**BLACK SOLDIER FLY (BSF) CHITOSAN VS COMMERCIAL CHEMICAL TREATMENTS FOR *DENDROBIUM* SONIA ORCHIDS**

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**A THESIS SUBMITTED TO THE FACULTY OF THE DEPARTMENT OF BIOLOGY AND ENVIRONMENTAL SCIENCE, COLLEGE OF SCIENCE**

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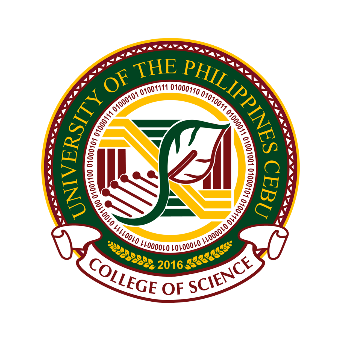
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**Black Soldier Fly (BSF) Chitosan vs Commercial Chemical Treatments for *Dendrobium* Sonia Orchids**

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**ACKNOWLEDGMENT**

>>Your text here…The first observed archaea were extremophiles, living in extreme environments, such as hot springs and salt lakes with no other organisms. Improved molecular detection tools led to the discovery of archaea in almost every habitat, including soil, oceans, and marshlands. Archaea are particularly numerous in the oceans, and the archaea in plankton may be one of the most abundant groups of organisms on the planet (Al-Otaibi & Wilbey, 2020).<<

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**ABSTRACT**

The body of your abstract starts here. The abstract of the thesis summarizes the background of the study, the research problem, the objectives of the research, the methodology or approaches to accomplish the objectives, the key results, the significance of the results, and the potential impact of the study. Do not include numbers, bullets, or lists in the abstract. It should be a single paragraph, without indentation, 1.5-spaced in 10-point Arial, justified, and containing 200-300 words. The first part of your abstract should state the background of the study, the problem or issue you have addressed, and your rationale for pursuing the study. The problem or issue might be a research question or a research gap. The purpose of your study is to solve this problem, eliminate the gaps in the literature, and/or add new knowledge or relevant information to the field. Your abstract should also describe the research methods/approaches that you carry out to solve the problem or the issue you identified. Your abstract also highlights your key results. Finally, your abstract should close with a statement of the implications and contributions of the study to the discipline. A good abstract will persuade readers that the research topic is important, interesting, and worth investigating further. These directions are written in the format required for the abstract for the Bio 200b thesis manuscript. Include 5 keywords separated by comma; do not use words found in the title. (Keywords are not included in the total word count.)

Keywords: keyword1, keyword2, keyword3, keyword4, keyword5

**INTRODUCTION**

**Rationale**

In tropical Asian countries like China, Philippines, Indonesia, and India, orchid farming is a large market that is growing annually. In 2002, it was reported that the orchid cut flower market is growing by approximately 10-20 % every year (Winrock International, 2002). In 2017, it was reported that 1.1 billion orchid plants were traded across countries from 1996 to 2015 (UNEP-WCMC, 2017). Reducing the cost of orchid farming by the application of a cheaper alternative fungicide, bactericide, and insecticide could help the industry thrive even more. This cheap alternative could be chitosan.

Chitosan is an *N*-deacetylated chitin derivative produced by alkaline deacetylation of chitin (Kaya et. al. 2014). Chitin is said to be the second most abundant polysaccharide next to cellulose and it can be found in the exoskeleton of most insects (Elieh-Ali-Komi & Hamblin, 2016). Several studies reported the antimicrobial and antifungal properties of chitosan, and it also has been reported that chitosan is effective in managing pests in some crops (Sharif et. al, 2018). However, chitosan production in the Philippines can be costly compared to the use of commercial chemical treatments. The price of fungicide and bactericidal products, for example, based on online markets platforms in the Philippines, is about ₱200 to ₱250 per pack. In local stores such as the ones found in Carbon Market Cebu City, Philippines, the price of these fungicide and bactericidal products can range from ₱300 to ₱500 per pack depending on the brand. Also, chitosan extraction from crustacean shells is said to be very labor-intensive and time-consuming (Philibert, Lee and Fabien, 2017). Not to mention, one must also find a big source of these crustaceans’ shells to harness chitosan's benefits and apply it to a medium-sized orchid farm. These factors can make the use of chitosan costly compared to buying and using commercial chemical treatments thereby hindering its possibility as a safer alternative. However, this can be resolved using black soldier fly (BSF) as the chitin source.

BSF as chitin source for chitosan production could also help resolve the two major problems of rapid population growth. These are the need to change the agriculture industry to sustain the population and the rapid increase in organic waste (Surendra et. al., 2020). The use of insects to convert organic waste into useful agricultural biomass (e.g., feeds and fertilizer) solves the previously stated two problems (Surendra and Kuehnle, 2019). The use of insects can reduce the dry mass of organic waste by approximately 25–72 % and convert the organic waste to usable nutrients such as nitrogen and phosphorus (Rehman et. al., 2019). In addition, many insects that have potential in this context or were already being used such as insects belonging to the order Lepidoptera, Diptera, Hymenoptera, Coleoptera, Trichoptera, Hemiptera, and Odonata have antifungal and antimicrobial properties (Elhag et. al., 2017). One of the insects of interest is the black soldier fly or BSF (*Hermetia illucens*) which belongs to the order Diptera and the family Stratiomyidae. BSF is a detritivore and can grow in various organic wastes. The diversity of organic wastes that it can grow into made it one of the popular insects used in converting organic wastes into useful biomass. BSF is said to grow in livestock manure (Mazza et. al., 2020), human feces (Nyakeri et. al., 2019), organic portion of municipal and city solid wastes (Sarpong et. al., 2019), food wastes (Nyakeri et. al., 2017), agricultural residues or materials that are left in the field after harvesting such as coconut husk and soybean curd (Lim et. al., 2019) and many others.

The use of BSF in converting organic wastes to useful products like insecticides, pesticides, or animal feeds are said to be not well examined and studied. Although there are already several farms that rears BSF, there are only a few facilities that are on a commercial scale in using BSF in organic waste to useful biomass conversion (Surendra et. al., 2020). Also, studies presenting the numerous benefits of BSF in agriculture could start to spark the trend of using BSF on a commercial scale. In this study, the chitosan extracted from BSF pupal cocoon will be applied to *Dendrobium* Soniaorchids. The benefits of BSF chitosan on *Dendrobium* Soniaorchids will then be compared to the benefits of using commercial chemical treatments. The result of this study could not only expand the knowledge on the possible applications of chitin and chitosan extracted from insects, but it could also help the farmers if it will be proven that the use of chitosan is an alternative to commonly used chemical treatments in growing crops.

**Review of Related Literature**

*Chitosan and its Possible Sustainable Sources*

BSF, besides being a viable solution to the problem of organic waste management, is used in European countries as an alternative to animal feeds since several studies have suggested that it is a great source of protein (Mazza et. al., 2020). BSF, shown in Figure 1, is usually found in Nearctic regions of the globe. This region is composed of countries that are mostly found in North America. However, due to technological advances, BSF can now be found in temperate and tropic regions (Oliveira et al., 2015). In the Philippines, there are already a few BSF farms and one of those is Chesed Farm which can be found in Purok Sandayung, Liloan, Cebu.

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**Figure 1.** Black soldier fly (BSF) (*Hermetia illucens)*. A. The five main stages of BSF lifecycle (Tomberlin et. al., 2002), B. BSF pre-pupal stage, C. BSF pupal stage, and D. The adult BSF.

