Effect of Cinnamon oil Nano-emulsion to Control the Foodborne Bacteria *Salmonella* spp. on Mung beans and Sprouts

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By

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Abstract

Foodborne outbreaks due to *Salmonella* in pulses and legumes have increased recently. Essential oils extracted from plants have been recognized for their effectiveness in food preservations. It is due to their strong antifungal, antiviral, and antibacterial properties. The utilization of Cinnamon oil Nanoemulsion may help to develop novel techniques to prevent foodborne outbreaks. The purpose of this study was to find the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of cinnamon oil nanoemulsions against *Salmonella* species. Furthermore, to check the efficacy of antimicrobial nanoemulsions as a decontaminate for reducing Salmonella on mung bean seeds with sprouting and post-harvest storage. Cinnamon oil nanoemulsion was prepared with ultrasonication using Tween 80 as an emulsifier. The particle size of oil droplets was characterized using dynamic light scattering. A minimum inhibitory concentration assay was performed on six of *Salmonella* strains to find out the antimicrobial efficacy of cinnamon oil nanoemulsion. Cell-membrane integrity has been performed to confirm the results of MIC and MBC. The minimum inhibitory concentration (MIC) of cinnamon oil nanoemulsion for *salmonella* was 0.03125%. The minimum bactericidal concentration was MIC i.e., 0.3125%. Scanning electron microscope images showed distortion of bacterial cell membrane with 0.3125% of Cinnamon oil nanoemulsion. These data suggest that cinnamon oil nanoemulsion can be used as an effective natural antimicrobial agent to decontaminate the pulses and legumes against *Salmonella* spp.

Keywords: Nanoemulsions, Essential oils, Antibacterial

1. Introduction

1.1 Introduction and Statement of the Problem

There is a rise in the usage of seeds and sprouts in recent years because of their nutritional value and health benefits. However, sprouts are categorized as high-risk food for causing foodborne outbreaks. Beginning around 1996, the FDA has reported 48 episodes related with sprouts outbreaks, bringing about 2499 cases, 179 hospitalizations, and 3 deaths. A major part of outbreaks was associated with alfalfa sprouts, mung beans, and clover. Salmonella was the most common causing pathogen, followed by E. coli and Listeria. Given epidemiological proof and quantitative risk assessments, seeds used for sprouting have been identified as the primary source of pathogens in most outbreaks.

In recent years, a few multistate outbreaks were reported in the US due to Salmonella contamination of raw sprouts (CDC online). This is of significant food safety concern since even when present in low numbers; pathogens can quickly increase to high numbers during sprouting. Consequently, seed disinfection before sprouting and antimicrobial barriers to prevent pathogen outgrowth during germination is critical to prevent sprout-associated foodborne illness. However, currently used disinfection techniques for seeds are ineffective in eliminating pathogenic microbes from sprouts. Furthermore, most approaches have limited application during sprouting due to their negative impact on germination, sprout yield, and retaining quality.

Therefore, the research community and the sprout industry have been searching for novel antimicrobial interventions to mitigate food safety risks associated with sprouts. In this regard, essential oils such as cinnamon are well-known for their antimicrobial properties. Despite having

strong antimicrobial activities, essential oils are not in use widely because of their lipophilic and strong aromatic nature.

Essential oils (EOs) are regular mixtures that have been demonstrated to be a promising treatment for food application considering their strong antifungal, antiviral, and antibacterial exercises (Burt, 2004; Ferreira et al., 2010). Cinnamon is a spice obtained from the tree species of the genus Cinnamomum's inner bark. Cinnamon oil contains two significant mixtures, cinnamaldehyde, and eugenol, which are acceptable inhibitors of microbial development (Burt, 2004; Lee and Ahn, 1998; Ooi et al., 2006). Likewise, cinnamon oil contains a wide range of antimicrobial impacts which makes it ideal for use in different produce items. These properties give an elective normal and safe antimicrobial to standard antimicrobial items.

The application of cinnamon oil is limited due to its lipophilic behavior and insolubility in water as is any essential oil (Donsì, Annunziata, Vincensi, & Ferrari, 2012). Because of this reality the utilization of cinnamon oil emulsion as an antimicrobial specialist is restricted because of high Minimum inhibitory Concentration (MIC) and absence of dissolvability in water. One of the procedures for managing such hydrophobic mixtures is by scattering them in nano-emulsion conveyance framework (Shah, Davidson, and Zhong, 2012). In this research, I centered around the usage of cinnamon oil as a nano-emulsion through the original course of ultrasonication in cinnamon oil and studied the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the viability of the pre-arranged cinnamon oil nano-emulsion against Salmonella artificially coated on mung beans seeds and sprouts.

Utilization of this natural antimicrobial nano-emulsion won't just lessen microbe load on seeds it has potential to forestall bacterial development and tainting that can happen during

production (germination and development), harvest, and postharvest storage. Subsequently, this review will assess the utilization of natural antimicrobial nano-emulsions as a protected and compelling antimicrobial hurdle to enhance the microbial safety of sprouts.

1.2 Hypothesis and Objectives of the Study

1.2.1 Hypothesis

Cinnamon oil nanoemulsions will reduce *Salmonella* contamination on mung bean seeds and prevents pathogen survival and proliferation during sprout growth and storage.

1.2.2 Objectives and Specific Aims

The main objective of this study is to utilize cinnamon oil nanoemulsion against pathogenic bacteria *Salmonella* which are of major concern in seeds & sprout industry. This study also aims to do the following things:

1. To design, fabricate & characterize cinnamon oil nanoemulsions
2. To determine the efficacy of cinnamon oil nanoemulsions as a decontaminate for reducing *Salmonella* on mung bean seeds.
3. To determine the efficacy of antimicrobial nanoemulsions for reducing *Salmonella* on mung bean sprouts during sprouting and post-harvest storage.

2. Review of Literature

2.1 Mung beans

Mung bean (Vigna radiata L. Wilczek) popularly referred to as green gram, believed to be a native crop of India, is a tiny circular-shaped bean in green color widely cultivated throughout Asia. Green gram (Vigna radiata) is a plant species of Fabaceae which is additionally referred to as mung. The mung is an annual vine with yellow flowers and fuzzy brown pods. There are three subgroups of Vigna radiata (mung beans), including one cultivated (Vigna radiata subsp. radiata) and two (Vigna radiata subsp. Sublobata and Vigna radiata subsp. glabra). It's a height of about 15–125 cm green gram contains a well-developed rootage. The lateral roots are many and slender, with root nodules grown. Stems are much branched, sometimes twining at the ends.

Mung beans are grown widely to be used as human food (as dry beans or fresh sprouts) but are often used as a manure crop and as forage for livestock. Oklahoma is the state where mung beans are highest grown in US. Fifteen to twenty million pounds of mung bean are consumed annually within the US and nearly 75 percent of this is often imported. Seeds of mung beans can be sprouted for fresh consumption or as a canned food shipment to food outlets. Sprouts are considered high in protein (21%–28%), calcium, phosphorus, and certain vitamins. Since these are easily digestible, they can be potential food to replace animal food in the human diet in the tropical areas of the globe. Due to their high consumption as sprouts, a high-quality seed with excellent germination is required. The food industry likes to get about 9 or 10 grams of fresh sprouts for every gram of seed. For sprouting a bigger seed with a glassy, green color is usually preferred for germination.

If seed of the mung beans does not meet the sprouting criteria it can be used as livestock food with about 1.5 times more protein content than soybean meal. Trials of feeding mung bean seeds as livestock food have been conducted at university of central Oklahoma for young calves and have shown good results.

2.1.1 Mung bean Distribution

The mung bean is thought to have started in the Indian subcontinent where it was trained as right on time as 1500 BC. Developed mung beans were acquainted with southern and eastern Asia, Africa, Austronesia, the Americas, and the West Indies. It is currently far and wide all through the Tropics and is found from ocean level up to an elevation of 1850 m in the Himalayas p (Lambrides et al., 2006; Mogotsi, 2006).

The mung bean is a quickly developing, warm-season vegetable. It arrives at development rapidly under tropical and subtropical conditions where ideal temperatures are around 28-30°C and consistently above 15°C. It tends to be planted during summer and fall. It doesn't need a lot of water (600-1000 mm precipitation/year) and is open-minded toward dry spells. It is touchy to waterlogging. High dampness at the development will in general ruin the seeds that might group before being reaped. The mung bean becomes on a wide scope of soils however favors very much depleted topsoil or sandy soil, with a pH going from 5 to 8. It is open-minded to saline soils (Mogotsi, 2006).

Mung bean creation is primarily (90%) arranged in Asia: India is the biggest maker with over half of world creation however devours nearly its whole creation. China creates a lot of mung beans, which addresses 19% of its vegetable creation. Thailand is the principal exporter and its creation expanded by 22% each year somewhere in the range between 1980 and 2000

(Lambrides et al., 2006). However, it is created in numerous African nations,and the mung bean is certainly not a significant yield there (Mogotsi, 2006).

2.1.2 Mung bean processes

Seeds Preparation and Germination:

Because the foremost use of mung bean is for sprouts; excellent germination must be maintained by careful harvesting and storage systems. Seed is not generally treated with fungicides, insecticides, or bactericides due to the chance of ingestion of treated seed. Because the seed is little, careful handling and a focus on planting machinery adjustments are important to make sure planting with little damage to the seed. If mung bean is being planted in an exceeding field for the primary time the right nitrogen-fixing bacteria must be provided. This inoculant may be applied to the seed just before planting or applied within the furrow in peat or granular form. Care must be taken to distribute this inoculant uniformly within the field. Make certain to use the bacteria that is specific for mung beans or closely related species. Only certified seed should be used so that quality and variety purity are guaranteed. Currently Oklahoma State University Crop Improvement Association is a source of foundation and authorized seed of certain varieties, several which may be adapted to this area.

