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Comparative Evaluation of Disinfectants' Efficacy in Reducing Bacterial and Fungal Contamination in Livestock Feed Productaaion

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ABSTRACT

Background: Disinfectants in feed factories have a crucial role in maintaining a clean, hygienic environment, preventing disease spread, controlling cross-contamination, and ensuring product quality, thereby ensuring food safety.

Objectives: The study aimed to assess the performance of multiple disinfectants in a factory producing livestock, poultry, and aquatic feed, as well as in the laboratory.

Methods: The microplate and agar-well diffusion methods were utilized to assess the efficiency of commercial chemical disinfectants (1 and 2) and formalin (37%) on the internal surfaces of the mixer, mill, extruder, dryer, and cooler in the factory and examine the performance of eight common disinfectants, including disinfectants 1, 2, 3, NaClO (10%), ethanol (70%), methanol (70%), povidone-iodine (10%), and formalin, against *Salmonella typhimurium*, *Escherichia coli*, and *Fusarium oxysporum*, in the laboratory.

Results: The extruder had the highest level of microbial contamination, while the cooler had the lowest. Disinfectant 2 and formalin had the most effective antibacterial and antifungal properties. Disinfectants 2 and 3 had the highest antibacterial effects in the laboratory, while other disinfectants had the lowest. Disinfectant 2 had the strongest antifungal effect, followed by formalin, povidone-iodine, and NaClO. Ethanol and methanol had the least effect.

Conclusions: The study emphasizes the importance of selecting effective disinfectants to reduce contamination in animal feed production facilities. Disinfectant 2 (Huwa-san), with its unique combination of hydrogen peroxide and silver-based ionic chemistry, is recommended as a powerful disinfectant solution for various applications. The findings can serve as a valuable guide for choosing appropriate disinfectants in similar industries.

Keywords: *E. coli*, feed factory, *Fusarium,* commercial disinfectant, *Salmonella*

Introduction

Feed is an integral part of the food chain, and its safety is a prerequisite for human health, animal health and welfare, income generation, and economic sustainability. Feed safety is a shared value and responsibility, and should be subject to quality assurance through integrated food safety systems, similar to food production (Negash, 2020). Maintaining a clean and hygienic environment in livestock, poultry, and aquatic feed production facilities is crucial in preventing the spread of disease and controlling cross-contamination between contaminated and non-contaminated materials. This prevents the colonization-infection-contamination cycle, ensuring the safety and quality of final products and reducing the risk of microbial agents entering human food sources. In this regard, effective disinfection protocols play a vital role in controlling microbial contamination and reducing the risk of pathogen transmission (Dvorak, 2008; Muckey, 2016). Some pathogens that can enter the human food supply through feed microbial contamination include *Salmonella enterica* serotypes, Shiga toxin-producing *Escherichia coli* strains, *Campylobacter* species, and *Yersinia enterocolitica* (Huss *et al.*, 2015). Since decontamination of facilities is an important step in preventing the spread of these diseases and controlling cross-contamination (Dvorak, 2008; Huss *et al.*, 2015; Muckey, 2021), it is therefore

necessary to evaluate the effectiveness of disinfectants in disinfecting facilities and removing or inhibiting the growth of microorganisms (Wales *et al.*, 2021). The assessment of disinfectant performance can be conducted through a variety of methods, all of which permit the investigation of antibacterial and antifungal effects with regard to specific pathogens. The judicious and effective selection of disinfectants assumes a critical role in the maintenance of a clean and secure environment, the reduction of the risk of disease transmission, and the guarantee of the quality of the final products (Abban *et al.*, 2013; Davies and Wales, 2019; Stringfellow *et al.*, 2009). The outcomes derived from this investigation will offer invaluable insights into the capacities of disinfectant agents for application in livestock, poultry, and aquatic feed production facilities. These findings can be utilized as a pragmatic guide by professionals within the industry for the selection of suitable disinfectant agents, thereby mitigating contamination and promoting food safety.

This study evaluated the performance of several common disinfectants in animal, poultry, and aquatic feed production facilities, focusing on their ability to remove feed microorganisms from surfaces. The research was conducted in the animal feed production factory and, at the same time, in a laboratory setting. Some of these disinfectants are commercially used on a large scale

in the animal feed industry, while others are used on a smaller scale to clean and disinfect specific small surfaces.

Materials and methods

Chemicals and media

Plate count agar (PCA), yeast extract glucose chloramphenicol (YGC), tryptic soy broth (TSB), potato dextrose agar (PDA), and Mueller-Hinton agar were produced from Mirmedia (Kardan Azma Co., Iran). Sabouraud dextrose broth (SDB), ethanol, methanol, and NaCl, were prepared from Merck (Germany); commercial disinfectants 1 (based on hydrogen peroxide, Iran); commercial disinfectants 2 (based on hydrogen peroxide with silver ions, Belgium); commercial disinfectants 3 (based on the composition of stabilized peroxyacetic acid and hydrogen peroxide, Iran); formalin (37% formaldehyde), sodium hypochlorite (NaClO), povidone-iodine 10%, and nalidixic acid are produced from Iran.

