

Summary: Chitin

High-level Review of Chitin as an Agricultural Product

Last updated 12/20/2024

Version 2.0B

CHITINS: What are they and why they matter

Chitin is a naturally occurring molecule found in crustacean & insect exoskeletons and fungal cell walls. It is a polymer, or chain, of smaller pieces, and provides natural support and rigidity. In recent years, there has been intensive interest in chitins as potential, organic, pest suppressants, growth regulators, and nutritional products.

The agricultural benefits of chitin are well documented. Scientific research studies have demonstrated that chitins promote plant defense and elicit immune response (Shibuy and Minami., 2001) and directly limit the growth of pathogenic fungi in fruit (Yu et al. 2008). When applied as a drench, chitin even reduced root rot caused by both Phytophthora and Rhizoctonia (Ahmed et al. 2003). When applied as a surface foliar, chitins form protective barriers that prevent pathogens from entering leaves (Hirano et al. 1996).

Agricultural studies have shown that chitin promotes plant growth, soil microbial communities, and plant defense responses (Shamshina, et al., 2020) and specifically promotes the growth of beneficial growthpromoting bacteria (Maximov et al. 2011). When applied to the soil, chitin is naturally degraded, by soil microbes, into larger pieces (oligomers) and finally its smallest subunit, N-acetylglucosamine. Research has shown that the oligomers, that result from chitin degradation, suppress pathogenic nematodes, and display nematicidal activity (Fan et al. 2019). N-acetylglucosamine further breaks down into ammonia, a readily available form of nitrogen (Andronopoulou and Vorgia et al., 2004). Making chitin an effective slow-release fertilizer.

Chitin has likewise been shown to act as a bio stimulant. Genetic studies indicate that chitin can promote genes associated with vegetative growth (Li, et al 2020; Winkler, Alexander et al. 2017). In fact, there are hundreds of scientific studies that demonstrate agricultural efficacy from chitin as well as related chitosan derivatives (Sharp et. al 2013). Finally, Chitin is considered safe by the USA Environmental Protection Agency (US EPA US 2018a, b, case #6063) under the authority of the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA, CFR 155.58 and CFR 152).

Key points:

- Chitin is a safe naturally occurring molecule.
- Chitin acts as a pest suppressant; inducing immune responses and deterring the growth of various pests and diseases.
- Chitin promotes the growth of beneficial soil microbes.
- Chitin acts as a plant growth regulator.

TYPES OF CHITINS

In broad strokes, there are two forms of chitin: alpha and beta. α -chitin is one of the most abundant macromolecules on earth. It is readily available from numerous sources. The agricultural research to date has been almost entirely conducted with α -chitin. The challenge with α -chitin is that it is very insoluble and heavily mineralized. In other words, it is basically solid. Despite the potential benefits of α -chitin in agriculture, it is hard to reliably deliver, to the plant, in a readily available form. α -Chitin and β -chitin differ in their crystalline structures. α -Chitin forms anti-parallel chains while β -chitin forms parallel chains. As a result, β -chitin is much more soluble, more bioactive, and more bioavailable (Cabrera-Barjas, et al. 2021).

 β -chitin, which is found in squid, is incredibly rare. In fact, it is only found in squid, marine worms, and other uncommon marine animals. Squid is the only natural, commercially viable, source of β -chitin. The total amount varies by species, but it is primarily found in their pen. The pen, which is the ancient remnant of a shell, is an internal, somewhat flexible, structural support organ. Studies suggest that the pen is anywhere between 26% (Pierfrancesco et al., 2009) and 40% chitin (Subhapradha, et al. 2013). The squid pen, being solid, presents a manufacturing challenge because it is hard to break down, or dissolve, into a liquid product. This is why other squid-based products typically remove the pens (and thus β -chitin) from their products. At GreenFlowCorp we have developed a proprietary process to hydrolyze squid pens, creating a β -chitin enriched product. Due to its rarity, there have been very few agricultural studies specifically on the β -form of chitin. Greenflow Corp. has executed several third-party studies on our Squid Juice product, that highlight the benefits of our β -chitin enriched products on crop yield, quality and performance.

Key points:

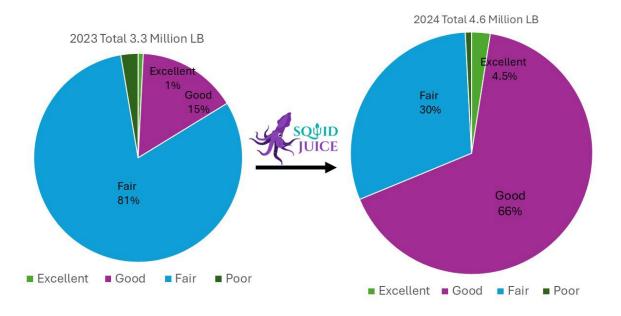
- Both chitins are good, but β -Chitin is more effective and much rarer than α -chitin.
- We have one of the very few products on the market that offer β-chitin formulated for agricultural products.

Our Findings

BLUEBERRY

Our β-Chitin enriched Squid Juice product was tested by one of the largest Blueberry Cooperatives in the United States, located in Georgia. This research was undertaken in conjunction with the University of Georgia. The preliminary results were astounding. When compared to the prior year (2023), fields that received regular, foliar, applications of the Squid Juice product showed a 27% increase in total fresh blueberry weight far outpacing the17% increase observed by adjacent farms and fields in the cooperative's region.

