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Investigating the Microbial Quality of Compost Products in Sri Lanka

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Abstract: Compost fertilizers offer numerous benefits to living organisms and eco systems and it was confirmed by many more research. However, despite their positive impacts, certain compost fertilizers may pose negative impact on the environment and health due to the composition of pathogenic microorganisms. The issue remains underexplored in existing studies. Therefore, this study analyzed the selected pathogenic microorganisms present in several compost fertilizer products sold in Sri Lankan markets. Both culture-based and molecular-based techniques were used to analyze four compost fertilizers. Gram staining identified both gram-positive and gram-negative bacteria, while morphological analysis of LB and SDA culture media revealed the *Aspergillus flavus, Aspergillus niger, Penicillium* sp., *Fusarium* sp., and *Candida* sp. as fungi, *Escherichia coli* and *Salmonella* as bacteria. The PCR analysis confirmed the presence of a few *Aspergillus* species and the absence of *Escherichia coli, Erwinia carotovora* & *Salmonella*. The findings underscore the need for quality control to ensure the safety of compost fertilizers, since *Aspergillus* sp. detected poses risks to public health and the environment. Continuous evaluation is essential to enhance microbial safety standards.

Keywords: Compost fertilizers, Aspergillus, Salmonella, Escherichia coli, Erwinia, Culture media, PCR

I. Introduction

Compost fertilizer offers numerous benefits, including improving soil structure, enhancing water retention, reducing the need for chemical fertilizers, and promoting a healthier ecosystem for plants and wildlife and it contain numerous microorganisms that help for the soil and plant improvement (Brandli *et al*., 2007; Samarasinghe *et al.,* 2015; Legein *et al.,* 2022). Therefore, compost fertilizers are crucial to sustainable agriculture and environment (Mamo *et al*., 2008). However, if compost fertilizer production is not managed properly, it can contain pathogenic microbes that pose serious dangers to the environment and public health because composition of materials used for composting, composting techniques, and composting environment all have an impact on the microbial content of the final product (Pell, 1997; Mamo *et al*., 2008). In compost, pathogenic microorganisms, especially plant pathogens, are multiplied if the temperatures are not adequate to destroy them (Singh *et al*., 2010). Thus, the transmission of these pathogens to crops due to their presence in compost fertilizers raises the possibility of foodborne diseases and other health problems.

Research studies conducted in several nations have shown that compost products contain human, animal & plant pathogenic bacteria such as *Salmonella* spp., *Escherichia coli*, *Yersinia enterocolitica, Listeria,* and fungi such as *Histoplasma capsulatum* (Kroupitski *et al*., 2009; Warriner & Namvar, 2010; Chapman *et al*., 1997; Paraguison *et al*., 2021; Gómez *et al*., 2018). These pathogens can have a significant impact on human health; *E. Coli O157:H7* can cause hemolytic uremic syndrome (HUS), gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, Crohn's disease (Tenaillon *et al.,* 2010); *Salmonella* can cause Salmonellosis (Chapman *et al*., 1997); *Aspergillus fumigatus* can cause aspergillosis and asthma (Adegunloye, 2007); *Mucor* species can cause mycosis (Rhode & Hartman, 1982). According to a study conducted by Adegunloye and colleagues, during the process of composting, *Bacillus sphearicus, Bacillus laterosporus, Micrococcus varians, Proteus mirabilis, Pseudomonas aeroginnosa, Enterobacter aerogenes, Aspergillus rapens, Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus, Mucor mucedo, Rhizopus stolonifer, Varicosporium, and Fusarium* like pathogens were reported to be present and, if the composting process does not effectively control the temperature at relevant stages, these pathogenic microorganisms can remain in compost fertilizers (Adegunloye, 2007). Furthermore, these pathogens, when present in compost, can have adverse impacts on wildlife, livestock (Maule, 2000; Guan *et al.,* 2007) and can also have significant agricultural impacts, leading to plant diseases and limiting crop production (Chapman *et al.,* 1997; Burgess, 2012).

