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**EFFICACY OF NANOMATERIALS AND BIORATIONALS ON THE BACTERIAL
LEAF STREAK MANAGEMENT IN WHEAT**

BY

ABRAHAM HANGAMAISHO

A thesis submitted in partial fulfillment of the requirements for the Master of Science

Major in Plant Science

South Dakota State University

2022

THESIS ACCEPTANCE PAGE
Abraham Hangamaisho

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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ABBREVIATIONS

Xtpvu: *Xanthomonas translucens* pv. *undulosa*

BLS: Bacterial leaf streak of wheat

NERF: Northeast Research Farm

USDA: United States Department of Agriculture

QS: Quorum sensing

NP: Nanoparticles

KB: King's B media

CFU: Colony forming unit

DSF: Diffusible signal factor

SDSU: South Dakota State University

MIC: Minimum inhibitory concentration

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ABSTRACT**EFFICACY OF NANOMATERIALS AND BIORATIONALS ON THE BACTERIAL LEAF
STREAK MANAGEMENT IN WHEAT**

ABRAHAM HANGAMAISHO

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Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the United States. Most wheat varieties are susceptible to bacterial leaf streak (BLS), a major disease caused by *Xanthomonas translucens* pv. *undulosa* (*Xtpvu*). BLS is challenging to manage since common chemicals do not provide adequate control. Nanomaterials and plant extracts have shown potential to provide a sustainable environmentally friendly control of animal and plant diseases. However, limited data are available on the efficacy of plant extracts and nanomaterials controlling BLS in wheat. The objective of this study was to evaluate the antibacterial activity of plant extracts and nanomaterials against *Xtpvu*. An in-vitro study was conducted with five treatments: nano-ZnO, nano-MgO, and nano-CuO amended with polyvinylpyrrolidone surfactant, were prepared at 2000 ppm each, with sterile water and Agrimycin as control checks. For the in-vitro study, a 100 μ L aliquot of each treatment was collected at 12, 24, 48, & 72 hours and plated on King's B agar medium, and colony forming units (CFUs)/mL were determined after three days to

quantify *Xtpvu* growth. The experiment was conducted twice. Results showed that; CuO, MgO and ZnO nanomaterials had antibacterial properties against *Xtpvu*. In the greenhouse and field, all plots were artificially inoculated with *Xtpvu* two days before treatments (CuO, MgO and ZnO nanomaterials and moringa, spirulina, ginseng, and tannic acid; with sterile water and Agrimycin as control checks) were applied. To assess BLS, 10 randomly selected plants per plot were rated based on the percentage of leaf area with BLS symptoms, and yield was determined by combine harvesting each plot and adjusted to bushels per acre at 13.5% moisture content. Nano-CuO, moringa and tannic acid had the highest antibacterial effect among all treatments tested. These results show that nano-CuO, tannic acid and moringa have potential to control *Xtpvu*.

Chapter 1

1.0 Literature review

1.1 Bacterial leaf streak disease in wheat

Wheat (*Triticum aestivum* L.) is one of the most important crops grown in the world (Calderini et al. 2020; Yamasaki et al. 2017; Singh et al. 2016). It is an important staple food crop for many countries including the USA, and grown on ~555 million acres worldwide. The world's largest producers are China, India, and the USA, producing 100, 70, and 64 million tons annually, respectively. The U.S is the second-largest exporter of wheat, behind only Russia (Bond & Liefert 2017). According to Bond & Liefert (2017), USA wheat food use was estimated at 949 million bushels and 131.7 pounds per capita consumption in 2016/17.

There are four major classes of wheat grown in the Great Plains, which include: hard red spring, durum, hard white, and hard red winter wheat. For states such as Minnesota, North Dakota, and South Dakota where spring wheat is the predominant wheat class, the production continues to be important because hard red spring wheat has a high protein content (Stanton 2019; Bond & Liefert 2017). It is conservatively estimated that pathogens and pests interfere with production by destroying between 10% and 30% of wheat produced worldwide.

Wheat production is highly affected by pests and diseases, most especially bacterial diseases of which bacterial leaf streak (BLS) is of great concern, since common chemicals do not provide effective control, and moreover these are also not eco-friendly (Rahman 2014).

Bacterial leaf streak is caused by *Xanthomonas translucens* pv. *undulosa* (Duveiller and Maraite 1995) and has led to economic losses in wheat (Stanton 2019; Liu et al. 2019) due to both decreased yield quantity and quality (Rahman et al. 2014; Duveiller and Maraite 1993; Pandey and Chatterjee 2022). *Xanthomonas translucens* pv. *undulosa* is classified under Kingdom Bacteria; Phylum Proteobacteria; Class Gammaproteobacteria; Order Xanthomonadales; Family Xanthomonadaceae; Genus *Xanthomonas*; Species *X. translucens*; *translucens* group pathovars: *undulosa*. *Xanthomonas* genus is a diverse and economically important group of bacterial phytopathogens (Pandey and Chatterjee 2022), belonging to the gamma-subdivision of the Proteobacteria (da Silva et al. 2002). BLS affects yield and grain quality by reducing photosynthetic leaf area and causing black chaff to form on wheat heads (Duveiller and Maraite 1993; Bamberg et al. 1936; Tillman 1999).

1.2 BLS symptoms

BLS first appears as water-soaking due to the multiplication of *Xanthomonas translucens* pv. *undulosa* (*Xtpvu*) in the intercellular spaces (Duveiller and Maraite 1995; Adhikari et al. 2012; Ramakrishnan et al. 2019), then lesions progressively turn yellow and eventually brown and necrotic (Curland et al. 2018; Petrova et al. 2021). These lesions can coalesce, forming large blotches that eventually kill the entire leaf. Almost all wheat varieties are susceptible to BLS.

Field infections are observed if the pathogen, present in the seed as a source of primary inoculum, is transmitted to the canopy under favorable weather conditions (Duveiller et al. 1991; Duveiller and Maraite 1995), by rain splash from soil and residue or by artificial inoculation of a highly concentrated bacterial suspension applied onto plants at the

tillering stage (Duveiller 1990a) and/or at flag leaf stage. It may take 8-10 days before first symptoms appear (Duveiller and Maraite 1995). BLS can occur over a range of very different conditions, such as in sprinkler-irrigated fields in temperate climates, high-rainfall subtropical highlands and warmer environments characterized by cool nights or frequent climatic changes and sudden temperature variations (Iqbal et al. 2013).

BLS is among the diseases which are globally challenging to control after field establishment, because of limited effective in-season chemical sprays (Li et al. 2020).

BLS has been shown to have variable, yet significant, economic impacts on wheat yields by impairing grain fill and/or reducing kernel number (Duveiller et al. 1997). However, the impact on yield varies with wheat cultivar, pathogen strain, environmental factors, and the combination of these factors (Stanton 2019). Cool nights and temperatures below 15°C are reported to reduce multiplication rate of the pathogen, although a few hours of temperatures above 20°C during the day can be enough to produce the critical population threshold for symptom expression (Duveiller and Maraite 1995).

1.3 Description of the causal pathogen in culture

The bacterium's colonies on laboratory media are usually yellow due to 'xanthomonadin' pigment production on King's B agar medium. When glucose or other sugars are added to the culture medium, colonies become very mucoid due to the production of an exopolysaccharide slime. A semi-selective medium can be prepared by adding an antibiotic to inhibit contaminants but not xanthomonads, and their maximum and optimum temperature ranges for growth are up to 39°C, and 28 to 30°C respectively (Gottwald, 2000). *Xanthomonas translucens* bacteria are Gram-negative non-spore

forming rods, $0.5\text{--}0.8 \times 1.0\text{--}2.5 \mu\text{m}$ in size, with a single polar flagellum, and form pale yellow colonies on *Pseudomonas F/ King's B* agar medium (Li et al. 2020).

Sapkota et al. (2020) observed (with transmission electron microscopy) that bacterial cells were mainly distributed in the mesophyll tissue of five-day post-inoculation diseased wheat leaf samples with the *Xtpvu* strain. Thus, the bacterial pathogen probably enters plant tissue through stomata and mainly colonizes mesophyll tissues.

1.4 Virulence mechanisms

Xanthomonas translucens utilizes a type II secretion system (T2SS) to secrete plant cell-degrading enzymes (Kaur et al. 2020), and a type III secretion system (T3SS) to deliver a suite of T3SS effectors (T3Es) inside plant cells (Liu et al. 2019; Ruh et al., 2017; Gürlebeck et al. 2006) which results in disease symptom development (Sunish and Sakthivel 2001; Ghosh, 2004). Lorenz et al. (2008) reported that binding of HpaA to HpaB within the bacterial cell favors secretion of extracellular components of the secretion apparatus, and thus promotes effector protein secretion after assembly of the T3S apparatus. High bacterial cell density increases production of extracellular polysaccharide (EPS) and adhesin which catalyzes biofilm formation, important for cell-cell and cell-plant attachment (Pandey and Chatterjee 2022; Rai et al. 2012, 2015; Legein et al. 2020). *Xtpvu* infects the plant through natural openings and wounds on the leaf surface and grows within the parenchyma of the host tissue (Stanton 2019).

Pandey and Chatterjee (2022) proposed that low cell density enhances diffusible signaling factor (DSF) levels which triggers chemotaxis, plus increased secretion of cell-wall hydrolyzing enzymes to degrade the plant cell walls for bacterial nutrition. In addition, quorum sensing helps phytopathogenic bacteria to measure their population size

for appropriate apoplast or plant cell entry (Kannan & Bastas 2015; Pfeilmeier et al., 2016; Legein et al. 2020). There are several methods that have been developed to detect the bacteria in seeds, including dilution plating with the use of selective media, seedling infection assays, serodiagnostic assays, PCR amplification, and loop-mediated isothermal amplification of DNA (Forster and Schaad 1985; Bragard and Verhoyen 1993; Maes et al. 1996; Langlois et al. 2017).

However, virulence differs among strains within a pathovar (Cunfer and Scolari 1982; Milus and Chalkly 1994; Adhikari et al. 2011; Sapkota et al. 2018). Using pathogenicity tests on several wheat genotypes, Adhikari et al. (2011) also confirmed relative divergence in *Xtpvu* strains collected from North Dakota.

Previous studies show that several triticale lines have dominant resistance genes to some *Xtpvu* strains, an indication of a gene-for-gene interaction in triticale (Johnson et al. 1987; Wen et al. 2018), but it remains unconfirmed if the wheat-*Xanthomonas translucens* pathosystem involves a gene-for-gene interaction. The main known virulence mechanisms are zinc uptake regulator (*Zur*) which regulates production of extracellular polysaccharides, iron uptake, detoxification, and multidrug resistance (Pandey and Chatterjee 2022).

1.5 Ecological factors influencing disease development

The populations of pathogenic *Xtpvu* in wheat plants vary depending on how seeds are produced, processed, and stored (Buck et al. 2003). Germinated seedlings with small initial population of bacteria can increase to high numbers during the growing season. It has been reported that if the number of bacteria is less than 1,000 CFU/g in the seed lots, no BLS symptoms can be observed in emerged plants (Klykov 1945; Duveiller et al.

1997). Other factors that support rapid bacterial multiplication include high relative humidity, optimum temperature, and nutrient-rich root exudates (Buck et al. 2003).

Taormina and Beuchat (1999) observed that pathogenic bacteria can survive for prolonged periods in or on stored dried seed, with long-term survival being higher at lower temperatures.

The environmental optimum conditions of BLS development are not well documented or known since BLS has been sporadic and can vary from year to year (Bamberg, 1936; Duveiller et al. 1991; Tubajika et al. 1998; Li et al. 2020). However, some information show that dry conditions are not a limiting factor for the multiplication of *Xtpvu* after its parenchyma invasion since water's role in the disease cycle is limited to the release and penetration of bacteria in the leaf (Duveiller et al. 1991; Duveiller and Maraite 1995; Pandey and Chatterjee 2022). Duveiller and Maraite (1995) observed that bacterial multiplication is faster, and the population can increase by more than three logs during the first 48 hours, with a maximum population (10^8 - 10^9 CFU/ leaf) reached after 4 days at 25°C.

Thus, BLS is difficult to induce in plants by spraying inoculum onto the leaf under controlled conditions (Duveiller and Maraite 1995), because infection process is mainly affected by presence of oxygen in the root zone, plant nutrition and inoculation timing which influence the size of stomatal opening (Randhawa and Civerolo 1985).

1.6 Life cycle, mode of infection and impact on yield

The survival rate of bacteria on seeds and transmission between seedlings are shown to be largely dependent on the storage conditions, the length of storage, and the level of genotype susceptibility (Boosalis 1951; Forster and Schaad 1990; Milus and Mirlohi

1995). Infected seeds have been found to be the major survival mechanism and source of transmission of *Xtpvu* (Milus & Mirlohi 1995), though crop debris and alternative hosts may also play a role (Curland et al. 2018; Darrasse et al. 2007). Previous studies show that weeds and grasses can be overwintering hosts or green bridges for bacteria to spread to the next growing season (Wallin 1946; Fang et al. 1950; Boosalis 1951; Thompson et al. 1989), since *Xtpvu* can survive in soil and crop debris for a short period of time (Milus and Mirlohi 1995; Duveiller et al. 1997; Stromberg et al. 2000; Li et al. 2020).