BSF belongs to the order Diptera and family Stratiomyidae. Its body is like a wasp, and it has a body color of mainly black (Tomberlin et al., 2002). BSF’s life cycle is composed of five life stages (Figure 1A). These five life stages are the egg, larval stage, prepupal stage, pupal stage, and adult stage. The larval stage lasts for about 13 to 18 days which is followed by pre-pupal and pupal stages from which the adult emerges (Figures 1B-C) (Tomberlin et. al., 2002). During the prepupal stage which lasts for about seven days, BSF empties its gut and searches for a dry place to undergo the pupation process. The pupal stage (the brown-colored pupa shown in Figure 1C) will last for about two weeks before becoming an adult (Figure 1D). With enough water, an adult BSF can live for about a week where males emerge first, two days before females emerge. BSFs usually mate after two days of metamorphosing into adults, and they mate in flight which is the reason that BSF farms require wide enclosure areas (Caruso et. al., 2014). BSF mate once within BSFs' lifespan and two to four days after mating, females lay one clutch of eggs (500-900 eggs per female) that hatch after four days.

The larval stage of BSF is greatly studied as an animal feed, specifically in the poultry industry. Studies have suggested that BSF larva is rich in protein necessary for the growth of poultry animals (Mazza et. al., 2020). Another use of BSF in agriculture is the important compounds that can be extracted from its body in its different life stages. One of these compounds is chitin, the raw material in producing chitosan.

Chitosan is a deacetylated form of chitin found in the exoskeleton of marine crustaceans and most insects (Elieh-Ali-Komi & Hamblin, 2016). Chitosan is a linear polymer composed of *D*-glucosamine and *N*-acetyl-*D*-glucosamine. The common sources of chitin and chitosan are marine crustaceans such as crabs and shrimps (Ghormade, Pathan & Deshpande, 2017). In fact, the largest chitin and chitosan source in the world is from the fishing industry with a total of about 15 % to 40 % chitin content (Hahn et. al., 2020) which accounts for approximately 1 × 1011 tons of chitin produced in 2006 (Je and Kim, 2006). However, marine crustaceans as a source are limited by seasonality, location, and cost of transport (Pittman and McAlpine, 2003).

With recent studies showing the potential of the use of chitin and chitosan across different industries, it is projected that their demand will increase at a rate of about 12.1 % annually from 2022 to 2032 which is based on 2012-2021 data ("Chitin Market ", 2022). The increasing demand and the unreliability of marine crustaceans as chitin and chitosan sources lead to the hunt for other more sustainable chitin sources.

Chitin can also be found in fungi as well as the exoskeletons of most insects. Chitin from both said sources is well-studied. However, one of the appealing candidates for chitin and chitosan sources are insects since it also solves the problem of organic waste management (Surendra et. al., 2020) while being sustainable as a chitin source. Among the insect candidates, BSF is the most used and studied (van Huis, 2019). The moldings from the prepupae and pupae stages of BSF were reported to be a great chitin source (Leong et. al., 2016). In addition, the chitin from the exoskeleton of BSF was reported to be composed of alpha-chitin which is the same to the commercially produced chitin (Soetemans et. al., 2022; Triunfo et. al., 2022).

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**Figure 2.** Chemical structures of chitin and chitosan. Chitosan is a deacetylated derivative of chitin.

*The Biological Activities of Chitosan on Plants*

Ngo and Kim (2014) stated that antioxidant, antihypertensive, anti-inflammatory, anticoagulant, antitumoral, antibacterial, hypocholesterolemic, and antidiabetic properties are among the biological actions that chitosan and its derivatives extracted from both crustaceans and BSF are thought to promote. Hence, chitosan can be used to treat chronic diseases. Chitosan could also be mixed in food to not only improve the health of consumers but also increase the shelf life of products (Ngo and Kim, 2014).

In plants, chitosan is reported to be an immune system elicitor. Doares *et. al.* (1995) reported that chitosan can activate genes for pathological defense in plants via the octadecanoid pathway. The octadecanoid pathway leads to the production of phytohormone jasmonic acid which is essential in the induction of the genes needed when the plant is under insect attack or pathological attack. Aside from this, chitosan was reported to activate the systemic wound response proteins (SWRPs). Stratmann and Ryan in 1997 reported that two minutes after a tomato plant’s leaf is wounded, 48-kDa myelin basic protein (48-kDa MBP) is activated that leads to the activation of systemic wound response proteins (SWRPs). According to Stratmann and Ryan (1997), chitosan acted like the plant wound signal called 8-amino acid polypeptide systemin. Chitosan, just like 8-amino acid polypeptide systemin, increased the activity of 48-kDa MBP thereby increasing SWRPs activity which leads to increased resistance to stress. These factors could be the explanation to what Eikemo *et. al.* had reported in 2003. Chitosan is reported to induce resistance to crown rot disease in strawberries (Eikemo et. al., 2003).

Hence, chitosan protects plants from pathogenic attacks and stress via acting as an immune system elicitor, by activating immune responses like octadecanoid pathway and SWRPs. However, the main reason that chitosan in plants leads to resistance and protection against pathogenic attacks could be due to its antibacterial and antifungal properties (Ben-Shalom e. al., 2003).

There are a lot of studies that reported that chitosan and its derivatives have antibacterial and antifungal properties. These studies have suggested many theories on the mechanism of chitosan’s antibacterial activity. The three most probable theories are the ionic interaction of chitosan to microbial cell walls leading to leakage of the microbial cell, chitosan hindering the bacterium's mRNA and protein synthesis, and chitosan forming a protective film on the plant's surface which inhibits the access of harmful microbes (Goy et. al., 2009). The first theory was proven to be true by the more recent studies like the study of Goy et. al. in 2016. The study of Goy *et. al.* (2016) revealed that the application of chitosan resulted in hydrolysis of the bacterial cell's peptidoglycan. On the other hand, the antifungal properties of chitosan were first reported in 1979 (Allan and Hadwiger, 1979). Since then, there have been a lot of studies proving and applying the antifungal properties of chitosan in plants or crops. Based on the collected data by Sharif *et. al.* in 2018, 0.1 % (w/v) of chitosan is effective in the management of *Fusarium oxysporum* f. sp. *Lycopersici* in tomatoes (Sathiyabama et. al., 2015), 0.2 g per liter of chitosan is effective in the management of *Botrytis cinerea* in cucumbers (Ben-Shalom and Fallik, 2003), and 1 % (w/v) of chitosan is effective in preventing fungus growth in post-harvest mangoes (Jitareerat et. al., 2007). These are just some of the studies showing the antifungal properties of chitosan as recorded or tabulated by Sharif et. al. in their paper. Chitosan is also used to coat fruits, seeds, and vegetables. A study by Photchanachai *et al*. in 2006 showed that chitosan-coated seeds of *Capsicum annuum* L.have reduced fungal infection leading to increased success in germination and seed performance. In addition, chitosan is also used in a device that controls the agrochemical release of fertilizer which improves the sustained availability of fertilizers or nutrients for the crops. This minimizes fertilizer loss and maximizes the treatment given to the crop (Atalay, Sargin & Arslam, 2022).

Several studies have also reported that chitosan can improve plant growth. For instance, a study published two decades ago by Ohta *et. al.* (1999) reported that soil mixed chitosan during sowing time increased the growth rate of *Eustoma grandiflorum (Raf.) Shinn*. In addition to the increased growth rate, the rate at which the plant flowered also increased. Ohta *et al.* reported that chitosan-treated *Eustoma grandiflorum* (Raf.) Shinn flowered 15 days earlier than those that were not treated. The inflorescence has more florets as well and is heavier compared to the inflorescence in plants that are not treated with chitosan. A similar study conducted by Ohta *et. al.* in 2004 has similar results. Soil mixed with chitosan leads to flowering at an earlier date than usual in *T. fournieri* Linden ex E. Fourn., *E. affine* Balf., *B. hiemalis* Fotsch, *S. speciosa* (Lodd.), *L. erinus* L., and *M. hybridus* hort. ex A. *Siebert et Voss* (Ohta et. al., 2004).