Culture-based methods, biochemical assays and molecular-based detection methods like PCR, microarrays, DNA sequencing can be used to detect those pathogens in compost fertilizers (Paraguison *et al.,* 2021; Franke *et al*., 2005; Albrecht *et al.,* 2007). Culture-based techniques enable the detection of types of microorganisms by using their morphologies. However, these techniques take a long time to provide results and sometimes the results cannot be distinguished due to mixed populations. In contrast, biochemical assays employ chemical reactions to determine the enzymes or metabolites of microorganisms. These techniques can be quicker than culture-based techniques and reveal details about the microorganisms' metabolic potential. Nevertheless, they might not have the sensitivity and specificity required for precise detection because they still depend on the organisms' development. Molecular-based detection techniques, such as DNA sequencing, microarrays, and Polymerase Chain Reaction (PCR), provide several advantages over conventional techniques. By amplifying DNA sequences, PCR makes it possible to identify even minuscule amounts of microbial DNA. This technique yields results in a matter of hours with high sensitivity and specificity. Several infections can be detected simultaneously by microarrays through the hybridization of DNA samples to a chip that has probes for different microbial genes. Comprehensive knowledge about the microbial population can be obtained through DNA sequencing, which also allows for the identification of unidentified or uncultivable organisms. With the use of molecular techniques, a wide range of microbial pathogens

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and in particular, species that may have gone undetected by conventional culture-based methods, can frequently be specifically identified.

Safety requirements for microbial contamination have been established in different countries, specifying the limit or absence of certain pathogenic species. *Salmonella* and faecal coliform colonies must not be present per gram of dry weight, according to Sri Lankan requirements (Sri Lanka Standard, 2019). According to Brazilian requirements, faecal coliforms must have a concentration of fewer than 1000 CFU/g and be free of *Salmonella*, *Phytophthora*, *Rhizoctonia*, *Pythium*, and *Fusarium* (Cancelado *et al.*, 2014). According to European standards, *Salmonella* must not be present, and *Escherichia coli/ Enterococcaceae* must not exceed 1000 CFU/g (OJ, 1998). *Erwinia carotovora* must be absent according to US regulations (USEPA, 1994).

Research on the microbial composition and safety of compost fertilizers has been scarce in Sri Lanka, despite being a widely recognized issue overseas. The lack of studies to identify the microbes present in compost fertilizers sold in the Sri Lankan markets is a significant concern, with no studies having been conducted to evaluate the extent to which these products adhere to safety guidelines and for ensuring consistent microbial quality in the process of compost production. Therefore, the identification of pathogenic microorganisms present in compost fertilizers is warranted to determine whether these products conform to the safety standards stipulated in Sri Lanka and to assess the suitability of utilizing molecular detection methods such as PCR to specifically and sensitively identifying the presence of pathogenic microorganisms from these products.

The main goal of the study is to identify the microbial diversity of compost fertilizers available for sale in the Sri Lankan market and to ascertain whether these adhere to the stipulated safety regulations. Specifically, the research will look for the presence of pathogenic bacteria that may be harmful to agriculture, environment and human health. The findings of this study will be beneficial for comprehending the safety of compost fertilizers sold in Sri Lankan markets and for formulating plans to reduce any possible health hazards related to their use.

II. Materials and Methods

Four compost fertilizers products were collected from four different brands sold in markets across Sri Lanka. The samples were prepared in a sterile environment to prevent contamination. For bacterial and fungal growth, the samples were cultured separately in LB (Lysogeny broth) agar medium and SDA (Sabouraud Dextrose Agar) medium, respectively. DNA was extracted from the bacterial and fungal colonies using the CTAB (Cetyltrimethylammonium Bromide) and boiling method, respectively, and the DNA extracts were quantified using the NanoDrop One Spectrophotometer. The fungal DNA extracts were subjected to an *Aspergillus* genus detection PCR assay to amplify a 521bp segment of the ITS region of the fungal genome and subsequently subjected to a nested PCR to detect three specific *Aspergillus* species (*Aspergillus fumigatus, Aspergillus niger* and *Aspergillus flavus*) by amplifying a 308 bp, 307 bp or 305 bp section of this amplified segment, respectively (Sugita *et al.*, 2004). The bacterial DNA extracts were subjected to three separate PCR assays were conducted for the identification of selected pathogenic bacteria; to detect *Escherichia coli* by amplifying a 101 bp segment of the 16S rRNA gene (Spano *et al.*, 2004), to detect *Erwinia carotovora* by amplifying a 119 bp segment of the 16S rRNA gene (Toth, 1999), and to detect *Salmonella* spp., by amplifying a 244bp segment of the InvA gene (Chiu *et al.*, 1996). The amplified PCR products were evaluated using Agarose Gel Electrophoresis (AGE) to visualize and confirm the presence of amplification of the target DNA fragments. Additionally, bacterial colonies were subjected to Gram staining to differentiate between gram-positive and gram-negative bacteria and visualized under 100X magnification using a light microscope.

