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CAN DATES VENDED IN SOUTH MUNICIPAL CORPORATION OF DHAKA DURING RAMADAN BE CONSUMED SAFELY?

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Abstract

In Bangladesh, during Ramadan for fulfilling a Muslim Sunnah, breaking fast with dates is considered divine. Consequently, its demand surges. An investigation was conducted into searching biological contaminations (microbial load) in dates from Dhaka South City Corporation (DSCC). Total Aerobic Bacterial Count (TABC) was found in all the samples with highest count in Boroi (BabuBazar) (7.44± 0.44 log CFU/ g) and lowest count in Boroi (Sadarghat) (<1.0). Significant difference was observed in different market places irrespective of date types at $p < .05$.Yeast and mold was detected between 6.88± 0.24 log CFU/g (Dhapas- Gulistan)- 2.07± 0.00 log CFU/ g (Morium- Gulistan) with significant market to marketvariation ($p<0.05$). Except Boroi (BDL) noteworthy extent of coliform colony was counted in Nagal ((1.2± 0.10 log CFU/ g), Dhapas (2.25± 0.90 log CFU/ g), Morium (1.17± 0.09 log CFU/ g and Tunisia (1.57± 0.21 log CFU/ g) from Babubazar collection point. Most of the samples crossed safety limits set by regulatory authority. A straight threat to food safety and public health safety is manifested. Hence, considering risks associated, employment of the standards for selling food products is recommended.

Keywords: *Date1, microbial load2, food safety3.*

Introduction

Dates carry various nutritive components (1-3) show antioxidant (4,5), anti viral(6) property and fight cancer(7).More than 300 varieties of dates (8,9) are available. Although fairly dry (10,11), microbial contaminants like yeasts, molds, lactic acid bacteria and some potential pathogens like Staphylococcus aureus, E. coli, and A. flavus/parasiticus (12-16) have been reported in date, responsible for fruit spoilage (17). Microbes have the ability to deposit in body organs which possess a great threat to the human health (18).The rate of contamination depends on several critical factors like cultivation weather, size, ripening stage, postharvest conditions like hygienic practices during processing and handling, storage, and transportation.

Sweetened dates are highly consumed by different age group people in Bangladesh. During Ramadan breaking fast with dates is considered holy. No stringent monitoring is in place on selling fruit hygiene. It may put people who are taking these dates for longer periods at greater health risks. Scanty published data are available on microbial count in the date fruits from the market sites in Bangladesh. This study aims to assess the biological contamination in terms of microbial load in dates available in Dhaka South City Corporation (DSCC). This investigation may help food safety authorities and the policy makers to heed the safety level of these fruits and take necessary measures in this regard.

Methods

Study area, Sample collection and Preservation

Jointly Waffen Research Lab, Gulshan-1; Centre for Advance Research & Science (CARS), Shahbagh, Dhaka; Daffodil International University (DIU), Dhaka and Jashore University of Science & Technology (JUST) in Bangladesh conducted this investigation. Under the area of Dhaka South City Corporation (DSCC) five sample collection zones (Motijhil, Hazaribagh, BabuBazar, Sadarghat and Gulisthan) were created. Locations are tagged (Figure: 1). Sampling locations have been selected on the basis of date fruits (Nagal, Dhapash, Boroi, Morium, and Tunisia) selling hotspots during July-August2018. During the collection time the ambient environmental temperature was fluctuating between 28°C to 36.8°C (Bangladesh Meteorological Department, Climate Division, Agargaon, Dhaka). The samples were collected by maintaining aseptic manners in sterile plastic sample bags (3M, USA) and carried to the laboratory within 4 hours and stored at 4°C until microbiological analysis.

Materials

Chemicals and Medium: Nutrient broth medium, Nutrient agar medium, MRS agar medium, saline water (0.85% NaCl solution), distilled water, Sorbital Dextrose Agar, BSA and Chromocult.

Culture media preparation

Nutrient broth medium

Nutrient broth media are favorable for bacterial growth and are composed of several nutrients. Approximately 50 ml distilled water was poured into 250 mL conical flux with the help of a measuring cylinder. Then peptone 1.00 g, yeast extract 0.50 g and sodium chloride 0.50 g were weighted for 100 mL medium by using electric balance, followed by water dissolution with the aid of a magnetic stirrer. Solution pH was maintained at 7.4 by adding base (0.1 M sodium hydroxide) or acid (0.1 M hydrochloric acid solution). Medium volume was adjusted to 100 mL. Conical flask was aluminum foil sealed and autoclaved (121° C and 1.5 atmosphere pressures) for 15 minutes.