In addition to improved immunity against pathogens, improved growth rate, and increased rate of flower production, chitosan was reported to affect the stomata of a plant. The stomata are the small openings in the leaf where the gas exchange between the plant and the environment happens. These openings, while used in gas exchange, is also an opening that pathogens take advantage of to enter the inner leaf tissue. A study by Lee *et. al.* in 1999 reported that the stomatal aperture or opening of tomato and Asiatic dayflower (*Commelina communis*) was reduced after treatment with chitosan. This narrowing of the stomatal aperture can be advantageous for plants in terms of defense against pathogens as well as in terms of water loss from transpiration (Limpanavech et al., 2008). It was reported that the application of chitosan on the leaves of pepper plants leads to a decrease in water use by 26 % to 43 % without affecting the biomass and yield (Bittelli et. al., 2001). This could mean that chitosan can also be used to counter the effect of excessive water loss during drought season.

Therefore, chitosan can be used in improving overall plant health by being an immune system elicitor, having antimicrobial properties, improving plant growth, improving rate of flower production, and lessens water loss due to transpiration.

*Effects of Chitosan on Dendrobium Orchids*

Chitosan has benefits to plants and application of chitosan has many benefits to *Dendrobium* orchids. Studies by Ohta *et al.* in 1999 and 2004 suggest that chitosan can be used to improve the orchid industry by improving the number and quality of inflorescence, improving the growth rate, and improving the overall health of orchids.

Perhaps one of the major effects of chitosan on orchids is the improvement in the number and quality of inflorescence. For instance, in Thailand, one of the countries where orchid farming is a major part of the economy, chitosan is used to increase the flowers produced per orchid plant. Chitosan (1-100 mg/L, molecular weight 45 kDa, ≥ 90 % deacetylation) has been reported to increase the number of flowers or inflorescence produced while also making the inflorescence more vibrant in a species of *Dendrobium* orchids called 'Earsakul' (Limpanavech et. al., 2008). Another study Kumari (2017) showed that the application of 7.5 ppm of chitosan to *Dendrobium* Sonia 17 increases the number of spikes per orchid plant, spike length, number of florets per spike, diameter of the flower, internodal length and vase life of the orchid flower. Another study by Chandrkrachang *et. al.* in 2005 reported that there is an increase in inflorescence shoots and yield in *Dendrobium sensational ‘Purple’* when treated with chitosan. Uthairatanakij *et. al.* in 2008 observed similar results in *Dendrobium* Sonia ‘No. 17’and reported that the biomass of inflorescence increased when sprayed six times of 400 mg per liter of chitosan at weekly intervals while the petal width of inflorescence increased when sprayed with 600 mg per liter of chitosan six times at weekly intervals. In *Dendrobium* Misteen, the application of chitosan leads to an increase in the length of the inflorescence while reducing the level of severity of leaf spot disease (Win. et. al., 2005). However, contrary to other studies, Win *et. al.* (2005) reported that chitosan has no effect on floret size and shoot growth which disagrees with the studies previously mentioned.

The benefits of chitosan in *Dendrobium* and other species of orchids are not limited to inflorescence. For example, Chandrkrachang in 2002 reported that spraying 10 mg per liter of chitosan increased the growth rate of orchids. Chandrkrachang also added that spraying chitosan with a concentration of 2.5 mg to 40 mg per liter leads to an increased length of leaves of *Paphiopedilum* orchids. Pornpeanpakdee *et. al.* in 2006 also reported that the growth of protocorm-like bodies (that are essential in orchid propagation) is enhanced when 10 mg and 20 mg per liter of chitosan with polymer units of 70 and 90 were applied. A similar study by Rahmah *et al.* in 2015 reported that the application of 1 mg per liter of chitosan in *Dendrobium* *mannii* *in vitro* leads to an increase in weight by 29.52 %, an increase in clump diameter by 33.00 % and an average number of protocorm-like bodies by 35.88 % after 14 weeks of cultivation. In addition, the application of 1 mg and 2 mg per liter of chitosan in *Dendrobium mirbelianum* leads to an increase in the number of leaves by up to 65.71 %. Rahmah *et al.,* however, added that 4 mg per liter of chitosan decreases the growth rate of the said two species of *Dendrobium* but 4 mg per liter of chitosan induces the proliferation of protocorm-like bodies only in *Dendrobium mannii. In vitro*, chitosan extracted from shrimp with 1 kDa of molecular weight was reported to stimulate the growth rate and rate of development of meristematic tissue from explants to protocorm-like bodies in *Dendrobium phalaenopsis* (Nge et. al., 2006). Nge *et al.,* however, added that chitosan extracted from fungi with a molecular weight of 10 kDa at a concentration of 15 mg per liter is more effective in stimulating growth compared to the chitosan oligomer extracted from shrimp with the same molecular weight. Hence, these studies would suggest that chitosan application could improve *Dendrobium* sp. orchid's overall health, propagation, and inflorescence production. However, the exact dose of chitosan needed to improve the overall health, propagation, and inflorescence production of *Dendobium* sp. orchid is still unclear.

*Dendrobium Sonia*

*Dendrobium* Sonia is an epiphytic orchid (Figure 3). It belongs to the clade Angiosperms, Order Asparagales, Genus Dendrobium. Specifically, it is a hybrid of two Dendrobiumspecies (Arrifin et. al., 2010). It is one of the *Dendrobium* species that are included in the 70% to 80% orchid trade in the tropics in 2006 (Kuehnle, 2006). It is a common ornamental plant in Malaysian households and offices (Arrifin et. al., 2010). Since it is heavily traded across Asia, it is not shocking that it is also present in the Philippines as recorded by Co’s Digital Flora of the Philippines (Barcelona et. al, 2013). Indeed, most orchid farmers in Sitio Tiguib, Barangay Malubog, Cebu City have this species of *Dendrobium.* The localities, in general, include this species of orchid to the umbrella term 'dendro' (orchids that do not belong to the genus *Oncidium* or dancing lady and genus *Vanda*).



**Figure 3.** The general characteristics of *D.* Soniaorchids. The image is a live *D.* Soniaorchid grown on an open farm in Barangay Malubog, Cebu City.

Generally, *D.* Sonia has a stem that is cylindrical. It has distichous ovate to oblong, leathery leaves. The leaves have a base that is attached to the stem via clasping sheaths. The apex of the leaves is obtuse and bilobed. Inflorescences most of the time is solitary and will arise from the node before the topmost pair of leaves. The floral bracts are elliptic with an area and the pedicels are pale red to violet in color. The inflorescence is a raceme that usually lasts for more than a week before withering and inflorescence is produced all year round (Kuenhle, 2006). The inflorescence also has a distinguishable smell. For the most common variation of *D.* Sonia, the sepals and petals are violet or indigo. However, for the variety presented in Figure 3, the three sepals and two petals are white but, in some cases, there can be observed stripes of pink at the tip. The lip of the flower is white while the keels are yellowish to light green. The dorsal sepal is ovate to lanceolate in shape. The lateral sepals are oblique ovate to lanceolate in shape which is slightly larger than the dorsal sepals. The flower also has a narrow conical mentum. The petals are obovate to oblong in shape. The margin of the petals is crenulated with an apex that is muconate (WFO, 2022).

