



SHIELDING BANGLADESH'S EXPORTABLE BETEL LEAVES: MANAGING *E. coli* AND *Salmonella* CONTAMINATION

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[Citation: Islam, M.M., Islam, M.N., Islam, M.N., Ali, M.R., Kabir, M.H. and Jamal Uddin, A.F.M. (2024). Shielding Bangladesh's Exportable Betel Leaves: Managing *E. coli* and *Salmonella* Contamination. *Int. J. Bus. Soc. Sci. Res.* 12(1): 55–61. Retrieve from <http://www.ijbssr.com/10.55706/ijbssr12111>]

Received Date: 08/10/2023

Acceptance Date: 15/05/2024

Published Date: 16/05/2024

Abstract

The study examined the presence of *E. coli* and *Salmonella* spp. in soil, water, and mature betel leaf samples at preharvest stages across three upazilas in Kushtia district: Kushtia Sadar, Mirpur, and Daulatpur, although these pathogens do not typically affect betel leaf and other fresh vegetables. Researchers tested the samples before and after sanitization with calcinated calcium (CCa) from May to September 2020. They collected soil, water, and betel leaf samples from 15 farmers' fields, and initially, all samples were contaminated with *E. coli* and *Salmonella* spp. After sanitization, *E. coli* contamination persisted in a few samples: 22.22% in Sadar, 28.89% in Mirpur, and 35.56% in Daulatpur, while *Salmonella* spp. was eradicated. Potential contamination vectors included dogs (86.67%), birds (77.78%), and cats (59.26%). In Dhaka, betel leaves from the central pack house showed significant contamination differences post-washing. Before washing, 51.67% of leaves had *E. coli* and 41.50% had *Salmonella* spp. After three washes with CCa, contamination was completely eliminated for both pathogens. The study highlights the effectiveness of CCa in reducing microbial contamination but also points out the need for better contamination control strategies and training for farmers to mitigate vector-related contamination. This comprehensive study highlights the effectiveness of CCa in reducing microbial contamination in betel leaf production and underscores the need for ongoing management to mitigate recontamination risks from environmental vectors.

Key words: Betel leaf, food safety, hygiene practices, sanitizer.

Introduction

Betel leaf (*Piper betel*) is a perennial climbing plant in the family Piperaceae (Nahar *et al.*, 2018). Bangladesh is the second-largest producer of betel leaves globally (Jahan *et al.*, 2016), with a total annual production of 206,993.70 MT from 21,247.77 hectares in 2020-21, averaging 9.63 tons per hectare (Yearbook of Agricultural Statistics, 2021). Betel leaf is a significant cash crop in Bangladesh, with over 20 million domestic consumers and substantial export potential, particularly to the Middle East and Europe (Islam, 2019). Major export destinations include the UK, Italy, France, Germany, and Saudi Arabia, among others (BFVAPEA, 2018). Despite high demand, exports to Europe have declined due to microbial contamination with *Salmonella*, leading to a ban by the European Union and the UK in 2014 (Islam, 2019; DG-SANTE, 2016). The EU requires that betel leaves be free from quarantine microbes, especially *Salmonella* (Nahar *et al.*, 2018; Fakruddin *et al.*, 2017). Zaman *et al.* (2021) emphasized the importance of microbial safety in soil and water to ensure contamination-free produce. Contaminated water and soil are primary sources of microbial contamination (Lapidot *et al.*, 2006; Jongman and Korsten, 2018). Nahar (2018) explained that the betel leaf and other fresh produce may be contaminated with *Salmonella* by pollution of air, water, soil at the time of production to harvest and post-harvest processing up to consumer table. (Hamilton *et al.* 2006) explained that risk of microbial contamination of different produce is mainly involved with water quality that are using the and also he suggested that to make good quality maintained need to established guide line first. Scientists found that the remarkable sources of produce contamination in the supply chain both at the pre-harvest (in the field) and post-harvest (carrying & processing) stages (Bari 2018). Proper washing can reduce contamination but not eliminate it entirely (FAO/WHO, 2008). Contaminated irrigation water and manure are significant contamination sources (Chang and Fang, 2007). Holvo *et al.* (2012) reported that at the time of production and after harvest when the produce is washed it can be the main road to contamination of the produce and then end user can be affected by the contaminated *Salmonella* through the produce. (Wachtel and Charkowaski 2002) stated that the after harvest the fresh produce when it is taking to the storage and then can be contaminated with *Salmonella*. Packaging and storage also play a crucial role in preventing contamination (Burdon, 2001; Goldberg *et al.*, 2011). Montanari, (2015) reported that due to contamination of