Harvesting:

The maturity of pods in the mung beans is not uniform due to the entered period of the plant to flower. This makes it difficult to plan when to harvest. In general harvesting starts when one-half to two-thirds of the pods are mature. Seeds could be between 13%−15% moisture at this point. Many grower swath the plants to permit further maturity of the pods and then the combine employing a pickup header on grain combine. This is often an especially useful harvest system

for the vine-type varieties of the beans or when there is delayed maturity or the presence of problem weeds. Swathing should be done earlier within the day to prevent severe shatter losses.

Direct combining is often worn out weed-free, uniformly mature fields of the upright growth habit kind of mung bean. It is also important to regulate the cylinder speed and concave clearance for complete threshing with a minimum of seed breakage. After combining the seed should be quickly cleaned to get rid of green pods, leaf material, debris, etc. which could create drying and storage problems. In developing countries, mung beans are handpicked because the pods mature.

Drying and Storage:

Before storing, remove all leaf material, stems, immature pods, dirt, insect parts and other debris. Mung beans at about 12% moisture can then be stored in regular grain bins previously fumigated to regulate bean weevils. If beans are higher in moisture, then 12% will be dried slightly by moving unheated air through thin layers until they are near the 12% value. Because they are going to be sprouted and eaten direct, care should be taken to stay all possible contaminants far away from the cargo area.

2.2 Overview of Foodborne illness in the United States

Fruits and vegetables are recognized as important sources of nutrients, fibers, and vitamins for humans. The production of fruits and vegetables increased by 94% between 1994-2004 in the world and the import of fresh fruits and vegetables doubled from 1994-2004 (Olaimat & Holley, 2012). Because of the expanded utilization, there is worry over the security of new products because of the flare-ups and diseases caused or identified with new leafy foods. Fruits and vegetables from the ground have been a developing wellspring of episodes in ongoing

history. There have been increasingly reported outbreaks in the United States, Australia, Europe, and the rest of the world (M. F. Lynch, R. V. Tauxe, & C. W. Hedberg, 2009). As per Rangel, Sparling, Crowe, Griffin, and Swerdlow (2005) flare-ups from produce-related sources were accounted for in 1991 interestingly and would typically top in the mid-year and fall months. They also mentioned that the occurrence of produce-related outbreaks was most common at eateries.

From 1990-2005 13 % of outbreaks and 21% of illnesses were associated with produce (DEWAAL & BHUIYA, 2007). Other outbreaks were associated with ground beef where E. coli was the main culprit, other meat products such as roast beef, steak, sirloin tips and salami, and dairy products such as raw milk, cheese curds, butter, and some commercial ice-cream bars (Rangel et al., 2005). The increased rate of outbreaks may be due to improvements in surveillance. It might be due to the increase in consumption, distribution systems and increase in consumer habits, increased intensity of livestock production near produce production areas, greater availability of produce, and increased numbers of immune-compromised consumers (Larry R Beuchat, 2002; Harris et al., 2003; Warriner, Huber, Namvar, Fan, & Dunfield, 2009).

Between 1998-2008 46% of outbreaks were linked to or were a direct consequence of produce elated outbreaks out of more than 9 million estimated foodborne illnesses each year in the United States (J. A. Painter, R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin, 2013). Painter et al. (2013) also attributed contamination of produce to 38% of hospitalizations and 23% of the deaths associated with food-borne outbreaks between 1998- 2008. The foodborne pathogens usually associated with products are Cyclospora cystinosis, Campylobacter, Coliforms, Enterococcus, Escherichia coli 0157:H7, Hepatitis A, Listeria monocytogenes, Norovirus, Salmonella spp., and Shigella spp. (L. Beuchat, 1998; L.R.

Beuchat, 1996; De Roever, 1998; Ebel et al., 2016; FDA, 2008; Newman et al., 2017; Taormina, Beuchat, & Slutsker, 1999).

Between 1996 and 2010, there have been 34 reported foodborne illness outbreaks associated with the consumption of sprouts. These outbreaks resulted in 2,150 cases of illness, out of which 123 hospitalizations and one death (Centers for Disease Control and Prevention (CDC). 2016. List of selected multistate foodborne outbreak investigations. Atlanta, GA: Center for Disease Control and Prevention). Within the past few years, the Center for Disease Control and Prevention reported 9 foodborne illness outbreaks that resulted from the consumption of sprouts. Because of the high number of outbreaks, sprouts are labeled as a "high risk" food. this implies that individuals with compromised immune systems, like children, the elderly, pregnant women, and people who are sick or taking medications that impair the immune system, should avoid eating sprouts. CDC reported average 1.35 million gets affected due to salmonella, out of 26500 were hospitalized and 420 deaths annually.

In 2011, the Food Safety Modernization Act (FSMA) was assigned as a law to ensure the safety of the food. This act may be a complete overhaul of the United States' food safety system shifting the main target from responding to foodborne illness outbreaks to prevention. Several components of this law are intended to prevent foodborne illness in the USA in manufactured foods and produce; however, one aspect of the law directly addresses the safety associated with the production of sprouts. Those that are producing sprouts for sale must comply with the regulations stated within the FSMA produce safety rules also with four additional requirements that are specific to growing sprouts. These requirements are: (1) taking steps to stop microorganisms on seeds; (2) testing irrigation water drained from growing sprouts; (3) testing the sprout production areas (growing, harvesting, packing, and holding) for Listeria

monocytogenes; and (4) if any test leads to a positive reading, then corrective actions must be put into place in so that laminated sprouts are not released purchasable. Additional educational training is being given to sprout producers to show them the new regulations and the way to grow sprouts safely.

2.3 *Salmonella*

Salmonella is facultative anaerobic Gram negative, pole molded microorganisms. They can be found in the digestion tracts of warm and cold-blooded creatures. They are large around 2-5 microns in length and 0.5-1.5 microns wide. They move with the assistance of peritrichous flagella and have a place with the family Enterobacteriaceae and are vital pathogenic organic entities therapeutically to the two creatures and people (Andino and Hanning, 2015; Farrar et al., 2013; Sorensen et al., 2002; Wells, Fedorka-Cray, Dargatz, Ferris, and Green, 2001). They comprise of two species and six subspecies. The two species are S. enterica and S. bongori and the subspecies are enterica Americaona, arizonae, houtenae, and indica and comprises of more than 2,579 serovars or serotypes which are generally equipped for causing human infections (Andino and Hanning, 2015; Yaun, 2002).

As per the Center for Disease Control and Prevention (CDC) (2016) Salmonella causes an expected 1,000,000 foodborne ailments in the United States. There are 19,000 hospitalizations and 380 passing identified with Salmonella, but there are more speculated gentle cases that may not be accounted for so the real number of pollution or disease might be way higher than revealed. Indications of Salmonella disease might be looseness of the bowels, fever, and stomach cramps 12 to 72 hours after the contamination. It might endure between 4 to 7 days and normally

the vast majority recuperate without treatment, yet a few people may have extreme symptoms and may require hospitalization (CDC, 2016).

The long-haul impact of *Salmonella* disease might cause receptive joint inflammation and agonizing solid discharges and pee entry as per the CDC. Salmonellosis is seen more in the late spring than the colder time of year and youngsters are bound to get tainted than grown-ups. Treatment is typically identified with soothing the indications and the anti-toxins are not for the most part utilized. This is because *Salmonella* has been known to be impervious to anti-infection agents. The *Salmonella Typhimurium DT104*, which arose in the ‘80s and ‘90s in the United States, has been known to be impervious to ampicillin, chloramphenicol, streptomycin, Sul-isoxazole, and antibiotic medication (CDC, 2001; Wedel et al., 2005).

Salmonella is by and large connected with dairy, meat, and poultry however there have been instances of late flare-ups in the produce also. Episodes have also identified with melons in 2012 with Salmonella Typhimurium and Salmonella Newport as per the CDC. Different flare-ups related were in Alfalfa sprouts in 2016 which were sullied with Salmonella Reading, Salmonella Abony, Salmonella München, and Salmonella Kentucky and there were flare-ups identified with cucumbers in 2014 and 2015 identified with Salmonella Newport and Salmonella Poona individually (CDC, 2016).

Salmonella Enteritidis (SE) is one of the foremost common serotypes of Salmonella isolated from humans worldwide. Within the US this serotype accounts for about 25% of human salmonellosis cases. In the US, additionally to livestock, SE commonly colonizes rodents, reptiles, and amphibians. Most SE outbreaks within the US are linked to undercooked eggs. Within the United States *Salmonella Enteritidis* infections related to eggs have been because by

certain phage types (PT). Progressive research on microbial phages is going on at the University of Central Oklahoma for Salmonella & Coliforms.

2.4 Current trends for antimicrobial treatments

There are traditional sanitizers developed to disinfect fresh produce like seeds and leafy foods. The purpose is to prevent cross-contamination during the washing process of them. Chlorine is a sanitizer being used widely as of now. However, Chlorine has limitations due to its loss of impact in the presence of organic matter. Other sanitizers like Hydrogen peroxide, Calcium Hypochlorite, Organic acids, Ozone, peroxyacetic acids, electrolyzed water etc. have also been for the treatment of seeds and leafy foods. The results of these treatments range from 1.4-6.5 log reduction depending on fresh produce type, treatment method and different concentrations used in it. The US sprout industry has many small farms and these methods, and the technology required for decontamination are expensive for them. Additionally, the use of calcium hypochlorite with a 20,000-ppm concentration causes a major problem for organic seeds and sprouts. As the certifying agencies will not allow producers to use such a high level of Chlorine resulting in the use of ongoing methods to control pathogen contamination on seeds and sprouts, with this there are many chances that they give a detrimental effect on viability of seeds, sprout yield and germination as where there is scope for the development of effective, user friendly, environment friendly, low-cost antimicrobial methods for the seeds and sprout industry.

2.5 Essential Oils

Humans have used EOs for centuries, not only as constituents of scents or as seasonings for the aromatization of food, but also in medicine as well because of their numerous & versatile natural attributes, including antimicrobial effects. The antimicrobial effect of EOs and using that

for the treatments can be essential and effective solution for the drastically growing issue of the pathogens causing foodborne outbreaks.

