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Risk assessment for ingestion of off-season longan stimulated by potassium chlorate

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ABSTRACT

Introduction: Production of off-season longan in Thailand is stimulated by potassium chlorate ($KClO_3$) which is a hazardous chemical and prohibited in food. This research analyzed the chemical residues in longan fruits and performed risk assessment for health effects from ingestion. **Materials and Methods:** Forty-two longan trees (*Dimocarpus longan* Lour.) were separated into eight study groups (n = 5) and a control group (n = 2). Four dosages of $KClO_3$ from 25 g/m to 127 g/m were used through either roots or leaves. After application, longan trees were observed every 30 days for effects on growth, and fruits were collected to analyze for chemical residues at 150, 180, and 210 days. Ultraviolet-spectrophotometry was used for ClO_3^- and Cl^- analyses, and atomic absorption spectrophotometry was used for K^+ and Na^+ analyses. **Results and Discussion:** $KClO_3$ was observed to have a dose-dependent effect on the growth of longan trees and fruits. $KClO_3$ at 43 g/m was the maximum useful dose for increasing the growth yields and 25 g/m was sufficient for off-season stimulation without significant reduction of yields. Higher doses were found to result in higher accumulation of ClO_3^-, Cl^-, K^+, and Na^+ residues in the peel, but accumulation of related chemical residues in the flesh was inconclusive. Application of $KClO_3$ through roots gave a higher accumulation of ClO_3^- and Cl^- residues in the flesh compared to application through leaves. However, application through roots resulted in better flesh weights and less adverse effects on plants. The risk assessment for chemical residues found that ingestion of 1000 g of longan flesh per day was safe with Hazard quotient << 1.00 and Margin of safety >> 1.00 for all chemical residues. **Conclusion:** $KClO_3$ used in agricultural production left little residue and the produce is safe for consumption by the general population.

Keywords: Chloride, Fruit growth, Potassium, Sodium, Stimulation through leaves, Stimulation through

INTRODUCTION

Potassium chlorate ($KClO_3$) is a hazardous chemical and harmful to health. As a strong oxidizer, $KClO_3$ is used in insecticides and pesticides, matchstick heads, explosives, flares, bleaches, disinfectants, dyes, and prints. By announcement of the Department of Agriculture of Thailand, $KClO_3$ is classified as an agricultural toxic substance.[1]

Due to its oxidizing property, ingestion of $KClO_3$ can lead to the destruction of erythrocytes, disseminated intravascular coagulation, kidney injury, or death.[2] *In vitro* studies using human erythrocytes found a dose-dependent decrease in the activity of G6PD and glutathione peroxidase, which resulted in a decrease in glutathione (GSH) levels by 50% within the first 60 min of exposure.[3] Animal studies showed that chronic ingestion over 3–9 months resulted in decreased GSH levels and subsequent hemolysis.[4] In human case reports, a 48-year-old active duty American soldier was discovered with renal insufficiency from ingesting one pack of matchstick heads every 4 days during field training to prevent insect bites for an estimated total 5.8 g of $KClO_3$ during field practices.[5] A 29-year-old pregnant woman developed pica during the third trimester and ingested >300 burned matchstick heads per week (cautopyreiophagia). Since she was unable to modify her behavior, childbirth was induced at term. The infant was vigorous at delivery with Apgar score = 8–9 but became hemolytic with subsequent hyperbilirubinemia within 24 h. The mother also showed evidence of hemolysis, hepatic injury, and hyperbilirubinemia within 24 h.[2] A 21-year-old man who attempted suicide by swallowing three boxes of matchstick heads (40 matchsticks per box) was referred to hospital with decreased consciousness 24 h after ingestion. The magnetic resonance imaging showed symmetric hyperintense signals within the deep gray matter and medial temporal lobes indicating higher cellular activity and higher oxygen requirement.[6]

In Thailand, $KClO_3$ is a prohibited substance in the food according to the Ministry of Public Health notification vol.151, B.E. 2536.[7] Nevertheless, $KClO_3$ is commonly used in agriculture to stimulate off-season longan production in Thailand. The application can be through either roots or leaves.[8] Therefore, this study analyzed chlorate (ClO_3^-) residue in longan fruits to assess the risk of health effects from ingestion of these longan fruits. The study also analyzed chloride (Cl^-) the final metabolite of chlorate,[9] potassium (K^+), and sodium (Na^+) residues in the fruits as well as observing for effects of $KClO_3$ stimulation on the growth of longan trees.

MATERIALS AND METHODS

Longan Tree Samples

Forty-two longan trees, *Dimocarpus longan* Lour., 10–15 years old (bush size 5–7.7 m) in Chiang Rai Province, Thailand, were used in this experiment.

Chemicals and Instruments

$KClO_3$ 99.9% commercial grade (The Sun, China) was used for off-season longan stimulation. Analysis chemicals were $KClO_3$ 99.5% AR (Univar, APS Ajax Finechem, Australia), KCl 99.8% AR (Ajax Finechem, Australia), Indigo Carmine AR (Loba, Italy), hydrochloric acid 12 M AR (RCI Labscan, Indonesia), ferric ammonium sulfate ($NH_4Fe(SO_4)_2$) 98.5% AR (Ajax Finechem, Australia), mercuric thiocyanate ($Hg(SCN)_2$) 99% AR (Loba, Italy), nitric acid 65% AR (RCI Labscan, Indonesia), sodium chloride 99.5% AR (Loba, Italy), and K^+ 1000 μg/ml of atomic absorption spectrophotometer (AAS) standard grade (PerkinElmer, USA). Analysis instruments were ultraviolet (UV)-Spectrophotometer (Pharmaspace UV-1700, Shimadzu) and AAS (PerkinElmer, USA).