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Salmonella spp. the export of betel leaf to Europe has tremendously declined from Bangladesh.

Sunzid *et al.*, (2017) reported that non chlorine sanitizer can be used for remove the pathogen from the betel leaf. Betel leaf that contaminated with the *Salmonella* spp. is dangerous for consumers. To control the microorganism on contaminated betel leaf it is to be needed of approved sanitizing water for washing the betel leaf properly and it is helping also further control of further cross contamination (FDA 2008). Winniczuk, (1994) reported that the efficiency of sanitizer water is to control of *Salmonella* and other microorganism is depend on type of soil time and water temperature. For export betel leaf in Europe country Bangladesh has to ensure that production of exportable vegetables comply with European countries requirement following Good Agriculture Practice (GAP), Good Hygiening Practice (GHP) as well as Good Manufacturing mean packaging Practice (GMP) from field production to till export. Sawai *et al.* (2002) stated that the Calcinated calcium which is made with 98% CaO and 2% CaO which can work as an anti-bacterial and antifungal activities against big number of microorganism. Non-chlorine sanitizers, like calcinated calcium, are effective against various microorganisms and are environmentally friendly (Sawai *et al.*, 2002). Considering all above in mind this study aims to identify trade barriers, sources of microbial contamination, and effective management practices for producing safe betel leaves for international and domestic consumers.

Materials and Methods

The study aimed to identify preharvest and postharvest contamination sources of betel leaf by *Salmonella* and develop effective management strategies. Conducted in Kushtia Sadar, Daulatpur, and Mirpur upazilas of Kushtia district, 45 betel leaf farmers were selected, with fields sized 50-80 decimals, and aided by the Upazila Agriculture Office. From May to September 2020, samples of soil, water, and mature betel leaves were collected from 45 fields, with replication in each upazila. Samples were tested for *Salmonella* and *E. coli* before and after sanitization with calcinated calcium (CCa). Postharvest samples were collected from local centers and central packhouses, also tested for microbial contamination. Sanitizer solutions included 0.01% CCa, 200 ppm chlorine, and 0.5% hydrogen peroxide. Betel leaves were dipped in these solutions for one minute at room temperature, then drained. Microbiological analysis determined the presence of *Salmonella* spp. and *E. coli*, with trials replicated three times. Statistical analysis ensured accuracy by observing colonies grown in culture media.

Results and Discussion

a. Presence of *E. coli* and *Salmonella* spp. preharvest stages at Sadar Upazilla in Kushtia

Status of *Salmonella* spp. and *E. coli* in soil sample, water sample and mature betel leaf sample before using sanitizer and after using sanitizer of 15 selected cluster farmers of preharvest stages in Kushtia Sadar Upazilla in Kushtia were recorded. The result was found that has shown on Table no 1 in below.

Soil sample Results: From the table 1 observed that, the soil test reports for the presence of *E. coli* and *Salmonella* spp. at before and after sanitization of the betel leaf field. From the table, the pH of the soil of the betel leaf field soil was ranged from 6.69 to 7.10. In case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization i.e. calcinated calcium (CCa), among 15 samples 3 samples showed the presence of *E. coli* at In case of *Salmonella* spp., out of 15 soil samples 3 samples were not contaminated with *Salmonella* spp. (<1.0) before using sanitizer (Calcinated calcium). After using sanitization by calcinated calcium (CCa), among 15 samples no contamination with *Salmonella* spp. was observed.