There are 300 well-known commercially used EOs in the flavor and aroma industry. Guenther, (1948) first reported that EO has antimicrobial properties that can be benefited to green revolution afterward. Past research shows that Gram-positive bacteria have a much more susceptibility to EOs in comparison with Gram-negative bacteria (Reyes-Jurado, Franco-Vega, Ramírez-Corona, Palou, & López-Malo, 2015). This is because the cellular walls of Gram-negative bacteria develop a protecting layer from the hydrophobic compounds in the presence of lipoproteins and lipopolysaccharides (Semeniuc, Pop, Rotar, & analysis, 2017).

Additionally, EOs have very strong antimicrobial properties. (Knobloch, Pauli, Iberl, Weigand, & Weis, 1989). Researchers reported that EOs can inhibit *Salmonella typhimurium* (Hammer, Carson, & Riley, 1999), *Bacillus cereus, Staphylococcus aureus* (El-Baroty, Abd El-Baky, Farag, & Saleh, 2010), *Listeria monocytogenes* (Bhargava et al., 2015) and Zygosaccharomyces bailli. A study reported a 2-4-fold log reduction for *P. aeruginosa, 2–8 fold for S. aureus, and 2–4 for E. coli.* (Atki Y, Aouam I, El-Kamari, Taroq A., Nayme K, Timinouni M, Lyoussi B, Abdellaoui A.). Thus, there is a chance to have similar antimicrobial effect of cinnamon oil on foodborne pathogens like *Salmonella spp*. and *Listeria spp.* as well.

2.6 Cinnamon

Cinnamon is a spice obtained from the bark of the tree. The species Cinnamomum but nowadays the species is referred to as cassia (Santich, Toussaint-Samat, & Bell, 2009). Few species of Cinnamomum are grown commercially for spice. It has been known and used from ancient times and has been known to be brought to Egypt as early as 2000 BCE (Santich et al., 2009) but the species Cinnamomum is indigenous to Sri Lanka and India (Paranagama et al.,

2010). Every part of the cinnamon tree including bark, leaves, flowers, fruits, and roots can be used in some way (Ranasinghe et al., 2013). Cinnamon contains different hydrocarbons, the main constituents being cinnamaldehyde, eugenol and camphor (Gruenwald, Freder, & Armbruester, 2010).

Cinnamon oil is mainly derived from the leaf or the bark. The bark contains a higher amount of cinnamaldehyde, and the leaf contains eugenol (Gruenwald et al., 2010). Cinnamon oil is an essential oil. Plant essential oils have been used to preserve food, alternative medicine, and pharmaceutical therapies (Jones, 1996; Ranasinghe et al., 2013), and many of them have antimicrobial properties against a range of bacteria (Bassyouni et al., 2016). Like other plant derived-oils, cinnamon oil has been shown to have antibacterial, antiviral, antifungal, and insecticidal properties. The main reason for these properties is thought to be cinnamaldehyde (Shan, Cai, Brooks, & Corke, 2007). Cinnamaldehyde is thought to cause inhibition of the proton motive force, respiratory chain, electron transfer, and substrate oxidation. (Nuryastuti et al., 2009). The result of these inhibitions causes uncoupling of oxidative phosphorylation, inhibition of active transport, loss of pool metabolites, and disruption of DNA, RNA, protein, lipid, and polysaccharide synthesis (Denyer, 1995; Farag, Daw, Hewedi, & El-Baroty, 1989; Nychas, 1995).

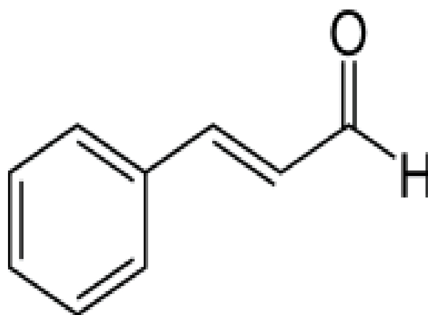

Figure 1: Structure of cinnamaldehyde (Inuzuka, 1961)

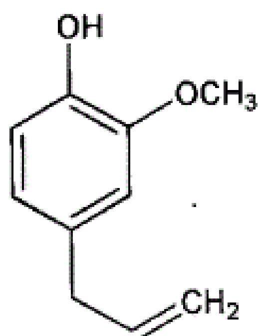

Figure 2: Structure of eugenol (Ito, Murakami, & Yoshino, 2005)

After cinnamaldehyde, eugenol is one other important compound found in cinnamon. It is a colorless to pale yellow phenolic compound found in essential oils (Mallavarapu et al., 1995; Pavithra, 1981). Eugenol is effective against *Sitophilus zeamais* and *Tribolium castaneum* in research conducted by Huang, Ho, Lee, and Yap (2002). It possesses antioxidant properties (Gordon, 1996) and anti-inflammatory action (Wargovich, Woods, Hollis, & Zander, 2001) but is also known to have antibacterial properties against *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), and *Saccharomyces cerevisiae* (ATCC 9763) according to research done by De, Krishna De, and Banerjee (1999).

2.7 Nanoemulsion Technology

Antimicrobial nanoemulsions are mixtures of detergent, oil, and water (particle size, 100 to 800 nm) that have been emulsified and has shown to have vast antimicrobial activity against bacteria, viruses, and fungi at a proportional level that is nontoxic in animals. Nanoemulsions work by fusing with lipid bilayers of cell membranes. Thus, the energy stored in the oil-and-detergent emulsion is released and ruptures the lipid cell membrane of the bacteria, i.e., they

have their strong antimicrobial activity. The antimicrobial activity of nanoemulsions is undefined, unlike that of antibiotics, thus allowing broad-spectrum research scope.

Several studies have been carried out on the application of nanoemulsion technology to test the efficacy of nanoemulsion-based delivery systems. Some researchers have demonstrated the use of essential oils in different food delivery systems and showed more promising results respectively. According to Zhou et al., (2007), the application of thymol nanoemulsion exhibited highly encouraging results against E. coli and Listeria monocytogenes. Treatment of oregano oil nanoemulsion on fresh lettuce against the various foodborne pathogens, Listeria monocytogenes, Salmonella typhimurium and E. coli O157:H7 was demonstrated by Bhargava et al., (2015). The findings of the study revealed that bioactive compounds in nanoemulsion disrupted the membrane of bacteria present in fresh lettuce. Oregano oil nanoemulsion has great potential to be used as a natural food delivery system against various foodborne pathogens. Nanoemulsion of basil oil prepared by ultra-sonication has been found to have strong antibacterial activity against E. coli (Ghosh, Mukherjee, & Chandrasekaran, 2013). Preparation of emulsion containing the essential oil, surfactant and emulsifier can act as an alternative approach to stabilizing the bioactive compounds by increasing the release of these bioactive compounds and whole coverage of fresh produce. Appropriate surfactants can be utilized to stabilize the oil droplets kinetically in the aqueous phase of the nanoemulsion system.

3. Methodology

3.1 Materials

The physio-chemical experiments were carried out in the laboratory of the Department of Huma Environmental Science. The microbial experiments were done in the Biosafety Level 2 (BSL2) hood in the microbiology lab at the Department of Biology, STEM building at University of Central Oklahoma, Edmond, OK. Antioxidant potential together with stability of CONE was measured in the laboratory of the Department of Human Environmental Sciences. The particle size of the nanoemulsion was measured using dynamic light scattering technology by DynaPro Plate Reader II, Wyatt Technology provided by Stanton Young Biomedical Research Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK. Mung beans were purchased from the Amazon online market. The Cinnamon oil was purchased from Fisher Chemical, Inc.

The materials used in this experiment were BD Difco TM Dehydrated Culture Media: Xylose Lysine Deoxycholate (XLD) Agar (Fisher Chemical, Inc.), TWEEN®-80 (Sigma-Aldrich, Inc.), pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd), ultra sonicator (QSONICA, Q700, Hudson Fusion LLC.), magnet stirrer (MS-H280-Pro, Scilogex, LLC.), PBS, distilled water, Themo Scientific alamarBlue TM Dye (Fisher Chemical, Inc.), 96-well plate, Absolute Alcohol 200 (Fisher Chemical Inc.), Glutaraldehyde 25% (Fisher Chemical Inc.), Phosphate Buffer Saline Solution (Fisher Chemical Inc).Pure bacterial cultures of *Salmonella entrica* (strain- 4293), *Salmonella entrica subspecies enterica serovar newport* (strain- 2725), *Salmonella entrica* (strain-1708), *Salmonella entrica* (strain-1975)*, Salmonella entrica* (strain-0172)*, Salmonella entrica* (strain-20740) were provided by Dr. Hari Kotturi, Department of Biology, the University of Central Oklahoma, Edmond, OK.

3.2 Preparation of Nanoemulsions

5% v/v oil-in-water emulsion of Cinnamon oil was prepared using high energy ultrasonication approach. Cinnamon oil was crudely mixed with Tween 80, a food-grade non-ionic surfactant using a magnetic stir plate for 30 minutes at a constant speed (700 rpm). 46.5 ml of sterile DI water was measured using a pipette, 1.0 ml of Tween 80 was pipetted, and 2.5 ml of Cinnamon oil was mixed and stirred.