The pathogen can also survive in extracellular polysaccharide matrices for several weeks, and later develop epiphytically on host and non-host plants (Timmer et al., 1987; Beattie & Lindow 1999). The yellow exudates formed on the lesions can be spread by rain, wind, and insects to the new site of infection on plant parts having micro injuries caused by rain and/or wind leading to the penetration of bacteria into leaf blades (Kaur et al. 2020).

Xanthomonads also infect plants through hydathodes in case there is no wounding on the plant (McElhaney et al. 1998).

The BLS disease cycle is completed when bacteria successfully contaminate the seed of their host, or when they survive on a substrate that facilitates subsequent infections of a new host (Stanton 2019).

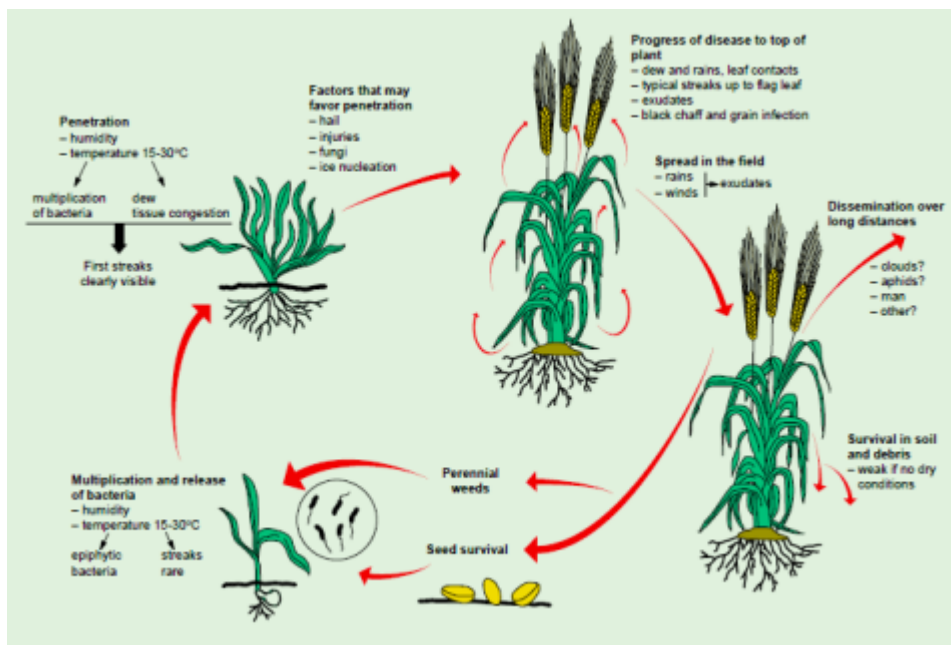


Figure 1.1 Disease cycle of bacterial leaf streak (BLS) and proposed routes of spread.

(Reproduced courtesy of E. Duveiller).

BLS negatively affects yield and grain quality by reducing photosynthetic leaf area and causing black chaff to form on wheat heads, yet it is difficult to manage since common chemicals do not provide effective control (Li et al. 2020). Field studies have shown that the *Xanthomonas translucens* pv. *undulosa* pathogen may cause up to 40% yield loss (Dill-Macky 2011).

1.7 Isolation and identification

Hauben, et al. (1997) noted that *Xanthomonas* species exhibited relatively high levels of overall genome sequence similarity, with a mean similarity value of 98.2%, which corresponded to an average of 14 mutual nucleotide differences. According to Iqbal et al. (2013), *Xtpvu* can be isolated from a diseased portion of leaf of an affected plant and cultured on yeast dextrose calcium agar medium, and bacterial colonies can be further purified and streaked on nutrient agar plates. *Xtpvu* may also be characterized through

pathogenicity, hypersensitivity, biochemical and molecular assays (Iqbal et al. 2014). Bacteria in plant samples can also be isolated on Wilbrink's Agar (WBA) (Sands et al. 1986). WBA is important for *Xtpvu* since *Xtpvu* can be easily differentiated from many saprophytic bacteria by observing yellow mucoid colonial growth of *Xanthomonas translucens* (Kaur et al. 2020).

Iqbal et al. (2014) noted that the 300 bp product amplified by a C1 and C2 primer pair confirmed the presence of *Xanthomonas*, while specific primers T1 and T2 amplified a product of 200 bp, which confirmed the presence of *Xtpvu*. Furthermore, biochemical characterization, Gram staining, KOH test, catalase and Kovac's oxidase test can be performed for characterization of the BLS-causing pathogen (Schaad 1980).

1.8 Disease management and control

The first step in managing BLS is starting with pathogen-free seed. Buck et al. (2003) noted that finding the optimum storage conditions that promote the desiccation and ultimate reduction of bacterial populations without compromising seed quality is a viable option for reducing bacterial populations. Seed certification can also be used to minimize the disease; thus, seed treatment may serve as a preventive measure (Iqbal et al. 2014; Duveiller 1994; Duveiller and Bragard 1992). For field control, Stanton (2019) reported that inoculation applied at booting growth stage (Z49) resulted in the highest center score (7.0), which was significantly higher than all other treatments examined at $p < 0.008$. Yet early inoculations at the three- leaf, four-leaf, or five-leaf growth stages (Z13, Z14, Z15) increased chances of uniform and consistently high levels of BLS in inoculated research plots (Stanton 2019). Therefore, spraying with bactericides and pesticides at these stages has always been suggested for controlling bacterial diseases. It should be noted that some

antibiotics and synthetic pesticides used to control bacterial pathogens in crops are restricted in many countries because of their negative impact, due to their high and acute toxicity, long degradation periods and accumulation in the food chain (Rahman et al. 2014).

Some pathovars of *Xanthomonas* with resistance to commonly used antibiotics have been reported (Rodriguez et al. 1997; Rahman et al. 2014). This has complicated BLS management, and hence there is a need to search for non-conventional chemicals for BLS management having an eco-friendly nature for sustainable environmental ecosystem quality and resilient BLS control mechanisms (Bolkan and Reinert 1994).

Breeding resistant wheat varieties is among the most cost-effective control options of BLS (Stanton 2019; Tillman et al. 1996) although breakthrough of resistance gene for BLS is still a major challenge. Classic genetic analysis has shown that BLS resistance can be quantitative or qualitative. Duveiller et al. (1992) reported a total of five genes (*Bls1*, *Bls2*, *Bls3*, *Bls4*, and *Bls5*) conferring BLS resistance in three resistant wheat cultivars, with *Bls1* present in all three partially resistant wheat cultivars with the largest effect (Li et al. 2020).

1.9 Use of plant extracts in BLS management

Naturally occurring biologically active plant products such as organic extracts can be explored as a source of new environmentally friendly pesticides for controlling plant-pathogenic microorganisms (Rahman et al. 2014). Plant extracts have always been used in traditional medicine as a source of antimicrobial compounds for disease treatment, food safety and shelf-life extension (Nabavi et al. 2015). All plant extracts tested are believed to have high levels of secondary metabolites which may directly target bacterial

pathogens (Blanc & Cock 2021). Among organic extracts, plant extracts from moringa (*Moringa oleifera*), Spirulina, Ginseng and Tannic acid have been explored (Farooq et al. 2012; El-Mohamedy & Abdalla 2014; Szczuka et al. 2019; Hlima et al. 2019).

1.9.1 Moringa

Moringa (*Moringa oleifera*) leaf extract (MLE) is known as a good source for phytohormones, phenolics and minerals (Nasir et al. 2016). MLE has also been reported as having antimicrobial, antioxidant, antiurolithiatic, and antihelminthic properties, which was supported after the discovery of inhibitory activity against several microorganisms (Farooq et al. 2012; El-Mohamedy & Abdalla 2014). It has also been reported that carboxymethyl cellulose containing moringa extract can suppress postharvest diseases and maintain quality of avocados (Rikhotso et al. 2019; Tesfay & Magwaza 2017). El-Mohamedy & Abdalla (2014) reported that moringa roots at 15% and 20% were most effective in decreasing spore/sclerotia germination (ranging from 53.4% to 81.4% and 67.0% to 94.2% decrease) of all tested pathogens (*Fusarium oxysporum*, *Fusarium solani*, *Alternaria solani*, *Alternaria alternata*, *Rhizoctonia solani*, *Sclerotium rolfsii* or *Macrophomina phaseolina*).

Nasir et al. (2016), reported an increase in fruit set and decrease in fruit drop as compared with control trees after foliar application of moringa leaf extract. While testing of moringa against plant fungal pathogens has been done, testing against plant pathogenic bacteria has not to our knowledge been done in wheat.

1.9.2 Spirulina

Spirulina (*Arthrospira platensis*, *Arthrospira maxima*) is known to have polyphenols and polysaccharides which are responsible for its antimicrobial activities (Hlima et al. 2019; Bajpai, 2016; Pagnussatt et al. 2016). Hlima et al (2019) demonstrated the capacity of *Spirulina* to inhibit all members of the studied panel of fungal strains (*Fusarium oxysporum*, *Fusarium culmorum*, *Fusarium graminearum*, *Aspergillus niger* and *Alternaria alternata*), particularly the *Fusarium* genus. Battah et al. (2014) also found that *Spirulina maxima* showed a broad spectrum of antifungal activity, with an average activity of 26% against five tested human and plant pathogenic fungi compared to the three tested commercial pesticides.

1.9.3 Ginseng

Ginseng (*Panax quinquefolius*) is an important medicinal plant as reported in several medical efficacy trials (Kim & Park 2011; Thomson 2010; Kitts & Hu 2000), showing antimicrobial activity against different pathogenic strains (Szczuka et al. 2019; Mehta et al. 2021). Kim & Yang (2018) stated that ginseng's effects not only directly kill bacteria but also work against the regulation of bacterial adhesion, inflammation, cytotoxicity, and hemagglutination. This is mainly due to their major pharmacological component (ginsenosides) which are specific secondary metabolites of *Panax sp.* (Kim & Yang 2018; Shahrajabian et al. 2019). It is also considered to be a food additive (Gillis 1997) in many countries including the USA.

1.9.4 Antibacterial activity test of individual and combined crude extracts

The antibacterial activity of the individual crude extracts and their combination have been studied by using agar diffusion methods. Minimum Inhibitory Concentration (MIC) can be analyzed using descriptive statistics (Mummed et al. 2018).

Although plant extracts have been studied extensively in both animal and plant disease management, there is not sufficient information on the effectiveness of these extracts on bacterial leaf streak of wheat.

1.10 Use of nanomaterials in BLS management

Nanotechnology is the science of manipulating matter at the atomic and molecular level, that deals with matter at the scale of one-billionth of a meter (Raliya et al. 2013). Size-related properties of nanoparticles offer innumerable opportunities for their diverse applications in the scientific world (Manojkumar et al. 2016). Another proposed definition is that nanomaterials exhibit a specific surface area to volume ratio greater than or equal to $60 \text{ m}^2/\text{cm}^3$ (Kreyling et al. 2010). This unique property of nanomaterials (high ratio of surface area to volume) further enhances their capability to penetrate cell membranes and to affect biochemical activities (Singh et al. 2019; Zhang et al. 2008). The reduction in size alters the electronic structure of the material, resulting in novel quantum effects. Therefore, the concentration of particles determines interparticle distance and is an important parameter to determine stability (Shrestha et al. 2020). The American Society for Testing and Materials categorizes nanoparticles based on their having two or more dimensions at the nanometer scale, and their having distinctive improved physical/chemical properties compared with their bulk counterpart (Limongi et al. 2019).

Recently, nanomaterials have been reported to minimize the number of chemical applications needed for plant disease management, which results in decreased toxicity and reduced cost of production (Worrall et al. 2018; Liao et al. 2019). They have been vigorously studied because they can be used as a novel, green and eco-friendly approach for managing diseases in plants very effectively (Singh et al., 2019; El-Argawy et al. 2017; Fu et al. 2020). For instance, nanoparticles have minimized bacterial leaf spot disease in tomatoes (Liao et al. 2019). The timing of nanomaterial application is very critical. Worrall et al. (2018) found out that application of nanomaterial after inoculation was more effective than application before infection or simultaneous application at the time of inoculation.

