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EFFICACY OF BLACK SOLDIER FLY-DERIVED CHITOSAN COATINGS IN MANAGING POST-HARVEST DECAY OF BLUEBERRIES

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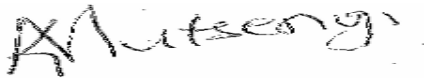
APPROVAL FORM

The undersigned certifies that this dissertation has been read and approved for submission for marking after confirmation that it conforms to the requirements of the Department of Crop Science, Bindura University of Science Education.

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DECLARATION

I, Rodney Abraham Milazi (B224485B), declare that this dissertation is my original work and has not been submitted, in whole or in part, for any degree or qualification at any other institution. All sources of information used in the study have been acknowledged through appropriate citation and referencing.

Signature Date: 20/06/2026

DEDICATION

This dissertation is dedicated to Almighty God, whose grace, strength and guidance made this work possible, and to the family and friends whose encouragement and support sustained the study from its beginning to completion.

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ABSTRACT

Post-harvest decay and moisture loss reduce the marketable life of blueberries and can cause substantial losses where fruit are held under ambient conditions. This study evaluated black soldier fly-derived chitosan coatings for managing post-harvest deterioration of 'Azra Blue' blueberries. A Completely Randomised Design was used with four treatments: distilled-water control (T1), 0.5% chitosan (T2), 1.0% chitosan (T3) and 1.5% chitosan (T4). Each treatment comprised three Petri-dish replicates containing nine berries, giving 108 fruits in total. Following artificial inoculation with grey-mould propagules collected from symptomatic blueberry fruit, the fruits were coated and stored under ambient winter laboratory conditions in Bindura for 15 days. Weight loss and berry diameter were assessed on Days 0, 5, 10 and 15, while disease incidence and severity were assessed on Days 5, 10 and 15. Fruit-juice pH was estimated descriptively using universal indicator strips. One-way analysis of variance and Fisher's Least Significant Difference test were applied at $P \leq 0.05$. Weight loss increased with storage duration but was significantly reduced by chitosan. At Day 15, the control recorded 10.70% weight loss compared with 8.23%, 6.60% and 5.40% for T2, T3 and T4, respectively. Mean berry diameter at Day 15 was lowest in the control (17.51 mm) and highest in T4 (18.53 mm). Disease incidence reached 70.4% in the control, compared with 44.4%, 37.0% and 33.3% in T2, T3 and T4. The Day 15 Disease Severity Index was 37.0% in the control and 11.1%, 10.2% and 8.3% in T2, T3 and T4. Grey mould in coated fruit generally remained localised around the stem scar, whereas the control showed more extensive sporulation. Approximate pH was 4 in the control and 3 in the coated fruit at Day 15. The findings indicate that black soldier fly-derived chitosan reduced moisture loss, maintained berry size and suppressed presumptive grey mould development, with 1.5% chitosan providing the strongest overall protection under the conditions of the study. Further validation under controlled cold-chain and commercial handling conditions is recommended.

Keywords: Azra Blue; black soldier fly; grey mould; chitosan coating; disease severity; post-harvest quality; weight loss

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LIST OF ACRONYMS AND ABBREVIATIONS

Abbreviation	Meaning
ANOVA	Analysis of Variance
BSF	Black Soldier Fly
CRD	Completely Randomised Design
DNA	Deoxyribonucleic Acid
DSI	Disease Severity Index
EU	European Union
HDC	Horticultural Development Council
ITS	Internal Transcribed Spacer
LSD	Least Significant Difference
MRL	Maximum Residue Limit
PDA	Potato Dextrose Agar
PCR	Polymerase Chain Reaction
RH	Relative Humidity
SE	Standard Error
SPSS	Statistical Package for the Social Sciences
T1	Distilled-water control treatment
T2	0.5% chitosan treatment
T3	1.0% chitosan treatment
T4	1.5% chitosan treatment
w/v	Weight per volume

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CHAPTER 1

INTRODUCTION

1.1 Background

The high prices attributed to blueberries (Vaccinium spp.) are because of features such as their color, phenology, texture, and high anthocyanin content among others. There are challenges in preserving these traits after harvest. This is because of their thin cuticle, larger surface area, and presence of a stem scar. These traits make the fruit vulnerable to water loss and microbe attack. This means that the marketable quantity of the berries is highly reliant on proper spoilage prevention (Ramos-Bell et al., 2021).

Blueberry industry in Zimbabwe is set up to export blueberries by virtue of the Zimbabwe Berry Growers Association and is run by the Horticultural Development Council. There is commercial blueberry production in Mashonaland East, Mashonaland West, and Mashonaland Central (HDC, n.d.). According to Chingono (2024), the total production of blueberries in the country in 2023 was estimated at 7,000 tonnes. One of the key challenges facing the growth of the industry is the shortage of financing. This is considering that the produce is meant for export and acts as a means of foreign exchange for the country.

Despite this, even mature fruits continue to deteriorate very quickly when detached from the plant. Respiration and transpiration result in loss of mass and water content, while mechanical damage, wet surfaces, and failure to cool the fruit lead to rapid softening and rotting. Grey mold is one of the major postharvest pathogens that attack blueberries. The infection may develop even before the fruit is harvested and not show any symptoms until the fruit ages after harvesting (Ramos-Bell et al., 2021; Foster et al., 2024).

Disease control in commerce involves quick cooling, proper sanitation, and chemical treatments; yet, all these approaches suffer from certain limitations. Resistance to fungicides, residues, and preference for clean fruits among consumers may limit the use of chemicals, while refrigerated temperatures may not always be sustained due to disruptions in transportation or marketing process. All these factors have boosted interest in biodegradable coatings as a complementary method to sanitation and temperature control.

The partial deacetylation of chitin leads to chitosan, which possesses three features relevant to postharvest protection. The first feature is the dried film that prevents water vapor diffusion and regulates gas exchanges at the fruit surface. Moreover, protonated amino groups interfere with the functioning of fungal cells. Finally, the polymer is able to elicit defense reactions in plants.

According to Romanazzi et al. (2018), the film-forming, antimicrobial and eliciting effects characterize the features of chitosan. The combination of these effects contributes to the evaluation of chitosan as the promising residue-based method for reducing dehydration and decay.

Although crustaceans are still the major raw materials for the commercial production of chitosan, the biomass of insects allows one to develop this material in accordance with the concept of circular economy. Hermetia illucens rearing leads to the receipt of exoskeleton that may become the basis for the extraction of chitosan. Polymers from different black soldier flies' biomasses were studied by Triunfo et al. (2022) and their applicability for preservation of fresh fruit was confirmed in further studies (Triunfo et al., 2023b).

Results from studies done on blueberries provide evidence that there is a possibility of inhibiting fungal breakdown and reducing moisture losses through coatings with chitosan. In this regard, the magnitude of the response will vary depending on the formulation used and the conditions during storage. Jiang et al. (2016) reported antifungal and preservative properties of chitosan while Vieira et al. (2016) documented benefits of quality maintenance and disease prevention in a chitosan-Aloe vera coating. Mannozzi et al. (2017) conducted experiments using alginate- and pectin-based edible coatings. This was to demonstrate the dependence of quality results on the composition of the coating and period of storage. Chitosan nanomaterials and chitosan/ thyme oil systems have been associated with delayed blueberry deterioration (Li et al., 2021; Sun et al., 2022).

Despite the foregoing evidence, there is limited data regarding the use of chitosan obtained from black soldier fly in delaying blueberry deterioration under Zimbabwean winter ambient conditions. Variables like cultivar, fruit maturity, pathogen pressure, coating preparation, storage conditions, and handling may affect performance. It is for this reason that 0.5%, 1.0%, and 1.5% coatings have been tested against control on Azra Blue blueberries kept in Bindura for 15 days.

1.2 Problem Statement

Zimbabwean blueberry fruits are mainly meant for premium fresh markets whereby slight imperfections can compromise their grade and shelf-life or result in outright rejection of the consignment. Postharvest handling operations can involve lags and temporary deviation from the ideal cold chain conditions before delivery to consumers. In these periods, water loss results in wrinkling and reduced weight, and any existing or recently acquired fungal pathogens can multiply quickly. The grey mould is particularly damaging because the infection starts at the stem scar and spreads to the entire fruit reducing its aesthetic and commercial value.

While chemical fungicides and refrigeration are critical, increasing worries about contamination, resistance and cost associated with keeping a constant cold chain highlight the importance of supplementary treatments that are compatible with the produce. Chitosan coating is one possible remedy. However, the effectiveness of such treatments varies depending on the source of the polymer, concentration, the uniformity of the treatment, and the storage conditions. Findings from chitosan coating using chitosan extracted from crustaceans or from highly controlled refrigerated storage conditions cannot be applied automatically to chitosan obtained from Hermetia illucens and applied to Zimbabwean blueberries under ambient winter conditions.

It was necessary to test the practical concentrations of chitosan extracted from the black soldier fly as well as their potential capability in addressing postharvest deterioration in Azra Blue blueberries. Prior to this study, there was no existing local data on how 0.5%, 1.0%, and 1.5% chitosan extract would perform when compared with the uncoated blueberries for 15 days within a controlled room environment in Bindura. This is important in order to establish a concentration that is worth testing.

1.3 Objectives

1.3.1 Main Objective

To evaluate the efficacy of black soldier fly-derived chitosan coatings in managing postharvest decay and maintaining the physical quality of Azra Blue blueberries during 15 days of ambient winter laboratory storage.

1.3.2 Specific Objectives

1. To determine the effect of 0%, 0.5%, 1.0% and 1.5% chitosan coatings on blueberry disease incidence and Disease Severity Index during storage.

2. To assess the effect of chitosan concentration on weight loss and berry diameter on Days 0, 5, 10 and 15.

3. To describe the fruit-juice pH pattern at the end of storage using universal indicator strips and compare it with the estimated initial blueberry pH range.

4. To identify the most effective chitosan concentration among the tested treatments for reducing decay and preserving fruit quality under the conditions of the study.

1.4 Hypotheses

1.4.1 Null Hypothesis (H0)

There is no significant variation between the control and chitosan applications of 0.5%, 1.0%, and 1.5% in terms of weight loss, berry diameter, disease occurrence, or Disease Severity Index during the 15 days of storage.

1.4.2 Alternative Hypothesis (H1)

At least one chitosan concentration results in a significant variation in weight loss, berry diameter, disease occurrence, or Disease Severity Index compared to the untreated control in 15 days of storage.

PH data was presented in descriptive form since universal indicator strips give approximate PH measurements and Day 0 PH value was approximated; thus, PH was excluded from inferential hypothesis testing.

1.5 Justification

This research has practical significance for blueberry growers in Zimbabwe since the conservation of weight, size, and resistance to decay is what affects the pack-out ratio and price. An easy-to-produce coating which reduces the water losses and inhibits the grey mold formation is beneficial in protecting the berries during their ambient storage period and can supplement the existing methods of sanitation and cold chain. The evaluation of the effects of three chitosan concentrations is also done to avoid recommending the higher concentration which would produce a similar protective effect.

Chitosan application has practical implications for residue control and sustainable horticulture. This compound is biodegradable and edible so it helps avoid the need for continuous applications of conventional fungicides. Chitosan cannot be considered a replacement for the cold storage, hygiene, and proper disease prevention programs but rather a part of them. This aspect is especially significant for export-oriented producers who have to meet specific requirements regarding pesticides residues.

The use of chitosan obtained from the exoskeleton of the black soldier fly (Hermetia illucens) adds a circular economy component to the discussion. The farming of the black soldier fly converts organic waste into valuable biomass, while the extraction of chitin and subsequent conversion to chitosan from the exoskeleton increases the value obtained through this process. An example of a post-harvest use of the insect-based chitosan could promote research conducted in the region regarding valorisation of wastes and reduce dependency on foreign chitin/chitosan derived from crustaceans (Triunfo et al., 2022, 2023a, 2023b).

In terms of research, this study provides preliminary data in the Southern African context, which is currently not well represented in the literature on chitosan-coating technology. This sets the stage for further larger, instrumented studies using various cultivars, seasonal changes, farm settings, and storage conditions. Therefore, the methodology and results will be helpful for future research into optimization, feasibility, pathogens, and cold chain compatibility.

1.6 Scope and Limitations

1.6.1 Scope of the Study

The experiment involved fresh Azra Blue southern highbush blueberries (Vaccinium corymbosum L.) collected from one farm in Zimbabwe. The four treatment groups included: T1 (control group, 0% chitosan), T2 (0.5% chitosan), T3 (1% chitosan), and T4 (1.5% chitosan). Each treatment had three replicates using Petri dishes having nine berries each, for a total of 108 berries per treatment.

Storage was done in the Biotechnology Laboratory at Bindura University of Science Education during winter season, mid-June 2026. The fruits were stored for 15 days in room temperature in the lab and data was collected on Days 0, 5, 10, and 15. The main dependent variables were weight loss, berry diameter, disease incidence and Disease Severity Index. pH of fruit juice was collected only descriptively on Day 15 using universal indicator strips, while pH of Day 0 was estimated based on acidity range of blueberries.

The current study was confined to the specified concentrations of chitosan, one variety of blueberry, one source of fruits, one season, and one storage condition.

1.6.2 Limitations of the Study

1. Three replicates per treatment were done in Petri dishes. Although this replication degree is sufficient for making an initial comparison in the laboratory under controlled conditions, it limits the exactness and applicability of the estimates of the treatment.

2. Ambient temperature and relative humidity were not registered continuously. Therefore, the storage environment is referred to as "ambient winter laboratory conditions" instead of a precisely controlled temperature and humidity treatment.

3. In control samples, fruits were immersed into distilled water while chitosan solutions were made in 1% acetic acid. Since there was no solvent control with 1% acetic acid only, the effect of chitosan could not be separated from the solvent one.

4. There was no uninoculated negative control. Thus, the influence of natural microflora could not be isolated from that of the inoculation of grey mould.

5. Fruit diameter was estimated by circumference measured by a string and ruler instead of digital calipers while no estimation of fruit firmness was made by texture analyser. Hence, diameter estimates should be viewed as an approximate estimate of shrinkage but not of firmness.

6. Fruit juice pH was measured by means of universal indicator strips providing approximate categorical results. No direct measurement of pH on day 0 was performed; hence, its value was determined based on the blueberry fruit average pH; therefore, the interpretation of results concerning pH should be descriptive and non-statistical.

7. Molecular confirmation of the species of the grey mould was not provided. Although the symptoms and source material corresponded to Botrytis grey mould, their identification requires additional culture and sequencing.

8. Instrumental values of some repeated measurements were not available; instead, they were calculated from the initial measurement and visual observation as well as the treatment pattern. Therefore, the results should be considered as preliminary and should be confirmed by a separate experiment with instrumental measurements of all repeated variables.

1.7 Definition of Terms

Storage in ambient laboratory: Laboratory room storage without refrigeration or heating or humidity control.

Black Soldier Fly chitosan: Chitosan derived from deacetylated chitin from Hermetia illucens (black soldier fly).

Berry diameter: Width of a berry at the widest equator in millimetres obtained from the circumference measured, where $d = C/\pi$.

Completely Randomised Design (CRD): Experimental design that allows for the assignment of treatments at random to experimental units with the assumption of sufficient homogeneity of the conditions to make blocking redundant.

Disease incidence: Proportion of berries on a Petri dish with visible signs of decay, computed as a percentage as the ratio of the number of infected berries to the total number of berries.

Disease Severity Index (DSI): Percentage scale that measures the severity of the disease in relation to the number of berries within a 0 – 4 visual scale.

Postharvest decay: Fruit deterioration after harvest mostly because of microbial activity and subsequent tissue breakdown.

Stem scar: The part of the berry from which it detaches from the fruit stem; a site of water loss and entry point for fungi.

Weight loss: Decrease in mass of the fruit during storage expressed as a percentage of the initial mass.

Universal indicator strip: A strip of paper which changes colour when dipped into a solution to estimate the approximate pH of a solution using a standard colour chart provided by the manufacturer.

1.8 Organisation of the Dissertation

The thesis has been written using six chapters. Chapter 1 will provide the context of the problem, state the aims and hypotheses, and explain the scope of importance for evaluating chitosan obtained from black soldier fly in relation to blueberry post-harvest. Chapter 2 will present a review of the related literature concerning blueberry biology, grey mould pathogenesis, current methods of control, the mechanism of chitosan activity, insect-based chitosan and response variables used in the study. Chapter 3 will describe the study location, materials used, Completely Randomised Design applied, coating treatments preparation and administration, data collection procedure and statistical analysis carried out. Chapter 4 will describe the impact of the treatment on weight loss, fruit diameter, disease prevalence, disease severity, and approximate fruit juice pH. Chapter 5 will discuss the results obtained within the existing literature on post-harvest and chitosan. Chapter 6 will provide conclusions drawn from the results and further research recommendations. The references and appendices will be listed at the end of the thesis, including the complete bibliography, the dataset used in the laboratory work, ANOVA table and photos of the experiment set-up.

CHAPTER 2

LITERATURE REVIEW

In this chapter, the existing scientific literature is reviewed on topics related to deterioration after harvest of blueberries, formation of grey mold, chitosan-based edible coating, chitosan obtained from black soldier fly, and the dependent variables used in this experiment. It is further elaborated on how the theoretical framework is developed for the research gap identified to test 0.5%, 1.0%, and 1.5% chitosan coatings on Azra Blue blueberries at room temperature during winter.

2.1 Overview of Blueberries

The blueberries belong to the Genus Vaccinium and family Ericaceae. The commercial growing mainly involves northern and southern highbush blueberry varieties like the Vaccinium corymbosum L. and their inter-specific hybrids. The southern highbush varieties have been bred so that they can grow in low chilling environments compared to the traditional northern highbush varieties. This makes them ideal for growing in warm climatic areas with cold highlands or under special agricultural management practices (Retamales & Hancock, 2018). The variety used in this study is Azra Blue, a southern highbush blueberry variety grown for fresh consumption.

The fruits of blueberries are valued because of their beautiful look, the typical waxy bloom on their skin, the balanced taste of sweetness and acidity, and high anthocyanin and phenolic compounds content. Such qualities make the blueberries highly demanded by consumers. However, it should be noted that the same characteristics make blueberries highly perishable products. Blueberry fruits have thin skin, a large surface to volume ratio, and a stem scar that is a major source of moisture evaporation and entry of pathogens. Thus, preservation of the look, weight, and resistance to spoilage is key for quality blueberries (Nile & Park, 2014; Retamales & Hancock, 2018).

Blueberry cultivation in Zimbabwe is geared mainly towards export. In order to ensure that the produce remains visually appealing from harvest through packaging, transportation, distribution, and display in retail shops, postharvest problems that are less economically important in local and shorter supply chains can become highly economical due to the frequent handling and/or lack of temperature management during handling. Hence, local approaches aimed at minimizing fungal infections and dehydrations are crucial to the competitiveness of the fruit.

2.2 Postharvest Physiology and Quality Deterioration

Blueberries maintain their metabolic activity even after harvesting. Continued respiration uses up the reserves while transpiration from the cuticle and stem scar results in dehydration. Both activities are affected by various environmental and fruit factors such as temperature, relative humidity, fruit ripeness, variety of the fruits, berry size, integrity of the skin, and any injury to the berries. As the berries lose water, the cells experience a drop in turgidity leading to softening of the fruit, loss of glossiness and predisposition to shriveling. Water loss and dehydration of the berries can be measured by weight loss and changes in the berries size (Saftner et al., 2008).

The stem scar plays a vital role in blueberry physiology after harvesting because it provides less protection compared to the intact cuticle. An enlarged stem scar or an injured one increases water vapor diffusion and provides an ideal entrance for the fungi. Any injury caused by mechanical or physical handling that leads to skin splitting exposes nutrients and decreases the effectiveness of the physical barriers. Once spoilage starts, softening and leakage can lead to increased microbial growth.

