

A Solution for Basal Stem Rot (BSR) Disease in Oil Palm Plantation

Ganoderma Adjuvant



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BASAL STEM ROT (BSR) DISEASES IN OIL PALM

BASAL STEM ROT (BSR) disease in oil palm is caused by the attack of a group of wood decaying fungi called Ganoderma. This fatal disease is considered as the most serious disease of oil palm where losses can reach up to 80% after repeated planting cycles.

The fungal genus Ganoderma is a group of wood-decaying fungi that are found throughout the world on all types of wood — gymnosperms, woody dicots, and palms. There are many different species of this fungus in Malaysia and Indonesia, but only one is a pathogen of oil palm. That fungus is Ganoderma boninense. Palms are considered the primary hosts of this fungus. In general, if a basidiocarp (conk) on a palm trunk is observed, it is probably safe to assume it is G. boninense and not some other Ganoderma species. Likewise, the Ganoderma species often observed on hard-wood trees, such as oak, are rarely observed on living palms. These other Ganoderma species may occur on dead palm trunks and stumps, but they are present simply as saprobes (fungi that live off dead plant material).

Ganoderma (white rot fungi) produces enzymes that will degrade the oil palm tissue and affect the infected oil palm xylem thus causing serious problems to the distribution of water and other nutrients to the top of the palm tree. Ganoderma boninense is an extraordinary organism capable exclusively of degrading lignin to carbon dioxide and water; celluloses are then available as nutrients for the fungus. Oil palm which is a highly significant crop in Malaysia and Indonesia is prone to basal stem diseases rot caused by this fungus.

Ganoderma biophysical composition and description

Fruiting Body – the fruiting body is the visible part of the Ganoderma. Most of Ganoderma's description pertains to this part of the fungi. It is the umbrella like structure common to most mushrooms. Its shade ranges from reddish purple to reddish brown. The cap-like structure is called the pileus. When young, its texture is meaty but turns woody when it matures.

Basidiocarp (Conk) - the conk can be dull red to reddish brown and sometimes black in color. It features pores on its underside, whitish, and brownish when touched. Areas of new growth are whitish, darken to yellow brown and eventually reddish brown at maturity, often with zonations of concentric growth patterns. Spores dispersed from the underside, collected on the surface of the cap giving the powdery brown appearance when dry.

- a. **Crust Layer** thick and closely arranged hyphae makes up the outermost layer. The glossy appearance of the cap is due to its thick, resin filled cell wall. Interwoven thick walled hyphae makes up the middle layer. Inside these hyphae are reddish-brown resins which produce the color of the cap. Inner layer have same interwoven structure but this time without resins or pigments. The cell wall in this layer is also thick and it is the transition stage from the crust to become flesh.
- b. **Flesh Layer** has a cork-like texture because the hyphae in this layer are pack loosely and have many voids in-between. These interwoven hyphaes contain large vacuoles.

c. Tube Layer – composed of parallel-arranged tubes of hyphae. The end of the hypha expands to basidium which is like an "eggplant" in appearance. Basidium has a thin cell wall, dense cytoplasm with two nucleuses. These nucleuses fuse, as the basidium gradually matures, through the process of karyogamy. Through mitosis, these fused nucleuses divide into four sub-nucleuses twice. Coinciding with this process is the formation of sterigmas which produces basidiospores. Upon maturation of the spores, is ejected to the air by means of mechanical force in the process called spore spreading.

Symptoms, Signs and Diagnosis

Ganoderma is a white rot fungus that produces numerous enzymes that allow it to degrade (rot) woody tissue, primarily lignin and cellulose. As the fungus destroys the palm wood internally, the xylem (water-conducting tissue) will eventually be affected. Therefore, the primary symptom that may be observed is a wilting, mild to severe, of all leaves but the spear leaf (Figures 1 and 2).



Figure 1. Oil palm with wilted and dessicated leaves due to Ganoderma boninense infection.

The basidiocarp or conk is the most easily identifiable structure associated with the fungus. The conk originates from fungal growth inside the palm trunk. Figure 3 illustrates different stages in the development of the conk. When the conk first starts to form on the side of a palm trunk or palm stump, it is a solid white mass that is relatively soft when touched. It will have an irregular to circular shape and is relatively flat on the trunk or stump.