However, the main challenge is to achieve well-dispersed nanoparticles to facilitate their use both *in vitro* and *in vivo* (Limongi et al. 2019). The presence of short inter-particle distance between the metal nanoparticles leads to an attraction between them due to the influence of van der Waals forces. This usually happens in the absence of repulsive forces between the two particles, leading to their aggregation (Manojkumar et al. 2016). Among the nanomaterials studied, ZnO nanomaterials are the most unstable in suspension, mainly due to the dissolution of particles to form high concentrations of ions, resulting in enhanced aggregation of particles (Tso et al. 2010). To address this challenge there are several approaches that can be used in the process of treatment preparation, including: (i) ultrasound pretreatment with pressure frequency greater than 20 KHz (sonication) to improve nanomaterial dispersion in order to minimize chances of agglomeration by inertial cavitation, (ii) redispersion of nanoparticles using 50 μm bead milling (Sato et al. 2008), and (iii) use of dispersion stabilizers as a chemical

modification of the nanoparticles' surfaces. Such chemical functionalization includes the use of organic coatings, comprising various natural and synthetic polymeric layers (Phan and Haes 2019; Limongi et al. 2019; Hidehiro and Motoyuki 2010). In addition, Phan and Haes (2019) noted that the nanostructures stabilized by the mixed monolayer were deemed to exhibit anti-aggregation behavior, due to the interruption of crystalline packing of ligands on particle surfaces. However, Zhang et al. (2007) reported that the use of ultrasonication does not seem to be effective in breaking down nanoparticle agglomerates, and the use of dispersants does not enhance the size reduction.

Generally, the stability of a nanofluid depends upon various factors such as particle concentration, solution chemistry, particle size, surfactant, and ultrasonication (Singh et al. 2020).

1.10.1 Nanomaterial activity

The detailed mechanisms describing the specific metallic nanostructure actions causing harm to bacteria remain uncertain; however, attention has been given to morphological alterations (Singh et al. 2019). Metal nanoparticles such as silver, copper, zinc oxide, and titanium dioxide have been intensively researched for their antibacterial and antifungal properties, and are known for their antiviral properties (Worrall et al. 2018). Much research has been carried out on copper nanoparticles; for instance, as reported by Rai et al. (2018), they have the ability to control fungal pathogens at 15 mgL^{-1} . They also exhibit deleterious effects on *E. coli* (Deryabin et al. 2013; Jamshidi and Jahangiri-Rad 2014; Harikumar and Aravind 2016; Chatterjee et al. 2014), *Bacillus subtilis* (Yoon et al. 2007), *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Azam et al. 2012; Singh et

al. 2019) which shows that they can be effective against both Gram-negative and Gram-positive bacteria.

Agricultural agronomic nanotechnology research and development is very promising (Worrall et al. 2018). Recently, Elmer et al. (2018) noted that nano-copper products significantly reduced bacterial spot disease severity caused by Cu – resistant *Xanthomonas perforans* in the greenhouse and field trials compared to copper fungicide treatments. It was also reported that the zinc – based nanoparticle - Zinkicide™ suppressed citrus scab (*Elsinoe fawcetti*) and melonase (*Diaporthe citri*) on grapefruit. Zinc oxide nanoparticles have also been observed to provide efficient control of pathogen growth (Dimkpa et al. 2013). Dimkpa et al. (2013) stated that zinc oxide nanoparticles have better pathogen suppression, lesser toxicity and soil fertility enhancement compared to silver nanoparticles, and they are considered as bio-safe material (Liu et al. 2019). Nano-zinc oxide's antibacterial activity varies based on its concentration and surface area, and like other nanoparticles, it damages bacterial membranes and walls (Zhang et al. 2007). ZnO particles are effective in inhibiting both Gram-positive and Gram-negative bacteria, and they are also effective against spores that are high-temperature and high-pressure resistant (Zhang et al. 2008).

However, the lipopolysaccharides of the outer membrane of Gram-negative Bacteria may provide resistance against nanoparticles (Yoon et al. 2007; Baek & An 2011; Suresh et al. 2013). The use of polyvinylpyrrolidone surfactant can increase nanoparticle activity and has been reported to improve nano ZnO suspension stability (Zhang et al. 2007). This is due to the surfactant's physicochemical properties that enables it to be an effective anti-

biofilm agent by penetrating and disrupting hydrophobic structures (Anestopoulos et al. 2020).

The registering of new nano–zinc products for crop disease management shows the recognition of nanoparticles as a viable alternative to conventional strategies (Elmer et al. 2018). This approach is increasingly being adopted into crop production. Nanoparticles alone have the potential to be directly applied to seeds, foliage, or roots for protection against pests and pathogens, such as insects, bacteria, fungi, and viruses. Elmer et al. (2018) also reported that nano CuO increased crop biomass in six of eight experiments, and it also increased fruit yield and disease suppression in greenhouse egg plants (Elmer et al. 2021; Elmer and White 2016).

1.10.2 Nanomaterial mode of action against bacterial pathogens.

Nanoparticles accumulate and dissolve in the bacterial cell membrane that leads to alterations in membrane permeability and dissipation of the proton motive force (McQuillan 2010; Singh et al. 2019; Rai et al. 2018). Bacteria are known to play important roles in ecosystems (Singh et al. 2019). Being present at the bottom of the food chain, they become a key point for entry of nanomaterials to interact with organisms present at higher trophic levels (Suresh et al. 2013). When nanostructures first interact with the cell wall, this results in a disaggregated exopolysaccharide matrix, and separated cells, followed by their elongation and re-arrangement of cells into smaller groups. These changes allow the physical association of bacteria and nanostructures on available surfaces. The completely disrupted cell wall is the predominant step in the second phase of interaction between nanostructures and bacteria, resulting in the development of

perforated and thickened cell walls (Singh et al. 2019). This is supported by Singh et al. (2019), who reported that nanoparticles affected plant–microbe interaction by exposure of the plant growth-promoting bacterium, *P. chlororaphis* O₆, to zinc oxide and copper oxide nanoparticles. The nanoparticles are thought to bind with thiol moieties of bacterial proteins which disrupts their functioning, and their attachment to the cell membrane alters its permeability by changing the cell electrical potential, hence affecting the respiration process leading to cell death (Radzig et al. 2013; Singh et al. 2019).

Nanoparticle properties such as high tensile strength, high conductivity, and other physiochemical features make it possible for them to interact with prokaryotic cells, which affects cell morphology by cell membrane alterations and cytoplasmic accumulation of nanoparticles within the cell (Sinha et al. 2011; Jain et al. 2018). In addition, Rai et al. (2018) noted that toxic ions (Ag^+ , Cu^{2+} , Cd^{2+} , Zn^{2+}) bind to sulfhydryl groups of sulfur-containing proteins and affect protein functioning which disrupts membrane and cell permeability.

Another suggested mechanism is dissolution of nanoparticles into ions and their ability to generate reactive oxygen species (ROS) which also mediate microbial toxicity (Rai et al., 2018; Choudhury et al. 2013). This is due to the nanomaterials' physical or chemical redox active surfaces, which can react with molecular oxygen to generate ROS leading to toxicity in some biological systems (Suresh et al. 2013). The created imbalance in downstream pathways triggers DNA damage, altered cellular signaling, and programmed cell death (Jain et al. 2018; Rai et al. 2018). In an assessment on the antibacterial effect of zinc oxide nanoparticles on *E. coli*, bactericidal activity increased with a decrease in particle size (Zhang et al. 2007; Zhang et al. 2008; Suresh et al. 2013), mainly because of

ROS-induced membrane lipid oxidation systems leading to the dose-dependent antibacterial action of ZnO nanoparticles against *E. coli* (Liu et al. 2019; Suresh et al. 2013; Dutta et al. 2013).

Unlike prokaryotes, the eukaryotic semipermeable plasma membrane selectively permits a few important nano-sized chemical species across the lipid membrane, either by specific membrane transport protein channels or by endocytosis (Alberts et al. 1997; Conner and Schmid 2003), and this explains the absence of phytotoxicity after nanomaterial foliar application.

1.10.3 Nanomaterial toxicity to bacteria

Toxicity of nanomaterials is mostly attributed to the small size and large surface area of nanomaterials (El-Argawy et al. 2017), but the unique physio-chemical properties like size, shape, charge, area, and reactivity of nanoparticles makes the investigation of their toxicity complicated (Jain et al. 2018). It is believed that significant physical, chemical, and electrical changes could occur when materials are prepared in their ultra-fine particulate form (Povey et al. 2008). The molecular mechanism of toxicity normally occurs by dissolution of nanoparticles into ions. This has many examples including microbial toxicity of zinc, copper, silver, and nickel ions (Suresh et al. 2013), and the toxicity rate differs for different ions and target pathogen species. The correlation between nanoparticle toxicity and its dissolved ion was observed in studying comparative toxicity of nano-crystallites of ZnO, CuO and TiO₂ against *V. fischeri*. The ZnO and CuO nanoparticles were found to be toxic with LC₅₀ values of 1.9 and 79 mg L⁻¹, as compared to TiO₂ nanoparticles which were not toxic even at high concentrations of 20 g L⁻¹

(Suresh et al. 2013), and the toxicity was mainly due to the release of soluble ions (Suresh et al. 2013).

According to Rai et al. (2018), zinc oxide nanoparticles have an inhibitory effect on hyphal growth of *B. cinerea*, and conidia germination in *P. expansum* at an application rate of 12 mmol L⁻¹. Suresh et al. (2013) also reported the bactericidal potential of 500–1000 nm diameter CuO nanoparticles against several disease-causing bacterial strains of *E. coli*, *S. typhi*, *S. aureus* and *B. subtilis*. A few studies show its antibacterial activity against all the bacterial strains that were assessed (Pandey et al. 2012; Suresh et al. 2013). Factors such as the method of nanomaterial synthesis, dose, the presence or absence of additives, and the solubility of the material can also influence the biological impact of the nanomaterial (Suresh et al. 2013), and surface charge as dictated by chemical coatings has also been found to be important in controlling the toxicity of other nanoparticles (Suresh et al. 2013). Nanoparticles of less than 100 nm are more toxic than larger particles of identical chemical composition (Jain et al. 2018; Liao et al. 2019). However, Brownian motion increases with reduction in particle size and high temperature, which influences the stability of nanofluids because surface energy and cohesion may lead to nanoparticle aggregation. However, nanoparticle preparation techniques like sonication and application of surfactants can minimize aggregation (Singh et al. 2020; Liu et al. 2019).

The agricultural adoption of nanotechnology research has not yet reached its full potential for commercial applications (Worrall et al. 2018). Moreover, the effect of nanoparticles depends upon the species of plant and type of nanoparticle. Nanomaterials can also be used as potential carriers, as modulating active ingredients of pesticides so that they can

be released at the appropriate time for better disease control (Rai et al. 2018; El-Argawy et al. 2017). Application of slow-release fertilizer coated with nanomaterials was reported to improve grain yield, with minimal increase in protein content plus decreased soluble sugar content in wheat compared to NPK treatment (Qiang et al. 2008; Elmer et al. 2018).

A number of studies have been conducted to establish the efficacy of metal oxide and metal nanoparticle treatments to suppress pathogens and benefit crop yield (Singh et al. 2019) but no data are available on the efficacy of nanoparticle application controlling BLS in wheat. Therefore, this study focused on the influence of nanomaterials on bacterial growth and BLS severity, with the goal of developing sustainable eco-friendly biopesticides for the management of wheat diseases and increasing scientific information availability of this practice for phytopathologists, extensionists and agronomists.

Researchers have tested several products to combat BLS, but no product is currently known to provide effective protection against BLS disease. Therefore, the study's main goal was to determine effectiveness of eco-friendly biopesticides, and nanomaterials as part of integrated BLS management. The specific objectives of the study were:

- i) To determine antibacterial activity of nanomaterials on *Xtpvu* in-vitro.
- ii) To evaluate the efficacy of nanomaterials and plant extracts on BLS development in wheat under field conditions.
- iii) To determine the influence of nanomaterials and plant extracts on wheat crop yield.

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Chapter 2

2.0 Efficacy of nanomaterials on bacterial leaf streak in wheat

ABSTRACT

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the United States. Most wheat varieties are susceptible to bacterial leaf streak (BLS), a major disease caused by *Xanthomonas translucens* pv. *undulosa* (*Xtpvu*). BLS is challenging to manage since common chemicals do not provide adequate control. Nanoparticles present a novel eco-friendly approach for plant disease management because of their high surface area and antibacterial properties. However, no data are available on the efficacy of nanoparticles controlling BLS in wheat. The objective of this study was to evaluate the antibacterial activity of nanoparticles against *Xtpvu* growth and BLS severity. In-vitro, greenhouse and field studies were conducted with five treatments: nano-ZnO, nano-MgO, and nano-CuO amended with polyvinylpyrrolidone surfactant, were prepared at 2000 ppm, with sterile water and Agrimycin as control checks. For the in-vitro study, a 100 μ L aliquot of each treatment was collected at 12, 24, 48, & 72 hours and plated on King's B agar medium and colony forming units (CFUs)/mL were determined after three days incubation to quantify *Xtpvu* growth. The experiment was conducted twice. Results showed that; CuO, MgO and ZnO nanomaterials had antibacterial properties against *Xtpvu*. In greenhouse and the field, all plots were artificially inoculated with *Xtpvu* two days before treatments were applied. To assess development of BLS, 10 randomly selected plants per plot were rated based on the percentage of leaf area with BLS symptoms.