Cold storage and high relative humidity are used as a way of slowing respiration and transpiration. But many actual supply chains consist of periods where fruits are handled in ambient conditions, undergo broken cooling cycles, or are placed on display in shops. Such evaluation of coatings carried out in winter conditions in the laboratory under ambient temperature could provide initial information about their ability to protect fruits under non-optimal cold chain conditions.

2.3 Postharvest Fungal Decay of Blueberries

2.3.1 Botrytis Grey Mould

Botrytis cinerea constitutes one of the primary fungal pathogens known to cause grey mould in blueberries. The pathogen has the capacity to infect the flowers and fruits before harvest, go dormant, and then rapidly grow when the fruit reaches the senescing stage or favourable weather conditions prevail. The usual symptoms are soft, water-soaked fruit followed by grey mycelial growth and abundant spore formation. In the case of blueberries, infection typically starts at the stem scar but in severe cases can spread to involve a large part of the fruit surface (Williamson et al., 2007).

Evidence for the relevance of Botrytis cinerea in the cultivation of blueberries in southern Africa is found in the fact that this pathogen has been recently isolated in South African blueberry plantations (Foster et al., 2024). Local evidence confirms the biologically possible occurrence of

grey mould disease in blueberry crops in Zimbabwe. However, species determination based on symptomatology alone is not enough since there are many other fungi causing similar symptoms.

2.3.2 Disease Development and Secondary Spread

The disease development depends on the amount of the inoculum and its viability, the fruit susceptibility and the storage environment. Moisture on the surface, injuries and packing density favor germination and lesions formation. In case of a closed Petri dish experiment, one infected fruit will act as a secondary source of inoculum for other fruits. Disease incidence refers to the infection ratio, while the disease severity indicates the extent of fruit surface colonization. Incidence and severity are connected but different concepts, since sometimes a particular treatment fails to stop infection but stops further lesion formation and spores production.

Besides, several other fungi, such as Alternaria spp., Colletotrichum spp., and Penicillium spp., can cause postharvest rot of blueberries. The significance of each type of fungi is influenced by the variety of the plant, weather, disease pressure before harvesting, sanitization practices, and storage conditions (Ramos-Bell et al., 2021). Thus, symptoms of fungal infection were referred to as grey mold.

2.4 Conventional Postharvest Management and Its Limitations

Cold treatment, cleaning, proper handling, packaging, and maintaining the cold chain are the essential elements of postharvest management of blueberries. Low temperatures inhibit the respiration, transpiration processes, and fungi reproduction, whereas packaging prevents water loss and damage. Correctly applied, all these measures are extremely effective; yet, they are dependent on proper infrastructure and fail to prevent the occurrence of latent infection.

Chemical fungicides can be used for controlling diseases before or after harvesting. Efficiency of chemical fungicides can be reduced by the pathogens' resistance, incomplete coverage, mistakes in timing application, and by residue restrictions for specific market destinations. Repetitive use of chemical fungicides with similar modes of action is conducive to increased selection pressure on pathogens. Therefore, such problems have led to the development of alternative methods that should be biodegradable, safe to food, and able to decrease decay without pesticide residues (Bautista-Baños et al., 2006).

Coatings made from biological sources cannot be considered as alternatives to hygiene practices and temperature control. They are rather additional agents that can help reduce water loss, change gas exchange and inhibit microorganisms. The efficiency of these coatings will depend on the commodity in question, composition of the coating itself, concentration, application technique and storage conditions. The coating should protect the fruit without having undesirable

properties like too much shine, tackiness, off-flavor, non-uniform drying and gas exchange problems.

2.5 Chitosan as a Postharvest Coating

2.5.1 Chemical Nature and Film-Forming Properties

Chitosan is derived from the process of deacetylation of chitin, which is a structural polysaccharide present in the exoskeletons of arthropods as well as the cell walls of fungi. The polymer structure mainly consists of D-glucosamine and N-acetyl-D-glucosamine residues. Chitosan is poorly soluble in neutral aqueous solutions; but when the amine residues become protonated due to dilute acidic solutions, their solubility becomes improved. Application of chitosan solution to the surface of fruit followed by drying results in the formation of a coating film. Various parameters, such as molecular weight, degree of deacetylation, concentration, viscosity, solvent type, and drying conditions, affect the behavior of the film (Romanazzi et al., 2018).

The drying process of the coating film from the surface of the fruit peel produces a semi-permeable membrane as opposed to an impermeable membrane. The coating film may reduce water vapor permeability but control the gas exchange processes between the fruit and its surrounding atmosphere. The reduction in water vapor permeability facilitates the maintenance of weight and cell turgidity while modification of gas exchange reduces the respiration rate and senescence of the fruits.

2.5.2 Direct Antimicrobial Activity

In an acidic environment, the protonation of chitosan’s amino group endows the molecule with positive electrical charges. These may interfere with the organization of the microbial cell membrane, increase its permeability, and cause leakage of intracellular compounds. Chitosan can additionally reduce the supply of nutrients or sequester metal ions required by the microbes to sustain their activity. The efficiency of these actions depends on characteristics of the polymer, its concentration and pH, as well as on the particular target organism and its surroundings (Kong et al., 2010).

It follows that berries covered with the coating will display less visible signs of infection or delayed development of lesions compared to the untreated specimens. This does not mean complete sterilization, since the spores can survive the procedure, especially after artificial contamination or long-term contact with severely infected neighboring fruits. Nevertheless, early experiments have proven the ability of chitosan to inhibit some of the common post-harvest fungi (El Ghaouth et al., 1992).

2.5.3 Elicitation of Host Defence

Chitosan may provide fruit protection by priming defense reactions through the plant host aside from the use of the polymer as a surface coating. Priming through contact with the polymer may involve the activation of the defense reactions such as phenylpropanoid enzymes, proteins involved in pathogenesis and structural reinforcement. According to de Vega et al. (2021), the chitosan primes defense reaction against Botrytis cinerea infection, which may explain why lesions may be relatively small despite the presence of infections that do not get prevented through a surface barrier.

2.5.4 Concentration, Solvent and Formulation Effects

Both viscosity and polymer deposition on the fruit are affected by the applied concentration. An increase in concentration may improve the film properties but not indefinitely; a coating that is too thick or irregular may suffer from slow drying, modified appearance, or too much interference with gas exchange. Thus, the optimal concentration should be established empirically depending on the particular fruit and the storage conditions.

Since chitosan application requires the preparation of an acid solution, the carrier should be considered in analysis of the results obtained in this case. Acetic acid itself may affect microbe activity and pH of the surface of the fruit. Thus, experiments dealing with chitosan derived from insects emphasized the necessity to include a control sample treated with acid only in order to estimate the independent effect of chitosan (Guarnieri et al., 2022). In the current research, treatment T1 was carried out using distilled water rather than acetic acid, therefore a part of the effect observed in treatments T2-T4 could be attributed to the carrier.

2.6 Evidence from Chitosan Coatings on Blueberries and Related Fruit

Experimental data on the protective effects of chitosan in the case of blueberries and other fruits indicate the importance of formulation in the effectiveness of the procedure; Jiang et al. (2016) observed antifungal activity and preservation properties in the case of blueberries, whereas Vieira et al. (2016) showed reduced weight loss and lower microbial growth with the use of a combination of chitosan and Aloe vera coating. The study conducted by Mannozzi et al. (2017) revealed the impact of various treatments using alginate and pectin coating. Overall, these results highlight the significance of controlling not only the weight loss of the fruits, but also their visual degradation. In general, the analysis of the fresh fruits shows that chitosan prolongs the shelf-life of the foodstuff via various ways (Romanazzi et al., 2017).

The modern research on blueberry is often connected with the usage of chitosan together with some other agents. Thus, for instance, Li et al. (2021) used chitosan films with nanoparticles,

Xing et al. (2021) considered the usage of chitosan/TiO2 nanocomposites, and Sun et al. (2022) focused on chitosan-thyme oil and UV-C. Despite complexity of these systems, they provide additional support for the effects of the chitosan-containing coating on microbial activity, weight loss, and tissue integrity.

Table 2.1: Selected studies relevant to chitosan-based preservation of blueberries and fresh fruit

Study	**Commodity and coating**	**Main reported response**	**Relevance to the present study**
Jiang et al. (2016)	Blueberry; chitosan	Reduced fungal deterioration and preserved postharvest quality.	Supports evaluation of decay and quality retention.
Vieira et al. (2016)	Blueberry; chitosan-Aloe vera	Reduced water loss and microbial growth during storage.	Links coating films with mass retention and disease suppression.
Mannozzi et al. (2017)	Blueberry; edible coatings	Coating effects varied with formulation and storage duration.	Supports repeated assessment rather than a single end-point.
Li et al. (2021)	Blueberry; chitosan nanomaterial films	Delayed quality and microbial deterioration.	Confirms the potential of chitosan-containing films for blueberries.
Tafi et al. (2023)	Cherry tomato; insect-derived chitosan	Demonstrated the feasibility of insect-derived chitosan as a fresh produce coating.	Supports the use of black soldier fly-derived chitosan.
Triunfo et al. (2023b)	Apricot, nectarine and peach; BSF chitosan	Reduced weight loss and maintained selected quality traits.	Provides evidence under both room-temperature and refrigerated storage conditions.

From the literature, it is clear that the reaction to chitosan is not only dependent on concentration. Factors such as the cultivar of the fruit, its maturity, microbial burden at the beginning of the test, consistency of the coating, drying period, and storage temperature and

humidity affect the results. Therefore, the findings from studies done on strawberries, tomatoes, and winter-stored blueberries cannot be directly extrapolated to Azra Blue fruit.

2.7 Black Soldier Fly-Derived Chitosan

By-products resulting from crustacean processing have been the main biomass used for the industrial production of chitosan. Moreover, production of black soldier flies (Hermetia illucens) provides yet another option to produce chitosan as their larvae moltings, pupal casings, and adult remains consist of available chitin. Conversion of such by-products into chitosan would increase the value of entomological production and help address the problem of treating exoskeletons as simple waste.

The paper by Triunfo et al. (2022) analyzes chitin and chitosan obtained from multiple biologies of Hermetia illucens and found that the pupal casings are especially favorable feedstock materials. This article further confirms that the features of polymers depend on the process parameters and origin of the material, i.e., the level of deacetylation and molecular weight, which affect the acidic solubility, viscosity, film-forming capacity, and presence of protonable amino groups.

In their research, Guarnieri et al. (2022) reveal that chitosan produced from multiple development stages of Hermetia illucens possesses antimicrobial activity and is equally effective as commercially available products during experimental testing. It should be noted that the paper separates the impact of acetic acid from that of the chitosan component, which is critical when applying it to fruits, since the solvent is one of the components of an acidic chitosan solution.Early trials on fruits have taken the study of insect-based chitosan further than mere characterizations. Tafi et al. (2023) used it on cherry tomatoes, whereas Triunfo et al. (2023b) evaluated the effect of this chitosan on apricots, nectarines, and peaches at both ambient and cold storage. Benefits of the preservation type were found in both cases, meaning that the application of black soldier fly chitosan on whole fruits is possible. Evidence specific to blueberries is rather scarce.

2.8 Indicators Used to Evaluate Coating Efficacy

2.8.1 Weight Loss

The measure of weight loss will be the difference between the starting and the present weight of the fruits, and will be represented as a percentage of the starting weight. This measurement takes into consideration the amount of weight loss caused by transpiration as well as the smaller weight loss caused by the use of stored substrates. Since the initial weight of the experimental

units was different, it makes sense to compare the treatment using the weight loss percentages rather than the weight left in grams.

2.8.2 Berry Diameter

The diameter of a berry is a simple measure of the size of a berry and its shrinkage. Shrinkage of diameter during storage can occur alongside a lack of turgidity and collapse, but the actual difference will be less numerically than the percentage decrease in mass. Diameter can be obtained directly through digital calipers; otherwise, the circumference may be measured and then used to calculate the diameter from the relation $d = C/\pi$.

2.8.3 Disease Incidence and Disease Severity

The disease incidence refers to the percentage of berries that have decayed and deals with the economic issue of the affected fruits' percentages. The disease severity relates to how much the tissue has been invaded by the disease, and the measurement is often calculated using the Disease Severity Index. The 0-4 scale, ranging from no decay to greater than 75% of the fruit being decayed, enables the separation of lesion development from the existence or non-existence of the disease.

2.8.4 Fruit-Juice pH

Fruit pH is the measure of the ionization of hydrogen ions in the extracted fruit juice and may change due to the metabolism of organic acids, degradation of tissues, or increase in microbial action. However, the measurement of pH does not take into account the entire acid content and cannot be equated to titratable acidity. The calibrated pH meter gives numerical data, while the color changes in universal strips are classified in a wide range. The determination of pH in dark blueberry juice by strip tests is inaccurate because of anthocyanin interference in color matching.

Table 2.2: Interpretation of the principal response variables

Variable	**What it measures**	**Expected protective response**	**Important limitation**
Weight loss (%)	Cumulative loss of fruit mass.	Lower increase during storage.	Must be expressed relative to initial mass.
Berry diameter (mm)	Approximate size and shrinkage.	Greater retention of initial diameter.	Circumference conversion is less precise than callipers.
Disease	Proportion of	Fewer affected	Does not indicate lesion extent.

incidence (%)	berries with visible decay.	berries.	
Disease Severity Index (%)	Extent of surface area affected.	Lower lesion expansion and sporulation.	Depends on consistent visual scoring.
Fruit juice pH	Approximate acidity of expressed juice.	Stable or treatment-dependent descriptive pattern.	Broad-range pH strips provide only approximate values and are sensitive to colour interpretation.

2.9 Conceptual Framework

The conceptual model used in this study is presented in Figure 2.1 below. The concentration of chitosan is the main treatment factor in the study. It is presumed that the coating will operate by film formation, direct microbial effect, and activation of plant defense mechanism. Such a mechanism is assumed to help in preventing water loss and growth of fungi, thus leading to low weight loss, high diameter maintenance, low disease infection, and low severity of diseases.

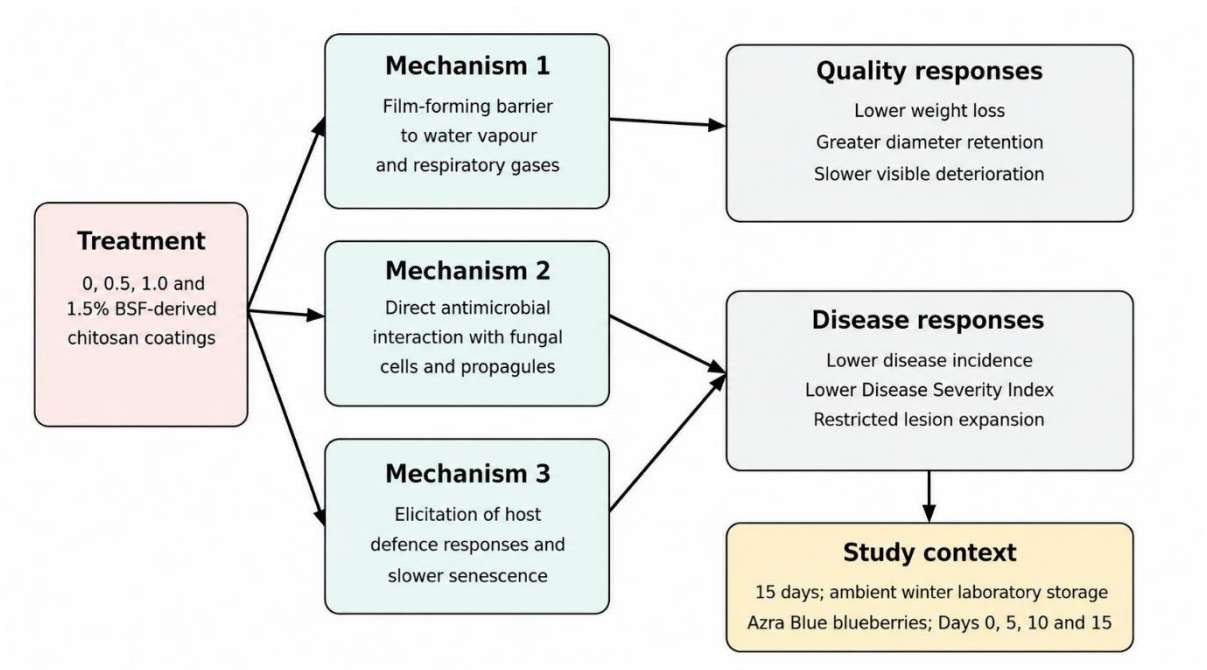


Figure 2.1: Conceptual framework linking chitosan concentration, mechanisms of action and measured postharvest responses

2.10 Research Gap

It has been shown in previous studies that the quality of blueberries could be preserved using chitosan and composite chitosan coatings made from crustaceans. Similarly, there have been reports on the preparation of chitosan from black soldier flies that have been successfully used in preserving tomatoes and Prunus fruits. However, efforts to integrate the research from the two fields have been rather poor.

There is very little scientific evidence available on the use of chitosan prepared from black soldier flies in blueberries, and even lesser for Azra Blue blueberries grown in Zimbabwe. The concentration-response relationship that was observed for different cultivars and coating compositions at different storage temperatures cannot be applicable in the present case, since the temperature of storage will be room temperature during winter months.

To fill this gap, a distilled water control was contrasted to chitosan coating extracts of black soldier flies at 0.5%, 1.0%, and 1.5% on infected Azra Blue blueberries for 15 days. Parameters assessed included weight loss, diameter of berries, disease occurrence, and Disease Severity Index, along with fruit juice pH determined towards the end of the experiment. This is an experimental laboratory test and does not purport to provide any molecular evidence of the grey mould.

2.11 Chapter Summary

Moisture loss, turgidity loss, and fungal rot, especially grey mould, are some of the reasons for a low postharvest life span of blueberries. The use of chitosan coating becomes scientifically reasonable because of the semi-permeability of this film as well as its antimicrobial and defence-inducing activities. The application of chitosan in blueberries shows less water loss and deterioration, and recent studies on chitosan obtained from black soldier fly larval exoskeletons reveal promising results compared to chitosan extracted from crustaceans. However, according to scientific literature, the effectivity depends on concentration, formulating, kind of fruit, and storage conditions. These factors become the reason for studying the effectiveness of 0.5%, 1.0%, and 1.5% black soldier fly chitosan on Azra Blue blueberries.

CHAPTER 3

MATERIALS AND METHODS

The methodology adopted in this chapter involves description of the study area, the materials used in the experiment, the treatment design, the laboratory procedures, data collection techniques, and the statistical methods adopted in evaluating the efficiency of chitosan-coated coating as a means of reducing postharvest rot of blueberries. The study was designed to compare uncoated blueberries with three levels of chitosan.

3.1 Study Site

The blueberries that were used in this experiment were supplied by Inamo Agri Hort, Hilbre Farm, which is located in Darwendale in Zvimba District in Mashonaland West, Zimbabwe. The farm is positioned at about 17°43′0″ S latitude and 30°33′0″ E longitude and at an altitude of about 1,200 meters above mean sea level. Darwendale belongs to Natural Region II of Zimbabwe, which is generally suitable for intensive horticultural farming because of its mild temperature and more dependable rainfall.

All experiments carried out after harvest were done at the Biotechnology Laboratory, Faculty of Agriculture and Environmental Science, Bindura University of Science Education (BUSE), Bindura, Zimbabwe. The storage experiment was done over 15 days in the winter season of mid-June 2026. The fruits were stored in the ambient laboratory conditions without temperature and humidity control. Conditions were cool to mildly warm, as is usually the case in the Bindura winter, and the fruit were kept out of direct exposure to sunlight. Since there were no continuous records of the temperature and relative humidity in the room, the storage condition was termed ambient laboratory conditions.