As the conk matures, a small shelf or bracket will start to form as the basidiocarp begins to extend or protrude from the trunk. It will still be white, both on the top and bottom surfaces. Eventually, it will form a very distinct shelf-like structure that is quite hard with a glazed reddish-brown top surface and a white undersurface (Figure 4). A mature conk will have distinct zones, hence the name G. boninense. The conk will have a half-moon shape with the relatively "straight" side directly attached to the trunk.

Figure 5 shows an example of the tomography of a cross section of an infected stem. Note that in this figure the lesion appears as a light brown area of rotting tissue with a distinctive irregularly shaped darker band at the borders of this area. A yellow reaction zone is also clearly identified. As the infection moves upwards from the roots, this zone can be first observed in a cross section prior to the appearance of the light brown infected area.



Figure 2. Queen palm is dying from Ganoderma. Only the spear and one other leaf remain green.



Figure 3. Three phases of basidiocarp (conk) development of Ganoderma. The white "button" near the top of the picture is the beginning stage of the conk. The lower-right structure is a mature conk. The lower-left structure is also a mature conk, but it is an old one; the underside of this conk is no longer white.



Figure 4. Basidiocarp (conk) of Ganoderma boninense. Note glazed reddish-brown top surface and white undersurface. The "straight" side of the conk is directly attached to the trunk. There is no "stem" or "stalk" that attaches the conk to the trunk.

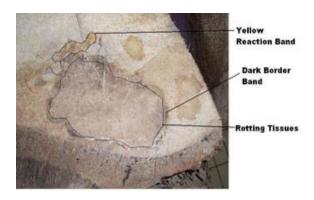


Figure 5. Cross-sections of lower trunk of oil palm stem infected Ganoderma boninense.

If a conk is present on the trunk at the same time the wilt or decline symptoms appear, then it is safe to diagnose ganoderma butt rot. However, it is not uncommon for conks not to appear prior to severe decline and death of a palm. In that situation, the only way to determine if Ganoderma butt rot is the cause is to cut cross-sections through the lower 4 feet of the trunk after the palm is cut down (Figures 5 and 6). Conks may form on the palm stump after the diseased palm is removed. Conks of G. boninense can be up to 8 inches at their widest point and 2 inches thick. However, conks will take on the shape and size of the area in which they are growing (Figure 7). Microscopic basidiospores are produced in the "pores" present on the underside of the conk. When basidiospores are dropped en mass on a white surface, they will appear brownish-red in color (Figure 8). Objects immediately around a conk that has dropped its spores may appear to be covered with a rusty colored dust. One conk can produce 3 cups of spores.



Figure 6. Comparison of oil palm sections that are healthy (left) and diseased (right) caused by Ganoderma boninense.



Figure 7. Cut palm stump with numerous basidiocarps (conks) of Ganoderma forming on it. The conks in the palm stump's center are crowding each other and thus are forming into shapes different from those on the outer edges of the stump.



Figure 8. Spore release from mature conks (same stump as Figure 7) has resulted in reddish-brown appearance of conks and surrounding area.



Figure 9. Longitudinal section through oil palm trunk and root system. The trunk is darkened due to infection with Ganoderma. The fungus is not rotting the roots but was isolated from the roots.

Disease and Fungus Life Cycle

The fungus is spread primarily by the spores produced in the basidiocarp (conk). The spores become incorporated into the soil, germinate and the hyphae (fungal threads) then grow over the palm roots. The fungus does not rot the palm roots, it simply uses the roots as a means of moving to the woody trunk

tissue (Figure 9). Once a palm is infected with G. boninense, the fungus will move with that palm to the location in which it is transplanted. It is also possible that soil associated with transplanted palms is infested with the fungus.

It is quite impossible to know exactly how many months or years pass between initial infection of a palm and development of the conk. There is no method that can determine if a palm is infected with G. boninense. Until the conk forms, there can be no confirmation of this disease. Therefore, it is not possible to guarantee that a palm is free of Ganoderma when first planted in the oil palm plantation.