III. Results and Discussion

The culture-based analysis revealed a diverse range of microbial communities in the tested compost fertilizers. Using LB media, bacterial colonies were successfully grown, however, there were no differentiable macroscopic morphologies among the bacterial colonies obtained in culture for the four fertilizers samples. Sub cultured colonies displayed two distinct types of colony morphologies in all fertilizer samples and those were coded as A and B (Figure 1). Type A colonies appeared pale in color, flatten or nonconvex, opaque with either irregular or circular shape, potentially indicating *Escherichia coli* or *Bacillus* sp. Type B colonies were mucoid, convex, translucent colonies with either irregular or circular shape, potentially indicating *Salmonella* sp.

Figure 1 : Appearance of subculcured colonies

Gram staining of these two types of colonies in each fertilizer sample showed the presence of gram positive and gram-negative bacteria. Both Gram-negative rods and cocci were observed in fertilizer sample 1 and 2, Gram-negative rods were observed in

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fertilizer sample 3, and Gram-positive rods were observed in fertilizer sample 4. According to a study conducted by Chapman and colleagues, Gram-negative bacteria are the most harmful microbes. Common Gram-negative bacteria include bacilli of the genera *Escherichia, Salmonella,* and *Klebsiella,* and cocci of the genera *Neisseria,* and *Chlamydia* (Chapman *et al.*, 1997). *Streptococcus* and *Staphylococcus* genera are Gram-positive cocci and *Corynebacterium, Bacillus,* and *Clostridium* are Gram-positive bacilli genera. In addition to *Listeria* is gram-positive coccobacillus genera. Further studies are needed to identify the specific bacteria species isolated from the tested compost fertilizer samples.

SDA media supported the growth of various types of fungi, potentially indicating *Aspergillus flavus, Aspergillus niger, Penicillium* sp., *Fusarium* sp., and *Candida* sp., based on their macroscopic morphologies observed in the culture plates. The morphology of fungal growths observed for Fertilizer sample 1 displayed a cotton like appearance and black colour, unclear marks which potentially indicate *Rhizopus* sp., *Aspergillus niger, Penicillium* sp., or certain *Fusarium* sp. The fungal growth observed in Fertilizer sample 2 displayed a morphology having a cotton like appearance with greenish yellow colonies and raised white colonies that potentially indicate *Rhizopus* sp., *Aspergillus niger, Penicillium* sp., certain *Fusarium* sp, or *Aspergillus flavus*. Notably, both fertilizer sample 1 and 2 appeared to contain a mix of morphologically distinct fungal growths. The fungal growth observed from Fertilizer sample 3 displayed a morphology that was a floccose olive-green center with filamentous yellow color surround, and it eventually covered by white color conidia circle, indicating *Aspergillus flavus* as the species responsible for the growth. The fungal growth observed from Fertilizer sample 4 displayed a morphology that was raised opaque with a white color center which was surrounded by flat transparent white color edges, possibly indicating *Fusarium sp.*, or *Aspergillus fumigatus* (Figure 2).

