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Studies on the efficacy of natural, biodegradable, biosecurity agent SANGUARD

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Abstract
The studies were conducted to determine the efficacy of SANGUARD, a biosecurity product. In the first study, SANGUARD efficacy was tested by spraying SANGUARD @ 10 ml/Litre of water on the empty shed and the shed in the presence of 2000 birds at 39 days of age. Nutrient agar and Yeast and Mold Chloramphenicol agar plates were exposed for 1 minute in the empty shed and shed with birds and tested for the total bacterial and mold counts both before and after spraying SANGUARD. The total bacterial count and mold count were significantly reduced after spraying SANGUARD. In the second study, the water from different areas was collected and tested for pH and Most Probable Number (MPN) of organisms before and after adding SANGUARD @ 1 ml/10 L of water at 0, 2, 4, and 12 hrs time intervals. The result depicts that pH and MPN were reduced and maintained after adding SANGUARD @ 1 ml/ 10 L of water. In the third study, the efficacy of SANGUARD was tested against chlorine in Reverse Osmosis (RO) water. The colony-forming units (CFU) are observed at different time intervals by taking the sample from the RO water treated with chlorine tablet @ 45 mg and SANGUARD @ 2 ml separately. Results showed that the efficacy of SANGUARD was similar to that of chlorine.

Keywords: SANGUARD, biosecurity agent, colony forming units, most probable number, pH, chlorine

1. Introduction
Water is one of the primary nutrients for poultry and livestock; it is impossible to state its exact requirements. Poultry birds generally drink approximately twice as much water as the feed consumed (Hess & Macklin, 2019) [1]. Profitability in poultry production can be optimised only when everything goes right, which includes bird health, hygienic water lines, and biosecurity. During extreme heat stress, birds water requirements may increase (Bruno *et al.*, 2011) [2]. Water is also the medium for administering medicines, vaccines and feed additives. It is essential to provide enough, plentiful and good quality water to the birds (Pesti G.M. *et al.*, 1984) [3] because water is one of the routes that can transport pathogens like bacteria, viruses and protozoa into poultry farms. Water quality can directly or indirectly affect the bird's performance. Good quality water is vital to enhance immunity, beneficial bacterial growth and profitable production in poultry. High levels of pathogens, contaminants, minerals and other pollutants can adversely affect the body's physiological functions, directly affecting growth performance. Ideally, bacteria should not be present in the poultry drinking water. Their presence indicates contamination by organic matter (Hess & Macklin, 2019) [1]. Water with a pH between 6.2 and 6.8 seems to be ideal for hens. For birds, water with a slightly acidic pH is preferable than water with a basic pH (Tabler *et al*., 2017) [4]. Generally, poultry farmers use various water sources such as underground, municipal, and sometimes surface and rainwater. Therefore, water sanitation is essential in poultry production and should be followed correctly. It is difficult to determine the good water quality for poultry because many of the standards determining water quality and quantity consumed differ between livestock and poultry and among different poultry species in different growth and physiological stages (Saqib Mukhtar, 1914) [5]. Testing a water sample regularly is an integral part of good water management.

Table 1: Naturally occurring contaminants and pH in water (*Hess & Macklin*, 2019) [1]

Contaminant	Average considerable level	Maximum acceptable level
Total bacteria (CFU/ml)	0/ml	1000/ml
Coliform bacteria (CFU/ml)	0/ml	50/ml
pH	6.3-7.5	6-8