The studies previously mentioned suggest that chitosan has benefits when applied to *Dendrobium* Sonia. Chitosan can stimulate the growth and development of orchid tissue which could also lead to the increased speed in generating inflorescence with bigger, heavier, and more florets. Spraying chitosan in *Dendrobium* leads to controlled levels of severity of leaf spot disease, increased growth rate, increased size of florets, and increased length of inflorescence (Uthairatanakij et. al., 2007). Uthairatanakij *et. al.* in 2008 reported that chitosan increased the quality of inflorescence of a variety of *D.* Sonia called ‘No. 17’. However, Nge *et. al.* (2006) reported that the benefits of chitosan in *Dendrobium* orchids are dependent on molecular size, the origin, and the concentration of the chitosan. This would mean that chitosan extracted from crustaceans would have a different effect on orchids compared to chitosan from insects such as BSF. There is only limited data on the effects of the application of chitosan extracted from BSF on orchids such as *Dendrobium* Sonia.

In addition, the specific dose of chitosan needed to produce the best result is still unclear. Different studies used different concentrations of chitosan and produced different improvements on *Dendrobium* orchids. Hence, there is also no data that shows at what specific concentrations of chitosan extracted from BSF would be advisable to use to improve the overall health as well as the inflorescence production of *Dendrobium* Sonia orchids. Lastly, there is no available data that assesses the effectiveness of BSF chitosan compared to commercial chemical treatments.

**Objectives**

This study aims to:

1. Determine the chitin and chitosan content per gram of BSF sample;
2. Determine the concentration of BSF chitosan (2.0 ppm, 4.0 ppm, 8 ppm, 16 ppm, 32 ppm, 64 ppm and 128 ppm) that leads to highest plant height, best root-shoot ratio, best leaf traits, and best inflorescence characteristics of *Dendrobium* Sonia orchids compared to the control group; and
3. Compare the plant height, root-shoot ratio, leaf traits, and characteristics of inflorescence of *Dendrobium* Sonia orchids sprayed with a concoction of chemical treatments, and BSF-derived chitosan alone.

**MATERIALS AND METHODS**

Chitosan was extracted from Black Soldier Fly (BSF) pupal cocoons. The cocoons were provided by Chesed Farm located in Liloan, Cebu. The chitosan extraction was done at the University of the Philippines laboratory. Seven concentrations of chitosan were prepared where each concentration had a total volume of six liters. The extracted chitosan was then applied to *D.* Sonia*.* orchids planted and grown in an open space farm in Sitio Tiguib, Barangay Malubog, Cebu City, Cebu (10°22'45.7" N 123°52'19.0" E).

**Sample preparation**

*Identification and Preparation of Dendrobium Sonia orchids*

The orchids used in this study were identified to be a variation of *D.* Sonia by using the Co’s Digital Flora of the Philippines, a website made by Pelser, Barcelona, and Nickrent in 2011. It is a website that compiles digital pictures of all vascular plants in the Philippines. At first, the website only contains the list made by Merrill (1920) and was later updated by the late Dr. Leonard Co. Today, the website contains photographs of different vascular plants that can be found in the Philippines that came from different contributors. The identification of the plants on the website is done by experts (Barcelona et. al, 2013). By comparing the morphology of the flower shown in Figure 3 and the morphology of *D.* Sonia found in Co’s Digital Flora of the Philippines website, the species of the orchids used in this study was identified to be *Dendrobium* Sonia which is a hybrid of two *Dendrobium* sp.

After confirming the species of orchids used in this study, 206 *D.* Soniaorchids of approximately the same size germinated from late December 2022 to early January 2023. These 206 samples were provided by the farmer that was interviewed on what chemical treatments are commonly used in growing *D.* Sonia orchids. In addition, the orchids were grown for about five months on similar materials, coir pasted on an elevated nine wood plank erected on an open space farm (Figure 4). The orchids were also watered daily using ‘hiringga’, a metal syringe like apparatus commonly used by the locals when spraying chemical treatments and water to the plants. It was ensured that all orchids received enough water daily. In this study, the orchid samples germinated late December 2022 asexually reproduced and produced two to nine keikis. Hence, all the individual keikis were observed instead of one individual sample. This brings the total number of orchid samples observed to about 575. Lastly, the 575 orchid samples were grouped into nine groups which correspond to the nine different treatment groups.

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**Figure 4.** The orchid samples used in this study were grown on an elevated wood plank erected on an open space farm. The orchids were attached to the elevated wood plank by tying the orchids with the coir. The orchids were grown for about five months and produced multiple keikis. Hence, there are multiple keikis per coir instead of only one. In addition, 20 to 29 orchid samples were attached on one elevated wood plank.

*Preparation of Seven Different Chitosan Concentrations*

BSF larvae cocoons, provided by Chesed Farm in Liloan, Cebu, were washed with tap water, rinsed with distilled water, and dried using a dehydrator at 60 °C for at least 3 hours. This drying method is a modified method of Zlotko et. al. (2021). After drying, the BSF cocoons were then pulverized using a blender followed by sieving (~0.1 mm) to eliminate solid contaminants.

The extraction of chitosan has three main stages: demineralization, deproteination, and deacetylation. The product of the deproteination stage is chitin while the product of deacetylation stage is chitosan. In the demineralization stage, 0.10 L of 1 M of HCl was added to 10 g of the dried and pulverized BSF cocoon sample (Soetemans, Uyttebroek, and Bastiaens, 2020; Marie et. al. 2016). The solution was allowed to react at room temperature for about 1 hour with frequent stirring, and then filtered under a vacuum to remove the excess acid. The filter paper used throughout the extraction process is a grade 1 qualitative filter paper with a pore size of about 11 µm. The filtrate was discarded, and the residue was washed by at least 0.40 L of distilled water to ensure that all the excess acid was removed. After washing with distilled water, the residue was then placed back to the beaker and 0.25 L of 1 M of NaOH was then added (Soetemans, Uyttebroek, and Bastiaens, 2020). The solution was allowed to react for about 1 hour at 80 °C with constant stirring, and then filtered under a vacuum to remove the excess base. The residue was then washed with at least 0.40 L of distilled water to remove all the excess base. This stage was repeated for at least 15 times or until the filtrate becomes clear. Afterwards, the sample was dried via a 6-tray commercial food dehydrator for 3 hours at 90 °C. The dried sample, which is chitin, was collected and weighed. About 1 mg of the extracted chitin was then subjected to Fourier-transform infrared spectroscopy (FTIR) at 4000 ~ 625/cm resolution (Kaya et. al., 2015). For the deacetylation, 0.30 L of 50 % NaOH was added to the extracted chitin. The solution was allowed to react for about 2.5 hours at 90 °C with constant stirring (Soetemans, Uyttebroek, and Bastiaens, 2020) and then filtered under a vacuum to remove the excess base. The residue was again washed with at least 0.40 L of distilled water to remove all the excess base. The deacetylation stage was repeated at most seven times or until the filtrate becomes clear. After the filtrate becomes clear, the residue was washed with 0.03 L of ethanol and then washed again with 0.10 L of distilled water. The residue, which is chitosan, was dried using the dehydrator for 6 hours at 90 °C. After drying, the extracted chitosan was weighed and about 1.0 mg of the extracted chitosan was then subjected to Fourier-transform infrared spectroscopy (FTIR) at 4000 ~ 625/cm resolution.

Enough extracted chitosan was then dissolved in 0.1 M acetic acid (1.0 g : 0.10 L) with continuous stirring at 55 °C for about 12 hours which was then diluted to 3.0 L of distilled water. There were seven concentrations prepared, 2.0 ppm, 4.0 ppm, 8.0 ppm, 16.0 ppm, 32 ppm, 64 ppm, and 128 ppm. These concentrations were based on the concentrations used by studies mentioned in the previous section like Kumari (2017), Uthairatanakij *et. al.* (2008), Pornpeanpakdee *et. al.* (2006), and Chandrkrachang (2002).