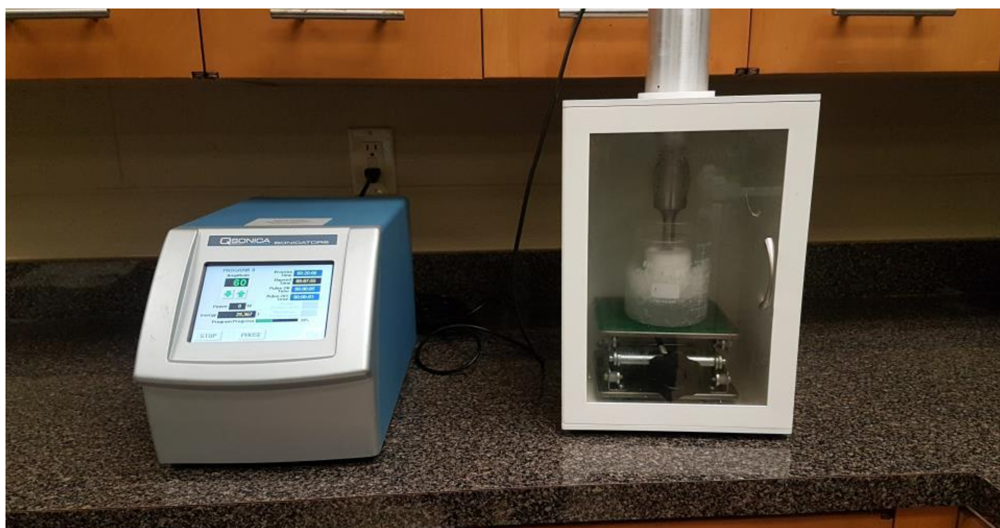

Figure 3: Ultra sonicator (QSONICA, Q700, Hfffudson Fusion LLC.)

Ultra sonicator (QSONICA, Q700, Hudson Fusion LLC.) was used to mix the nanoemulsion through sonication. The probe depth was maintained at 3/4th of an inch (the probe is 0.5 inch in diameter). The amplitude was set to 60. If there was splashing or foaming at this amplitude, the amplitude was lowered, and the probe depth was checked. The watts used were in a range from 50-70. The emulsion pulse was started for 5 seconds and stop for 3 seconds. Since the process of sonication produces heat, the experiment was carried out in a water bath to prevent the beaker from heating up. Process time was set at 20 minutes. Furthermore, control was also

prepared just using distilled water and Tween 80 without the use of oregano oil. Water and Tween 80 concentrations were adjusted accordingly.

Figure 4: Ultra sonicator Program Specifics (QSONICA, Q700, Hfffudson Fusion LLC.)

3.3 Formation of Nanoemulsions

Different ratio of Cinnamon oil & Tween 80 was carried out to get most stable composition for nanoemulsion. The proportionate of Cinnamon Oil & Tween 80 performed in 2:1,1:2,1:3,3:1,1:3 & 1:1 to analyze the stability of the emulsion. The stability of Cinnamon oil nanoemulsion was analyzed by keeping the samples for a period of 45 days. An emulsion having the best stability was considered as ideal for the research.

3.4 Emulsion Characterization

The particle size of emulsions was measured using dynamic light scattering (DynaPro Plate Reader II, Wyatt Technology). The smallest particle size formulation was used for my experiment.

Figure 5: Dynamic Light Scattering (DynaPro Plate Reader II, Wyatt Technology)

3.5 Antimicrobial Susceptibility Tests

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were determined by broth micro-dilution method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines for Salmonella spp. Prepared stock solutions of individual essential oils (100µl/L) were serially diluted in 96-well plate and fifty microliters of the inoculum (0.5 McFarland) were added to wells to obtain final concentrations of 2.5%, 1.25%, 0.625%, 0.3125%, 0.15625%, 0.078125%, 0.0390625%, 0.01953125%, 0.009765625%, 0.004882813%, 0.002441406%, and 0.001220703% v/v. Negative controls without the respective organism and tested oils were included to detect any cross-contamination from one

well to the other during the handling of plates. Plates were incubated at 37°C for 24hr. and streaked on XLD agar for growth. Experiments were performed in triplicate.

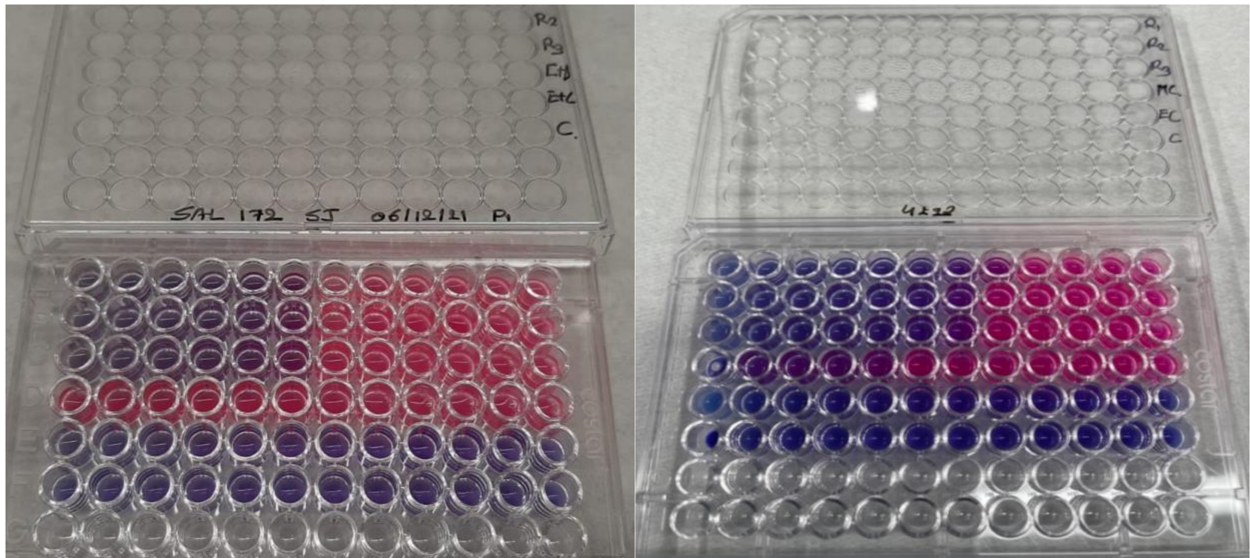

Figure 6: MIC on 96-well plate

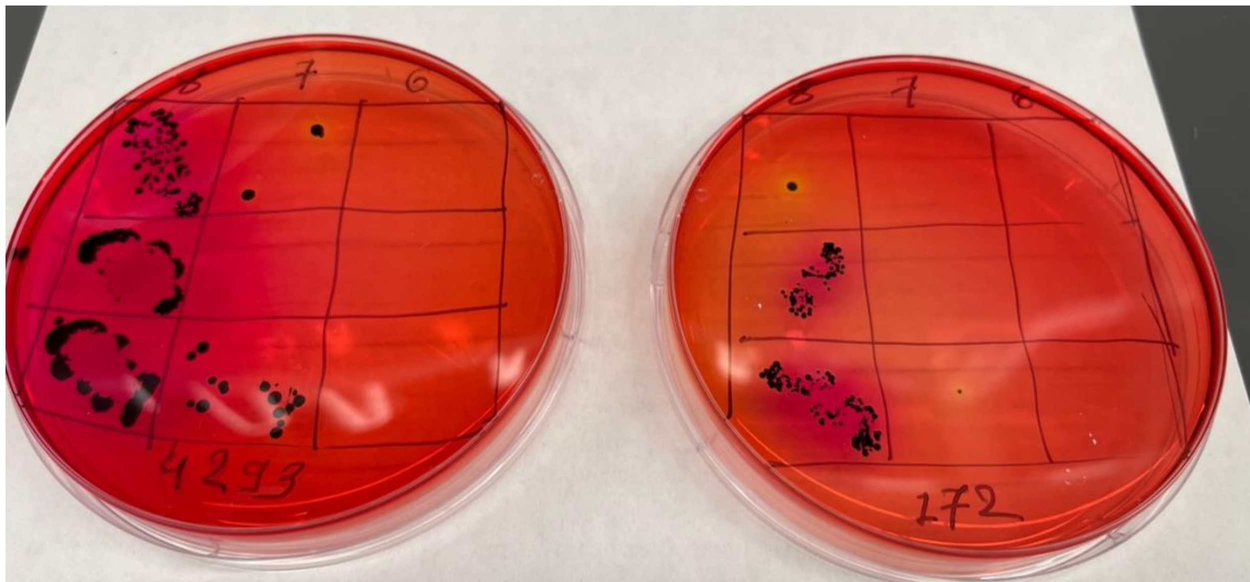

Figure 7: MBC Confirmatory Test After MIC

3.6 Preparation of different Cinnamon Concentrations

The smallest particle size formulation was then diluted to make two different cinnamon oil concentration of 0.5% and 1.0%. with use of Sterile DI water.

3.7 Kinetic Time-Kill Assay for Nanoemulsions against *Salmonella spp*.

Overnight bacterial cultures (200µl) were added to 1.8 ml of NB with 0.5%, 1.0% dilutions of 5% CONE. Control performed to get to know actual microbial pullulation. At 1, 3, 5, 15, and 30 min after mixing of bacterial culture and 5% CONE, aliquots of samples were collected and diluted accordingly in saline solution at room temperature for viability testing. Measured volumes (100µl) were spread onto XLD Agar using a disposable spreader and incubated at 37°C overnight for colony formation. Overnight colonies were counted, and average counts were determined from triplicates.

3.8 Integrity of Cell membrane due to Nanoemulsions

Overnight bacterial culture grown at 37°C in nutrient broth was harvested, washed, resuspended, and adjusted to 0.5 MacFarland with sterile saline. 500µl of the above adjusted into three tubes each containing 4.5 ml of the 0.5% nanoemulsion (tube-1) and 1% nanoemulsion (tube-2), emulsion control(tube-3) and a NB control then incubated at 37°C with agitation for 1h. The samples were then centrifuged at 3600×g for 10 min. Followed by fixing it with 2ml of 2.5% glutaraldehyde for 1 h. Afterwards successive wash with a gradient concentration (30, 50, 70, 90 and 100%) of ethanol carried out. Centrifugation after each wash were performed and suspended in 2mls of the next wash solution. 100µl of final suspension were fixed on a glass slide and stored at room temperature. Gold sputtered glass slides visualized using the Auto Fine Coater and SEM.

