

Low Usage Rate

**Effective Suppression/Control of
Plant-Parasitic Nematodes**

Apply at ANYTIME in the Growing Season

**Apply to Turf, Fruit, Vegetables,
Ornamentals, and Row Crops**

NEMASAN[®]

NEMATODE SUPPRESSION / CONTROL



EPA Registered

**Manufactured Exclusively For
The Pioneer in Agricultural Chitosan Technology**

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What is Nemasan?

Nemasan is an EPA-registered (# 92032-1) nematocide product from the Organisan Corporation. Nemasan is a unique, natural biochemical nematocide treatment formulation featuring Chitosan and Quillaja extract. Nemasan Chitosan and Quillaja extract have multiple mechanisms of effectiveness, acting both directly and indirectly in synergy to combat phytoparasitic nematodes. Nemasan Chitosan and Quillaja are obtained from commercially sustainable sources. Nemasan provides effective seasonal protection against many plant parasitic nematodes and plant diseases to give your crops the proper protection needed. Nemasan also helps establish a vigorous root system that results in greater yields, more uniform foliage, and overall enhanced plant health.

Nemasan: Your effective go to nematocide!

When choosing your nematocide, you may want to consider the following. Can your nematocide work with beneficial soil microbes to attack nematodes? Can your nematocide work with plant defense systems to influence plant parasitic nematode viability and reproducibility? Does your nematocide have the lowest REI allowed by EPA? Does your nematocide have active ingredients classed by the FDA as GRAS or active ingredients that are EPA exempt from the requirement of a tolerance? Is your nematocide miscible with most pesticides, adjuvants and nutritionals? Can your nematocide be applied without cumbersome PPE? Is your nematocide made in the USA from renewable, biodegradable, natural materials? Are you using an EPA-registered nematocide that costs you \$15/acre? You can have all of the above with Nemasan!



Chitosan

Chitosan is a natural fiber very similar to cellulose. Chitosan is derived from a parent substance, chitin, via a process called deacetylation. Most Chitosans are sourced predominantly from shellfish. Chitin is the second-most abundant biopolymer on the planet after cellulose. It is estimated that 10^{11} metric tonnes are produced per year globally. There are no accumulations of Chitosan or chitin in the environment as all of it gets recycled via biodegradation back through carbon and nitrogen cycles. Chitosan differs from cellulose in having a functional amine group ($-NH_2$). Chitin is fairly insoluble while Chitosan is soluble in mild solvents like weak organic acids. This facile solubility makes Chitosan a more valuable and applicable material than chitin. In acidic conditions, Chitosan becomes positively charged (cationic) and this charge is the basis of many of the polymer's activities. The polymeric nature, multiple amine groups and positive charge classify Chitosan as a polycationic aminopolysaccharide.

Quillaja

The Quillaja used in Nemasan is an extract from the soapbark tree *Quillaja saponaria* Molina. Plant saponins are either based on a steroidal or non steroidal (triterpene) core. Quillaja saponins are of the latter type and several forms are found

in Quillaja extract. The saponins are amphipathic meaning they have parts that are hydrophobic and other parts are hydrophilic. This amphipathic characteristic affords saponins a foaming ability and secondly, a surfactant activity. The latter is a very useful feature for agricultural applications. The different Quillaja saponin forms are identified generally by the type and quantity of the particular hydrophilic groups and where they attach to the triterpene core. The hydrophilic parts contribute to the surfactant properties and the triterpene core exhibits more of the pesticidal functions.

