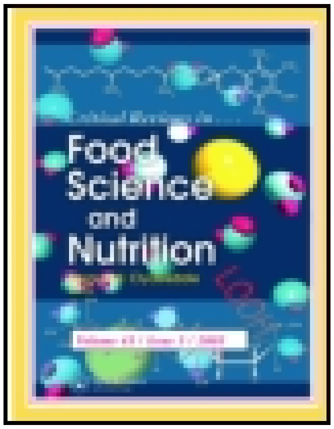

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Microbiology of Fresh Produce: Route of Contamination, Detection Methods and Remedy

Asmita Rajwar[ab], Pragati Srivastava[a] & Manvika Sahgal[a]

[a] Department of Microbiology

[b] Department of Post Harvest and Food Processing, G.B.P.U.A. & T. Pantnagar, Uddham Singh Nagar, Uttarakhand, India, 263145

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Microbiology of Fresh Produce: Route of Contamination, Detection Methods and Remedy

Asmita Rajwar*[1], Pragati Srivastava[1], and Manvika Sahgal[1]

1 Department of Microbiology, 2 Department of Post Harvest and Food Processing

G.B.P.U.A. & T. Pantnagar, Uddham Singh Nagar

Uttarakhand, India, 263145

***Asmita Rajwar**

asmitamicro@gmail.com

Room No-119 Golden Jubilee Hostel

G.B.P.U.A. & T. Pantnagar, Uddham Singh Nagar

Uttarakhand, India, 263145

Ph:- +91-9917797221

Fax:- +91-5944-233473

Abstract

Fresh fruits and vegetables are an important part of a healthful diet. They provide vitamins, minerals and fibre to help keep our body healthy. Occasionally, fresh fruits and vegetables can become contaminated with harmful bacteria or viruses, which are also known as pathogens. The major family of pathogen associated with food are members of Enterobacteriaceae which commonly form a part of microbiological criteria and their presence is traditionally related to hygiene and safety of foods. Organic fertilizers, irrigation water quality and soil are major source of contamination. For removal of pathogens, various decontamination procedures are also followed to reduce microbial load on the fruits. These are chemical preservatives and irradiation.

Microbiological study of fresh produce can be done by various phenotypic, biochemical and molecular techniques so that pathogen can properly processed. The World Health Organisation (WHO) developed global risk communication message and training materials to assist countries in strengthening their food educating programs. There is a need for improved surveillance systems on food-borne pathogens, on food products and on outbreaks so that comparable data are available from a wider range of countries.

Keywords

Fresh produce, Microbial contamination, Enteropathogen, Route of entry, Food safety.

Introduction

Fresh fruit and vegetables are important components of a healthy and balanced diet. They are a major source of fiber and micronutrients such as iron, manganese and copper. Compounds like polyphenolics, carotenoids and glucosinolates, present in fruits also have nutritional value. They are good source of vitamins.

Their consumption is encouraged in many countries by government health agencies to protect against a range of illnesses such as eye diseases, cancers and cardiovascular diseases. Moreover recent findings show that consumption of fast food delicacieslike Pizza, burger , chowmin are major reasons for high level of obesity and multivitamin deficiencies in children and young. **(Rickman *et al.*, 2007).**

However, fruits and vegetables, and in particular leafy greens that are consumed raw, are increasingly being recognized as important vehicles for transmission of human pathogens that were traditionally associated with foods of animal origin. There is currently limited knowledge about where in the supply chain contamination occurs or about the mechanism by which human pathogens colonize and survive on or in fruits and vegetables **(Berger *et al* ., 2010).**

Major Food- borne pathogens

Enterobacteriaceae

Members of the family *Enterobacteriaceae* are gram negative, straight rods, 0.3-1.5µm in diameter; facultative anaerobes, oxidase-negative and have a respiratory and fermentative metabolism. They ferment D-glucose to give acids and often gas. They often utilise glucose as sole source of carbon and grow well on peptone and meat extracts. They are non spore forming, non acid fast and non-halophilic but tolerate the presence of bile salts **(Brenner, 1984). They colonise the enteric system of animals** and their presence is traditionally related to hygiene and safety of foods and is an important microbiological criteria for assessing food quality. Methods for testing members of enterobacteriaceae were first applied to milk and later extended to all foods **(Ayers and Johnson, 1915; Shippen, 1915; Weinzirl and Harris, 1928; Tanner and Windsor, 1929).** *Escherischia coli* is an indicator micro-organism for water and food quality.