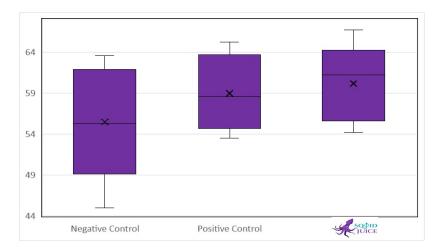




Moreover, in addition to the substantial increase in yield, a massive shift in quality was observed. The total percentage of blueberries that received a "good" rating increased from 15% in 2023 to 66% in 2024 when the same fields received the Squid Juice product. Likewise, the percentage of blueberries receiving the "Excellent" rating increased from 1% to 4.5% while those that received the "Fair" and "Poor" ratings decreased from 81% to 30% and 5% to 1.5%. As a result of these dramatic improvements in quality, only a single pallet was returned in 2024.

<u>SOYBEAN</u>

Third-party replicated field trials were conducted on soybean by '*Center of Grain Research*' (Cérom), in Québec, Canda. Three treatments were compared: No fertilizer (Negative Control), standard synthetic fertilizer (positive Control, 17.8 lbs phosphorus per acre) and a Squid Juice Treatment in which 25% of their standard fertilizer treatment was displaced with two foliar applications of the Squid Juice Product at the rate of 1.5 gallons per acre.



Although all plots were productive for the region, plots that received the β-Chitin enriched Squid had an 8% yield increase relative to the negative control (4.7 bushels an acre) and a 6% yield relative to plots that received the recommended dose of synthetic fertilizer (3.5 bushels an acre). Note that this increase happened in conjunction with a 25% decrease in synthetic fertilizer.

CORN

Foliar and soil application of Squid Juice resulted in consistent yield increases of three bushels an acre at two different sites. Trials were conducted by third-party research organizations in Woodland, California, USA and Ontario, Canada. Each condition was replicated three times.



As shown above, treatments with the Squid Juice product resulted in significantly greater yield, kernel set, and kernel row length. Positive results were obtained from both soil drenches and foliar applications. Yield was shown to increase with increasing concentrations of the Squid Juice product.

SCIENTIFIC LITERATURE

Andronopoulou, Evi, and Constantinos E. Vorgias. "Multiple components and induction mechanism of the chitinolytic system of the hyperthermophilic archaeon Thermococcus chitonophagus." *Applied microbiology and biotechnology* 65 (2004): 694-702.

Cabrera-Barjas, Gustavo, et al. "Utilization of marine waste to obtain β-chitin nanofibers and films from giant Humboldt squid Dosidicus gigas." *Marine drugs* 19.4 (2021): 184.

Chang, Wen-Teish, Yu-Chung Chen, and Chia-Ling Jao. "Antifungal activity and enhancement of plant growth by Bacillus cereus grown on shellfish chitin wastes." *Bioresource technology* 98.6 (2007): 1224-1230.

Fan, Zhaoqian, et al. "The bioactivity of new chitin oligosaccharide dithiocarbamate derivatives evaluated against nematode disease (Meloidogyne incognita)." *Carbohydrate polymers* 224 (2019): 115155.

Hirano, Shigehiro, et al. "Chitin biodegradation and wound healing in tree bark tissues." *Journal of environmental polymer degradation* 4 (1996): 261-265.

Li, Kecheng, et al. "Chitin and chitosan fragments responsible for plant elicitor and growth stimulator." *Journal of Agricultural and Food Chemistry* 68.44 (2020): 12203-12211. Winkler, Alexander J., et al. "Short-chain chitin oligomers: Promoters of plant growth." *Marine drugs* 15.2 (2017): 40.

Maksimov, I. V., R. R. Abizgil'Dina, and L. I. Pusenkova. "Plant growth promoting rhizobacteria as alternative to chemical crop protectors from pathogens." *Applied Biochemistry and Microbiology* 47 (2011): 333-345.



Messerli, Mark A., et al. "Construction and composition of the squid pen from Doryteuthis pealeii." *The Biological Bulletin* 237.1 (2019): 1-15.

Morganti, Pierfrancesco. "Chitin Nanofibrils in skin treatment." J. Appl. Cosmetol 27 (2009): 251-270.

Shamshina, Julia L., et al. "Agricultural uses of chitin polymers." *Environmental Chemistry Letters* 18.1 (2020): 53-60.

Sharp, Russell G. "A review of the applications of chitin and its derivatives in agriculture to modify plantmicrobial interactions and improve crop yields." *Agronomy* 3.4 (2013): 757-793.

Sid Ahmed, A., et al. "Effect of chitin on biological control activity of Bacillus spp. and Trichoderma harzianum against root rot disease in pepper (Capsicum annuum) plants." *European Journal of Plant Pathology* 109 (2003): 633-637.

Shibuya, N., and E. Minami. "Oligosaccharide signalling for defence responses in plant." *Physiological and Molecular Plant Pathology* 59.5 (2001): 223-233.

Subhapradha, Namasivayam, et al. "Preparation of phosphorylated chitosan from gladius of the squid Sepioteuthis lessoniana (Lesson, 1830) and its in vitro antioxidant activity." *Bioactive Carbohydrates and Dietary Fibre* 1.2 (2013): 148-155.

Yu, Ting, et al. "Effect of chitin on the antagonistic activity of Cryptococcus laurentii against Penicillium expansum in pear fruit." *International journal of food microbiology* 122.1-2 (2008): 44-48.