Disease Management

No environmental conditions or landscape management practices have been observed that favor the development of Ganoderma butt rot. The disease occurs in natural settings (palms never transplanted) and in highly-maintained, transplanted landscapes. It occurs on palms that have been maintained very well nutritionally (no nutrient deficiencies) and on palms that were severely stressed by deficiencies. The disease occurs in well-drained settings and in swamps. The fungus has killed trees that had no apparent mechanical injuries and those that had been severely damaged by, for example, weed trimmers. Soil type appears to have no relationship with disease either, as diseased palms have been observed on deep sands (both silica and calcareous), muck (peat), and limestone rock. There has been no discernible pattern to provide clues as to why some palms become infected and die from G. boninense, and others do not.

In general, the fungus will be located in the lower 4-5 feet of trunk. This has three implications. First, this means the fungus is not spread with pruning tools since the fungus is not associated with leaves. Second, this means that only the lower trunk portion should not be chipped and used for mulch. If possible, the diseased section should be placed in a landfill or incinerated. The remaining, fungus-free portion of the palm trunk could be chipped and used for mulch in the landscape.

Third, only the lower 4-5 feet of trunk will need to be protected from the fungus. However, typical xylemlimited, systemic fungicides will not be effective unless they are capable of spreading beyond the vascular tissue and protecting all the wood in the lower portion of the trunk. We know of no fungicide with these capabilities. Also, no fungicide will be effective once the conks have formed, since a large percentage of the trunk cross-sectional area has already been destroyed. Since we have no means of predicting or determining which palms are infected with G. boninense, this effectively eliminates the use of fungicides as a control method, either preventively or curatively, for the present time. Therefore, there are no fungicide recommendations for this disease.

Since basidiospores from the basidiocarps (conks) are probably the primary method of spreading the fungus, palms should be monitored closely, especially after a palm has died or been removed for any reason. The fungus will readily colonize and degrade palm stumps (See Figures 7 and 8). Once the fungus becomes established in this dead wood, it will normally produce conks with millions of basidiospores that are easily moved by wind and water.

Therefore, monitor palms and palm stumps for the conks. Remove the conk and place in a trash receptacle that will be incinerated or delivered to a landfill. Do not place in trash that will be recycled either in the

plantation or landscape. The earlier the conk is removed (i.e., before it becomes a distinct shelf-like structure), the less likely that spores will be released into the environment. If you have never observed Ganoderma butt rot on the property, monitoring the palms once every six months will be adequate. Once you have observed the conks on palms or have a palm cut or fall down for any reason, monitor your palms at least once a month. Also, monitor the entire neighborhood, not just your yard. These spores blow with the wind, so it should be a community effort to reduce the spread of the spores of this lethal fungus.

Once a conk is observed on a palm, the palm should be removed – primarily for safety reasons. This is especially important during the hurricane season. As indicated before, if conks are being produced on a live palm, it means that a significant portion of the trunk is already rotted. These palms are likely to be the first blown down in heavy winds. As much as you may want to keep the palm, it is probably best not to do so. When you remove the palm, remove as much of the stump and root system as possible. Any palm material left behind will be a host for the fungus.

The fungus survives in the soil. It has been observed that replacement palms planted into the same site where a palm died from Ganoderma butt rot also became diseased and died. Therefore, replanting with another palm is risky. We do not know how long you should wait before it is safe to plant another palm in a Ganoderma-infested site. We can say that the time is measured in multiple years, not months, since the fungus is probably capable of living in the soil almost indefinitely.

Fungi

"To fight fungal infection, one must understand fungal function."



A **fungus** is a member of a large group of **eukaryotic organisms** that includes microorganisms such as yeasts and molds, as well as the more familiar mushrooms. These organisms are classified as a kingdom, **Fungi**, which is separate from plants, animals, and bacteria. One major difference is that fungal cells have cell walls that contain glucans and chitin, unlike the cell walls of plants, which contain cellulose. These and other differences show that the fungi form a single group of related organisms, named the Eumycota (true fungi or Eumycetes), that share a common ancestor (a monophyletic group).

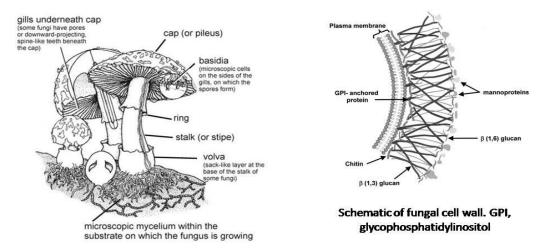
Most fungi are inconspicuous because of the small size of their structures, and their cryptic lifestyles in soil, on dead matter, and as symbions of plants, animals, or other fungi. They may become noticeable when fruiting, either as mushrooms or molds. Fungi perform an essential role in the decomposition of organic matter and have fundamental roles in nutrient cycling and exchange.