Plant-parasitic Nematodes

Plant-parasitic nematodes are divided into two main categories depending on their feeding mechanisms being either ectoparasitic or endoparasitic. There are around 27,000 known species occupying every known terrestrial and marine habitat, including polar and tropical regions as well as high and low elevations. Around 10 percent of this total are nematodes known to be parasites of plants. Estimates are that annual total crop production losses caused by plant-parasitic nematodes are 8.8%-14.6% which equates to \$100-\$150 billion worldwide. Plant-parasitic nematodes are one of the most devastating plant pests by utilizing more than 4,000 higher terrestrial plants as the main nutrition source. Nematodes feed on all parts of the plant, including roots, stems, leaves, flowers and seeds. One common feature of their feeding is a specialized spear structure called a stylet. Many plant-parasitic nematodes feed on the roots of plants, in some cases causing chronic damage to the plant's root system. This damage includes reduction of root mass or distortion of root structure and/or enlargement of the roots. This form of damage reduces the plant's ability to absorb water and nutrients. Additionally, this damage of the plant's root system also provides an opportunity for other plant pathogens to invade the root and thus further weaken the plant. Plant-parasitic nematodes also cause direct damage to other plant tissues by shoot-feeding. This damage also results in reduced vigor, distortion of plant parts, and death of infected tissues depending upon the nematode species.

Nemasan Chitosan

To better understand the various ways in which Nemasan Chitosan acts to provide protection to your crops, it is useful to have some appreciation of natural processes occurring with soil microbiota around the root zone. Chitosan's beneficial effects on plants can either be direct and/or indirect. The following discussion provides a basic, non-holistic description of a very complex ecosystem. No claim is made that Nemasan can cause everything mentioned. Rather, Nemasan facilitates and helps to establish conditions that are more favorable for beneficials and less favorable for pathogens. Briefly, Chitosan acts in an indirect manner through a variety of actions that mimic and enhance the natural processes taking place. In soil applications, Chitosan can activate certain beneficial microbes. This class of microbes, termed "chitinolytic" can recognize and digest Chitosan by producing enzymes called chitinases. In other words, the result is the soil levels of these chitinolytic enzymes increase, creating conditions that are unfavorable to pathogenic organisms. These microbes that produce chitinolytic enzymes are resistant to chitin and Chitosan. Nemasan Chitosan is not directly nematocidal but positively influences the soil-beneficial microbiota. Exogenously-



applied Chitosan (Nemasan) mimics the natural processes by modulating and influencing the existing native microbial communities associated with nematode control, especially those with chitinolytic activity. Additionally, some of the microbes that respond favorably to Chitosan also produce substances that positively influence the plant responses to biotic and abiotic stresses. The net effect is a shift towards beneficial microbes and creation of an environment in and around the root zone that is detrimental to nematode and microbial-pathogen establishment. Even in chronic situations, treatment with Nemasan has reversed severe infestations when more established treatments have come up short. In situations where fields have experienced successive seasons of synthetic nematicides, soil biology has been decimated. Initial Nemasan treatments may not be effective as there is a paucity of beneficial microbes available for Nemasan to interact with and exert its full effects. Because of this Nemasan does not work as well initially in soils that are sterilized such as that frequently used in greenhouses or soils that have experienced generations of synthetic nematode applications.

Chitosan and Chitinases

There are residual chitinous residues in the soils from fungal and arthropod cadavers and these constitute a portion of soil organic matter. Nematode eggshells also contain chitin. This collective residual material is not only an important organic carbon and nitrogen source, it also affects the structural composition of soil bacterial communities and contributes to natural soil suppression of plant pathogens. A wide variety of bacteria, some actinomycetes and fungi in soil are involved in the degradation of chitin and Chitosan. Example bacterial genera include *Bacillus spp.*, *Serratia spp.*, *Streptomyces spp.* and *Pseudomonas spp.*

With nematodes, there are at least three targets for chitinase attack. Nematode eggshells, pharynx and feeding stylet, as all have chitin components. Chitinases in the soil can attack eggshells directly causing premature hatch and resulting in fewer viable juveniles. Females and juveniles can ingest chitinases. Chitinase damage to the stylet can affect feeding. Ingested chitinases can attack the internal pharynx. The resulting damage can be lethal to embryonic nematodes and/or leading to females laying defective eggs and/or causing moulting failure. The net result is interruption of the lifecycle via increased egg mortality, interrupted feeding and overall decreased numbers and reduced active nematodes in the root zone.