Table 1. Presence of *E. coli* and *Salmonella* spp. in soil irrigated surface water and mature betel leaf at Kushtia Sadar upazila of Kushtia district

Sample No.	Soil sample					Water Sample				Mature B. leaf Sample				
	pH	<i>E. coli</i>		<i>Salmonella</i>		pH	<i>E. coli</i>		<i>Salmonella</i>		<i>E. coli</i>		<i>Salmonella</i>	
		BE	AE	BE	AE		BE	AE	BE	AE	BS	AE	BE	AE
1	6.88	P	A	P	A	6.89	P	A	P	A	P	A	P	A
2	6.90	P	A	P	A	6.91	P	P	A	A	P	P	P	A
3	6.79	P	A	A	A	6.88	P	A	P	A	P	A	A	A
4	6.69	P	P	P	A	6.88	P	P	P	A	P	A	P	A
5	7.10	P	A	P	A	6.94	P	A	P	A	P	A	P	A
6	6.91	P	P	P	A	6.91	P	A	P	A	P	P	P	A
7	6.86	P	A	P	A	6.86	P	P	P	A	P	A	P	A
8	6.72	P	A	P	A	6.79	P	A	A	A	P	A	P	A
9	6.70	P	A	P	A	6.80	P	A	P	A	P	A	P	A
10	7.07	P	A	A	A	7.01	P	A	P	A	P	A	P	A
11	6.75	P	A	P	A	6.85	P	A	P	A	P	A	P	A
12	6.71	P	A	P	A	6.83	P	A	P	A	P	A	P	A
13	6.76	P	A	P	A	6.82	P	A	P	A	P	A	P	A
14	6.67	P	P	A	A	6.87	P	P	P	A	P	P	P	A
15	6.92	P	A	P	A	6.89	P	A	P	A	P	A	P	A
Total		15	3	12	00		15	4	13	00	15	3	14	00

[Here, BE= Before Enrichment, AE= After Enrichment, P= Present, A= Absent]



Surface water sample Results: when the water used in surface water and before and after sanitization and Ph range was 6.79-7.01 which showed the favour of growth betel leaf and organism. From the Table 1 it was found that in case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 4 samples showed the presence of *E. coli*. In case of *Salmonella* spp., out of 15 water samples, 2 samples were not contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Kustia Sadar upazila under Kustia district.

Mature betel leaf sample Results: From the table 1 it was found that the presence of *E. coli* and *Salmonella* spp. at before and after sanitization. In case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 3 samples showed the presence of *E. coli* at Kustia Sadar upazila. In case of *Salmonella* spp., out of 15 mature betel leaf samples, only one sample was not contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed.

b. Presence of *E. coli* and *Salmonella* spp. preharvest stages at Mirpur Upazilla in Kushtia

Presence of *Salmonella* and *E. coli* of soil sample, water sample and mature betel leaf sample before using sanitizer and after using sanitizer of 15 selected cluster farmers of preharvest stages at Mirpur Upazilla in Kushtia were recorded. The result was found that has shown on Table no 2 in below.

Soil sample Results: About presence *E. coli* and *Salmonella* spp. in the soil sample before and after sanitizer used and the soil sample pH was 6.72 to 6.97. From Table 2 it was observed that In case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 5 samples showed the presence of *E. coli* was observed. In case of *Salmonella* spp., out of 15 soil samples, only one sample was not contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Mirpur upazila under Kustia district

Surface water sample Results: About presence *E. coli* and *Salmonella* spp. in the water sample before and after sanitizer used and the soil sample pH was 6.8 to 6.81. From Table 2 it was observed that In case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 4 samples showed the presence of *E. coli* at Mirpur upazila under Kustia district. In case of *Salmonella* spp., out of 15 water samples, only one sample was contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Mirpur upazila under Kustia district.