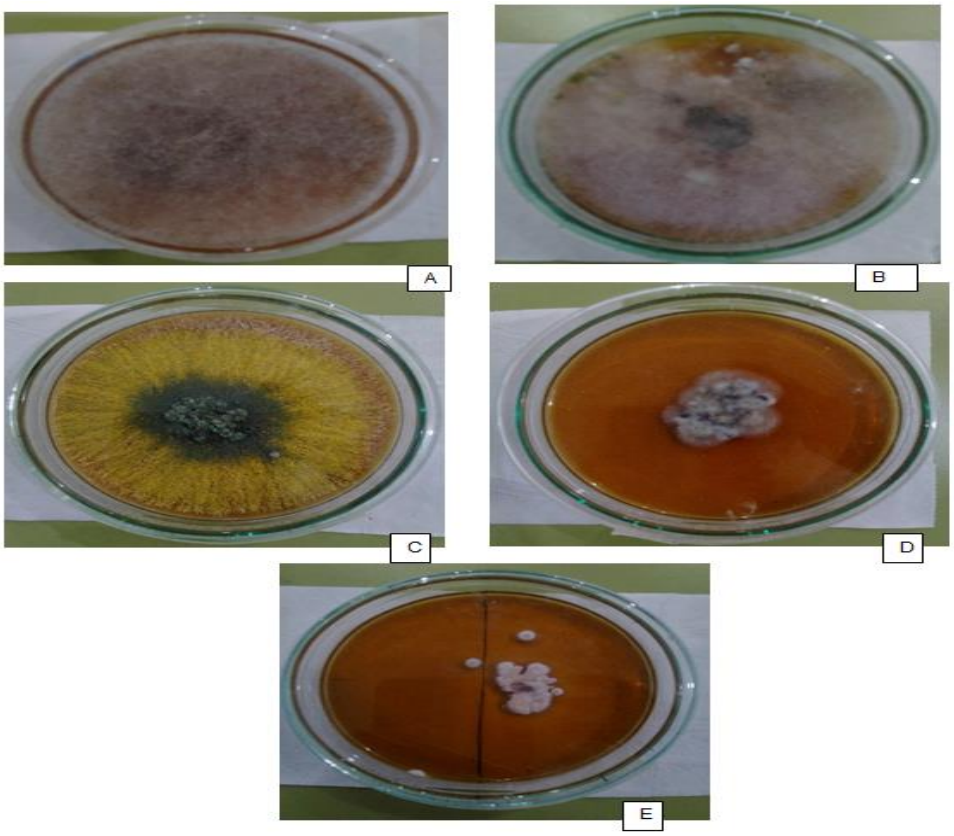

Figure 2. SDA plate images of cultured fungal colonies. A – Fertilizer sample 1, B – Fertilizer sample 2, C – Fertilizer sample 3, D – Fertilizer sample 4, E – Culture positive sample (Right) and Culture negative sample (Left).

The molecular-based analysis provided more specific results than the culture-based analysis, with the PCR assays carried out confirming the presence of *Aspergillus* genus in three of the four fertilizer samples (Figure 3). After that, from another species-specific PCR assay, *Aspergillus flavus* was detected in two fertilizer samples, and *Aspergillus niger* in two fertilizer samples (Figure 4). These findings are significant since *Aspergillus flavus* produces aflatoxins and the presence of aflatoxins in compost poses significant issues to agricultural hygiene and food safety because it can lead to the spread of the toxins to edible crops and it is endangering to the consumer health (Perrone *et al.*, 2007). *Aspergillus flavus* can negatively impact plant products such as corn, wheat, peanuts, rice, sorghum, wheat midds, cottonseed and tree nuts (Kosmidis & denning, 2015). The consumption of that contaminated food can pose serious health diseases, including aflatoxicosis, invasive aspergillosis, non-invasive aspergilloma and allergic bronchopulmonary aspergillosis. As well as aflatoxins are potential carcinogens that pose significant health issues, especially in children such as liver cancer, immune suppression, and growth retardation (IARC, 2012). In addition, *Aspergillus niger* can produce ochratoxins that can contaminate grapes, grains, legumes, coffee, dried fruits, etc. and can be harmful for consumption by humans (Perrone *et al.*, 2007). Other specific *Aspergillus* species indicated from *Aspergillus* genus detection PCR results can be

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confirmed using species-specific PCR assays or sequencing. If appropriate composting procedures are not followed, *Aspergillus* species may contaminate compost fertilizers. These dangerous bacteria can survive when animal dung, untreated organic waste, or poorly managed compost is used. They can then spread to crops. To reduce the risk of pathogen persistence and guarantee the safe use of compost in agricultural activities, effective composting techniques including appropriate temperature management are crucial (Reynolds *et al*., 2017). The detection of *Aspergillus* species, particularly *Aspergillus flavus* and *Aspergillus niger* highlights the potential for adverse impacts that can be caused, necessitating further evaluation to be carried out to determine the suitability of the products that are presently sold in the local market (Abbas *et al*., 2011; Wogen *et al*., 2012; Kosmidis & denning, 2015; Perrone *et al*., 2007).

According to the NCBI primer blast results, *Aspergillus* genus primers used in the study can also amplify certain *Penicillium* species, therefore, *Penicillium* specific PCR assays can be conducted to accurately confirm the presence of *Penicillium* species in the fungal growths observed in the cultured fertilizer samples. However, more comprehensive research needs to be done to confirm those results totally.