Nutrient agar medium

Distilled water (Approximately 50 mL) was poured in a 500 mL beaker. Then compositions (peptic digest of animal tissue 0.50 g, sodium chloride 0.50 g, beef extract 0.15 g, yeast extract 0.15 g and agar 1.5 g) were weighted for 100 mL medium by using electric balance and mixed it into distilled water. Solution pH was maintained at 7.4 by adding base (0.1 M sodium hydroxide) or acid (0.1 M hydrochloric acid solution). Gradual heating through a microwave oven was provided for 3 minutes for dissolving Agar. The aluminum foil sealed beaker was autoclaved (121° C and 1.5 atmosphere pressures) for 15 minutes. The medium was put in laminar air flow and poured into autoclaved petri dish under sterile condition. For 6 plates with 0.5 cm thickness, 100 mL of media

was sufficient. Incubation was done at 37°C for contamination check.

Sorbital dextrose agar medium
Distilled water (Approximately 100 mL) was poured in a 500 mL beaker. Gradual heating through a microwave oven was provided for 3 minutes for dissolving 4.2 g of SDA. The aluminum foil sealed beaker was autoclaved (121°C and 1.5 atmosphere pressures) for 15 minutes. Under laminar air flow the medium was taken in and poured into autoclaved petri dish maintaining sterile condition. 100 mL of media was sufficient for 7 plates having 0.5 cm thickness. Incubation was done at 28°C. Contamination was monitored.

Chromocult Coliform Agar (CCA)
Within 24 hours Chromocult Coliform Agar (CCA) medium enables the simultaneous detection, differentiation and enumeration of E. coli and coliform bacteria in drinking water or another sample. Counting of coliform bacteria is based on the ability of s-D-galactosidase, an enzyme which is characteristic of coliform bacteria, to cleave the substrate Salmon-GAL. The reaction produces salmon red colored coliform bacteria colonies. E. coli counting is based on the breakdown of both the substrates X-glucuronide by s-D-glucoronidase and Salmon-GAL by s-Dgalactosidase, an enzyme combination, which is characteristic of E. coli. In the presence of E. coli both substrates were cleaved, resulting in colonies that take on a dark blue to violet color as opposed to the salmon red of other coliform bacteria colonies. Non-coliform bacteria were appeared as colorless or in rare cases as turquoise colonies. The CCA formulation contains sodium heptadecylsulfate (e.g. TergitolR 7) as an inhibitor of Gram-positive bacteria with no negative effect on the growth of the targeted coliform bacteria / E. coli was observed.

Bismuth Sulphite Agar (BSA)
Suspension was made by taking 52.33 g of Bismuth Sulphite Agar (BSA) in 1000 mL distilled water. Suspension was boiled till dissolving into solution. As the sensitivity of the medium depends largely upon uniform dispersion of precipitated bismuth sulphite in the final gel, therefore, it was dispersed before pouring into sterile Petri plates.

Liquid medium culture
Previously ready 200 mL liquid nutrient broth medium was used in this culture. The broth conical flask was sealed with cotton plug and then parafilm. This culture was used as stock culture.

Serial dilution
1g bread sample was mixed with nutrient broth and a homogenization was carried out. Dilution plate method was conducted to enumerate the fungi, bacteria. The working surface was ethanol sterilized. 1ml was taken from the homogenate. Serial dilution was conducted.

Spreading
The dilute culture was spread toward the surface of plate Nutrient Agar medium (N/A) and Sorbital Dextrose Agar (SDA) with the help of germ-free glass made spreader. Hitting was followed by cooling, during the single step of task. After spreading petri dish was Para film sealed. Incubator was done for 72 hours at 37°C for PDA and 27°C of Nutrient Agar respectively i.e 1:10 (1+9). Dilution was made using nutrient broth. Dilutions up to 10^{-7} were conducted.

Bacteria colony count
After incubation of NA, BSA, Chromocult; bacteria colonies were totaled by naked eye. The number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was done for 1 mL of original sample, and plating was done in duplicate for each dilution. An average count was taken to obtain the total count.