Microbial strains

Fusarium oxysporum (PTCC-2112), obtained from the Iranian Research Organisation for Science and Technology (IROST), *Salmonella Typhimurium* (ATCC-14028) and *Escherichia coli* (ATCC-10698) were obtained from the Microorganisms Collection of the Food Microbiology Laboratory of the Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, prepared and activated according to the provided instructions.

Animal feed factory phase

Surface determination, preparation, and sample collection from factory facilities

The performance of disinfectant agents in a factory producing animal, poultry, and fish feed was studied. This study was conducted in a completely randomized design with four treatments and three replicates. The experimental treatments included two chemically-based disinfectant agents available on the market (2 treatments), formalin as a positive control, and a location without the use of a disinfectant agent as a negative control (sterile water spray).

As part of the Hazard Analysis and Critical Control Point (HACCP) program, certain areas in the factory producing animal, poultry, and fish feed were identified, which were as follows: 1) inside the mixer; 2) inside the mill; 3) inside the extruder area; 4) inside the dryer; and 5) inside the

cooler. After physically cleaning the designated areas (10 cm^2), disinfectants were applied to the surfaces in quantities consistent with the manufacturer's recommended concentrations. The treated surfaces were allowed to dry following the manufacturer's instructions. Sampling was then conducted using a swab, and the swabs were transferred to glass containers with screw lids containing 5.0 mL of normal saline. Subsequently, the samples were promptly sent to the laboratory.

Laboratory analysis

In the lab, the samples were diluted under aseptic conditions. PCA was used for total microbial enumeration, and YGC was used for mold and yeast enumeration. The cultivation was done in two layers. The mold and yeast counts were performed after three to five days of incubation at 25 °C, and the bacterial counts were performed after two days of incubation at 37 °C.

The laboratory phase

Microplate method

The microplate method was utilized to examine the performance of disinfectant agents. Nine common disinfectant agents on the market were used, including commercial disinfectants 1, 2,

and 3, sodium hypochlorite (10%), ethanol (70%), methanol (70%), povidone-iodine (10%), nalidixic acid (40 ppm), and formalin (37% formaldehyde). The tests were performed twice, with three replicates for each treatment.

Bactericidal tests: Following Farouk et al.'s (2020) method with minor modifications, the recommended amount of disinfectant was mixed with sterile distilled water, and then 100 µL of each disinfectant was added to 100 µL of TSB medium (double concentration) in each well. A volume of 10 µL of bacterial suspension (*Salmonella Typhimurium* and *E. coli*) equal to 0.5 McFarland standard (approximately 10^8 CFU/mL) was added to the wells. A row of culture medium and bacterial suspension was used as a positive control, while a row of culture medium without bacteria was a negative control. After inoculation with bacteria and disinfectants, the microplate was placed inside a microplate reader instrument (model: mqx200r2), and the data were obtained after 24 hours at 37 °C, the wavelength 600 nm, and the shaking intensity 10 seconds every 60 minutes, with a one-hour reading.

Fungacidal tests: The antifungal effects were studied using a 96-well microplate (Rahimi-Kakolaki *et al.*, 2023). To prepare a spore suspension, a sterile normal saline solution was pipetted onto a five-day-old PDA culture. After collecting the resulting solution, the number of

spores was adjusted to 2×10^6 spores per mL using a hemocytometer. The recommended amount of disinfectant was mixed with sterile distilled water. In each well, 100 µL of each disinfectant was added to 100 µL of SDB (double concentration). A volume of 10 µL of *Fusarium oxysporum* spore suspension (2×10^6 spores/ml) was added to the wells. After incubation at 25 °C for five to seven days, the wells were examined for fungal growth by visually observing the mycelium. The absence of fungal growth in the wells indicated the inhibitory effect of the tested substance in the respective culture. *Agar-well diffusion method*

The agar-well diffusion method was used to investigate the effects of the antimicrobial activity of the disinfectants in Mueller-Hinton agar, PCA and PDA.

Bactericidal tests: The bacteria were inoculated with 0.5 McFarland concentration (1×10^8 cfu/mL) of *Salmonella* and *E. coli*, following the method of Gomaa *et al.* (2020) with minor modifications. After bacterial inoculation, 5-mm-diameter wells were created in the agar plates. A volume of 50 µL of each sample was added to the wells. The plates were then incubated at 37 °C for 24 hours. Nalidixic acid antibiotics were used as the standard control in both methods. After incubation, the diameter of the created inhibition zone was measured.

