



A Review on Chitosan Nanoparticles as a Biostimulant and Their Applications in Agriculture

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ABSTRACT

The increasing worldwide demand for food production exerts a significant strain on agriculture, which faces ongoing challenges from biotic and abiotic stressors that restrict crop growth. Chitosan nanoparticles (ChNPs) have emerged as a sustainable biostimulant alternative to chemical agro-inputs owing to their biodegradability, biocompatibility, and multifunctional biological properties. Chitosan, obtained from the deacetylation of chitin, which is largely derived from crustacean waste, can be converted into nanoparticles via ionic gelation. This process results in increased surface area, better penetration, and superior biological efficacy. ChNPs act as an effective biostimulant by activating plant defence systems, enhancing photosynthesis, improving nutrient absorption, promoting root growth, and increasing resilience to abiotic stresses such as drought, salinity, and heat. They also improve yield characteristics and postharvest quality in various crops. Moreover, ChNPs exhibit antibacterial, antiviral, antifungal, and insecticidal properties, providing robust protection against a wide range of diseases and pests. In addition to regulating plant growth, ChNPs serve as non-viral gene delivery vehicles and contribute to soil health by enhancing microbial activity and enabling controlled-release fertiliser formulations. Hybrid ChNPs-based nanocomposites that incorporate metals, polymers, microalgae, and bacteria further enhance their agricultural efficacy by improving nutrient delivery, stress tolerance, and biocontrol effectiveness. Despite considerable progress, future research should prioritise field-scale validation and optimisation of hybrid nanostructures for broader agricultural applications. ChNPs represent a versatile, environmentally sustainable nanotechnology platform with broad potential for sustainable agriculture and soil management.

Keywords: biostimulant, chitosan nanoparticles, controlled-release fertiliser, nutrient uptake



1. INTRODUCTION

The modern world faces a strong demand for food crops, putting enormous pressure on agriculture to increase output. Agriculture, on the other hand, faces significant hurdles due to biotic and abiotic factors that affect yield (Shcherban, 2023). To address these issues, agrochemicals have been utilised; however, this has led to deterioration of soil health, agroecosystem problems, environmental pollution, and the development of chemical resistance in insects and pathogens. As a result, a transition from chemical to biological sources is required to address these negative effects (Kashyap et al., 2015). Biostimulants are appropriate choices in such an environment.

Biostimulants are substances or microbes that, when applied to plants or the rhizosphere, stimulate natural processes to improve nutrient uptake efficiency, boost stress tolerance, and augment crop quality features, regardless of their nutrient content (du Jardin, 2015). They work by modulating physiological processes in plants, making them more efficient in utilising available resources and more resilient to environmental stresses (Yakhin et al., 2017). Many biostimulants are available today that benefit crops. Among them, chitosan nanoparticles are a distinctive option because they are biocompatible, biodegradable, and eco-friendly (Nandini et al., 2025). Research has demonstrated that chitosan application can enhance photosynthetic efficiency, improve water relations, increase nutrient uptake, stimulate root growth, and boost the production of beneficial secondary metabolites. All these effects make chitosan a suitable biostimulant in agriculture (Emami Bistgani et al., 2017).

Chitosan is a linear polysaccharide obtained by deacetylation of chitin and can be extracted from fungal cell walls and crustacean shells (Orzali et al., 2017). The properties of chitosan, such as biocompatibility, non-toxicity, biodegradability, plant growth regulation, antimicrobial activity, and stress inhibition, make it suitable for agricultural applications (Ingle et al., 2022; Sangwan et al., 2023). When formulated into nanoparticles, chitosan exhibits enhanced surface area, improved penetration capabilities, and superior biological activity compared to its bulk form (Kumaraswamy et al., 2018). That is why the nanoparticle form is preferred over the bulk form.

This review highlights the characteristics and extraction methods of chitosan nanoparticles (ChNPs), emphasising their applications in crop production and soil conditioning. It also explores their potential for gene delivery and the exciting possibilities of creating hybrid ChNPs by combining organic and inorganic agents.