Treatment with nano CuO had the lowest BLS severity followed by nano ZnO and nano MgO and they were statistically significant from the control. These results show that nano CuO has potential to control *Xtpvu*.

2.1 Introduction

Wheat is a grass crop mainly grown for its cereal grain worldwide, occupying about 17% of all crop area. It plays many roles in nature, which include but are not limited to; making leaven flour and flat and steamed breads, providing livestock feed, being, starting materials in fermentations to make beer and other alcoholic beverages, and acting as a feed-stock to produce bioenergy. Thus, it is considered as a staple food crop globally (Mergoum et al. 2009). The rice-wheat (R-W) farming system is a major supplier of digestible energy, and it meets about 30 % of the total protein requirements of the world (Khanal & Maharjan 2015). In South Dakota, 0.77 million acres of spring wheat, which is more than half of 1.4 million acres of total wheat in the state were planted in 2020 (www.nass.usda.gov). Hard red spring wheat is the second largest class of US wheat produced. It is used to blend with other low protein wheat, making yeast breads and hard rolls because of its high protein (12.0 to 15.0%), strong gluten and high-water absorption. Other major products requiring it include pizza crust, bagels, buns, croissants, ramen noodles, and breads. In 2020, South Dakota spring wheat production was >\$180m out of close to \$3B USA total production in 2020 (www.nass.usda.gov).

Bacterial leaf streak (BLS) generally affects cereal crops, and was first observed on barley in 1917 and later in wheat (1919) and rye (1924) and other grass species (Kaur et al. 2020). BLS was first reported in the USA as black chaff in 1919 (Smith et al. 1919). It affects all classes of wheat, but has a great impact on spring wheat if favorable

environmental conditions are present. The most important environmental factors for disease development are high relative humidity and optimum temperature for *Xtpvu* growth. Development of BLS under field conditions is very sporadic, and its impact becomes more severe following storm occurrence in a particular region.

BLS of wheat has been reported in almost all six continents, with the exception of western Europe because of its relatively low temperatures in most wheat-growing areas (Kaur et al. 2020). It affects yield and grain quality in most wheat-growing regions in the USA (McMullen & Adhikari 2011). BLS is challenging to manage since there are no varieties with complete resistance, and yet common chemicals do not provide adequate control.

Although overall rates of wheat yield loss due to plant diseases in the USA have decreased over the last several decades, there have been problems with pesticide resistance, environmental safety, and sustainability issues due to high synthetic chemical application in commercial farming.

The use of nanomaterials is novel and has been found effective on horticultural crops (Singh et al., 2019; El-Argawy et al. 2017; Liao et al. 2019; Elmer et al. 2018; Elmer et al. 2021; Elmer and White 2016; Fu et al. 2020). Using nanomaterials minimizes the number of applications, because the nanomaterials directly target the pathogen, which results in decreased toxicity and reduced cost of production. They can be applied as a novel, green and eco-friendly approach for managing diseases in plants.

Although there is sufficient evidence for efficacy of nanomaterial application for plant disease management in other crops, no data are currently available on the efficacy of nanomaterials controlling BLS in wheat.

Thus, there is a critical need to develop biopesticides for all major staple food crops to reduce chemical toxication, and slow down development of pesticide resistance by having many alternatives, and help ensure climate-smart agriculture for environmental sustainability. The objectives of this study were:

- i) To determine antibacterial activity of nanomaterials on *Xtpvu* in-vitro.
- ii) To evaluate the efficacy of nanomaterials on BLS development in wheat plants.
- iii) To determine the influence of nanomaterials on wheat grain yield.

2.2 Materials and Methods

2.2.1 In-vitro assay

King's B (agar and broth) media (Becton, Dickinson and Company Sparks, MD 21152 USA) was used to culture *Xanthomonas translusens* pv. *undulosa* (*Xtpvu*) which was streaked on KB plates from cryovial stocks (isolated from a wheat field in Brookings County, South Dakota and stored at -80°C) and then transferred to KB broth for inoculum production. Serial dilutions of cells to get viable plate counts were used for establishing a Standard Curve in concert with turbidometric measurements at 546 nm wavelength to get 3×10^6 CFU/mL bacterial cell suspension.

Three nanomaterials were evaluated: nano-zinc oxide (ZnO, 99.8%, 10-30 nm), nano-magnesium oxide (MgO, 99.9%, 10-30 nm), and nano copper oxide (CuO, 99+%, 40 nm) (SkySpring Nanomaterials, Inc, Houston, TX, USA) at 2000 ppm, amended with polyvinylpyrrolidone surfactant (1g/L) in sterile distilled water and sonicated for one hour of continuous sonication at 18 kHz (The Virtis Company, inc. Gardiner, NY. 12525

USA) before application. Sterile H₂O and Agrimycin were control checks. Two mL of each treatment were applied to 18 mL of 0.01M MgSO₄ solution inoculated with 200 µL *Xtpvu* suspension and incubated at 25°C on an orbital shaker at 150 rpm. Treatments were applied in a completely randomized design with four replicates, and the experiment was repeated once.

A 100 µL aliquot of each treatment was collected at 12, 24, 48, and 72 hours and plated on King's B agar medium and CFUs/mL were determined to quantify *Xtpvu* growth.

2.2.2 Greenhouse study

Hard red spring wheat cultivar “ND Frohberg” was planted in the Plant Science greenhouse at South Dakota State University in 2021. Seeds were planted in Coex thermoform square pots of a cell diameter of 10.16 cm and a depth of 12.7 cm, filled with a soil mix PRO-MIX®. Six seeds per pot were planted and kept at 26°C – 30°C with 12 hours light/day.

Xtpvu was streaked on KB plates to get isolated colonies after incubation at 28°C ± 2 for 72 hours and then transferred to KB broth for inoculum production. Serial dilutions to obtain viable plate counts were used for establishing a Standard Curve in concert with turbidometric measurements at 546 nm wavelength to obtain a cell suspension of 3x10⁹ CFU/mL. The inoculum was amended with carborundum at a rate of 1 g/L, and then applied using a mist blower at flag leaf stage to cause mechanical injury on plants for bacterial entry during inoculation, two days before product application. The nanomaterials used were nano ZnO (2000 ppm), nano MgO (2000 ppm), and nano CuO (1000 ppm) amended with polyvinylpyrrolidone surfactant (1 g/L). These were sonicated

for one hour before application. Agrimycin (standard check) and nanomaterial treatments were applied using plastic spray atomizers until leaves were wet with continuous shaking of the products in the sprayer bottle.

Treatments were applied to plants in pots in a completely randomized design with four replicates, and the experiment was repeated twice. Plants were kept in a plastic chamber with a humidifier providing 95% humidity at night for 10 days, and the temperature was kept at 26°C – 30°C to enhance disease development.

To assess BLS, one randomly selected plant per pot/plot was rated based on the percentage of leaf area with BLS symptoms (Bock et al. 2021) 14 days after inoculation. *Xtpvu*'s presence was detected from plant samples, using 0.7 cm discs in symptomatic leaves (Pothier et al., 2011).

2.2.3 Field trial

Hard red spring wheat cultivar “Select” (known for its high susceptibility to BLS in the field) was planted at the Volga Research Farm and Northeast Research Farm (NERF) near Watertown, SD in the spring of 2020 and 2021. Planting of seeds was done using a 7-row tractor-mounted small grain planter fitted with cone units at a seeding rate of 323 / m². The plot size was 1.5 m wide and 4.6 m long. All plots were artificially inoculated at tillering and flag leaf stage (using a mist blower) with *Xtpvu* (3×10^8 CFU/mL) and later amended with carborundum just before spray application at a rate of 1 g/L to cause mechanical injury on plants for bacterial entry during inoculation, two days before treatments were applied. The Volga plots had continuous misting during flowering growth stage in 2021.

Three nanomaterials--nano ZnO (1000 ppm), nano MgO (1000 ppm), and nano CuO (1000 ppm), were sonicated for one hour before application for 2020. In 2021, treatments of nano ZnO (2000 ppm), nano MgO (2000 ppm), and nano CuO (1000 ppm) amended with polyvinylpyrrolidone surfactant and sonicated for one hour before application & agrimycin (standard check) were applied with continuous shaking. Non-inoculated plants and inoculated but not treated plants were also used as control checks. The experiment was conducted as a randomized complete block design with four replications.

To assess BLS, 10 randomly selected plants per plot were rated based on the percentage of leaf area with BLS symptoms, and yield was determined by combine-harvesting each plot and adjusted to bushels per acre at 13.5% moisture content.

2.3 Data analysis

BLS severity data were subjected to analysis of variance using linear mixed model in R-program (software Version 4.0.5) to get the P value of treatments, and then Fisher's Least Significant Difference (LSD) procedure was performed for treatment mean comparisons.

2.4 Results

2.4.1 In-vitro efficacy of nanoparticles against *Xtpvu*

Nano CuO completely inhibited bacterial growth after 24 hours, whereas nano ZnO slightly inhibited growth, while nano MgO inhibited growth the least compared to agrimycin standard check which also completely inhibited bacterial growth at all time intervals. Thus, nano-CuO had the lowest CFUs followed by nano-ZnO and nano-MgO at all time intervals (Figure 2.1).

2.4.2 Efficacy of nanoparticles on BLS in spring wheat greenhouse trial

All nanomaterial treatments were significantly different from control, with ($P < 0.0001$) for BLS severity in the greenhouse experiments.

Nano CuO significantly reduced BLS severity (3.00%), followed by nano ZnO (6.25%) and then nano MgO (7.25%) which had the least effect on BLS severity. Agrimycin (standard check) had the lowest BLS severity (2.75%), and the untreated check had the highest BLS severity (22.50%) (Table 2.1).

2.4.3 Efficacy of nanoparticles on BLS severity in spring wheat under field conditions.

Overall, nanomaterials reduced BLS severity in all site years, with nano CuO and nano ZnO being the most effective. There was an increased BLS severity in 2021 at Volga due to addition of misting which provided a favorable environment for BLS development (Table 2.3 and Table 2.5).

There was higher BLS severity at Volga than NERF in both 2020 and 2021. No statistical differences were observed among treatments at Volga in 2020. Nano ZnO (11.66%), and nano CuO (14.18%) had numerically low BLS severity in 2020. Also, no statistical differences were observed at NERF in 2020, with nano CuO (7.6%), having numerically lowest BLS severity (Table 2.3).

In 2021, statistical differences were observed among treatments at Volga, with nano CuO (10.03%) being the most effective in reducing BLS severity. Statistical differences were also observed at NERF in 2021, with nano MgO (5.7%), and nano CuO (6.78%) being the most effective in reducing BLS severity (Table 2.5).

Yields were not significantly different among nanomaterial treatments, for both locations in 2020 and 2021. However, nano MgO and nano CuO had numerically higher yields at Volga, and nano MgO had the highest yield at NERF (Table 2.3 and Table 2.5).

2.5 Discussion

This study focused on evaluating the influence of nanomaterials on bacterial growth, and their efficacy on bacterial leaf streak severity and impact on yield.

For the in-vitro assay, nanomaterial treatments caused statistically significant reduction of *Xtpvu* in-vitro growth. Hence, we have sufficient evidence to accept that at least there is an interaction among these nanomaterial treatments and *Xtpvu*. Nano CuO, nano MgO, and nano ZnO had antibacterial properties against *Xtpvu* under greenhouse and field conditions, and these results are consistent with previous research (Liao et al. 2019). Zinc oxide nanofluids have been reported to have bacteriostatic activity against *E. coli* (Zhang et al. 2007; Povey and York 2008). Pandey et al. (2012) reported that a sonicated CuO nanoparticle suspension enhanced bactericidal efficacy against Gram-positive and Gram-negative waterborne disease-causing bacteria such as *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus* and *Bacillus subtilis*. This is consistent with the findings of this study. Like our study, Elmer et al. (2021) also reported that CuO nanoparticles treatments were associated with an increase in fruit yield and disease severity suppression. Similar results were obtained in the study of Elmer et al. (2018) where plants treated with CuO nanoparticles yielded 39%, and 53% more fruit in Hamden, CT. It was further shown that all nanoparticle treatments (B, CuO, MnO, and ZnO) significantly reduced the disease ratings relative to the control, with CuO nanoparticles having significantly lower rank.