Experimental Procedures and $KClO_3$ Dosages

Eight groups of longan trees (n = 5 trees) were treated with four doses of $KClO_3$ and a control group (n = 2 trees) without $KClO_3$. The two trees outside (but next to) the farmer's orchard were used as a control to avoid contamination of $KClO_3$. The fruit from these two trees was not intended to be sold. From the farmer's view, they were household trees (located between the house and the orchard). Each dose of $KClO_3$ was applied through the roots (dissolving $KClO_3$ in water then pouring on the soil under the tree's bush) or through the leaves (dissolving $KClO_3$ in water then spraying over the tree's bushes). The first dose of $KClO_3$ was the farmer's own choice: 125 g/tree for 5 m average bush size (25 g/m) and was classified as Group 1 when applied through the roots and Group 2 when applied through the leaves. The second dose of $KClO_3$ was the recommended dose from the Office of Agricultural Research and Development Region 1, Chiang Mai, Thailand,[10] $KClO_3$ 640 g/tree for 7.55 m average bush size (85 g/m) and was classified as Group 3 when applied through the roots and Group 4 when applied through the leaves. The third dose was 50% of the recommended dose,[10] $KClO_3$ 300 g/tree for 7.22 m average bush size (43 g/m) and was classified as Group 5 when applied through the roots and Group 6 when applied through the leaves. The last dose was 150% of the recommended dose,[10] $KClO_3$ 960 g/tree for 7.56 m average bush size (127 g/m) and was classified as Group 7 when applied through the roots and Group 8 when applied through the leaves.

Observation for Effects on Growth of Longan Trees After $KClO_3$ Application

The observations were recorded 30, 60, 90, 120, 150, 180, and 210 days after application of $KClO_3$.

Sample Preparation for Chemical Analyses

Longan fruit samples were collected 150 days (baby longan fruits), 180 days (medium size fruits), and 210 days (ready-to-eat fruits) after $KClO_3$ application. The longan fruit samples were separated into flesh, seed and peel portions, except for the samples collected at 150 days which were too young to be separated. Thus, the whole fruits were used. After separation, the flesh, seed, and peel portions were blended and extracted in Milli-Q water (1:10 w/v) for 30 min at room temperature using a magnetic stirrer. After that, the extracts were centrifuged at 7500 rpm/min for 15 min before filtration. The filtrates were then lyophilized and reconstituted back by Milli-Q water (20:1 v/v).

Chemical Analysis

UV-spectrophotometry was used to detect chloride at 460 nm[11] and chlorate at 610 nm.[12] AAS was used for Na^+ and K^+.[13]

Risk Assessment

Risk assessment for ingestion of off-season longan was estimated from three ingestion rates of longan flesh; 10 fruits/day, 500 g flesh/day, and 1000 g flesh/day. These ingestion rates were used to calculate the amount of Cl^-, K^+, and Na^+ consumed as ingestion doses/day (daily ingestion dose [DDs]) without considering body weight, except for ClO_3^- where body weight was included in the DD calculation. For males, the body weight was 60 kg and for females 50 kg. The reference doses (recommended doses) or RfDs were: Cl^- 2300 mg/day,[14] K^+ 3800 mg/day,[14] and Na^+ 2400 mg/day.[14] The RfD of chlorate was 30 μg/kg/day.[15]

The results of risk assessment are presented as Margin of safety (MOS) and Hazard quotient (HQ).

$$MOS = RfD/DD, \text{ and } HQ = DD/RfD$$

An MOS>1 and HQ<1 means the consumption of the longan fruits should give no adverse effects on consumer health, and an MOS<1 and HQ>1 means that adverse effects are expected as for threshold toxicity risk assessment.[16,17]

Statistical Analysis

Differences between groups were tested by one-way analysis of variance, *post hoc* multiple comparisons were by Scheffe's method, linear correlations were by Pearson correlation, using IBM SPSS Statistics 19.

RESULTS AND DISCUSSION

Growth of Longan Trees After $KClO_3$ Application

At 30 days, all $KClO_3$ treated longan trees had dropped leaves with a burned appearance (leaf chlorosis). Group control also had dropped leaves but without the burned appearance. The burned appearance was likely due to the oxidizing effect of $KClO_3$ on the biology and physiology of the trees.[18-20] Therefore, Groups 1 and 2 showed lesser burned effect than the other $KClO_3$ treated groups, presumably due to the lesser $KClO_3$ dose applied. Groups 1 and 2 also showed new leaves growing earlier than the other $KClO_3$ treated groups. The groups with high doses of $KClO_3$ (Groups 6, 7, and 8) had overall leaves dropped and not yet grown new leaves.

At 60 days, the longan trees in group control had started growing a lot of flowers, while the longan trees of Groups 1 and 2 had grown sufficient leaves to become an extended bush and the leaf color was dark green. The leaves in Groups 3, 4, and 5 were still a pale green color. Groups 6, 7, and 8 had started growing new leaves, with Group 8 having the least new leaves.

At 90 days, group control had started growing fruit buds. Groups 1, 2, 3, 4, and 5 had started flowering with Groups 1, 2, and 4 showing dense flowering. Group 7 had started flowering, with few flowers and a lot of leaves. Groups 6 and 8 had started growing leaves to become bush-like.

At 120 days, group control had grown bigger fruits. Groups 1, 2, 3, 4, and 5 had dense flowering. Group 7 had increased more flowering than at 90 days. Group 6 had grown a lot of flowers. Group 8 had no flowers, only growing young leaves.

At 150 days, the group control fruits had grown to medium size. Groups 1, 2, 3, 4, 5, and 7 had grown baby fruits (too small to separate peel, flesh, and seed). Group 6 had lost all of its flowers. Group 8 had still only grown leaves.

At 180 days, group control had full-size fruits. Groups 1, 2, 3, 4, 5, and 7 had medium size fruits (could separate peel, flesh, and seed), with Groups 1 and 2 having bigger sized fruits.