3.9 Practical Application of Cinnamon Oil Nanoemulsions on Mung beans

3.9.1 Germination effect on Mung beans in the presence of Nanoemulsions

Three batches of 25 mung bean seeds treated with 1% and 0.5% concentration of Cinnamon oil nano emulsion. One batch with emulsion solution of tween 80 in distilled water served as control (C1) and another with distilled water considered as C2. Seeds treated for 30 min with a solution of 1% and 0.5% NE, following by rinsing with sterilized water. Twenty-five mung bean seeds were taken in the petri dishes and added 15 ml of water. The petri dishes were kept in a growth/germination chamber at 25 ± 2 °C for 24h. Seeds showing protruding radicle of 1 mm were recorded as germinated. Seeds were monitored daily to observe the germination after 24 hours, 72 hours. After 7 days, all the germinated and non-germinated seeds were counted.

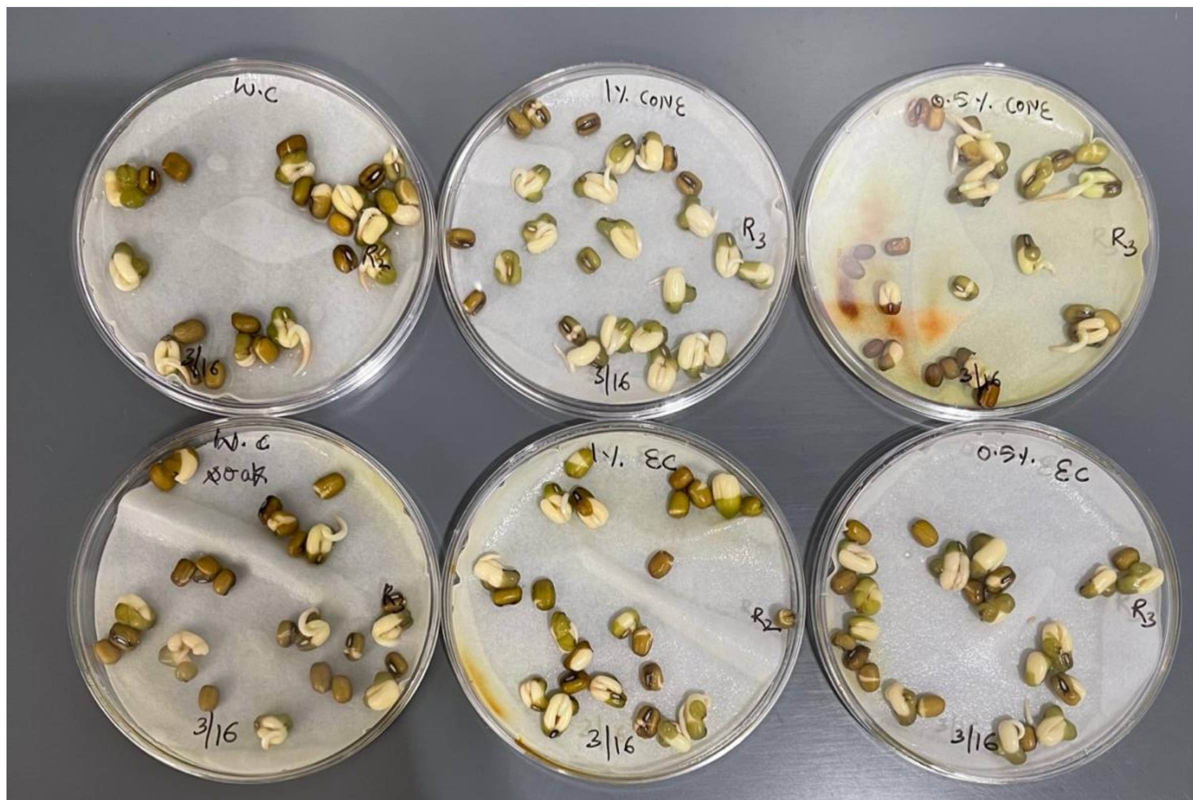

Figure 8: Germination Study

3.9.2 Antimicrobial efficacy of Nano emulsions on Mung bean seeds: Pre-harvest study

Surface sterilized with the use of Calcium Hypochlorite (20,000 ppm); Mung beans seeds were added to Salmonella cocktail suspension, gently agitated, and soaked for 3 minutes at room temperature. Seeds were drained and dried in biosafety cabinet for 24 hours. Dried seeds were divided in total 7 groups of 25 counts. The control sample was taken after drying to determine the initial level of bacterial inoculation. Seeds in group-1 were treated/ washed in sterile water for 30 minutes. Seeds in group-2,3,4 were submerged into emulsion control for 30 minutes with the concentration of 0.5%, 1.0% respectively. Seeds in remaining groups were submerged into Cinnamon oil Nano emulsion with the concentration of 0.5%, 1.0% respectively for 30 minutes with gentle agitation. Sampling time was (day 0, 3, 5), Every sampling time mung-beans were submerged in 50 ml of 1 % Phosphate Buffer solution & vortexed multiple times. Diluted samples from each treatment were plated on XLD agar for the enumeration of the Salmonella species respectively. Enumeration of surviving pathogens were performed, to elucidate the antimicrobial efficacy of the nanoemulsion in controlling Salmonella contamination on Mung bean seeds.

3.9.3 Antimicrobial efficacy of Nano emulsions on Mung bean Sprouts: Post-harvest study

Mung bean seeds were taken in the petri dishes and added 15 ml of water. The petri dishes were kept in a growth/germination chamber at 25 ± 2 °C for 24h-48h. Seeds showing protruding radicle of 1 mm were recorded as germinated. Surface sterilized with the use of Calcium Hypochlorite (20,000 ppm); Mung beans sprouts were added to Salmonella cocktail suspension, gently agitated, and soaked for 3 minutes at room temperature. Sprouts were drained and dried in biosafety cabinet for 24 hours. Dried sprouts were divided in total 7 groups of 25 counts. The control sample was taken after drying to determine the initial level of bacterial

inoculation. Sprouts in group-1 were treated/ washed in sterile water for 30 minutes. Sprouts in group-2,3,4 were submerged into emulsion control for 30 minutes with the concentration of 0.5%, 1.0% respectively. Sprouts in remaining groups were submerged into Cinnamon oil Nano emulsion with the concentration of 0.5%, 1.0% respectively for 30 minutes with gentle agitation. Sampling time was (day 0, 3, 5), Every sampling time mung-beans were submerged in 50 ml of 1 % Phosphate Buffer solution & vortexed multiple times. Mung bean sprouts were stored in refrigerated temperature for the study. Diluted samples from each treatment were plated on XLD agar for the enumeration of the Salmonella species respectively. Enumeration of surviving pathogens were performed, to elucidate the antimicrobial efficacy of the nanoemulsion in controlling Salmonella contamination on Mung bean sprouts.

Figure 9: Treatment with Cinnamon oil Nanoemulsion

3.10 Microbial counts

The bacterial counts were processed in triplicate immediately after culture inoculation or after 24 hrs., 48 hrs., 72hrs storage. Every procedure was carried out in sterile conditions. 1.5 gm

of mung bean seeds and sprouts were used for each treatment. Samples were then diluted and plated into XLD agars for the enumeration of *Salmonella* species. All the experiments were performed in triplicate.

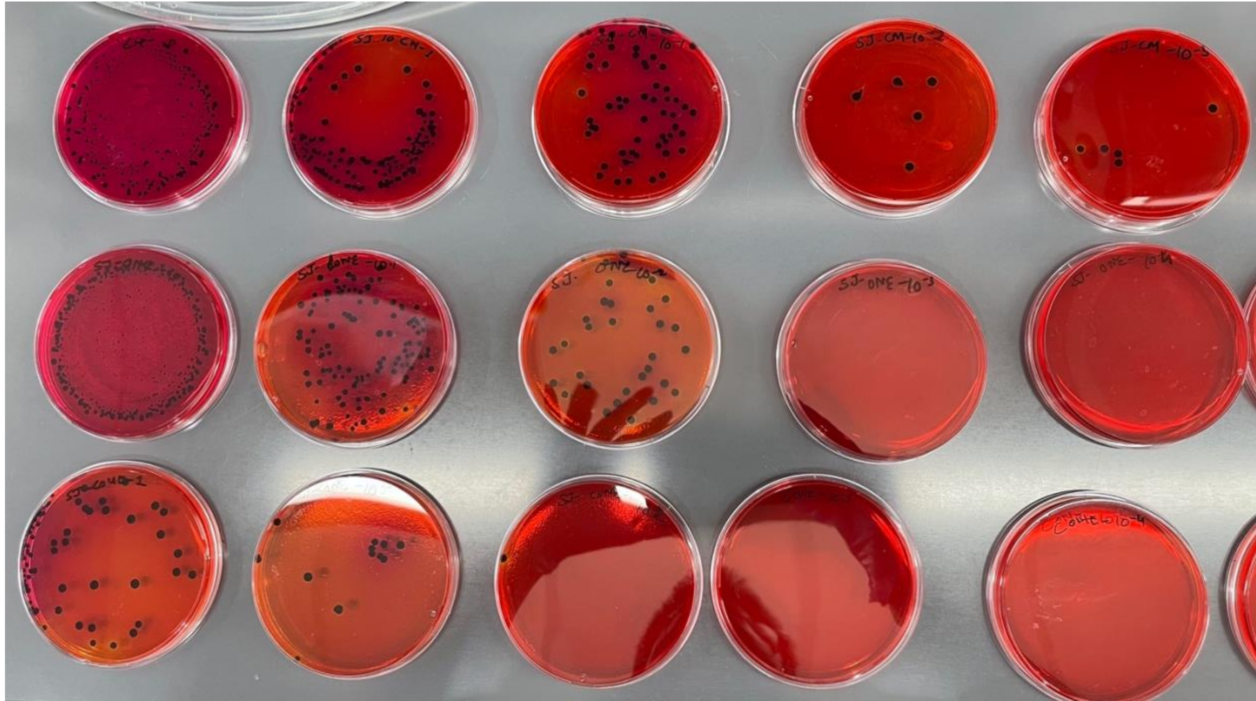

Figure 10: Enumeration of Salmonella colonies

3.11 Statistical Analysis

All experiments were carried out in triplicates. Reduction in population of *Salmonella* on was determined and their time treatment combination was compared respectively. To determine the significant difference between all different type of treatments and control samples, simple statistics analysis was performed using a general Microsoft office excel program.

