of Electrolytes Washing on Imidacloprid Removal

Tomato fruits treated with imidacloprid at two concentrations were subjected to decontamination by washing with household electrolytes viz., NaCl, $NaHCO_3$ and their combinations at different concentrations and various time intervals. After washing both the fruits and washing solutions were analysed for imidacloprid residues to understand the decontamination rate and also the interaction of it with washing solutions. The results obtained are presented in Fig. 3. It was observed that the imidacloprid residue was removed by both the electrolytes irrespective their concentrations and also by their combinations within 5 and 10 minutes. However the residue was detected when time of washing has been increased to 15 minutes at higher concentrations of both the electrolytes irrespective of washing interval and their combinations. Imidacloprid concentration detected in tomato fruits after washing with electrolytes was 0.022, 0.011, 0.013, 0.016 and 0.019 mg/kg respectively with 3% NaCl, 2 and 3% $NaHCO_3$, 1% NaCl + 1% $NaHCO_3$ and 1% NaCl + 2% $NaHCO_3$, solutions. When concentration of imidacloprid sprayed over fruit was double the recommended dose (40 g ai/ha), then the washing solutions gave different results and residue detected ranged from 0.04 to 0.44 mg/kg. Tap water and various concentration of NaCl didn't remove the residue to BDL in tomato fruits at 5 and 10 minutes where it was removed to BDL after 15 minutes washing. Whereas $NaHCO_3$ or its combined washing with NaCl showed different trend that the washing of residue contaminated fruits upto 10 minutes removed the residue to BDL while washing up to 15 minutes were loaded with imdacloprid residue from 0.04-0.16 mg/L. This showed that the increased washing time have negative effect on decontamination.

To understand the efficiency of electrolytes residue removal, the decontamination rate (%) and processing factor were calculated and the results obtained were presented in Table 1. The decontamination rate increased as the time interval increased across the dose of imdacloprid and was mean value ranged from 87-100% and 85-91% respectively for X and 2X doses irrespective of washing interval. These results are aligned with the findings of Sharma and Anil [14] who stated that removal of residues due to washing ranged from 32.5 to 72.7 per cent. The results are in agreement with the findings of Deka et al. [15] Zhang et al. [16] who reported 50% and 73.3% removal of cypermethrin residues from cauliflower by salt solution. Among the electrolytes, washing of tomato fruits for 5–10 minutes with various concentrations of NaCl and $NaHCO_3$, either alone or in combination, achieved 100% decontamination to BDL. For double the recommended (2X) dose, the decontamination rate ranged from 53-100, 75-100 and 59-100 % at 5, 10 and 15 minutes respectively and showing that the increased washing period beyond 10 minutes decreased the decontaminate rate. Similarly, Wheeler et al. [17] also reported that washing of cauliflower with salt solution for 10 min was capable of removing 28-93% of triazophos and quinalphos residues. There was slight reduction of decontaminations rate among some treatments when longer washing times were used. For example, the treatment with 3% NaCl had a 95% decontamination rate, 1% $NaHCO_3$ with 71% decontamination rate and 3%$NaHCO_3$ with 97% decontamination rate. Tap water washing also