Escherichia coli

This bacterium was first described by Theodor Escherich (1885). Its common occurrence in faeces, generally non pathogenic character and survival characteristics in water led to the adoption of *E.coli* as an indicator of faecal contamination **(Adams and Moss, 1995).**

Escherichia is the type genus of family Enterobactetiaceae and *E.coli* is the type species of the genus. It is a catalase-positive, oxidase-negative, fermentative short, gram negative, non spore forming rods. *Escherichia coli* is a typical mesophile growing from 7^{o}C upto 50^{o}C with an optimum around 37^{o}C. A near-neutral pH is optimal for growth.

Important measures to prevent food poisoning include educating food workers in safe food handling techniques and proper personal hygiene, properly heating food to kill pathogens and holding foods under appropriate conditions to avoid bacterial multiplication. Additionally, untreated human sewage should not be used to fertilize vegetables and crops used for human consumption nor should non-chlorinated water be used for cleaning food processing equipment and food contact surfaces **(Doyle 1990).**

Salmonella

Most species of genus *Salmonella* are regarded as human pathogens, though they differ in the characteristics and the severity of the illness they cause. The paratyphoid bacilli were isolated by **Achard and Bensaud (1897),** and confirmed as culturally and serologically distinct from the typhoid bacilli by **Schottmuller (1911).** Salmonellas are established as one of the most important causes of food borne illness worldwide. It is estimated that 2-4 million cases of Salmonellosis occur in USA annually.

Salmonellas are gram negative, non spore forming rods (typically 0.5µm x 1-3µm) which are facultatively anaerobic, catalase-positive, oxidase-negative and generally motile with peritrichous flagella. They are heat-sensitive and readily destroyed by pasteurization temperatures **(Adams and Moss 1995)**.

***Campylobacter* spp.**

Campylobacter spp. and in particular, *Campylobacter jejuni,* are thought to be the most frequent bacterial cause of gastrointestinal disease in humans **(Allos and Taylor 1998).** Animals and birds are the main reservoir of human pathogenic *Campylobacter* **(Stern 1992; Altekruse *et al*., 1994)** although water is also a potential source for contamination with these organisms **(Buswell *et al*., 1999; Mason *et al*., 1999).** There is also potential forcross-contamination of fresh produce with *Campylobacter* from meat and poultry during food preparation **(Beuchat, 1995).** Although campylobacters are an important cause of sporadic human infections they are rarely reported as causing outbreaks of gastrointestinal disease. *Campylobacter* infections usually present as abdominal pain profuse diarrhoea and malaise with a duration of 2-7 days. Bacteraemias are rare and the casefatality rate in industrialised countries is about 0.05%.

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***Shigella* spp.**

Shigella sonnei is primarily spread by the person-to-person route although food and waterborne transmission can occur. Shigellosis can be endemic in institutional settings such as prisons, mental hospitals and nursing homes, where population densities are high and/or poor hygiene conditions may be present. *Shigella* infection usually presents as abdominal cramps, fever and diarrhoea, which may contain blood and mucus. The duration of illness is 4-7 days **(Anon 2001).** The case-fatality rate in industrialised countries is estimated to be 0.1%.

Vibrio cholerae

Vibrio cholerae serogroups O1 and O139 are the causes of epidemic cholera. This is predominantly a waterborne infection and high numbers of organisms are necessary to cause infection. Nevertheless, a significant number of fruit and vegetable borne outbreaks have been reported **(Faruque *et al.,* 1998).** The characteristic profuse watery diarrhoea of cholera is due mainly to the effects of a heat labile enterotoxin elaborated by the organism in the intestine. Cholera is of rapid onset and can lead to severe dehydration and death within hours if left untreated. The illness usually lasts for 3-7 days **(Anon., 2001).**

Contamination Route

1. **Agricultural Practices**

1.1 Organic fertilizers

Use of organic fertilizers, such as animal manures and slurries **(Beuchat, 1996),** abattoir wastes **(Avery *et al.,* 2005)** and sewage sludge introduce pathogens directly to the field, and run-off can contaminate irrigation water.