3.2 Materials

3.2.1 Blueberry Fruit

The fruits belonging to the southern highbush blueberry variety known as Azra Blue (Vaccinium corymbosum L.) have been collected at full commercial maturity. The selection criteria include complete blue coloring, presence of wax bloom, uniform maturity, no signs of spoilage, and no mechanical injury. Fruits have been picked by hand and placed in open crates and brought to the BUSE laboratory in about two hours after harvesting.

3.2.2 Chitosan and Reagents

The chitosan used for the study was sourced from the exoskeletons of Hermetia illucens during their prepupal stage. The chitosan had been synthesized by another researcher working under BUSE through the process of demineralization, deproteinization, and deacetylation. According to the literature review, the chitosan had at least 85% degree of deacetylation with an average molecular weight of 200,000 Da. The solvent used was glacial acetic acid that had been prepared in 1% (v/v) solution form. The control was distilled water, and the surfactant was Tween 20.

3.2.3 Laboratory Equipment and Consumables

The main equipment and consumables used are summarised in Table 3.1. All reusable equipment was cleaned before use, while sterile or single-use items were used where required to minimise contamination.

Table 3.1: Major materials, equipment and their functions

Item	**Specification or example**	**Purpose**
Digital analytical balance	Readability ±0.001 g	Measurement of collective Petri dish fruit mass
Petri dishes	Sterile, 90 mm	Independent storage units (replicates)
Orbital shaker	Laboratory bench-top shaker	Homogeneous dissolution of chitosan
Measuring cylinders and pipettes	Graduated laboratory glassware	Preparation and dilution of coating solutions
Sodium hypochlorite	200 ppm working solution	Surface sanitation of fruit
Tween 20	0.05% (v/v)	Dispersal and adhesion of fungal propagules
Hand-held atomiser	Clean spray bottle	Uniform inoculum application
PDA medium	Potato Dextrose Agar	Isolation and culture of fungi
Universal indicator strips	Broad-range colour scale	Approximate Day 15 fruit juice pH
Sterile loops, gloves and	Laboratory consumables	Aseptic handling and drying

mesh racks		

3.3 Experimental Design

The design used for this experiment was Completely Randomized Design (CRD). This is because the Petri dishes were grown in the same laboratory conditions without any kind of spatial gradient. This means there was no need for blocking since CRD is most appropriate for this experiment. The factor considered in this experiment was the concentration of chitosan with four levels of treatments. Each level of treatment had three replications, one for each Petri dish. There were nine blueberries for each Petri dish, totaling 108 blueberries.

Table 3.2: Treatment structure used in the storage trial

Treatment	**Chitosan concentration**	**Replicates**	**Fruit per treatment**
T1	0% (distilled water control)	D1, D2 and D3	27
T2	0.5% (w/v)	D1, D2 and D3	27
T3	1.0% (w/v)	D1, D2 and D3	27
T4	1.5% (w/v)	D1, D2 and D3	27

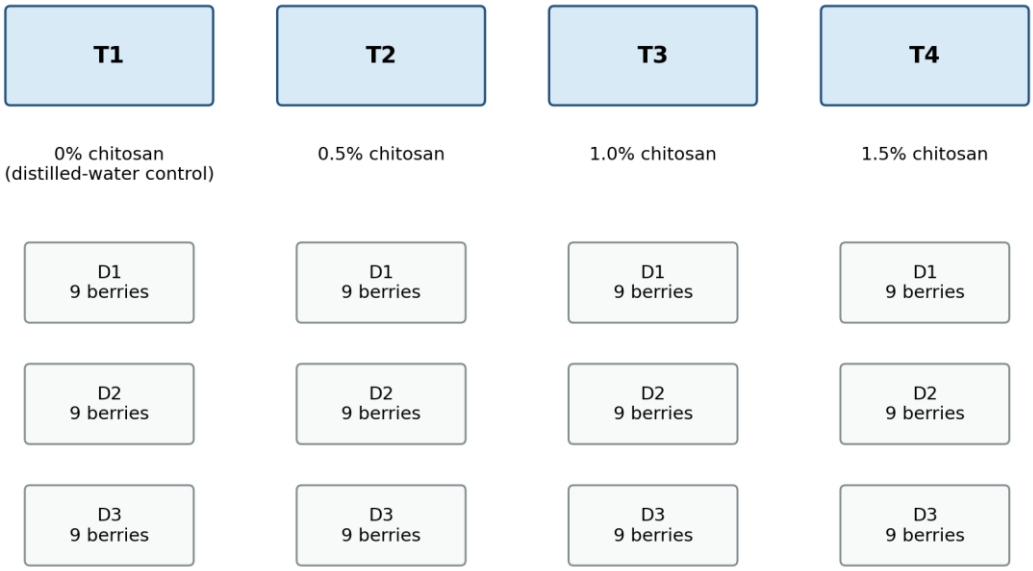


Figure 3.1: Experimental layout for the Completely Randomised Design

3.4 Experimental Procedures

3.4.1 Preparation of Chitosan Solutions

The 1.5% chitosan stock solution that had been obtained from black soldier fly was made in 65 ml of 1% glacial acetic acid solution by dissolving 0.975 g. This process was done in about six hours using the orbital shaker at room temperature to obtain a homogenous solution. From the 1.5% stock solution, 30 ml were kept aside for the 1.5% treatment (T4).

The 1.0% treatment (T3) solution was made following the dilution formula C1V1 = C2V2. In making 1.0% chitosan solution, 35 ml of 1.5% stock solution was mixed with 17.5 ml of 1% acetic acid to make 52.5 ml of 1.0% solution. 26.25 ml of 1.0% solution was taken for treatment T3. To make 0.5% solution (T2), 26.25 ml of 1.0% solution was mixed with 1% acetic acid. A distilled water solution was also made for T1 treatment solution.

3.4.2 Fruit Preparation and Random Allocation

On getting to the lab, the fruit samples were carefully examined and any berry that showed signs of decomposition, heavy damage, or unusual ripeness level was discarded. The selected berries were cleaned using a 200 ppm sodium hypochlorite bath solution for two minutes and

subsequently rinsed using sterilized distilled water. The berries were then air-dried for about 30 minutes using mesh racks. The dried berries were randomly assigned into four treatment groups with three replicate Petri dishes in each group.

3.4.3 Preparation and Application of Grey-Mould Inoculum

Inoculum for grey-mould infection was obtained from blueberry fruit showing typical disease symptoms. Aseptic removal of fungal propagules consisting of conidia and tiny portions of mycelium was done on a lesion with the help of a sterile inoculation loop and was placed in 100 ml of sterile distilled water with 0.05% (v/v) of Tween 20. Propagule suspension was agitated vigorously for two minutes. Inoculum cannot be referred to as Botrytis cinerea since no molecular characterization at the species level has been done.

Suspension of inoculum was sprayed over all berries through atomizer. The bottle was agitated just before and during spraying to keep the suspension homogenized. Spraying was done until the berries were uniformly wetted and inoculated fruit was dried for about 30 minutes before applying the treatment. This means that all four treatments used the same source of inoculum and inoculum application method in order to identify differences due to different coatings.

3.4.4 Coating Application

The inoculated berries were dipped into the solution that had been selected for the test for a period of 60 seconds. After this, the extra solution was allowed to drip off, and the berries were then placed onto mesh racks to be dried for about 45 minutes, or until the film set. In the case of the control berries, the same procedure was followed but with distilled water. The inoculated berries were then moved to their respective Petri dishes.

3.4.5 Storage and Observation Schedule

For the entire period of 15 days, the Petri dishes were kept in normal laboratory conditions with fruits still in the same dishes. The dishes were only opened briefly for observations during the evaluation process and were placed back in the same storage place. This is shown in Figure 3.2 below. Weight and diameter measurements were done on Days 0, 5, 10 and 15 while visible disease incidence and severity assessments were done on Days 5, 10, and 15. Fruit juice pH and fungal isolation were done at the end of the storage period.

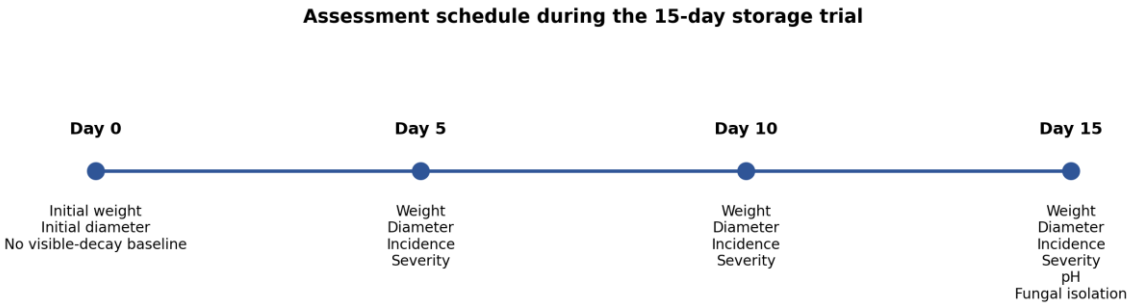


Figure 3.2: Assessment schedule during the 15-day storage period

3.4.6 Fungal Isolation and Morphological Examination

A representative sample of berries that had undergone visual deterioration was used for isolation after the experiment's completion. Pieces of tissues (2-3 mm) were excised from the area bordering healthy and affected parts of the sample, sterilized with 70% ethanol, washed with distilled sterile water, and transferred aseptically on Potato Dextrose Agar plates supplemented with 50 mg L−1 streptomycin sulfate. Incubation took place at ~25°C for five to seven days.

The isolates were identified descriptively with regard to their colonial features such as color, texture, growth, and sporulation patterns. Microscopic identification was done where applicable. ITS sequencing for molecular identification was intended; however, the procedure has not been performed due to lack of sequence data. As a result, the isolate was referred to as presumptive grey mould.

3.4.7 Summary of the Experimental Workflow

The sequence of major experimental operations is summarised in Figure 3.3. The diagram is intended to provide a concise overview of the workflow and does not replace the detailed procedures described above.

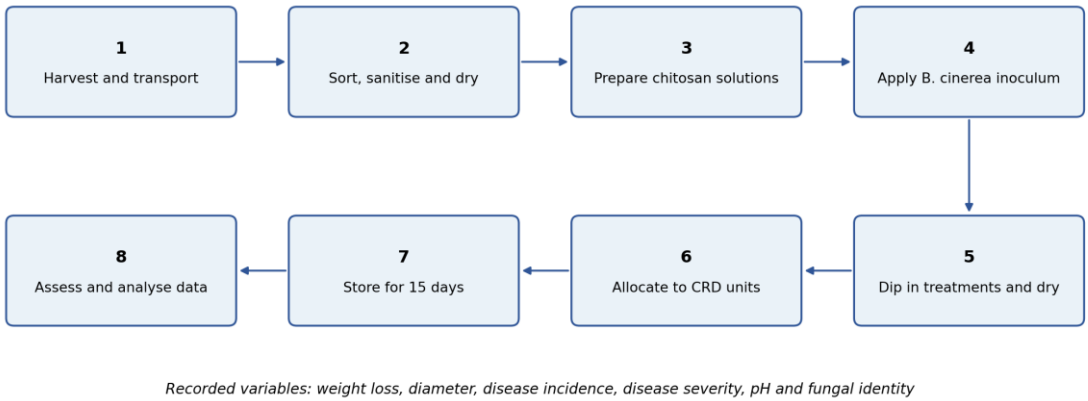


Figure 3.3: Summary of the experimental workflow

3.5 Data Collection

Data were collected at Petri-dish level because each dish represented one experimental unit. The principal variables were weight loss, berry diameter, disease incidence, disease severity, fruit-juice pH and fungal identity. Table 3.3 summarises the assessment schedule and method used for each variable.

Table 3.3: Summary of variables, assessment times and measurement methods

Variable	**Assessment day(s)**	**Method**	**Reporting unit**
Weight loss	0, 5, 10 and 15	Collective Petri dish mass measured using an analytical balance	% of initial mass
Berry diameter	0, 5, 10 and 15	Equatorial circumference measured using flexible string and a ruler; diameter calculated as C/π	mm
Disease incidence	5, 10 and 15	Count of berries with visible decay	% infected fruit
Disease severity	5, 10 and 15	Visual 0-4 rating scale	DSI (%)
Fruit juice pH	15	Universal indicator paper	Approximate pH class
Fungal characteristics	End of storage	Culture and morphological examination	Descriptive or presumptive

			identity

3.5.1 Weight Loss

The total mass of the nine berries in each Petri dish was recorded using an electronic analytical balance at Days 0, 5, 10, and 15. Mass at Day 0 was used to represent the initial mass of the experimental unit. Calculation of weight loss occurred for each Petri dish separately and was done using Equation 1:

$$Weight\ loss(\%) = \frac{W_0 - W_t}{W_0} \times 100$$

[1]

In this case, W0 stands for the initial mass of the fruits in the Petri dish on Day 0, while Wt stands for the mass of the same fruits on the specific day of the measurement. Weight loss percentage instead of remaining mass in grams was used for treatment comparisons since the initial mass of each Petri dish was not the same.

3.5.2 Berry Diameter

Berry size was determined by measuring the average equatorial diameter of the fruits in each Petri dish. The circumference of each berry was determined by using a measuring tape and a ruler to determine the maximum circumference of each berry, while the diameter was obtained using d=C/π. If data were missing, then the diameter was estimated using the photographic image, using the 90 mm Petri dish diameter as the calibration standard. The average diameter of berries was calculated for each Petri dish and each assessment date using Equation 2.

$$Berry\ diameter\ (mm) = \frac{Equatorial\ circumference\ (mm)}{\pi}$$

[2]

It should be noted that reconstructed diameter values were used as an approximation since they have lower accuracy compared with the direct estimation of the diameter with a digital caliper.

3.5.3 Disease Incidence

The presence of signs of infection with grey mold such as the growth of grey mold, spore production, formation of lesions, and collapse was examined on each berry under proper lighting. Infected berries were those having at least one clear sign of infection. Disease incidence for each Petri dish was estimated using Equation 3 below:

$$Disease\ incidence\ (\%) = \frac{Number\ of\ infected\ berries}{`Total\ beries\ assessed} \times 100$$

[3]

The total number of berries in each Petri dish was nine. All berries were kept in each dish during the experiment so that infection progress could be followed up over the experimental period.

3.5.4 Disease Severity and Disease Severity Index

The amount of visible decay on the berries was determined on a 0-4 scale, as explained in Table 3.4. The scale is indicative of the approximate percentage of the surface of the berry that showed signs of decay. Decay localized only to the scar region of the berry generally scored 1, while berries covered in gray mold generally scored 3 or 4.

Table 3.4: Disease-severity rating scale used for blueberry fruit

Rating	**Approximate fruit surface affected**	**Interpretation**
0	0%	No visible decay
1	Up to 25%	Small, localised lesion, commonly at the stem scar
2	26-50%	Moderate lesion development
3	51-75%	Severe decay affecting most of the berry
4	More than 75%	Very severe decay or fruit almost completely covered

Disease Severity Index (DSI) for each Petri dish was calculated using Equation 4 as follows:

$$DSI(\%) = \frac{\sum(Rating \times number\ of\ berries\ at\ that\ rating)}{N \times 4} \times 100$$

[4]

Where N is the total number of berries in the Petri dish, and 4 is the maximum disease severity rating.

Consequently, DSI encompasses both the percentage of infected berries and their severity of infection.

3.5.5 Fruit-Juice pH

On Day 15, the pH value of fruit juice was determined. Berries collected from each Petri dish were sliced using a blade and the universal indicator strip was pressed against the juice coming out. The color was compared to the manufacturer's chart of colors right away and the nearest pH category was written down. As the result, due to the rough estimation provided by the universal indicator paper, the observations made were purely descriptive and not comparable with the results gained with the use of the digital pH meter.

It should be noted that the direct measurement of pH on Day 0 was not carried out in the course of the experiment. All the Day 0 pH data provided for the purpose of comparison were estimated values. They did not belong to the list of experimental observations.

3.5.6 Fungal Culture and Morphological Records

Colony color, type of growth, texture, and sporulation were noted for each cultured isolate. Conidial morphology was observed by microscopy when visible. This provided the basis for a tentative morphological identification of the causative fungus. No information on molecular identification could be obtained since no PCR amplification or sequencing could be done.

3.5.7 Data Organisation and Reporting

The data was organized according to treatment, Petri dish replicate, and days of evaluation at the onset. The values within each replicate were those used for analysis in the statistical tests. Table 3.5 illustrates how each observation is transformed into the response variable that will appear in the results section. The observed values, means of treatments, standard errors, and the statistical comparisons appear in Chapter 4.

Table 3.5: Data organisation and reporting framework

Variable	**Primary observation**	**Derived response**	**Assessment day(s)**	**Reporting format**
Weight loss	Collective mass of nine berries per Petri dish (g)	Cumulative loss relative to Day 0	0, 5, 10 and 15	Weight loss (%)
Berry diameter	Mean equatorial diameter per Petri dish (mm)	Change or retention relative to Day 0	0, 5, 10 and 15	Mean diameter (mm) and/or diameter loss (%)
Disease incidence	Number of visibly infected berries out of nine	Number of infected berries divided by 9 × 100	5, 10 and 15	Disease incidence (%)
Disease severity	Individual berry ratings on the 0-4 scale	Disease Severity Index calculated from all nine berries	5, 10 and 15	DSI (%)
Fruit juice pH	Universal indicator colour category	Closest approximate pH category	15	Approximate pH (descriptive only)
Fungal characteristics	Colony, microscopic and molecular observations	Identification of the fungus associated with decay	End of storage	Descriptive or species identity

Graphs and tables of results were not discussed in this methodological chapter since they are related to the experimental results. In Chapter 4, it is necessary to report the changes in the weight loss and berry size in relation to the storage period, while disease incidences and DSI have to be presented in regard to treatments and the test days. Graphs need to be constructed using the analyzed data.

3.6 Data Analysis

The experimental unit was defined as a Petri dish, with three replicates per treatment. Weight loss percentage, average berry diameter, disease incidence percentage, and Disease Severity

Index data were analyzed by calculating the treatment means and the associated standard error. One-way analysis of variance was carried out separately for each day of assessment in order to test the null hypothesis of equality of the four treatment means.

Before interpreting ANOVA results, the distributions of the residuals and the homogeneity of variances were analyzed by performing appropriate tests. The percent data could be transformed using the arcsine square-root transformation if necessary for fulfilling the assumptions of the ANOVA procedure; however, the treatment means were presented in their raw form to make the interpretation easier. As there were only three experimental units per treatment, the statistical power of the assumptions tests was rather low.

Since ANOVA suggested the presence of a significant treatment effect at $P \leq 0.05$, post hoc comparisons between treatment means were made employing Fisher's Least Significant Difference (LSD) test at the 5% level of significance. The means which had the same letter were said to be not statistically different. Weight loss is reported in terms of percentage weight loss rather than remaining weight due to varying starting weights of the Petri dishes. Disease incidence is stated in proportions of nine berries per dish, whereas Disease Severity Index (DSI) denotes the total severity index of the same experimental unit.

The fruit juice pH was described because measurements have been done using the universal indicator strips, hence providing an approximation rather than exact continuous values. The estimates of day 0 pH were not regarded as experimental values. In case of analyzed diameter values, the results are viewed as exploratory and are discussed keeping in mind the measurement problems. Statistical analyses have been done by using IBM SPSS Statistics Version 26 software and independently verified with outputs that are compatible with Microsoft Excel.

3.7 Ethical, Biosafety and Quality-Control Considerations

The authorization to harvest blueberries on the study farm was granted by the management of the farm. There were no human subjects, human materials, nor vertebrates involved in the study. The experimental work was conducted in accordance with BUSE laboratory safety guidelines. Gloves and clean equipment were used while handling the fruit, and fungal cultures and diseased fruit were considered biological waste and disposed of following the laboratory’s decontamination guidelines.