2. CHITOSAN: AN OVERVIEW OF THE EXTRACTION, STRUCTURE AND PROPERTIES

Chitosan is obtained from chitin, a structural polysaccharide that is the primary component of arthropod exoskeletons, especially in crustaceans such as prawns, crabs and lobsters, as well as in the cell walls of fungi and certain algae (Elieh-Ali-Komi & Hamblin, 2016). The chitosan used in industrial applications is predominantly sourced from crustacean shells, particularly those of crabs, shrimps, and prawns, which are abundant byproducts of the food processing sector (Pellis et al., 2022). The global seafood processing sector produces millions of tonnes of crustacean shell waste each year, providing an abundant, renewable source for chitin extraction (Xu et al., 2013). The commercial manufacture of chitosan from this waste stream is an outstanding example of circular economy principles, transforming what would otherwise be discarded material into valuable products (Kandra et al., 2012).

In crustacean shells, chitin is complexed with protein and calcium carbonate, and several stages are involved in the extraction of chitin and its conversion into chitosan (Younes & Rinaudo, 2015).

The synthesis of chitosan from crustacean shells includes multiple consecutive procedures (No & Meyers, 1995). *Figure 1* demonstrates the process. First, shells are demineralised using mild hydrochloric acid to remove calcium carbonate and other minerals. The proteins are then deproteinised with sodium hydroxide, and the chitin is obtained (Abdou et al., 2008). The resultant chitin is subsequently deacetylated, often using concentrated sodium hydroxide at high temperatures, converting N-acetyl-glucosamine units to glucosamine units (Sini et al., 2007). The degree of deacetylation, which represents the proportion of deacetylated units, is a crucial characteristic that determines chitosan's properties and biological activities.

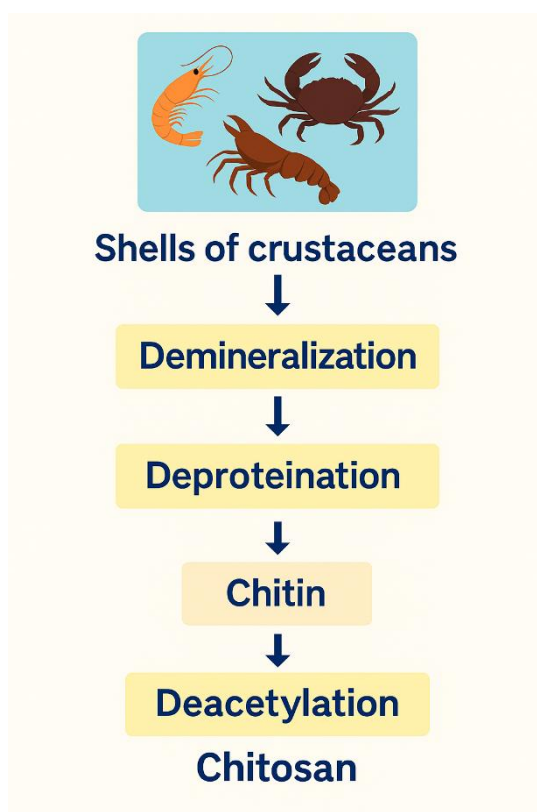


Figure 1: Extraction procedures of chitosan from crustacean shells

Chitosan is a linear polysaccharide consisting of randomly arranged β -(1 \rightarrow 4)-linked D-glucosamine and N-acetyl-D-glucosamine units. The chemical structure comprises three functional groups: a primary hydroxyl group at C-6, a secondary hydroxyl group at C-3, and an amino group at C-2 (Aranaz et al., 2021). These functional groups, particularly the amino group, are responsible for chitosan's distinctive properties and its capacity to undergo various chemical modifications (Mourya & Inamdar, 2008). The presence of free amino groups endows chitosan with polycationic properties in acidic conditions, distinguishing it from its parent molecule, chitin, which is electrically neutral (*Figure 2*).