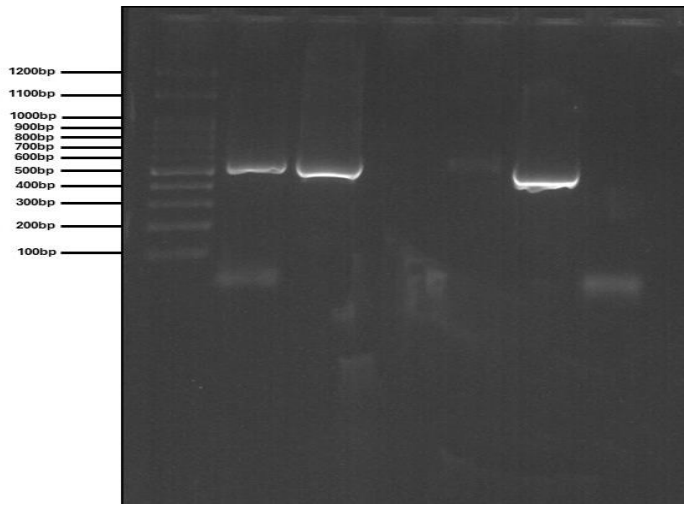

Figure 3. Agarose gel image of PCR-amplified products targeting the *Aspergillus* genus. Lane 1 - 100 bp ladder, lane 02 to 05 - amplified products from fertilizer samples 01 to 04, lane 06 – culture positive samples , lane 07 -Negative control.

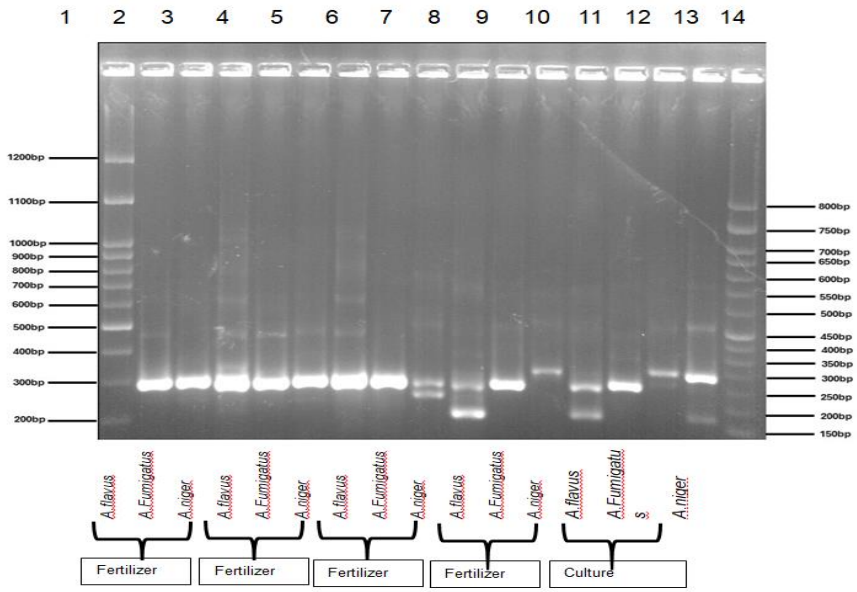

Figure 4. Agarose gel image of nested PCR-amplified products targeting the *Aspergillus* species; *Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger*. Lane 01 - 100 bp ladder, lane 02 to 04 – Amplicons from fertilizer sample 01 (for 03 *Aspergillus* species respectively), lane 05 to 07 – Amplicons from fertilizer sample 02 (for 03 *Aspergillus* species respectively), lane 08 to 10 – Amplicons fertilizer sample 03 (for 03 *Aspergillus* species respectively), lane 11 to 13 – Amplicons from fertilizer sample 04 (for 03 *Aspergillus* species respectively), lane 14 to 16 – Amplicons from culture positive sample (for 03 *Aspergillus* species respectively), lane 17 - 50bp ladder.