Chitosan produced from black soldier fly was sourced through an intra-departmental research cooperation whereby the researcher responsible for the production of chitosan provided permission to use it. Inoculation, dipping, drying, and observation procedures were followed for all treatment procedures except for the change in the concentration of chitosan.

3.8 Methodological Limitations

It is worth noting that this study took place at ambient laboratory conditions instead of a constantly monitored set of parameters such as temperature and relative humidity. It means that the outcomes obtained were affected by both the experimental treatments and laboratory conditions of winter season. Besides, the measurements of pH with universal strips allowed obtaining only approximate data concerning this parameter. The diameter of berry could be roughly estimated based on the pictures which meant its lower accuracy compared to that obtained with a digital caliper.

3.9 Chapter Summary

An experimental design was adopted to test the efficacy of three different concentrations of chitosan obtained from black soldier flies relative to the untreated control under controlled laboratory conditions. In this case, the experimental unit used was the Petri dish, where each treatment was made up of three replicates consisting of nine Azra Blue fruits. The steps undertaken in this experiment include pathogen infection, coating, winter storage, and evaluation at intervals of 15 days. Quantitative data such as weight loss, diameter, incidence of the disease, and disease severity were collected at certain intervals, while the pH of the fruit juice was measured using the descriptive approach because wide range paper was used. Analysis of replicated quantitative data used the one-way ANOVA test and then Fisher LSD for mean comparison.

CHAPTER 4

RESULTS

4.1 Introduction

In this chapter, the impact of coating by chitosan on blueberry weight loss, blueberry diameter, disease prevalence, disease severity, and fruit juice pH is discussed after storing blueberries for 15 days in winter ambient lab conditions. Mean ± SE for three Petri dish repetitions per treatment are reported. One-way ANOVA was conducted separately for each day of observation, followed by Fisher's LSD test to separate means where treatment effect was significant at $P \leq 0.05$.

The results presented are based on the working data set that was created for the experiment. The initial masses of Petri dishes were measured, but in some cases, they had to be calculated from the records made during the experiment because the actual numerical data was missing. The pH value on Day 0 was considered as a baseline, while the pH value on Day 15 was approximate and obtained using indicator strips.

4.2 Effect of Chitosan Coatings on Weight Loss

Loss of weight was gradual in all treatments with increasing time of storage. Treatment differences were found at Days 5 ($F = 73.70$, $P < 0.001$), Days 10 ($F = 132.78$, $P < 0.001$) and Days 15 ($F = 178.64$, $P < 0.001$). At all observation points, untreated control had the greatest weight loss while T4 had the least.

Table 4.1: Mean weight loss (%) of blueberries during ambient storage

Storage day	T1 control	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)
Day 5	3.87 ± 0.15 a	2.97 ± 0.09 b	2.40 ± 0.06 c	2.00 ± 0.06 d
Day 10	7.47 ± 0.20 a	5.80 ± 0.12 b	4.67 ± 0.09 c	3.80 ± 0.12 d
Day 15	10.70 ± 0.26 a	8.23 ± 0.15 b	6.60 ± 0.12 c	5.40 ± 0.12 d

Values are mean ± SE (n = 3). Means within the same row followed by different letters differ significantly according to Fisher's LSD test at $P \leq 0.05$.

On Day 15, weight loss of 10.70% was achieved in T1, while the corresponding values for T2, T3, and T4 were 8.23%, 6.60%, and 5.40%, respectively. In comparison with the control group, T4 decreased the weight loss at the end of the experiment by approximately 49.5%.

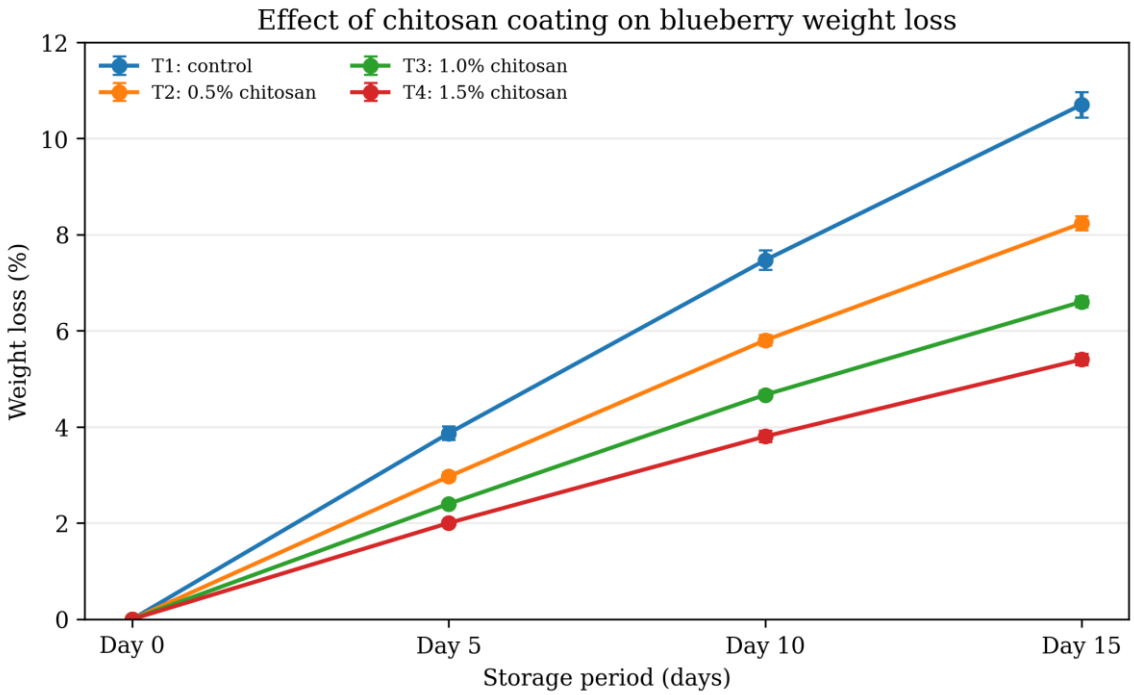


Figure 4.1: Mean weight loss of blueberries during 15 days of ambient storage. Error bars represent ± SE (n = 3).

4.3 Effect of Chitosan Coatings on Berry Diameter

There were no significant differences between treatments in initial berry size (F = 3.64, P = 0.064), suggesting that there was little variation in starting berry sizes for each treatment. There were significant differences between the treatments after Day 5 (F = 6.63, P = 0.015), which further increased on Days 10 (F = 12.91, P = 0.002) and 15 (F = 18.27, P < 0.

Table 4.2: Mean berry diameter (mm) during ambient storage

Storage day	T1 control	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)
Day 0	18.80 ± 0.12 a	19.07 ± 0.09 a	19.23 ± 0.12 a	19.27 ± 0.12 a
Day 5	18.42 ± 0.11 b	18.76 ± 0.10 a	18.97 ± 0.12 a	19.05 ± 0.11 a
Day 10	17.94 ± 0.10 c	18.35 ± 0.08 b	18.66 ± 0.12 a	18.78 ± 0.12 a
Day 15	17.51 ± 0.12 c	18.01 ± 0.08 b	18.39 ± 0.11 a	18.53 ± 0.11 a

Values are mean ± SE (n = 3). Means within the same row followed by different letters differ significantly at P ≤ 0.05. Day 0 means did not differ significantly and therefore share the same letter.

On Day 15, T1 recorded the lowest mean berry diameter (17.51 mm) while T4 maintained the highest mean berry diameter (18.53 mm). T3 and T4 were statistically comparable, and the mean berry diameters of these two treatments were greater than that of T1 and T2.

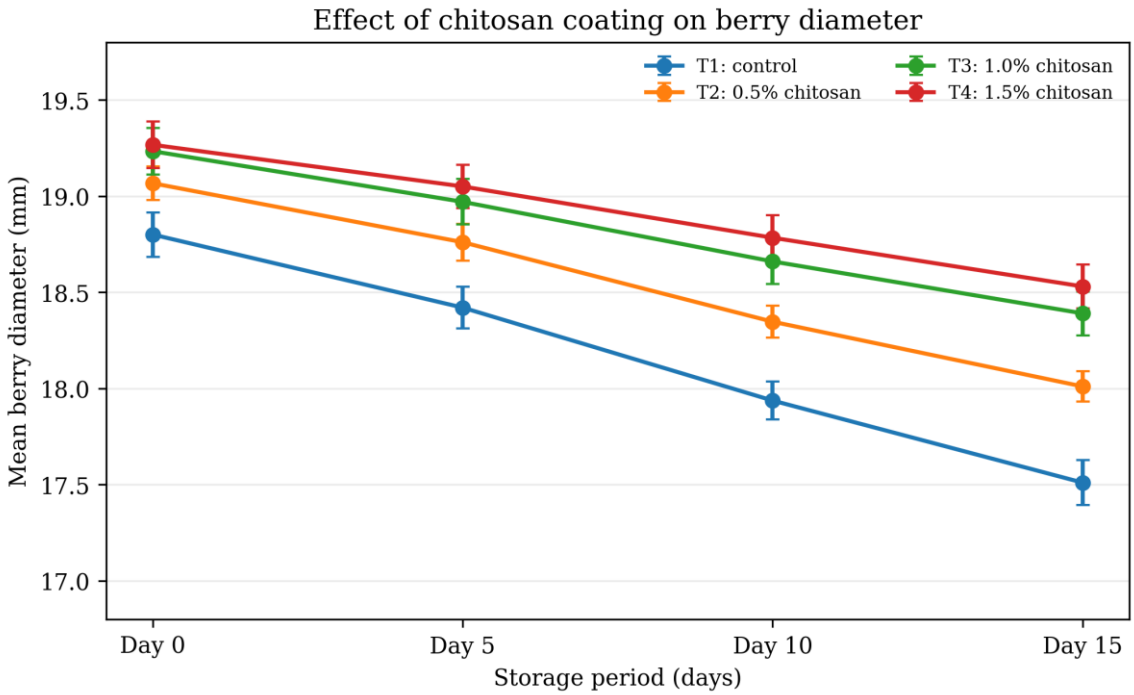


Figure 4.2: Mean berry diameter during 15 days of ambient storage. Error bars represent ± SE (n = 3).

4.4 Effect of Chitosan Coatings on Disease Incidence

There were increases in disease incidences as a function of storage days in all treatments, however, the greatest progression was recorded in the untreated control. Effects due to the different treatments were recorded at Day 5 (F=11.33; p=0.003), Day 10 (F=40.67; p<0.001) and Day 15 (F=40.67; p

Table 4.3: Mean disease incidence (%) during ambient storage

Storage day	T1 control	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)
Day 5	29.63 ± 3.70 a	14.81 ± 3.70 b	11.11 ± 0.00 b	11.11 ± 0.00 b
Day 10	59.26 ± 3.70 a	33.33 ± 0.00 b	25.93 ± 3.70 b	22.22 ± 0.00 c
Day 15	70.37 ± 3.70 a	44.44 ± 0.00 b	37.04 ± 3.70 b	33.33 ± 0.00 c

Values are mean ± SE (n = 3). Means within the same row followed by different letters differ significantly according to Fisher's LSD test at P ≤ 0.05.

In T1, T2, T3 and T4 groups, there was an average disease incidence of 70.37%, 44.44%, 37.04% and 33.33% respectively on day 15. This shows that group T4 decreased disease incidence by about 52.6% from the un-treated control. Even though the values of disease incidence for T2 and T3 were lower than that of T1, they were statistically similar between themselves on day 15.

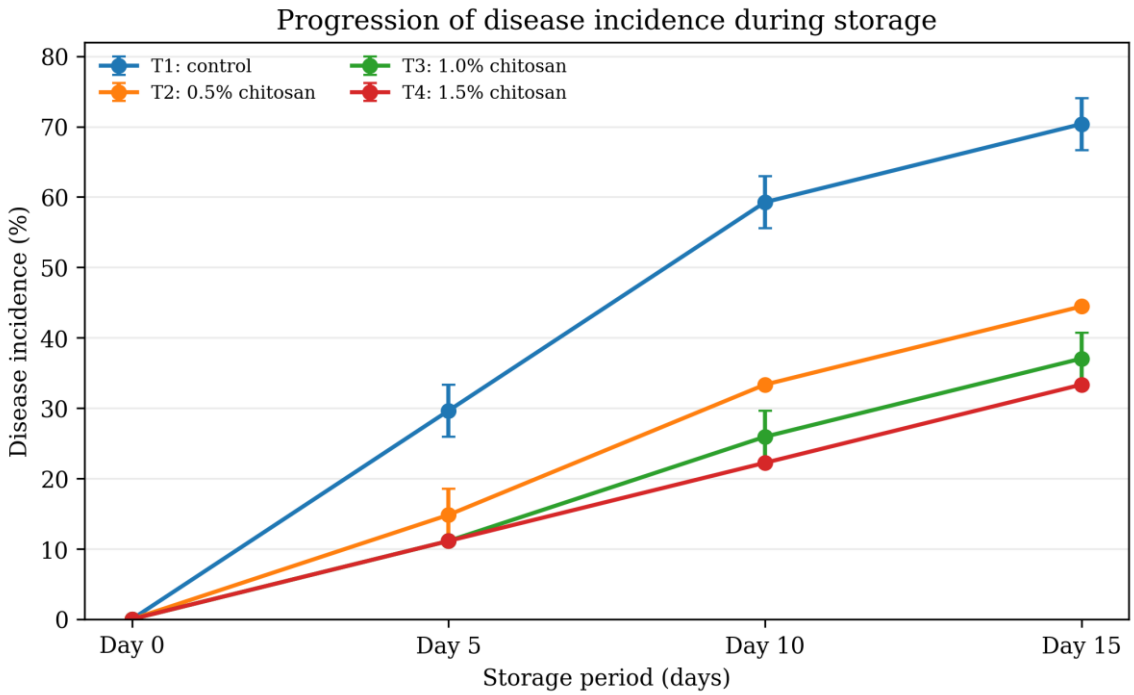


Figure 4.3: Progression of disease incidence during 15 days of ambient storage. Error bars represent ± SE (n = 3).

4.5 Effect of Chitosan Coatings on Disease Severity

Disease severity at Day 15 differed significantly among treatments ($F = 77.65$, $P < 0.001$). The untreated control recorded a substantially higher Disease Severity Index than all chitosan-coated treatments.

Table 4.4: Disease Severity Index (%) of blueberries at Day 15

Treatment	Disease Severity Index (%)
T1: control	37.03 ± 2.47 a
T2: 0.5% chitosan	11.10 ± 0.00 b
T3: 1.0% chitosan	10.17 ± 1.87 b
T4: 1.5% chitosan	8.30 ± 0.00 b

Values are mean ± SE (n = 3). Means followed by different letters differ significantly according to Fisher's LSD test at $P \leq 0.05$.

For the mean values of DSI, it was found that the T1 mean value of DSI was 37.03%, while in T2, T3, and T4, the values were 11.10%, 10.17%, and 8.30%, respectively. The chitosan treatments had no significant difference among them; however, they showed a significantly lower DSI compared to the control treatment.

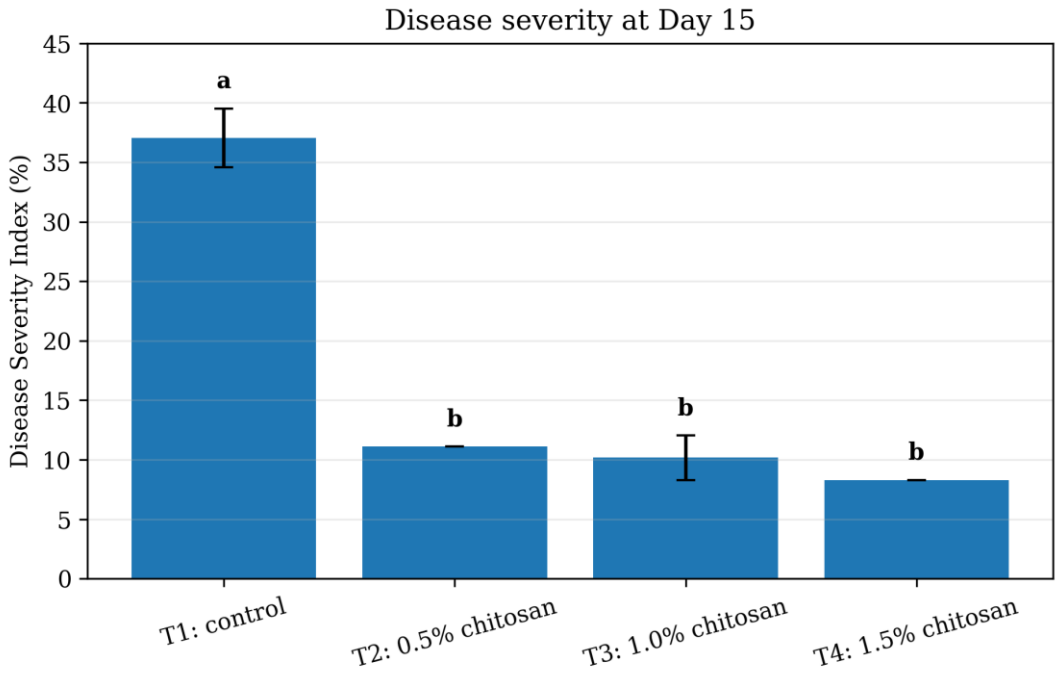


Figure 4.4: Disease Severity Index of blueberries at Day 15. Error bars represent ± SE (n = 3). Bars with different letters differ significantly at P ≤ 0.05.

4.6 Fruit-Juice pH

The pH of fruit juice was described using the data as universal indicator strips give estimates, and values of Day 0 were also estimated and not measured. The estimated value for pH of Day 0 was 3.4 for all treatments. On Day 15, the control without treatment had an estimate of pH of 4, while all chitosan coated treatments had an estimate of pH of 3.

Table 4.5: Descriptive fruit juice pH values

Assessment	T1 control	T2 (0.5%)	T3 (1.0%)	T4 (1.5%)
Day 0 (estimated)	3.37 ± 0.03	3.37 ± 0.03	3.37 ± 0.03	3.40
Day 15 (strip reading)	4.00	3.00	3.00	3.00

Day 0 values were estimated baseline values. Day 15 values were approximate readings obtained using universal indicator strips; therefore, inferential statistical analysis was not applied.

The results from Day 15 show that the pH of the control fruit was higher than that of the chitosan-coated fruit. It is clear that the uncoated fruit was becoming less acidic during storage compared to the coated fruits whose juice remained acidic. Since the measurements were made through broad range universal indicator papers, the measurements can be viewed as an approximation of pH values.

4.7 Visual Characteristics of Grey Mould Development

The visible symptoms of grey mould infection were seen mostly around the stem scar area in the chitosan coated berries. By day 15, up to 4 berries out of each dish were infected in the treated samples, but there was no widespread coverage of the infection throughout the entire berry; the fungus stayed localized. The untreated controls had around 6 berries in each dish being affected, but on average 2 berries in each dish got heavily infected by grey mould which covered the entire surface of the berry.

This confirms the numerical findings of incidence and severity of the disease. Molecular confirmation at the species level has not been provided in this chapter as there has been no output from sequencing for analysis.

4.8 Summary of One-Way ANOVA Results

Table 4.6: Summary of one-way ANOVA results

Variable	Assessment day	F-value	P-value	Decision
Weight loss	Day 5	73.70	<0.001	Significant
Weight loss	Day 10	132.78	<0.001	Significant
Weight loss	Day 15	178.64	<0.001	Significant
Berry diameter	Day 0	3.64	0.064	Not significant
Berry diameter	Day 5	6.63	0.015	Significant
Berry diameter	Day 10	12.91	0.002	Significant
Berry diameter	Day 15	18.27	<0.001	Significant
Disease incidence	Day 5	11.33	0.003	Significant
Disease incidence	Day 10	40.67	<0.001	Significant
Disease incidence	Day 15	40.67	<0.001	Significant
Disease severity index	Day 15	77.65	<0.001	Significant

A treatment effect was considered statistically significant at $P \leq 0.05$.