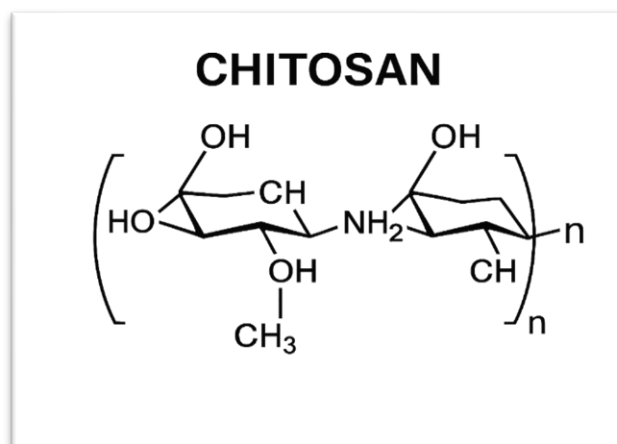


Figure 2: Structure of chitosan

Chitosan's molecular weight varies widely depending on source and manufacturing method, typically ranging from 10 to 1000 kDa. The physicochemical properties of chitosan, such as viscosity, solubility, and biological activity, are greatly influenced by its molecular weight. Lower molecular weight chitosan has greater water solubility and cellular permeability than high molecular weight chitosan (Zhang & Neau, 2001).

3. CONVERSION OF CHITOSAN INTO NANOPARTICLE FORM

The transformation of chitosan from bulk polymer to nanoparticle form is a pivotal innovation that markedly enhances its functional capabilities and broadens its applicability across several domains (Sreekumar et al., 2018). Nanoparticles ranging from 1-100 nanometres display superior characteristics attributable to their elevated surface area-to-volume ratio, enhanced bioavailability and cellular absorption, improved penetration of biological barriers, regulated and sustained release capabilities, and increased stability and protection of encapsulated substances (Hoang et al., 2022; Sreekumar et al., 2018). The ionic gelation method is the most widely used procedure for this conversion.

The ionic gelation process involves dissolving chitosan in an aqueous acidic solution to yield chitosan cations, which are subsequently added to a polyanionic tripolyphosphate (TPP) solution while continuously stirring. Chitosan undergoes ionic gelation and precipitates into spherical particles due to the complexation of opposing charges (positive and negative). Cross-linking chitosan with an agent such as TPP results in inter- and intra-molecular cross-linkages due to their opposing charges, yielding chitosan nanoparticles (Gutiérrez-Ruiz et al., 2024; Hoang et al., 2022).

4. BIOSTIMULANT ACTION OF CHITOSAN NANOPARTICLES IN AGRICULTURAL CROPS

ChNPs have emerged as promising biostimulants in modern agriculture, offering sustainable alternatives to conventional chemical inputs for enhancing crop productivity and resilience. The utilisation of ChNPs in agriculture has garnered considerable interest owing to their diverse modes of action, which encompass eliciting plant defence responses, stimulating growth and development, augmenting nutrient uptake, and enhancing stress tolerance (Kashyap et al., 2015). ChNPs can be administered by several means, including seed treatment, foliar application, soil soaking, and root immersion. These application techniques provide accurate and



effective delivery of ChNPs, optimising their protective advantages while reducing environmental impact (Nandhini et al., 2025).

4.1 Elicitation of plants' defence by ChNPs

ChNPs are recognised by plant cell receptors as pathogen-associated molecular patterns (PAMPs), triggering a series of defence signalling pathways (Pichyangkura & Chadchawan, 2015). Chitosan, upon detection by pattern recognition receptors (PRRs) on the plant cell surface, triggers the generation of reactive oxygen species, which act as secondary messengers in defence signalling and activate mitogen-activated protein kinase cascades (Iriti & Faoro, 2009). These signalling events lead to the upregulation of defence-related genes and the production of pathogenesis-related proteins, such as chitinases, β -1,3-glucanases, and peroxidases, which collectively bolster the plant's resistance to pathogen infection (Hadwiger, 2013).

Research has shown that ChNPs treatment elicits systemic acquired resistance (SAR) in multiple crops, conferring enduring immunity against future pathogen assaults (Benhamou & Thériault, 1992). The induction of defence responses by ChNPs has been demonstrated to augment cell wall thickness via increased lignification and callose deposition, thereby forming physical barriers against pathogen invasion (El Hadrami et al., 2010).

4.2 ChNPs in plant growth and development

ChNPs play a key role in the growth and development of several plants. Chitosan foliar spray enhances okra growth, yield, and photosynthesis rate (Mondal et al., 2012). In strawberry plants, the treatment helps boost leaf growth, yield, and root biomass (El-Miniawy et al., 2013).