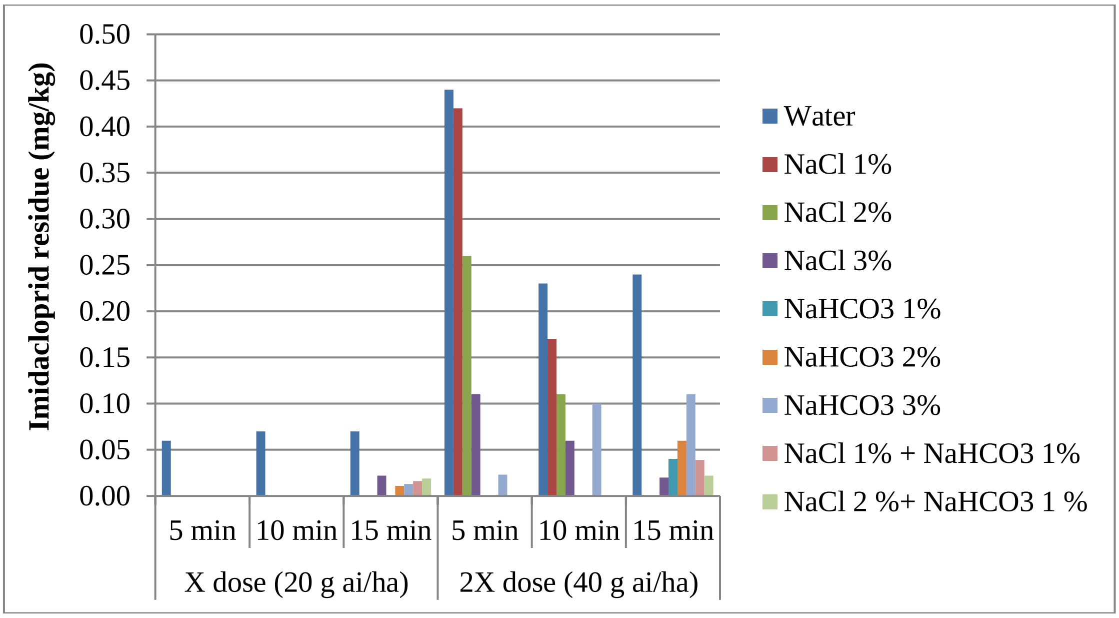

Fig. 3. Imidacloprid residue on tomato fruits after washing with various concentrations of electrolytes at various period as determined with UFLC-DAD

Table 1. Efficiency of electrolytes, their concentrations and washing period on Imidacloprid residue decontaminate rate (%) from tomato fruits

Treatments	X dose (20 g ai/ha)			2X dose (40 g ai/ha)		
	5 min	10 min	15 min	5 min	10 min	15 min
Water	87	85	85	53	75	48
NaCl 1%	100	100	100	55	82	100
NaCl 2%	100	100	100	72	88	100
NaCl 3%	100	100	95	88	94	96
$NaHCO_3$ 1%	100	100	100	100	100	91
$NaHCO_3$ 2%	100	100	98	100	100	87
$NaHCO_3$ 3%	100	100	97	98	89	76
NaCl 1% + $NaHCO_3$ 1%	100	100	97	100	100	92
NaCl 2 %+ $NaHCO_3$ 1 %	100	100	96	100	100	95

showed significant residue decontamination from tomato fruits (87-100 and 53-79% at X and 2X doses, respectively).

Interestingly, the decontamination rate was lower for the 2X dose compared to the X dose. This may be attributed to the double dose having a diminishing effect on the decontamination rate. In 2X dose the effectiveness of NaCl treatments varied with concentration. The 2% and 3% NaCl treatments showed better decontamination rates as time increased, ranging from 61% to 100% and 88% to 100%, respectively. However, the 3% NaCl treatment had a decreasing decontamination rate as the washing time increased, indicating that lower concentrations were more effective. The 1% and 2% $NaHCO_3$ treatments were highly effective, achieving 100% decontamination when tomatoes were washed for 5-10 minutes. These results are similar to findings of Yang et al. [18] who stated that 2% $NaHCO_3$ reduces residue upto 50%-90%. The 3% $NaHCO_3$ treatment had a high initial decontamination rate of 98% at 5 minutes, but this rate dropped to 89% at 10 minutes and further to 65% at 15 minutes. These results align with the findings of Anil reddy et al. [19] who reported that 0.1% baking soda solutions was found to be more effective than salt solutions in removing fipronil, imidacloprid residues in green chilli.. Similarly, the combination treatments showed excellent results when used for shorter duration up to 10 minutes, achieving a 100% decontamination rate. The findings concur with those of Balkan and Yılmaz [20] who claimed that combination of salt and soda water removed dimethoate residue up to 50%. However, as the washing time increased the decontamination rate for these combination treatments decreased slightly, reaching 96 - 97% for the X dose and 92 - 95% for 2X dose. Finally, the washing with inorganic solutions demonstrates that the effectiveness of the treatments (water, NaCl and $NaHCO_3$) in removing imidacloprid residue on tomatoes depends on both the concentration of the electrolyte and the washing duration. Lower concentrations of NaCl and $NaHCO_3$ were generally more effective, especially when used for shorter time intervals. Additionally, using a higher dosage (2X) did not necessarily result in better outcomes and, in some cases, led to reduced decontamination rate.

3.3 Interaction of Imidacloprid with Electrolytes

The washings of the imidacloprid treated fruits were extracted and analysed for confirms the imidacloprid residue removal and its decontamination mechanism by the electrolytes (Fig. 4). Irrespective of electrolytes and its concentration, the increase in period of washing increased the imidacloprid residue removal and higher concentration of imidacloprid residue was extricated by $NaHCO_3$ than NaCl or their combinations. While increased residue removal was observed with increase in period of washing by NaCl and $NaHCO_3$ or their combinations at X dose, it decreased after 10 minutes with 2X notice. This showed that the quantity of residue bound to the fruits also decides the period of washing beside the electrolytes concentration. It could also be attributes to the degradation of the parent imidacloprid to its metabolites like 6-chloronicotinic acid {1-[(6-chloro-3-pridinyl) methyl]-2-imidazolidone} favoured by the higher pH of the increased electrolytes concentration viz., 7.9 and 8.7 for 3% NaCl and 3% $NaHCO_3$, respectively. Similar degradation of imidacloprid at higher solution pH was reported by Yari et al. [21] and could be attributed to the enhanced hydrolysis of imidacloprid. Thuyet et al. [22], stated that the –C=N– bond of imidacloprid has small positive charge by coupling with a strong electron-with drawing group ($–NO_2$) and an imidazolidine ring. This positive charge can interact with the OH^- ion in the solution when its pH is higher and hence it increases its hydrolysis to metabolites.