Fungacidal tests: Applying the method introduced by Kavitha and Satish (2016) with slight modifications to investigate the antifungal effects of disinfectants, the surface of the PDA culture medium was inoculated with the appropriate amount (in this test, 100 µL) of *Fusarium oxysporum* spores with a concentration of 2 x 10^6 spores/mL. After drying the surface, five-millimeter-diameter wells were made in the agar plates. A volume of 50 µL of each disinfectant sample was added to the wells, and the plates were incubated at 25–28 °C for three days. After incubation, the diameter of the created inhibition zone was measured.

Statistical analysis

Statistical analysis was performed using SPSS 2016 to compare mean values using Duncan's multiple range test with a significance level of less than 0.05 and Graph Pad Prism 8 software for laboratory data analysis and graph drawing.

Results

Animal feed factory phase

Samples were collected post-physical cleaning to minimize surface contamination (Figure 1) and promptly transported to the laboratory for further analysis. The antibacterial and anti-mold and anti-yeast effects of disinfectants in samples obtained from feed factory surfaces are shown in Tables 1 and 2. Examination of factory sections showed high microbial contamination in the extruder and low contamination in the cooler. Disinfectant 2 and the positive agent (formalin 37%) had the best antimicrobial effects. Commercial disinfectant 1 only had a good effect in the most contaminated area (extruder).

The extruder had the highest mold and yeast contamination in the factory equipment and facilities, while the cooler had the least contamination. Disinfectant 2 and the positive agent (formalin 37%) had the best antifungal effects. Disinfectant 1 had no effect in different sections of the facilities. Formalin and disinfectant 2 had the greatest antimicrobial effects. Disinfectant 1 had no antimicrobial effect in some places (sampling locations 1, 3, etc.) and had a minimal antimicrobial effect in some other places. The comparison of antifungal effects in sampling location 5 (cooler) showed no significant difference ($P > 0.05$), but formalin and disinfectant 2 had a greater antifungal effect compared to disinfectant 1 and the control group. The disinfectants showed inhibitory effects on *E. coli*. However, disinfectant 1 had no inhibitory

effect on this bacterium. The results of the inhibitory effects on *E. coli* were similar to the results of the inhibitory effects on *Salmonella Typhimurium*.

The laboratory phase

Microplate method

Formalin and disinfectant 2 had antifungal effects at different concentrations, but disinfectant 1 only had antifungal effects at 5% and 10% concentrations. Disinfectants 2, 3, and nalidixic acid had the highest effects, while 70% ethanol, 70% methanol, 10% povidone-iodine, and 10% sodium hypochlorite had the least antimicrobial effects against *Salmonella Typhimurium* (Figure 2). Nalidixic acid had the highest effect, and disinfectants 2 and 3 had good antimicrobial effects against *E. coli* compared to other substances, while 70% ethanol, 70% methanol, 10% povidone-iodine, and 10% sodium hypochlorite had the least antimicrobial effects. Disinfectant 2 had the greatest antifungal effect against the *Fusarium oxysporum* fungus. formalin (37% formaldehyde), 10% povidone-iodine, 10% sodium hypochlorite, and disinfectant had good antifungal effects compared to other substances, while 70% ethanol and 70% methanol had the least antifungal effects.

Agar-well diffusion method

Table 3 shows the results of a comparison of disinfectant inhibitory effects on *E. coli*, *Salmonella Typhimurium*, and *Fusarium oxysporum* using the agar well diffusion method. Disinfectants 2, disinfectants 3, and nalidixic acid had the highest inhibitory effects, while 70% ethanol, 70% methanol, 10% povidone-iodine, and 10% sodium hypochlorite had the least antimicrobial effects against *Salmonella Typhimurium* (Figure 3).

Nalidixic acid had the highest effect, and disinfectants 3 and 2 had good antibacterial effects against *E. coli* compared to other substances, while 70% ethanol, 70% methanol, 10% povidone-iodine, and 10% sodium hypochlorite had the least antibacterial effects (Figure 4, A and B). Disinfectant 2 had the greatest antifungal effect against the *Fusarium oxysporum* fungus (Figure 4, C). Formalin and disinfectant 3 have good antifungal effects; 10% povidone-iodine and 10% sodium hypochlorite were next, while 70% ethanol and 70% methanol had the least antifungal effects.

Discussion

In this study, the internal surfaces of five important parts of livestock, poultry, and aquatic feed production facilities that were identified in research as potential sources of contamination (Davies and Wales, 2010; Huss *et al*., 2015; Jones, 2011; Muckey, 2016) and HACCP programs have been evaluated, selected, and reviewed. Sampling was done after physical cleaning so that the presence of organic substances inside and on the surfaces of the equipment does not affect the effectiveness of disinfectants. Organic matter can deactivate chemical disinfectants such as sodium hypochlorite (Huss *et al*., 2015).