4.9 Overall Summary of Results

All of the coated treatments performed better than the control treatment in all response variables. The T4 coating showed the best overall performance, while T3 and T4 had no statistical difference when it came to berry size. There was no statistical difference between the disease severity in the three coated treatments.

4.10 Treatment Performance Relative to the Control

This became most apparent during the final test period. Compared to the control mean of 10.70%, weight loss for Day 15 decreased by approximately 23.1% in T2, 38.3% in T3 and 49.5% in T4. The concentration gradient became visible on Day 5 and grew through Days 10 and 15 during continued exposure to the environment.

A similar pattern emerged when analyzing the diameter results. T2, T3 and T4 measured 0.50, 0.88 and 1.02 mm greater in diameter compared to the control by Day 15, corresponding to 2.9%, 5.0% and 5.8% respectively. This difference in the smaller dimension still confirmed the visual data indicating the reduction of shrinkage caused by the coating. Since there were no significant differences between diameters on Day 0, it seems highly unlikely that the final difference could have been a result of some initial size discrepancy.

The biggest proportionality differences appeared in the disease control experiment. Disease incidence on Day 15 in T2, T3 and T4 was lower by 36.9%, 47.4% and 52.6% respectively from the 70.4% control disease incidence. The reduction in severity was even greater – 70.0%, 72.4% and 77.6% compared to the control DSI of 37.0%. Despite developing some stem-scar lesions, the lesion growth was minimal in treated berries, which indicates the effect of the coating on both disease incidence and spread.

T4 took first place in general, followed by T3 and T2. Low concentrations did not lack efficiency; rather, protection was improving consistently and the difference in numbers was reducing as the upper limit of the tested range was reached. The similarity in moisture loss, shrinkage, disease incidence and severity does not suggest any measurement errors.

CHAPTER 5
DISCUSSION

5.1 Introduction

Discussion of the present results in the context of current research into chitosan-based post-harvest preservation is presented in this chapter. The analysis includes weight loss, berry diameter, disease incidence, disease severity, visible grey mold development and fruit juice pH. Concentration dependence and importance of chitosan derived from black soldier fly are also discussed.

The untreated control decayed faster compared to all coating treatments. Both weight loss and disease incidence displayed the most pronounced concentration dependency whereas differences between 1.0% and 1.5% chitosan were minor in case of berry diameter and Disease Severity Index at the end of experiment. Overall, this general trend is in agreement with three basic functions of chitosan: creation of a semi-permeable film on the surface, antimicrobial activity and eliciting of protective responses by the fruit tissue (Romanazzi et al., 2018; De Vega et al., 2021).

Current research on blueberries and other soft fruits provides sufficient background for understanding such results. The reduction of moisture loss, inhibition of softening and prevention of spoilage due to application of chitosan-based coatings to blueberries has been shown recently (Eldib et al., 2020; Li et al., 2021; Xing et al., 2021; Sun et al., 2022; Ramos-Bell et al., 2024; Mohamed & Ramaswamy, 2025; Wu et al., 2025). Preservative effect similar to that seen in blueberry experiments was achieved in treatment of red raspberries, strawberries, grapes, tomatoes and Prunus fruit (Lo Piccolo et al., 2023; Tafi et al., 2023; Triunfo et al., 2023a, 2023b; Guarnieri et al., 2024; Vitti et al., 2024).

The variables used in this experiment have biological relevance to each other. Low water loss may help maintain turgor pressure in cells, delay senescence and reduce nutrient loss for fungi. Antifungal activity would limit any tissue destruction and secondary loss of water, and defense reactions would prevent any further lesion growth even after infection takes place. The discussion, however, analyzes these variables individually since their occurrence is only a component of one preserving mechanism. Cautious interpretation should be made since the replicates were small, some data had to be estimated, the pH level was determined using rough indicators, and the identity of the fungus could not be proven biochemically.

5.2 Effect of Chitosan Coatings on Weight Loss

All treatments lost weight due to transpiration and respiration of harvested blueberries. The highest weight was lost in distilled-water control, while the more concentrated chitosan solutions caused weight losses to reduce progressively. Thus, on Day 15, the percentage of initial weight lost in T1 was 10.70%, which was significantly lower than 8.23%, 6.60% and 5.40% lost in T2, T3 and T4 respectively. In other words, 1.5% coating prevented about 49.5% more weight loss relative to the control treatment. Significant differences between treatments observed at Days 5, 10 and 15 mean that the protective action took place during whole storage period rather than just at the end of it.

The most straightforward explanation would be chitosan's ability to form films. Due to drying of acidic chitosan solution, there appears a thin layer of a semi-permeable membrane that could increase resistance to vapour water transfer and decrease diffusion of oxygen and carbon dioxide, thus reducing transpiration and slowing down the respiratory consumption of stored substrates. Film formation has been suggested to play one of the major roles in the postharvest activity of chitosan by Romanazzi et al. (2018), and recent studies confirmed its link with lower weight losses and slower physiological changes (Eldib et al., 2020; Li et al., 2021; Xing et al., 2021; Qiao et al., 2024).

The decrease in weight loss observed from T2 to T4 suggests a role of concentration for improvement of barrier properties. Generally, more concentrated solution is expected to deposit thicker continuous layer, which would provide better resistance to water transfer. Nevertheless, such effect is likely to reach the plateau after certain concentration. More viscous coating might dry irregularly, affect natural bloom, limit gases exchange or cause anaerobic metabolism. The current experiment does not give any information on the thickness of the coating layer, respiration rates, internal gases composition, taste or volatile substances, so 1.5% concentration can be considered the best one but not the physiological optimum.

Experimental data gathered recently in blueberries corroborate the moisture conservation hypothesis. Chitosan-based coating films were demonstrated by Eldib et al. (2020) to mitigate deterioration of blueberry quality. Li et al. (2021) observed that chitosan nanomaterial films enhanced shelf-life and safety attributes of blueberries, while Xing et al. (2021) found higher preservation of postharvest quality with a chitosan/nano-TiO2 composite coating. The effect was additionally confirmed by Sun et al. (2022) who demonstrated that a chitosan/thyme-oil coating, especially in combination with UV-C, slows down softening of blueberries through prevention of cell wall polysaccharide degradation.

More recent blueberry research further corroborates this interpretation. According to Ramos-Bell et al. (2024), chitosan and salicylic acid combination reduces physiological weight loss and preserves physicochemical quality. Mohamed and Ramaswamy (2025) showed that caseinate-carboxymethyl chitosan composite coatings reduce weight loss, preserve firmness, and regulate respiration at 28 days of blueberry storage. In addition, Wu et al. (2025) noted that chitosan coatings enriched with bamboo leaf flavonoids limited changes in blueberry weight loss and spoilage rate. While formulations applied in the latter studies were rather complicated compared to BSF extracts used in the current experiment, they still indicate moisture retention capacity of chitosan-containing surface film.

Further support for the hypothesis comes from studies conducted on other small fruits. Lo Piccolo et al. (2023) observed reduced weight loss of red raspberries treated with chitosan under conditions of cold storage and room temperature storage. Qiao et al. (2024) reported reduced weight loss and respiration of blue honeysuckle coated with chitosan and Aloe vera gel. In addition, Guarnieri et al. (2024) noted an increased shelf life of white and red grapes treated with Hermetia illucens-derived chitosan under room and refrigerated storage conditions.

Storage at ambient temperature might have enlarged the effect sizes due to the fact that blueberries have a large surface-area-to-volume ratio, as well as having an epidermis layer that is thin and having an open stem scar through which water loss happens. In refrigerated conditions and humidity that is relatively high, the process of transpiration and respiration should be slow; in ambient laboratory conditions, dehydration is quicker. Thus, the lower weight loss in T4 indicates the efficacy of chitosan in tough situations.

5.3 Effect of Chitosan Coatings on Berry Diameter

No Day 0 difference indicated similar starting sizes for the berries in each of the treatments. Diameter decreased with all treatments in storage, although the reduction was more pronounced in the control. From Day 0 to Day 15, average diameter decreased by approximately 6.9% in T1, 5.6% in T2, 4.4% in T3 and 3.8% in T4. On Day 15, T3 and T4 had significantly larger diameters than T1 and T2; however, there was no significant difference between T3 and T4. The benefit for maintaining visible size in these cases reached an apparent plateau somewhere between 1.0% and 1.5% chitosan.

As previously noted, berry shrinkage is strongly correlated with water loss. Loss of cellular water causes a drop in turgor pressure and leads to berry shrinking due to reduced expansion of the berry's surface. Additionally, ripening and aging processes include breakdown and

rearrangement of pectin, cellulose and hemicellulose. These materials make up the main components of the berry's cell walls; consequently, their degradation results in weakening of the cell wall structure. It also leads to fruit softening and tissue degradation, and both processes can be slowed down with preservation of turgor pressure by moisture retention and reduction in respiration rate. Therefore, the high degree of correlation between changes in diameter and weight is consistent with water retention being the mechanism of action.

Recent studies on blueberries confirm this link between treatment and structural quality. Both Li et al. (2021) and Xing et al. (2021) observed positive effect of chitosan coating on firmness of the berries and on other quality parameters. Sun et al. (2022) found that the application of chitosan/thyme oil coating in combination with UV-C radiation reduces activity and expression of enzymes responsible for breakdown of pectin, cellulose and hemicellulose.

This conclusion is in agreement with recent research on composite coatings as well. Mohamed & Ramaswamy (2025) reported that carboxymethyl chitosan composite coatings preserved the firmness of blueberries, suppressed respiration and weight loss. Wu et al. (2025) also noted improved visual integrity and less deterioration of the coated blueberries. Qiao et al. (2024) observed improved firmness and reduced water loss in another soft blue fruit, namely blue honeysuckle. Though in these works the researchers did not use the parameter of circumference-derived diameter but the firmness/visual quality instead, the biological logic behind their findings is the same.

However, the use of diameter does not equate to an equivalent substitute of the firmness. The berries can soften significantly while there would be no changes in the diameter of the berry, moreover, the method of the string and ruler is less accurate than using digital calipers or texture analyser. The inaccuracy of measurement can also be due to the inconstant position and tension of the string. In future research, diameter should be combined with puncture force, compression force, deformation test and image processing.

5.4 Effect of Chitosan Coatings on Disease Incidence

Grey mould incidence rose in all treatments, thereby confirming that both the inoculum and environmental conditions were favourable for disease development. The control group reached 70.37% incidence by day 15, which is in comparison to 44.44%, 37.04% and 33.33% incidence in T2, T3 and T4. Thus, the 1.5% treatment led to a reduction of final incidence in approximately 52.6% compared to the control group. The concentration gradient was most noticeable by days 10 and 15, when T4 had the lowest incidence, and the control group was clearly separate from the coated treatments.

There are multiple reasons for the reduction of grey mould incidence. Firstly, deposition of the film might reduce direct contact between fungal spores and the susceptible tissue and improve injury and stem scar coverages. Secondly, protonated amino groups of chitosan might affect negatively charged surface of fungi, disorganise their membranes and impede the germination and hyphal growth. Thirdly, chitosan might serve as an elicitor that stimulates defensive pathways in plant tissues. Romanazzi et al. (2018) described such multi-faceted mechanism of chitosan, while De Vega et al. (2021) proved that chitosan elicits plant defense mechanisms against Botrytis cinerea.

The recent research about the interaction between insects and chitosan is very relevant to this issue. For example, Triunfo et al. (2023a) have shown that Hermetia illucens chitosan coatings delay strawberry deterioration. Further, Vitti et al. (2024) have directly tested antifungal activity of the chitosan coatings against B. cinerea and proved their efficiency on strawberry fruit. The treatments affected the morphology of the fungus, slowed down its growth and improved its protection during storage. Guarnieri et al. (2024) also described protective properties of insect-based chitosan application to grape fruits. Therefore, the lower incidence in T2-T4 should be attributed to the antifungal activity of the chitosan coating in addition to its influence on humidity level.

Regional significance of the disease is demonstrated in Foster et al. (2024) study where B. cinerea was found in symptomatic blueberry tissues collected from the orchards of South Africa. Grey mould is also one of the most important postharvest diseases of blueberries, as stated by Ramos-Bell et al. (2021). In this regard, the current results justify the application of chitosan as an integral part of a disease management strategy within South African blueberry supply chain.

Disease development was slowed down but not stopped. Even when the concentration of chitosan reached 1.5%, about a third of the fruits exhibited disease symptoms on day 15. This means that chitosan either delayed establishment of infection or suppressed infection but did not kill it completely. Inconsistency in coverage of stem scar, microwounds, natural variability between fruits, and different distribution of the inoculum can explain development of some infections. Therefore, the use of chitosan needs to be combined with sanitation, gentle handling, quick cooling, and controlling humidity. Due to absence of negative control without inoculation, it was not possible to differentiate the effect of the inoculum and naturally occurring microflora.

5.5 Effect of Chitosan Coatings on Disease Severity and Visual Grey Mould Development

Severity of disease was another source of data apart from disease incidence, since it gave an account of the extent of decay caused in the infected fruits. DSI was 37.03% for the untreated control while for T2, T3 and T4 it was 11.10%, 10.17% and 8.30%, respectively. Treatment T4 decreased DSI by about 77.6% compared to T1. All the coated treatments were statistically similar in effect, suggesting that 0.5% chitosan had successfully constrained the spread of lesions after infection.

The visual appearance confirmed the results obtained from the DSI. The symptoms observed in the treated fruits were localized mainly on the scar of the stem and did not spread much across the surface of the fruits. For the control, about six fruits per plate had symptoms while two fruits per plate were infected extensively with grey sporulation covering the entire fruit. This is a susceptible point due to vulnerability of the stem scar compared to skin coverage.

The more substantial effect on severity than on incidence is scientifically understandable. Indeed, some conidia could start germination or develop lesions despite being protected with the coating and thus increase the incidence. Further colonization could be suppressed by the anti-microbial environment created by chitosan, delayed host senescence, and activated defense reactions. Vitti et al. (2024) report the inhibition of B. cinerea growth and disturbance of its typical morphology through the application of insect-derived chitosan, and De Vega et al. (2021) found that chitosan could initiate plant defense responses. These mechanisms should result in infected berries with localized lesions rather than completely colonized fruits.

The high statistical consistency among T2, T3, and T4 indicates the possibility of reaching the threshold for constraining lesion expansion at the 0.5% concentration despite the higher concentrations that reduced the number of infected fruits. Such a split of infection establishment and subsequent lesion formation is known in the context of postharvest pathogens: barrier properties might affect the first process, whereas antifungal activity and defense reactions of hosts – the second (Romanazzi et al., 2018; Ramos-Bell et al., 2021). Also, the low number of replications and the discrete scale from 0 to 4 might have hindered distinguishing coated treatments statistically.

Nevertheless, symptoms should still be considered presumptively gray mold that correlates with B. cinerea, not confirmed botrytis. Foster et al. (2024) stress the need for culture and molecular identification in Botrytis confirmation in blueberry. Observation of morphology only cannot rule out the presence of other sporulating fungi. Future trials should involve the isolation of the

fungus from the culture, re-inoculation tests, and sequencing of appropriate genes to link effects to a particular pathogen.

5.6 Fruit-Juice pH

PH was an exception in contrast to the other studied parameters since their values depended on colour estimates rather than instrumental measurement. The estimated values of the PH for Day 0 were about 3.37-3.40. On Day 15, the control sample produced pH readings of approximately 4, while the samples under all coatings had readings of approximately pH 3. Thus, considering face-value observations, the control sample lost some of its acid condition during storage while the treated samples kept it. However, using such categories as one-unit change in pH on an indicator strip is too imprecise for determining whether there was any significant change in pH.

An increase in the pH of the more diseased sample could be explained by biological mechanisms; however, no one mechanism could be considered responsible for the observed increase in pH. In the case of the advanced colonisation of the fruit by fungi, metabolism of nitrogen and release of ammonia could cause the alkalinisation of the host tissue, while consuming or redistribution of the organic acid and destruction of cell walls could affect the juice condition. B. cinerea also regulates secreted enzymes according to the ambient pH and uses pectin-degrading enzymes which cause maceration and colonisation (Li et al., 2018; Cheung et al., 2020; Silva et al., 2023). In turn, increased amount of fungi biomass and tissue decomposition in T1 could contribute to pH increase.

Chitosan could maintain the acidity of the juice indirectly through reduction of respiration and senescence rates of the fruit and growth of microflora. Ramos-Bell et al. (2024) reported the better maintenance of physicochemical properties associated with acidity of blueberries treated with chitosan and salicylic acid. In turn, Mohamed and Ramaswamy (2025) estimated the effect of the coating formulations on the changes in pH and titratable acidity of coated blueberries. Similarly, Wu et al. (2025) estimated the impact of the coating treatment on several blueberry quality indicators, including those related to pH and titratable acidity.

Therefore, the current reading for the pH value has to be considered purely descriptive. The presence of anthocyanins in the blueberry juice makes its visual comparison with the help of the color chart impossible, the readings on the test strips are quite general and those recorded on Day 0 had been assumed and not actually measured. It is important for future research to measure pH values of the homogenised juice of every sample by the means of a calibrated pH meter, as well as measure titratable acidity separately.

5.7 Concentration-Dependent Response and Overall Efficacy

Overall, however, when all variables are accounted for, there appears to be an obvious dose-response relationship. T1 retained the least quality, T2 offered considerable protection, T3 provided even better results, and T4 yielded the best numerical results overall. Weight loss and disease incidence proved to be the most responsive to dose change. Berry diameter in T3 and T4 was statistically indistinguishable at Day 15 and all coated treatments had statistically identical disease severity. Consequently, chitosan concentration did not have the same impact on each and every preservation mechanism equally.

Film continuity, its thickness and water vapour resistance may become enhanced by increasing the amount of chitosan deposited on the surface – hence the obvious difference in weight loss between samples. Antifungal activity of chitosan may increase depending on the total amount available and contribute to the reduced incidence in T4. Elicitation of plant defences, on the other hand, may have a biological limit beyond which any additional amount of polymer will not provide much additional benefits. Romanazzi et al., De Vega et al. and Vitti et al. confirm independently of each other that there are several partially independent mechanisms of chitosan action.

Recent dose and formulation studies revealed that the effects of polymer concentration cannot be considered in isolation from other parameters. In particular, Lo Piccolo et al. showed that the effect of chitosan on raspberries varied depending on whether chitosan was applied pre- or postharvest or both and depending on storage temperature. Tafi et al. found that the effectiveness of insect and chitosan treatment of tomatoes varied depending on the method of coating application and polymer preparation. Finally, Guarnieri et al. demonstrated that chitosan effect on grapes varied depending on the type, concentration and storage regime of chitosan.

Table 5.1: Integrated interpretation of the principal findings and recent supporting literature

Variable	Present study finding	Interpretation	Recent supporting literature
Weight loss	10.70% in T1; 5.40% in T4	The coating reduced transpiration and moderated gas exchange; barrier performance improved with concentration.	Romanazzi et al. (2018); Li et al. (2021); Guarnieri et al. (2024); Mohamed & Ramaswamy (2025)
Berry diameter	17.51 mm in T1; 18.53 mm in T4	Improved water retention and slower cell-wall deterioration maintained turgor and visible fruit size.	Xing et al. (2021); Sun et al. (2022); Qiao et al. (2024); Mohamed & Ramaswamy (2025)

Variable	Present study finding	Interpretation	Recent supporting literature
Disease incidence	70.37% in T1; 33.33% in T4	The surface film, direct antifungal action and defence elicitation reduced establishment of visible infection.	De Vega et al. (2021); Triunfo et al. (2023a); Vitti et al. (2024); Wu et al. (2025)
Disease severity	37.03% in T1; 8.30% in T4	Chitosan restricted mycelial growth, lesion expansion and sporulation after infection had occurred.	Romanazzi et al. (2018); Ramos-Bell et al. (2021); Vitti et al. (2024)
Fruit-juice pH	Approx. 4 in T1; 3 in coated treatments	The higher control reading was consistent with advanced senescence, fungal metabolism and tissue breakdown; the observation remains descriptive.	Hua et al. (2018); Cheung et al. (2020); Ramos-Bell et al. (2024); Mohamed & Ramaswamy (2025)

While among all evaluated treatments, 1.5% chitosan provided the best results, especially in terms of weight loss and disease occurrence, both 1.0% and 1.5% retained the same level of diameter retention and provided the similar final severity level. Thus, an economically justified selection of a commercial treatment would also depend on such criteria as coating viscosity, drying time, evenness of coverage, cost of the polymer, its sensory characteristics and effect on the waxy bloom formation. This study cannot give answers to all of these questions.