In the work of Liao et al. (2019), for in-vitro assay of Cu – tolerant *Xanthomonas perforans* strain GEV485, nano CuO was the most effective in limiting bacterial growth. Similar results were reported by Baek & An (2011) where nano MgO and nano ZnO showed little inhibition. The limited bacterial growth inhibition by nano ZnO and nano MgO may be due to differences in the time of exposure to nanomaterials and the bacterial sensitivity to copper. This is further confirmed in the same study which showed a similar trend to this study's result with Cu – sensitive *Xanthomonas perforans* strain 91 - 118.

Increasing the rate of nano ZnO and nano MgO to 2000 ppm, with addition of polyvinylpyrrolidone surfactant improved the efficacy of these treatments in 2021 compared to 2020 with only nano ZnO and nano MgO at 1000 ppm. This is similar to the results of Dutta et al. (2013) who observed that the choice of capping agent, and presence of hydroxyl groups were important parameters for synthesizing nano ZnO and their consequential antibacterial activity. This is consistent with our study's results in 2021 which had improved antibacterial efficacy due to addition of a surfactant.

Treatments did not significantly impact yield in both years. This most likely was due to relatively low BLS pressure in all plots for both our elevated level of BLS inoculum and naturally occurring BLS inoculation.

Our results show that nano CuO has potential to control *Xtpvu* based on the in-vitro, greenhouse, and field results. However, in-vitro conditions had higher antibacterial activity for all treatments when compared with their efficacy under field conditions. This may be due to the influence of uncontrolled environmental conditions. Addition of a misting system increased BLS severity due to the presence of favorable environmental conditions. However, more testing on the different application rates and timing may be

needed. Also, more studies on the effect of nanomaterials on other epiphytic and endophytic microbiomes in wheat are needed.

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Table 2.1 Mean comparisons for bacterial leaf streak (BLS) severity caused by *Xanthomonas translucens* pv. *undulosa* in spring wheat ‘ND Frohberg’ under greenhouse conditions after application of nanomaterials in 2022.

Treatment	BLS severity (%) ^a
Control (untreated)	22.50a
NanoMgO	7.25b
NanoZnO	6.25bc
NanoCuO	3.00cd
Agrimycin	2.75d

^a For each treatment within a column, means with different letters are significantly different, according to Fisher’s Least Significant Difference (LSD) procedure at $P = 0.05$.

Table 2.2 Analysis of variance of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of nanomaterials at two South Dakota State University agricultural research stations in 2020.

	Volga				Northeast research farm			
	BLS severity (%)		Yield (bu/acre)		BLS severity (%)		Yield (bu/acre)	
	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)
Treatment	1.369	0.31	1.34	0.30	0.52	0.76	0.75	0.60

Least significance level of $\alpha = 0.05$.

Table 2.3 Mean comparisons of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of nanomaterials at two South Dakota State University agricultural research stations in 2020.

Treatment	Volga		Northeast research farm	
	BLS severity (%)	Yield (bu/acre)	BLS severity (%)	Yield (bu/acre)
NanoZnO	11.66a	11.62ab	14.20a	50.80a
Check-Inoculated	16.88a	12.87ab	10.80a	50.40a
Check-NonInoculated	8.80a	9.80b	10.50a	50.10a
NanoMgO	14.48a	15.07ab	7.80a	52.20a
NanoCuO	14.18a	15.50a	7.60a	50.10a
Agrimycin	9.32a	12.24ab	4.00a	48.80a

“Select” as the Cultivar was planted at both locations. For each treatment within a column, means with different letters are significantly different, Fisher’s Least Significant Difference (LSD) procedure at $p = 0.05$.

Table 2.4 Analysis of variance of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of nanomaterials at two South Dakota State University agricultural research stations in 2021.

	Volga				Northeast research farm			
	BLS severity (%)		Yield (bu/acre)		BLS severity (%)		Yield (bu/acre)	
	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)
Treatment	1.51	0.244	1.53	0.24	3.14	0.04*	0.30	0.91

Least significance level of $\alpha = 0.05$.

Table 2.5 Mean comparisons of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of nanomaterials at two South Dakota State University agricultural research stations in 2021.

Treatment	Volga		Northeast research farm	
	BLS severity (%)	Yield (bu/acre)	BLS severity (%)	Yield (bu/acre)
NanoZnO	11.70ab	15.09a	19.18a	35.23a
Check-Inoculated	23.95a	10.99a	17.58ab	33.73a
Check-NonInoculated	6.45b	19.26a	0.28c	35.06a
NanoMgO	9.53ab	17.16a	5.70bc	35.01a
NanoCuO	10.03ab	18.28a	6.78abc	39.11a
Agrimycin	10.75ab	11.23a	3.79c	35.18a

“Select” as the Cultivar was planted at both locations. For each treatment within a column, means with different letters are significantly different, Fisher’s Least Significant Difference (LSD) procedure at $p = 0.05$.

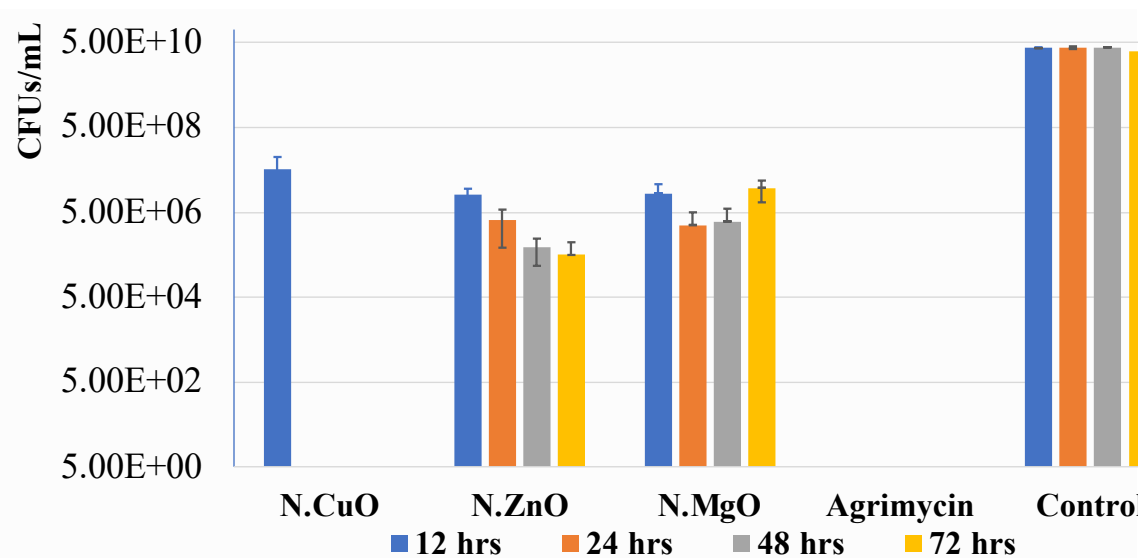


Figure 2.1 In vitro effect of Nanomaterials (copper oxide, zinc oxide, magnesium oxide) on *Xanthomonas translucens* pv. *undulosa* growth after 12 hours, 24 hours, 48 hours, and 72 hours.

CFU = colony forming units, N.CuO = Nano Copper oxide, N.ZnO = Nano Zinc oxide, N.MgO = Nano Magnesium oxide, under in-vitro conditions. Error bars represent Standard Error of the Mean (SEM).

Chapter 3

3.0 Efficacy of biorationals on bacterial leaf streak in wheat

ABSTRACT

Bacterial leaf streak (BLS) is a challenging disease to manage since common chemicals do not provide adequate control. Some plant extracts have been shown promise to provide a sustainable environmentally friendly control of animal and plant diseases. However, limited data are available on the efficacy of plant extracts controlling BLS in wheat. The objective of this study was to evaluate the antibacterial activity of selected biorationals against *Xtpvu*. Field studies were conducted with seven treatments: moringa, spirulina, ginseng, and tannic acid, with Agrimycin as a standard check, and inoculated and non-inoculated controls. All plots were artificially inoculated with *Xtpvu* two days before treatments were applied at flag leaf growth stage. To assess BLS, 10 randomly selected plants per plot were rated based on the percentage of leaf area with BLS symptoms. Yield for each plot was determined at harvest.

Moringa and tannic acid had the highest antibacterial effect among all plant extracts tested. Although there were no statistical differences between the effect of different biorationals on BLS severity and yield, these results show that tannic acid and moringa may have potential to control *Xtpvu* and warrant further research on rates and timing of these plant extracts.

3.1 Introduction

Biorational pesticides are natural products and chemical compounds which were developed due to the discovery of pathogen molecular mechanisms and biological control, and their efficacy can be determined by methods including observing zones of growth inhibition in confluent lawns of target microbe grown on an agar medium (Gardener & Fravel 2002).

Ginseng inhibits/controls bacterial diseases through its anti-quorum sensing activity (Song et al. 2010). In addition to affecting quorum sensing (QS) and biofilm formation, it was reported that American Radix Ginseng inhibited in-vitro growth of *Pseudomonas aeruginosa* (Wu et al. 2014).

Tannins are polyphenolic secondary plant metabolites with metal-chelating and antimicrobial properties which can be applied to control plant pathogens (Lim et al. 2013). Tannins can be derived from plant leaves, roots, wood, bark, fruits, and buds (Kraus et al. 2003), and includes tannic acid. They have strong antimicrobial properties through inhibiting biofilm formation, and they also affect the synthesis of cell wall (Payne et al. 2013; Dong et al. 2018). Tannins also work by protecting tissues from microbial attack, having direct toxicity to microbes, deactivating microbial exoenzymes, and by precipitation of essential metal ion micronutrients to starve plant pathogens (Kraus et al. 2003; Field & Lettinga 1992; Scalbert 1991; McDonald et al. 1996; Smith et al. 2005).

Funatogawa et al. (2004) reported that plant-derived hydrolysable tannins have antibacterial effects against *H. pylori*, and Kraus et al. (2003) also reported that the tannin concentrations required to inhibit microbial populations varied. A range of (0.31– 12 g

L⁻¹) inhibited fungi, (0.01–20 g L⁻¹) was effective against bacteria, and concentrations required to induce enzyme inhibition ranged from (0.1 to 2 g L⁻¹) and varied by both the type of enzyme and tannin. While Wu et al (2010) stated that tannic acid showed the strongest inhibition on FabG with a half inhibition concentration of 0.78 mM (0.81 mg/mL), tannic acid inhibited Gram-positive bacteria more effectively than Gram-negative bacteria.

Moringa (*Moringa oleifera*) leaf extract (MLE) is a potential antibacterial agent because of its high content of phytohormones, phenolics and minerals (Nasir et al. 2016). MLE has also been reported as an effective agent against most microbes (Farooq et al. 2012; El-Mohamedy & Abdalla 2014; Rikhotso et al. 2019; Tesfay & Magwaza 2017).

Spirulina (*Arthrospira platensis*, *Arthrospira maxima*) contains polyphenols and polysaccharides that are responsible for its antimicrobial activities (Hlima et al. 2019; Bajpai, 2016; Pagnussatt et al. 2016; Battah et al. 2014). Spirulina's low toxicity (Ali & Saleh 2012) makes it a good candidate for BLS management in wheat.

Ginseng (*Panax quinquefolius*) is an important medicinal plant in many aspects, ranging from its use as an antimicrobial agent to its health promotion benefits as a food additive (Gillis 1997; Kim & Park 2011; Thomson 2010; Kitts & Hu 2000; Kim & Yang 2018; Szczuka et al. 2019; Mehta et al. 2021). Ginsenosides are the major secondary metabolites of *Panax sp.* Ginsenosides are known to be involved in all beneficial effects of ginseng (Kim & Yang 2018; Shahrajabian et al. 2019).

However, there is not sufficient information on bacterial leaf streak of wheat management using these biorationals. The objectives of this study were:

- i) To evaluate the efficacy of biorationals on BLS development in wheat plants.
- ii) To determine the influence of biorationals on wheat crop yield.

3.2 Materials and methods

3.2.1 Field trial

Hard red spring wheat cultivar “Select” (known for its high susceptibility to BLS in the field) was planted at the Volga Research Farm and Northeast Research Farm (NERF) in the spring of 2020 and 2021. Planting of seeds was done using a 7-row tractor-mounted small grain planter fitted with cone units at a seeding rate of 323 m⁻². The plot size measured 1.5 m wide and 4.6 m long. All plots were artificially inoculated at tillering and flag leaf stage (using a mist blower) with *Xtpvu* (3x10⁸ CFU/mL), and was later amended with carborundum at a rate of 1 g/L to cause mechanical injury on plants for bacterial entry during inoculation, which was two days before treatments were applied (Volga plots had continuous misting in 2021).

The efficacy of plant extracts including ginseng, moringa, & spirulina (purchased from MAJU super foods, Amazon), and tannic acid (Sigma Aldrich) were evaluated.

Four plant extracts (ginseng, spirulina, moringa, and tannic acid) and agrimycin (standard check) were spray-applied in the field with continuous shaking. Non-inoculated and inoculated but not treated were also used as control checks.