There are comprehensive guidelines available to growers that advise on sufficient treatment of wastes and correct timing of application, with the aim of limiting contamination of crops. In UK, these guidelines are set out in the Safe Sludge Matrix (ADAS) **(Anon 2001)** and the Codes of Good Agricultural Practice (Department of the Environment, Food and Rural Affairs). The Safe Sludge Matrix, for example, states that even when enhanced-treated sludge is applied to land, a 10-month harvest interval is necessary and the use of conventionally treated sludge requires a 30-month harvest interval for salad crops.

1.2 Irrigation water quality

Faecal material, soil and other inputs such as sewage overflow introduce enteropathogens directly to watercourses from which irrigation water may be extracted. In UK, 71% of irrigation water is obtained from surface waters, which receive treated sewage effluent **(Tyrell *et al.*, 2006). Islam *et al.* (2004)** demonstrated that a single application of *S. typhimurium* inoculated irrigation water resulted in contamination of carrot and radish at harvest, with *Salmonella* surviving for 203 days in soil post application. Lettuce plants irrigated with a single application of *E. coli* O157:H7 contaminated water tested positive for presence of *E. coli* O157:H7 at harvest (30 days post inoculation) and plants contaminated at days 7 and 14 of the study were shown to yield increased populations **(Solomon *et al.*, 2003).**

A survey of UK-based salad vegetable producers showed that over 50% growers will harvest baby-leaf crops within 24 h of the last irrigation **(Tyrell *et al.*, 2006).** A number of outbreaks have been traced to the use of contaminated water in irrigation. Water may also act as a vehicle for the dissemination of viral particles. **Beuchat (1996)** reports a nontypical outbreak of norovirus linked to celery and irrigation with sewage-contaminated water has resulted in the outbreaks of hepatitis A linked to lettuce consumption **(Seymour and Appleton, 2001)** and spring onions. **Solomon *et al.* (2002)** showed that *E.coli* O157:H7 in contaminated water can enter the vascular system of lettuce and reach the edible parts of the plant.

1.3 Soil

Pathogens may be naturally present in soil, for example *Listeria* spp. **(Nicholson *et al.*, 2005),** or may become incorporated in the soil matrix from organic wastes added as fertilizer. Pathogens within soil may contaminate crops directly when heavy rain or water gun irrigation causes leaf splash. Initially, the pathogen must survive in the propagation environment until crops are planted out, or in organic wastes applied to the land. **Kudva *et al.* (1998)** demonstrated that aeration of ovine manure decreased survival of *E. coli* O157:H7 from >365 to 120 days. *Escherichia coli* and *Salmonella* will exhibit the general stress response, producing a range of stress proteins which can confer cross-resistance to a range of stresses **(Barker *et al.*, 2007). Leyer and Johnson (1993)** report that after acid adaptation, *S.typhimurium* displayed increased tolerance of heat and osmotic stress, whilst **Hartke *et al.* (1996)** demonstrated that pre-irradiation of *Lactococcus lactis* increased resistance to lethal challenges of acid. The stress response of *L. monocytogenes* is similar to that of *E. coli*, but is regulated by the sigma factor rB, which has been suggested to increase virulence **(Wonderling *et al.*, 2004).** Campylobacter may enter a viable but nonculturable stage **(Buswell *et al.*, 1998),** but the main mechanism of survival is production of large numbers of cells within the host **(Jones, 2001)**.

1.4 Spray of Pesticides and insecticides

Various disinfectants can be used to reduce the microbial load on fruits and vegetables. The purpose of using these agents is to control plant pathogens (plant protection) or food pathogens or spoilage organisms (preserving additive). The effect of disinfectants on contaminants depends on many factors including the concentration used, treatment time, temperature, pH and sensitivity of the target organism(s). Chlorine is the major compound used for disinfection of fresh produce. During sprouting of seeds chlorine can be used in the water to prevent growth of contaminating microorganisms. The most effective form is hypochlorous acid (HOCl) **(Simons and Sanguansri, 1997)** and chlorine concentration of 100 ppm is frequently used. However, the use of chlorine does not ensure elimination or even an efficient reduction in pathogen levels. Other substances may be used including organic acids, chlorine dioxide, hydrogen peroxide and ozone **(Beuchat, 1998)**. Organic acids alone, or in combination with chlorine, have been shown in experimental designs to effectively reduce the number of pathogens for example, *Yersinia enterocolitica* and *Listeria monocytogenes* in parsley **(Zhang and Farber, 1996).** Ozone has been cited as a safe alternative for disinfecting fresh produce. Several studies suggest that hydrogen peroxide has potential as a sanitising agent for fruits and vegetables **(Beuchat, 1998).** It is classified in the US as Generally Recognised As Safe (GRAS) for use in food products **(Sapers and Simmons, 1998)**. **Beuchat (1998)** concluded that prevention of contamination at all points of the food chain is preferred over the application of disinfectants.