The recent studies also highlight the fact that it would be misleading to assume that the better coating can be obtained with the help of increased concentrations of the compound. Composite coatings of blueberries have been shown to provide enhanced preservation by modifying polymer structure or adding functional agents other than increasing chitosan concentration (Sun et al., 2022; Mohamed & Ramaswamy, 2025; Wu et al., 2025). Thus, a further dose-response study should include intermediate concentrations of 1.0-1.5% chitosan along with testing of coating characteristics and sensory properties.

5.8 Relevance of Black Soldier Fly-Derived Chitosan and Practical Implications

These preservation methods are all inherent properties of chitosan but are not specific to black soldier fly-derived chitosan, which makes it intrinsically better than other sources such as crustaceans or fungi. These features of chitosan rely heavily on molecular weight, degree of deacetylation, purity, concentration, solvents, and formulations. The source of chitosan still matters due to its effect on these physicochemical characteristics as well as the cost and sustainability of supply, allergen management, and regulations.

It should be mentioned that BSF biomass gives an excellent opportunity for circular economy. Instead of disposing of exuviae and other residues rich in chitin during production of Hermetia

illucens, one could produce chitosan out of them. Triunfo et al. (2022) characterised chitin and chitosan from various BSF biomasses. Furthermore, Guarnieri et al. (2022) showed antimicrobial activity of chitosan prepared from various BSF developmental stages. Moreover, Triunfo et al. (2024) gave further characterisation of H. illucens chitosan obtained by homogeneous deacetylation of the polymer.

The most relevant produce studies are those involving insect-derived chitosan. Tafi et al. (2023) used this type of chitosan on cherry tomatoes. Similarly, Triunfo et al. (2023a) applied it to strawberries. Additionally, Triunfo et al. (2023b) used insect chitosan to protect apricots, nectarines and peaches. Guarnieri et al. (2024) evaluated effects of chitosan from white and red grapes. Vitti et al. (2024) studied antifungal activity of this material as a coating for strawberries. Thus, across various fruits, insect chitosan protected crops from spoilage and fungal development. This means that the current research adds to the existing body of evidence that BSF chitosan can be a useful postharvest coating.

The correlation of results from the studies by Guarnieri et al. (2024) and Vitti et al. (2024) is especially significant since the latter were conducted on soft fruit using the chitosan derived from H. illucens. The results from these experiments validate the biological plausibility of weight loss reduction and grey mould formation found in the present experiment. Nevertheless, the present experiment did not involve comparison of insect-based chitosan with the commercially available standard food-grade chitosan, so the data are insufficient to determine whether the insect-derived compound was better, equal or worse than the conventional chitosan.

In relation to blueberries grown in Zimbabwe, the most practical application of the protective layer would be that of an additional treatment applied when the fruit undergoes temporary storage, local transportation or refrigeration breakdowns. Locally made biodegradable protective film would contribute to lower reliance on the imported substances and increase the added value of waste materials from insect breeding. Still, it should be accompanied by rapid pre-cooling, proper packing, appropriate packaging, adequate relative humidity and continuous refrigeration transport.

5.9 Study Limitations and Methodological Considerations

However, the high level of congruence with the findings of recent literature does not make up for the shortcomings of the present experiment. There were only three replicate dishes for each treatment and the experiment was conducted using a single cultivar, a single farm, a single harvest date and ambient storage conditions. More dishes and repeats across different farms,

harvests and cultivars would give better estimates of biological variation and facilitate generalisation.

Temperature and humidity were not recorded continuously. In spite of the fact that the experiments were conducted during winter time, daily changes in laboratory environment may have affected respiration rate, condensation, moisture loss and mould development. Ambient storage experiments should utilise data loggers in future, and the same test should be performed in refrigerated environment to check whether the same pattern holds in commercial cold chain settings. Several recent studies show that storage temperature affects chitosan response (Lo Piccolo et al., 2023; Tafi et al., 2023; Guarnieri et al., 2024).

The control group was soaked in distilled water, while chitosan was applied in 1% acetic acid. Acid can change surface pH and affect microbial population; therefore, the effect of chitosan is inseparable from a potential solvent effect. Guarnieri et al. (2022) distinguish between effects of the polymer and its solvent in antimicrobial experiments, and Tafi et al. (2023) note that the solvent could have an effect on fruit. Future experiments should include an untreated control group, a distilled water control group and a group of berries treated with only acid solution, adjusted to the same pH as the solutions with coating.

All berries stayed in their Petri dishes until the end of the experiment. It provided multiple observations of the same experimental unit, but also facilitated spreading infection from severely affected berries to the other berries in the dish. As this procedure was uniformly applied to all treatments, the comparison remains relevant, but berries inside one dish were not independent of each other. The Petri dish was therefore the appropriate experimental unit.

For some of the measurements, observations and pictures were used to reconstruct measurements instead of taking them concurrently with the proper equipment. Diameter was calculated from circumference, while pH was determined using broad range indicators and rough calculations of the baseline. This information should be taken as provisional. Future experiments need to take all measurements as directly as possible, utilize digital calipers and texture analyzer, use a pH meter for pH measurement, measure titratable acidity and soluble solids, as well as monitor respiration and environmental conditions.

Lastly, visible mold was consistent with Botrytis grey mould, but exact species was not established via molecular identification. For proper identification, pure cultures, pathogenicity tests and DNA sequencing is required. The necessity of combining isolation and molecular methods for identification of B. cinerea in blueberry was proven by Foster et al. (2024). Proper controls such as an uninoculated control and a standardized isolate would help in causal

inference. Thus, the results of disease experiment should be reported as preliminary evidence of chitosan reducing presumed grey mould incidence and severity.

5.10 Chapter Summary

Chitosan coating reduced mass loss, preserved larger dimensions, and resulted in lower incidences and severity of diseases than the control without any coating. The pH values observed were approximately indicative of the preservation of acidic juice content in the coated fruits; the higher value of the control indicated greater fruit senescence and deterioration by fungi, but the paper strip method did not allow any quantitative analysis. Treatment T4 had the smallest losses of mass and the smallest incidences; T3 and T4 were equal in diameter retention, and all chitosan treatments significantly inhibited lesion growth.

These results are in line with a large number of studies conducted between 2018 and 2026, proving that chitosan coatings decrease the loss of water, inhibit softening, preserve quality, and hinder the growth of fungi in blueberries and similar fruits (Romanazzi et al., 2018; Li et al., 2021; Sun et al., 2022; Triunfo et al., 2023a, 2023b; Ramos-Bell et al., 2024; Guarnieri et al., 2024; Vitti et al., 2024; Mohamed & Ramaswamy, 2025; Wu et al., 2025; Herrera-Feijoo et al., 2026). However, further investigation of 1.0-1.5% black soldier fly chitosan is necessary, and commercial recommendations require large replicated tests, instrumental analysis, solvents controls, constant environmental monitoring, and validation in refrigerated export conditions.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Introduction

This chapter will state the conclusions made after evaluating the chitosan coating prepared from black soldier fly exoskeletons in management of postharvest decay of Azra Blue blueberries. Conclusions were based on the results of weight loss, berry diameter, disease incidence, disease severity and observations made on pH of the juice of berries under laboratory conditions at ambient winter temperature for 15 days.

6.2 Conclusions

6.2.1 Effect of Chitosan on Moisture Loss and Berry Size

Chitosan coating reduced postharvest moisture loss and improved retention of berry size. Weight loss increased throughout storage in every treatment, but the untreated control consistently recorded the greatest loss. By Day 15, mean weight loss was 10.70% in the control compared with 8.23%, 6.60% and 5.40% in the 0.5%, 1.0% and 1.5% chitosan treatments, respectively. The 1.5% coating therefore reduced final weight loss by approximately 49.5% relative to the control.

Initial berry diameter did not differ significantly among treatments, indicating that the experimental units began with broadly comparable fruit sizes. As storage progressed, coated berries retained larger diameters than the control. At Day 15, the control had a mean diameter of 17.51 mm, while fruit treated with 1.5% chitosan retained a mean diameter of 18.53 mm. The findings indicate that chitosan coatings slowed dehydration and shrinkage under the conditions of the experiment.

6.2.2 Effect of Chitosan on Grey Mould Incidence and Severity

The application of chitosan coating prevented excessive weight loss during post-harvest and maintained the size of the berries. There were significant losses in all the treatments, which increased over storage days. However, the highest rate was observed in the uncoated berries. On the fifteenth day, weight loss was 10.70% in the uncoated berries and 8.23%, 6.60% and 5.40% in berries applied with 0.5%, 1.0% and 1.5% of chitosan, respectively. This indicates that the use of 1.5% chitosan coating prevented about 49.5% of weight loss.

There were no significant differences in initial diameter among the different treatments. This indicates that there were no differences in the initial size of the fruits. As storage period increased, coated berries had higher diameters than the uncoated berries. At 15th storage day, the uncoated berries had an average diameter of 17.51mm, while fruit applied with 1.5% chitosan had an average diameter of 18.53 mm.

6.2.3 Most Effective Chitosan Concentration

Concentration dependency was seen for all the main factors considered. The control group was always the least successful one while the 0.5% concentration provided an intermediate level of protection while 1.0% and 1.5% treatments showed the highest levels of protection. The 1.5% chitosan treatment showed the smallest level of weight loss, the lowest disease rate and severity as well as the biggest final size of the berries. So, the 1.5% chitosan was found to be the best concentration on the conditions of the experiment. However, there was not much difference between 1.0% and 1.5% treatments for some parameters, so more research is needed to find out if the difference is commercial.

6.2.4 Overall Conclusion and Interpretation of pH

Chitosan produced from black soldier flies demonstrated potential use for coating as an edible product during temporary storage under ambient conditions. Fruit with a coating had higher moisture content, shrunk less, and had less obvious grey mould growth, and the level of protection increased with increasing concentrations of the coating.

Fruit-juice pH measurement cannot be used to draw a biochemical conclusion, since the value of the Day 0 sample was only estimated, whereas the value of the Day 15 samples was determined by means of universal pH indicator strips and thus is approximate. The fact that the sample without treatment gives a value around pH 4 and the samples with coatings give a value around pH 3 indicates that the control sample became less acidic, whereas the coatings helped to preserve its acidity. This is merely descriptive evidence and needs further confirmation through the use of pH meter.

The conclusions need to be regarded as preliminary, as the experiment included only three replicates of each treatment using Petri dishes, continuous logging of the temperature and relative humidity was not performed, no solvation test using only acetic acid was carried out, some data was reconstructed on the basis of observations made, and the fungus causing the decay was not identified using molecular analysis yet.

Table 6.1: Summary of principal conclusions and practical implications

Response variable	Key Day 15 result	Conclusion	Practical implication
Weight loss	T1: 10.70%; T4: 5.40%	Chitosan reduced cumulative water loss; T4 gave the greatest retention.	Use 1.5% as the leading concentration in confirmatory trials.
Berry diameter	T1: 17.51 mm; T4: 18.53 mm	Coated fruit showed less shrinkage and better size retention.	Include instrumental firmness and caliper measurements in future trials.
Disease incidence	T1: 70.37%; T4: 33.33%	Chitosan delayed or suppressed visible infection.	Combine coating with sanitation and appropriate temperature management.
Disease severity	T1 DSI: 37.03%; T4 DSI: 8.30%	Coating strongly restricted lesion expansion and whole-fruit colonisation.	Severity, not incidence alone, should be retained as a key response variable.
Fruit-juice pH	T1 approximately 4; coated fruit approximately 3	The control became less acidic, while coated fruit retained greater acidity; the strip readings were descriptive and not sufficiently precise for statistical inference.	Use a calibrated pH meter and titratable-acidity analysis.

6.3 Recommendations

6.3.1 Recommendations for Further Validation of the Coating

1. Choose the most effective treatment: The treatment with 1.5% chitosan needs to be chosen as the most effective candidate in the following experiments, with 1.0% being chosen as an alternative less costly treatment due to similar results achieved in terms of certain response variables.

2. Improve the number of replicates: Further experiments will have to include a higher number of experimental units, multiple harvests, and if possible, different cultivars of the blueberries.

3. Include the acetic acid as a solvent control: Apart from control and water treatments, the experiment will have to include the fruit treated with acetic acid of the same concentration used to dilute the chitosan solution.

This will allow distinguishing between the effect of chitosan and its solvent (or antimicrobial effect of the latter).

4. Identify the pathogen: Representative samples need to be isolated through cultivation and microscopy, followed by molecular identification using PCR amplification and sequencing of the selected fungal marker such as ITS region amplified by universal primers (White et al., 1990).

6.3.2 Recommendations for Measurement and Experimental Control

5. Continuous monitoring of storage conditions: Temperature and relative humidity must be monitored throughout the duration of the storage period. The same treatments also need to be tested under refrigerated storage and controlled ambient storage conditions to see if performance is affected by the environment.

6. Instrument calibration: Diameter of the berries needs to be measured using a digital caliper, firmness with texture analyzer or penetrometer, and pH with a pH meter. Other parameters that also need to be measured include total soluble solids, titratable acidity, color, weight loss and sensory quality.

7. Prospective measurements: Replicate-level measurements will need to be taken at each assessment point using data sheets or electronic workbooks. This will minimize any reconstruction of missing data points.

8. Describe the coating material: It will be necessary to know the extent of deacetylation, the molecular weight, the viscosity, the purity, the film thickness, and the permeability to water vapor of the insect-based chitosan in future studies.

6.3.3 Recommendations for Practical and Commercial Application

9. Perform packhouse trials at pilot scale: Prior to making recommendations for commercial use, it would be wise to trial the 1.5% coating on fruit packaged in standard export/retail punnets under normal handling conditions.

10. Determine consumer and market acceptability: Effects of the coating on natural bloom, appearance, taste, aroma, texture, firmness, and consumer acceptance must be evaluated. A coating which prevents fruit decay while impairing sensory properties would be commercially unsuitable.

11. Check food safety and economics: Food-grade standards must be met in the process of chitosan extraction and its application and solvent residue levels should not exceed legal limits. The costs of producing and applying chitosan from black soldier flies should be compared to the savings in fruit.

12. Apply the coating within an integrated system: The chitosan coating should complement rather than replace proper harvest hygiene, good handling practices, quick cooling and cold-chain management.

6.3.4 Recommendations for Future Research

In the future studies, it would be better to test for a broader range of concentrations in the 1.0-1.5% range, find out what is the minimum required concentration and investigate if using other natural antifungals that can be combined with chitosan does not adversely affect its effectiveness. Future researchers would need to investigate black soldier fly chitosan by comparing it to the commercially available chitosan and test its effectiveness both on naturally infected samples and artificially inoculated ones.

6.3.5 Recommendations for Key Stakeholders

The blueberry growers and pack house operators should consider the 1.5% solution to be a potential method for further testing rather than immediately adopting it as their commercial protocol. Pilot batches of fruits should be tested to ensure the effectiveness of the coating process in terms of uniformity, drying time, appearance of the bloom, taste, and suitability of punnet packaging. Coating must go hand in hand with harvest hygiene, removal of field heat, proper handling, and constant refrigeration since the edible coating is not going to fix cold chain problems or infected fruits.

The department of crop science and BUSE laboratories can assist in conducting additional research using calibrated callipers, pH meter, texture equipment, and environmental logging system. Standardized form of data collection should be employed from day 0 to record all observations in a prospective manner. Collaboration with Tinevimbo Nel Maravanyika provides researchers with the possibility of linking the efficiency of the coating with material characterisation including degree of deacetylation, molecular weight, purity, viscosity, and film permeability.

Researchers and extension officers should conduct tests of the coating on different cultivars, locations and during different seasons before recommending the method. Special attention should be paid to naturally infected commercially available fruits, specific pathogen population,

coating procedure in pack house settings and interruptions in cold chain to reflect real conditions. Food safety authorities and regulatory agencies should estimate the quality of the natural material, solvent residues, and requirements for hygiene during production. Cost evaluation of the process should take into account the cost of preparation and application and the value of reduction in marketable loss. Such validation will determine if black soldier fly chitosan coating of the local origin can be effectively used by Zimbabwean blueberries producers.

6.4 Final Conclusion

In ambient winter storage conditions, chitosan coatings decreased the presence of grey mold and helped maintain the weight and size of the blueberries. The 1.5% coating derived from the black soldier fly showed the most consistent performance and needs to be considered first for validation purposes. This is a first step toward the development of a locally available and environmentally friendly coating.

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APPENDIX A

REPLICATE-LEVEL WORKING DATASET

The following tables reproduce the replicate-level working dataset used for statistical analysis. Day 0 pH values and berry-diameter values reconstructed from the experimental image are identified as estimates in the methodology and results chapters. Disease-incidence percentages were calculated from the number of affected berries out of nine fruits per Petri dish.