*Comparison of Effects of Different Concentrations of BSF Chitosan on D. Sonia orchids*

Nine set-ups were prepared corresponding to nine different treatments used in this study (Table 1). Orchids belonging to seven of the nine set-ups were sprayed weekly for four weeks with nine different concentrations of BSF-derived chitosan (T1 to T7). Orchids belonging to the fifth set-up were sprayed weekly for four weeks with concoction of chemical treatments that are commonly applied by the farmers in Sitio Tiguib, Barangay Malubog, Cebu City (T5). Farmers in Sitio Tiguib, Barangay Malubog Cebu City apply the chemical treatments in one go or in one concoction instead of applying the chemical treatments individually. Spraying the chemical treatments individually was said to be time-consuming and very tedious. The concoction is composed of three types of foliar fertilizer (2 tablespoons each of the two types and one tablespoon of the third type), two types of insecticide (two tablespoons each), a flower inducer (2 tablespoons), and a fungicide (1 tablespoon). One of the foliar fertilizers has 19 % nitrogen, 19 % phosphorus, and 19 % potassium while the other has 4 % nitrogen and 48 % potassium. The third type of foliar fertilizer is mainly a chelated calcium nitrate in granular form.  One of the insecticides is 20 SC or composed of 20 % suspended concentration of the active ingredient (clothianidin) while the other has 31.5 % emulsified concentration of the active ingredient (21 % chlorpyrifos and 10.5 % fenobucarb dissolved in xylene which is 45 % of the insecticide). The fungicide is 80 % wettable powder which suggests that 80 % of the powder is the active ingredient (mancozeb). These chemical treatments were mixed in one container with a volume of 26 liters and about three liters of this mixture was used during the whole duration of the study. The orchids in the last set-up were only sprayed with water (T0). In addition, the treatments were sprayed from the leaves to the roots of the orchid samples.

**Table 1.** The total number of orchid samples per treatment.

|  |  |  |  |
| --- | --- | --- | --- |
| **Set-up number** | **Treatment Code** | **General Description of the Treatment** | **Total Number of Orchid Samples** |
| 1 | T1 | 2 ppm chitosan | 63 |
| 2 | T2 | 4 ppm chitosan | 58 |
| 3 | T3 | 8 ppm chitosan | 55 |
| 4 | T4 | 16 ppm chitosan | 61 |
| 5 | T5 | 32 ppm chitosan | 66 |
| 6 | T6 | 64 ppm chitosan | 73 |
| 7 | T7 | 128 ppm chitosan | 72 |
| 8 | T8 | Concoction of Chemical Treatments | 57 |
| 9 | T0 | Water  (Negative Control) | 70 |

Ten (10) orchids were randomly selected per treatment for the measurement of root-shoot ratio. This was based on "New handbook for standardised measurement of plant functional traits worldwide" by Pérez-Harguindeguy *et.* al. (2013) and the request of the orchid farmer that provided the orchids used in this study.

**Data Collection**

*Chitin and Chitosan Content per gram of BSF Cocoon Sample and % Deacetylation*

There were five chitosan extraction trials done. The last trial was not applied to the orchids. One (1) mg of extracted chitin and chitosan of one of the trials was sent to University of San Carlos Cebu for Fourier-transform infrared spectroscopy (FTIR) at 4000 ~ 450/cm resolution (KBr pellet). Also, another 1 mg of extracted chitosan of the last trial was sent to University of San Carlos Cebu for Attenuated Total Reflectance - FTIR (ATR-FTIR) at 4000 ~ 600/cm resolution. The absorbance of the extracted chitosan samples at 1655 cm-1 and 3450 cm-1 was determined. These absorbances are characteristic absorbance of primary amide group and hydroxyl group, respectively (Kaya et. al., 2015). The % deacetylation (% DD) was calculated using Equation 3 which is adapted from Fatima (2020). In addition, the chitin and chitosan content per gram of BSF pupal cocoon sample was calculated using Equations 1 and 2. Lastly, the number of repetitions of deproteination and deacetylation stages were determined.

Equation 1

Equation 2

Equation 3

where,

*Leaf Traits, Plant Height, and Root-Shoot Ratio*

There are three main orchid parameters measured in this study, the leaf traits, the plant height, and the root-shoot ratio. Leaf traits include the average number of leaves per plant, average leaf mortality, and description of the presence of leaf disease. These three main parameters, leaf traits, plant height, and root-shoot ratio, mainly describe the growth and health of the growing orchid samples.

*I. Leaf Traits*

There were three parameters measured to describe the leaf traits of each orchid sample, the average number of leaves per plant, average leaf mortality, and description of the presence of leaf disease. These parameters were measured pre- and post-treatment.

*a. Average number of leaves per plant*

This parameter was measured by directly counting the number of leaves in each sample pre- and post-treatment (Pérez-Harguindeguy et. al., 2013).

*b. Leaf mortality*

This parameter was measured by counting the number of leaves pre- and post-treatment then calculating the leaf mortality per one month (Equation 6) (Charrier et. al., 2018).

Equation 6

*c. Description of presence of leaf disease*

The physical appearance of leaves of orchid samples were described and the possible diseases present were determined based on the description of symptoms provided by Prasartporn et. al. (2018) via https://doi.org/10.1007/978-3-319-39670-5\_21.

*II. Plant height*

Plant height was measured by determining the shortest distance between the topmost photosynthetic tissues excluding the inflorescences and the base of the orchid attached to the coir (Pérez-Harguindeguy et. al., 2013). This parameter was measured two times, pre- and post-treatment.

*III. Root-Shoot ratio*

Ten (10) randomly selected orchids in each treatment were carefully uprooted from its respective coir. The samples were then wrapped with moist newspaper and then placed into a plastic zip lock. Before closing the zip lock, the experimenter breathed into the zip lock. The zip locks that contained the samples were then placed in a dark container and brought to the laboratory. In the laboratory, the root system was carefully separated from the shoot system. For the shoot system, the stem was cut horizontally into very thin pieces while the leaves were separated from the stem and cut in half. This was intended so that that the drying duration will be cut to only 24 hours to 36 hours instead of 72 hours. The dry mass of the samples’ root and shoot systems were then determined by drying at 70 °C for more than 36 hours and weighing the dried samples (Pérez-Harguindeguy et. al., 2013).

*Inflorescence Yield, Inflorescence Height, and Number of flowers per Raceme*

The effect of different concentrations of BSF-derived chitosan on the characteristics of inflorescence was also measured in this study. Two main inflorescence characteristics were the focus of this study, the height of the inflorescence and the number of flowers per raceme. The quality and market value of *D.* Sonia flowers are dependent to these factors. The inflorescences in each treatment groups produced in the entire duration of the experiment were compared.

1. *Effect on the Number of Orchids that Developed Inflorescence*

The number of orchids that produced an inflorescence in each treatment group was measured by directly counting how many orchids produced an inflorescence after the treatment.

1. *Inflorescence length*

Inflorescence height was determined by measuring the shortest distance between the tip of the topmost flower and the point of attachment of the entire inflorescence. The height of the inflorescences produced post-treatment were measured.

1. *Number of flowers per inflorescence*

This parameter was measured by directly counting the total number of flowers each inflorescence has. The number of inflorescences of collected post-treatment were determined.

**Data Analysis**

Tests for normality, homoscedasticity, and power analysis were done for all the data of each measurement parameter. The different data analyses done to answer every question of each objective are summarized in Table 2. All analyses were performed using R version 4.2.2.