Drinking water must be clear, odourless, colourless and tasteless. (Hess & Macklin, 2019) [1]. Sanitisation, checking of regular biofilm in pipes, regular testing and rigorous cleaning of the water system between the flocks can ensure the water quality is as high as possible. An enhanced biosecurity product will deliver additional benefits and eliminate bacterial growth in the water. Selection of a good biosecurity product is difficult because no product has efficacy against all pathogens, or some have resistance or explosive characteristics and are difficult to use. Commonly used chemical substances as water sanitisers are chlorine, hydrogen peroxide, chlorine dioxide, copper sulphate, antibiotics and organic acids. However, these products are sometimes incompatible, and their properties make other micronutrients unavailable. Chlorine products, such as sodium hypochlorite or calcium hypochlorite, used in the poultry industry long ago, are unsuccessful as microbes become resistant to these products as they have not been appropriately used (Maharjan, P. 2013) [6]. Chlorine is effective against gram-positive and gram-negative bacteria but hardly effective against spores and viruses. Chlorine requires more contact time, i.e., 20 min, and it is alkaline, increasing water pH and microbial growth in water. It has a strong smell, so it may modify the water smell and affect water intake and feed consumption. Excess chloride intake by poultry leads to wet feces, extreme water consumption, ascites, edema etc (Baloš *et al*., 2016) [7]. Hydrogen peroxide does not drop the pH and does not kill the spores. Chlorine dioxide was highly sensitive to UV light, Water temperature and low pH. So, it was needed to administer higher levels of chlorine dioxide to provide its efficacy. Higher levels of chlorine dioxide may also increases the risk of the byproducts like chloritre and chlorate. Doxycycline, tylosin, neomycin, amoxicillin etc are the commonly used antimicrobials in poultry farms (Imam *et al*., 2020) [8] which causes Antimicrobial resistance (AMR), causing treatment failure in birds (Weese *et al*., 2015) [9] and also a serious threat to public health (WHO, 2021) [10]. Organic acids use to drop the water pH, but adding an effective dosage reduces water pH<4, which is extremely low for a bird's digestive system, and it may affect bird's performance (*Kaoud*, 2016) [11].

Providing good biosecurity agents is the most cost-effective method to prevent infectious diseases in poultry farms (Robertson (2020) [12]. The ideal disinfectant should be able to kill microorganisms, be stable, be non-explosive, have shown a positive effect on bird's performance and be effective in controlling microbial growth in water lines (*Jiang et al*., 2018) [13]. SANGUARD is one of the best natural biosecurity enhancers with zero contact time and an eco-friendly product to enhance water quality. SANGUARD is a non-toxic, non-corrosive, biodegradable product with no risk of resistance and has multipurpose use and is safe in the presence of birds. Several trials were conducted on broiler farms in India to check the efficacy of SANGUARD on bacterial and mold growth.

2. Materials and Methods

The present studies were performed in different regions of India. The first study was conducted to determine the effect of SANGUARD on microbial levels when it was used as a spray in an empty shed and in the presence of birds. SANGUARD was used as spray @ 10 ml/L of water in the empty shed and in the shed of 2000 birds at 39 days of age, and samples were collected before and after spraying SANGUARD. Results of the samples tested for the total bacterial count and total mold count on nutrient agar and Yeast mould chloramphenicol agar plates respectively exposed for 1 minute before and after spraying of disinfectant SANGUARD.

The second study was conducted *in vitro* to evaluate the efficacy of SANGUARD on the quality of water with dosage @ 1 ml/10 L of water in different water samples over time. The parameters studied include pH and MPN at different time intervals. The MPN technique is a method for getting quantitative estimations of bacteria in food or water samples (Michael and Busta, 1999) [14]. MPN is a laboratory microbiological test which uses replicated tubes or wells containing known reducing amounts of sample. These replicated tubes are observed in sets called 'Dilution Series'. The positive results obtained in the test are related to the most probable number of microorganisms via a standard table, and this finally determine the efficacy of SANGUARD, and interprets the results of the test (McBride, G. B. 2003) [15].

The third study was conducted as a challenge model to compare the efficacy of SANGUARD with chlorine tablets in RO water. In this study, two beakers with 5L capacity filled with 5L of RO water are used. *E. coli* sample was added to both the beakers and after proper mixing plating was done in LB Media plates. After that to the Beaker-1 containing 5L RO water, 45 mg chlorine tablet was added and for beaker-2 containing 5L RO water 2 ml SANGUARD was added and mixed properly. After adding the disinfectants, in between time intervals, 1 ml water samples were taken into LB Media plates and tested for no. of CFU and observed the results.

3. Results

In the first study, the total bacterial count in the empty shed before spraying SANGUARD was 293 cfu/ml, and after spraying, it was reduced to 139.3 cfu/ml within 1 min (Figure1). The results showed that after using SANGUARD, there was a significant reduction in bacterial count (CFU) in the empty shed. Moreover, in the shed, with the presence of 2000 birds which are at 39 days of age, the bacterial count before spraying SANGUARD was 625.25 cfu/ml, and after spraying, there was a significant reduction in the bacterial count to 170.25 cfu/ml. The results showed a significant reduction in the total bacterial count after using SANGUARD. The total mold count in the empty shed before the spraying of SANGUARD was 11.33 cfu/ml, and after the spraying of SANGUARD @ 10 ml/L, there was a significant reduction in the mold count to 4.33 cf/ml. And in the presence of birds, the mold count was 23.33 cfu/ml at the age of birds 39 days; after spraying SANGUARD @ 10 ml/L of water, the mold count was significantly reduced to 7.33 cfu/ml (Figure 2).