4. Result & Discussion

4.1 Nanoemulsion formulation

Table 1: Radius and polydispersity of different nanoemulsion formulations based on percentage of tween and ultrasonication time

Formulation	Radius nm	% Polydispersity
2.5%T 10min S control	1279.16±196.3	347.07±29.1
2.5%T 10min S nanoemulsion	9.63±0.3	10.43±0.8
2.5%T 20min S control	33.57±2.3	60.20±9.5
2.5%T 20min S nanoemulsion	10.60±0.4	10.87±0.8
5% T 10 min S control	1729.23±201.8	300.27±12.4
5% T 10 min S nanoemulsion	38.23±3.8	8.27±0.9
5% T 20 min S control	62.27±3.4	35.03±5.0
5% T 20 min S nanoemulsion	9.30±2.5	25.73±5.0

Legend: T= tween 80, S= ultrasonication, nm= nanometer

All samples contain 5% cinnamon oil

A high energy ultrasonic emulsification method was used to develop cinnamon oil nanoemulsion. This emulsion has smaller particle size and prolonged stability. The particle size and polydispersity were calculated by using dynamic light scattering method. Polydispersity depends on the size homogeneity of the particle. Emulsion are most homogenous when it has smaller value of particle size. Therefore, the formulation with 2.5% tween 80, 5% cinnamon oil with an ultrasonication time of 10 mins. It has a radius of 9.6±0.3 nm and a polydispersity percentage of 10.4±0.8%. The standard deviations for radius and polydispersity of the selected sample were calculated as 0.5 and 1.4 respectively.

Polydispersity index is the ratio of molecular weight averages and used as a measure of molecular weight distributions (MWD) (Rogošić, Mencer, & Gomzi, 1996). Thus, a polydispersity index closes to 1 or 100% indicates that there is a heterogenous distribution between the oil droplet size of a nanoemulsion (Salvia-Trujillo, Rojas-Graü, Soliva- Fortuny, & Martín-Belloso, 2013). Additionally, polydispersity tells us the extent consistency and cohesion of the droplet size in the emulsion (Sugumar, Ghosh, Nirmala, Mukherjee, & Chandrasekaran, 2014). From our evaluation, A polydispersity index was 10.4±0.8% or 0.104±0.008 which is a highly acceptable to describe it being as homogeneous as according to Flores et al. (2011) the polydispersity index below 0.25 indicates adequate homogeneity. A parallel study done while formulating essential oil based nanoemulsion of basil oil yielded a droplet size of 30 nm and a polydispersity index of 0.234. Similarly in another experiment done in a blended cloves/cinnamon essential oil nanoemulsion, the researchers reported a polydispersity index in the range of 0.22-0.29 (Zhang, Zhang, Fang, & Liu, 2017). Our droplet size and polydispersity index of the nanoemulsion used were lower than these values.

4.2 Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration

Table 2: The MICs and MBCs for cinnamon oil nanoemulsion against all six pathogenic strains.

Strain	MIC (v/v)	MBC (v/v)
Salmonella - 4293	0.3125%	0.3125%
Salmonella - 2725	0.3125%	0.3125%
Salmonella - 1708	0.3125%	0.3125%
Salmonella - 1975	0.3125%	0.3125%
Salmonella - 0172	0.3125%	0.3125%
Salmonella – 20740	0.3125%	0.3125%

The average MIC of cinnamon oil nanoemulsion for *Salmonella* were 0.039% v/v and the average MBCs for *Salmonella* strains were 0.039% as well. In parallel study by Bhargava et al. (2015) fount that the MIC value of oregano oil nanoemulsion against L. monocytogenes, S. Typhimurium, and E. coli was 0.0625% which is greater than what we got for cinnamon oil nanoemulsion against *Salmonella*. Similarly, Bhargava et al. (2015) reported the MBC values for *S. Typhimurium* at 0.625 % which is higher than what we have for Salmonella but reported a lower MBC of 0.125% for E. Coli at 0.125% which is much lower than ours. Studies also confirm than tween 80 had no effect on bacterial growth.

It is known that Gram-positive bacteria are more affected by essential oil than Gram-negative bacteria. Gram-positive bacteria do not have lipopolysaccharide (LPS) protection payer from hydrophobic compounds as investigated by M. Hyldgaard, T. Mygind, and R. L. Meyer (2012). Due to the process of emulsification through sonication the cinnamon oil nanoemulsion has reduced hydrophobic property and will increase the antibacterial effect on Gram-negative bacteria as well.

The major compounds found in cinnamon oil are Cinnamaldehyde and eugenol. Cinnamaldehyde inhibits ATPase and perturbs cell membrane as well as inhibit cytokinesis (Morten Hyldgaard, Tina Mygind, & Rikke Louise Meyer, 2012; Kwon, Yu, & Park, 2003). Eugenol permeabilizes the cell membrane interacting with proteins (Morten Hyldgaard et al., 2012) by altering the cell membrane which results in the release of cellular content (Bennis, Chami, Chami, Bouchikhi, & Remmal, 2004). Bhargava et al. (2015) observed an identical MIC for both Gram-positive and Gram-negative bacteria. Cinnamaldehyde was found to be more potent against *Salmonella typhimurium* in a study done by (Helander et al., 1998) and it appeared

to be less potent than eugenol against *L. monocytogenes* (Gill & Holley, 2004). Cinnamon oil contains higher amounts of cinnamaldehyde than eugenol. This may explain lower MIC for Gram-negative bacteria in these studies.

4.3 Kinetic Time killing

The kinetics of antimicrobial activity of Cinnamon oil nanoemulsion was evaluated against *Salmonella enterica* (strain- 4293), *Salmonella enterica subspecies enterica serovar newport* (strain- 2725), *Salmonella enterica* (strain-1708), *Salmonella enterica* (strain-1975), *Salmonella enterica* (strain-0172), *Salmonella enterica* (strain-20740) at dilution ranges of 0.5% and 1.0%. Collective presentative data from the study are shown in below chart.

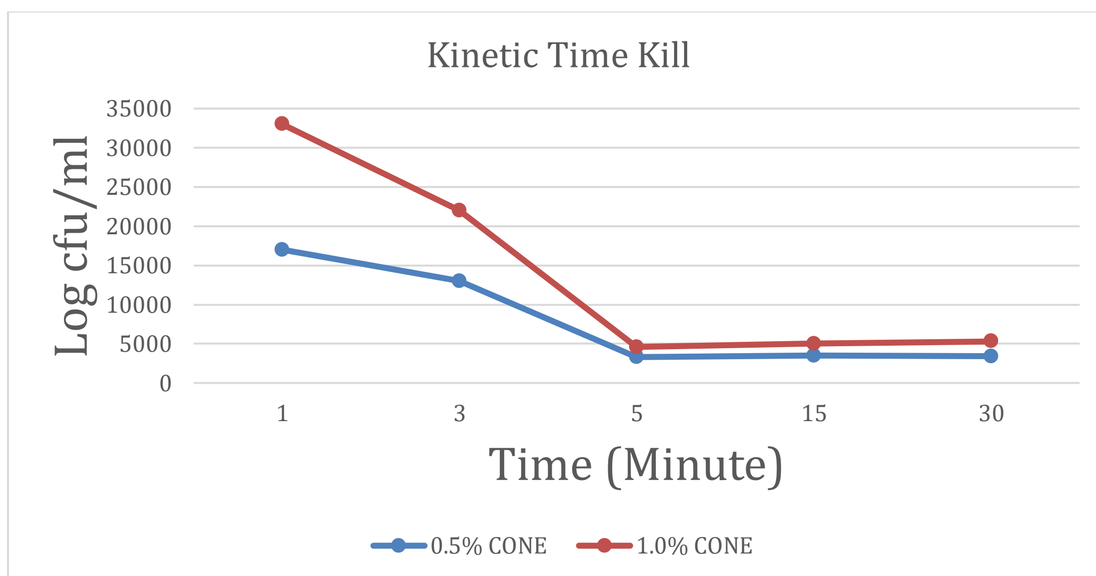

Figure 11: Kinetic Time Killing with nanoemulsions

CONE: Cinnamon Oil Nanoemulsions

At a 0.5% dilution of CONE, bacterial colony counts were reduced >2 logs for all the salmonella strains at 5 min. By 30 min, all bacterial strains had been reduced by ≥2 logs. At dilutions of 1.0% the viability of all bacterial strains was reduced by ≥2 logs by 5 min. Data also shows that the cinnamon oil Nanoemulsion are effective as an antimicrobial at 3 minutes as well.

4.4 Integrity of Cell membrane due to Nanoemulsions

Nanoemulsions fuses with the lipid-bilayer of bacterial cell membrane and destabilize its integrity and it leads to cell lysis (Ghosh et al., 2013b). Cinnamon oil Nanoemulsion caused damage to the Salmonella bacterial cell membranes, SEM images also showed alteration of the surface morphology of the bacterial cells upon treatment with 0.5% and 1.0% CONE. The control (untreated) cells of *Salmonella* showed rod shaped structures with an intact cell membrane. A distortion of the membrane was seen in bacterial cells treated with CONE than tween 80.