In this study, the pellet cooler had the lowest levels of contamination, while the extruder had the highest levels of mold, bacteria, and yeast. Our results are in contradiction with the results of the study by Davies and Wray (1997), who observed that 85% of the samples collected from coolers were contaminated with *Salmonella*. Parker *et al.* (2019) also reported that the probability of a positive *Salmonella* sample from the cooler is twice the probability of its detection in the final feed ($P \leq 0.05$). This can be due to the increase in moisture density in the pellet cooler. Moisture added to the powder feed to generate steam during pellet preparation is removed through a pellet cooler at the end. However, condensation on pellet cooler indoor surfaces can lead to increased humidity and microbial growth such as *Salmonella* (Jones, 2008). The lower contamination observed in the pellet cooler in this study may be attributed to the implementation of adequate

ventilation. Additionally, in the studied factory, the production line involves a dryer where pellets are dried for 30 minutes at 100 degrees before entering the cooler. This process eliminates many existing microorganisms. The hot pellets, which are still hot when entering the cooler, reduce the microbial load in the cooler area. These results are consistent with Jones' (2011) findings, which indicated that maintaining a temperature of 46°C at the top of the pellet cooler can effectively reduce Salmonella growth. All three commercial disinfectants, 1, 2, and 3, utilize hydrogen peroxide in their structure. Hydrogen peroxide (H_2O_2) is a disinfectant with bactericidal and sporicidal properties, effective against most chlorine-resistant bacteria (Linley *et al.*, 2012) and effectively combats biofilms by producing free radicals that affect the biofilm matrix (Farjami *et al.*, 2022). Unlike peracetic acid and aldehydes, which require disruption of the biofilm matrix before use, hydrogen peroxide can be effective without this process (Wirtanen and Salo, 2003). The superior performance and more effective efficiency of commercial disinfectant 2 compared to commercial disinfectants 1 and 3 can be attributed to the presence of colloidal silver in commercial product 2. By adding a silver stabilizer to hydrogen peroxide, a complex salt mixture containing ionic silver is formed. This mixture plays a crucial role in stabilizing the hydrogen peroxide and augmenting its effectiveness (Martin *et al.*, 2015).

Our results are consistent with previous research on the antimicrobial effects of formalin (Chen *et al*., 2016; Ricke *et al*., 2019), but there are also reports that show that some microorganisms, including *Pseudomonas* species, members of the *Enterobacteriaceae* family, and *Escherichia coli* strains, have shown resistance to formalin (Chen *et al*., 2016; Nikolic *et al*., 2019). Resistance to formaldehyde has often been observed in gram-negative bacteria (Nikolic *et al*., 2019). Although formaldehyde is one of the most effective antibacterials available (Ricke *et al*., 2019), concerns have been raised about its safety, especially for people working in closed environments (Carrique-Mas *et al*., 2007; Ricke *et al*., 2019). The European Food Safety Authority considers formaldehyde safe for humans when used as an additive in animal feed products, but warns against inhalation and skin and eye contact (Resae *et al*., 2023; EFSA, 2014). The results of the present study regarding 70% ethanol, 70% methanol, 10% povidone iodine, and 10% sodium hypochlorite against *salmonella* are consistent with the results of Abed and Hussein's study in 2016. In their research, the disinfectant chemicals used (0.5% NaClO, 70% ethanol, 1% iodine, and 10% potassium permanganate) had the lowest antimicrobial effect against the studied microorganisms compared to formalin and the commercial disinfectant Dettol®. In contrast to our findings, in the study of Møretrø *et al.* (2009), 70% ethanol and alcoholic compounds were more effective in controlling *Salmonella* strains in animal feed

production facilities in Norway compared to acids, aldehydes, peroxides, and chlorine-based surface disinfectants.

The observed differences can be attributed to the variety of disinfectant compounds used. In the present study, while most of the commonly used compounds demonstrated effectiveness, they ranked lower when compared to formalin and disinfectant 2 and 3. The insufficient efficacy of disinfectants such as povidone-iodine 10%, sodium hypochlorite 10%, 70% ethanol, and 70% methanol can be attributed to the emergence of resistance in the studied microbes, which has become a serious concern and highlights the need for more effective and sustainable solutions (Tong *et al.*, 2021). Continuous exposure to disinfectants increases adaptation and tolerance in microorganisms through phenotypic adaptation, gene mutation, and horizontal gene transfer (Cloete, 2003). The rapid growth of disinfectant-resistant bacteria is alarming and reduces the killing efficiency of disinfectants (Zhu *et al.*, 2021), which poses challenges for medical treatment and foodborne diseases. These concerns have led to extensive research into safer alternatives to disinfectant chemicals, including formaldehyde, in the animal feed industry. Effective plant essential oils have been identified as a potential solution to combat microbial resistance (Vidács *et al.*, 2018; Rahimi Kakolaki *et al.*, 2023). The antimicrobial effects of some probiotics (Soltani *et al.*, 2023; Rahimi-Kakolaki and Omidi, 2020; Zhang *et al.*, 2012; Zhao *et*

al., 2017) and the interaction of probiotics and bacteria to remove biofilms have also been confirmed, making them potential alternatives to disinfectants (Tong *et al.*, 2021; Hassanzadeh and Mohammadzadeh, 2022; Asad Salman *et al*., 2023). Anyway, the food industry employs new technologies, such as nanotechnology, precise methods, and high-quality ingredients, to fulfill global requirements for extended storage, stringent quality control, and international hygiene standards (Peidaei et al., 2023).