**Table 2.** The questions and objectives, and the different statistical analyses done to answer the questions and objectives.

| **Objectives number** | **Questions** | **Statistical Analyses** | |
| --- | --- | --- | --- |
| **Parametric test** | **Non-parametric test** |
| 1 | Do the number of repetitions of deproteination and deacetylation stages affect the chitin and chitosan % yield per BSF cocoon sample? | Pearson Correlation Test | |
| 2 | At what concentration of BSF-derived chitosan produced higher leaf traits, plant height, root-shoot ratio, and best inflorescence characteristics? |  |  |
|  | a. Do application of different concentrations of BSF-derived chitosan improved the number of leaves, plant height, and root-shoot ratio of *D.* Sonia orchids? | ANOVA, Tukey test | Kruskal-Wallis test, Welch’s ANOVA, Wilcoxon rank sum test |
|  | b. Do application of different concentrations of BSF-derived chitosan effective in managing leaf diseases of *D.* Sonia orchids? | Chi Square test | |
|  | c. Do application of different concentrations of BSF-derived chitosan increase the number of orchids that produce inflorescence and the number of flowers per inflorescence of *D.* Sonia orchids? | Chi Square test | |
| 3 | Which among the nine treatments produced the best leaf traits, highest plant height, root-shoot ratio, and best inflorescence characteristics? | ANOVA, Tukey test | Kruskal-Wallis test, Welch’s ANOVA, Wilcoxon rank sum test |

**RESULTS**

*Hermetia illucens*, commonly known as Black Soldier Fly (BSF), is a viable solution to organic waste management. BSF can convert organic waste into products needed in agriculture. In Europe, it is proven to be a great source of protein and hence, it is being used mainly as animal feed specially for poultry animals (Mazza et. al., 2020). However, several studies have reported that useful compounds can be extracted from the body of BSF in its different life stages. This paper investigates whether the BSF can be a viable source of chitosan and whether the BSF-derived chitosan is effective and comparable to the chemical treatments in improving overall health and inflorescence quality of *Dendrobium* Sonia orchids.

*Chitin Content and Chitosan Content of BSF Pupal Cocoons*

This study performed five trials of chitosan extraction from BSF pupal cocoons. The chitosan extracted from BSF pupal cocoons were whitish and is dusty in appearance (Figure 5).



**Figure 5.** The appearance of chitosan extracted from BSF. BSF-derived chitosan is white in color with a dusty texture.

On average, the chitin content of the BSF pupal cocoons is about 10.47 ± 4.884 %. On the other hand, the chitosan content of the BSF pupal cocoons is about 4.158 ± 1.870 % (Table 3). This chitin content is lower than those reported by Soetemans, Uyttebroek, and Bastiaens (2020) of 23 ± 1.5 % chitin despite using the same method in chitin and chitosan extraction.

**Table 3.** The chitin and chitosan content of BSF pupal cocoon at 95% confidence.

|  |  |  |
| --- | --- | --- |
|  | **Chitin Content per Gram\* of BSF Cocoon Sample (%)** | **Chitosan Content per\* Gram of BSF Cocoon Sample (%)** |
| Confidence Interval (95%) | 10.47 ± 4.884 | 4.158 ± 1.870 |

\*values are reported as mean ± SD (n = 5)

The difference in chitin yield could be due to constant washing with NaOH during the deproteination stage. The deproteination process in this study was repeated more than 15 times compared to 12 times in the study of Soetemans, Uyttebroek, and Bastiaens (2020). The Pearson correlation test revealed that the number of repetitions of deproteination stage and chitin yield has a -0.4097 correlation with a p-value of 0.4933 and 95 % confidence. This suggests that increasing the number of repetitions of deproteination stage decreases the chitin yield. However, given p-value equal to 0.4933, this relationship is not significant. Hence, it is safe to say that chitin yield is independent of the number of repetitions of the deproteination stage.

In addition, the % deacetylation (% DD) of the extracted chitosan was also different to that of the study of Soetemans, Uyttebroek, and Bastiaens (2020) despite having similar methods. In this study, two extracted chitosan samples were subjected to FTIR analysis (Appendix A). The first sample has a % DD of approximately 66.62 % while the second sample has a % DD of approximately 74.48 %. Each sample has a different number of repetitions of deacetylation stage and different chitosan yield (Table 4).

**Table 4.** Different number of repetitions of deacetylation stage leads to different % DD and chitosan yield (g).

|  |  |  |
| --- | --- | --- |
|  | **Sample 1** | **Sample 2** |
| Number of Repetition of Deacetylation stage | 7 | 4 |
| %DD | 66.62 % | 74.48 % |
| Chitosan Yield (g) | 0.3483 | 0.4846 |

The Pearson correlation test revealed that there is a weak negative relationship between the number of repetitions of deacetylation stage and chitosan yield (r = -0.1614, α = 0.05, p-value = 0.7954, power = 0.1105). This suggests that there is no significant relationship between the number of number of repetitions of deacetylation stage and chitosan yield.

*Comparison Between the Effect of Different Concentrations of 66.62 % DD Chitosan and Chemical Treatments on Dendrobium orchids.*

Different studies have shown that different concentrations of BSF-derived chitosan have different effects on *Dendrobium* orchids. However, there is no studies that compare the effect of chitosan, especially BSF-derived chitosan, to the effect of commercial chemical treatments on *Dendrobium* orchids. In this study, the effect of different concentrations of 66.62 % DD BSF-derived chitosan (2.0 ppm, 4.0 ppm, 8.0 ppm, 16 ppm, 32 ppm, 64 ppm, and 128 ppm) with unknown molecular weight on *Dendrobium* Sonia’s leaf traits, height, root-shoot ratio, and inflorescence characteristics were compared with the commercial treatments.

*Effect on Leaf Traits*

1. *Effect on average number of leaves*

Chitosan improves tissue development of *Dendrobium* orchids (Nge et. al., 2006). Tissue development could mean an increase in number of leaves, increase in shoot height or development of new shoots. In this study, it was observed that there were orchids in each treatment group that grew new leaves.

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**Figure 6.** The number new leaves that every orchid in every treatment group grew after a span of a month. The line shows the standard deviation and at the center is the calculated mean. Orchids sprayed with 32 ppm of 66.62 % DD BSF-derived chitosan has the highest mean number of newly grown leaves after a span of a month (Welch’s ANOVA, p-value = 4.36 × 10-05, α = 0.05, n ≈ 60, power = 0.61).

Orchids sprayed with 32 ppm of 66.62 % DD BSF-derived chitosan has the highest mean number of newly grown leaves with a mean of about 0.7879 with a standard deviation of 1.409. Welch’s ANOVA revealed that there is a significant (p-value = 4.36 × 10-05 < 0.05, power = 0.61) difference between the number of new leaves produced in each treatment group. A post-hoc Wilcoxon rank sum test (α = 0.05) revealed that orchids sprayed with 32 ppm of 66.62 % DD BSF-derived chitosan (T5) grew significantly higher numbers of new leaves than orchids sprayed with the chemical concoction (x̄ = 0.1250, sd = 0.6049042).

1. *Effect on production of new buds for asexual reproduction*

Budding is a type of asexual reproduction where an organism produces a bud at a specific area. The offspring is an exact clone of the parent. In plants, the bud is produced at a specific surface of the stem. *D.* Sonia orchids used in this study produced asexual buds at the bottom most region of the stem. Asexual reproduction of new orchids via the growing buds could mean increase in number of orchids per treatment as well as increase in number of leaves per treatment.