2. Post Harvest Handling, Collection, Transportation and Packaging

Post harvest treatment of fruits and vegetables includes handling, storage, transportation and

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cleaning. During these practices conditions may arise which lead to cross contamination of the produce from other agricultural materials or from the workers. Environmental conditions and transportation time will also influence the hygienic quality of the produce prior to processing or consumption.

Poor handling can damage fresh produce, rendering the product susceptible to the growth/survival of spoilage and pathogenic microorganisms. This damage can also occur during packaging and transport. The presence of cut and damaged surfaces provides an opportunity for contamination and growth of microorganisms and ingress into plant tissues **(Francis and O'Beirne, 1999).**

Fruits and vegetables can become contaminated with pathogenic microorganisms during harvesting through faecal material, human handling, harvesting equipment, transport containers, wild and domestic animals, air, transport vehicles, ice or water **(Beuchat, 1995).** Fruits and vegetables can become contaminated whilst growing in fields, or during harvest, handling, processing, distribution and use **(Beuchat, 1995).**

Fresh fruits and vegetables continue to respire, consuming oxygen and producing carbon dioxide and water vapour. Modified Atmosphere Packaging (MAP) of fresh fruits and vegetables results in an extended shelf-life. A great deal of work has been carried out to assess the effect of MAP on the growth of pathogens associated with minimally processed fruits and vegetables, especially *Listeria monocytogenes.* The effect of MAP in inhibiting the

growth of pathogens is influenced more by the type of vegetable than by the atmosphere used **(Jacxsens *et al.*, 1999).**

Doses of irradiation, that would be required to inactivate human pathogens, are known to have an adverse effect on fruits and vegetables, and relatively little commercial activity is carried out to control foodborne pathogens in these foods. Irradiation up to a maximum dose of 1 kGy has been approved in the US for the disinfestation of grains, fruits and vegetables for the inhibition of sprouting and for delaying ripening in order to extend the shelf-life.

Microbial load on fresh produce:- Microorganisms form part of the epiphytic flora of fruits and vegetables and many will be present at the time of consumption. The majority of bacteria found on the surface of plants are usually Gram-negative and belong either to the *Pseudomonas* group or to the *Enterobacteriaceae* **(Lund, 1992).** Many of these organisms are normally non-pathogenic for humans. The numbers of bacteria present will vary depending on seasonal and climatic variation and may range from 10^4 to 10^8 per gram. The inner tissues of fruits and vegetables are usually regarded as sterile**.** Microbial population of about $<10^3$ on the fruit surface is regarded within the safe limits **(Gilbert et al., 2000).**

Methods for Identification of Microbes associated with food

Bacteria can be characterised by phenotypic, chemotaxonomic and genetic methods.

(1) Phenotypic

The phenotypic variability among microorganisms can be assessed phenotypically by simple plating methods. **Sorheim *et al.* (1989)** isolated soil bacteria on three different media for studying phenotypical divergence among them. **Deleij *et al.* (1993)** conducted a study on the use of colony development for the characterization of bacterial communities on roots and in soil and suggested that simple agar plating can be used to quantify microbial communities in different habitats. Similarly, **Hattori *et al.* (1997)** isolated soil bacteria from paddy as well as grassland soils and categorized them into colony forming curves (CFC) of different growth rates.

(2) Biochemical

Until 1960s morphological aspects together with biochemical and physiological properties were basis for characterization of organism. Despite the advent of molecular methods including whole genome sequencing, biochemical characterization of micro-organisms is important. Tests like Indole, methy red, vogues proskaeur, catalase, nitrate reduction, determination of sugar and aminoacid utilization pattern of micro-organisms are also important. These days biochemical test strips, each having a combination of tests important in identification of specific group of bacteria are available.