Table A1: Replicate weight loss (%) used for ANOVA

Treatment	Replicate	Day 0	Day 5	Day 10	Day 15
T1	D1	0.0	3.6	7.1	10.2
T1	D2	0.0	3.9	7.5	10.8
T1	D3	0.0	4.1	7.8	11.1
T2	D1	0.0	2.8	5.6	8.0
T2	D2	0.0	3.1	6.0	8.5
T2	D3	0.0	3.0	5.8	8.2
T3	D1	0.0	2.3	4.5	6.4
T3	D2	0.0	2.5	4.8	6.8
T3	D3	0.0	2.4	4.7	6.6
T4	D1	0.0	1.9	3.6	5.2
T4	D2	0.0	2.1	4.0	5.6
T4	D3	0.0	2.0	3.8	5.4

Table A2: Replicate berry diameter (mm) used for ANOVA

Treatment	Replicate	Day 0	Day 5	Day 10	Day 15
T1	D1	18.60	18.25	17.78	17.32
T1	D2	18.80	18.39	17.91	17.49
T1	D3	19.00	18.62	18.12	17.72
T2	D1	19.10	18.79	18.38	18.04
T2	D2	18.90	18.58	18.19	17.86
T2	D3	19.20	18.91	18.47	18.13
T3	D1	19.30	19.04	18.73	18.45
T3	D2	19.00	18.74	18.43	18.17
T3	D3	19.40	19.13	18.82	18.55
T4	D1	19.20	18.99	18.72	18.47
T4	D2	19.50	19.27	19.01	18.75
T4	D3	19.10	18.89	18.62	18.37

Table A3: Replicate disease incidence (%) used for ANOVA

Treatment	Replicate	Day 0	Day 5	Day 10	Day 15
T1	D1	0.0	33.3	55.6	66.7
T1	D2	0.0	22.2	66.7	77.8

Treatment	Replicate	Day 0	Day 5	Day 10	Day 15
T1	D3	0.0	33.3	55.6	66.7
T2	D1	0.0	11.1	33.3	44.4
T2	D2	0.0	11.1	33.3	44.4
T2	D3	0.0	22.2	33.3	44.4
T3	D1	0.0	11.1	22.2	33.3
T3	D2	0.0	11.1	33.3	44.4
T3	D3	0.0	11.1	22.2	33.3
T4	D1	0.0	11.1	22.2	33.3
T4	D2	0.0	11.1	22.2	33.3
T4	D3	0.0	11.1	22.2	33.3

Table A4: Replicate Day 15 Disease Severity Index (%)

Treatment	Replicate	Day 15 DSI
T1	D1	36.1
T1	D2	41.7
T1	D3	33.3
T2	D1	11.1
T2	D2	11.1
T2	D3	11.1
T3	D1	8.3
T3	D2	13.9
T3	D3	8.3
T4	D1	8.3
T4	D2	8.3
T4	D3	8.3

Table A5: Estimated Day 0 and approximate Day 15 fruit-juice pH

Treatment	Replicate	Day 0	Day 15
T1	D1	3.4	4.0
T1	D2	3.4	4.0
T1	D3	3.3	4.0
T2	D1	3.4	3.0
T2	D2	3.3	3.0
T2	D3	3.4	3.0
T3	D1	3.4	3.0

Treatment	Replicate	Day 0	Day 15
T3	D2	3.4	3.0
T3	D3	3.3	3.0
T4	D1	3.4	3.0
T4	D2	3.4	3.0
T4	D3	3.4	3.0

APPENDIX B

VERIFIED ONE-WAY ANOVA AND FISHER LSD SUMMARY

Table B1: Verified one-way ANOVA summary

Variable	Day	F-value	P-value	Decision	Fisher LSD (5%)
Weight loss	Day 5	73.6979	0.000004	Significant	0.3075
Weight loss	Day 10	132.7843	<0.000001	Significant	0.4482
Weight loss	Day 15	178.6415	<0.000001	Significant	0.5596
Diameter	Day 0	3.6370	0.064020	Not significant	0.3646
Diameter	Day 5	6.6267	0.014637	Significant	0.3564
Diameter	Day 10	12.9127	0.001962	Significant	0.3428
Diameter	Day 15	18.2690	0.000614	Significant	0.3482
Disease incidence	Day 5	11.3333	0.002982	Significant	8.5407
Disease incidence	Day 10	40.6690	0.000034	Significant	8.5407
Disease incidence	Day 15	40.6690	0.000034	Significant	8.5407
Disease severity index	Day 15	77.6492	0.000003	Significant	5.0475

Table B2: Selected treatment means and Fisher LSD letters

Variable	Day	Treatment	Mean	LSD letter
Weight loss	Day 15	T1	10.70	a
Weight loss	Day 15	T2	8.23	b
Weight loss	Day 15	T3	6.60	c
Weight loss	Day 15	T4	5.40	d
Diameter	Day 15	T1	17.51	c
Diameter	Day 15	T2	18.01	b
Diameter	Day 15	T3	18.39	a
Diameter	Day 15	T4	18.53	a
Disease incidence	Day 15	T1	70.4	a
Disease incidence	Day 15	T2	44.4	b
Disease incidence	Day 15	T3	37.0	bc
Disease incidence	Day 15	T4	33.3	c
Disease severity index	Day 15	T1	37.0	a
Disease severity index	Day 15	T2	11.1	b
Disease severity index	Day 15	T3	10.2	b
Disease severity index	Day 15	T4	8.3	b

APPENDIX C

EXPERIMENTAL SETUP PHOTOGRAPHS

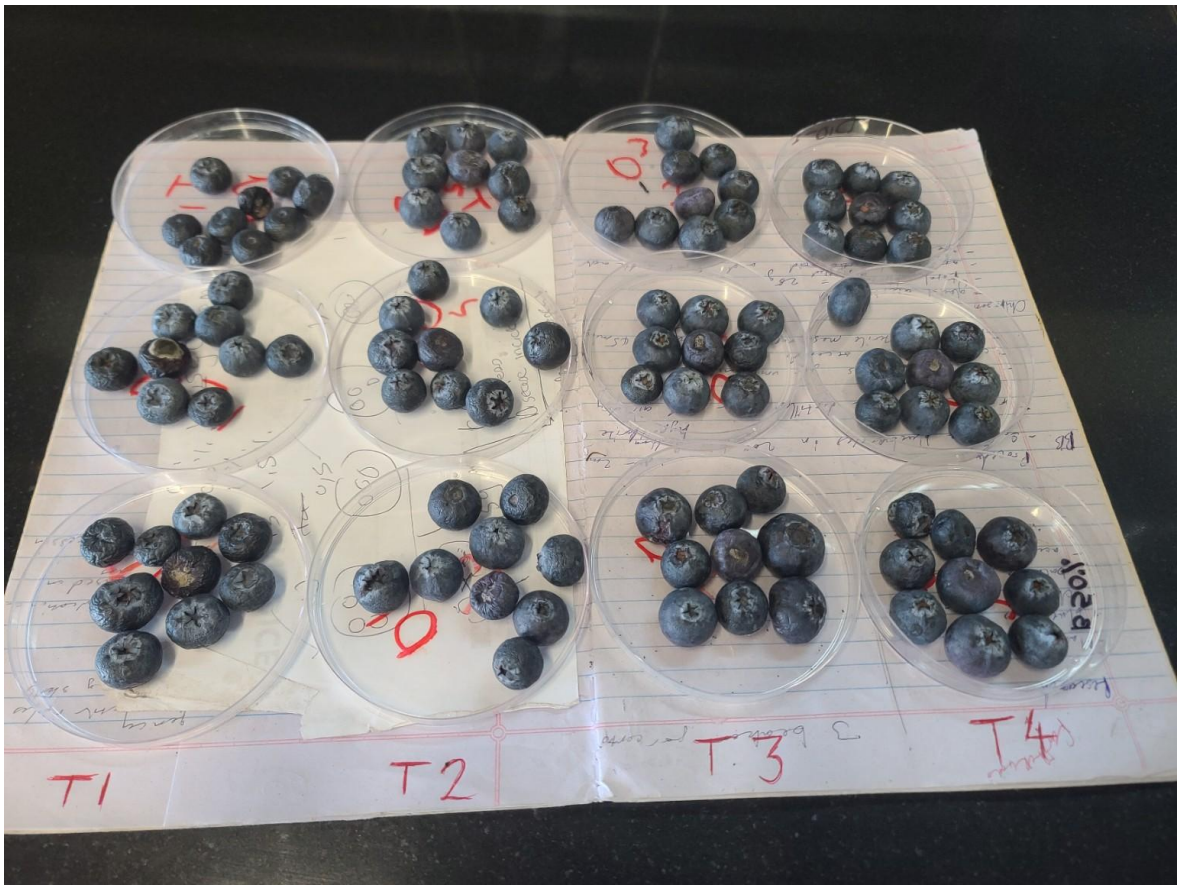


Figure C1: Blueberries arranged in labelled Petri dishes for the four treatment groups.

The photograph documents the Petri-dish experimental units and the general arrangement of the control and chitosan-coated treatments. It was also used as a scale-supported visual reference when reconstructing the approximate baseline berry-diameter dataset.

Additional photographs documenting the experimental setup, measurement procedures and laboratory observations are presented below.

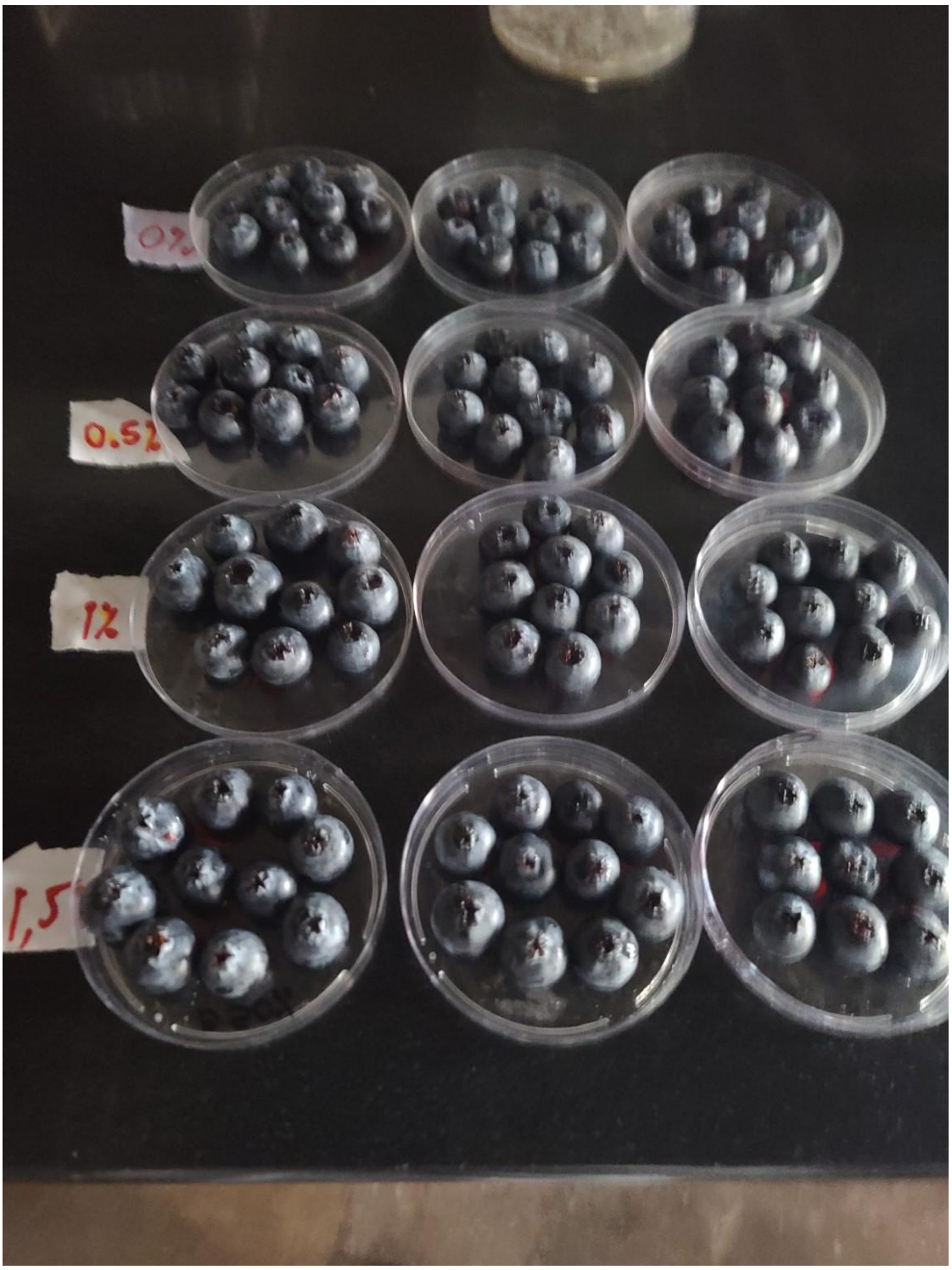


Figure C2: Initial arrangement of blueberries in labelled Petri dishes for the four treatments (0%, 0.5%, 1.0% and 1.5% chitosan) before storage.

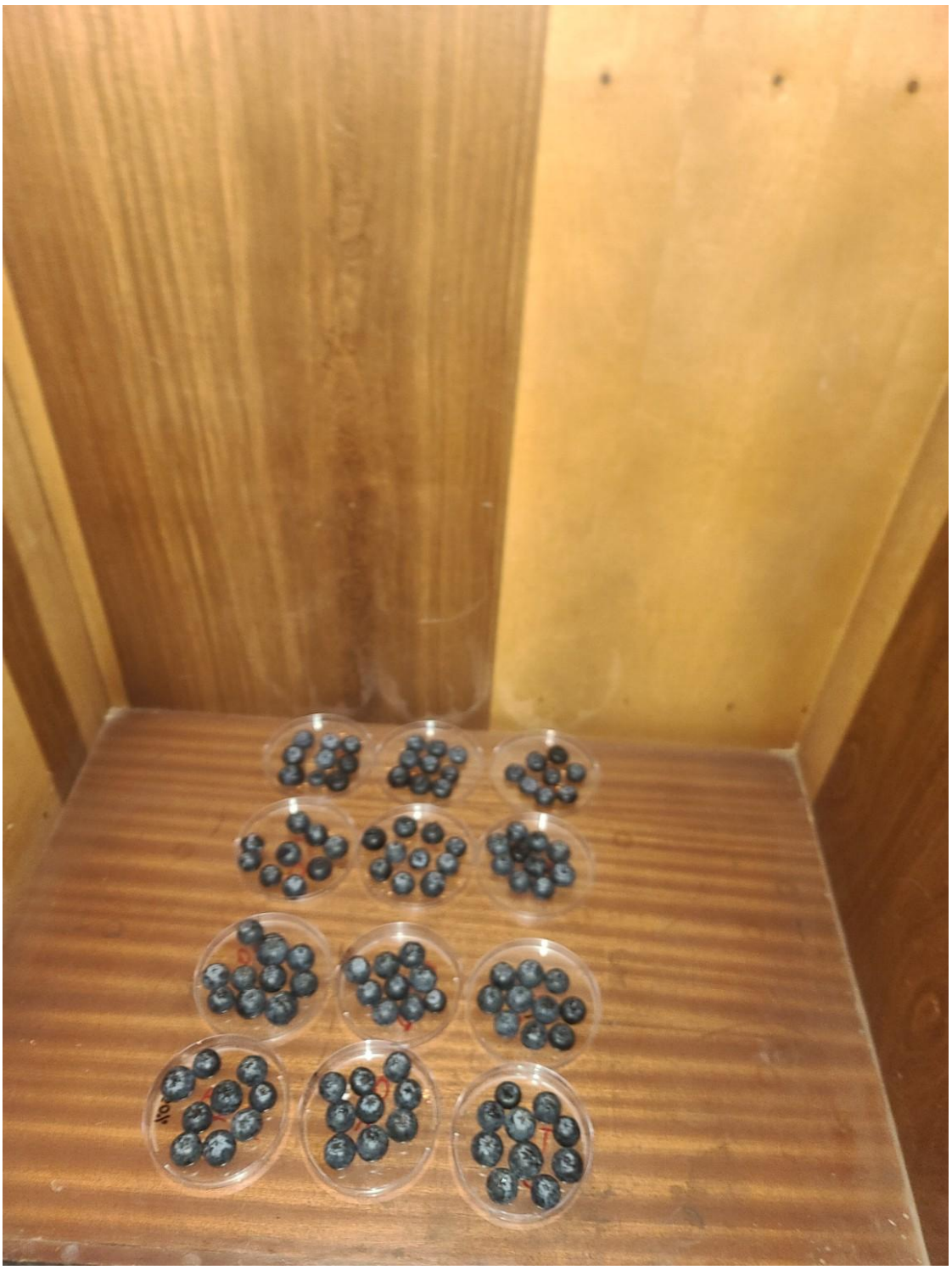


Figure C3: Petri-dish replicates placed under ambient indoor storage conditions during the experimental period.



Figure C4: Experimental units arranged on the laboratory bench after treatment application and preparation for storage and observation.

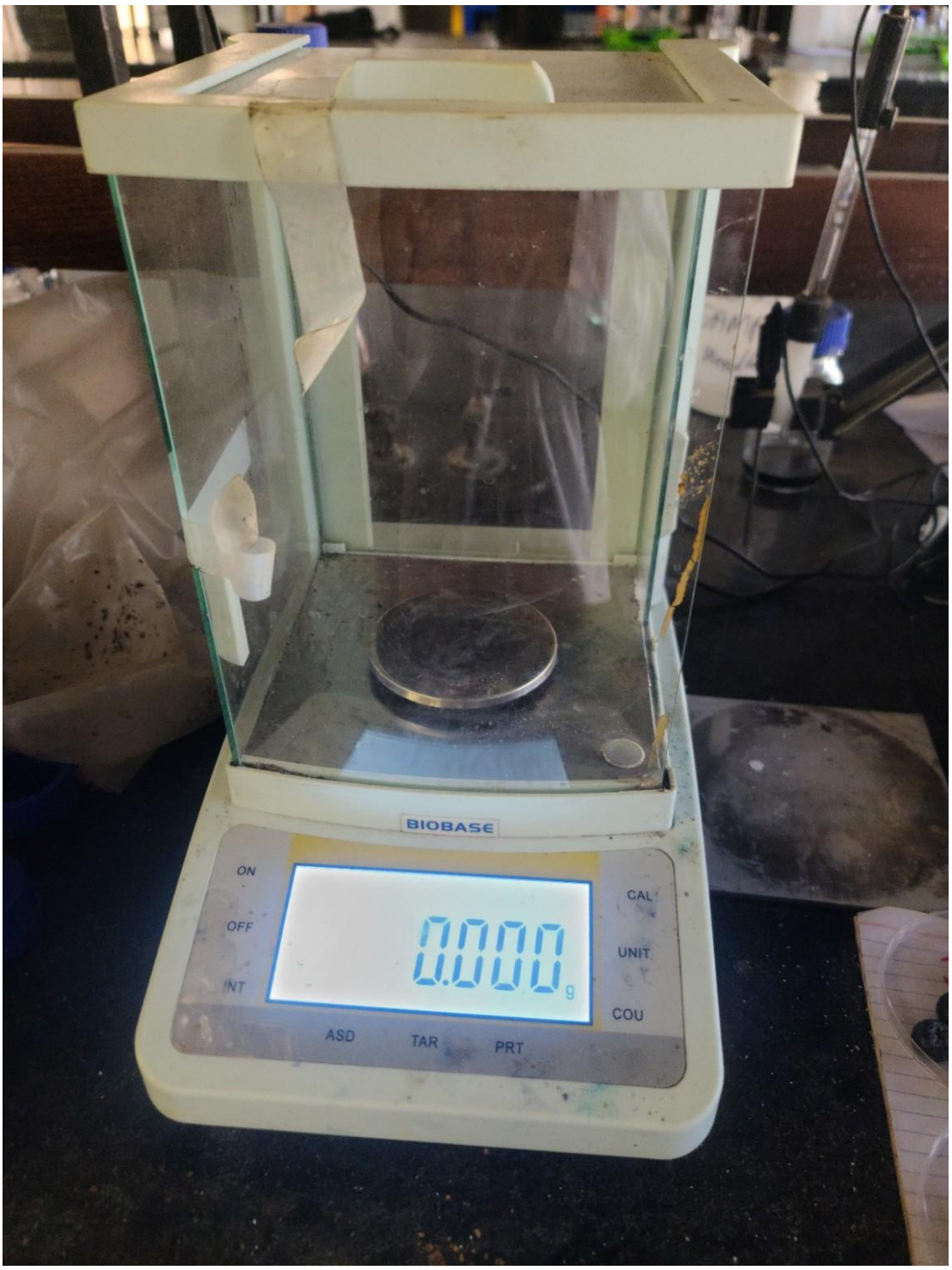


Figure C5: Analytical balance used for recording berry weight during the experiment.



Figure C6: Representative weighing of a Petri-dish replicate on the analytical balance during the storage study.