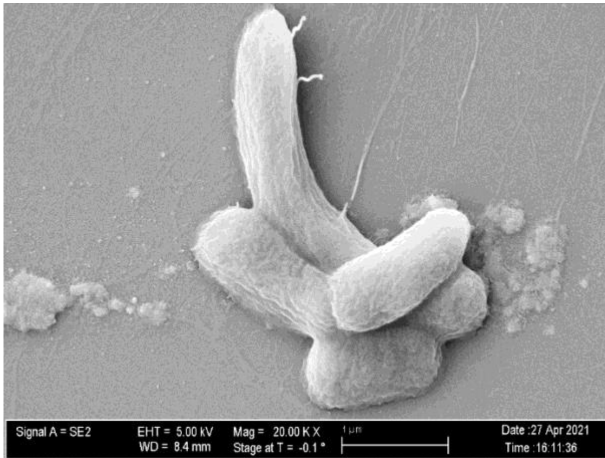

Figure 12: Control

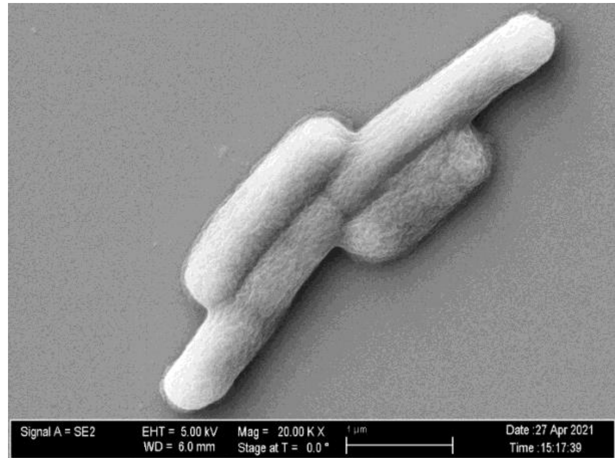

Figure 13: Emulsion Control (Only Tween80)

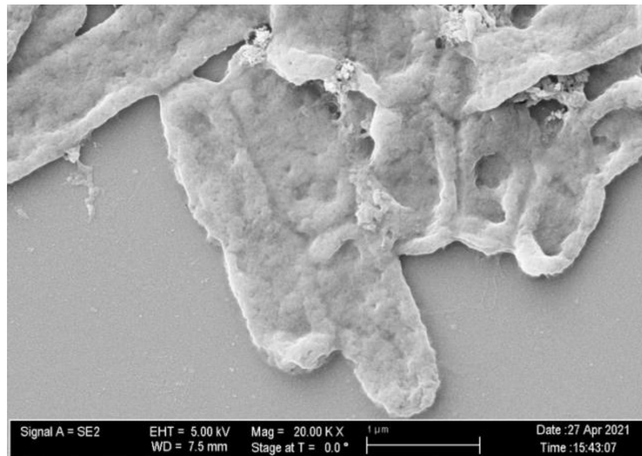

Figure :14 Treated with CONE

4.5 Germination effect on Mung beans in the presence of Nanoemulsions

Nanoemulsions do not give any effect on the germination process of sprouts. Our results showed partial increase in germination percentage. Mung bean seeds were germinated with very identical speed with water control and with emulsion control as well. The data and charts are confirmed that.

Hours	1% CONE	0.5% CONE	1 % ONE	0.5% ONE	Water control 1 (30 min Soak)	Water control 2
24	7	6	7	6	6	5
48	14	14	15	13	13	13
72	22	21	22	21	20	20
GP	88	84	88	84	80	80
GS	7	7	7	7	7	7

Table 3: Germination Speed & Percentage in presence of Nanoemulsions

CONE: Cinnamon Oil Nano Emulsion

GP=Germination percentage:

GP = $\frac{n}{N} \text{x}100$

n = Number of newly germinating seeds.

N = Total number of seeds.

GS=Germination Speed:

GS = $R_s = \sum_{i=1}^{n} Si/Di$

R_s = germination speed (number of seeds per day).

Si = Number of germinated seeds.

Di = Number of days.

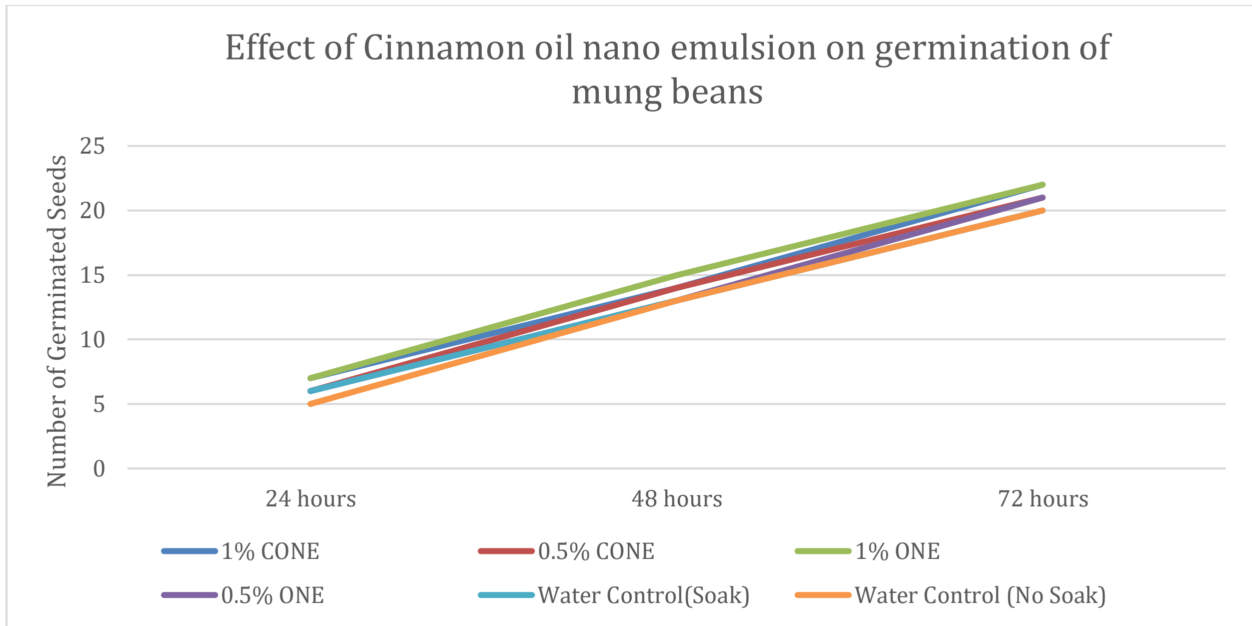

Figure 15: Graphical Representation of Germination Speed

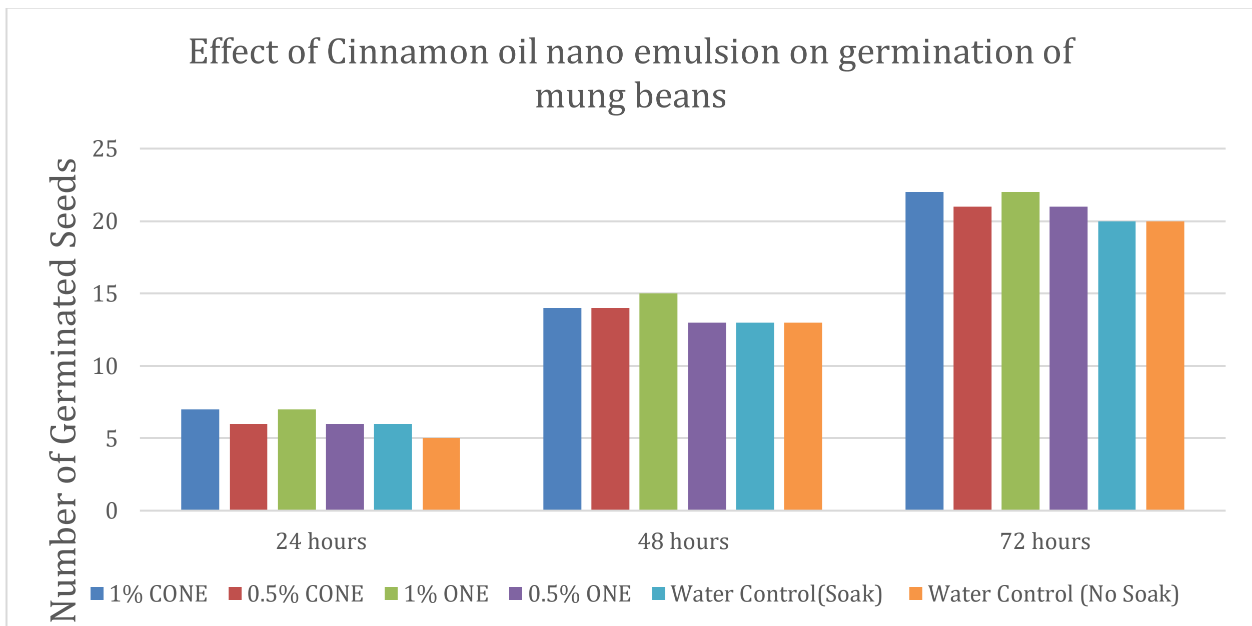

Figure 15: Graphical Representation of Germination Percentage

Germination speed showed that mung bean seeds got geminated fully after 72 hours, however, in the presence of 1.0% cinnamon oil nano emulsion seeds showed higher germination

percentage at regular time interval. There is not such significant difference for germination speed in water soak samples as well.

4.6 Antimicrobial efficacy of Nano emulsions on Mung bean seeds: Pre-harvest study

The initial concentration of Salmonella was 8.0 CFU/gm for mung beans. Microbial populations were calculated at three different time intervals (24hrs, 72hrs, and 120hrs) after treatment (Figure). Both the concentrations of cinnamon oil nanoemulsion were able to inhibit microbial growth. The treatments 0.50% and 1.0% at 24h and 72h showed significant difference ($p<0.05$) compared to control for Salmonella. However, there was no significant difference ($p>0.05$) between both the treatments and controls at 120hrs.