Modes of action-Chitosan: Beneficials Synergy

In essence, Chitosan fosters a symbiotic mutualism between plants and beneficial microbiota. Chitosan application promotes beneficial fungi and bacteria that are chitinolytic. Bacteria are more populous and establish quicker than fungi. Bacteria are more opportunistic, exploiting resources where they find them. In this regard, bacteria treat Chitosan and soil chitin as valuable organic substrates of carbon and nitrogen. Some beneficial fungi are nematophagous (nematode eating) and act as nematode control agents. There are approximately 700 species of nematophagous fungi. Of these, five groups are recognized based on how they attack nematodes. These are nematode trappers or predators, egg parasites, endoparasites, toxin-producers or those forming special structures to attack nematodes. Common examples include *Pochonia chlamydosporia*, *Trichoderma harzianum* and *Hirsutella minnesotensis*. The manner of attack is not focused solely on chitin. These fungi produce a variety of hydrolytic enzymes like proteases along with chitinases targeting multiple components of nematodes. *P. chlamydosporia* parasitizes eggs of *Meloidogyne spp.*, *Heterodera spp.*, *Globodera spp.*, *Nacobbus spp.* and *Rotylenchulus spp.* among others. *P. chlamydosporia* has already been used to manage nematodes in crops such as tomato, potato, soybean, cucumber, banana and beetroot. *T. harzianum* directly infects and kills nematode eggs and juveniles, particularly *Meloidogyne spp.*



H. minnesotensis is an endoparasite controlling cyst nematodes, particularly juveniles (J2) in soybeans. Their spores attach to the outside and penetrate the internal tissues. The fungus feeds off of juveniles, killing them and producing more conidia (spores) to repeat the lifecycle. All three show some commonality in that their actions are synergistic with Chitosan where the combined activity is greater than the fungus or Chitosan acting alone. All are resistant to Chitosan exposure and this is partially due to their plasma membranes having a low content of polyunsaturated free fatty acids. They are all chitinase producers and the abundance of these fungi increase with Chitosan application. This would suggest that Chitosan may play a role in suppressing competing organisms and/or promote these chitinolytic microbes.

The favorable balance of beneficials over pathogens via Chitosan application also has indirect benefits back to the



Nemasan Quillaja

To date, Quillaja extract has at least 100 different identified saponins. In addition to saponins, the extract also contains polyphenolics and oligosaccharides and these provide additional, non-nematicidal benefits. Quillaja saponins have a hydrophobic triterpene core and attached to this are a variety of hydrophilic side chains. This amphipathic nature imparts several activities to the saponins.

Quillaja saponins act as a natural wetting agent, reducing the surface tension of water and helping it penetrate hydrophobic mediums like dried-out soil and peat or coco coir-based substrates. This improved infiltration and moisture retention helps combat issues like poor soil health and water scarcity which is especially beneficial for drought-prone areas.

Studies have shown that Quillaja extract can increase soil water retention by approximately 25%. By improving soil health and structure, Quillaja saponins indirectly enhance nutrient uptake by plants. Improved water distribution and soil biology facilitate the movement and availability of nutrients for plant roots. Saponins can positively influence soil microbial communities, contributing to overall soil health and promoting a thriving ecosystem within the soil. Quillaja saponins, particularly triterpenoid saponins, have been shown to improve soil structure. While the exact mechanism might be complex, it's thought that their influence on soil biology and water dynamics contributes to this improvement, providing additional benefits for plants and agriculture. Quillaja saponins are effective as a natural wetting agent and can significantly improve the efficacy of foliar sprays by enhancing spreading and leaf coverage. Saponins can act as elicitors, triggering the plant to recognize them as a threat and activate its defenses. Saponins can activate a plant's natural immune system, known as systemic acquired resistance (SAR), potentially increasing their resistance to pathogens and pests. Saponins can activate the salicylic acid (SA) signaling pathway, a key component of SAR, which in turn leads to the production of various defense-related compounds and proteins. Quillaja extracts can stimulate plant growth and development, including promoting root development and enhancing resilience against environmental stressors like drought and heat.

The saponins exert a direct toxic effect on parasitic nematodes, causing cellular breakdown and affecting their motility. Quillaja saponins can also act as repellents and deterrents, discouraging nematodes from infesting plant roots. They can limit nematode development past the egg stage, leading to a reduction in nematode populations over time. Being a botanical extract, Quillaja saponins are considered a more sustainable and environmentally conscious approach to nematode control, posing less harm to humans, non-target organisms, and the environment.