Conclusion

This research was focused on evaluating the effectiveness of several common disinfectants in animal, poultry, and aquatic feed production facilities. The findings of this research highlight the importance of selecting factors that can effectively reduce and control microbial contaminants in the sensitive area of livestock, poultry, and aquatic feed. In the present research, as a baseline study, by analysing the performance of several common chemical disinfectants and three new commercial disinfectants, commercial disinfectant 2 (Huwa-san) was identified as a broad-spectrum disinfectant with high reliability in the factory environment, and it was observed in the laboratory that it can compete with formaldehyde in the parameters investigated in this study.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ارزیابی مقایسه‌ای کارایی ضدعفونی کننده‌ها در کاهش آلودگی باکتریایی و قارچی در تولید خوراک دام

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چکیده

زمینه مطالعه: ضدعفونی‌کننده‌ها در کارخانه‌های خوراک، نقش مهمی در حفظ یک محیط پاکیزه و بهداشتی، جلوگیری از گسترش بیماری، کنترل آلودگی متقاطع و تضمین کیفیت محصول و در نهایت تضمین ایمنی مواد غذایی دارند.

هدف: این مطالعه با هدف ارزیابی عملکرد چند ضدعفونی کننده شیمیایی، در کارخانه تولید کننده خوراک دام، طیور و آبزیان و همچنین در آزمایشگاه انجام گرفت.

روش کار: از روش‌های میکروپلیت و انتشار درچاهک آگار به منظور ارزیابی کارایی ضدعفونی‌کننده‌های شیمیایی تجاری (1 و 2) و فرمالین (37٪) بر روی سطوح داخلی میکسر، آسیاب، اکسترودر، خشک‌کن و کولر در کارخانه و بررسی عملکرد هشت ضدعفونی کننده رایج شامل ضدعفونی کننده‌های 1، 2، 3، NaClO (10٪)، اتانول (70٪)، متانول (70٪)، پوویدون آیودین (10٪) و فرمالین (37٪) در برابر *سالمونلا تیفی موریوم*، اشریشیا کولای و *فوزاریوم اکسیسپوروم* در آزمایشگاه استفاده شد.

نتایج: اکسترودر بیشترین میزان آلودگی میکروبی و کولر کمترین میزان آلودگی را داشت. ضدعفونی کننده 2 و فرمالین بیشترین اثرات ضدباکتریایی و ضدقارچی را داشتند. ضدعفونی کننده‌های 2 و 3 بیشترین اثرات ضد باکتریایی را در آزمایشگاه نشان دادند؛

در حالی که سایر ضدعفونی کننده‌ها کمترین میزان کارایی را داشتند. ضدعفونی کننده 2 قوی‌ترین اثر ضدقارچی را داشت و پس از آن فرمالین، پوویدون آیوداین و NaClO قرار گرفتند. اتانول و متانول کمترین اثربخشی را داشتند.

نتیجه گیری نهایی: این مطالعه بر اهمیت انتخاب مواد ضد عفونی کننده مؤثر به منظور کاهش آلودگی در تأسیسات تولید خوراک دام، طیور و آبزیان تأکید می‌کند. ضدعفونی‌کننده 2 (Huwa-san)، با ترکیب منحصر به فرد پراکسید هیدروژن و شیمی یونی مبتنی بر نقره، به عنوان یک محلول ضدعفونی‌کننده قوی برای کاربردهای مختلف توصیه می‌شود. یافته‌های این مطالعه می‌تواند به عنوان راهنمای ارزشمندی برای انتخاب ضدعفونی‌کننده‌های مناسب در صنایع مشابه باشد.