Fungi colony count
After incubation of NA, bacteria colonies and fungi were calculated by eye observation. Number of colonies on the plate was multiplied by the reciprocal of the dilution factor and calculation was done for 1 mL of original sample, and plating was done in duplicate for each dilution. Total count was found from average count.

Result

25 samples of five different varieties (Nagal, Dhapash, Boroi, Morium and Tunisia) collected from five different markets (Motijhil, Hazaribagh,

BabuBazar, Sadarghat and Gulisthan) within Dhaka South City Corporation (DSCC) were examined for microbial load as biological contamination. Every samples were tested 3 times (Number of replications, n=3) and Mean value was accepted as a standard value for comparison with international regulatory bodies for safety.

Highest Total Aerobic Bacterial Count (TABC) was found in Boroi (BabuBazar) 7.44±0.44 log CFU/ g and lowest count was observed in Boroi (Sadarghat) which was Below Detection Limit (BDL) (<1.0). Mean yeast and mold count was found to be highest in Dhapas from Gulisthan (6.88± 0.24) log CFU/ g) with nearing yeast and mold count about same in Dhapas (BabuBazar) 6.62± 0.37 log CFU/ g and Borai (Motijheel) 6.77± 0.05 log CFU/ g and Boroi from babubazar (6.82± 0.11 log CFU/ g). Mean count of yeast and mold vary from 6.88± 0.24 log CFU/ g (Dhapas- Gulistan) to 2.07± 0.00 log CFU/ g (Morium-Gulistan). The maximum value of Coliform count was 2.25± 0.90 (log CFU/ g) in Dhapas variety from Babubazar and lowest value was BDL (<1.0) in most of the samples under investigation as none samples from Motijheel, Hazaribagh, Sadarghat and Gulistan hotspots were found to be contaminated with TCC (BDL) (Figure: 2-4).

Discussion

Most of the samples from all collection hubs were found contaminated with bacteria. However, just one type of date sample (Boroi) from Sadarghat was least TABC containing date sample (<1.0 log CFU/ g). Date samples of Nagal origin was minimally contaminated with TABC in Sadarghat outlets (<3.5 log CFU/ g). Dhapash, Morium, and Tunisia of Gulisthan outlet and, all samples of BabuBazar outlet were contaminated significantly with TABC.Significant difference was observed in different market places (Motijhil, Hazaribagh, BabuBazar, Sadarghat and Gulisthan) irrespective of date type (Nagal, Dhapash, Boroi, Morium and Tunisia) as the difference was significant at 5% significant level (the F-ratio is 4.88924 at p-value .00161, the result is significant at $p < .05$).

Unsatisfactory level of TABC was found in this investigation according to Woolworths Quality Assurance Standard, 2009 (18). Samples exceeding safety limit by Guidelines for the microbiological quality of various ready-to-eat foods have been summarized in Figure 2 (19) with 80% samples from Motijheel and Gulistan, 100% samples from Hazaribag and Babubazar and 20% samples from Sadarghat crossed safety limits.

The bacteria isolated were Staphylococcus aureus, Staphylococcus species, Streptococcus species, Proteus mirabilis, Enterobacter species, Escherichia coli and Salmonella species. The Total Aerobic Bacterial Count (TABC) directs the overall microbiological quality of date fruits (20). The environment where the dates were being sold was unsanitary and unhygienic as it was found that majority of these fruits selling points of Babubazar, Gulistan, Sadarghat and Hazaribagh were close to waste disposal point or dusty or street with human and vehicular traffic (Babubazar, Gulistan and Motijheel). There deposition of bio aerosol on the exposed fruits transfer from one hand to other. Transformation also takes place through flies and contributes to contamination (21). Nagal, Dhapas, Boroi, Morium and Tunisia dates collected from New Market and Karwan Bazar area (areas under Dhaka North City Corporation) were found containing significant amount of TABC (aerobic bacteria) ranging from 4.34- 5.65 and 4.08-4.58 log CFU/ gm respectively in our previous investigation (18which is less than the TABC found in current investigation conducted on date samples collected from DSCC, can lead to health hazard (22).