The performance of each electrolyte was assessed using mean data (Fig. 5) and observed that the $NaHCO_3$ or its combination with NaCl was efficient in removing the imidacloprid residue from tomato fruits irrespective of its concentration while the NaCl was efficient only at the recommended low concentration. Zhang et al. [23] also reported that due to the use of non-chemical cleaning agents like detergent or potassium permanganate, washing vegetables in soda-salt solution won't result in secondary pollution Mostly farmers apply pesticides

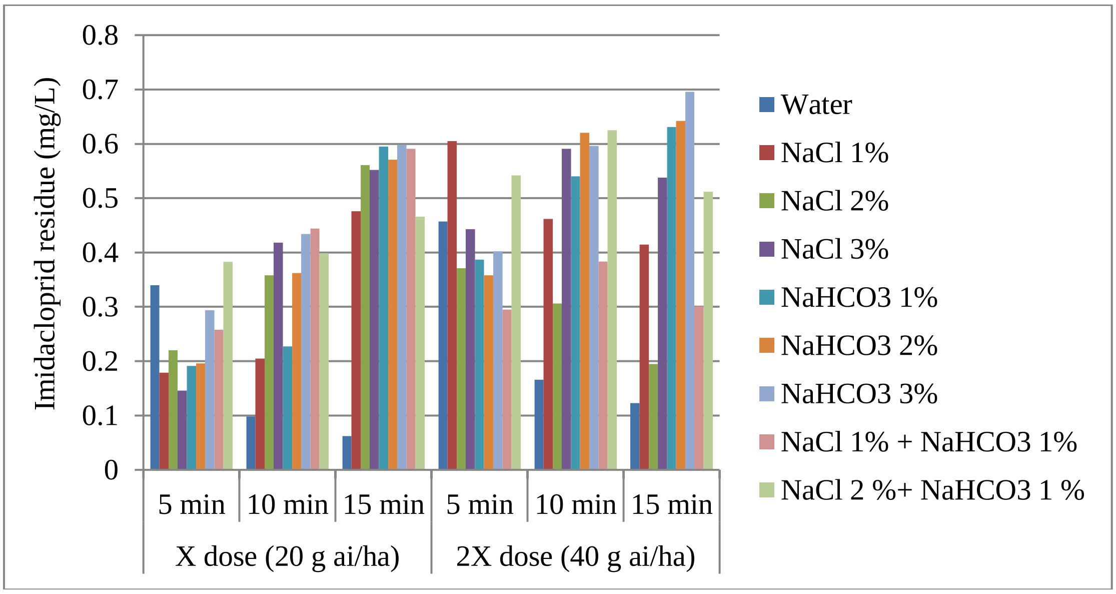

Fig. 4. Imidacloprid residue in washing solutions after washing of the contaminated tomato fruits at various period as determined with UFLC-DAD