کلید واژه‌ها: *اشریشیا کولای*، *سالمونلا*، ضدعفونی‌کننده تجاری، *فوزاریوم*، کارخانه خوراک دام

Table 1: Comparative evaluation of antibacterial effects of disinfectants (Total count, CFU/10 cm^2)

Sampling Location	Negative Control (No Disinfectant)	Positive Control (Formalin)	Disinfectant 1	Disinfectant 2
Mixer	30.67±10.78 [a]	7.67±2.51 [b]	29.67±4.72 [a]	14.67±2.30 [b]
Hammer mill	37.33±7.02 [a]	1.00±0.0 [b]	37.33±8.02 [a]	5.33±1.52 [b]
Extruder	61.00±7.0 [a]	5.67±1.52 [c]	20.67±5.85 [b]	17.00±4.35 [b]
Dryer	34.33±3.05 [a]	6.00±1.73 [b]	34.00±1.0 [a]	9.33±3.51 [b]
Cooler	5.67±2.08 [a]	1.33±0.57 [c]	3.00±2.0 [ab]	1.33±0.57 [c]

Dissimilar letters in each row represent differences between groups ($P \leq 0.05$).

Table 2: Comparative assessment of disinfectants' anti-mold and anti-yeast effects (Total count, CFU/10 cm^2)

Sampling Location	Negative Control (No Disinfectant)	Positive Control (Formalin)	Disinfectant 1	Disinfectant 2
Mixer	8.33±2.51 [a]	2.00±2.0 [b]	8.00±2.0 [a]	2.33±1.52 [b]
Hammer mill	8.00±2.0 [a]	3.00±1.0 [c]	6.00±1.0 [ab]	3.67±1.52 [bc]
Extruder	33.67±10.21 [a]	6.67±2.88 [b]	32.67±5.03 [a]	7.67±2.51 [b]
Dryer	8.67±2.30 [a]	3.00±1.0 [b]	5.33±2.08 [b]	4.00±1.0 [b]
Cooler	1.67±1.15 [a]	0.33±0.57 [a]	0.67±0.57 [a]	0.033±0.57 [a]

Dissimilar letters in each row represent differences between groups ($P \leq 0.05$).

Table 3: Comparative evaluation of disinfectant performance in laboratory using agar well diffusion method (cm^2)

Disinfectants	*E.coli*	*Salmonella Typhimurium*	*Fusarium oxysporum*
Disinfectant 1	0.00±0.00 ^{e}	0.00±0.00 ^{e}	0.00±0.00 ^{d}
Disinfectant 2	1.7±0.89 ^{c}	2.4±0.11 ^{a}	2.2±0.28 ^{a}
Disinfectant 3	1.8±0.15 ^{bc}	2.6±0.30 ^{a}	1.6±0.15 ^{b}
Sodium hypochlorite (10%)	0.7±0.00 ^{d}	0.5±0.43 ^{d}	1.4±0.05 ^{bc}
Ethanol (70%)	0.5±0.45 ^{de}	0.8±0.05 ^{cd}	0.1±0.00 ^{d}
Methanol (70%)	0.2±0.35 ^{de}	0.9±0.25 ^{c}	0.1±0.00 ^{d}
Betadine (10%)	0.8±0.11 ^{d}	0.8±0.11 ^{cd}	1.3±0.15 ^{c}
Formalin (37%)	2.43±0.05 ^{ab}	1.9±0.05 ^{b}	1.6±0.11 ^{bc}
Nalidixic acid (40ppm)	2.5±0.00 ^{a}	2.5±0.05 ^{a}	-

Dissimilar letters in each row represent differences between groups ($P \leq 0.05$).