At 210 days, group control had reached the stage of fully mature fruit. Groups 1, 2, 3, 4, 5, and 7 had grown ready-to-eat fruits. Groups 6 and 8 had no fruits at all; they only developed bushes of leaves over the season.

Validation of Chemical Analysis Methods

Methods validation found the detection linearity range for chlorate residues 0.001–2 ppm, precision by %relative standard deviation (RSD) < 5, R^2 = 0.9991; for chloride residues 0.1–2 ppm, precision by %RSD < 5, R^2 = 0.9948; for potassium residues 0.2–1.0 ppm, precision by %RSD < 5, R^2 = 0.9986; and for sodium residues 0.1–0.5 ppm, precision by %RSD < 5, R^2 = 0.9982.

Growth of Longan Fruits After $KClO_3$ Application

At 150 and 180 days after the $KClO_3$ application, the growth of fruits was highest in group control [Figures 1 and 2]. However, at 210 days, the highest growth was found in Group 5 (43 g/m $KClO_3$) in terms of flesh and peel weights [Figures 3 and 4] (seed weights were not reported since the seeds were mistakenly discarded). While the flesh weights among Groups 1 and 2 (25 g/m), Group 5 (43 g/m), and Group 3 (85 g/m) were not statistically different, Groups 1 and 5 gave the highest flesh weights [Figures 3 and 4 and Table 1]. Therefore, we concluded that 43 g/m of $KClO_3$ should be the maximum stimulation dose to use for increasing the growth yields, higher doses than this showed reduced benefits. As the farmer's own choice of dose (25 g/m) was not statistically different from 43 g/m, this reflected the value

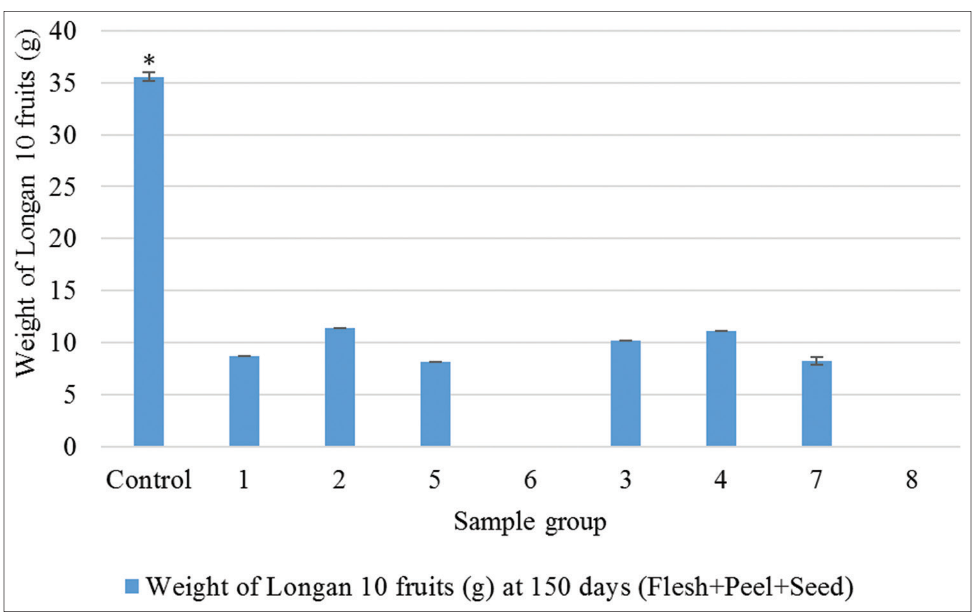

Figure 1: Comparison for weight of longan fruits (mean±SD) at 150 days after $KClO_3$ stimulation among nine groups with four different doses – group control (0 g/m), Groups 1 and 2 (25 g/m), Groups 5 and 6 (43 g/m), Groups 3 and 4 (85 g/m), Groups 7 and 8 (127 g/m). *Group control was significantly higher fruit growth of all treated groups at $P<0.001$

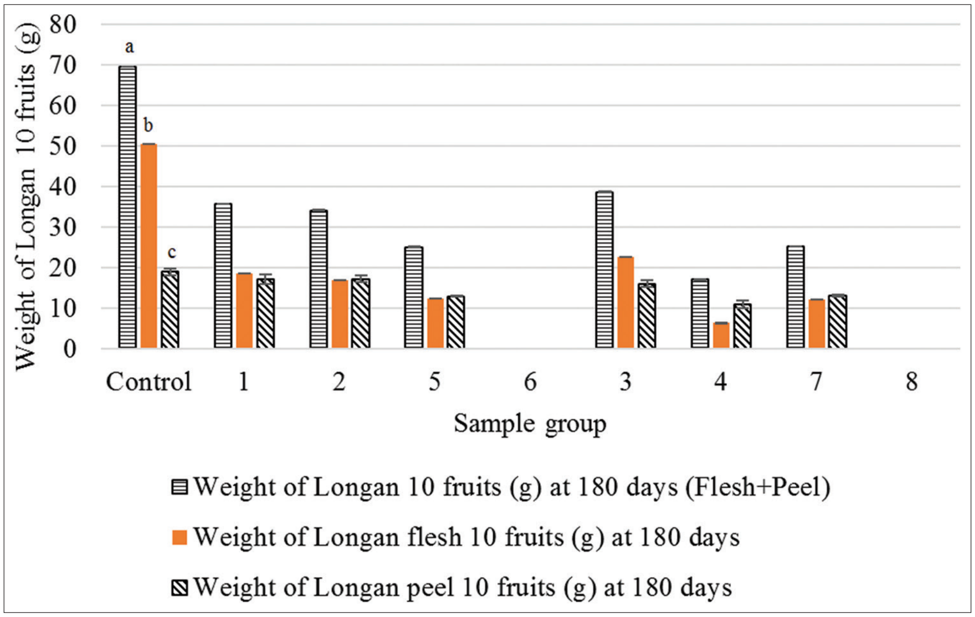

Figure 2: Comparison for weight of longan fruits (mean±SD) at 180 days after $KClO_3$ stimulation among nine groups with four different doses – group control (0 g/m), Groups 1 and 2 (25 g/m), Groups 5 and 6 (43 g/m), Groups 3 and 4 (85 g/m), Groups 7 and 8 (127 g/m). Group control was significantly higher fruit growth of all treated groups, i.e., higher flesh + peel weight[a] at $P<0.001$, higher flesh weight[b] at $P<0.001$ and higher peel weight[c] at $P<0.001$