Although the precise mechanisms underlying the nematicidal activity of Quillaja saponins remain under investigation, several pathways have been proposed. Owing to their amphiphilic nature, saponins can interact with biological membranes by inserting into the lipid bilayer. Cholesterol plays a significant role in cell membrane composition, particularly in the plasma membrane, where it can account for

plant. Beneficials minimize potential harm to the plant by space and resource competition, by providing higher nutrient and water uptake to the plant, or by modifying the root morphology and/or rhizosphere interactions, that constitutes an advantage for plant growth. *P. chlamydosporia* colonizes plant roots endophytically, stimulating plant defenses by induction of salicylic and jasmonic acid and favors plant growth and development. Similarly with *T. harzianum*, the fungus establishes itself in the rhizosphere, penetrates and colonizes plant roots. This "infection" is actually beneficial as this affords the plant greater access to water and nutrients. In addition to improving nutrient uptake by the plant, *T. harzianum* and *P. chlamydosporia* stimulate plant growth by producing their own phytohormones and phyto regulators. These fungal products function identically to their plant counterparts, influencing various stages of plant development.

Combinations of Chitosan and biocontrol (entomopathogenic and nematophagous) fungi are currently being developed for enhancing fungal pathogenicity and plant defense abilities. Nemasan is an excellent Chitosan source for this strategy and has demonstrated not only exceptional compatibility but impressive efficacy with these biocontrol fungi and bacteria.

Foliar Applications

Foliar applications of Nemasan mimic the fungal infections and plants respond to this exposure by producing several defense-related enzymes, including chitinases. Naturally, plants release chitinases as part of their defense mechanism against various pathogens and plant-parasitic nematodes. Induction of plant defenses by Chitosan also reduces the severity of root-knot nematodes. In addition to defense enzymes, Chitosan also induces plants to synthesize defense related hormones like jasmonic acid, which is a signaling molecule. Jasmonic acid targets the phenylpropanoid pathway, upregulating it to generate higher levels of numerous secondary products, including flavonoids. Flavonoids are detrimental to nematodes in numerous ways, including as a direct kill, repellent and inhibitor of egg hatch. Some flavonoids accumulate at nematode feeding sites and they may negatively affect nematode fertility and fecundity by limiting egg production. Flavonoids can also influence the ratio of males to females, as more females are formed under abundant nutrition and vice versa.

up to 40% of the lipids. This high concentration of cholesterol is crucial for maintaining membrane structure, flexibility, and various cellular functions. Nematodes are incapable of *de novo* cholesterol biosynthesis and must acquire sterols from dietary sources. This makes cholesterol a vital nutrient for nematodes. This interaction by Quillaja saponins disrupts membrane integrity, increases permeability, and causes leakage of cellular contents, ultimately leading to cell lysis and nematode mortality. Quillaja saponins may impair cholesterol metabolism by interfering with biosynthetic pathways or by forming insoluble complexes with sterols, thereby inhibiting absorption and utilization. Disruption of cholesterol availability could impair nematode egg development and larval viability.

The nematode cuticle, composed primarily of collagen and other structural proteins, serves as a critical protective barrier. It has been hypothesized that saponins interact with cuticle components, leading to degradation or destabilization of its structural integrity, thereby weakening the nematode's defenses. Triterpenoid saponins have been observed to induce ultrastructural alterations in nematode larvae, including damage to multiple cellular components, which compromises essential cellular processes and development.

In addition to direct nematicidal effects, Quillaja saponins may exert indirect influences through their immunomodulatory properties. In plants, saponins are known to activate defense responses, which could enhance resistance to nematode infection and contribute to population suppression.

In summary, the nematicidal action of Quillaja saponins likely involves multiple complementary mechanisms: disruption of cell membranes, interference with sterol metabolism, structural compromise of the cuticle, alteration of subcellular architecture, and induction of host-plant defenses. Further research will clarify the relative contributions of these pathways across different nematode species.