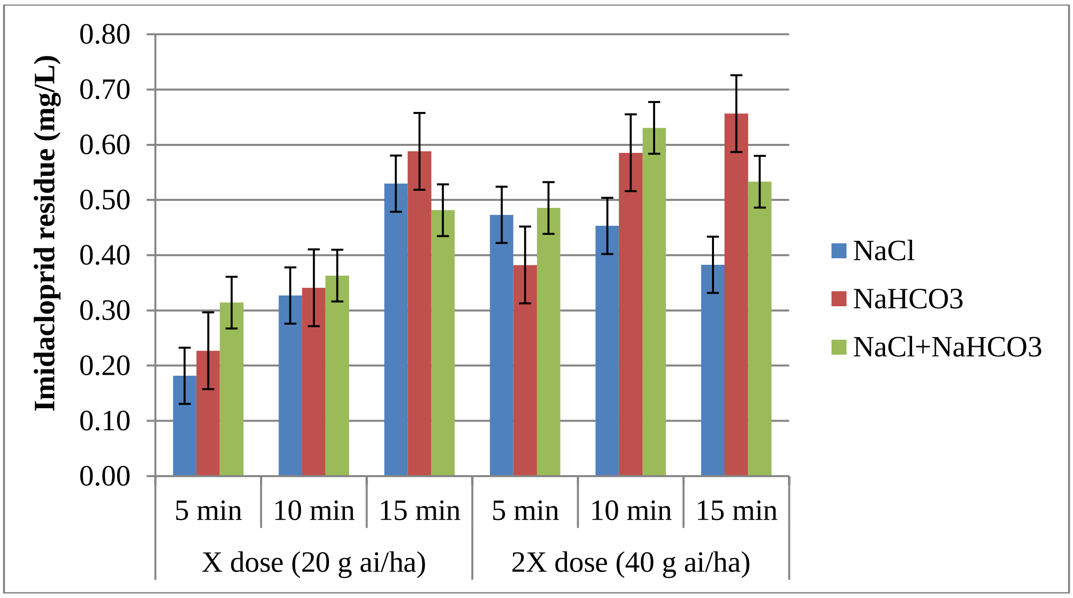

Fig. 5. Efficiency of electrolytes on extracting the imidacloprid residue on tomato fruits at various period as determined with UFLC-DAD

repeatedly to manage insect pests, it is wise to use 2 % $NaHCO_3$ or 1%NaCL+1%$NaHCO_3$ as washing solution for 10 minutes to decontaminate the imidacloprid residues from the tomato fruits. Excess period of washing also must be avoided to prevent the re-sorption of residue by the fruits from washing solution.

3.4 Toxicity Assessment

The residue detected in tomato fruits after washing with electrolyte solutions of different concentrations were used to assess the dietary risk assessment by calculating the hazard quotient (Table 2). At the recommended level of imidacloprid spray, all concentrations of both the electrolytes and their combinations recorded HQ of 0.08 to 0.18 and tap water recorded 0.50-0.58. Whereas at double the recommended rate, HQ was > 1.0 or equal to 1 for NaCl viz., 0.92-3.50, 0.50-1.92 and 0.08-0.17, respectively for 5, 10 and 15 minutes. This suggested that the removal efficiency increased with increased washing time. The $NaHCO_3$ recorded the HQ <1.0 irrespective of imidacloprid dose, washing period and electrolyte concentration and similar is the results for the combined electrolytes washed fruits. Similar hazard index of <1.0 for imidacloprid in okra and brinjal fruits was reported by [11].

Table 2. Hazard quotient for imidacloprid residue in tomato fruit after decontamination with electrolytes at different concentration and period

Treatments	X dose (20 g ai/ha)			2X dose (40 g ai/ha)			Mean
	5 min	10 min	15 min	5 min	10 min	15 min	
Water	0.50	0.58	0.58	3.67	1.92	2.00	1.54
NaCl 1%	<0.10	<0.10	<0.10	3.50	1.42	<0.10	0.88
NaCl 2%	<0.10	<0.10	<0.10	2.17	0.92	<0.10	0.57
NaCl 3%	<0.10	<0.10	<0.10	0.92	0.50	0.17	0.32
$NaHCO_3$ 1%	<0.10	<0.10	<0.10	<0.10	<0.10	0.33	0.13
$NaHCO_3$ 2%	<0.10	<0.10	0.09	<0.10	<0.10	0.50	0.15
$NaHCO_3$ 3%	<0.10	<0.10	0.11	0.19	0.83	0.92	0.37
NaCl 1% + $NaHCO_3$ 1%	<0.10	<0.10	0.13	<0.10	<0.10	0.33	0.13
NaCl 2 %+ $NaHCO_3$ 1 %	<0.10	<0.10	0.16	<0.10	<0.10	0.18	0.11
Mean	<0.10	<0.10	0.18	3.50	1.42	0.92	

4. CONCLUSION

Even after harvest, tomatoes are sprayed with imidacloprid, which results in a large buildup of residue on the fruits and may provide a toxicity risk to consumers. In order to disinfect the residue, non-thermal decontamination utilizing common electrolyte solutions was explored. It was discovered that washing fruits in NaCl at 1 to 3% for 10 minutes washed off the imidacloprid residue from BDL at the suggested spray rate. While imidacloprid spray with a doubled imidacloprid concentration was shown to be effective (92–100%) when combined with NaHCO3 for 10 minutes of washing. A dietary risk assessment was conducted to demonstrate the effectiveness of the decontamination techniques used, and it was discovered that washing with NaHCO3 and mixing it with NaCl for 10 minutes only had a hazard quotient of 1.0. Study findings suggested that washing of tomato fruits with 2%$NaHCO_3$ or 1%NaCl+2% $NaHCO_3$ for 10 minutes is the efficient decontamination way to ensure safety to the consumers.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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