Like plants, fungi often grow in soil, and in the case of mushrooms form conspicuous fruiting bodies, which sometimes bear resemblance to plants such as mosses. The fungi are now considered a separate kingdom, distinct from both plants and animals, from which they appear to have diverged around one billion years ago. Some morphological, biochemical, and genetic features are shared with other organisms, while others are unique to the fungi, clearly separating them from the other kingdoms:

As other eukaryotes, fungal cells contain membrane-bound nuclei with chromosomes that contain DNA with noncoding regions called introns and coding regions called exons. In addition, fungi possess membrane-bound cytoplasmic organelles such as mitochondria, sterol-containing membranes, and ribosomes of the 80S type.

Fungi lack chloroplasts and are heterotrophic organisms, requiring preformed organic compounds as energy sources. Fungi possess a cell wall and vacuoles. They reproduce by both sexual and asexual means, and like basal plant groups produce spores. Similar to mosses and algae, fungi typically have haploid nuclei.

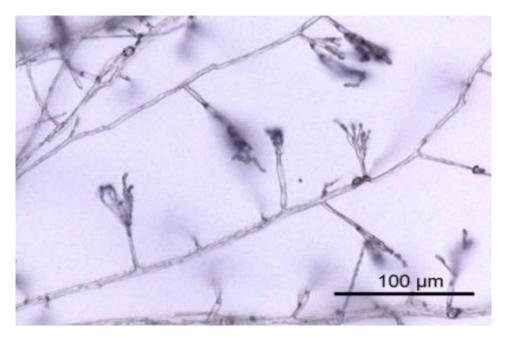
The cells of most fungi grow as tubular, elongated, and thread-like (filamentous) structures and are called hyphae, which may contain multiple nuclei and extend at their tips. Each tip contains a set of aggregated vesicles—cellular structures consisting of proteins, lipids, and other organic molecules. Fungi grow as filamentous hyphal cells.



Hyphae

A **hypha** (plural **hyphae**) is a long, branching filamentous structure of a fungus. In most fungi, hyphae are the main mode of vegetative growth, and are collectively called a mycelium; yeasts are unicellular fungi that do not grow as hyphae.

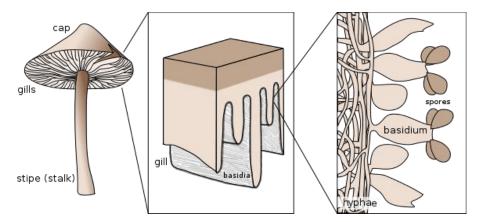
A hypha consists of one or more cells surrounded by a tubular cell wall. In most fungi, hyphae are divided into cells by internal cross-walls called "septa" (singular septum). Septa are usually perforated by pores large enough for ribosomes, mitochondria and sometimes nuclei to flow between cells. The major structural polymer in fungal cell walls is typically chitin, in contrast to plants that have cellulosic cell walls. Some fungi have aseptate hyphae, meaning their hyphae are not partitioned by septa.



Hyphae of Penicillium

Basidium

A **basidium** is a microscopic, spore-producing structure found on the hymenophore of fruiting bodies of basidiomycete fungi. The presence of basidia is one of the main characteristic features of the Basidiomycota. A basidium usually bears four sexual spores called basidiospores; occasionally the number may be two or even eight. In a typical basidium, each basidiospore is borne at the tip of a narrow prong or horn called a sterigma, and is forcibly discharged upon maturity.



Schematic showing a basidiomycete mushroom, gill structure, and spore-bearing basidia on the gill margins.

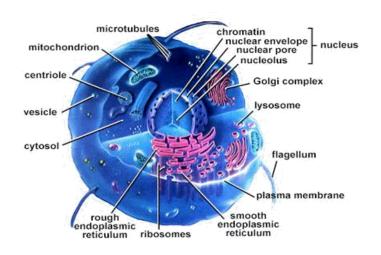
Most basidiomycetes have single celled basidia, but in some groups basidia can be multicellular. In most basidiomycetes, the basidiospores are **ballistospores**--they are forcibly discharged. The propulsive force is derived from a sudden change in the center of gravity of the discharged spore. Important factors in forcible discharge include **Buller's drop**, a droplet of fluid that can be observed to

accumulate at the proximal tip of each basidiospore; the offset attachment of the spore to the subtending sterigma, and the presence of hygroscopic regions on the basidiospore surface.