At the molecular level, chitosan nanoparticles enhance the expression of genes involved in photosynthesis, carbon metabolism, and nitrogen assimilation, thereby improving overall plant metabolic efficiency (Abuelsoud et al., 2023). Furthermore, chitosan enhances the activity of essential enzymes in nitrogen metabolism, including nitrate reductase and glutamine synthetase, hence promoting superior nitrogen utilisation and protein synthesis (Górnik et al., 2008).

4.3 Nutrient uptake stimulation

ChNPs have demonstrated the capacity to enhance the bioavailability of vital macro- and micronutrients by inhibiting their precipitation or fixation in soil and facilitating their absorption by plant roots (Abdel-Aziz et al., 2016). In coffee plants, treatment with ChNPs increased uptake rates and improved biophysical activity (Nguyen Van et al., 2013).

ChNPs treatment has been shown to enhance the expression of genes encoding high-affinity nitrate, phosphate, and metal ion transporters, resulting in improved nutrient uptake in both optimal and deficient environments (Li et al., 2020). Moreover, chitosan nanoparticles enhance root growth and proliferation, specifically the formation of lateral roots and root hairs, hence augmenting the root surface area for nutrient absorption (Dzung et al., 2011). The augmented nutrient profile from ChNPs application results in superior crop quality metrics, including elevated protein levels, improved oil quality, and higher concentrations of vitamins and minerals in harvested produce (Maluin & Hussein, 2020).



4.4 Stimulation of yield

Yield is a primary concern in agriculture, and ChNPs significantly enhance production across various plants. In one study, foliar application of ChNPs on wheat grown in sandy soil increased yield compared with the control group treated with conventional fertiliser (Abdel-Aziz et al., 2016). In strawberry plants, spraying with ChNPs increases yield without affecting fruit quality (El-Miniawy et al., 2013). Another study on lupine plants under drought conditions indicates that the treatment enhances both yield quantity and quality (Bakhoum et al., 2022). From all this, it can be concluded that ChNPs are suitable for increasing yield in various plants.

4.5 ChNPs against abiotic and biotic stress

Under stress, ChNPs modulate plants' antioxidant defence system, increasing the activity of enzymatic antioxidants such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase, which collectively scavenge excess reactive oxygen species and mitigate oxidative damage. Chitosan treatment also increases the accumulation of non-enzymatic antioxidants such as ascorbic acid, glutathione, and phenolic substances, which provide additional protection against oxidative stress (Hidangmayum et al., 2019; Zeng & Luo, 2012).

During drought stress, plants treated with chitosan close their stomata, reducing water loss. It has also been observed that chitosan treatment increases abscisic acid levels, which assist stomatal closure (Iriti et al., 2009). In heat-stress conditions, ChNPs promote the synthesis of heat shock proteins, which function as molecular chaperones to prevent protein aggregation, facilitate proper protein folding, and protect cellular structures from thermal damage (Malerba & Cerana, 2016). In response to salinity stress, antioxidant enzyme activities increase and malondialdehyde levels decrease, reducing the deleterious effect of salt stress in rice plants treated with ChNPs (González et al., 2015).

The protective activity of chitosan nanoparticles against biotic stress begins with their molecular identification by plant defence systems. Through PRRs on the plant plasma membrane, plants recognise ChNPs as PAMPs or microbe-associated molecular patterns (Pichyangkura & Chadchawan, 2015). ChNPs treatments trigger the generation and transmission of signals to distant tissues, preparing the entire plant for enhanced defence responses upon subsequent pathogen attack (Malerba & Cerana, 2016).

It was discovered that ChNPs inhibit the replication of plant viruses, halting growth and spread. This may be because when chitosan nanoparticles penetrate plant tissues, they bind strongly to nucleic acids, causing various types of damage and specific inhibition (El Hadrami et al., 2010; Kulikov et al., 2006). In potato and tomato, foliar treatment with ChNPs for bacterial wilt, caused by the bacterium *Ralstonia solanacearum*, decreased bacterial populations, disrupted bacterial cell structure, and reduced disease incidence (Khairy et al., 2022). Furthermore, ChNPs can penetrate fungal hyphae and interfere with DNA transcription and protein synthesis, disrupting critical cellular processes (Saharan et al., 2013). ChNPs have been shown to act as potential insecticides against soybean pests, namely *Aphis gossypii* and *Callosobruchus maculatus*, as they drastically reduce the number of eggs laid by females (Sahab et al., 2015). Thus, ChNPs are a suitable substitute for plants under both abiotic and biotic stress.