Among all types of date samples, Tunisia dates from all markets under investigation (Motijheel, Hazaribagh, Sadarghat, BabuBazar and Gulisthan) were found least containing yeast and molds (<5 log CFU/ g). The F-ratio value is 3.42587 which was significant at 95% level of significance (The p-value is .013043), meaning differenceson contamination from market to market.The limit for yeast contamination in date fruits according to the Saudi standard specification are in 2 out of 5 replicates tested from a sample the targeted limit is 1 log CFU/ g and no implicate should reach a load of 2 log CFU/ g (23). Yeast and Mold cells counts in dates fruits were between 2.07± 0.00 log CFU/ g to 6.88± 0.24 log CFU/ g which were high and above

recommended value, thus making it unfit for human consumption (Figure: 3). In our previous investigation on Dhaka North City Corporation (DNCC) date samples were found contaminated with unsatisfactory level of yeasts and molds according to the guidelines provided by WQAS, 2009 which is in accordance with our current investigation (18).

The high level of contamination may have come from two different pathways. Contamination from the source during harvesting and dry-windy month (24-28) and the selling environment of date fruits with unhygienic handling. The yeast cells and mold motives food poisoning. Therefore, the date fruits are dangerous for human consumption.

Packaging and storing at refrigeration temperature (25) ceases microbial contamination of date fruits and presence of antimicrobial components in date fruits also serves as natural preservative (29-34). Although presence of high sugar content and antimicrobial components like tannins (around 2.5%) in date flesh exerts inhibitory effect on fungi population (35), presence of yeast and mold in unsatisfactory level in current investigation is definitely indicating poor hygiene and handling practices during post-harvest processing of the fruits. From our visual observation during sample collection the unsanitary conditions of the marketing areas is considered to contribute airborne microorganisms including fungal spores to the date samples were displayed openly.

Presence of Total Coliform Count (TCC) and E. coli were threat in all types of dates collected from BabuBazar as except Boroi (BDL) variety significant amount of colony was counted (1.2± 0.10 log CFU/ g in Nagal, 2.25± 0.90 log CFU/ gm in Dhapas, 1.17± 0.09 log CFU/ g in Morium and 1.57± 0.21 log CFU/ g in Tunisia) in other diversities of dates. Coliform bacteria predominantly fecal coliform are enteric bacteria, whose natural habitat is the intestinal tracts of humans and animals (28). The coliform counts were between BDL-2.25± 0.90 log CFU/ g in date fruit which was above recommended values for coliform count in foods (36).The coliform count was above recommended values for coliform count in foods (log1cfu/ gm) (Figure, 4) (37).

However, our previous probing on microbial contamination (18) in date samples were found zero contamination with coliform bacteria and E. coli. Surprisingly, the processing, storage and transportation in that investigation and in current investigation is same. The only difference is selling spots which may have open routes for the entrance of E. coli and coliform bacteria. Incidence of fecal coliform in date fruits designates the presence of fecal contamination from the unsanitary environment of fruit selling hotspots (Figure: 1) or via human handler (28). This incidence turns the fruits into hazardous category for human consumption.

Most of the date samples under investigation were found to have biological contamination in ranges higher than permissible limit recommended by authority concerned with food safety. Therefore, similar assessment for other date samples and in other market places need to be carried out to make a broad spectrum of quality assurance assessment. This will lead us to get a complete scenario of date fruit quality in terms of microbial load. In conclude minimization and elimination of microbial load should be ensured incorporating Government agencies by means of ensuring standards for selling date fruits in the local market.

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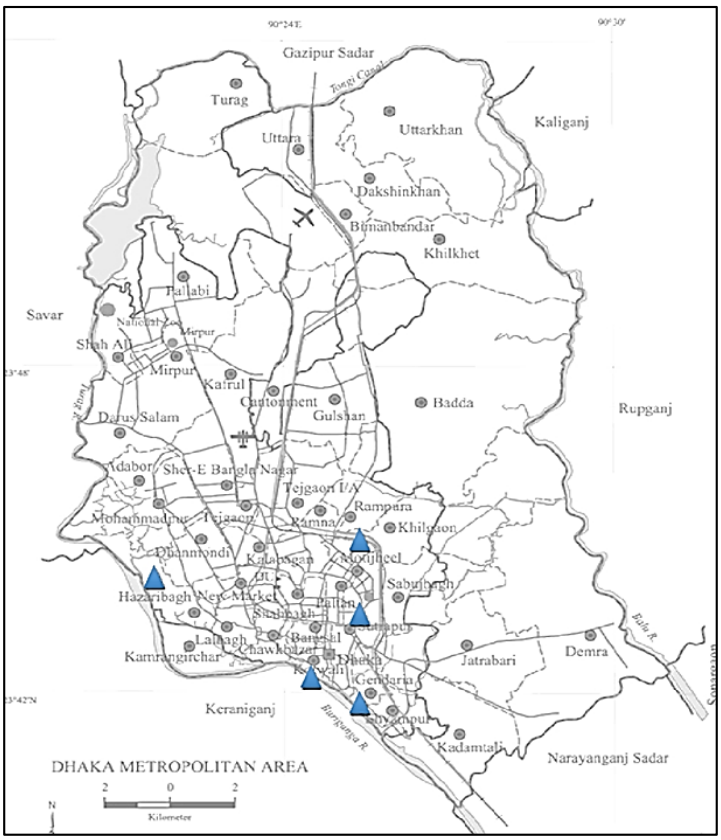