Mature betel leaf sample Results: About presence *E. coli* and *Salmonella* spp. in the soil sample before and after sanitizer used and the mature betel leaf sample. From Table 2 it was observed that in case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 3 samples showed the presence of *E.* at Mirpur upazila under Kustia district. In case of *Salmonella* spp., all samples were contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Mirpur upazila under Kustia district

Table 2. Presence of *E. coli* and *Salmonella* spp. in soil irrigated surface water and mature betel leaf at Mirpur Upazila of Kustia district

Sample No.	Soil sample						Water Sample				Mature B. leaf Sample			
	pH	<i>E. coli</i>		<i>Salmonella</i>		pH	<i>E. coli</i>		<i>Salmonella</i>		<i>E. coli</i>		<i>Salmonella</i>	
		BE	AE	BE	AE		BE	AE	BE	AE	BE	AE	BE	AE
1	6.87	P	A	P	A	6.68	P	A	P	A	P	P	P	A
2	6.93	P	A	A	A	6.75	P	A	P	A	P	A	P	A
3	6.73	P	P	P	A	6.73	P	A	P	A	P	A	P	A
4	6.87	P	A	P	A	6.78	P	A	A	A	P	A	P	A
5	6.97	P	A	P	A	6.74	P	P	P	A	P	A	P	A
6	6.94	P	A	P	A	6.74	P	A	P	A	P	A	P	A
7	6.85	P	P	P	A	6.75	P	A	P	A	P	A	P	A
8	6.74	P	P	P	A	6.73	P	P	P	A	P	P	P	A
9	6.79	P	P	P	A	6.76	P	P	P	A	P	A	P	A
10	6.95	P	A	P	A	6.81	P	A	P	A	P	A	P	A
11	6.97	P	A	P	A	6.77	P	A	P	A	P	P	P	A
12	6.76	P	P	P	A	6.72	P	P	P	A	P	A	P	A
13	6.78	P	A	P	A	6.73	P	A	P	A	P	A	P	A
14	6.72	P	A	P	A	6.69	P	A	P	A	P	A	P	A
15	6.93	P	A	P	A	6.78	P	A	P	A	P	A	P	A
Total		15	5	14	00		15	4	15	00	15	3	15	00

[Here, BE= Before Enrichment, AE= After Enrichment, P= Present, A= Absent]

c. Presence of *E. coli* and *Salmonella* spp. preharvest stages at Daulatpur Upazilla in Kushtia

Presence of *Salmonella* and *E. coli* of soil sample, water sample and mature betel leaf sample before using sanitizer and after using sanitizer of 15 selected cluster farmers of preharvest stages at Mirpur Upazilla in Kushtia were recorded. The result was found that has shown on Table no 3 in below.

Soil sample Results: About presence *E. coli* and *Salmonella* spp. in the soil sample before and after sanitizer used and the soil sample pH was 6.71 to 6.71. From Table 3 it was observed that in case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 5 samples showed the presence of *E. coli* at Daulatpur upazila of Kustia district. In case of *Salmonella* spp., out of 15 soil samples, only 2 samples were not contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Daulatpur upazila under Kustia district.

Surface water sample Results: About presence *E. coli* and *Salmonella* spp. in the soil sample before and after sanitizer used and the water sample pH was 6.66 to 6.68. From Table 3 it was observed that in case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 5 samples showed the presence of *E. coli* at Daulatpur upazila under Kustia district. In case of *Salmonella* spp., out of 15 water samples, only 2 samples were not contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Daulatpur upazila under Kustia district.