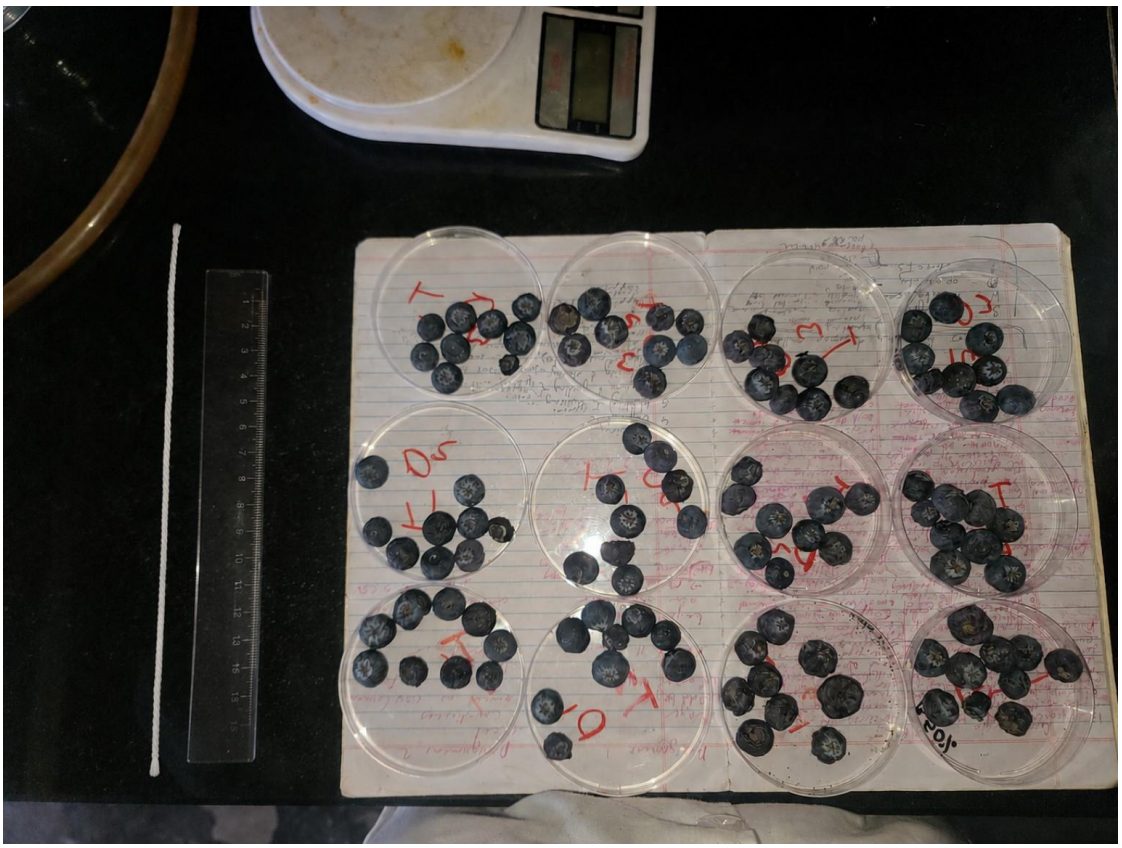


Figure C7: String and ruler used to estimate berry diameter from circumference measurements, shown alongside the Petri-dish replicates.

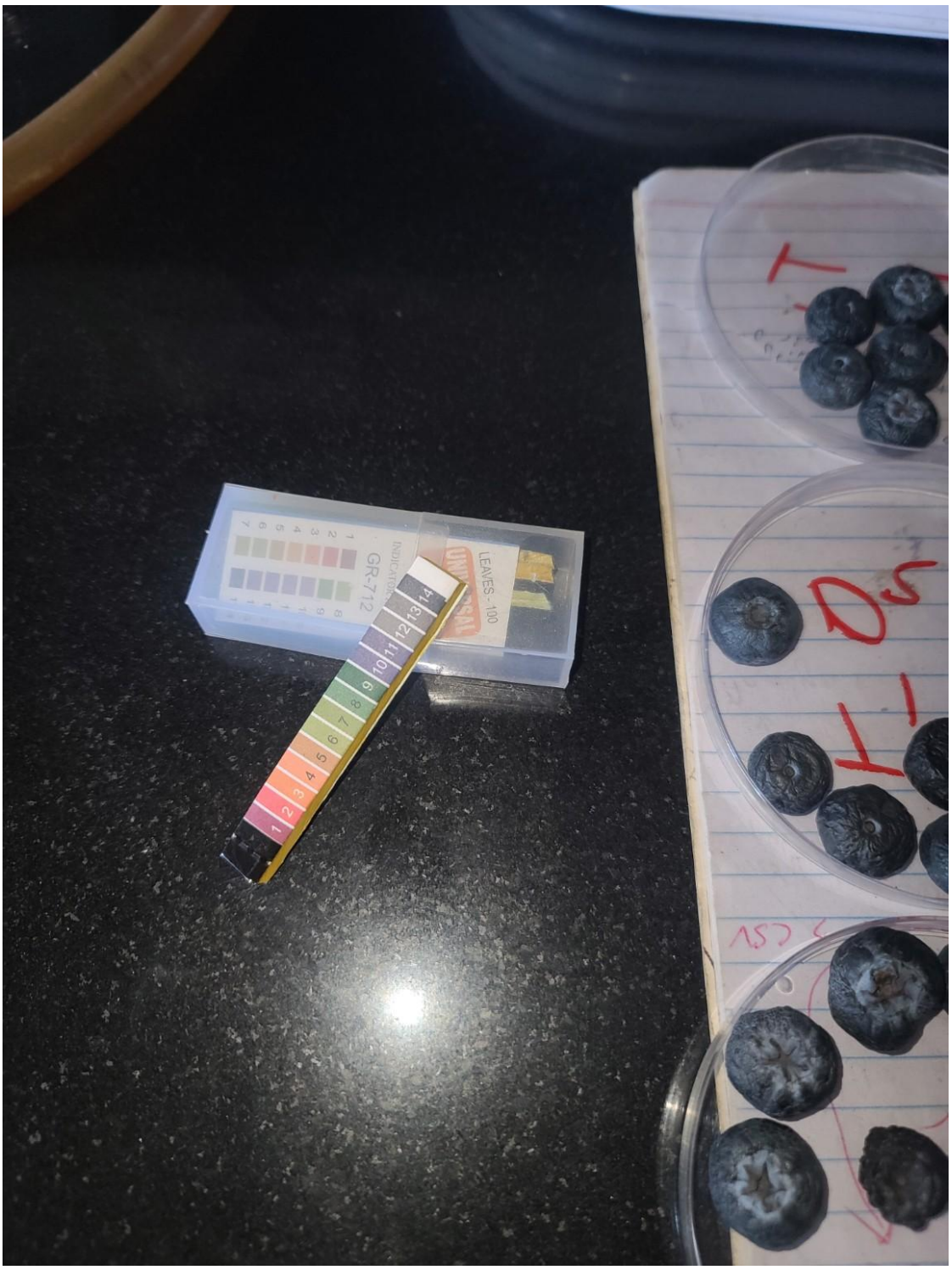


Figure C8: Universal indicator strips and colour chart used for approximate fruit-juice pH determination at the end of storage.

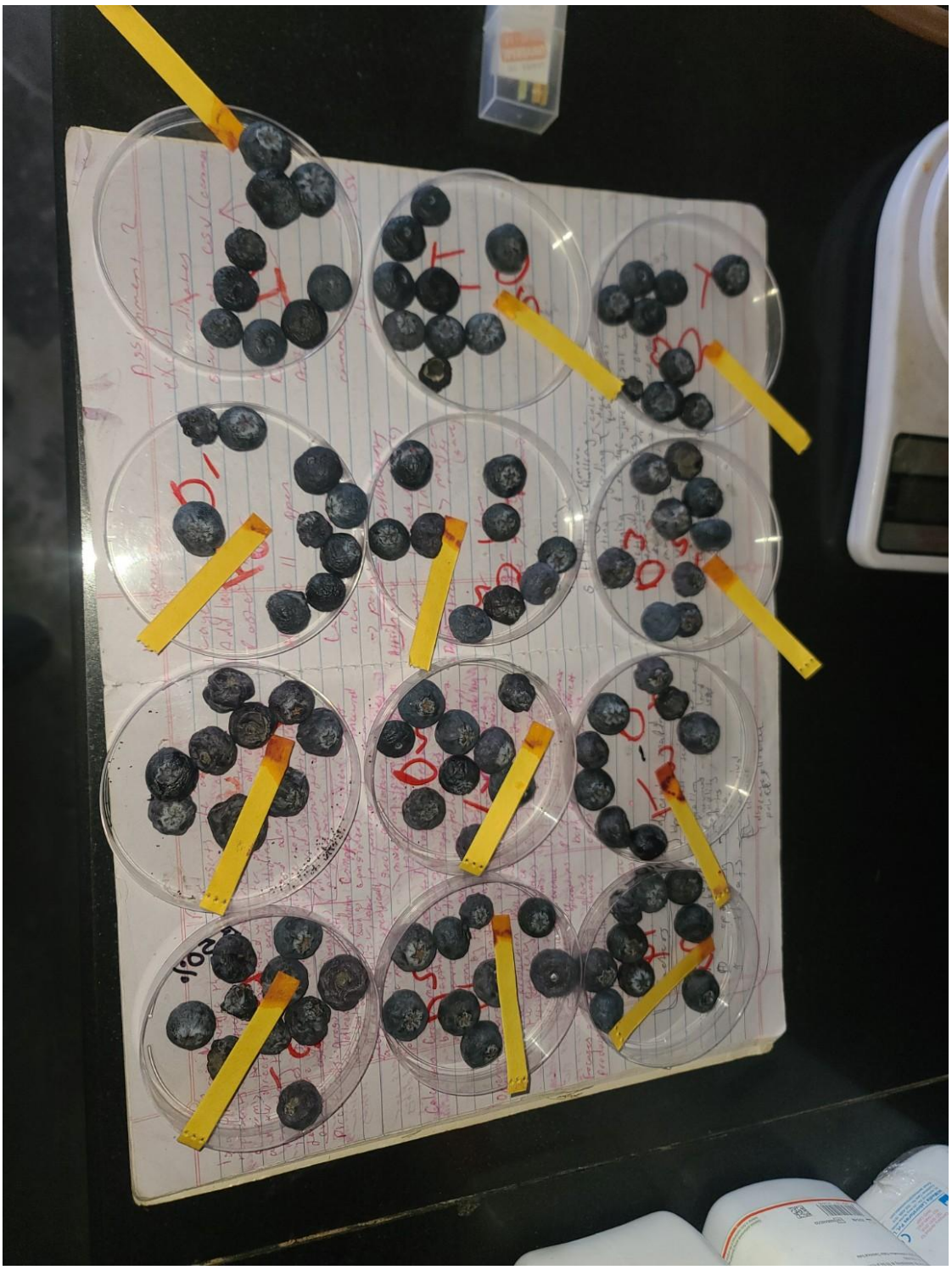


Figure C9: Day-15 Petri-dish replicates showing fruit condition during disease assessment and pH evaluation.

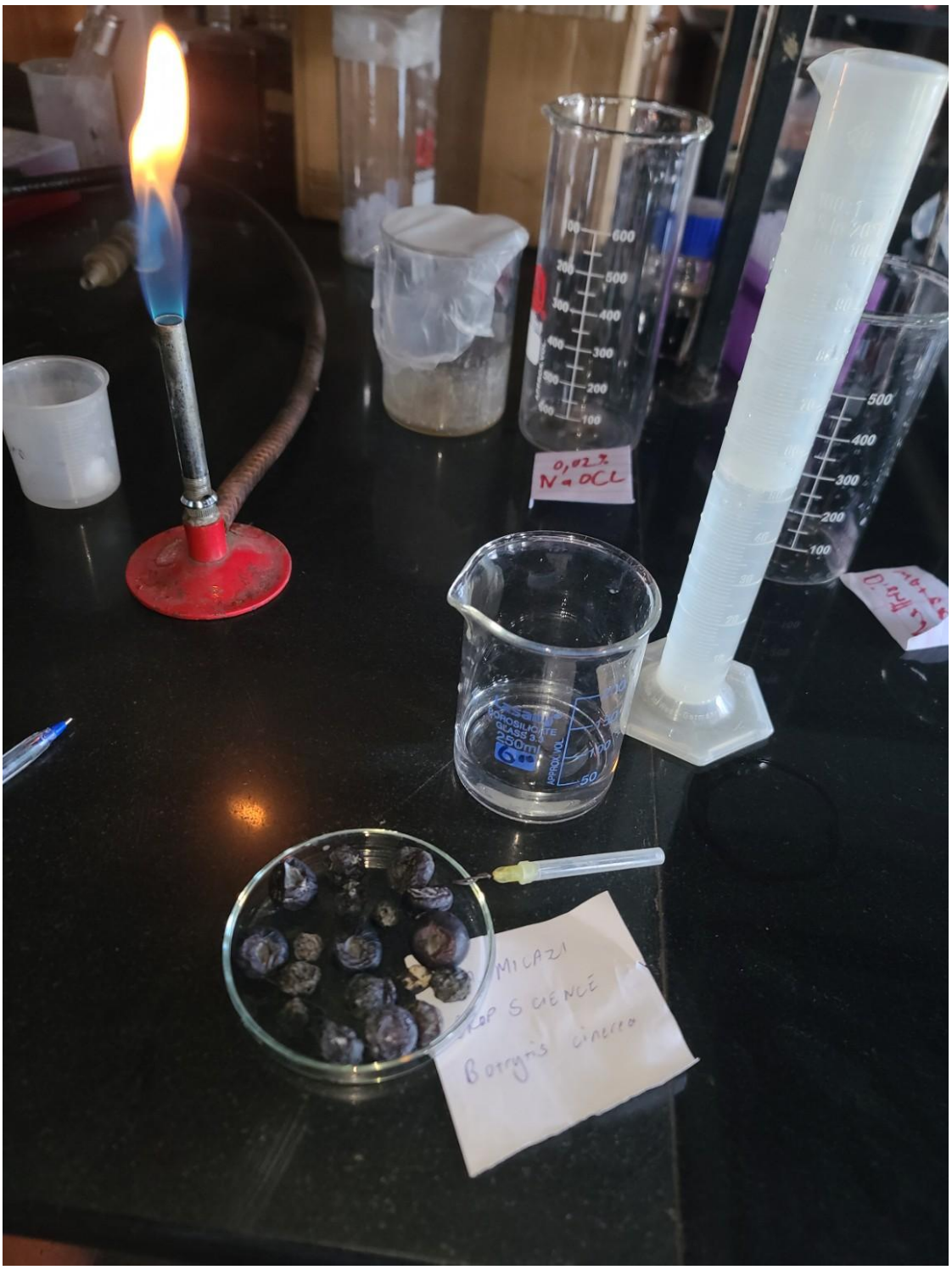
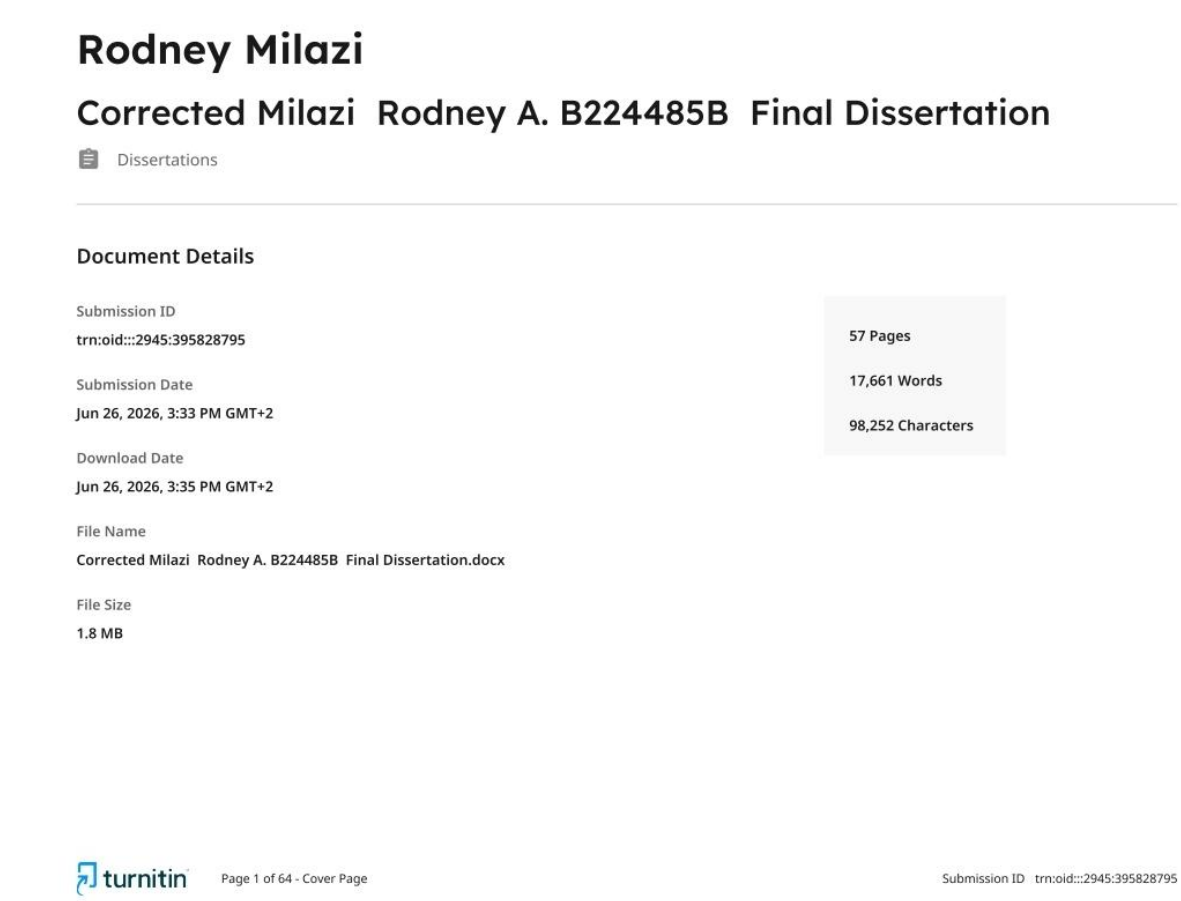


Figure C10: Laboratory materials and symptomatic blueberry samples used during fungal isolation and related observations.



Figure C11: Handling of a symptomatic berry during fungal examination and isolation procedures.

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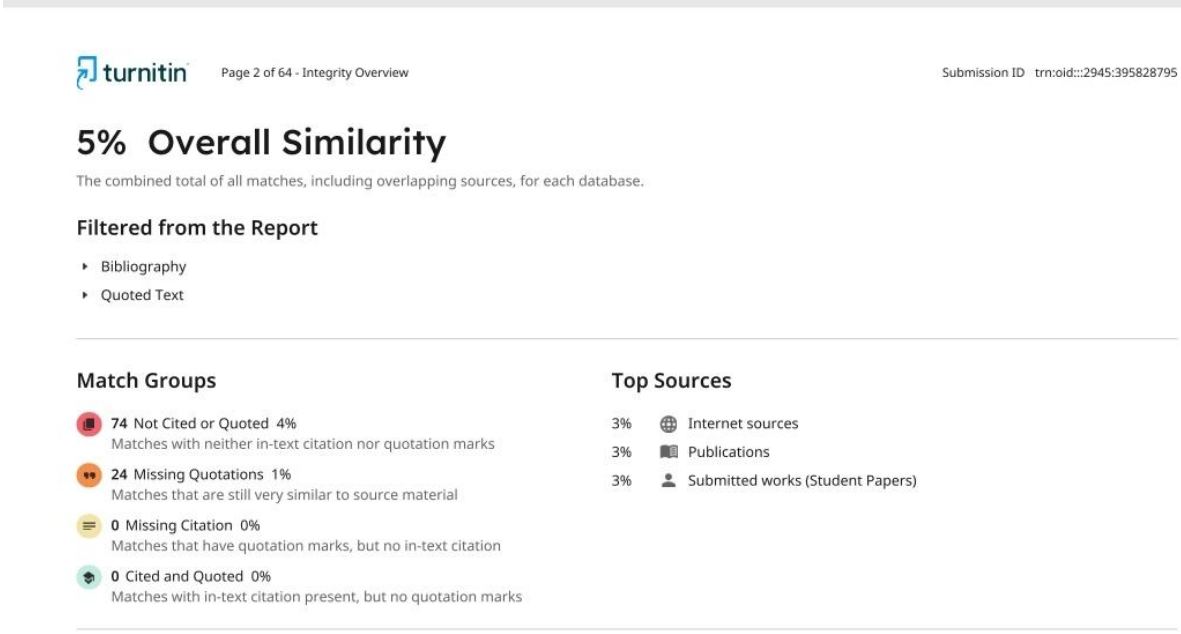
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