(3) Chemotaxonomic

Qualitative analysis of different cell components provides sufficient information for discrimination among certain groups of organisms **(Schleifer and Kandler, 1972).** The comparison of whole cell protein pattern obtained by SDS-PAGE is a reliable method for comparing and grouping large number of closely related strains **(Vauterin *et al.*, 1993)**. RNA polymerase and protein synthesis machinery can also be used as method for differentiation between Archaea, Bacteria and Eukarya. Hence, chemotaxonomic markers are used for taxon, genus and species differentiation.

(4) Phylogenetic

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DNAï DNA hybridization (DDH) values have been used by bacterial taxonomists since the 1960s to determine relatedness between strains and are still the most important criterion in the delineation of bacterial species. Analysis of DNAï DNA hybridization values remains the ógold standardô for defining bacterial species **(Wayne *et al.*, 1987)**. Techniques required to obtain these values, however, tend to be expensive and time consuming and often require specialized instruments or radioactive labels.

Amplified Ribosomal DNA Restriction Analysis (ARDRA)

This technique involves an enzymatic amplification using primers directed at the conserved regions at the ends of the 16S rDNA, followed by digestion using tetracutter restriction enzymes. The pattern obtained is said to be representative of the species analysed. Patterns obtained from three or more restriction enzymes can be used to phylogenetically characterize cultured isolates and 16Sr RNA genes obtained through cloning from community DNA **(Vaneechoutte *et al.,* 1993).** Since the morphological and nutritional criteria used to describe microbes failed to provide a natural taxonomy revealing evolutionary relationships, the use of molecular tools and including 16S rDNA sequencing were applied into microbial diversity studies **(Pace, 1997). Woese and Fox (1977)** used small subunit (SSU) rRNA as a phylogenetic tool. Since rRNA molecule is present in all cellular life forms, comparative analysis of these ribosomal RNA sequences has indicated that all cellular life belongs to one of the three domains: Bacteria, Archaea and Eukarya **(Woese, 1984).**

ARDRA is an alternative to more laborious and expensive methods, e.g., DNA-DNA hybridisation for the identification of eubacteria. It is a good criterion for microbial classification at both genus and species level **(Massol-Deya *et al.*, 1995)**. ARDRA has also been used in the analysis of mixed bacterial populations from different environments **(Moyer *et al.*, 1994).**

Cebula *et al.* (1995) developed a Multiplex PCR assay that simultaneously identifies isolates of O157:H7 and the type of Shiga like toxins. The assay uses three set of primers, from which two detected conserved region within the gene encoding for Shiga like toxins-I and Shiga like toxin II **(Jackson *et al.*, 1987).** The third set of primers is directed to the *uid*A gene **(Feng, 1993)** .

A multiplex PCR procedure was established to detect *Escherichia coli, Listeria monocytogenes* and *Salmonella typhimurium* in artificially inoculated wheat grain **(Kim *et al.*, 2006). Ziemer and Steadham (2003)** designed nine set of primers targeting *Salmonella* were evaluated for their specificity with pure cultures of intestinal associated bacteria prior to their application to *Salmonella.*

Consumers at risk

Groups vulnerable for infection with pathogens and opportunistic pathogenic organisms

The young, the old, the pregnant and the immune compromised consumers potentially have a higher risk of bacterial, viral or protozoan infection than other groups by consuming fresh fruits raw. This factor is important in risk assessment and management relating to the consumption of fruits and vegetables. A particular concern relates to infection of young children with *E. coli* O157:H7 leading to Haemolytic Uraemic Syndrome (HUS) **(Parry and Palmer, 2000).** Diarrhoea is a significant cause of morbidity and mortality in the elderly**. Lew *et al.* (1991)** noted that 51% of deaths caused by diarrhoea over a 9-year period occurred in individuals