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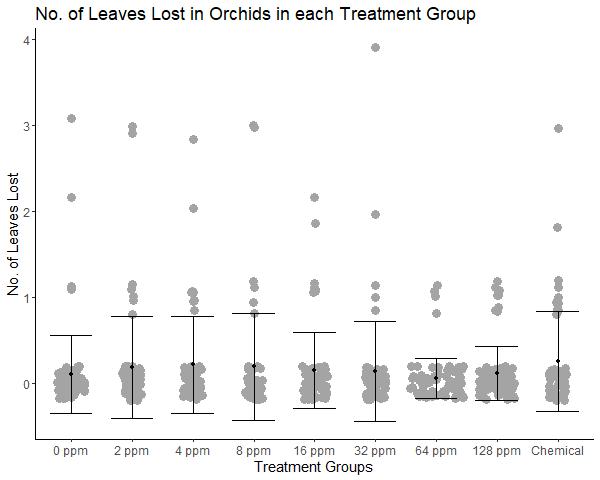
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**Figure 7.** The number of *D.* Sonia orchids that produced new buds after applying the treatments for one month. The highest number of orchids that developed buds are orchids sprayed with 16 ppm of 66.62 % DD BSF-derived chitosan. However, there is no significant difference between treatment groups (Chi square test, p-value = 0.8698, α = 0.05, power = 0.06755).

There are more orchids that developed more buds when sprayed with 16 ppm and 128 ppm of 66.62 % DD BSF-derived chitosan compared to spraying other concentrations of chitosan and spraying chemical treatment. This could mean that spraying 16 ppm and 128 ppm of 66.62 % BSF-derived chitosan could affect asexual reproduction of *D.* Sonia orchids. However, chi square test revealed that the number of orchids that asexually reproduced and developed buds are not significantly different (p-value = 0.8698, α = 0.05, power = 0.06755). This means that treatments do not affect the asexual reproduction of *D.* Sonia orchids.

1. *Effect on leaf mortality*

Leaf mortality is the number of leaves lost during a specific period. Leaf mortality measurements could reveal the problems related to acquisition of nutrients necessary for tissue survival. It was observed in this study that the different treatments do not affect the number of leaves that an orchid lost for a span of a month.



**Figure 8.** The number of leaves that each orchid in each treatment lost in a month. The line shows the standard deviation with the mean at the center. *D.* Sonia orchids in each treatment groups lost equal number of leaves after a month (Kruskal-Walis test, p-value = 0.2323, α = 0.05, n ≈ 60, power = 0.05708)

At α = 0.05, the Kruskal-Wallis test revealed that there is no significant difference between the number of leaves lost after a month and treatment groups. This will mean that increasing the concentration of 66.62 % BSF-derived chitosan will not affect an orchid’s number of leaves it will lose in a month. Applying chemical treatment will also have the same effect.

1. *Effect on Leaf Health*

One of the probable reasons that a *D.* Sonia orchid can lose a leaf is the presence of leaf disease. Leaf disease in orchids can be caused by infection from pathogens such as fungi, bacteria, viruses, and other microbes including microscopic nematodes. Pests could also cause the loss of leaf tissue (Prasartporn et. al., 2018). Based on the study of Prasartporn *et.* al. (2018), five common orchid leaf disease were identified. These leaf diseases are most probably caused by fungi infection (Figure 7).

A hand holding a leaf

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A https://doi.org/10.1007/978-3-319-39670-5\_21

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A person measuring a leaf

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D https://doi.org/10.1007/978-3-319-39670-5\_21

A person measuring a plant

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E https://doi.org/10.1007/978-3-319-39670-5\_21

**Figure 9.** The five leaf diseases observed in *D.* Sonia orchids. The probable causes of these leaf diseases are determined using the description made by Prasartporn *et.* al. (2018). These leaf diseases are caused by fungi. A. Yellow spots are caused by *Cercospora*spp. / *Phyllosticta* spp. B. Yellow leaves with depressions are caused by *Cercospora* spp. / *Phyllosticta*spp. C. Black spots on both sides of leaf are caused by *Guignardia* spp. / *Fusarium*spp. */ Phyllosticta* spp. D. Leaf damage that could be caused by pests or by pathogenic attack. E. Sooty molds that only grow in superficial leaf tissue and have minimal to no effect on plant health. Sooty molds are caused by saprobic ascomycetes from families of Dothideales (Callan and Carris, 2004).

The five leaf diseases observed in the *D.* Sonia orchids across different treatment groups are the yellows spots (YS), black spots (BS), yellow leaves (YL), leaf damage (LD), and black fungus (BF). Based on the description of symptoms compiled by Prasartporn *et.* al. (2018), these leaf diseases, except BF, are caused by fungi infection in deep leaf tissues. The number of *D.* Sonia orchids having these diseases across different treatment groups are shown in Figure 8.

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**Figure 10.** The number of *D.* Sonia orchids in each treatment group that have leaf disease. There are lesser orchids having yellow spots when sprayed with 32 ppm and chemical treatment compared to other treatment groups. There are lesser orchids having black spots when sprayed with 16 ppm and chemical treatment compared to other treatment groups. There are lesser orchids having leaf damage when sprayed with 8 ppm and chemical treatment compared to other treatment groups. Spraying 128 ppm chitosan leads to lesser orchids having YL compared to other treatment groups.

There can be three things that can be observed on the effect of treatments on *D.* Sonia’s leaf health. One, increasing the concentration of 66.62 % DD BSF-derived chitosan leads to decreasing total number of orchids having any leaf disease. However, second, among all the treatments, the treatment group that has the least total number of orchids having leaf disease is the treatment group sprayed with chemical treatment. Treatment group sprayed with 128 ppm chitosan has comparable effect with treatment group sprayed with chemical treatment. Lastly, specific concentrations of BSF-derived chitosan are better in managing certain leaf diseases. For instance, 128 ppm chitosan is more effective in managing YL and 8 ppm is effective for LD compared to other treatments. This would mean that 128 ppm could be effective against *Cercospora*spp. and 8 ppm could be effective against pests and other pathogens. In addition, it is challenging to describe the possible effect of different treatments on BF since most of the treatment groups are the same to the negative control which have no orchids infected by BF. These observations can be supported by the fact that Chi square test revealed that the number of orchids having leaf disease is significantly different across different treatments (p value = 7.323 × 10-7, α = 0.05, df = 32, power = 0.2939).

*Effect on Plant Height*

The change in plant height at a certain time interval will give a glimpse of the rate of growth of samples in response to nutrient availability and presence of diseases (Wang et. al., 2013). Observing the change in plant height after one month of application of the treatment could give a glimpse of the nature of the treatment whether it could affect the rate of growth of *D.* Sonia orchids.

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**Figure 11.** The change in height of *D.* Sonia orchids after one month of application of each treatment. The point at the center is the mean orchid height. Orchids sprayed with 4 ppm of 66.62 % DD of BSF-derived chitosan has the highest mean of change in height followed by orchids sprayed with 128 ppm. However, orchids sprayed with 2 ppm of 66.62 % DD BSF-derived chitosan are the only orchids that have significantly higher change in height when compared with the control group (Welch’s ANOVA, p-value = 0.027, α = 0.05, power = 0.3212)

Orchids sprayed with 4 ppm of 66.62 % DD BSF-derived chitosan has the highest average plant height among orchids in other treatment groups. Orchids sprayed with 128 ppm has the next highest average plant height. Both treatment groups have higher average plant height compared to orchids sprayed with chemical treatment and other concentrations of chitosan. The Welch’s ANOVA revealed that this differences in average plant height is significant at α = 0.05. There is a significant difference between the mean plant height of orchids sprayed with different treatments (p-value = 0.003056, α = 0.05, n ≈ 60, power = 0.3212). However, a post-hoc Wilcoxon rank sum test revealed that the difference between the mean plant height of orchids sprayed with 2 ppm chitosan and control are the only groups that are significantly different from each other (p-value = 0.027, α = 0.05, power = 0.3212). This could mean that spraying 2 ppm of 66.62 % DD BSF-derived chitosan can significantly increase the plant height of orchids. Other concentrations of chitosan and chemical treatments have approximately the same effect on plant height with not applying any treatment at all. Hence, only 2 ppm chitosan has a significant effect on *D.* Sonia’s plant height.