Mature betel leaf sample Results: About presence *E. coli* and *Salmonella* spp. in the soil sample before and after sanitizer used. From Table 3 it was observed that in case of *E. coli*, all samples were contaminated with *E. coli* (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples 6 samples showed the presence of *E. coli* at Daulatpur upazila under Kustia district. In case of *Salmonella* spp., out of 15 mature betel leaf samples, only one sample was not contaminated with *Salmonella* spp. (<1.0) before sanitization. After using sanitization by calcinated calciam (CCa), among 15 samples no contamination with *Salmonella* spp. was observed among the samples at Daulatpur upazila under Kustia district.

Table 3: Presence of *E. coli* and *Salmonella* spp. in soil irrigated sarface water and mature betel leaf at Daulatpur upazila of Kustia district

Sample No.	Soil sample					Water Sample					Mature B. leaf Sample			
	pH	<i>E. coli</i>		<i>Salmonella</i>		pH	<i>E. coli</i>		<i>Salmonella</i>		<i>E. coli</i>		<i>Salmonella</i>	
		BE	AE	BE	AE		BE	AE	BE	AE	BE	AE	BE	AE
1	6.79	P	A	P	A	6.73	P	A	P	A	P	A	P	A
2	6.82	P	P	P	A	6.81	P	A	P	A	P	A	P	A
3	6.88	P	A	A	A	6.75	P	P	P	A	P	P	P	A
4	6.71	P	A	P	A	6.68	P	P	P	A	P	A	P	A
5	7.03	P	P	P	A	6.80	P	A	A	A	P	P	P	A
6	6.93	P	P	P	A	6.87	P	A	P	A	P	A	P	A
7	6.96	P	A	A	A	6.75	P	A	P	A	P	A	P	A
8	6.75	P	A	P	A	6.71	P	P	P	A	P	P	P	A
9	6.80	P	A	P	A	6.66	P	A	P	A	P	A	A	A
10	6.87	P	P	P	A	6.69	P	A	P	A	P	P	P	A
11	6.85	P	A	P	A	6.73	P	A	P	A	P	A	P	A
12	6.74	P	A	P	A	6.78	P	P	P	A	P	P	P	A
13	6.77	P	A	P	A	6.72	P	A	P	A	P	P	P	A
14	6.84	P	A	P	A	6.69	P	P	P	A	P	A	P	A
15	6.86	P	P	P	A	6.73	P	A	A	A	P	A	P	A
Total		15	4	2	00		15	5	13	00	15	6	14	00

[Here, BE= Before Enrichment, AE= After Enrichment, P= Present, A= Absent]

d. Evaluate the effectiveness of sanitizer (CCa) on the *Salmonella* spp. and *E. coli*

Effectiveness of *Salmonella* spp. and *E. coli* has been shown in Table 4 in below. From the table 4 we can observe that, *Salmonella* spp. was not present in the samples after using calcinated calcium as a sanitizer at Kustia Sadar, Mirpur and Daulatpur upazilas of Kustia district. But in case of *E. coli*, 22.22% sample was contaminated with *E. coli* which was comparatively low than the other Upazilas of Kustia district. In Mirpur upazila, 28.89% sample was contaminated with *E. coli*. On the other hand, highest infestation by *E. coli* was observed in Daulatpur upazila of Kustia district. Overall, 35.56% samples collected from the betel leaf field were contaminated with microorganism like *E. coli* at Kustia district.



Table 4. Upazila wise presence of *E. coli* and *Salmonella* spp. in the selected field at preharvest level