All products were mixed with water in conical flasks at a rate of 1:10 (v/v) product/water, except tannic acid which was applied at 285.7 mg/mL, and were then placed on a rotary shaker at 150 rpm at 25 ° C for 24 hours. The extracts were later filtered through

cheesecloth in a chemical fume hood to produce the filtrate for field application. The experiment was conducted as a randomized complete block design with four replications. To assess BLS, 10 randomly selected plants per plot were rated based on the percentage of leaf area with BLS symptoms, and yield was determined by combine-harvesting each plot and adjusted to bushels per acre.

3.3 Data analysis

BLS severity data were subjected to analysis of variance using linear mixed model in R-program (software Version 4.0.5) to get the P value of treatments, and then Fisher's Least Significant Difference (LSD) procedure was performed for treatment mean comparisons.

3.4 Results

3.4.1 Efficacy of biorationals in spring wheat field experiment

There was higher BLS severity at Volga than NERF in both 2020 and 2021. Statistical differences were observed among treatments at Volga in 2020 (Table 3.2 and Table 3.4). Moringa (11.73%), spirulina (8.2%) and tannic acid (14.43%) were the most effective in reducing BLS severity in 2020 (Table 3.2).

However, no statistical differences were observed at NERF in 2020, with moringa (8.975%), spirulina (6.2%) and tannic acid (0.75%) having numerically low BLS severity (Table 3.2).

In 2021, statistical differences were observed among treatments at Volga, with moringa (16.13%), and tannic acid (16.75%) being the most effective in reducing BLS severity. Statistical differences were also observed at NERF in 2021, with moringa (4.1%),

spirulina (6.488%) and tannic acid (3.34%) being the most effective in reducing BLS severity (Table 3.4).

Yields were not significantly different among treatments, for both locations in 2020 and 2021, except higher yield was observed in non-inoculated plots, and the lowest yield in inoculated-not treated plots at Volga in 2021 (Table 3.2 and Table 3.4).

3.5 Discussion

Plant extracts have been extensively used in traditional medicine globally, although their application to control wheat diseases is not yet extensively studied.

We found statistically significant differences among the treatments, where tannic acid was the most effective in reducing disease severity and increasing yield in three out of the four site years. Its inefficiency in one of the site years could be due to the BLS bacterium's ability to overcome tannin's inhibition/bactericidal effect by tannin degradation, dissociation of tannin–substrate complexes, tannin inactivation by high-affinity binders, membrane repair and/or metal ion sequestration (Smith et al. 2005). Moringa extract also reduced disease severity and improved yields as compared to the control, and this agrees with Rikhotso et al. (2019) who reported that carboxymethyl cellulose (CMC) incorporated with moringa leaf extracts (M) significantly reduced peteca spot incidence on 'Eureka' lemon. Nasir et al. (2016) also observed that combined application of moringa leaf extract, K and Zn in 'Kinnow' mandarin trees at the fruit set stage resulted in significantly lower fruit drop and higher fruit set, yield, fruit weight, juice weight, soluble solid contents, vitamin C, sugars, total antioxidants, and total phenolic contents. This could be a result of moringa leaf extract's enrichment with phytohormones, phenolics and minerals. It was further shown that all fungal mycelial

growth gradually decreased with an increase in concentration of moringa roots extract and moringa leaves extract (El-Mohamedy & Abdalla 2014).

Spirulina was another plant extract that showed significant effect on disease severity and yield. There was reduced *Fusarium* spp mycelial growth rate in the presence of free phenolic extract obtained from Spirulina (3% and 8%) which showed inhibition of 37% and 68%, respectively (Pagnussatt et al. 2016). Furthermore, Battah et al. (2014) reported that partially purified agent of *Spirulina maxima* showed a broad spectrum of antifungal activity, with an average activity of 26% inhibition against five tested human and plant pathogenic fungi compared to the three tested commercial drugs. The most inhibited fungus was *P. oxalicum* (91%) followed by *F. solani* (65%) and *R. solani* (20%) compared to the tested antifungal drugs. Blanc & Cock (2021) also reported that aqueous spirulina extract was a particularly good inhibitor of *P. mirabilis*, with MIC values as low as 220 µg/mL, although it was found ineffective against other bacterial species tested, which shows its potential to be selective.

Therefore, results of our study support similar research where plant extracts have antimicrobial potential, especially if applied in combination with other products that can boost their efficacy.

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Table 3.1 Analysis of variance of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of plant extracts at two South Dakota State University agricultural research stations in 2020.

	Volga				Northeast research farm			
	BLS severity (%)		Yield (bu/acre)		BLS severity (%)		Yield (bu/acre)	
	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)
Treatment	2.75	0.06	2.95	0.05	0.86	0.54	0.42	0.86

Least significance level of $\alpha = 0.05$.

Table 3.2 Mean comparisons of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of plant extracts at two South Dakota State University agricultural research stations in 2020.

Treatment	Volga		Northeast research farm	
	BLS severity (%)	Yield (bu/acre)	BLS severity (%)	Yield (bu/acre)
Ginseng	15.45ab	11.21b	9.30a	48.27a
Check-Inoculated	16.88a	15.83ab	10.80a	50.42a
Check-NonInoculated	8.80c	12.628b	10.48a	50.08a
Moringa	11.73abc	14.29b	8.98a	48.79a
Spirulina	8.20c	13.86b	6.20a	49.68a
Agrimycin	9.32bc	15.44b	4.03a	48.80a
TannicAcid	14.43abc	21.84a	0.75a	47.02a

“Select” as the Cultivar was planted at both locations. For each treatment within a column, means with different letters are significantly different, Fisher’s Least Significant Difference (LSD) procedure at $p = 0.05$.

Table 3.3 Analysis of variance of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of plant extracts at two South Dakota State University agricultural research stations in 2021.

	Volga				Northeast research farm			
	BLS severity (%)		Yield (bu/acre)		BLS severity (%)		Yield (bu/acre)	
Treatment	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)	F value	Pr(>F)
	1.46	0.25	1.92	0.01	2.05	0.11	0.36	0.89

Least significance level of $\alpha = 0.05$.

Table 3.4 Mean comparisons of spring wheat ‘Select’ bacterial leaf streak (BLS) severity (percentage) caused by *Xanthomonas translucens* pv. *undulosa* and yield (bu/acre) after application of plant extracts at two South Dakota State University agricultural research stations in 2021.

Treatment	Volga		Northeast research farm	
	BLS severity (%)	Yield (bu/acre)	BLS severity (%)	Yield (bu/acre)
Ginseng	18.55ab	11.78b	7.63ab	34.38a
Check-Inoculated	23.95a	10.99b	17.58a	33.73a
Check-NonInoculated	6.45b	19.26a	0.28b	35.06a
Moringa	16.13ab	11.81b	4.10b	32.61a
Spirulina	19.00ab	112.87b	6.49ab	33.58a
Agrimycin	10.75ab	11.23b	3.79b	35.18a
TannicAcid	16.75ab	13.07b	3.34b	29.86a

“Select” as the Cultivar was planted at both locations. For each treatment within a column, means with different letters are significantly different, Fisher’s Least Significant Difference (LSD) procedure at $p = 0.05$.

Chapter 4

4.0 Aggressiveness of *Xanthomonas translucens* pv. *undulosa* isolates and differential reaction among spring wheat varieties in controlled environment.

ABSTRACT

Xanthomonas translucens pv. *undulosa* (*Xtpvu*) causes bacterial leaf streak (BLS) in wheat which has caused up to 40% losses globally. Understanding the aggressiveness of isolates from different locations in the state may help in screening for BLS resistance in wheat breeding programs. A greenhouse study was conducted to determine aggressiveness of isolates collected from South Dakota on spring wheat ‘SY Rockford’ and differential reaction of *Xtpvu* on 21 spring wheat varieties at the SDSU Plant Science greenhouse in 2021 and 2022. The 17 isolates were prepared as inocula amended with carborundum and inoculated using mist blower at flag leaf growth stage. The experiment was conducted as a randomized complete design with four replications. To assess BLS severity, one randomly selected plant per pot was rated based on the percentage of leaf area with BLS symptoms.

The results show significant differences among the bacterial isolates, with *Xtpvu*21OC (23.33%) as the most aggressive in causing BLS in wheat. ‘MN Washburn’ (25.0%) and ‘ND Frohberg’ (22.5%) were the most susceptible wheat varieties. These results show differences in aggressiveness among the *Xanthomonas translucens* pv. *undulosa* strains, and the most aggressive isolate should be used for screening resistance.

4.1 Introduction

Wheat is severely affected by BLS in the USA's Northern plains. Natural variation in aggressiveness of pathogen isolates among different geographic regions could be a reason for severe outbreaks in certain areas. Aggressiveness of isolates could have differences due to genetic and environmental effects (Finckh & Nelson 1999; Cowger & Mundt 2002; Pariaud et al. 2009). Bragard et al. (1997) observed that xanthomonads isolated from small grains are phylogenetically closely related bacteria, and the pathovar and the pathogenicity type groupings still correspond to true biological entities.

Virulence assay of *Xtpvu* was assessed by using a leaf-clipping method with scissors dipped in bacterial suspension, and using sterile water as a control (Hu et al. 2007; Ray et al. 2000), and employing infiltration method and foliar inoculation. However, Shah et al. (2021) observed a greater diversity in the virulence determinants and pathogenicity repertoires among the worldwide population of *Xanthomonas translucens* than the one that had been described before. For example, significant variations were found among the 14 isolate *Xanthomonas translucens* dataset in membrane fusion and ABC transporter genes, where these genes were lacking in the *Xanthomonas translucens* pv. *translucens* strains (XtKm8 and XtKm34) but present in XtKm9 and the reference strain DSM 18974T (Shah et al. 2021).

Xtpvu isolates from different wheat fields were evaluated for their relative pathogenicity on spring wheat, and all isolates were pathogenic (Raja et al. 2010). Kaewnum et al. (2005) reported the diversity of *Xanthomonas axonopodis* pv. *glycines* with regard to pathogenicity on soybean and induction of the hypersensitive response (HR) on several plant species. A similar study was conducted on rice where all isolates of *Xanthomonas*

oryzae were pathogenic on all tested varieties of rice, with differences in their aggressiveness (Jabeen et al. 2011). Stromberg et al. (1999) observed that pathogen inoculum density determined the time required for visible BLS symptoms to develop on wheat leaves. Various *Xanthomonas* strains such as *Xanthomonas campestris* pv. *graminis*, *Xanthomonas campestris* pv. *phleipratensis*, and *Xanthomonas campestris* pv. *poae* are related, but they were also found distinguishable by RFLP patterns, serology, and pathogenicity on bread wheat (Bragard et al. 1995).

The goal of this study was to assess aggressiveness of different isolates of *Xanthomonas translucens* pv *undulosa* on spring wheat. While the assessment of variation in aggressiveness of *Xtpvu* and wheat variety susceptibility has been studied in other regions, no data are available on *Xtpvu* aggressiveness among local isolates in South Dakota.

4.2 Materials and Methods

A collection of 17 *Xtpvu* isolates were obtained within South Dakota as follows: 10 *Xtpvu* isolates were collected from winter wheat breeding lines having variation in BLS symptom expression at Volga Research Farm, South Dakota State University. Seven isolates were collected from spring wheat across the state of South Dakota. Hard red spring wheat cultivar “SY Rockford” (standard susceptible variety) was planted in the Plant Science greenhouse at South Dakota State University in 2021.

10 mm leaf discs with BLS symptoms were surface sterilized in 70% ethanol, placed on King’s B (KB) agar medium using sterile forceps, and incubated at 28°C for 72 hours to obtain *Xtpvu* growth. *Xtpvu* was streaked on KB plates to get isolated colonies, and then

transferred to KB broth for inoculum production, as described in previous sections of this thesis.

For the variety screening experiment, 21 spring wheat varieties were planted in the Plant Science greenhouse at South Dakota State University in 2021 and 2022. Seeds were planted in Coex thermoform square pots with a cell diameter of 10.16 cm and a depth of 12.7 cm, filled with a soil mix PRO-MIX®. Six seeds per pot were planted and kept at 26°C – 30°C with 12 hours light/day.

To prepare bacterial inoculum, serial dilutions were performed to get 3×10^9 CFU/mL and were amended with carborundum at a rate of 1 g/L and then used to inoculate plants using a mist blower at flag leaf stage.

Treatments (isolates) were applied to plants in pots in a completely randomized design with four replicates, and the experiment was repeated twice. Plants were kept in a plastic chamber with a humidifier providing 95% RH at night for 10 days, and the temperature was kept at 26°C – 30°C to enhance disease development.

To assess BLS, three upper leaves of one randomly selected plant per pot were rated based on the percentage of leaf area with BLS symptoms (Bock et al. 2021) 14 days after inoculation. *Xtpvu*'s presence was detected from plant samples, using 0.7-cm discs from symptomatic leaves (Pothier et al. 2011).