In the second study water samples collected from different areas and tested for pH and MPN. The pH of water no 1 was

7.8 at the time of testing, after adding SANGUARD @ 1 ml/10 L of water, the water pH at different time intervals like 0, 2, 4, and 12 hrs was 7, 7, 7, and 6.9 respectively. The pH of water no 2 was 7.9, and after using SANGUARD @ 1 ml/10 L of water, the pH of water at 0, 2, 4, and 12 hrs was 7.2, 7.2, 7.2 and 7.3, respectively. The pH of water number 3 was 7.9, and after adding SANGUARD @ 1 ml/10 L of water, the pH at different time intervals 0, 2, 4, and 12 hrs was 7.1, 7.1, 7.1, and 7.2, respectively (Figure3). The MPN of water no 1 was 650, and after adding SANGUARD @ 1 ml/10 L of water the MPN at different time intervals 0, 2, 4, and 12 hrs was observed as 23, 122, 256, and 278 respectively. The MPN of Water no 2 was 754 at the time of testing before adding SANGUARD, and after adding SANGUARD, the MPN was 39, 156, 199, and 356, at 0, 2, 4, and 12 hrs time intervals respectively. The MPN of Water no 3 was 695 at the time of testing before adding SANGUARD and after adding SANGUARD the MPN was 44, 128, 196 and 347 respectively, at 0, 2, 4 and 12 hrs time intervals.

In the third study, the sample results were tested for the total CFU count of plates exposed to the sample for different time intervals. RO water contaminated with *E. coli* sample and treated with chlorine tablet @ 45 mg, and then 1 ml sample was taken every time and growth in the plates was observed at different time intervals, i.e., overnight grown after adding *E.coli*, 0, 1, 2, and 3 hrs after adding Chlorine, the plates showed growth of *E. coli* Too Numerus To Count (TNTC), 0, 0, 2, and 0 colonies (Plates 2 to 6) respectively. At the same time intervals, RO water contaminated with *E. coli* and treated with SANGUARD @ 2 ml was tested, and the plates showed TNTC, 0, 0, 0, and 1 colony (Plates 2 to 6) at respective time intervals.

4. Discussion

The results of our first study demonstrated that there is a significant reduction in the bacterial and mold count after using SANGUARD at the proper dosage. In line to our results Burbarelli *et al*., (2015) [16] reported that European treatment of cleaning and disinfection procedures has reduced total microbial counts from 21010 CFU/cm^2 before treatment to 1466 CFU/cm^2 on poultry shed floor. Payne *et al*., (2005) [17] also demonstrated that application of different disinfectants on poultry house floor have significantly reduced the total aerobic bacterial, yeast and mold populations compared to control.

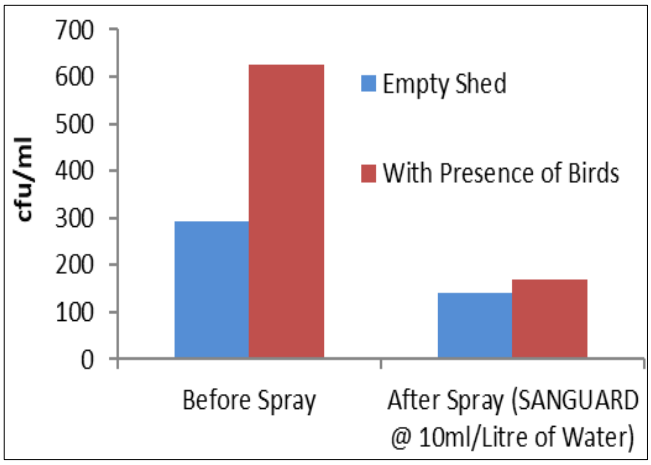

Fig 1: Total bacterial count (CFU/ml)

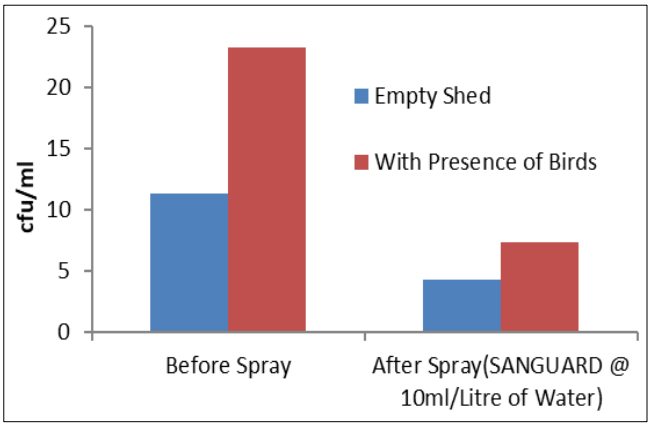

Fig 2: Total Mold count (CFU/ml)