4.6 Post-harvest application of ChNPs

ChNPs have valuable uses in postharvest management, with ChNPs-based coatings extending the shelf life of fruits and vegetables by creating a semi-permeable barrier that regulates gas exchange, reduces moisture loss, and inhibits microbial growth (Sharif et al., 2018). In bananas, they were applied to delay ripening. An increase in antioxidant and vitamin C content was also observed (Suseno et al., 2014). The application of ChNPs markedly improved the shelf life, aesthetic appeal, and many quality characteristics of refrigerated tomatoes (García et al., 2014).

5. GENE TRANSFER MECHANISM OF ChNPs

ChNPs are a popular tool for certain non-viral gene transfer techniques. Nanoparticles apply to both monocotyledonous and dicotyledonous plants, as well as all sorts of organs. Secondly, this gene-carrier form can efficiently bypass transgenic silencing by regulating the number of DNA copies associated with nanoparticles. In one experiment, they used ChNPs to transfer thionine genes into potato plants to protect them against fungal infection. As a result, the plants developed the ability to defend themselves against the fungal attack, becoming transgenic (Abdel-Razik et al., 2017).

6. SOIL HEALTH IMPROVEMENT BY ChNPs

Chitosan application can positively influence soil biological properties and overall soil health. Studies have shown that chitosan promotes beneficial soil microorganisms, including bacteria involved in nitrogen fixation and nutrient cycling, as well as fungi that form symbiotic associations with plant roots (Sharp, 2013). A study on the soil application of ChNPs showed increased microbial populations, thereby enhancing soil fertility (Divya et al., 2021). The augmentation of the microbial population was directly correlated with chitosan decomposition, indicating its impact on soil fertility (Sawaguchi et al., 2015).

Soil pollution, primarily caused by the application of chemical fertilisers, is a significant environmental concern. ChNPs can serve as a coating for this fertiliser to minimise environmental loss and regulate its release; this is known as a controlled-release fertiliser (Zahoor et al., 2025). Microencapsulation can also be achieved using ChNPs, which can protect active compounds and help control their release (García-Carrasco et al., 2023).

7. CHITOSAN NANOPARTICLE HYBRID NANOCOMPOSITES

Chitosan-based hybrid nanocomposites are formed by combining chitosan nanoparticles with various materials, including metal oxides, microalgae, bacteria, and other polymers, to enhance their functionality and broaden their agricultural applications (Kumaraswamy et al., 2018).

ChNPs-metal oxide nanocomposites are a promising category of hybrid nanomaterials for agricultural applications. Chitosan nanoparticles integrated with zinc oxide (ZnO) exhibit augmented antibacterial efficacy against plant diseases and facilitate enhanced nutrient delivery to crops (Xing et al., 2015). The integration of copper oxide nanoparticles into chitosan matrices has demonstrated efficacy in managing fungal infections while supplying vital micronutrients to plants (Saharan et al., 2015). Silver nanoparticles incorporated into chitosan matrices exhibit potent antibacterial activity and have been effectively utilised as seed coating agents to safeguard against seed-borne diseases (Shukla et al., 2013).



The combination of copper and zinc with ChNPs increased defence enzyme activity in plants (Karamchandani et al., 2024).

They can also be combined with biocontrol agents such as bacteria and microalgae to enhance their properties. Encapsulated plant growth-promoting rhizobacteria in ChNPs exhibit enhanced survival and regulated release, thereby augmenting their efficacy in fostering plant growth and safeguarding against soil-borne diseases (Divya & Jisha, 2018). In one study, microalgae were incorporated into chitosan, resulting in improved yield parameters and enhanced drought tolerance in winter wheat plants (Molnár et al., 2023).

Chitosan-alginate nanoparticles have been extensively researched for the encapsulation and controlled release of insecticides, fertilisers, and biopesticides (Kumaraswamy et al., 2018). Chitosan-cellulose nanocomposites exhibit improved water retention and have been utilized to control drought stress (Malerba & Cerana, 2019).

8. FUTURE PERSPECTIVES

ChNPs can be utilised more extensively in agriculture as an alternative to chemical biostimulants. Future studies should focus on their applications in open fields rather than in closed environments. The majority of the experiments are conducted on a small scale or under greenhouse conditions. Furthermore, there is significant potential for ChNPs in conjunction with other particles, as their combination performs more efficiently than standard ChNPs. There is also considerable scope for combining ChNPs with precision agriculture technologies.