The targeted PCR assays used in this study for the identification of pathogenic bacteria revealed that *Escherichia coli* (Figure 5), *Erwinia carotovora* (Figure 6) and *Salmonella* (Figure 7) were absent from all the compost fertilizers tested. This result indicates the

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safety of the compost fertilizers and conformance to the safety standards stipulated in Sri Lanka and other countries (Table 1) regarding those bacteria. However, *Salmonella* results should be confirmed by repeating the assay, due to the absence of amplification of the utilized positive controls and it was a limitation of the assay that identified through the research.

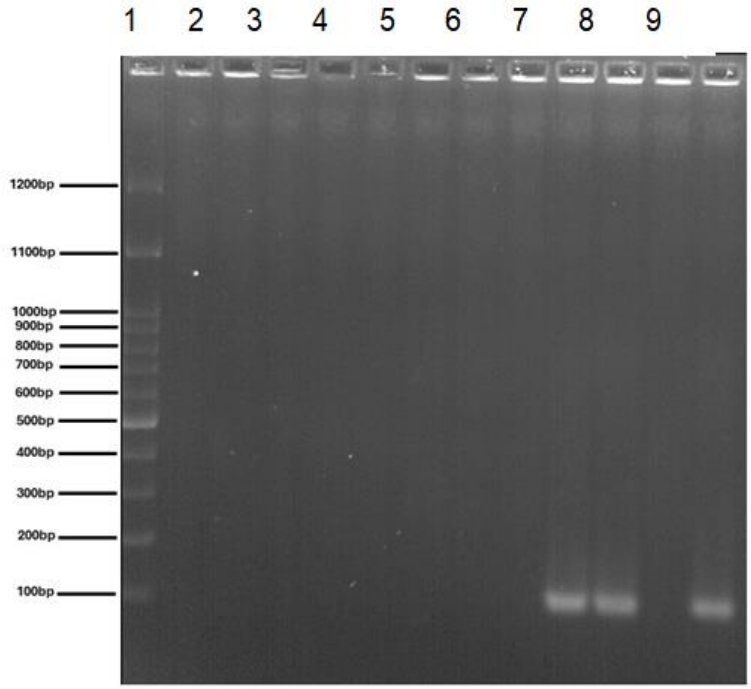

Figure 5. Agarose gel image of PCR-amplified products targeting the *Escherichia coli*. Lane 01 - 100 bp ladder ; lane 02 - fertilizer sample 1A, lane 03 – fertilizer sample 1B, lane 04 – fertilizer sample 2A, 05 - fertilizer sample 2B, lane 06 – fertilizer sample 3A, lane 07 – fertilizer sample 3B, lane 08 – fertilizer sample 4A, lane 09 – fertilizer sample 4B, lane 10 - culture positive sample, lane 11 - *E.coli* positive sample, lane 12 - negative control, lane 13 - positive control.