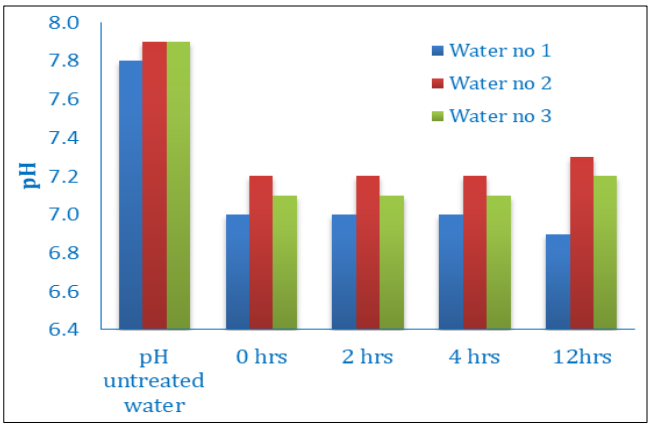

Fig 3: The pH of water at different time intervals after using SANGUARD @ 1 ml/10 L

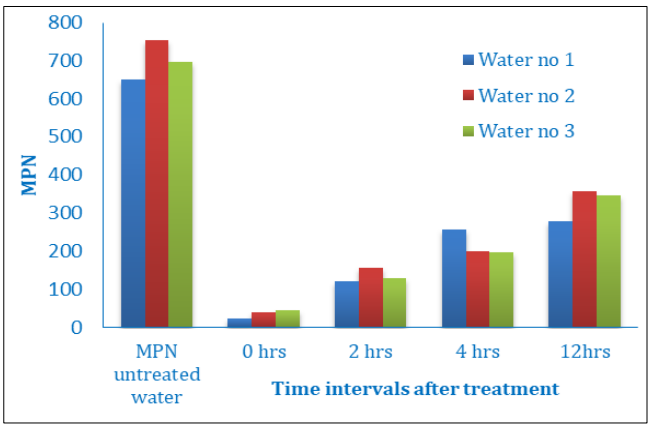

Fig 4: MPN of water at different time intervals after using SANGUARD @ 1 ml/10 L

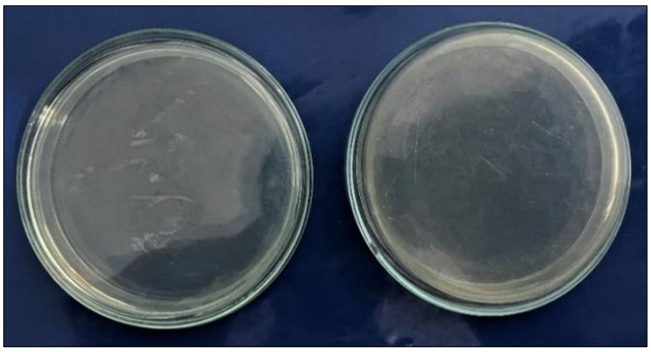

Plate 1: Blank plates

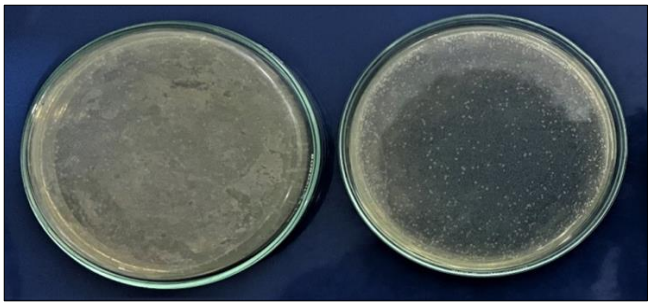

Plate 2: CFU units of the plate exposed to RO water sample overnight after adding 1 ml of E. *coli*

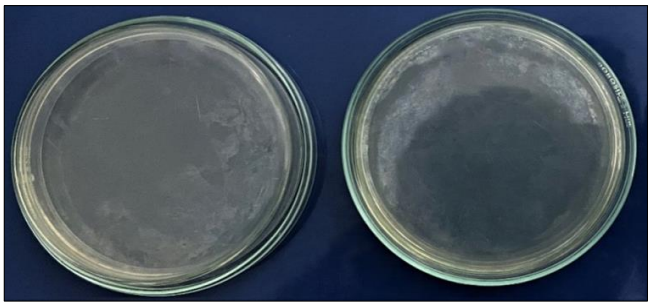

Plate 3: CFU units of the plate exposed to RO water sample overnight after adding chlorine & SANGUARD at 0hrs.