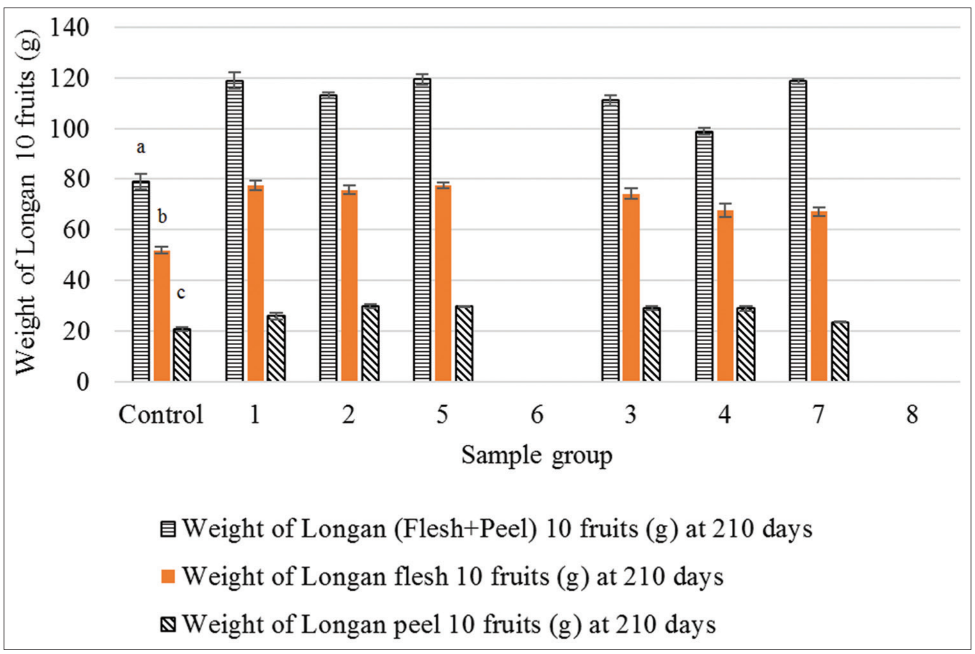

Figure 3: Comparison for weight of longan fruits (mean±SD) at 210 days after $KClO_3$ stimulation among nine groups with four different doses – group control (0 g/m), Groups 1 and 2 (25 g/m), Groups 5 and 6 (43 g/m), Groups 3 and 4 (85 g/m), Groups 7 and 8 (127 g/m). Group control was significantly lower fruit growth of all treated groups, i.e., lower flesh + peel weight[a] at $P<0.001$, lower flesh weight[b] at $P<0.001$ and lower peel weight[c] at $P<0.001$

of the farmer's experience that 25 g/m was sufficient for off-season stimulation without significant reduction in yield. Too high doses reduced the farmer's earnings significantly comparing 43 g/m (Group 5 vs. 127 g/m Group 7), $P < 0.001$.

Test for Pearson correlation for the flesh weights at 210 days versus $KClO_3$ doses, for all study groups found a high negative linear correlation when excluding group control ($r = -0.806$, $P < 0.001$ for flesh weight) [Table 1]. This finding reflected that increasing $KClO_3$ dose significantly reduced the yields. Moreover, since Pearson correlations were found only when excluding group control in the tests, it was possible that group control may have had confounding factor(s) such as inadvertent exposure to some level of $KClO_3$ (see more discussion in topic 3.4).

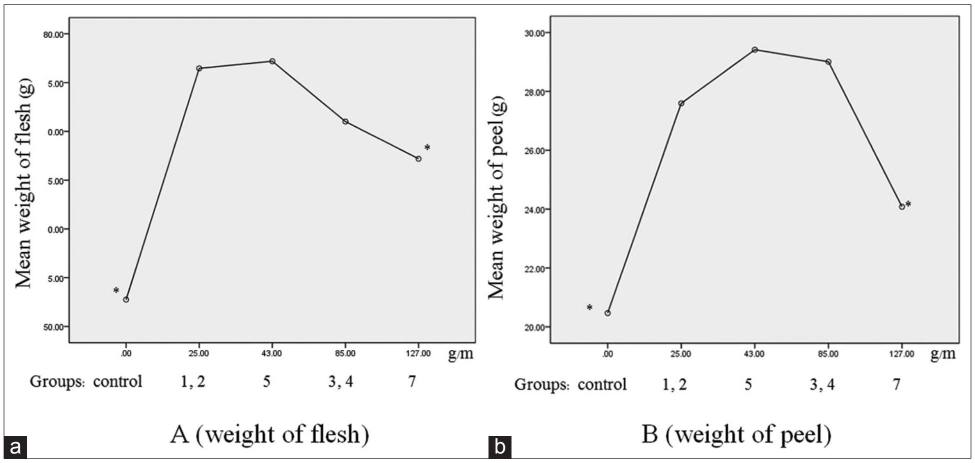

Figure 4: Comparison for growth of longan fruits at 210 days by means of fruit weight (a = weight of flesh and b =weight of peel) versus doses of $KClO_3$ stimulation, among 7 study groups: Control (0 g/m), Groups 1 and 2 (25 g/m), Group 5 (43 g/m), Groups 3 and 4 (85 g/m), Group 7 (127 g/m). *Groups 1 and 2 (25 g/m), Group 5 (43 g/m), and Groups 3 and 4 (85 g/m) were not significantly different both flesh and peel weights, but Group 5 (43 g/m) was significantly different from Group 7 (127 g/m) at P=0.008 for flesh, at P=0.017 for peel, and different from group control (0 g/m) at P=0.001 for flesh and P≤0.001 for peel