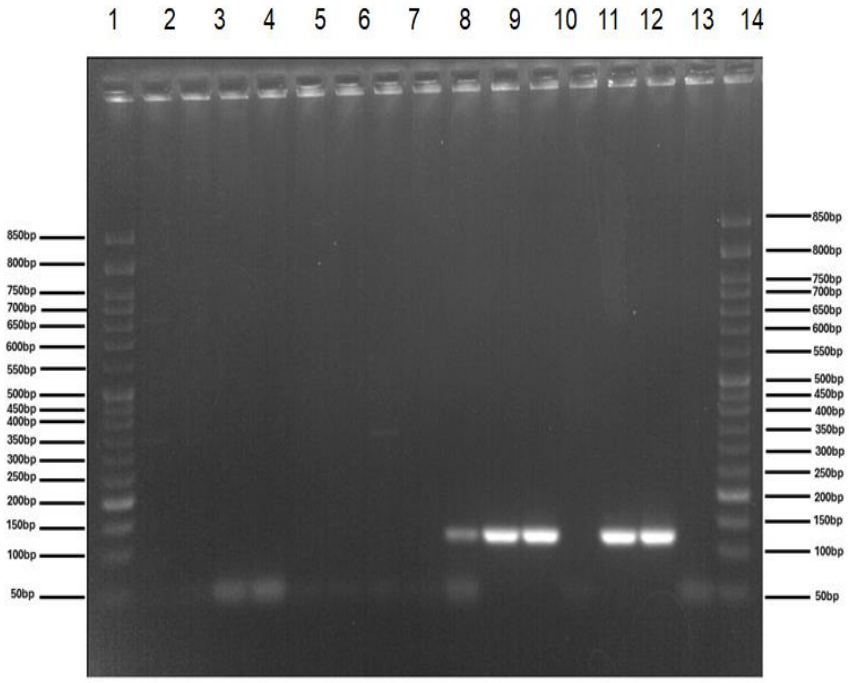

Figure 6. Agarose gel image of PCR-amplified products targeting the *Erwinia carotovora*. Lane 01 – 50 bp ladder, lane 02 – fertilizer sample 1A, lane 03 – fertilizer sample 1B, lane 04 – fertilizer sample 2A, lane 05 – fertilizer sample 2B (Diluted), lane 06 – fertilizer sample 3A, lane 07 – fertilizer sample 3B, lane 08 – fertilizer sample 4A, lane 09 – fertilizer sample 4B, lane 10 - culture positive sample, lane 11 – *Erwinia carotovora* positive control 01 (Diluted), lane 12 – *Erwinia carotovora* positive control 02 (Diluted), lane 13 - negative control, lane 14 – *Erwinia carotovora* positive control 01 (Undiluted), lane 15 - *Erwinia carotovora* positive control 02 (Undiluted), lane 16 – fertilizer sample 2B (Undiluted), lane 17 - 50bp ladder.

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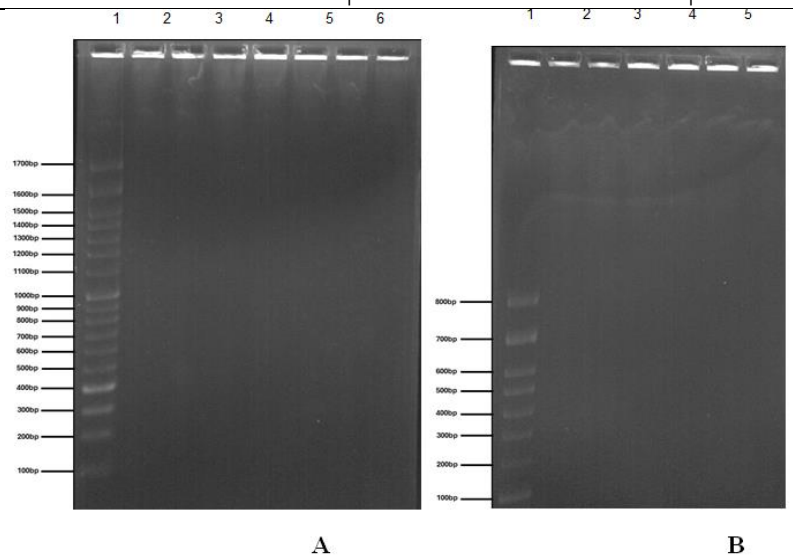

Figure 7. Agarose gel image of PCR-amplified products targeting Salmonella. (A) Lane 01 - 50 bp ladder, lane 02 - fertilizer sample 1A, lane 03 - fertilizer sample 2A, lane 04 - fertilizer sample 3A, lane 05 - fertilizer sample 4A, lane 06 - culture positive sample, lane 07 – Salmonella positive control 1, lane 08 – Salmonella positive control 2. (B) Lane 01 - 50 bp ladder, lane 02 - fertilizer sample 1B, lane 03 - fertilizer sample 2B, lane 04 - fertilizer sample 3B, lane 05 - fertilizer sample 4B, lane 06 - Salmonella positive control 03, lane 07 - negative control.

Table 1. Microorganisms requirements of compost production (Sri Lanka Standard, specification for compost made from raw materials of agricultural origin, 2019; OJ, 1998; Cancelado *et al*.,2014)

Characteristic	Requirement
Sri Lankan Standards	
Faecal coliform colonies MPN, g on dry weight basis	Free
Salmonella, per (MPN)/g on dry weight basis	Free
Europe Standards	
Salmonella spp.	Absent
Escherichia coli / Enterococcaceae (CFU/g)	<=1000
Brazilian Standards	
Fusarium, Phytophtora, Pythium, Rhizoctonia e Sclerotinia	Absence
Fecal coliforms	<1000 ufc/g
Salmonella	Absence in 10g
According to the USEPA 1994 recommendation the compost residual piles should maintain at above 55°C for at least 5 days, to eliminate Erwinia carotovora (USEPA, 1994)	