Upon maturity of a basidiospore, sugars present in the cell wall begin to serve as condensation loci for water vapor in the air. Two separate regions of condensation are critical. At the pointed tip of the spore (the hilum) closest to the supporting basidium, Buller's drop accumulates as a large, almost spherical water droplet. At the same time, condensation occurs in thin film on the adaxial face of the spore. When these two bodies of water coalesce, the release of surface tension and the sudden change in the center of mass leads to sudden discharge of the basidiospore. Successful basidiospore discharge can only occur when there is sufficient water vapor available to condense on the spore.

Differences among eukaryotic cells

There are many different types of eukaryotic cells, though animals and plants are the most familiar eukaryotes, and thus provide an excellent starting point for understanding eukaryotic structure. However, fungi and many protists have some substantial differences.



Eukaryotic cells

Animal cell

An animal cell is a form of eukaryotic cell that makes up many tissues in animals. The animal cell is distinct from other eukaryotes, most notably plant cells, as they lack cell walls and chloroplasts, and they have smaller vacuoles. Due to the lack of a rigid cell wall, animal cells can adopt a variety of shapes, and a phagocytic cell can even engulf other structures.

There are many different cell types. For instance, there are approximately 210 distinct cell types in the adult human body.

Plant cell

Plant cells are quite different from the cells of the other eukaryotic organisms. Their distinctive features are:

- A large central vacuole (enclosed by a membrane, the tonoplast), which maintains the cell's turgor and controls movement of molecules between the cytosol and sap;
- A primary cell wall containing cellulose, hemicellulose and pectin, deposited by the protoplast on the outside of the cell membrane; this contrasts with the cell walls of fungi, which contain chitin, and the cell envelopes of prokaryotes, in which peptidoglycans are the main structural molecules;
- The plasmodesmata, linking pores in the cell wall that allow each plant cell to communicate with other adjacent cells; this is different from the functionally analogous system of gap junctions between animal cells;
- Plastids, especially chloroplasts that contain chlorophyll, the pigment that gives plants their green color and allows them to perform photosynthesis;
- Higher plants, including conifers and flowering plants (Angiospermae) lack the flagellae and centrioles that are present in animal cells.

Fungal cell

Fungal cells are most similar to animal cells, with the following exceptions:

- A cell wall that contains chitin;
- Less definition between cells; the hyphae of higher fungi have porous partitions called septa, which allow the passage of cytoplasm, organelles, and, sometimes, nuclei;

Not all species of fungi have cell walls but in those that do, the plasma membrane is followed by three layers of cell wall material. From inside out these are:

- a chitin layer (polymer consisting mainly of unbranched chains of N-acetyl-D-glucosamine);
- a layer of β-1,3-glucan (zymosan) ;
- a layer of mannoproteins (mannose-containing glycoproteins) which are heavily glycosylated at the outside of the cell.

INTRODUCTION OF GANODERMA ADJUVANT

BASAL STEM ROT (BSR) diseases in oil palm is caused by the attack of a group of wood decaying fungi called Ganoderma. This fatal disease is considered as the most serious disease of oil palm where losses can reach up to 80% after repeated planting cycles. The fungus survives in the soil. It has been observed that replacement palms planted into the same site where a palm died from Ganoderma butt rot also became diseased and died. Therefore, replanting with another palm is risky. We do not know how long you should wait before it is safe to plant another palm in a Ganoderma-infested site. We can say that the time is measured in multiple years, not months, since the fungus is probably capable of living in the soil almost indefinitely.

Shemical International takes the challenge of totally new approach to destroy Ganoderma and control Basal Stem Rot diseases affecting oil palm without destroying plant health and the environment. It is a quick acting, effective, residually non- toxic and biodegradable for combating Ganoderma.