*Effect on Root-Shoot Ratio*

The root-shoot ratio could summarize the effect of treatments on *D.* Sonia’s material accumulation leading to growth. Big root-shoot ratio means higher root dry mass and higher growth rate (Pérez-Harguindeguy et. al., 2013). Like the observations on plant height, orchids sprayed with 128 ppm chitosan have the highest root-shoot ratio (Figure 12).

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**Figure 12.** The root-shoot ratio of *D.* Sonia orchids sprayed with different treatments. The red point at the center is the mean root-shoot ratio per treatment group. Orchids sprayed with 128 ppm of 66.62 % DD of BSF-derived chitosan has the highest mean of root-shoot ratio. However, there is no significant difference between the root-shoot ratio between treatment groups (Kruskal-Walis test, p-value = 0.07956, α = 0.05, n ≈ 9, power = 0.1364).

Orchids sprayed with 128 ppm of 66.62 % DD BSF-derived chitosan has the highest mean root-shoot ratio among different treatment groups. However, the 128-ppm group also has the highest variance. Indeed, Kruskal-Walis test revealed that the root-shoot ratio between different treatments are not significantly different (p-value = 0.07956, α = 0.05, n ≈ 9, power = 0.1364). This means that increasing the concentration of 66.62 % BSF-derived chitosan will not affect an orchid’s root-shoor ratio. This will also mean that spraying BSF-derived chitosan and chemical treatment will have the same effect with spraying no treatment at all.

*Effect on the Number of Orchids that Developed Inflorescence*

The number of orchids that produced inflorescence answers the question whether different treatments could possibly induce the development of an inflorescence. This study suggests that increasing the concentration of 66.62 % DD BSF-derived chitosan does not affect the number of orchids that developed inflorescence (Figure 13).

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**Figure 13.** The number of *D.* Sonia orchids that developed inflorescence. If 66.62 % BSF-derived chitosan could induce inflorescence development, it would be expected that orchids sprayed with BSF chitosan would have higher number of orchids that developed inflorescence compared to control group (0 ppm). However, there is no significant difference in the number of orchids that produce inflorescence between treatment groups (Chi square, p-value = 0.8376, α = 0.05, power = 0.06906).

Figure 13 shows that there were more orchids that produced inflorescence that weren’t sprayed with any treatment. However, the chi square test suggests that there is no significant difference in the number of orchids that developed inflorescence between different treatment groups (p-value = 0.8376, α = 0.05, power = 0.06906). This would mean that different concentrations of 66.62 % DD BSF-derived chitosan as well as the chemical treatment did not induce development of inflorescence.

*Effect on Inflorescence Characteristics*

Several studies have shown that spraying BSF-derived chitosan could improve the quality of inflorescence characteristics of *Dendrobium* orchids. A more recent study by Kumari (2017) suggested that 7.5 ppm of ≥ 90 % DD chitosan leads to increase in number of flowers per inflorescence, increase in length of inflorescence, increase in weight of inflorescence, and improvement of color of flowers per inflorescence. All these characteristics are the characteristics of inflorescence that affects the market value of orchids flowers. In this study, the effect of 66.62 % BSF-derived chitosan on two inflorescence characteristic, height, and number of flowers, was examined.

1. *Inflorescence length*

Several studies, like that of Kumari in 2017, suggested that chitosan increases inflorescence height of *Dendrobium* orchids. This study suggests otherwise (Figure 14).

A picture containing text, diagram, screenshot, plan

Description automatically generated

**Figure 14.** The inflorescence length of *D.* Sonia orchids sprayed with different concentrations of 66.62 % DD BSF-derived chitosan and chemical treatment. 2 ppm, 64 ppm and 128 ppm treatment groups only have sample size each. Orchids sprayed with 128 ppm of BSF-derived chitosan produced inflorescence with highest length compared to other treatment groups. However, there is no significant difference in the length of inflorescence in each treatment group (ANOVA, p-value = 0.719, α = 0.05, power = 0.09041).

Orchids sprayed with 128 ppm of 66.62 % DD BSF-derived chitosan have inflorescences with the highest length compared to other orchids sprayed with other concentrations of BSF-derived chitosan. Orchids sprayed with 128 ppm chitosan also have higher inflorescence length than orchids sprayed with chemical treatment. However, due to high variances in some treatment groups and small sample size, one-way ANOVA test revealed that there is no significant difference in the height of inflorescences of orchids in each treatment group. This means that different concentrations of 66.62 % DD BSF-derived chitosan do not have any effect on the length of inflorescence that *D.* Sonia orchids will produce. Effect of different concentrations of 66.62 % DD BSF-derived chitosan on the length of inflorescence are also comparable the effect of spraying chemical treatment. Both BSF-derived chitosan and chemical treatment do not have effect on the length of inflorescence of *D.* Sonia orchids.

1. *Numbers of Flowers per Inflorescence*

Chitosan was reported to increase the number of flowers per inflorescence in *Dendrobium* orchids (Kumari, 2017). In this study, it was observed that orchids sprayed with 128 ppm of 66.62 % DD BSF-derive chitosan produce inflorescence with the highest number of flowers compared to orchids sprayed with other concentrations of chitosan and chemical treatment.

A graph of flowers in each treatment group

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**Figure 15.** The number of flowers per inflorescence of orchids sprayed with different concentrations of 66.62 % DD BSF-derived chitosan and chemical treatment. Orchids sprayed with 128 ppm chitosan produced inflorescence with a mean number of flowers higher than inflorescences produced by orchids sprayed with other concentrations of BSF chitosan and chemical treatment. However, there is no significant difference in the number of flowers per inflorescence in each treatment group (Kruskal-Walis, p-value = 0.4886 α = 0.05, power = 0.1115).

Kruskal-Walis test revealed that the difference in number of flowers per inflorescence between treatment groups is not significant. This means that spraying 66.62 % DD BSF-derived chitosan and chemical treatment produces inflorescence that have the same number flowers compared to not spraying any treatment at all. Hence, BSF-derived chitosan does not affect the number of flowers that *D.* Sonia orchids produce.

**DISCUSSION**

**Focus on physiology**

1. **Short summary of result**
2. **Explain why or why not effective on**
   1. **Leaf traits**
   2. **Plant height**
   3. **Root-soot biomass**

>>Your text here…The first observed archaea were extremophiles, living in extreme environments, such as hot springs and salt lakes with no other organisms. Improved molecular detection tools led to the discovery of archaea in almost every habitat, including soil, oceans, and marshlands. Archaea are particularly numerous in the oceans, and the archaea in plankton may be one of the most abundant groups of organisms on the planet (Al-Otaibi & Wilbey, 2020).<<

As with the Materials and Methods section, you may add your subsections / subtopics accordingly.

**SUMMARY** or **CONCLUSIONS**

This section becomes CONCLUSIONS AND RECOMMENDATIONS when applicable. >>Your text here…The first observed archaea were extremophiles, living in extreme environments, such as hot springs and salt lakes with no other organisms. Improved molecular detection tools led to the discovery of archaea in almost every habitat, including soil, oceans, and marshlands. Archaea are particularly numerous in the oceans, and the archaea in plankton may be one of the most abundant groups of organisms on the planet (Leach et al., 2019).<<

**LITERATURE CITED**

Al-Otaibi, M.M. & Wilbey, R.A. (2020) Rethinking microbiology experiments. *Journal of Microbiology Research,* **72**(2), 234-242.

Leach, G.C., Pyle, D.L. & Niranjan, K. (2019) Recent advances in hedgerow surveying techniques. *International Journal of Ecological Surveying*, **29**(5), 547-558.

**CURRICULUM VITAE**