Figure 1: Date fruits collection hotspots in Dhaka South City Corporation (DSCC), Bangladesh

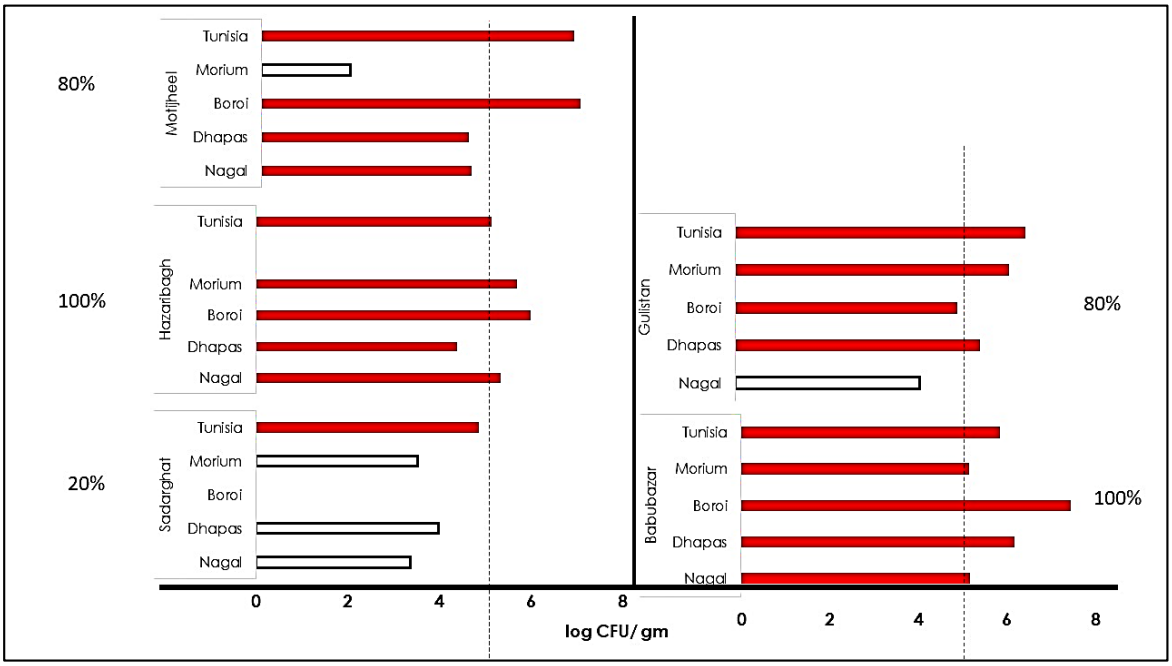

Figure 2: Date fruits above safety limit for Total Aerobic Bacterial Count.