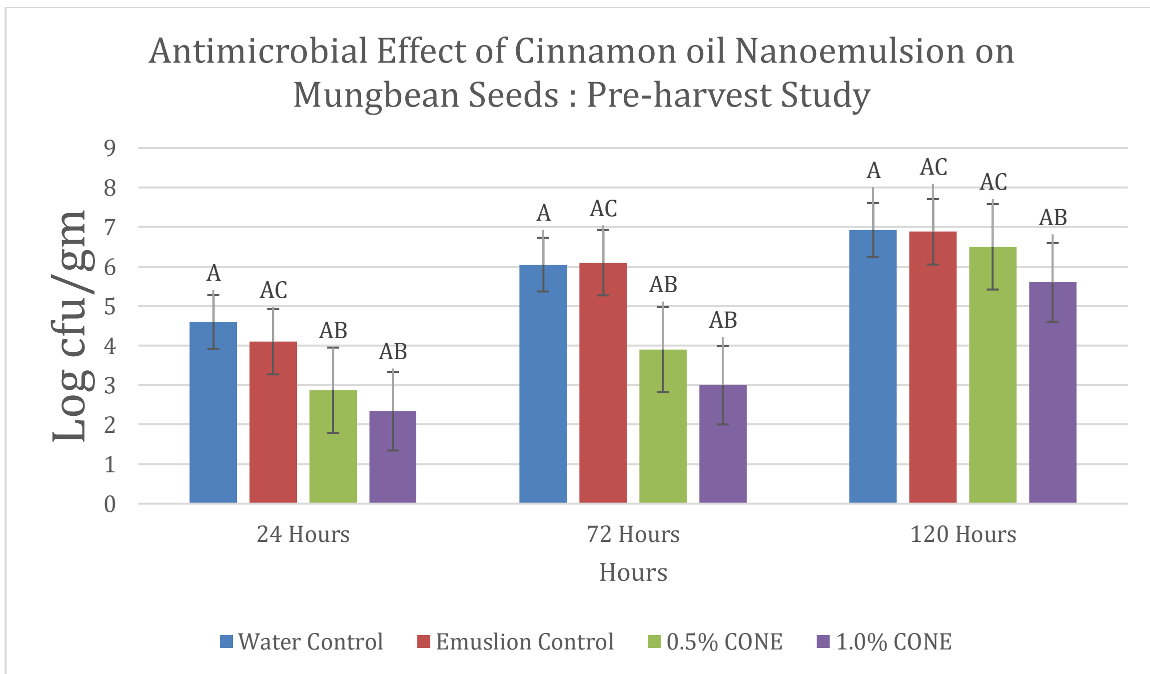

Figure 16: Graphical Representation of Pre-harvest treatment study

A=Control, AB=where p-value is less than 0.05 and AC=where p-value is greater than 0.05.

There was significant difference between the control and 1.0% formulation at 72hrs. The final log reductions for *Salmonella* after treatment at 72hrs were 3.05 log reduction and 2.04 log

reductions respectively in 0.5% formulation as well. Our results show a gradual decrease in bacterial samples in the control over time but there was increase in Salmonella count at 120hrs.

The increase in the bacterial count may be due to the seeds we kept at room temperature for the bacterium to survive, hence, increase the bacterial load. However, Bhargava et al. (2015) found a partial decrease in microbial load with time.

4.7 Antimicrobial efficacy of Nano emulsions on Mung bean Sprouts: Post-harvest study

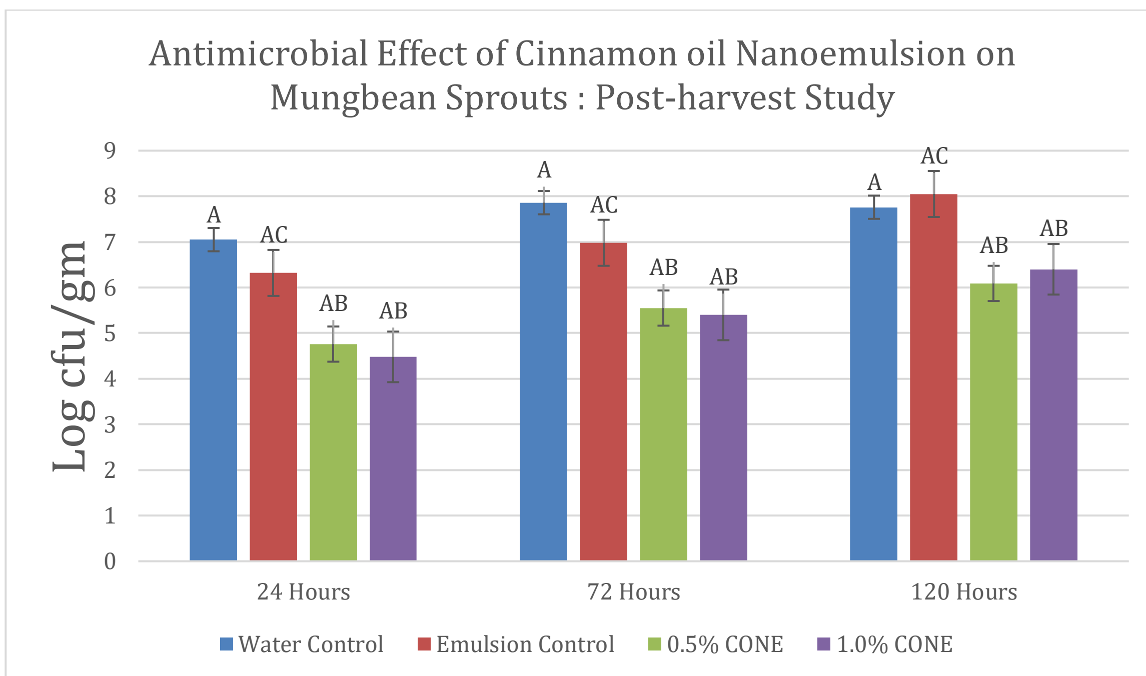

Figure 17: Graphical Representation of Post-harvest treatment study

A=Control, AB=where p-value is less than 0.05 and AC=where p-value is greater than 0.05.

The initial concentration of Salmonella was 8.0 CFU/gm for mung beans. Microbial populations were calculated at three different time intervals (24hrs, 72hrs, and 120hrs) after treatment (Figure). Both the concentrations of cinnamon oil nanoemulsion were able to inhibit microbial growth. The treatments 0.50% and 1.0% at 24h and 72h showed significant difference

($p<0.05$) compared to control for *Salmonella*. Additionally, there was also notable difference ($p<0.05$) between both the treatments and controls at 120hrs as well.

There was significant difference between the control and 1.0% formulation at 48hrs & 72hrs. The final log reductions for Salmonella after treatment at 48hrs & 72hrs were 2.57 log reduction and 2.46 log reductions respectively. However, there was only 1.67 in 0.5% formulation at 120hrs. Additionally, our results show a gradual decrease in bacterial samples in the control over time but there was increase in Salmonella count at 120hrs.

Studies on oregano oil nanoemulsion have resulted up to 3.57 log reductions (Bhargava et al., 2015). Our study ranges from 1.67-3.5 log reduction. This may support the possibility of cinnamon oil having stronger antimicrobial activity. In past studies on antimicrobial activity of cinnamon oil, allspice, and clove bud oils in apple puree edible films against *E. coli O157:H7, S. enterica,* and *L. monocytogenes*, the antimicrobial effect of cinnamon oil was found to be compellingly outstanding than allspice and clove bud oil (Du et al., 2009).

Similar research on nanoemulsion of clove/cinnamon mixture reported synergistic antimicrobial activity against bacterial strains of *E. coli, B. subtilis, S. typhimurium*, and *S. aureus* with 4% of the essential oil nanoemulsion (Zhang et al., 2017). Our concentration used was very low (0.50%, 1.0%) compared to this experiment.

5. Conclusion & Future directions

This research optimized the formulation of nanoemulsion by using different time, energy, and concentration of emulsifier. Radius size and polydispersity index were the basis for the optimal nanoemulsion. Based on this study, we concluded that the nanoemulsion preparation procedure which created the smallest radius size, and the lowest polydispersity index was the most optimized formulation process which will be our standard procedure for preparing cinnamon oil nanoemulsion. We were able to achieve a radius size of 9.63±0.3 nm and a polydispersity index of 10.43±0.8 % which is our best radius and polydispersity index.

We evaluated the antimicrobial properties of prepared cinnamon oil nanoemulsion against 6 strains of *Salmonella enterica*. Results were an average MIC value of 0.3125% v/v for Salmonella. Our MBC values for all strains same as MIC i.e., 0.3125% v/v. These were the most effective and lowest concentrations of nanoemulsions.

The different formulation of cinnamon oil nanoemulsion were prepared to test against Salmonella on mung bean seeds and sprouts which were control, emulsion control, 0.50% and 1.0% v/v. We evaluated a kinetic time required with minimum concentration of emulsion to inhibit the bacterial growth. We concluded that emulsion must be in contact with bacterial cell for minimum 5 minute to get at least 2 log reduction in bacterial count.

Mechanistic studies showed that the higher antibacterial activity of the 1.0% best due to its increased efficacy to disrupt the bacterial cell membrane. Additionally, 1.0% cinnamon oil nanoemulsion showed significant reduction on the log CFU/gm count in both mung bean seeds and sprouts against salmonella bacteria. 0.50% had a reduction of 1.8-3.0 log CFU/gm for Salmonella. Both 0.50% and 1.0% were effective in reducing the bacterial load but 1.0% concentration was the most effective.

We optimized a process of producing cinnamon oil nanoemulsion with a small radius and low polydispersity index. The cinnamon oil nanoemulsion was able to significantly reduce different strains of Salmonella sp. on mung bean seeds (pre-harvest) and sprouts(post-harvest) as well. With the germination study we concluded that the presence of nanoemulsion doesn't hinder the sprouting of the seeds. In fact, its presence partially supports the germination speed.

Future studies on the process or the mechanism by which the main compounds in cinnamon oil, cinnamaldehyde and eugenol, act against pathogens should be studied to realize the full potential of cinnamon oil nanoemulsion. The properties of cinnamon oil nanoemulsion on a range of different pathogenic bacterium can be an important study to further the knowledge on this area. Studies against potential risk produce types such as different sprouts and fresh leafy vegetables can be done to expand the horizon of impact of cinnamon oil nanoemulsion. Further sensory and texture study should be done to confirm no harm to the produce is done by the nanoemulsion.

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