The importance of pH

pH is a numerical scale describing acidity (or alkalinity) in aqueous solutions. pH is a term utilized by many who perhaps do not understand what the term or values mean. pH is an indication of the concentration of hydrogen ions (H^+) generally in an aqueous solution. The pH scale runs from 0 (most acidic) to 14 (most alkaline). The smaller the pH value the more acidic a solution is, so the pH scale is an inverse relationship with H^+ concentration. pH values are determined from a logarithmic scale where a pH change in one whole value is a 10-fold increase (or decrease) in H^+ concentration and vice versa. This relationship is expressed as follows:

$$pH = -\log_{10}[H^+]$$

square brackets indicate concentration and the negative sign indicates the inverse relationship between pH and H^+

So, a solution of pH 4 has 10 times more H^+ than a solution at pH 5. Conversely it can be stated that a solution of pH 5 has 10 times less H^+ than a solution at pH 4. Likewise, a pH of 2 has a 10^3 (thousandfold) greater concentration of H^+ than a pH 5 solution. pH is an important consideration for agriculturalists

with regards to soil and spray tank mixtures. The same applies to Chitosan in seed treatment.

From a Chitosan perspective, pH is a very important consideration to a spray tank mixture. Chitosan is a polycation. This means it carries multiple positive charges along the length of the polymer chains. The degree of this cationic nature is dependent on pH. Basically, the more acidic, the more cationic Chitosan becomes. It is the cationic nature of Chitosan that is at the heart of its amazing variable functionality. Obviously, there has to be a balance between practicality, applicability and potential harm to target crops (very low pH). With Chitosan, increasing pH (becoming more alkaline) causes the positive charges along the polymer chain to gradually disappear. At around pH 6.2



- 6.3 a significant proportion of these charges have been removed and Chitosan solubility diminishes fairly rapidly. An important point to note is, if the polycationic property of Chitosan is deprived or reversed (for example by elevating pH), the corresponding antimicrobial (and so many other) capacities will be weakened or lost. Chitosan's effective cationic functionality occurs at pH below 5.0. This is an important consideration for the spray tank pH (Chitosan solubility) and one of the main reasons Organisan Corporation recommends acidifying spray tank pH to 5.0 or below (to ensure optimal Chitosan functionality).

About Our Company

There are a lot of things to understand about our company and its products. First, we are a market leader. We innovate. We are not a “me too” company, which seems to be the trend with our competitors. Numerous aspects separate us from others in the market. Since Chitosan is such an important raw material for us, we don’t source from the cheapest vendor. Our Chitosans are manufactured from shellfish exoskeletons that are sustainably harvested. Our Chitosans are top quality food-grade material. In addition, our Chitosans enjoy a “Generally Recognized As Safe” (GRAS) status from the U.S. FDA. Our Chitosans are also compliant with California Proposition 65, that state’s Safe Drinking Water and Toxic Enforcement Act of 1986. Chitosan quality has a significant impact on performance.

Unlike others, we do not view Chitosan as just a name or the latest trend or buzzword we have encountered. We have been in this industry since 2012, actively pioneering the way forward with our Chitosan agricultural technology. We are not at the cutting edge. Our competitors may claim to be there, but we are out in front of that edge with a file, honing it, defining it with our collective technology, experience and expertise. Chitosan is a science and a technology with a history. Chitosan is not a monolithic, one-size-fits-all material. Far from it. Organisan Corporation has understood this from the beginning and is at the forefront, developing specific Chitosan-based products for a number of agricultural applications. We pioneered Chitosan agricultural technology.

Chitosan is known to be active in many areas. For example, Chitosan is recognized as a plant-growth regulator, a plant-defense booster, an elicitor, has fungicidal and antimicrobial properties and is also employed as a sticker agent in adjuvant formulations. Chitosan works well with the natural biology of the soil and has been employed with much success with several commercial beneficial biological agents such as *Beauveria bassiana* in combatting the deleterious effects of many plant pathogens.