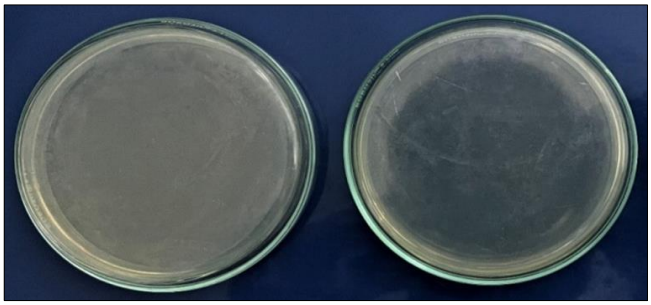

Plate 4: CFU units of the plate exposed to RO water sample overnight after adding chlorine & SANGUARD at 1 hrs

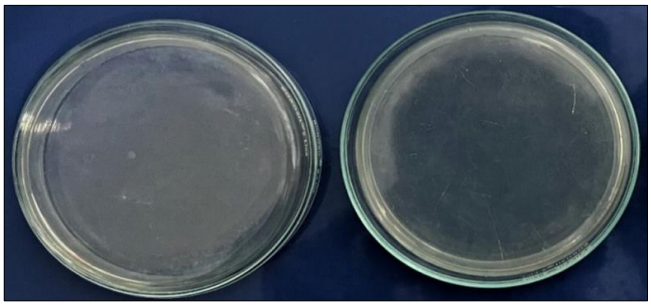

Plate 5: CFU units of the plate exposed to RO water sample overnight after adding chlorine & SANGUARD at 2 hrs

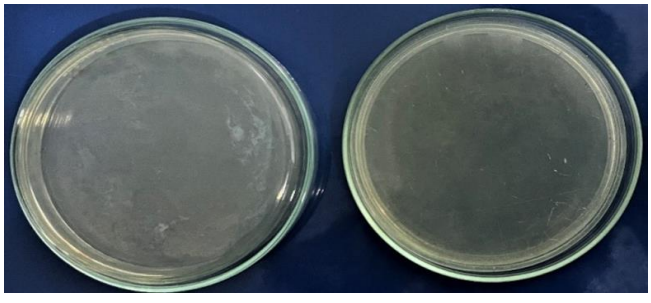

Plate 6: CFU units of the plate exposed to RO water sample overnight after adding chlorine & SANGUARD at 3 hrs.

The pH indicates the acidity or basicity of water; the water in our second study has basic pH. After using SANGUARD, there was a significant reduction in the pH of water to neutral. Supporting our study Karabayir *et al*., (2018) [18] stated that after adding disinfectant to the water the pH was maintained near neutral similar to that of control which is healthier to the poultry. Also, there was a significant reduction in the MPN after adding SANGUARD disinfectant to the water. Adding 1 ml/10 L of SANGUARD to water showed promising results by reducing the bacteria and mold growth.

In our third study results interpreted that RO water treated with SANGUARD @ 2 ml showed fewer colonies in lower dosages than Chlorine tablets. Excessive chlorine intake through water reduces water and feed consumption in birds due to low flavour perception and reduced taste buds (Schneider *et al.*, 2016) [19]. (Khan *et al.*, 2008) [20] reported that excess chlorine intake caused reduced testicular weight in male Japanese quails. Water represents 65% of the egg's weight, so that water restriction may result in lower egg weight and reduced egg production in layers (Leeson & Summers, 1997) [21] (Faria *et al.*, 2009) [22]. By the above postulates, we can say that using SANGUARD instead of chlorine in poultry drinking water will be beneficial.

5. Conclusion

The quality of poultry drinking water is an essential factor. It may increase microbial growth when the water quality is disturbed. In some farms, poor water quality may cause bird mortality, directly affecting the farm's overall performance. The microbiological quality of water must be regularly analysed to ensure its safety. By using SANGUARD as a spray or water disinfectant at different dosage, it improves water quality as well as reduce the number of microorganisms. It prevents the risk to poultry health and reduces digestive issues.

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