PRODUCT DESCRIPTION

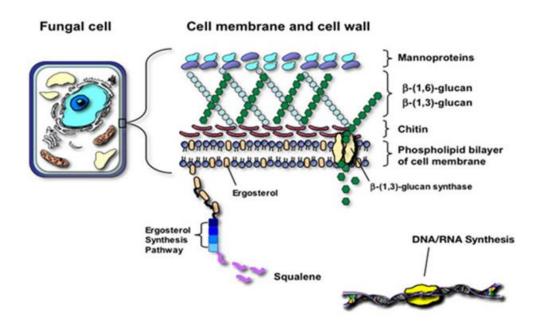
Ganoderma Adjuvant is a 100% formulation based on Safety, Health and Environment Friendly ingredients. It doesn't use any toxic chemicals, inert ingredients, halogenated hydrocarbon, enzymes, animal fatty acids, aliphatic and aromatic hydrocarbon toxic solvents, CFC and ozone depleting substances. The main ingredients are Nano Alpha 10, nano silver, food additives and plant base derivatives. In the formulation, there are various **Nano Colloidal Antifungal Agents (NCAA)** in nanometers which have unique properties and characteristics as following:

- a. Do not settle out of the suspension of gravity;
- b. Small enough to pass through the unreachable areas of the porous partitions called septa in fungi;
- c. Move in at least one dimension randomly;
- d. Have the velocity that will move efficiently;
- e. Have tremendous wetting capacity;
- f. Reduce the surface tension in water or water solutions;
- g. Have sterilizing effect by disrupting the DNA or RNA of the virus, prokaryotic cell of bacteria, and eukaryotic cell of algae, protozoa and fungi;
- h. Can bind to ergosterol in the fungal cell membrane;
- i. Resulting fungal cell in abnormally weak to withstand osmotic stress.

ANTIFUNGAL CAPACITY OF GANODERMA ADJUVANT

Fungal Cell Structure and Targets

Knowledge of fungal cell structure and function is essential for understanding the biochemical of antifungal agents. Like plant cells, fungi are eukaryotes with DNA organized into chromosomes within the cell nucleus and have distinct cytoplasmic organelles including endoplasmic reticulum, Golgi apparatus, mitochondria, and storage vacuoles. This homology to plant cells also extends to biosynthetic pathways, where fungi share similar mechanisms for DNA replication and protein synthesis.



The similarity of fungal and plant cells creates a number of problems for designing fungicides that are selectively toxic to fungal cells but not the plant host. Nano Colloidal Antifungal Agents (NCAA) currently utilized in Ganoderma Adjuvant for plant diseases attack various targets in fungal pathogens:

- A. Fungal Cell Membrane
 - Fungi and plant cells both contain a cell membrane that serves and important role in cell structure, division, and metabolism. Complex lipid particles, called sterols, account for approximately 25% of the weight of the cell membrane. However, the sterol content between plant cells and fungal cells is different. Whereas plant cell membranes contain primarily phytosterol and ergosterol is the predominant sterol in many pathogenic fungi. This difference in sterol content has been exploited as the target of several classes of antifungal agents used to treat invasive fungal infections in plants including Ganoderma disease in oil palm.

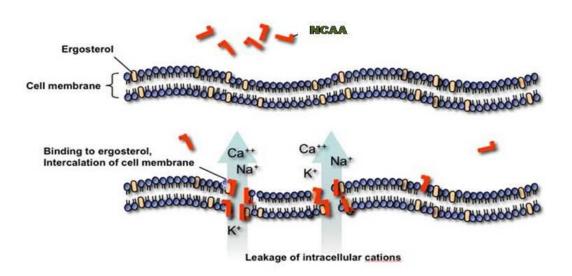
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Ergosterol

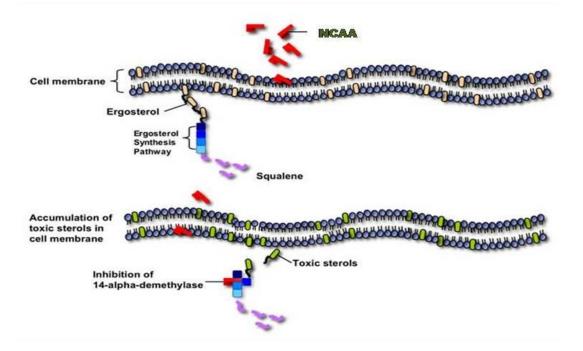
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Phytosterol