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over the age of 74. Older patients may be at increased risk of *Salmonella* infection because of achlorhydria, decreased intestinal motility associated with medications, gastrointestinal diseases prevalent in the elderly, and more frequent use of antibiotics **(Lew *et al.*, 1991; Russell *et al.*, 1993).** Infectious hepatitis is a common complication in patients with acute leukaemia and has been related to several agents, including hepatitis virus types A **(Dienstag, 1988).** The classic pathogens identified in patients with defects in cell mediated immunity are *Listeria monocytogenes*, *Salmonella* and non-tuberculous Mycobacteria **(Burckhardt *et al.*, 1997).** Transplant recipients, and also patients with AIDS, can acquire infections caused by a wide range of bacteria, viruses, protozoa and fungi **(Jurado *et al.* 1993).**

International Initiatives on Food Safety

A more reliable way to achieve the distribution of good quality product is to set up a quality management system that can be monitored effectively. A quality management system aimed specifically at hazard control in the food industry is the HACCP (Hazard Analysis Critcal Control Point) system. Laboratory based microbiological analyses of samples can be important in two stages. The first stage is identification of hazards of microbial origin. The second stage is evaluation of the effectiveness of monitoring of the critical control points (CCP).

The World Health Organisation (WHO) developed global risk communication message and training materials to assist countries in strengthening their food educating programs.

Glenda Lewis, an expert on food borne illness with the Food and Drug Administration, says produce can be contaminated in many ways. During the growing phase, fruits and veggies may be contaminated by the soil, water, or the fertilizer. After itø harvested, it passes through

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many hands, increasing the contamination risk. Contamination can also occur once the produce has been purchased, during the food prep or even through inadequate storage. With so many sources from where contamination can occur, safely preparing your produce before eating is especially important. Because cooking food kills harmful bacteria, raw veggies and fruits carry the biggest risk of contamination.

Conclusions

Published surveys of raw fruits and vegetables demonstrate that there is potential for a wide range of these products to become contaminated with microorganisms, including human pathogens. Almost any ready-to-eat fruit or vegetable that has been contaminated with pathogens either from the environment, from human or animal faeces or through storage, processing and handling could potentially cause diseases. However, epidemiological traceability is difficult for fruits and vegetables as carriers of foodborne pathogens. Outbreaks have been reported involving a range of bacterial, viral and protozoan pathogens. The frequency of foodborne outbreaks of gastrointestinal illness associated with fruits and vegetables appears to be low compared to products of animal origin. However, it appears that food-borne illnesses associated with fruit and vegetables are increasing. The reason for this is not clear but factors include increased consumption, new commodities and changes in production practices. The large outbreak of *E. coli* O157:H7 in Japan in 1996 was linked to sprouted radish illustrates the potential for serious public health problems arising from the contamination of fresh fruit and vegetables. Fruits and vegetables can be contaminated with pathogens from animal and human reservoirs and the environment as a result of production practices. A major source of contamination is organic fertiliser and faecal contaminated water. Harvesting at the appropriate time and keeping the harvested products under well-controlled conditions will help in restricting growth of pathogenic and post-harvest spoilage microorganisms.

Limited data exist on the safety of microorganisms used for bio-control of pests during production of fruit and vegetables. The use of additional post-harvest procedures can reduce the contamination level on vegetables and fruits. Chemical decontaminants are not widely used, with the exception of chlorine, and their impact on microbial food safety is not well established. Chemicals used for these purposes have to undergo a safety assessment.

Fruits and vegetables can be contaminated during harvest and further processing by food handlers. This problem is particularly associated with the occurrence of human intestinal viruses. Available data are insufficient to indicate clear trends in consumption of specific categories or types of fruits and vegetables. However, there is a marked increase in the production of cut and packed ready-to-eat fresh vegetables.

Recommendations

Public health professionals investigating incidents of food-borne illness, persons involved in the production chain and consumers should be made aware that contaminated fruits and vegetables eaten raw can be potential vehicles of pathogenic agents. There is a need for improved surveillance systems on food-borne pathogens, on food products and on outbreaks so that comparable data are available from a wider range of countries. Organic fertilisers and sewage sludge should be treated in such a way that they do not contaminate fresh produce with microorganisms at levels that might cause harm to the consumer. Further studies are required on the microbiological status and survival of various pathogens in the agricultural environment, in/on raw fruits and vegetables and the most efficient decontamination procedures. Safety of chemicals used for decontamination processes has to be assessed. National regulations that cover or impact on the production of fruits and vegetables need to be compiled in order to develop a harmonised Indian approach.

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