Comparison of yields at 210 days showed that using different routes of application for the same dose of $KClO_3$, i.e., Groups 1 versus 2 (25 g/m), Groups 3 versus 4 (85 g/m), and Group 5 versus Group 6 (43 g/m) had different effects. Comparing flesh weight, Group 1 and Group 2 were not significantly different, but Group 3 was significantly higher than Group 4 (P = 0.004) [Figure 3]. Comparing peel weight, these two doses and routes were not different. Thus, the application of lower dose either through roots or leaves did not affect flesh weight or peel weight, but application of higher dose through roots increased flesh weight. When looking at Groups 5 and 6 with intermediate dose, it was found that Group 5 (stimulation through roots, 43 g/m) produced the highest yield, but Group 6 (stimulation through leaves, 43 g/m) did not produce fruit. The reason why Group 4 (85 g/m through leaves) produced fruit, while Group 6 (43 g/m through leaves) did not produce fruit despite the lower dose than Group 4, may be due to interference or other confounding factors, as discussed in topic 3.4. From these findings, we concluded that stimulation of $KClO_3$ through roots gave better yields in term of flesh weights and less adverse effects on plants compared to stimulation through leaves.

Chlorate (ClO_3^-) and Chloride (Cl^-) Residues in Longan Fruits

Chemical residues in whole baby longan fruits at 150 days after $KClO_3$ application found that ClO_3^- concentrations were detectable only in Groups 4, 5, and 7 with the highest ClO_3^- concentration 0.0422 ± 0.0000 μg/g in Group 7 (data not shown). The highest Cl^- concentration was in Group 2 at 9.8531 ± 1.6623 μg/g, and the second highest concentration was in group control at 9.7600 ± 1.6349 μg/g (data not shown). Thus, group control had the highest total Cl^- in fruits since it had the highest fruit weight of all study groups. The amount of ClO_3^- andCl^- residues in fruits at 150 days did not correspond to the dose of $KClO_3$ applied.

At 180 days, the highest ClO_3^- concentration at 0.3170 ± 0.0001 μg/g and the highest total ClO_3^- in the flesh was found in fruits from Group 1. The highest Cl^- concentration in the flesh at 11.0386 ± 0.0000 μg/g was from Group 7, but the highest total Cl^- was from group control since the flesh weight of group control was much higher (data not shown). Again, the amount of ClO_3^- andCl^- residues in the flesh and the peel at 180 days did not reflect the dose of $KClO_3$ applied and did not have a clear pattern.

At 210 days, ClO_3^- residue was found at the highest concentration in the flesh of Group 5 fruits, with this group giving the highest flesh weights. The ClO_3^- residue concentrations of most study groups were significantly different in the flesh and the peel [Table 1]. Pearson correlation tests between $KClO_3$ doses and ClO_3^- concentrations or total ClO_3^- in the flesh of all study groups were found to be not significant. Conversely, significant Pearson positive correlations were found for ClO_3^- concentration and total ClO_3^- in the peel [Table 1]. Thus, $KClO_3$ doses affected the accumulation of ClO_3 residues in the peel, higher $KClO_3$ doses higher ClO_3^- residues in the peel, not the flesh.

However, for Cl^- residues, the highest concentrations were found in the flesh and the lowest concentrations were found in the peel of group control. Pearson correlation tests showed a moderate negative correlation between $KClO_3$ dose and Cl^- concentration or total Cl^- (r = −0.568, P = 0.004; r = −0.587, P = 0.003, respectively) in the flesh but a moderate positive correlation between $KClO_3$ dose and Cl^- concentration or total Cl^- (r = 0.550, P = 005; r = 0.530, P = 0.007, respectively) in the peel [Table 1]. These results indicated that higher $KClO_3$ doses would result in higher Cl^- residues in the peel but lesser Cl^- residues in the flesh.

These findings confirmed that increased $KClO_3$ doses did not have a corresponding effect on levels of ClO_3^- and Cl^- residues in the edible part of longan fruits (flesh). However, for those who make use of other parts of longan fruits, for example, seeds and flowers for Chinese traditional medicines,[21] more studies should be pursued.

Comparison between different routes of application for the same doses of $KClO_3$ produced inconclusive results when considering ClO_3^- concentrations in the flesh and the peel but when considering total ClO_3^-, application through roots

Table 1: Comparison of $KClO_3$ doses used in stimulation versus weight of flesh and peel, chemical concentrations, and total chemicals in longan fruits at 210 days after application, for ClO_3^- and Cl^- residues