We don’t stop there. We constantly refine our manufacturing processes to give you top quality, functional products tailored to your needs. We leverage our proprietary manufacturing, technical expertise and technology to modify our manufacturing processes to create specifically crafted products with tailored Chitosan properties. We constantly support and improve our manufacturing with lab work, testing formulas, testing new inerts and actives, and seeking efficiencies. All this builds on the already impressive experience we have with Chitosan. Unlike our competitors, our products are not just Chitosan solutions of various claimed concentrations. Chitosan is a functional foundational raw material for us. Our products are specifically formulated and feature Chitosan as a main active component along with other actives and proprietary inerts.

Our products feature Chitosan incorporated in a formulation that maximizes its bioavailability, its penetration in the soil and plant tissues. Our proprietary inerts maximally enhance Chitosan effectiveness that is not seen with Chitosan alone. Our Chitosan-based products work in concert with many soil beneficials. Together, this combined synergy creates a far more rhizosphere-friendly environment, keeping pathogens and pests at bay. Our Chitosan products are the result of extensive research, product refinement and field testing. This means, all Chitosan products ARE NOT the same, especially products we manufacture. All of our products are manufactured at our Broussard, Louisiana, facility and shipped to you from there. We take the time and effort to consult and listen to our customers and formulate Chitosan-based products that suit their needs. And we don’t walk away. We are committed to you, our customers, and we work closely through all aspects of your crop’s growing cycle. We listen to you, advise and recommend tailored strategies with tailored products to deliver the results you want.

By working with us, this is what you can expect, and we will deliver. We are your Chitosan experts. This is not a groundless claim. We are not newcomers. The Organisan team has over 80 combined years experience under one roof that comprehensively covers Chitosan from raw material acquisition, manufacturing, processing, product development, R&D, sales, marketing, agronomy, and application. We have sales support 24/7. We back that sales team with technical sales support. We have invested the last 13 years developing products and applying them with success all over the country and internationally, on various crop types, environments and geographies. Years of consistent results from the field attest to this. We know Chitosan. We know how to manufacture it, formulate products with it, sell in the markets we service, and we know how to apply it. We maintain an in-house library of over 6,000 publications gathered from the literature. This resource is available to our personnel and we are constantly searching the literature for applications, technology and other aspects of Chitosan that apply to our business. So when you do business with us, you are not just buying another Chitosan product, you are getting that specifically formulated product along with accessing our experience and expertise. We are on your side and want nothing more than to see your success. That is our commitment to you.

So, if you’ve tried “Chitosan” products and did not see the outcome you wanted, there’s a good chance your product was not made specifically for your use or made by a company that knows what it is doing. That’s why you should invest your hard-earned dollars with a team of 80+ year’s experience backing the right product to maximize your results. And you’ll only get that knowledge and experience with the Organisan team and/or our authorized representatives.

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The Pioneer in Agricultural Chitosan Technology

Our proprietary manufacturing operations are located in Broussard, Louisiana, close to where our scientist lives. The “science” behind the formula was created, developed and is manufactured under the supervision of Dr. André Blanchard. Originally from south Louisiana, André spent most of his young life growing up in Inverness, Scotland (you’ll be treated to both accents). He attended the University of the West of Scotland getting his bachelors in Applied Biology. André went on to gain his Ph.D. in Plant Molecular Biology at the University of Exeter in southwest England. From there, he returned home in 1992.

André brings a combined 30 years experience in academia and the private sector. André has worked with industrial scale recycling technologies and specialty chemicals manufacture. Within these industries, he has gained experience in directing product and process research and development. These efforts led to the technical development of a process (now a U.S. Patent) for manufacturing a key raw material. André is also experienced in small business management, consulting, technology transfer, commercializing technologies, project management, process design and manufacturing strategies. He also initiated several collaborative projects with leading universities involving several external grant funded efforts from Federal agencies.

André’s association with Chitosan over the past 18 years has involved researching and formulating new products, designing manufacturing processes, marketing and commercialization.

André is leveraging his experiences to leading future innovations of a variety of products, and constantly improving the manufacturing process.



Dr. André Blanchard, Ph.D., Plant Molecular Biology
Vice President, Technology and Operations, Organisan Corporation
Extensive Chitosan research and application for 30 years.