Upazila	Number of total samples	Number of samples presence of microorganism	
		<i>E. coli</i>	<i>Salmonella</i> spp.
Kustia Sadar	45	10 (22.22%)	0 (0.00%)
Mirpur	45	13 (28.89%)	0 (0.00%)
Daulatpur	45	16 (35.56%)	0 (0.00%)
Total	135	39 (35.56%)	0 (0.00%)

e. Possible vectors for *E. coli* and *Salmonella* contamination of betel leaf

After application of the sanitization at soil, irrigation water and betel leaves, *E. coli* and *Salmonella* spp. again found on the betel leaf samples. To find out the vector of these microbial organism researchers asked some questions among the selected betel leaf growers about the entrance of other animals, human being etc. occurred in the betel leaf field. And their perception was represented in the table 5 From the table, it can be observed that, among 45 betel leaf growers from the three upazilas like Kustia Sadar, Mirpur and Daulatpur upazila of Kustia district maximum 86.67% respondent farmers informed that dog casually enter into the betel leaf field, followed by bird (77.78%), cat (59.26%), hen/duck (37.04%), goat/sheep (21.48%), human (14.07%) and cow/buffalo (5.93%) entered in to the betel leaf field who were possible vector for *E. coli* and *Salmonella* spp. contamination. From the table it can be observed that, possible vectors for contamination of *E. coli* and *Salmonella* spp. in the betel leaf field could enter into the betel leaf field was low at Kustia Sadar upazila whereas the highest percent was observed at Daulatpur upazila of Kustia district. So, the betel leaf farmers of Kustia Sadar upazila were more aware and skilled to control the contamination of betel leaf field with *E. coli* and *Salmonella* spp. And to produce the microorganism contaminated free betel leaves the betel leaf farmers need more training.

Table 5. Possible vectors of *E. coli* and *Salmonella* to contaminate betel leaves

Possible sources	Farmers' response (%)						
	Human	Cow/ Buffalo	Hen/ Duck	Goat/ Sheep	Bird	Dog	Cat
Khulna Sadar (N=45)	8.89	2.22	15.56	11.11	57.78	77.78	37.78
Mirpur (N=45)	13.33	4.44	37.78	24.44	84.44	84.44	57.78
Daulatpur (N=45)	20.00	11.11	57.78	28.89	91.11	97.78	82.22
Total (N=135)	14.07	5.93	37.04	21.48	77.78	86.67	59.26

f. Presence of *E. coli* on harvested betel leaf that transported at central pack house in Dhaka

There was significant difference among the effect of treatments against the presence of *E. coli* on betel leaves collected from the collection center (Table 6). From the table we observed that, among 120 sampled harvested betel leaves from the collection center maximum 51.67% betel leaf was contaminated with *E. coli* before washing at T₁ followed by T₃ (50.00%) and T₁ (49.17%). On the other hand, lowest percent of betel leaves (48.33%) was contaminated with *E. coli*. at T₄. After 1st washing with the treatments, maximum 51.67% betel leaves were contaminated with *E. coli* at T₁ (washing with distilled water) after collection of betel leaves from collection center, followed by T₂ (42.50%) and T₃ (41.67%). On the other hand, lowest 11.67% of betel leaves were contaminated with *E. coli* at T₄ (washing with CCa) treatment which was statistically different from other treatments. Same trend was observed after 2nd wash with the treatments. After 3rd wash, no contamination of betel leaves with *E. coli* was observed in T₄ (washing with CCa) treatment which was statistically different from others and followed by T₃ (19.17%) treatment. On the other hand, maximum contamination of betel leaves 64.17% was observed in T₁ (washing with distilled water) treatment which was statistically different from other treatments and followed by T₂ (30.00%) treatment (Table 6) has shown in below.

Table 6: Evaluate the effect of treatments to management *E. coli* on betel leaves at central packaging house

Treatments	Number of leaves harvested	Percent leaf contamination by <i>E. coli</i> before washing (%)	Percent leaf contamination by <i>E. coli</i> (%)		
			After 1 st wash	After 2 nd wash	After 3 rd wash
T ₁	120	51.67 a	51.67 a	56.67 a	64.17 a
T ₂	120	49.17 c	42.50 b	35.83 b	30.00 b
T ₃	120	50.00 b	41.67 c	25.83 c	19.17 c
T ₄	120	48.33 d	11.67 d	2.50 d	0.00 d
CV (%)	-	3.73	2.13	11.12	9.87
LSD (0.05)	-	0.29	0.43	0.27	0.46