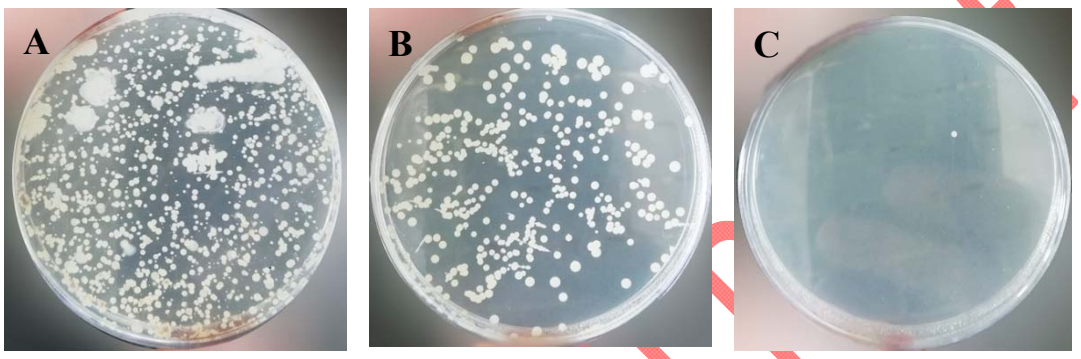
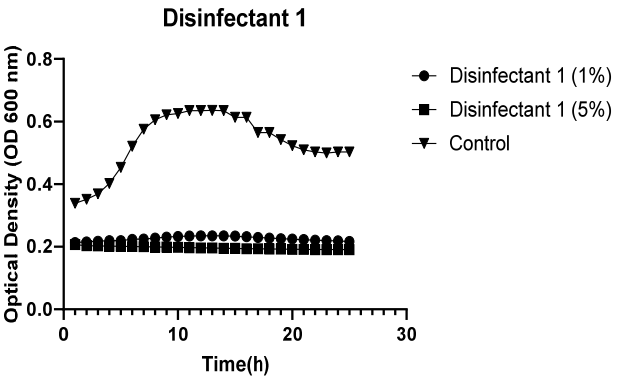
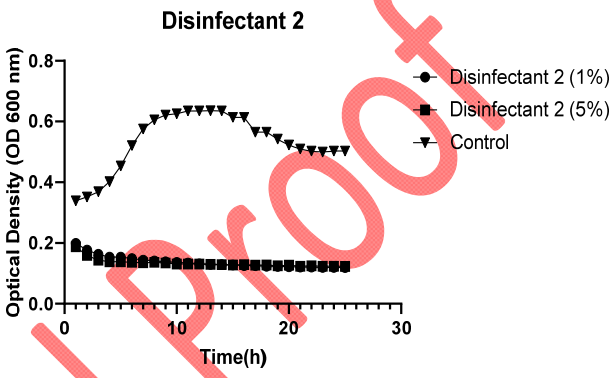
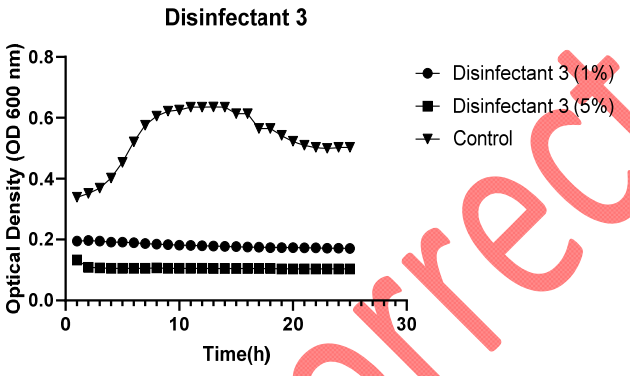
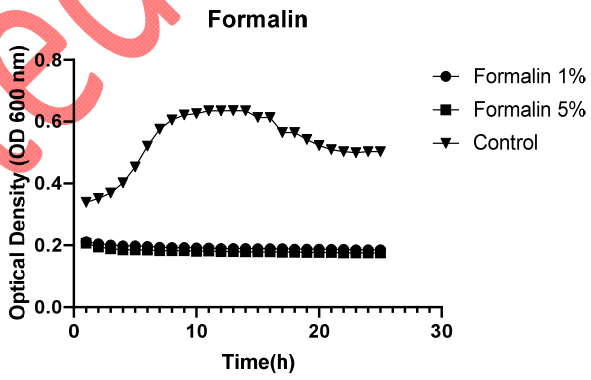


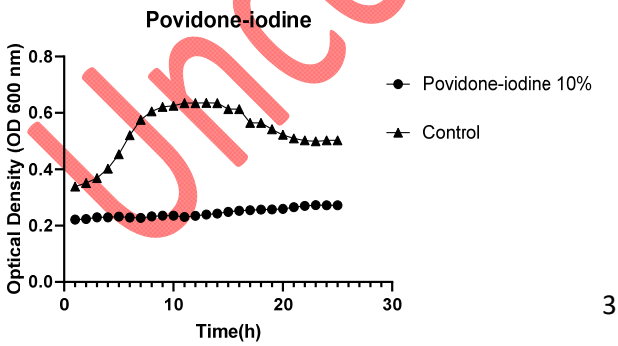
Figure 1: Investigating the effect of physical cleaning on reducing pollution; A:the number of bacteria before physical cleaning; B:the number of bacteria after surface cleaning; and C: the number of bacteria after disinfection.