Sample groups	$KClO_3$ Dose[#] (g/m)	Weight of flesh (g/fruit)	Weight of peel (g/fruit)	ClO_3^- conc. in flesh (µg/g)	ClO_3^- conc. in peel (µg/g)	Total ClO_3^- in flesh (µg/fruit)	Total ClO_3^- in peel (µg/fruit)	Cl^- conc. in flesh (µg/g)	Cl^- conc. in peel (µg/g)	Total Cl^- in flesh (µg/fruit)	Total Cl^- in peel (µg/fruit)
Control	0	5.19±0.93[b,1]	2.07±0.68[b,2]	0.033±0.000[b,1]	0.088±0.000[b,1]	0.16[b,1]	0.1[b,3]	6.207±0.008[b,1]	3.617±0.002[b,1]	32.23[b,1]	7.49[b,1]
1	25R	7.76±0.61	2.59±0.12	0.149±0.000	0.079±0.000	1.16	0.201	0.905±0.010	19.143±0.006	7.06	49.57
2	25L	7.57±0.56	2.96±0.16	0.049±0.000[a,1]	0.154±0.000[a,1]	0.38[a,1]	0.44[a,1]	0.420±0.003[a,1]	16.336±0.008[a,1]	3.18[a,1]	48.37
5	43R	7.76±0.75	2.96±0.15	0.238±0.000	0.108±0.000	1.72	0.33	1.113±0.000	11.770±0.008	7.95	34.84
6	43L	-	-	-	-			-	-		
3	85R	7.42±0.16	2.89±0.50	0.047±0.000	0.167±0.000	0.37	0.49	1.032±0.000	9.873±0.007	7.64	28.52
4	85L	6.76±0.64[a,2]	2.89±0.93	0.049±0.000[a,1]	0.189±0.000[a,1]	0.34	0.55[a,3]	0.367±0.014[a,1]	9.194±0.100[a,1]	2.50[a,1]	26.56
7	127R	6.72±0.75	2.34±0.25	0.225±0.000	ND	1.55	NA	0.593±0.000	30.166±0.000	3.96	70.60
8	127L	-	-	-	-			-	-		
Pearson[d]		NS	NS	NS	r=0.773, P<0.001	NS	r=0.796, P<0.001	r=−0.568, P=0.004	r=0.550, P=0.005	r=−0.587, P=0.003	r=0.530, P=0.007
Pearson[e]		r=−0.806, P<0.001	r=−0.389, P=0.055	NS	r=0.702, P=0.002	NS	r=0.681, P=0.003	NS	NS	NS	NS

[a]Comparing between stimulation through leaves and through roots of the same doses (Group 2 vs. 1, and group 4 vs. 3), they were significantly different at [1]$P<0.001$, [2]$P=0.004$, [3]$P=0.005$, [b]Comparing between group control versus all stimulated groups (Groups 1, 2, 3, 4, 5, and 7), they were all significantly different at [1]$P<0.001$, [2]$P<0.005$ (except to group 7), [3]$P<0.001$ (except to Group 1), [d]Test for Pearson correlation between doses of $KClO_3$ used versus outcomes among all groups (Groups 1, 2, 5, 3, 4, and 7, control), [e]Test for Pearson correlation between doses of $KClO_3$ used versus outcomes among 6 groups (Groups 1, 2, 5, 3, 4, and 7, excluding group control), [#]R: stimulation through root, L: stimulation through leaves, ND: Not detectable, NA: Not applicable, NS: Non-statistical significance. KClO3: Potassium chlorate

Table 2: Doses of $KClO_3$ used in stimulation, weight, chemical concentrations, and total chemicals in longan fruits at 210 days after application, for K^+ and Na^+ residues

Sample group	$KClO_3$ Dose (g/m)	Weight of flesh (g/fruit)	Weight of peel (g/fruit)	Weight of seed (g/fruit)	K^+ conc. in flesh (µg K^+/g)	K^+ conc. in peel (µg K^+/g)	K^+ conc. in seed (µg K^+/g)	Total K^+ in flesh (mg/fruit)	Na^+ conc. in flesh (µg Na^+/g)	Na^+ conc. in peel (µg Na^+/g)	Na^+ conc. in seed (µg Na_+/g)	Total Na^+ in Flesh (µg/fruit)
Control	0	5.19±0.93	2.07±0.68	2.13±0.21	98.78±0.45[#]	160.57±0.38	12.79±0.06	5.211±0.22	0.16±0.01	0.14±0.00	0.14±0.01	8.43±0.27
1	25R	7.76±0.61	2.59±0.12	1.69±0.32	261.80±0.46	204.28±0.00	252.24±0.26	20.20±0.04	0.15±0.00	0.10±0.00	0.19±0.00	11.66±0.27
3	85R	7.42±0.16	2.89±0.50	2.89±0.49	204.53±0.73	309.35±0.00	257.01±0.50	15.28±0.31	0.10±0.00	0.14±0.00	0.10±0.00	7.76±0.10
Pearson[a]		r=0.658, P=0.027	r=0.916, P<0.001	NS	NS	r=1.000, P<0.001	r=0.735, P=0.012	NS	r=−0.989, P<0.001	NS	r=−0.586, P=0.049	NS

All groups (values) were significantly different from each other at $P<0.05$, [a]Test for Pearson correlation between doses of $KClO_3$ used versus the outcome, R: Stimulation through root, NS: Not significant
$KClO_3$: Potassium chlorate

Table 3: Assessment result of ClO_3^- analysis and calculated from the ingestion route by MOS and HQ for men and women

IR	Value	ClO_3 [1]	DD[1] (μg/kg−d)	MOS[1]*	HQ[1]*	ClO_3 [5]	DD[5] (μg/kg−d)	MOS[5]*	HQ[5]*	ClO_3 [C]	DD[C] (μg/kg−d)	MOS[C]*	HQ[C]*
Men													
IR1	10 fruits/day	11.55	0.19	156	0.01	18.45	0.31	98	0.01	1.69	0.03	1,065	0.00
IR2	500 g/day	55.79	0.93	32	0.03	86.02	1.33	21	0.05	11.64	0.19	155	0.01
IR3	1000 g/day	111.57	1.86	16	0.06	172.03	2.87	10	0.10	23.29	0.39	77	0.01
Women													
IR1	10 fruits/day	11.55	0.19	130	0.01	18.45	0.30	81	0.01	1.69	0.03	888	0.00
IR2	500 g/day	55.79	0.93	27	0.04	86.02	1.72	17	0.06	11.64	0.19	129	0.01
IR3	1000 g/day	111.57	1.86	13	0.07	172.03	3.44	8.7	0.11	23.29	0.39	64	0.02