4.3 Data analysis

BLS severity data were subjected to analysis of variance using linear mixed model in R-program (software Version 4.0.5) to get the P value of treatments, and then Fisher's Least Significant Difference (LSD) procedure was performed for treatment mean comparisons.

4.4 Results

All isolates caused symptoms on 'SY Rockford' variety. Symptoms were observed 4 to 7 days after inoculation. Some isolates produced mild symptoms on the standard susceptible variety under greenhouse conditions.

There were significant differences in BLS severity among the isolates ($P < 0.0001$).

Xtpvu21OC had the highest BLS severity (most aggressive) (Table 4.1 below). 'MN Washburn' (25.0%) and 'ND Frohberg' (22.5%) experienced the highest BLS severity (were the most susceptible varieties) (Table 4.2).

The origin of the isolate did not have an influence on its aggressiveness (Table 4.1).

4.5 Discussion

Several bacterial isolates were significantly different in causing BLS in wheat. Our results are consistent with those of Curland et al. (2020) who observed that there was a significant population variance detected between populations of *Xanthomonas translucens* pv. *undulosa* collected from different wheat fields. Adhikari et al. (2012) also reported that all strains tested were highly aggressive on the susceptible wheat line 'ND495'. According to Khojasteh et al. (2020) *Xtpvu* strains showed severe aggressiveness on both barley and wheat plants, and this confirms higher genetic diversity of *Xtpvu*. It has been reported that genetically distinct strains exist within each group (Alizadeh et al. 1997; Alizadeh et al. 1995). Like our study, Kaewnum et al. (2005) observed that the geographical origin of isolates did not appear to be related to their relative pathogenicity.

Some isolates produced mild symptoms, which may not significantly affect yields as was observed in our field studies reported in Chapter Two and Chapter Three. Mild BLS symptoms result in low yield losses (Tillman 1994).

It was interesting to see bacterial isolates from one location with different breeding lines having differences in aggressiveness. This may be due to the genetics of the host; moderately resistant cultivars were reported to select for more aggressive isolates (Cowger & Mundt 2002).

In our study, some varieties were moderately susceptible. This correlates with Adhikari et al. (2012) who reported that wheat cultivars showed differential responses to several bacterial strains tested. This could be due to partial resistance to *Xtpvu*. Thus, wheat cultivars with partial BLS resistance, if integrated with other control measures, could minimize the impact of BLS on wheat.

Therefore, further molecular studies need to be conducted for detailed characterization of *Xanthomonas translucens* pv. *undulosa* strains in wheat for better disease control and management.

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Table 4.1 Mean comparisons for aggressiveness of *Xanthomonas translucens* pv. *undulosa* isolates in causing bacterial leaf streak (BLS) in spring wheat ‘SY-Rockford’ under greenhouse conditions in 2021.

Isolate	Source	BLS severity (%)
Xtpvu21OC	Brookings	23.33a
Xtpvu21X9	Volga	20.00ab
Xtpvu21CS	Brookings	18.33abc
Xtpvu21X2	Volga	18.33abc
Xtpvu21N	Watertown	16.67abcd
Xtpvu21W	Watertown	16.67abcd
Xtpvu21X7	Volga	15.00bcde
Xtpvu21PR	Pierre	11.67cdef
Xtpvu21X4	Volga	10.67def
Xtpvu21X6	Volga	10.67def
Xtpvu21X5	Volga	10.00def
Xtpvu21T	Brookings	8.33ef
Xtpvu21X1	Volga	8.33ef
Xtpvu21OCG	Brookings	7.33f
Xtpvu21X3	Volga	7.33f
Xtpvu21X10	Volga	6.67f
Xtpvu21X8	Volga	6.33f

Treatment means values at South Dakota State University plant science greenhouse using “SY-Rockford” as the Cultivar. For each treatment within a column, means with different letters are significantly different, same letters are not significantly different, multiple letters are not significantly different from treatments with those letters according to Fisher’s Least Significant Difference (LSD) procedure.

Table 4.2 Mean comparisons for bacterial leaf streak (BLS) severity caused by *Xanthomonas translucens* pv. *undulosa* in spring wheat varieties under greenhouse conditions in 2022.

Varieties	BLS severity(%age) ^a
MN Washburn	25.00a
ND Frohberg	22.50a
ND Vitpro	16.25b
Lang MN	15.00bc
Shelly	13.75bcd
Driver	11.75bcde
Select	11.75bcde
Surpass	10.50cde
Faller	8.75def
Focus	8.00efg
Bolles	6.75efgh
SY-Rockford	6.75efgh
SD4873	5.00fghi
Forefront	4.00fghi
WB9719	3.50ghi
Boost	3.25ghi
LSC Trigger	3.00ghi
Prevail	3.00ghi
Advance	2.50hi
MN-Torgy	2.00hi
SY Valda	1.50i

^a For each treatment within a column, means with different letters are significantly different, multiple letters are not significantly different from treatments with those letters according to Fisher's Least Significant Difference (LSD) procedure at $P = 0.05$.

Chapter 5

5.0 Conclusions and Recommendations

Nanomaterials had strong antibacterial activity under in-vitro, greenhouse, and field conditions. Nano CuO particles were the most effective. Therefore, nano CuO can be applied as a potential antibacterial agent to control BLS in wheat.

Amendment of nanoparticles with polyvinylpyrrolidone surfactant improved the nanoparticle's efficacy, thus pretreatment of nanoparticles with dispersing agents or coatings can reduce agglomeration and improve their efficacy.

Misting, higher humidity and warm temperatures provided a favorable environment for BLS development. Therefore, it's more appropriate to apply antibacterial sprays at flag leaf growth stage when the plants are more susceptible under favorable environmental conditions.

Addition of carborundum to the *Xtpvu* inocula increased chances of BLS development. This implies that environmental conditions such as storms which cause mechanical injuries on plants can increase BLS development in wheat.

No significant differences were observed in yields among nanomaterial treatments, which was likely due to low BLS severity. Therefore, controlling BLS to low disease pressure can result in low economic wheat grain yield loss.

Moringa, spirulina and tannic acid were the most effective in reducing BLS severity. Therefore, further research on these products on concentration and frequency of application to increase their efficacy should be done. These have potential for BLS management in wheat, especially when used concurrently with other cultural disease

control practices or when amended with other antibacterial agents in moderately resistant cultivars.

All pathogen isolates caused BLS symptoms on 'SY Rockford' variety, and the origin of the isolate did not have an influence on its aggressiveness.

'MN Washburn' and 'ND Frohberg' experienced the highest BLS severity (were the most susceptible varieties). Therefore, they can be used as control checks while screening for resistance to BLS in spring wheat breeding programs.

Further molecular studies need to be conducted for detailed characterization of *Xanthomonas translucens* pv. *undulosa* strains that are pathogens of wheat for better understanding of aggressiveness differences between strains. This could be due to different variants within the same species.

Additionally, detailed testing of the different nanoparticle materials regarding application rates and timing, of nanomaterials and plant extracts in combination with common pesticides applied in wheat such as fungicides and insecticides in tank mix is needed. This would allow for tank mixing and saving on application costs.

Appendix

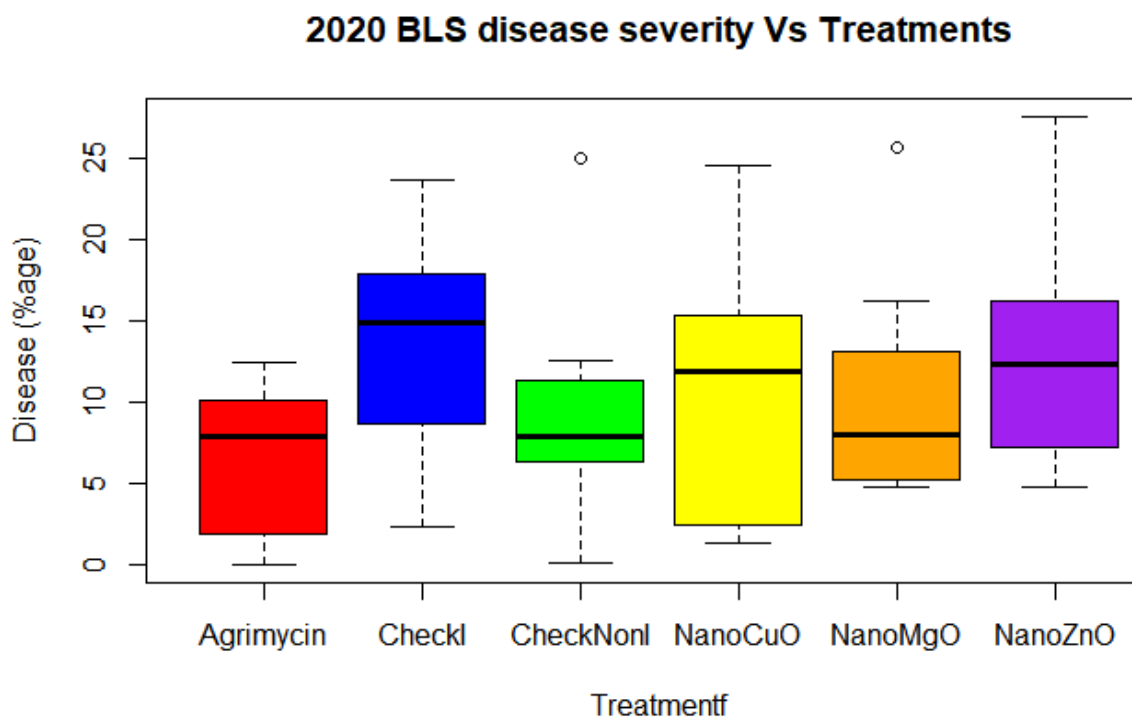


Figure 5.1 Effects of nanoparticle treatments on bacterial leaf streak (BLS) disease severity (percentage) at two locations in 2020

CheckI = Inoculated & not treated, CheckNonI = Non inoculated and not treated.

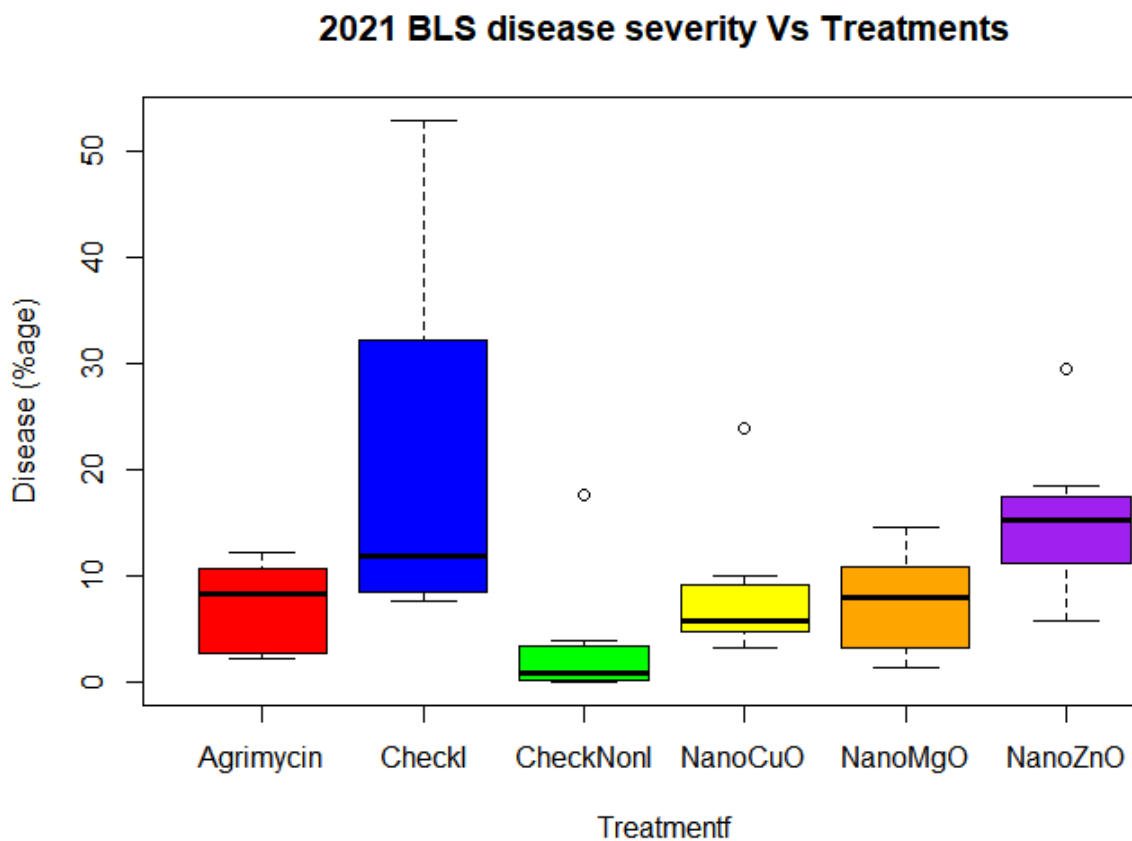


Figure 5.2 Effects of nanoparticle treatments on bacterial leaf streak (BLS) disease severity (percentage) at two locations in 2021

CheckI = Inoculated & not treated, CheckNonI = Non inoculated and not treated.