The absence of common bacterial pathogens in compost fertilizers illustrates a degree of conformance to above specific safety standards and stipulated guidelines. The presence of potentially harmful fungi indicates the need for improved safety measures to ensure the suitability of compost fertilizer samples sold in the local market. Further optimization of species-specific PCR assays is necessary to enhance the detection accuracy for various pathogens identified through Gram staining. PCR assays offer significant benefits in accurately detecting microbial contamination, including the ability to identify specific species with high sensitivity and specificity. By developing and utilizing targeted PCR assays for different pathogens, researchers can achieve more precise identification and quantification of microbial contaminants in compost fertilizers. This approach not only improves the reliability of detection methods but also aids in better understanding the prevalence and impact of different microbial species, ultimately contributing to safer agricultural practices and public health protection.

This study highlights the risks associated with the contamination of compost fertilizers with pathogenic microbes in Sri Lanka and the need for ongoing monitoring and improvement of safety standards to protect public health and the environment. Continuous evaluation of microbial content is essential to ensure that compost fertilizers are safe for agricultural use and do not pose a threat to consumers or the environment.

The findings of the study can benefit researchers, institutions, consumers, manufacturers, to give attention about safety standards regarding the microbes in compost and to do future investigations into microbial contamination in compost fertilizers, which will facilitate the creation of more sophisticated detection techniques and a more thorough comprehension of the mechanisms by which pathogenic microbes detected in fertilizer products can have an impact on agriculture, the environment and on human health. In

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addition to that, the results are useful to fertilizer manufacturers to enhance their quality control procedures and guarantee that their products are devoid of harmful microorganisms, thereby creating a safer product for sale in the local market and this may boost customer confidence and perhaps create new markets where strict safety regulations are necessary.

Most previous research on microbial diversity in composting systems has shown that the microbial community undergoes significant changes during the composting process, often leading to a reduction in pathogenic microorganisms. In comparison, this study found that certain pathogens, such as *Aspergillus* species, persisted in the compost samples even after several weeks of composting.

The study's overall findings emphasize the importance of the safety of compost fertilizers and the need for more comprehensive research to identify the safety of compost fertilizers.

IV. Conclusion

The outcomes of the study revealed the presence of different Gram-negative and Gram-positive bacteria, as well as indicated the morphologies of several fungi, namely *Rhizopus* sp., *Penicillium* sp., *Aspergillus niger*, and *Aspergillus flavus*, in the tested compost fertilizer samples using culture-based techniques. Furthermore, the presence of *Aspergillus* genus, *Aspergillus flavus* and *Aspergillus niger* as well as the absence of *Erwinia carotovora*, and *Escherichia coli* in the tested compost fertilizer samples were confirmed by molecular techniques related to PCR. The absence of *Salmonella* also was confirmed but another assay should be conducted because of the absence of amplification of the positive controls which were used to the assay. Compared to culture based traditional approaches, the use of PCR has shown to be more sensitive and reliable in identifying certain fungal species, highlighting the possible dangers of using contaminated fertilizers in agricultural settings. These findings emphasize the significance of strict monitoring and adherence to safety regulations to lessen the negative effects of microbial contamination on crop health and human safety.

The findings of the study can aid in raising awareness about the safety of compost fertilizers sold in the local market and lead to further investigation into discovering the different pathogenic microbes present in the products and identifying a suitable method for testing and monitoring these products for safety and suitability to be sold to consumers. To gain a more thorough understanding of microbial contamination in fertilizers, future research can involve the analysis of a greater range of samples from different producers as well as a larger sample size and use of specific primers to accurately identify other species of pathogenic bacteria and fungi that were detected in this study. Understanding potential contamination can empower consumers to make informed decisions regarding the use and handling of products, ultimately enhancing safety. It is feasible to create more reliable monitoring and detection methods for microbial contamination in fertilizers by building on the research's findings. With this information, customers may be able to make more educated choices, which may lead to more stringent safety regulations and testing. Producers will be compelled by more stringent testing and regulations to produce safer fertilizer products that meet these strict requirements. As a result, this will protect customer health and encourage increased confidence and dependability in fertilizer products. To guarantee the security of compost fertilizers, regulatory bodies can impose more stringent guidelines and quality assurance procedures. Overall, the potential actions that can result from the outcomes of this study can minimize the potential adverse impact on human health that use of compost fertilizer contaminated with pathogenic microbes can have, improve environmental sustainability and secure agricultural output.

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