9. CONCLUSION

Chitosan nanoparticles (ChNPs) are a highly promising, environmentally benign innovation for sustainable agriculture, offering numerous advantages beyond traditional chemical inputs. Their conversion from chitosan to a nanoscale form markedly amplifies their biological efficacy, facilitating superior plant defence activation, augmented nutrient absorption, enhanced photosynthetic performance, and heightened resilience to abiotic and biotic stressors. ChNPs enhance crop yields, elevate postharvest quality, and promote soil health by stimulating beneficial microbes and facilitating controlled-release fertiliser treatments. Their adaptability is further augmented by hybrid nanocomposites that integrate metals, polymers, microalgae, and beneficial microbes, thereby improving antibacterial efficacy, nutrient transport, and stress resistance. Moreover, their function as non-viral gene delivery vehicles creates new opportunities for sophisticated plant biotechnology. Although most existing discoveries originate in controlled laboratory or greenhouse settings, it is essential to extend research to open-field contexts to confirm their large-scale relevance. ChNPs serve as a sustainable, innovative tool that supports contemporary agriculture in addressing global food security issues while reducing ecological damage.



Kitozán nanorészecskék biostimuláns hatása és mezőgazdasági felhasználási lehetőségei: irodalmi áttekintés

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ÖSSZEFOGLALÁS

A világszerte növekvő élelmiszertermelési igény jelentős nyomást gyakorol a mezőgazdaságra, amely folyamatos kihívásokkal néz szembe a biotikus és abiotikus stresszorok miatt, amelyek korlátozzák a növények növekedését. A kitozán nanorészecskék (ChNPs) fenntartható biostimulánsként jelentek meg az agrokémiai inputok alternatívájaként, köszönhetően biológiai lebomlásuknak, biokompatibilitásuknak és multifunkcionális biológiai tulajdonságaiknak. A kitozán, amelyet elsősorban rákfélék hulladékából származó kitin deacetilezésével nyernek, ionos géllképzéssel nanorészecskékké alakítható. Ez a folyamat nagyobb felületet, jobb penetrációt és magasabb biológiai hatékonyságot eredményez. A ChNPs hatékony biostimulánsként működnek a növényi védelmi rendszerek aktiválásával, a fotoszintézis fokozásával, a tápanyag-felvétel javításával, a gyökérnövekedés elősegítésével, valamint a szárazság, sóstressz és hőstressz elleni ellenállóképeség növelésével. Emellett javítják a termésmennyiség jellemzőit és a betakarítás utáni minőséget különböző növényekben. Továbbá a ChNPs antibakteriális, antivirális, antifungális és rovarirtó tulajdonságokat mutatnak, így robusztus védelmet nyújtanak számos betegség és kártevő ellen. A növényi növekedés szabályozásán túl a ChNPs nem virális génszállító eszközként is szolgálnak, és hozzájárulnak a talaj egészségéhez a hasznos mikroorganizmusok tevékenységének fokozásával, valamint a kontrollált kiadású műtrágya-formulációk lehetővé tételével. A fémekeket, polimereket, mikroalgákat és baktériumokat integráló hibrid ChNPs-alapú nanokompozitok tovább növelik mezőgazdasági hatékonyságukat a tápanyag-szállítás, a stressztűrés és a biológiai védekezés javításával. A jelentős előrelépések ellenére a jövőbeli kutatásoknak elsőbbséget kell adniuk a hibrid nanostruktúrák nyílt terepi hitelesítésének és optimalizálásának a szélesebb körű mezőgazdasági alkalmazás érdekében. A ChNPs sokoldalú és környezetbarát nanotechnológiai platformot képviselnek, széles potenciállal a fenntartható mezőgazdaságban és a talajkezelésben.

Kulcsszavak: biostimuláns, kitozán nanorészecskék, lassan felszabaduló műtrágya, tápanyag-felvétel



Abbreviations

ChNPs: Chitosan nanoparticles

PAMPs: Pathogen-associated molecular patterns

PRRs: Pattern recognition receptors

TPP: Polyanionic tripolyphosphate

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