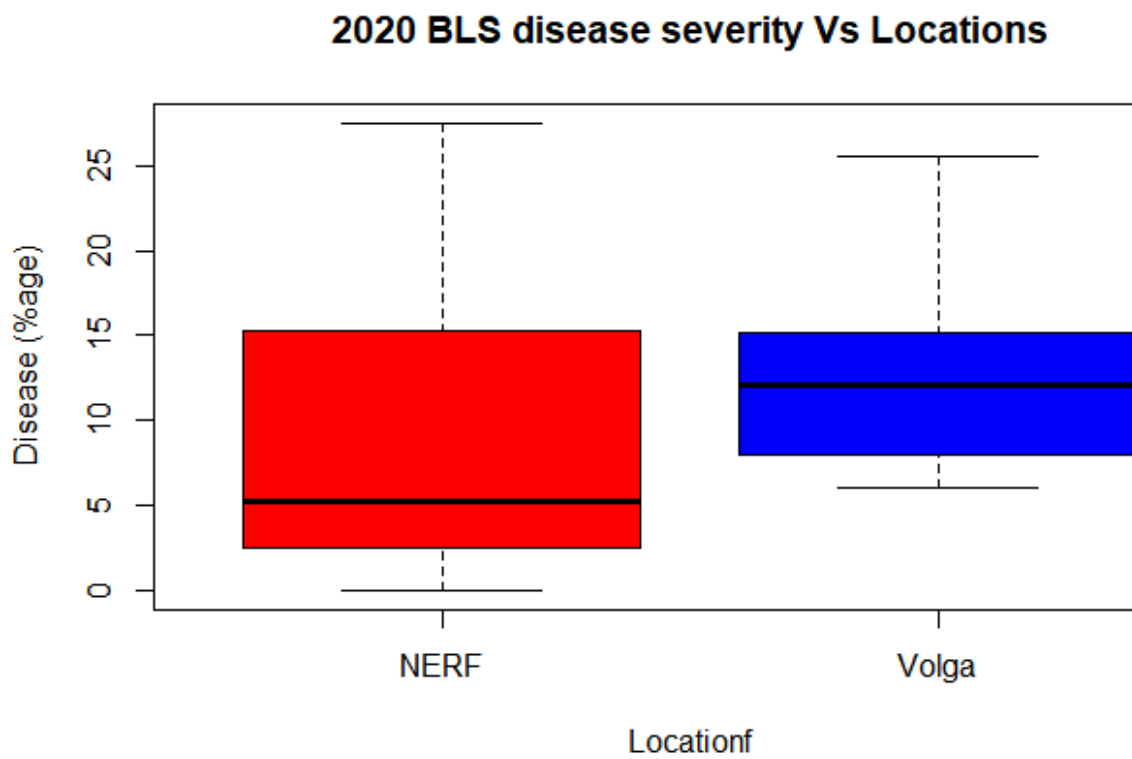


Figure 5.3 Boxplots of bacterial leaf streak (BLS) disease severity (percentage) at two locations in 2020

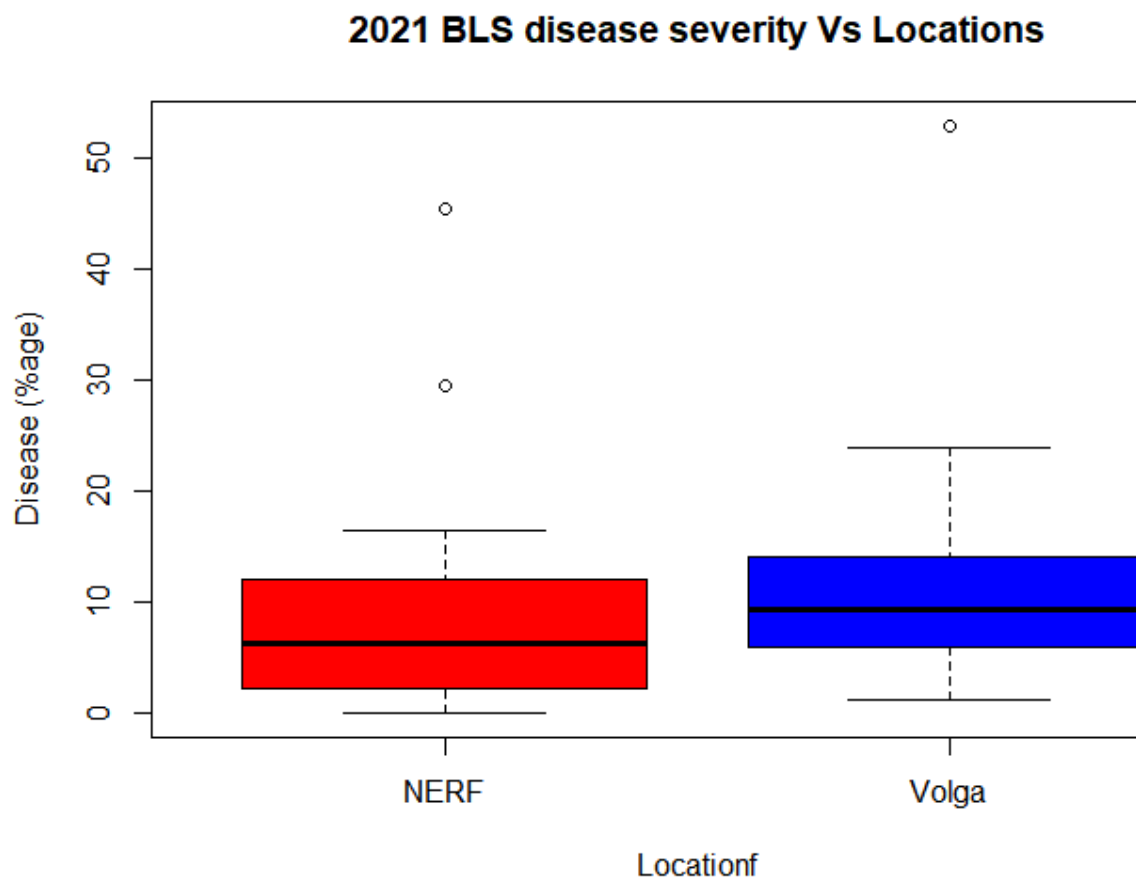


Figure 5.4 Boxplots of bacterial leaf streak (BLS) disease severity (percentage) at two locations in 2021

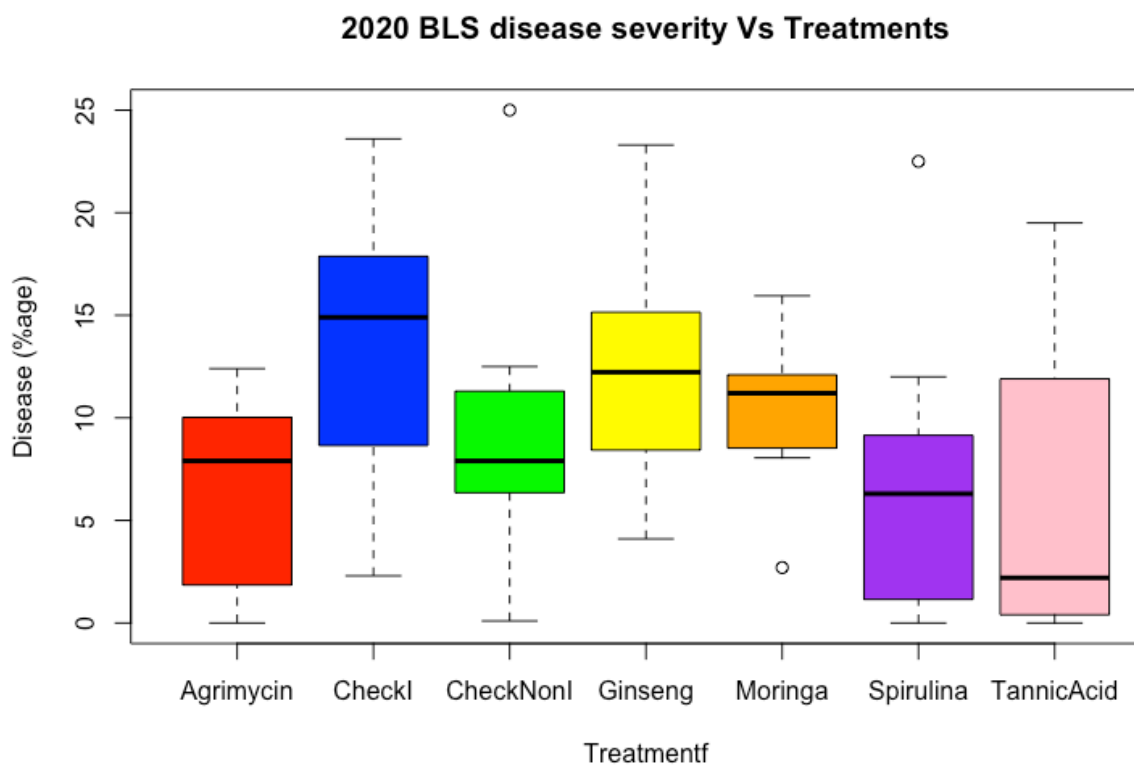


Figure 5.5 Effects of plant extracts treatments on bacterial leaf streak (BLS) disease severity (percentage) at two locations in 2020

CheckI = Inoculated & not treated, CheckNonI = Non inoculated and not treated.

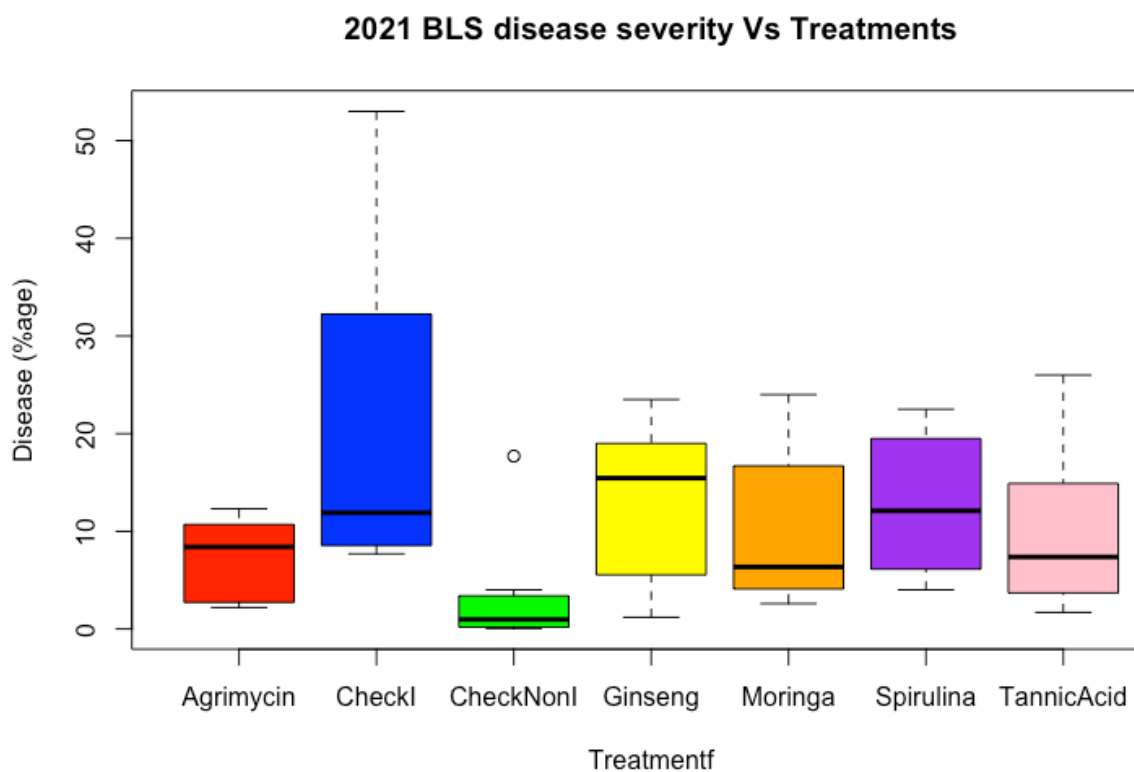


Figure 5.6 Effects of plant extracts treatments on bacterial leaf streak (BLS) disease severity (percentage) at two locations in 2021

CheckI = Inoculated & not treated, CheckNonI = Non inoculated and not treated.