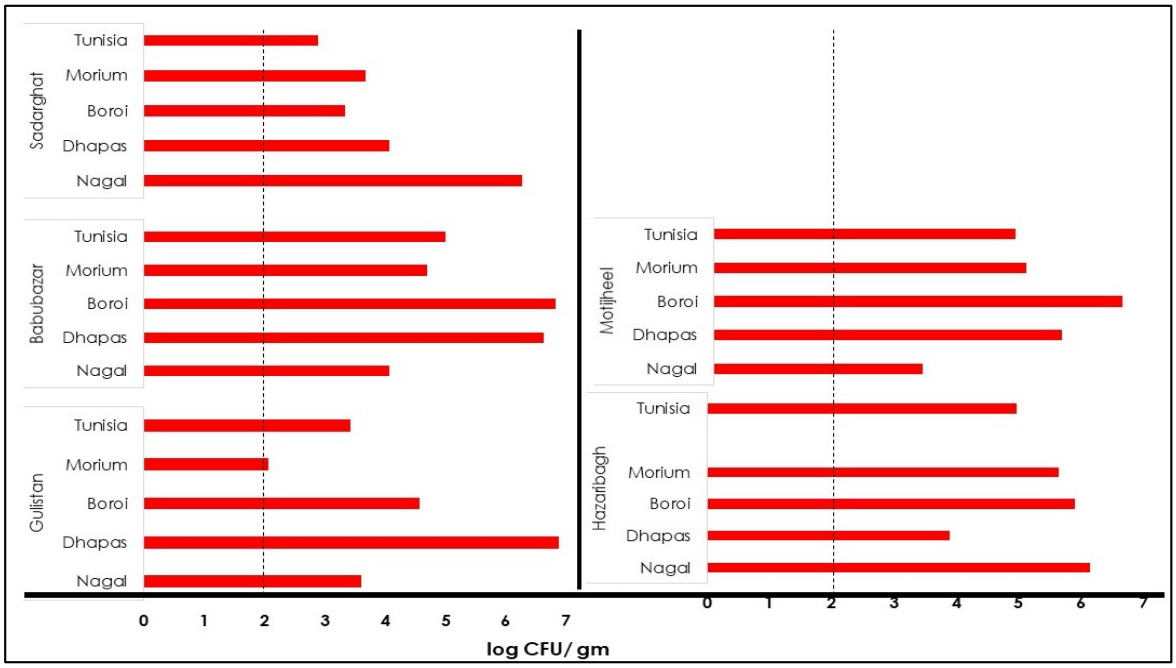

Figure 3: Date fruits above safety limit for Yeast and Mold count.

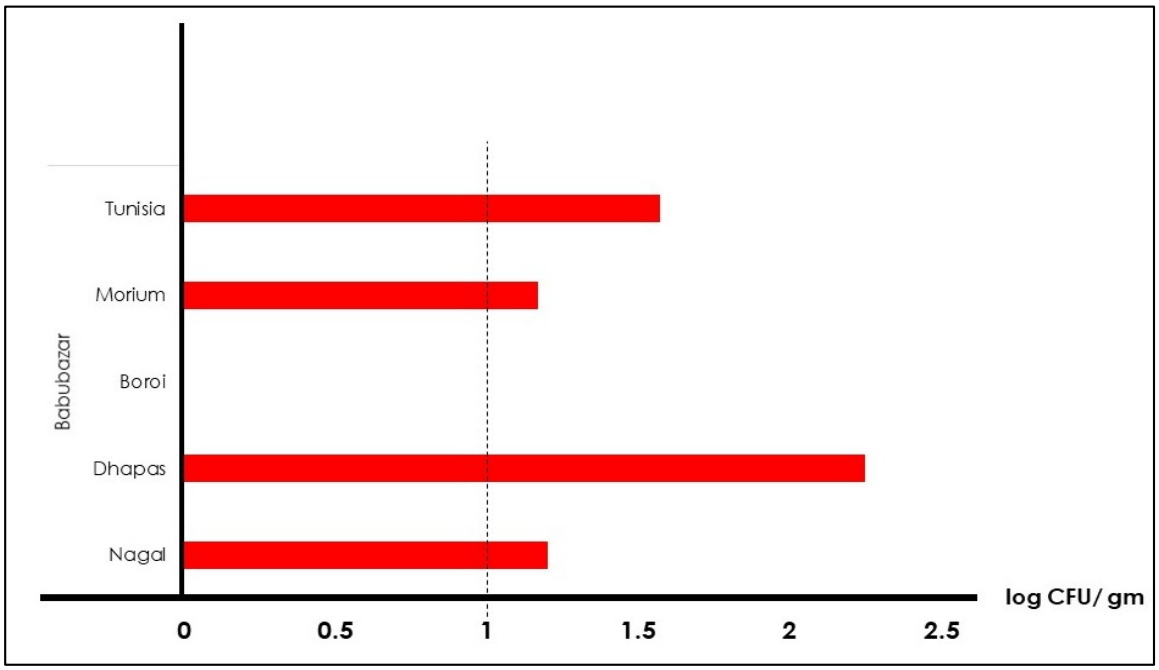

Figure 4: Total coliform count (TCC) exceeding safety limit