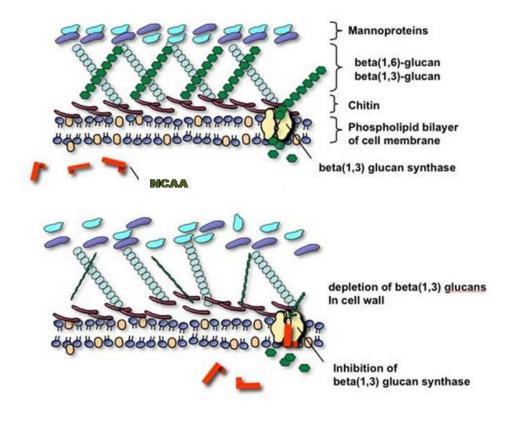
 Nano Colloidal Antifungal Agents (NCAA) act by binding to ergosterol in the fungal cell membrane. This binding results in depolarization of the membrane and formation of pores that increase permeability to proteins and monovalent and divalent cations, eventually leading to cell death. It may also induce oxidative damage in fungal cells.



 Nano Colloidal Antifungal Agents (NCAA) inhibit the fungal cytochrome P-450 3-A dependent enzyme 14-alpha demethylase, thereby interrupting the synthesis of ergosterol. Inhibition of this critical enzyme in the ergosterol synthesis pathway leads to the depletion of ergosterol in the cell membrane and accumulation of toxic intermediate sterols, causing increased membrane permeability and inhibition of fungal growth.



- B. Fungal Cell Wall
 - The fungal cell wall is critical for cell viability and pathogenicity. Beyond serving as a protective shell and providing cell morphology, the fungal cell wall is a critical site for exchange and filtration of ions and proteins, as well as metabolism and catabolism of complex nutrients. Because plant cells have walls that are strong enough to withstand the osmotic pressure, it also represents an ideal and specific target for antifungal application. Structurally, the fungal cell wall is composed of a complex network of proteins and polycarbohydrates that varies in composition depending on the fungal species. Disruption of this protein/carbohydrate matrix results in a structurally-defective cell wall, rendering the fungal cell sensitive to osmotic lysis.
 - Nano Colloidal Antifungal Agents (NCAA) present as being glucan synthesis inhibitors to block fungal cell wall synthesis by inhibiting the enzyme 1,3-beta glucan synthase. Inhibition of this enzyme results in depletion of glucan polymers in the fungal cell, resulting in an abnormally weak cell wall unable to withstand osmotic stress.





Nano Colloidal Antifungal Agents (NCAA) are able to penetrate the closely arranged hyphae make up outermost layer which is a thick resin filled cell wall.



Basidiocarp (conk) of Ganoderma outermost layer glazed (reddish-brown top surface) start to damage and deform after 30 minutes of spraying diluted Ganoderma Adjuvant.



Basidio is the microscopic cells on the bottom side of basidiocarp which spores form. It will absorbs Nano Colloidal Antifungal Agents (NCAA) into its thin wall very quickly which resulted severe damage to basidiospores.



Basidiocarp (conk) of Ganoderma under layer (white undersurface) start to damage and deform after 30 minutes of spraying diluted Ganoderma Adjuvant.



DIRECTION

Mix both SET A (900g) and SET B (1000g) of Ganoderma Adjuvant with 50-100 liters of clean water depending on the size of the Ganoderma. Spray thoroughly on the top and the bottom of the Ganoderma until it is fully wet. For better result and prevention purpose, spray the whole trunk area infected by Ganoderma.

FOR SERIOUS GANODERMA CASE: Mix both SET A (900g) and SET B (1000g) of Ganoderma Adjuvant with **50 liters** of clean water.

FOR PREVENTION OF GANODERMA: Mix both SET A (900g) and SET B (1000g) of Ganoderma Adjuvant with **100 liters** of clean water.

PRECAUTIONS

- 1. Store in original container, tightly closed in a safe place.
- 2. Wear protective gloves and face shield when handling the concentrate.
- 3. When using, do not eat, drink or smoke.
- 4. Wash hands and exposed skin before meals and after work.
- 5. Wash concentrate from skin or eyes immediately.
- 6. Wash out container thoroughly and dispose safely.
- 7. Keep away from food, drink and animal feeding stuffs.
- 8. Harmful to fish. Do not contaminate ponds, waterways or ditches with chemical or used container.
- 9. Keep out of reach of children.

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