IR1: Ingestion rate of 10 longan fruits/day, IR2: Ingestion rate of 500 g longan fruits/day, IR3: Ingestion rate of 1000 g longan fruits/day, [1]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 1, [5]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 5, [C]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of longan fruits of the control group, *Tolerable daily intake (TDI) value of reference dose (RfD) of chlorate group=30 μg/kg−d[14]

resulted in higher accumulation of ClO_3^- in the flesh but lower ClO_3^- in the peel. When considering Cl^- residues, it was found that application through roots gave a higher accumulation of Cl^- residues in both the flesh and the peel (and hence total Cl^-). Any explanation for the observed differences in accumulation of ClO_3^- and Cl^- residues in the flesh and the peel of the fruits from different stimulation routes will need a thorough understanding and investigation of plant biology. Therefore, we left this part for other experts to pursue.

As ClO_3^- residue was found in both the flesh and the peel of group control fruits; we proposed that ClO_3^- residues may have come from either $KClO_3$ used in the past growing season(s) that remained in the soil or from the use of $KClO_3$ in nearby areas. The study of Jantiang[22] reported that $KClO_3$ could have a half-life for 75–365 days in the soil before decomposition. $KClO_3$ from previous seasons and/or from nearby areas may be uncontrolled confounding factors in this study. The longan orchard used in this study is located 700 km far from the researchers' office, which means that the researchers could only travel back and forth between the orchard and the office at each 30-day observation. Therefore, some of the farmer's activities were uncontrollable factors. Our researcher observed that the study field had been covered with piles of old, dry leaves before $KClO_3$ application. Therefore, the amount of applied $KClO_3$ that reached the roots of longan trees could be lesser than expected since some of any applied $KClO_3$ left on top of the piles of leaves could have been lost or decomposed by environmental conditions (e.g. sunlight and rain) without reaching the roots. Similarly, $KClO_3$ applied to the leaves could also have been lost by environmental conditions. This factor was uncontrollable. Moreover, the growing areas of some study groups were on steeper slopes than others, so the chemicals may have washed away or runoff from high areas to lower areas. Therefore, the low areas may have received more chemicals than expected and the high areas received less chemicals than expected. In our study, the design of study longan trees was arranged to the line by group control, 1, 2, 3, 4, 5, 6, 7, and 8. This resulted as Groups 6, 7, and 8 were located at lower areas, with Group 8 the lowest. This may have contributed to Group 8 showing the most adverse effects from $KClO_3$; in addition to, the dose being the highest and the application being through the leaves. The lower growing area may also partly explain why Group 6 did not produce fruit despite receiving a lower dose than Group 4. Furthermore, differences in the biochemical composition of the soil in the field may have affected $KClO_3$. For example, a different amount of bacterial colonies reducing ClO_3^- (bacterium reductase) in the soil[23] could have affected the chemical. All of these uncontrolled confounding factors could affect the amount of ClO_3^- and Cl^- residues (the end product of $KClO_3$) detected in our study groups. Nevertheless, we believe that our major findings in the correlation between $KClO_3$ doses and amount of ClO_3^- and Cl^- residues in the edible part of longan fruits were still valid and that these uncontrolled confounding factors would not have changed our study results significantly.

3.5 K^+ and Na^+ Residues in Longan Fruits

In fruits collected from Groups 1 and 3, and control at 210 days, the K^+ concentration and total K^+ in the flesh were highest in Group 1, while the highest K^+ levels in the peel and seed were in Group 3. Group control had the lowest K^+ residues in the flesh, the peel and the seed [Table 2]. These findings indicate that K^+ residues found in longan fruit peel and seed came at least partly from $KClO_3$ used in off-season stimulation, as a higher dose of $KClO_3$ resulted in higher K^+ residues in the peel and the seed, with group control having the lowest levels.

For Na^+ residues, the concentration of Na^+ in the flesh and the peel was highest in group control, whereas the concentration of Na^+ in seeds was highest in Group 1.

In longan fruit flesh, K^+ was found at levels thousand-folds higher than Na^+. High K^+ was found even in group control. This confirmed that longan fruits are a good source of K^+.Since the dose of $KClO_3$ did not have a linear correlation with K^+ residues in the flesh, this confirmed that the dose of $KClO_3$ did not affect K^+ residues in the flesh but could affect levels in the peel and the seed.

The K^+ and Na^+ detected in longan fruits may have come from the soil or elsewhere in addition to the $KClO_3$ used for off-season stimulation. The tree's metabolism is likely to have a more significant contribution to the accumulation of K^+ and Na^+. One previous study reported that plants preferentially accumulate a lot of Na^+ in the root and only a little in the fruit.

Table 4: Assessment result of Cl^- analysis and calculated from the ingestion route by MOS and HQ

IR	Value	DD[1] (µg/day)	MOS[1]*	HQ[1]*	DD[5] (µg/day)	MOS[5]*	HQ[5]*	DD[C] (µg/day)	MOS[C]*	HQ[C]*
IR1	10 fruits/day	108.58	21×10^3	0.00	133.55	17×10^3	0.00	497.83	4.6×10^3	0.00
IR2	500 g/day	524.58	4.4×10^3	0.00	622.72	3.7×10^3	0.00	3,429	670	0.00
IR3	1000 g/day	1048.16	2.2×10^3	0.00	1,245.43	1.8×10^3	0.00	6,859	335	0.00

IR1: Ingestion rate of 10 longan fruits/day, IR2: Ingestion rate of 500 g longan fruits/day, IR3: Ingestion rate of 1000 g longan fruits/day, [1]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 1, [5]Daily exposure dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 5, [C]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of longan fruits of the control group, *Tolerable daily intake (TDI) or reference dose (RfD) of chloride=2300 mg/day[13]