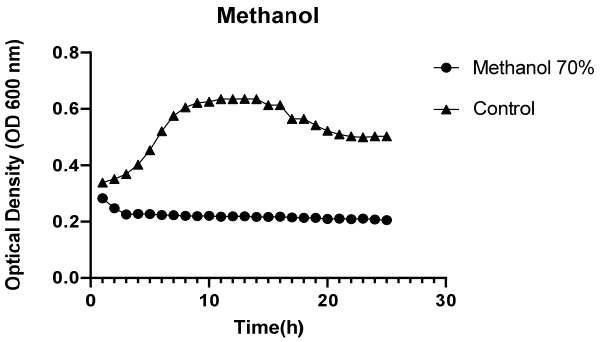












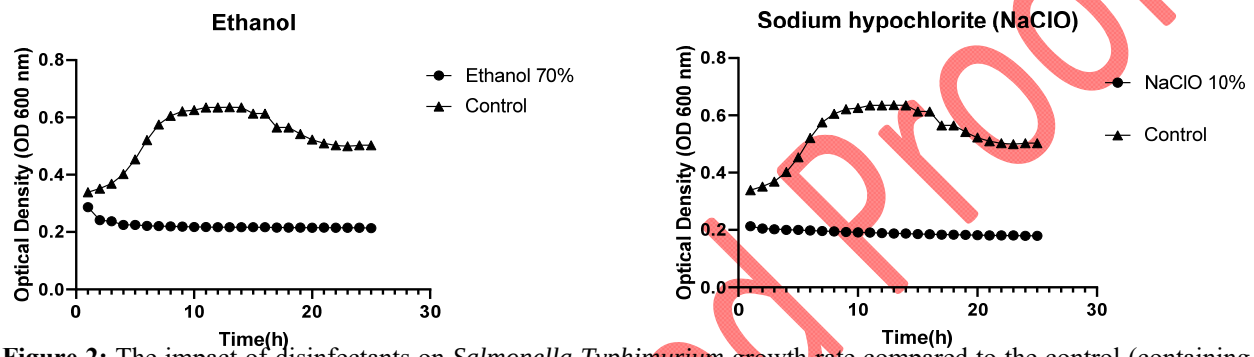


Figure 2: The impact of disinfectants on *Salmonella Typhimurium* growth rate compared to the control (containing bacteria) at 600 nm wavelength and 37°C for 24 hours

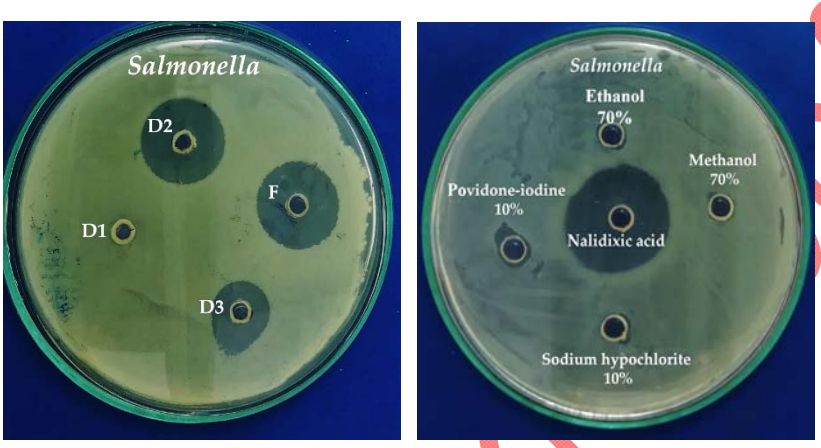


Figure 3: Comparison of disinfectant inhibitory effects on *Salmonella Typhimurium* using the agar-well diffusion method; D1: disinfectant 1 ; D2: disinfectant 2; D3: disinfectant 3 and F: formalin.

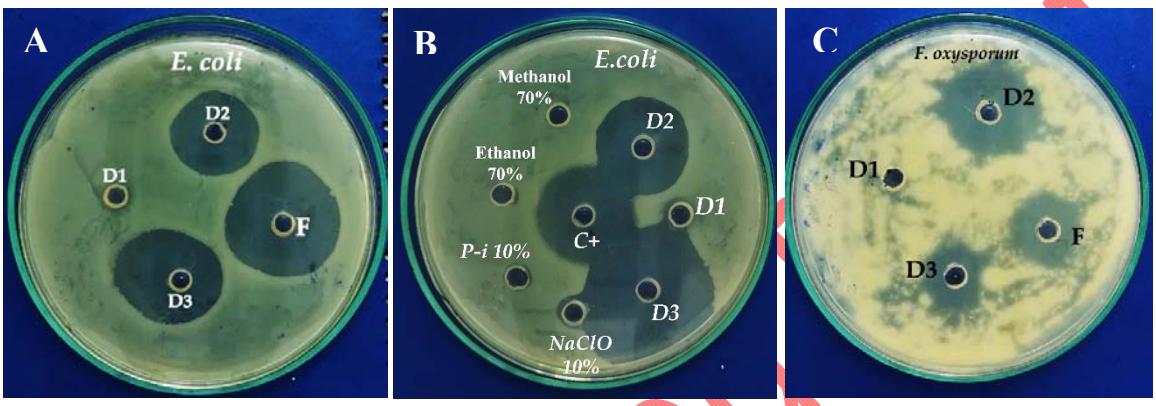


Figure 4: Comparison of disinfectant inhibitory effects on *E. coli* (A, B) and *Fusarium oxysporum* (C) using the agar-well diffusion method; D1: disinfectant 1; D2: disinfectant 2; D3: disinfectant 3; F: formalin; P-i 10%: 10% povidone-iodine; NaClO10%: 10% sodium hypochlorite; Ethanol 70%: 70% ethanol; Methanol 70%: 70% methanol; and C+: Nalidixic acid (positive control).