[Here, T₁= Washing with distilled water, T₂= Washing with H₂O₂ (Hydrogen per oxide), T₃= Washing with ClO₂ (Chlorine dioxide), T₄= Washing with CCa (Calcinated calcium)]

g. Presence of *Salmonella* spp. on harvested betel leaf that transported at central pack house in Dhaka

There was significant difference among the effect of treatments against the presence of *Salmonella* spp. on betel leaves collected from the central packaging house (Table 7). From the (Table 7) we can observe that, among 100 sampled collected betel leaves from the central packaging house maximum 41.50% betel leaf was contaminated with *Salmonella* spp. before washing at T₂ followed by T₄ (39.50%) and T₁ (39.00%). On the other hand, lowest percent of betel leaves (41.50%) was contaminated with *Salmonella* spp. at T₂. After 1st washing with the treatments, maximum 43.00% betel leaves were contaminated with *Salmonella* spp. at T₁ (washing with distilled water) after collected betel leaves from the collection center, followed by T₂ (24.00%) and T₃ (19.50%). On the other hand, lowest 9.50% of betel leaves were contaminated with *Salmonella* spp. at T₄ (washing with CCa) treatment which was statistically different from other treatment. Same trend was observed after 2nd wash with the treatments. After 3rd wash, no contamination of betel leaves with *Salmonella* spp. was observed in T₄ (washing with CCa) treatment which was statistically different from others and followed by T₃ (6.50%) treatment. On the other hand, maximum contamination of betel leaves 47.00% was observed in T₁ (washing with distilled water) treatment which was statistically different from other treatments and followed by T₂ (18.00%) treatment (Table 4.4.6).

Table 7: Evaluate the effect of treatments to management *Salmonellas* pp. on betel leaf at central packaging house

Treatments	Number of leaves harvested	Percent leaf contamination by <i>Salmonella</i> spp. before washing (%)	Percent leaf contamination by <i>Salmonella</i> spp. (%)		
			After 1 st wash	After 2 nd wash	After 3 rd wash
T ₁	120	39.00 c	43.00 a	46.00 a	47.00 a
T ₂	120	41.50 a	24.00 b	20.50 b	18.00 b
T ₃	120	38.00 d	19.50 c	12.00 c	6.50 c
T ₄	120	39.50 b	9.50 d	3.00 d	0.00 d
CV (%)	-	6.34	5.23	6.33	7.12
LSD (0.05)	-	0.93	1.12	1.03	0.71

[Here, T₁= Washing with distilled water, T₂= Washing with H₂O₂ (Hydrogen per oxide), T₃= Washing with ClO₂ (Chlorine dioxide), T₄= Washing with CCa (Calcinated calcium)]

Conclusions

Betel leaf, as a high-value cash crop in Bangladesh, has proven to be one of the most commercially viable crops with significant export potential, crucial for earning foreign remittances essential for the country's economic development. Despite the high export demand for Bangladeshi betel vine, the export of betel leaf has seen a steep decline due to the presence of *Salmonella* and *E. coli* contamination. Microbial contamination, specifically *E. coli* and *Salmonella* spp., remains the primary barrier preventing the export of betel leaves. The sanitizer (CCa) was highly effective against *Salmonella* spp., eliminating contamination in all samples tested across different upazilas. However, it showed moderate effectiveness against *E. coli*, with significant reduction but not complete elimination in all samples. Animals like dogs, birds, and cats were identified as potential vectors for microbial contamination, highlighting the need for improved control measures to prevent their entry into betel leaf fields. Overall, while the sanitizer was successful in controlling *Salmonella* spp., further measures are needed to fully eliminate *E. coli* contamination, especially considering potential sources from animal entry into betel leaf fields.

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