Table 5: Assessment result of K^+ analysis and calculated from the ingestion route by MOS and HQ

IR	Value	DD[1] (µg/day)	MOS[1]*	HQ[1]*	DD[3] (µg/day)	MOS[3]*	HQ[3]*	DD[C] (µg/day)	MOS[C]*	HQ[C]*
IR1	10 fruits/day	10,842.63	350.47	0.00	8595.65	450.47	0.00	2868.87	1325	0.00
IR2	500 g/day	104,769.27	36.27	0.00	81,850.98	46.43	0.00	13,860.53	274.2	0.00
IR3	1,000 g/day	209,538.53	18.14	0.00	163,701.97	23.21	0.00	27,721.05	137.1	0.00

IR1: Ingestion rate of 10 longan fruits/day, IR2: Ingestion rate of 500 g longan fruits/day, IR3: Ingestion rate of 1000 g longan fruits/day, [1]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 1, [3]Daily exposure dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 3, [C]Daily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of longan fruits of the control group, *Tolerable daily intake (TDI) or reference dose (RfD) of K^+ =3800 mg/day[13]

Table 6: Assessment result of Na^+ analysis and calculated from the ingestion route by MOS and HQ

IR	Value	DD[1] (µg/day)	MOS[1]*	HQ[1]*	DD[3] (µg/day)	MOS[3]*	HQ[3]*	DD[C] (µg/day)	MOS[C]*	HQ[C]*
IR1	10 fruits/day	4.77	5031×10^3	0.00	23.05	7346×10^3	0.00	46.10	678.7×10^3	0.00
IR2	500 g/day	3.27	520.6×10^3	0.00	15.85	757.1×10^3	0.00	31.70	98.6×10^3	0.00
IR3	1,000 g/day	3.54	260.3×10^3	0.00	24.36	378.5×10^3	0.00	48.72	49.3×10^3	0.00

IR1: Ingestion rate of 10 longan fruits/day, IR2: Ingestion rate of 500 g longan fruits/day, IR3: Ingestion rate of 1000 g longan fruits/day, [1]Daily ingestion dose (DD), Margin of safety (MOS) and Hazard quotient (HQ) of longan fruits consumption of Group 1, [3]Daily exposure dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of Group 3, CDaily ingestion dose (DD), Margin of safety (MOS), and Hazard quotient (HQ) of longan fruits consumption of longan fruits of control group, *Tolerable daily intake (TDI) or reference dose (RfD) of Na^+=2400 mg/day[13]

Risk Assessment for Consumption of Longan Flesh

Daily consumption levels of chemical residues from the ingestion of longan flesh were compared at three consumption rates, i.e., 10 fruits/day, 500 g flesh/day, and 1000 g flesh/day. The sample groups used for different residue levels were; sample Group 1 (farmer's own choice of $KClO_3$ dose and the lowest stimulation dose in this study), the sample groups that gave the highest chemical residues among the $KClO_3$ stimulated groups, i.e., Groups 5 for ClO_3^- and Cl^- residues, and 3 for K^+ and Na^+ residues, and the control group (no $KClO_3$ stimulation). The highest risk was HQ = 0.11 and MOS = 10 for men, and HQ = 0.11 and MOS = 8.7 for women, for ClO_3^- residue ingested in 1000 g of longan flesh collected from Group 5 (the highest consumption rate from the highest residue, Table 3). For Cl^- residue, the highest risk was HQ = 0.00, MOS = 1.8×10^3 [Table 4]. For K^+ residue the highest risk was HQ = 0.00, MOS = 23.21 [Table 5], and for Na^+ residue that the highest risk was HQ = 0.00 and MOS = 378.5×10^3 [Table 6] from ingestion of 1000 g of longan flesh collected from Group 3. All combinations had HQ much <1.00, or MOS much >1.00 indicating that there were no toxicity concerns for the general population ingesting off-season longan flesh. Nevertheless, groups of people with chronic health conditions such as diabetes, hypertension, anemia, and kidney insufficiency should be cautious about consuming excess off-season longan flesh.

Since $KClO_3$ is a legally prohibited substance in food, we had long been curious about the safety of its use in longan production. The discovery that $KClO_3$ use in agricultural production leaves little residue in the produce means that the fruit is safe for consumption by the general population.

CONCLUSION

We conclude that 43 g/m of $KClO_3$ (Group 5) should be the maximum useful dose in the off-season stimulation of longan production. Higher doses than 43 g/m of $KClO_3$ reduced the benefits, and 25 g/m of $KClO_3$ (the farmer's own choice) was sufficient for off-season stimulation without significant reduction in yield.

$KClO_3$ did not affect the accumulation of ClO_3^- residues in the flesh but did in the peel. Higher $KClO_3$ doses resulted in higher ClO_3^- residues in the peel. Higher $KClO_3$ doses also resulted in higher Cl^- residues in the peel but lesser Cl^- residues in the flesh.

Overall, our study found that higher $KClO_3$ doses resulted in higher ClO_3^-, Cl^-, K^+, and Na^+ in the peel, but our findings of the accumulation of related chemical residues in the flesh were inconclusive. Application of $KClO_3$ through roots gave a higher accumulation of ClO_3^- and Cl^- in the flesh compared to application through leaves for the same dose. Nevertheless, the stimulation of $KClO_3$ through roots resulted in better flesh weights and less adverse effects on plants.

The risk assessment for chemical residues found that ingestion of 1000 g of longan flesh was safe since all risk determinants were clearly in the safe range (HQ <<1.00, or MOS >>1.00). Therefore, we conclude that there is no need for concern among general consumers about ingestion of off-season longan flesh within our study settings.

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